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C. H. Lochmuller[a]; P. M. Gross[a]
[a] Chemical Laboratory, Duke University Durham, North Carolina

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GAS CHROMATOGRAPHIC SEPARATION OF ENANTIOMERS

C. H. Lochmüller
P. M. Gross Chemical Laboratory
Duke University
Durham, North Carolina 27706

I. INTRODUCTION

Separation methods for the resolution of "optical isomers" (especially enantiomers) are developed to solve either of two basic problems: 1) the need to obtain, in optically pure" form, one antipode or the other on a scale suitable for further chemical use - i.e. - "preparative" or 2) the analysis of the extent of racemization or "optical resolution" of a mixture of enantiomorphs on a scale sufficient for quantitative analysis - i.e. - "analytical". The most economical, large-scale separation methods in use today are not chromatographic in nature but rather range from such common, general unit operations as recrystallization to very specific biological degradation.[1,2] Chromatographic methods are generally limited to systems in which analytical data is the desired end product or in which only relatively small amounts of resolved material are needed.

Chromatographic methods for optical isomers offer distinct advantages to the analyst. In addition to the generally recognized advantages of speed, large dynamic range etc. normally associated with chromatography, direct methods of enantiomer resolution greatly reduce the potential for interference and the limit of detection common to the conventional optical methods. Despite this fact, the attainment of generalized methods for direct resolution has proven a chimerical chase despite the efforts of many inspired researchers. This paper deals with gas chromatographic methods but recent advances in high-performance liquid chromatographic methods by Cram,[2] Gil-Av,[4] Lochmüller[5] and, particular, Pirkle (6) should not be overlooked.

II. The Problem

The terminology "optical isomer" is not suitable for the discussion of chromatographic methods and will not be used further here. The term enantiomer refers to a molecule which is one of a pair of mirror images distinguished by their chirality or handness. Stereoisomers which are not enantiomorphic (or geometrical isomers) often are refererred to as diastereomers. Therefore any non-enantiomorphic pair of the thirty-two aldohexoses can be considered diastereomers but malic and fumaric acids are not.

The separation of diastereomers is generally straightforward as they need not have (and do not have in the chromatographic sense) similar physical and/or chemical properties. Indeed, they

can be as different as structural isomers. This is explainable in the fact that diastereomers cannot be identical with each other under any circumstances. Enantiomers are identical in all ways except chirality and therefore exhibit identical physical properties in any isotropic medium.

Chromatographic resolution can occur only when the resolution factor α is non-unity.

$$\alpha = \frac{K_2}{K_1} = \frac{\gamma_1 p_1^\circ}{\gamma_1 p_2^\circ}$$

where K partition coeff., activity coefficient and p° saturation vapor pressure of components 1 and 2 resp.

In the case of diastereomers, $p_1^\circ \neq p_2^\circ$ and resolution is possible. As shown by Karger[7] resolution can be enhanced by interaction close to the assymetrically-substituted carbon of an ester but this enhancement disappears if the main interaction [solute-stationary phase] occurs more than one C-C bond remote from this site. Enantiomers resolution is not possible via simple vapor pressure differences since $p_1^\circ = p_2^\circ$. It is therefore necessary to design stationary phases such that $\gamma_1 \neq \gamma_2$ and this can occur only in a phase which is in itself chiral.

II. RESOLUTION BY DIASTEREOMER FORMATION

Gas chromatographic resolution of enantiomers via diastereomer formation requires a chemical reaction of the racemate with a suitable, "optically"-resolved reagent. This reagent should:

1. React rapidly and quantitatively with the racemic substance(s)
2. Form diastereomers of acceptable volatility
3. Be sufficiently different, in the chromatographic sense, from the diastereomers formed as to be cleanly resolved from them.

