Antimalarial Activity of Tropical Meliaceae Extracts and Gedunin Derivatives

S. MacKinnon, T. Durst,* and J. T. Arnason*

Ottawa-Carleton Institutes of Chemistry and Biology, University of Ottawa, Ottawa, Ontario K1N 6N5, Canada

C. Angerhofer and J. Pezzuto

Program for Collaborative Research in the Pharmaceutical Sciences, University of Illinois at Chicago, Chicago, Illinois 60612-7231

P. E. Sanchez-Vindas and L. J. Poveda

Facultad de Ciencas de la Tierre y el Mar, Universidad Nacional, Heredia, Costa Rica

M. Gbeassor

Faculté des Sciences, Université du Bénin, Lomé, Togo

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Extracts of 22 species of Meliaceae were examined for antimalarial activity using *in vitro* tests with two clones of *Plasmodium falciparum*, one sensitive to chloroquine (W2) and one chloroquine-resistant (D6). Twelve extracts were found to have activity, including extracts of *Cedrela odorata* wood and *Azadirachta indica* leaves, which contained the limonoid gedunin. These extracts were more effective against the W2 clone than the D6 clone, suggesting there is no cross-resistance to chloroquine. Gedunin was extracted in quantity, and nine derivatives prepared for a structure−activity study, which revealed essential functionalities for activity. The study also included four other limonoids derived from related Meliaceae. Only gedunin had better activity than chloroquine against the W2 clone. This active principle could be used to standardize a popular crude drug based on traditional use of *A. indica* in West Africa.

The increase in incidence of malaria in recent years has been attributed to the development of resistance of the malarial protozoa (*Plasmodium falciparum*) to chloroquine and other drugs and to the development of resistance of the vector mosquitoes to presently used insecticides. In tropical America, members of the Meliaceae family, *Cedrela odorata*, *Carapa guianensis*, and *Swietenia mahagoni*, have been used in traditional medicine for the treatment of fevers,[1] a characteristic symptom of malaria. In West and East Africa, a well-known member of this family, *Azadirachta indica* "neem", has been used as a traditional antimalarial.[2] The bark and aqueous decoctions of the twigs, stembark, and roots have been administered for the treatment of malaria.[3] In Nigeria the leaves of *A. indica* are boiled, and the extract is given in uncontrolled doses for the treatment of fevers thought to be due to malaria.[4] Tropical Meliaceae have not been extensively screened for antimalarial activity, and the first part of the present study presents the results of a screening of more than 60 extracts, which suggested that extracts of neem and *C. odorata* containing gedunin (**1**) warranted further evaluation.

Previous phytochemical investigations directed at the characterization of the antimalarial principle of the neem tree were initially conducted by Rochanakij *et al.* in 1985.[5] Examination of the leaves of *A. indica* var. *siamensis* resulted in the isolation of nimbolide, which they associated with neem's antimalarial activity. In 1986, Khalid *et al.* screened various limonoid, including compound **1**, nomilin, and limonin for antimalarial activity using *P. falciparum* in a radioisotope microdilution bioassay to determine IC_{50} values (50% inhibitory concentration).[6] For the FCR_{3TC} strain of this plasmodium, the IC_{50} values for **1**, nomolin, and limonin were determined to be 800, 84 100, and >100 000 ng/mL, respectively. The value for **1** in this particular screen was roughly equivalent to that of quinine, suggesting a possible new antimalarial lead. Three years later, using the same bioassay procedure, they determined that it was the most active antimalarial principle of the bark of *A. indica* and *Melia azadarach*.[7]

A study that involved the *in vitro* testing of 27 limonoids for antimalarial activity against *P. falciparum* revealed that **1** was the most active, having an IC_{50} value of 720 ng/mL.[2] It was three and 20 times less active than chloroquine and quinine, respectively. Nimbolide had an IC_{50} value of 1740 ng/mL; 1,2-dihydrogedunin (**2**) displayed an IC_{50} value of 2630 ng/mL, and limonin displayed no antimalarial activity when tested at 50 000 ng/mL[2]. Unfortunately, there were not enough gedunin-related limonoids tested at this time to allow for a more detailed evaluation of the structure−activity relationships of this group of D-*seco*-limonoids. The second part of the present study, therefore, examines the antimalarial of a series of nine derivatives of **1**, to define structure−activity relationships for this compound.

