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IN VITRO ANTIVIRAL ACTIVITY OF DAMMAR RESIN TRITERPENOIDS

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ABSTRACT.—Nine triterpenes with antiviral activity against *Herpes simplex* virus types I and II in vitro were isolated from dammar resin. Each compound caused a significant reduction in viral cytopathic effect when Vero cells were exposed continuously to 1-10 μg/ml of compound for 48 h after viral challenge. The triterpenes were identified as dammaradienol [**1**], dammarenediol-II [**2**], hydroxydammarenone-I [**3**], ursonic acid [**5**], hydroxyhopanone [**11**], dammarenolic acid [**15**], shoreic acid [**16**], eichlerianic acid [**17**], and a novel compound, hydroxyoleanonic lactone [**7**], on the basis of their chromatographic, spectroscopic, and physical properties.

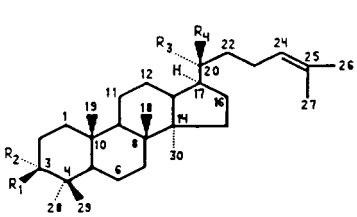
In the course of our continuing investigation of biologically active compounds from natural sources, a sample of dammarenediol-II [**2**], isolated from a hexane extract of twigs and leaves of *Cowania mexicana* (Rosaceae), was observed to exhibit activity in our in vitro antiherpes bioassay (unpublished results). Subsequently, isofouquierol [**21**] (1) was identified as the major antiherpes active compound obtained from a hexane extract of twigs, leaves, and flowers of *Gierocarpus intricatus* (Rosaceae) (unpublished results). Because these structurally similar triterpenoids both exhibited antiherpes activity, our attention was drawn to dammar resin as a natural phytochemical source of additional dammarane-type compounds.

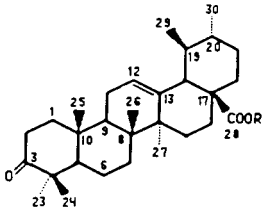
Commercial dammar resin is a soft, clear to yellow, solid resin obtained primarily from the following genera of the family Dipterocarpaceae (order Theales): *Hopea, Shorea, Balanocarpus,* and *Vateria*. The genus *Canarium* of the family Burseraceae (order Rutales) also yields a commercial product considered to be a dammar resin (2). Members of the family Dipterocarpaceae are generally described as tall, woody trees distributed throughout the rain forests of Southeast Asia. The resin is used in microscopy to preserve specimens. It has also been used as a component of experimental insecticide formulations (3).

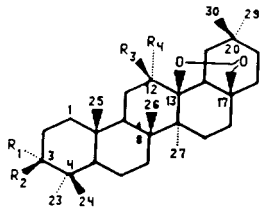
The earliest systematic investigation of dammar resin was reported by Tschirch and Glimann who described a compound called dammarolic acid (4), a name also given to a different compound isolated by Mladenovic and Barkovic (5) and subsequently shown by Brewis and Halsall to be asiatic acid (6). Mills and Werner performed an in-depth study of dammar resin in which they identified ten triterpenoids (7) as well as the "dammarolic acid" of Mladenovic and Barkovic. This work was followed up by further studies on the chemistry and structural details of the neutral and acidic triterpenes of dammar resin (8,9). Bisset *et al.* (10) reported an interesting study in which the shoreic and dammarenolic acid content, as well as the neutral terpene content of the resin, was used as a means of chemotaxonomic differentiation of the genus *Shorea* from several related subgenera.

RESULTS AND DISCUSSION

The chemical fractionation of dammar resin was monitored and guided by in vitro antiherpes testing of fractions produced by extraction or column chromatography. In vitro antiherpes activities of the nine dammar resin triterpenoids thus isolated are listed in Table 1. Unpublished preliminary data suggested that compounds of this type with IC_{50} values in the 2-10 μg/ml range may be expected not to have significant activity in vivo against *Herpes simplex* virus Type 1.



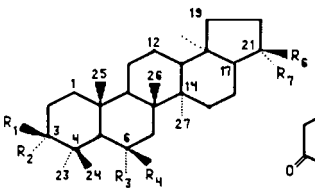


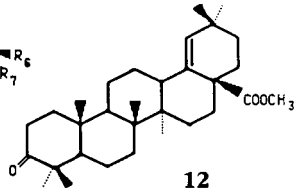


1 R_1=OH, R_2=H, R_3,R_4==CH_2
2 R_1=OH, R_2=H, R_3=CH_3, R_4=OH
3 R_1,R_2==O, R_3=OH, R_4=CH_3
4 R_1,R_2==O, R_3=CH_3, R_4=OH

