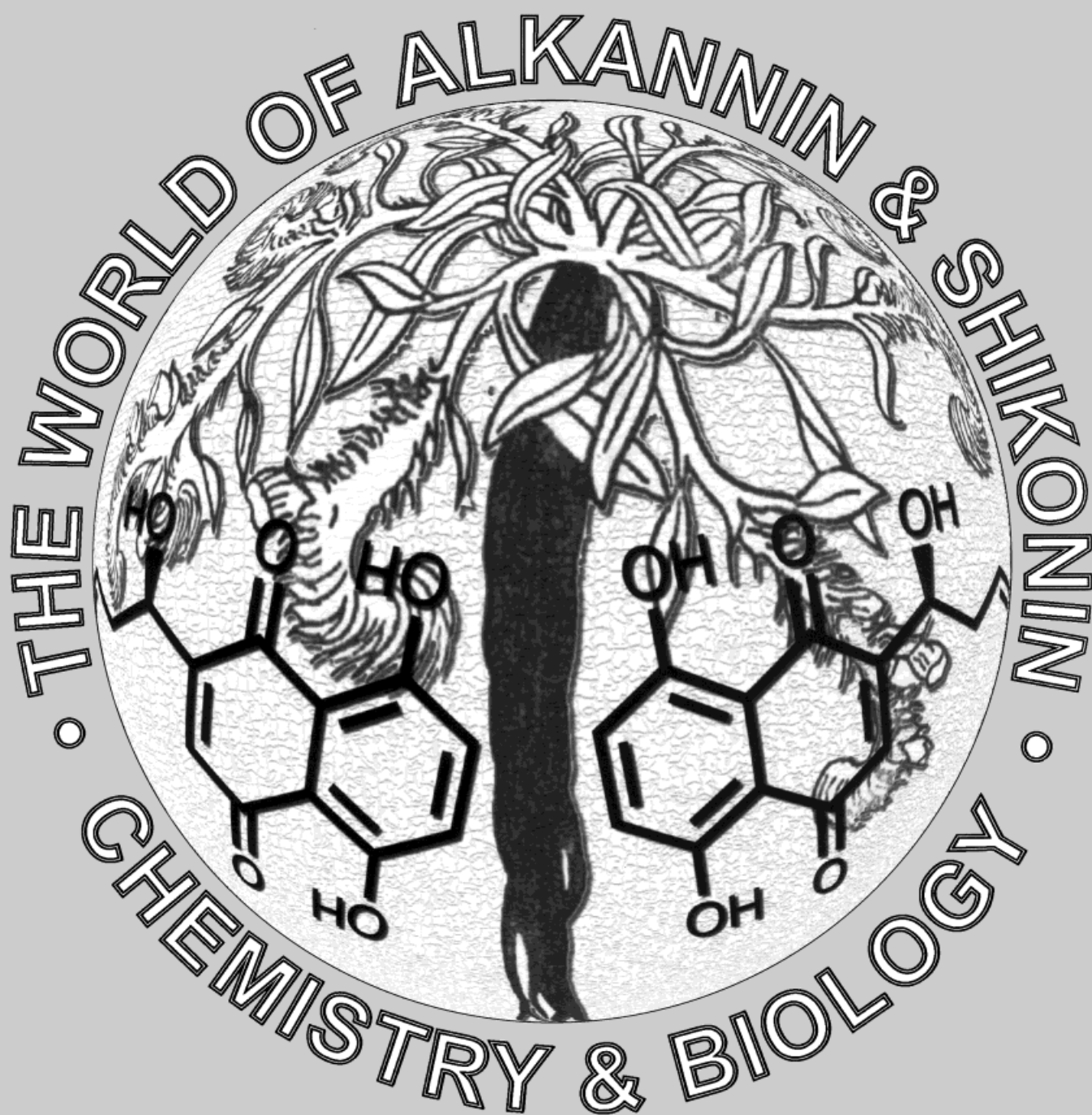




ANGEWANDTE
CHEMIE — © WILEY-VCH

The Chemistry and Biology of Alkannin, Shikonin, and Related Naphthazarin Natural Products**

Vassilios P. Papageorgiou,* Andreana N. Assimopoulou, Elias A. Couladouros, David Hepworth, and K. C. Nicolaou*

There can be few natural products with histories as rich as those of the enantiomeric naphthoquinones alkannin (**1**) and shikonin (**2**). Extracts from the roots of *Alkanna tinctoria* in Europe and *Lithospermum erythrorhizon* in the Orient have been used independently for many centuries as natural red dyes and as crude drugs with the magic property of accelerating wound healing. It was only in 1935 that the major constituents of these extracts, **1** and **2**, were correctly identified as

enantiomers, and in 1976 that the wound healing properties of alkannin (**1**) were clinically demonstrated. Today shikonin (**2**) has a high value both for its medicinal and coloring properties, especially in Japan. With synthetic chemists unable, until recently, to devise an efficient synthetic route, and sources from the plants unable to meet demands, it was left to biotechnologists to develop the first commercial plant-cell culture manufacturing process. In Europe, alkannin derivatives are the

active principles of a pharmaceutical ointment used for the treatment of ulcers and burns. This review is an attempt to tell the “whole story” of **1** and **2**, from the 5th century BC and presents up to date aspects of plant biology, pharmacology, and bioorganic, synthetic, and medicinal chemistry.

Keywords: bioorganic chemistry • natural products • phytochemistry • quinones • wound healing

1. Historical Introduction

The story of the enantiomeric naphthoquinone natural products alkannin (**1**) and shikonin (**2**; Figure 1) can be traced back many centuries. In fact, the story comprises two sub-

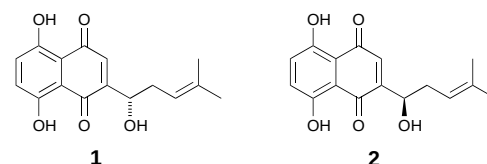


Figure 1. Structures of alkannin (**1**) and shikonin (**2**).

[*] Prof. V. P. Papageorgiou, A. N. Assimopoulou
Organic Chemistry Laboratory, Chemical Engineering Department
Aristotle University of Thessaloniki, 54006 Thessaloniki (Greece)
Fax: (+30)31-996252
E-mail: vaspap@eng.anth.gr
Prof. K. C. Nicolaou, Dr. D. Hepworth
Department of Chemistry and
The Skaggs Institute for Chemical Biology
The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, California 92037 (USA)
Fax: (+1)619-784-2469
E-mail: kcn@scripps.edu
and

Department of Chemistry and Biochemistry
University of California, San Diego
9500 Gilman Drive, La Jolla, California 92093 (USA)

Prof. E. A. Couladouros
Chemical Laboratories
Agricultural University of Athens, Iera Odos 75, 118.55 (Greece)
and
Organic and Bio-organic Laboratories, NCSR “DEMOKRITOS”
153.10 Ag. Paraskevi Attikis, POB 60228 (Greece)

[**] A list of abbreviations not defined in the main text can be found at the end of the article.

plots that have run remarkably parallel courses in Europe and the Orient, being united only in the 20th century.

In Europe, the *S* enantiomer, alkannin (**1**, Figures 1 and 2), is found in the roots of the plant *Alkanna tinctoria* TAUSCH (*AT*) (Figure 3a), also known as *Anchusa tinctoria*, and in common English as alkanet.^[1] The compound is a major component of the deep red pigments that are easily extracted from the roots of the plant.^[2] It was probably the highly colored nature of alkannin that first captured the attention of botanists. Its use as a dyestuff for fabrics almost certainly dates back centuries BC.^[3] Perhaps the first recorded use of *AT* roots is found in the works of the Greek doctor and philosopher Hippocrates (4th and 5th centuries BC) who described their use for the treatment of ulcers.^[4] The botanist and scholar Theophrastus (3rd and 4th centuries BC) also alluded to their application as dyes and medicines.^[5] Alexander the Great was known to have employed doctors trained in herbal medicine,^[6] and it thus seems likely that the medicinal

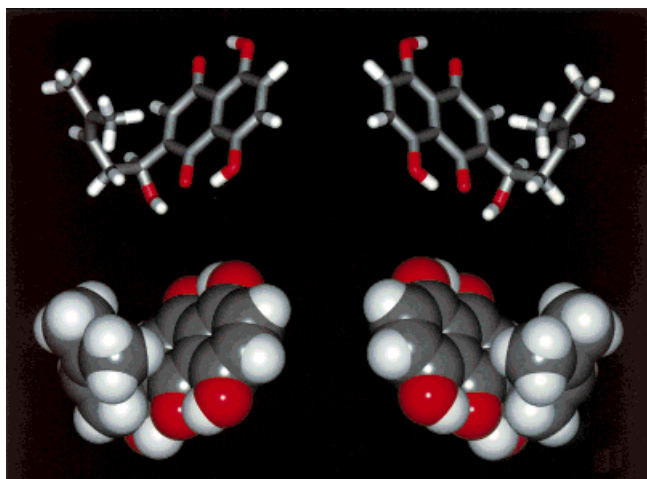


Figure 2. Computer-generated molecular models of alkannin (1) (left) and shikonin (2) (right). Color code: gray: carbon; white: hydrogen; red: oxygen.

properties of *AT* root were used to treat the sick and injured during his campaigns. Dioscorides (Figure 4), who many consider to be the founder of pharmacognosy, described the properties in more detail in his *De Materia Medica* around 77 AD (Figure 5).^[7]

While discussing the properties of the related species *Anchusa eteria*, Dioscorides goes on to describe rather more bizarre effects:^[8] “.ye roots red,.having something like blood in them. But ye faculty of it, and ye leaves is to help ye venemous-beast-bitten, especially ye viper-bitten, being eaten or drank or hanged about one. And if one having chewed it, do spitt it out into ye mouth of ye venemous beast, it will kill him.”

Despite this blatant detour into fiction, the wound healing properties of *AT* have been reported by others. Pliny, a Roman herbalist contemporary with Dioscorides, independently made very similar claims for the medicinal properties of *AT*:^[9] “.It stains the hands the colour of blood; it is used for imparting rich colours to wool. Applied with cerate it heals



V. P. Papageorgiou



A. N. Assimopoulou



E. A. Couladouros



D. Hepworth



K. C. Nicolaou

Vassilios P. Papageorgiou was born in 1942 in Greece and received his Ph.D. and D.Sc. degree from the Aristotle University of Thessaloniki working on the isolation and structure elucidation of wound-healing agents of plant origin. After postdoctoral work at the Pharmacy School, University of Kentucky, he was elected Professor of Organic Chemistry and then Head of the Department of Chemical Engineering at the University of Thessaloniki. He is currently Dean at the School of Engineering. His research interests include the isolation and study of biologically active molecules of plant origin and the development of novel wound-healing agents.

Andreana N. Assimopoulou was born in Greece in 1974. She received her B.Sc. in Chemical Engineering in 1997 from the Aristotle University of Thessaloniki. She is currently a postgraduate student in the Papageorgiou group.

Elias A. Couladouros, born in 1956 in Greece, received his B.Sc. from the University of Athens and his Ph. D. from the Agricultural University of Athens under the direction of Professor M. P. Georgiadis. After a postdoctoral stint in the Nicolaou group at the University of Pennsylvania (1988–89), he joined the faculty of the Agricultural University of Athens, where he is currently Associate Professor of Chemistry. He is also a Visiting Professor at the research center “Demokritos” in Athens. His research interests focus on new synthetic methods and the total synthesis of natural products.

David Hepworth, born in 1970 in England, studied Chemistry at the University of Oxford. He completed his D.Phil. under the supervision of Prof. S. G. Davies in 1996, working on asymmetric synthesis with organochromium chemistry and chiral hydroxylamines. Since joining the Nicolaou group in 1997, he has completed a synthesis of alkannin and shikonin, and worked in the epothilone team.

K. C. Nicolaou is the Chairman of the Department of Chemistry at The Scripps Research Institute, La Jolla, California where he holds the Darlene Shiley Chair in Chemistry and the Skaggs Professorship of Chemical Biology. He is also Professor of Chemistry at the University of California, San Diego.

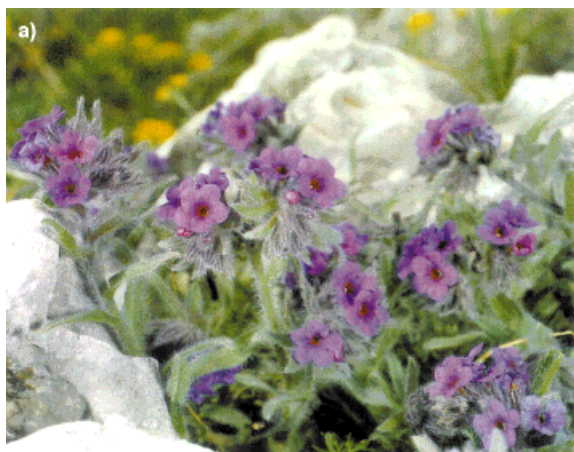


Figure 3. The plants a) *Alkanna tinctoria* TAUSCH, a source of alkannin (1), and b) *Lithospermum erythrorhizon* SIEB. ET ZUCC., a common source of shikonin (2) (courtesy of Medpharm Scientific Publications (a) and Kyoto Pharmaceutical University (b)).

ulcerous sores, those of the aged in particular: it is employed also for the cure of burns.”

For centuries Dioscorides *De Materia Medica* remained one of the standard botanical texts in Europe, with many other herbals being developed from it. Thus, the properties of alkanet became known across Europe, and possibly even further afield.^[10] The English “gentleman student of Physick and astrology” Nicolas Culpeper, from the 17th century AD, stated that alkanet helps to cure “old ulcers, hot inflammations, and burning by common fire”.^[11] Furthermore, the medical doctor William Salmon described in *English Herbs* (ca. 1710),^[12] a formula for: “...deep wounds and punctures of the nerves made with thrusts, stabs or pricking with any pointed weapon.” The recipe is as follows: “Olive oil 2lbs., Alkanet Root 3 or 4 ounces, Earthworms cleaned and purged number 40. Boil them together, then strain out whilst hot and keep it close for use.”



Figure 4. The founder of pharmacognosy Dioscorides collecting botanical samples (courtesy of Parke Davis).

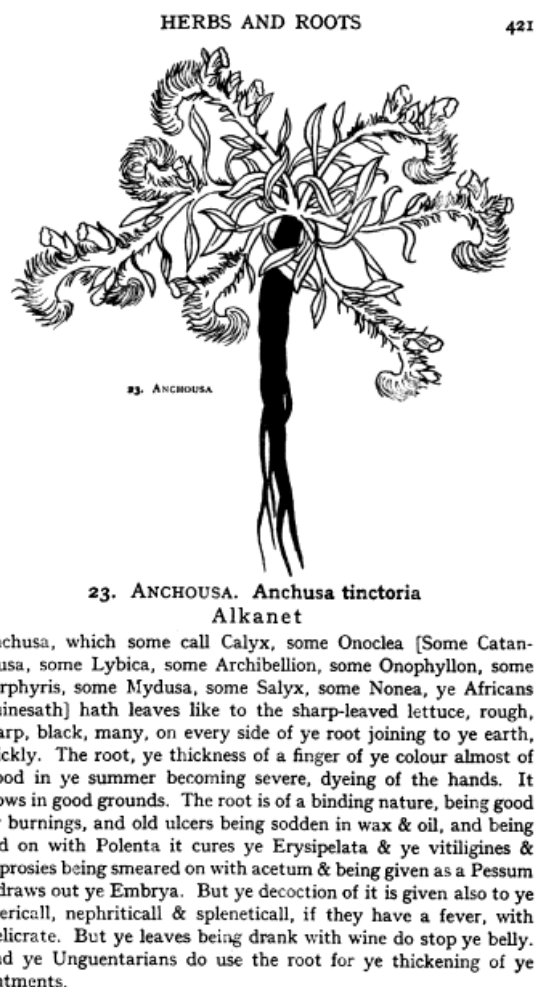


Figure 5. English translation of a passage from Dioscorides' *De Materia Medica*.

Since then, however, the medicinal properties of the plant seem either to have drifted into folklore, or been forgotten. In fact, many modern accounts of herbal medicine describe the roots as being; “rarely used for medicinal purposes”,^[12] “not widely available”,^[13] and as having “no medicinal importance”.^[14] However, alkannin (**1**) is still listed in the *Merck Index* as an astringent.^[15]

Nevertheless, in 1976 Papageorgiou described the results of experiments, which confirmed the wound healing properties and antimicrobial properties of *AT* root extracts (Sections 5.1 and 5.3, respectively) and was the first to identify alkannin derivatives as the active components. More recently, such compounds have also been shown to exhibit significant antitumor, antibacterial, and anti-inflammatory activities. These aspects will be dealt with in Section 5.

The tale of shikonin (**2**), the *R* enantiomer, is set in the Orient, originally in China. This molecule is the major constituent of the red pigment extracts from the roots of the plant *Lithospermum erythrorhizon* SIEB. ET ZUCC. (*LE*) (Figure 6), known in Chinese by various names including *tzu ts'ao*, *tzu-ken* (purple root), and *hung-tzu ken* (reddish-purple root). The use of **2** for dyeing silk probably dates back as far as



Figure 6. Roots of the plant *Lithospermum erythrorhizon* SIEB. ET ZUCC. (courtesy of Kyoto Pharmaceutical University).

the use of **1** in Europe. Its application to traditional Chinese medicine may have originated with the great surgeon Hua To (born ca. 136–141 AD), who is considered to be among the first to have used crude drugs such as antiseptics and anti-inflammatory ointments.^[16] Its use can be traced back with certainty to the latter days of the Ming dynasty, as it is included in the classic compilation of traditional Chinese medicine *Pen Ts'ao Kang Mu*, which was written in 1596 AD.^[17]

The development of Chinese medicine is considered to be a process of constant distillation and selection of all that has gone before, rather than the linear path taken by Western medicine, whereby recent developments are built upon by others, and what has gone before becomes assumed or forgotten. It is thus testimony to the medicinal properties of *LE* roots that they are still included in compilations of ingredients for traditional Chinese and other oriental medi-

cines.^[18] The treatable indications claimed for *LE* roots include: burns, anal ulcers, hemorrhoids, infected crusts, bedsores, external wounds, and oozing dermatitis. These are notably similar to the medicinal properties claimed independently for *AT* in many of the ancient herbal texts. Various preparations that contain **2** and its derivatives are still used today for medicinal purposes in China, Japan, and Korea. In addition, they are also used in Japan as cosmetics^[19] and dyestuffs.^[20] As with alkannin (**1**), the medicinal properties claimed for shikonin (**2**) have more recently been tested scientifically. The interesting findings of these experiments will also be described in Section 5.

In Europe and North America, **1** is mainly used today as a pigment for food coloring^[13, 21] and cosmetics.^[13, 22] Worldwide, however, there has been extensive scientific research concerning these natural products in many areas throughout the disciplines of chemistry and biology. This review is an attempt to bring together material from over 400 articles and patents that have been written in this field, most of which have appeared since the first review in 1980.^[23]

2. Isolation and Identification of Alkannin and Shikonin

Shikonin (**2**) was first isolated as its acetate (**15**) from the roots of *LE* by the Japanese chemists Majima and Kuroda in 1922.^[24] These workers determined many of the chemical properties of **2**, and noting its physical and chemical similarities to the known compound naphthazarin (Section 4.1), proposed structure **3** (Figure 7). Unfortunately, they failed to

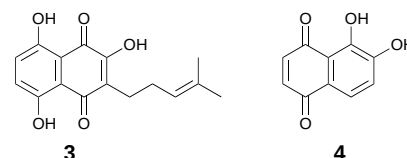


Figure 7. Wrongly assigned structures for shikonin (**3**) and naphthazarin (**4**).^[24]

realize that **2** was optically active, and were probably further confused by the widely accepted, but incorrect, structural assignment for naphthazarin (**64**) as 5,6-dihydroxy-1,4-naphthoquinone (**4**). Brockmann reinvestigated **2** after the structure of naphthazarin (**64**) was correctly revised to 5,8-dihydroxy-1,4-naphthoquinone.^[1] He was the first to identify alkannin (**1**) and shikonin (**2**) as enantiomers, to correctly assign their structures, and to finally unite the stories of these natural products, which had for so long run in parallel. The absolute configurations of **1** and **2** were later determined by degradation to malic acid.^[25]

Brockmann found that the specific rotations of **1** and **2** were not of identical magnitude, but that the values for **1** were generally greater than those for **2**. He suggested that this was a consequence of the presence of a quantity of the racemate, which he termed shikalkin (**1/2**), in the extracts of *LE*. More recently, the ratio of enantiomers in various sources of alkannin (**1**) and shikonin (**2**) have been examined by circular

dichroism^[26] and chiral stationary-phase HPLC.^[27] Interestingly, it was found that the ratio of **1**:**2** varies with the species of plant from which it was extracted.^[26] In this respect, **1** and **2** may be unique among natural products.

In addition to being found in the plants *Alkanna tinctoria* and *Lithospermum erythrorhizon*, **1**, **2**, and related compounds have been isolated from many other *Boraginaceae*. Table 1 gives an up-to-date compilation of selected natural products in the alkannin and shikonin class, together with their occurrence and the biological activities investigated for each.

3. Production of Alkannin and Shikonin

For organic chemists uninitiated in the chemistry of quinones, the structures of alkannin (**1**) and shikonin (**2**) may look misleadingly simple. However, in spite of great efforts over many years by several research groups worldwide, a much needed viable synthetic route to these enantiomers has remained elusive until very recently (Section 4.3.4).

At present we have to rely upon nature, the master organic chemist, for commercial sources of shikonin (**2**). The attempted cultivation of *LE* plants in Japan failed to meet the demand for this valuable natural product. Plants must grow for five to seven years before the shikonin concentration in their roots reaches one to two percent. Formerly, the entire supply of **2** in Japan had to be imported from China and Korea. These difficulties of supply motivated research into producing **2** from plant cell cultures. The fruits of these studies are a highly effective commercial production method for **2**, together with a wealth of information concerning the biosynthesis of **2** and related molecules.

3.1. Production of Alkannin and Shikonin from Cell Tissue Cultures

The first successful production of shikonin (**2**) and a range of ester derivatives from callus cultures was achieved by Tabata and co-workers in 1974.^[91] Since the natural product accumulates only in cork layers in the roots of the plant, they reasoned that the biosynthesis may be triggered by certain environmental factors associated with the production of cork cells.

The culturing of callus tissues, originally derived from germinating *LE* seeds, could be initiated on a Linsmaier–Skoog (LS) medium^[92] that contained kinetin (10^{-6} M) and 2,4-dichlorophenoxyacetic acid (10^{-6} M), but these cultures failed to produce any naphthoquinone pigments. However, transfer of these callus tissues after three months to a culture medium that contained the auxin 3-indoleacetic acid (IAA) stimulated pigment production. It was necessary to conduct the whole tissue culture in the dark for successful naphthoquinone pigment formation, which is consistent with the location of the natural products in the plant roots, and in line with the authors original hypothesis. The possibility that failure of **2** to accumulate in cells illuminated with white light was a result of decomposition of the photosensitive product was ruled out.

When the cells were illuminated only in the period prior to the triggering of pigment formation with IAA, complete inhibition of shikonin production was still observed. In fact, it was found that light disrupts one of the key enzymes in the biosynthetic route (Section 3.2).

Further experimentation identified additional controlling factors in the biosynthesis of **1** and **2**. While pigment formation occurred when callus cultures were grown in LS agar medium, the process failed completely when conducted in a LS liquid medium in the absence of agar. It was suggested that this effect arose from a triggering of the secondary metabolism by a polysaccharide present in the agar. This hypothesis has been supported by experimentation.^[93] More significantly, upon transfer of cells initially cultured in LS liquid medium to the specially developed production medium M9, which contains nitrate ions as the source of nitrogen in place of ammonium ions (which have been shown to inhibit shikonin biosynthesis), the cultures started to produce large quantities of shikonin.^[94] This medium also contains Cu^{2+} ions, which activate the biosynthesis.^[94] Polysaccharides, similar to those identified in agar that activate secondary metabolism, have been isolated from these shikonin-producing cells cultured in the M9 medium.^[95] Gibberelin A_3 deactivates shikonin biosynthesis even at concentrations as low as 10^{-7} M without affecting cell growth. It was suggested that this may be one of the important endogenous regulators of the biosynthesis.^[96] Furthermore, glutamine has been shown to inhibit pigment formation,^[97] while activated charcoal promotes it.^[98]

Further improvements in shikonin production have been made by selection of the most active variant cell lines with improved stability, a process assisted by the brightly colored nature of the natural product,^[99] and by modification of the culture medium.^[100]

These advances have enabled the efficient commercial production of **1** and **2** by the Mitsui Petrochemical Company.^[101, 102] Remarkably, **2** can be produced in upwards of 4 g L^{-1} of liquid culture by this method. This process was the world's first commercial application of plant tissue culture for the production of a secondary metabolite, and remains as one of the few that have been operated on a commercial scale. It has, consequently, been covered in many reviews,^[99c, 103] hence its rather brief coverage here.