Results and Discussion

Sixty EtOH extracts of 22 Meliaceae were evaluated for antimalarial activity against *Plasmodium falciparum* (clone D6 and W2). Twelve species provided extracts that showed significant activity with IC_{50}

* To whom correspondence should be addressed. Phone: (613) 564-3458. FAX: (613) 564-5014.

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Table 1. Ethanolic Extracts of Meliaceae with Antimalarial Activity at < 20 (μg/mL)[a]

species	part extracted	collection site	IC_{50} (μg/mL) clone D6	IC_{50} (μg/mL) clone W2
Aphanamixis polystachya	wood	Miami	16.8	> 20
Azadirachta indica[b]	leaves	Togo	2.5	2.48
Cedrela salvadorensis	bark	Costa Rica	> 20	15.0
	fruits		18.7	15.3
	leaves		8.3	> 20
	wood		13.8	13.2
Cedrela odorata[b]	wood	Costa Rica	1.37	1.25
	wood	Belize	1.20	1.11
	wood	Togo	9.29	2.77
Chukrasia tabularis	leaves	Miami	6.36	3.39
Dysoxylum fraseranum	leaves	Costa Rica	13.1	> 20
	wood		10.2	16.4
Guarea glabra	bark	Costa Rica	19.7	> 20
Guarea pyriformis	wood	Costa Rica	13.9	13.3
Trichilia glabra	bark	Costa Rica	8.75	19.8
Trichilia hirta	leaves	Costa Rica	16.2	> 2 0
Trichilia trifolia	leaves	Costa Rica	10.3	15.2
artemisinin			2.8×10^{-3}	2.9×10^{-3}
mefloquine			4.4×10^{-3}	2.0×10^{-3}
chloroquine			$1-3 \times 10^{-3}$	23.7×10^{-3}
quinine			8.5×10^{-3}	56.6×10^{-3}

[a] Inactive extracts (IC_{50} > 20 μg/mL) include extracts of other plant parts of species listed above and extracts of all parts tested of *Carapa guianensis, Cedrela fissilis, Cedrela tonduzii, Cedrela toona, Swietenia macrophylla, Swietenia mahogoni, Trichilia americana, Trichlia havanensis, Trichilia martiana, Trichilia pleena,* and *Trichilia quadrajuga.* [b] Data from a separate trial with comparable controls.

values of 20 μg/mL or lower (Table 1). Extracts exhibiting the highest activity against the chloroquine-sensitive clone D6 were the leaves of *Azadirachta indica*, *Cedrela salvadorensis,* and *Chukrasia tabularis*; the bark of *Trichilia glabra;* and wood of *Cedrela odorata* and *Dysoxylum fraseranum*. The most active extracts against the chloroquine-resistant clone W2 were the leaves of *A. indica, C. tabularis,* and *C. salvadorensis* and the wood of *C. odorata* and *Guarea pyriformis*. The extract of the leaves of *C. tabularis* was active against both strains of plasmodium and was therefore submitted for cytotoxicity evaluation against a number of human tumor cell lines, as a preliminary attempt to assess selectivity. The antimalarial activity of this extract was determined to be selective because it was inactive against the tumor cell lines.

Some of the active species mentioned above have been investigated for limonoid content. The wood and roots of *C. tabularis* contain limonoids chukrasines A−E and cedrelone.[8,9] Investigations of the genus *Dysoxylum* have resulted in the isolation of limonoids, but no work has been reported on the constituents of *D. fraseranum*.[10] Recently, phytochemical investigations of the stem bark of *C. salvadorensis* resulted in the isolation of cedrelanolide.[11] The remaining species, *Trichilia glabra* and *Guarea pyriformis*, have not yet been investigated, but other species in these genera are known to contain limonoids. Therefore, it is plausible to suggest that the limonoid constituents could be responsible for the antimalarial activity exhibited. Thus far, no further fractionation of the extracts has been carried out, but the extract of the leaves of *C. tabularis* certainly warrants further investigation.

