EDITOR'S NOTE

Chirality is fundamentally important to all life on Earth, yet its role remained largely unappreciated and unrecognized until the late nineteenth century. Since then, chirality has become a subject of an unusually wide range of multidisciplinary studies embracing biological, pharmacological, chemical, cosmetic, forensic and even outer space studies. Chirality is also one of the most frequently encountered catchwords in contemporary scientific publications. However, it suffers from much inappropriate usage and confused meanings surrounding this key concept are disappointingly commonplace.

We feel that there is a need for a forum where free discussion of the various aspects of chirality can take place, and where open dialog can foster an improved mutual understanding between scientists working in the various fields relating to chirality. This journal is certainly best suited to accommodate such a "*Chirality Forum*", where discussion-oriented papers and correspondence related to chirality in its broadest meaning can be published, and where they can be read and appreciated by the unique cross section of scientists constituting the readership of *Chirality*.

In this issue, we present our inaugural "*Chirality Forum*" article, in which the views of Pedro Cintas are offered on nomenclature and terminology relating to chirality. For future "*Chirality Forum*" articles and correspondence, we invite interested authors to respond, rebut, or present topics of their own choosing relating to stereochemistry or chirality.

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Chirality Forum

Chirality and Chemical Processes: A Few Afterthoughts

PEDRO CINTAS

Departamento de Química Orgánica e Inorgánica, Facultad de Ciencias-UEX, Badajoz, Spain

ABSTRACT Chirality and chiral have become terms that pervade a wide range of disciplines in physical and life sciences. Although such terms are precisely defined, their use often engenders confusion and ambiguity. Perhaps, the most improper use of chirality, yet widely accepted, is related to its association with stereodynamics and physico-chemical transformations, such as chiral discrimination, chiral resolution, chiral recognition, chiral synthesis, and so on. Even though this conceptual perversion has been highlighted by renowned stereochemists, it has become a recurring keyword and a hot message in modern literature. It is timely to renew the correct use and context in forums such as the present journal, adding further reflections that may help both beginners and practitioners. This short article is not intended to criticize or highlight errors, but rather to encourage a level of rigor and the use of statements, which should be universally correct. *Chirality 20:2–4, 2008.* © 2007 Wiley-Liss, Inc.

KEY WORDS: asymmetry; chirality; enantioselective; diastereoselective; stereodiscrimination; synthesis; terminology; transformation

INTRODUCTION

If nomenclature jeopardizes a scientific discipline, this should most likely be stereochemistry. Its terminology possesses a strong ideographic content, i.e., representing concepts or ideas that are intellectually apprehended as precise structural information and molecular relationships. Stereochemical nomenclature is by no means pedantic stuff as a given audience or readership should interpret a concept and its implications regardless of the context, and not what the speaker or writer chooses it to mean.

The paradigmatic misuse of stereochemical terms is best exemplified by chirality and related terms. This is largely influenced by the fact that chirality itself and the occurrence of chiral structures and morphologies have become hot topics linked to exciting and far-reaching fields, ranging from biomedicine to the origin of life. But in such an interdisciplinary scenario, the appropriate training in organic stereochemistry may be actually scarce. Some authors have already dealt with the common misinterpretation of chiral and homochiral, especially as equivalent or associated to single enantiomers.[1–4] The situation is even worse when the term chiral is accompanied by adjectives such as nonracemic intended to draw attention to an enantiomeric imbalance.[5] But, perhaps the most important semantic aberration that has found widespread use through papers and monographs, often written and reviewed by chemists and stereochemists, is the use of the word chiral, or chirality, applied to processes and reactions: chiral amplification, chiral catalysis, chiral chromatography, chiral discrimination, chiral inversion, chiral recognition, chiral resolution, chiral synthesis, chiral transformation, etc. The present note re-examines again the conceptual basis of chirality and emphasizes the proper interpretation recommended by some leading scholars and stereochemistry pioneers. Their lessons should provide a fresh look at chirality as a linguistic tool for communicating precise terminology, useful to all of us.

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CHIRALITY AND DISCRIMINATION

In their authoritative manual on organic stereochemistry, Eliel and Wilen discourage the use of chirality or chiral for the separation or transformation of stereoisomers, while such terms should be restricted to molecules and models.[5] The obvious reason lies in the concept itself, as chirality simply describes the nonidentity of two-mirror image objects by translation and rotation in a given Euclidean space (if one disregards other topological and statistical aspects). Accordingly, chirality constitutes a geometrical property and as such, it should only (let us say, strictly) applied to molecules or objects in general, i.e., chiral substrate, chiral catalyst, or chiral stationary phase. Before going any further, it should also be argued that, when applied to processes, the term chiral engenders the same ambiguous connotation as the situation with a mac-

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Correspondence to: Prof. Pedro Cintas, Departamento de Química Orgánica e Inorgánica, QUOREX Research Group, Facultad de Ciencias, Universidad de Extremadura, Avenida de Elvas s/n, E-06071 Badajoz, Spain. E-mail: pecintas@unex.es
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roscopic sample of molecules (in stark contrast to an individual entity that may be labelled as left- or right-handed). If a reaction is claimed to be chiral, it is not completely clear what we are talking about. It is not the reaction that is chiral, but the material present (substrates, products, or both). In most cases, the authors refer to an asymmetric induction or asymmetric transformation (accepted names in the IUPAC glossary, even though numerous reagents possess C_2 symmetry and are not asymmetric in an etymological sense)[6] leading to an enantioenriched sample (i.e., with complete or partial enantiomeric excess). Moreover, one often discloses that behind expressions like chiral discrimination or chiral resolution there is actually formation of homochiral or heterochiral aggregates. Note that the term homochiral does certainly imply a nonracemic sample (made up of molecules with the same sense of chirality), whereas a heterochiral sample is still chiral, but racemic. It is equally relevant in this context the conceptual difference between enantiomorphism (or enantiomerism) and chirality, again misused as synonyms. The former refers to the relationship between two nonsuperposable mirror-image objects, while chirality denotes the phenomenon that indicates the existence of such objects.[7] In the light of this observation, expressions like chiral synthesis, chiral reaction, or chiral transformation are extremely confusing as only a careful reading reveals the meaning intended by the authors; formation of a single chiral stereoisomer or, of various molecules that are chiral but they may or may not be enantiomers.

Another compelling reason to use the never-obsolete concepts of asymmetric synthesis and asymmetric transformation is their functional character. They tell us how an achiral precursor or a mixture of stereoisomers give rise to a single stereoisomer, for instance on introducing new chiral elements (e.g., reactions at prochiral centers) as well as by separation following equilibration such as in crystallization-induced enrichments. It is in such contexts that the terms enantioselective or diastereoselective appear to be the correct adjectives for the intended meaning. The term chiral has merely a structural connotation (a feature equally applicable to the cases of chiral or asymmetric centers)[8] as chirality does not involves any idealized conversion leading to a stereoisomer, but the distinction between the self and nonself; i.e., one knows what stereoisomers, chiral or achiral, may exist as the result of a given transformation.

It is also pointed out that the imperfect use of chiral discrimination, chiral recognition, or chiral amplification to name a few, stems from the fact that there is nothing chiral per se about these processes, which are exhibited by chiral substances but caused by diastereomeric, not enantiomeric interactions.[9] It is perhaps an irony that, while stereochemical education emphasizes the role of diastereomeric transition structures in asymmetric syntheses or biological activity resulting from drug-enzyme interactions,[10] research scientists have acquired a particular amnesia overlooking the origin of such molecular interactions. The molecular situation should therefore be denoted as stereoisomer discrimination (vide infra), either enantiomer or diastereomer discrimination.[11]

Yet another potential problem linked to the term chiral discrimination is that discrimination may actually occur in the presence of achiral species. Thus, enantiomer discrimination refers to measurable differences between homochiral and heterochiral interactions ($R\cdots R$ against $R\cdots S$), while diastereomer discrimination alludes to differences between the interactions of one enantiomer of a given species with the two enantiomers of a different species (i.e. $R^{I}\cdots R^{II}$ versus $R^{I}\cdots S^{II}$). Collectively, both can be denoted as manifestations of stereoisomer discrimination and revealed in both 2D and 3D spaces. However, heterochiral interactions may lead to a *meso* aggregate and as a result, enantiomer discrimination would involve a difference between chiral and achiral entities. Agranat and Sarel have suggested to replace chiral discrimination by chiral distinction invoking that, while discrimination is associated with differences between two things as being equal, distinction implies the lack of resemblance between two like things.[12] Chiral distinction might then be equivalent to diastereomeric interactions in a stereochemical context. However, as noted by these authors, these meanings appear to be rooted in human psychology and are too broad to be universally accepted in a scientific context.

Since chirality can be observed at different levels of structure and organization, processes leading to chiral supramolecules from achiral precursors or having chiroptical properties different from those of their chiral monomers are likewise viewed in the literature as examples of chiral amplification or discrimination. We however know well that the properties of chiral substances depend on the composition and proportion of their components and the state of the system, in other words, the system does not behave as the sum of the individual components. As a representative example, formation of helical structures with a particular handedness is again a manifestation of stereoisomer discrimination where homo- and hetero-chiral interactions may differ substantially with respect to their source and energy. This situation does not imply a chirality change, but a different stereochemical outcome (hence asymmetric or stereoselective, not chiral). There is no such a thing called chirality transfer as we cannot transfer handedness on shaking hands.

ARE THERE MEASURABLE CHIRALITY CHANGES?

A final point that deserves attention and discourages the use of chiral still further when transformations or syntheses are involved, refers to the chiral content. This subject obviously requires an analysis beyond the scope of this article, although a few considerations also evidence the improper usage of, for instance chiral reaction or chiral amplification. Intuitively, one could interpret that such transformations involve measurable changes in chirality content or, alternatively in symmetry contents according to the symmetry principle: the elements of symmetry of the causes must be found in the effects produced; although the effects produced may be more symmetrical that the causes that produce them.[13–16]

However, quantification of chirality in terms of chiroptical or thermodynamic parameters is not immediately

obvious as chirality is by definition a geometrical property of molecules or objects, thereby requiring an algebraic treatment of models viewed as collections of points considered without any mass. Only the magnitude of a chirality function could be used as a quantitative measure of chirality.[17,18] Arguments based on enantiomeric excesses as well as chiroptical and other spectroscopic measures as evidence of chirality contents, and hence of chiral change, amplification, or discrimination, may certainly be flawed. One should note the dichotomy between chirality per se and the physical observables that support the existence of that concept.[19] Consider for instance the fact that chiral molecules may have null optical rotation (within conventional detection); reductions in symmetry going from molecules to crystals; or processes, often highlighted as chirality memory reactions, where the enantiomer excess of substrate and product does not match at all.[20] Semantics may be particularly problematic with chiral polymers, in which a particular handedness is usually influence by the statistical domain of chiral or achiral units. However, a majority rule could lead to decreased control of the helical sense and therefore of optical activity. The conflict suggests that chirality is not an intrinsic property of the chiral moiety but rather depends on the molecular environment.[21]

Chirality has also remarkable relationships with concepts such as similarity and entropy, not usually handled in a chemical context. According to a more general symmetry evolution principle, for an isolated physical system the degree of symmetry cannot decrease as the system evolves, but either remains constant or increases; the degree of symmetry has to do with the initial and final symmetry groups, it does not refer to any symmetry of the states of the physical system as the latter evolves.[15,22] As a result, this symmetry evolution principle and the second law of thermodynamics are isomorphic; paradoxically a monotonically ascending correlation exists between entropy and degree of symmetry. Thus, even though the chirality of a system implies the lack of certain symmetry elements, chirality and degree of symmetry of an evolving system may be very different things.[18]

CONCLUSIONS

We have long misused the terms chiral and chirality to denote systems evolution and molecular dynamics, which deviate from the conceptual basis of chirality embedded in geometry. Some readers and practitioners will feel that, at least intuitively, expressions such as chiral reaction, chiral recognition, or chiral discrimination, which inundate the chemical literature, suggest any enantiomeric (perhaps stereoisomeric?) bias without major headaches. But, why ignoring the more accurate and precise terms that characterize stereoselective and asymmetric transformations, as well as the nature of molecular interactions? Chirality is certainly the hallmark of stereochemistry, but it can also be too much of a good thing. A few adjectives (enantio selective, diastereoselective, enantiomer, diastereomer, stereoisomer), combined as judiciously as possible provide the best illustration in the present context. In doing so, one does justice to the original Kelvinian concept, emphasizes the true origin of the stereochemical outcome, and uses clear and consistent definitions as properly vindicated by stereochemistry educators.

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WILEY InterScience® DISCOVER SOMETHING GREAT CHIRALITY 20:5–19 (2008)

Review Article

The Discovery of Biological Enantioselectivity: Louis Pasteur and the Fermentation of Tartaric Acid, 1857—A Review and Analysis 150 Yr Later

JOSEPH GAL*

Division of Clinical Pharmacology and Toxicology, University of Colorado School of Medicine, Denver, Colorado

ABSTRACT Nearly a decade after discovering molecular chirality in 1848, Louis Pasteur changed research direction and began investigating fermentations. Conflicting explanations have been given for this switch to microbiology, but the evidence strongly suggests that Pasteur's appointment in 1854 to the University of Lille—an agricultural-industrial region where fermentation-based manufacturing was of great importance—and an appeal for help in 1856 by a local manufacturer experiencing problems in his beetroot-fermentation-based alcohol production played a significant role. Thus began, in late 1856, Pasteur's pioneering studies of lactic and alcoholic fermentations. In 1857, reportedly as a result of a laboratory mishap, he found that in incubations of ammonium (±)-tartrate with unidentified microorganisms (+)-tartaric acid was consumed with considerable preference over (−)-tartaric acid. In 1860, he demonstrated a similar enantioselectivity in the metabolism of tartaric acid by *Penicillium glaucum*, a common mold. Chance likely played a significant role both in Pasteur's shift to microbiology and his discovery of enantioselective tartrate fermentations, but he rejected pure serendipity as a significant factor in experimental science and in his own career. Pasteur's milestone discovery of biological enantioselectivity began the process that in the long run established the fundamental importance of molecular chirality in biology. *Chirality 20:5–19, 2008.* © 2007 Wiley-Liss, Inc.

KEY WORDS: chance; chirality; dissymmetry; history; microbiology; paratartaric acid; racemic acid; serendipity; fermentation; enantioselectivity

INTRODUCTION

To most readers of *Chirality*, the name Louis Pasteur (1822–1895) evokes the discovery of molecular chirality by the French chemist in 1848.[1] Less-well appreciated is the fact that in late 1857—by which time he had switched research fields from investigations of the chemistry and crystallography of molecular chirality to microbiological studies—he made another milestone discovery: that of the enantioselective "fermentation" of tartaric acid by microorganisms. The vast importance of molecular chirality in biology is well established today,[2–6] but 150 yr ago Pasteur's discovery of the enantioselective microbial metabolism of tartaric acid was the first observation of enantioselectivity in a biological (biochemical) process.

When considering Pasteur's studies of the fermentation of tartaric acid, an important and intriguing question arises: why did he switch from the field of his brilliant discoveries in the chemistry and crystallography of molecular dissymmetry (his term for chirality[7]) to investigations of fermentations and microbiology? In the middle of the nineteenth century fermentations were a subject of interest to many chemists (see below), but Pasteur had no training in biology and no experience in fermentations, while he was spectacularly successful in his work on molecular chirality, a branch of chemistry he had practically created himself (on the basis, to be sure, of the work of his predecessors, e.g., Haüy, Malus, and Biot, pioneers of crystallography, polarized light, and optical rotation, respectively). Pasteur's discovery of molecular chirality brought him, at the age of 25, attention and recognition from the French scientific establishment.[8] Why, then, would he desert the field of his great achievements and the promise of further significant discoveries in chemistry and crystallography for the sake of what appeared to be an uncertain future in biology? Conflicting answers to this question have been given by a variety of sources and the matter is controversial.[9]

In the section "Pasteur's Shift from Molecular Chirality to Fermentations" of the present article, then, the reasons

*Correspondence to: Prof. Joseph Gal, Division of Clinical Pharmacology C 237, University of Colorado Health Sciences Center, 4200 East 9th Avenue, Denver, CO 80262. E-mail: joe.gal@uchsc.edu
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and events associated with Pasteur's change of research fields from molecular chirality to the study of fermentations—the basis of his discovery of biological enantioselectivity—will be critically examined. In the section "Pasteur and the Discovery of Enantioselective Tartrate Fermentations," the background, nature, and significance of his discovery of the enantioselective fermentation of tartaric acid will be analyzed. Finally, Pasteur's obsession with minimizing the role of serendipity in the scientific discovery process in general and in his own career in particular, and the resulting distorted accounts given by him of the events that are the subject of this article will be discussed in the section "Pasteur and the Role of Serendipity in Science."

PASTEUR'S SHIFT FROM MOLECULAR CHIRALITY TO FERMENTATIONS

Background

First, it will be useful to discuss briefly the nomenclature of the tartaric acids, which are the central compounds in this article. When Pasteur began his studies on "tartaric acid," two relevant compounds were known. One of them was referred to as "tartaric acid" or "ordinary tartaric acid" and these names denoted what we call today (+)-tartaric acid or *dextro*-tartaric acid. This compound was obtained from tartar, a material deposited in the containers of fermenting grape juice during the wine-making process. When Pasteur initiated his investigations it was known that "tartaric acid" was dextrorotatory. The other compound known at the time, also an acid, was first isolated ca. 1820 in a factory producing the above-mentioned tartaric acid, in Alsace, France. This second compound was found to have the same elemental composition as ordinary tartaric acid and very similar chemical and physical properties except that it differed from (+)-tartaric acid in that it had no optical rotation and had different crystal morphology. This acid was referred to as "racemic acid" or "paratartaric acid." In today's nomenclature, racemic or paratartaric acid is called racemic tartaric acid or (±)-tartaric acid. On the basis of the similarities and differences between the two acids, in the middle of the nineteenth century they were considered structural isomers.[10]

As we shall see shortly, Pasteur soon discovered (−)-tartaric acid, and this made the nomenclature somewhat more complicated. However, in the material included in this article, Pasteur's nomenclature for the compounds remained relatively simple. In addition to "tartaric acid" and "ordinary tartaric acid," he also used "right tartaric acid" to refer to (+)-tartaric acid; for (−)-tartaric acid he used "left tartaric acid," and for (±)-tartaric acid he used the above-mentioned two names, "racemic acid" and "paratartaric acid." It should be emphasized that when Pasteur carried out his studies of the tartaric acids their chemical structures were not known. For the sake of clarity and consistency, in this article the modern nomenclature for these compounds will be used, but, obviously, any direct quote from Pasteur reproduced in this article will retain his nomenclature.

In 1848 Pasteur found that the sodium ammonium salt of (±)-tartaric acid crystallized as a mixture (conglomerate) of enantiomorphous (i.e., nonsuperposable-mirror-image) crystals, and this finding led him in turn to the recognition of the existence of molecular chirality.[1] Although almost nothing was known at the time about the details of molecular structure and atomic bonding,[11] Pasteur correctly inferred that molecular chirality could be the result of a tetrahedral or a helical arrangement of the atoms within the molecule.[12] He continued his studies of the crystallography, chemistry, and optical activity of chiral natural products for about 10 yr and made additional important contributions to early stereochemistry, e.g. the recognition of the phenomenon of diastereoisomerism in molecular chirality; the discovery of the resolution of racemic mixtures via fractional crystallization of diastereoisomeric salts; the racemization of the tartaric acid enantiomers; the existence of *meso*-tartaric acid, etc.[13] (Pasteur referred to *meso*-tartaric acid as "inactive tartaric acid"). Importantly, Pasteur concluded that chiral molecules could only be formed in living beings and that therefore molecular chirality was a manifestation of life.[13] Then, in 1856, rather suddenly, he shifted his attention to biology and began studying phenomena of fermentations.[14]

In the middle of the nineteenth century, when Pasteur began his studies of fermentations, the nature of the phenomenon was not well understood. The prevailing views were chemical, championed by prominent chemists such as Justus von Liebig (1803–1873) and Jöns Jakob Berzelius (1779–1848). In these chemical theories of fermentation no significant role was granted to live microorganisms and the transformations seen in fermentations were attributed to chemical action, catalytic or otherwise, by organic material.[15–17] There existed, however, a few claims[18,19] that fermentations were phenomena due to the vital activity of live microorganisms, but such views were held by a small minority and were firmly dismissed by the chemists. Such was the state of knowledge when Pasteur arrived on the scene; he soon became the leading proponent of live microorganisms as the agents of fermentations.[20]

Pasteur's First Explanation of His Switch to the Study of Fermentations

The first reason Pasteur gave for switching from the chemistry and crystallography of chirality to the study of fermentations invoked an optically active substance he called amyl alcohol. His attention had been drawn[21] to the optical activity of this substance in 1849 by Jean-Baptiste Biot (1774–1862), one of the pre-eminent physicists and astronomers of the nineteenth-century, a pioneer of the study of optical activity, and Pasteur's mentor and backer. In Pasteur's first memoir on fermentations, which was about lactic fermentation, he stated that he was drawn to the study of fermentations by the fermentation origins of optically active amyl alcohol.[14] In lactic fermentation milk sugar is converted to lactic acid, and Pasteur stated that amyl alcohol is usually found as a product of the fermentation of sugars. Pasteur began the introductory section of that first memoir[14] on fermentations with the following sentence: "Je crois devoir indiquer en quelques mots comment j'ai été conduit à m'occuper de recherches sur les fermentations." ("I believe I need to indicate in a few

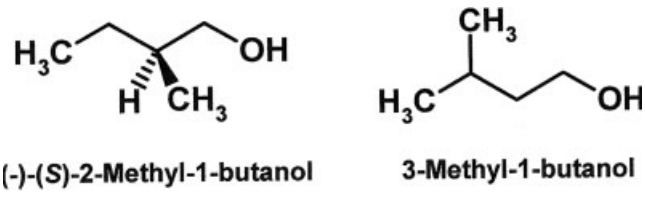


Fig. 1. The two alcohols in "amyl alcohol" that Pasteur studied.

words how I became engaged in research on fermentations"; all translations are by the present author unless otherwise indicated). After that sentence there follows a long discussion in two paragraphs (one of which takes a whole page) of his "preconceived ideas" about the nature of fermentations and how amyl alcohol is relevant to the subject. However, the memoir on lactic fermentation focused on the formation of lactic acid and neither amyl alcohol nor chirality is mentioned after the introductory section (see later).[14]

Pasteur published two articles on amyl alcohol.[21,22] The first,[21] a short summary of some of his work on the substance presented to the *Académie des Sciences* (Academy of Sciences, *Académie* henceforth;) in 1855, dealt with the chemical composition and purification of commercial samples of amyl alcohol. In the summary[21] Pasteur reported that commercially available "amyl alcohol" was a mixture of two isomeric alcohols that were extremely similar, even indistinguishable, in most of their chemical and physical properties. After a great deal of effort, he achieved the separation of the two alcohols via fractional crystallization of the barium salts of their sulfate monoesters and found that one of them was optically active while the isomeric component was inactive,[21] but their chemical structures were not known at the time. He reported that the optically active alcohol was levorotatory.[21] By 1888, long after Pasteur had abandoned this work, the optically active component in his amyl alcohol had been identified as (−)-2-methyl-1-butanol.[23] Its absolute configuration has been determined[24] as S (Fig. 1). By 1888 it was also known that the optically inactive isomer from Pasteur's "amyl alcohol" was 3-methyl-1-butanol[23] (Fig. 1). The two alcohols are major components of fusel oil, a product of the fermentation of carbohydrates to form ethanol.[25]

At the end of his first article[21] on amyl alcohol, Pasteur pointed out that his interest in these compounds stemmed from crystallographic considerations. The two alcohols are liquids, but he prepared a variety of crystalline derivatives, and found that the derivatives of the optically active alcohol violated his laws[21,22] on the crystallography of chiral substances. According to these laws, in crystals of optically active compounds there is an obligatory presence in the crystal habit (morphology) of "non-superposable hemihedrism," as he put it, i.e., the presence of hemihedral facets that render the crystals chiral. Crystalline derivatives of his optically active amyl alcohol were the first examples he found that violated the hemihedrism requirement,[21,22] and this was a radical challenge to his laws governing the relationship of molecular chirality and crystallography.

In his second and final publication[22] on the subject of amyl alcohol, Pasteur took up the crystallographic problem connected to the derivatives of the optically active component of his amyl alcohol, i.e., the absence of hemihedrism in the crystals of these optically active compounds. He attempted, unsuccessfully, to induce hemihedrism in the crystals by varying the conditions of crystallization, an approach that had served him well in earlier cases where hemihedrism at first did not appear in the crystals of optically active compounds. His conclusion at the end of the work on amyl alcohol was that crystal hemihedrism is not a necessary manifestation of molecular chirality.[22] Pasteur did not pursue his studies of optically active amyl alcohol farther, and there is no evidence that he had a significant interest in exploring its biological origins. Moreover, even in some of his subsequent papers[14,26] on alcoholic and lactic fermentations, Pasteur emphasized the extraordinary crystallographic interest that the amyl alcohol derivatives represented, thereby reaffirming the basis for his interest in the compounds.

Pasteur's grandson, Louis Pasteur Vallery-Radot[27,28] (1886–1970; LPVR henceforth), was a physician and accomplished medical researcher, writer, member of the *Académie de Médecine* (Academy of Medicine) and the *Académie française* (French Academy [of letters]). He devoted a great deal of effort to the preservation and dissemination of Pasteur's work, and edited the monumental 7-volume *Œuvres de Pasteur* (collected works of Pasteur, *Œuvres* henceforth) and *Correspondance de Pasteur* (Pasteur's collected correspondence, *Correspondance* henceforth). Significantly, in the extensive subject index to Pasteur's writings and lectures (published or unpublished) in volume 7 of the *Œuvres*, under the heading "alcool amylique" (amyl alcohol) the only references listed are to the above-discussed two articles[21,22] on the chemistry and crystallography of the amyl alcohol compounds and to a brief mention in an unpublished note in 1863 of amyl alcohol as one of the products of the fermentation of glycerol.[29]

Thus, the evidence clearly indicates that Pasteur's main interest in amyl alcohol stemmed not from its fermentation origins but from the crystallographic properties of its derivatives. The discrepancy between this conclusion and Pasteur's own assertion of the importance of amyl alcohol in his interest in fermentations derives fundamentally from his firm belief that serendipity had little significance in science (see below).

The Amyl-Alcohol-Based Theory Persists

As discussed earlier, the evidence does not support a significant role for amyl alcohol in Pasteur's change of research direction toward fermentations. Nevertheless, amyl alcohol appears frequently in works on Pasteur as an important element in his switch to microbiology, and several authors have made amyl alcohol the central reason in Pasteur's shift to fermentations. Jacques, for example, presents, without critical examination, Pasteur's own version that amyl alcohol was the real reason for his venture into fermentations.[30] The reasoning used by several of Pasteur's biographers[30–32] has been that since amyl alcohol was a product of fermentation and optically active, Pasteur concluded that fermentations must be acts of living

organisms (since he believed that chiral molecules could only arise in living beings) and that this was therefore the driving force for his shift to the study of fermentations. However, no such explicit reasoning appears in Pasteur's writings.

Particularly surprising, in this context, is Geison's treatment of the amyl-alcohol matter. Geison, who was a noted historian of science, wrote extensively and at times highly critically on Pasteur's life and work,[33–36] When it came to the analysis of Pasteur's move to microbiology, however, Geison accepted Pasteur's own amyl-alcohol-based explanation.[37] Geison produced no evidence, however, that Pasteur actually examined the biological formation of amyl alcohol or even that the fermentation origins of amyl alcohol seriously influenced Pasteur's thinking in the direction of fermentations.

Geison's main argument in support of the chiral-amyl-alcohol-to-fermentation move by Pasteur is limited to emphasizing Pasteur's well-known belief that molecular chirality is associated with life, with living organisms, i.e., plants or animals.[31] When Pasteur discovered molecular chirality in 1848, in addition to (+)-tartaric acid many other optically active compounds, all natural products, were known,[38] e.g., amygdalin, various sugars, essential oils, mandelic acid (known as "amygdalic acid"), camphor, morphine, strychnine, brucine, cinchonine, quinine, albumin, etc. However, none of these compounds prompted him to study their biological genesis, and the evidence indicates that neither did amyl alcohol.

Pasteur's lack of interest in the details of the biological mechanisms of the formation of optically active natural products may find an explanation in the complexity of his views on the causes of molecular chirality. While it is abundantly clear that he recognized that molecular chirality in the known optically active compounds was associated with their origins in living organisms, he also believed that the ultimate cause of chirality in natural products was not biological but physical ("cosmic" as he put it), e.g., forces of magnetic or electric fields, or sunrays, which he thought would act on life forms to induce molecular chirality in the substances within them.[39,40] Indeed, in the early 1850s, while he was at the University of Strasbourg, he invested a great deal of effort and resources in attempts to prove his "cosmic" hypothesis.[41] For example, he studied plants grown in magnetic fields of varying polarity or in sunlight reflected by mirrors[41,42] (however, importantly in the present context, he did not include investigations of the effects of the physical forces on fermentations).

Pasteur's attempts to induce molecular chirality by the action of physical forces produced no useful results.[41,42] Nevertheless, he did not abandon his belief in the fundamental role of such forces in the genesis of molecular chirality and later even undertook experiments to generate optically active compounds using only electromagnetic fields, with the total exclusion of biological factors.[42,43] Moreover, as late as 1871, ca. 15 yr after he had stopped active work on the chemistry and crystallography of chirality and while he was fully occupied with microbiological investigations, he still believed in the "cosmic" basis of molecular chirality and resumed experiments to prove it.[43] Even in 1883, he continued to assert his firm belief in the "cosmic," physical, origins of molecular chirality.[44]

Clearly, then, the claim that the fermentation origins of amyl alcohol could have served as the driving force that pushed Pasteur to fermentation is supported neither by his view of the ultimate origins of molecular chirality nor by his actions to elucidate those origins.

An Alternative Explanation: The Lille Industries and Bigo's Problem

If amyl alcohol cannot in fact explain Pasteur's switch to microbiology, what alternative explanations can be offered instead? In 1854, Pasteur, who was then professor of chemistry at Strasbourg, accepted an appointment as professor of chemistry and dean of the newly opened Faculty of Sciences at the University of Lille, in northern France.[45] This was an industrial region where agricultural and food industries had considerable economic significance, and fermentation-based manufacturing, such as the production of ethanol from sugar beets and the production of beer, were of particular importance.[46–48] Some of Pasteur's biographers believe that his move to Lille played a significant role in his shift to studies of fermentations.[46–48]

The desirability of teaching practical applied science useful to the industry of the region was emphasized by the French minister of education when Pasteur became dean of the Faculty Sciences at Lille.[49] Pasteur was well aware of this concern and made considerable efforts to assure that his students would be taught not only basic or theoretical science but would also be trained in the applied aspects. For example, he organized field trips for the students to visit various factories[50,51] and taught a course in applied chemistry on the production of alcohol by fermentation of beetroot juice.[52] Figure 2 shows a page from his lesson plan for the course.[53]

The date visible at top left of the page is February 2, 1857, and the first three lines of the plan read as follows:

- Continuation of the study of 'fermentation.'
- The phenomenon is known to us in its major features.
- Its importance requires more details.

A critical event in the story occurred when, shortly after the opening of the academic year in the autumn of 1856, a certain Mr. Bigo, manufacturer of alcohol by fermentation of beetroot juice in Lille and father of one of Pasteur's students, approached him with a plea for help in tackling a mysterious new problem in the fermentations which was causing the spoiling of the product ethanol.[54–57] According to this account of Pasteur's path from chirality to fermentations, despite his lack of both training in biology and direct experience in fermentations, he agreed to examine Bigo's problem, thereby becoming directly and intensively involved in studies of fermentations.[54–57]

There is in fact clear evidence that Pasteur did respond to Bigo's appeal for help and initiated studies of the alcoholic fermentation of beetroot sugar. Thus, Bigo's name and Pasteur's work in Bigo's factory appear in Pasteur's

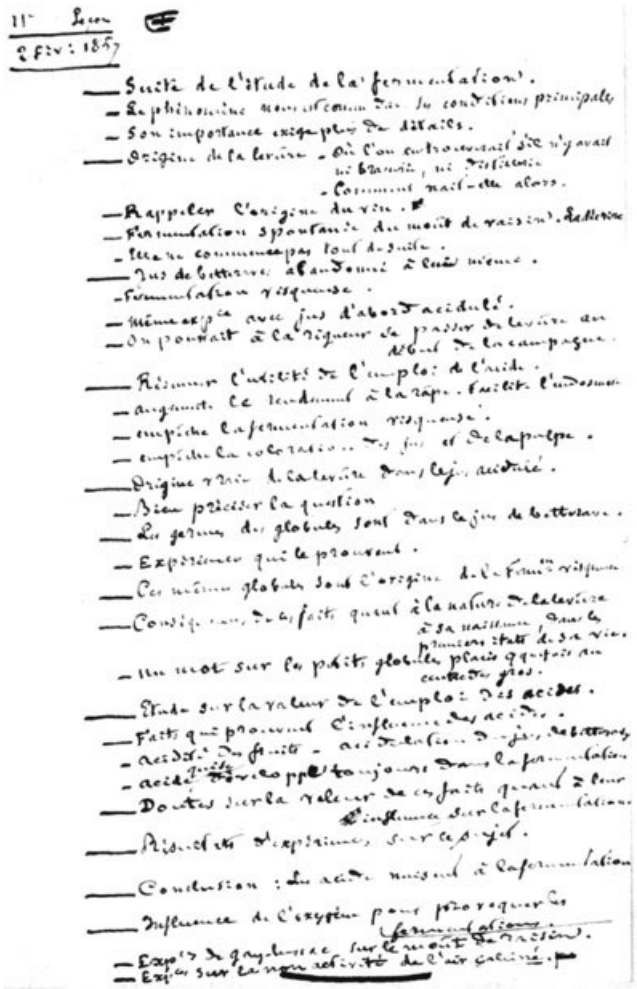


Fig. 2. Page from Pasteur's lesson plan for a lecture in a course on fermentations at the Faculty of Sciences in Lille. See text for more details. (Reprinted from "IMAGES DE LA VIE ET DE L'ŒUVRE DE PASTEUR," by Pasteur Vallery-Radot, Flammarion, 1956).

laboratory notebook of the period.[58] Figure 3 is the image of a page from the notebook as given by LPVR; the first line on the page gives the date (November 4); "Esquermay," i.e., the name of the street where Mr. Bigo's factory was located; and "factory of Mr. Bigo." In the legend[58] LPVR indicates that the page concerned Pasteur's first investigation of fermentations (Fig. 3).