Much work has been done regarding the selection of appropriate derivatizing agents[8] and has generally been directed towards increasing the chromatographic α value for a given class or subclass of molecules. the commercialization of high resolution (large effective plate number) gas chromatography in the last few years has reduced the importance of these efforts to some extent in so far as analysis is concerned.

the formation of diastereomeric derivatives of racemic compounds is less desirable than direct resolution methods for obvious reasons. First,reactions prior to analysis can,and often do, increase the final error in an analytical scheme. Second, even if the reaction is rapid and quantitative, the automation of the analysis is a more complex problem. Direct methods are, therefore, more desirable from a practical analysis viewpoint and, equally importantly, their development presents a theoretical challenge to separation science.

III. DIRECT METHODS OF RESOLUTION

In principle,the introduction of a chiral stationary phase should be sufficient to permit direct resolution by gas-liquid chromatographic methods. In point of practical fact. the differ-

ence in solution behavior of the two antipodal solutes must be large enough, in the energetic sense, to reduce the plate number requirements to a reasonable one. That is to say, the difference in the standard free - energy change for the solution process must correspond to a reasonable α value ($\Delta(\Delta G^{o})=RT \ln \alpha$). Therefore, a great deal of research has been done to determine the minimum requirements for suc a practical goal to be acheived. The majority of the emphasis has been towards engineering of stationary phase structures to provide the proper interactions. The rest of this review will deal with the historical development of direct methods with emphasis on the principal successes. It is, therefore, a selective, not an encyclopedic document.

A better understanding of the course of development of indirect methods over the last two decades can be achieved best by first examining the model used to plan the successful design of the first stationary phases. The earliest model, still used by some investigators today, was first proposed by Dahlgleish[9]. Dahlgleish observed that certain "optically-active" hydroxyphenylglycine amino acids could be resolved by paper chromatography on optically active cellulose. In fact, the order of decreasing resolution was o >m >p-hydroxyphenylglycine with the last isomer showing no apparent resolution. Treating cellulose as a true adsorbent - i.e. - a surface not a liquid - Dahlgleish explained this variation through the use of a very early enzyme-substrate model originally proposed to explain the production or conversion of specific enantiomorphs. This model assumes that such a

reaction requires three significant points of "attachment" between the substrate and the enzyme in a chiral region of the enzyme substructure. Schematically, the model requires a interaction geometry as shown in Figure 1.

The reader may wish to confirm the fundamental requirement of at least three significant interactions by examining how many fingertip interactions uniquely define a right or left hand in contact with his own right hand.

Unfortunately, early work using this model in chromatography assumed that three interactions implied three strong, directed interactions between functional groups, principally the formation of hydrogen bonds. Working models ignored the fact that a chiral environment could of itself provide chiroselective interactions

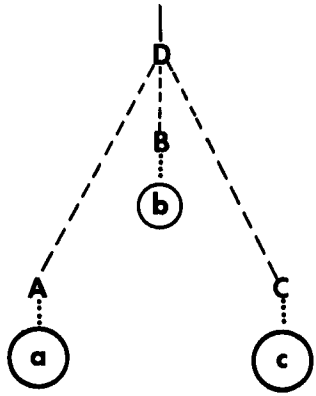

FIGURE 1. The "three point" model. Sites a, b, c, can only interact with their corresponding substrate functionality. Hence only a substrate which has a counter clockwise A B C sense viewed along D axis can completely bind.

in number far greater than three. One therefore finds structures in the literature which require that known conformations and configurations be distorted in order to fit in a possible third interaction or that a solute break a solvent-solvent bond whose heat of formation exceeds that of the new bond to be formed.

The first truly successful report of a direct separation of enantiomers was reported by Gil-Av and coworkers.[10] A number of N-trifluoroacetyl-α-amino acid esters showed varying degrees of resoltuion on a 100 meter glass open tubular column coated with S-(N-tufloroacetyl)isoleucine lauryl ester. Except for analysis (neglecting time), the separations were of little practical value in terms of preparative-scale separation and Gil-Av and Feibush[11] examined a dipeptide phase-S(N-trifluoroacetyl)valyl-S-valine cyclohexyl ester. This phase separated N-trifluoroacetyl alanine t-butyl ester enantiomers on a 2 m packed column. The enhanced resolution was attributed to the formation of an additional hydrogen bond in the solute-solvent molecular complex (see Figure 2).

