Irradiation of 1b in Acetone. In a typical run, a solution of **1b** (200 mg) in acetone (30 mL) was irradiated at room temperature for 10 h. After removal of the solvent under reduced pressure, the residue was chromatographed with chloroform as eluant to afford β-lactam **2** and 1,3-dioxolane **6** (27% and 45% yield, respectively). NMR of **2** ($CDCl_3$ δ 3.22 (2 H, t, J = 2 Hz), 2.95 (2 H, t, J = 2 Hz), 2.83 (3 H, s). NMR of **6** ($CDCl_3$) δ 4.21 (1 H, s), 3.18 (3 H, s), 2.84 (3 H, s), 1.41 (3 H, s), 1.39 (3 H, s), 1.30 (3 H, s), 1.13 (3 H, s).

Irradiation of 1e in Methanol. A solution of the diazo amide **1e** (200 mg) in methanol (30 mL) was photolyzed and worked up in the usual way to give β-lactam **9** and the ester **10** (16% and 30% yield, respectively). NMR of **9** ($CDCl_3$) δ 4.05 (1 H, q, J = 4.2 Hz, of d, J = 2.0 Hz), 3.70 (1 H, d, J = 2.0 Hz), 3.41 (2 H, m), 2.19 (3 H, s), 1.35 (3 H, d, J = 4.2 Hz), 1.17 (3 H, m). NMR of **10** ($CDCl_3$) δ 3.72 (3 H, s), 3.38 (2 H, m), 3.35 (1 H, q, J = 4.5 Hz), 1.41 (3 H, d, J = 4.5 Hz), 1.21 (3 H, m).

Irradiation of 1f in Methanol. A solution of **1f** (200 mg) in methanol (30 mL) was photolyzed and worked up in the usual way to afford oxindole **11** (mp 99-101 °C), ester **12**, and acetoacetamide **13** (25%, 12%, and 18% yield, respectively). NMR of **11** ($CDCl_3$) δ 7.42-6.89 (4 H, m), 3.90 (2 H, q, J = 3.8 Hz), 3.47 (1 H, s), 2.43 (3 H, s), 1.32 (3 H, t, J = 3.8 Hz). NMR of **12** ($CDCl_3$) δ 7.60-7.03 (5 H, m), 3.81 (2 H, q, J = 3.8 Hz), 3.64 (3 H, s), 3.35 (1 H, q, J = 3.7 Hz), 1.30 (3 H, d, J = 3.7 Hz), 1.14 (3 H, t, J = 3.8 Hz).

Registry No. **1a**, 6112-00-1; **1b**, 62285-47-6; **1c**, 76269-81-3; **1d**, 38118-70-6; **1e**, 76269-82-4; **1f**, 76269-83-5; **2**, 2679-13-2; **9**, 76269-84-6; **10**, 76269-85-7; **11**, 76269-86-8; **12**, 76269-87-9.

Antitumor Plants. 11.[1,2] Diterpenoid and Flavonoid Constituents of *Bromelia pinguin* L.

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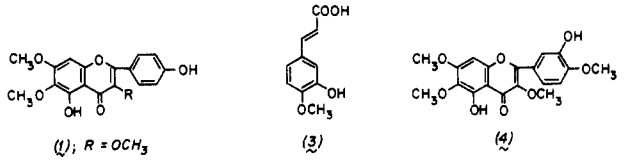
The roots and basal stems of *Bromelia pinguin* L. contain the flavonoids penduletin, cirsimaritin, and casticin, as well as isoferulic acid and three new diterpenoids. Two of these are the novel phyllocladane derivatives 3-oxophyllocladan-16ξ-ol and phyllocladan-16α,19-diol, and the third is 3-oxopimar-15-ene-7β,8β-diol. The major cytotoxic activity of the extracts of *B. pinguin* is probably due to the flavonoids.

The family Bromeliaceae is a mostly tropical group comprising some 45 genera and 1900 species, restricted to the New World except for one West African *Pitcairnia* species. The family has not been thoroughly investigated chemically, except for pineapple (*Ananas comosus*, Bromelioideae) and Spanish moss (*Tillandsia usneoides*, Tillandsioideae). We now report our investigation of the chemical constituents of the roots and basal stems of the pineapple relative *Bromelia pinguin* L. The fruits of this species, known as maya fruit, are a foodstuff throughout the Caribbean area, Mexico, and southward to Panama and Guiana. Aqueous methanolic extracts of the stems and leaves showed cytotoxicity in the KB assay[3] (ED_{50} 2 and 9 μg/mL; two tests) which prompted the present work.