5 R=H
6 R=CH_3

7 R_1,R_2==O, R_3=H, R_4=OH
8 R_1=H, R_2=OH, R_3=H, R_4=OH
9 R_1=H, R_2=OH, R_3,R_4==O
10 R_1,R_2==O, R_3,R_4==O





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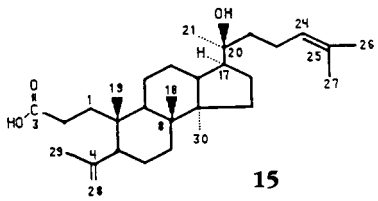
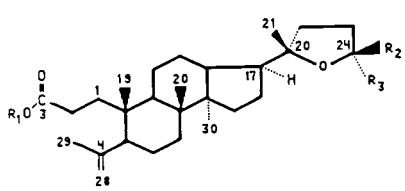
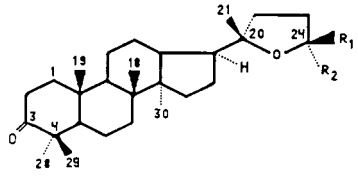


15

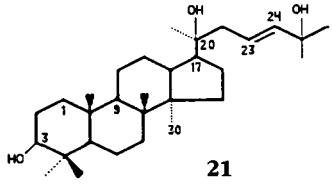
11 R_1,R_2==O, R_3=R_4=R_5=H, R_6=$-C(CH_3)_2OH$
13 R_1=R_2=R_3=R_4=R_6=H, R_5=$-C(CH_3)(CH_2OAc)OH$
14 R_1=R_2=R_4=R_6=H, R_3=OAc, R_5=$-C(CH_3)_2OH$





16 R_1=R_2=H, R_3=$-C(CH_3)_2OH$
17 R_1=R_3=H, R_2=$-C(CH_3)_2OH$
20 R_1=CH_3, R_2=H, R_3=$-C(CH_3)_2OH$

18 R_1=H, R_2=$-C(CH_3)_2OH$
19 R_1=$-C(CH_3)_2OH$, R_2=H



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Dammaradienol [**1**] was identified by comparing its ^{13}C-nmr spectrum with that of dammarenediol-II [**2**] (11). The chemical shifts in the ^{13}C-nmr spectra were very similar to the corresponding values in dammarenediol except for C-20 and C-21. Dehydration of the C-20 carbinol shifted the dammarenediol singlet at 75.4 ppm downfield to 152.7, while the dammarenediol C-21 methyl signal at 24.9 ppm was correspondingly shifted downfield to become a triplet at 107.5 in dammaradienol. Dammarenediol-II [**2**] was identified by its physical properties, tlc comparison with an authentic sample, and comparison of its ^{13}C-nmr spectrum with published data for the *S* configuration at C-20 (11). Identification of hydroxydammarenone-I [**3**] was made by comparing its ^{13}C-nmr spectrum with those of hydroxydammarenones-I and -II [**4**] (11), and the *R* configuration at C-20 was confirmed by the optical rotation value. Ursonic acid [**5**] was readily identified on the basis of its physical properties and a comparison of its ^{13}C-nmr spectrum to a published spectrum of methyl ursonate [**6**] (12).

The hydroxyoleanonic acid lactone [**7**] has not been previously reported, although

TABLE 1. In Vitro Antiherpes Activity of Dammar Resin Components

Compound	IC_{50} (μg/ml)	
	HSV-1	HSV-2
Dammaradienol [**1**]	2.5	3.0
Hydroxydammarenone-I [**3**]	2.0	5.0
Ursonic acid [**5**]	2.5	8.0
Hydroxyoleanonic lactone [**7**]	5.0	5.0
Hydroxyhopanone [**11**]	7.0	5.0
Dammarenediol-II [**2**]	7.0	7.0
Dammarenolic acid [**15**]	3.0	2.0
Shoreic acid [**16**]	7.0	8.0
Eichlerianic acid [**17**]	7.0	8.0

the analogous oleanolic acid lactones **8** and **9** are known (13,14), and the 3,12-diketolactone **10** has been synthesized (15). Absorption signals in the ir spectrum corresponding to a cyclic ketone (1705 cm^{-1}) and to a lactone or ester carbonyl (1737 cm^{-1}) were correlated in the ^{13}C-nmr spectrum by signals at 218 ppm and 180.7 ppm, respectively. A cursory examination of the ^{13}C-nmr spectrum of **7** in comparison to typical ursane, oleanane, dammarane, and hopane-type triterpenoids suggested the presence of a saturated oleanane-type structure containing two possible hydroxyl moieties in adddition to the two carbonyls. The ^{13}C-nmr spectrum (Table 2) was useful for assignment of the ester carbonyl, but alone was insufficient to permit unambiguous assignment of the locations of the remaining carbonyl or the two presumed hydroxyl functions. X-ray diffraction data (16) indicated that the ester carbonyl was part of a lactone and facilitated the placement of the hydroxyl and ketone functionalities.