IV. STUDIES OF THE EFFECT OF STRUCTURE ON SELECTIVITY

The largest number of studies have centered around the three types of stationary phases first produced in the laboratories of Gil-Av and his co-workers. The general structures for these types are shown in Figure. 3. The amino acid ester amides and, in particular the dipeptide ester amides have been the focus

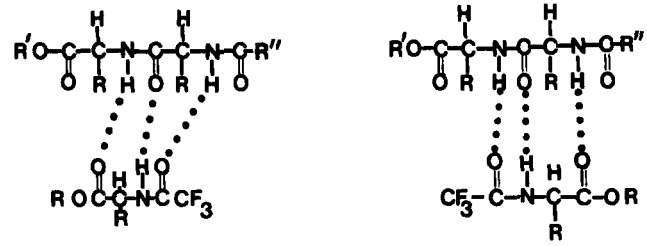

FIGURE 2. Proposed models for H-bond association between N-TFA-aminoacid ester phase molecules and N-TFA-aminoacid ester solutes.

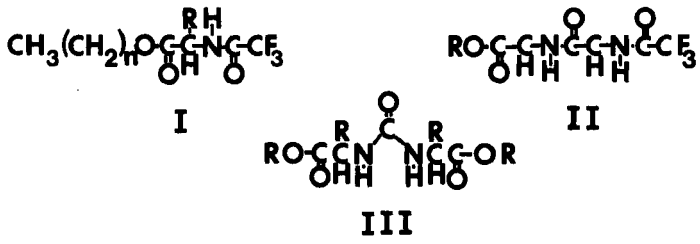

FIGURE 3. Basic structures of the "Gil-Av" phase for direct resolution of chiral amides and amino acid derivatives by gas chromatography.

of much attention as these phase types yield the largest resolution factors and thus provided the greatest practical promise in the initial experiments. The Type III molecules are carbonyl-bis(amino acid esters) and were not the object of much early interest because of their qualitatively poorer resolving power as liquid phases and the low upper-temperature limit they provided. In the earliest studies and in commercial literature these com-

pounds are often and erroneously referred to as "uerides" which, of course, they are not.

The dipeptide phases give progressively greater resolution as the ester function of the solute is changed from primary to secondary to tertiary.[12] Similar, perhaps more subtle effects are seen as the structure of the alkyl substituent at the α position is varied. Substitution at the β position causes a relative loss in resolution factor while substitution the γ position results in the reverse effect. A val-val-val tripeptide phase prepared by Gil-Av and Feibush yielded relatively smaller but useable resolution factors compared to the classic val-val phase.[13] A new phase, N-lauroyl- S-valyl-tert.-butylamide (a Type I phase) was reported by Feibush and, compared to the dipeptides, the new phase showed improved resolution factors, increased thermal stability and better selectivity for a number of N-trifluoro acetyl amino acid methyl esters. Some of the typical resolution factors for this type of phase are shown in Table I.[14]

Gil-Av and his coworkers were the first to postulate that the separation mechanism for these systems involved a "three-point" mechanism involving the formation of hydrogen bonds between the solute and solvent molecules. They felt that the complex thus formed is, in fact, a transient, conformational stereoisomer and the the interaction of an R configuration solute with an S configuration solvent would yield a complex with different physical properties than the corresponding S-S inter-

TABLE I

RESOLUTION FACTORS α (S/R) of N-TFA α-AMINO ACID METHYL ESTERS

Column: 150 ft. x 0.02 in I.D., WCOT Column: N-lauroyl S-valyl- tert.-butylamide at 130°C

Amino acid	α(S/R)
Alanine	1.188
Valine	1.170
O-TFA threonine	1.117
tert.-Leucine	1.084
Alloisoleucine	1.186
Isoleucine	1.159
Leucine	1.280
Proline	1.057
O-TFA serine	1.101
Aspartic acid	1.078
Glutamic acid	1.170
Methionine	1.215
Phenylalanine	1.198
O-TFA tyrosine	1.262

action. The work of Rogers[15] and of Paar[16] provided further information toward an understanding of the molecular basis for these separations.