Dried, ground roots and basal stems were extracted with methanol, and the extract was partitioned in the usual manner.[4] The aqueous fraction, which was inactive vs. KB, gave positive tests for tannins and reducing substances. It was not otherwise investigated. The methanolic fraction, which showed activity vs. KB at 2.1 μg/mL, contained flavonoids (Willstätter test) and polyphenolic substances. The polyphenolics were removed with lead(II) acetate,[5] and the fraction was separated into sodium bicarbonate soluble, sodium carbonate soluble, and nonacidic subfractions. The sodium bicarbonate soluble subfraction was inactive vs. KB and yielded no homogeneous characterizable compounds on chromatography, but the sodium carbonate soluble fraction, which showed activity vs. KB at 4.9 μg/mL, yielded the flavonoids penduletin (**1**) and



(1); R = OCH_3 (2); R = H (3) (4)

cirsimaritin (**2**), as well as a small amount of isoferulic acid (**3**). Taken together with our recent considerations of the cytotoxicity of flavonoid compounds[6] and in view of the fact that chromatography of the nonacidic subfraction yielded no materials with activity >15 μg/mL, we infer that the cytotoxicity of *B. pinguin* extracts arises mostly from the flavonoids.

The nonacidic fraction gave the flavonoid casticin (**4**). The occurrence of compounds **1**, **2**, and **4** in *B. pinguin* is of considerable chemotaxonomic interest in view of Williams' recent studies of leaf flavonoids of the Bromeliaceae.[7] The occurrence of 6-hydroxylated flavonoids in *B.*

(1) For part 10 in this series, see: Le Quesne, P. W.; Larrahondo, J. E.; Raffauf, R. F. *J. Nat. Prod.* **1980**, *43*, 353.

(2) The word "antitumor" as used in this title signifies no more than the fact that this plant was regarded by the National Cancer Institute as being of sufficient potential interest in this respect to warrant investigation.

(3) Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep.* **1972**, 3(2), 1.

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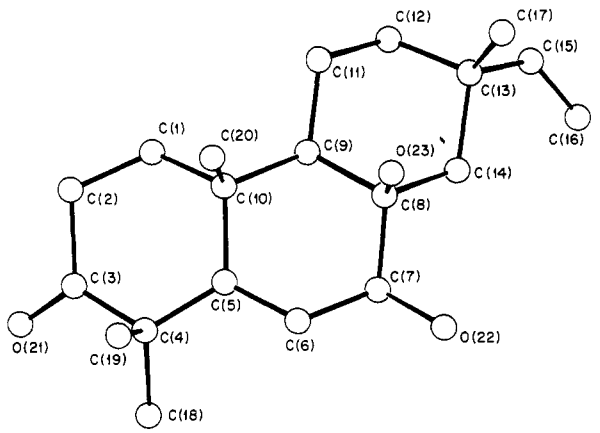
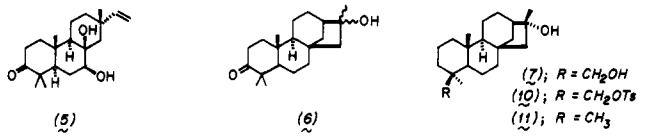


Figure 1. Computer-generated drawing of pimarene ketone diol **5**. Hydrogen atoms are omitted for clarity, and no absolute configuration is implied.

pinguin is much more in accord with the subfamily Tillandsioideae than with the Bromelioideae, although it should be noted that our plant material was comprised of roots and basal stems rather than leaves.

Gradient-elution column chromatography of the nonacidic subfraction of the methanol extract yielded, as well as casticin (**4**), mentioned above, three diterpenoids. The first of these ($C_{20}H_{32}O_3$, mp 155 °C, $[\alpha]_D$ −23°) could be seen from ultraviolet, infrared, and ^1H NMR spectra to contain a nonconjugated ketone group, a vinyl group, and secondary and tertiary alcohol functions. On this basis we assumed a tricyclic diterpenoid structure, and in view of the small quantities of material available, the structure was determined by crystallographic means. A computer-generated perspective drawing of the structure of this compound, **5**, is shown in Figure 1. The absolute configuration



shown in structure **5** and Figure 1 is an arbitrary choice, since the X-ray experiment did not define the absolute configuration. However, on the basis of indirect evidence discussed below, we favor this absolute configuration. The compound would thus be named 7β,8β-dihydroxypimar-15-en-3-one. The relative stereochemical designators are *R** (C(5)), *S** (C(7)), *R** (C(8)), *R** (C(9)), *S** (C(10)), and *S** (C(13)). The cyclohexane rings all have the chair conformation and are joined in a standard trans-anti-trans fashion. All bond lengths agree well with generally accepted values except for the exocyclic ethylidene group which is short. We attribute this to the large torsional displacement about the C(13)–C(15) bond. There appears to be a hydrogen bond between O(22)H and O(21) of 2.79 Å. No other abnormally short intermolecular contacts were noted.