Hydroxyhopanone [**11**] was initially identified on the basis of its physical properties and characteristic eims fragmentation pattern. Typical eims fragmentation patterns for various substituted hopanes have been reported (17) with major peaks at *m/z* 59, 149, 189, 207, and 384 being especially characteristic for the 22-hydroxyhopanes (18). The hydroxyhopanone identity without reference to C-21 stereochemistry was supported by assignment of the ^{13}C-nmr spectrum in comparison with published

TABLE 2. Proposed ^{13}C-nmr Chemical Shifts for Hydroxyoleanonic Lactone [**7**]

Carbon No.	Chemical Shift	Carbon No.	Chemical Shift
1	39.0 t[a]	16	27.2 t
2	33.9 t[b]	17	46.8 s
3	217.0 s	18	43.4 d
4	44.3 s	19	38.9 t[a]
5	54.4 d	20	31.0 s
6	18.7 t	21	33.6 t[b]
7	33.0 t	22	27.6 t
8	41.7 s[c]	23	26.3 q
9	51.0 d	24	20.6 q
10	35.9 s	25	15.8 q
11	20.9 t	26	17.9 q[d]
12	74.8 d	27	18.0 q[d]
13	90.9 s	28	179.5 s
14	41.9 s[c]	29	32.8 q
15	28.8 t	30	23.3 q

[a,b,c,d]Assignments may be interchanged.

spectra of methyl moronate [**12**] (19), 29-acetoxyhopan-22ϵ-ol [**13**] and zeorin 6-acetate [**14**] (20). The β-configuration of the C-21 hydroxyisopropyl side chain was established by physical properties (melting point, optical rotation) which vary markedly from the α- form. In addition, spectroscopic evidence was obtained that suggested hydroxyhopanone might exist as a dimeric complex held together by hydrogen bonds. The ir spectrum in KBr showed a medium intensity peak at 3470 cm^{-1} (free OH) and twin carbonyl absorptions at 1686 and 1708 cm^{-1}, as might be expected for a roughly equal mixture of dimeric and monomeric forms. The 22-wavenumber difference between the two carbonyl peaks provided a qualitative estimate of the strength of the bonds involved in the solid state, placing them in the medium-weak category (21). Compared with the solid state spectrum, the ir spectrum in solution displayed a free OH peak whose intensity was drastically diminished, and the split carbonyl absorption had become a single intense peak at 1698 cm^{-1}. These observations were consistent with our proposal of an extensively hydrogen-bound species in solution, presumably the dimeric form of hydroxyhopanone.

The ^{1}H-nmr spectrum of dammarenolic acid[1] [**15**] resembled published data for the methyl ester (22). The ^{13}C-nmr spectrum of **15** closely resembled that of dammarenediol, with the exception of the A ring which was cleaved to a 3,4-seco system. The C-3 carbinol signal was shifted to a downfield acid carbonyl, and the C-4/C-28 signals appeared downfield in the unsaturated region. The eims fragmentation pattern exhibited good agreement with published data (23), and physical properties were in general agreement with literature values (7).

Although shoreic acid[1] [**16**] has been isolated from the resin of a *Shorea* species (24), its epimer eichlerianic acid[1] [**17**] has not been previously reported as a constituent of dammar resin. We report here as well the first isolation of these compounds in the free acid form rather than the methyl esters reported by earlier investigators (24,25). Given the high degree of physical and chemical similarity of these two epimers, it was serendipitous that they could be separated from each other so readily using only a single-component isocratic mobile phase on a silica hplc support. The presence of a 3,4-seco system in **16** and **17** was established by comparison of their ^{13}C-nmr spectra with the spectrum of dammarenolic acid. The attachment of the substituted tetrahydrofuran ring at C-17 and the stereochemical configuration at C-24 was established by comparing the ^{13}C-nmr spectra of the two compounds with the spectra of cabraleone [**18**] and ocotillone [**19**] (25) whose absolute configurations have been determined by comparison to the X-ray crystallographic structure of methyl shoreate [**20**] (26). Slight deviations in the chemical shifts of C-24, C-25, and C-26 were sufficiently characteristic to permit correct identification as shown in Table 3. Although the eims could not be used to distinguish between the epimers, it did establish the presence of the shoreic/eichlerianic 3,4-seco system typical of dammarenolic acid. The mass spectrum of each epimer displayed a strong base peak at *m/z* 143 with other significant fragments at 109 and 125 amu, and the molecular ion in both cases was weak or absent. These observations were in general agreement with reported spectra of 3,4-seco-triterpenoids (23). Other physical and spectroscopic data were consistent with the proposed structures.

