BIOLOGICALLY ACTIVE COMPOUNDS FROM PLANTS WITH REPUTED MEDICINAL AND SWEETENING PROPERTIES[1]

A. Douglas Kinghorn

Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612

ABSTRACT.—Examples are presented of plants with folkloric reputations that have recently afforded constituents with great potential for use as drugs or pharmaceutical excipients. Described are the results of laboratory investigations at this institution on the biologically active compounds of four plants with reputed medicinal properties and of six sweet plants used by local populations in various regions of the world. It is concluded that folklore information should be considered seriously in programs designed to yield prototype, biologically active molecules from plant sources.

Since the beginning of the 19th century, a large number of biologically active secondary metabolites of plant origin have been found to have commercial application as drugs, flavors, pesticides, or as other types of speciality chemicals. Natural products obtained as plant isolates may be useful directly or may serve as starting materials for the synthesis of active agents (1-4). In addition, natural products are suitable as lead compounds for the subsequent design of structurally related molecules that are more active or less toxic (1,4,5).

Recently, there has been an upsurge in interest in the use of plants with folkloric reputations or with application in traditional medicine as sources of potentially useful compounds (6-9). The term *folklore* in regard to the discovery of vegetal antitumor agents has been put forth as "encompassing all reports implying that a plant produces a physiologic effect against any animal organism and includes plants used for medicinal purposes, insecticides, fish or arrow poisons, and those suspected to be poisonous to man or livestock" (10). The following examples of promising biologically active molecules, derived in recent years from plants with histories of folkloric use, show the potential value of this type of information.

The sesquiterpene endoperoxide, qinghaosu (artemisinin) [1], is a constituent of the Chinese medicinal herb, *Artemisia annua* L. (Compositae), that has been employed in China for centuries as an anti-infective and for the treatment of malaria. This compound was first isolated by Chinese investigators in 1972, and has since been used successfully in the treatment of several thousand patients in China where it was revealed to have potency as an antimalarial comparable to chloroquine (11,12). The drug has been
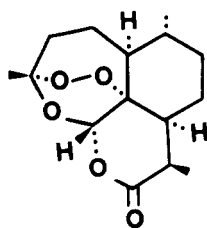

1   2
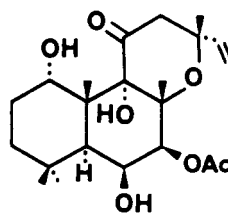
totally synthesized, and several of its derivatives also exhibit potent, drug-resistant, plasmodicidal activity (11-13).

A further terpenoid with outstanding potential for drug use is the labdane diterpenoid, forskolin [2], an isolate of *Coleus forskohlii* Briq. (Labiatae). The use of *Coleus* species for the treatment of ailments including heart diseases, respiratory problems, and convulsions was described in ancient Hindu Ayurvedic texts. Forskolin [2] was isolated by two groups in India and is an antihypertensive agent, with spasmodic, positive inotropic, and platelet aggregation inhibitory activities. The compound decreases intraocular pressure and, thus, may have use in the therapy of glaucoma and is a powerful activator of adenylate cyclase in various tissues (14-16).

Garlic (*Allium sativum* L., Liliaceae) has been used around the world for many years to treat ailments such as heart problems, and for the prevention of strokes, coronary thrombosis, and atherosclerosis (17). Block and co-workers have isolated several sulfur-containing constituents of garlic, including (*E,Z*)-ajoene [3] which is a potent inhibitor of platelet aggregation. The antithrombotic activity of ajoene has been attributed to its ability to alter platelet membranes by capturing sulfhydryl groups (18).
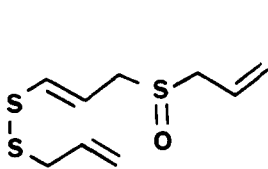

3
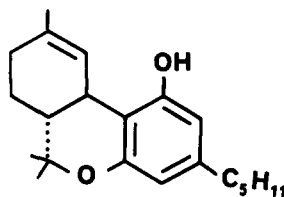
4

Extracts of the seeds of *Thaumatococcus daniellii* (Bennett) Benth. (Marantaceae) have been used by local populations in West Africa for sweetening sour fruits and for making over-fermented palm wine more palatable (19). Two sweet principles, thaumatins I and II, were isolated and characterized from this plant in 1972 and found to be similar proteins with almost identical molecular weights of 22,000 (20,21). Thaumatin I is about 3,000 times sweeter than sucrose on a weight basis, and its three-dimensional structure was recently established (21). Talin® protein, a mixture of the aluminum salts of several thaumatin proteins extracted from *T. daniellii*, is now available as a high-intensity sweetening agent in several countries including Australia, Japan, and the United Kingdom. Talin® protein has been accorded Generally Regarded as Safe (GRAS) status in the United States as a flavor adjunct in chewing gum. In the United Kingdom the Committee on the Safety of Medicines has approved Talin® protein as a safe excipient for sweetening and improving the flavor of medicines (22).

A very recent addition to the drug market in the United States is dronabinol (Marinol®), which is the synthetic form of Δ^9-tetrahydrocannabinol [4], the principal, psychoactive constituent of marihuana (*Cannabis sativa* L., Cannabinaceae) (23). This compound is available for its antiemetic effects during cancer chemotherapy that has not responded to conventional drugs used to suppress emesis. Although the antiemetic effect of marijuana has been known since the 19th century (24,25), the introduction of 4 into current drug therapy may be directly attributed to more recent anecdotal reports suggesting that smoking marijuana decreases the nausea and vomiting experienced during cancer chemotherapy (25,26).

Etoposide (VePesid®) [5] is an example of a synthetic compound modeled on a natural product, podophyllotoxin [6], a constituent of *Podophyllum peltatum* L. (Berberidaceae), a plant with folkloric reputation in the treatment of cancer (27,28). Etoposide is now clinically available in the United States for the treatment of testicular

5   6
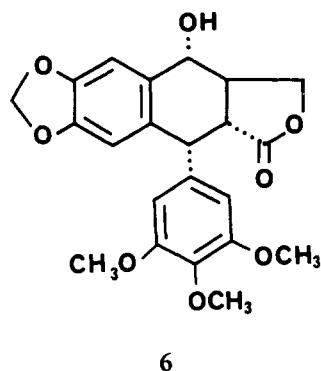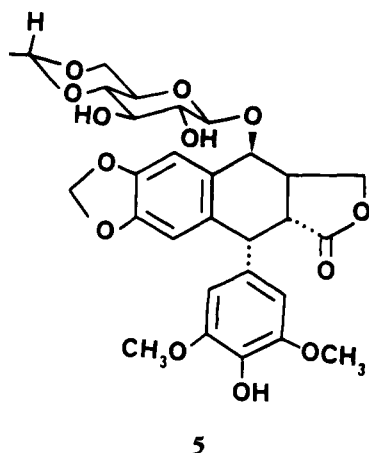
cancer and small-cell lung carcinoma (23). Compound **5** is a less toxic and more potent antineoplastic agent than **6** and was developed in the Basel laboratories of Sandoz, Ltd. (28).