Structural variation in a variety of dipeptides was examined by Paar,[17] who concluded that the "amide end" of the dipeptide contributes the most to separation in that interactions at that end are critical to resolution. Parr[18] investigated a series of dipeptide phases in which the substituent on the α-carbon of the amino acid residue was systematically varied from methyl to n-butyl with the result that resolution of both the amino acids and their respective enantiomers improved as the net

"bulk" at the α position increased. Parr also demonstrated that increasing the size of the substituent on the solute molecules, holding the dipeptide structure constant, results in a loss of chiral recognition and separation degrades. It is important to recognize two distinct problems in this area of separations: First, the enantiomers must be resolved to a sufficient degree for the experimental requirements. Second, if a complex mixture of amino acids is to be examined, the stationary phase must not only resolve the enantiomers of each acid but also the individual acids must be resolved as well. The fact is that these two goals are not often achieved in practice. The commonly expressed surprise at this result has its origins in the natural tendency to consider the amino acid as a type of homologous series which of course they are not.

Rogers[15] and co-workers have also examined effects of systematic structural variation on the resolving power of the dipeptide phases. These workers sought to demonstrate the importance of the ester vs. amide end in resolution through an interesting approach. They synthesized dipeptide phases using racemic amino ester and resolved amido acids. This reaction yield a mixture of R,S-diastereomer and the S,S-enantiomer (assuming S amidoacid is used). The reasoning was that if the primary interaction of the solute aminoacid ester amide occurred at the ester end of the peptide then on the average a solute would "see" an equal number of right-and left-handed "sites" and no resolution would occur. In fact, resolution is observed with

such phases and is not seen with phases prepared from resolved amino acid ester and racemic amido acid. These authors also report steadily poorer resolution as the peptide side group bulk was increased. A tripeptide phase was examined and gave essentially the same relative retention values as the homologous dipeptide. This was considered significant as it indicated that the ester and amide functions need not be in close proximity for resolution to occur. In addition, phases prepared with N.-pentafluoropropionyl (PFP) amides were seen to have superior performance compared to N-trifluoroacetyl (NTFA) amides.

The question as to the number and location of the significant interactions in these phases has been partially clarified by the spectroscopic work of Lochmüller[19] and the more recent work of Feibush[20] again using NMR methods. Both of these workers rcognized the partial "double bond" character of the amide bond to be an important factor in determining the geometry of the environment around the region in which the proposed conformational, diastereoisomeric association occurs. If one recognizes that the bulk of the evidence indicates that the carbonyl oxygen of a peptide linkage is most like to be trans to the N-H hydrogen, then many of the two-dimensional structures one finds in the literature can be recognized as impossible. In addition, once one recognizes that the normal, self-association of peptides (and ureas) involves a fairly strong hydrogen bond between the peptide functions of adjacent molecules, the likelyhood of three hydrogen bonds in the solute-solvent complex becomes small.

The initial impetus for the preparation of the carbonyl-bis-(amino acid ester) phases was to increase the probability of a three-point interaction around the asymmetrically substituted carbons of the solvent molecules. The classic phase is carbonyl-bis(S-valine isopropyl ester) and was shown to have resolving power for enantiomorphic alkyl and aryl amides. The relative retention values obtained were significantly smaller than those obtained with dipeptide phases and Gil-Av and Feibush[21] did not pursue this direction much further. Rogers and Corbin conducted a systematic study of the chromatographic properties of this phase and reported that when this phase was used below its melting point greatly enhanced separations were obtained. The authors attributed the enhancement of resolution to the increased structural rigidity of the solid phase which causes a more fixed interaction geometry than is available with the liquid phase.[22] The chromatograms reported show anomalous peak shapes in that the leading peak of the racemate is much sharper than the more retained antipode; on the liquid phase the same peaks have essentially equal widths.