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[1]Solutions of dammarenolic, shoreic, and eichlerianic acids should be handled with great care. During the course of this investigation one of our workers was inadvertently exposed to a few drops of CH_2Cl_2 solution containing a mixture of these acids. Although the affected area was cleansed immediately, symptoms of severe contact dermatitis manifested in the exposed skin area after a 48-h delay. Small red lesions appeared, accompanied by itching and burning characteristic of exposure to poison ivy. Swelling of lesions continued until they were lanced, whereupon recovery commenced and was complete by 4 weeks following exposure. Treatment consisted of periodic soaking of the affected area in salt baths and oral administration of steroids.

TABLE 3. ^{13}C-nmr Identification of Shoreic [**16**] and Eichlerianic Acids [**17**]

Carbon No.	Chemical Shift Values (67.8 MHz in Pyridine-d_5)[a]			
	Cabraleone [**18**]	Shoreic Acid [**16**]	Ocotillone [**19**]	Eichlerianic Acid [**17**]
24	87.4	87.3	84.1	84.3
25	70.4	70.5	71.1	71.3
26	26.8	26.9	26.0	26.4

[a]Values for cabraleone and ocotillone taken from Lantz and Wolff (24).

In summary, the antiherpes virus bioactivity of these nine triterpenes is the first report of any such activity for components of dammar resin. Of these, **7** is a novel structure, **17** has not been previously reported in dammar resin, and the epimers **16** and **17** were isolated for the first time in their free acid forms. Finally, it would seem worthwhile to characterize the physical constants associated with the apparent dimerization of hydroxyhopanone.

EXPERIMENTAL

APPARATUS/METHODS.—Mps were obtained on a Thomas-Hoover melting point apparatus and are uncorrected. Optical rotations were performed in $CHCl_3$ relative to the D line of sodium using a Jasco DIP-360 digital polarimeter equipped with a constant temperature bath held at 20°. Uv spectra for all compounds were uncharacteristic, exhibiting only end absorption. Ir spectra were recorded on a Nicolet 20DXB ftir in KBr. ^{1}H- and ^{13}C-nmr spectra were obtained on a JEOL GX-270 instrument (H, 270 MHz; C, 67.8 MHz) employing the GASPE technique for carbon spectra with INEPT experiments to distinguish multiplicities. The ^{13}C-nmr spectra were run in the same solvent as the ^{1}H- spectra unless specified, and ^{1}H-nmr spectra are reported as δ ppm values downfield from TMS. Mass spectra were recorded on a Finnigan 4610 quadrupole instrument with sample introduction via direct probe. Merck Si gel 60 (230-400 mesh) was used for all open column and rapid elution silica chromatography. Bio-Rad AG-7 neutral alumina (100-200 mesh) was used for alumina rapid elution chromatography. Gravity columns were monitored by uv at 254 nm and/or by tlc on Merck alumina or Si gel 60 F-254 precoated plastic sheets (0.2 mm layer thickness) with ceric sulfate/H_2SO_4 or H_3PO_4/vanillin visualization. Semi-preparative hplc purifications were performed using two tandem connected Rainin Dynamax 8u silica columns (21.4 mm ID×25 cm each) equipped with silica guard module. A Beckman 112 pump fitted with a preparative head was employed for solvent metering, and detection was by simultaneous uv (225 nm) and refractive index. Reagent grade solvents were adequate for open columns, but only glass-distilled or hplc-grade solvents were used for preparative lc.

ANTIHERPES VIRUS BIOASSAY.—Assay is based upon observing the cytopathic effect (cpe) due to viral infection of cultured Vero cells (African green monkey kidney) and scoring the extent to which this infection process is inhibited. Each of two immunologically distinct types of herpes simplex virus (HSV-1, HSV-2) causes specific morphological changes in the host cell during replication. The HSV-1 virus (MacIntyre strain, ATCC VR-539) causes host cells to round up and form syncytia; HSV-2 (MS strain, ATCC VR-540) causes cell round-up within defined plaque areas characterized by central cell-loss as the plaque area grows outward. Additionally, both strains cause buildup of eosinophilic inclusion bodies.

Vero cells were plated at a density of 15,000 cells/cm^2 and incubated overnight at 37° in Dulbecco's minimal essential medium containing 4.5 mg/ml glucose (Gibco 60K8326), 10% v/v fetal bovine serum, and 50 μg/ml gentamycin (Gibco 600-5750). Virus (20 plaque forming units of HSV-1 or 50-75 pfu of HSV-2) was added to host cell medium and allowed to remain for 1 h at 37°. Medium was then removed and replaced with medium containing test substance or control (acyclovir 10 μg/ml or bromovinyldeoxyuridine 1 μg/ml). The test/control medium contained only 3% fetal bovine serum to minimize overgrowth of host cell monolayer. Samples were tested in duplicate at two or more dose levels. After 48 h the plates were examined visually for relative amount of cpe in tests and controls. Results were expressed as percent cpe relative to controls, and IC_{50} values are reported as concentration of material required to produce a 50% reduction in cpe.