The great success of this method has prompted investigations into shikonin (**2**) and alkannin (**1**) production by culturing cells of many other *Boraginaceae* species, which were met with varying degrees of success.^[26, 104–107] Improvement of the culture process with *LE* cells is still the subject of intense research and many publications appear each year in this area.^[108]

3.2. Biosynthesis of Alkannin and Shikonin

The extensive research into the production of **1** and **2** from cell cultures has facilitated the elucidation of several stages in the biosynthesis of these and related natural products.^[109] The generally accepted biosynthetic route is shown in Scheme 1. The two key precursors are 4-hydroxybenzoic acid (PHB, **45**)

Table 1. Selected members of the alkannin/shikonin family of natural products.^[a]

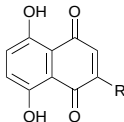
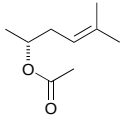
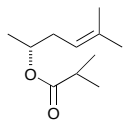
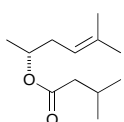
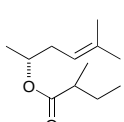
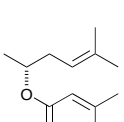
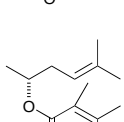
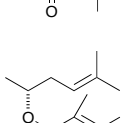
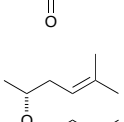
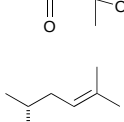
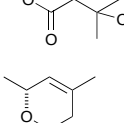
Com-pound	R	Name	Occurrence and biological properties
1		alkannin (also arnebin-4)	roots: <i>Alkanna tinctoria</i> , ^[1, 2, 28–31, 159] <i>Arnebia hispidissima</i> , ^[2, 32, 33] <i>A. nobilis</i> , ^[171] <i>A. tinctoria</i> , ^[34] <i>Macrotomia cephalotes</i> , ^[35] <i>M. euchroma</i> , ^[36] <i>Onosma echioides</i> , ^[37] <i>O. paniculata</i> , ^[105g] <i>Plagiobotrys arizonicus</i> . ^[26] cell cultures: <i>Alkanna tinctoria</i> , ^[104b] <i>Echium lycopsis</i> . ^[26] biological activity: wound healing (Section 5.1), antiinflammatory (Section 5.1), antibacterial (Section 5.3), inhibition of topoisomerase-I (Section 5.2), antithrombotic (Section 5.4).
5		acetylalkannin or arnebin-3	roots: <i>Alkanna tinctoria</i> , ^[38, 241a] <i>Arnebia euchroma</i> , ^[44] <i>A. hispidissima</i> , ^[33] <i>A. nobilis</i> , ^[171] <i>Macrotomia cephalotes</i> . ^[35, 39] cell cultures: <i>Alkanna tinctoria</i> , ^[104a] <i>Echium lycopsis</i> , ^[26] <i>Onosma paniculatum</i> . ^[105g] biological activity: antimicrobial (Section 5.3), inhibition of topoisomerase-I (Section 5.2), antithrombotic (Section 5.4), antitumor (Section 5.2).
6		isobutyrylalkannin	roots: <i>Alkanna tinctoria</i> , ^[40] cell cultures: <i>Echium lycopsis</i> . ^[26]
7		isovalerylalkannin	roots: <i>Alkanna tinctoria</i> , ^[38, 47, 202] <i>Arnebia hispidissima</i> , ^[33] <i>A. tinctoria</i> , ^[34] <i>Macrotomia cephalotes</i> , ^[35, 39] <i>Onosma heterophylla</i> . ^[41] cell cultures: <i>Echium lycopsis</i> . ^[26] biological activity: inhibition of topoisomerase-I (Section 5.2).
8		α -methylbutyrylalkannin	roots: <i>Alkanna tinctoria</i> , ^[38] <i>Macrotomia cephalotes</i> . ^[35, 39] biological activity: antimicrobial (Section 5.3).
9		β,β -dimethylacrylalkannin or arnebin-1	roots: <i>Alkanna tinctoria</i> , ^[159] <i>Arnebia euchroma</i> , ^[42–45] <i>A. gutatta</i> , ^[42] <i>A. nobilis</i> , ^[171] <i>Lithospermum erythrorhizon</i> , ^[42] <i>Macrotomia cephalotes</i> , ^[39] <i>Onosma heterophylla</i> , ^[41] <i>O. hookeri</i> , ^[42] <i>O. paniculata</i> . ^[42] cell cultures: <i>Alkanna tinctoria</i> , ^[104a] <i>Echium lycopsis</i> , ^[26] <i>Onosma paniculatum</i> . ^[105g] biological activity: inhibition of topoisomerase-I and anticancer (Section 5.2), antimicrobial (Section 5.3), antithrombotic (Section 5.4), antiinflammatory (Section 5.1).
10		teracrylalkannin	roots: <i>Arnebia densiflora</i> . ^[46] biological activity: antimicrobial (Section 5.3).
11		angelylalkannin	roots: <i>Alkanna tinctoria</i> . ^[30, 38, 47, 202]
12		β -hydroxyisovalerylalkannin	roots: <i>Arnebia euchroma</i> , ^[42, 44] <i>A. hispidissima</i> , ^[33] <i>Macrotomia cephalotes</i> . ^[35] cell cultures: <i>Echium lycopsis</i> . ^[26] biological activity: antimicrobial (Section 5.3).
13		β -acetoxyisovalerylalkannin	roots: <i>Alkanna tinctoria</i> , ^[203] <i>Arnebia euchroma</i> , ^[42–44] <i>Moltkiopsis ciliata</i> , ^[85] <i>Onosma heterophylla</i> . ^[41] cell cultures: <i>Onosma paniculatum</i> . ^[105g] biological activity: antimicrobial (Section 5.3).
14		unnamed	roots: <i>Cynoglossum officinale</i> , ^[36] <i>Eritrichium incanum</i> , ^[36] <i>E. sichotense</i> , ^[36] <i>Lappula con-sanguinea</i> , ^[36] <i>Lappula echinata</i> , ^[36] <i>Mertensia maritima</i> . ^[36]

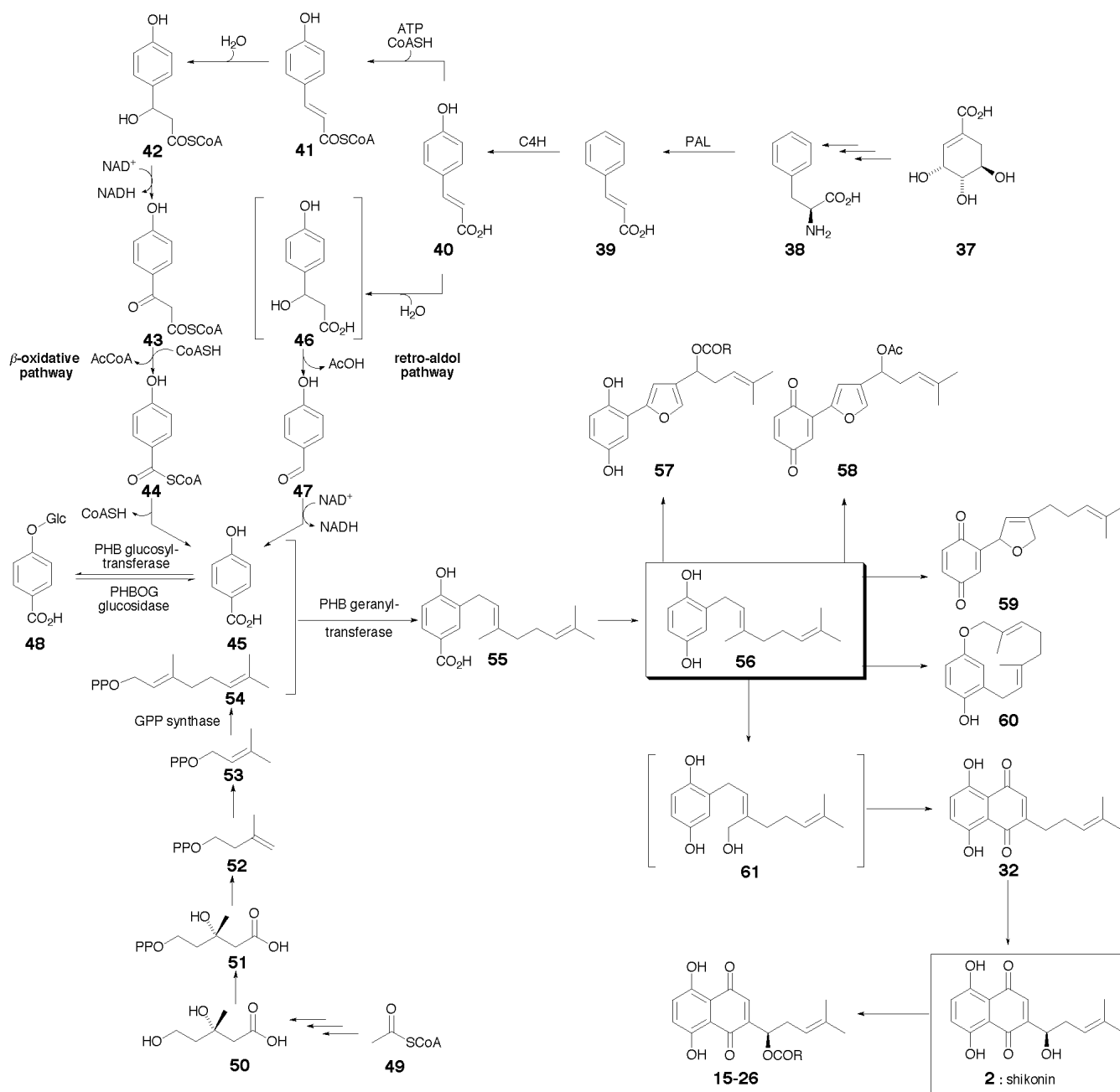
Table 1. (Continued)

Com- pound	R	Name	Occurrence and biological properties
2		shikonin	roots: <i>Arnebia euchroma</i>,^[42–44, 48, 49] <i>A. hispidissima</i>,^[32] <i>A. guttata</i>,^[49, 50] <i>A. tibetiana</i>,^[51–53] <i>Cynoglossum officinale</i>,^[36] <i>Echium lycopsis</i>,^[54] <i>E. rubrum</i>,^[55, 69] <i>E. vulgare</i>,^[36] <i>Eritrichium incanum</i>,^[36] <i>E. sichotenze</i>,^[36] <i>Jatropha glandulifera</i>,^[56] <i>Lappula consanguinea</i>,^[36] <i>L. echinata</i>,^[36] <i>Lithospermum erythrorhizon</i>,^[48, 57–61, 77] <i>L. officinale</i>,^[58–62] <i>Macrotomia echinoides</i>,^[63] <i>M. ugamensis</i>,^[52, 53, 64] <i>M. euchroma</i>,^[36, 65, 66] <i>Mertensia maritima</i>,^[36] <i>Onosma caucasicum</i>,^[52, 55] <i>O. confertum</i>,^[78] <i>O. hookeri</i>,^[42] <i>O. livanovii</i>,^[63] <i>O. polyphyllum</i>,^[67] <i>O. tauricum</i>,^[68] <i>O. sericum</i>,^[63] <i>O. setosum</i>,^[63] <i>O. visianii</i>,^[69] <i>O. zerizaminium</i>.^[52–53, 70] cell cultures: <i>Arnebia euchroma</i>,^[107e,f] <i>Echium lycopsis</i>,^[26] <i>Lithospermum erythrorhizon</i>,^[91, 99a] <i>Onosma paniculatum</i>.^[105] biological effects: antitumor (Section 5.2), antiamebic (Section 5.3), antipyretic and analgesic (Section 5.1), antifungal and antibacterial (Section 5.3), wound healing (Section 5.1), chemopreventive (Section 5.2), antiinflammatory (Section 5.1), inhibition of topoisomerase-II (Section 5.2), inhibition of microsomal monooxygenase,^[71] stimulation of peroxidase,^[72] protection from uv-radiation,^[73] inhibition of testosterone-α-reductase,^[19f] induction and secretion of nerve growth factor.^[74]
15		acetylshikonin	roots: <i>Arnebia decumbens</i>,^[75] <i>A. euchroma</i>,^[36, 42–44, 49] <i>A. guttata</i>,^[42, 49, 50] <i>Cynoglossum officinale</i>,^[36] <i>Echium vulgare</i>,^[36] <i>Eritrichium incanum</i>,^[36] <i>E. sichotenze</i>,^[36] <i>Jatropha glandulifera</i>,^[56] <i>Lappula consanguinea</i>,^[36] <i>L. echinata</i>,^[36] <i>Lithospermum arvense</i>,^[76] <i>L. erythrorhizon</i>,^[42, 57, 77] <i>Mertensia maritima</i>,^[36] <i>Onosma confertum</i>,^[78] <i>O. hookeri</i>,^[42] <i>O. paniculatum</i>,^[42] cell cultures: <i>Arnebia euchroma</i>,^[107e,f] <i>Echium lycopsis</i>,^[26] <i>L. erythrorhizon</i>,^[91, 99a] <i>O. echinoides</i>.^[106]
16		1-methoxyacetylshikonin	roots: <i>Arnebia euchroma</i> . ^[44]
17		propionylshikonin	cell cultures: <i>Lithospermum erythrorhizon</i> . ^[79]
18		isobutyrylshikonin	roots: <i>Cynoglossum officinale</i>,^[36] <i>Echium vulgare</i>,^[36] <i>Eritrichium sichotenze</i>,^[36] <i>Lappula consanguinea</i>,^[36] <i>L. echinata</i>,^[36] <i>Lithospermum arvense</i>,^[76] <i>L. erythrorhizon</i>,^[57, 80] <i>Macrotomia euchroma</i>,^[36] <i>Mertensia maritima</i>.^[36] cell cultures: <i>Arnebia euchroma</i>,^[107f] <i>Echium lycopsis</i>,^[26] <i>Lithospermum erythrorhizon</i>.^[91, 99a]
19		isovalerylshikonin	roots: <i>Arnebia decumbens</i>,^[81] <i>Cynoglossum officinale</i>,^[36] <i>Echium vulgare</i>,^[36] <i>Lappula consanguinea</i>,^[36] <i>L. echinata</i>,^[36] <i>Lithospermum arvense</i>,^[76] <i>L. erythrorhizon</i>,^[36, 57, 82] <i>Macrotomia euchroma</i>.^[36] cell cultures: <i>Echium lycopsis</i>,^[26] <i>L. erythrorhizon</i>.^[91, 99a] biological effects: inhibition of topoisomerase-I (Section 5.2).
20		α -methylbutyrylshikonin	roots: <i>Cynoglossum officinale</i>,^[36] <i>Echium vulgare</i>,^[36] <i>Eritrichium incanum</i>,^[36] <i>E. sichotenze</i>,^[36] <i>Lappula consanguinea</i>,^[36] <i>L. echinata</i>,^[36] <i>L. erythrorhizon</i>,^[77, 82] <i>Mertensia maritima</i>.^[36] cell cultures: <i>L. erythrorhizon</i>.^[99a] biological activity: antimicrobial (Section 5.3).
21		β,β -dimethylacrylshikonin	roots: <i>Alkanna hirsutissima</i>,^[83] <i>Arnebia euchroma</i>,^[36, 49, 84] <i>A. guttata</i>,^[49, 84] <i>A. tibetiana</i>,^[53] <i>Cynoglossum officinale</i>,^[36] <i>Echium vulgare</i>,^[36] <i>Eritrichium incanum</i>,^[36] <i>E. sichotenze</i>,^[36] <i>Jatropha glandulifera</i>,^[56] <i>Lappula consanguinea</i>,^[36] <i>L. echinata</i>,^[36] Other <i>Echium</i> species,^[53] <i>L. erythrorhizon</i>,^[36, 80, 82, 84] <i>Macrotomia ugamensis</i>,^[53] <i>Mertensia maritima</i>,^[36] <i>Moltkiopsis ciliata</i>,^[85] <i>Onosma confertum</i>,^[78] <i>O. paniculatum</i>,^[84] <i>O. hookeri</i>,^[84] <i>O. zerizaminium</i>.^[53] cell cultures: <i>Arnebia euchroma</i>,^[107e] <i>Echium lycopsis</i>,^[26] <i>L. erythrorhizon</i>.^[91, 99a]
22		teracrylshikonin	roots: <i>Arnebia euchroma</i>,^[49] <i>A. guttata</i>,^[49] <i>Lithospermum erythrorhizon</i>,^[86] <i>L. euchromum</i>.^[86] cell cultures: <i>Arnebia euchroma</i>.^[107e] biological activity: antimicrobial (Section 5.3).
23		angelylshikonin	roots: <i>Alkanna hirsutissima</i> . ^[87, 88]
24		β -hydroxyisovalerylshikonin	roots: <i>Arnebia euchroma</i>,^[49] <i>A. guttata</i>,^[49, 50] <i>Lithospermum arvense</i>,^[76] <i>L. erythrorhizon</i>,^[57, 61, 77, 86] <i>L. euchromum</i>.^[86] cell cultures: <i>Arnebia euchroma</i>,^[107e] <i>Echium lycopsis</i>,^[26] <i>L. erythrorhizon</i>.^[91, 99a] biological activity: antimicrobial (Section 5.3).

Table 1. (Continued)

Compound	R	Name	Occurrence and biological properties
25		β -acetoxy-isovalerylshikonin	roots: <i>Macrotomia euchroma</i> . ^[89]
26		benzoylshikonin	roots: <i>Moltkiopsis ciliata</i> . ^[85]
27		unnamed	roots: <i>Lithospermum erythrorhizon</i> . ^[61]
28		arnebin-2	roots: <i>Arnebia nobilis</i> . ^[171b]
29		acetylarnebin-2	roots: <i>Onosma heterophylla</i> . ^[41]
30		arnebin-5	roots: <i>Arnebia nobilis</i> . ^[171b]
31		arnebin-6	roots: <i>Arnebia nobilis</i> . ^[171b]
32		deoxyalkannin, deoxyshikonin, or arnebin-7	roots: <i>Alkanna tinctoria</i> , ^[241a] <i>A. hirsutissima</i> , ^[88] <i>Arnebia decumbens</i> , ^[75] <i>A. euchroma</i> , ^[42–44, 49] <i>A. hispidissima</i> , ^[33] <i>A. guttata</i> , ^[42, 49, 50] <i>A. nobilis</i> , ^[90] <i>Cynoglossum officinale</i> , ^[36] <i>E. vulgare</i> , ^[36] <i>Eritrichium incanum</i> , ^[36] <i>E. sichotense</i> , ^[36] <i>Lappula consanguinea</i> , ^[36] <i>L. echinata</i> , ^[36] <i>Lithospermum erythrorhizon</i> , ^[36, 42, 77, 82] <i>Macrotomia cephalotes</i> , ^[35, 39] <i>M. euchroma</i> , ^[82] <i>Mertensia maritima</i> , ^[36] <i>Onosma confertum</i> , ^[78] <i>O. heterophylla</i> . ^[41] cell cultures: <i>Arnebia euchroma</i> , ^[107e,f] <i>Echium lycopsis</i> , ^[26] <i>L. erythrorhizon</i> , ^[91, 99a] <i>O. paniculatum</i> . ^[105g] biological effects: antidermatophytic and antibacterial (Section 5.3), antitumor (Section 5.2).
33		alkannan	roots: <i>Alkanna tinctoria</i> , ^[1] <i>Cynoglossum officinale</i> , ^[36] <i>Echium vulgare</i> , ^[36] <i>Eritrichium incanum</i> , ^[36] <i>E. sichotense</i> , ^[36] <i>Lappula consanguinea</i> , ^[36] <i>L. echinata</i> , ^[36] <i>Lithospermum erythrorhizon</i> , ^[36] <i>M. euchroma</i> , ^[36] <i>Mertensia maritima</i> . ^[36]
34		anhydroalkannin	roots: <i>Lithospermum erythrorhizon</i> , ^[61] <i>Macrotomia euchroma</i> . ^[82]
35		lithospermidin-A	roots: <i>Lithospermum erythrorhizon</i> . ^[77]
36		lithospermidin-B	roots: <i>Lithospermum erythrorhizon</i> . ^[77]

[a] 1) ester hydrolysis may occur during some extraction procedures, which leads to the isolation of alkannin (**1**) and shikonin (**2**) in addition to, or rather than, their ester derivatives; 2) in many cases, no attempt was made to assign the absolute stereochemistry of each derivative, so alleged alkannin derivatives may be shikonin derivatives, or vice versa, or even racemates. Recent circular dichroism (CD)^[26, 166f, 172] and chiral HPLC^[27] experiments have shown that *Arnebia euchroma* contains mainly alkannin derivatives, while *Onosma confertum* contains mainly shikonin derivatives. However, the stereochemistry may vary between ester derivatives from the same plant, and even between plants from different locations.^[26] Differences may be noted between extracts from plants and those from cell cultures.^[26]

Scheme 1. Biosynthesis of shikonin (**2**).

and geranyl pyrophosphate (GPP, **54**). These components are unified in a reaction mediated by the enzyme PHB geranyltransferase to yield *m*-geranylPHB (**55**). In general, far more is known about the early stages of the biosynthetic route to **2** than the later ones.

GPP (**54**) is derived from the well-defined isoprenoid pathway^[110] that starts from acetyl-CoA (**49**), and proceeds via mevalonic acid (**50**), while PHB (**45**) is formed from phenylpropanoid metabolites. L-Phenylalanine (**38**), originally derived from shikimic acid (**37**), is converted into cinnamic acid (**39**) by the enzyme phenylalanine ammonia lyase (PAL), with the transformation to coumaric acid (**40**) then being mediated by cinnamate 4-hydroxylase (C4H). There is some dispute as to whether the biosynthetic conversion of coumaric acid (**40**)

into **45** occurs by the β -oxidative pathway^[111] or the retroaldol pathway.^[112] This question has implications beyond the biosynthesis of shikonin since PHB (**45**) is a biosynthetic intermediate for many metabolites, such as the ubiquinones (**62**)^[113] and vitamin E (**63**)^[114] (Figure 8).

A large volume of evidence has been gathered in favor of this biosynthetic route. Feeding experiments have been used to show that shikimic acid (**37**), L-phenylalanine (**38**), cinnamic acid (**39**), PHB (**45**), *m*-geranylPHB (**55**), *m*-geranylhydroquinone (**56**), and mevalonic acid (**50**) are all intermediates in the biosynthetic route.^[115] Furthermore, PHB (**45**), *m*-geranylPHB (**55**), and *m*-geranylhydroquinone (**56**) have been isolated from shikonin-free cell cultures of *LE* grown in LS liquid medium (in which shikonin biosynthesis is

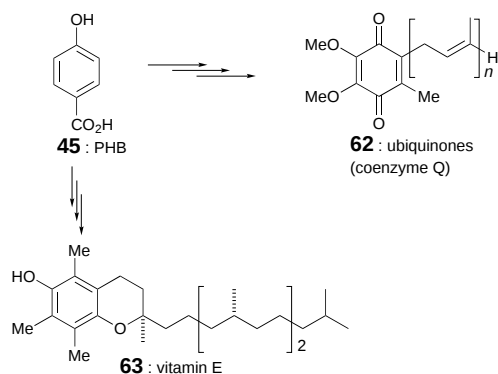


Figure 8. 4-Hydroxybenzoic acid (PHB, **45**) as a versatile biosynthetic intermediate.

inhibited).^[115b] PHB (**45**) actually accumulates in the vacuoles of shikonin-free cells as its β -D-glucoside PHBOG (**48**),^[116] which is speculated to be a defense mechanism since **45** is known to be toxic to the cells.^[117] Intermediates up to *m*-geranylPHB (**55**) have been detected from these shikonin-free cells, from which it was suggested that its conversion into *m*-geranylhydroquinone (**56**) is repressed by ammonium ions.^[118] Once transferred to the production medium M9, which induces shikonin biosynthesis, the amount of PHBOG (**48**) decreased rapidly, which thus implicated PHB (**45**) or PHBOG (**48**) as intermediates.^[116] It has been shown more recently that deoxyshikonin (**32**) is in fact a biosynthetic precursor to shikonin (**2**).^[115c] This provides the only direct evidence for any of the steps that follow the production of *m*-geranylhydroquinone (**56**). The other steps from **56** to shikonin (**2**) are speculative only.

Indirect evidence in favor of the biosynthetic route has been gained by the isolation of structurally related secondary metabolites from various species of *Boraginaceae*, both from plants and cell cultures. Evidence in favor of intermediate **61** comes from the isolation of the furan and dihydrofuran products, shikonofurans (**57**),^[119] echinofuran (**58**),^[120] and dihydroechinofuran (**59**).^[121] Presumably, the cyclic arylether arnebinol (**60**) is derived from an allylic hydroxylation at the *trans* terminal methyl group of **59** prior to macroetherification.^[122]

Several of the enzymes involved in shikonin biosynthesis have been identified and their location within the cell defined. PHB geranyltransferase is the enzyme that is repressed upon exposure of *LE* cell cultures to white light,^[94c] which thus halts shikonin production. It has been isolated from cell-free extracts of *LE* and its properties were investigated.^[123] Similar enzymes have been identified and characterized in ubiquinone biosynthesis,^[114] and the utility of a prenyltransferase, formed by *E. coli* overproduction, in organic synthesis has been demonstrated.^[124] It has also been suggested that the biosynthesis may be inhibited by light through the photo-degradation of flavin mononucleotide (FMN), a cofactor that may be required for one or more of the oxidation processes that lead to shikonin. In support of this, the FMN photo-degradation product lumiflavin was shown to inhibit shikonin biosynthesis, most likely through disruption of PHB geranyltransferase.^[125]

PHB geranyltransferase has been localized to a microsomal fraction of *LE* cells that contain specific vesicle membranes derived from the endoplasmic reticulum.^[126] PHB glucosyltransferase, which catalyses the conversion of PHB (**45**) into PHBOG (**48**), has also been partially purified and its characteristics assessed.^[127] It has been localized to the cytosol,^[128] as has the glucosidase responsible for the reverse transformation, and the enzyme responsible for GPP (**54**) production, GPP synthase.^[129] Thus, it appears that PHBOG (**48**) is stored in the vacuoles prior to triggering shikonin biosynthesis, possibly to protect it from the glucosidase.^[128] Upon transfer of the cells to the production medium, this store of PHBOG (**48**) may be utilized for shikonin biosynthesis. It has been suggested that all steps subsequent to, and including the coupling of PHB (**45**) and GPP (**54**), take place in a special vesicle or biosynthetic site, which facilitates not only the orderly biosynthetic reactions, but also the transport and secretion of the secondary metabolite.^[126]

Publications concerning shikonin biosynthesis continue to appear at a steady rate, most notably from the laboratories of Mamoru Tabata at Kyoto University, Japan. Studies in this area are presumably on-going and we wait with anticipation for more detailed information on the later stages of the biosynthetic route.

4. Chemistry of Alkannin and Shikonin

Before discussing the chemistry of alkannin and shikonin, it will be instructive to consider first the chemical properties of the parent naphthoquinone, naphthazarin (**64**).

4.1. Chemistry of Naphthazarin

Naphthazarin (commonly considered as 5,8-dihydroxy-1,4-naphthoquinone, **64a**; Figure 9) has been used historically as a rather expensive purple dye. It occurs naturally in the wood bark of *Lomatia obliqua*^[130] and in walnut husks of *Juglans mandschurica maxim* var. *Sieboldianna* Makino.^[131] It can be prepared directly either by heating 1,5-dinitronaphthalene with sulfur and fuming sulfuric acid,^[132] or by double Friedel–Crafts acylation of either hydroquinone,^[133] or 1,4-dimethoxybenzene (**65**),^[134] with maleic anhydride in fused AlCl_3 – NaCl . Yields for both processes are generally low. An indirect, three-step procedure with dichloromaleic anhydride is usually employed today to produce larger quantities of naphthazarin for laboratory use (Scheme 2, Table 2).^[135]

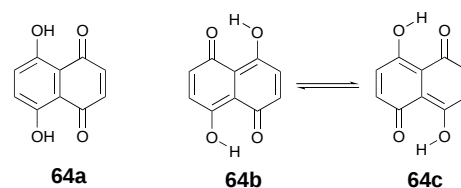


Figure 9. Structures of naphthazarin (**64**).

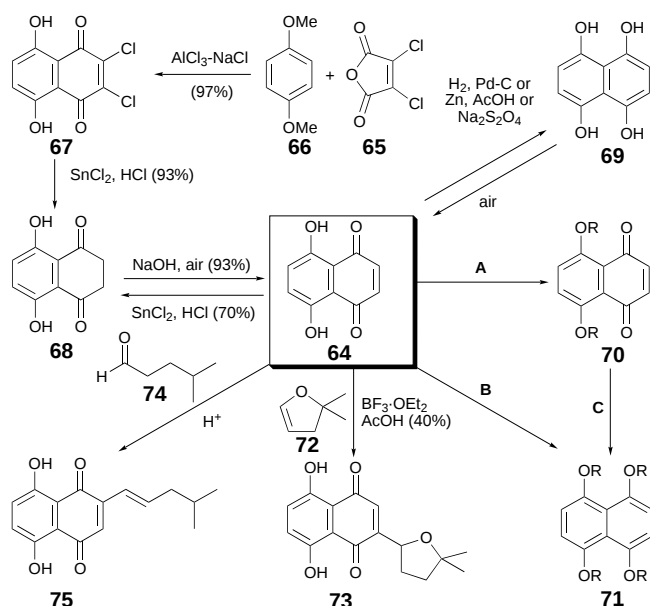
Scheme 2. Chemistry of naphthazarin (**64**).