The second diterpenoid, to which structure **6** is assigned, was obtained as a colorless powder: mp 173 °C; $[\alpha]_D$ +41°. The high-resolution mass spectrum of this compound indicated the formula $C_{20}H_{32}O_2$. The infrared spectrum showed absorptions at 3600 (OH) and 1700 cm^{-1} (C=O). In the ^1H NMR spectrum a multiplet at δ 2.42 (2 H) was assigned to methylene protons α to the carbonyl group. A methyl group on a hydroxyl-bearing carbon gave rise to a singlet at δ 1.34, and three other tertiary methyl groups

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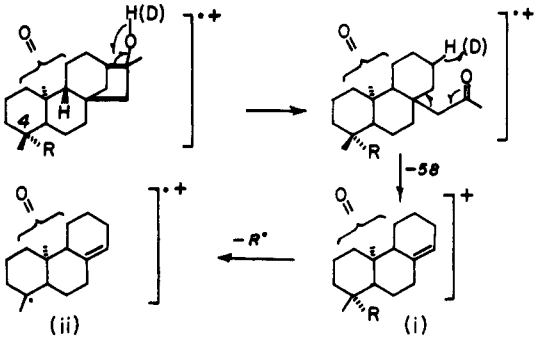
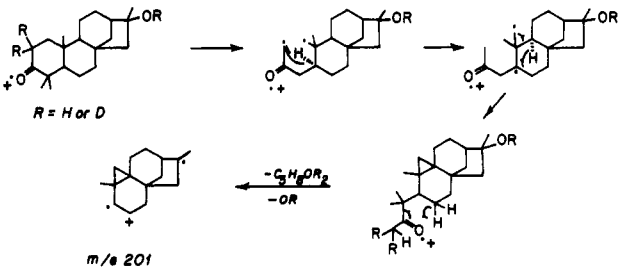
Scheme I. Fragmentation Pattern of Kauran-16-ol Derivatives



Scheme II. A-Ring Fragmentations of Compound 6



gave signals at δ 1.06, 1.02, and 0.98. Facile elimination of water and CH_3 from the molecular ion together with the presence of a characteristic (M^+ − 58) fragment suggested the presence of a structure resembling that of kauran-16-ol (Scheme I; cf. ref 8). The fact that the M − 58 peak at *m/e* 246 further loses 15 units to give a peak at *m/e* 231 suggests that this conversion may be represented by i → ii (R = CH_3) in Scheme I, with, however, a carbonyl group located in either ring A, B, or C.

Deuteration[9] of this compound gave mixed dideuterio and trideuterio derivatives $C_{20}H_{30}O_2D_2$ (*m/e* 306) and $C_{20}H_{29}O_2D_3$ (*m/e* 307). On electron impact these species gave a strong peak at *m/e* 248, in accord with the loss of C_3H_6O and C_3H_5OD from these ions (Scheme I) and retention of the other two deuterium atoms in tricyclic daughter ions i-d_2 and ii-d_2. Strong daughter ions at *m/e* 200 and *m/e* 201 were also seen. This can be interpreted in terms of the fragmentations outlined in Scheme II, which are analogous to those proposed for 4,4-dimethyl-5α-androstan-3-one.[10] These data strongly indicate that the carbonyl group is located at C-3. The stereochemistry proposed for **6** is discussed below.

The third diterpenoid obtained from the nonacidic subfraction was a crystalline compound: $C_{20}H_{34}O_2$; mp 203 °C; $[\alpha]_D$ +9.0°. The presence of a hydroxyl group could be inferred from the infrared spectrum (3320, 1050 cm^{-1}). The NMR spectrum gave signals (δ 3.57 and 3.41, each 1 H, d, J = 10 Hz) corresponding to the prochiral hydrogen atoms of a CH_2OH group attached to a chiral, quarternary carbon. In the mass spectrum, an intense peak for M^+ − CH_2OH at *m/e* 275.238 ($C_{19}H_{31}O$ requires *m/e* 275.237) indicated an axial hydroxymethyl group at C-4 in a tetracyclic diterpenoid (see ref 8 and Scheme I, i → ii). The mass spectrum in general ($M^+ - H_2O$, $M^+ - CH_2OH - H_2O$, $M^+ - C_3H_6O$) strongly supported a tetracyclic diterpenoid skeleton as in compound **6**, with an axial C-4 hydroxy-

