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A MODERN LOOK AT FOLKLORIC USE OF ANTI-INFECTIVE AGENTS[1]

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ABSTRACT.—Infectious diseases are of ancient origin, and mankind has a venerable history of use of higher plant extracts for the therapy of such infections. Some such agents survive in use from earlier times—quinine, emetine, and sanguinarine, for example—but the modern use of fermentation-based antibiotics has greatly overshadowed work on agents from other sources. After a brief review of the present status of the field of antibiotics, this review focuses upon the present status of antimicrobial agents from higher plants with particular reference to agents from plants with a folkloric reputation for treatment of infections. In particular, recent work on the tropical genus *Erythrina* is emphasized. The use of modern microbiological techniques demonstrates that higher plants frequently exhibit significant potency against human bacterial and fungal pathogens, that many genera are involved, that many folkloric uses can be rationalized on this basis, that the active constituents are readily isolated by bioassay-directed techniques, that their chemical structures are types uncommon amongst fermentation-based agents but are familiar to natural product chemists, that their antimicrobial spectra are comparatively narrow but that their potency is often reasonable, that they are comparatively easy to synthesize and the unnatural analogues so produced can possess enhanced therapeutic potential and, thus, it is concluded that such work generates a gratifying number of novel lead structures and that the possibility of finding additional agents for human or agricultural use based upon higher plant agents is realistic.

More than 46 years have passed since the modern antibiotic era opened with the first clinical trial of penicillin in early 1941. In the intervening years medical practice has been transformed, and the use of antibiotics has grown to enormous proportions. In 1985, for example, the world market for antibiotic drugs amounted to $5,670,000,000—about 20% of the total market for pharmaceuticals (1). In the United States this is distributed among 114 antibiotics and anti-infective agent, 34 of which (17%) are listed among the top 200 most frequently prescribed drugs in the USA (2). This list does not give the total picture, as parenteral agents utilized in an institutional setting and those agents used in agricultural practice are not considered in the tabulations.

In addition, it is variously estimated that between 5,000 and 10,000 natural antibiotics have been isolated and characterized and that at least 50,000 to 100,000 analogues have been synthesized (3). Clearly, the vast majority fail to find medicinal use.

The bulk of the natural antibiotics have been isolated from soil microorganisms through intensive screening and dereplication schemes. As seen in Figure 1, in 1952, the bulk of the 58 agents reported in the literature were derived from the streptomycetes with most of the remainder coming from bacteria and fungi. By 1982, the total number of new agents had increased to 220, but the percentage derived from the streptomycetes had declined as had the number derived from bacteria and fungi. One observed instead a dramatic increase in the use of rarer microorganisms. Such organisms were drawn from the *Actinomadura, Actinoplanes, Dactylosporangium, Kitasatoa, Saccharopolyspora, Streptoalloteichus, Streptosporangium,* and *Streptoverticillium* (4). The reasons for this shift lie largely in the perception that the point of diminishing returns had been reached using classical methodology, and if newer agents were to be discovered, fishing in a different gene pool was more likely to prove useful. A close look at the identity of

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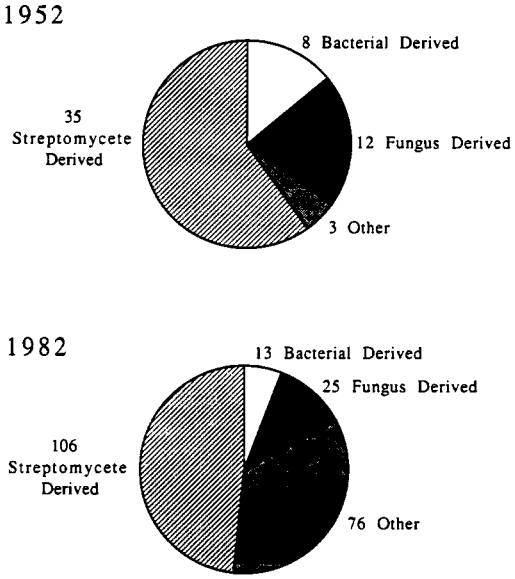


FIGURE 1. The pattern of new antibiotic discovery in 1952 and 1982

the antimicrobial agents produced by this shift in program reveals that, whereas a significant number of structurally new agents were uncovered in this way, the newer agents still belonged primarily to the same chemical families as had been seen in 1952. Thus, these agents are variants on a well-known theme rather than representatives of dramatically new structural families with dramatically novel biological properties (4).

Some other microbiology-based avenues explored in attempts to breathe significant novelty into antibiotic discovery include search into novel environments (5), directed screening methods (specific enzyme inhibition, comparative activity against resistant and supersensitive strains, addition of enzymes to the media, and so on) (6), directed biosynthesis (including mutasynthesis) (7,8), biochemical screens directed toward a specific mode of action (9), and genetic engineering (10).

Disappointingly, almost totally absent from this list is the design of novel antimicrobial agents from first principles based upon our ever-expanding knowledge of comparative biochemistry (11). Screening, by whatever methodology, remains the most effective way to find new antibiotics.

Other fruitful avenues under present exploration include the search for antibiotics from the sea (12) and from higher animals (13). In these cases quite novel findings are being made. No new agent has reached the market yet from these studies, but a number of analogues appear quite promising.

The remainder of this report will deal with various aspects of the search for novel antimicrobial agents from higher plants.

The use of higher plants and preparations taken from them for the treatment of infections predates written records. Certainly some of the earliest surviving accounts of medical practice [Pen Tsao of 3000 BC, the Ebers Papyrus of 1500 BC, and Celsus' "De Medicina," for example (14)] record such usage. From the vantage point of modern knowledge, most other early reports seem full of fanciful nonsense. Man knew nothing reliable about the nature of infectious disease until the 1800s, so it would be more remarkable if this were not the case.