Early November, 1856, was shortly after Bigo had first approached Pasteur about his problem with the fermentation of beet juice, and, in agreement with this general timeframe, on December 10, 1856, Pasteur's wife Marie wrote[59] to her father-in-law about her husband's work: "Louis continues to work zealously. He is now immersed up to his neck in beet juice. He spends his days in an alcohol factory." LPVR explained in an editorial footnote to Marie's letter[56] that the factory was that of Mr. Bigo. Pasteur identified lactic acid as a major product in Bigo's defective alcoholic fermentations, and expanded his studies to include lactic fermentation.[60–62]

The Demise of the Amyl-Alcohol Theory

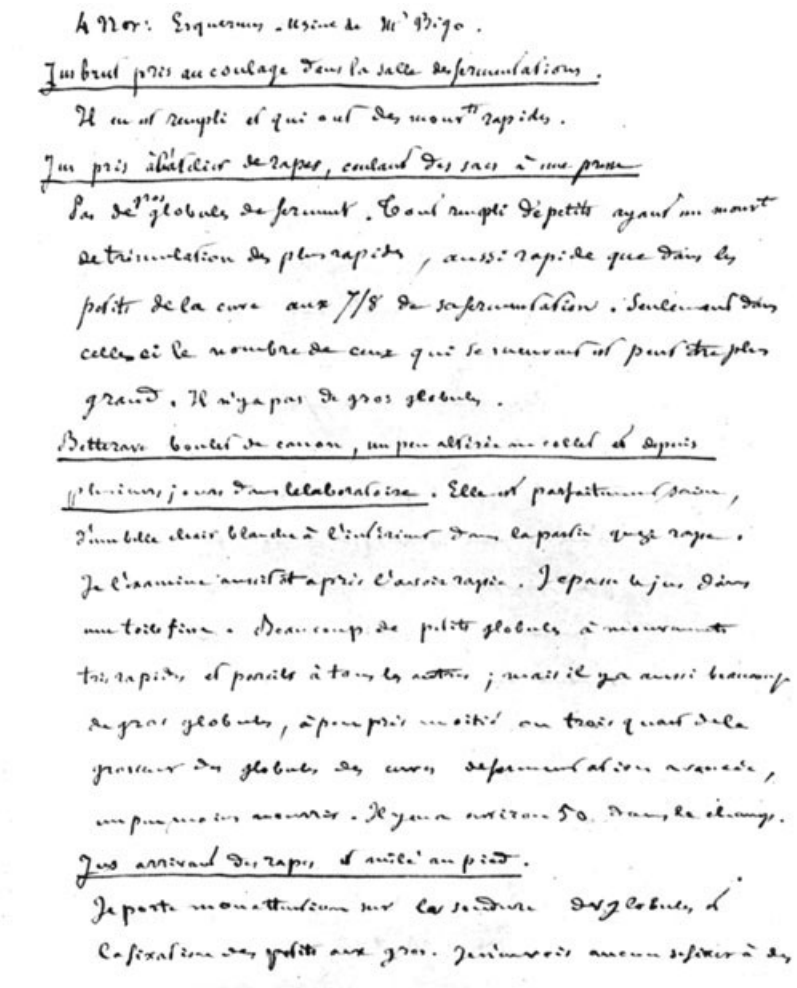
As discussed earlier, there is simply insufficient concrete evidence for a significant role of amyl alcohol and its chirality in Pasteur's shift to fermentations, and there is no tangible evidence of any significant involvement by Pasteur in any aspect of the fermentation origins of amyl alcohol.

Moreover, considerable additional evidence has accumulated against amyl alcohol as a significant factor in Pasteur's shift to fermentations. For example, Émile Duclaux (1840–1904), one of Pasteur's closest collaborators who worked extensively on fermentations, stated in his detailed scientific biography of Pasteur—which appeared in 1896, the year following Pasteur's death and which is considered a valuable resource on Pasteur's work[63]—that the major factor in Pasteur's embarking on the investigation of lactic fermentation was "more profound" than the origin of amyl alcohol: the study of lactic fermentation was Pasteur's first campaign to assert his vital (i.e., live-microorganism-based) theory in the war on the purely chemical view of fermentations.[64] According to Duclaux, Pasteur chose this battlefield over alcoholic fermentation because the latter had been extensively examined and debated, while lactic fermentation was essentially virgin territory.[64] In these considerations amyl alcohol played no role.

Most importantly, almost from the very beginning, Pasteur himself began backing off from amyl alcohol as the explanation for his move from chirality to fermentations, as indicated by several lines of evidence. Thus, in his first memoir on fermentations[14] mentioned above—in which he first claimed amyl alcohol as his motivation for the change in research focus—he stated the following in the third and final paragraph of the introduction:

> "Those were the opportunity and the motive for me for new experiments on fermentations. But, as often happens in such circumstances, my work expanded little by little and deviated from its original direction, in such a way that the results that I publish today appear unrelated to my previous studies [i.e., of dissymmetry]. The link [between dissymmetry and fermentations] will become more evident in results which will follow later. I hope to be able in the future to establish a relationship between the phenomena of fermentations and the character of molecular dissymmetry of organic substances."

In the *Œuvres* LPVR added an editorial footnote to the effect that the future studies Pasteur promised in the above-quoted paragraph referred to his investigations of the fermentation of tartaric acids (which, however, began well after he had started his studies on alcoholic and lactic fermentations in Bigo's factory). Surely, this is a clear acknowledgment at the outset by Pasteur that amyl alcohol had little to do with his move from chirality to fermentations, despite his statement to the contrary a few lines earlier in the article. We must also remember here that the memoir in question (on lactic fermentation) was the result



PAGE D'UN CAHIER DE LABORATOIRE
Premières recherches de Pasteur sur les fermentations (4 novembre 1856).

Fig. 3. A page from one of Pasteur's laboratory notebooks. The legend by LPVR: "PAGE FROM A LABORATORY NOTEBOOK First investigations of Pasteur on fermentations (November 4, 1856)." "Esquermay" refers to the name of the street where the factory was located. (Reprinted from "IMAGES DE LA VIE ET DE L'ŒUVRE DE PASTEUR," by Pasteur Vallery-Radot, Flammarion, 1956).

of Bigo's fermentation problem and was not related to any chirality considerations. Moreover, the memoir on lactic fermentation mentioned neither amyl alcohol nor dissymmetry after that introductory section discussed above. Finally, Pasteur's statement about future studies foreshadows his later claim of the fermentation of tartaric acids as the reason for his move from chirality to fermentations (see below).

Pasteur's contradictory statements in the introductory section of the memoir on lactic fermentations discussed earlier appear, on first reading, difficult to reconcile, but when all the evidence is taken into account the only reasonable conclusion is that he himself implicitly acknowledged that amyl alcohol had precious little to do with his change in research focus. His disingenuous use of amyl alcohol in the memoir to connect his chirality work with his switch to fermentations is a matter rooted in Pasteur's rejection of serendipity in science, a view that induced him to provide at times disingenuous explanations of the development of his scientific path. In this case he seems to have been unable to acknowledge that his focus on fermentations was the result of his fortuitous appointment to Lille coupled with the serendipitous fermentation problem in Bigo's factory. Pasteur therefore sought a "logical scientific" reason for his change of research fields, i.e., the amyl-alcohol link, but the evidence clearly indicates that this was a disingenuous connection. His adamant rejection of serendipity in science was in turn the result of his view of the role of science in the life of the nation and society's responsibility to provide adequate support for the scientific research enterprise (see the section "Pasteur and the Role of Serendipity in Science").

As we shall see in detail shortly, well after he had started his investigations of alcoholic and lactic fermentations, Pasteur returned to his beloved tartaric acids (Fig. 4) and showed that microorganisms consumed one

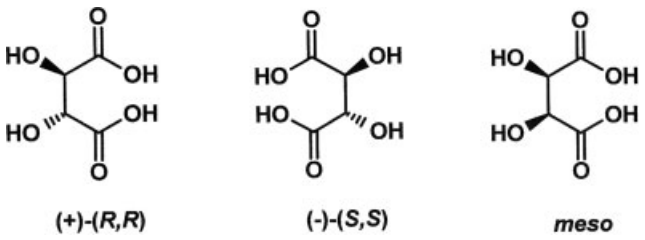


Fig. 4. The stereoisomeric forms of tartaric (2,3-dihydroxybutanedioic) acid.

enantiomer preferentially over the other. In the first announcement[65] of those results, in December, 1857 (see later), he wrote: "And thus my earlier studies [i.e., of dissymmetry] are broadened by these mysterious phenomena of fermentations, which at first seemed to distance me from my earlier work." This is another clear acknowledgement by Pasteur that his move into the field of fermentations was not linked to his chirality work, and he identifies here, for the first time, a later connection between his earlier work on molecular chirality and his subsequent studies of fermentations, that connection being the enantioselective fermentation of tartaric acid (the future connection he promised in the first memoir on lactic fermentation, see earlier). Amyl alcohol no longer figures in this explanation of his move from chirality to fermentations.

Within just two-and-a-half years after the events in question, in his famous two lectures of 1860 to the Chemical Society of Paris, Pasteur nearly completely ignored amyl alcohol while he dwelled on the enantioselective tartrate fermentation and emphasized the importance of chirality in the fermentation process as demonstrated by the fermentation of (±)-tartaric acid. His only reference to amyl alcohol was in the second lecture and consisted of the following single sentence: "I also discovered inactive amyl alcohol which provides a whole series of inactive derivatives corresponding to the series of derivatives of active amyl alcohol."[66] ("Active" and "inactive" refer to optical rotation). It is indeed noteworthy that in the lectures no mention whatsoever is made of optically active amyl alcohol as a factor in his interest in fermentations, while the tartrate fermentation is emphasized[67] from the point of view of the connection between chirality and fermentations.

Another clear indication of Pasteur's abandonment of amyl alcohol comes from a lecture, he presented[68] to the *Société centrale de médecine vétérinaire* (Central Society of Veterinary Medicine) in 1880. While tracing the evolution of his career in the lecture, Pasteur said nothing about amyl alcohol and stated explicitly and unequivocally that it had been his observation of the enantioselective metabolism of (±)-tartaric acid by microorganisms that had prompted him to move from chirality into the field of fermentations. The problem with this assertion by Pasteur is, of course, that, as we have seen above, his fermentation studies began, in Bigo's factory, with lactic and alcoholic fermentations—which were unrelated to chirality—in 1856, well before he initiated investigations of the fermentation of tartaric acid in 1857 (see below); (parenthetically, it can be safely assumed that in the lecture of 1880, i.e., nearly 25 yr after the events in question, Pasteur's audience of veterinarians was not likely to have been aware of the chronological inconsistencies inherent in Pasteur's claim).

Finally, a few years later, in the mid-1880s, Pasteur's abandonment of amyl alcohol was again confirmed. The confirmation came in Pasteur's first biography,[69] authored anonymously by his son-in-law, René Vallery-Radot (1853–1933; RVR henceforth). The biography, published in 1883, is generally recognized to have been produced under Pasteur's control.[70,71] In the biography Pasteur's switch to microbiology is stated to have been the result of the discovery of the paratartrate fermentation, and nothing is mentioned in it about amyl alcohol.[69]

Pasteur's Move to Fermentations: Conclusions

At the outset of the preparation of this article, it was clear that the literature on Pasteur's shift from chirality to fermentations was complex and often confused and contradictory. Many writers on Pasteur's work have recognized the importance of the Lille industrial context (including Bigo's role), but some have not. Jacques, for example, is silent on the Lille context and gives only the amyl alcohol version,[30] and Geison explicitly rejects the Lille industries/Bigo version of the events while he fully accepts the amyl alcohol story.[72] Conversely, some biographers simply do not mention amyl alcohol and accept only the Lille context. For example, RVR, in his *La Vie de Pasteur* (Life of Pasteur), the renowned biography of Pasteur first published in 1900,[73] and in his 1883 anonymous biography of Pasteur (see above), makes no mention of amyl alcohol.[69] Still others, e.g., Robbins,[74] grant some role to both amyl alcohol and the Lille industrial influence. It appeared, therefore, that a comprehensive, detailed, and critical analysis of this controversial issue was needed to reach a conclusion that would best fit the known facts.

As discussed earlier, all in all, it is difficult to avoid the conclusion that amyl alcohol played no significant role and that it was in fact the impetus provided by the agricultural and food industries in the Lille region, coupled with Bigo's manufacturing problems that set in motion Pasteur's move to the field of fermentations. In this context it is also probable that Pasteur's applied-chemistry course on fermentations was useful to him when he initiated his research on fermentations, i.e., the studies of Bigo's manufacturing problem in the production of alcohol from beetroot.

As discussed earlier, Pasteur's work on amyl alcohol was brief and focused on questions unrelated to fermentation. It is also firmly established that he did not begin investigating fermentations until late 1856, i.e., about 2 yr after his arrival in Lille and 7 yr after he became aware of the optical activity of amyl alcohol. When he finally did begin his fermentation studies, they involved lactic- and alcoholic fermentations, with no link to chirality. Moreover, as we have seen earlier, Pasteur quickly backed away from amyl alcohol and began using his tartrate fermentation studies as the precise cause of his shift to fermentations. As we have also seen above, however, neither

amyl alcohol nor the tartrate fermentation is supported by the totality of the evidence as the explanation for his change of research focus. As mentioned above also, this discrepancy derives from Pasteur's view of the nature of science and his rejection of chance in scientific discovery (see later).

PASTEUR AND THE DISCOVERY OF ENANTIOSELECTIVE TARTRATE FERMENTATIONS

The First Report: The Memoir of December, 1857

After 3 yr in Lille, Pasteur moved again, this time to Paris: on October 22, 1857, he was appointed Administrator of the École normale supérieure (ENS) and Director of Scientific Studies there. On December 21, 1857, shortly after his arrival in Paris, he presented a communication[65] to the *Académie* entitled "Memoir on Alcoholic Fermentation." As its title indicates, the memoir dealt with certain aspects of alcoholic fermentation, but near the end of the communication Pasteur said the following:

> "Before concluding, I ask for the permission of the Academy to present results to which I attach great importance. I have discovered a means of fermenting tartaric acid which readily affects ordinary right tartaric acid but involves left tartaric acid very poorly or not at all. Now, a remarkable thing, predictable from the preceding fact, is that when paratartaric acid, formed by the combination, molecule for molecule, of the two tartaric acids, right and left, is subjected to the same method of fermentation, it is resolved into the right acid which is fermented and left acid which remains intact, in such a way that the best means of obtaining left tartaric acid I know of today is to resolve paratartaric acid by fermentation."[65]

The memoir was published[65] in the proceedings of the *Académie*, the *Comptes rendus des séances de l'Académie des Sciences* (*Comptes rendus* henceforth), and the original passage whose translation is given above is shown in Figure 5.

The description of the fermentation of "paratartaric acid" in the memoir of December, 1857, constitutes the first published observation of enantioselectivity in a biological process. About 3 mo after that brief announcement[65] of the enantioselective microbial metabolism of tartaric acid, Pasteur presented to the *Académie* a communication[75] devoted entirely to the subject.

"Memoir on the Fermentation of Tartaric Acid"

The new communication, bearing the title in quotation marks above, was presented to the *Académie* on March 29, 1858, and, as usual, was published[75] in the *Comptes rendus*. Memoirs of original research appearing in the *Comptes rendus* were often relatively short, with few experimental details, and concentrated mainly on the essence and interpretation of the work. Pasteur sometimes followed up a presentation to the *Académie* with a full paper in another journal, as he did, for example, for his announcement to the *Académie* of his discovery of molecular chirality in 1848, which was followed up with a full paper[76] in the *Annales de chimie et de physique*. He did not, however, publish a full paper on the fermentation of the tartaric acids after his memoir[75] of March, 1858, to the *Académie*.

» Avant de terminer, je demande à l'Académie la permission de lui annoncer un résultat auquel j'attache une grande importance. J'ai découvert un mode de fermentation de l'acide tartrique, qui s'applique très-facilement à l'acide tartrique droit ordinaire, et très-mal ou pas du tout à l'acide tartrique gauche. Or, chose singulière, mais que le fait précédent permet de prévoir, lorsque l'on soumet l'acide paratartrique formé par la combinaison, molécule à molécule, des deux acides tartriques, droit et gauche, à ce même mode de fermentation, l'acide paratartrique se dédouble en acide droit qui fermente et en acide gauche qui reste intact, de telle sorte que le meilleur moyen que je connaisse aujourd'hui pour isoler l'acide tartrique gauche consiste à dédoubler l'acide paratartrique par la fermentation.

Fig. 5. Passage from Pasteur's memoir in the *Compte rendu* of December 21, 1857, announcing the enantioselective fermentation of (±)-tartaric acid. (See translation in text; reprinted from *Comptes rendus des séances de l'Académie des sciences*).

The memoir[75] does not appear to have been translated into English. Its historically important contents suggest, however, that the availability of an English translation would be desirable, and therefore such a translation is provided in the appendix below.

Interestingly, in the title of the memoir Pasteur seems to use "tartaric acid" in a general sense rather than designating the dextrorotatory compound. For (±)-tartaric acid, in the memoir Pasteur abandoned "paratartaric acid" that he had used in the earlier communication[65] and employed instead the other common name for the compound at the time, "racemic acid" (see above). It should also be noted that while the stereochemical course of Pasteur's tartrate fermentation is described today as *enantioselective*, this (or any other) *enantio*-based term does not appear in his lectures and writings. The first *enantio*-based terms were introduced by Carl Friedrich Naumann (1797–1873), a German mineralogist, in 1856, but Pasteur did not adopt this terminology.[77]

The memoir[75] is divided into two parts (see Appendix). Part one dealt with the fermentation of (+)-tartaric acid and Pasteur pointed out that the spontaneous fermentation of this acid had been known for a long time as a result of manufacturing accidents. He also gave some of the experimental details of the fermentation as conducted in his laboratory. The fermentation mixture contained ammonium (+)-tartrate, nitrogenous "albuminoid" material from plant or animal sources, and material from a previous active fermentation of tartaric acid.

In part two the analogous incubation of (±)-tartaric acid is described. The fermentation was carried out in the same manner as that of the dextrorotatory acid, and the key experimental tool was the monitoring of the optical rotation of the mixture as the fermentation proceeded. It was found that the reaction mixture, which showed no optical rotation at first, became levorotatory as the fermentation progressed over several days. The rotation continued to increase and eventually reached a maximum, at which point the fermentation stopped. The dextrorotatory acid

was no longer present in the mixture, having been destroyed in the fermentation. (−)-Tartaric acid, which was not affected by the "ferment," could then be readily isolated in pure form from the mixture. Pasteur pointed out that this was the best method he knew to prepare the levorotatory acid. In the remainder of the memoir[75] he proposed an explanation for the selective destruction of (+)-tartaric acid in the fermentation (see below).

Analysis of the Discovery

As we have seen, when Pasteur undertook to study the fermentation of tartaric acid he was already in the midst of investigations of lactic and alcoholic fermentations. What, then, prompted him to also examine the fermentation of tartaric acid? Some of his biographers have stated that it was the result of a chance discovery: an impure aqueous solution of (+)-tartaric acid (or a salt thereof) abandoned on a bench in Pasteur's laboratory for a period of time in warm weather became turbid, a telltale sign of fermentation.[78,79] It had long been known that solutions of the calcium salt of (+)-tartaric acid would ferment under such conditions, and, therefore, according to the story, most other investigators would have thrown out the spoiled tartrate solution without giving it another thought. Pasteur, on the other hand, had the insight to consider the implications of this laboratory mishap, and he followed up the accidental observation with imaginative experiments that led to the discovery of biological enantioselectivity.

That account of the accidental fermentation of (+)-tartaric acid in Pasteur' laboratory and the resulting discovery of microbial enantioselectivity in fermentations appears to have been first given by Duclaux in his scientific biography of Pasteur.[79] On the basis of Pasteur's rejection of serendipity in science (see below), however, predictably, the story of the accidental finding of the fermentation of (+)-tartaric acid in his laboratory and its serendipitous role in the discovery of the enantioselectivity of the fermentation of (±)-tartaric acid is not confirmed by Pasteur. Indeed, in his publications and lectures on the subject he did not mention any such laboratory accident and never portrayed his discovery as the outcome of a chance observation of an accidental fermentation of (+)-tartaric acid. For example, in one his two famous lectures to the Chemical Society of Paris in 1860 he said the following[80]:

> "It had been known for a long time, from an observation by a manufacturer of chemical products in Germany, that impure commercial calcium tartrate [i.e., calcium (+)-tartrate], contaminated with material from live organisms and abandoned in water in the summer, could ferment and produce various products.
>
> With that established, I carried out the fermentation of ordinary tartrate [i.e., a salt of (+)-tartaric acid] in the following manner: [here Pasteur gave the experimental details; they will be omitted in the present recounting].
>
> So far, nothing unusual, it is a tartrate [i.e., (+)-tartrate] that ferments. It is a known fact.
>
> But let us apply this method of fermentation to ammonium paratartrate . . ."

Similarly, in his memoir[75] of March, 1858, on the tartrate fermentations (see Appendix), he did not refer to a laboratory accident as the triggering event of those studies. Pasteur's implications are clear: he had been aware of the earlier observations on the spontaneous fermentation of impure (+)-tartrate, confirmed it in his laboratory, and then applied it to (±)-tartaric acid. There is no hint of serendipity in his account, which obviously differs from the version given by some of Pasteur's biographers who specifically describe an initial *chance* observation in his laboratory as the trigger for these studies.[78,79] Here too, Pasteur's aversion to acknowledging a significant role to serendipity in science seems to have played a role (see below).

When and where were the experiments on the fermentation of tartaric acid carried out? In this matter also, contradictory information has appeared in the literature. In *La Vie de Pasteur*, the experiments in question are said to have taken place during a period which appears to correspond to late 1853 or early 1854, while Pasteur was still in Strasbourg, but no documentation or supporting information is provided by the author for this claim.[81] In his first volume[82] on Pasteur, published in 1950, Dubos is conflicted on the date of the tartrate-fermentation experiments: on p 41 of the volume he gives 1854 as the period in question and indicates that Pasteur was still in Strasbourg at the time, whereas on p 106–107 of the same volume the year is given as 1857.

Several credible sources in fact agree that the experiments were carried out in 1857. In his second work on Pasteur 10 yr after the first volume, Dubos settles on 1857 as the period of the tartrate-fermentation work.[83] LPVR stated on the basis of Pasteur's laboratory notebooks that Pasteur began his investigations of the fermentation of (+)-tartaric acid in April of 1857 and that on August 27 of that year the first experiments on the fermentation of paratartaric acid were undertaken.[84] Moreover, in a letter dated September 7, 1857, Pasteur announced[85] to Biot his new finding on the enantioselective fermentation of (±)-tartaric acid, stating, after a brief summary of the results: "These are the facts that I wished to notify you of, as soon as they were beyond doubt . . ."

It seems clear therefore that (a) the tartrate-fermentation experiments were carried out in 1857, and that (b) while the memoir of December, 1857, was presented after Pasteur's appointment to the ENS in Paris, the key experiments were performed in Lille, before he moved to Paris.

Pasteur did not identify a specific microorganism in the memoir on the fermentation of tartaric acid, although he referred to the organism as "yeast." He also described it as resembling the lactic ferment, i.e., the microorganism he had identified as responsible for lactic fermentation. In his scientific biography of Pasteur, Duclaux suggested that the microorganism of the tartrate fermentation may have been a species of *Penicillium*, a fungal microorganism.[86] In fact, in 1860 Pasteur reported in a brief note that *Penicillium glaucum*, a common mold, enantioselectively

metabolized paratartaric acid in a manner very similar to the earlier fermentation: here too, (+)-tartaric acid was consumed and (−)-tartaric acid was left behind largely untouched.[87]

The nature of the products of the fermentation of tartaric acid was not addressed by Pasteur in his memoir of March, 1858. He indicated in the memoir that he would soon publish information on this matter (see Appendix), but no such publication ever appeared. He did mention in the memoir an earlier report from the literature that identified metacetonic acid as a product of the fermentation of calcium (+)-tartrate. "Metacetonic acid" is an old name for propionic acid.[88]

No indication is given in the memoir of March, 1858, whether (−)-tartaric acid was separately incubated under the conditions of the fermentation, although the brief statement in the first report (of December, 1857) suggests that such an experiment had in fact been carried out (see above). In a related matter, in 1853 Pasteur had discovered[89] the racemization of tartaric acid when he heated (+)-tartaric acid with a cinchona alkaloid, e.g., cinchonidine or quinine. Among the products of the reaction, he found not only (±)-tartaric acid but also *meso*-tartaric acid (Fig. 4). He recognized that this molecule was inherently achiral and that therefore the substance was non-resolvable. Interestingly, however, he did not include the *meso* acid in the investigation of the tartrate fermentation. This is surprising indeed, inasmuch as Pasteur emphasized[90] the importance of the role of chirality as a modifier of affinity in "physiological chemistry."

Also relevant in this context is that in 1863 Pasteur reported[91] that an anaerobic microorganism fermented calcium (+)-tartrate, and he stated that the behavior of the other stereoisomers of tartaric acid (he listed them: "left, inactive, and paratartaric") under the same conditions would be described in a subsequent memoir. However, no such investigation was published by Pasteur and no documents relating to such a study were found by LPVR among Pasteur's unpublished papers.

Pasteur's explanation in the memoir of March, 1858, for the observed enantioselectivity in the tartrate fermentations is of considerable historical importance and is also highly relevant for today. He proposed that chiral optically active compounds within the constitution of the microorganism are involved in the utilization of the tartrate molecules as nutrients, and stated the fundamental principle that two enantiomerically related molecules (the tartrate enantiomers in this case) can interact differently with a third chiral molecule (i.e., a constituent of the microorganism), explaining that the chiral constituent of the microorganism does not "accommodate" equally well the left- and right-tartrate molecules (see Appendix). This was the first enunciation of that principle, which is generally accepted today as the basis of enantioselectivity in biology and chemistry.[2] Pasteur's words "we see here the property of molecular dissymmetry possessed by natural materials intervening in a physiological phenomenon as a modifier of affinity" (see Appendix) show a clear recognition of the essence of chirality as a modulator of molecular recognition in biology, as we would put it today.

Almost 30 yr later, in 1886, Pasteur was asked to present to the *Académie* work by Arnaldo Piutti, an Italian chemist, concerning a striking difference in the taste of the enantiomers of asparagine. Natural (−)-asparagine was without taste while the synthetic (+)-enantiomer was sweet. After presenting the work, Pasteur added his own comments in which he repeated the above principle that explains biological enantioselectivity. He proposed that a chiral molecule in the "nervous system of taste,"—a *receptor* by today's understanding—must be the mediator of the enantioselectivity in the taste.[92] In this instance too, Pasteur was farsighted, providing the first and correct explanation of receptor-mediated enantioselective pharmacological action.

The Impact of Pasteur's Discovery

The 1860 note[87] on the action of *Penicillium glaucum* on the tartrates was Pasteur's last investigation of enantioselective fermentations. Thus, the tartrate-fermentation study was but a brief episode in the midst of his first studies of lactic and alcoholic fermentations. Nevertheless, the tartrate-fermentation work caught the attention of the scientific establishment. The Prize for Experimental Physiology for 1859 was awarded by the *Académie* to Pasteur for his fermentation studies, and the fermentation of the tartrate isomers was explicitly included in the award statement.[93] Another recognition came in 1861 when he was the winner of the Jecker Prize of the Chemistry Section of the *Académie*, and here too, the tartrate-fermentation work was explicitly praised.[94]

Pasteur's discovery of the enantioselective metabolism of tartaric acid had a considerable impact on the further development of the field. His finding has often been acclaimed as another ("Pasteur's third") method for resolving racemic mixtures (albeit with the loss of one of the enantiomers),[95] but its significance as a demonstration of the role of chirality in biological phenomena slowly gained attention during the second half of the nineteenth century. During this period many studies of the stereochemical course of microbiological reactions were stimulated by Pasteur's tartrate-fermentation work. These early studies examined tartaric acid or other compounds as substrates, and eventually included the monumental work[96] of Fischer on the stereoselectivity of enzymatic reactions of sugars. Early reviews of the microbiological and enzymatic work were published by Fajans,[97] Hirsch,[98] and Cushny[99]; a more recent review has been provided by Nicolle.[100] As Nicolle pointed out, the methodology available to the early workers was at times unreliable, but it was nevertheless convincingly demonstrated that, depending on the microorganism, the substrate, and the conditions used, the stereochemical course could be in favor of one or the other enantiomer, or displayed no enantioselectivity.[100]

It is worth recalling that on the day Pasteur first announced his finding of the enantioselective fermentation of (±)-tartaric acid, in December, 1857, he was 6 days short of his 35th birthday; his portrait from that year is shown in Figure 6.

Fig. 6. Pasteur as Dean of the Faculty of Sciences in Lille in 1857, the year he discovered biological enantioselectivity (reprinted from "IMAGES DE LA VIE ET DE L'ŒUVRE DE PASTEUR," by Pasteur Vallery-Radot, Flammarion, 1956).

PASTEUR AND THE ROLE OF SERENDIPITY IN SCIENCE

Finally, as discussed earlier, chance likely played an important role both in Pasteur's decision to study fermentations and in his discovery of biological enantioselectivity, but he himself did not acknowledge any involvement of serendipity in those events in his career. For example, as we have seen, Bigo's name and Pasteur's investigation of his fermentation problem appear in Pasteur's laboratory notebook, but they are nowhere to be found in his published writings and lectures. As we have seen also, both Duclaux, a biographer intimately familiar with Pasteur's work, and others reported the accidental fermentation of (+)-tartaric acid in his laboratory as a serendipitous hint for the investigation of the fermentation of (±)-tartaric acid but Pasteur made no mention of such a laboratory accident in his writings and lectures.

Pasteur's reluctance to acknowledge the intercession of serendipity in crucial events in his scientific life was most likely a reflection of his mind-set in which pure chance had little relevance to discoveries in experimental science in general and to the shaping of his own scientific career in particular. Concerning the former, his statement[101] that "in the field of experimentation, chance favors only the prepared mind" has been widely quoted, Pasteur expressed this view many times, in the form given above or in some modifications thereof, but it appears that the first recorded instance of his using the above statement was in his inauguration speech when he became Dean of the Faculty of Sciences in Lille, on December 7, 1854.[101] At first reading, his famous statement may seem to stress the importance of chance in science, but on closer examination it becomes clear that Pasteur's intent was to downplay the importance of serendipity and to elevate the essential role of preparation (i.e., education, training, culture, etc) in the scientific discovery process. As for his own career, he expressed his firm belief that the evolution of his scientific path was determined by a series of compelling logical interconnections that inexorably led from one investigation to the next.[102] In this vein, he said the following about his move from crystallography and chirality to fermentations: "Carried along, enchained should I say, by the almost inflexible logic of my studies, I have gone from investigations on crystallography and molecular chemistry to the study of microorganisms" (translation by Dubos).[102]

In this regard, Pasteur's disingenuous assertion first of optically active amyl alcohol and later of the enantioselective tartrate fermentation as the cause of his move from chirality into microbiology (see above) may be ascribed to the need he must have felt to demonstrate that "inflexible logic" in the changing focus of his research career. Obviously, such rationalization also relieved Pasteur of the necessity to acknowledge serendipity as a significant factor in the evolution of his scientific path.

The basis for Pasteur's rejection of chance as a significant factor in scientific discovery may be traced to the broader framework he constructed for the role of experimental science in the life of a country. He viewed science as a rational and universal force for peace and progress, and believed that society had a responsibility to provide sufficient financial support for scientific research.[103–107] After the defeat of France by Prussia in the war of 1870–1871, he even expressed his belief that it was because of the neglect of science during the previous 50 yr that France had lost its military might and the war.[106] The fallacy of this claim has been pointed out by Rocke.[108]

Not surprisingly, Pasteur's view of the role science—a rational system promising progress and security for the nation derived from taxpayer-funded research and its practical technological fruits—could not include a priori reliance on the unpredictable device of chance. It is also clear, however, that even when serendipity did demonstrably play a role in his work, Pasteur could not countenance its a posteriori public acknowledgment. Little wonder Bigo appeared only in his laboratory notebook.

CONCLUSIONS

Pasteur's move into fermentations in 1856 eventually resulted in his luminous career in microbiology and infectious diseases for which he has been widely recognized and honored. In the present context, his venture into fermentations led him to the discovery of the phenomenon of enantioselectivity in biology when he examined the fer-

mentation of tartaric acid in 1857. Serendipity most likely played an important role in those turns of his career, but, on the basis of his view of the nature and role of experimental science, Pasteur's rejected chance as a significant factor in the scientific discovery process. The result was his prejudiced account of both the reasons for his shift from molecular chirality to microbiology and for his undertaking studies on the fermentation of tartaric acid. These conclusions must be viewed, however, in the broader context of Pasteur's life and work. There is no doubt that he was a brilliant scientific mind and one of the greatest scientists humanity has known, but it is also clear that, despite more than a 100 yr of hagiographic literature about him that turned him into a "saint laïque" (lay saint),[109] he was a human being with both admirable qualities and human weaknesses.