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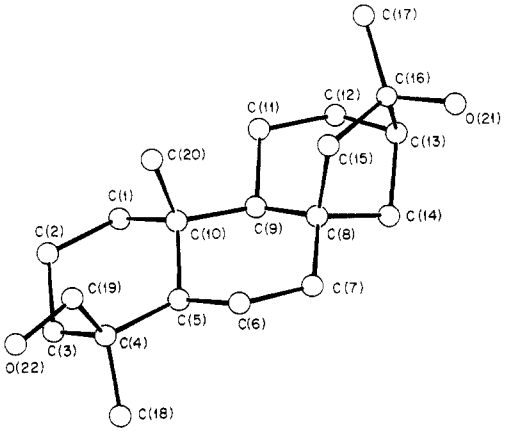
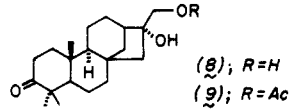


Figure 2. Computer-generated perspective drawing of phyllocladanediol (7). Hydrogen atoms are omitted for clarity, and no absolute configuration is implied.

methyl function and a tertiary OH group at C-16. Since the data available did not correspond with any known tetracyclic diterpenoid diol, the structure was determined by crystallographic means. The structure and probable absolute configuration 7 were assigned to this compound.

Figure 2 presents a computer-generated perspective drawing of the final X-ray model. This X-ray experiment, similarly to that outlined above, did not define the absolute configuration, but the enantiomer shown is favored for reasons to be discussed below. The relative stereochemical designators are *S** (C(4)), *R** (C(5)), *S** (C(8)), *S** (C(9)), *R** (C(10)), *R** (C(13)), and *R** (C(16)). The three cyclohexane rings are all in the chair conformation and are joined in a trans-anti-trans fashion. The cyclopentane ring is well described as having the envelope conformation with C(14) serving as the flap and 0.70 Å removed from the plane of the other four atoms. In general, the molecular parameters agree well with generally accepted values. There are no abnormally short intermolecular contacts in the unit cell. There is an infinite helix of hydrogen bonds along the screw axis along *Z* between O(21) and O(22). The hydrogen bond distances are 2.73 and 2.76 Å.

The occurrence of 7, a phyllocladane diol, in *B. pinguin* is of considerable interest for two reasons. First, no diterpenoids have, to our knowledge, been reported hitherto from the family Bromeliaceae; second, the natural occurrence of oxygenated phyllocladanes appears so far to be confined to calliterpenone (8) and its monoacetate 9,



(8); R=H
(9); R=Ac

constituents of *Callicarpa macrophylla* Vahl (Verbenaceae).[11] We attempted to define unequivocally the absolute configuration of 7 by conversion to the monotosylate **10** and reduction of this to a phyllocladan-16α-ol, since the alcohol of structure and absolute configuration 11 is well-known from structural and stereochemical studies in the kaurane and phyllocladane series.[12] In the event, attempted reduction of the tosylate **10** either with lithium aluminum hydride or lithium triethylborohydride[13] gave only traces of material corresponding by TLC to 11 and generally returned starting material. Undoubtedly severe 1,3-diaxial interactions suffered by the hydroxymethyl group in 7 dispose the tosylate **10** to S–O fission rather than C–O fission as the hydride reagents attack. Lack of material has prevented our pursuit of less direct routes for this conversion. It should be noted, however, that the $[\alpha]_D$ of our diol (+9.0°, in ethanol) is very similar to that reported for 11 (+16°, in chloroform). Since it is highly unlikely that the conversion 7 → 11 would alter the sign of rotation, we favor the absolute configuration indicated for 7, which is the same as for all other natural phyllocladane derivatives known to us. Compound 6 is assigned the phyllocladane structure and stereochemistry (except for C-16) shown by analogy with 7, and on biogenetic analogy the absolute configuration of 5 is suggested to be as shown.

1-Monoarachidin was obtained from more polar fractions of the nonacidic subfraction of the methanol-soluble fraction. In the petroleum ether soluble fraction, stearic, palmitic, and arachic acids were detected by mass spectrometry.

Oxygenated phyllocladanes are extremely rare in nature. The discovery of compounds 6 and 7, together with the probably related pimarene keto diol 5, is of considerable interest in this regard. The wide variety of physiological activities found among oxygenated kauranoids adds significance to oxygenated derivatives of phyllocladane; the scantily explored Bromeliaceae may be good potential sources of such compounds.

Experimental Section

General Methods. General experimental directions are given in ref 2.

A. Extraction and Preliminary Fractionation of *Bromelia pinguin* L. The dried, ground roots and basal stems (31 kg) were extracted to exhaustion at room temperature with methanol. The extract was concentrated under reduced pressure below 45 °C, and the precipitated material was filtered off (160 g). The filtrate was partitioned between chloroform (4 L) and methanol–water (1:9, 4 L). The aqueous layer was washed with 1 L of equilibrated lower phase. This aqueous fraction was concentrated to a small volume (rotary evaporator) and finally freeze-dried to give a brown solid (A, 372 g). The chloroform fraction was concentrated to a syrup and partitioned between petroleum ether (5 L) and methanol–water (9:1, 5 L). Concentration of these extracts gave, after freeze-drying, a methanolic fraction (125 g), and after removal of solvent under reduced pressure and air drying, a petroleum ether soluble fraction (18.4 g).