STUDIES ON THE CONSTITUENTS OF SOME PLANTS WITH REPUTED MEDICINAL PROPERTIES.—The seed of *Croton tiglium* L. (Euphorbiaceae), the source of croton oil, is used in traditional Chinese medicine as a purgative, revulsive in colds and for apoplexy, paralysis, scabies, throat infections, toothache, and schistosomiasis (29). Croton oil produces severe symptoms of toxicity when taken internally or applied externally to the skin (30). However, croton oil is perhaps of greatest interest to the scientific community as a result of the discovery of its tumor-promoting activity for mouse skin in 1941 (31), after which the pure biologically active constituents were demonstrated in the 1960s to be esters of the diterpene, phorbol (32). Hecker and colleagues eventually isolated eleven phorbol 12,13-diesters from croton oil that were extractable into hydrophilic solvents, as well as a further three such compounds obtained after the partial hydrolysis of a mixture of phorbol 12,13,20-triesters present in a lipophilic extract of the seed oil (32). In addition, phorbol esters have been implicated as inducers of the Epstein-Barr virus with which there is a strong etiological relationship in some human tumors such as nasopharyngeal carcinoma. Hirayama and Ito have correlated the geographical areas with the highest incidence of nasopharyngeal cancer in the People's Republic of China with a correspondingly high usage of *C. tiglium* and other euphorbiaceous herbs that are employed for the treatment of gastrointestinal disturbances (33).

In this laboratory we have shown the use of droplet counter-current chromatography (dccc) for the purification of several diterpene components of both unhydrolyzed and hydrolyzed *C. tiglium* seed oil (34-37). In addition to obtaining several of the phorbol diesters previously characterized by Hecker, we were able to isolate by dccc seven short-chain esters of phorbol [**7-13**] and four short-chain esters of 4-deoxy-4α-phorbol [**14-17**] that had not been obtained from croton oil previously (35-37). Several of these compounds were new, and neither diterpene monoesters such as **12**, **13**, and **17**, nor 4-deoxy-4α-phorbol esters had been known as croton oil constituents before. These various short-chain phorbol and 4-deoxy-4α-phorbol esters occurred in a combined yield of 0.258% w/w in croton oil and may have considerable biological significance when croton oil is used in two-stage carcinogenesis experiments, inasmuch as they may interfere with the tumor-promoting activity of the major phorbol ester constituent of *C. tiglium*, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) [**18**]. Several synthetic, short-chain, phorbol esters, while inactive as mouse skin tumor promoters themselves, have been
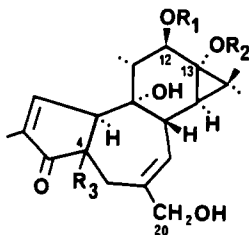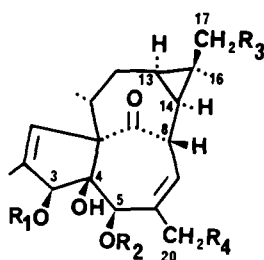
Compound	R_1	R_2	R_3
7	tiglate	isobutyrate	β-OH
8	tiglate	Ac	β-OH
9	2-methylbutyrate	isobutyrate	β-OH
10	2-methylbutyrate	Ac	β-OH
11	Ac	Ac	β-OH
12	tiglate	H	β-OH
13	H	Ac	β-OH
14	tiglate	isobutyrate	α-H
15	tiglate	Ac	α-H
16	2-methylbutyrate	Ac	α-H
17	H	Ac	α-H
18	tetradecanoate	Ac	β-OH

shown by others to drastically inhibit in a dose-response fashion the activity of TPA (38). Dccc is a method usually employed for the separation of polar, plant secondary metabolites (39), although its applicability to the resolution of the croton oil, phorbol ester toxins suggests that this procedure may be useful for the separation of other fixed oil constituents.

We have recently demonstrated the effectiveness of overpressure layer chromatography (oplc) for the preparative purification of TPA [18] and other phorbol diester constituents of croton oil (40). The oplc stage was carried out after a single preliminary separation step using low-pressure column chromatography, and the homogeneity of the eluted TPA was confirmed by chemical ionization ms (40). Oplc offers a much more rapid method for the purification of the croton oil, biologically active principles than either the multiple liquid-liquid distribution techniques used traditionally (32) or dccc (36,37). This procedure should be much more widely applied for the analysis and purification of natural products in future years.

Euphorbia hermentiana Lem. (syn. *E. trigona* Haw.) (Euphorbiaceae) has folk use as a medicinal plant in southern Asia and is used for the treatment of earache and boils (41). Our group encountered *E. hermantiana* as a houseplant sold in the United States and decided to perform a controlled human dermatological experiment on the latex of this species. Small (5-μl) portions of the undiluted latex placed on flexor forearms of volunteer subjects in open (non-occluded) patch tests resulted in the generation of irritant follicular dermatitis with residual hyperpigmentation persisting for over a week following application. Closed (occluded) testing to the upper surfaces of the forearm produced bullae and severe vesiculation. These results indicate that *E. hermentiana* represents a potential health hazard if purchased as a houseplant, and great care should be exercised when moving or pruning this plant (42).

The skin-irritant latex of *E. hermentiana* proved to contain a complex mixture of diterpene esters that was painstakingly separated using dccc as the primary isolation technique. The compounds obtained were the 17-hydroxyingenol esters [19-21], the ingenol ester [22], the 20-deoxy-17-hydroxyingenol ester [23], the 8-methoxyingol esters [24-26], the 19-hydroxyingolester [27], and the ingol esters [28-30] (43-45). A number of these diterpenoids had not been purified or characterized previously. The positions of ester substitution of these isolates were established by stepwise, hydrolysis

Compound	R_1	R_2	R_3	R_4
19	deca-2,4,6-trienoate	H	O-Angelate	OH
20	angelate	Ac	OAc	OAc
21	angelate	H	OAc	OAc
22	angelate	H	H	OAc
23	angelate	H	OAc	H
31	Ac	Ac	H	OAc
32	Ac	Ac	OAc	H

experiments. The skin-irritant activity of the latex of *E. hermentiana* is due primarily to the 17-hydroxyingenol and ingenol derivatives [19-22]; the 20-deoxy-17-hydroxy-ingenol derivative [23] would not be expected to be active in this regard (44,45). While compounds 19-21 were originally assigned as esters of 16-hydroxyingenol (44), the subsequent availability of high-field nmr instrumentation permitted the running of an nOe difference experiment on their common diterpene product, 17-hydroxyingenol-3,5,17,20-tetraacetate [31], obtained in each case on the hydrolysis of 19-21. Therefore, it was concluded that the C-16 methyl group of 31 has the same configuration as the C-13 and C-14 protons and that the C-17 acyloxymethyl group is β-oriented (45). A similar nOe difference experiment was performed on the hydrolyzed, acetylated product of compound 23 in which the presence of 20-deoxy-17-hydroxyingenol-3,5,17-triacetate [32] was confirmed (45).