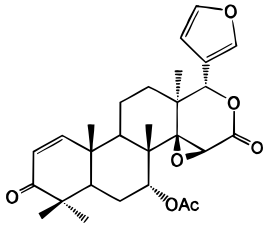
Because extracts made from plant materials containing **1** were especially active, including leaf extracts of *A. indica* from Togo and wood extracts of *C. odorata* (Table 1), they received further investigation. EtOH extracts of wood of *C. odorata* from different countries were all active with increasing activity in the order Togo, Costa Rica, and Belize (EC_{50} = 3.26, 1.25, and 1.1 μg/mL, respectively, for the W2 clone). The yield of **1** in the wood increased in the same order (0.1%, 0.3%, and 0.4% dry wt). Toluene extracts were found to give a cleaner extract with a slightly higher content of **1** to EtOH extracts. All toluene wood extracts were found to be active, with better efficacy against the W2 clone increasing in the order Togo, Costa Rica, and Belize (EC_{50} = 2.77, 0.70, and 0.65 μg/mL). All wood extracts, except the Costa Rica toluene extract, were inactive in cytotoxicity tests with KB cells (IC_{50} > 20 μg/mL). These gedunin-containing materials are widely available in both Africa and tropical America and suggest that there are a number of practical sources of this compound. A botanical crude drug could possibly be standardized based on its content of **1**. *C. odorata* is a major commercial timber species, and its wood-waste is a convenient source of this substance.

Table 2. Antimalarial IC_{50} Values of Derivatives of **1**

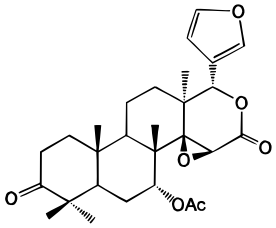
	Plasmodium falciparum IC_{50} (ng/mL)		relative activity to **1**[a]	
compd	clone D6	clone W2	clone D6	clone W2
1	39	20	100	100
2	> 10 000	840	< 0.39	2.38
3	2580	980	0.93	2.04
4	4210	2440	0.93	0.82
5	2610	1280	1.50	1.56
6	> 10 000	> 10 000	< 0.39	< 0.0005
7	2500	900	1.56	2.22
8	133	39	29.38	51.3
9	832	156	4.69	12.8
10	10 000	2130	0.39	0.94
11	173	96	22.6	20.8
chloroquine	1.3	29.5	3000	67.8
quinine	14.8	34.9	264	57.3
mefloquine	7.5	1.4	521	1429
artemisinin	1.8	0.5	2170	4000

[a] Values are normalized, relative to the potency of **1** (= 100%).

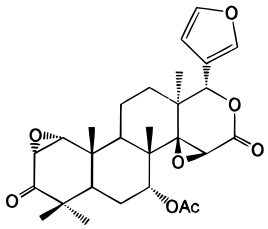
Investigation of the antimalarial activity of the gedunin derivatives (**2**−**10**) did not yield a compound with a higher activity than **1** (Table 2). However, the study did provide insight with respect to functionalities required for activity and in terms of structure−activity relationships for antimalarial activity of D-seco limonoids.



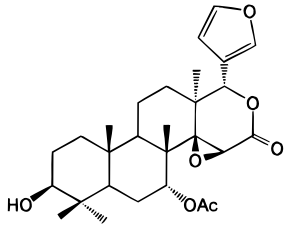
Gedunin (**1**)



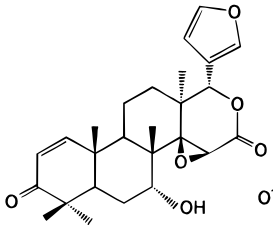
1,2-Dihydrogedunin (**2**)



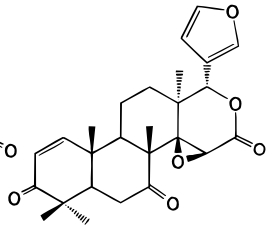
1,2-Epoxygedunin (**3**)