Lochmüller *et al.*[19] initially reported NMR studies of the sites of hydrogen bond formation with two carbonyl-bis-(amino acid esters) and various solutes. Association chemical shifts with carbon tetrachloride as the solvent medium showed (by ^{13}C and by ^{1}H NMR) that the only significant hydrogen-bonding interaction occurred between the N-H portion of the amide solute and the ester carbonyl of the carbonyl-bis-(amino acid

ester). This spectroscopic evidence was interpreted to indicate that only one significant point of "attachment" is involved in formation of the diastereomeric association complexes. The effect of substituent changes on the donor or acceptor strength of functional groups is also important in determination of the strength of hydrogen bonding, and in the case of the carbonyl-bis-(amino acid esters) the effect on the ester carbonyl of changing the ester substituent from methyl to ethyl to isopropyl and finally to tert.-butyl was spectroscopically shown to be inductive in nature. The effect of these changes on the α-values is complex, with the α-values rising from methyl to ethyl, remaining nearly constant at isopropyl and then decreasing with tert.-butyl. These variations are shown in Table II. In a series of publications Lochmüller and Souter[23,24,25] demonstrated that carbonyl-bis(S-valine-and S-leucine aminoacid esters) exhibit a liquid-crystalline-line state at temperatures below their "normal" melting point. The nature of the mesophase type was determined by diferential scanning calorimetry as being a unique type of smectogenic state.

Carbonyl-bis-(S-valine isopropyl ester), the compound studied by Corbin and Rogers[22] as a solid stationary phase, exhibits two stable smectic states prior to melting. The chromatographic behavior of this and other closely related stationary phases were studied. Resolution was achieved in all cases on short packed columns, and the resolving power of the

Table II.
VARIATION OF α WITH ESTER SUBSTITUENT STRUCTURE OF STATIONARY PHASE FOR ACYLATED α-METHYLBENZYLAMINE SOLUTES
Stationary phases as isotropic liquids.

Compound	α
Carbonyl-bis-(L-valine methyl ester)	
N-TFA α-methylbenzylamine	1.065
N-PFP α-methylbenzylamine	1.060
N-HFB α-methylbenzylamine	1.066
Carbonyl-bis-(L-valine ethyl ester)	
N-TFA α-methylbenzylamine	1.096
N-PFP α-methylbenzylamine	1.106
N-HFB α-methylbenzylamine	1.105
Carbonyl-bis-(L-valine isopropyl ester)	
N-TFA α-methylbenzylamine	1.099
N-PFP α-methylbenzylamine	1.100
N-HFB α-methylbenzylamine	1.108
Carbonyl-bis-(L-valine tert.-butyl ester)	
N-TFA α-methylbenzylamine	1.017

smectic phases was found generally superior to that of the "liquid" state. Typical results are seen in Table III.

As can be seen in Table III, resolution by the liquid crystalline phase is more sensitive to the nature of the amide function than in the isotropic temperature range and that energetically the difference in the R,S and S,S interaction is enhanced by an order of magnitude. The anomalous lack of resolution of N-TFA-2-amino- 3-phenyl propane has been further examined in a recent report by Lochmüller and Hinshaw.[23] It appears that in the series $C_6H_5(CH_2)_n(CH_3)CHN$-amide, there is a

Table III. RESOLUTION OF ENANTIOMORPHIC SOLUTES ON A.2 METER X 4 MM PACKED COLUMN. CARBONYL-BIS(R-LEUCINE ISOPROPYL ESTER) AS A FUNCTION OF TEMPERATURE.