It has been proposed that diterpenes of the phorbol ester and biogenetically related types (daphnane, ingenane, lathyrane) are plant irritant, defense substances (37). Because no previous work showing the effects of these toxins on insects had apparently been performed, we tested compounds of this type against a number of insect pests. Twenty diterpene esters were assayed for growth-inhibitory and insecticidal effects against newly hatched larvae of the lepidopterous organism, *Pectinophora gossypiella* Saunders (pink bollworm). The most potent compound tested was 12-0-tetradecanoyl-
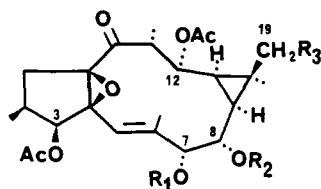

Compound	R_1	R_2	R_3
24	benzoate	Me	H
25	tiglate	Me	H
26	angelate	Me	H
27	Ac	benzoate	OH
28	Ac	benzoate	H
29	Ac	tiglate	H
30	tiglate	H	H

phorbol-13-acetate (TPA) [**18**] which also is the most potent tumor-promoting princi-
ple of croton oil (32). TPA was found to cause 100% mortality on second-stadium lar-
vae of *Culex pipiens* L. (house mosquito) at a dose of 0.6 ppm, but either negligible or no
effects occurred when TPA was tested at 25-32 μg/insect on the milkweed bug (*Oncopel-
tus fasciatus* Dallas; second-stadium nymph) and the confused flour beetle (*Tribolium con-
fusum* Jacquelin du Val; adults). Only the phorbol esters with long-chain, acyl sub-
stituents and the daphnane esters tested were active as growth-inhibitory compounds
against *P. gossypiella*; the ingenane and ingol (lathyrane) derivatives evaluated showed
no effects in this regard at the maximum dose tested (50 ppm) (37). A close correlation
was observed between results obtained in this *P. gossypiella* growth inhibition bioassay
and cytotoxicity data determined for the same test compounds against P-388 lym-
phocytic leukemia cells in culture (37,45). Therefore, this insect bioassay might well
be of future use as an inexpensive and convenient prescreen for cytotoxic phorbol and
daphnane esters.

The bark of *Acosmium panamense* (Bentham) Yakovlev (syn. *Sweetia panamensis*
Mohlenbrock) (Leguminosae) was formerly official in the "National Formulary" of the
United States as Cascara Amarga, Bitter Bark, or Honduras Bark, during the period
1926-1942 (46,47). In the form a fluidextract, Cascara Amarga was used as an altera-
tive for the treatment of chronic cases of syphilitic tubercles and eruptions, as well as
gummy tumors, chronic eczema, and chronic nephritis (48). In Mexico and certain
Central American countries the bark itself or a decoction of the bark has been used
folklorically as an anticatarrhal, antimalarial, antipyretic, antitussive, bitter tonic, and
a remedy for diabetes and scrofula (48). The bitter nature of the bark of *A. panamense* can
be attributed to the abundant and varied quinolizidine alkaloids that accumulate to the
extent of about 1.5% w/w. These compounds were found to be of three types, namely,
quinolizidines of the lupine, *Ormosia*, and of a new type, which we have called *Acosmium*
alkaloids. Among the lupine-type alkaloids was the new compound, (−)-4α-hydroxy-
sparteine [**33**], whose structure was proven by ms after derivatization (49). The *Ormosia*
alkaloid, sweetinine, originally obtained from this plant source by Beal and co-workers
(47), was reisolated and assigned the structure (±)-6-*epi*-podopetaline [**34**] (50). A
close derivative of compound **34**, (±)-homo-6-*epi*-podopetaline [**35**], was also isolated
from *A. panamense* bark and characterized as a new natural product (48,51). However,
the major alkaloids proved to be based on a new C_{20} skeleton and have been named acos-
minine [**36**] and acosmine (4-deoxyacosminine) [**37**]. The structure of acosminine [**36**]
was established by spectroscopic studies, incorporating uv, ir, ms, and one- and two-
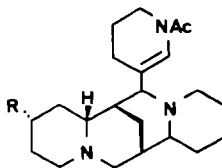dimensional nmr (52).
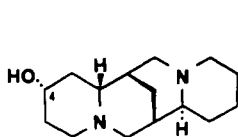
33
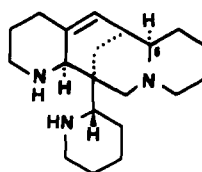
34
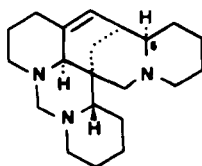
35

36   R=OH
37   R=H

The genus *Acosmium* is the most primitive taxon of the legume subfamily Papilionoideae known to biosynthesize quinolizidine alkaloids, and, thus far, the *Acosmium* alkaloids **36** and **37** have not been found in any other legume species (48,51,52). Notably absent from the bark of *A. panamense* were α-pyridone quinolizidine bases, which are considered the most biogenetically advanced members of the lupine alkaloids, and it may be suggested that oxidative pathways leading to the pyridones must have occurred subsequent to the evolution of the primitive, rain forest trees that constitute the genus *Acosmium* (48,51,52). Pyridone quinolizidine bases are also known to be more acutely toxic than saturated lupine quinolizidines (51), and the absence of these compounds from the bark of *A. panamense* is consistent with the fact that no cases of acute toxicity were reported during the former wide use of Cascara Amarga in the United States (48).

A final example of a medicinal plant we have encountered with a folklore use is *Ottonia frutescens* (C. DC.) Trel. (Piperaceae). This was obtained from northeastern Paraguay, where it is known locally as "Anestesia," and is used to relieve toothache pains and to treat sore throats. When the fresh or dried stems or roots are chewed, a prolonged numbness of the tongue results. Activity-guided fractionation revealed that the tongue-numbing principle of *O. frutescens* was the known *N*-isobutylamide, piperovatine [**38**] (53). The local anesthetic activity of piperovatine has long been appreciated, and a solution of this compound in almond oil was once used in the United Kingdom to afford temporary relief from painful, superficial oral lesions. The rather short-term nature of its effect, however, coupled with its tendency to cause excess salivation, prevented its successful application in minor dental operations (53,54).
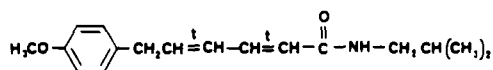
38

THE SEARCH FOR HIGH-INTENSITY SWEETENERS FROM PLANTS WITH FOLKLORIC USE.—Among sweet substances, sucrose is paramount in that it produces sweetness that is unmasked by any other taste sensation. However, when this caloric substance is used as a bulk sweetener, relatively large amounts need to be incorporated into foods, beverages, and medicines. In addition, sucrose is now recognized as the major dietary component tending to produce dental caries in developed societies. Therefore, much effort has been spent in research laboratories to discover compounds of either natural or synthetic origin that may serve as non-caloric and/or non-cariogenic sucrose substitutes. The search for natural, high-intensity sweeteners, which are compounds hundreds or even thousands of times sweeter than sucrose, has been stimulated by perceived problems with the safety, chemical instability, unpleasant taste characteristics, or the high cost of production of existing sucrose substitutes (55).