If the need for such agents was pressing and the search was on among sources we now know to contain such materials, why did man not find such substances? A partial answer is that he did. Before the discovery of penicillin, such agents as allyl isothiocya-

nate, berberine, chelidonine, conessine, cepharanthine, emetine, harmine, kawain, pinosylvine, quinine, sanguinarine, thujaplicins, and thymol, and the plant extracts containing them, were known to have antimicrobial properties (14). Berberine, emetine, quinine, and sanguinarine still find specialized uses. On balance, however, agents derived from higher plants, just as those found in the average fermentation, most often represent novel structural leads for possible further chemical improvement rather than competitively significant therapeutic agents, and most have been discarded in preference to more potent or more selective agents. This is not a particular characteristic of the antibiotic field but represents the general fate of the majority of biologically active natural products in every therapeutic category. Another relevant observation is that penicillin itself, the first of the fermentation-derived antimicrobial agents, was itself not found until 1929. After the discovery of penicillin and, beginning in the 1940s and 1950s, of a host of other highly potent and often broad spectrum agents, interest shifted dramatically away from other lines of enquiry. There is also a residual antipathy against higher plant products resulting from the excesses of hucksterism and quackery associated with unregulated and unscientific use of higher plant remedies in the past century.

It has only been in the past decade or so that interest in higher plant antimicrobial agents has been reawakened world wide, and the literature in the area is now becomming substantial (4).

In addition to an intact integument and environmental and personal sanitation, animals utilize a functioning immune system to produce high molecular weight immune bodies as primary defenses in warding off infectious disease. Production of low molecular weight compounds by animals for antimicrobial purposes is rare. By contrast, higher plants supplement their outer defenses with the capacity to elaborate both high and low molecular weight antimicrobial agents.

These macromolecules include protease inhibitors, glycosidase inhibitors, lectins, and phytohemagglutinins. These agents have not found much interest as potential medicinal agents. Of greater apparent potential for human use are the two classes of inhibitors with low molecular weight, the constituitive (preinfectional) agents and the phytoalexins (postinfectional agents) (15). Although the biology associated with these classes is strikingly different, their chemical structures are very similar, and, in some cases, the same compound is a constituitive agent in some strains and a phytoalexin in others. Phytoalexins are antimicrobial compounds that are either not present or are present only in very small quantities in uninfected plants. After microbial invasion, however, in plants with this capability, enzymes which catalyze the formation of phytoalexins that are toxic to the invading organism become activated. In plants having constituitive antimicrobial agents, field resistance to infection is often a consequence of this feature of their biosynthetic machinery. In searching for antimicrobial agents from higher plants it is technically much easier to screen for constituitive agents than for phytoalexins. Also, the quantity of phytoalexins is often very small even in infected plants as compared with the amount of constituitive agents.

In our experiments we have found that the residues from ethanolic extracts of dried, ground plants can be tested conveniently against a battery of screening organisms used industrially as indicators of potential in treatment of human infections of commercial importance. In these agar-dilution streak assays, seven different organisms can be screened simultaneously on a given Petri dish at single, fixed concentrations of extract. There is no difficulty in detecting constituitive agents at dilutions of 1:1000 and 1:100 of extract in agar, and, in our hands, even relatively weak antimicrobial agents found at $<1\%$ concentration in extracts can be detected successfully. *Staphylococcus aureus* represents the Gram-positives; the Gram-negatives are represented by *Escherichia coli*, *Sal-*

monella gallinarum, Klebsiella pneumoniae, and *Pseudomonas aeruginosa. Mycobacterium smegmatis* stands in for the acid-fasts and *Candida albicans* for yeasts and fungi (16,17).

Table 1 gives a list of the relative potency of 18 commercially significant antibiotics against these screening organisms. Whereas agents like cephalothin, streptomycin, and tetracycline would easily be detected even in the crude state, some agents like spectinomycin and sulfisoxazole would probably be missed, and methicillin, oleandomycin, penicillin V, and spiramycin would not be regarded as impressive and would not have been given high priority for fractionation.

TABLE 1. In vitro Activity of Standard Antibiotics Against Representative Screening Microoganisms

Antibiotic	Organism[a] (μg/ml)						
	1	2	3	4	5	6	7
Amphotericin B	>100	>100	>100	>100	>100	0.39	>100
Carbenicillin	<0.1	50	3.12	50	i[b]	i	i
Cephalothin sodium	0.78	6.25	1.56	6.25	0.39	>100	25
Chloramphenicol	5	10	2.5	1.25	10	>100	>100
Erythromycin	0.2	>100	25	>100	0.78	>100	100
Gentamicin	0.78	1.56	0.78	0.78	0.78	>100	1.56
Hetacillin	<0.63	>10	<0.63	>10	>10	>10	>10
Lincomycin	0.78	>100	100	>100	1.56	>100	>100
Methicillin sodium	<0.63	>10	>10	>10	>10	>10	>10
Nalidixic acid	100	12.5	12.5	3.13	>100	>100	>40
Oleandomycin	<0.63	>10	>10	>10	>10	>100	>100
Oxolinic acid	3.13	0.78	0.78	<0.10	50	>100	>100
Phenoxymethylpenicillin sodium	<0.1	>100	50	>100	>100	>100	>100
Spectinomycin dihydrochloride	>10	>10	>10	10	>10	>10	>100
Spiramycin	2.5	>10	>10	>10	>10	>100	>100
Streptomycin sulfate	5	5	50	2.5	1.25	>100	>100
Sulfisoxazole	100	>100	100	>100	>100	>100	>100
Tetracycline	<0.1	6.25	1.56	12.5	>100	>100	>100

[a]Microorganism 1=*Staphylococcus aureus* ATCC 13709, 2=*Escherichia coli* ATCC 9637, 3=*Salmonella gallinarum* ATCC 9184, 4=*Klebsiella pneumoniae* ATCC 10031, 5=*Mycobacterium smegmatis* ATCC 607, 6=*Candida albicans* ATCC 10231, and 7=*Pseudomonas aeruginosa* ATCC 27853.

[b]Inactive.