Racemic Solute	T(°C)	α	Δ(ΔG°) cal/mole
N-TFA-α methyl benzylamine	88.6	1.119	81
B-PFP-α methyl benzylamine	(smectic)	1.658	363
N-HFB-α methyl benzylamine		1.399	241
N-TFA-α methyl benzylamine	122.5	1.073	55
N-PFP-α methyl benzylamine	(isotropic)	1.080	61
N-HFB-α methyl benzylamine		1.079	60
N-TFA-2-amino-3-phenyl propane		unresolved	--
N-TFA-3-amino-4-phenyl butane		1.050	38

Note: TFA, PFP, HFB refer to CF_3CO, CF_3CF_2CO and $CF_3CF_2CF_2CO$, respectively
$\Delta(\Delta G°) = RT \ln\alpha$

pronounced odd- even effect in the capacity factor of the amide solutes of the same absolute configuration as the stationary phase. Plots of log k' vs C# are linear for a homologous series of enantiomorphs of absolute configuration opposite to the stationary phase. The loss of resolution is therefore the result of the unpredicted loss in capacity of odd-n enantiomorphs in this series.

Figure 4. shows the typical anomalous peak shape relation observed in experiments with carbonyl-bis (amino acid ester

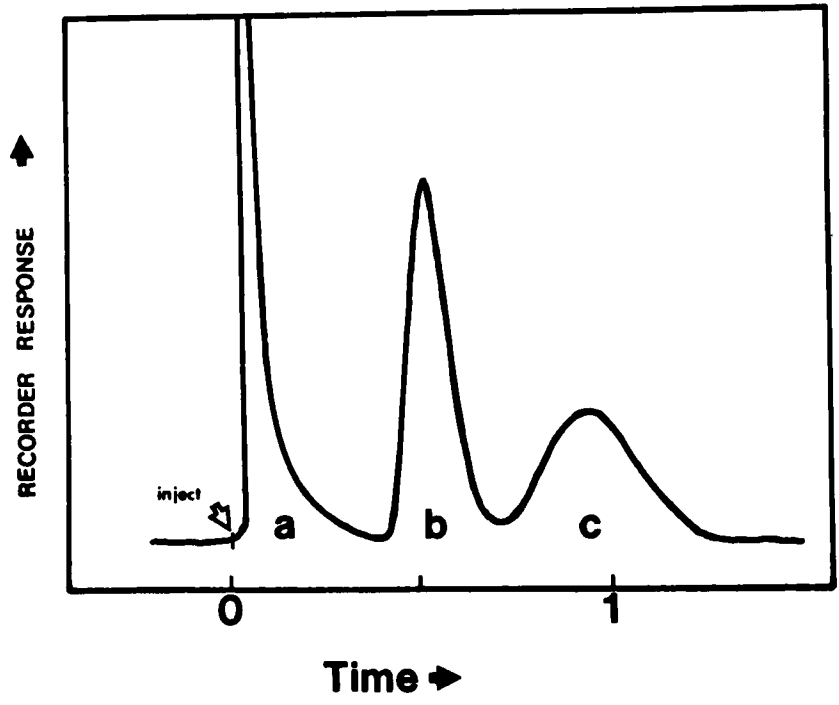

Figure 4. Typical chromatogram obtained in smectogenic region with carbonyl-bis(S-valine isopropyl ester). T= 96.9 C(b,c are peaks for the racemic pair

phases). The relation is anomalous because one does not expect that two solutes, identical in all ways except chirality, with relatively large and nearly identical capacity factors would show such behavior. In all cases, the solute to elute last results in a peak with significantly fewer plates than its antipode; in some cases, as little as one-half the plate number. Since this phenomena must be stationary phase mass transport related, two possible causes can be proposed: 1. That significant liquid surface sorption occurs in these systems and that the magnitude of the relative adsorption/solution contribution to retention has a dependence chirality. In this case, the time constant for desorption could be different than for evaporation from the dissolved state resulting in the apparent difference in mass-transport effects. 2. That the solute-solvent interdiffusion rate of R solutes in an S solvent is significantly different from that of S solutes in an S solvent (by as much as a factor of 4).