A summary of the known classes of high-intensity plant-derived sweeteners is given in Table 1. Analysis of the table shows the chemical diversity of these sweet substances. These compounds are not restricted to a few taxonomic groups, and many have been reported only very recently (56). Several of the compounds mentioned in Table 1 have commercial value in one or more countries of the world (22,55,56). Given that at the moment there is a lack of convenient bioassay systems to screen for potential sweeteners, and given that there is insufficient knowledge to rationally design sweeteners not related structurally to existing compounds, work on plant-derived sweet substances has been very useful in elucidating information about the relationship of sweetness to struc-

TABLE 1.   Sweetness Relative to Sucrose of Various Plant-Derived Intense Sweetening Agents[a,b]

Compound Class	Compound Name	Approximate Sweetness[c] Relative to Sucrose
Monoterpenoid	Perillartine[d]	370
Sesquiterpenoid	Hernandulcin [39]	1500
Diterpene Glycosides	Stevioside [44]	210
	Steviolbioside[e] [45]	90
	Rebaudioside A [46]	242
	Rebaudioside B[e] [47]	150
	Rebaudioside C [48]	30
	Rebaudioside D [49]	221
	Rebaudioside E [50]	174
	Dulcoside A [51]	30
	Rubusoside [52]	114
	Baiyunoside	500
Triterpene Glycosides	Glycyrrhizin	93
	Ammonium glycyrrhizin[d]	50-100
	Periandrin I	90
	Periandrin II	95
	Periandrin III	92
	Periandrin IV	85
	Mogroside V [58]	256
Steroidal Saponin	Osladin	300
Aromatic Aldehyde	Cinnamaldehyde	50
Dihydroisocoumarin	Phyllodulcin[d]	400
Dihydrochalcones	Naringin dihydrochalcone[d]	300
	Neohesperidin dihydrochalcone[d]	2000
Proteins	Thaumatin	3000
	Monellin	3000

[a]Relative intensity data taken from Kinghorn and Soejarto (56) and Hussain *et al.* (57).
[b]It should be noted that not all of these quantitative data have been obtained using the same sensory techniques and that sweetness intensity may vary with several factors including sweetener concentration.
[c]Sucrose = 1.0; figures are expressed on a weight comparison basis.
[d]Derivative of natural product.
[e]Sodium salt.

ture (55). Folkloric information on the use of sweet plants, whether obtained in the field or via literature reports, has been extremely valuable in leading to the discovery of additional examples of natural sweeteners (56). Work performed at this institution on several plants with a history of use for sweetening and other purposes by native populations will be reviewed in the following paragraphs.

The intensely sweet, bisabolane sesquiterpene, hernandulcin [39], was isolated and characterized from *Lippia dulcis* Trev. (Verbenaceae), a plant native to tropical America. In his monograph entitled "Natural History of New Spain," written between 1570 and 1576, the Spanish physician Francisco Hernández described and illustrated a sweet plant that was known to the Aztec people by the Nahuatl name "Tzonpelic xihuitl" (58). Based on this monograph and on other literature sources, "Tzonpelic xihuitl" was identified as *L. dulcis* (58,59). Samples of this plant were collected in central Mexico, and the plant was, indeed, found to be sweet and sold in market-places under the name "Hierba dulce." The sweet constituent of this plant was found to be a volatile oil component and, therefore, soluble in nonpolar solvents. Its structure was established as 39 after the application of various spectroscopic techniques and synthesis from two ketone precursors by a directed-aldol condensation (58-60). Compound 39 was named hernan-

39   40
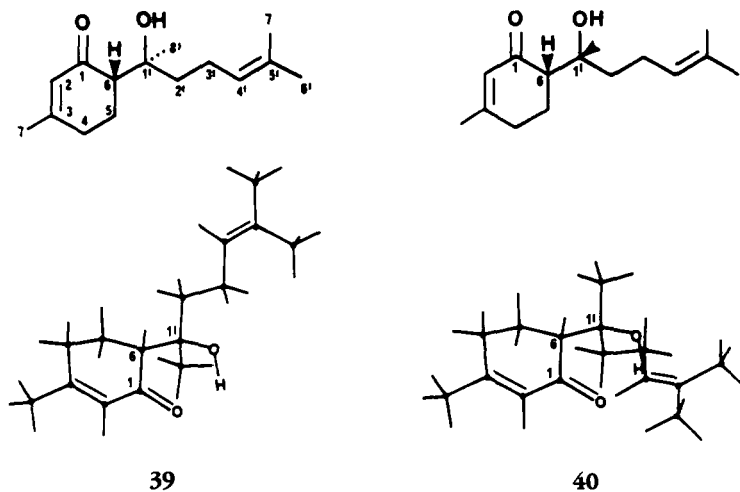
dulcin in honor of Hernández. The synthetic (±)-hernandulcin was not mutagenic when examined in a forward mutation assay utilizing *Salmonella typhimurium* strain TM677 and was not acutely toxic for mice at doses up to 2 g/kg body weight. Preliminary studies with a human taste panel indicated that natural (+)-hernandulcin was about three orders of magnitude sweeter than 0.25 *M* sucrose on a molar basis or about 1,500 times sweeter on a weight basis. Unfortunately, this isolate proved to exhibit perceptible off- and after-tastes and was somewhat bitter (58).

Molecular mechanics calculations performed on hernandulcin [39] have shown that the C-1' hydroxyl and the C-1 carbonyl groups are arranged about 2.6 Å apart in its preferred conformation (shown beneath the structure of 39). Therefore, 39 closely fits the model of Shallenberger *et al*. (61) for sweet-tasting compounds. The C-1' epimer of 39, epihernandulcin [40], obtained during our synthesis of hernandulcin, also fits the AH,B model proposed by Shallenberger *et al*. (61) for sweet compounds but is not sweet. In the case of 40, the preferred conformation, as determined by molecular mechanics calculations (shown beneath the structure of 40), shows a lack of linearity when compared to 39, and it may be concluded that the bulky hydrophobic group prevents the correct receptor fit in order to elicit sweetness (60). Mori and Kato (62) have recently synthesized both the (*S**,*S**)- and (*R**,*R**)- forms of hernandulcin [39] and found that only the naturally occurring (+)-(*S**,*S**)- form is intensely sweet. It has been found at this institution that alterations in the basic structure of hernandulcin [39] lead to either bitter or neutral tasting compounds (60,63).

While hernandulcin [39] continues to be evaluated for its potentiality for commercialization, it seems unlikely that crude or partially purified extracts of the plant could be used for sweetening purposes for two reasons. First, there was a very low natural abundance of (+)-hernandulcin [39] in the *L. dulcis* samples we examined. Second, this sweet constituent would need to be separated from camphor, the main component of the volatile oil of *L. dulcis*. Camphor is regarded as a very toxic compound with a reported lethal dose as low as 50 mg/kg. Field work carried out by members of our group indicated that *L. dulcis* is now sold in Mexico for a reputed abortifacient effect rather than for sweetening purposes. The high levels of camphor in the plant are consistent with its present folk use as an abortifacient, because this compound crosses the placenta and has been associated with neonatal death (59).