Table 2. Reaction conditions for pathways A–C in Scheme 2.

R	A	B	C
Me	MeOTs, K ₂ CO ₃ (44 %) ^[147–148]	Na ₂ S ₂ O ₄ , NaOH MeI (44 %) ^[149] or 1) H ₂ Pd-C, DMF 2) NaH, Me ₂ SO ₄ , DMF (72 %) ^[150]	Na ₂ S ₂ O ₄ , NaOH MeI (48 %) ^[149]
Ac	Ac ₂ O, NaOAc ^[133]	Ac ₂ O, NaOAc, Zn ^[133]	Ac ₂ O, NaOAc Zn ^[133]
TMS	MTMSTFA (100 %) ^[151a]	MTMSTFA, NH ₄ I (100 %) ^[151b]	–
TBS	MTBSTFA (94 %) ^[152]	MTBSTFA, NH ₄ I (89 %) ^[152]	–

The symmetry of naphthazarin (**64**) has been a matter of some debate.^[136] The most important aspect from the synthetic viewpoint is that rapid “tautomerization” occurs, which leads to a structure that is essentially centrosymmetric as evidenced from its ¹H NMR spectrum, which exhibits only a single signal at $\delta_{\text{H}} = 7.13$, midway between the signals expected for the aromatic (ca. $\delta_{\text{H}} = 7.25$) and quinoid protons (ca. $\delta_{\text{H}} = 6.95$).^[137] In fact, the interconversion of naphthazarin structures may be more accurately described as a tunneling process, since it has not been possible to split the aromatic and quinonid signals below a coalescence temperature. Furthermore, the spectroscopic properties of naphthazarin (**64**), including its intense color in solution, appear to be more consistent with a 1,5-quinoid structures (**64b, c**; Figure 9) than the more commonly depicted 1,4-quinoid structure (**64a**).^[138]

Naphthazarin (**64**) has been described in the crystalline state as deep brown–red crystals with a green–golden luster and as deep green crystals with a metallic reflex.^[139] It is readily soluble in organic solvents such as benzene and dichloromethane to form blood-red solutions. Upon treatment of these solutions with strong alkali, the quinone is extracted into the aqueous phase as its deep “cornflower

blue” dianion. In fact, this property has allowed the use of naphthazarin (**64**) as a pH indicator.^[139] Upon immediate acidification of the blue solution, naphthazarin can be recovered, while prolonged exposure to atmospheric oxygen prior to acidification causes oxidation of the aromatic nucleus to give naphthopurpurin (2,5,8-trihydroxy-1,4-naphthoquinone).^[132]

Naphthazarin (**64**) may be oxidized to the naphthodiquinone (**124**) (see Scheme 12) upon treatment with Pb(OAc)₄,^[133] or more effectively, phenyliodonium bis(trifluoroacetate).^[140] Highly reactive diquinone (**124**) has been used for the synthesis of cage compounds,^[140] and in a synthesis of cycloshikonin (Section 4.3.3, Scheme 12).^[141]

Reduction of naphthazarin (**64**) may be effected with a number of reagents. Use of catalytic hydrogenation, sodium dithionite, or zinc, all give rise to the highly air-sensitive 1,4,5,8-tetrahydroxynaphthalene (**69**).^[139] In particular, alkaline solutions of **69** are rapidly oxidized to the naphthazarin dianion upon exposure to atmospheric oxygen.^[135] Reduction of **64** with tin(II) chloride in hydrochloric acid affords the more air-stable crystalline diketo tautomer of leuconaphthazarin (**68**).^[142–143] Reduction of halogenated naphthazarins by this method occurs with concomitant dehalogenation and formation of **68**. This feature has allowed the more efficient large-scale preparation of naphthazarin (**64**) by a three-step sequence. Friedel–Crafts acylation of 1,4-dimethoxybenzene (**66**) and dichloromaleic anhydride (**65**) proceeds, in contrast to the reaction with maleic anhydride, in very high yield.^[144] Tin(II) chloride reduction of 2,3-dichloronaphthazarin (**67**),^[142] followed by air oxidation of an alkaline solution of leuconaphthazarin (**68**), affords naphthazarin (**64**) in very good overall yield.^[135]

Methods for the bis-protection, and reductive tetra-protection of naphthazarin (**64**) are summarized in Scheme 2. Etherification of the phenolic groups is rather difficult, presumably due to the stability of the naphthazarin dianion.

Naphthazarin (**64**) reacts with only a limited range of electrophiles. The scope of these reactions is probably limited by the tendency of **64** to bind Lewis acids. Satisfactory yields of alkylated products are obtained upon reaction with oxonium ions derived from cyclic enolethers, as exemplified by the synthesis of cycloshikonin (**73**) (Scheme 2).^[145] During unsuccessful attempts to synthesize shikonin (**2**), Brockmann demonstrated that condensation reactions of aldehydes with **64** under acidic conditions provided complex mixtures of products, which included alkene derivatives such as **75** (Scheme 2).^[1]

The adjacent keto and phenolic oxygen atoms make naphthazarins ideal substrates for the formation of metal chelates.^[136, 146] These have been studied in some detail with regards to a number of specific properties such as magnetic exchange interactions between paramagnetic ions.^[136, 146] See also Section 5.2.

Controlled nucleophilic addition reactions with naphthazarin (**64**) have not yet been achieved. Complex mixtures generally result, including both 1,2- and 1,4-addition products.^[153] One of the only useful compounds in this respect is the bisulfite addition compound Alizarin Black S, which was formerly used in printing.^[142] Recently, an efficient procedure

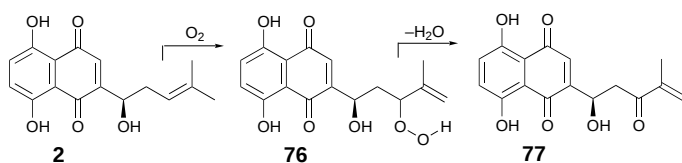
for formal 1,4-addition has been reported via the leuco form of the respective bromo derivative.^[153]

Cycloaddition reactions are generally the most useful and successful reactions of naphthazarin (**64**). In particular, Diels–Alder reactions with **64** have found extensive use in the construction of anthracycline antibiotics. This aspect has been dealt with extensively elsewhere^[154] and will not be discussed here.

4.2. Reactivity of Alkannin and Shikonin

In general, the chemistry of alkannin (**1**) and shikonin (**2**) has many similarities to the chemistry of naphthazarin (**64**). The major tautomeric form of the quinone nucleus substituted by an electron-donating group is that depicted in **1** and **2**.^[137] The hydroxyl-containing side-chain introduces some interesting properties that render **1**, **2**, and their derivatives far more sensitive than **64**.

In acidic conditions (either Brønsted or Lewis), cyclization of the side chain to the tetrahydrofuran isomer **73** (cycloshikonin) occurs readily.^[1, 2, 155, 156] Quinones **1** and **2** are also susceptible to photooxidation by exposure to air and light.^[157] The major photolytic product **77** is thought to arise through the mechanism indicated in Scheme 3. Polymerization of **1** and **2** occurs readily by the action of acids, bases, heat, and light.^[158, 159] Compounds **1** and **2** have also been shown to undergo racemization under strongly acidic conditions.^[1]

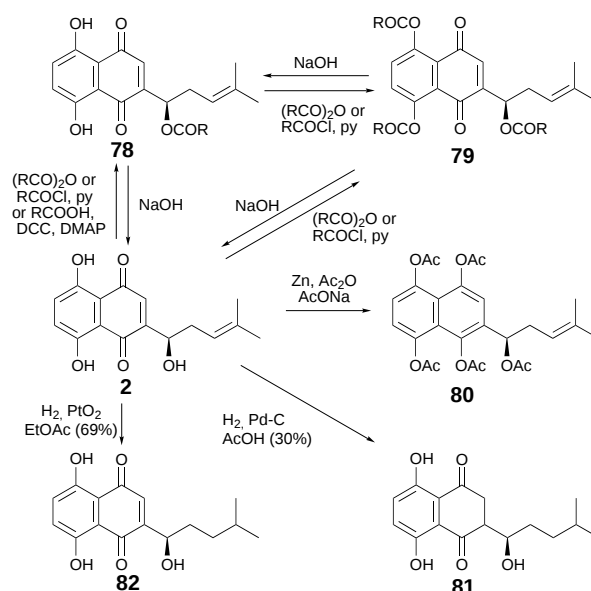


Scheme 3. Photooxidation of shikonin (**2**).^[157]

The aliphatic hydroxyl group in shikonin may be selectively acylated in the presence of the phenolic hydroxyl groups by standard esterification methods.^[160] Conversely, acylshikonin derivatives **78** can be produced through the selective hydrolysis of triacylated shikonin derivatives **79** (Scheme 4).^[161] All other protection-deprotection chemistry resembles that of naphthazarin (**64**). Hydrogenation of **2** with Pd catalysis causes reduction of both the quinone and the alkene groups to afford **81**, while use of catalytic PtO₂ allows for selective alkene reduction to dihydroshikonin **82** (Scheme 4).^[156a] The quinone may be selectively reduced with Zn or Na₂S₂O₄^[139] to provide a highly air-sensitive tetrahydroxynaphthalene derivative, which has been trapped as the pentaacetyl derivative **80** (Scheme 4).^[162]

The acid/base color chemistry of **1** and **2** (Figure 10) resembles that of naphthazarin (**64**).

The only examples of ring functionalization of **1**, **2**, and its derivatives were undertaken by Shukla and co-workers, who performed a double Mannich-type addition to afford **83**, and a reaction with diazomethane, which proceeds by a sequence of cycloaddition followed by N-methylation, to provide **85**



Scheme 4. Selected reactions of shikonin (**2**).

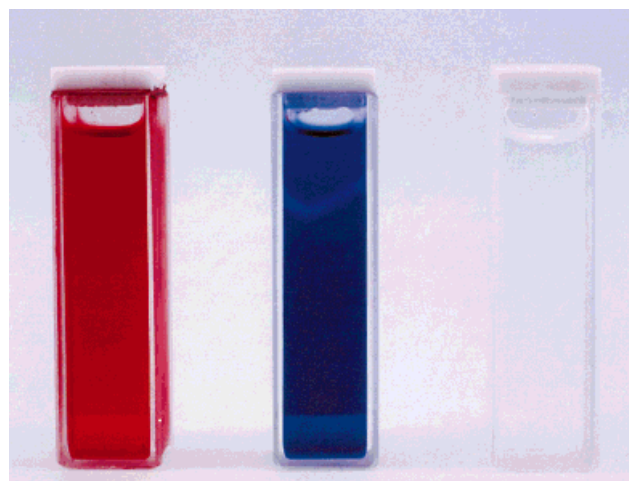
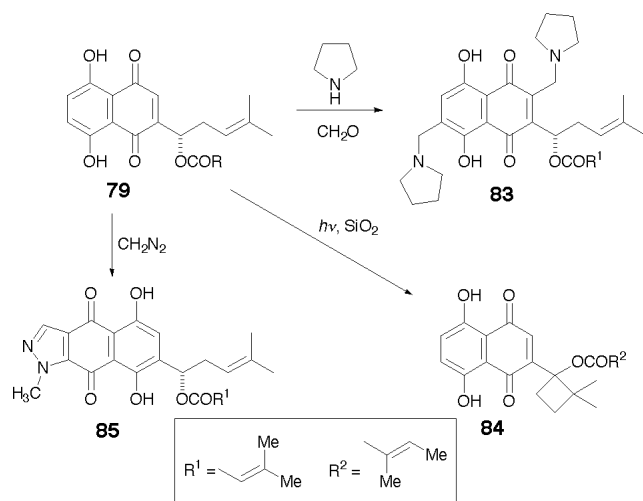


Figure 10. Solutions of alkannin (**1**) in CH₂Cl₂ (red) and in 1N aqueous NaOH (cornflower blue) as contrasted with a solution of pentaacetyl derivative **80** in CH₂Cl₂ (colorless, barely visible).

(Scheme 5).^[163] An interesting rearrangement to the cyclobutane derivative **84** has been observed during the isolation of certain alkannin esters.^[87]

A large number of physical techniques have been applied for the analysis and study of **1**, **2**, and their derivatives. Chromatographic techniques used include TLC,^[87, 164] densitometry,^[165] gel permeation,^[159] and HPLC.^[27, 42, 43, 49, 50, 166] The properties studied include UV/Vis,^[33, 52, 53, 77, 166c, 167, 168] IR,^[44, 50, 52, 53, 77, 167] ¹H NMR,^[33, 44, 50, 77, 167] ¹³C NMR,^[169a, 170] mass spectrometry,^[38, 40, 44, 89, 166c,h, 171a] circular dichroism,^[26a, 166f, 170, 172, 173] and indirect atomic absorption.^[174] Polarography,^[175] voltammetry,^[176] differential pulse voltammetry,^[177] and even a photoacoustic technique for transdermal adsorption measurements,^[178] have been utilized for qualitative and quantitative determinations of **1**, **2**, and their derivatives.

Scheme 5. Chemistry of alkannin ester **79**.^[87, 163]

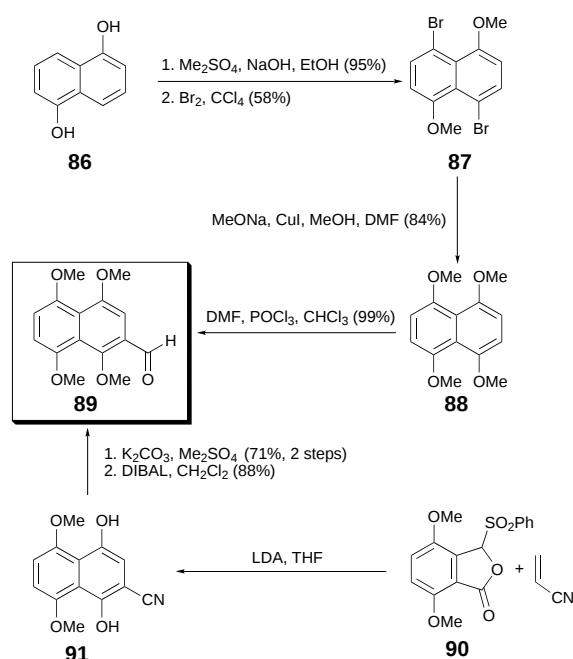
4.3. Total Syntheses of Alkannin and Shikonin

4.3.1. Introduction

There are several problems that must be overcome in order to accomplish an efficient synthesis of alkannin (**1**) and/or shikonin (**2**). As mentioned above, the natural product is sensitive to Brønsted and Lewis acids, light, and oxygen. There are further practical difficulties, such as the strong affinity of naphthazarins for both silica and alumina, which makes chromatographic purification difficult. Clearly, the most general approach to substituted naphthazarins, the Friedel–Crafts acylation of hydroquinones with maleic anhydride derivatives (Section 4.1) is not applicable here. Naturally, an ideal synthesis of **1** and **2** would be one in which protection of the naphthazarin system was not required. So far, this has not been realized. Perhaps the most challenging aspect of this synthetic problem, however, is the development of an effective protecting system for the naphthoquinone core. The sensitivity of the natural product to a variety of reagents and conditions besides acids, light, and oxygen (for example, with reducing agents and nucleophiles) severely limits the options available for the final deprotection steps of the synthesis.

Most of the successful syntheses of **1** and **2** have been achieved through the use of 1,4,5,8-tetramethoxynaphthalene (**88**, Scheme 6) and its derivatives, as a protected form of the naphthazarin core. While **88** can be prepared directly from naphthazarin (**64**) by reductive methylation (Scheme 2), a more economical approach, which avoids the use of this costly material, has been devised by Terada and co-workers.^[149] Thus, the cheap starting material 1,5-dihydroxynaphthalene (**86**, Scheme 6) was O-methylated before bromination, and subsequent copper(i)-assisted methoxylation afforded **88**.^[149]

Formylation of 1,4,5,8-Tetramethoxynaphthalene (**88**) by the Vilsmeier method produces **89** (Scheme 6) in almost quantitative yield.^[149] This intermediate is common to most of the syntheses of **1** and **2** reported thus far. An alternative approach to **89** has recently been described by the Coula-douros group (Scheme 6).^[179] This method employs an annu-

Scheme 6. Two syntheses of key intermediate **89**.^[149, 179]

lation reaction of the phthalide anion derived from **90** with acrylonitrile to afford **91**.^[180] Subsequent O-methylation and DIBAL reduction provided **89**. This method is notable for the high overall yield obtained and the possibility that hydroquinone **91** may be used to produce a naphthalene intermediate with orthogonal protection of the two aromatic rings. This may be of assistance for improving the efficiency of its subsequent demasking to a naphthazarin (see below).

Methods for the release of naphthazarins from tetramethoxynaphthalene derivatives have been thoroughly investigated by Tanoue and Terada.^[181] They found that boron tribromide, one of the standard reagents for aromatic ether cleavage, successfully converts tetramethoxynaphthalene (**88**) into naphthazarin (**64**) in 68 % yield after acid oxidation of the originally produced tetrahydroxynaphthalene.^[149] This method is, however, unsuitable for the preparation of 2-substituted derivatives of **64**, since polymeric materials are formed. Instead, a two-step procedure was developed (Scheme 7, Table 3). Initial oxidation with ceric ammonium nitrate (CAN) affords two regioisomeric products, the selectivity being dependent upon the nature of the substituent R. For

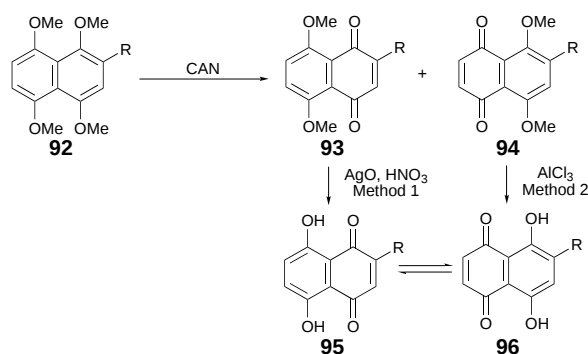
Scheme 7. Deprotection of tetramethoxynaphthalene derivatives.^[181]

Table 3. Yields for the reactions shown in Scheme 7.

R		CAN oxidation		Demethylation	
		93 [%]	94 [%]	method	yield [%]
a	H	70	–	–	–
b	CH ₂ OH	75	13	1	53
c	CH(OH)CH ₃	61	13	1	52
d	CH(OH)(CH ₂) ₃ CH ₃	42	16	1	22
e	CH(OH)(CH ₂) ₃ CH(CH ₃) ₂	52	17	1	22
f	CH(OH)(CH ₂) ₃ C(CH ₃) ₂ OH	70	15	1	28
g	CH(OH)(CH ₂) ₃ COCH ₃	33	33	1	27
h	COCH ₃	3	77	2	71
i	CHO	0	85	2	43

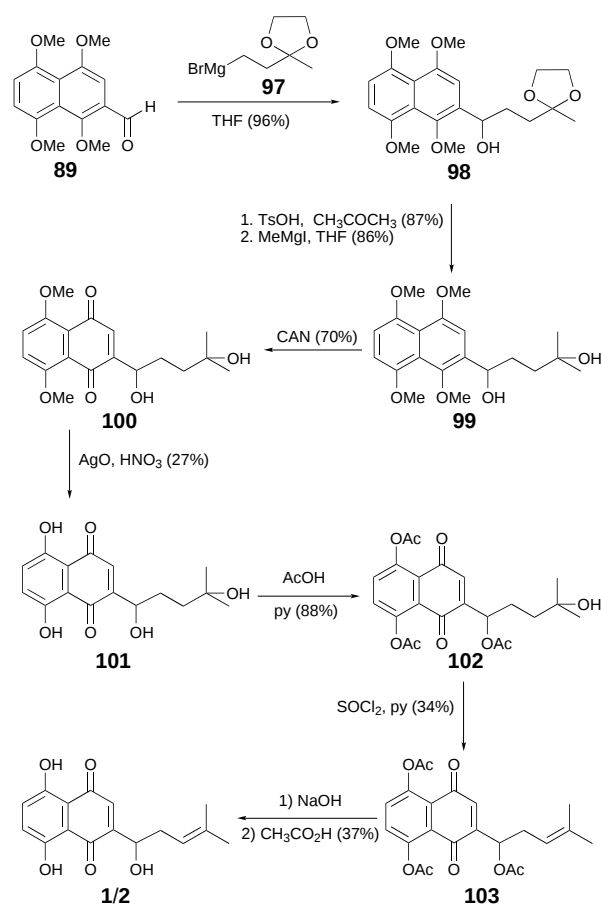
electron-donating groups such as hydroxyalkyl (Table 3, entries **b–g**), the substituted ring is preferentially oxidized, while electron-withdrawing groups such as acetyl and formyl, favor isomer **94** as the major product. In the former case, regioselectivity is usually modest. Methoxyquinones of form **93** can be further demethylated by treatment with AgO/HNO₃, although generally in low yield. The regioisomeric quinones **94** failed to react under these conditions, and required the action of AlCl₃. These latter conditions would be unsuitable in a synthesis of **1** and **2**. Tautomerization between **95** and **96** occurs readily, with **95** being favored when R is electron donating, and **96** the major isomer when R is electron withdrawing.^[137]

Despite being far from ideal, tetramethoxynaphthalene derivatives were, until recently, the only protected form of naphthazarins suitable for the synthesis of **1** and **2**.

4.3.2. Syntheses of Shikalkin (1/2)

After Terada and co-workers had developed a method for the conversion of tetramethoxynaphthalenes into naphthazarins they were able to complete the first synthesis of shikalkin **1/2** in 1983 (Scheme 8).^[182] Addition of the Grignard reagent **97** to the formyl derivative **89** produced intermediate **98** in good yield. Ketal hydrolysis and subsequent addition of methylmagnesium iodide afforded diol **99**. At this stage, these researchers opted to release the naphthoquinone from its protected form by the two-step CAN/AgO/HNO₃ sequence, as described previously. In this case, the initial CAN oxidation proceeded with reasonably high selectivity in favor of the desired isomer **100**, and in moderate yield, although the AgO/HNO₃ demethylation, as ever, gave only a poor yield of the naphthoquinone **101**. Treatment of **101** with acetic anhydride in pyridine selectively produced triacetate **102**. This allowed subsequent elimination with thionyl chloride and pyridine to afford **103**, the low yield being a consequence, in part, of the production of significant amounts of the terminal alkene regioisomer. The synthesis was completed by alkaline hydrolysis of the acetate groups to yield shikalkin (**1/2**). This research group also used this method for the preparation of isomers of **1**.^[183]

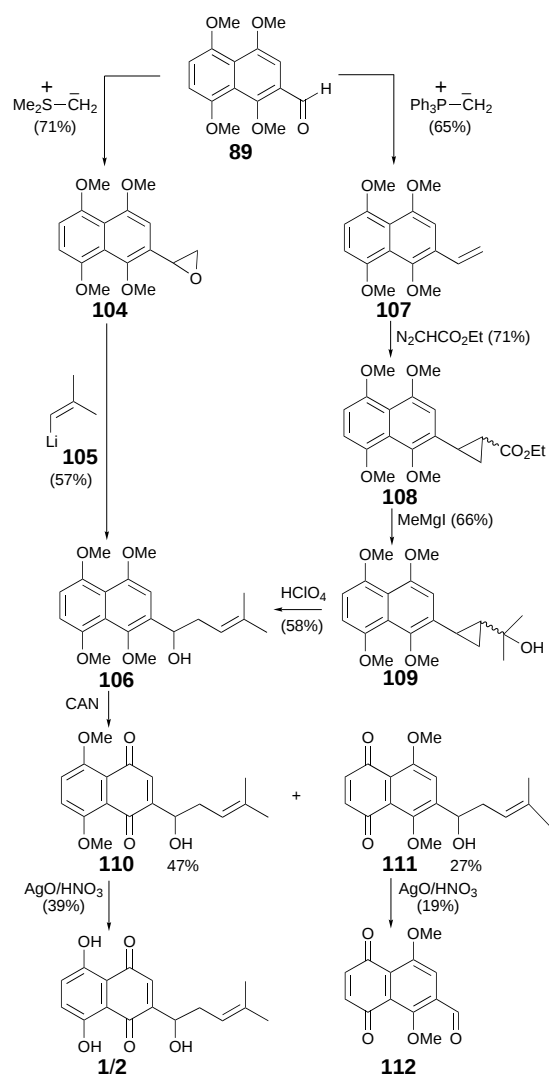
Researchers from Russia have reported two approaches to **1/2** from the formyl derivative **89** (Scheme 9). Conversion of aldehyde **89** into epoxide **104** was achieved in good yield with standard sulfur ylid methodology.^[184] The epoxide was



Scheme 8. The first synthesis of shikalkin (**1/2**) by Terada and co-workers.^[149, 182]

opened by treatment with the organolithium reagent **105** to afford **106**. This intermediate was common to their alternative approach, which utilized a Wittig olefination, a cyclopropanation, and a double Grignard addition to form the key intermediates **109**.^[185] The transformation into **106** was accomplished by an intriguing acid-catalyzed rearrangement. Conversion of **106**, obtained from both routes, into **1/2** was effected by the usual two-step sequence. In this case, the regioselectivity of the initial CAN oxidation was negligible, and once again the second demethylation reaction was low-yielding. Attempts to convert the undesired regioisomer **111** into **1/2** by AgO/HNO₃ treatment led, rather curiously, to the aldehyde product **112**, and not to **1/2**.

More recently, Torii and co-workers have reported a synthesis of **1/2** also starting from formyl derivative **89** (Scheme 10).^[186] The initial step is a McMurry-type coupling between **89** and the unsaturated aldehyde **113** mediated by a low-valent vanadium species.^[187] Closure of diols **114** to the cyclic carbonates **115** (presumably a mixture of diastereoisomers) set the stage for a palladium-catalyzed allylic reduction. Unfortunately, this reaction afforded a significant amount of a regioisomeric alkene (ca. 28%), and a rather elaborate reaction sequence was required before acetate **116** could be obtained in a pure form. These workers developed an alternative method for the deprotection of the tetramethoxynaphthalene core, which employed an anodic oxidation.