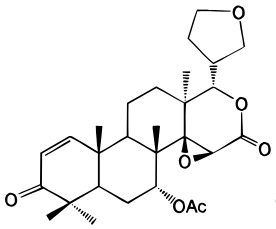
1,2-Dihydro-3β-gedunol (**4**)



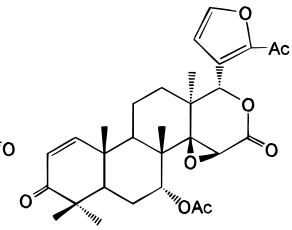
7-Deacetylgedunin (**5**)



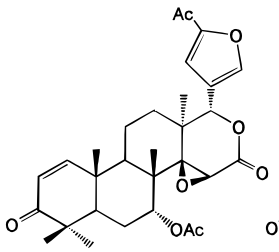
7-Ketogedunin (**6**)



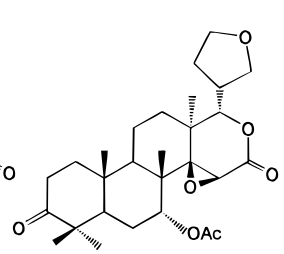
Tetrahydrogedunin (**7**)



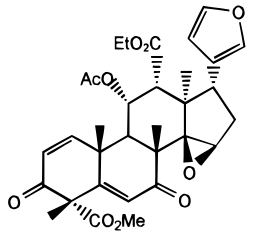
21-Acetylgedunin (**8**)



23-Acetylgedunin (**9**)



Hexahydrogedunin (**10**)



Hirtin (**11**)

Not surprisingly, changes in the α,β-unsaturated ketone moiety in ring A of **1** revealed its importance for antimalarial activity. When the double bond was reduced, as in **2**, a large loss in activity was observed in both clones but most notably with the chloroquine-sensitive clone (D6). Epoxidation to the 1,2-β-epoxy derivative **3** also resulted in loss of activity. Reduction of both the double bond and the ketone, to 1,2 dihydro-3β-gedunol (**4**) resulted in substantial losses of activity against both clones, again most dramatically in clone D6.

Another structural feature of **1** that was investigated was the 7-acetate of ring B. This was examined through the testing of 7-deacetylgedunin (**5**) and 7-ketogedunin (**6**). Again, losses of activity were observed for both clones. However, it seems that the presence of a 7-keto functionality eliminates all the antimalarial activity of gedunin. The difference in activity between the 7-acetoxy (**1**) and 7-hydroxy analogs (**5**) in both clones may be due to differences in bioavailability. Limonin, obacunone, and nomilin also possess 7-keto functions and similarly exhibited no antimalarial activity (data not shown). The absence of an OH group or a potential OH group at C-7 could be preventing the interconversion of these limonoids, *in situ*, to their mero-analogs and therefore rendering them inactive. The hypothesis that the mero-interconversion products are related to the antimalarial activity of the limonoids[12] needs to be further investigated. This could be initiated by testing merogedunin for antimalarial activity.

The relatively high level of activity exhibited by hirtin, **11**, was not anticipated because it did not contain an epoxylactone in ring D and a 7-OH function in ring B. The potential antimalarial activity of related tetracarbocyclic limonoids needs to be investigated in the future.

Alterations to the furan moiety were also investigated. Tetrahydrogedunin (**7**) exhibited a loss in activity comparable to that observed for **3**. With other derivatives, even though losses of activity were observed, the magnitude was much less than what was seen previously. This is seen especially with 21-acetylgedunin (**8**) and 23-acetylgedunin (**9**). Comparing **2** with hexahydrogedunin (**10**) revealed that reduction of the double bonds in the furan resulted in only a small change in loss of activity in strain W2. From the data obtained with modified furan moieties of **1**, it seems that this section of the molecule is less important for antimalarial activity than the α,β-unsaturated ketone in ring A and the 7-acetate function in ring B.

Most of the compounds tested, especially **1** and **11**, were found to be more active against the chloroquine-resistant clone W2. Because of the apparent lack of cross resistance, these limonoids warrant further investigation as a potential source of new antimalarial agents. The IC_{50} value of **1** is lower than that of quinine and chloroquine for the chloroquine-resistant clone of *P. falciparum*. For the chloroquine-sensitive clone, it is 2 and 21 times less active than quinine and artemisinin, respectively.