Another sweet plant sold in a marketplace under the name "Hierba dulce" is *Tessaria dodoneifolia* (Hook. & Arn.) Cabrera (Compositae) which was purchased in Asuncion, Paraguay. In this case, the plant is used by the local population as an em-

menagogue. The plant was grown from seed at the University of Illinois Pharmacognosy Field Station, and it was found that only the young, growing shoots of the plant were sweet. After a standard extraction scheme and preliminary toxicity and mutagenicity assessment, the sweetness of this acquisition was found to concentrate into EtOAc. The sweet principle was found to be the known compound dihydroquercetin-3-acetate [41], which represents the first example of a new class of intensely sweet compounds, the dihydroflavonol sweeteners. Compound 41 was obtained originally from *T. dodoneifolia*, although it was not recognized as being sweet at the time of its first isolation (64). The absolute stereochemistry of the sweet principle of this plant was established in our laboratory as $(2R^*,3R^*)$-, by conversion to $(+)$-pentaacetoxydihydroquercetin [42] and comparison with an authentic sample (65). While compound 41 was rated as about 50 times sweeter than sucrose by a human taste panel, a simple synthetic modification of this isolate, the novel 5,7,3'-trihydroxy-4'-methoxydihydroflavonol-3-acetate [43], was assessed as possessing about 400 times the sweetness intensity of a 2% sucrose solution (65). Thus far, the mixtures of diastereoisomers of 43 produced by synthesis have not been resolved, and due to the strict stereospecificity of the sweetness receptors in the papillae of the tongue, it may be expected that the active $(2R^*,3R^*)$- form of 43 is some 800 times sweeter than sucrose. Neither compound 41 nor 43 was found to be mutagenic or acutely toxic for mice. Assessment of the dihydroflavonol sweeteners in regard to their sensory, stability, and solubility parameters, as well as their cost of production, is still at an early stage. However, these sweet compounds represent a further class of intensely sweet, natural products that have been elucidated as a result of following up a folklore lead on a sweet plant.

41   R=H
42   R=Ac

43
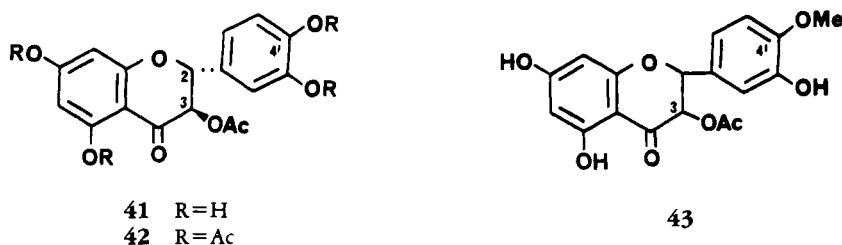
We have found that laboratory studies on plants with a folk reputation for sweetening purposes do not always result in the isolation of intense sweeteners. For example, the bark of *Boscia salicifolia* Oliv. (Capparidaceae) is pounded and used as a sweetening agent for soup in Nigeria (66). Work-up of a sample of *B. salicifolia* bark showed the presence of large amounts of sucrose (10.09% w/w) as the sole sweet compound present (67). In this case, the folklore use of this plant part as a nutritive sweetener is, indeed, vindicated, and *B. salicifolia* bark contains sucrose at only slightly lower levels than either sugar cane (15-20%) or sugar beet (10-17%) (68).

Stevia rebaudiana (Bertoni) Bertoni (Compositae) has been used for centuries in Paraguay, its country of origin, to sweeten beverages such as maté (*Ilex paraguayensis* St.-Hil.). Stevioside [44] is the major sweet *ent*-kaurene glycoside constituent of *S. rebaudiana*, and this compound and extracts of the leaves of its plant of origin are accepted for general use as sucrose substitutes in a wide variety of foods and beverages in Japan. In addition to being intensely sweet, stevioside [44] offers several other advantages in being stable to heat and acids and nonfermentive. *S. rebaudiana* is cultivated commercially for the Japanese market not only in Paraguay and Japan but in several other Asian countries (69). In Paraguay teas made from *S. rebaudiana* are currently prescribed by physicians for the treatment of hyperglycemia (69,70).

Nearly 60 years elapsed between the initial isolation of stevioside [44] and its final
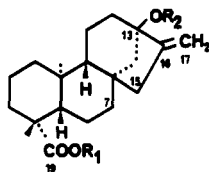
	R_1	R_2
44	β-glc	β-glc^2-β-glc
45	H	β-glc^2-β-glc
46	β-glc	β-glc^2-β-glc |3 β-glc
47	H	β-glc^2-β-glc |3 β-glc
48	β-glc	β-glc^2-α-rha |3 β-glc
49	β-glc^2-β-glc	β-glc^2-β-glc |3 β-glc
50	β-glc^2-β-glc	β-glc^2-β-glc
51	β-glc	β-glc^2-α-rha
52	β-glc	β-glc
53	H	H

structural determination at the National Institute of Arthritis and Metabolic Diseases, NIH (69,71). Seven additional sweet *ent*-kaurene glycosides [45-51] were also identified as *S. rebaudiana* constituents in the 1970s (72,73). Certain of these compounds occur in remarkably high concentrations in the plant. For example, a sample of dried *S. rebaudiana* leaves cultivated in the People's Republic of China that we recently examined contained stevioside [44], rebaudioside A [46], rebaudioside C [48], and dulcoside A [51] in respective yields of 6.6, 3.7, 2.1, and 0.53% w/w (74). Despite occurring in such high abundance in *S. rebaudiana*, these compounds appear to be very rare in the genus *Stevia*, which is composed of about 200 species. When 110 *Stevia* leaf herbarium specimens were examined phytochemically, *ent*-kaurene glycosides were detected in only two species, namely, *S. rebaudiana* collected in Paraguay in 1919, and *Stevia phlebophylla* A. Gray collected in Mexico in 1889. Only trace amounts of this compound were found in the latter species which may now be extinct (75). Considerable efforts have been expended in attempts by several groups to produce semi-synthetic analogues of stevioside [44] that do not have the bitterness characteristic of the natural compound (56,69).

A plant used as a sweet, caffeine-free tea by local populations of the Guangxi Autonomous Region in the People's Republic of China has been found to biosynthesize an intensely sweet compound, rubusoside [52], which is structurally related to the sweet *ent*-kaurenoid glycosides of *S. rebaudiana* (76). The leaves of this plant, *Rubus suavissimus* S. Lee (formerly *R. chingii* Hu, Rosaceae), contain over 5% w/w rubusoside [52] (76,77). Compound 52 is based on the same diterpene aglycone as the *S. rebaudiana* sweet glycosides, namely, steviol [53]. As was the case with the sweet steviol glycosides of *S. rebaudiana*, rubusoside [52] is rare in the genus *Rubus* and was found to occur in only one of 39 species examined (77).