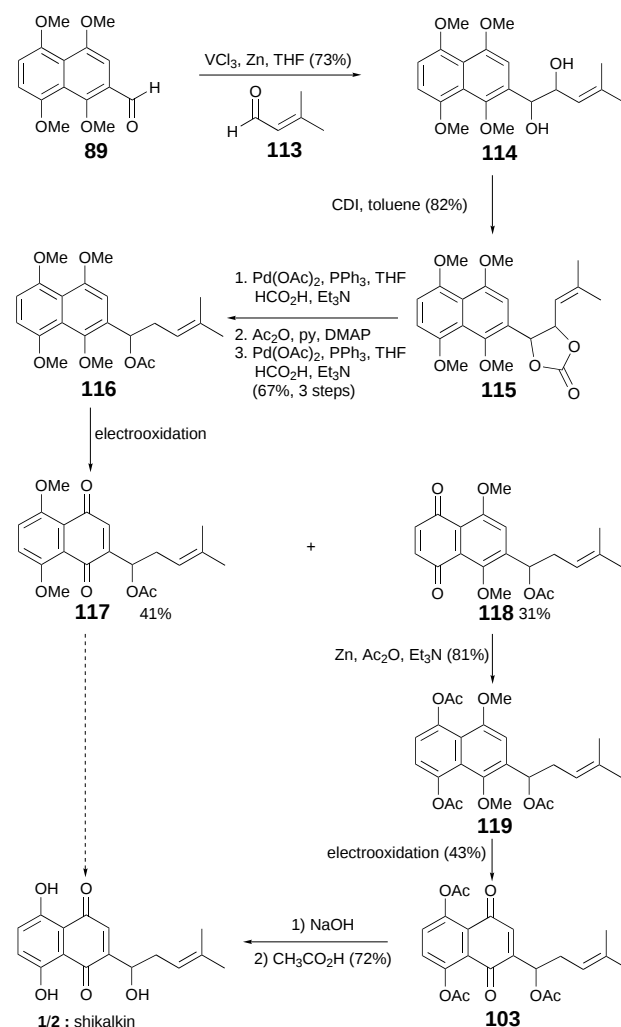


Scheme 9. Further syntheses of shikalkin (**1/2**) from the formyl derivative **89**.^[184, 185]

Despite significant experimentation, however, they were unable to improve the regioselectivity of the initial oxidative demethylation. In fact, the desired isomer **118** (which would be the unwanted one for a AgO/HNO₃ demethylation) was the minor product of this reaction. In order to achieve the second demethylation, a reductive acetylation to provide **119** was required, whereupon selective oxidation of the methoxylated ring could be achieved by a second anodic oxidation. Alkaline hydrolysis of **103** completed the synthesis. These researchers apparently made no attempt to demethylate their unwanted regioisomer **117**, although its transformation into acetylshikonin (**15**), and then to **1/2**, should be possible by AgO/HNO₃ treatment. Thus, the anodic oxidation method is complementary to the previously described two-step demethylation procedure.^[181]

4.3.3. Approaches via Cycloshikonin

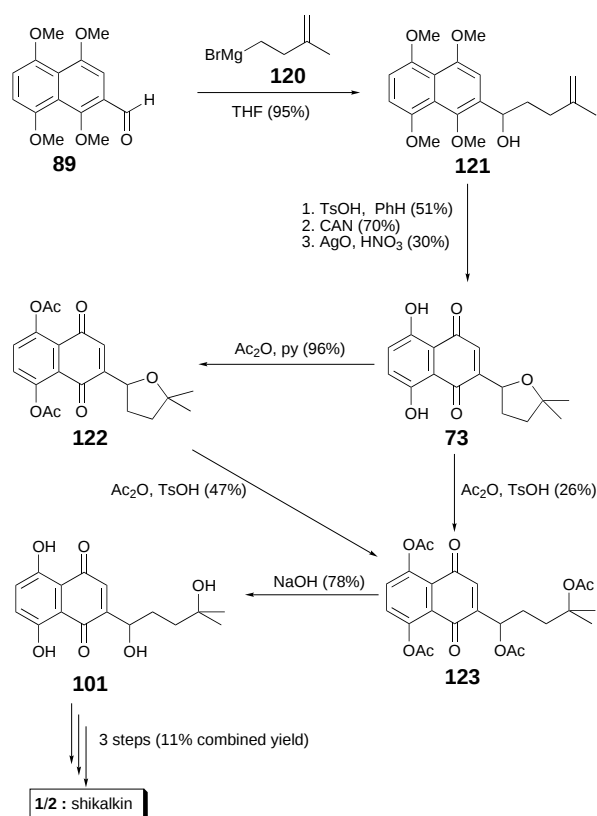
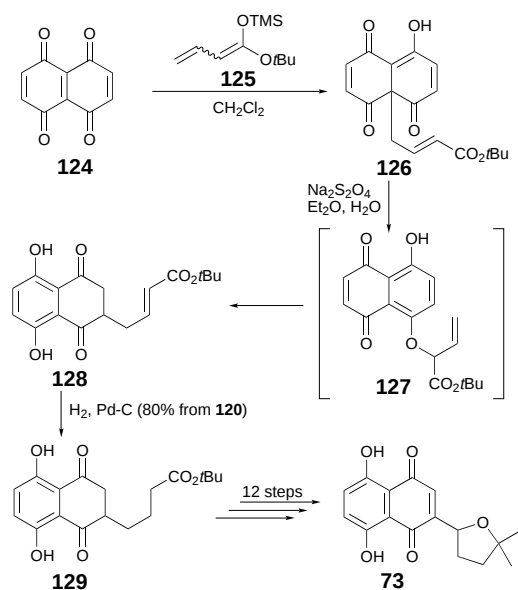
Terada and co-workers have also shown that cycloshikonin (**73**) may be converted into shikalkin (**1/2**) itself (Scheme 11).^[188] Thus, a synthesis of **73** constitutes a formal



Scheme 10. The approach of Torii and co-workers for a total synthesis of shikalkin (**1/2**).^[186]

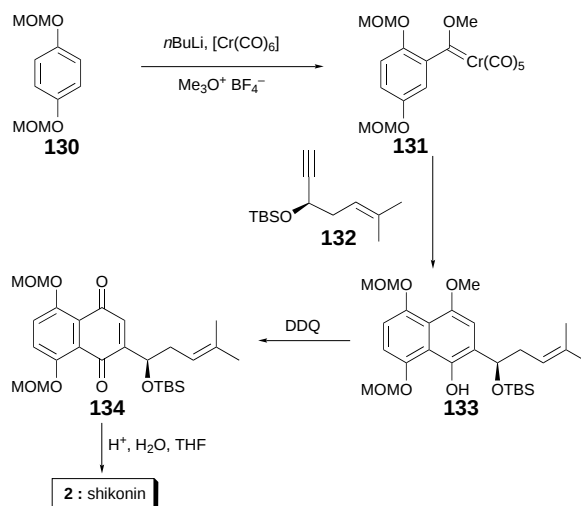
synthesis of **1/2**. The key step is the opening of the tetrahydrofuran ring to **123** with *p*-toluenesulfonic acid in the presence of acetic anhydride, starting either from **73** itself or more efficiently from the diacetate **122**. Alkaline hydrolysis of **123** provided **101**, which was an intermediate in their earlier synthesis of shikalkin.^[149, 182] The initial approach to cycloshikonin (**73**) developed by this group (Scheme 11), in which the key steps are addition of the Grignard reagent **120**, followed by acid-catalyzed ring closure,^[188] has been superseded by the report of the direct synthesis of **73** from naphthazarin (**64**) (Scheme 2).^[145]

An interesting, although rather long, route to cycloshikonin (**73**) has recently been reported by Aso and Kanematsu (Scheme 12).^[141] The γ -addition of silyl keteneacetal **125** to the highly reactive naphthodiquinone **124** afforded **126**, which underwent two consecutive reductive [3,3] sigmatropic rearrangements to produce, after hydrogenolysis, leuconaphthazarin (**129**) in 80% overall yield. Conversion into cycloshikonin (**73**) was then achieved in a rather inefficient 12-step linear sequence. Although impractical for the synthesis of shikonin (**1**) and cycloshikonin (**73**), this route may be of some utility for the synthesis of analogues.


 Scheme 11. Terada's approach to shikalkin (**1/2**) via cycloshikonin (**73**).^[188]

 Scheme 12. Kanematsu's synthesis of cycloshikonin (**73**).^[141]

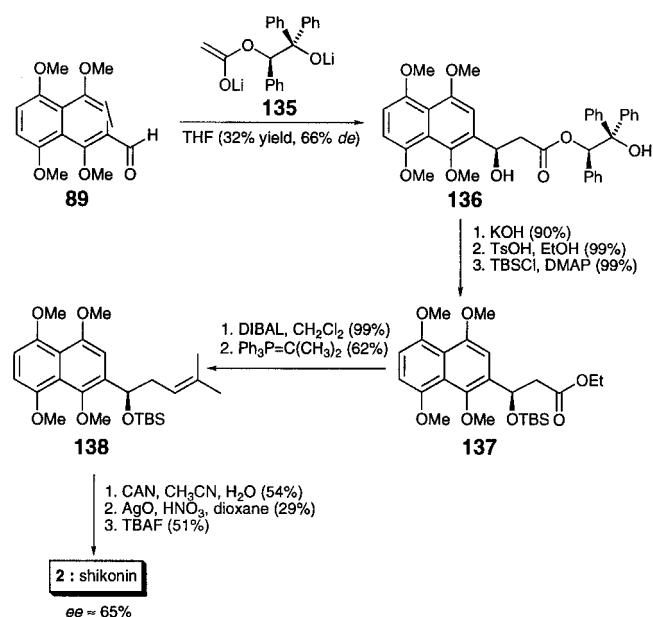
4.3.4. Asymmetric Approaches

The first asymmetric synthesis of **2** was disclosed in a patent,^[189] and involves an elegant application of the Dötz annulation reaction (Scheme 13).^[190] Thus, MOM-protected hydroquinone **130** was converted into the chromium carbene intermediate **131**, by the standard method, after *ortho* lithiation of **130**. Thermolysis with enantiomerically pure alkyne **132** provided the protected naphthazarin **133**. The


 Scheme 13. A stereoselective synthesis of shikonin (**2**) by the Dötz annulation reaction.^[189]

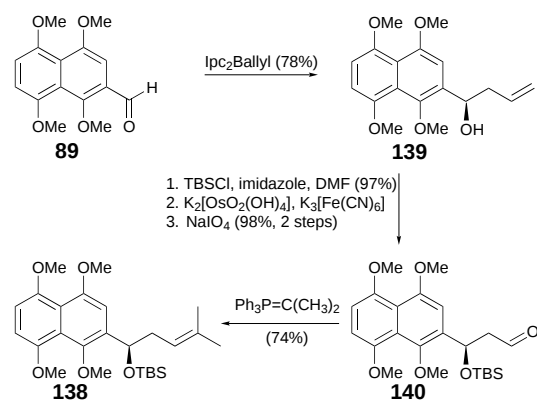
phenolic group in **133** allowed selective oxidation of the more functionalized ring of the naphthalene to produce quinone **134**, which was then converted into shikonin (**2**) by acid hydrolysis. Unfortunately, the authors failed to quote yields for the synthesis, so it is not possible to comment on the efficiency of their route. It should be noted, however, that the published synthesis of alkyne **132** involves a five-step linear sequence from commercially available starting materials and uses a Sharpless asymmetric epoxidation to secure the required stereochemistry.^[191]

The asymmetric approach to **1** and **2** taken by Braun and Bauer was to perform an asymmetric aldol addition onto the formyl derivative **89**,^[192] by using their asymmetric acetate enolate equivalents derived from (*R*)- and (*S*)-(2-hydroxy-1,2,2-triphenylethyl) acetate (Scheme 14).^[193] While these reagents generally give very high stereocontrol, the selectivity


 Scheme 14. Braun's synthesis of shikonin (**2**) by an asymmetric aldol reaction.^[192]

in this case was rather modest (ca. 5:1 after purification). An early transition state is usually invoked to explain the asymmetric induction obtained with these reagents, but the electron-rich nature of aldehyde **89** may disfavor this scenario, and lead to the poor selectivity. Conversion of the initial aldol adduct **136** into **138** was achieved by standard methods. These workers considered it prudent to protect the aliphatic hydroxyl group in an attempt to improve the efficiency of the final deprotection steps. However, no improvement in yield for the demethylation sequence was observed. The enantiomeric excess (ee) of shikonin (**2**) produced by this route was determined to be approximately 65% by CD measurements.

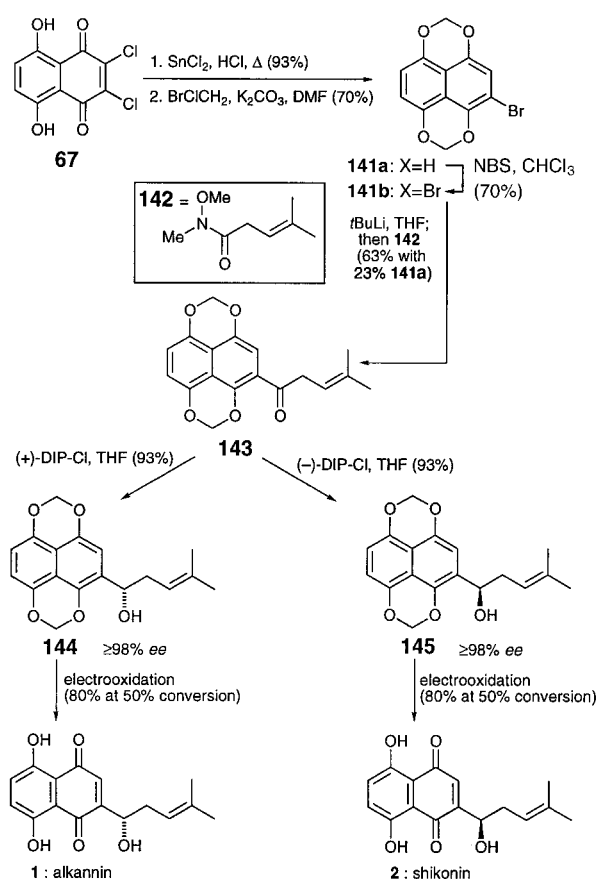
The Coulaudouros group has recently achieved a synthesis of alkannin intermediate **138**,^[179] by an asymmetric allylation reaction with allyldiisopinocampheylborane (Scheme 15).^[194] Thus, alcohol **139** was produced in 82% ee (based on an 80% ee of the chiral reagent). Construction of the side-chain was completed after hydroxyl protection by a high-yielding three-step sequence.



Scheme 15. Improved approach to **138** by asymmetric allylation (Coulaudouros and co-workers).^[179]

Recently, the Nicolaou group reported highly efficient total syntheses of both alkannin (**1**) and shikonin (**2**) (Scheme 16).^[195] This strategy was designed with three considerations in mind: 1) to use a commercially viable source of the naphthazarin core; 2) to produce a late-stage intermediate from which it would be possible to form both alkannin (**1**) and shikonin (**2**) in high ee; and 3) to devise a novel protecting system for the naphthazarin core, which could be cleaved at the end in one step, and under mild conditions.

The first aim was achieved by using 2,3-dichloronaphthazarin (**67**) as the readily available starting material (Section 4.1).^[144] It was reasoned that ketone **143** should be an ideal intermediate to access both enantiomeric series, since arylalkyl ketones of this type are generally considered to be excellent substrates for many asymmetric reducing agents.^[196] Furthermore, it was anticipated that bis(methyleneacetal) derivatives such as **A** (Figure 11) would provide a solution to the deprotection problem. In this case, oxidative treatment may still produce two regioisomeric intermediates **B** and **C**, but spontaneous elimination of formaldehyde should afford



Scheme 16. Nicolaou's stereocontrolled total synthesis of alkannin (**1**) and shikonin (**2**).^[195]

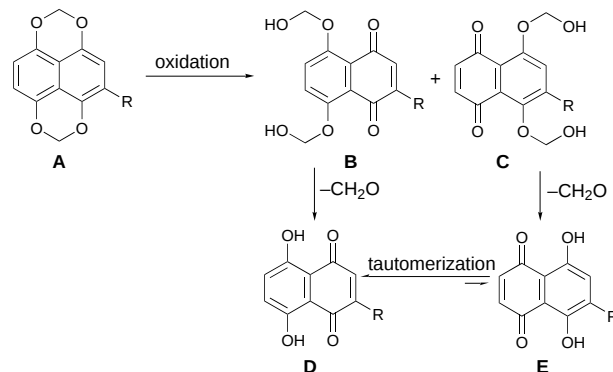


Figure 11. Projected deprotection of bis(methyleneacetal) derivatives, and preferential formation of naphthazarin isomer **D** (Nicolaou and Hepworth).^[195]

the two naphthazarin isomers **D** and **E**. These isomers should rapidly tautomerize in favor of the desired form **D** by virtue of the stabilizing effect of the electron-donating alkyl group.^[137]

The plans came to fruition in a concise and highly efficient synthesis. Thus, bromine-substituted methyleneacetal **141b** (Scheme 16) was prepared from 2,3-dichloronaphthazarin (**67**) by tin(II) chloride reduction,^[142] O-alkylation with bromochloromethane,^[197] and subsequent bromination with NBS. Halogen–metal exchange with *tert*-butyllithium followed by the addition of Weinreb amide **142** afforded ketone **143**. Separate reduction of **143** with each enantiomer of DIP-Cl^[198]

afforded the enantiomeric alcohols **144** and **145** in high yield and excellent ee. Cleavage of the methyleneacetal protecting groups was achieved by anodic oxidation. The yields of shikonin and alkannin were high (about 80%, based on recovered starting alcohol) provided the reaction was taken no further than 50% conversion. Furthermore, the oxidation did not require any elaborate electrochemical equipment. A power supply that forced a constant potential difference across the cell of 3 V, and low-cost graphite-rod electrodes were used.^[199] This sequence may provide a commercially viable synthetic route to **1** and **2**.

5. Biological Activity of Alkannin, Shikonin, and Their Derivatives

Biological investigations over the last 25 years have shown that many of the medicinal properties claimed for *AT* and *LE* in the historical texts do indeed have a sound scientific basis, and that the active components are **1**, **2**, and their derivatives. Each of the major biological effects, namely: wound healing, antitumor, antimicrobial, and antithrombotic properties will be considered in turn. References for other biological properties not discussed are given in Table 1.

5.1. Wound Healing and Anti-Inflammatory Effects

As described in the introduction, *AT* and *LE* roots have been used for the treatment of wounds since ancient times. Not until 1976, however, was this medicinal property confirmed experimentally, and the active components identified by Papageorgiou.^[200]

Many samples of *AT* taken from different locations were obtained, and the roots extracted.^[159] After further extraction and chromatographic separation, the initial hexane extract was divided into: waxes,^[201] fluorescent compounds, natural polymers,^[159] and pigments.^[2] These extracts were tested by topical application on skin ulcers induced in laboratory animals (for example, rats, cats, dogs). The pigment-containing fraction showed excellent healing effects, with the other fractions being completely inactive. Chemical analysis of the pigments identified the following esters of alkannin: β , β -dimethylacrylate (**9**),^[159] angelate (**11**),^[202] isovalerate (**7**),^[202] and the novel β -acetoxyisovalerate (**13**).^[203] Interestingly, free alkannin (**1**) was not detected during the isolation.^[204]

An ointment containing the alkannin esters extracted from *AT* root (HISTOPLASTIN RED and afterwards HELI-DERM) was formulated and patented following these successful animal trials.^[47, 205] This preparation was then tested clinically on patients suffering from indolent ulcers of the lower leg, which had resisted all previous attempts at treatment, being applied topically each day around the edge of the ulcer.^[206] The results were dramatic (Figure 12). After three to four weeks of treatment, there was proliferous growth of granulation tissue, and within five to six weeks, complete healing, or at least considerable reduction in ulcer size, was observed. The success rate was 80%, with no skin inflammation being observed at any stage of the treatment.



Figure 12. An indolent ulcer before (right) and after (left) four weeks of treatment with HISTOPLASTIN RED. The patient was a 62 year old woman and the ulcer on her right leg was previously unresponsive to treatment for ten years.

The efficacy of this ointment has been further tested for the treatment of burns, in which it was shown to be significantly superior to the drugs Betadin and Fucidin.^[207] It has also been applied successfully to the treatment of leprosy ulcers, decubitus, traumatic ulcers,^[208] and acute anal fissures.^[209]

Several in vivo animal studies have been undertaken in Japan to assess the efficacy of shikonin (**2**) and its derivatives on various aspects of wound healing. In 1977 Hayashi reported the results of extensive tests on rats with **2**, acetylshikonin (**15**), the Japanese preparation Shiunko, and crude ether extracts of “shikon” (*LE* root).^[210] Each increased proliferation of granuloma tissue induced by a subcutaneous cotton pellet implant. These preparations, when applied topically, also showed anti-inflammatory effects against acute edema induced by several methods, including histamine, serotonin, heat, and uv radiation, and promoted wound healing. Mild antipyretic and analgesic effects were also observed. He concluded that these drugs were effective for the treatment of cutaneous injuries.

Ozaki and co-workers compared Japanese “Shikon”, “Koushikon” (both of which are *LE* roots that contain mainly shikonin (**2**), and its esters), and “Nanshikon” (the root of *Macrotomia euchroma* PAULS which contains mainly alkannin esters). They found that the proliferation of granulation tissue induced by the ether extracts from these roots correlated well with the total naphthoquinone pigment content, and seemed to be independent of both the side-chain stereochemistry and the aliphatic ester group.^[211] A separate comparison of the anti-inflammatory activities of alkannin (**1**) and shikonin (**2**) also led to the conclusion that the absolute configuration has little influence on biological activity.^[212]

Seto and co-workers studied the effects of shikonin and several derivatives as pure compounds on various aspects of wound healing, including anti-inflammatory effects.^[162] Shikonin (**2**) was shown to be more active than acetylshikonin (**15**) with respect to acceleration of granuloma formation, but equally active to the pentaacetyl derivative MDS-004 (**80**; Figure 13). Furthermore, in contrast to **2** and **15**, MDS-004

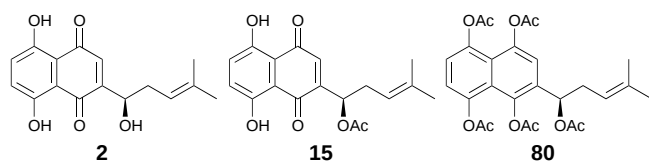


Figure 13. Structures of shikonin (**2**), acetylshikonin (**15**), and MDS-004 (**80**).

(**80**) showed strong inhibition when applied topically against delayed-type allergies (ear edema) induced by oxazolone and dinitrofluorobenzene. MDS-004 (**80**) has the significant marketing advantage of being a colorless compound, rather than a brightly colored pigment. Again, in contrast to **2** and **15**, MDS-004 was also orally active against carrageenan-induced hind paw edema, and exhibited a tendency to heal gastric ulcers induced by acetic acid. Thus, in these model studies, MDS-004 (**80**) was shown to have improved pharmacological properties relative to shikonin (**2**). Interestingly, and in contrast to the study by Hayashi,^[210] none of the derivatives tested showed any healing ability for incised and open wounds. No reports of clinical trials for either **80** or **2** have yet been published.

The anti-inflammatory effects of extracts from *Arnebia euchroma* (alkannin esters),^[213] and β,β -dimethylacrylalkannin (**9**) in particular,^[214] have been studied and have shown similar results to those previously described.^[162, 210, 212] More recently, shikonin (**2**) has been shown to inhibit the biosynthesis of leukotriene B_4 and 5-hydroxyeicosatetraenoic acid, which the authors suggested may play a major role in the mechanism of its anti-inflammatory effects.^[215]

In recent years, significant advances have been made in understanding the biochemistry of wound healing and tissue repair, which is a highly complex and delicate balance of degradative and regenerative processes.^[216] Although the efficacy of alkannin and shikonin derivatives have been demonstrated in vivo, their precise mode of action remains undetermined.

5.2. Antitumor Activity

In addition to the wound healing properties described above, *LE* root extracts have been used in Chinese traditional medicine for many years as a cancer treatment.^[217] Its use for this purpose, however, is missing from several current pharmacopoeias of Chinese medicine,^[18] and may be less common than the applications already described. While various types of *Anchusa* have been used for cancer treatment, dating back as far as the 12th century AD,^[218] *AT* appears not to have been used in this regard. Nevertheless, **1**, **2**, and their derivatives have been investigated as potential drug candidates for various aspects of cancer treatment within the last 25 years.

The increasing problem of cancer in the developed world, it now being second only to cardiovascular disease as a cause of death, has motivated the extensive growth of cancer research in recent years. Mass screening programs of natural products and synthetic compounds by the National Cancer Institute of

the USA have identified the quinone moiety as a pharmacophore that commonly affords cytotoxic activity.^[219] Of over 1500 quinones screened in one campaign in 1974, more than 10% exhibited significant in vivo (mice or rats) activity against at least one of the following cancer cells: L1210 leukemia, Walker carcinosarcoma 256 (W256), Adenocarcinoma 755, Sarcoma 180, Lewis lung carcinoma, and in vitro cytotoxicity against KB cells in culture. Among the active quinones tested, alkannin (**1**), its acetate (**5**), its isobutyrate (**6**), and its β,β -dimethylacrylate (**9**), all exhibited in vitro cytotoxicity against KB cells.^[219]

Alkannin derivatives **1**, **5**, **9**, and **28** (Table 1) isolated from the roots of *Arnebia nobilis* were previously shown to be active against W256 in rats and P388 lymphoid leukemia in mice.^[163, 220] The synthetic derivatives **83** and **85** (Scheme 5) were shown to have comparable activities.

Similar results were obtained by Sankawa et al.^[156] in Japan. Shikonin (**2**) and a range of simple derivatives completely inhibited tumor growth in mice at a dose of 5–10 mg kg⁻¹ day⁻¹. They found that **2** caused acute toxicity at higher doses (see Section 5.5), but was inactive at lower doses. More recently, however, it has been shown that while alkannin (**1**) and shikonin (**2**) both exhibit cytotoxic activity at high concentrations (100 μ g mL⁻¹–10 ng mL⁻¹), they displayed immunostimulating activities at extremely low concentrations (10 ng mL⁻¹–10 fg mL⁻¹) in vitro on human granulocytes and lymphocytes.^[221]

The chemopreventive properties of alkannin (**1**) and shikonin (**2**) derivatives have been examined by several other groups. Chemoprevention is based on the idea that non-carcinogenic, synthetic or natural products can inhibit the process of carcinogenesis. The mutagenic effects of picrolonic acid, benzo[a]pyrene,^[222] and 4-nitroquinoline-1-oxide^[223] on the bacterium *Salmonella typhimurium* TA98 was inhibited in vitro by extracts from *LE* root. Shikonin (**2**) has been shown to inhibit the early antigen activation by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) of the Epstein–Barr virus in Raji cells,^[224] although significant cytotoxicity was also noted at the concentrations tested. Chemopreventive effects have also been demonstrated in vivo, with shikonin (**2**) reducing the incidence of azoxymethane-induced intestinal tumors in rats.^[225]

Platinum complexes of naphthazarins, including **1**, **2**, and their derivatives, exhibit similar antitumor activities to cisplatin, but with lower nephrotoxicity.^[226] Copper complexes of shikonin (**2**) have been patented as DNA cleaving agents.^[227]

Several research groups have investigated the biological mechanism through which **1**, **2**, and their derivatives produce their cytotoxic activity. There are a number of ways in which quinones can disrupt cellular processes.^[228] One of the most commonly proposed mechanisms of quinone cytotoxicity is through “oxidative stress”, which arises from the capacity of these compounds to enter redox cycles. For example, quinones can undergo a one-electron reduction, catalyzed by enzymes such as NADPH-cytochrome P-450 reductase, to form a semiquinone radical.^[228] This radical is thought to initiate a redox cycle since it can readily autooxidize in the presence of dioxygen to regenerate the quinone and form the

superoxide radical anion. The generation of free radicals from this, or the semiquinone, by a chain mechanism may also cause cellular damage, which could contribute to their cytotoxicity.