The IC_{50} values that were determined for **1** and **2** by Bray *et al.*[2] agreed with those obtained in this study, but only qualitatively. A probable reason for this is that the clone of *P. falciparum* used by Bray *et al.* was K1, not the D6 and W2 clones used in the present study.

The results of the cytotoxicity screening obtained with derivatives of **1** and KB cells, human epidermoid carcinoma cells, are presented in Table 3. Compounds were initially tested at 20 000 ng/mL, to give percent viability data, and select compounds were then evaluated for the determination of IC_{50}. Compounds **1**, **8** , **9**,

Table 3. The Cytotoxicity of Derivatives of **1**

compounds	KB screen (% viability)	KB IC_{50} (ng/mL)
1	32.0	2300
2	71.0	
3	81.0	
4	69.0	
5	39.0	
6	99.0	
7	81.0	
8	31.0	9400
9	38.0	10 900
10	99.0	*a*
11	4.9	500

[a] Amount was not toxic at 20 000 ng/mL, so IC_{50} was not determined.

and **11** all exhibited some degree of cytotoxicity (Table 3). As reported previously,[13] the IC_{50} values for chloroquine, quinine, mefloquine, and artemisinin were 17 400, > 20 000, 3500, and > 20 000 ng/mL, respectively. The cytotoxicity of **11**, a limonoid belonging to the havanensis group, was much greater than that of the other gedunin analogs and drugs that are currently used to treat malaria. Therefore, **11** appears to be nonselective in its mode of antimalarial activity and exhibits the qualities of a general toxin. On the other hand a number of analogs have reduced cytotoxicity, relative to **1**, and may be of value.

An in vivo study of **1** administered orally or subcutaneously to mice infected with *Plasmodium berghei*, a murine malaria parasite, in a 4-day test resulted in no inhibition of parasitemia.[2] The differences between rat and human systems and differences between *Plasmodium* species could possibly account for this observation, but problems related to reaching the target site or metabolism are also likely causes of differences between the in vitro and in vivo studies. Nonetheless, due to the importance placed on the traditional neem remedies in Africa, we are reinvestigating the biological potential of **1** and selected analogs in different in vivo systems.

Experimental Section

General Experimental Procedures. Melting points were measured on a Thomas Hoover capillary melting point apparatus and are uncorrected. Mass spectra were obtained using an VG 7070E or a Kratos concept 2H instrument. IR spectra were recorded in CH_2Cl_2, employing a Bomem−Michelson MB-100 FT/IR spectrophotometer. Optical rotation values were determined using a Perkin−Elmer polarimeter (model 241) set at the sodium D line(589 nm). The samples were, for the most part, analyzed in spectroscopic grade $CHCl_3$. ^{1}H- and ^{13}C-NMR spectra were obtained on a Bruker AMX-500 spectrometer with the shifts reported in ppm. The assignments shown are based on the analysis of the ^{1}H and ^{13}C spectra, as well as the ^{1}H−^{1}H, and ^{1}H−^{13}C correlation spectra.

Plant Material and Extracts for Screening. Plant samples (wood, leaf, and bark specimens) for screening in Table 1 were collected directly into EtOH at the collection sites in various field sites in Costa Rica and Togo or at Fairchild Tropical Garden, Miami, FL. Voucher specimens were collected and are maintained in the herbarium at the University of Ottawa. Extracts were filtered, the residue washed in EtOH, and the combined extracts reduced to dryness first in a rotary evaporator followed by freeze drying.

Antimalarial Microdilution Assay. The antimalarial screening bioassay was originally developed by Desjardins *et al.*[14] and has become one of the most widely used test systems for the evaluation of potential antimalarial compounds.[14,15] The procedure was conducted using two *P. falciparum* clones: clone D6 was a chloroquine-sensitive clone from CDC Sierra Leone, whereas clone W2 was a chloroquine-resistant clone obtained originally from CDC Indochina III. The initial screening involved the determination of the parasite viability, expressed as percent of zero-drug control, to each drug tested at 10 000 ng/mL. The IC_{50} values were determined only for the drugs that exhibited a percentage of parasite viability below 75% for one of the clones. The concentration required to inhibit [^{3}H]-hypoxanthine incorporation by 50% (IC_{50} value) was determined by linear regression analysis of the dose−response curves generated by the serial dilution series of each drug.