Pasteur's discovery of biological enantioselectivity was, first, a confirmation of his exceptional ability to identify new and fundamentally important directions for scientific inquiry and to design crucial experiments for the testing and development of new ideas. His finding was also a revolutionary observation that pointed the way for early investigations of the potential influence of chirality in biological phenomena. In the final analysis, Pasteur's discovery of the enantioselective tartrate fermentation began a process that, over a period of more than a century, established the fundamental importance of molecular chirality in a variety of fields of biology.[2–6]

APPENDIX: ENGLISH TRANSLATION OF PASTEUR'S MEMOIR PRESENTED TO THE ACADÉMIE ON MARCH 29, 1858 (COMPTES RENDUS DES SÉANCES DE L'ACADÉMIE DES SCIENCES 1858;46:615–618)

Memoir on the Fermentation of Tartaric Acid

Part One—Just as there is an alcoholic ferment, yeast of beer, which is found everywhere where sugar is converted to alcohol and carbonic acid and which is an organized being according to the observations of Mr. Cagniard de Latour, so there is lactic yeast, always present when sugar is converted to lactic acid. If all decomposable nitrogenous matter can transform sugar into lactic acid, it is because such matter is, for the development of this ferment, suitable food for its constitution. These were the results of work that I had the honor of communicating to the Academy during the session of last November 30. I will now show that the fermentation of tartaric acid leads to entirely analogous conclusions.

It has been known for a long time as a result of mishaps during manufacturing, that crude calcium tartrate mixed with organic matter and kept in water could ferment. Mr. Nœllner, a manufacturing chemist, studied the products of this fermentation and found among them an acid that he believed to be new. The exact composition of this acid was given by Mr. Nicklés, and the same acid was found by Messrs. Dumas, Malaguti, and Leblanc, in their fine study of cyanohydrin ethers, to be identical with metacetonic acid, which Mr. Gottlieb had obtained from the action of potash on sugar.

Today I will not deal with the products which arise from the fermentation of tartaric acid. I will revisit this matter in the near future in a particular study. Here I will only state that my experiments involved ammonium tartrate and not calcium tartrate. This change in the nature of the base causes changes in the composition of the products, together with some other very strange particulars, but these details would complicate the examination of the cause of the phenomenon to which I want to devote the first part of this communication.

I operate in the following manner:

Pure ammonium tartrate is dissolved in distilled water to which I add nitrogenous albuminoidal matter soluble in water, such as the extract of the juice of a plant, of any humor of animal economy, or the soluble part of ordinary yeast of beer. It is sufficient that the tartrate solution contain such matter to the extent of two-to-three thousands of its total weight. The perfectly clear liquid, while very warm, is placed in a flask, filling it to the neck. When it has cooled to about 30 degrees, one adds a few cubic centimeters of turbid liquid from a fermentation of tartaric acid that has been proceeding for several days and which was initiated in the ordinary way, so to speak. The quantity of solid matter that is introduced in this way is unknowable. However, it has a great effect. If the conditions of temperature and neutrality or slight alkalinity are properly observed the entire liquid becomes turbid in a few hours and the next day the fermentation manifests itself by the release of gas.

Here are some characteristics of the fermentation carried out as I have just described.

The turbidity of the liquid and the release of gas increase little by little, and one observes the progressive formation of a precipitate at the bottom of the flask. The quantity of precipitate is extremely small in relation to the weight of the tartrate. As in all fermentations, the release of gas decreases after reaching a maximum. Moreover, the gradual conversion of tartaric acid to products inactive on polarized light can be easily followed by optical examination of the liquid. The material that is deposited during the fermentation appears in the microscope to be formed of small rods or granulations of very small diameter, collected in a mass, in irregular fragments, and as though attached together with a glutinous substance. But a more careful examination shows that this attachment together of the granules is probably due to an entanglement of small filaments formed by the granulations arranged like the beads of a rosary. The diameter of the small granulations or globules is noticeably the same as in the lactic yeast, and the general appearance in the microscope of these two organisms shows great analogies. The deposits in question here, washed abundantly with water and placed in a solution of ammonium tartrate in pure water, assure its fermentation. After a few hours of contact, one can show that some tartrate has been transformed, that is, that the fermentation occurs immediately.

Part Two—The Academy will remember the unique nature of racemic acid. The Academy knows that this acid is formed by the combination of one molecule of right tartaric acid —which is the ordinary tartaric acid—and one

molecule of left tartaric acid, which differs from right tartaric acid only in that these two otherwise identical forms are not superposable, and also in that the optical rotation by the former is to the right and in the latter to the left, to an equal extent in absolute value. Moreover, the Academy knows that the chemical properties of these two acids are so completely identical that it is impossible to distinguish them, unless they are placed in the presence of substances active on polarized light, for then their behavior will be fundamentally different.

There was a great deal of interest therefore to investigate whether racemic acid would undergo the same fermentation as the right acid, or, in other words, whether the yeast whose preparation I provided above would transform the left acid as easily and in the same manner as it would the right acid. Ammonium racemate was subjected to fermentation by following the directions I gave above for the right tartrate. The fermentation materialized equally readily, with the same characteristics and yeast deposition. But if the progress of the phenomenon is followed with the aid of a polarimeter, it becomes clear that the course is very different here. After a few days of fermentation, the initially inactive liquid acquires noticeable optical rotatory power to the left, and this power increases progressively as the fermentation continues, in such a way that it reaches a maximum. At that point the fermentation stops. There is no trace of the right acid remaining in the liquid, which, after it is evaporated and treated with an equal volume of alcohol, immediately gives an abundant crystallization of ammonium left-tartrate.

Here, then, is without doubt an excellent means of preparing left tartaric acid. But all the interest in the preceding facts derives from the physiological role of the fermentation, which behaves in my experiments as a phenomenon with characteristics of life. Indeed, we see here the property of molecular dissymmetry possessed by natural materials intervening in a physiological phenomenon as a modifier of affinity. There is no doubt that it is the kind of dissymmetry possessed by the molecular arrangement of the left tartaric acid that is the unique, exclusive, cause of the non-fermentation of this acid under conditions where the right acid is destroyed.

To be sure, certain philosophical ideas on the sustenance by all things necessary to the harmony of the universe allow the affirmation that the dissymmetric character of natural organic products which is so general plays a role in the plant and animal economies. But science requires more than a priori views. Now, I note that for the first time, in the phenomenon which I have just exposed, the property of right or left dissymmetry of natural products intercedes clearly as a modifier of chemical reactions of a physiological nature.

As for the intimate cause of the difference that I demonstrate in the fermentation of the two tartaric acids, it appears plausible to me to attribute it to the rotatory power of the materials that are part of the constitution of the yeast. It is logical that if the yeast is by nature made up of dissymmetric materials, it does not accommodate to an equal degree the two forms of a nutrient which is itself dissymmetric in the same or in the inverse sense, more or less as we have seen in my previous investigations that the right tartrate of quinine differs fundamentally from the left tartrate of the same base, which is optically active, while the left and right tartrates of potassium or any other inactive base are chemically identical.

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Absolute Configuration of Tropane Alkaloids Bearing Two α,β-Unsaturated Ester Functions Using Electronic CD Spectroscopy: Application to (*R,R*)-*trans*-3-Hydroxysenecioyloxy-6-Senecioyloxytropane

MUNIR HUMAM,[1] PHILIPPE CHRISTEN,[1] ORLANDO MUÑOZ,[2] KURT HOSTETTMANN,[1] AND DAMIEN JEANNERAT[3]*

[1]*Laboratory of Pharmacognosy and Phytochemistry, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, CH-1211 Geneva 4, Switzerland*

[2]*Departamento de Química, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile*

[3]*Department of Organic Chemistry, University of Geneva, CH-1211 Geneva 4, Switzerland*

ABSTRACT The absolute configuration of heterocyclic natural products substituted with two mobile α,β-unsaturated esters was studied using electronic circular dichroism (CD) spectroscopy. The conformational flexibility of the side chains imposed the use of density functional theory calculation to determine the set of the most probable conformations in solution. The electronic CD and UV spectra were calculated by Boltzmann-weighted average of the simulated spectra using the results of the excited states calculation of a set of simplified structures. Comparison with the experimental CD spectrum allowed to determine whether the calculations were made with the right enantiomer. The method was applied to the determination of the absolute configuration of (*R,R*)-*trans*-3-hydroxysenecioyloxy-6-senecioyloxytropane. *Chirality 20:20–25, 2008.* © 2007 Wiley-Liss, Inc.

KEY WORDS: tropane alkaloids; *Schizanthus tricolor*; (*R,R*)-*trans*-3-hydroxysenecioyloxy-6-senecioyloxytropane

INTRODUCTION

Tropane alkaloids are natural compounds having in common the 8-aza-bicyclo[3.2.1]octane structure. They mainly occur in the Solanaceae, Erythroxylaceae, and Convolvulaceae families. Some of these alkaloids such as (−)-hyoscyamine, the more stable racemate atropine and (−)-scopolamine have important pharmacological properties and are widely used in therapeutics. The majority of these compounds are esters of 3-hydroxy, di-(3*R*,6*R*; 3*S*,6*S*) hydroxy or tri-(3,6,7)hydroxytropanes with various organic acids.[1] In particular, the genus *Schizanthus* (Solanaceae) is characterized by the presence of a wide range of α,β-unsaturated ester substituents, Scheme 1.

Because of the difficulty to distinguish the enantiomers, the drawing and the numbering of tropane alkaloid molecules is often confusing in the literature and it is in many cases an opened question whether naturally occurring disubstituted tropanes are (3*R*,6*R*) or (3*S*,6*S*). It is still to be verified whether it is general, but the currently available data[2] indicate a majority of compounds to be (3*R*,6*R*). The relative stereochemistry is based on NMR data and the fact that all naturally occurring tropanes are either (*R,R*) or (*S,S*). In this article, we shall demonstrate that the new disubstituted alkaloid isolated from the aerial parts of *Schizanthus tricolor* Grau et Gronbach[3] is indeed (*R,R*)-*trans*-3-hydroxysenecioyloxy-6-senecioyloxytropane (**A**, Scheme 2).

The rationalization of the NMR chemical shift induced by a chiral auxiliary reagent such as Mosher acid[4] is a mean to determine the absolute configuration of such compounds, but it imposes the chemical saponification of the side chains and functionalization of the alcohol with the shift reagent. To avoid having to sacrifice often precious material, we propose to apply the much more sensitive exciton circular dichroism (CD) spectroscopy in combination with computer simulation of CD spectra.

Exciton coupling is a very useful tool to determine the absolute configuration and conformation of organic compounds.[5] It allows determining the absolute sense of twist between two UV-active chromophores provided the electronic transition moment is known and that they are not accidentally orthogonal, collinear, or coplanar. For example, the relative configuration of adjacent secondary hydroxyl groups can be determined when derivatized with benzoates.[5] When the molecules are conformationally rigid and the chromophores have no or little mobility with respect to each other, a simple model allows to predict whether the experimental CD cotton effect should be positive or negative. In this article we will discuss the case of

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*Correspondence to: Damien Jeannerat, Department of Organic Chemistry, University of Geneva, Quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland. E-mail: damien.jeannerat@chiorg.unige.ch

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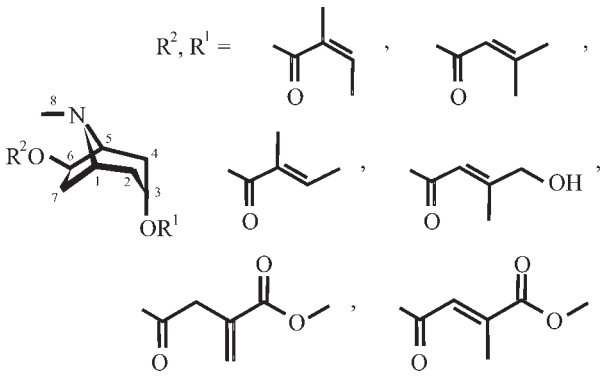


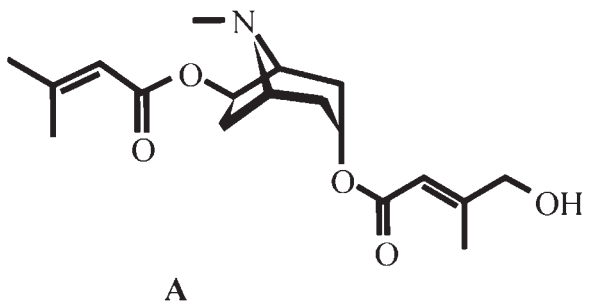
Scheme 1. Common substitutants of natural tropanes encountered in genus *Schizanthus*.

molecules containing pairs of mobile α,β-unsaturated esters, a chromophore with more than one stable conformation and more than one important electronic transition contributing to the CD spectrum. Because of the high energy barrier of the s-*cis* to s-*trans* conformational change, we could not apply the method based on molecular dynamic developed by Bringmann et al.[6,7] Note that a method using vibrational CD spectroscopy has been recently developed to ascertain the absolute configuration of diastereoisomers of monosubstituted 6β-hydroxyhyoscyamine.[8]

MATERIALS AND METHODS

(3*R*,6*R*)-*trans*-hydroxysenecioyloxy-6*R*-senecioyloxytropane (**A**) was isolated from the aerial parts of *Schizanthus tricolor* Grau et Gronbach (Solanaceae) and characterized as a new alkaloid.[3] The electronic CD spectrum of **A** (0.1 mM in 1 ml MeOH) was measured on a Jasco J-715 spectropolarimeter.

All density functional theory (DFT) calculations were carried out with the program Gaussian 03[9] using the B3LYP functional. The geometry optimizations were done using the 6-31G(d) basis set. All the energies found in the tables correspond to values obtained after zero point correction. The simulated CD spectrum was obtained as follows: 30 transition states were calculated for each of the 36 geometries of the generic structure, using the B3LYP/6-311 + G(d,p) and ZINDO for comparison. One calculation using the pVDZ basis was used to show the similarity



Scheme 2. (*R,R*)-*trans*-3-hydroxysenecioyloxy-6-senecioyloxytropane.

TABLE 1. Energy differences after geometry optimization of the main piperidine ring with R^1, R^2 = H

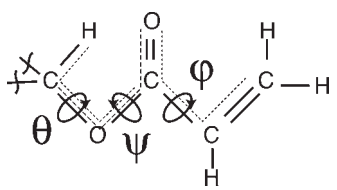
Six-membered ring conformation	Me–N orientation	Energy (kcal/mol)
Chair	Equatorial	0.0^{ref}
	Axial	1.5
Boat	Equatorial	4.6
	Axial	7.4

with the 6-311 + G(d,p) calculations (see supplementary material). For each geometry, the result is a spectrum consisting of 30 delta functions located at the excitation energies with amplitudes equal to the velocity rotational strengths of the transitions. The convolution product of this spectrum with a 14.4 nm half-width at full height Gaussian function results in the CD spectra of the individual geometries. It was assumed that the CD spectra do not differ significantly upon substitutions of the vinylic hydrogens with methyl and hydroxymethyl groups. The Boltzmann-weighted average spectra were calculated based on the energy profiles calculated using 6-31G(d) and taking into account the effect of substitution to the double bonds.

RESULTS AND DISCUSSION

DFT geometry optimization of partially constrained structures allowed mapping the energy profile of the mobile elements. The calculations started with the determination of the conformation of the main tropane skeleton. The piperidine six-membered ring alone (R^1 = R^2 = H) has the methyl group in equatorial position. This is in agreement with the ^{13}C chemical shift expected for this conformation.[10] Table 1 shows that the structure with the Me group in axial position has the second lowest energy (+1.5 kcal) representing less than 10% population. It was neglected as well as the two higher-energy structures in the boat conformation. For relevance with our situation, the 6-OH group was not allowed to turn towards the nitrogen and make hydrogen bond with the free pair of electrons of the N when the Me is axial. This type of stabilization is known to occur in both enantiomers of the 6-hydroxy compounds where the CH_3 is in axial conformation.[8]

Then the side chains were added and their conformations studied. To reduce the number of degrees of freedom because of the methyls and $-CH_2OH$, we replaced these groups with hydrogens and used as generic side chains R^1, R^2 = O—CO—CH=CH_2. The different conformations of the side chains were studied as a function of the dihedral angles φ, ψ, and θ (Scheme 3).



Scheme 3. Definition of the dihedral angles ψ, φ, θ.

TABLE 2. Comparison of the electronic energy as a function of ψ, the CHO—CO dihedral angle

R^1	R^2	ψ	Energy (kcal/mol)
CO—CH=CH_2	H	0	0.0^{ref}
CO—CH=CH_2	H	180	8.6
H	CO—CH=CH_2	0	0.0^{ref}
H	CO—CH=CH_2	180	9.3

The first dihedral angle optimized was ψ. Calculations were made only for the geometries with π-conjugation, i.e., where ψ = 0° and 180°. Table 2 shows a clear advantage for the carbonyl pointing towards the hydrogens on C(3) and C(6) allowing to safely ignore the case with ψ = 180°.

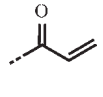
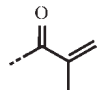
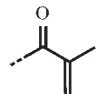
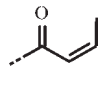
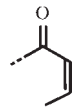
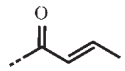
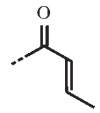
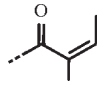
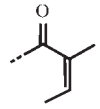
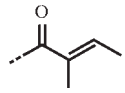
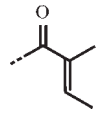
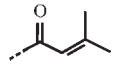
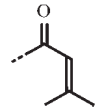
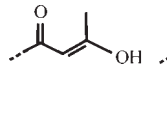
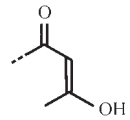
The second optimized dihedral angle was φ. The difference in energy between the s-*cis* (φ = 0°) and s-*trans* (φ = 180°) conformations was calculated independently for the 3- and 6-monosubstituted tropanes. Table 3 reveals a small energy difference corresponding to an abundance ratio of 3.5:1, at 25°C, in favor of the s-*cis* conformation. But this ratio is not large enough to neglect the s-*trans* structure especially when methyls or hydroxymethyls are added to the double bond. When considering the C(3) and C(6) disubstituted tropanes, four possibilities (*cis/cis*, *cis/trans*, *trans/cis*, and *trans/trans*) have to be taken into account in the simulations of the CD spectrum. To determine the *cis/trans* ratio in mono- and di-substituted structures that are quite common in natural products, we calculated the energy differences in a model structure where the tropane is replaced with a methyl group. The results (Table 4) indicate that in the cases where a methyl is bond in the *E* configuration with respect to C=C, the s-*trans* configuration can be neglected because of a low probability of occurrence.

Finally the energy profiles as a function of θ were calculated for both the s-*cis* (Figs. 1A and B) and s-*trans* conformations (Figs. 1C and D) of tropane substituted only at C(3) and C(6) separately for both the generic structures and methylated double bonds. The energy minimum of the C(6) substituted structure corresponds to an *anti* relationship between the O—CO and the C(6)—C(7) bonds. At the C(3) position, the profile is symmetrical with respect to the local environment of the structure, and the minima correspond to dihedral angles of ±35°, i.e., 10° off the *anti* relationship with respect to the C(3)—C(2) and C(3)—C(4) bonds. Note that the assumption used in similar problems involving carbohydrates[5] that the dihedral angle is fixed at a value close to zero is not fulfilled in our case. At C(3), the range of angles corresponding to electronic energies below 0.5 kcal goes from −50° to +50°. The only exception corresponds to the steric hindrance of the methyl group with the ester in the structure shown in the second column of entry 3 in Table 4. This means that the chromophore has a large degree of freedom which justifies to take the structure dynamics into account.

TABLE 3. Comparison of relative energies as a function of the φ angle

R^1	R^2	Conformation	Energy (kcal/mol)
CO—CH=CH_2	H	s-*cis* (φ = 0°)	0.0^{ref}
CO—CH=CH_2	H	s-*trans* (φ = 180°)	0.8
H	CO—CH=CH_2	*s-cis*	0.0^{ref}
H	CO—CH=CH_2	*s-trans*	0.7

TABLE 4. Comparison of the relative energies of diverse methylacrylamides as a function of the φ angle

	s-*cis*[a]	s-*trans*[b]	ΔE [kcal/mol][c]	*cis/trans* ratio[d]	CH_3 rotation[e] cis/trans
1			0.71	3.3	-
2			−0.07	0.9	2.06/1.52
3			1.83	22.0	0.78/0.57
4			0.90	4.6	1.93/1.94
5			1.14	6.9	[f]
6			0.29	1.63	[f]
7			2.29	47.7	[f]
8			2.16	38.3	3.31/3.13[g]

[a](φ = 0).
[b](φ = 180).
[c]of the s-*trans* conformation relative to the s-*cis*.
[d]at 25°C.
[e]Rotation barrier of the allylic methyl. The lowest energy corresponds to 180° C=C−C−H dihedral angles.
[f]The barriers were not calculated but the fact that the lowest energies correspond 180° C=C−C−H dihedral angles was checked.
[g]The barriers correspond the C=C−C−O dihedral angles.

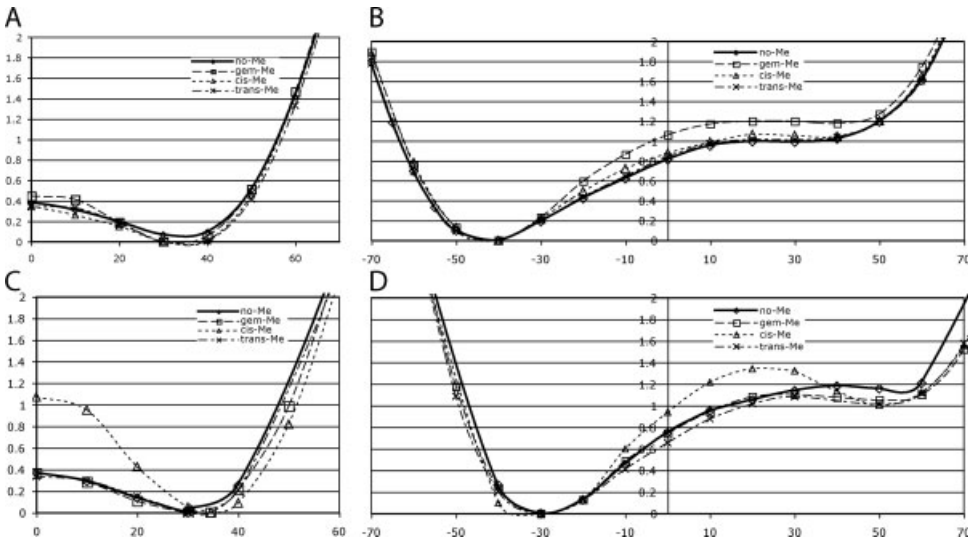


Fig. 1. Energy profiles of tropane monoderivatized at C(3) (**A**, **C**) and C(6) (**B**, **D**) with constrained H—C—O—CO dihedral angles with CO—CH=CH_2 in the s-*cis* conformation at (A, B) and s-*trans* conformation at (C, D). The minima of the non-methylated structure correspond to θ equal to 35.2 (A) −42.7 (B) 34.26 (C) and −46.82 (D).

At C(6) the mobility is slightly smaller: the range of dihedral angle below 0.5 kcal is only −55° to −20° but reaches a broad plateau up to about +55° and is only 1 kcal/mol above the minimum corresponding to structures with probabilities that cannot be neglected. Assuming independent dynamics of the two side chains in disubstituted tropanes, the bidimensional energy profile of the biderivatized model compound can be approximated to be the sum of the two profiles of Figure 1.

Population analysis of molecular orbitals of methylacrylamide showed that two electronic absorptions contribute significantly to the UV spectrum around 200 nm (see Supplementary material). When comparing the excited states using ZINDO and DFT calculations one can observe very different electric dipoles directions for one of the relevant excited states (38° and 97° differences for the s-*cis* and s-*trans* structures respectively—see Supplementary material). This probably explains why the ZINDO-based CD spectra match poorly the experimental data. Analysis of excited states of the disubstituted tropanes showed that the important Kohm-Sham orbitals (see Supplementary material) are almost exclusively of the π and π* type allowing us to conclude that the mechanism is mainly excitonic.

DFT calculation of the excited states (TDDFT) allowed to simulate the CD spectra of the most probable structures with the generic side chains R^1, R^2 = O—CO—CH=CH_2. This simplification of the structure is expected to cause down shift of the chromophore absorption energy of about 10 cm^{-1} per removed methyl[11] but this shift causes no difficulties because the general shape of the spectrum is more important than the position of spectral features along the abscissa. To correctly simulate the experimental CD of disubstituted tropanes, one has to consider the Boltzmann-weighted average simulations as a function of the two independent dihedral angles θ_1 and θ_2. We therefore calculated four set of spectra corresponding to the 3-*cis*/6-*cis*, 3-*cis*/6-*trans*, 3-*trans*/6-*cis*, and 3-*trans*/6-*trans* conformations (φ_1, φ_2 = 0/180°). Each set was composed of 3 × 3 spectra to fractionate the motion of the θ angles in three (θ_1 = −35, 0, +35° and θ_2 = −45, 0, +45°). In the lowest energy structure shown in Figure 2, the dihedral angles are θ_1 = +33.8°, θ_2 = −42.3° and φ_1, φ_2 = 0° corresponding to the 3-*cis*/6-*cis*. Because the amplitudes of the CD spectra can vary significantly as a function of θ_1 and θ_2 (see supplementary material), the contributions of relatively low-probability conformations with strong CD spectra may contribute more to the weighted average CD spec-

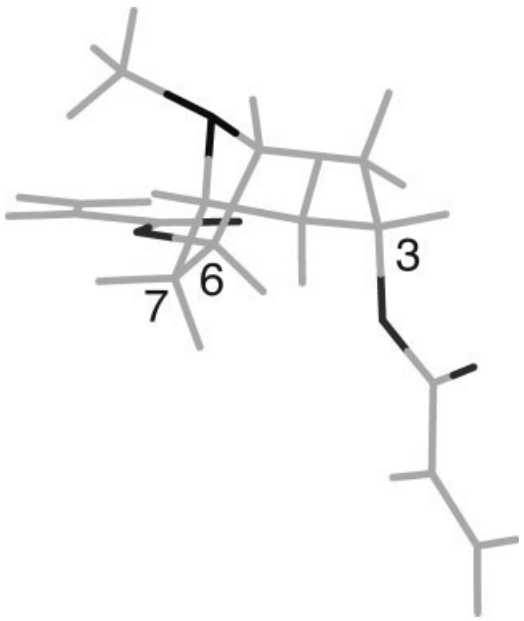


Fig. 2. 3D structure of the lowest energy 3,6-disubstituted model tropane. The side chains are flat and the CO of the C(3) bond ester points towards us.

trum than the spectrum of the lowest energy structure. This explains why we calculated the CD spectra of geometries corresponding to what could otherwise appear too low probabilities to be taken into account. The calculated spectra for the 3-*cis*/6-*cis*, 3-*cis*/6-*trans*, 3-*trans*/6-*cis*, 3-*trans*/6-*trans* are shown in Figure 3.

The application of the CD calculation to the determination of the absolute configuration of our *trans*-(3)-hydroxy-senecioyloxy-(6)-senecioyloxytropane only required to take into account the CD spectra of the 3-*cis*/6-*cis* conformation (see broad continuous lines in Fig. 3A) because the three "s-*trans*" structures having methyls in *E*-configuration with respect to C=C are too high in energy to be significantly populated as shown in Table 4. The Boltzmann-weighting of the calculated spectra shown in Figure 4A is only slightly different from the broad line in Figure 3A because the energy profiles of s-*cis*- and s-*trans*- disubstituted double bonds are very similar to the one of the generic structure as shown in Figures 1A and B. A comparison of the experimental spectra of (**A**) (Fig. 4) with the calculated spectrum using B3LYP/6-311 + G(d,p) allows to conclude that the simulated spectrum corresponds to

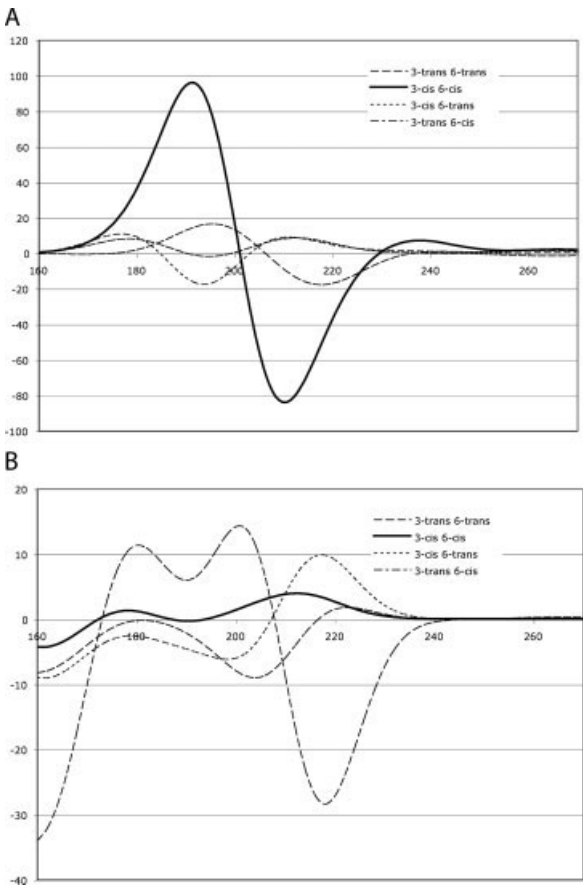


Fig. 3. Calculated CD spectra after Boltzmann weighting of the mobility along θ_1 and θ_2. Calculations are made separately for the s-*cis* and s-*trans* side-chains corresponding to $\varphi = 0$ and 180° respectively using (A) B3LYP/6-311+G(d,p) and (B) ZINDO techniques. The units are the same as the ones of the spectra of each considered geometry (see Supplementary material).

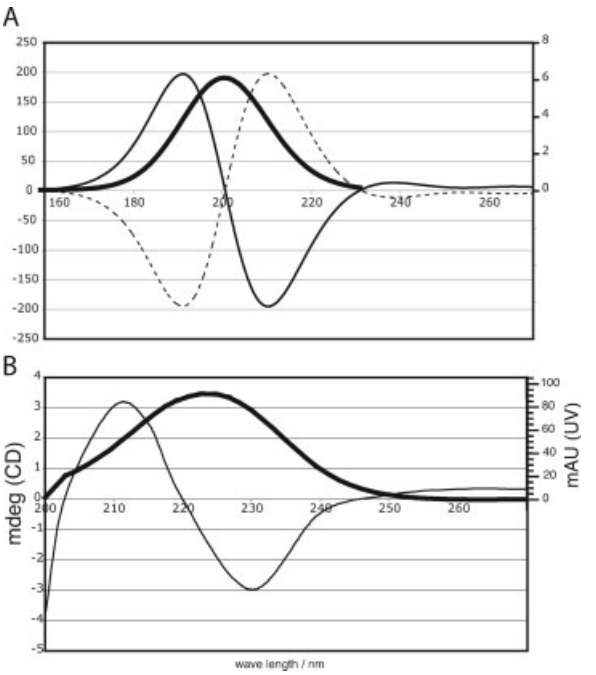


Fig. 4. (A) Calculated CD (normal line) and UV (broad line) spectra based on TDDFT of the generic structures after Boltzmann weighting of the nine *cis*/*cis* spectra with the energies of the *cis*-methylated structures (triangles in Figs. 1A and B). (B) Experimental spectrum of (*R*,*R*)-trans-3-hydroxysenecioyloxy-6-senecioyloxytropane clearly corresponds to the calculation of Fig. 3A.

the structure found in solution and that the absolute configuration is indeed (3*R*,6*R*) and not (3*S*,6*S*). The fact that ZINDO calculation cannot be applied to such calculation is clear from the bad correspondence with the experimental data and B3LYP/6-311 + G(d,p) calculations. Further confirmation that this family of molecules correspond to (3*R*,6*R*) has been given by NMR experiments of Mosher derivatized[4] C(6)-alcohols (unpublished results).

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CHIRALITY 20:26–34 (2008)

Chiral Mono and Diamide Derivatives of Calix[4]arene for Enantiomeric Recognition of Chiral Amines

ERDAL KOCABAS, MUSTAFA DURMAZ, SABRI ALPAYDIN, ABDULKADIR SIRIT,* AND MUSTAFA YILMAZ
Department of Chemistry, Selçuk University, Konya, Turkey

ABSTRACT Novel chiral mono and diamide derivatives of calix[4]arene have been prepared from the aminolysis reaction of 5,11,17,23-tetra-*tert*-butyl-25,27-diethoxycarbonyl-methoxy-26,28-dihydroxycalix[4]arene **1** and 25,27-diethoxycarbonyl-methoxy-26,28-dihydroxycalix[4]arene **2** with chiral (*S*)-(−)-1-phenylethylamine (PEA) and (1*S*,2*S*)-(+)-2-amino-1-(4-nitrophenyl)-1,3-propanediol, respectively. Spectrophotometric titrations have been performed in $CHCl_3$ at 20–30°C in order to obtain the binding constants (K) and the thermodynamic quantities (ΔH and ΔS) for the stoichiometric 1:1 inclusion complexation of various chiral amines with these new host compounds. Preliminary experiments were undertaken to confirm the complexation properties of receptors **9** and **13** with PEA by ^1H NMR in $CDCl_3$ at room temperature. The molecular recognition abilities and enantioselectivities for guests (*R* and *S*)-α-PEA and (*R* and *S*)-cyclohexylethylamine (CHEA) are discussed from a thermodynamic point of view. *Chirality 20:26–34, 2008.* © 2007 Wiley-Liss, Inc.

KEY WORDS: chiral calix[4]arene; monoamide; diamide; molecular recognition; UV–vis titration

INTRODUCTION

Chiral recognition is the crucial process in biological systems such as enzymes, antibodies or genes, and is also the fundament of supramolecular chemistry.[1–3] In this process, the functional groups of molecular receptors form complexes preferentially with one of the enantiomers of a chiral molecule by noncovalent interaction such as hydrogen bonding, electrostatic interaction, and hydrophobic interaction.[4–7] Although these interactions, when considered individually, are weak in comparison with covalent bonds, the concerted action of several of these bond types often leads to complexes with very high stability.