Using this methodology, we have screened 1248 extracts of higher plant species and found 338 (26%) of these to be active. Of the 129 genera represented, 75 (58%) were active. Particularly fruitful were representatives of the Leguminosae, Rutaceae, and the Compositae. Extracts are generally richest in antimicrobial agents after the flowering (sexual) stage of their growth is complete, and plants taken from stressful environments were also particularly useful. As can be seen from the data in Table 2, anti-Gram negative activity was comparatively rare, but activity against *S. aureus* (187/

TABLE 2. Incidence of Antibiotic Activity of Higher Plant Extracts Against Primary Screening Microorganisms

	Active	%
Staphylococcus aureus ATCC 13709	187	15[a]
Escherichia coli ATCC 9637	2	<1[a]
Salmonella gallinarum ATCC 9184	14	1[a]
Klebsiella pneumoniae ATCC 10031	7	0.5[a]
Mycobacterium smegmatis ATCC 607	236	19[a]
Candida albicans ATCC 10231	87	7[a]
Pseudomonas aeruginosa ATCC 27853	44	6[b]

[a]Of 1,248 tests.

[b]Of 746 tests.

1248=15%), *M. smegmatis* (236/1248=19%), *C. albicans* (87/1248=7%), and *P. aeruginosa* (44/746=6%) was usefully frequent.

Antimicrobial agents from higher plants can be assumed to be useful to the producing plant in warding off infectious disease, but because the infecting microorganisms are rarely the same as those infecting higher animals, there is no compelling reason to suppose that plant antiinfective agents would be active against human or veterinary pathogens. It is comforting, therefore, to find that the spectrum of activity of higher plant antimicrobial agents is broad enough to include significant human pathogens as was suggested by folkloric and historic accounts.

Initial experiments followed up the folkloric reports of use of plant extracts for antimicrobial activity, and successes were encountered with *Ptelea trifoliata* (18), *Strobilanthes cusia* (19), *Zanthoxylum elephantiasis* (20), and the like. The incidence of confirmation of such activity was only a bit higher than that with semi-random acquisition of plants. Thus, although one has an interesting story to tell when one confirms a folkloric use, for practical purposes one can do nearly as well by spending the time in the field rather than long hours in the library.

From the recent literature it is clear that the chemical structures of the antimicrobial agents found in higher plants belong to most commonly encountered classes of higher plant secondary metabolites so their chemistry is familiar to most natural product chemists. Recent examples include alkaloids (21-24), bibenzyls (25), coumarins (26), chromans (27), dihydrophenanthrenes (28), flavanoids (29-32), quinones (33,34), saponins (35), terpenes (36-42), and the like. These chemical structures are significantly different from those of the antibiotics of fermentation origin. As there is overwhelming evidence that chemical structure and biological activity are intimately related, this means that the spectrum and mode of action of higher plant extracts will often be different from that of the fermentation-derived antibiotics. Whether the differences are usefully exploitable in human medicine must be established by further experimentation.

One of the primary factors that has enabled rapid progress in the field of antibiotics has been the extensive employment of bioassay-directed fractionation methodology. In this type of work, one employs a convenient bioassay to guide the chemist to the active constituents regardless of their chemical properties or their relative concentrations in the extracts. By contrast, traditional phytochemistry involves the isolation of abundant or readily crystallized substances or compounds belonging to a preselected chemical class followed, when the investigator desires, by a search for activity or utility when the product is already in hand. The substances so produced may or may not reflect the biological activity of the crude extract. Thus, whereas the phytochemist is in a position to make a contribution to phytochemistry, his contribution to medicine will often be fortuitous. The bioassay-directed natural product chemist, on the other hand, faces only a small risk of being diverted to the study of extraneous substances, and is just as likely to find, in passing, significant phytochemicals, and is well situated to make a simultaneous contribution to both chemistry and biology.

To illustrate the results one might expect from such work, we summarize here our recent experiences and those of others with the tropical genus *Erythrina* (Leguminoseae). World wide, the *Erythrina* have a significant history of folkloric medicinal use. A number of examples are included in Table 3 in which one sees a number of applications that could be interpreted as related to infectious disease.

In addition to these folkloric uses, several recent studies have focused upon the antimicrobial agents found in these species. The phytoalexins of *Erythrina sandwicensis* have been shown to be sandwicarpin [**1**], sandwicensin [**2**], dimethylmedicarpin [**3**], 3,6a,9-trihydroxypterocarpan [**4**], phaseollidin [**5**], and cristacarpin [**6**] (43).

TABLE 3. Folkloric Uses Worldwide of Various *Erythrina* Species

Erythrina species	Part	Origin	Disease
E. corallodendron	leaves	Haiti	asthma, venereal disease, neuralgia, hysteria (48)
	bark	Brazil	asthma, bronchitis, insomnia (49)
	leaves	Brazil	relieve toothaches, skin diseases (49)
E. berteroana	flowers	Guatemala	nervousness, hemorrhages, dyssentery (50)
E. folkersii	seeds	Columbia	diuretic, eye infections (51)
E. pallida	leaves	Trinidad	poultice for fresh wounds (52)
E. variegata	bark	China	antipyretic, liver ailments (53)
	bark	Indochina	fever, rheumatism (53)
E. subumbrans	bark	S.E. Asia	cough, poultice, postpartum vomiting (53)
E. abyssinica	roots	E. Africa	malaria, syphilis (54)

Phytoalexins have also been isolated from *Erythrina crista-galli* (phaseollidin [**5**], dimethylmedicarpin [**3**], and cristacarpin [**7**] (44). Constituitive agents have been isolated from *Erythrina abyssinica* (erythrabyssins I [**6**] and II [**8**], abyssinones I [**9**], II [**10**], III [**11**], IV [**12**], V [**13**], VI [**14**], phaseollin [**16**], and phaseollidin [**5**]) (45), *Erythrina sigmoidea* (sigmoidin A [**15**] and B [**17**]) (46), and *E. crista-galli* (erycristagallin [**18**]) (47).

Thus, we have focused a part of our effort on a systematic study of these plants, selecting species based upon literature reports and local advice.