Cytotoxicity Assay. Evaluation of the cytotoxicity of the plant extracts and the gedunin derivatives was also conducted using cultured KB cells, as described previously by Likhitwitayawuid *et al.*[16]

Isolation of 1. Planks of *C. odorata* wood obtained from Belize, Costa Rica, and Togo were ground into sawdust using a benchsaw. Three methods were used to obtain **1**.

In method A, the sawdust (726 g) was extracted three times with 95% EtOH over a period of 1 week. The combined extracts were condensed using a rotary evaporator and then freeze dried. The residue was reconstituted in a 1:1 mixture of 95% EtOH−H_2O and then extracted three times with hexane in a separatory funnel. The remaining EtOH−H_2O layer was evaporated on a rotary evaporator until the volume was reduced by half. The resulting aqueous solution was then extracted four times with CH_2Cl_2 and the organic layer reduced to dryness. Gedunin-rich fractions were obtained by repeated flash chromatography of the CH_2Cl_2 extract over Si gel (mesh 270−400) eluted with CH_2Cl_2−EtOAc mixtures of 100:0 to 95:5. Quartz-shaped crystals of gedunin (283 mg) were obtained by crystallization of the combined fractions containing **1** from MeOH. The spectroscopic data were in agreement with those reported in the literature.[18]

In method B, the sawdust (1.82 kg) obtained from Costa Rica was covered with approximately 16 L of toluene, and the mixture was stored at room temperature for 3 days. The mixture was filtered and the sawdust washed with 3 L of fresh toluene. Evaporation of the combined toluene afforded 50 g of a light brown viscous oil. To this oil was added about 200 mL of a 4:1 mixture of hexane−Et_2O and the mixture kept at 4 °C overnight. Filtration yielded 10.6 g of a solid that was chromatographed using 500 g of Si gel and 3:1 hexane−EtOAc as eluent. The yield of pure **1** was 3.9 g.

In method C, *C. odorata* sawdust (30 g each from Belize, Costa Rica, and Togo) was suspended in 300 mL of toluene and the mixture heated for 8 min in a kitchen-type microwave oven that had been modified with a vent hole to provide access for a condenser attached to the extraction flask. The mixture was allowed to cool, and the sawdust was filtered and washed with fresh toluene. The yield of crude product after evaporation was 770, 860, and 990 mg for the three wood samples, respec-

tively. The crude product was chromatographed as in method B. The yield of essentially pure **1** was 120 mg (0.4%), 105 mg (0.35%), and 40 mg (0.1%), respectively.

Preparation of Gedunin Derivatives. Preparation of **2**,[18] **3**,[19] **4**,[20] **5**,[21] and **6**[22] was according to the literature cited. The synthesis of new derivatives is described below. The AB junction in all the derivatives is *trans*.

Preparation of 10. Hydrogenation of **1** in EtOAc at 1 atm of H_2 for 48 h in the presence of 5% Rh/carbon afforded **10**: mp 253−255 °C in 36% yield after purification; IR (CH_2Cl_2) ν (cm^{-1}) 2939, 1737, 1704; ^1H NMR ($CDCl_3$) δ (ppm) 2.45 and 2.55 (m, H-1), 1.50 and 1.90 (m, H-2), 1.82 (m, H-5), 1.72 and 1.82 (m, H-6), 4.47 (m, H-7), 2.26 (m, H-9), 1.72 and 1.92 (m, H-11), 1.54 (m, H-12), 3.40 (s, H-15), 4.47 (m, H-17), 2.40 (m, H-20), 3.34 (t, J = 8.6 Hz, H-21), 3.85 (t, J = 8.0 Hz, H-21), 1.92 and 2.07 (m, H-22), 3.69 (q, J = 6.7, 8.5 Hz, H-23), 3.80 (m, H-23), 0.99, 0.97, 1.04, 1.04, 1.27 (s, Me × 5), 21.0 (s, Me-acetate); ^{13}C NMR ($CDCl_3$) δ (ppm) 33.7 (C-1), 38.9 (C-2), 215.8 (C-3), 46.7 (C-4), 47.8 (C-5), 23.8 (C-6), 73.7 (C-7), 42.0 (C-8), 43.8 (C-9), 37.3 (C-10), 15.1 (C-11), 25.8 (C-12), 39.0 (C-13), 69.6 (C-14), 56.3 (C-15), 167.6 (C-16), 83.2 (C-17), 39.6 (C-20), 70.7 (C-21), 29.5 (C-22), 67.7 (C-23), 15.7, 17.9, 18.2, 21.0, 26.0 (Me × 5), 20.1 (Me-acetate), 169.9 (C═O-acetate).