It has been proposed that the reaction of quinones as electrophiles with cellular nucleophiles may be, at least partly, responsible for their cytotoxic behavior.^[228] Perhaps the most appealing of the cytotoxicity mechanisms for quinones, at least from a chemists viewpoint, is that of bioreductive alkylation.^[229] In this mechanism reduction of the molecule in vivo promotes the elimination of a suitably positioned leaving group to produce a highly potent alkylating agent. This electrophile may serve to capture cellular nucleophiles, such as glutathione or DNA, which may eventually lead to cell death. The process, shown in Figure 14 for **1**, **2**, and their derivatives, is well established, perhaps most famously for the antitumor natural product mitomycin C.^[230] Moore was the first to suggest that **1**, **2**, and their derivatives could function in this manner, although no evidence was presented to support this hypothesis.^[229]

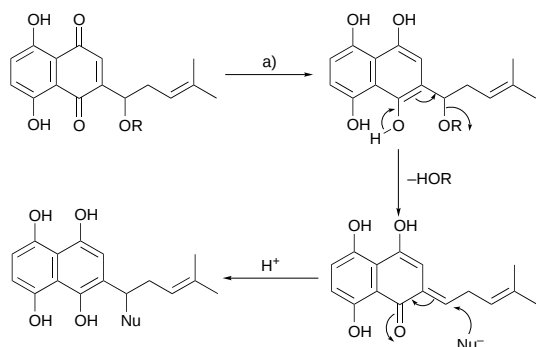


Figure 14. A plausible bioreductive alkylation mechanism of alkannin (**1**) and shikonin (**2**) (Moore).^[229] a) Reduction, Nu = nucleophile.

Inhibition of enzymes vital for cell metabolism or replication may also be a process through which quinones are toxic to cells. In this regard it has been demonstrated in vitro that shikonin (**2**) and its derivatives can interact strongly with DNA topoisomerases.^[160, 231] These enzymes control the topological state of DNA by concerted breaking and rejoining of DNA strands.^[232] Topoisomerases have been identified as targets for chemotherapy since they are involved in many important processes such as DNA replication, transcription, and recombination. A range of shikonin esters have been tested for their inhibitory effects on topoisomerase-I.^[160] The mechanism of action of the well-known anticancer agent camptothecin involves inhibition of this enzyme. A selection of typical examples is given in Table 4, which also shows that several of these derivatives are more potent in vitro inhibitors than camptothecin. In general, the shorter chain esters (C_2 – C_6) were more potent than longer chain derivatives. The selective inhibition of topoisomerase-I with no activity against topoisomerase-II (from Hella cells) was reported in this study.^[233] However, a separate study showed shikonin (**2**) can induce DNA cleavage mediated by topoisomerase-II (from calf thymus), through the formation of a cleaveable complex.^[231] Through the application of a DNA unwinding assay, these authors were also able to establish that **2** did not

Table 4. Inhibition of topoisomerase-I by shikonin esters.^[161]

Compound	R	IC ₅₀ [μM]
15	CH ₃	45 ± 3
146	CH ₂ =CHCH ₂	40 ± 3
147	CH ₃ (CH ₂) ₂	144 ± 10
148	CH ₃ (CH ₂) ₄	207 ± 15
149	CH ₃ (CH ₂) ₆	> 625
shikonin (2)	–	208 ± 10
camptothecin	–	127 ± 9

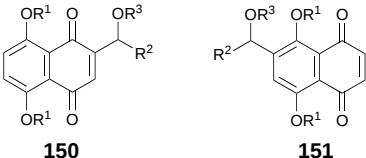
intercalate with the DNA. These studies suggest that the cytotoxic activity of **1**, **2**, and their derivatives may be due, at least in part, to their interactions with these important enzymes.

Recently, Ahn and co-workers have undertaken more in-depth investigations to elucidate the mechanism of cytotoxicity for **1**, **2**, and their derivatives.^[234] Furthermore, these researchers have prepared a series of analogues in an attempt to identify structure–activity relationships and subsequently to produce more active derivatives. These racemic analogues were synthesized by a “modified Terada method”.^[149, 153]

To test whether electrophilic arylation was an important mechanism for these compounds, Ahn and coworkers prepared 5,8-dimethoxy-1,4-naphthoquinone (DMNQ) derivatives and compared the biological data with those of the corresponding naphthazarin derivatives. They reasoned that this structural modification should increase the electrophilicity of the quinone moiety (as it prevents the rapid “tautomerization” present in the naphthazarins),^[137] but decrease the susceptibility of the naphthoquinone to one-electron reduction (since the semiquinone produced on reduction of the latter should achieve greater delocalization than the corresponding radical from a DMNQ derivative). Their ability to capture cellular nucleophiles was assessed by incubation of the quinones with glutathione. As predicted, the general order was: 6-substituted DMNQs > 2-substituted DMNQs > naphthazarins. When the cytotoxicity of 2-substituted naphthazarins **150a–c**, and DMNQ derivatives **150d–f** against L1210 cells were compared, however, the latter were found to be three- to tenfold less active (Table 5). From these experiments the authors deduced that redox cycling plays a prominent role in the cytotoxicity of 2-substituted naphthazarins.

In a protection study, tocopherol, an antioxidant which has been used to scavenge radicals derived from redox cycling, reduced the cytotoxicity of shikonin (**2**) by 25%, while aurintricarboxylic acid (ATA), a general inhibitor of endonucleases, gave a protective effect of 50% at 1 μM (Table 6).^[235] A combination of ATA and tocopherol produced a protective effect of 80%. These results suggest that at least two different mechanisms of cytotoxic action may be operating with these agents.

Further trends in cytotoxicity can be deduced from Table 5. Acetylation of the 2-substituted DMNQ derivatives **150d–f**

Table 5. Cytotoxicity and in vivo activity of shikonin analogues.^[234–238]


Compound	R ¹	R ²	R ³	ED ₅₀ [μg mL ⁻¹] ^[a]	T/C (%) ^[b]
2	H	3-methyl but-2-enyl	H	0.02	92.5
150 a	H	Bu	H	0.03	–
150 b	H	pentyl	H	0.04	–
150 c	H	<i>i</i> -pentyl	H	0.05	–
150 d	Me	Bu	H	0.41	–
150 e	Me	pentyl	H	0.37	–
150 f	Me	<i>i</i> -pentyl	H	0.15	–
150 g	Me	Bu	Ac	0.07	–
150 h	Me	pentyl	Ac	0.06	–
150 i	Me	<i>i</i> -pentyl	Ac	0.05	–
150 j	H	pentyl	Ac	–	255
150 k	H	nonyl	Ac	–	197
151 a	Me	Bu	H	0.05	141
151 b	Me	pentyl	H	0.06	110
151 c	Me	<i>i</i> -pentyl	H	1.10	–
151 d	Me	Me	Ac	0.09	–
151 e	Me	Bu	Ac	0.03	–
151 f	Me	<i>i</i> -pentyl	COPr	0.03	–
151 g	Me	<i>i</i> -pentyl	Ac	0.02	148
151 h	Me	<i>i</i> -pentyl	COPr	0.03	198
151 i	Me	<i>i</i> -pentyl	COpentyl	0.03	217
151 j	Me	<i>i</i> -pentyl	COheptyl	0.10	205

[a] Cytotoxicity was measured in vitro against L1210 cancer cells. [b] Life-span increase, relative to a control, for mice bearing S-180 cells (dose 10 mg kg⁻¹ day⁻¹).

Table 6. Protection from shikonin cytotoxicity.^[235]

Protective agent	Concentration ^[a]	Protection [%]
tocopherol	0.3 mM	25 ± 0.23
ATA	1 μM	50 ± 0.81
tocopherol and ATA	not given	80

[a] Concentration of protective agent. Shikonin concentration was 60 nM throughout.

leads to compounds **150 g–i**, which showed an approximately threefold increase in activity. The 6-substituted derivatives of DMNQ **151 a–c** showed cytotoxicities similar to those of the corresponding naphthazarin derivatives (**150 a–c**) (Table 5). By variation of R¹ and R³ a range of 6-substituted DMNQ analogues (**151 d–j**) were investigated. None of these were significantly more potent in vitro than **2** or any of the other analogues examined.

In vivo tests for many of the analogues were undertaken on mice with S-180 cells. A dose of 10 mg kg⁻¹ day⁻¹ was administered and the increase in life-span relative to a control was recorded. The results (Table 5) can be compared with the cytotoxicity values from in vitro screens, and those for shikonin (**2**) obtained from a previous study^[156a] with an identical dosage. There was no correlation between in vitro cytotoxicity and life-span increase. Nevertheless, the analogues tested, which have recently been patented,^[236] showed

improved antitumor properties over shikonin (**2**). The investigators concluded that binding to endonucleases, such as topoisomerase-I, may be the most plausible mode of in vivo antitumor action of these compounds. They suggest that making structural modifications that increase selective binding to endonucleases while decreasing the nonselective binding to cellular nucleophiles and participation in redox cycling may be the best approach for future developments. In order to achieve this, the question of stereochemistry should no longer be ignored.

Recent reports from the same group have concentrated along these lines in the study of the antitumor activity of arylacetyl esters of shikonin.^[237, 238] Cytotoxicities for 17 analogues were determined in vitro against A549, L1210, and K562 cancer cells. The analogues were found to be much less active against the solid tumor cell line A549 than the leukemic cells L1210 and K562. The cytotoxicity of these analogues against A549 cells was, however, correlated to their anti-cell adhesive properties,^[238] which they suggest may be their mode of action. The 4-(*N,N*-dimethylamino)phenylacetyl derivative of shikonin (**2**), which was synthesized with the aim of improving the water solubility, was one of the most active, showing a T/C value of 192 %.

While further research in the field is certain to continue, the emergence of a clinically useful anticancer agent remains to be seen.

5.3. Antimicrobial Activity

Over the past 30 years the antimicrobial activity of **1**, **2**, and their derivatives has been investigated by a number of groups. Crude preparations derived from plant roots containing these substances were initially shown to exhibit antibacterial and antifungal properties.^[239] This was not sufficient, however, to implicate **1**, **2**, and their derivatives as the active components of the plant roots, and systematic studies for each of the common sources of **1** and **2**, namely *LE*,^[82, 240] *AT*,^[164, 241] and *Arnebia nobilis*,^[171] were therefore undertaken to show that this is, indeed the case. Extracts from pigment-producing callus cultures of *LE* showed antibacterial activity similar to the plant roots.^[242]

In general, **1**, **2**, and their derivatives were found to be active against Gram-positive bacteria such as *Staphylococcus aureus*, *Enterococcus faecium*, and *Bacillus subtilis* with minimum inhibitory concentrations (MIC) of between 0.3 and 6.25 μg mL⁻¹.^[231, 240a,b, 241a,b, 243] They were inactive against Gram-negative bacteria, such as *Escherichia coli* and *Pseudomonas aeruginosa*.^[231, 240a,b, 241b] Plant extracts containing **1**, **2**, and their derivatives have also been shown to act against various species of lactic acid bacteria.^[244]

Although initial reports indicated that **1**, **2**, and their derivatives exhibit a bacteriostatic effect,^[245] a recent kinetic study has demonstrated that the action is, in fact, bactericidal.^[246] This same study showed that such derivatives are selectively toxic to methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* (MRSA and MSSA, respectively). Because of its low toxicity, it was suggested that shikonin (**2**) may be a useful therapeutic agent against MRSA.

There is generally less agreement in the literature concerning the antifungal properties of **1**, **2**, and their derivatives. Honda and co-workers have shown that shikonin (**2**) and deoxyshikonin (**32**), extracted from the cell cultures of *LE*, exhibited significant antidermatophytic properties against five fungi tested: *Saccharomyces sake*, *Trichophyton rubrum*, *T. mentagrophytes*, *T. tonsulans* var. *sulfureum*, *Microsporum gypseum*, and *Epidermophyton floccosum* (MIC < 25 $\mu\text{g mL}^{-1}$).^[247] The action was shown to be fungistatic rather than fungicidal. Deoxyshikonin (**32**) was generally more active. An ointment for the control of athlete's foot with this as the active component has been patented.^[248] Other antimicrobial preparations containing shikonin as the active principle have also been patented.^[249] Shikonin (**2**) and deoxyshikonin (**32**) were shown to be inactive (MIC > 200 $\mu\text{g mL}^{-1}$) against several other fungi, including *Candida albicans*.^[247] Several groups have also shown that **1**, **2**, and their derivatives were inactive against *Candida albicans*,^[231, 243, 247] while other researchers have shown them to be active.^[171a, 239, 241a] The crude ethanol extract from *Arnebia nobilis* has been tested in vivo on guinea pigs against experimentally induced *Candida albicans* infection.^[250] By topical application twice daily of 0.1 mL of a 5 % solution of the arnebines in polyethylene glycol, the infection was cleared within six days.

Shikonin (**2**) has been shown to exhibit a significant antiamebic effect in vitro on *Entamoeba histolytica*, but the therapeutic effect was weak when administered orally to rats with experimental intestinal amebiasis.^[251] Its activity against *Aedes aegypti* larvae has also been demonstrated.^[252]

Papageorgiou and co-workers studied structure–activity relationships for the antimicrobial properties of the constituents of *AT*,^[241b] and reached the following conclusions: 1) the antibacterial effect of the hexane extract arises from the naphthoquinone pigment, with the naphthazarin system being necessary for activity; 2) alkylation of the phenolic groups leads to complete loss of activity; 3) polymerization results in complete loss of activity; and 4) since **1**, **2**, and their esters are all active, the aliphatic side chain may act as a delivery system for the naphthazarin. It was thus suggested that screening a variety of ester derivatives may lead to products with greater potency, which is a goal that remains to be reached.

5.4. Antithrombotic Activity

Thrombus formation in humans can restrict blood flow to vital tissues or organs and cause peripheral, cerebral, or coronary ischemia. Embolism of the thrombosis can be catastrophic, and is a common cause of death. Platelet aggregation is considered to be a major pathogenic mechanism that leads to arterial thrombosis.

Arnebia euchroma is one of the so-called “vasoactive-antithrombotic” herbs of Chinese medicine. Recently, four compounds were isolated from crude extracts and shown to inhibit platelet aggregation induced by collagen. Although their structures were assigned as shikonin (**2**), acetylshikonin (**15**), β,β -dimethylacrylshikonin (**21**), and teracrylshikonin (**22**),^[253] they remain under suspicion, since alkannin deriva-

Table 7. Inhibition of collagen-induced platelet aggregation by alkannin (**1**) and esters.^[253]

Compound	Platelet aggregation [%] ^[a]	IC ₅₀ [$\mu\text{g mL}^{-1}$]
Control	89.0 $\pm$ 0.3	–
β,β -dimethylacrylshikonin (21)	21.0 $\pm$ 18.1	4.2
teracrylshikonin (22)	0	2.8
acetylshikonin (15)	0	2.1
shikonin (2)	67.5 $\pm$ 4.9	10.7

[a] Measured with a substrate concentration of 20 $\mu\text{g mL}^{-1}$.

tives (**1**, **5**, **9**, **10**) were isolated from this source (see Table 1, note 2).^[42, 44] Results of in vitro tests are presented in Table 7.

Acetylshikonin **15** also inhibits arachidonic acid induced platelet aggregation. It was suggested that **15** acts through suppression of phosphoinositide breakdown.^[253b]

5.5. Toxicity, Pharmacokinetics, and Other Pharmacological Aspects

In vitro and in vivo potencies are not the only factors to be considered if **1**, **2**, and/or their derivatives are to become therapeutically useful drugs. Aspects such as toxicity, metabolism, and formulation are also vitally important.

Alkannin (**1**) was shown to have low toxicity when administered orally in a feeding study.^[254] The LD₅₀ was 3 g kg^{−1} in mice and less than 1 g kg^{−1} in rats. No evidence of toxicity was observed when **1** was fed to mice for 15 weeks at 1 % of their diet (average total intake 3.38 g). Autopsies showed the animals had no Heinz bodies and their vital organs were of unchanged morphology. Alkannin was excreted in the urine and not deposited in abdominal fat. Similar results were demonstrated for shikonin (**2**) and acetylshikonin (**15**).^[210] Shikonin **2** and its derivatives are rather more toxic to mice by intraperitoneal administration. Results are summarized in Table 8.

Table 8. Toxicity of shikonin and esters to mice by intraperitoneal administration.

Compound	LD ₅₀ [mg kg ^{−1}]
shikonin (2)	20 $\pm$ 5 ^[210b]
acetylshikonin (15)	41 $\pm$ 10 ^[210b] ; 22.75 ^[213]
β,β -dimethylacrylshikonin (21)	48 $\pm$ 5 ^[214]

In addition, during in vivo testing of **1**, **2**, and their derivatives for antitumor effects, toxicity has been observed. Table 9 indicates the derivatives for which this was the case (dose administered intraperitoneally). Alkannin (**1**)^[255] and

Table 9. Toxic doses of **1**, **2** and their derivatives in mice and rats.

Compound	Toxic dose
shikonin (2)	10 $\times$ 5 mg kg ^{−1} day ^{−1} ^[156a]
shikonin (2)	30 mg kg ^{−1} day ^{−1} ^[156a]
alkannin (33)	30 mg kg ^{−1} day ^{−1} ^[156b]
alkannin leucoacetate	30 mg kg ^{−1} day ^{−1} ^[156b]
cycloalkannin diacetate	30 mg kg ^{−1} day ^{−1} ^[156b]
β,β -dimethylacrylalkannin (9)	10 mg kg ^{−1} day ^{−1} ^[220]
α -methoxyphenylacetyl shikonin	7.75 mg kg ^{−1} day ^{−1} ^[257]

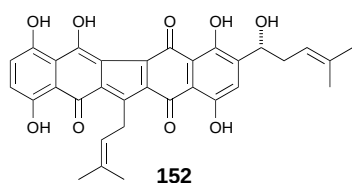
alkannin esters (**5–13**)^[200] are nonmutagenic according to the Ames test.^[256]

A pharmacokinetic study has been described using [³H]shikonin in mice.^[257] The absorption was rapid after both oral and intramuscular administration, with radioactivity detectable in plasma after about one minute. Other results are presented in Table 10. Within 96 hours of intravenous injection, the total radioactivity excreted was 40.5 % in the urine and 40.3 % in feces, with 3.6 and 7.7 %, respectively, being as the unchanged drug.

Table 10. Pharmacokinetic data for shikonin (**2**) in mice.^[257]

Administration	Peak level in plasma [min]	Bioavailability [%]
oral	5.78	34.3
intramuscular	7.62	64.7

As a predictive model to mimic the transformation of shikonin (**2**) in the human gastrointestinal tract, Kadota and co-workers have incubated **2** with *Bacteroides fragilis* subsp. *thetaotus* from human feces.^[258] Ten metabolites were detected: five monomers, including anhydroalkannin (**34**), deoxyshikonin (**32**), cycloshikonin (**73**), and five dimeric structures such as shikometabolin A (**152**).



Cheng and co-workers were able to dramatically improve the pharmaceutical properties of **1** and **2** through the formation of a 1:1 inclusion complex with hydroxypropyl- β -cyclodextrin.^[259] Water solubility was improved 200-fold and photochemical decomposition rate constants decreased from 0.329 to 0.156 h⁻¹. Furthermore, the release rate of **1** and **2** was enhanced dramatically at one tenth of the control preparation Shiunko ointment.

6. Summary and Outlook

The ancient medicinal properties claimed for *AT* and *LE* have, at least in part, been confirmed by scientific experimentation within the last 25 years. These investigations resulted in the identification of alkannin (**1**), shikonin (**2**), and related derivatives as the active components. The clinical application of preparations that contain ester derivatives of **1** extracted from *AT* for the treatment of burns and ulcers is, perhaps, the most dramatic development. The use of **1**, **2**, and related derivatives in other therapeutic areas, particularly in cancer chemotherapy, is still in development. Significant developments are to be expected in this area in the future.

The value of **2** in Japan motivated biotechnologists to develop the world's first manufacturing process utilizing plant cell cultures. The research in this area has provided a wealth of knowledge to the field of biotechnology. In addition, great

insights into the biosynthesis of these natural products and to our understanding of plant secondary metabolism in general, has been gained from this work.

The challenge of synthesizing these deceptively simple-looking compounds has kept a number of synthetic chemists occupied worldwide for many man-years. The pioneering work of Terada, and others in the field, laid the foundations upon which recent advances in the chemical synthesis of these substances relied. These developments should allow for many future adventures in the chemistry, biology and medicine of alkannin (**1**), shikonin (**2**), and related naphthazarins.

Addendum

Since the initial submission of this review a number of papers concerning various aspects of the chemistry and biology of alkannin and shikonin have appeared in the literature. To provide literature coverage up to November 30, 1998 these are included here under the following categories: medicinal properties,^[260] physical properties and analysis,^[261] cell cultures and biosynthesis,^[262] dyeing,^[263] and miscellaneous biological properties.^[264]

Recently, the wound-healing ability of HELIXDERM has been evaluated in further clinical trials. This study was undertaken at the Freie Universität of Berlin under the supervision of Prof. C. E. Orfanos, and showed that HELIXDERM gave very good results in terms of granulation and epithelization.

In addition, it has come to our attention that further research concerning the anticancer properties of "shikonin mixture" has been conducted in China. In vitro experiments were conducted which indicated that the pigment mixture was active against stomach and esophagus cancer cell lines.^[265] Perhaps more significantly, clinical trials have been conducted using shikonin mixture for 19 patients with later stage lung cancer. From this study the authors concluded that "Shikonin mixture is safe and effective for later-stage cancer".^[266] Further studies to test these claims are certainly necessary.

Appendix: List of Abbreviations Used

CDI	<i>N,N'</i> -carbonyldiimidazole
DCC	dicyclohexylcarbodiimide
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIBAL	diisobutylaluminum hydride
DIP-Cl	<i>B</i> -chlorodiisopinocampheylborane
DMAP	4-dimethylaminopyridine
DMF	<i>N,N</i> -dimethylformamide
<i>hν</i>	light
Ipc	isopinocampheyl
LDA	lithium diisopropylamide
MOM	methoxymethyl
MTBSTFA	<i>N</i> -methyl- <i>N</i> - <i>tert</i> -butyldimethylsilylacetamide
MTMSTFA	<i>N</i> -methyl- <i>N</i> -trimethylsilylacetamide
NBS	<i>N</i> -bromosuccinimide
py	pyridine
TBAF	tetrabutylammonium fluoride

TBS	<i>tert</i> -butyldimethylsilyl
THF	tetrahydrofuran
TMS	trimethylsilyl
Ts	<i>p</i> -toluenesulfonyl

V.P.P. greatly appreciates the help of Professor A. Sagredos and Assistant Professor A. Mellidis for their contribution during this project. Additionally, much credit is due to Dr. Christos Verelis, Under-Secretary of the Environment, Physical Planning, and Public Works of Greece, for his continuing interest and valuable assistance for the development of the pharmaceutical preparations HISTOPLASTIN RED and HELIXDERM. K.C.N and D.H. would like to thank Dr. Paul King, Mr. Christopher Boddy, and Mr. Nicolas Winssinger for assistance in the preparation of this manuscript. We thank Mr. K. Goto and Dr. T. Konoshima of Kyoto Pharmaceutical University, Parke-Davis, and Medpharm Scientific Publications for the use of illustrations. This work was financially supported by the National Institutes of Health (USA) and The Skaggs Institute for Chemical Biology.