The amines and substituted ammonium compounds are among the most important molecules in natural living systems. They are also present as intermediate and synthetic products in the chemical, pharmaceutical, and dyes industries. The study of the enantiomeric recognition of these compounds by artificial receptors was of critical importance in the preparation, separation, and analysis of enantiomerically pure amines and disclosing the mechanism of interaction of the amines with biological systems.[8,9] The rational design of receptors with recognition ability for chiral amines and their ammonium derivatives is still receiving considerable attention, although numerous chiral macrocyclic receptors have been developed for amines, amino acids, and related compounds.

Among the several types of synthetic receptors for recognition, calixarenes offer a number of advantages in terms of their selectivity and efficiency of binding.[10,11] The introduction of chiral substituents on the lower rim through the phenolic oxygens or at the para positions of the calix[4]arene skeleton or by synthesizing "inherently" chiral derivatives could, in turn, lead to the chirality of the artificial receptors. Chiral receptors that are based on the calixarene platform may have potential applications in the preparation, separation, and analysis of enantiomers. In this regard, investigations conducted on the synthesis and chiral recognition properties of chiral calix[4]arene derivatives have attracted considerable attention.[12–14]

Since the synthesis of calixarenes bearing chiral residues were first reported by Shinkai and coworkers,[15] a large number of calix-[4]arene derivatives have been prepared by attaching chiral units at one of the calix rims.[16–18] Among the most popular chiral building blocks used, amino acids,[19,20] peptides,[21,22] amino alcohols,[23,24] sugars,[25–27] tartaric acid esters,[28,29] binaphthyl,[30] glycidyl,[31] menthone,[32] and guanidinium[33] groups offer a wide range of possibilities for providing calix[4]arenes with asymmetric features. Many of the chiral calix[4]arene derivatives have shown remarkable recognition properties toward achiral cations and anions,[34,35] however, more interestingly, some of them have exhibited significant chiral discrimination abilities for chiral guests such as amines,

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*Correspondence to: Abdulkadir Sirit, Department of Chemistry, Selçuk University, 42031 Konya, Turkey. E-mail: asirit42@yahoo.com
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organic ammonium salts, amino alcohols, and amino acids.[36–38]

Our recent studies in this field were mainly dedicated to synthesis and complex formation of chiral calix[4]arene derivatives with amino acids[39] and amines[40] in chloroform. Also the extraction abilities and the transport through chloroform liquid membrane[41] of some functionalized calix[4]arene derivatives upon some anions[42] and cations[43] were performed. The synthesis of calix[4]arene amide derivatives can be achieved by following two synthetic pathways: the first is the reaction of acid chloride derivative of calix[4]arene, prepared in two steps starting from calix[4]arene diester, with a primary amine as reported earlier[44] and the aminolysis[45,46] reaction of calix[4]arene esters is the second and easy alternative route.

Herein, we report the synthesis of new chiral calix[4]arene amide derivatives from the aminolysis reaction of calix[4]arene diester and the binding properties of these new receptors with various chiral amines based on UV–visible spectrophotometric titrations and by ^{1}H NMR spectroscopy.

MATERIALS AND METHODS

General

^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker spectrometer in $CDCl_3$ with TMS as an internal standard. Chemical shifts were reported in ppm. IR spectra were obtained on a Perkin Elmer 1605 FTIR spectrometer using KBr pellets. Optical rotations were measured on Atago AP-100 digital polarimeter. UV–visible spectra were obtained on a Shimadzu 160A UV–visible recording spectrophotometer. The HPLC measurements were carried out on Agilent 1100 equipment connected with a Zorbax RX-C18 column. Elemental analyses were performed using a Leco CHNS-932 analyzer. FAB-MS spectra were taken on a Varian MAT 312 spectrometer.

Analytical TLC was performed using Merck prepared plates (silica gel 60 F_{254} on aluminum). Flash chromatography separations were performed on a Merck Silica Gel 60 (230–400 Mesh). All reactions, unless otherwise noted, were conducted under a nitrogen atmosphere. All starting materials and reagents used were of standard analytical grade from Fluka, Merck, and Aldrich and used without further purification. Toluene was distilled from CaH_2 and stored over sodium wire. Other commercial grade solvents were distilled, and then stored over molecular sieves. The drying agent employed was anhydrous $MgSO_4$.

Analytical grade amines were purchased from Aldrich or Fluka and employed without further purification as guest molecules for the experiments: i.e, (*S*)-(−)-1-phenylethylamine (*S*-PEA), (*R*)-(−)-1-phenylethylamine (*R*-PEA), (*S*)-(+)-cyclohexylethylamine (*S*-CHEA) and (*R*)-(−)-cyclohexylethylamine (*R*-CHEA) (Fig. 1).

UV Spectral Measurement

The recognition abilities of chiral calix[4]arenes with amines were determined on the basis of the differential UV spectrometry in chloroform. The UV–vis spectra were measured at 20, 25, and 30°C with a thermostated cell compartment by Shimadzu 160 UV spectrometer. The same concentrations of guest solution were added to the sample cell and reference cell (light path = 1 cm). The association constants were determined at 242 nm. The concentration of the hosts is between 0.71 and 1.33 × 10^{-4} mol dm^{-3} with the increasing concentration between 2.5 and 13 × 10^{-3} mol dm^{-3} of the added guest.

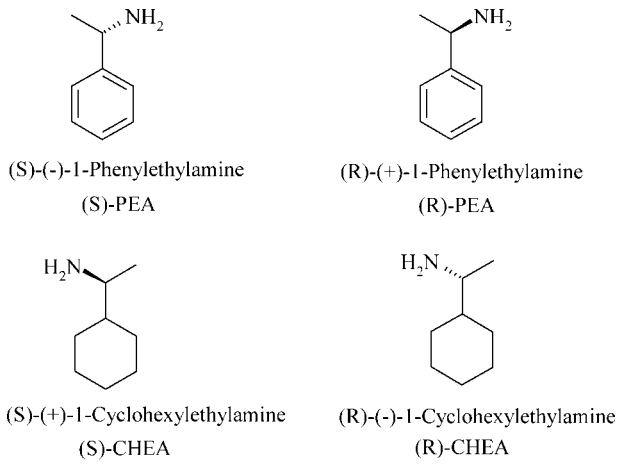


Fig. 1. Chemical structures of the guests employed.

^{1}H NMR Experiments

In NMR experiments, host compounds **9** and **13** were dissolved in dry $CDCl_3$ and the stock solutions of guest (*rac*-PEA) was prepared by dissolving in dry $CDCl_3$. Upon addition of the guest solution (10 mM) to the 10 mM host solution, upfield or downfield chemical shifts and the resolutions of resonances for various protons of guest and host compounds were observed.

Synthesis

Compounds **1–2** and **8–13** were synthesized according to previously described methods.[47–49]

General procedure for the synthesis of compounds 3–7. Appropriate chiral primary amine or amino alcohol (20.0 mmol) was dissolved in 1:2 toluene/MeOH mixture (60 ml) and added dropwise into a solution of 5,11,17,23-tetra-*tert*-butyl-25,27-diethoxycarbonylmethoxy-26,28-dihydroxycalix-[4]arene **1** or 25,27-diethoxy-carbonylmethoxy-26,28-dihydroxycalix[4]arene **2** (4.0 mmol) in 20 ml toluene with continuous stirring at room temperature for about 30 min. Then the reaction mixture was refluxed and the reactions were monitored by TLC. After the substrate had been consumed the solvent was evaporated under reduced pressure and the residue was triturated with MeOH to give a crude product.

Chiral p-tert-butylcalix[4]arene monoamide [3]. The crude product was purified by flash chromatography on silica gel (EtOAc/Hexane 1:5) to afford **3** as a white solid (2.61 g, 74%). m.p. = 185–187°C; $[\alpha]_D^{20}$ = + 19.2 (c = 1, $CHCl_3$); IR ν_{max} (KBr)/cm^{-1}: 3357 (OH), 1761 (COO), 1686 (CO) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.81

(s, 9H, C(CH_3)$_3$), 0.87 (s, 9H, C(CH_3)$_3$), 1.24 (s, 18H, C(CH_3)$_3$), 1.60 (d, 3H, CHCH_3), 3.51 (s, 3H, OCH_3), 4.01 (d, 2H, J = 13.4 Hz, ArCH_2Ar), 4.15 (d, 2H, J = 13.1 Hz, ArCH_2Ar), 4.37 (d, 4H, J = 13.3 Hz, ArCH_2Ar), 4.46 (s, 2H, OCH_2CO), 4.57 (d, 2H, J = 15.6 Hz, OCH_2CO), 5.25 (p, 1H, NH—CH—CH$_3$), 6.60–6.80 (m, 4H, ArH), 6.90–7.03 (m, 4H, ArH), 7.07–7.12 (m, 3H, ArH, ph), 7.20 (d, 2H, ArH, ph), 7.40 (s, 2H, ArOH), 8.65 (d, 1H, J = 8.4 Hz NH); ^{13}C NMR (100 MHz, CDCl$_3$) δ = 22.42, 30.91, 30.97, 31.51, 31.76, 31.91, 33.90, 33.98, 51.79, 72.34, 74.36, 125.29, 125.62, 125.79, 125.88, 126.01, 126.21, 126.76, 127.18, 127.52, 127.80, 127.99, 128.32, 131.76, 132.06, 132.22, 142.13, 144.05, 147.38, 147.72, 148.93, 150.08, 150.37, 167.30, 168.87; FAB-MS (*m/z*): 905.1 [M + Na]$^+$. Anal. Calcd for $C_{57}H_{71}NO_7$ (882.18): C, 77.60%; H, 8.11%; N, 1.59%. Found: C, 77.44%; H, 8.35%; N, 1.40%.

Chiral calix[4]arene monoamide [4]. The crude product was purified by flash chromatography on silica gel (EtOAc/Hexane 1:7) to afford **4** as a white solid (0.84 g, 32%). m.p. = 215–217°C; $[\alpha]_D^{20}$ = + 11.4 (c = 0.5, CHCl$_3$); IR ν_{max} (KBr)/cm^{-1}: 3355 (OH), 1758 (COO), 1680 (CO); ^{1}H NMR (400 MHz, CDCl$_3$) δ = 1.63 (d, 3H, CHCH_3), 3.50 (s, 3H, OCH_3), 4.05 (d, 2H, J = 13.4 Hz, ArCH_2Ar), 4.20 (d, 2H, J = 13.2 Hz, ArCH_2Ar), 4.38 (d, 4H, J = 13.6 Hz, ArCH_2Ar), 4.45 (s, 2H, OCH_2CO), 4.58 (d, 2H, J = 15.8 Hz, OCH_2CO), 5.27 (p, 1H, NH—CH—CH$_3$), 6.59–6.68 (m, 4H, ArH), 6.72–6.80 (m, 4H, ArH), 6.93–7.03 (m, 2H, *ArH*), 7.10 (t, 2H, *ArH*), 7.18 (t, 3H, ArH, ph), 7.27 (d, 2H, ArH, ph), 7.38 (s, 2H, ArOH), 8.67 (d, 1H, J = 8.3 Hz NH); ^{13}C NMR (100 MHz, CDCl$_3$) δ = 22.84, 31.37, 31.75, 32.58, 52.14, 73.47, 75.40, 125.31, 125.94, 126.17, 126.28, 126.57, 126.92, 127.38, 127.63, 127.96, 128.06, 128.57, 129.18, 132.43, 132.89, 133.68, 142.96, 145.17, 147.97, 148.14, 149.42, 150.37, 150.83, 166.75, 167.93; FAB-MS (*m/z*): 680.7 [M + Na]$^+$. Anal. Calcd for $C_{41}H_{39}NO_7$ (657.75): C, 74.87%; H, 5.98%; N, 2.13%. Found: C, 73.67%; H, 6.63%; N, 1.97%.

Chiral calix[4]arene diamide [5]. The crude product was purified by flash chromatography on silica gel (EtOAc/Hexane 1:7) to afford **5** as a yellow solid (1.82 g, 40%). m.p. = 239–241°C; $[\alpha]_D^{20}$ = + 9.7 (c = 0.5, CHCl$_3$); IR ν_{max} (KBr)/cm^{-1}: 3347 (OH), 1682 (CO) cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ = 1.58 (d, 6H, CHCH_3), 3.26 (d, 2H, J = 13.4 Hz, ArCH_2Ar), 3.39 (d, 2H, J = 13.5 Hz, ArCH_2Ar), 3.93 (d, 2H, J = 13.4 Hz, ArCH_2Ar), 3.99 (d, 2H, J = 13.6 Hz, ArCH_2Ar), 4.20 and 4.49 (d, 2H each, J = 15.2, OCH_2CO), 5.09 (p, 2H, NH—CH—CH$_3$), 6.65 (t, 4H, ArH), 6.78 (d, 4H, *ArH*), 7.00 (d, 4H, *ArH*), 7.05–7.20 (m, 10H, ArH, ph), 7.52 (s, 2H, ArOH), 8.95 (d, 2H, NH); ^{13}C NMR (100 MHz, CDCl$_3$) δ = 21.76, 32.19, 32.67, 50.85, 74.62, 125.38, 125.73, 126.04, 126.67, 126.80, 126.93, 127.78, 127.84, 129.06, 132.18, 133.95, 143.72, 144.39, 147.86, 149.13, 149.84, 167.93,; FAB-MS (*m/z*): 769.89 [M + Na]$^+$. Anal. Calcd for $C_{48}H_{46}N_2O_6$ (746.89): C, 77.19%; H, 6.21%; N, 3.75%. Found: C, 77.89%; H, 5.94%; N, 3.08%.

Chiral p-tert-butylcalix[4]arene diamide [6]. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/Acetone 10:1) to afford **6** as a yellow solid (1.85 g, 61%). m.p. = 220–222°C; $[\alpha]_D^{20}$ = +34.5 (c = 0.3, CHCl$_3$); IR ν_{max} (KBr)/cm^{-1}: 3360 (OH), 1662 (CO) cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ = 1.12 (s, 18H, C(CH_3)$_3$), 1.28 (s, 18H, C(CH_3)$_3$), 3.35 (d, 2H, ArCH_2Ar, J = 13.5 Hz), 3.54 (d, 2H, ArCH_2Ar, J = 14.0 Hz), 4.16–4.27 (m, 4H, OCH_2CO), 4.35–4.41 (m, 2H, CH$_2$OH), 4.55 (d, 2H, CHOH), 4.60 (d, 2H, ArCH_2Ar, J = 13.5 Hz), 4.70 (d, 2H, ArCH_2Ar, J = 14.0 Hz), 4.94 (d, 2H, CH_2OH), 5.17 (d, 2H, CHOH), 6.98–7.18 (m, 8H, ArH), 7.53 (d, 2H, ArH, ph), 7.94 (d, 2H, ArH, ph), 8.06 (d, 2H, ArH, ph), 8.32 (d, 2H, ArH, ph), 8.84 (s, 1H, ArOH, ph), 8.94 (s, 1H, ArOH), 9.28 (d, 1H, NH, J = 10.4 Hz), 9.40 (d, 1H, NH, J = 10.5 Hz); ^{13}C NMR (100 MHz, CDCl$_3$) δ = 30.62, 31.1, 31.8, 32.1, 52.7, 67.0, 72.1, 123.90, 125.5, 126.22, 126.33, 126.79, 126.89, 128.1, 128.4, 129.3, 132.38 132.54, 138.02, 147.6, 149.3, 167.7; FAB-MS (*m/z*): 1176.26 [M + Na]$^+$. Anal. Calcd for $C_{66}H_{80}N_4O_{14}$ (1153.36): C, 68.73%; H, 6.99%; N, 4.86%. Found: C, 69.17%; H, 7.21%; N, 4.46%.

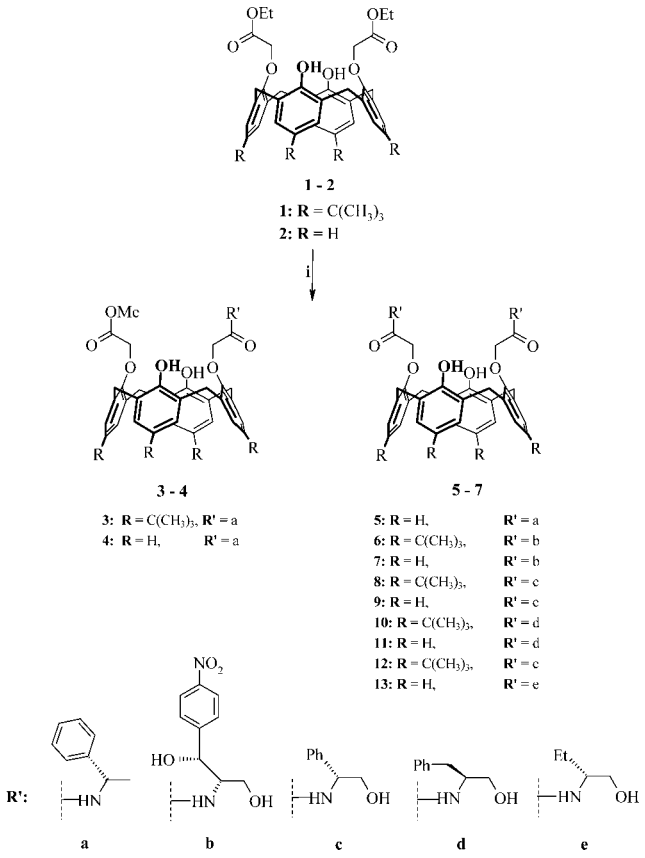
Chiral calix[4]arene diamide [7]. The crude product was purified by flash chromatography on silica gel (EtOAc/Hexane 1:5) to afford **7** as a white solid (2.38 g, 64%). m.p. = 178–180°C; $[\alpha]_D^{20}$ = + 36.1 (c = 1, EtOAc); IR ν_{max} (KBr)/cm^{-1}: 3358 (OH), 1657 (CO) cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ = 3.43 (d, 2H, ArCH_2Ar, J = 13.7 Hz), 3.63 (d, 2H, ArCH_2Ar, J = 14.2 Hz), 4.18 (s, 4H, OCH_2CO), 4.39–4.46 (m, 2H, CH$_2$OH), 4.52 (d, 2H, CHOH), 4.63 (d, 2H, ArCH_2Ar, J = 13.8 Hz), 4.72 (d, 2H, ArCH_2Ar, J = 14.1 Hz), 4.83 (d, 2H, CH_2OH, J = 10.4 Hz), 5.22 (d, 2H, CHOH), 6.94–7.14 (m, 8H, ArH), 7.49 (d, 2H, ArH, ph), 7.90 (d, 2H, ArH, ph), 8.09 (d, 2H, ArH, ph), 8.28 (d, 2H, ArH, ph), 8.79 (s, 1H, ArOH), 8.90 (s, 1H, ArOH), 9.32 (d, 1H, NH, J = 10.5 Hz), 9.46 (d, 1H, NH, J = 10.5 Hz); ^{13}C NMR (100 MHz, CDCl$_3$) δ = 31.3, 32.2, 52.1, 66.3, 70.1, 122.40, 124.8, 125.72, 125.64, 125.48, 126.10, 127.21, 127.82, 128.6, 131.90, 132.12, 138.02, 146.9, 148.7,167.0; FAB-MS (*m/z*): 951.83 [M + Na]$^+$. Anal. Calcd for $C_{50}H_{48}N_4O_{14}$ (928.93): C, 64.65%; H, 5.21%; N, 6.03%. Found: C, 65.40%; H, 5.83%; N, 5.76%.

RESULTS AND DISCUSSION

Design and Synthesis of the New Chiral Receptors

The synthetic route for the preparation of chiral calix[4]-arene mono and diamide derivatives is described in Scheme 1; 5,11,17,23-tetra-*tert*-butyl-25,27-diethoxy-carbonylmethoxy-26,28-dihydroxycalix[4]arene **1** or 25,27-diethoxycarbonyl-methoxy-26,28-dihydroxycalix[4]arene **2** was refluxed with (*S*)-(–)-1-PEA and (1*S*,2*S*)-(+)-2-amino-1-(4-nitrophenyl)-1,3-propanediol, respectively, to give corresponding amide derivatives of calix[4]arene **3–7** in 32–74% yields. Toluene–methanol solvent mixture was employed as toluene facilitates the dissolution of diester while methanol is beneficial to transforming the ethyl ester to the more reactive methyl ester prior to aminolysis.[50]

The new compounds **3–7** were characterized by FTIR, ^{1}H NMR, ^{13}C NMR, FAB MS, and elemental analysis. ^{1}H NMR spectra of the monoamide calix[4]arenes showed a singlet for methoxy protons at 3.50, 3.51 ppm while IR



Scheme 1. Chiral mono and diamide derivatives of calix[4]arene, (i) Appropriate primary amine or amino alcohol, Toluene/MeOH (1:1), reflux.

spectra showed both characteristic amide and ester carbonyl bands about 1680 cm^{-1} and 1760 cm^{-1}, respectively. ^1H NMR spectra of the calix[4]arene diamide derivatives showed only the amide protons at 8.65–9.46 ppm, while IR spectra showed only characteristic amide bands about 1682 cm^{-1} and the disaappearance of ester carbonyl band at 1760 cm^{-1}.

Complexation Studies

The binding constants (K) of inclusion complexes of aforementioned chiral calix[4]arene receptors with amines were determined on the basis of the differential UV spectrometry in chloroform.[51-53] The titration experiments showed that the absorption maximum of all hosts gradually decreased with the addition of various concentrations of amines (Fig. 2).

$$H + G \overset{K}{\rightleftharpoons} H \cdot G \tag{1}$$

With the assumption of a 1:1 stoichiometry, the complexation of amines (G) with chiral amide derivative of calix[4]-arene (H) is expressed by eq. 1:

Under the conditions employed, the concentration of calix[4]arene derivatives (0.71–1.33 $\times$ 10^{-4} mol dm^{-3}) is much smaller than that of amines, i.e. $[H]_0 << [G]_0$. Therefore, the stability constant of the supramolecular system formed can be calculated according to the modified Hildebrand–Benesi equation,[54,55] eq. 2, where $[G]_0$ denotes the total concentration of amine, $[H]_0$ refers to the total concentration of calix[4]arene derivative, $\Delta\varepsilon$ is the difference between the molar extinction coefficient for the free and complexed calix[4]arene derivative, ΔA denotes the changes in the absorption of the modified calix[4]arene on adding amines.

$$1/\Delta A = 1/K\Delta\varepsilon[H]_0[G]_0 + 1/\Delta\varepsilon[H]_0 \tag{2}$$

For all guest molecules examined, plots of calculated $1/\Delta A$ values as a function of $1/[G]_0$ values give good straight lines, supporting the 1:1 complex formation. Typical plots are shown for the complexation of compound **3** with (*S*)-PEA (Fig. 3).

The free-energy change (ΔG) for inclusion complexes formed by chiral calix[4]arene amide derivatives and guest amines is calculated from the equilibrium constant K by eq. 3 and is related to

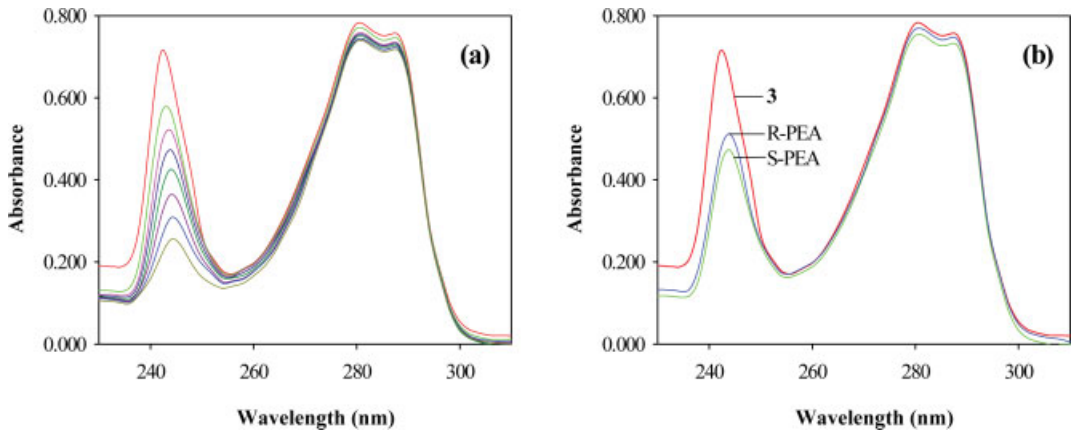


Fig. 2. (**a**) UV–vis spectra of **3** (1.33 $\times$ 10^{-5} mol dm^{-3}) in the presence of (*S*)-(−)-1-phenylethylamine (4–12 $\times$ 10^{-2} mol dm^{-3}) in $CHCl_3$. (**b**) Spectral changes upon the addition of phenylethyl amine ((*R*)-PEA; (*S*)-PEA) (5.3 $\times$ 10^{-2} mol dm^{-3}) to an $CHCl_3$ solution of **3** (1.33 $\times$ 10^{-2} mol dm^{-3}) at 25°C. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

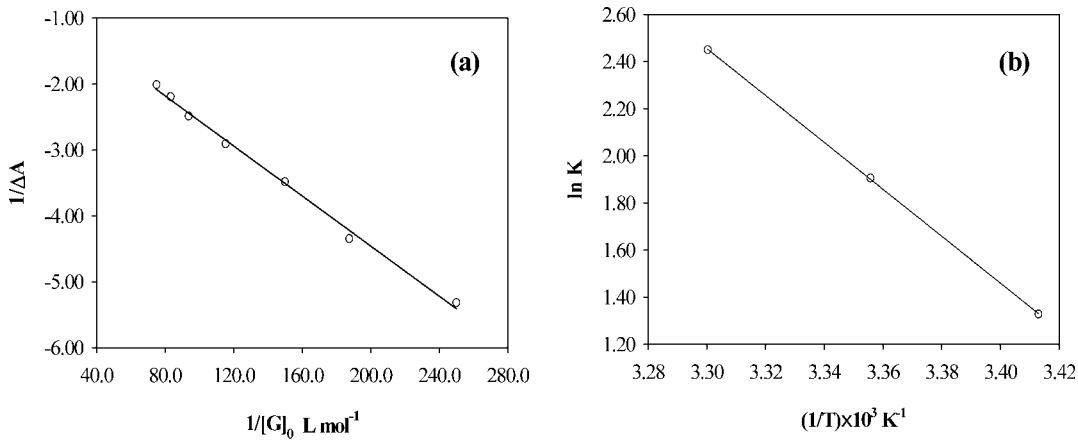


Fig. 3. (**a**) Typical Benesi-Hildebrand plot of $1/\Delta A$ versus $1/[G]_0$ and (**b**) The plot of ln K versus $1/T$ for the host-guest complexation of **3** and (*S*)-(−)-1-phenylethylamine in $CHCl_3$ at 25°C.

$$\Delta G = -RT \ln K \tag{3}$$

The enthalpic and entropic changes (ΔH and ΔS) through the Gibbs–Helmholtz equation (eq. 4). Combining eqs. 3 and 4, we obtain eq. 5, which describes the temperature dependence of K.

Thus, plots of the ln K values, as a function of the inverse of temperature gave good linear relationships for working temperature range (Fig. 3).

$$\Delta G = \Delta H - T\Delta S \tag{4}$$

$$\ln K = -\Delta H/RT + \Delta S/R \tag{5}$$

The association constants (K), the free-energy change ($-\Delta G_0$) calculated from the slope and the intercept, and the thermodynamic parameters are shown in Table 1, along with the enantioselectivity K_R/K_S for the complexation of *R*/*S*-amines by these hosts. Inspection of Table 1 shows that these chiral receptors can recognize not only differences between the molecular size and shape of amines, but also the chirality of the *R*- or *S*-isomer.

From the data shown in Table 1, all hosts have greater K values toward the enantiomers of PEA than CHEA, nonaromatic analog of PEA, due to π-π interactions as well as hydrogen bonding. The chiral calix[4]arenes having an aromatic group at lower rim could have π-π interaction with that of PEA as an additional binding force. As a result, stronger binding was realized as seen for the cases of **3–11**. Furthermore, fairly greater K values were obtained for chiral receptors **6–13**, bearing the alcohol–OH group. Such additional functional group seems to work for better complexation and chiral recognition because of hydrogen bonding with —OH groups, while chiral host **6** exhibits relatively stronger binding ability for chiral guests, although no enantioselectivity was observed.

As reported previously,[56,57,58] the complexation of amines with *p*-substituted calix[*n*]arenes occurs in a two-step process involving a proton-transfer reaction from the calixarene to the amine (K_1) followed by the ion-pair formation (K_2) between the protonated amine and the deprotonated host as described by eq. 6.

$$\text{Calix}\text{—}\text{OH} + R\text{—}\text{NH}_2 \overset{K_1}{\rightleftharpoons} \text{Calix}\text{—}\text{O}^- + R\text{—}\text{NH}_3^+ \overset{K_2}{\rightleftharpoons} \text{Calix}\text{—}\text{O}^- \cdot R\text{—}\text{NH}_3 \tag{6}$$

In case of chiral guests, the equilibrium constant of the first step, K_1, is in principle the same for reactions with enantiomer guest pairs, whereas the latter one, K_2, is different and is a measure of the enantiodiscrimination.

The complexation of the lower rim substituted calixarenes with amines possibly occurs without a proton transfer, because the acidity of OH groups is significantly lower and p*K*a values of amine and the calixarene in this case are comparable. Thus, only polarization of O—H bond and formation of hydrogen bonded complex without charge separation may be observed.

Noncovalent interactions between the nitrogen atom of the guest and hydrogen bonding sites defined by carbonyl oxygen, amide nitrogen, and hydroxy groups at roughly similar positions with respect to the phenoxy oxygen at each pendant group contribute to the stabilization of these complexes as well as π-π interactions (Fig. 4).

Preliminary experiments were undertaken to confirm the complexation properties of receptors **9** and **13** with PEA by ^{1}H NMR in $CDCl_3$ at room temperature. The spectra of receptor **9** and its complex with equimolar amounts of *rac*-PEA are shown in Figures 5A–5C. When treated with equimolar amounts of receptor **9**, the —CH proton of *rac*-PEA cleaved into more complicated signals (Fig. 5B) with a downfield shift, while that of some protons of the host moved upfield or downfield without displaying any resolution (Table 2).

The similar phenomenon was observed when adding equimolar amounts of the *rac*-PEA to a solution of **13** (Figs. 5D and 5E). The signals of —CH proton of *rac*-PEA underwent downfield shift, while that of various protons of the host were shifted upfield (Table 2). These shifts are

TABLE 1. Binding constants (K), enantioselectivities (K_R/K_S), and thermodynamic parameters for the complexation of chiral amines with the chiral receptors 3–6 and 8–13 in $CHCl_3$ at 25°

Host	Guest	$K \times 10^{-3}$ ($dm^3\ mol^{-1}$)	K_R/K_S	$-\Delta G$ (kJ mol^{-1})	$-\Delta\Delta G$[a]	ΔH (kJ mol^{-1})	$\Delta\Delta H$[b]	ΔS (J mol^{-1})	$\Delta\Delta S$[c]
3	*R*-CHEA	5.75	1.46	4.33	0.93	92.22	5.89	324.00	22.88
	S-CHEA	3.95		3.40		86.33		301.12	
	R-PEA	34.72	5.19	8.81	4.10	108.46	25.62	393.51	99.72
	S-PEA	6.69		4.71		82.84		293.79	
4	*R*-CHEA	5.52	2.52	4.24	2.30	114.16	13.47	397.31	52.89
	S-CHEA	2.19		1.94		100.69		344.42	
	R-PEA	14.71	1.08	6.67	0.20	88.36	2.97	318.92	10.67
	S-PEA	13.62		6.47		85.39		308.25	
5	*R*-CHEA	3.94	0.62	4.39	−0.20	58.05	−6.12	209.52	−21.23
	S-CHEA	6.37		4.59		64.17		230.75	
	R-PEA	6.91	0.30	4.78	−3.01	99.46	−15.74	349.81	−62.90
	S-PEA	23.12		7.79		115.20		412.71	
6	*R*-CHEA	44.24	1.04	9.40	0.08	60.85	1.43	235.73	5.06
	S-CHEA	42.67		9.32		59.42		230.67	
	R-PEA	114.89	1.02	11.74	0.05	106.98	2.49	398.37	8.48
	S-PEA	112.34		11.69		104.49		389.89	
8	*R*-CHEA	27.70	3.77	8.20	3.23	47.96	22.38	188.47	85.98
	S-CHEA	7.35		4.97		25.58		102.49	
	R-PEA	76.86	1.89	10.75	1.56	80.93	9.98	307.67	38.75
	S-PEA	40.62		9.19		70.95		268.92	
9	*R*-CHEA	28.40	1.66	8.27	1.24	21.11	9.42	98.60	35.78
	S-CHEA	17.10		7.03		11.69		62.82	
	R-PEA	97.38	1.90	11.35	1.60	31.22	16.23	142.88	59.87
	S-PEA	51.12		9.75		14.99		83.01	
10	*R*-CHEA	9.73	1.83	5.63	1.48	36.43	5.62	141.14	23.81
	S-CHEA	5.31		4.15		30.81		117.33	
	R-PEA	46.15	2.82	9.50	2.58	36.28	16.74	153.61	64.82
	S-PEA	16.35		6.92		19.54		88.79	
11	*R*-CHEA	15.49	2.50	6.80	2.28	98.48	14.36	353.29	55.82
	S-CHEA	6.20		4.52		84.12		297.47	
	R-PEA	62.07	2.79	10.24	2.58	125.45	14.02	455.33	55.67
	S-PEA	22.21		7.66		111.43		399.66	
12	*R*-CHEA	3.36	0.48	3.01	−1.79	62.65	−15.55	220.33	−58.21
	S-CHEA	6.93		4.80		78.20		278.54	
	R-PEA	17.15	0.69	7.15	−0.78	38.44	−20.51	152.99	−71.45
	S-PEA	24.72		7.93		58.95		224.44	
13	*R*-CHEA	2.35	0.38	2.11	−2.42	73.27	−12.42	252.95	−49.82
	S-CHEA	6.24		4.53		85.69		302.77	
	R-PEA	17.61	0.52	7.11	−1.63	64.00	−13.85	238.62	−51.95
	S-PEA	34.08		8.74		77.85		290.57	

[a]$\Delta\Delta G = \Delta G_R - \Delta G_S$.
[b]$\Delta\Delta H = \Delta H_R - \Delta H_S$.
[c]$\Delta\Delta S = \Delta S_R - \Delta S_S$.

due to specific host-guest complexation and indicated that the interaction between the host and guest also happened by multiple hydrogen bondings.