There is now an extensive, though by no means exhaustive, literature on safety studies concerning stevioside [44] and extracts of *S. rebaudiana*, although little or nothing appears to have been published in the primary literature in this regard on rubusoside [53]. Crude and purified *S. rebaudiana* extracts, as well as crystalline

stevioside [**44**], have been tested on rodents for acute and subacute toxicity with no evidence of adverse effects. Also, no toxicological evidence has been published indicating that extracts or constituents of *S. rebaudiana* produce teratogenic effects in experimental animals (69). However, despite reports of negative results on *S. rebaudiana* extracts and several of its sweet constituents in a variety of mutagenicity assay systems (69,78), steviol [**53**] (*ent*-13-hydroxykaur-16-en-19-ol), the aglycone of sweet compounds **44-52**, was found to be mutagenic toward *S. typhimurium* strain TM677, when metabolically activated (78). Using appropriately substituted derivatives, it has been shown that the C-13 hydroxy group of steviol is required for the exhibition of mutagenicity, as is the C16-C17 exomethylene group functionality (78,79). Interestingly, these very same structural features must be present for glycosides of steviol [**53**] to produce a sweet effect (69). Steviol [**53**] can be activated to mutagenic substances by human liver microsomes (80). After incubation in the presence of a rat liver preparation, under conditions similar to those found to cause a mutagenic response, three in-vitro metabolites of steviol [**53**] have been identified by gc/ms, namely, compounds **54-56** (81). The most abundant metabolite detected under these conditions was 15α-hydroxysteviol [**54**], indicating that the major pathway of steviol [**53**] in mammalian metabolism is allylic oxidation. Although compound **54** was not mutagenic, the closely related derivative, 15-oxosteviol [**57**] was a direct-acting mutagen (80-81). Additionally, it was found that this compound was highly toxic to bacteria used in the mutagenicity assay (80,81). While compound **57** was not detected among the products of steviol [**53**] metabolism, it is possible that his compound is indeed formed during the incubation, and, due to its high reactivity, it could be trapped by a component of the mixture such as a cysteine of the activating proteins (81).
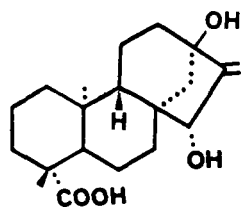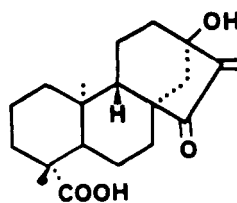

54
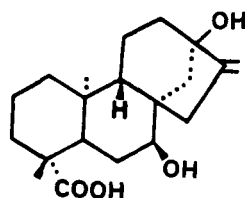
55
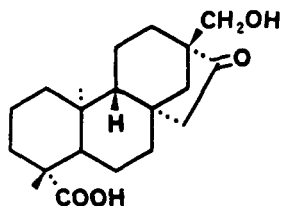
56

57

It may be pointed out that, thus far, there have been no adverse effects reported from the human consumption of either *S. rebaudiana* or *R. suavissimus* products, despite the high levels of steviol [**53**] glycosides present in each case. The discovery of the mutagenicity of steviol [**53**], however, does suggest that additional safety assessments, including in-vivo metabolism studies, should be performed on these natural, sweet glycosides before they can be considered safer for human consumption (69,80).

In a recent study, we have shown that steviol [**53**] possesses feeding deterrent activ-

ity against the aphid pest, *Schizaphis graminum* Rondani (greenbug). A total of 16 analogues of this compound were characterized and tested, and it was found that a loss of feeding deterrent activity was observed after the acetylation or glycosylation of the C13-tertiary hydroxy group or on methylation of the C19-carboxylic acid substituent. In contrast to effects on its mutagenicity, the antifeedant activity of steviol [**53**] was not unduly affected by structural modification of the C16-C17-exomethylene group (82). Stevioside [**44**] had a weak but discernible feeding deterrent effect on *S. graminum*. The demonstration of this type of adverse effect on an insect species may provide a clue to the reason why such large amounts of steviol glycosides accumulate in both *S. rebaudiana* and *R. suavissimus*.

A final example of a sweet plant with a folkloric reputation is *Thladiantha grosvenorii* (Swingle) C. Jeffrey (syn. *Momordica grosvenorii* Swingle; Cucurbitaceae). This is an intensely sweet-tasting plant, known as "lo han kuo" in the south of the People's Republic of China where it occurs, that has been used for centuries as a household remedy for colds, sore throats, and minor stomach and intestinal problems (83). *T. grosvenorii* was unknown to western botanists until the mid 1930s, and chemical work on its constituents did not begin until the 1970s. Despite apparently not being used for sweetening purposes, the fruits of this vine contain an abundant, sweet, triterpene glycoside, mogroside V [**58**], which was characterized by Takemoto *et al.* (84). Mogroside V [**58**] was found to occur in concentration levels of about 1.0% w/w in dried *T. grosvenorii* fruits and was determined as nonmutagenic and produced no mortalities in acute toxicity experiments on mice at doses up to 2 g/kg body weight (85). As shown in Table 1, the compound is somewhat more intensely sweet than stevioside [**44**]. Therefore, mogroside V [**58**] deserves a more thorough assessment as to its feasibility for commercial use as a sweetener. In addition, it may be possible to use extracts of *T. grosvenorii* directly for sweetening purposes, since they seem to have a history of safe use among human populations. *T. grosvenorii* extracts should, thus, be subjected to more intensive laboratory investigation from both the phytochemical and toxicological standpoints.
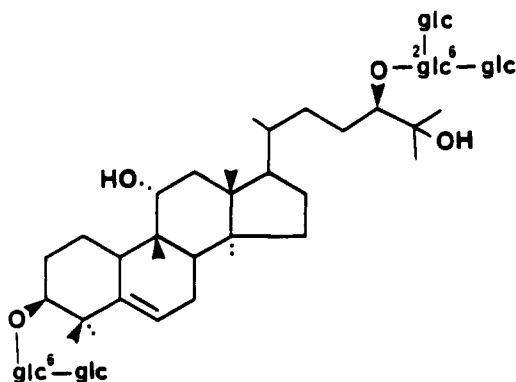
58

While the viewpoint has been expressed that all of the possible therapeutic natural products have largely been discovered (86), it would seem that greater opportunities than ever before exist to discover new prototype, biologically active molecules from the plant kingdom, given the development of improved isolation methods and the more ready availability of high-resolution spectroscopic techniques for structure determination on ever smaller amounts of sample. In addition, bioactivity-guided fractionation is now possible with increasingly ingenious and specific types of bioassay. Many of the plants mentioned in this review that have demonstrated folkloric reputations as medici-

nal or sweetening agents have afforded interesting, biologically active compounds on subsequent investigation. Although folkloric claims for a given species may not be substantiated when extracts of the plant are subjected to laboratory examination, such information should play a prominent role in programs directed towards the isolation and characterization of biologically active substances from plants for the judicious selection of material for study. A consideration of plants used for sweetening purposes by local populations is vital to the success of any project designed to elucidate the structures of further examples of intensely sweet compounds that are potentially non-cariogenic and/or non-caloric. This information may be obtained either from the literature or by field observations, and, provided sufficient leads of this type are established, a systematic search for sweet molecules may be conducted in this manner.