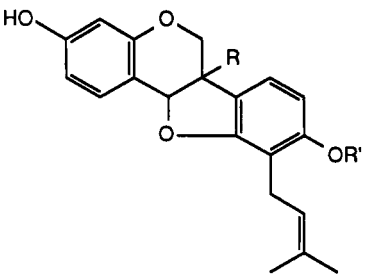
In our own laboratory we collected samples of *E. crista-galli* (from Bolivia), *Erythrina variegata* (syn. *Erythrina indica*) (from India), *Erythrina glauca* and *Erythrina falcata* (from Panama), *Erythrina fusca, Erythrina berteroana, Erythrina costaricans, E. variegata,* and *E. fusca* (from Honduras), and *Erythrina mildbraedii* (from Nigeria), ex-

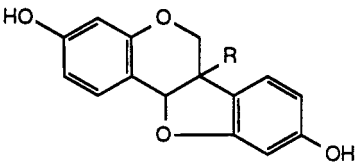
TABLE 4. In vitro (Agar-Streak Dilution) Antibacterial Activity of Various *Erythrina* spp. Crude Extracts

Erythrina species	Fraction	Microorganism[a] (μg/ml)						
		1	2	3	4	5	6	7
E. berteroana	leaf	i[b]	i	i	i	i	i	i
E. berteroana	bark	1000	i	i	i	1000	i	i
E. costaricans	root	1000	i	i	i	1000	i	i
E. crista-galli	root bark	100	i	i	i	100	i	i
E. crista-galli	stem bark	1000	i	i	i	100	i	i
E. crista-galli	flowers	i	i	i	i	i	i	i
E. crista-galli	twigs	i	i	i	i	i	i	i
E. crista-galli	leaves	i	i	i	i	i	i	i
E. falcata	bark	i	i	i	i	i	i	i
E. falcata	leaves	i	i	i	i	i	i	
E. fusca	roots	100	i	i	i	100	i	i
E. glauca	roots	i	i	i	i	i	i	i
E. indica	roots	1000	i	i	i	100	i	i
E. mildbraedii	roots	100	i	i	i	100	i	i
E. variegata	seed	i	i	i	i	i	i	i
E. variegata	bark	1000	i	i	i	100	i	i

[a]Microorganism 1=*Staphylococcus aureus* ATCC 13709; 2, *Escherichia coli* ATCC 9637; 3, *Salmonella gallinarum* ATCC 9184; 4, *Klebsiella pneumoniae* ATCC 10031; 5, *Mycobacterium smegmatis* ATCC 607; 6, *Candida albicans* ATCC 10231; 7, *Pseudomonas aeruginosa* ATCC 27853.

[b]Inactive.





1 R=OH, R′=H
2 R=H, R′=Me
5 R=R′=H
6 R=OH, R′=Me

3 R=H
4 R=OH

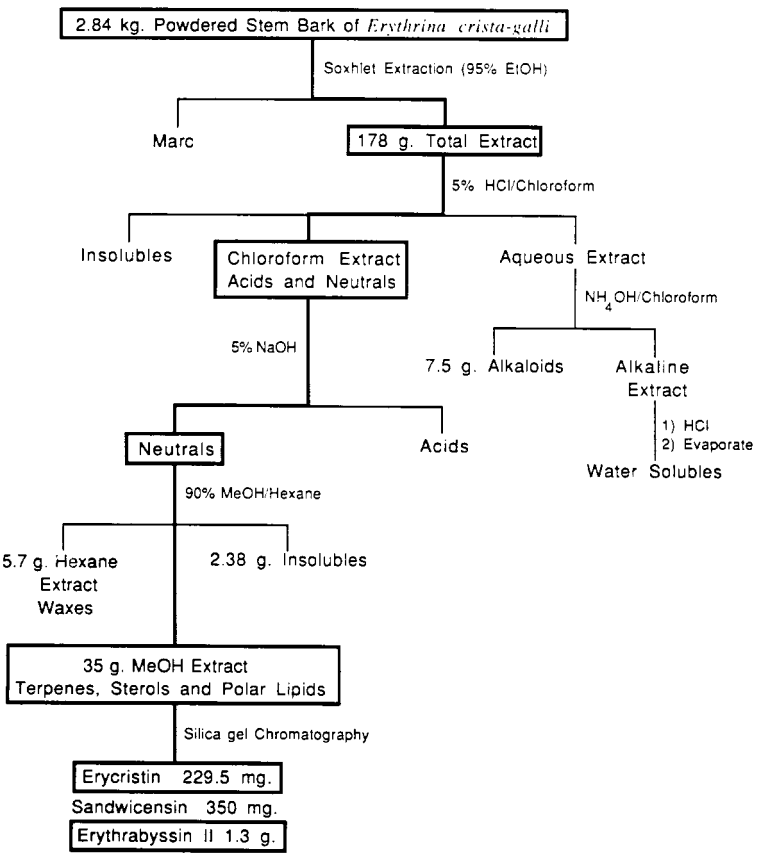
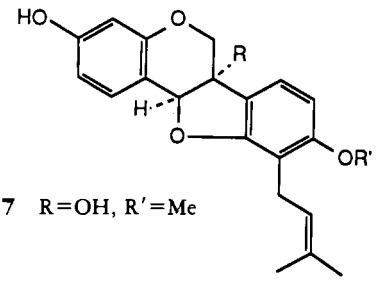
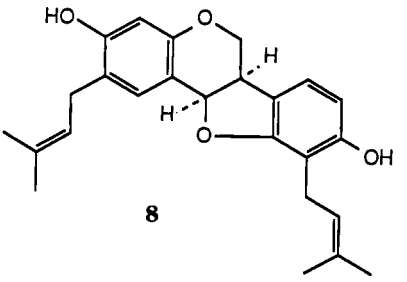


FIGURE 2. Bioassay-directed fractionation of *Erythrina crista-galli* extracts. Antimicrobially active materials are enclosed in boxes.