Received: November 27, 1997

Revised version: February 26, 1998 [A261 IE]

German version: *Angew. Chem.* **1999**, *111*, 280–311

- [1] H. Brockmann, *Justus Liebigs Ann. Chem.* **1936**, 521, 1–47.
- [2] a) R. H. Thomson, *Naturally Occurring Quinones*, 2nd ed., Academic Press, New York, **1971**, pp. 248–251; b) R. H. Thomson, *Naturally Occurring Quinones III, Recent Advances*, Chapman & Hall, **1987**, pp. 219–223.
- [3] P. Weigle, *Ancient Dyes for Modern Weavers*, Watson-Guptil, New York, **1974**, pp. 50–52.
- [4] Translations of the works of Hippocrates by Francis Adams can be found in the *Internet Classics Archive*. For the use of alkanet, see <http://webatoms.com/Classics/Hippocrates/ulcers.10.10.html>.
- [5] Theophrastus, *Enquiry into Plants and Minor works on Odours and Weather Signs*, Vol. 2 (Translation by A. Hort), Harvard University Press, Cambridge, Massachusetts, **1949**, chap. 7.9.3, p. 113.
- [6] M. Wood, *In the Footsteps of Alexander the Great*, University of California Press, Los Angeles, **1997**, pp. 143–144.
- [7] P. Dioscorides in *De Materia Medica*, Vol. IV.23 (Ed.: Max Wellman), Berloni Apud Weidmannos, Berlin, **1958**, pp. 187–188; b) R. T. Gunther, *The Greek Herbal of Dioscorides*, Vol. IV, Hafner, New York, **1968**, p. 421.
- [8] The effect has also been written about for alkanet.^[11a]
- [9] Pliny, *The Natural History*, Vol. 4, Book XXII, chap. 23 (Translation by J. Bostock), H. T. Riley, George Bell, London, **1890**, p. 409.
- [10] Its use for wound healing is apparently recorded in an ancient Arabic treatise.^[220] Also see L. Boulous, *Medicinal Plants of North Africa*, Reference Publications, Algonac, Michigan, **1983**, pp. 35–37.
- [11] a) N. Culpeper, *The English Physitian Enlarged*, Churchill, London, **1695**, pp. 3–4; b) N. Culpeper, *Pharmacopoeia Londinensis*, Streeter, London, **1672**, *Roots*, p. 1.
- [12] R. C. Wren in *Potters New Cyclopaedia of Botanical Drugs and Preparations* (Ed.: R. W. Wren), Health, Devon, **1975**, pp. 11–12.
- [13] A. Y. Leung, S. Foster, *Encyclopedia of Common Natural Ingredients*, Wiley, New York, **1996**, pp. 19–20.
- [14] a) M. Wichtl, *Herbal Drugs and Phytopharmaceuticals: A Handbook for Practice on a Scientific Basis* (Ed.: N. G. Bisset), Medpharm, Stuttgart, **1994**, pp. 55–56; b) *Teedrogen und Phytopharmaka 3* (Ed.: M. Wichtl), Medpharm, Stuttgart, **1997**.
- [15] *The Merck Index*, 11th ed. (Ed.: S. Budavari), Merck, Rahway, NJ, **1989**, p. 43.
- [16] R. Hyatt, R. Feldman, *Chinese Herbal Medicine: Ancient Art and Modern Science*, Schocken Books, New York, **1978**, pp. 17–28.
- [17] B. E. Read, *Chinese Medicinal Plants from the Pen Ts'ao Kang Mu (AD 1596)*, 3rd ed., Peking Natural History Bulletin, Peking, **1936**, p. 38.
- [18] *A Barefoot Doctor's Manual* (Translation of a Chinese Instruction to Certain Chinese Health Personnel), US Government Printing Office, Washington, **1974**, p. 908; b) H.-Y. Hsu, W. G. Preacher, *Chinese Herb Medicine and Therapy*, Oriental Healing Arts Institute of USA, Los Angeles, **1982**, p. 147; c) *Handbook of Commonly Used Chinese Herbal Prescriptions*, (Translation by W. Yu-Ching), Oriental Healing Arts Institute, **1983**, p. 59; d) *The Pharmacopoeia of Japan*, Yakuji Nippo, Tokyo, **1982**, p. 1115.
- [19] For recent patents and publications related to this aspect, see a) S. Ootani, I. Takagishi (Pentel K. K.) JP-B 06336411, **1994** [*Chem. Abstr.* **1994**, 122, 142067p]; b) A. Hashimoto, A. Kusumi, K. Yamaguchi (Sunstar) JP-B 04029915, **1992** [*Chem. Abstr.* **1992**, 116, 241988x]; c) S. Shimoyama, T. Shimoyama, (Ihara Chemical Industry) WO-B 9201022, **1992** [*Chem. Abstr.* **1992**, 116, 196225h]; d) T. Okuda, (Amato Pharmaceutical Products) JP-B 01215861, **1989** [*Chem. Abstr.* **1990**, 112, 185548g]; e) S. Mori, H. Otsuke, S. Kida, H. Bando (Sunstar; Sanwa Chemical Industry) JP-B 01275520, **1989** [*Chem. Abstr.* **1990**, 112, 164775p]; f) M. Tsuboi, K. Matsui, Y. Ando, (Ichimura) JP-B 63083017, **1988** [*Chem. Abstr.* **1989**, 111, 45072b]; g) K. Hara, S. Murata, (Kao) JP-B 62270545, **1987** [*Chem. Abstr.* **1988**, 109, 197176k]; h) M. Tsuboi, Y. Ando, K. Matsui, (Ichimura) JP-B 62116520, **1987** [*Chem. Abstr.* **1988**, 108, 26820r]; i) K. Matsui, M. Tsuboi, Y. Ando, (Ichimura Co) JP-B 62089772, **1987** [*Chem. Abstr.* **1987**, 107, 204937y]; j) T. Morimoto, H. Ikeda, E. Inui, M. Hara, Y. Omura (Mitsui Petrochemical Industries) JP-B 62070305, **1987** [*Chem. Abstr.* **1987**, 107, 64658f]; k) T. Morimoto, H. Ikeda, E. Inui, M. Hara, (Mitsui Petrochemical Industries) JP-B 62070315, **1987** [*Chem. Abstr.* **1987**, 107, 46072f]; l) T. Morimoto, H. Ikeda, E. Inui, M. Hara, (Mitsui Petrochemical Industries; Sanwa Kagaku Kogyo K. K.) JP-B 61252299, **1986** [*Chem. Abstr.* **1987**, 106, 143807m]; m) T. Morimoto, H. Ikeda, E. Inui, M. Hara, (Mitsui Petrochemical Industries; Sanwa Kagaku Kogyo K. K.) JP-B 61252298, **1986** [*Chem. Abstr.* **1987**, 106, 143806k]; n) T. Morimoto, H. Ikeda, E. Inui, M. Hara, (Mitsui Petrochemical Industries; Sanwa Kagaku Kogyo K. K.) JP-B 61252297, **1986** [*Chem. Abstr.* **1987**, 106, 143805j]; o) T. Suzuki, S. Abe, (Shiesido) JP-B 61130233, **1986** [*Chem. Abstr.* **1986**, 105, 139645u]; p) T. Suzuki, S. Abe, (Shiesido) JP-B 61130232, **1986** [*Chem. Abstr.* **1986**, 105, 139644j]; q) A. Yamagata, H. Tezuka, (Guerlain Kaihatsu Kenkyusho K. K.) JP-B 61118306, **1986**, 105, 120746f]; r) T. Morimoto, H. Ikeda, (Mitsui Petrochemical Industries) JP-B 60169413, **1985** [*Chem. Abstr.* **1985**, 104, 24086z]; s) Mitsui Petrochemical Industries, JP 60094499, **1985** [*Chem. Abstr.* **1985**, 103, 106631s]; t) S. Kubo, E. Kawada (Shiesido) DE-B 3433648, **1985** [*Chem. Abstr.* **1985**, 103, 92640e]; u) Shiesido, JP-B 60058911, **1985** [*Chem. Abstr.* **1985**, 103, 76074t]; v) Ichimaru Farukosu K. K., JP 59010507, **1984** [*Chem. Abstr.* **1984**, 100, 144835g]; w) K. Matsui, H. Ando, T. Endo, JP-B 52094432, **1977** [*Chem. Abstr.* **1978**, 88, 27680a]; x) S. Kishimoto, K. Aota (Kanebo) JP-B 52027692, **1977** [*Chem. Abstr.* **1977**, 87, 189309i].
- [20] For some patents and publications, see a) C. Suga (Ichimaru Co.), JP-B 07216249, **1995** [*Chem. Abstr.* **1995**, 123, 259672]; b) C. Suga (Mitsui Petrochemical Industries) JP-B 06248581, **1994** [*Chem. Abstr.* **1994**, 122, 33501]; c) I. Fumoto, C. Suga (Mitsui Petrochemical Industries) JP-B 03012458, **1991** [*Chem. Abstr.* **1991**, 114, 249246]; d) M. Schallies, *Prax. Naturwiss. Chem.* **1989**, 38, 31–36; e) C. Suga (Mitsui Petrochemical Industries) JP-B 63156741, **1988** [*Chem. Abstr.* **1988**, 110, 40446]; f) E. Benk, *Riechst. Aromen Körperpflege*, **1967**, 17, 3–4.
- [21] R. Winter, *A Consumer's Dictionary of Food Additives*, Crown, New York, **1984**, p. 25.
- [22] a) R. Winter, *A Consumer's Dictionary of Cosmetic Ingredients*, Crown, New York, **1984**, p. 23; b) For a recent patent, see H. Lorenz (Goldwell) DE-B 4201749 [*Chem. Abstr.* **1993**, 119, 146341].
- [23] V. P. Papageorgiou, *Planta Med.* **1980**, 38, 193–203.
- [24] R. Majima, C. Kuroda, *Acta Phytochim. (Tokyo)* **1922**, 1, 43–65.
- [25] H. Arakawa, M. Nakazaki, *Chem. Ind. (London)* **1961**, 947.
- [26] H. Fukui, M. Tsukada, H. Mizukami, M. Tabata, *Phytochemistry* **1983**, 22, 453–456.
- [27] Y. Ikeda, N. Ishida, C. Fukaya, K. Yokoyama, M. Tabata, H. Fukui, G. Honda, *Chem. Pharm. Bull.* **1991**, 39, 2351–2352.

- [28] a) J. Pelletier, *Ann. Chim. Phys.* **1832**, 51, 191–192; b) J. Pelletier, *Ann.* **1833**, 6, 27.
- [29] P. Bolley, R. Wydler, *Justus Liebigs Ann. Chem.* **1847**, 62, 141.
- [30] K. Brand, A. Lohmann, *Ber. Dtsch. Chem. Ges. B* **1935**, 68, 1487–1494.
- [31] H. Raudnitz, W. Stein, *Ber. Dtsch. Chem. Ges. B* **1935**, 68, 1479–1484.
- [32] A. C. Jain, S. K. Mathur, *Bull. Nat. Inst. Sci. India* **1965**, 28, 52–56 [*Chem. Abstr.* **1967**, 66, 26143m].
- [33] H. A. Khan, I. Chandrasekharan, A. Ghanim, *Phytochemistry* **1983**, 22, 614–615.
- [34] T. S. El-Afly, N. D. El-Tanbouly, N. M. Sokkar, *Egypt. J. Pharm. Sci.* **1996**, 37, 65–70 [*Chem. Abstr.* **1997**, 126, 328044s].
- [35] R. Chaisuksant, I. Niopas, A. Voulgaropoulos, A. S. Mellidis, V. P. Papageorgiou, *Pharmazie* **1995**, 50, 363–364.
- [36] S. A. Fedoreev, O. E. Krivoshekova, V. A. Denisenko, P. G. Gorovoi, O. B. Maksimov, *Khim. Prir. Soedin* **1979**, 625–630 [*Chem. Abstr.* **1981**, 94, 80213a].
- [37] a) N. N. Boldyrev, *Farmatsiya* **1939**, 10, 24–25; *Khim. Ref. Zh.* **1940**, 5, 108 [*Chem. Abstr.* **1942**, 36, 39103].
- [38] P. De Leo, A. Miceli, L. Ronzini, L. Sanasi, R. Sgarra, *Agro Food Ind. Hi-Tech* **1996**, 7, 23–25.
- [39] V. P. Papageorgiou, *Planta Med.* **1979**, 37, 259–263.
- [40] V. P. Papageorgiou, A. S. Mellidis, A. N. Assimopoulou, A. Tsarbo-poulos, *J. Mass Spectrom.* **1998**, 33, 89–91.
- [41] A. S. Mellidis, V. P. Papageorgiou, *J. Nat. Prod.* **1987**, 50, 618–619.
- [42] S. Fu, T. Shang, P. Xiao, *Yaoxue Xuebao* **1984**, 19, 921–925 [*Chem. Abstr.* **1985**, 103, 11307b].
- [43] L. Shurong, M. Lu, *Yaowu Fenxi Zazhi* **1986**, 6, 280–282 [*Chem. Abstr.* **1986**, 106, 50609h].
- [44] S. Fu, P. Xiao, *Zhongcaoyao* **1986**, 17, 434–437 [*Chem. Abstr.* **1987**, 106, 55728f].
- [45] G.-S. Liu, *Yao Hsueh T'ung Pao* **1981**, 16, 14–15 [*Chem. Abstr.* **1981**, 95, 156425q].
- [46] N. Kirimer, B. Boazan, K. H. C. Baser, *Fitoterapia* **1995**, 66, 499–500 [*Chem. Abstr.* **1996**, 124, 284323j].
- [47] V. P. Papageorgiou, DE-B 2829744, **1979** [*Chem. Abstr.* **1979**, 91, 181441s].
- [48] M. Zhang, Y. Jin, L. Guo, Y. Cai, *Zhongcaoyao* **1989**, 20, 449–450 [*Chem. Abstr.* **1990**, 112, 33368k].
- [49] F. Zhu, F. Lu, G. Xiang, *Sepu* **1984**, 1, 131–133 [*Chem. Abstr.* **1985**, 103, 59142w].
- [50] F. Lu, Q. Xiang, F. Zhu, *Zhiwu Xuebao* **1983**, 25, 455–459 [*Chem. Abstr.* **1984**, 100, 99960f].
- [51] A. S. Romanova, A. I. Ban'kovskii, K. I. Boryaev, SU-B 200737, **1967** [*Chem. Abstr.* **1968**, 68, 62695t].
- [52] A. S. Romanova, A. I. Ban'kovskii, N. V. Tareeva, L. D. Marenova, K. I. Boryaev, *Lek. Rast.* **1969**, 15, 529–537 [*Chem. Abstr.* **1971**, 75, 85209w].
- [53] N. V. Tareeva, A. S. Romanova, A. I. Ban'kovskii, *Sb. Nauch. Rab., Vses. Nauchno-Issled. Inst. Lek. Rast.* **1970**, 1, 175–181 [*Chem. Abstr.* **1972**, 76, 70042h].
- [54] L. R. Shcherbanovskii, Yu. A. Luks, *Khim. Prir. Soedin.* **1974**, 513–514 [*Chem. Abstr.* **1975**, 82, 28587p].
- [55] A. S. Romanova, N. V. Tareeva, A. I. Ban'kovskii, *Khim. Prir. Soedin.* **1967**, 3, 71 [*Chem. Abstr.* **1967**, 67, 18548c].
- [56] J. A. Ballantine, *Phytochemistry* **1969**, 8, 1587–1590.
- [57] O. E. Krivoshekova, S. A. Fedoreev, V. A. Denisenko, O. B. Maksimov, P. G. Gorovoi, *Khim. Prir. Soedin* **1976**, 726–730 [*Chem. Abstr.* **1977**, 86, 86122b].
- [58] N. V. Tareeva, A. S. Romanova, A. I. Ban'kovskii, P. N. Kibal'chich, *Khim. Prir. Soedin.* **1966**, 2, 359–360 [*Chem. Abstr.* **1967**, 66, 79524t].
- [59] a) M. Kuhara, *J. Chem. Soc.* **1879**, 35, 22–25; b) M. Kuhara, *Ber. Dtsch. Chem. Ges.* **1878**, 11, 2143–2147.
- [60] C. Kuroda, *J. Tokyo Chem. Soc.* **1918**, 39, 1051–1115.
- [61] G. Bai, X.-J. Jin, *Chem. Res. Chin. Univ.* **1994**, 10, 263–265 [*Chem. Abstr.* **1995**, 122, 183154h].
- [62] S. Miura, *Shoyakugaku Zasshi* **1963**, 17, 45–49 [*Chem. Abstr.* **1965**, 61, 3065c].
- [63] A. S. Romanova, N. V. Tareeva, L. N. Pervykh, G. K. Kalashnikova, K. I. Boryaev, D. Pakalns, A. V. Patudin, *Khim. Prir. Soedin.* **1981**, 96 [*Chem. Abstr.* **1981**, 95, 3375y].
- [64] A. S. Romanova, A. I. Ban'kovskii, *Khim. Prir. Soedin.* **1965**, 226–227 [*Chem. Abstr.* **1965**, 63, 16773e].
- [65] A. S. Romanova, A. I. Ban'kovskii, N. V. Tareeva, K. I. Boryaev, I. A. Gubanov, SU-B 240933, **1969**, [*Chem. Abstr.* **1969**, 71, 53591r].
- [66] M. E. Pimenova, N. V. Tareeva, *Rastit. Resur.* **1980**, 16, 82–86 [*Chem. Abstr.* **1980**, 92, 143310m].
- [67] L. R. Shcherbanovskii, *Khim. Prir. Soedin.* **1972**, 8, 666 [*Biol. Abstr.* **1974**, 57, 4830].
- [68] L. R. Shcherbanovskii, *Khim. Prir. Soedin.* **1972**, 8, 238 [*Biol. Abstr.* **1973**, 55, 16427].
- [69] L. R. Shcherbanovskii, *Khim. Prir. Soedin.* **1971**, 4, 517–518 [*Chem. Abstr.* **1972**, 54, 51321].
- [70] A. S. Romanova, A. I. Ban'kovskii, K. I. Boryaev, N. V. Tareeva, SU-B 200735, **1967** [*Chem. Abstr.* **1969**, 68, 62694s].
- [71] M. Tsuboi, Y. Ando, K. Matsui, JP-B 62116520, **1985** [*Chem. Abstr.* **1988**, 108, 26820r].
- [72] L. N. Blagonravova, L. R. Shcherbanovskii, *Prikl. Biokhim. Mikrobiol.* **1974**, 10, 666–669 [*Chem. Abstr.* **1975**, 82, 27824b].
- [73] A. Crespa Ayuso, L. Crespa Ayuso, ES-B 356371, **1970** [*Chem. Abstr.* **1970**, 73, 44118f].
- [74] Y. Hashimoto, Y. Myama, A. Mizuchi, N. Fukazawa (Mitsui Toatsu Chemicals) JP-B 06340526 [*Chem. Abstr.* **1995**, 122, 178412m].
- [75] M. Afzal, G. Al-Oriquat, *Agric. Biol. Chem.* **1986**, 50, 1651–1652.
- [76] W. Cisowski, W. Dembska-Migas, J. Dzikowska, *Acta Pol. Pharm.* **1993**, 50, 443–446 [*Chem. Abstr.* **1994**, 121, 200860j].
- [77] S. Hisamichi, F. Yoshizaki, *Shoyakugaku Zasshi* **1982**, 36, 154–159 [*Chem. Abstr.* **1982**, 97, 178739p].
- [78] K.-H. Ai, F.-Y. Li, W. Wang, Y. Y. Wu, *Zhiwu Xuebao* **1989**, 31, 549–553 [*Chem. Abstr.* **1990**, 112, 115753w].
- [79] S. A. Fedoreyev, V. A. Denisenko, N. I. Kulesh, N. P. Krasovskaya, M. M. Kozynenko, V. P. Bulgakov, Yu. N. Zhuravlev, *Khim. Farm. Zh.* **1993**, 27, 33–37 [*Chem. Abstr.* **1994**, 120, 240061k].
- [80] I. Morimoto, T. Kishi, S. Ikegami, Y. Hirata, *Tetrahedron Lett.* **1965**, 4737–4739.
- [81] M. Afzal, G. Al Oriquat, *Agric. Biol. Chem.* **1986**, 50, 759–760.
- [82] K. Kyogoku, H. Terayama, Y. Tachi, T. Suzuki, M. Komatsu, *Shoyakugaku Zasshi* **1973**, 27, 24–30 [*Chem. Abstr.* **1974**, 80, 112549u].
- [83] M. Afzal, N. Muhammad, *Agric. Biol. Chem.* **1983**, 47, 411–412.
- [84] C. W. Sung, K. S. Liu, N. W. Li, *Yao Hsueh T'ung Pao* **1980**, 15, 3–5 [*Chem. Abstr.* **1981**, 94, 71295b].
- [85] These workers actually found the quinone pigments in aerial parts of the plant. N. A. Salam, T. Sarg, Y. Ibrahim, S. Khafagy, *Acta Pharm. Jugosl.* **1981**, 31, 237–241 [*Chem. Abstr.* **1982**, 96, 118993m].
- [86] I. Morimoto, Y. Hirata, *Tetrahedron Lett.* **1966**, 3677–3680.
- [87] M. Afzal, M. Tofeeq, *J. Chem. Soc. Perkin Trans 1* **1976**, 15, 1579–1582.
- [88] M. Afzal, M. Tofeeq, *J. Chem. Soc. Perkin Trans 1* **1975**, 14, 1334–1335.
- [89] P. Cong, *Yaoxue Xuebao* **1984**, 19, 450–454 [*Chem. Abstr.* **1985**, 103, 104350a].
- [90] Y. N. Shukla, J. S. Tandon, M. M. Dhar, *Indian J. Chem.* **1973**, 11, 528–529.
- [91] a) M. Tabata, H. Mizukami, N. Hiraoka, M. Konoshima, *Phytochemistry* **1974**, 13, 927–932; b) M. Konoshima, H. Mizukami, M. Tabata, *Shoyakugaku Zasshi* **1974**, 28, 74 [*Biol. Abstr.* **1976**, 61, 51525].
- [92] E. M. Linsmaier, F. Skoog, *Physiol. Plant* **1965**, 18, 100–127.
- [93] M. Tabata, H. Mizukami, N. Hiraoka, M. Konoshima, *Phytochemistry* **1983**, 22, 2451–2453.
- [94] a) Y. Fujita, Y. Hara, C. Suga, T. Morimoto, *Plant Cell Rep.* **1981**, 1, 59–60; b) Y. Fujita, Y. Hara, T. Ogino, C. Suga, *Plant Cell Rep.* **1981**, 1, 61–63; c) L. Heide, N. Nishioka, H. Fukui, M. Tabata, *Phytochemistry* **1989**, 28, 1873–1877; d) H. Mizukami, M. Konoshima, M. Tabata, *Phytochemistry* **1977**, 16, 1183–1186.
- [95] M. Tani, H. Fukui, M. Shimomura, M. Tabata, *Phytochemistry* **1992**, 31, 2719–2723.
- [96] N. Yoshikawa, H. Fukui, M. Tabata, *Phytochemistry* **1986**, 25, 621–622.
- [97] K. Yazaki, H. Fukui, M. Kikuma, M. Tabata, *Plant Cell Rep.* **1987**, 6, 131–134.
- [98] Y. Fujita, N. Yoshikawa, M. Tabata, *Phytochemistry* **1984**, 23, 301–305.

- [99] a) H. Mizukami, M. Konoshima, M. Tabata, *Phytochemistry* **1978**, *17*, 95–97; b) Y. Fujita, S. Takahashi, Y. Yamada, *Agric. Biol. Chem.* **1985**, *49*, 1755–1759; c) Y. Fujita, M. Tabata, *Plant Biol.* **1987**, *3*, 169–185 (Plant Tissue Cult.); d) Y. Fujita, S. Takahashi, Y. Yamada, *Eur. Congr. Biotechnol.*, 3rd, Vol. 1, VCH, Weinheim, **1984**, pp. 161–166.
- [100] Y. Fujita, Y. Hara, *Agric. Biol. Chem.* **1985**, *49*, 2071–2075.
- [101] M. E. Curtin, *Biotechnology* **1983**, *1*, 649–657.
- [102] For patents relating to this process, see a) Y. Motoyama (Mitsui Petrochemical Industries) JP-B 03176454, **1991** [*Chem. Abstr.* **1991**, *115*, 231894d]; b) Mitsui Petrochemical Industries, JP-B 58101687, **1983** [*Chem. Abstr.* **1983**, *99*, 156862n]; c) Mitsui Petrochemical Industries, JP-B 57063082, **1982** [*Chem. Abstr.* **1982**, *97*, 108555b]; d) Mitsui Petrochemical Industries, JP-B 57063081, **1982** [*Chem. Abstr.* **1982**, *97*, 90440y]; e) Mitsui Petrochemical Industries, EP-B 71999, **1983** [*Chem. Abstr.* **1983**, *98*, 196387]; f) Mitsui Petrochemical Industries, JP-B 57039779 **1982** [*Chem. Abstr.* **1982**, *97*, 4700d]; g) Mitsui Petrochemical Industries, JP-B 57039778, **1982** [*Chem. Abstr.* **1982**, *97*, 4699k].
- [103] a) H. N. Chang, S. J. Sim, *Biotechnol. Agric. For.* **1996**, *38*, 233–242 (Plant Protoplasts and Genetic Engineering VII); b) W. W. Su, *Appl. Biochem. Biotechnol.* **1995**, *50*, 189–230; c) H. J. Chi, *Hwahak Sekye* **1994**, *34*, 1063–1064 [*Chem. Abstr.* **1995**, *123*, 54169p]; d) O. Sahai, *Bioprocess Prod. Flavor Fragrance Color Ingredients* **1994**, 239–275; d) D. Fang, S. Hou, X. Li, *Zhongcaoyao* **1992**, *23*, 209–212 [*Chem. Abstr.* **1992**, *117*, 76285b]; f) Y. L. Chan, L. F. Liu, *Kexue Nongye (Taipei)* **1991**, *39*, 181–185; g) Y. Fujita, C. Suga, *Fragrance J.* **1991**, *19*, 33–36; h) Y. Fujita, *Kagaku to Kagyo (Tokyo)* **1990**, *43*, 1102–1104 [*Chem. Abstr.* **1990**, *113*, 170344j]; i) T. Vanek, *Chem. Listy* **1989**, *83*, 287–300 [*Chem. Abstr.* **1989**, *110*, 189343t]; j) H. X. Fan, W. H. Zhu, *Xiaoxue Xuebao* **1988**, *23*, 716–720 [*Chem. Abstr.* **1989**, *110*, 91988g]; k) Y. Fujita, *Ciba Found. Symp.* **1988**, *137*, 228–238 (Appl. Plant Cell Tissue Cult.); l) V. Petiard, P. Steck, *NATO ASI Ser. Ser. A* **1987**, *139*–153 (Perspect. Biotechnol.); m) Y. Fujita, *Kagaku Kogyo* **1987**, *38*, 405–409 [*Chem. Abstr.* **1987**, *107*, 57303m]; n) Y. Fujita, C. Suga, K. Matsubara, Y. Hara, *Nippon Nogei Kagaku Kaishi* **1986**, *60*, 849–854 [*Chem. Abstr.* **1987**, *106*, 16951d]; o) M. Tabata, Y. Fujita in *Biotechnology in Plant Science* (Eds.: P. R. Day, M. Zaitlin, A. Hollaender), Academic Press, Florida, **1985**, p. 207; p) M. Tabata, *Yaoxue Tongbao* **1985**, *20*, 519–521 [*Chem. Abstr.* **1986**, *104*, 166825p]; q) P. Brodelius, *Hereditas (Lund Swed.)* **1985**, (Suppl. 3), 73–81; r) Y. Fujita, T. Morimoto, *Saibo Kogaku* **1985**, *4*, 405–411 [*Chem. Abstr.* **1985**, *103*, 140168f]; s) Y. Fujita, *Yuki Gosei Kagaku Kyokashii* **1985**, *43*, 1003–1012 [*Chem. Abstr.* **1986**, *104*, 49737a]; t) P. F. Heinsteins, *J. Nat. Prod.* **1985**, *48*, 1–9.
- [104] From *Alkanna tinctoria*, see a) H. Urbanek, K. Bergier, S. Katarzynna, M. Saniewski, J. Patkowski, *Plant Cell Rep.* **1996**, *15*, 637–641; b) G. Mita, C. Gerardi, A. Miceli, R. Bollini, P. De Leo, *Plant Cell Rep.* **1994**, *13*, 406–410.
- [105] From *Onosma paniculatum*, see a) W. Ning, T. Yu, R. Cao, *Zhiwu Shengli Xuebao* **1996**, *22*, 74–80 [*Chem. Abstr.* **1996**, *125*, 190281n]; b) W. Wen, R. Cao, *Nanjing Daxue Xuebao, Ziran Kexue* **1995**, *31*, 334–337 [*Chem. Abstr.* **1996**, *124*, 82234v]; c) W. Ning, R. Q. Cao, *3rd Met. Ions Biol. Med. Proc. Int. Symp.* (Libbey, Montrouge, France) **1994**, 473–476 [*Chem. Abstr.* **1995**, *122*, 286710r]; d) R. Cao, Q. Zhao, *Biochem. Eng. 2001, Proc. Asia-Pac. Biochem. Eng. Conf.* (Eds.: S. Furusaki, I. Endo, R. Matsuno), Springer, Tokyo, **1992**, pp. 286–288 [*Chem. Abstr.* **1993**, *119*, 137416k]; e) L. Zhou, G. Zheng, S. Wang, *Yunnan Zhiwu Yanjiu* **1991**, *13*, 315–320 [*Chem. Abstr.* **1992**, *117*, 44645c]; f) L. Zhou, G. Zheng, S. Wang, F. Gan, *Tianran Chanwu Yanjiu Yu Kaifa* **1991**, *3*, 34–38 [*Chem. Abstr.* **1991**, *115*, 252160b]; g) R. Zhu, R. Cao, M. Wang, D. Pan, Z. Du, *Zhiwu Xuebao* **1990**, *32*, 749–752 [*Chem. Abstr.* **1991**, *114*, 225685z].
- [106] From *Onosma echioides*, see S. Koul, M. Sambyal, R. K. Khajuria, S. M. Jain, *Fitoterapia* **1993**, *64*, 552–553 [*Chem. Abstr.* **1994**, *121*, 78272b].
- [107] From *Arnebia euchroma*, see a) S. Chen, S. Huo, *Tianran Chanwu Yanjiu Yu Kaifa* **1994**, *6*, 97–100 [*Chem. Abstr.* **1995**, *122*, 185416u]; b) L. V. Kozlovitseva, G. V. Zaitseva, S. E. Strogov, *Biotechnologia* **1994**, *3*, 24–26 [*Chem. Abstr.* **1994**, *121*, 228870m]; c) O. V. Zakhlenjuk, *Biopolim. Kletka* **1994**, *10*, 32–37 [*Chem. Abstr.* **1995**, *122*, 235505v]; d) J.-W. Dong, H.-C. Ye, X. Wu, G.-F. Li, Z.-R. Wu, L.-M. Gu, J.-L. Chen, *Zhiwu Xuebao*, **1993**, *35*, 57–61 [*Biol. Abstr.* **1993**, *96*, 60965]; e) G. Xiang, F. Lu, F. Zhu, G. Li, H. Ye, J. Dong, X. Wu, J. Chen, *Zhiwu Xuebao* **1992**, *34*, 470–474 [*Chem. Abstr.* **1993**, *118*, 98068f]; f) V. N. Davydenkov, A. V. Patudin, Yu. G. Popov, S. A. Rabinovich, A. I. Miroshnikov, *Khim. Farm. Zh.* **1991**, *25*, 53–55 [*Chem. Abstr.* **1991**, *115*, 47729c]; g) H. Ye, Z. Yin, G. Li, X. Wu, J. Dong, Z. Wu, *Zhiwu Xuebao* **1991**, *33*, 927–931 [*Chem. Abstr.* **1992**, *117*, 23461z].
- [108] For advances since 1990, see a) P. Lui, Z. Wei, Z. Xu, X. Chu, R. Cao, *Zhiwu Shengli Xuebao* **1996**, *22*, 243–250 [*Chem. Abstr.* **1997**, *127*, 92755s]; b) W. Ning, R. Cao, *Zhiwu Xuebao* **1996**, *38*, 367–374 [*Chem. Abstr.* **1997**, *126*, 211065h]; c) S. Liang, J. Gao, K. Gao, S. Qi, L. Lin, *Yaowu Shengwu Jishu* **1996**, *34*, 229–234 [*Chem. Abstr.* **1997**, *126*, 143342u]; d) X.-M. Luo, L. Yuan, M. Zhou, P. Quyang, *Nanjing Huangong Daxue Xuebao* **1996**, *18*, 12–16 [*Chem. Abstr.* **1997**, *126*, 88324n]; e) V. P. Bulgakov, M. V. Khodakovskaya, M. M. Kozyr-enko, N. V. Labetskaya, Yu. N. Zhuravlev, *Prikl. Biokhim. Mikro-biol.* **1996**, *32*, 453–457 [*Chem. Abstr.* **1997**, *126*, 6523q]; f) X. Luo, L. Yuan, M. Zhou, P. Ouyang, *Huaxue Gongye Yu Gongcheng (Tianjin)* **1996**, *13*, 1–6 [*Chem. Abstr.* **1996**, *125*, 56298v]; g) X. Luo, L. Yuan, M. Zhou, P. Ouyang, *Nanjing Huangong Xueyuan Xuebao* **1995**, *17*, 60–64 [*Chem. Abstr.* **1996**, *124*, 53782s]; h) L. Yuan, X. Luo, M. Zhou, P. Ouyang, *Nanjing Huangong Xueyuan Xuebao* **1995**, *17*, 78–82 [*Chem. Abstr.* **1996**, *124*, 53780q]; i) H. Lu, Q. Zhao, R. Cao, Z. Xia, *Zhiwu Shengli Xuebao* **1995**, *21*, 111–116 [*Chem. Abstr.* **1995**, *123*, 254619x]; j) X. Luo, L. Yuan, P. Ouyang, *Huaxue Fanying Gongcheng Yu Gongyi* **1995**, *11*, 192–197 [*Chem. Abstr.* **1995**, *123*, 196682z]; k) G. V. Zaitseva, S. E. Strogov, D. B. Travnikov, N. V. Dudnik, E. M. Fetisova, *Biotechnologia* **1994**, 24–27 [*Chem. Abstr.* **1995**, *122*, 131066m]; l) L. Yuan, M. Zhou, P. Ouyang, *Nanjing Huangong Xueyuan Xuebao* **1994**, *16*, 16–19 [*Chem. Abstr.* **1994**, *121*, 253761j]; m) W. Ning, R. Cao, *Shengwu Gongcheng Xuebao* **1994**, *10*, 76–80 [*Chem. Abstr.* **1994**, *121*, 155797f]; n) L. Yuan, P. Ouyang, *Nanjing Huangong Xueyuan Xuebao*, **1992**, *14*, 44–47 [*Chem. Abstr.* **1994**, *120*, 75536j]; o) L. Yuan, P. Ouyang, *Huaxue Fanying Gongcheng Yu Gongyi* **1992**, *8*, 379–384 [*Chem. Abstr.* **1994**, *120*, 52716d]; p) V. Srinivasan, D. D. Y. Ryu, *Biotechnol. Bioeng.* **1993**, *42*, 793–799; q) L. Yuan, P. Ouyang, *Biochem. Eng. 2001, Proc. Asia-Pac. Biochem. Eng. Conf.* (Eds.: S. Furusaki, I. Endo, R. Matsuno), Springer, Tokyo, **1992**, pp. 258–261 [*Chem. Abstr.* **1993**, *119*, 137409k]; r) H. N. Chang, S. J. Sim, *Biochem. Eng. 2001, Proc. Asia-Pac. Biochem. Eng. Conf.* (Eds.: S. Furusaki, I. Endo, R. Matsuno), Springer, Tokyo, **1992**, pp. 237–241 [*Chem. Abstr.* **1993**, *119*, 137405f]; s) W. T. Seo, Y. H. Park, T. B. Choe, *J. Microbiol. Biotechnol.* **1992**, *2*, 41–45; t) S. J. Sim, H. N. Chang, *Biotechnol. Lett.* **1993**, *15*, 145–150; u) D. J. Kim, H. N. Chang, *5th Proc. Eur. Congr. Biotechnol.* (Munksgaard, Copenhagen) **1990**, *1*, 124–127; v) V. Srinivasan, D. D. Y. Ryu, *Biotechnol. Bioeng.* **1992**, *40*, 69–74; w) K. Shimomura, H. Sudo, H. Saga, H. Kamada, *Plant Cell Rep.* **1991**, *10*, 282–285; x) Y. H. Park, T. Woen, J. R. Liu, *J. Ferment. Bioeng.* **1990**, *70*, 317–321; y) D. J. Kim, H. N. Chang, *Biotechnol. Bioeng.* **1990**, *36*, 460–466; z) D. J. Kim, H. N. Chang, *Biotechnol. Lett.* **1990**, *12*, 443–446; aa) D. J. Kim, H. N. Chang, *Biotechnol. Lett.* **1990**, *12*, 289–294.
- [109] For a recent and more detailed review, see M. Tabata, *Shokubutsu Soshiki Baiyo* **1996**, *13*, 117–125 [*Chem. Abstr.* **1996**, *125*, 243054z].
- [110] J. Mann, *Chemical Aspects of Biosynthesis*, Oxford Chemistry Primers, No. 20 (Ed.: S. G. Davies), Oxford University Press, Oxford, **1994**, pp. 31–52.
- [111] R. Löschler, L. Heide, *Plant Physiol.* **1994**, *106*, 271–279.
- [112] a) K. Yazaki, L. Heide, M. Tabata, *Phytochemistry* **1991**, *30*, 2233–2236; b) J.-P. Schnitzler, J. Madlung, A. Rose, H. U. Seitz, *Planta* **1992**, *188*, 594–600; c) C. J. French, C. P. Vance, G. H. N. Towers, *Phytochemistry* **1976**, *15*, 564–566.
- [113] R. E. Olsen, H. Rudney, *Vitam. Horm. (NY)* **1983**, *40*, 1–43.
- [114] a) R. E. Beyer, K. Nordenbrand, L. Ernstner, *Chem. Scr.* **1987**, *27*, 145–153; b) G. W. Burton, K. U. Ingold, *Acc. Chem. Res.* **1986**, *19*, 194–201; c) S. Kasperek in *Vitamin E: A Comprehensive Treatise, Vol. 1* (Ed.: L. J. Machlin), Marcel Dekker, New York, **1980**, pp. 7–65.
- [115] a) H. V. Schmidt, M. H. Zenk, *Tetrahedron Lett.* **1971**, 4151–4155; b) H. Inouye, S. Ueda, K. Inoue, H. Matsumura, *Phytochemistry*