B. Constituents of Aqueous Fraction. Qualitative Tests. The aqueous–methanolic (9:1) residue (1 g) was dissolved in distilled water (10 mL) and boiled for 10 min. Positive gelatin and ferric chloride tests suggested the presence of tannins and a positive Fehling's test the presence of reducing substances.

C. Constituents of Methanolic Fraction. The methanol fraction showed in vitro antitumor activity in the KB assay (ED_{50} 2.1 μg/mL). The fraction (125 g) was dissolved in chloroform (2.5 L) and extracted successively with 5% sodium bicarbonate and 5% sodium carbonate solutions (2.5 L each). The neutral organic phase was then washed with distilled water and evaporated to a syrup (23.4 g).

(a) Constituents of Bicarbonate-Soluble Subfraction. The aqueous sodium bicarbonate extract was acidified with concentrated hydrochloric acid and back-extracted with ether (3 × 1 L). The ether phase was washed with distilled water, dried over sodium sulfate, filtered, and concentrated to a thick syrup (6.1 g; ED_{50} >100 μg/mL). Column chromatography of this fraction on silica gel 60 (Woelm, activity II or III, 250 g) yielded no homogeneous characterizable compounds among the oils obtained. This material was not investigated further.

(b) Constituents of Sodium Carbonate Soluble Subfraction. This fraction was acidified with concentrated HCl and back-extracted with ether as for part a above. The material (5.5 g) so obtained was dissolved in ethyl acetate–methanol, from which crystals were obtained. A second crystallization from ethyl acetate gave yellow crystals (170 mg) which showed two spots on TLC

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(13) Brown, H. C.; Krishnamurthy, S. *J. Org. Chem.* **1976**, *41*, 3064.

(hexane–ethyl acetate, 2:1). Purification by preparative TLC (Merck 0.25-mm plates, same system) gave a yellow crystalline compound from acetone–methanol which was identified as penduletin (4′,5-dihydroxy-3,6,7-trimethoxyflavone, **1**): 130 mg; mp 214 °C (lit.[14] mp 216–217 °C); positive Willstätter test;[15] UV (nm, log ε in parentheses) λ_{max} (MeOH) 233 sh (4.19), 258 sh (4.16), 272 (4.26), 340 (4.29); λ_{max} (MeOH–$AlCl_3$) 236 sh (4.19), 268 (4.16), 280 (4.18), 300 sh (4.05), 372 (4.31); λ_{max} (MeOH–$AlCl_3$–HCl) 245 sh (4.22), 276 sh (4.15), 282 (4.21), 303 sh (4.08), 365 (4.30); λ_{max} (MeOH–NaOMe) 237 sh (4.21), 257 sh (4.19), 272 (4.23), 296 (4.41); λ_{max} (MeOH–unfused NaOAc) 270 (4.26), 340 (4.24); λ_{max} (MeOH–NaOAc–H_3BO_3) 271 (4.31), 340 (4.30); IR (Nujol) 3160 (OH), 1645 (C=O), 1595, 1570, 1550 cm^{-1} (aromatic); NMR ($CDCl_3$) δ 3.86 (3 H, s), 3.92 (3 H, s), 3.94 (3 H, s) 6.49 (1 H, s) 6.90 (2 H, d, J = 9 Hz, part of AB system), 8.03 (2 H, d, J = 9 Hz, part of AB system);[16] mass spectrum, m/e (relative intensity) 344 (M^+, 100), 329 (M^+ – 15, 84), 314 (M^+ – OCH_3, 18), 301 (M^+ – CH_3C=O, 23).