The chiral receptors **3–13** are structurally similar in that all contain a group capable of hydrogen bonding as part of a chiral center attached directly to a phenyl, benzyl, or ethyl group. Though the number of recognition sites that are necessary for producing chiral recognition is due to the shape of the receptor, three ordinary recognition sites are required in the receptor molecules.[58] In this study, Chiral calix[4]arenes **3–13** interact with minimum three of the possible recognition groups (carbonyl oxygen, amide nitrogen, phenoxy oxygen, phenyl group, and hydroxy groups) in order to exhibit enantioselective binding to the chiral amines.

CONCLUSION

Novel chiral mono and diamide derivatives of calix[4]arene have been synthesized and examined for their binding abilities by UV–vis absorption and ^1H NMR spectroscopy. All chiral receptors have greater K values toward the enantiomers of PEA than CHEA, nonaromatic analog of PEA, due to π-π interactions as well as hydrogen bonding. The hosts bearing an aromatic group at lower rim showed π-π

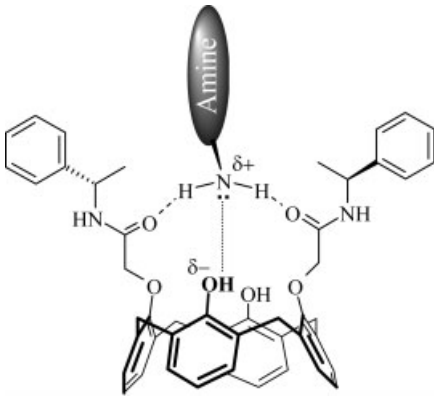


Fig. 4. Proposed recognition mode of chiral calix[4]arene **5** toward a chiral amine.

interaction with that of PEA as an additional binding force. Thus, stronger binding was realized as seen for the cases of **3–11**. Furthermore, fairly greater *K* values were obtained for chiral receptors **6–13**, bearing the alcohol–OH group. Such additional functional group seems to work for better complexation and chiral recognition because of multiple hydrogen bonding.

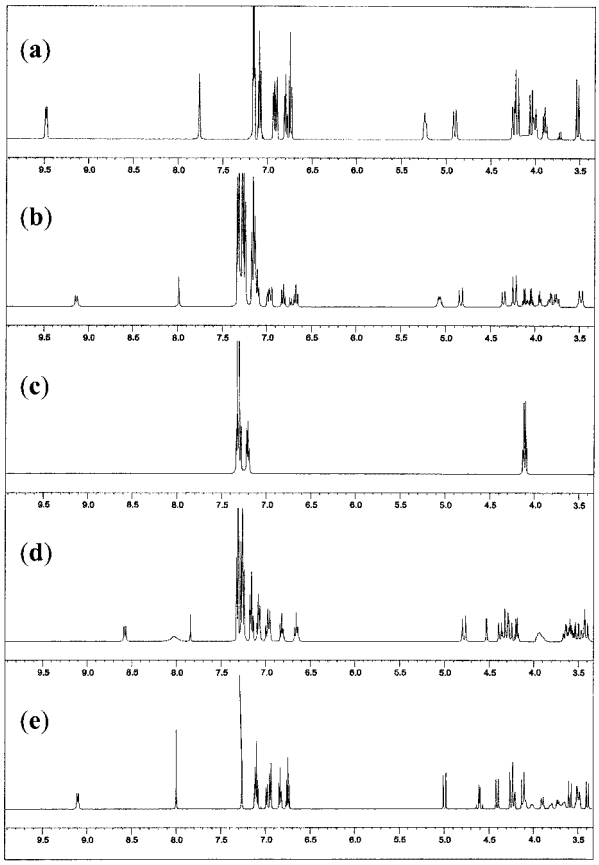


Fig. 5. Partial ^{1}H NMR spectra of receptors **9** and **13** and their guest complexes with equimolar mixtures (10 mM each) at 25°C in $CDCl_3$ at 400 MHz. (**a**) host **9**; (**b**) *rac*-PEA with host **9**; (**c**) *rac*-PEA; (**d**) *rac*-PEA with host **13**; and (**e**) host **13**.

TABLE 2. Chemical shift values for the complexation of *rac*-phenylethylamine with calix[4]arene diamides 9 and 13 in $CDCl_3$ at 25°

Host	Guest	Free[a]	Complex[b]	Δδ (ppm)[c]
9 (phenolic —O*H*)	PEA	7.76	7.98	−0.22
9 (—N*H*)	PEA	9.48	9.13	0.35
9 (—C*H*)	PEA	5.24	5.07	0.17
9 (CH_2OH)	PEA	4.90	4.83	0.07
13 (phenolic —O*H*)	PEA	8.00	7.84	0.16
13 (—N*H*)	PEA	9.1	8.58	0.52
13 (—C*H*)	PEA	4.60	4.52	0.08
13 (CH_2OH)	PEA	5.00	4.78	0.22
9	PEA (—C*H*)	4.10	4.15	−0.05
13	PEA (—C*H*)	4.10	4.20	−0.10

[a][9], [13], and [*rac*-PEA] = 10 mM.
[b]The chemical shifts were based on the spectrum of *rac*-PEA in the presence of **9** and **13**.
[c]Obtained by subtracting the complex value from the free value.

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Chiral Resolutions of (9-Anthryl)methoxyacetic Acid and (9-Anthryl)hydroxyacetic Acid by Capillary Electrophoresis

BAO-LIN LI,[1,2]* ZHEN-GUO ZHANG,[1,2] LI-LI DU,[1,2] AND WEI WANG[1,2]

[1]*School of Chemistry and Materials Science, Shaanxi Normal University, Xi'an, China*

[2]*The Key Laboratory of Medicinal Plant Resource and Natural Pharmaceutical Chemistry of Ministry of Education, Shaanxi Normal University, Xi'an, China*

ABSTRACT The resolutions of (9-anthryl)methoxyacetic acid (9AMAA) and (9-anthryl)hydroxyacetic acid (9AHAA) were performed by capillary electrophoresis using hydroxypropyl-β-cyclodextrin (HP-β-CD) as a chiral selector. Various factors affecting migration time and resolutions of these compounds were investigated with a run voltage of 20 kV, column temperature 20°C and 20 mM Tris-H_3PO_4 buffer (pH 6.5) containing 5 mM HP-β-CD for 9AMAA, or 10 mM HP-β-CD for 9AHAA, (±)-9AMAA and (±)-9AHAA were successfully separated at Rs 3.27 and 1.92, respectively. *Chirality 20:35–39, 2008.* © 2007 Wiley-Liss, Inc.

KEY WORDS: chiral derivatizing agent; hydroxypropyl-β-cyclodextrin; chemical resolution; CE analysis; resolution

INTRODUCTION

Some arylmethoxyacetic acids containing naphthyl or anthryl systems[1–4] are used as chiral derivatizing reagents for assigning absolute configuration of chiral organic compounds by NMR in the "Modified Mosher Method."[5] In general, all these compounds produce more-intense shielding than α-methoxy-α-phenylacetic acid which was used as a chiral derivatizing reagent in the original method,[2,6] thus better separation of the ^{1}H NMR signals observed. This effect is particularly important when (9-anthryl)methoxyacetic acid (9AMAA) or ethyl (9-anthryl)-hydroxyacetate (9AHA) are used to assign absolute configuration of α-chiral secondary alcohols, α-chiral primary amines or α-chiral carboxylic acids by reacting these chiral compounds with (*R*)- and (*S*)-9AMAA, or (*R*)- and (*S*)-9AHA, and comparing the ^{1}H NMR spectra of the diastereomeric products. The $\Delta\delta^{RS}$ values obtained are 3–4 times higher than those with other chiral derivatizing reagent.[6] Therefore, recently we have used a solvent-free Friedel-Crafts reaction to synthesize ethyl (9-anthryl)glyoxylate (**1**) in high regioselectively, and successively transformed **1** to the excellent chiral derivatizing reagents 9AMAA and 9AHA as in Scheme 1, and resolved racemic 9AMAA and its analogue (9-anthryl)hydroxyacetic acid (9AHAA) using chemical methods. In order to determine the results of synthesis and chemical resolution of (±)-9AMAA and (±)-9AHAA, we needed to establish an analytical method. Capillary electrophoresis (CE) has been shown to be a powerful tool for the chiral analysis of amino acids, dipeptides,[7] chiral drugs,[8] natural products, and other chiral substances[9] due to its very high resolution capability, short analysis time, use of minute amounts of both samples and buffer, etc.[10–13] This article presents here the resolutions of (±)-9AMAA and (±)-9AHAA by capillary electrophoresis.

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MATERIALS AND METHODS

Apparatus

CE was performed using a Lumex CAPEL 105 capillary electrophoresis instrument equipped with an on-line UV detector. An uncoated fused-silica capillary tube (length 60 cm, effective length 55 cm, id 75 μm) was employed. Before use, the capillary was successively flushed with 0.1 M NaOH for 5 min, and de-ionized water twice for 5 min, followed by running buffer for 5 min. The capillary was rinsed with de-ionized water twice for 3 min and running buffer for 3 min between each injection, respectively. The run voltage was 20 kV and the column temperature was set at 20°C. UV detection was performed at 254 nm. Samples were injected by applying a pressure of 3 kPa for 5 sec.

pH of Solutions were determined with pHS-3C-meter.

The NMR spectra were recorded with a Bruker AVANCE300 spectrometer at 300 MHz for ^{1}H and at

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*Correspondence to: Bao-Lin Li, Southern Changan Road 199#, Xi'an 710062, Shaanxi, China. E-mail: baolinli@snnu.edu.cn
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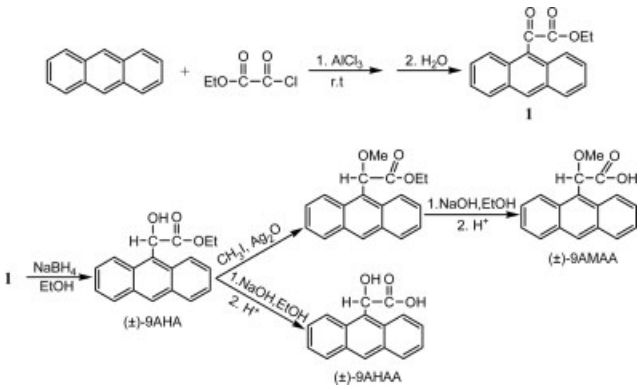

Scheme 1. Synthesis of (9-anthryl)methoxyacetic acid and its analogues.

75 MHz for the ^{13}C nucleus. Chemical shifts are reported in ppm using TMS as an internal standard. The IR spectra were recorded with a Nicolet 170SX FT-IR spectrometer using KBr pellets. The optical rotation was measured with a JASCO-20C polarimeter. Melting points were determined using a WRS-113 digital melting point instrument (the thermometer was not corrected).

Reagents

Hydroxypropyl-β-CD (HP-β-CD) (degree of substitution 10) was synthesized by a literature method.[14] The other chemicals were analytical grade. Water was purified with a Milli-Q Academic instrument.

Synthesis of ethyl (9-anthryl)glyoxylate (1). Anthracene (17.82 g; 0.10 mol), 26.67 g (0.20 mol) anhydrous $AlCl_3$, and 1.98 g (0.02 mol) 1-methyl-2-pyrrolidone were triturated in a porcelain mortar in an anhydrous operator chest with silica gel for absorbing moisture and as a moisture indicator, and solid NaOH for absorbing the HCl gas released from the reaction. To this mixture was added 20.48 g (0.15 mol) ethyl oxalyl chloride at 3–6 drops per minute with trituration. After leaving the dark green reaction mixture for 4.5 h, 130.00 g crushed ice was added to the reaction mixture. After the ice thawed, the mixture was filtered to give a brown solid as the crude product. This was purified by recrystallizing from acetone to yield 25.70 g yellow solid **1** (92.5% yield). M.p. 83.8°C–84.7°C. ^1H NMR (300 MHz, acetone-d_6) δ: 1.30(t, 3H), 4.37(q, 2H), 7.61(m, 2H), 7.67(m, 2H), 7.99(m, 2H), 8.19(m, 2H), 8.79(t, 1H). ^{13}C-NMR (75 MHz, acetone-d_6) δ: 14.3, 63.5, 124.8, 126.8, 129.1, 130.0, 130.1, 131.2, 131.9, 132.1, 162.8, 193.1. IR (KBr): 3051, 2948, 2301, 1725, 1600, 1451, 1400, 1302, 1250, 1100 cm^{-1}.

Synthesis of 9AHA and 9AHAA. Ten grams of **1** was dissolved in 350 ml ethanol and 1.00 g $NaBH_4$ was added gradually with stirring in ice-water bath until **1** was exhausted. The process was tracked by TLC. Then, to the reaction mixture, 10 ml dilute hydrochloric acid was slowly added with stirring. Ethanol was removed in vacuo and a yellow solid was obtained. The solid was dissolved with acetone. After filtration, the acetone was removed in vacuo, giving 9.81 g 9AHA as straw yellow solid at 97.4% yield. ^1H NMR (300MHz, $CDCl_3$) δ: 1.05(t, 3H), 3.71(s, 1H), 4.16(q, 2H), 6.61(s, 1H), 7.55(m, 4H), 8.03(m, 2H), 8.37(m, 2H), 8.51(m, 1H).

9AHAA was obtained by the hydrolysis of 9AHA. Six grams of 9AHA was dissolved with 100 ml ethanol and 20 ml 30% aqueous solution of NaOH was added and refluxed until 9AHA was exhausted (by TLC). Ethanol was removed in vacuo. After acidification with dilute hydrochloric acid, 5.17 g of a straw yellow solid was filtered off (95.8% yield). ^1H NMR (300 MHz, DMSO-d_6) δ: 3.41(s, 1H), 6.18(s, 1H), 6.55(s, 1H), 7.53(m, 4H), 8.11(d, 2H), 8.57(m, 3H), 12.69(s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 67.4, 125.4, 125.6, 126.3, 128.1, 129.3, 130.0, 131.6, 132.7, 175.5.

Synthesis of 9AMAA. The mixture of 14.00 g 9AHA, 82 ml CH_3I, and 8.87 g Ag_2O was refluxed until 9AHA was exhausted (by TLC). Ethyl (9-anthryl)methoxyacetate (13.30 g) was obtained as yellow solid (90.5% yield) after filtering off Ag_2O, and removing CH_3I. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.94(t, 3H), 3.39(s, 3H), 4.02(q, 2H), 6.48(s, 1H), 7.55(m, 4H), 8.10(d, 2H), 8.58(s, 1H), 8.65(m, 2H).

One gram of ethyl (9-anthryl)methoxyacetate was dissolved in 30 ml ethanol, 10 ml 30% aqueous NaOH added, and refluxed until ethyl (9-anthryl)methoxyacetate was exhausted (monitored with TLC). Ethanol was removed in vacuo. After acidification with dilute hydrochloric acid, the mixture was filtered to give 0.85 g straw yellow solid 9AMAA (93.9% yield). ^1H NMR (300 MHz, DMSO-d_6) δ: 3.30(s, 3H), 3.35(s, 1H), 6.04(s, 1H), 7.48(m, 4H), 8.08(t, 2H), 8.53(s, 1H), 8.68(m, 2H).

Resolution of (±)-9AHAA by chemical method. Four grams (20 mmol) (−)-ephedrine hydrochloride was dissolved in 10 ml H_2O and 5 ml 40% aqueous NaOH was added with stirring. The mixture was extracted twice with 10 ml ethyl ether. The extract was dried over anhydrous Na_2SO_4, and the ether was removed to give 3.20 g (−)-ephedrine. Ethanol (30 ml) was added to a 50 ml flask including 3.20 g (20 mmol) (−)-ephedrine, which was added 5.04 g (20 mmol) racemic 9AHAA, and refluxed for 2 h. After cooling to room temperature, 1.41 g of yellow crystals of the salt of (+)-9AHAA and (−)-ephedrine was obtained from this solution by slow evaporation at room temperature. This salt was dissolved in water, acidified with dilute hydrochloric acid, and the mixture was filtered to give 0.83 g straw yellow solid of (+)-9AHAA, $[\alpha]_D^{20} = +169^\circ$ ($c = 0.2197$, methanol).

Buffer and Sample Preparation

Background electrolyte (BGE) was made by adding an appropriate amount of HP-β-CD to 20 mM Tris-H_3PO_4 solution, giving the final pH values of 5.0, 5.5, 6.0, 6.5, 7.5, and 8.0, respectively. Stock solutions were prepared by dissolving 9AMAA or 9AHAA in water:ethanol (1:1, v:v) at a concentration of 0.02 g/l. All the solutions were stored at 4°C, and filtered using a membrane filter (0.45 μm) before use.

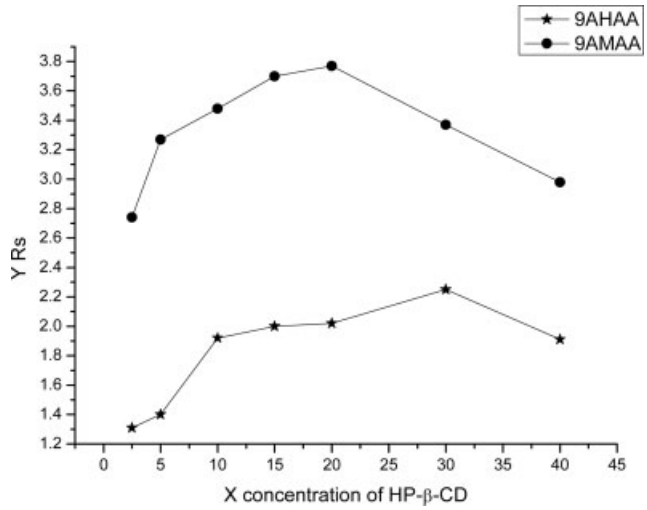

Fig. 1. The effect of the concentration of HP-β-CD on the resolution of 9AMAA and 9AHAA. Separating conditions: 20 mM Tris-H_3PO_4 (pH 6.50); uncoated capillary: length 60 cm, effective length 55 cm, id 75 μm; run voltage: 20 kV; column temperature: 20°C; pressure injection: 3 kPa × 5 sec.

RESULTS AND DISCUSSION

In chiral CE analysis, cyclodextrins (CDs) and their derivatives have been widely applied as chiral selectors in BGE for the separation of enantiomers of many chiral compounds.[15,16] CDs and their derivatives are successful chiral separation resulting from the varieties of their structures and molecular recognition by host-guest phenomena, which concerns inclusion complexes formed through noncovalently controlled interactions between the host and guest.[17,18] In the previous study, we used β-CD, and some its derivatives, such as dimethyl-β-CD, trimethyl-β-CD, carboxymethyl-β-CD and sulfated β-CD as chiral selectors in Tris-H_3PO_4 buffer system (pH 2.5) for the resolution of 9AHAA by CE, respectively.[19] The enantiomers of 9AHAA were resolved (Rs 0.93) (run voltage −15 kV) with sulfated β-CD as chiral selector but they could not be separated with other chiral selectors.[19] HP-β-CD in the BGE provided sufficient resolution for the chiral CE analysis of the enantiomers of a series of organic acids.[20,21] In this experiment, we found that HP-β-CD was an effective chiral selector for the separations of 9AMAA and 9AHAA. We then investigated various factors which would affect migration time and resolutions of 9AMAA and 9AHAA. These factors include the concentration of HP-β-CD, the pH of BGE, the run voltage and the temperature of capillary column.

Effect of HP-β-CD Concentration

Tris-H_3PO_4 buffers (20 mM; pH 6.5) containing various concentrations of HP-β-CD (2.5–40 mM) were used as chiral mobile phases in CE. The separations of 9AMAA and 9AHAA are shown in Figure 1. As the HP-β-CD concentration increased from 2.5 to 20 mM, an increase in the separations of 9AMAA enantiomers was observed. Optimum separation of 9AMAA was obtained at 20 mM HP-β-CD in the run buffer. With increasing concentration of HP-β-CD, a longer migration time was observed, especially when the concentration of HP-β-CD changed from 20 to 30 mM, the increasing resolution was not seen, but the migration time became longer obviously for 9AMAA. According to the experiment, when the HP-β-CD concentration is too high (>30 mM), the Rs will be greatly decreased. On the basis of Rs and migration time, we selected the concentration of HP-β-CD at 5 mM to further optimize other separation conditions of 9AMAA. By the same means, we selected that 10 mM HP-β-CD in BGE was as an optimizing concentration of HP-β-CD of separation 9AHAA for optimizing other conditions.

Effect of BGE pH

The BGE pH is a very important parameter when chiral CE analysis of organic acids use CDs or their derivatives as chiral selectors,[22] because the analytes, organic acids, possess ionizable carboxyl groups. The change of BGE pH influences the charge of analytes, thus, the electrostatic interactions of analyte and chiral selector, the electropho-

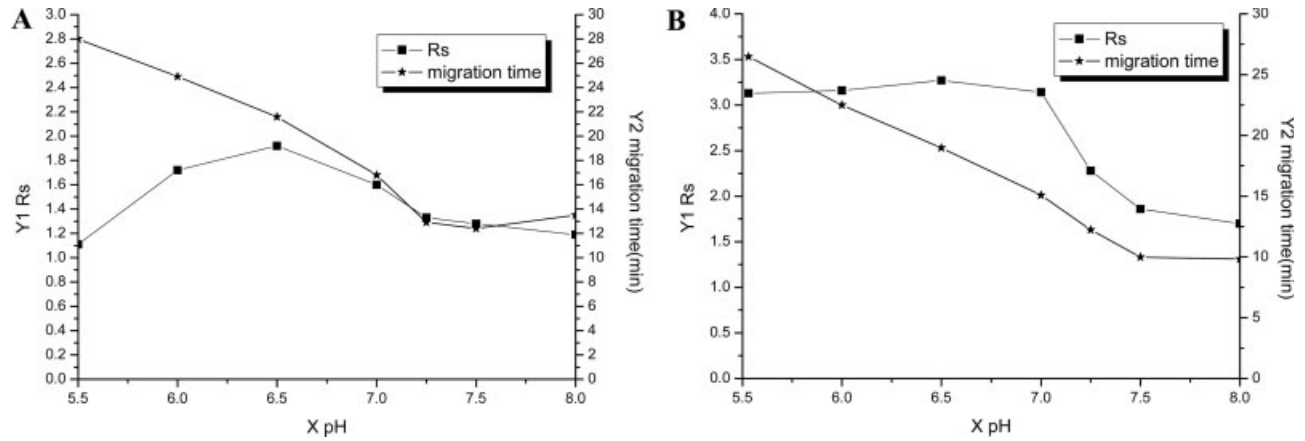

Fig. 2. The effect of pH on the resolutions of 9AHAA (**A**) and 9AMAA (**B**). Separation condition: 20 mM Tris-H_3PO_4 buffer (pH 6.5) containing 10 mM HP-β-CD for 9AHAA (A) and 5 mM HP-β-CD for 9AMAA (B), respectively; run voltage: 20 kV; column temperature: 20°C; Detection wavelength: 254 nm.

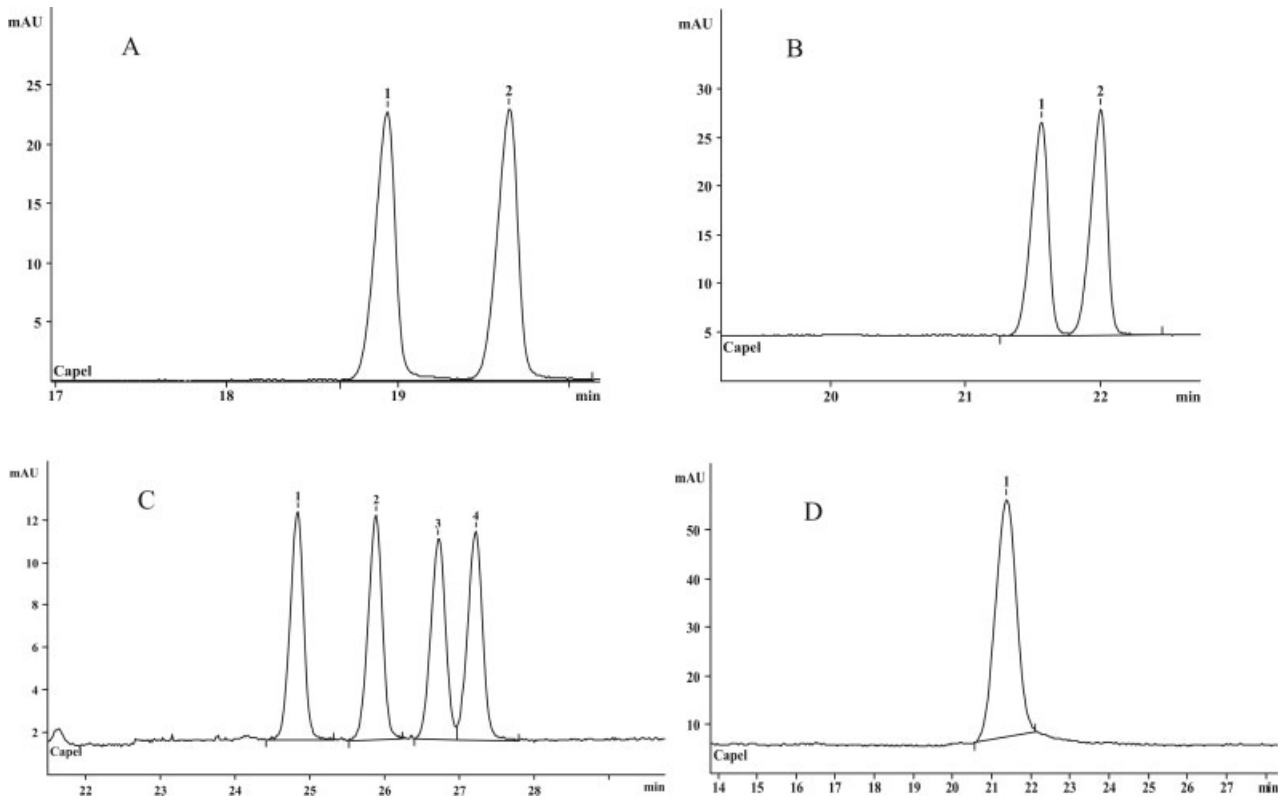

Fig. 3. Eletrophorograms from the chiral separation of 9AMAA and 9AHAA using the selected CE conditions. CE conditions—run voltage: 20 kV; column temperature: 20°C; detection wavelength: 254 nm; pressure injection: 3 kPa × 5 sec; 20 mM Tris-H_3PO_4 buffer (pH 6.5) containing 5 mM HP-β-CD for the racemic 9AMAA (Rs = 3.27) (**A**), or containing 10 mM HP-β-CD for the racemic 9AHAA (Rs = 1.92) (**B**), the mixture of racemic 9AHAA and 9AMAA as BGE containing 10 mM HP-β-CD in pH 6.5 (**C**), and enantiomer (+)-9AHAA from the chemical resolution as BGE containing 10 mM HP-β-CD in pH 6.5 (**D**).

retic motilities and the stability of analytes are also influenced. Therefore, the CE behavior of 9AMAA and 9AHAA enantiomers were investigated at different pHs of 20 mM Tris-H_3PO_4 buffer containing 5 mmol/l HP-β-CD for 9AMAA and 10 mmol/l HP-β-CD for 9AHAA, respectively. Figure 2 shows the effect of pH on the chiral separation of the analytes. According to the results of the experiments, it was impossible to detect these acid enantiomers at pH 5.0 in the limited time. Increasing BGE pH from 5.5 to 6.5, increasing resolutions of 9AMAA and 9AHAA, and decreasing migration time were observed. However, further increasing the pH of the BGE decreased the resolution of these acidic enantiomers, and the peak shapes of eletrophorograms were not as good. A BGE pH of 6.5 was selected as optimum for the separation of these analytes for run buffer containing HP-β-CD.

Effect of Run Voltage and Capillary Column Temperature

Run voltage plays a major role in determining times in CE analyses. It can also affect the efficiency of analysis, since efficiency is directly proportional to the voltage.[23] The effect of the run voltage on the resolutions of 9AMAA and 9AHAA enantiomers was examined in the selected conditions of HP-β-CD and the buffer pH. Run voltages from 10 to 22 kV were investigated to obtain the optimum run voltage. There was an increase in resolution with the increasing run voltage from 10 to 20 kV. The preferable resolution was obtained at 20 kV of run voltage, but further increases in voltage led to the decrease of separation efficiencies.

The effect of column temperature on the resolutions of 9AMAA and 9AHAA enantiomers was investigated in the range of 15–30°C at optimum HP-β-CD concentration, buffer pH, and run voltage. The results indicated that the capillary temperature does not influence the resolution obviously in this system. According to Heuermann and Blaschke,[24] lower capillary temperature increases both resolution and migration time, and with the increase of the capillary temperature, the decrease of the stability constant of cyclodextrin inclusion complex leads to decrease in resolution.[25] So the column temperature of 20°C was selected for the analyses.

Application

In selected conditions, run voltage 20 kV, column temperature 20°C, 20 mM Tris-H_3PO_4 buffer (pH 6.5) containing 5 mM HP-β-CD for 9AMAA, or 10 mM HP-β-CD for 9AHAA, (±)-9AMAA and (±)-9AHAA were successfully separated at Rs 3.27 and 1.92, respectively. The electropherograms of 9AMAA and 9AHAA are presented in Figures 3A and 3B. We also tried the simultaneous determination of the enantiomers of 9AHAA and 9AMAA, and

obtained good results (Fig. 3C) when the BGE contained 10 mM HP-β-CD in pH 6.5. When using the established analytical method to determine the result of chemical resolution of 9AHAA, the CE analysis in Figure 3D showed the resolution of (±)-9AHAA gave optically pure 9AHAA.

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CHIRALITY 20:40–46 (2008)

The Chiral Resolution of Pesticides on Amylose-tris(3,5-dimethylphenylcarbamate) CSP by HPLC and The Enantiomeric Identification by Circular Dichroism

PENG WANG,[1] DONGHUI LIU,[1] SHUREN JIANG,[1] YANGGUANG XU,[2] XU GU,[1] AND ZHIQIANG ZHOU[1]*

[1]*Department of Applied Chemistry, China Agricultural University, Beijing, China*

[2]*Chinese People's Armed Police Force Academy, Langfang, China*

ABSTRACT Amylose-tris(3,5-dimethylphenylcarbamate) (ADMPC) was synthesized and coated on γ-aminopropylsilica to prepare a chiral stationary phase (CSP). The chiral resolutions of seven pesticide enantiomers including fenoxaprop-ethyl, quizalofop-ethyl, lactofen, metalaxyl, benalaxyl, hexythiazox and fluroxypyr-meptyl on the CSP by high-performance liquid chromatography were performed. Mobile phase was *n*-hexane and isopropanol with a flow rate of 1.0 ml/min. The influences of isopropanol content in the mobile phase and temperature on the resolutions were investigated. Under the optimized conditions the enantiomers could obtain complete resolutions except that metalaxyl got partial resolution. Decreasing the content of isopropanol increased the retention and the resolutions. Temperature was an important chromatographic parameter for optimization, and the results showed that low temperature was not always good to the resolutions. The enantiomers were identified by a circular dichroism (CD) detector which could provide the CD signals [(+) or (−)] and the CD spectra in the range of 220–420 nm by online scanning. *Chirality 20:40–46, 2008.* © 2007 Wiley-Liss, Inc.

KEY WORDS: ADMPC; chiral pesticide; enantiomer; resolution; HPLC

INTRODUCTION

The chirality of drugs, pesticides, cosmetics, aromas, food additives, and sweeteners attracts increasing attention in the recent years because of its importance in many biological processes. Chiral compounds accounts for more than one quarter of the commercialized pesticides.[1] The two enantiomers often show great differences in terms of toxicity, bioavailability, distribution, metabolism, and excretion.

Chiral resolution of enantiomers is of great importance and often difficult because they have almost the same physical and chemical properties.[2] HPLC, GC, and CE are the most popular means for this work, in which HPLC based on chiral stationary phases (CSPs) is used widely because of the powerful separation ability and both for analytical and preparation purpose. Many new CSPs have been developed in the past 30 years and are still a hot topic, in which polysaccharide based CSPs such as OD and AD are among the most powerful CSPs.

In this work, the ADMPC CSP was prepared for the chiral separation of pesticide enantiomers by HPLC. The samples were fenoxaprop-ethyl, quizalofop-ethyl, lactofen, metalaxyl, benalaxyl, hexythiazox (*R,R*; *S,S*), and fluroxypyr-meptyl, in which fenoxaprop-ethyl, quizalofop-ethyl, lactofen, and fluroxypyr-meptyl are four herbicides; metalaxyl and benalaxyl are fungicides; hexythiazox is an acaricide. The R-enantiomers of fenoxaprop-ethyl, quizalofop-ethyl, metalaxyl, benalaxyl are the active enantiomers, and optical pure products of metalaxyl, fenoxaprop-ethyl, and quizalofop-ethyl had been produced and used.