DAMMAR RESIN.—Material used in this investigation was obtained from Fluka Chemical Corp. (Hauppauge, NY 11788) catalog no. 30424.

EXTRACTION.—Dammar resin (1000 g) was powdered in a mortar and triturated once with 6 liters

MeOH and a second time with 3 liters MeOH. MeOH solubles were combined and evaporated (711 g) and found to contain most of the HSV-2 activity. MeOH insolubles (260 g) containing most of the HSV-1 activity were not further investigated. The MeOH soluble residue (600 g) was twice triturated with 3 liters of petroleum ether; upon evaporation a tlc spot with Rf identical to dammarenediol-II (Et_2O-hexane, 1:1) was observed in a sample of the petroleum ether solubles (315 g) which also exhibited HSV-2 activity. The petroleum ether insolubles (257 g) also exhibited HSV-2 activity but have not as yet been investigated.

COLUMN CHROMATOGRAPHY.—A sample of petroleum ether soluble material (120 g) was adsorbed onto 250 g coarse silica and subjected to rapid elution chromatography in a 2-liter medium pore glass frit funnel half filled with silica. Elution was performed with 1.5-liter solvent portions in a step gradient starting with hexane and ending with Et_2O in approximately 10% increments, plus a final wash with 1.5 liters each of EtOAc and MeOH. Eluents were evaluated by tlc (Et_2O-hexane, 1:1) and pooled into seven fractions. Two of these (A, 65.6 g; B, 29.6 g) exhibited strong HSV-1 and HSV-2 activity. A 60 g sample of A was chromatographed on silica in a manner similar to that just described. Nine fractions were obtained, of which two were both active and present in sufficient quantity (12.3 g, 7.4 g) for further fractionation. Two other fractions (2.6 g, 12.8 g), initially inactive, appeared crystalline. A 27-g sample of B, chromatographed on silica in the same fashion as sample A, produced three active fractions (2.8 g, 14.5 g, and 5.6 g).

DAMMARADIENOL [**1**] (3β-HYDROXY20,24-DAMMAREDIENE).—The 12.3 g fraction from sample A contained a major tlc component (Rf=0.72, CH_2Cl_2-MeCN-iPrOH, 9:1:0.2) which was chromatographed three times on silica using various gradient schemes employing CH_2Cl_2, hexane, Et_2O, MeOH, and MeCN to yield 1.3 g of enriched material, which was precipitated from Et_2O/MeCN to give 940 mg of material which by tlc appeared >90% pure. A sample of this material (150 mg) was purified by repeated prep-hplc injections (Et_2O-CH_2Cl_2, 4:96; *10 ml/min*). The chromatogram showed two peaks that were collected (82 mg and 27 mg, respectively), of which only the first was active. The 82-mg fraction was crystallized from Et_2O/MeCN to yield 73 mg of dammaradienol as fluffy colorless needles, mp 135° sharp, [α]=+52.0° (*c* 0.88); ir ν max 3367 broad (-OH), 3078 (olefinic C-H), 2943 broad (CH), 1643 (C=C), 1442, 1387 and 1375 (di-Me), 1091, 1043 and 1030 (C-O, β-OH), 985, 887 (olefinic C-H) cm^{-1}; ^1H nmr ($CDCl_3$/CD_3OD) 0.78 (s, 3H), 0.85 (s, 3H), 0.87 (s, 3H), 0.98 (s, 6H), 1.62 (s, 3H), 1.69 (s, 3H), 3.20 (dd, 1H, *J*=10.5, 5.9 Hz), 4.72 (d, 2H, *J*=9 Hz), 5.13 (t, 1H, *J*=1.4 Hz); ^{13}C nmr 152.7s, 124.5d, 107.5t, 79.0d, 56.1d, 51.1d, 49.5s, 47.8d, 45.5d, 40.6s, 39.3t, 39.1s, 37.4s, 35.6t, 34.4s, 31.5t, 29.1t, 28.1q, 27.5t, 27.2t, 25.6q, 25.1t, 21.5t, 18.4t, 17.7q, 16.0q, 16.0q, 15.7q, 15.4q; eims *m/z* 426 (M^+).