- 1979**, 18, 1301–1308; c) T. Okamoto, K. Yazaki, M. Tabata, *Phytochemistry* **1995**, 38, 83–88.
- [116] K. Yazaki, H. Fukui, M. Tabata, *Phytochemistry* **1986**, 25, 1629–1632.
- [117] S. Gaisser, L. Heide, *Phytochemistry* **1996**, 41, 1065–1072.
- [118] K. Yazaki, H. Fukui, M. Tabata, *Chem. Pharm. Bull.* **1986**, 34, 2290–2293.
- [119] a) F. Yoshizaki, S. Hisamichi, Y. Kondo, Y. Sato, S. Nozoe, *Chem. Pharm. Bull.* **1982**, 30, 4407–4411; b) X.-S. Yao, Y. Ebizuka, H. Noguchi, F. Kiuchi, H. Seto, U. Sankawa, *Tetrahedron Lett.* **1983**, 24, 2407–2410.
- [120] H. Inouye, H. Matsumura, M. Kawasaki, K. Inoue, M. Tsukada, M. Tabata, *Phytochemistry* **1981**, 20, 1701–1705.
- [121] H. Fukui, M. Tani, M. Tabata, *Phytochemistry* **1992**, 31, 519–521.
- [122] X.-S. Yao, Y. Ebizuka, H. Noguchi, F. Kiuchi, M. Shibuya, Y. Itaka, H. Seto, U. Sankawa, *Chem. Pharm. Bull.* **1991**, 39, 2956–2961.
- [123] a) L. Heide, M. Tabata, *Phytochemistry* **1987**, 6, 1645–1650; b) L. Heide, M. Tabata, *Phytochemistry* **1987**, 6, 1651–1655; c) R. Boehm, S.-M. Li, M. Melzer, L. Heide, *Phytochemistry* **1997**, 44, 419–424.
- [124] L. Wessjohann, B. Sonntag, *Angew. Chem.* **1996**, 108, 1821–1823; *Angew. Chem. Int. Ed. Engl.* **1996**, 35, 1697–1699.
- [125] M. Tabata, K. Yazaki, Y. Nishikawa, F. Yoneda, *Phytochemistry* **1993**, 32, 1439–1442.
- [126] Y. Yamaga, K. Nakanishi, H. Fukui, M. Tabata, *Phytochemistry* **1993**, 32, 633–636.
- [127] A. Bechthold, U. Berger, L. Heide, *Arch. Biochem. Biophys.* **1991**, 288, 39–47.
- [128] K. Yazaki, K. Inushima, M. Kataoka, M. Tabata, *Phytochemistry* **1995**, 38, 1127–1130.
- [129] S. Sommer, K. Severin, B. Camara, L. Heide, *Phytochemistry* **1995**, 38, 623–627.
- [130] M. Moir, R. H. Thomson, *Phytochemistry* **1973**, 12, 1351.
- [131] A. Ohta, P. M. Sivalingham, S. Lin, N. Ikekawa, N. Yaginuma, Y. Inada, *Toxicon* **1973**, 11, 235–241.
- [132] L. F. Fieser, *J. Am. Chem. Soc.* **1928**, 50, 439–465.
- [133] K. Zahn, P. Ochwat, *Justus Liebigs Ann. Chem.* **1928**, 462, 72–97.
- [134] H. Brockmann, K. Müller, *Justus Liebigs Ann. Chem.* **1939**, 540, 51–72.
- [135] J. R. Lewis, J. G. Paul, *Z. Naturforsch. B* **1977**, 32, 1473–1475.
- [136] C. A. Tsipis, M. P. Sigalas, V. P. Papageorgiou, M. N. Bakola-Christianopoulou, *Can. J. Chem.* **1983**, 61, 1500–1504, and references therein.
- [137] R. E. Moore, P. J. Scheuer, *J. Org. Chem.* **1966**, 31, 3272–3283.
- [138] S. Bratan, F. Strobusch, *J. Mol. Struct.* **1980**, 61, 409–414.
- [139] *Elsevier's Encyclopaedia of Organic Chemistry, Series III, Vol. 12B*, (Ed.: F. Radt), pp. 3184–3188, and references therein.
- [140] S. Yoshino, K. Hayakawa, K. Kanematsu, *J. Org. Chem.* **1981**, 46, 3841–3846.
- [141] M. Aso, K. Kanematsu, *Chem. Pharm. Bull.* **1993**, 41, 1549–1556.
- [142] D. B. Bruce, R. H. Thomson, *J. Chem. Soc.* **1952**, 2759–2766.
- [143] M. S. Pearson, B. J. Jensch, F. X. Greer, J. P. Hagstrom, N. M. Wells, *J. Org. Chem.* **1978**, 43, 4617–4622.
- [144] R. Huot, P. Brassard, *Can. J. Chem.* **1974**, 52, 838–842.
- [145] Y. Tanoue, A. Terada, H. Taniguchi, T. Okuma, H. Kaai, M. Anan, Y. Kakara, M. Doi, S.-I. Morishita, *Bull. Chem. Soc. Jpn.* **1993**, 66, 3712–3715.
- [146] C. G. Pierpont, L. C. Francesconi, D. N. Hendrickson, *Inorg. Chem.* **1978**, 17, 3470–3477.
- [147] D. B. Bruce, R. H. Thomson, *J. Chem. Soc.* **1955**, 1089–1096.
- [148] T. H. Smith, H. Y. Wu, *J. Org. Chem.* **1982**, 47, 1974–1976, and references therein.
- [149] A. Terada, Y. Tanoue, A. Hatada, H. Sakamoto, *Bull. Soc. Chem. Jpn.* **1987**, 60, 205–213.
- [150] M. Kawasaki, F. Matsuda, S. Terashima, *Tetrahedron Lett.* **1986**, 27, 2145–2148.
- [151] a) M. N. Bakola-Christianopoulou, V. P. Papageorgiou, K. K. Apazidou, *Phosphorous Sulfur Silicon Relat. Elem.* **1994**, 88, 53–65; b) M. N. Bakola-Christianopoulou, V. P. Papageorgiou, K. K. Apazidou, *J. Chromatogr.* **1993**, 645, 293–301.
- [152] M. N. Bakola-Christianopoulou, K. K. Apazidou, *J. Org. Chem.* **1996**, 61, 1850–1853.
- [153] E. A. Couladouros, Z. F. Plyta, V. P. Papageorgiou, *J. Org. Chem.* **1996**, 61, 3031–3033.
- [154] a) J. F. M. de Bie, R. M. Peperzak, M. J. Daenen, H. W. Scheeren, *Tetrahedron* **1993**, 49, 6463–6472, and references therein; b) Y. Naruta, K. Maruyama in *The Chemistry of the Quinonoid Compounds, Vol. II* (Ed.: S. Patai, Z. Rapaport), Wiley, New York, **1988**, pp. 277–303.
- [155] C. Coffey in *Rodd's Chemistry of Carbon Compounds, IIIG*, Elsevier, Amsterdam, **1978**, p. 238.
- [156] a) U. Sankawa, Y. Ebizuka, T. Miyazaki, Y. Isomura, H. Otsuka, S. Shibata, M. Inomata, F. Fukuoka, *Chem. Pharm. Bull.* **1977**, 25, 2392–2395; b) U. Sankawa, H. Otsuka, Y. Kataoka, Y. Iitaka, A. Hoshi, K. Kureitani, *Chem. Pharm. Bull.* **1981**, 29, 116–122.
- [157] a) H.-W. Cheng, F.-A. Chen, H.-C. Hsu, C.-Y. Chen, *Int. J. Pharm.* **1995**, 120, 137–144; b) F.-A. Chen, H.-W. Cheng, A.-B. Wu, H.-C. Hsu, C.-Y. Chen, *Chem. Pharm. Bull.* **1996**, 44, 249–251.
- [158] L. M. Van Der Vijver, K. W. Gerritsma, *J. Chromatogr.* **1975**, 114, 443–450.
- [159] V. P. Papageorgiou, *Chem. Chron.* **1978**, 7, 45–54.
- [160] B.-Z. Ahn, K.-U. Baik, G.-R. Kweon, K. Lim, B.-D. Hwang, *J. Med. Chem.* **1995**, 38, 1044–1047.
- [161] J. Otsuki, K. Otsuki, JP-B 7240788, **1972** [*Chem. Abstr.* **1973**, 78, 15906j].
- [162] Y. Seto, S. Motoyoshi, H. Nakamura, J. Imuta, T. Ishitoku, S. Isayama, *Yakugaku Zasshi* **1992**, 112, 259–271 [*Chem. Abstr.* **1992**, 117, 124158].
- [163] S. B. Katti, Y. N. Shukla, J. S. Tandon, *Indian J. Chem. Sect. B* **1979**, 18, 440–442.
- [164] V. P. Papageorgiou, DSc thesis, Polytechnic School Thessaloniki (Greece), **1976**.
- [165] V. P. Papageorgiou, M. Liakopoulou-Kyriakides, C. Papadakis, *Flavour Fragrance J.* **1985**, 1, 21–24.
- [166] a) E. Yesilada, E. Sezik, M. Aslan, A. Yesilada, *J. Liq. Chromatogr. Relat. Technol.* **1996**, 19, 3369–3381; b) R. Chaisuksant, PhD thesis, Aristotle University of Thessaloniki (Greece), **1994**; c) Y. Arai, T. Hanai, A. Nosaka, K. Yamaguchi, *J. Liq. Chromatogr.* **1990**, 13, 2449–2464; d) L. Snyder, J. Gajch, J. Kirkland, *Practical HPLC Method Development*, Wiley, Toronto, **1988**; e) S. Nickel, T. Carrol, *J. Chromatogr.* **1984**, 295, 521; f) M. Tsukada, H. Fukui, C. Habara, M. Tabata, *Shoyakugaku Zasshi* **1983**, 37, 299–306 [*Chem. Abstr.* **1984**, 101, 97723c]; g) L. X. Lin, J. W. Lan, Y. Han, M. F. Han, K. D. Lin, *Yaoxue Xuebao* **1995**, 30, 123–126 [*Chem. Abstr.* **1995**, 122, 248473h]; h) N. Shibayama, R. Yamaoka, M. Sato, J. Iida, *Shitsuryo Bunseki*, **1991**, 39, 123–131 [*Chem. Abstr.* **1992**, 116, 131028b].
- [167] V. P. Papageorgiou, DE-B 2829744, **1979** [*Chem. Abstr.* **1979**, 181441s].
- [168] T. Tani, Y. Sakakibara, K. Yamamoto, *Jpn. J. Appl. Phys. Suppl.* **1989**, 28–3, 239–245.
- [169] a) K. Inoue, M. Akaji, H. Inouye, *Chem. Pharm. Bull.* **1985**, 33, 3993–3997; b) V. P. Papageorgiou, *Planta Med.* **1979**, 37, 185–187.
- [170] V. P. Papageorgiou, *Planta Med.* **1980**, 40, 305–307.
- [171] a) Y. N. Shukla, J. S. Tandon, D. S. Bhakuni, M. M. Dhar, *Experientia* **1969**, 25, 357–358; b) Y. N. Shukla, J. S. Tandon, D. S. Bhakuni, M. M. Dhar, *Phytochemistry* **1971**, 10, 1909–1915.
- [172] K. H. Ai, J. Jin, H. Liu, *Yaoxue Xuebao* **1993**, 28, 282–285 [*Chem. Abstr.* **1993**, 119, 82263a].
- [173] A. A. Nabiullin, S. A. Fedoreev, T. N. Deshko, *Khim. Prir. Soedin.* **1983**, 568–573 [*Chem. Abstr.* **1984**, 100, 117799s].
- [174] A. V. Kovatsis, V. P. Papageorgiou, M. Christianopoulou, *Toxicol. Aspects (9th Int. Congr. Eur. Assoc. Poison Control Cent.)* **1980**, 43–54.
- [175] W. Otake, N. Sugiyama, K. Saito, H. Sato, *Nihon Daigaku Nojuigakubu Gijutsu Kenkyu Hokoku* **1990**, 218–223 [*Chem. Abstr.* **1991**, 114, 198810c].
- [176] R. Chaisuksant, A. Voulgaropoulos, A. S. Mellidis, V. P. Papageorgiou, *Analyst* **1993**, 118, 1346.
- [177] R. Chaisuksant, A. Voulgaropoulos, A. S. Mellidis, V. P. Papageorgiou, *Analysis* **1994**, 22, 400–403.
- [178] a) R. Takamoto, R. Namba, S. Yamamoto, T. Takamatsu, M. Matsuoka, T. Sawada, *Bunseki Kagaku* **1996**, 45, 225–238 [*Chem. Abstr.* **1996**, 124, 211615w]; b) R. Takamoto, R. Namba, M. Matsuoka, T. Sawada, *Anal. Chem.* **1992**, 64, 2661–2663; c) R. Takamoto, R. Namba, O. Nakata, T. Sawada, *Anal. Chem.* **1990**, 62, 674–677.

- [179] E. A. Couladouros, Z. F. Plyta, A. T. Strongilos, V. P. Papageorgiou, *Tetrahedron Lett.* **1997**, 38, 7263–7266.
- [180] a) F. M. Hauser, D. J. Mal, *J. Am. Chem. Soc.* **1983**, 105, 5688–5690; b) M. D. Shair, T. Y. Yoon, K. K. Mosny, T. C. Chou, S. J. Danishefsky, *J. Am. Chem. Soc.* **1996**, 118, 9509–9525; c) B. L. Chenard, M. G. Dolson, A. D. Sercel, J. S. Swenton, *J. Org. Chem.* **1984**, 49, 318–325.
- [181] Y. Tanoue, A. Terada, *Bull. Chem. Soc. Jpn.* **1988**, 61, 2039–2045.
- [182] A. Terada, Y. Tanoue, A. Hatada, H. Sakamoto, *J. Chem. Soc. Chem. Commun.* **1983**, 987–988.
- [183] Y. Tanoue, A. Terada, H. Taniguchi, S.-I. Morishita, *Suisan Daigakko Kenkyu Hokoku* **1994**, 42, 157–162.
- [184] A. M. Moiseenkova, N. N. Balaneva, V. L. Novikov, G. B. Elyakov, *Dokl. Akad. Nauk. SSSR* **1987**, 295, 614–617.
- [185] V. L. Novikov, N. N. Balaneva, A. M. Moiseenkova, G. B. Elyakov, *Izv. Akad. Nauk SSSR Ser. Khim.* **1992**, 1901–1910.
- [186] S. Torii, K. Akiyama, H. Yamashita, T. Inokuchi, *Bull. Chem. Soc. Jpn.* **1995**, 68, 2917–2922.
- [187] a) J. H. Freudenberger, A. W. Konradi, S. F. Pedersen, *J. Am. Chem. Soc.* **1989**, 111, 8014–8016; b) A. W. Konradi, S. F. Pedersen, *J. Am. Chem. Soc.* **1994**, 116, 1316–1323.
- [188] Y. Tanoue, A. Terada, Y. Sugyo, *J. Org. Chem.* **1987**, 52, 1437–1439.
- [189] Y. Shimai, T. Koga (Pias) JP-B 63156741, **1988** [*Chem. Abstr.* **1989**, 110, 23567u].
- [190] a) K. H. Dötz, *Angew. Chem.* **1984**, 96, 573; *Angew. Chem. Int. Ed. Engl.* **1984**, 23, 587–608; b) K. H. Dötz in *Organometallics in Organic Synthesis* (Eds.: A. de Meijere, H. Tom Dieck) Springer, Berlin, **1988**, pp. 85–104; c) P. J. Harrington, *Transition Metal in Total Synthesis*, Wiley, New York, **1990**, pp. 346–399; d) W. D. Wulff in *Comprehensive Organic Synthesis, Vol. 5* (Eds.: B. M. Trost, I. Fleming, L. A. Paquette), Pergamon, New York, **1991**, pp. 1065–1113.
- [191] J. S. Yadav, P. K. Deshpande, G. V. M. Sharma, *Tetrahedron* **1990**, 46, 7033–7046.
- [192] M. Braun, C. Bauer, *Liebigs Ann. Chem.* **1991**, 1157–1164.
- [193] a) M. Braun, R. Devant, *Tetrahedron Lett.* **1984**, 25, 5031–5034; b) R. Devant, U. Mahler, M. Braun, *Chem. Ber.* **1988**, 121, 397–406.
- [194] a) P. K. Jadhav, K. S. Bhat, P. T. Perumal, H. C. Brown, *J. Org. Chem.* **1986**, 51, 432–439; b) Y. Yamamoto, N. Asao, *Chem. Rev.* **1993**, 93, 2207–2293.
- [195] K. C. Nicolaou, D. Hepworth, *Angew. Chem.* **1998**, 110, 864–866; *Angew. Chem. Int. Ed.* **1998**, 37, 839–841.
- [196] “Stereoselective Synthesis”: M. M. Midland, L. A. Morrel, *Methoden Org. Chem. (Houbden-Weyl) 4th ed.* 1952–, VI, E21, D, chap. 2.3.3.2, pp. 4049–4066.
- [197] F. Dallacker, J. Jacobs, W. Coerver, *Z. Naturforsch. B* **1983**, 38, 1000–1007.
- [198] a) H. C. Brown, J. Chandrasekharan, P. V. Ramachandran, *J. Am. Chem. Soc.* **1988**, 110, 1539–1546; b) P. V. Ramachandran, A. V. Teodorovic, M. V. Rangaishenvi, H. C. Brown, *J. Org. Chem.* **1992**, 57, 2379–2386; c) R. K. Dar, *Aldrichimica Acta* **1994**, 27, 43–51.
- [199] D. A. Frey, N. Wu, K. D. Moeller, *Tetrahedron Lett.* **1996**, 37, 8317–8320.
- [200] V. P. Papageorgiou, *Experientia* **1978**, 34, 1499–1501.
- [201] V. P. Papageorgiou, *Chem. Chron.* **1977**, 6, 365–374.
- [202] V. P. Papageorgiou, G. A. Digenis, *Planta Med.* **1980**, 39, 81–84.
- [203] V. P. Papageorgiou, *Planta Med.* **1977**, 31, 390–394.
- [204] It is thought that the isolation procedure adopted by Brockmann^[1] may have caused ester hydrolysis. See Table 1, note 1.
- [205] V. P. Papageorgiou, DE-B 2700448, **1978**, US-A 4282250, **1981**, [*Chem. Abstr.* **1978**, 89, 152707m].
- [206] The clinical trials were undertaken at Heidberg Dermatological Clinic, Hamburg by Prof. A. Winkler.
- [207] C. Michaelides, C. Striglis, P. Panayotou, I. Ioannovich, *Ann. Medit. Burns Club* **1993**, VI, 24–25.
- [208] M. Owili, Clinical Report to the Pharmaceutical Company CHROPI S.A., Kenyatta National Hospital, Nairobi, Kenya, October 17, **1986**.
- [209] G. Karkanas, N. Alexandropoulos, N. Haritopoulos, *Abstr. Pap. 45th Hellenic Natl. Conf. (Athens)* **1991**, p. 61.
- [210] a) M. Hayashi, *Nippon Yakurigaku Zasshi* **1977**, 73, 177–191 [*Chem. Abstr.* **1977**, 88, 44862d]; b) M. Hayashi, *Nippon Yakurigaku Zasshi* **1977**, 73, 193–203 [*Chem. Abstr.* **1977**, 88, 44863e]; c) M. Hayashi, *Nippon Yakurigaku Zasshi* **1977**, 73, 205–214 [*Chem. Abstr.* **1977**, 88, 44864f].
- [211] a) Y. Ozaki, A. Ohno, K.-I. Abe, Y. Saito, M. Satake, *Biol. Pharm. Bull.* **1993**, 16, 683–685; b) Y. Ozaki, A. Ohno, Y. Saito, M. Satake, *Biol. Pharm. Bull.* **1994**, 17, 1075–1077; c) Y. Ozaki, L. Xing, M. Satake, *Biol. Pharm. Bull.* **1996**, 19, 233–236.
- [212] S. Tanaka, M. Tajima, M. Tsukada, M. Tabata, *J. Nat. Prod.* **1986**, 49, 466–469.
- [213] Z.-B. Lin, B.-L. Chai, P. Wang, Q.-X. Guo, F.-S. Lu, G.-Q. Xiang, *Peiching I Hsueh Yuan Hsueh Pao* **1980**, 12, 101–106 [*Chem. Abstr.* **1980**, 93, 143008q].
- [214] Z.-B. Lin, P. Wang, Y. Ruan, Q.-X. Guo, *Acta Pharmacol. Sin.* **1980**, 1, 60–63 [*Biol. Abstr.* **1981**, 73, 34163].
- [215] W. J. Wang, J. Y. Bai, D. P. Lui, L. M. Xue, X. Y. Zhu, *Yaoxue Xuebao* **1994**, 29, 161–165 [*Chem. Abstr.* **1994**, 121, 148550s].
- [216] P. Martin, *Science* **1997**, 276, 75–81.
- [217] F. Hübötter, *Beiträge zur Kenntnis der chinesischen sowie der tibetisch-mongolischen Pharmakologie*, Urban und Schwarzenberg, Berlin, **1913**, p. 324.
- [218] J. L. Hartwell, *Plants Used Against Cancer: A Survey*, Quarterman, Lawrence, MA, **1982**, pp. 68–72.
- [219] J. S. Driscoll, G. F. Hazard, Jr., H. B. Wood, Jr., A. Goldin, *Cancer Chemother. Rep. Part 2*, **1974**, 4(2), 1–362.
- [220] S. K. Gupta, I. S. Mathur, *Indian J. Cancer* **1972**, 9, 50–55.
- [221] H. Wagner, B. Kreher, K. Jurcic, *Arzneim. Forsch.* **1988**, 38, 273–275.
- [222] a) H. Lee, J.-Y. Lin, *Mutation Res.* **1988**, 204, 229–234; b) T. Hamano, I. Yasuda, Y. Watanabe, T. Konoshima, T. Harukuni, *Kenkyu Nenpo Tokyo-toristu Eisei Kenkyusho* **1992**, 43, 46–50 [*Chem. Abstr.* **1993**, 118, 204762q].
- [223] A. Ohtsuka, T. Nunoshiba, K. Nakayama, R. Namiuchi, S. Saigusa, T. Sotani, H. Nishioka, *Sci. Eng. Rev. Doshisha Univ.* **1986**, 27, 65–73.
- [224] a) T. Konoshima, M. Kozuka, J. Koyama, T. Okatani, K. Tagahara, H. Tokuda, *J. Nat. Prod.* **1989**, 52, 987–995; b) G. J. Kapadia, V. Balasubramanian, H. Tokuda, T. Konoshima, M. Takasaki, J. Koyama, K. Tagahara, H. Nishino, *Cancer Lett.* **1997**, 113, 47–53.
- [225] N. Yoshimi, A. Wang, Y. Morishita, T. Tanaka, S. Sugie, K. Kawai, Y. Yamahara, H. Mori, *Jpn. J. Cancer Res.* **1992**, 83, 1273–1278.
- [226] a) V. P. Papageorgiou, M. N. Christianopoulou, L. L. Boutis, A. Papageorgiou, C. A. Tsipis, *Inorg. Chem. Acta* **1986**, 124, 203–206; b) A. N. Sagredos, V. P. Papageorgiou, A. S. Mellidis, EP-B 144809, **1985** [*Chem. Abstr.* **1985**, 103, 196266d].
- [227] H. Sakurai, N. Hashimoto, JP-B 06100569, **1992** [*Chem. Abstr.* **1994**, 121, 244290c].
- [228] M. G. Miller, A. Rodgers, G. M. Cohen, *Biochem. Pharmacol.* **1986**, 35, 1177–1184.
- [229] H. W. Moore, *Science* **1977**, 197, 527–531.
- [230] M. Tomasz, C. M. Mercado, J. Olson, N. Chatterjee, *Biochemistry* **1974**, 13, 4878–4887.
- [231] N. Fujii, Y. Yamashita, Y. Arima, M. Nagashima, H. Nakano, *Antimicrob. Agents Chemother.* **1992**, 36, 2589–2594.
- [232] For a review, see S. J. Froelich-Ammon, N. Osheroff, *J. Biol. Chem.* **1995**, 270, 21429–21432.
- [233] Earlier, these authors had reported activity of acetylshikonin (**15**) against both topoisomerase-I and -II: K. R. Kweon, K. U. Baik, K. Lim, B. D. Hwang, B. Z. Ahn, *Proc. Am. Assoc. Cancer Res. Annu. Meet.* **1993**, 34, 328.
- [234] B.-Z. Ahn, D.-E. Sok, *Curr. Pharm. Des.* **1996**, 2, 247–262, and references therein.
- [235] B.-Z. Ahn, G.-Y. Song, K. U. Baik, D. E. Sok, *Korean J. Med. Chem.* **1996**, 6, 98–109.
- [236] a) B. Z. Ahn, Y. Kim, K. U. Baik, WO-B 9703940, [*Chem. Abstr.* **1997**, 126, 199354s]; b) B. Z. Ahn, K. U. Baik, WO-B 9502572 [*Chem. Abstr.* **1995**, 123, 227829j].
- [237] S.-H. Kim, G.-Y. Song, G.-Z. Jin, B.-Y. Ahn, *Arch. Pharm. Res.* **1996**, 19, 416–422.
- [238] S.-H. Kim, G.-Y. Song, D.-E. Sok, B.-Y. Ahn, *Arch. Pharm. Res.* **1997**, 20, 155–157.
- [239] D. S. Bhakuni, M. L. Dhar, M. M. Dhar, B. N. Dhawan, B. N. Mehrotra, *Indian J. Exp. Biol.* **1969**, 7, 250–262 [*Biol. Abstr.* **1970**, 53, 43416].
- [240] a) Y. Tanaka, T. Odani, *Yakugaku Zasshi* **1972**, 92, 525–530 [*Chem. Abstr.* **1972**, 92, 97680t]; b) K. Kyogoku, H. Terayama, Y. Tachi, T.