Previous research showed that fenoxaprop-ethyl was resolved on β-CD derivatives CSP by GC.[3] The enantiomers of lactofen, fluroxypyr-meptyl, and benalaxyl were reported to be separated on HPLC OD column by our group.[4] The enantiomers of metalaxyl was analyzed by chiral GC,[5,6] HPLC with OD and OJ,[7,8] and CE.[9]

Although ADMPC was a popular CSP, it has not been used widely for chiral resolutions of pesticides. The literature shows it could separate the enantiomers of fenamiphos, fensulfothion, profenofos, crufomate,[10] *o,p*-DDT, *o,p*-DDD,[11] and heptachlor epoxide.[12]

The development of analytical methods for the efficient resolution of enantiomers is one of the most important tasks in the field of pesticides. A systematic study of the enantioselective HPLC chromatographic separation of the seven chiral pesticides on ADMPC CSP was carried out here with the aim of setting up methods for chiral analysis of single enantiomers.

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*Correspondence to: Zhiqiang Zhou, Department of Applied Chemistry, College of Science, China Agricultural University, Beijing 100094, China. E-mail: zqzhou@cau.edu.cn

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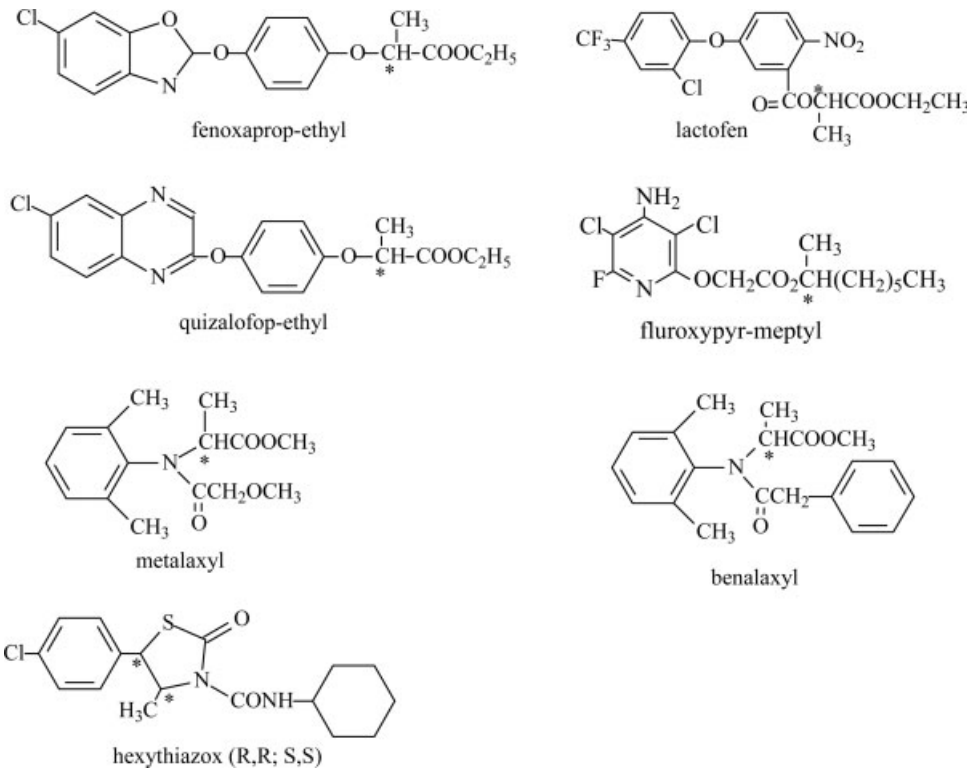


Fig. 1. The chemical structures of the pesticides. Asterisk denotes the chiral center.

MATERIALS AND METHODS

Chemicals and Materials

Amylose and 3,5-dimethylphenylisocyanate (99%) were purchased from Sigma-Aldrich, and γ-aminopropyltriethoxysilane (99%) from Acros Organics (Belgium). Macrospherical silica was prepared in this laboratory with the following properties: particle size, 5–7 μm, average pore diameter 6.7 nm, specific surface area 110 m^2/g.

Pesticide samples were provided by Institute for Control of Agrichemicals, Ministry of Agriculture (Beijing, China), Laboratory of Pesticide Residual Analysis and Environmental Toxicology of China Agricultural University (Beijing, China), and several pesticide manufacturers (China), and the purity was more than 95%. The sample solutions were prepared in isopropanol. All eluents were of analytical grade (Beijing Chemical Reagents Company, Beijing, China), distilled and filtered by 0.45-μm filter before use.

Apparatus

System 1: Agilent 1100 Series HPLC (Agilent Technologies Palo Alto, CA) equipped with G1311A pump, G1322A degasser, G1316A COLCOM, G1328A injector, a 20-μl sample loop, and G1315B DAD. The signal was acquired and processed by an HP1100 workstation. This system was used to separate the enantiomers and optimize the chromatographic conditions.

System 2: JASCO 2000 HPLC (Jasco, Tokyo, Japan), equipped with PU-2089 plus pump, CD-2095 plus circular dichroism (CD) detector, a 20-μl sample loop, and a Chrompass workstation. This system was used to identify the eluting enantiomers and determine the elution orders.

Chromatographic Conditions

Mobile phase was *n*-hexane/isopropanol, flow rate of 1.0 ml/min, and injection volume was 20 μl. The detection wavelength was 230 nm. Column was 250 mm × 4.6 mm (I.D.). The following parameters were calculated: k' (capacity factor): $\frac{t-t_0}{t_0}$; α (separation factor): $\frac{k'_1}{k'_2}$; Rs (resolution factor): $\frac{2(t_2-t_1)}{w_1+w_2}$.

Synthesis of ADMPC and APS

Preparation of the Chiral Column. The CSP was synthesized according to the procedures described in Refs 13–15. ADMPC (0.45 g) was dissolved in 60 ml tetrahydrofuran (THF), and this solution was added to APS (2.55 g) drop by drop. The mixture was stirred, and after evaporating the solvent, dried at 60°C for 8 h.

The slurry of the CSP in hexane–isopropanol (90:10 v/v) was packed into stainless steel column (250 mm × 4.6 mm i.d.) under 4.0×10^7 Pa.

RESULTS AND DISCUSSION

The ADMPC CSP was prepared to investigate the resolution of chiral pesticides. The chemical structures of the chiral pesticides are listed in Figure 1. Hexythiazox had two chiral centers, but the technical product contains one

TABLE 1. The chiral resolutions and the effect of the isopropanol content

Compound	Content of isopropanol (%)	k_1'	k_2'	α	Rs
Fenoxaprop-ethyl	15	2.37	2.58	1.09	0.50
	10	3.03	3.34	1.10	0.65
	5	4.46	4.98	1.12	0.92
	2	7.04	8.25	1.17	1.35
	1	8.25	9.86	1.20	1.43
	0.5	21.62	36.23	1.68	1.75
Quizalofop-ethyl	15	2.57	2.86	1.11	0.87
	10	3.18	3.67	1.16	1.20
	5	4.83	5.53	1.15	1.26
	2	7.09	8.28	1.17	1.71
	1	9.50	11.34	1.19	1.64
Lactofen	15	2.54	2.93	1.16	1.06
	10	3.45	4.04	1.17	1.22
	5	5.17	6.12	1.18	1.52
	2	8.00	9.64	1.20	1.84
	1	10.59	13.10	1.24	2.26
Metalaxyl	15	3.44	3.54	1.03	0.28
	10	5.15	5.43	1.05	0.57
	5	9.22	9.90	1.07	0.80
	2	16.59	18.21	1.10	1.13
Benalaxyl	15	2.19	2.87	1.31	2.05
	10	3.12	4.20	1.35	2.50
	5	4.99	6.86	1.37	2.76
Hexythiazox	5	4.45	4.73	1.06	0.64
	2	7.06	7.76	1.10	0.96
	1	8.27	9.20	1.11	1.34
	0.5	18.48	21.59	1.17	1.75
Fluroxypyr-meptyl	15	3.28	3.82	1.16	1.14
	10	5.25	6.19	1.18	1.31
	5	11.46	13.78	1.20	1.72

Mobile phase *n*-hexane/isopropanol, flow rate 1.0 ml/min, 20°C.

pair of enantiomers [the mixture of (2*R*, 3*R*)- and (2*S*, 3*S*)-enantiomer]. Other samples had one chiral center and consisted of a pair of enantiomers.

For normal-phase mode, *n*-hexane–isopropanol was used as the mobile phase, flow rate 1.0 ml/min throughout.

Three parameters capacity factor (k'), separation factor (α), and resolution factor (Rs) were used to evaluate the resolution. Rs value of 1.5 was used as a criterion for complete separation. The void time t_0 was 2.06 min determined by tri-t-butylbenzene.

Chiral Resolutions

The chiral resolutions of the pesticides were performed at 20°C and at monitored 230 nm. The results of the chiral resolutions and the effect of the content of isopropanol are listed in Table 1. Fenoxaprop-ethyl, quizalofop-ethyl, lactofen, benalaxyl, fluroxypyr-meptyl, and hexythiazox (*R,R*; *S,S*) obtained baseline resolutions, while metalaxyl gave partial separation.

Fenoxaprop-ethyl (ethyl-2-[4-(6-chloro-2-benzoxazolyloxy)phenoxy]phenoxy)propionate) and quizalofop-ethyl (ethyl-2-[4-(6-chloro-2-quionoxalinyloxy)phenoxy]phenoxy)-propionate) are phenoxy propanoate herbicides which have very similar chemical structures and molecular size. The retention and resolution behaviors of the two herbicides on the CSP were similar too from the capacity factors, separation factors, and resolution factors at the same chromatographic conditions. The retention of quizalofop-ethyl on the CSP was better than that of fenoxaprop-ethyl, perhaps because the polarity of quizalofop-ethyl was a little greater. Fenoxaprop-ethyl gave the best resolution using *n*-hexane/isopropanol 99.5:0.5 with the Rs value of 1.75, and quizalofop-ethyl gave the best Rs of 1.71 with *n*-hex-

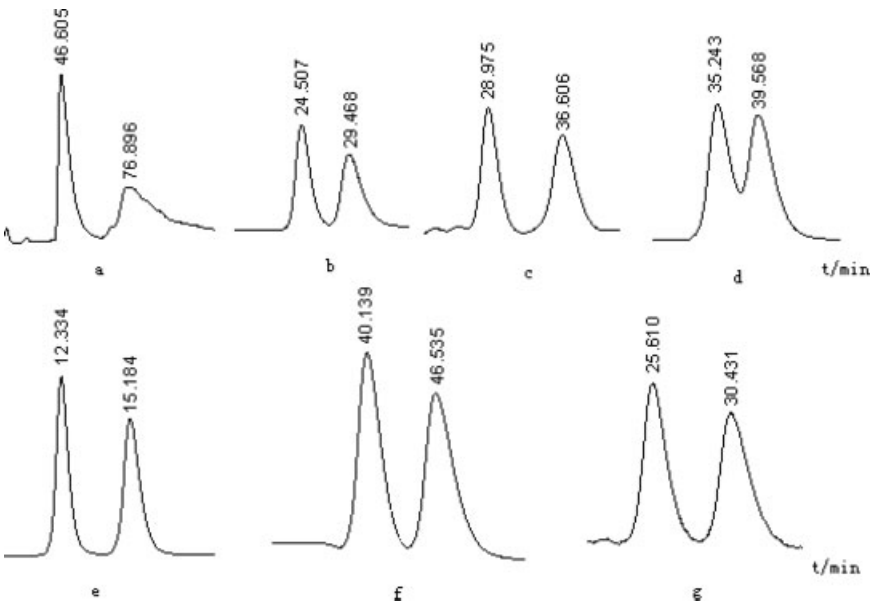


Fig. 2. Chromatograms for the chiral resolutions. Flow rate 1.0 ml/min, wavelength 230 nm (**a**) fenoxaprop-ethyl, 0.5% isopropanol, 20°C; (**b**) quizalofop-ethyl, 2% isopropanol, 0°C; (**c**) lactofen, 2% isopropanol, 0°C; (**d**) metalaxyl, 2% isopropanol, 20°C; (**e**) benalaxyl, 5% isopropanol, 20°C; (**f**) hexythiazox, 0.5% isopropanol, 20°C; (**g**) fluroxypyr-meptyl, 5% isopropanol, 20°C.

ane/isopropanol 98:2 as mobile phase. For fenoxaprop-ethyl, decreasing the percentage of isopropanol resulted in a higher resolution and retention. For quizalofop-ethyl, the retention increased with the decreasing content of isopropanol while the resolution did not follow this trend, reaching a maximum at 2%, after which the resolution decreased because of peak tailing.

The structure of lactofen (ethyl-*O*-[5-(2-chloro-α,α,α-trifluoro-*P*-tolyloxy)-2-nitrobenzoyl]-lactate) was similar to the above herbicides in terms of functional groups and molecular size. Its two enantiomers showed higher enantioselectivity on the CSP. Reducing isopropanol content from 15% to 1% on increased retention and resolution. Baseline resolution was achieved with 5% isopropanol, and the Rs value reached 2.26 when the content of isopropanol was 1%. Fluroxypyr-meptyl (1-methylheptyl[(4-amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy]) was completely resolved at 5% isopropanol, where the Rs value was 1.72. Its two enantiomers showed the strongest retention on the CSP.

Benalaxyl (methyl-*N*-(2′-phenylacetyl)-*N*-(2,6-dimethyl phenyl)-α-alaninate) and metalaxyl (methyl-*N*-(2′-methoxyacetyl)-*N*-(2,6-dimethyl phenyl)-α-alaninate) are two acetamide fungicides. Although they have very similar chemical structures, the retention and resolutions were greatly different. Benalaxyl was excellently resolved, but the two enantiomers of metalaxyl could not be separated completely. The Rs value of benalaxyl reached 2.05 with 15% isopropanol, and 2.76 when the isopropanol content was 5%. The Rs value of metalaxyl was only 1.13 with 2% isopropanol. The metalaxyl enantiomers showed strong retention on the CSP while the retention of benalaxyl enantiomers was weak. With 5% isopropanol the capacity factors (k_1) of the first eluted enantiomer were 9.22 and 4.99 for metalaxyl and benalaxyl respectively. The retention and chiral resolution were not consistent because the retention was related to the interactions of the enantiomers with CSP, while the resolution was related to the difference between the interactions of the two enantiomers with the CSP. The structural differences between the two chiral fungcides are in the phenylacetyl group of benalaxyl, and the methoxyacetyl group of metalaxyl. Although the phenylacetyl group could form π–π interactions, it may give a poorer fit with the helical ADMPC, thus causing weaker retention. While the metalaxyl enantiomers had the smaller methoxyacetyl group, resulting in better fit in the ADMPC CSP, so both the enantiomers could have strong interactions with the CSP. However the difference in these interactions was small, and as a result the resolution was poorer.

Hexythiazox (2*R*,3*R*; 2*S*,3*S*) (5-(4-chlorophenyl)-*N*-cyclohexyl-4-methyl-2-oxo-3-thiazolidinecarboxamide) was resolved only at low isopropanol concentrations, with complete resolution only when the content of isopropanol was 0.5%.

The trend in chiral resolution was benalaxyl > fluroxypyr-meptyl > lactofen > quizalofop-ethyl > fenoxaprop-ethyl > metalaxyl. The chromatograms are shown in Figure 2.

The chiral resolution mechanisms of polysaccharide CSP have not been satisfactorily elucidated. It is commonly considered that a combination of attractive forces such as hydrogen bonding, hydrophobic interactions, dipole–dipole interactions, and charge transfer complex (π–π) formation play the most important roles, and the main chiral adsorbing sites in ADMPC are the polar carbamate groups.[16] The pesticide samples in the study contain phenyl rings, C=O, and N or S atoms which could form the attractive forces. Furthermore, the comparison of the chiral resolutions of metalaxyl and benalaxyl indicated that an appropriate molecular size to the CSP was also important.

TABLE 2. The influence of temperature on the resolutions

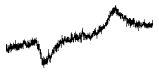
Compound *n*-hexane: isopropanol	Temperature (°C)	k_1'	k_2'	α	Rs
Fenoxaprop-ethyl 99:1	0	10.56	13.18	1.248	1.63
	10	8.97	10.75	1.198	1.62
	20	8.25	9.86	1.195	1.43
	30	7.97	9.89	1.241	2.37
	40	7.27	9.23	1.269	2.42
Quizalofop-ethyl 98:2	0	10.90	13.31	1.22	1.87
	10	7.80	9.24	1.18	1.65
	20	7.09	8.28	1.17	1.71
	30	6.62	7.68	1.16	1.55
	40	6.41	7.38	1.15	1.62
Lactofen 98:2	0	13.07	16.77	1.28	2.77
	10	8.83	10.87	1.23	1.82
	20	8.00	9.64	1.20	1.84
	30	7.06	8.41	1.19	1.63
	40	6.71	7.95	1.18	1.77
Fluroxypyr-meptyl 90:10	0	6.28	7.76	1.23	1.64
	10	5.62	6.73	1.20	1.44
	20	5.25	6.19	1.18	1.31
	30	4.97	5.85	1.18	1.37
	40	4.95	5.75	1.16	1.39
Metalaxyl 95:5	0	12.40	13.36	1.08	0.81
	10	10.65	11.46	1.08	0.80
	20	9.22	9.90	1.07	0.80
	40	7.75	8.15	1.05	0.64
Benalaxyl 85:15	0	2.97	4.06	1.37	2.36
	20	2.19	2.87	1.31	2.05
	30	2.04	2.64	1.29	2.10
	40	1.90	2.39	1.26	1.87
Hexythiazox (R,R; S,S) 99:1	0	9.70	11.02	1.14	1.09
	10	8.75	9.75	1.11	1.07
	20	8.27	9.20	1.11	1.16
	30	7.57	8.34	1.10	1.10
	40	7.15	7.84	1.10	1.04

Mobile phase *n*-hexane/isopropanol, flow rate 1.0 ml/min.

Effect of Temperature

The impact of temperature on the resolutions is listed in Table 2. As in traditional chromatographic analysis, the retention of the enantiomers decreased with increasing temperature, but the separation factors and resolutions did not follow this trend. The impact of temperature in the range of 0°–40°C on the resolution of fenoxaprop-ethyl was examined using *n*-hexane/isopropanol 99:1 as mobile phase. The k' values decreased when the temperature increased, and the separation factor and the resolution factor showed the minimum value at 20°C. The Rs gave a maximum value of 2.42 at 40°C where peak tailing is smaller. Figure 4 shows the influence of temperature on

TABLE 3. The elution orders of the enantiomers on the CSP obtained by CD on-line scanning

Compound	W.L. (nm)	Elution order Pk1/Pk2	CD signal
Fenoxaprop-ethyl	220–250	−/+	
	250–420	no absorbance	
Quizalofop-ethyl	220–290	−/+	
	290–420	no absorbance	
Lactofen	220–254	−/+	
	254–300	+/−	
	300–400	−/+	
	400–420	no absorbance	
Benalaxyl	220–230	+/−	
	230–280	−/+	
	280–420	no absorbance	
Metalaxyl	220–260	−/+	
	260–420	no absorbance	
Hexythiazox	220–275	−/+	
	275–420	no absorbance	
Fluroxypyr-meptyl	225–240	+/−	
	220–225, 240–420	no absorbance	

Mobile phase *n*-hexane/isopropanol, flow rate 1.0 ml/min.

the separation of fenoxaprop-ethyl. For the chiral resolution of quizalofop-ethyl, capacity factors, separation factors and resolution factor seem not very susceptible to temperature. The k' and α values decreased slightly with increasing temperature, and resolutions did not change very much.

The resolution of lactofen was greatly affected by temperature, with better resolution at low temperature with the highest Rs value of 2.77. The mobile phase of *n*-hexane/isopropanol 90:10 was adopted for investigation of the effect of temperature from 0°–40°C on the separation of fluroxypyr-meptyl. The k' and α values gradually decreased with increasing temperature but Rs only reached baseline separation at 0°C and did not change regularly with temperature. Benalaxyl gave excellent resolution. Capacity factors, separation factors, and resolution factors all increased with decreasing temperature for both metalaxyl and benalaxyl, indicating that the optimized conditions should be at a low temperature. For hexythiazox (2*R*,3*R*; 2*S*,3*S*), the retention and resolution both increased with decreasing temperature.

It was considered that two different effects of temperature could affect enantioselectivity.[17] One was a kinetic effect that influences the viscosity and the diffusion coefficient of the solute. Another was the thermodynamic effect that changed the Gibbs free energy change (ΔG) of transfer of the enantiomer between the stationary phase and the mobile phase, which could be denoted as enthalpic and entropic contributions. The fact that the separation factor usually decreased with increasing temperature may be due to the Gibbs free energy change (ΔG) of transfer of the analyte between the two phases decreased at high temperature.

Elution Orders

HPLC-CD is a useful technique for identifying low amounts of enantiomer.[18,19] The elution orders of the two enantiomers (CD signals) were determined and the CD spectra of the single enantiomers from 220 to 420 nm were also studied by on-line scanning on the JASCO 2000 HPLC system. The results are listed in Table 3 which sug-

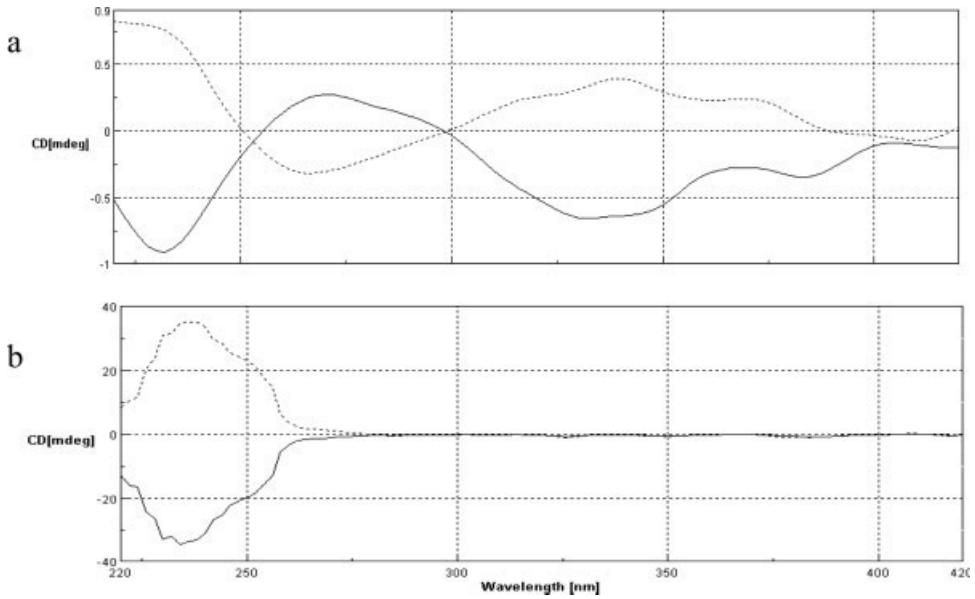


Fig. 3. The CD spectra of the eluted enantiomers of **(a)** lactofen and **(b)** hexythiazox. Real line represents the first eluted enantiomer and the broken line represents the second eluted enantiomer.

gests that the CD signal (the differential absorbance for right circularly polarized light versus left circularly polarized light) and the absorbance intensity of an enantiomer are related to wavelength. For fenoxaprop-ethyl, quizalofop-ethyl, metalaxyl and hexythiazox (2*R*,3*R*; 2*S*,3*S*) the CD signals of the two enantiomers did not change with wavelength but absorbance intensity changed. As Figure 3b showed, the scanning CD spectra curve of one enantiomer was always above the "0" axis which denoted that the CD signal was (+) and the other was always below, a (−) CD signal; the absorbance intensity changed with the wavelength and there was a maximum value. The CD signals and absorbance intensity of lactofen and benalaxyl both changed with wavelength. For example, lactofen for example (shown in Fig. 3a), in the wavelength range of 220–254 nm and 300–400 nm the elution order (CD signal) was −/+ and the maximum absorbance wavelength were 230 nm and 330 nm, and in the range of 254–300 nm the elution order was +/− and the maximum absorbance wavelength was 260 nm. Both enantiomers of fluroxypyrmeptyl did not show symmetrical CD absorbance, and only the wavelength 225–240 nm was appropriate for identifying the enantiomers. It is this very important to denote the wavelength for determination of elution orders using CD signals. For most of the samples, there was no or weak CD absorbance above 300 nm. Figure 4 shows the CD spectra of lactofen and hexythiazox.

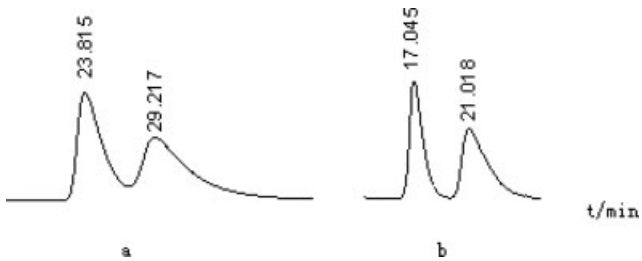


Fig. 4. The effect of temperature on the chiral resolution of fenoxaprop-ethyl. *n*-Hexane/isopropanol 99:1, **(a)** 0°C, **(b)** 40°C.

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CHIRALITY 20:47–50 (2008)

Synthesis and Optical Resolution of an Allenoic Acid by Diastereomeric Salt Formation Induced by Chiral Alkaloids

JONAS NYHLÉN,[1] LARS ERIKSSON,[2] AND JAN-E. BÄCKVALL[1]*

[1]*Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, Stockholm, Sweden*
[2]*Department of Structural Chemistry, Arrhenius Laboratory, Stockholm University, Stockholm, Sweden*

ABSTRACT A synthetic procedure for the preparation of 4-cyclohexyl-2-methyl-buta-2,3-dienoic acid in the two optically active forms has been developed. Synthesis of the racemic allenoic acid was made by an efficient route with good overall yield. Resolution of the enantiomers was achieved by forming the cinchonidine and cinchonine diastereomeric salt, respectively, and the enantiomers were isolated in up to 95% enantiomeric excess. The absolute configuration of the allenoic acid was determined by X-ray crystallography. *Chirality 20:47–50, 2008.* © 2007 Wiley-Liss, Inc.

KEY WORDS: cinchonidine; cinchonine; optical resolution; dienoic acid; butenolides; enantiomers

INTRODUCTION

Chiral allenoic acids are important intermediates in the synthesis of optically active butenolides.[1] The structures of these butenolides are found in many natural products and have therefore received much attention. Despite this interest, there are not many reports in the literature on the preparation of enantiomerically pure allenoic acids.[2,3]

Separation of enantiomers with a carboxylic acid moiety can be done in a few different ways,[4,5] perhaps the most well-known method is the crystallization with a chiral alkaloid where the enantiomers are separated by their difference in solubility.[6,7]

The bark from the Cinchona tree is a source of a variety of alkaloids that has long been used as chiral auxiliaries in organic synthesis. It has previously been shown that the Cinchona alkaloid cinchonidine (Fig. 1) has affinity for one of the enantiomers of a similar acid.[2,3] In this report we show how both enantiomers of an allenoic acid **1** (Fig. 1) can be crystallized with a good overall yield due to effective recovery of the starting material. The absolute configuration of **1** was determined by an X-ray crystal structure of one of the diastereomeric salts.

EXPERIMENTAL SECTION

General Methods

Analytical TLC was performed using aluminum plates with silica gel 60 F_{254} using UV-light and charring with AMC [ammonium molybdate, 10 g, Cerium(IV)sulfate, 2 g, dissolved in 10% H_2SO_4 (200 ml)] for visualization. Flash chromatography was carried out on 60 Å (35–70 μm) silica gel. Organic phases were dried over anhydrous sodium sulfate. Removal of solvent was done at reduced pressure employing a bath temperature of 40°C. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. Chemical shifts (δ) are reported in ppm using residual solvent peak in $CDCl_3$ (δH 7.26 and δC 77.16) or CD_3OD (δH 3.31 and δC 49.00) as reference. Enantiomeric excess (ee) was determined by analytical gas chromatography employing an IVADEX-1 chiral column (25 m, 0.25 mm, 0.15 μm). Optical rotations were measured in $CHCl_3$ at ambient temperature using a Perkin-Elmer 241 polarimeter equipped with a sodium lamp.

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[(Methoxycarbonyl)methylene]triphenylphosphorane (2)

To a stirring solution of PPh_3 (23.65 g, 90.1 mmol) in EtOAc (135 ml), a solution of 2-bromo-acetate (9 ml, 85.8 mmol) in EtOAc (25 ml) was added. After 17 h the white precipitate was collected by filtration, washed three times with Et_2O, and dried. The solid was dissolved in 200 ml DCM and NaOH (7.6 g, 190 mmol) in 160 ml H_2O was added. After 15 min of vigorous stirring, the phases were separated. The aqueous phase was washed twice with DCM and the organic phases were combined, dried, filtered, and evaporated. The white solid **2** was obtained in quantitative yield. Spectral data were in accordance to those reported in the literature.[8]

[1-(Methoxycarbonyl)ethyl]triphenylphosphorane (3)

Compound **2** (28.68 g, 85.8 mmol) was dissolved in 150 ml DCM and cooled on ice. Methyl iodide (8.0 ml, 128.6 mmol) was added and the solution was stirred overnight.

CCDC 649345 contains the supplementary crystallographic data of compound (+)-(S_a)-1 for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk

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*Correspondence to: Jan-Erling Bäckvall, Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, SE-10691 Stockholm, Sweden. E-mail: jeb@organ.su.se
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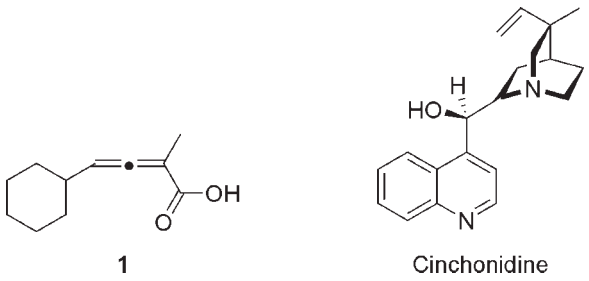


Fig. 1. Structure of the allenoic acid and cinchonidine.

After the solvent was evaporated, yellow foam was obtained. The foam was dissolved in 300 ml DCM and stirred vigorously for 2 h with NaOH (9.5 g, 237 mmol) in 200 ml H_2O. The phases were separated and the aqueous phase was washed twice with DCM. The organic phases were combined, dried, filtered, and evaporated. Compound **3** was obtained as a yellow solid in quantitative yield. Spectral data were in accordance to those reported in the literature.[8]

Cyclohexylacetyl Chloride (4)

Cyclohexyl acetic acid (16.4 g, 115 mmol) was dissolved in thionyl chloride (25.9 ml, 356 mmol) in a round-bottomed flask and the solution was stirred overnight at room temperature in a water bath. The acid chloride **4** was obtained from vacuum distillation as a colorless liquid (15.9 g, 99.3 mmol) in 86% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.75 (d, J = 7.0 Hz, 2H), 1.84–1.96 (m, 1H), 1.61–1.80 (m, 5H), 1.22–1.35 (m, 2H), 1.08–1.21 (m, 2H), 0.93–1.05 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.1, 54.6, 35.3, 32.6, 26.0, 25.9.

4-Cyclohexyl-2-methyl-buta-2,3-dienoic Acid Methyl Ester (5)

Compound **3** (12.5 g, 78.1 mmol) and Et_3N (15.5 ml, 117 mmol) were stirred for 10 min in dry DCM (240 ml) together with molecular sieves in a flask fitted with a gas bubbler outlet. The acid chloride **4** dissolved in DCM (40 ml) was added and after a strong exothermic reaction the stirring continued overnight. The solvent was evaporated until a precipitate was formed. Et_2O was then added and the bottle was put in the freezer for 40 min. The solution was filtered and the solvent was again evaporated until a precipitate was formed. The above procedure was repeated. After two filtrations, all of the remaining solvent was evaporated and the crude product was purified with flash chromatography (pentane to pentane:EtOAc 80:20 gradient). The allenoic acid methyl ester **5** was obtained as a colorless liquid (14.47 g, 0.745 mol) in 95% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44 (dq, J = 2.9, 5.8 Hz, 1H), 3.72 (s, 3H), 2.07–2.18 (m, 1H), 1.86 (d, J = 2.9 Hz, 3H), 1.67–1.81 (m, 3H), 1.58–1.65 (m, 1H), 1.08–1.34 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 209.5, 168.7, 99.8, 96.2, 52.3, 37.2, 33.0, 32.8, 26.1, 26.0, 25.9, 15.6.

4-Cyclohexyl-2-methyl-buta-2,3-dienoic Acid (1)

The allenoic acid methyl ester **5** (4.5 g, 23.2 mmol) was dissolved in dry DCM (225 ml) and cooled to –70°C. One molar BCl_3 in hexanes (93 ml, 92.7 mmol) was added over 5 min and the solution was stirred for another 10 min before the reaction was quenched with 2 M NaOH. When the solution reached room temperature, Et_2O was added and the solution was extracted with basic water until no product was in the organic phase. The aqueous phases were combined and acidified with HCl to a pH of ~3 and extracted twice with DCM. The organic phases were dried, filtered, and evaporated. The crude product was purified by filtration through a silica plug (pentane:EtOAc 4:1) and the allenoic acid **1** (2.24 g, 12.4 mmol) was obtained in 54% yield as yellowish solid. ^{1}H NMR (400 MHz, CD_3OD): δ = 5.47 (dq, J = 2.9, 5.7 Hz, 1H), 2.08–2.18 (m, 1H), 1.81 (d, J = 2.9 Hz, 3H), 1.70–1.80 (m, 3H), 1.60–1.67 (m, 1H), 1.12–1.39 (m, 5H); ^{13}C NMR (100 MHz, CD_3OD): δ = 210.1, 171.4, 100.3, 97.3, 38.2, 33.9, 33,8, 27.2, 26.9, 26.9, 15.6.