The mother liquor, after the separation of penduletin, was evaporated to dryness under reduced pressure and air dried. This material (5.35 g) was chromatographed on silica gel 60 (activity II or III, 300 g; 4 cm × 120 cm column). Elution with toluene–ethyl acetate (4:1) gave more penduletin (30 mg). The second compound eluted was cirsimaritin (4′,5-dihydroxy-6,7-dimethoxyflavone, **2**): 98 mg; mp 258 °C (lit.[17] mp 262–263 °C); UV (nm, log ε in parentheses) λ_{max} (MeOH) 274 (4.40), 334 (4.49); λ_{max} (MeOH–$AlCl_3$) 264 sh (4.16), 286 (4.30), 330 (4.33), 364 (4.52); λ_{max} (MeOH–$AlCl_3$–HCl) 264 sh (4.21), 286 (4.36), 300 (4.38), 358 (4.51); λ_{max} (MeOH–$NaOCH_3$) 235 sh (4.32), 272 (4.27), 389 (4.59); λ_{max} (MeOH–unfused NaOAc) 273 (4.39), 333 (4.44); λ_{max} (MeOH–NaOAc–H_3BO_3) 273 (4.43), 333 (4.50); IR (Nujol) 3160 (OH), 1650 (C=O), 1600, 1570, 1500 cm^{-1} (aromatic); NMR ($CDCl_3$) δ 3.80 (3 H, s), 3.98 (3 H, s), 6.66 (1 H, s) 6.83 (1 H, s), 7.00 (2 H, d, J = 9 Hz, part of AB system), 7.92 (2 H, d, J = 9 Hz, part of AB system); mass spectrum, m/e (relative intensity) 314 (M^+, 100), 299 (M^+ – CH_3, 85), 271 (M^+ – CH_3C=O, 25).[16] The compound was identical with an authentic sample.

A third compound, eluted from the column with toluene–ethyl acetate (1:1), was identified as isoferulic acid (**3**): 2 mg; mp 228 °C (lit.[18] mp 228 °C); UV (nm, log ε in parentheses) 241 sh (4.40), 291 (4.43), 322 (4.44); IR (film) 3600–2500 (C(O)OH), 1670 (α,β-unsaturated C=O), 1630 (C=C), 1610, 1580, and 1510 cm^{-1} (aromatic); NMR ($CD_3C(O)CD_3$) δ 3.89 (3 H, s), 6.30 (1 H, d, J = 16 Hz), 7.02–7.16 (3 H, m), 7.56 (2 H, d, J = 16 Hz); mass spectrum, m/e (relative intensity) 194 (M^+, 100), 179 (M^+ – CH_3, 35); mixture melting point with authentic sample of isoferulic acid, 228 °C.

(c) Nonacidic Subfraction. This subfraction (23.4 g) was chromatographed on silica gel 60 (Woelm activity II or III, 1 kg; 4.5 cm × 150 cm column) by employing gradient elution (hexane, toluene, ethyl acetate, methanol). Toluene–ethyl acetate (9:1) yielded a fraction which on crystallization from acetone gave mixed crystals and powder (674 mg). The crystals were separated manually and recrystallized five times from acetone to yield the pure pimarenediol **5**: mp 155 °C; $[\alpha]^{23}_D$ –23° (c 0.7, EtOH); UV λ_{max} 262 (ε 254); IR (film) 3555, 3400 (OH), 1685 (C=O), 1630 (C=C), 910 cm^{-1} (CH=CH_2); NMR ($CDCl_3$) δ 5.86 (1 H, dd, J = 17, 10 Hz), 4.90 (2 H, complex dd, J = 17 Hz, 2 Hz), 3.37 (1 H, m), 2.49 (2 H, dd), 1.24 (3 H, s), 1.09 (6 H, s), 1.07 (3 H, s); high-resolution mass spectrum gave M^+ at m/e 320.237 (calcd for $C_{20}H_{32}O_3$ m/e 320.234) and M^+ – C_5H_{11} at m/e 249.149 (calcd for $C_{15}H_{21}O_3$, m/e 249.148).

Elution with toluene–ethyl acetate (8:1) gave greenish material (410 mg) which after repeated recrystallizations from acetone, ethyl acetate and acetone gave compound **6** as a colorless powder: 17 mg; mp 173 °C; $[\alpha]^{23}_D$ +41° (c 1.2 EtOH); IR (film) 3600 (OH), 1700 cm^{-1} (C=O); NMR ($CDCl_3$) δ 2.42 (2 H, m), 1.34, 1.06, 1.02, 0.98 (3 H each, 4 s); high-resolution mass spectrum, m/e 304.242 (M^+; calcd for $C_{20}H_{32}O_2$ m/e 304.239).

Deuterium exchange of the enolizable hydrogen atoms[9] was carried out by adding 5.5 mg of the compound to a solution of 5.7 mg of sodium in a mixture of MeOD (0.7 mL) and D_2O (0.7 mL) and refluxing the mixture for 1 h. After evaporation of solvents under reduced pressure and addition of D_2O (0.7 mL) the mixture was extracted with ether. A conventional workup of the product gave the deuterated derivative which on electron impact gave the molecular ion at m/e (relative intensity) 307 (28) (calcd for $C_{20}H_{29}D_3O_2$ m/e 307), M^+ – HOD at m/e 288 (40), $C_{20}H_{20}D_2O_2$–C_3H_6O at m/e 248 (38), and M^+ – $C_5H_8O_2D_3$ at m/e 201 (55).