Preparation of 7. The preparation of **7** was performed in two steps starting with **10**. The 3-keto group in **10** was α-phenylselenylated as reported by Hernandez *et al.*[23] Oxidative deselenylation by stiring overnight with Oxone and $NaHCO_3$ in MeOH yielded **7** (mp 260−262 °C) in an overall yield of 11%: IR (CH_2Cl_2) ν (cm^{-1}) 3092, 3015, 2956, 1738, 1669; ^1H NMR ($CDCl_3$) δ (ppm) 7.12 (d, J = 10.2 Hz, H-1), 5.87 (d, J = 10.2 Hz, H-2), 2.15 (dd, J = 2.4, 13.3 Hz, H-5), 1.78 and 1.92 (m, H-6), 4.49 (br s, H-7), 2.44 (m, H-9), 1.83 and 2.07 (m, H-11), 1.61 (m, H-12), 1.63 (d, J = 8.8 Hz, H-12), 3.42 (s, H-15), 4.51 (m, H-17), 2.46 (m, H-20), 3.37 (t, J = 8.6 Hz, H-21), 3.87 (t, J = 8.0 Hz, H-21), 1.92 and 2.07 (m, H-22), 3.71 (dt, J = 6.7, 8.5 Hz, H-23), 3.83 (dt, J = 3.9, 8.4 Hz, H-23), 1.04, 1.05, 1.08, 1.20, 1.29 (s, Me × 5), 2.06 (s, Me-acetate); ^{13}C NMR ($CDCl_3$) δ (ppm) 156.8 (C-1), 126.1 (C-2), 203.9 (C-3), 44.1 (C-4), 46.1 (C-5), 23.3 (C-6), 73.2 (C-7), 42.6 (C-8), 39.6 (C-9), 40.0 (C-10), 15.1 (C-11), 26.0 (C-12), 38.8 (C-13), 69.6 (C-14), 56.4 (C-15), 167.4 (C-16), 83.1 (C-17), 39.3 (C-20), 70.7 (C-21), 29.5 (C-22), 67.7 (C-23), 18.2, 18.6, 19.7, 21.2, 27.1 (Me × 5), 21.0 (Me-acetate), 169.8 (C═O-acetate); MS (CI/ISO) 487 $[MH]^+$ (80), 427 (35), 371 (9.2), 279 (29.2), 257 (10.5).

Preparation of 8 and 9. The acetylation of the furan ring of **1** at the 21 and 23 positions was carried out for 21 h in CH_2Cl_2 at 0 °C using $BF_3{\cdot}Et_2O$−$(CH_3CO)_2O$. The reaction mixture was worked up by washing with 10% Na_2CO_3 solution. Chromatography over Si gel gave the final products, **8** (17% yield) and **9** (49% yield). The procedure for the acetylation of furan rings has been reported previously.[21]