General Procedure for Diastereomeric Salt Formation

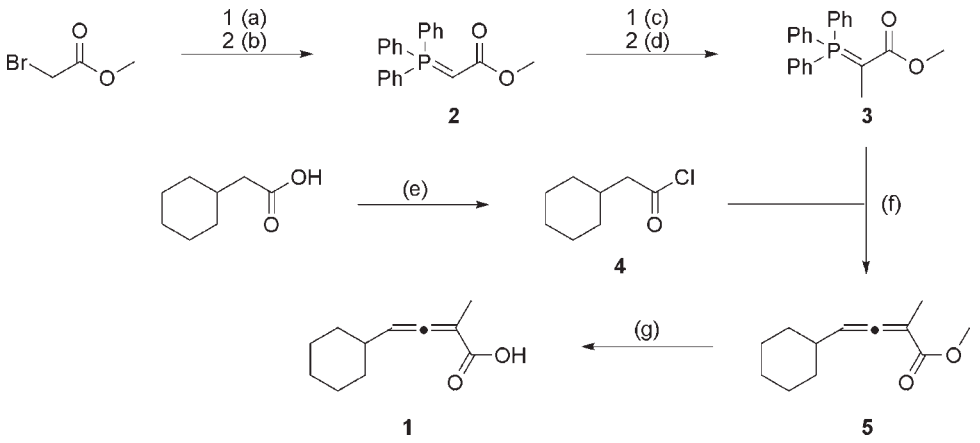
Cinchonidine salt. EtOAc (10 ml) was added to a vial containing cinchonidine (246 mg, 0.83 mmol) and heated to 60°C in an oil bath. The acid (200 mg, 1.11 mmol) was dissolved in 0.7 ml EtOAc and was added to the cinchonidine solution. The stirring bar was removed and the vial was left overnight at 6, 21, or 30°C (Table 1). A white precipitate formed and was collected by filtration and recrystallized twice from EtOAc; the filtrates were collected and reused. The mother liquors were concentrated and the allenoic acid was extracted using the release procedure (see Results and Discussion).

Cinchonine salt. The allenoic acid (120 mg, 0.67 mmol, 35% ee) obtained from the mother liquors of the above experiment and the chiral alkaloid (137 mg, 0.47 mmol) were dissolved in 38 ml EtOAc and heated to 80°C in a oil bath. When the solution was clear it was allowed to cool to ambient temperature and the salt was formed after 24–48 h in the freezer. The precipitate was recrystallized once from EtOAc, the filtrates were combined with the mother liquors, and the remaining allenoic acid was extracted using the release procedure.

TABLE 1. Diastereomeric salt formation of (+)-(S_a)-1

Entry	Volume (ml)	Temperature (°C)	Base (equiv)	Salt formed (mg)	% ee[a]
1	10.7	6	0.5	167	29
2	16.7	6	0.5	140	27
3	10.7	6	0.65	164	30
4	10.7	21	0.5	120	43
5	19.7	21	0.5	50	52
6	10.7	30	0.65	52	55
7	10.0	30	0.75	202	60
8	10.0	30	1.0	290	30[b]

[a]Determined by chiral GC. Refers to the released acid which is equal to the de of the salt.
[b]Possibly because the solution is too saturated or the salt formation is too fast.



Scheme 1. Reagents and conditions: (a) PPh_3, EtOAc, rt, 17 h; (b) NaOH, H_2O, DCM, rt, 15 min, quant.; (c) MeI, DCM, rt, 17 h; (d) NaOH, H_2O, DCM, rt, 2 h, quant.; (e) $SOCl_2$, rt, 17 h, 86%; (f) Et_3N, Mol. Sieves, DCM, rt, 17 h, 95%; (g) BCl_3, DCM, 10 min, −70°C, 54%.

RESULTS AND DISCUSSION

We recently studied the kinetic resolution of allenoic acid **1** using mutant enzymes obtained from directed evolution of *Pseudomonas aeruginosa*.[9] Until now, the absolute configuration of the hydrolyzed enantiomer had been unknown.

Preparation of the Allenoic Acid (Scheme 1)

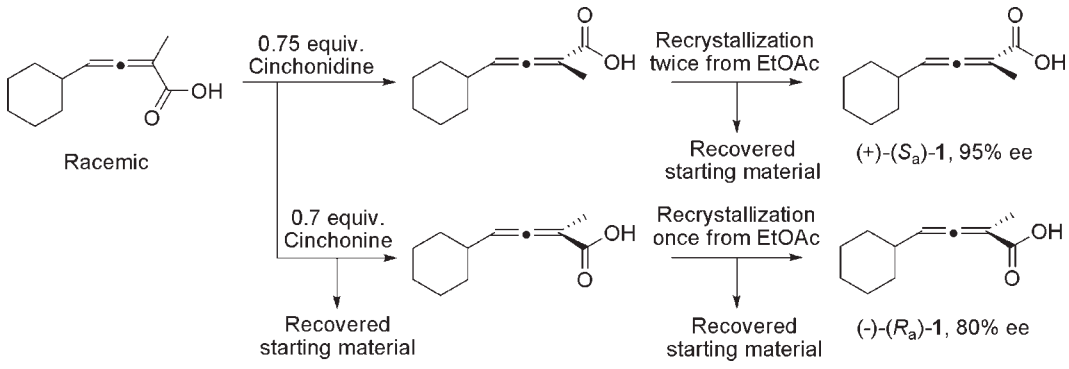
According to previously known procedures,[8] bromoacetic acid methyl ester was stirred with triphenylphosphine to afford the phosphorane **2**, which was then reacted with methyl iodide to produce the Wittig reagent **3**. The allenoic acid methyl ester **5** was prepared by reacting **3** with the acid chloride **4** obtained from cyclohexyl acetic acid and thionyl chloride.[10] Hydrolysis of the methyl ester turned out to be a bigger problem than we first expected because of the acidic alkene hydrogen. The use of sodium hydroxide produces the alkyne and in a typical experiment the alkyne product and the allenoic acid **1** were obtained in approximately a 1:1 ratio. Some mild hydrolysis methods[11,12] were tried but did not afford the desired product. We finally decided to use the method described by Marshall et al.,[13] in which the allenoic acid was formed in moderate yield (54%) but no extensive purification was needed.

Optical Resolution

The separation of the enantiomers was carried out by the use of two different alkaloids, cinchonidine and cinchonine (Scheme 2). The use of cinchonidine led to higher yields and was therefore chosen for the first step.

Diastereomeric Salt Formation of (+)-(S_a)-1

The first salt formation seemed to be crucial to avoid a large number of recrystallization steps and to obtain a good yield. The diastereomeric salt was crystallized from EtOAc. It was shown that the enantiomeric excess could be increased by raising the temperature without affecting the yield (Table 1). The best result, 202 mg with 60% de (60% ee of released acid), was obtained at 30°C with 10 ml of solvent using 0.75 equiv of base. Increase of base gave a higher yield (290 mg) but with a much lower selectivity. Use of more solvent (10.7 ml) gave a dramatic drop in yield (entry 6, Table 1). The use of 12 ml of solvent gave no precipitation at 30°C (not shown). After two recrystallizations the (+)-allenoic acid **1** was obtained in 20% yield (the low yield is due to low efficiency in the diastereomeric salt formation) and 95% ee, $[\alpha]_D^{20} = +131°$. By collecting the filtrates, 90% of the remaining starting material was recovered. The absolute configuration of (+)-**1** was



Scheme 2. Diastereomeric salt formation scheme for both enantiomers using two different alkaloids.

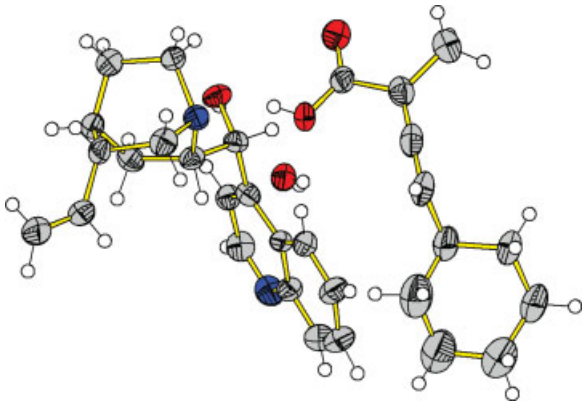


Fig. 2. X-ray structure of (+)-(S_a)-**1** and cinchonidine. Ellipsoids drawn at 50% probability. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

established from the X-ray crystal structure of the diastereomeric acid salt with cinchonidine (Fig. 2).

Diastereomeric Salt Formation of (−)-(R_a)-1

Isolation of the (R_a)-enantiomer turned out to be harder then expected. At first, several different conditions using quinine as the chiral base were tried but without success. Cinchonine was neglected at first because of the low solubility in ethyl acetate but was later tried and showed to have a good selectivity for the desired enantiomer with the drawback that larger amounts of solvent had to be used. Using ethyl acetate in ethanol or isopropanol mixtures could increase solubility but no salt was formed using these conditions. After one recrystallization from EtOAc the (–)-allenoic acid **1** was obtained in 15% yield and 80% ee, $[\alpha]_D^{20} = -106^\circ$. Recovery of the filtrates yielded 64% of the remaining starting material. One additional recrystallization improved the enantiomeric excess to about 85%.

Recovery of the Starting Material

All the mother liquors were evaporated after removing the salt by filtration. By using the release procedure described below, the acid could be extracted and collected in the organic phase. If needed, the acid was purified by filtration through a silica plug.

Release of the Allenoic Acid from the Salt

The cinchonidine and cinchonine salts with the allenoic acid were dissolved in methanol and ethanol, respectively. A 20% H_2SO_4 aqueous solution was added and the solution was extracted four times with ethyl acetate. The organic phases were combined and dried. Evaporation of the solvent afforded the acid. If needed, the acid was purified by filtration through a silica plug.

CONCLUSION

Both enantiomers of allenoic acids (+)-(S_a)-**1** and (–)-(R_a)-**1** were successfully separated and isolated in good enantiomeric excess after only 1–2 recrystallization steps. The overall yield for the resolution was good because of the efficient recovery of the starting material. An interesting temperature dependence of the salt purity was observed.

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CHIRALITY 20:51–53 (2008)

Determination of Enantiomeric Excess of Ethyl 3,5-dihydroxy-6-benzyloxy hexanoate by Chiral Reverse Phase High Performance Liquid Chromatography

JI-HUA LIU, BO-YANG YU, AND YI-JUN CHEN*

School of Life Science and Technology, China Pharmaceutical University, Nanjing, People's Republic of China

ABSTRACT A simple and reliable chiral HPLC method was developed for the determination of enantiomeric excess of a chiral dihydroxy intermediate for the chemoenzymatic synthesis of side chain of statin drugs. After evaluating different columns and conditions, the four stereoisomers of ethyl 3,5-dihydroxy-6-benzyloxy hexanoate were well resolved by a simple gradient elution on OD-RH column, and the enantiomeric excess of the desired 3*R*,5*S*-enantiomer was accurately measured. This study provides a simple, rapid, accurate, and reliable method to assess the enantiomeric quality of such important intermediates. *Chirality 20:51–53, 2008.* © 2007 Wiley-Liss, Inc.

KEY WORDS: chiral intermediate; enantiomeric excess; 3*R*,5*S*-dihydroxyhexanoate side chain; statins

INTRODUCTION

The enantioselective synthesis of dihydroxyhexanoate side chain of synthetic statins such as Lipitor® has been a very competitive research area due to a high cost by the current synthetic route. Compared to chemical synthesis,[1–3] microbial/enzymatic approach has shown certain advantages based on its high enatioselectivity. Several biocatalytic strategies have been explored and reported.[4–10] Among different routes, the reduction of a diketone substrate, ethyl 3,5-diketo-6-benzyloxy hexanoate, to the desired enantiomer, ethyl 3*R*,5*S*-dihydroxy-6-benzyloxy hexanoate (Fig. 1), by a single step in a microbial system is very unique and attractive, which may offer an efficient route for the synthesis of chiral side chain to significantly reduce the cost of manufacturing synthetic statins.[6,8] Obviously, the quality of final drug products is dependent upon the enantiomeric purity of the chiral side chain, and determination of enantiomeric excess (*ee*) and diastereomeric excess (*de*) is essential for the assessment of the enantiomeric purity because greater than 99.5% *ee* and 99% *de* are required for the dihydroxyhexanoate side chain. While *de* could be determined easily by conventional high performance liquid chromatography (HPLC) method, normal phase chiral HPLC was used to determine *ee* in the earlier report.[6] Later, it was reported to use reverse phase chiral HPLC for the determination of *ee* following the advancement and availability of columns with different chiral-stationary materials.[8] Considering the use of volatile and more toxic solvents for the safety concerns and the difficulty of accurate integration of broad peaks produced, in some cases, by normal phase HPLC, a reproducible reverse phase HPLC method is preferable and advantageous. In addition, since reverse phase-HPLC has been a routine analytical method for the quality control in pharmaceutical industry, it is definitely an easy choice for the analyses of enantiomeric excess of the dihydroxy products without a dedication of an instrument under anhydrous condition that a normal phase HPLC is required. Surprisingly, when we utilized the method reported in the literature[8] to assess the *ee* values of this chiral intermediate, four stereoisomers of the racemic ethyl dihydroxyhexanoate produced by chemical reduction could not be resolved at all (Fig. 2), which was very strange and unexpected. To investigate such unusual phenomenon and to fulfill the requirement of an accurate and reliable analytical method, we examined different columns and solvent systems in the aim of developing a precise and reproducible method for the analyses of *ee* values of the chiral dihydroxy intermediates. In the present report, a simple reverse phase-HPLC method with a chiral OD-RH column has been developed, and its accuracy, reproducibility, and working range have also been investigated.

MATERIALS AND METHODS

Materials

Racemic ethyl 3,5-dihydroxy-6-benzyloxy hexanoate and ethyl 3*R*,5*S*-dihydroxy-6-benzyloxy hexanoate were prepared according to the literature.[8] All other chemicals and solvents were analytical grade and purchased from Sigma-Aldrich Chemical (Shanghai, China).

Analytical Method

Chromatographic studies were conducted on Agilent 1100 HPLC system (Palo Alto, CA) equipped with autosampler,

*Correspondence to: Yi-Jun Chen, School of Life Science and Technology, China Pharmaceutical University, Nanjing 210009, People's Republic of China. E-mail: yjchen_cpu@yahoo.com.cn

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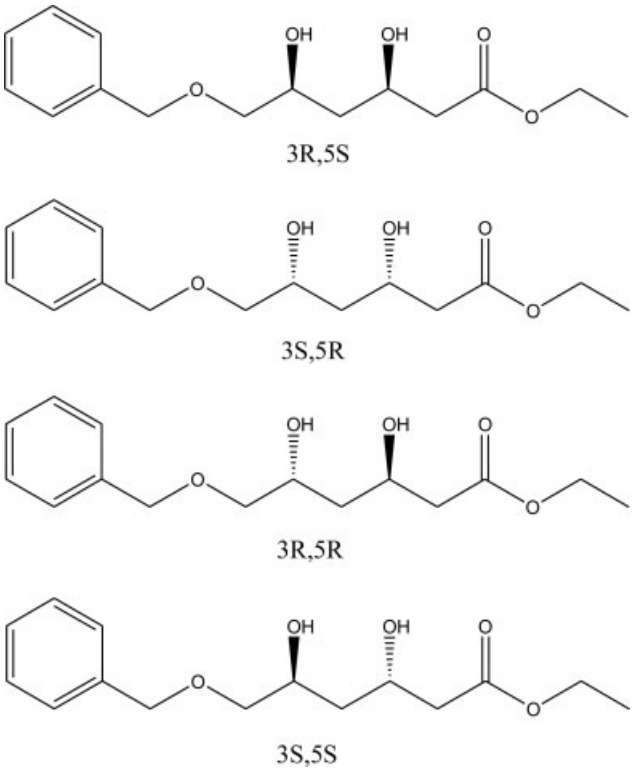

Fig. 1. Chemical structures of stereoisomers of ethyl 3,5-dihydroxy-6-benzyloxy hexanoate.

thermostated-column device, and a variable-wavelength UV detector set at 220 nm. Samples of 20 μl were chromatographed on a Chiralcel OD-RH column (4.6 mm × 150 mm, 5 μm particle size, Daicel Chemical Industries, Exton, PA) with a flow rate of 0.5 ml/min at 25°C. The mobile phase consisted of a mixture of A—0.1% trifluroacetic acid in water; B—0.1% trifluroacetic acid in acetonitrile. Elution was achieved with a gradient of 25–30% B in 25 min and kept at 30% B for additional 5 min.

RESULTS AND DISCUSSION

Biocatalytic approach has been demonstrated to be a cost-effective method for the preparation of chiral compounds.[4,5] The enantioselective reduction of ethyl 3,5-diketo-hexanoate to desired single enantiomer, ethyl 3*R*,5*S*-dihydroxy-6-benzyloxy hexanoate, has shown promises and advantages for the synthesis of chiral side chain of synthetic statin drugs that have the most patients and largest market worldwide. The enantiomeric quality of the side chain is essential for the final drug products, and determination of the enantiomeric excess with an appropriate method is vital for the chiral intermediate. Since the reported method was unable to resolve the four stereoisomers of dihydroxy product, as shown in Figure 2, development of a simple and reliable HPLC method to assess the enantiomeric excess has become highly demanding.

A variety of HPLC columns with different chiral-stationary phase, including OD, AD, and OJ, were examined, and elution with both normal and reverse phases was also evaluated. Both OD and AD columns (10 μm particle size) with normal phase (heptane/ethanol) elution were able to yield baseline separation of the stereoisomers (data not shown), but the peaks were very broad and difficult to integrate. Thus, accurate determination of *ee* values was not possible with normal phase elution in the case of enzymatic products having higher optical purity. With the reasons and advantages stated earlier, we decided to pursue our study with a reverse phase-HPLC to establish a simple, reliable, and reproducible method. After a thorough investigation, differing from the reported method,[9] simply omitting methanol from the solvent system on an OD-RH

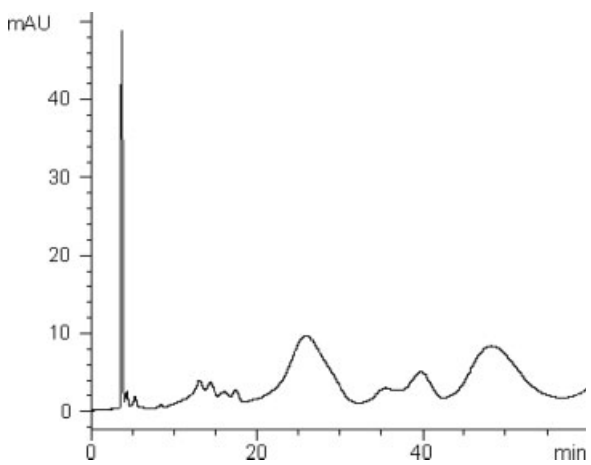

Fig. 2. Chromatogram of racemic ethyl 3,5-dihydroxy-6-benzyloxy hexanoate (four stereoisomers) based on reported method.[8] Column: Chiralcel OD-RH (4.6 mm × 150 mm, 5 μm particle size); temperature: 25°C; injection volume: 20 μl; mobile phase: 0.05% trifluroacetic acid in water/methanol 80:20 (A) and 0.05% trifluroacetic acid in acetonitrile/methanol 80:20 (B); elution: isocratic composition of A/B (90:10) for 60 min; flow rate: 0.5 ml/min; detection: UV 220 nm.

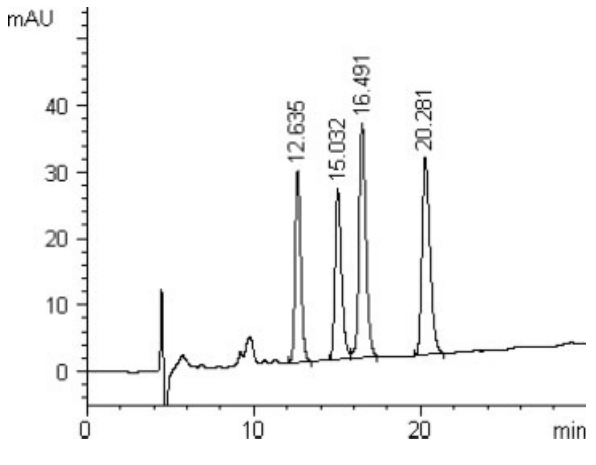

Fig. 3. HPLC chromatogram of racemic ethyl 3,5-dihydroxy-6-benzyloxy hexanoate. Retention times of (3*R*,5*S*), (3*S*,5*R*), (3*R*,5*R*), and (3*S*,5*S*)-dihydroxy stereoisomers are 20.3, 16.5, 12.6, and 15.0 min, respectively. Column: Chiralcel OD-RH (4.6 mm × 150 mm, 5 μm particle size); temperature: 25°C; injection volume: 20 μl; mobile phase: 0.1% trifluroacetic acid in water (A) and 0.1% trifluroacetic acid in acetonitrile (B); elution: gradient of 25–30% B in 25 min and kept at 30% B for additional 5 min; flow rate: 0.5 ml/min; detection: UV 220 nm.

column (5 μm particle size) with isocratic elution of acetonitrile–water system containing 0.1% TFA resulted in four individual peaks (data not shown), but the *ee* values were still difficult to measure because they were not completely baseline separated. Further modification with a linear gradient (25–30% solvent B) produced a well-resolved baseline separation of the four stereoisomers (Fig. 3). Under the experimental conditions, the retention times for (3*R*,5*S*), (3*S*,5*R*), (3*R*,5*R*), and (3*S*,5*S*)-dihydroxy stereoisomers were 20.3, 16.5, 12.6, and 15.0 min, respectively. Figure 4 shows the chromatogram of the product that was reduced from the diketone substrate by enzymatic catalysis, indicating that desired 3*R*,5*S*-dihydroxy enantiomer was produced with high enantiomeric excess. Although there was a slight shift of retention times from sample to sample, relative position, baseline separation, and accurate quantification were not affected by the present method. Such simple reverse phase-HPLC method can be widely adapted without switching routine solvent systems by any analytical laboratories for quality control of the dihydroxy product, and the 30 min run is also reasonable and easy to program and operate. Even though the exact reasons for the inability of reproducing reported method[8] are not clear, based on our findings, it is quite reasonable to speculate that methanol in the solvent system may have played a critical role for the retention and separation of the stereoisomers on the chiral-stationary phase.

To validate the present method, different concentrations of enzymatic product were compared to determine the minimum concentration required for an accurate and reproducible method. As shown in Table 1, there was a significant difference between 0.25 and 0.5 mg/ml, while similar numbers were obtained from the same sample above 0.5 mg/ml with small variations. Moreover, sample quantification above 0.5 mg/ml was much more consistent and reproducible. Therefore, it should be recommended that a minimal concentration of 0.5 mg/ml is required for this assay in order to accurately assess and determine the enantiomeric excess of the dihydroxy products.

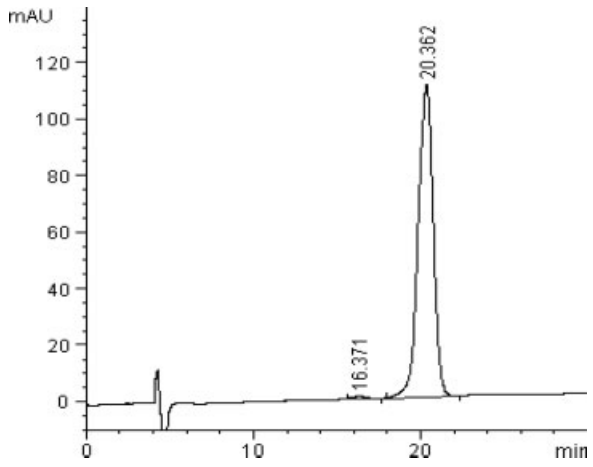

Fig. 4. Chromatogram of ethyl 3*R*,5*S*-dihydroxy-6-benzyloxy hexanoate enzymatic produced. Same conditions were used as Figure 3.

TABLE 1. Relationship between sample concentration and *ee* value[a]

Sample concentration (mg/ml)	*ee*% ± SD (*n* = 5)
0.25	95.12 ± 2.57
0.50	97.75 ± 0.64
1.0	97.38 ± 0.16
2.0	97.37 ± 0.11

[a]At a condition of 20 μl injection and 220 nm detection.

CONCLUSIONS

We have developed a simple reverse phase-HPLC method for the determination of enantiomeric excess of ethyl 3,5-dihydroxy-6-benzyloxy hexanoate, an important intermediate for the chemoenzymatic synthesis of synthetic statin drugs. The present method has been confirmed to be simple, rapid, reliable, and reproducible, which can be effectively utilized for the assessment of enantiomeric excess of microbial/enzymatic products.

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Theoretical Insight into the Influences of α-Substituents in Aliphatic Aldehydes on the Enantioselectivities of Aldol Reactions

JIAN-FEN FAN,* LIANG-JUN HE, AND YUN-PENG SUN

Key Laboratory of Organic Synthesis of Jiangsu Province, Faculty of Chemistry and Chemical Engineering, Suzhou University, Suzhou, People's Republic of China

ABSTRACT Density functional theory has been applied to study the influences of α-substituents in aliphatic aldehydes on the enantioselectivities of the (*S*)-proline-catalyzed direct aldol reactions. Reaction scenarios of three kinds of aliphatic aldehydes were investigated. Four transition states associated with the stereocontrolling step of each reaction have been obtained. They are corresponding to the syn and anti arrangements of enamine intermediates and the *si* and *re* attacks to the carbonyl group of an aldehyde. The solvent effect of DMSO was investigated using self-consistent reaction field method based on the polarizable continuum model. The computed energies of transition states explain the origin of the catalysis and enantioselectivities for these (*S*)-proline-catalyzed aldol reactions and reveal the influences of α-substituents in aliphatic aldehydes on the enantioselectivities of these reactions. *Chirality 20:54–61, 2008.*
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KEY WORDS: DFT; aliphatic aldehydes; α-substituents; enantioselectivities; aldol reactions

INTRODUCTION

As a fundamental C—C bond formation reaction, the catalytic asymmetric aldol reaction is widely utilized, promoting the rapid evolution of efficient catalysts in organic synthesis.[1] Although (*S*)-proline-catalyzed enantioselective aldol reactions were first reported in 1971,[2] the real breakthrough came from the work by List et al. in 2000.[3] They found that the simple amino acid (*S*)-proline catalyzes the aldol reactions between acetone and a variety of aldehydes in good yields and with high enantioselectivities, and proposed the enamine mechanism, suggesting that the stereocontrolling step is the C—C bond formation between enamine intermediate and aldehyde.[3,4] Houk, Domingo, and Boyd et al.[5–8] theoretically studied the transition structures associated with the stereocontrolling steps of various (*S*)-proline-catalyzed intermolecular aldol reactions, and the energy results well explained the enantioselectivities of these reactions, supporting the enamine mechanism.

It was reported that the (*S*)-proline-catalyzed direct aldol reactions between acetone and aliphatic aldehydes usually give good enantioselectivities,[3] and α-substituent in an aldehyde influences the enantioselectivity greatly.[9] Among 2,2-dimethyl propyl aldehyde, 2-methyl propyl aldehyde, and 3,3-dimethyl butyric aldehyde, the first one gives the best enantioselectivity with 98% ee and the last one gives the worst value with only 68% ee.[9] That is the more the substituents on α-site in an aldehyde, the higher the enantioselectivity of the reaction.[9]

Our interest in the enzymatic-type catalyzed aldol reactions has prompted us to carry out a theoretical investigation on the influences of α-substituents in aliphatic aldehydes on the enantioselectivities of aldol reactions. Three kinds of aliphatic aldehydes shown in Scheme 1 were adopted. The (*S*)-proline-catalyzed aldol reactions between acetone and the aldehydes were studied. Density functional theory (DFT)[10] calculations for the C—C bond formation steps of these reactions reveal the origins of the enantioselectivities and the influences of α-substituents in these aliphatic aldehydes on the enantioselectivities of the reactions.

COMPUTATIONAL METHODS

Based on enamine mechanism proposed by List et al. and Sakthivel et al.,[3,4] the initial interaction between (*S*)-proline **1** and acetone **2** generates an enamine intermediate **3**, which reacts further with an aldehyde **4** to form a zwitterion intermediate **5** and finally yields the aldol product **6** (see Scheme 2). The enamine intermediate has two conformers named as *syn*- and *anti*-one, corresponding to two arrangements of the active methylene group relative

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*Correspondence to: Fan Jian-Fen, Key Laboratory of Organic Synthesis of Jiangsu Province, Faculty of Chemistry and Chemical Engineering, Suzhou University, 215123 Suzhou, People's Republic of China.
E-mail: jffan1965@163.com
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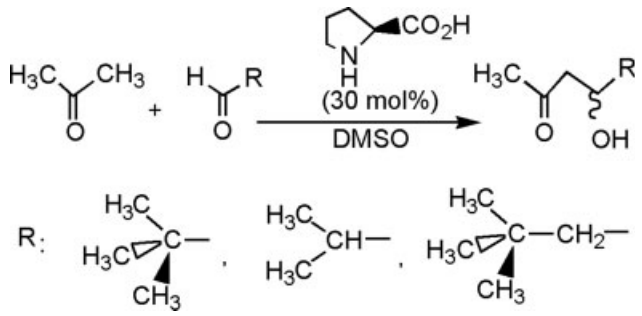


Scheme 1. Direct intermolecular aldol reaction.

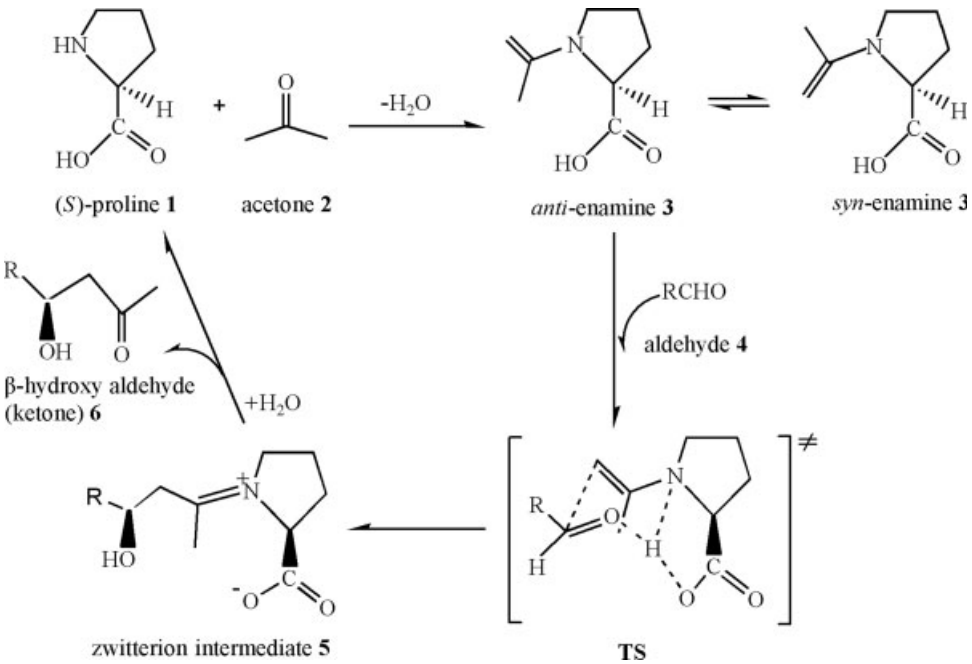
TABLE 1. The energies (Hartree) of *anti*- and *syn*-enamines at various levels and their differences ΔE (kJ/mol)

Item	Energy of *anti*-enamine (Hartree)	Energy of *syn*-enamine (Hartree)	ΔE (kJ/mol)
B3LYP/6-31G*	−517.864501	−517.866829	6.1
B3LYP/6−31G**	−517.886999	−517.889316	6.1
B3LYP/6-31G** in DMSO	−517.899690	−517.902800	8.2
B3LYP/6-311++G** //B3LYP/6-31G**	−518.022189	−518.025103	7.7
B3LYP/6-311++G** in DMSO//B3LYP/ 6-31G**	−518.028614	−518.030955	6.1

to carboxyl one. The active methylene carbon in *syn*- and *anti*-enamines can both attack to the *si* and *re* faces of aldehyde carbonyl group to form the C—C bond, respectively. Thus, there are four reaction channels in the C—C bond-formation step, forming four transition state (TS) structures, named as *anti*-(*R*)-TS, *anti*-(*S*)-TS, *syn*-(*R*)-TS, and *syn*-(*S*)-TS, respectively.

Three scenarios of (*S*)-proline-catalyzed intermolecular aldol reactions between acetone and 2,2-dimethyl propyl aldehyde, 2-methyl propyl aldehyde, and 3,3-dimethyl butyric aldehyde have been studied, respectively. Molecular species involved in the C—C bond-formation step of each reaction scenario were studied using DFT[10] in Gaussian 98 suite of program.[11] Becke's three-parameter hybrid exchange functional (B3)[12,13] and the correlation functional of Lee, Yang, and Parr (LYP)[14] were used. Geometry optimizations for all involved species were performed with the 6-31G* and 6-31G** basis sets, using default values as convergence criteria. The stationary points were characterized by frequency calculations in order to verify that minima and transition structures have zero and one imaginary frequency, respectively.[15] The intrinsic reaction coordinate (IRC)[16] paths were traced in order to check the energy profiles connecting each transition structure to the two associated minima of the proposed mechanism by using the second-order Gonzalez–Schlegel integration method.[17,18] Single-point energies were calculated with the larger basis set 6-311++G** using the 6-31G** geometries. The optimized structures and energies of *syn*- and *anti*-enamine intermediates, four TSs of the stereocontrolling step in each reaction scenario, have been obtained and are available from the authors.

DMSO, a moderate ionizing solvent of dimethyl sulfoxide with dielectric constant of 46.7, is widely used in aldol reactions.[3,4] As solvents can modify both the reaction activation energies and enantioselectivities, these aldol reactions were also studied in the solvent of DMSO to simulate the usual environments of the species and reactions. The



Scheme 2. Reaction mechanism of (*S*)-proline-catalyzed aldol reaction.