Material from a later toluene–ethyl acetate fraction on recrystallization from acetone gave yellowish shining prisms along with a yellow powder. These substances were separated manually and the prisms recrystallized from acetone to give a pure compound, identified as casticin 4 (100 mg) on the basis of the following data: mp 186 °C (lit.[19] mp 186–187 °C); positive Willstätter test; UV (nm, log ε in parentheses) λ_{max} (MeOH) 257 (4.01), 267 sh (3.94), 347 (4.04); λ_{max} (MeOH–$AlCl_3$) 267 (4.05), 280 (4.01), 298 sh (3.82), 384 (4.07); λ_{max} (MeOH–$AlCl_3$–HCl) 267 (4.00), 280 (3.97), 297 sh (3.82), 375 (4.04); λ_{max} (MeOH–$NaOCH_3$) 233 sh (4.06), 268 (4.17), 335 (3.83), 375 (3.76); λ_{max} (MeOH–unfused NaOAc) 257 (4.00), 270 (3.93), 348 (4.03); λ_{max} (MeOH–NaOAc–H_3BO_3) 257 (4.03), 272 (3.98), 345 (4.02); IR (film) 3480 (OH), 1650 (C=O), 1600 (C=C), 1580, 1550, and 1510 cm^{-1} (aromatic); NMR ($CDCl_3$) δ 7.76–7.64 (2 H, m), 6.93 (1 H, d, J = 9 Hz), 6.48 (1 H, s), 5.75 and 12.57 (2 H, br s), 3.98, 3.94, 3.91, 3.87 (12 H, s) (ref 16); mass spectrum, m/e 374 (M^+).

This compound (20 mg) was treated with 0.5 mL of acetic anhydride and 1 drop of 70% perchloric acid at room temperature. After 0.5 h the reaction was worked up in the usual manner, and the product was crystallized from ethanol and then from ethyl acetate. Casticin diacetate appeared as colorless crystals: mp 179–180 °C (lit.[19] mp 178–179 °C); UV (nm, log ε in parentheses) λ_{max} (MeOH) 258 (4.45), 324 (4.59); no color with ferric chloride solution.

From an immediately following fraction, eluted with toluene–ethyl acetate (1:1), was obtained a crude compound (211 mg). Three recrystallizations from acetone–methanol gave compound **7** as colorless needles: mp 203 °C; $[\alpha]^{24}_D$ + 9.0° (c 1, EtOH); IR (KBr) 3320 and 1050 cm^{-1} (OH); NMR ($CDCl_3$) δ 3.57, 3.41 (each 1 H, d, J = 10 Hz), 1.32 (3 H, s), 0.95 and 0.85 (each 3 H, s); high-resolution mass spectrum, M^+ at m/e 306.254 (calcd for $C_{20}H_{34}O_2$ m/e 306.255); low-resolution mass spectrum, m/e (relative intensity) 306 (M^+, 8), 288 (M^+ – H_2O, 11), 275 (M^+ – CH_2OH, 73), 257 (M^+ – CH_2OH – H_2O, 100), 248 (M^+ – C_3H_6O, 49).

A later fraction, also eluted with toluene–ethyl acetate (1:1), gave colorless crystals of 1-monoarachidin from acetone: mp 80–80.5 °C; (lit.[20] mp 83.5–84 °C); IR (KBr) 3320 (OH), 1725 cm^{-1} (C=O); NMR ($CDCl_3$) δ 4.16 (2 H, m), 3.83–3.46 (3 H, m), 2.34 (2 H, m), 1.26 (34 H, br s); mass spectrum, m/e 386.339 (calcd for $C_{23}H_{46}O_4$ m/e 386.338).

D. Constituents of the Petroleum Ether Fraction. The crude fraction obtained by partition between 5% aqueous sodium carbonate solution and ether gave acidic (27%) and neutral material (73%).

Chromatography of the acidic material (3.3 g) on silica gel 60 (Woelm, activity II or III, 200 g; 3 cm × 60 cm column) yielded, by elution with hexane–ethyl acetate (3:1 and 1:1), a mixture of carboxylic acids. From the high-resolution mass spectrum, the mixture of acids was seen to contain mainly stearic, palmitic, and arachic acids.

E. X-ray Crystallographic Analysis of 5. A large, roughly cubic crystal of ketone diol **5** was chosen for a single-crystal X-ray diffraction analysis. Preliminary X-ray photographs revealed monoclinic symmetry, and accurate cell constants of a = 10.197 (3) Å, b = 16.960 (3) Å, c = 6.046 (1) Å, and β = 118.95 (2)° were determined from a least-squares fitting of 15 diffractometer-measured, moderate, 2θ values. The presence of chirality and

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systematic extinctions ($0k0$, absent if $k = 2n + 1$) were uniquely accommodated by space group $P2_1$. A calculated density indicated one molecule of composition $C_{20}H_{32}O_3$ formed the asymmetric unit.