ACKNOWLEDGMENTS

LITERATURE CITED

1. A.B. Svendsen and J.J.C. Scheffer, *Pharmaceut. Weekbl., Sci. Ed.*, **4**, 93 (1982).
2. N.R. Farnsworth, in: "Natural Products and Drug Development. Alfred Benzoin Symposium 20," Ed. by P. Krogsgaard-Larsen, S.B. Christensen, and H. Kofod, Munksgaard, Copenhagen, Denmark, 1984, pp. 17-30.
3. N.R. Farnsworth, O. Akerele, A.S. Bingel, D.D. Soejarto, and Z. Guo, *Bull. World Health Org.*, **63**, 965 (1985).
4. M.F. Balandrin, J.A. Klocke, E.S. Wurtele, and W.H. Bollinger, *Science*, **228**, 1154 (1985).
5. N.J. de Souza, B.N. Ganguli, and J. Reden, *Ann. Rep. Med. Chem.*, **17**, 301 (1982).
6. A.N. Dohadwalla, *Trends Pharmacol. Sci.*, **6**, 49 (1985).
7. R.P. Steiner, Ed. "Folk Medicine. The Art and the Science," American Chemical Society, Washington, D.C., 1986.
8. D.M. Hosler and M.A. Mikita, *J. Chem. Ed.*, **64**, 328 (1987).
9. R.E. Christoffersen, *Pharm. Technol.*, (April issue), 16 (1987).
10. R.W. Spjut and R.E. Perdue, Jr., *Cancer Treat. Rep.*, **60**, 979 (1976).
11. D.L. Klayman, *Science*, **228**, 1049 (1985).
12. Z-G. Wang and G-Z. Liu, *Trends Pharmacol. Sci.*, **6**, 423 (1985).
13. G. Schmid and W. Hofheinz, *J. Am. Chem. Soc.*, **105**, 624 (1983).
14. N.J. de Souza, A.N. Dohadwalla, and J. Reden, *Med. Res. Rev.*, **3**, 201 (1983).
15. K.B. Seamon, *Ann. Rep. Med. Chem.*, **19**, 293 (1984).
16. H.P.T. Ammon and A.B. Müller, *Planta Med.*, 473 (1985).
17. E. Block, *Sci. Am.*, **252**, 114 (1985).
18. E. Block, S. Ahmad, J.L. Catalfamo, M.K. Jain, and R. Apitz-Castro, *J. Am. Chem. Soc.*, **108**, 7045 (1986).
19. J.D. Higginbotham, in: "Developments in Sweeteners—1," Ed. by C.A.M. Hough, K.J. Parker, and A.J. Vlitos, Applied Science, London, 1979, pp. 87-123.
20. H. van der Wel and K. Loeve, *Eur. J. Biochem.*, **31**, 221 (1972).
21. A.M. de Vos, M. Hatada, H. van der Wel, H. Krabbendam, A.F. Peerdeman, and S-H. Kim, *Proc. Natl. Acad. Sci., USA*, **82**, 1406 (1985).
22. J.D. Higginbotham, in: "Alternative Sweeteners," Ed. by L. O'Brien-Nabors and R.C. Gelardi, Marcel Dekker, New York, 1986, pp. 103-134.
23. D.A. Hussar, *Am. Pharm.*, **NS27** (No. 3), 26 (1987).
24. J. Aulde, *Ther. Gaz.*, **14**, 523 (1890).
25. M. Levitt, in: "Cannabinoids as Therapeutic Agents," Ed. by R. Mechoulam, CRC Press, Boca Raton, Florida, 1986, pp. 71-83.
26. S.E. Sallan, N.F. Zinberg, and E. Frei, III, *New Engl. J. Med.*, **293**, 795 (1975).