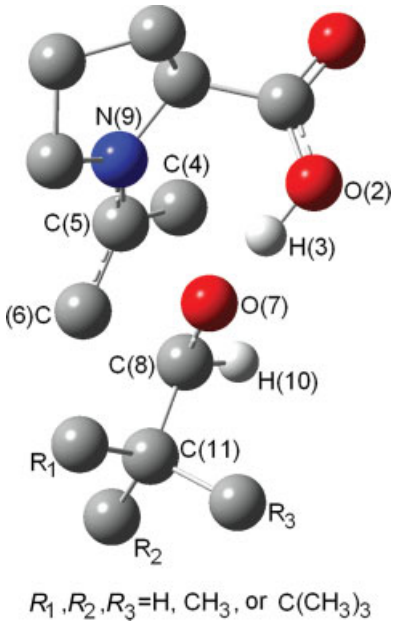


Fig. 1. The atomic numberings in the structures of the transition states formed in the C—C bond formation step. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

solvent effects of DMSO were investigated using a relatively simple self-consistent reaction field method,[19] based on the polarizable continuum model (PCM) of Tomasi's group.[20]

RESULTS AND DISCUSSIONS

Enamine Intermediate 3

(*S*)-Proline-catalyzed intermolecular direct aldol reaction starts with the nucleophilic attack of N atom in proline **1** to the carbonyl C atom in acetone **2** (shown in Scheme 2), which converts to the enamine intermediate **3** by proton transfer from the methyl group to the carbonyl group. For the enamine intermediate **3**, two conformational structures (*syn*- and *anti*-enamines) are possible owing to the restricted rotation around the C—N bond.

Table 1 lists the energies of *syn*- and *anti*-enamines at B3LYP/6-31G* and B3LYP/6-31G** levels and their differences (ΔE). Also listed are the single-point energy results at the diffusion basis set of 6-311++G** with 6-31G** geometries and the results in DMSO with PCM computations. Obviously, the syn arrangement is preferred over the anti one by 7.7 and 6.1 kJ/mol at the level of B3LYP/6-311++G**//B3LYP/6-31G** in gas and DMSO, respectively. This result is possibly caused by the lower barrier for proton transfer from the methyl group cis to α-carbon of proline to the carboxyl group. It is in agreement with the calculation reported by Boyd and coworkers,[8] in which C—C double bond in enamine more easily locates on the same side of carboxylic acid group. However, the easy C—N bond rotation allows the equilibrium between *syn*- and *anti*-enamine conformers. Boyd and coworkers[8] reported that *syn*-enamine may convert to *anti*-one by undergoing a barrier of 25 kJ/mol, computed at the level of B3LYP/6-31G**.

Optimized Structures of TSs

Via the addition of enamine **3** to aldehyde **4**, the C—C bond is formed, which is the stereocontrolling step for a proline-catalyzed aldol reaction.[8] Four reactive channels of this step related to the nucleophilic attacks of C(6) atom in the active methylene group of the *syn*- and *anti*-enamines to carbonyl C(8) from the *si* or *re* faces of the carbonyl group of an aldehyde have been investigated. The atomic numberings in these TSs are depicted in Figure 1. The optimized structures of four TSs, i.e., *anti*-(*R*)-TS, *syn*-(*R*)-TS, *anti*-(*S*)-TS, and *syn*-(*S*)-TS in the reaction scenarios of 2,2-dimethyl propyl aldehyde, 2-methyl propyl aldehyde,

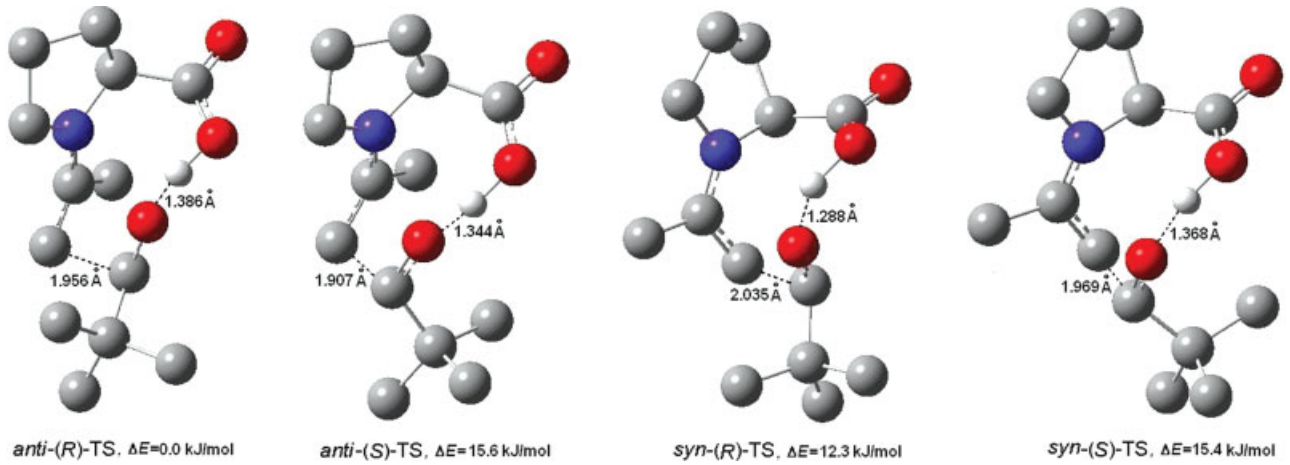


Fig. 2. (*R*)- and (*S*)-transition states at the level of B3LYP/6-31G**, formed in the stereocontrolling step of (*S*)-proline-catalyzed aldol reaction between acetone and 2,2-dimethyl propyl aldehyde. Hydrogen atoms except H(3) were omitted. Also listed is the relative energies (ΔE) computed at the level of B3LYP/6-311++G**//B3LYP/6-31G** in DMSO. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

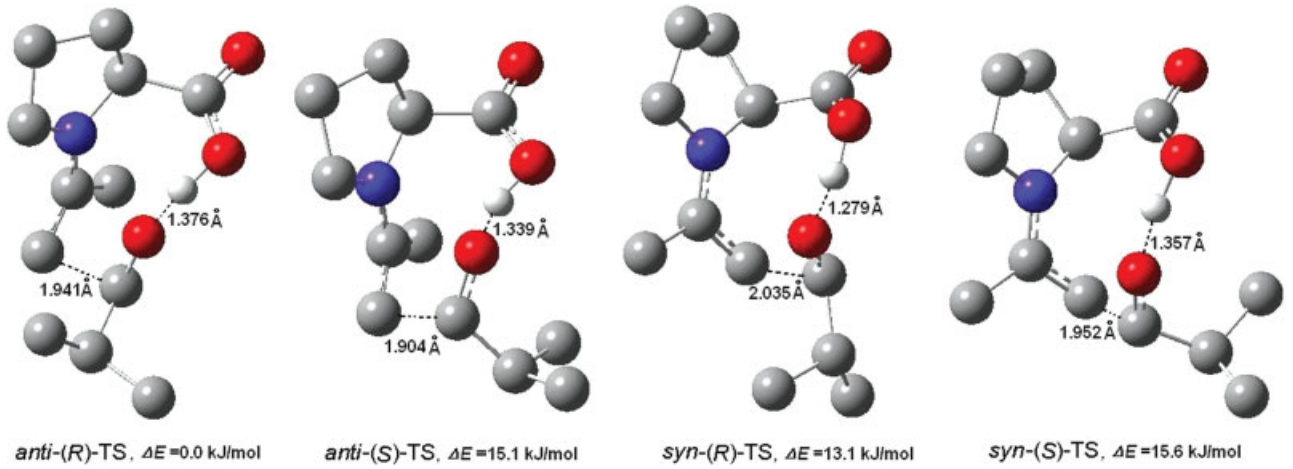


Fig. 3. (*R*)- and (*S*)-transition states at the level of B3LYP/6-31G**, formed in the stereocontrolling step of (*S*)-proline-catalyzed aldol reaction between acetone and 2-methyl propyl aldehyde. Hydrogen atoms except H(3) were omitted. Also listed is the relative energies (Δ*E*) computed at the level of B3LYP/6-311++G**//B3LYP/6-31G** in DMSO. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

and 3,3-dimethyl butyric aldehyde were illustrated in Figures 2–4, respectively. Tables 2–4 list the main geometric parameters of the four ^{TM}s formed in these three reaction scenarios, respectively.

The C(6)—C(8)—O(7)—C(11) dihedral angle reflects the chirality of TS. The angles in *anti*-(*R*)-TS and *syn*-(*R*)-TS are 114–123° and in *anti*-(*S*)-TS and *syn*-(*S*)-TS are −128° to −115°, respectively.

The C—C bond formation between an enamine intermediate and an aldehyde is the stereocontrolling step. In these TSs, the newly formed C(6)—C(8) bonds are about 1.9–2.0 Å.

The intermolecular hydrogen bond in proline-catalyzed process is responsible for the large catalytic effect observed for this kind of catalyzed aldol reaction. The newly formed H(3)—O(7) bonds in these TSs are 1.3–1.4 Å. The formation of an intermolecular hydrogen bond between the acidic H(3) of (*S*)-proline and the carbonyl O(7) atom of the aldehyde in an early stage of the process catalyzes very effectively the C—C bond formation by a large stabilization of the negative charge that is developing at the carbonyl O(7) atom along the nucleophilic attack.

In all these transition structures, the distances between N(9) and H(3) atoms are about 2.5–2.6 Å. Thus, there exists weak hydrogen bond N(9)···H(3)—O(2). Such hydrogen bond originally exists in *syn*- or *anti*-enamines, with 2.0–2.1 Å of the bond length.[22] Obviously, it is largely weakened in the C—C bond formation step. Integrated with the newly formed H(3)—O(7) bonds in these TSs, it can be concluded that H(3) is tricoordinated in these TSs.

The N(9)—C(5)—C(4)—C(6) dihedral angle indicates that C(5) atom in enamine moiety deviates slightly from

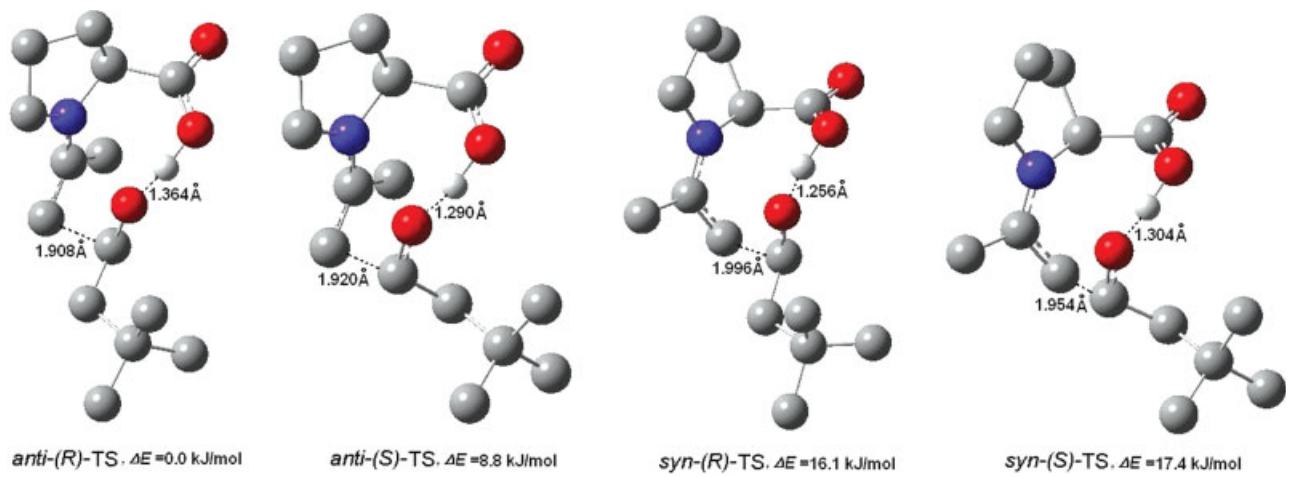


Fig. 4. (*R*)- and (*S*)-transition states at the level of B3LYP/6-31G**, formed in the stereocontrolling step of (*S*)-proline-catalyzed aldol reaction between acetone and 3,3-dimethyl butyric aldehyde. Hydrogen atoms except H(3) were omitted. Also listed is the relative energies (Δ*E*) computed at the level of B3LYP/6-311++G**//B3LYP/6-31G** in DMSO. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE 2. Main geometric parameters of the four transition states at the level of B3LYP/6-31G, formed in the stereocontrolling step of (*S*)-proline-catalyzed aldol reaction between acetone and 2,2-dimethyl propyl aldehyde**

Selected parameters	*anti*-(*R*)-TS	*anti*-(*S*)-TS	*syn*-(*R*)-TS	*syn*-(*S*)-TS
Bond length (Å)				
C(6)—C(8)	1.956	1.907	2.035	1.969
H(3)—O(7)	1.386	1.344	1.288	1.368
H(3)—O(2)	1.079	1.102	1.130	1.076
N(9)—H(3)	2.496	2.475	2.602	2.608
O(7)—C(8)	1.293	1.305	1.295	1.289
Dihedral angle (°)				
C(6)—C(8)—O(7)—C(11)	119.1	−128.4	123.1	−121.6
N(9)—C(5)—C(4)—C(6)	168.2	171.4	−167.8	−167.6
O(7)—C(8)—H(10)—C(11)	136.0	−133.5	138.2	−137.9
C(7)—C(8)—O(6)—C(5)	47.2	29.6	−3.5	−37.9
C(11)—C(8)—O(6)—C(5)	172.7	−103.2	125.4	−167.8

sp^2 hybridization. The O(7)—C(8)—H(10)—C(11) dihedral angle shows that the carbonyl C(8) atom has a large deviation from sp^2 hybridization. The O(7)—C(8) bond lengths in these TSs increase to 1.3 Å, whereas those in the three investigated aldehydes are about 1.2 Å.

Conformational analysis along the newly formed C(6)—C(8) shows that the O(7)—C(8)—C(6)—C(5) and C(11)—C(8)—C(6)—C(5) dihedral angles in *anti*-(*R*)-TSs of the three reaction scenarios are 47.2°, 48.0°, 49.7° and 172.7°, 173.1°, 173.1°, respectively. These results reveal that *anti*-(*R*)-TS presents one gauche arrangement between the carbonyl oxygen atom and the enamine framework, However, these dihedral angles in *anti*-(*S*)-TSs are 29.6°, 28.0°, 46.0° and −103.2°, −104.4°, −83.1°, respectively, showing two gauche arrangements existing in *anti*-(*S*)-TS. Thus, the steric hindrance between the alkyl group of an aldehyde and the methyl group of *anti*-enamine moiety in *anti*-(*S*)-TS is responsible for its less stability than *anti*-(*R*)-TS, as what will be shown in later section. Nevertheless, the O(7)—C(8)—C(6)—C(5) and C(11)—C(8)—C(6)—C(5) dihedral angles in *anti*-(*S*)-TS for the reaction scenario of 3,3-dimethyl butyric aldehyde are 46.0° and −83.1°, respectively. They are much different from those in the reaction scenarios of the other two aldehydes. This result is probably caused from the less steric hindrance between the methyl group of *anti*-enamine and the alkyl group of 3,3-dimethyl butyric aldehyde, as for C(11) is secondary carbon. Thus, the stability difference between *anti*-(*R*)-TS and *anti*-(*S*)-TS for the reaction scenario of 3,3-dimethyl butyric aldehyde is smallest.

Identify of TSs

The two lowest vibration frequencies (ν_1 and ν_2)of the four TSs in each of the three reaction scenarios are listed in Table 5. Each TS is characterized with unique imaginary frequency ν_1. IRC calculation was carried out for each TS to confirm that the TS is at the saddle point on the potential energy surface. As an example, Figure 5 depicts the IRC calculation result at the level of B3LYP/6-31G* for *anti*-(*S*)-TS in the stereocontrolling step of the reaction scenario of 3,3-dimethyl butyric aldehyde. Along the reaction path, it can be easily found that the acidic H(3) is gradually breaking away from the carboxyl O(2) and moving toward the carbonyl O(7), and finally forms zwitterion intermediate **5**. With the GaussView software, the only imaginary frequency of each transition structure can be confirmed to be mainly associated with the movements of C(6) and C(8) atoms along the newly formed C(6)—C(8) bond and the coupled movement of the acidic H(3) to O(7) atom along the proton-transfer direction. Therefore, the C(6)—C(8) bond formation and the H(3) proton transfer are concerted processes in these TSs.

Influences of α-Substituents in Aliphatic Aldehydes on the Enantioselectivities of Aldol Reactions

Tables 6–8 list the results of energies at various levels of the four TSs formed in the stereocontrolling step of (*S*)-proline-catalyzed aldol reaction between acetone and 2,2-dimethyl propyl aldehyde, 2-methyl propyl aldehyde, and

TABLE 3. Main geometric parameters of the four transition states at the level of B3LYP/6-31G, formed in the stereocontrolling step of (*S*)-proline-catalyzed aldol reaction between acetone and 2-methyl propyl aldehyde**

Selected parameters	*anti*-(*R*)-TS	*anti*-(*S*)-TS	*syn*-(*R*)-TS	*syn*-(*S*)-TS
Bond length (Å)				
C(6)—C(8)	1.941	1.904	2.035	1.952
H(3)—O(7)	1.376	1.340	1.279	1.357
H(3)—O(2)	1.084	1.103	1.136	1.082
N(9)—H(3)	2.503	2.484	2.611	2.624
O(7)—C(8)	1.294	1.305	1.294	1.291
Dihedral angle (°)				
C(6)—C(8)—O(7)—C(11)	116.7	−126.3	122.0	−119.6
N(9)—C(5)—C(4)—C(6)	167.7	171.7	−168.2	−166.9
O(7)—C(8)—H(10)—C(11)	137.2	−134.6	138.6	−138.9
O(7)—C(8)—C(6)—C(5)	48.0	28.0	−3.9	−38.7
C(11)—C(8)—C(6)—C(5)	173.1	−104.4	124.3	−168.2

TABLE 4. Main geometric parameters of the four transition states at the level of B3LYP/6-31G, formed in the stereocontrolling step of (*S*)-proline-catalyzed aldol reaction between acetone and 3,3-dimethyl butyric aldehyde**

Selected parameters	*anti*-(*R*)-TS	*anti*-(*S*)-TS	*syn*-(*R*)-TS	*syn*-(*S*)-TS
Bond length (Å)				
C(6)—C(8)	1.908	1.920	1.996	1.954
H(3)—O(7)	1.364	1.290	1.256	1.304
H(3)—O(2)	1.089	1.137	1.146	1.114
N(9)—H(3)	2.497	2.495	2.609	2.626
O(7)—C(8)	1.298	1.304	1.298	1.298
Dihedral angle (°)				
C(6)—C(8)—O(7)—C(11)	114.4	−122.0	118.5	−115.5
N(9)—C(5)—C(4)—C(6)	166.8	170.2	−167.1	−166.3
O(7)—C(8)—H(10)—C(11)	138.5	−138.7	140.8	−140.8
O(7)—C(8)—C(6)—C(5)	49.7	46.0	−18.0	−32.2
C(11)—C(8)—C(6)—C(5)	173.1	−83.1	109.0	−159.1

TABLE 5. The two lowest vibration frequencies (ν_1 and ν_2) of the four transition states in the stereocontrolling step of (*S*)-proline-catalyzed aldol reaction between acetone and three different aldehydes, obtained at the level of B3LYP/6-31G**

	2,2-Dimethyl propyl aldehyde		2-Methyl propyl aldehyde		3,3-Dimethyl butyric aldehyde	
Reaction scenarios	ν_1 (cm^{-1})	ν_2 (cm^{-1})	ν_1 (cm^{-1})	ν_2 (cm^{-1})	ν_1 (cm^{-1})	ν_2 (cm^{-1})
anti-(*R*)-TS	322.95i	46.47	337.20i	41.48	337.66i	18.58
anti-(*S*)-TS	411.63i	43.99	416.71i	54.97	656.50i	29.34
syn-(*R*)-TS	559.91i	36.59	584.50i	33.70	650.34i	32.65
syn-(*S*)-TS	356.93i	31.89	371.14i	31.13	524.05i	27.50

3,3-dimethyl butyric aldehyde, respectively. Also listed are the relative energies of the four TSs in each reaction. Obviously, the relative stabilities of the four TSs in each of the three reaction scenarios are all in the sequence of *anti*-(*R*)-TS > *anti*-(*S*)-TS > *syn*-(*S*)-TS > *syn*-(*R*)-TS at the level of B3LYP/6-31G* in gas phase. B3LYP/6-31G** computations slightly alter the relative stabilities of *anti*-(*S*)-TS and *syn*-(*S*)-TS in the reaction scenario of 2,2-dimethyl propyl aldehyde. Single-point energy calculations with larger basis set of 6-311++G** obviously exchange the order of the relative stabilities of *anti*-(*S*)-TS and *syn*-(*S*)-TS for the reaction scenarios of 2,2-dimethyl propyl aldehyde and 2-methyl propyl aldehyde in gas phase. Solvent effect calculations with PCM model indicate that the least stable *syn*-(*R*)-TS in gas phase become a little bit more stable than *syn*-(*S*)-TS in DMSO for all the three reaction scenarios. Nevertheless, along the *anti* channels, the attack of the active methylene on the *si* face of the aldehyde is favored to the attack on the *re* face. *anti*-(*R*)-TS is the most stable transition isomer either in gas phase or in DMSO solvent in each reaction scenario. As mentioned in the former section, this is mainly caused by the less steric hindrance between the alkyl group of an aldehyde and the methyl group of *anti*-enamine moiety. Although the *syn*-enamine is energetically favorable to the *anti*-one either in gas phase or in DMSO, the C—C bond formation takes place along the *anti* arrangement of enamine. The Curtin–Hammett principle[21] is operative in these proline-catalyzed aldol reactions, that is the C—C bond formation takes place via the more unfavorable anti arrangement of enamine.

As we know, the relative yield of *R* and *S* transition stereoisomers in the stereocontrolling step, mainly controlled by their energy difference, determines the final enantioselectivity of the whole reaction and the absolute configuration of the final product. The larger the energy difference, the higher the enantioselectivity of the reaction. Single point energy computations at the level of B3LYP/6-311++GG**//B3LYP/6-31G** in gas phase show that the most stable *R* and *S* transition isomers for the reaction scenarios of 2,2-dimethyl propyl aldehyde, 2-methyl propyl aldehyde are *anti*-(*R*)-TS and *syn*-(*S*)-TS, respectively. The most stable *S* transition isomer changes to be *anti*-(*S*)-TS in the reaction scenario of 3,3-dimethyl butyric aldehyde. The energy differences of the most stable *S* and *R* transition isomers in the reaction scenarios of 2,2-dimethyl propyl aldehyde, 2-methyl propyl aldehyde, and 3,3-dimethyl butyric aldehyde in gas phase are 17.5, 18.4, and 8.0 kJ/mol at the level of B3LYP/6-311++GG**//B3LYP/6-31G**, respectively.

The solvent effect of DMSO on these reactions is great, stabilizing all the transition isomers more or less. In DMSO, the most stable *R* and *S* transition isomers in the reaction scenario of 2,2-dimethyl propyl aldehyde change to be *anti*-(*R*)-TS and *syn*-(*S*)-TS based on the single-point PCM computations with the diffusion basis set of 6-311++G** (shown in Table 9). PCM computations for the reaction scenarios of 2-methyl propyl aldehyde and 3,3-dimethyl butyric aldehyde show that *anti*-(*R*)-TS and *anti*-(*S*)-TS are the most stable *R* and *S* transition isomers, respectively. The energy differences between the most stable *S* and *R* transition isomers in the reaction scenarios of 2,2-dimethyl propyl aldehyde, 2-methyl propyl aldehyde, and 3,3-dimethyl butyric aldehyde are computed to be 15.4, 15.1, and 8.8 kJ/mol, respectively. Generally speaking,

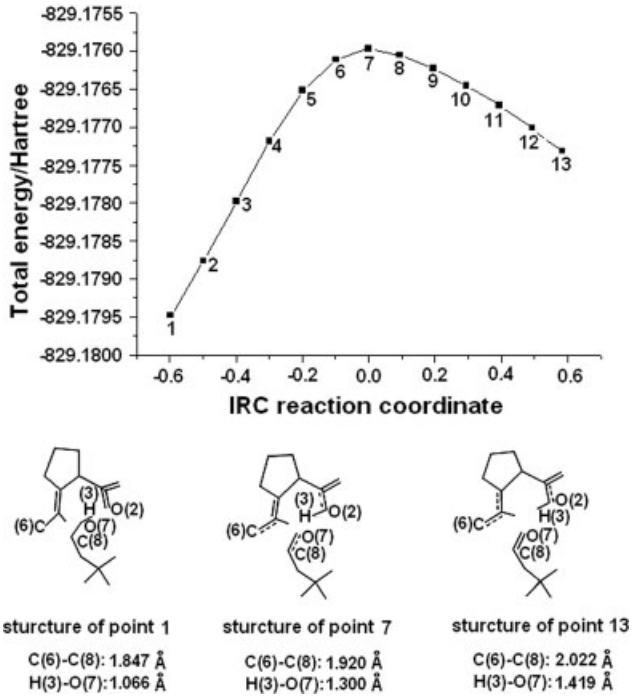


Fig. 5. The result of IRC calculation at the level of B3LYP/6-31G** for *anti*-(*S*)-TS in the stereocontrolling step of (*S*)-proline-catalyzed aldol reaction between acetone and 3,3-dimethyl butyric aldehyde.

TABLE 6. The results of energies (hartree) at various levels of the four TSs in the stereocontrolling step of (*S*)-proline-catalyzed aldol reaction between acetone and 2,2-dimethyl propyl aldehyde

Species	*anti*-(R)-TS	*anti*-(*S*)-TS	*syn*-(R)-TS	*syn*-(*S*)-TS
B3LYP/6-31G*	−789.631541 (0.0)	−789.624379 (18.8)	−789.623044 (22.3)	−789.624069 (19.6)
B3LYP/6-31G**	−789.669526 (0.0)	−789.662704 (17.9)	−789.661365 (21.4)	−789.662118 (19.4)
B3LYP/6-31G** in DMSO	−789.674790 (0.0)	−789.669336 (14.3)	−789.670417 (11.5)	−789.668658 (16.1)
B3LYP/6-311++G**//B3LYP/6-31G**	−789.866530 (0.0)	−789.859095 (19.5)	−789.858772 (20.4)	−789.859859 (17.5)
B3LYP/6-311++G** in DMSO//B3LYP/6-31G**	−789.875110 (0.0)	−789.869167 (15.6)	−789.870441 (12.3)	−789.869228 (15.4)

The data in parentheses represent the results of relative energies (kJ/mol).

TABLE 7. The results of energies (hartree) at various levels of the four TSs in the stereocontrolling step of (*S*)-proline-catalyzed aldol reaction between acetone and 2-methyl propyl aldehyde

Species	*anti*-(*R*)-TS	*anti*-(*S*)-TS	*syn*-(*R*)-TS	*syn*-(*S*)-TS
B3LYP/6-31G*	−746.232521 (0.0)	−746.227321 (17.6)	−746.223214 (23.5)	−746.181324 (20.3)
B3LYP/6-31G**	−750.354413 (0.0)	−750.347637 (17.8)	−750.345389 (23.7)	−750.346719 (20.2)
B3LYP/6-31G** in DMSO	−750.360885 (0.0)	−750.355937 (13.0)	−750.355884 (13.1)	−750.354317 (17.2)
B3LYP/6-311++G**//B3LYP/6-31G**	−750.544079 (0.0)	−750.536548 (19.8)	−750.535720 (21.9)	−750.537057 (18.4)
B3LYP/6-311++G** in DMSO//B3LYP/6-31G**	−750.554065 (0.0)	−750.548300 (15.1)	−750.549070 (13.1)	−750.548128 (15.6)

The data in parentheses represent the results of relative energies (kJ/mol).

the energy difference larger than 10 kJ/mol usually can give quite good enantioselectivity. For the reaction scenarios of 2,2-dimethyl propyl aldehyde and 2-methyl propyl aldehyde, the energy differences of 15.4 and 15.1 kJ/mol reveal that these two reactions would give high enantioselectivity, which is in agreement with the experimental results of 98% and 96% ee, respectively.[3,9] The reaction scenario of 3,3-dimethyl butyric aldehyde only gives 8.8 kJ/mol of this energy difference, which is in agreement with the experimental result of ee being only 68%.[9]

As discussed in the former section, the large energy differences between the most stable *S* and *R* transition structures in the reaction scenarios of 2,2-dimethyl propyl aldehyde and 2-methyl propyl aldehyde are mainly caused by the large differences of the steric hindrances between the methyl group in enamine moiety and the alkyl group of aldehyde. However, such steric hindrance is weak in the reaction scenario of 3,3-dimethyl butyric aldehyde, as for C(11) is secondary carbon.

In each of the three reaction scenarios, *anti*-(*R*)-TS is always the most stable transition structure either in gas phase or in DMSO solvent. This result is consistent with the experimental fact of the absolute configuration *R* of the final product in each of the three reaction scenarios.

TABLE 8. The results of energies (hartree) at various levels of the four TSs in the stereocontrolling step of (*S*)-proline-catalyzed aldol reaction between acetone and 3,3-dimethyl butyric aldehyde

Species	*anti*-(*R*)-TS	*anti*-(*S*)-TS	*syn*-(*R*)-TS	*syn*-(*S*)-TS
B3LYP/6-31G	−828.917182 (0.0)	−828.915146 (5.3)	−828.910949 (16.4)	−828.911305 (15.4)
B3LYP/6-31G**	−829.178574 (0.0)	−829.175961 (6.9)	−829.173015 (14.6)	−829.173417 (13.5)
B3LYP/6-31G** in DMSO	−829.184677 (0.0)	−829.182320 (6.2)	−829.180083 (12.1)	−829.179567 (13.4)
B3LYP/6-311++G**//B3LYP/6-31G**	−829.191640 (0.0)	−829.188609 (8.0)	−829.186008 (14.8)	−829.186316 (14.0)
B3LYP/6-311++G** in DMSO//B3LYP/6-31G**	−829.201164 (0.0)	−829.197831 (8.8)	−829.195019 (16.1)	−829.194521 (17.4)

The data in parentheses represent the results of relative energies (kJ/mol).

TABLE 9. The energy differences ΔE (kJ/mol) between the most stable *S* and *R* transition isomers in the stereocontrolling step of the three reaction scenarios of 2,2-dimethyl propyl aldehyde, 2-methyl propyl aldehyde, and 3,3-dimethyl butyric aldehyde, respectively, computed at the level of B3LYP/6-311++G//B3LYP/6-31G** in DMSO**

Reaction scenario	The most stable *R* transition isomer	The most stable *S* transition isomer	ΔE (kJ/mol)	Exptl. ee%[9]
2,2-Dimethyl propyl aldehyde	*anti*-(*R*)-TS	*syn*-(*S*)-TS	15.4	98 [*R*]
2-Methyl propyl aldehyde	*anti*-(*R*)-TS	*anti*-(*S*)-TS	15.1	96 [*R*]
3,3-Dimethyl butyric aldehyde	*anti*-(*R*)-TS	*anti*-(*S*)-TS	8.8	68 [*R*]

CONCLUSIONS

The transition structures associated with the C—C bond formation steps of the proline-catalyzed intermolecular aldol reactions between acetone and three kinds of aliphatic aldehydes with different α-substituents have been studied using DFT. The reaction of acetone with (*S*)-proline affords an enamine that is in equilibrium between two planar conformers, i.e., *syn*- and *anti*-enamines. The former is energetically preferable. For the stereocontrolling step, TSs in the four reactive channels corresponding to the two diastereoisomeric approach modes to the *si* and *re* faces of the carbonyl group of the aldehyde by the active methylene carbon in *syn*- and *anti*-enamines were studied.

The C(6)—C(8) bond formation and the H(3) proton transfer are concerted process in these TSs. The bond lengths of the newly formed C(6)—C(8) and H(3)—O(7) bonds are 1.9–2.0 Å and 13–1.4 Å, respectively.

The relative stabilities of the four TSs in each of the three reaction scenarios are all in the sequence of *anti*-(*R*)-TS > *anti*-(*S*)-TS > *syn*-(*S*)-TS > *syn*-(*R*)-TS at the level of B3LYP/6-31G* in gas phase. Inclusion of solvent DMSO deduces the energies of all the investigated transition structures more or less. *syn*-(*S*)-TS instead of *anti*-(*S*)-TS becomes the most stable *S* transition isomer in the reaction of 2,2-dimethyl propyl aldehyde. The energy differences between the most stable *S* and *R* transition isomers in the reaction scenarios of 2,2-dimethyl propyl aldehyde, 2-methyl propyl aldehyde, and 3,3-dimethyl butyric aldehyde are 15.4, 15.1, and 8.8 kJ/mol based on the single-point calculations with the diffusion basis set of 6-311++G** in DMSO, respectively. The results revealed the experimental fact that the former two reaction scenarios can give much higher stereoselectivities than the last one, which accords with the experimental results that the ee values of these three reaction scenarios are 98%, 96%, and 68%, respectively.[9]

The large energy differences between the most stable *S* and *R* transition structures in the reaction scenarios of 2,2-dimethyl propyl aldehyde and 2-methyl propyl aldehyde are mainly caused by the large differences of the steric hindrances between the methyl group in enamine moiety and the alkyl group of aldehyde. However, such steric hindrance is weak in the reaction scenario of 3,3-dimethyl butyric aldehyde, as for C(11) is secondary carbon.

anti-(*R*)-TS is always the most stable TS in each of the three reaction scenarios either in gas phase or in DMSO, which accords with the experimental result of the absolute configuration *R* of the final product for each of the three reaction scenarios. The stability of *anti-(R)*-TS is mainly caused by the less steric hindrance between the alkyl group of an aldehyde and the methyl group of *anti*-enamine moiety.

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