- Suzuki, M. Komatsu, *Shoyakugaku Zasshi* **1973**, 27, 31–36 [*Chem. Abstr.* **1974**, 80, 52315c]; c) Y. Tanaka, T. Odani, T. Kanaya, *Shoyakugaku Zasshi* **1974**, 28, 173–178 [*Chem. Abstr.* **1975**, 83, 152418u].
- [241] a) V. P. Papageorgiou, A. S. Mellidis, A. N. Sagredos, *Chem. Chron.* **1980**, 9, 57–63; b) V. P. Papageorgiou, A. Winkler, A. N. Sagredos, G. A. Digenis, *Planta Med.* **1979**, 35, 56–60.
- [242] M. Tabata, H. Mizukami, S. Naoe, K. Masao, *Yakugaku Zasshi* **1975**, 95, 1376–1379 [*Biol. Abstr.* **1976**, 62, 18482].
- [243] M. Tabata, M. Tsukada, H. Fukui, *Planta Med.* **1982**, 44, 234–236.
- [244] a) L. R. Shcherbanovskii, G. I. Nilov, Z. D. Rabinovich, V. A. Gorina, *Rast. Resur.* **1972**, 8, 112–115 [*Chem. Abstr.* **1972**, 76, 136281u]; b) L. P. Sherbanivskii, *Ukr. Bot. Zh.* **1971**, 28, 504–508 [*Chem. Abstr.* **1971**, 76, 68687d]; c) L. R. Shcherbanovskii, A. Yu. Luks, I. G. Kapelev, *Mikrobiol. Zh. (Kiev)* **1975**, 37, 629–634 [*Chem. Abstr.* **1976**, 84, 728c]; d) L. R. Shcherbanovskii, *Abstr. Pap. 8th Fitontsidy: Rol Biogeotsenozakh, Znach. Med. Mater. Soveshch. (Kiev, USSR)* **1979**, pp. 121–126 [*Chem. Abstr.* **1982**, 97, 89062v].
- [245] I. Inagaki, M. Yamazaki, A. Takahashi, K. Ooye, S. Shibata, S. Takada, *Nagoya Shiritsu Daigaku Yakugakubu Kenkyu Nempo* **1967**, (15), 27–32 [*Chem. Abstr.* **1968**, 69, 41955h].
- [246] M. Liu, T. Ohuchi, T. Ieiri, M. Ohe, S. Matsuzaki, *Dokkyo J. Med. Sci.* **1996**, 23, 63–69.
- [247] G. Honda, F. Sakakibara, K. Yazaki, M. Tabata, *J. Nat. Prod.* **1988**, 51, 152–154.
- [248] M. Tabata, Y. Honda, (Mitsubishi Petrochemical) JP-B 62289516, **1987**, [*Chem. Abstr.* **1988**, 109, 806m].
- [249] a) V. V. Gusev, A. A. Alekseev, E. V. Makarov, A. V. Barinov, SU-B 1829942, **1993** [*Chem. Abstr.* **1995**, 123, 179515e]; b) V. V. Gusev, A. A. Alekseev, E. V. Makarov, A. V. Barinov, SU-B 1829944, **1993** [*Chem. Abstr.* **1995**, 123, 179516f].
- [250] S. Wahab, R. N. Tandon, Z. Jacob, B. Chandra, O. P. Srivastava, *Indian J. Med. Res. Suppl.* **1979**, 76, 77–82.
- [251] M. A. Rubinchik, *Abstr. Pap. Mater. Vses. Konf. Issled. Lek. Rast. Perspekt. Ikh Isol'z Proivod. Lek. Prep. (Bittsa, USSR)* **1970**, pp. 236–237 [*Chem. Abstr.* **1975**, 83, 671h].
- [252] a) H. M. S. Sulaiman, S. N. H. Naqvi, A. M. S. Mohammad, *Curr. Sci.* **1978**, 47, 743–744 [*Chem. Abstr.* **1979**, 90, 1653k]; b) S. N. H. Naqvi, H. M. S. Sulaiman, M. Afzal, A. M. S. Mohammad, *Pak. J. Zool.* **1984**, 16, 175–180 [*Chem. Abstr.* **1986**, 104, 2173y]; c) N. S. Gorgefs, S. N. H. Naqvi, L. J. Rashan, S. J. Zakaria, *Folia Histochem. Cytochem.* **1978**, 16, 51–55 [*Chem. Abstr.* **1978**, 89, 18317f].
- [253] a) Y.-S. Chang, S.-C. Kuo, S.-H. Weng, S.-C. Jan, F.-N. Ko, C.-M. Teng, *Planta Med.* **1993**, 59, 401–404; b) F.-N. Ko, Y.-S. Lee, S.-C. Kuo, Y.-S. Chang, C.-M. Teng, *Biochem. Biophys. Acta.* **1995**, 1268, 329–334.
- [254] L. Majlathova, *Nahrung* **1971**, 15, 505–508 [*Chem. Abstr.* **1971**, 76, 12251j].
- [255] L. Tikkanen, T. Matsushima, S. Natori, K. Yoshihira, *Mutation Res.* **1983**, 124, 25–34.
- [256] B. N. Ames, J. McCann, E. Yamasaki, *Mutation Res.* **1975**, 31, 347–363.
- [257] W. J. Wang, M. G. Yi, X. Y. Zhu, *Yaoxue Xuebao* **1988**, 23, 246–251 [*Chem. Abstr.* **1988**, 109, 47792u].
- [258] a) M. R. Meselhy, S. Kadota, K. Tsubono, A. Kusai, M. Hattori, T. Namba, *Tetrahedron Lett.* **1994**, 35, 583–586; b) M. R. Meselhy, S. Kadota, K. Tsubono, M. Hattori, T. Namba, *Tetrahedron* **1994**, 50, 3081–3098.
- [259] C. Y. Chen, F.-A. Chen, A.-B. Wu, H.-C. Hsu, J.-J. Kang, H. W. Cheng, *Int. J. Pharm.* **1996**, 141, 171–178.
- [260] a) “Inhibition of Topoisomerase I by Naphthoquinone Derivatives”: Z. F. Plyta, T. Li, V. P. Papageorgiou, A. S. Mellidis, A. N. Assimopoulou, E. N. Pitsinos, E. A. Couladouros, *Bioorg. Med. Chem. Lett.* **1998**, 8, 3385–3390; b) “Evaluation of antibacterial effect of shikonin ointment against methicillin-resistant *Staphylococcus aureus*”: T. Sekine, K. Kojima, S. Sasaki, T. Matsumoto, T. Yamamoto, Y. Maitani, T. Nagai, *S.T.P. Pharma Sci.* **1998**, 8, 255–259 [*Chem. Abstr.* **1998**:770483];^[267] c) “Preparation and evaluation of shikonin ointment for wound healing: effectiveness in an experimental wound healing model in rats”: T. Sekine, K. Kojima, S. Ota, T. Matsumoto, T. Yamamoto, Y. Maitani, T. Nagai, *S.T.P. Pharma Sci.* **1998**, 8, 249–253 [*Chem. Abstr.* **1998**:770482];^[267] d) “Evaluation of Shikonin on granulation tissue formation compared with carrageenan”: T. Sekine, K. Kojima, T. Matsumoto, T. Yamamoto, Y. Maitani, T. Nagai, *Biol. Pharm. Bull.* **1998**, 21, 950–952; e) “Heat-shock protein formation inhibitors containing dihydroxynaphthoquinones”: Y. Kiyosuke, S. Tsuzuki, T. Shirakami, M. Morino, C. Yoshikumi, (Kureha Chemical Industry Co.) JP 10212230, **1998** [*Chem. Abstr.* **1998**, 129, 144853]; f) “Some phytochemicals and related compounds in vegetables as potent inhibitors of human DNA topoisomerase II”: M. Miyahara, M. Kawasaki, H. Akiyama, T. Narui, M. Toyoda, T. Okuyama, Y. Saito, *Food Factors Cancer Prev. [Int. Conf.]* **1997**, 182–187 [*Chem. Abstr.* **1998**, 129, 197664]; g) “Apoptosis inducer”: M. Nakayama, N. Ikeda, Y. Kato, *Frangrance J.* **1998**, 26, 72–80; h) “Shikonin, an ingredient of *Lithospermum erythrorhizon*, inhibits angiogenesis in vivo and in vitro”: T. Yuki Hisa, Y. Kimura, K. Takada, F. Suzuki, M. Takigawa, *Anticancer Res.* **1998**, 18(2A), 783–790; i) “Haloacetylshikonin derivatives: synthesis and evaluation of antitumor activity”: X.-G. Zheng, G.-Z. Jin, G.-Y. Song, C. Hoon, B.-Z. Ahn, *Yakuhak Hoechi* **1998**, 42, 159–164 [*Chem. Abstr.* **1998**, 129, 16007]; j) “Study of the accelerating effect of shikonin and alkannin on the proliferation of granulation tissue in rats”: Y. Ozaki, I. Sakaguchi, M. Tujimura, N. Ikeda, M. Nakayama, Y. Kato, H. Suzuki, M. Satake, *Biol. Pharm. Bull.* **1998**, 21, 366–370; k) “Antiviral and antifungal activities of some naphthoquinones isolated from the roots of *Lithospermum erythrorhizon*”: C. Li, Y. Fukushi, J. Kawabata, S. Tahara, J. Mizutani, I. Uyeda, *Nippon Noyaku Gakkaishi* **1998**, 23, 54–57 [*Chem. Abstr.* **1998**, 128, 241823]; l) “Heat-shock protein 47 (HSP 47) formation inhibitors containing shikonin”: Y. Kiyosuke, S. Tsuzuki, T. Shirakami, M. Morino, C. Yoshikumi, (Kureha Chemical Industry Co.) JP 10045575, **1998** [*Chem. Abstr.* **1998**, 128, 158908]; m) “Heat-shock protein 27 (HSP 27) formation inhibitors containing shikonin”: M. Morino, S. Tsuzuki, T. Shirakami, Y. Seiho, C. Yoshikumi, (Kureha Chemical Industry Co.) JP 10045574, **1998** [*Chem. Abstr.* **1998**, 128, 158907]; n) “Shikonin for wound treatment”: A. A. Adamyan, S. V. Dobysh, G. V. Polikakhina, I. A. Argunovskij, P. M. Golovanova, L. R. Makarova, N. N. Tuzova, (Aktzionernoe Obshchestvo Zakrytogo Tipa Mnogoprofilnyj Kompleks “universal”, Russia; Obshchestvo S Ogranichennoj Otvetstvennostyu Nauchno-Tekhnicheskij Tsentr “riza”): RU 2071788, **1997** [*Chem. Abstr.* **1997**, 127, 351235]; o) “Inhibition of xanthine oxidase by hydroxylated anthraquinones and related compounds”: S.-Y. Sheu, H.-C. Chiang, *Anticancer Res.* **1997**, 17, 3293–3297; p) “Study on baths with crude drug. III. The effect of *Ligustici chuanxiong rhizoma* extract on the percutaneous absorption of some natural compounds”: K. Sekiya, S. Kadota, K. Katayama, T. Koizumi, T. Namba, *Biol. Pharm. Bull.* **1997**, 20, 983–987.
- [261] a) “Determination of shikonin content with thin layer chromatography-chemiluminescence”: H. Li, B. Wang, Z. Pang, *Fenxi Huaxue* **1998**, 26, 1282 [*Chem. Abstr.* **1998**, 129, 347370]; b) “Enantiomeric ratio of shikonin derivatives as a possible key for the determination of the origin of *Lithospermum Radix*”: J. S. Kang, B. Z. Ahn, G. Blaschke, *Arch. Pharmacol. Res.* **1998**, 21, 565–569; c) “In vitro biotransformation of shikonin by LC-MS”: H. Li, S. Luo, T. Zhou, S. Yang, *Zhongguo Yaoxue Zazhi (Beijing)* **1997**, 32(Suppl.), 37–39 [*Chem. Abstr.* **1998**:660197];^[267] d) “Evaluation of superoxide anion radical scavenging activity of shikonin by electron spin resonance”: T. Sekine, T. Masumizu, Y. Maitani, T. Nagai, *Int. J. Pharm.* **1998**, 174, 133–139 [*Chem. Abstr.* **1998**:655481];^[267] e) “Quantitative determination of naphthoquinones of *Arnebia densiflora* (Nordm.) Ledeb. by an improved high-performance liquid chromatographic method”: B. Bozan, K. H. C. Baser, S. Kara, *J. Chromatogr. A* **1997**, 782(1), 133–136 [*Chem. Abstr.* **1997**, 127, 298820].
- [262] a) “Shikonin production by hairy roots of *Lithospermum erythrorhizon* in bioreactors with in situ separation”: S. J. Sim, H. N. Chang, *Hairy Roots* (Ed.: P. M. Doran), Harwood, Amsterdam, **1997**, pp. 219–225; b) “Changes of intracellular Ca^{2+} and cAMP during the formation of shikonin derivatives promoted by *Aspergillus oryzae* elicitor”: W. Ning, R. Cao, J. Wang, Y. Liu, X. Lu, *Zhiwu Shenglixue Tongxun* **1998**, 34, 194–196 [*Chem. Abstr.* **1998**, 129, 328285]; c) “The effects of Ca^{2+} during the elicitation of shikonin derivatives in *Onosma paniculatum* cells”: W. Ning, J.-X. Wang, Y.-M. Liu, N. Li, R.-Q. Cao, *In Vitro Cell. Dev. Biol.* **1998**, 34, 261–265 [*Chem. Abstr.* **1998**, 129, 313453]; d) “Effects of fungal

- elicitors on the cell growth and the shikonin biosynthesis in *Arnebia euchroma* cells in suspension culture" C.-J. Liu, S.-S. Hou, *Zhiwu Shengli Xuebao* **1998**, *24*, 6–10 [*Chem. Abstr.* **1998**, *129*, 148102]; e) "4-Hydroxybenzoate 3-geranyltransferase from *Lithospermum erythrorhizon*. Purification of a plant membrane-bound prenyltransferase": A. Muehlenweg, M. Melzer, S.-M. Li, L. Heide, *Planta* **1998**, *205*, 407–413; f) "Shikonin: a geranyl diphosphate-derived plant hemiterpenoid formed via the mevalonate pathway": S.-M. Li, S. Hennig, L. Heide, *Tetrahedron Lett.* **1998**, *39*, 2721–2724; f) "Formation and secretion of a new brown benzoquinone by hairy root cultures of *Lithospermum erythrorhizon*": H. Fukui, A. F. M. F. Hasan, T. Ueoka, M. Kyo, *Phytochemistry* **1998**, *47*, 1037–1039; g) "Enhancement of shikonin production in cell suspension cultures of *Arnebia euchroma* employing two-liquid-phase systems": X. Fu, D. Lu, *Chin. J. Chem. Eng.* **1998**, *6*, 86–90 [*Chem. Abstr.* **1998**, *128*, 307562]; h) "Isolation of shikonin derivatives from organic solvent by macroporous adsorption resin": Q. Men, D. Lu, G. Zhang, *Gaoxiao Huaxue Gongcheng Xuebao* **1998**, *12*, 23–27 [*Chem. Abstr.* **1998**, *128*, 307561]; i) "Shikonin production by cell suspension culture of *Onosma paniculatum* in turbine stirred bioreactor": H. Zhang, W. Ning, Q. Zhao, R. Cao, Y. Che, *Nanjing Daxue Xuebao, Ziran Kexue* **1997**, *33*, 455–458 [*Chem. Abstr.* **1998**, *128*, 229385]; j) "Plant cell and tissue culture. Industrial applications": C. Ponzzone, *Chim. Ind. (Milan)* **1997**, *79*, 1217–1221; k) "Manufacture of plant secondary metabolites using coronatines": T. Gyoso, Y. Hara, (Mitsui Petrochemical Industries, Ltd., Japan) JP 10033190, **1998** [*Chem. Abstr.* **1998**, *128*, 139817]; l) "Regulatory role of microsomal 3-hydroxy-3-methylglutaryl-coenzyme A reductase for shikonin biosynthesis in *Lithospermum erythrorhizon* cell suspension cultures": B. M. Lange, K. Severin, A. Bechthold, L. Heide, *Planta* **1998**, *204*, 234–241; m) "Relationship between active oxygen burst and shikonin derivatives formation in cultured *Onosma paniculatum* cells induced by *Aspergillus* elicitor": W. Ning, H. Xu, R. Cao, *Nanjing Daxue Xuebao, Ziran Kexue* **1997**, *33*, 259–264 [*Chem. Abstr.* **1998**, *128*, 137643]; n) "cDNA cloning and gene expression of phenylalanine ammonia-lyase in *Lithospermum erythrorhizon*": K. Yazaki, M. Kataoka, G. Honda, K. Severin, L. Heide, *Biotechnol. Biochem.* **1997**, *61*, 1995–2003; o) "*Arnebia euchroma*: in vitro culture and the production of shikonin and other secondary metabolites": O. V. Zakhlenjuk, V. A. Kunakh, *Biotechnol. Agric. For.* **1998**, *41* (*Medicinal and Aromatic Plants X*), 28–44 [*Chem. Abstr.* **1998**, *128*, 101107]; p) "*Alkanna tinctoria* T. (alkanets): in vitro culture and the production of alkannin and other secondary metabolites": C. Gerardi, G. Mita, E. Grillo, G. Giovinazzo, A. Miceli, P. De Leo, *Biotechnol. Agric. For.* **1998**, *41* (*Medicinal and Aromatic Plants X*), 14–27 [*Chem. Abstr.* **1998**, *128*, 74326]; q) "Measurement of phenolic compounds and their effect on shikonin production in *Lithospermum* cultured cells": K. Yazaki, H. Fukui, Y. Nishikawa, M. Tabata, *Biosci. Biotechnol. Biochem.* **1997**, *61*, 1674–1678 [*Chem. Abstr.* **1998**, *128*, 1945]; r) "Induction of phytoalexin by uptake of naphthoquinones in cell cultures of petunia": M.-J. Kim, S.-S. Kwak, *Han'guk Nonghwa Hakhoechi* **1997**, *40*, 352–356 [*Chem. Abstr.* **1997**, *127*, 245602]; s) "Biotechnology in detergents and cosmetics": S. Ito, *Baioisaiensu to Indasutori* **1997**, *55*, 541–545 [*Chem. Abstr.* **1998**, *127*, 189671]; t) "Effects of methyl jasmonate on shikonin and dihydroechinofuran production in *Lithospermum* cell cultures" K. Yazaki, K. Takeda, M. Tabata, *Plant Cell Physiol.* **1997**, *38*, 776–782; u) "Plant regeneration from callus cultures of *Lithospermum erythrorhizon*": H. J. Yu, S. K. Oh, M. H. Oh, D. W. Choi, Y. M. Kwon, S. G. Kim, *Plant Cell Rep.* **1997**, *16*, 261–266; v) "4-Hydroxybenzoate prenyltransferases in cell-free extracts of *Lithospermum erythrorhizon* cell cultures": R. Boehm, S.-M. Li, M. Melzer, L. Heide, *Phytochemistry* **1997**, *44*, 419–424.
- [263] a) "Chemical properties of shikonin from the view point of dyeing": N. Nagashima, K. Sakata, A. Katayama, *Nippon Sanshigaku Zasshi* **1998**, *67*, 123–127 [*Chem. Abstr.* **1997**, *129*, 150067]; b) "Red colorants, their production and cosmetics containing the colorants": M. Oinuma, S. Shimoyama, Y. Noda, (Den Material K. K.) JP 09296125 **1998** [*Chem. Abstr.* **1998**, *128*, 26758].
- [264] a) "The ability of the naphthoquinone shikonin to influence microorganisms in the rhizosphere of *Lithospermum erythrorhizon*": L. A. Brigham, P. J. Michaels, H. E. Flores, *Curr. Top. Plant Physiol.* **1998**, *18* (*Radical Biology: Advances and Perspectives on the Function of Plant Roots*), 451–453 [*Chem. Abstr.* **1998**, *129*, 300083]; b) "Effect of substituent and ring changes in naturally occurring naphthoquinones on the feeding response of larvae of the Mexican bean beetle, *Epilachna varivestis*": M. Weissenberg, J. Meisner, M. Klein, I. Schaeffler, M. Eliyahu, H. Schmutterer, K. R. S. Ascher, *J. Chem. Ecol.* **1997**, *23*, 3–18 [*Chem. Abstr.* **1997**, *126*, 167886].
- [265] G. Lu, J. Liao, *Chung Hsi I Chieh Ho Tsa Chih (Chinese Journal of Modern Developments in Traditional Medicine)* **1990**, *10*, 422–5, 390.
- [266] X. P. Guo, X. Y. Zhang, S. D. Zhang, *Chung Hsi I Chieh Ho Tsa Chih (Chinese Journal of Modern Developments in Traditional Medicine)* **1991**, *11*, 598–9, 580.
- [267] Accession number for CAPLUS database. Not yet published in *Chemical Abstracts*.