All unique diffraction maxima with $2\theta \leq 114°$ were surveyed on a computer-controlled, four-circle diffractometer by using graphite-monochromated Cu Kα radiation (1.541 78 Å) and a variable-speed, 1° ω scan technique. Periodically monitored check reflections showed no significant crystal decomposition. Of the 1285 reflections surveyed in this manner, 1153 (90%) were considered observed ($|F_o| \geq 3\sigma(F_o)$) after correction for Lorentz, polarization, and background effects.

A phasing model was achieved by using a standard multisolution tangent formula approach, and an E synthesis revealed a plausibly connected 15-atom fragment.[21] The remainder of the nonhydrogen atoms were located on an F synthesis phased by these atoms. Block-diagonal, least-squares refinement followed by a ΔF synthesis revealed the hydrogen atoms which were assigned fixed isotropic temperature factors (4.1 Å^{-2}). Full-matrix, least-squares refinements with anisotropic thermal parameters for the nonhydrogen atoms have currently converged to a standard, unweighted, crystallographic residual of 0.045 for the observed data. Further crystallographic details can be found in the supplementary material described in the paragraph at the end of this paper.

(ii) X-ray Crystallographic Analysis of 7. A clear rectangular parallelepiped of phyllocladane diol **7** with dimensions 0.7 × 0.2 × 0.2 mm was chosen for single-crystal X-ray diffraction analysis. Preliminary X-ray photographs indicated orthorhombic symmetry, and accurate lattice parameters of $a = 17.582$ (4), $b = 14.606$ (4), and $c = 7.025$ (1) Å were determined by a least-squares fit of 15 moderate 2θ values. The systematic extinctions ($h00$, h = $2n + 1$; $0k0$, $k = 2n + 1$; $00l$, $l = 2n + 1$) and presence of chirality were uniquely accommodated by the choice of $P2_12_12_1$ as a space group. A calculated density was also consistent with one molecule of $C_{20}H_{34}O_2$ in the asymmetric unit.

All unique diffraction maxima with $2\theta \leq 60°$ were collected on a computer-controlled, four-circle diffractometer by using graphite-monochromated Mo Kα radiation (0.710 69 Å) and a variable-speed, 1° ω scan technique. Of the 2729 reflections surveyed in this manner, 2325 (85%) were considered observed ($|F_o| \geq 3\sigma(F_o)$) after correction for Lorentz, polarization, and background effects. No crystal decomposition was observed in periodical monitoring of check reflections.

The structure was easily solved by using a multisolution, weighted tangent formula approach for phase determination.[21] An E synthesis calculated from the set of phases with the most favorable figures of merit revealed the entire nonhydrogen framework except the C(18) methyl group. The structure was routinely completed by Fourier methods and all the hydrogen atoms were located in a difference Fourier synthesis calculated from a partially refined ($R \approx 0.10$) set of phases. Full-matrix, least-squares refinements with anisotropic thermal parameters for the nonhydrogen atoms and isotropic thermal parameters for the hydrogen atoms have converged to a standard crystallographic residual of 0.045 for the observed data (0.060 weighted residual). Further crystallographic details can be found in the supplementary material described in the paragraph at the end of this paper.

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Registry No. 1, 569-80-2; 2, 6601-62-3; 3, 537-73-5; 4, 479-91-4; 4 diacetate, 76215-22-0; 5, 76215-21-9; 6, 76215-23-1; 7, 76248-59-4; 1-monoarachidin, 50906-68-8.

Supplementary Material Available: Tables of fractional coordinates and thermal parameters (Tables I and IV), bond distances (Tables II and V), and bond angles (Tables III and VI) for compounds 5 and 7, respectively (8 pages). Ordering information is given on any current mast head page.

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Diterpenes from the Sponge *Dysidea amblia*

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The marine sponge *Dysidea amblia* contained two major metabolites, ambliol-A (8) and ambliol-B (18), and three minor metabolites, ambliofuran (15), ambliolide (13), and dehydroambliol-A (12). The diterpenes are the first to be isolated from a *Dysidea* species. Examination of individual animals indicated that some contained ambliol-A (8) while others contained ambliol-B (18), although the individuals could not be distinguished by means of classical taxonomy.

An unusually diverse array of secondary metabolites has been isolated from *Dysidea* species. Various samples of *Dysidea herbacea* contained brominated diphenyl esters,[1] chlorinated metabolites such as dysidin (1),[2] dysidenin (2),[3] isodysidenin[4] and the dioxopiperazine derivative 3,[5] and some unusual sesquiterpenes.[6] An Australian *Dysidea*

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