HYDROXYDAMMARENONE-I [**3**] [(20*R*)-HYDROXY-24-DAMMAREN-3-ONE].—A 7-g sample of the 7.4 g active component of sample A was chromatographed on silica (400 g) in CH_2Cl_2-MeCN (9:1). Eluents were analyzed by tlc (Et_2O-hexane, 70:30), pooled into five fractions, and evaporated. Two fractions (Rf=0.77, 900 mg; Rf=0.58, 297 mg; CH_2Cl_2-MeCN-iPrOH, 9:1:0.2) were active. Upon crystallization from Et_2O/MeCN, the 900 mg fraction yielded 453 mg hydroxydammarenone-I as colorless needles, mp 136-137°, [α]=+60.6° (*c* 1.08); ir ν max 3493 (O-H), 2980-2940 (CH), 1694 (C=O), 1455, 1379, 1183 (C-O), 1122, 1082, 988, 928; ^1H nmr ($CDCl_3$) 0.89 (s, 3H), 0.95 (s, 3H), 1.01 (s, 3H), 1.04 (s, 3H), 1.08 (s, 3H), 1.13 (s, 3H), 1.63 (s, 3H), 1.69 (s, 3H), 5.12 (t, 1H, *J*=7.3 Hz); eims *m/z* 442 (M^+).

URSONIC ACID [**5**] (3-OXO-12-URSEN-28-OIC ACID).—The second active fraction (297 mg) remaining after isolation of hydroxydammarenone-I (above) was crystallized from Et_2O/MeCN to yield 109 mg of ursonic acid as colorless rosette clusters, mp 265-269° dec, [α]=+82.0° (*c* 0.94); ir 3426 broad (O-H), 3000-2900 (CH), 1703 very intense (C=O), 1458, 1385; ^1H nmr ($CDCl_3$) 0.82 (s, 3H), 0.85 (s, 3H), 0.87 (s, 3H), 0.96 (s, 3H), 1.03 (s, 3H), 1.05 (s, 3H), 1.08 (s, 3H), 5.26 (m, 1H); ^{13}C nmr 217.7s, 184.0s, 138.0s, 125.5d, 55.2d, 52.5d, 48.0s, 47.3s, 46.7d, 42.0s, 39.4s, 39.2t, 39.0t, 38.7d, 36.6t, 34.0t, 32.4t, 30.5t, 27.9t, 26.5q, 24.0t, 23.5q, 23.4q, 21.4q, 21.1q, 19.5t, 16.9q, 15.1q; eims *m/z* 454 (M^+).

HYDROXYOLEANONIC LACTONE [**7**] (12α,13-DIHYROXY-3-OXO-28-OLEANANOIC ACID, γ-LACTONE).—The 2.6-g semicrystalline fraction from sample A was crystallized once from CH_2Cl_2/Me_2CO to yield 564 mg fine colorless prisms, of which 220 mg was recrystallized from CH_2Cl_2/MeOH to yield 200 mg of somewhat larger prisms mp 304-306°, [α]=+60.4° (*c* 1.08); ir 3514 (O-H), 2950 broad (CH), 1737 (O-*C*=*O*), 1705 (C=O), 1458, 1395 (di-Me), 1256 (C-O), 1226 (C-O), 1144, 1069 (CH-OH), 946, 909; ^1H nmr ($CDCl_3$/CD_3OD) 0.90 (s, 3H), 0.99 (s, 6H), 1.05 (s, 3H), 1.10 (s, 3H), 1.19 (s, 3H), 1.32 (s, 3H), 3.88 (t, 1H, *J*=2.7 Hz); ^{13}C nmr ($CDCl_3$-pyridine-d_5, 4:1) see Table 2; eims *m/z* (%rel. int.) 41 (55), 49 (62), 69 (62), 81 (44), 84 (42), 93 (25), 95 (37), 107 (28), 109 (27), 121 (23), 135 (16), 147 (13), 163 (18), 175 (17), 177 (36), 187 (14), 189 (21), 205 (100), 218 (28), 234 (16), 246 (16), 250

(16), 264 (10), 300 (4), 409 (21), 452 (14), 455 (13), 470 (m^+, 8); hrms ($C_{30}H_{46}O_4$ mw=470.3396): found, 470.3397.