27.   J.L. Hartwell, *Lloydia*, **31**, 71 (1968).
28.   I. Jardine, in: "Anticancer Agents Based on Natural Product Models," Ed. by J.M. Cassady and J.D. Douros, Academic, New York, 1980, pp. 319-351.
29.   J.A. Duke and E.S. Ayensu, "Medicinal Plants of China," Reference Publications, Inc., Algonac, Michigan, 1985, p. 303.
30.   A.D. Kinghorn, in: "Plant Toxicology," Ed. by A.A. Seawright, M.P. Hegarty, L.F. James, and R.F. Keeler, Queensland Poisonous Plants Committee, Yeerongpilly, Queensland, Australia, 1985, pp. 357-366.
31.   I. Berenblum, *Cancer Res.*, **1**, 44 (1941).
32.   E. Hecker and R. Schmidt, *Fortschr. Chem. Org. Naturst.*, **31**, 377 (1974).
33.   T. Hirayama and Y. Ito, *Prev. Med.*, **10**, 614 (1981).
34.   G.T. Marshall and A.D. Kinghorn, *J. Chromatogr.*, **206**, 421 (1981).
35.   A.D. Kinghorn and G.T. Marshall, U.S. Patent 4,468,328, Aug. 28 (1984).
36.   G.T. Marshall and A.D. Kinghorn, *J. Am. Oil Chem. Soc.*, **61**, 1220 (1984).
37.   G.T. Marshall, J.A. Klocke, L-J. Lin, and A.D. Kinghorn, *J. Chem. Ecol.*, **11**, 191 (1985).
38.   R. Schmidt and E. Hecker, in: "Carcinogenesis—A Comprehensive Survey," Ed. by E. Hecker, N.E. Fusenig, W. Kunz, F. Marks, and H.W. Thielman, vol. 7, Raven Press, New York, 1982, pp. 57-63.
39.   K. Hostettmann, M. Hostettmann, and A. Marston, *Nat. Prod. Rep.*, **1**, 471 (1984).
40.   C.A.J. Erdelmeier, P.A.S. Van Leeuwen, and A.D. Kinghorn, *Planta Med.*, in press.
41.   I.H. Burkill, "A Dictionary of the Economic Products of the Malay Peninsula," vol. I, Ministry of Agriculture and Co-operatives, Kuala Lumpur, Malaysia, 1966, p. 998.
42.   S.M. Worobec, T.A. Hickey, A.D. Kinghorn, D.D. Soejarto, and D. West, *Contact Dermatitis*, **7**, 19 (1981).
43.   L-J. Lin and A.D. Kinghorn, *Phytochemistry*, **22**, 2795 (1983).
44.   L-J. Lin, G.T. Marshall, and A.D. Kinghorn, *J. Nat. Prod.*, **46**, 723 (1983).
45.   A.D. Kinghorn, L-J. Lin, and G.T. Marshall, *Rev. Latinoamer. Quím.*, in press.
46.   A. Osol and G.E. Farrar, Jr., Eds., "The Dispensatory of the United States of America, 25th ed.," J.B. Lippincott Co., Philadelphia, 1955, p. 1616.
47.   T.J. Fitzgerald, J.B. LaPidus, and J.L. Beal, *Lloydia*, **27**, 107 (1964).
48.   M.F. Balandrin, "Structure Elucidation of Some Biologically Active Constituents of the Genus *Acosmium* (Leguminosae)," Ph.D. Thesis, Chicago: University of Illinois at the Medical Center, 1982, pp. 13-17.
49.   M.F. Balandrin and A.D. Kinghorn, *Heterocycles*, **19**, 1931 (1982).
50.   M.F. Balandrin and A.D. Kinghorn, *J. Nat. Prod.*, **44**, 619 (1981).
51.   A.D. Kinghorn and M.F. Balandrin, in: "The Alkaloids: Chemical and Biological Perspectives," Ed. by S.W. Pelletier, vol. 2, Wiley-Interscience, New York, 1984, pp. 105-148.
52.   M.F. Balandrin and A.D. Kinghorn, *Phytochemistry*, submitted.
53.   H.C. Makapugay, D.D. Soejarto, A.D. Kinghorn, and E. Bordas, *J. Ethnopharmacol.*, **7**, 235 (1983).
54.   W.R. Dunstan and H. Garnett, *J. Chem. Soc.*, **67**, 94 (1895).
55.   A.D. Kinghorn and C.M. Compadre, *Pharm. Int.*, **6**, 201 (1985).
56.   A.D. Kinghorn and D.D. Soejarto, *CRC Crit. Rev. Plant Sci.*, **4**, 79 (1986).
57.   R.A. Hussain, J. Kim, T-W. Hu, J.M. Pezzuto, D.D. Soejarto, and A.D. Kinghorn, *Planta Med.*, 403 (1986).
58.   C.M. Compadre, J.M. Pezzuto, A.D. Kinghorn, and S.K. Kamath, *Science*, **227**, 417 (1985).
59.   C.M. Compadre, E.F. Robbins, and A.D. Kinghorn, *J. Ethnopharmacol.*, **15**, 89 (1986).
60.   C.M. Compadre, R.A. Hussain, R.L. Lopez de Compadre, J.M. Pezzuto, and A.D. Kinghorn, *J. Agric. Food Chem.*, **35**, 273 (1987).
61.   R.S. Shallenberger, T.S. Acree, and C.Y. Lee, *Nature*, **221**, 555 (1969).
62.   K. Mori and M. Kato, *Tetrahedron*, **42**, 5895 (1986).
63.   C.M. Compadre, R.A. Hussain, R.L. Lopez de Compadre, J.M. Pezzuto, and A.D. Kinghorn, Abstract No. 32 presented at the 27th Annual Meeting of the American Society of Pharmacognosy, Ann Arbor, Michigan, July 27-30, 1986.
64.   J. Kavka, E. Guerreiro, and O.S. Giordano, *An. Quím.*, **73**, 305 (1977).
65.   N.P.D. Nanayakkara, R.A. Hussain, J.M. Pezzuto, D.D. Soejarto, and A.D. Kinghorn, Abstract No. 116 presented at the 28th Annual Meeting of the American Society of Pharmacognosy, Kingston, Rhode Island, July 19-22, 1987.
66.   F.R. Irvine, "Woody Plants of Ghana," Oxford University Press, London, 1961, p. 42.
67.   R.A. Hussain, Y-M. Lin, J.M. Pezzuto, D.D. Soejarto, and A.D. Kinghorn, unpublished results.
68.   M. Windholz, Ed., "The Merck Index," 10th ed., Merck & Co., Rahway, New Jersey, 1983, p. 1273.

69. A.D. Kinghorn and D.D. Soejarto, in: "Economic and Medicinal Plant Research," Ed. by H. Wagner, H. Hikino, and N.R. Farnsworth, vol. 1. Academic, London, 1985, pp. 1-52.

70. D.D. Soejarto, C.M. Compadre, P.J. Medon, S.K. Kamath, and A.D. Kinghorn, *Econ. Bot.*, **37**, 71 (1982).

71. E. Mosetigg, U. Beglinger, F. Dolder, H. Lichti, P. Quitt, and J.A. Waters, *J. Am. Chem. Soc.*, **85**, 2305 (1963).

72. O. Tanaka, *Saengyak Hakhoe Chi*, **11**, 219 (1980); *Chem. Abstr.*, **95**, 113426y, 1981.

73. M. Kobayashi, S. Horikawa, I.H. Degrandi, J. Ueno, and H. Mitsuhashi, *Phytochemistry*. **16**, 1405 (1977).

74. H.C. Makapugay, N.P.D. Nanayakkara, and A.D. Kinghorn, *J. Chromatogr.*, **283**, 390 (1984).

75. A.D. Kinghorn, D.D. Soejarto, N.P.D. Nanayakkara, C.M. Compadre, H.C. Makapugay, J.M. Hovanec-Brown, P.J. Medon, and S.K. Kamath, *J. Nat. Prod.*, **47**, 439 (1984).

76. T. Tanaka, H. Kohda, O. Tanaka, F-H. Chen, W-H. Chou, and J-L. Leu, *Agric. Biol. Chem.*, **45**, 2165 (1981).

77. T. Seto, T. Tanaka, O. Tanaka, and N. Naruhashi, *Phytochemistry*, **23**, 2829 (1984).

78. J.M. Pezzuto, C.M. Compadre, S.M. Swanson, N.P.D. Nanayakkara, and A.D. Kinghorn, *Proc. Natl. Acad. Sci., USA*, **82**, 2478 (1985).

79. J.M. Pezzuto, N.P.D. Nanayakkara, C.M. Compadre, S.M. Swanson, A.D. Kinghorn, T.M. Guenthner, V.L. Sparnins, and L.K.T. Lam, *Mutation Res.*, **169**, 93 (1986).

80. J.M. Pezzuto, in: "New Trends in Natural Products Chemistry 1986," Ed. by Atta-ur-Rahman and P.W. Le Quesne, vol. 26, Elsevier Science, Amsterdam, Netherlands, 1986, pp. 371-386.

81. C.M. Compadre, R.A. Hussain, N.P.D. Nanayakkara, J.M. Pezzuto, and A.D. Kinghorn, *Biomed. Environ. Mass Spectrom.*, in press.

82. N.P.D. Nanayakkara, J.A. Klocke, C.M. Compadre, R.A. Hussain, J.M. Pezzuto, and A.D. Kinghorn, *J. Nat. Prod.*, **50**, 434 (1987).

83. W.T. Swingle, *J. Arnold Arbor.*, **22**, 197 (1941).

84. T. Takemoto, S. Arihara, T. Nakajima, and M. Okuhira, *Yakugaku Zasshi*, **103**, 1167 (1983).

85. H.C. Makapugay, N.P.D. Nanayakkara, D.D. Soejarto, and A.D. Kinghorn, *J. Agric. Food Chem.*, **33**, 348 (1985).

86. J. Vane and P. Cuatrecasas, *Nature*, **312**, 303 (1984).