7 R=OH, R′=Me



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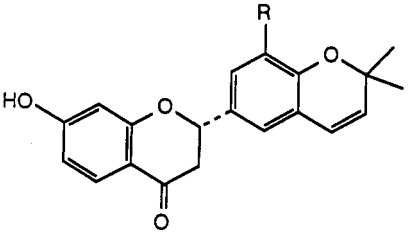
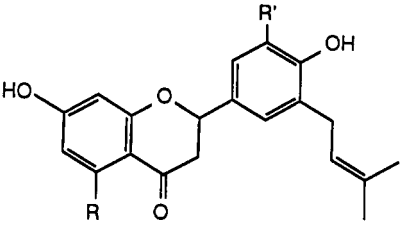
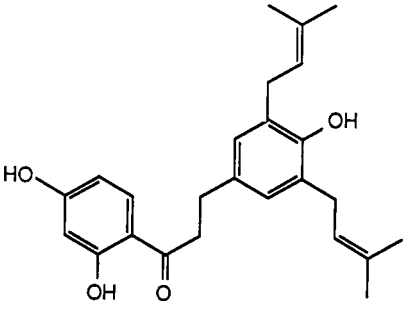


9 R=H
11 R=prenyl



10 R=R′=H
12 R=H, R′=prenyl
13 R=OH, R′=prenyl



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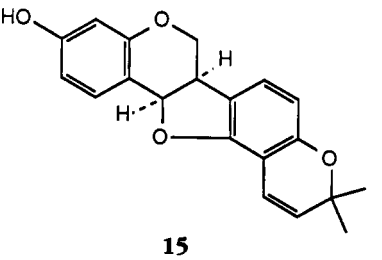

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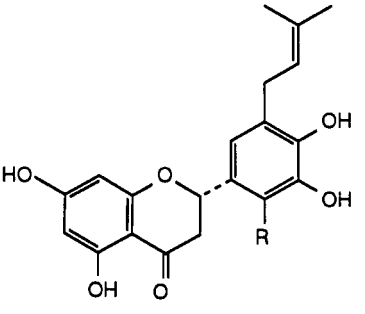


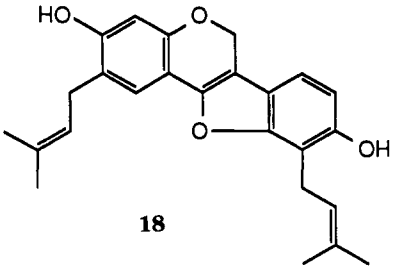
16 R=prenyl
17 R=H

tracted samples exhaustively with hot EtOH, and obtained the testing results in Table 4. *E. falcata* and *E. glauca* extracts were inactive. The other plants were active against *S. aureus* and *M. smegmatis*, but not all portions of the plants showed activity. Generally, the bark and the roots had the activity of the plants.

Each specimen was then fractionated systematically by bulk transfer methodology into groups of related polarity as illustrated for *E. crista-galli* in Figure 2. Each subfraction was bioassayed against all of the organisms, and the activity was found to be limited to the phenolic portion.

The phenolic portions were then subjected to Si gel column chromatographic resolution, and each fraction was tested for its biological activity. The active substances were purified to homogeneity and identified from their physical and chemical properties or by chemical degradation until an unambiguous structure emerged. The structures were confirmed by chemical interconversions.

With *E. crista-galli*, sandwicensin [**2**] (43), and erythrabyssin II [**8**] (44) were previously known compounds whose structures had been established by spectroscopy. The structures of erycristagallin [**18**] (47) and erycristin [**19**] were first assigned by spectroscopic considerations and then confirmed by a network of reactions through which all four compounds were interconverted. This leaves no reasonable doubt of their structural identity.



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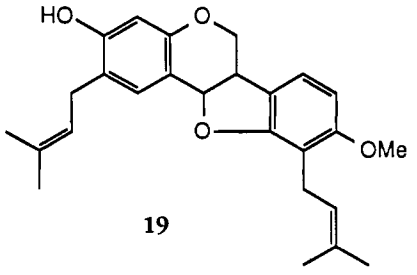

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As seen in Figure 3, prenylation of the free *ortho*-positions adjacent to the solitary phenolic OH of sandwicensin [**2**] produced a mixture of erycristin [**19**] and isoerycristin [**20**]. These two were easily distinguished by ^{1}H nmr because erycrestin has a *para*-AB Ar-H pair, while those of isoerycristin are *ortho*-orientated. Interconversion of erycristin [**19**] and erythrabyssin II [**8**] was readily accomplished by methylation compound [**21**]. The location of the free phenolic OH of erycrestin was obvious from the *ortho* shifts seen upon its acetylation. When the diacetyl ester of erythrabyssin II [**22**] was dehydrogenated, it was smoothly converted to erycristagallin diacetate [**23**]. Thus, the structures of these compounds are in agreement with both spectroscopic and chemical findings including those of erythrabyssin II and erycristagallin whose structures previously depended entirely upon spectroscopic findings.

It is interesting to note that the roots and stem bark of *E. crista-galli* contain closely related but chemically distinct antimicrobial agents. Their antimicrobial spectra (Table 5) are identical, but their potencies are rather different. The pterocarpan (erythrabyssin II) is roughly equipotent to erycristagallin, while the other compounds are significantly less active in vitro.

While working with the mother-liquors containing a number of minor constituents, the inactive isoflavenone derrone [**24**], previously known from *Derris robusta*, was isolated and characterized (55).

From an investigation of the constituents of Honduran *E. fusca* roots, the active principle identified in this plant was found to be 5,7,4′-trihydroxy-6,8-diprenylisoflavone [**25**], previously identified in *Euchresta japonica* (56) and *Millettia pachycarpa* (57) but not previously known to have antimicrobial activity.

The activity of extracts of the roots of *E. variegata* is attributable to the presence of five phenolic components. Four of these proved to be known substances. Erycristagallin [**18**] and erythrabyssin II [**8**] have just been discussed as components of *E. crista-galli* as

well as has 5,7,4′-trihydroxy-6,8-diprenylisoflavone [**25**] from *E. fusca*. Warangalone [**26**] has recently been reported as a constituent of African *Erythrina senagalensis*, although no biological activity was reported (58). Warangalone is about half as potent as **25**, suggesting the importance of a free C-7 OH group in isoflavanoids for antimicrobial potency.