HYDROXYHOPANONE [**11**] (21β,22-HYDROXY-3-HOPANONE).—The 12.8-g fraction from sample A was dissolved in Et_2O/MeCN, with the MeCN added dropwise until a heavy white precipitate (700 mg) was obtained. This precipitate was recrystallized from CH_2Cl_2/MeOH to yield 297 mg of hydroxyhopanone as silky, filamentous needles mp 253-254° (lit. β 252-256°, α 214-215°), [α]=+61.1° (*c* 0.82) (lit. β +64°, α +50°) (27); ir (KBr) 3470 (OH), 2947 (CH), 1708 (C=O), 1443, 1377; ir ($CHCl_3$) 3602 very weak (OH), 2950 (CH), 1698 (C=O), 1461, 1386; ^{13}C nmr ($CDCl_3$/CD_3OD) ca. 220s, 73.3s, 54.4d, 53.5d, 50.6d, 49.6d, 49.2d, 47.0s, 43.6s, 41.5s, 41.2s, 40.8t, 39.0t, 36.3s, 33.9t (two signals), 32.1t, 29.5q, 27.6q, 26.1t, 25.9q, 23.6t, 22.0t, 21.1t, 20.4q, 19.3t, 16.3q, 15.8q, 15.6q, 15.2q. A second isolation of hydroxyhopanone (97 mg) was made from the 2.8-g fraction of sample B by precipitation and crystallization from hexane/MeCN, identical to the first isolation by tlc, physical, and spectroscopic properties.

DAMMARENEDIOL-II [**2**] [(20*S*)-DAMMAR-24-ENE-3β,20-DIOL].—The 14.5-g fraction from sample B contained a tlc spot whose Rf (silica, 100% Et_2O) was identical to that of authentic dammarenediol but heavily streaked with contaminants. When chromatographed on alumina in a variety of solvent systems the dammarenediol spot moved while leaving a large amount of material apparently irreversibly bound at the origin. The entire sample was fractionated using rapid elution chromatography on alumina (400 ml bed volume) and eluted with 500 ml each of CH_2Cl_2, CH_2Cl_2-EtOAc (1:1), EtOAc, EtOAc-MeOH (1:1), MeOH. Activity eluted in the EtOAc and EtOAc/MeOH fractions which were combined (4.9 g) and rechromatographed on silica isocratically in CH_2Cl_2-MeCN-iPrOH (9:1:0.2). A fraction (1.3 g) with a tlc spot corresponding to dammarenediol was crystallized from Et_2O/MeCN to yield 507 mg dammarenediol-II as fine colorless needles mp 130-136°, [α]=+32.8° (*c* 1.05); ir 3500-3300 broad (O-H), 2943 (CH), 1636 (C=C), 1450, 1375, 1134 (C-O), 1044 and 1029 (C-O, β-OH), 984, 921; ^1H nmr ($CDCl_3$) 0.77 (s, 3H), 0.84 (s, 3H), 0.88 (s, 3H), 0.97 (s, 6H), 1.14 (s, 3H), 1.62 (s, 3H), 1.69 (s, 3H), 2.05 (m, 2H), 3.20 (dd, 1H, *J*=5.6, *J*=10.5 Hz), 5.12 (t, 1H, *J*=6.6 Hz); eims *m/z* 444 (M^+).

DAMMARENOLIC ACID [**15**] [(20*S*)-HYDROXY-3,4-SECO-4(28),24-DAMMARADIEN-3-OIC ACID].—A 3.4-g portion of the 5.6-g fraction from sample B was chromatographed on silica (300 g) using a complex gradient employing CH_2Cl_2, MeCN, and MeOH starting at 100% CH_2Cl_2, then CH_2Cl_2-MeCN-MeOH (8:2:0.1) ending with CH_2Cl_2-MeOH (8:2). After pooling eluents according to bioassay data two active fractions were obtained (1.2 g, 0.48 g). The 1.2 g fraction was crystallized several times from Et_2O/MeCN to yield 137 mg of dammarenolic acid as colorless needles, mp 146-148°, [α]=+43.3° (*c* 1.28); ir 3408 broad (O-H), 2931 broad (CH), 1708 (C=O), 1640 (C=C), 1667 (di-Me-*C*=*C*), 1453, 1379, 1309 (C-O acid), 1179 (C-O alc.), 1113, 1080, 924, 894 (olefinic C-H); ^1H nmr ($CDCl_3$) 0.85 (s, 3H), 0.89 (s, 3H), 1.00 (s, 3H), 1.15 (s, 3H), 1.62 (s, 3H), 1.69 (s, 3H), 1.73 (s, 3H), 4.66 broad (s, 1H), 4.85 broad (s, 1H), 5.11 (t, 1H, *J*=7.1 Hz); ^{13}C nmr 179.8s, 147.4s, 131.5s, 124.6d, 113.4t, 75.6s, 50.8d, 50.6s, 49.7d, 42.3d, 41.0d, 40.6t, 40.0s, 39.0t, 34.2t, 33.8t, 31.2t, 28.3t, 27.4t, 25.7q, 25.3q, 24.7t, 24.5t, 23.2q, 22.5s, 22.0t, 20.1q, 17.7q, 16.3q, 15.3q; eims *m/z* (%rel. int.) 43 (36), 55 (25), 69 (70), 81 (25), 95 (32), 109 (100), 121 (14), 125 (3), 127 (15), 143 (3), 149 (12), 161 (7), 175 (6), 189 (5), 205 (4), 215 (3), 233 (3), 289 (4), 329 (8), 357 (4), 371 (20), 397 (1.5), 425 (2), 440 (12).