Compound 8: mp 245−247 °C; $[\alpha]^{23}_D$ +118.8° (*c* 0.0032, $CHCl_3$); MS (CI/ISO) 525 $[MH]^+$ (42.5), 509 (13.8), 481 (20.6), 465 (51.7), 139 (100); HRMS calcd for $C_{30}H_{36}O_8$ 524.6155, found 524.2406; IR (CH_2Cl_2) ν (cm^{-1}) 3056, 2976, 1741, 1673, 1586, 822; ^1H NMR ($CDCl_3$) δ (ppm): 7.05 (d, J = 10.2 Hz, H-1), 5.83 (d, J = 10.2 Hz, H-2), 2.14 (m, H-5), 1.75−1.95 (m, H-6), 4.52 (br s, H-7), 2.46 (dd, J = 7.1, 12.3 Hz, H-9), 1.75−1.91 (m, H-11), 1.22 and 2.14 (m, H-12), 3.51 (s, H-15), 6.48 (s, H-17), 6.68 (d, J = 1.7 Hz, H-22), 7.49 (m, H-23), 1.04, 1.05, 1.19, 1.20, 1.23 (s, Me × 5), 2.09 (s, Me-acetate), 2.49 (s, Me-acetyl); ^{13}C NMR ($CDCl_3$) δ (ppm): 157.1 (C-1), 125.9 (C-2), 204.0 (C-3), 44.0 (C-4), 46.1 (C-5), 23.2 (C-6), 73.2 (C-7), 42.8 (C-8), 39.4 (C-9), 40.6 (C-10), 15.0 (C-11), 24.6 (C-12), 40.2 (C-13), 69.4 (C-14), 57.0 (C-15), 167.6 (C-16), 76.0 (C-17), 128.2 (C-20), 148.6 (C-21), 114.1 (C-22), 144.6 (C-23), 17.6, 18.5, 19.8, 21.2, 27.1 (Me × 5), 21.1 (Me-acetate), 27.2 (Me-acetyl), 169.9 (C═O-acetate), 188.7 (C═O-acetyl).

Compound 9: mp 268−271 °C $[\alpha]^{23}_D$ +75.3° (*c* 0.0068, $CHCl_3$) MS (CI/ISO) 525 $[MH]^+$ (43.2), 481 (39.3), 465 (26.4), 423 (26.1), 422 (30.9), 421 (100); HRMS calcd $C_{30}H_{36}O_8$ for 524.6155, found 524.2421; IR (CH_2Cl_2) ν (cm^{-1}) 2928, 1744, 1676, 1596, 855; ^1H NMR ($CDCl_3$) δ (ppm) 7.06 (d, J = 10.2 Hz, H-1), 5.85 (d, J = 10.2 Hz, H-2), 2.15 (dd, J = 2.4, 13.3 Hz, H-5), 1.79 and 1.95 (m, H-6), 4.54 (dd, J = 2.1, 3.4 Hz, H-7), 2.45 (m, H-9), 1.84 and 2.00 (m, H-11), 1.55 and 1.76 (m, H-12), 3.53 (s, H-15), 5.61 (s, H-17), 7.53 (t, J = 0.8 Hz, H-21)*, 7.06 (m, H-22)*, 1.04, 1.05, 1.14, 1.20, 1.20 (s, Me × 5), 2.08 (s, Me-acetate), 2.46 (s, Me-acetyl); ^{13}C NMR ($CDCl_3$) δ (ppm) 156.7 (C-1), 126.1 (C-2), 203.8 (C-3), 44.0 (C-4), 46.0 (C-5), 23.3 (C-6), 73.2 (C-7), 42.7 (C-8), 40.0 (C-9), 39.6 (C-10), 14.9 (C-11), 26.0 (C-12), 38.7 (C-13), 69.7 (C-14), 56.7 (C-15), 166.9 (C-16), 77.7 (C-17), 123.3 (C-20), 144.4 (C-21), 116.0 (C-22), 153.1 (C-23), 17.7, 18.3, 19.7, 21.2*, 27.1 (Me × 5), 21.0 (Me-acetate)*, 26.1 (Me-acetyl), 169.8 (C═O-acetate), 186.9 (C═O-acetyl). The asterisk (*) indicates that the assignments are interchangeable.

Other Limonoids. Seeds that had been removed from Florida oranges purchased from a local fruit market were dried at 40 °C for 3 days and then ground up using a Wiley Mill. The Me_2CO extraction of the seeds and isolation of limonin has been previously published in the literature.[25] Epiluminol was prepared according to the literature[25] via a $NaBH_4$ reduction of limonin. Nomolin, obacunone, and hirtin were provided by colleagues working in the area.

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