Eryvarigatin [**27**] is structurally quite interesting because relatively few bibenzyls have as yet been reported in the chemical literature, and it is a new compound to the lit-

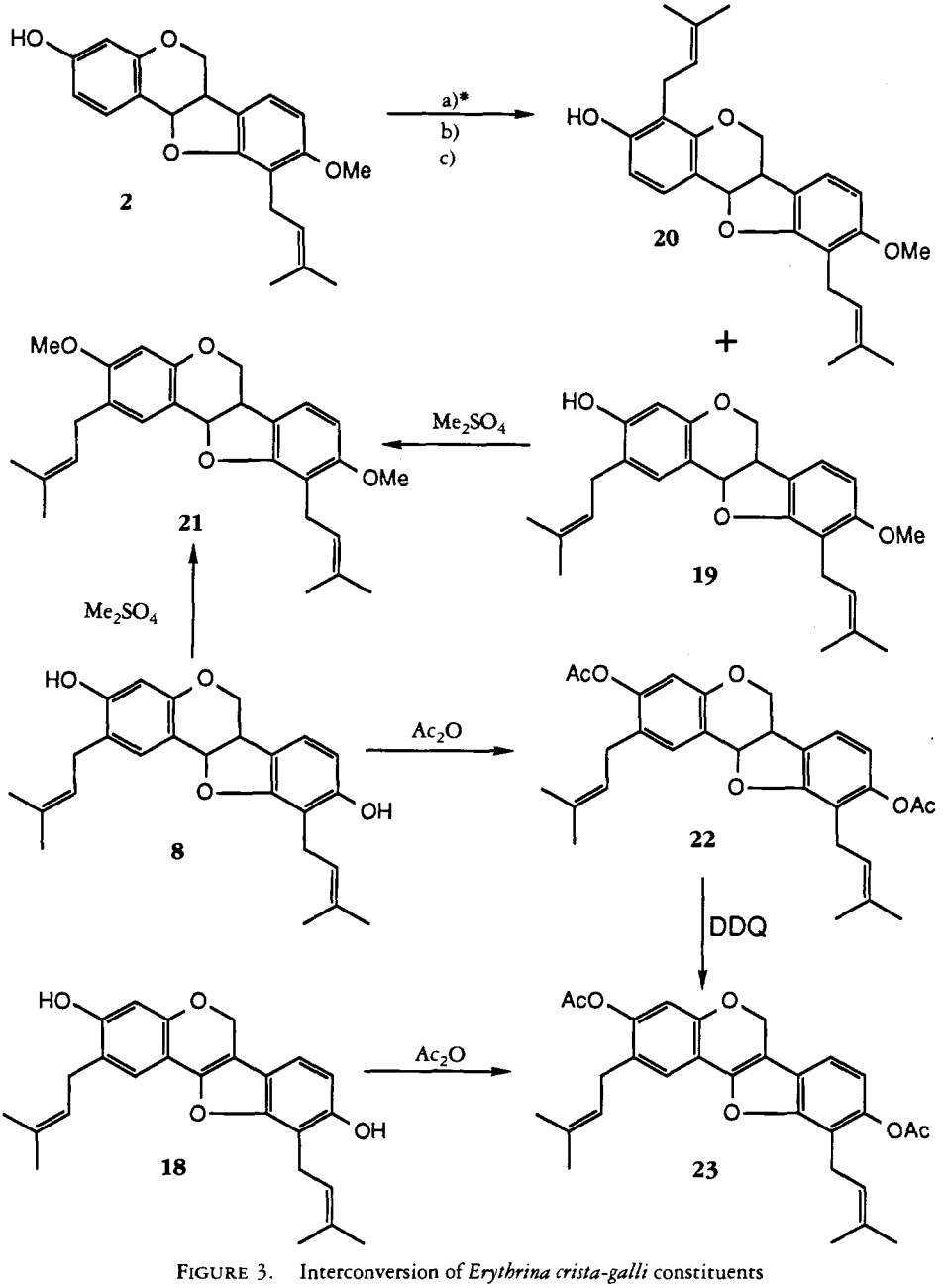


FIGURE 3. Interconversion of *Erythrina crista-galli* constituents

*(a) 3-Chloro-3-methylbutyne/K_2CO_3/KI/Me_2CO; reflux 24 h; (b) Pd-C/$BaSO_4$ quinoline/H_2; (c) *N,N*- diethylaniline/reflux 1 h.

TABLE 5. Antimicrobial Potency of Various *Erythrina* Components in vitro

Compound	Microorganism[a] (μg/ml)						
	1	2	3	4	5	6	7
Sandwicensin [**2**]	i[b]	i	i	i	i	i	i
Erythrabyssin II [**8**]	3.12	i	i	i	0.78	i	i
Erycristagallin [**18**]	1.56	i	i	i	1.56	i	i
Erycristin [**19**]	6.25	i	i	i	6.25	i	i
Derrone [**24**]	i	i	i	i	i	i	i
5,7,4′-Trihydroxy-6,8-diprenylisoflavone [**25**]	6.25	i	i	i	6.25	i	i
Warangalone [**26**]	12.5	i	i	i	12.5	i	i
Eryvarigatin [**27**]	50	i	i	i	25	i	i
Phaseolin [**15**]	25	i	i	i	25	i	i
Erybraedin A [**28**]	12.5	i	i	i	6.25	i	i
Erybraedin B [**29**]	12.5	i	i	i	12.5	i	i
Erybraedin C [**30**]	12.5	i	i	i	12.5	i	i

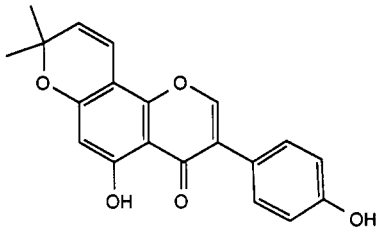
[a]Micoorganism 1=*Staphylococcus aureus* ATCC 13709; 2, *Escherichia coli* ATCC 9637; 3, *Salmonella gallinarum* ATCC 9184; 4, *Klebsiella pneumoniae* ATCC 10031; 5, *Mycobacterium smegmatis* ATCC 607; 6, *Candida albicans* ATCC 10231; 7, *Pseudomonas aeruginosa* ATCC 27853.