Recent work by Lochmüller and Deutsch[27] has examined the extent of liquid surface sorption of solute molecules on smectogenic-and isotropic-state phases. This work demonstrates that for lightly loaded columns as mush as 60% of the net retention may arise from surface sorption. Even in such extreme cases however the α values for the adsorption process (R/S) is unity; that is, resolution depends on the partition mechanism. The observed α value is, therefore, a strong function of film thickness-growing larger as the film thickness increases due to the r^2/r^3 relation of area to volume of phase (since $V_R' = K_S A_S + K_l V_l$ in this case).

V. RECENT ADVANCES

The work discussed so far has dealt with stationary phase types which are themselves low molecular weight species. A recent advance in the laboratories of Bayer[28] has significantly overcome some of the problems associated with the more classic phase types; namely pronounced "bleed", decomposition and consequent short column life. These workers have produced a novel, polysiloxane containing N-propionyl-S-valine tert.-butyl amide side groups. Although these phases exhibit somewhat lower resolution factors than the more common phases containing this function, most protein amino acid enantiomers are resolved in the range between 70 and 240°C. In addition these phases have been shown by Bayer to be useful for the resolution of chiral drugs such as the ephedrines and epinepherines[29] and for the GCMS analysis of these materials and their metabolites.[30] These new phases provide a powerful tool for the deterimination of optical purity, of metabolic racemisation and of configuration of drugs where this is not already known. Table IV. contains some typical results obtained with such phases.

V. LITERATURE SEARCH

In preparing this article a computer-assisted search of the chemical literature was carried out. The following represents the dialogue of the search and is reproduced here for the reader interested in a general approach to the retrieval of specific resolution methods.

Table IV. SEPARATION FACTORS FOR N,O-PFP DERIVATIVES OF THE PARENT COMPOUND

separated on the Bayer phase.[28,29]

Cpd.	R	R'	R"	R"'	Name	Separation factor $\alpha_{L/D}$ at 110°C	at 160°C
(1)	H	C_2H_5	H	OH	Etilefrine (effortil)	1.014	
(2)	H	C_2H_5	H	OCH_3	"Oψ-Methyleffortil"	1.024	
(3) erythro	CH_3	CH_3	H	H	Ephedrine	1.028	
(4)	H	CH_3	OH	OCH_3	Metanephrine	1.031	
(5) erythro	CH_3	CH_3	OH	H	ρ-Hydroxyephedrine (suprifen)	1.040	
(3) threo	CH_3	CH_3	H	H	Pseudophehdrine	1.050	
(5) threo	CH_3	CH_3	OH	H	ρ-Hydroxyephedrine (suprifen)	1.053	
(6)	H	H	H	H	2-Amino-1-phenylethanol	1.059	1.038
(7)	H	CH_3	OH	H	Synephrine		1.014
(8)	H	H	OH	OH	Norephinephrine		1.018
(9)	H	H	H	OH	Norfenefrine		1.027
(10)	H	H	OCH_3	OCH_3	2-Amino-1-(3,4-dimethoxyphenyl)ethanol		1.032
(11)	H	H	H	OCH_3	"Oψ-Methyl-norfenefrine"		1.042
(12) erythro	CH_3	H	H	H	Norephedrine		1.058
(13)		[a]			N-Cyclohexyllactamide	1.102	

[a] Examined as the O-pentafluorpropionyl derivative.

The mechanics of searching will vary somewhat from system to system but the use of key words and logical operators is almost universal. The system used here is DIALOG.

Set	Items	Description	
1	12852	CHROMATOG	
2	309	ENANTIO	
3	9727	OPTICAL	Period: 1977-78
4	5822	ISOMER	
5	215	3AND4	
6	485	2OR5	
7	52	1AND6	
8	28163	GAS	
9	23	7AND8	

Using this strategy one finds the following number of citations containing GAS CHROMATOG ENANTIO/OPTICAL/ ISOMER:

Period	"Hits"
1976-1978	23
1973-1976	46
1970-1973	15

COPIES OF THE TITLES OF THESE ARTICLES ARE AVAILABLE FROM THE AUTHOR UPON REQUEST.

VI. ACKNOWLEDGMENTS

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