SHOREIC ACID [**16**] [(20*S*,24*R*)-EPOXY-25-HYDROXY-3,4-SECO-4(28)-DAMMAREN-3-OIC ACID].—The 480-mg fraction remaining from the purification of dammarenolic acid above was observed by tlc (silica, Et_2O-hexane, 9:1) to contain two spots with nearly the same Rf. The entire sample was purified by prep-hplc (Dynamax silica, 100% Et_2O hplc grade without preservative, flow rate 10.0 ml/min, 360 psi, 100 mg/injection) via repeated injections. Two major peaks were collected, yielding upon evaporation 85 mg and 81 mg, respectively, of clear films with pungent odor. Upon crystallization from MeCN/H_2O the 85 mg sample yielded 71 mg of shoreic acid as rectangular plates mp 102-104°, [α]=+38.4° (*c* 1.0); ir 3446 broad (O-H), 3070 (olefinic C-H), 2957 broad (CH), 1717 (C=O), 1636 (C=C), 1457, 1375, 1301 broad (C-O acid), 1203 (C-O alc.), 1123 (C-O-C), 1060, 1011, 952, 895 (olefinic C-H); ^1H nmr ($CDCl_3$) 0.86 (s, 3H), 0.89 (s, 3H), 1.02 (s, 3H), 1.12 (s, 3H), 1.15 (s, 3H), 1.20 (s, 3H), 1.74 (s, 3H), 3.66 (m, 1H), 4.67 (s, 1H), 4.85 (s, 1H); ^{13}C nmr (pyridine-d_5) 176.3s, 148.1s, 113.7t, 87.3d, 86.4s, 70.5s, 51.0d, 50.8s, 50.4d, 43.3d, 41.6d, 40.4s, 39.6s, 35.8t, 35.7t, 34.4t, 31.8t, 29.4t, 27.3t, 27.1t, 20.0q, 26.9q, 26.3q, 26.2t, 25.2t, 23.7q, 22.8t, 20.6q, 16.7q, 15.6q; eims major fragments *m/z* (%rel. int.) 41 (24), 43 (57), 55 (39), 59 (35), 67 (22), 69 (22), 71 (32), 81 (32), 85 (35), 93 (29), 95 (31), 107 (27), 109 (20), 125 (37), 143 (100), 161 (12), 375 (1), 387 (0.1), 397 (8), 415 (19), 459 (3), 474 (M^+, 0.14); cims (NH_3) *m/z* 474 (M^+).

EICHLERIANIC ACID [**17**] [(20*S*,24*S*)-EPOXY-25-HYDROXY-3,4-SECO-4(28)DAMMAREN-3-OIC ACID].—The oily film (81 mg) remaining from the purification of shoreic acid above was crystallized with

difficulty from MeCN/H_2O to yield 45 mg of colorless cubical clusters mp 101-102°, [α]=+40.0° (*c* 0.96); ir 3410 broad (O-H), 3074 (olefinic C-H), 2972 broad (CH), 1703 (C=O), 1636 (C=C), 1463, 1376, 1298 broad (C-O acid), 1171 (C-O alc.), 1122 (C-O-C), 1073, 1019, 945, 890 (olefinic C-H); ^{1}H nmr ($CDCl_3$) 0.85 (s, 3H), 0.89 (s, 3H), 1.00 (s, 3H), 1.13 (s, 3H), 1.14 (s, 3H), 1.21 (s, 3H), 1.73 (s, 3H), 3.74 (t, 1H, *J*=7.2 Hz), 4.67 (s, 1H), 4.85 (s, 1H); ^{13}C nmr (pyridine-d_5) 176.4s, 148.2s, 113.7t, 84.3d, 86.3s, 71.3s, 51.0d, 50.8s, 50.4d, 43.4d, 41.6d, 40.4s, 39.6s, 36.4t, 36.3t, 34.4t, 31.9t, 29.5t, 27.5t, 26.9t, 26.4q, 26.1t, 25.2t, 23.7q, 22.5t, 21.2q, 16.7q, 15.6q; eims *m/z* (%rel. int.) 43 (13), 55 (8), 59 (31), 67 (6), 69 (5), 71 (7), 81 (9), 85 (12), 93 (8), 95 (7), 107 (9), 109 (5), 125 (13), 143 (100), 161 (5), 175 (3), 375 (1), 397 (3), 415 (5), 459 (1); cims (NH_3) *m/z* 474 (M^+).

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