[b]Inactive.

erature of natural products. Unfortunately, it has only weak antimicrobial activity. Its chemical structure relies, for the moment, entirely upon spectroscopic findings and must be regarded as tentative. A synthesis is in progress and will be reported separately.

E. mildbraedii roots are used in Nigeria for the treatment of infections. Fractionation of the bioactive root extracts led to the isolation of two known active compounds, phaseolin [**15**] and erythrabyssin II [**8**], and the new compounds, erybraedins A-C [**28-30**]. The structures of these new substances were established by spectroscopy and proven by chemical interconversions.

The flavanoids isolated from various tropical *Erythrina* species that possess antimi-



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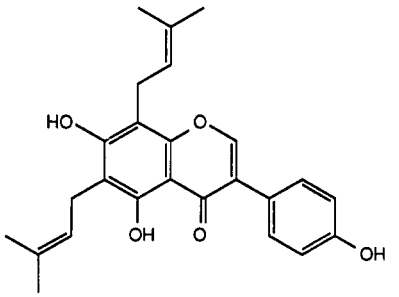

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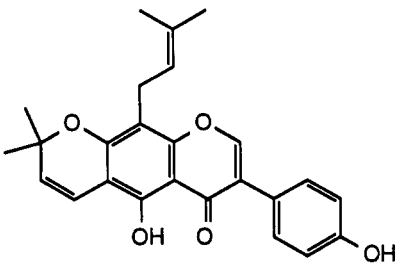

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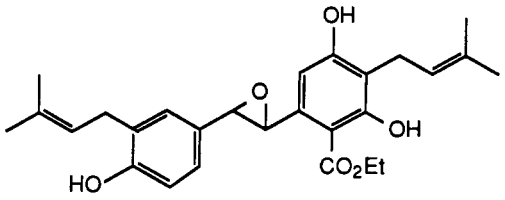

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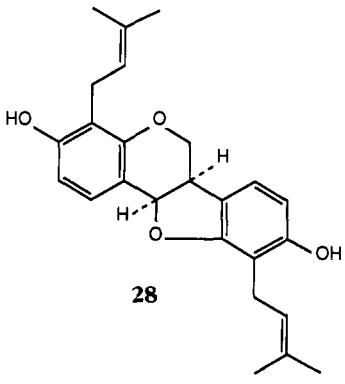

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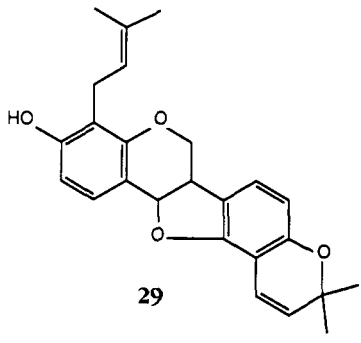

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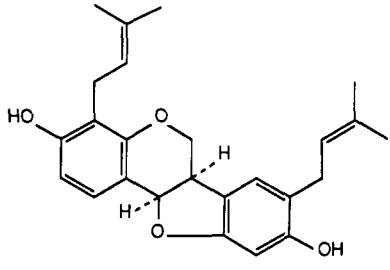

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crobial activity are sufficient to rationalize the folkloric use of these plants for the control of certain infections. The antimicrobial spectra and potencies of these compounds are recorded in Table 5.

Studies with *Glycyrrhiza glabra* demonstrated that even thoroughly studied plants contain new constituents that can be uncovered by the use of bioassay-directed fractionation methodology (59). Subsequent work with *Glycyrrhiza lepidota* demonstrated that bioactive plants in the same genus may well have different active constituents and be worthy of fractionation (60).

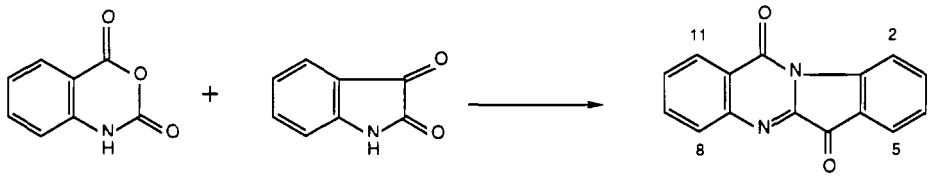
The active constituent of Taiwanese *Strobilanthes cusia*, a species used locally for der-

TABLE 6. Antimicrobial Activity of Various Synthetic Analogues of Tryptanthrin

Tryptanthrin	[1][a]50 [2]100 [3]6.25 [4]100	Tryptanthrin	[5]3
11-Cl	i[b]	8-CH_3	[5]1
3-Cl	i	4-Br	[5]0.3
3,11-diCl	i	10-Cl	[5]0.1
11-Br	[1]25 [2]25 [3]25 [4]50	10-Me	[5]1
11-NO_2	i	5-Me	[5]10
3-NO_2	[1]12 [3]12	6-Me	[5]10
3-NO_2-11-Cl	i	2-Me	[5]1
3-NO_2-11-Br	[1]6.25 [3]25 [4]50	7-NO_2	[5]10
3,4-diCl	[3]6.25	4-Cl	[5]1
5-Cl	[3]6.25		
4,5-diCl	[3]12.5		
10-Br	[1]25 [2]25 [3]25 [4]50		
4-NO_2-10-Br	[1]3.12 [3]25 [4]50		
4-NO_2	[1]12 [3]12		
4,10-diNO_2	[1]100 [3]12.5		

[a]Test microorganisms designated in brackets [1]=*Staphylococcus aureus* ATCC 13709, [2]=*Klebsiella pneumoniae* ATCC 10031, [3]=*Mycobacterium smegmatis* ATCC 607, [4]=*Candida albicans* ATCC 10231, [5]=*Bacillus subtilis* (E. Fiedler *et al.*, *Arch. Microbiol.*, 1976, **107**, 249).

matophytes, proved to be the previously known alkaloid tryptanthrin [**31**] (61). A facile synthesis was subsequently developed utilizing substituted isatoic anhydrides [**32**] and isatins [**33**], which allowed the production of numerous analogues (Table 6).



32 **33** **31**

In our hands as well as in the hands of others (62), it was clear that potency against any individual microorganism could be intensified by the appropriate choice of substituent and that the antimicrobial spectrum could be modified at the same time. As seen in Table 7, tryptanthrin was superior in potency to nystatin in vitro against a number of dermatophytic fungi (63). This certainly rationalizes the folkloric use of this material, and the search for a clinically useful agent based upon the structure of tryptanthrin continues.

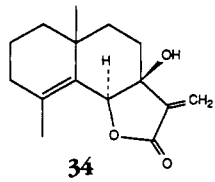
TABLE 7. Antidermatophytic Activity of Tryptanthrin and Some Analogues (in μg/ml)

Dermatophytes	Nystatin	Tryptanthrin	4-NO_2	4-NO_2-10-Br	4-Cl
Epidermaphyton floccosum ATCC 290	6.2	1.56	6.2	i[a]	i
Microsporum audouini ATCC 10216	3.1	1.56	6.2	i	i
Microsporum canis	3.1	1.56	1.56	i	i
Microsporum gypseum ATCC 1236	6.2	>1000	>1000	i	i
Trichophyton mentagrophytes ATCC 9533	3.1	1.56	6.2	i	i
Trichophyton tonsurans ATCC 10217	0.78	0.39	3.1	i	i
Trichophyton rubrum	0.78	0.78	3.1	i	i
Pityrosporum ovale ATCC 14521	0.78	12.5	—	—	—

[a]Inactive.

A somewhat wider sense of the structural range of materials that can be encountered in such studies can be gained by considering the structures of the active constituents of Indian *Sphaeranthus indicus* roots (64) (the sesquiterpene lactone, sphaerindicin [**34**]), *Amorpha nana* fruit from South Dakota (65) (the stilbene, amorphastilbol [**35**]), Kansan *Amorpha fruiticosa* fruit (66) (the bibenzyls, amorfruitins A [**36**] and B [**37**]), Australian *Boehmeria cylindrica* (67) (the intensely antifungal phenanthroquinolizidine alkaloid, cryptopleurine [**38**] and its seco analogue, julandine [**39**]), Texan *Karwinskia humboldtiana* (68) (the dimethylbenzisochromans karwinaphthols A [**40**] and B [**41**]), Indian *Flemingia stricta* (32) (the flavanoid, flemiflavanone D [**42**]), and Southeast Asian *Daemonorops draco* (69) (the flavanoids, dracorhodin [**43**] and dracorubin [**44**]).

From the studies cited and the numerous works of others in this expanding field, the following generalizations emerge. Antimicrobial agents from higher plants are plentiful. In many cases, investigation with modern methodology confirms folkloric accounts of the use of higher plant preparations for the treatment of infections. The



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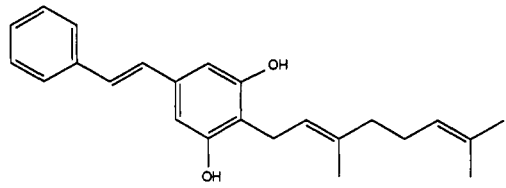

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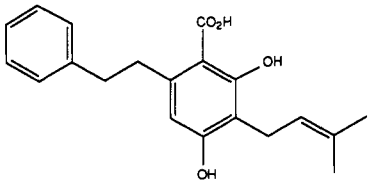

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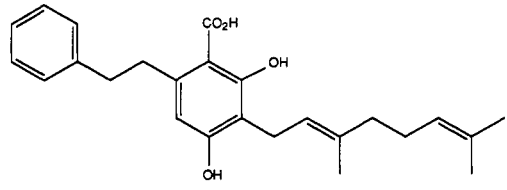

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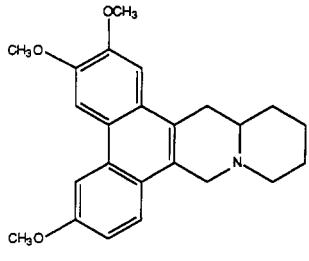

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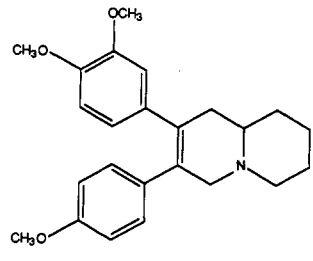

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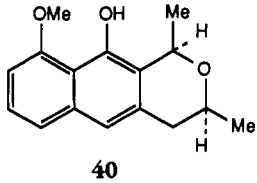

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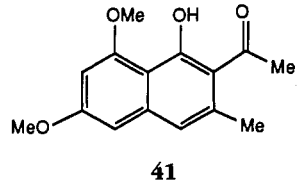

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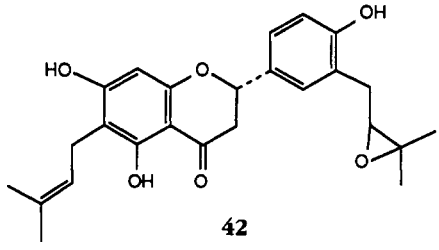

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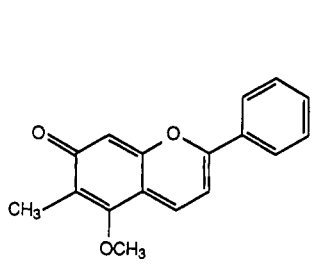
screening methodology for detection of such agents is simple: The compounds are easy to isolate; they are usually easy to synthesize for the production of analogues, and the synthetic analogues can have superior properties even with respect to potency and spectrum. Their potency in vitro is often as intense as that of commercialized agents from other sources; they usually have relatively narrow antimicrobial spectra; such agents represent novel structural leads; many known natural products must have unestablished antimicrobial activity which could be detected by study, thoroughly studied plants often contain new constituents when studied using bioassays, and the agents detected have a finite potential for use in the treatment of human or agricultural infections.



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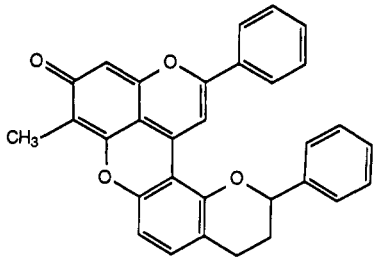

44

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