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PLANT ANTICANCER AGENTS, XLVI. CYTOTOXIC CASBANE-TYPE CONSTITUENTS OF *AGROSTISTACHYS HOOKERI*[1]

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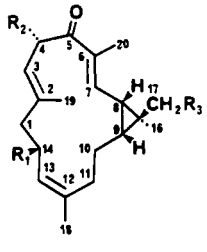
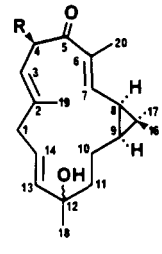
ABSTRACT.—In addition to the previously characterized agrostistachin [**1**], three additional cytotoxic macrocyclic casbane-type diterpenes were isolated from a $CHCl_3$ extract of *Agrostistachys hookeri*, by bioactivity-guided fractionation using the P-388 lymphocytic leukemia cell culture system. These novel compounds, 14-dehydroagrostistachin [**2**], 17-hydroxyagrostistachin [**3**], and agroskerin [**4**], were identified by spectral data comparison to agrostistachin and demonstrated ED_{50} values of 2.8, 0.8, and 1.4 μg/ml, respectively, with cultured P-388 cells.

Agrostistachys hookeri Benth. & Hook. f. (Euphorbiaceae) is part of a genus of eight or nine species distributed from India and Sri Lanka to west Malaysia (2). This species is indigenous to Sri Lanka, especially moist regions below 1000 feet in altitude, where it is found as a small tree (3). Our study on *A. hookeri* was initiated when, as part of a continuing search for plant-derived antineoplastic agents, it was found that a $CHCl_3$ extract of the twigs of this species displayed significant activity against the P-388 lymphocytic leukemia cell culture system, when tested using standard protocols (4). In a preliminary investigation, the novel casbane derivative, agrostistachin [**1**], was found to be a cytotoxic constituent of *A. hookeri* twigs, and the structure of this compound was proposed by the application of one- and two-dimensional high-field nmr techniques and confirmed by single-crystal X-ray crystallography (1). In the present communication, we wish to report the isolation and characterization of three further cytotoxic plant constituents of *A. hookeri*, namely, 14-dehydroagrostistachin [**2**], 17-hydroxyagrostistachin [**3**], and agroskerin [**4**], which have been identified on the basis of spectral data comparison with compound **1**. Prior to our work on this species, no previous phytochemical or biological studies had been performed on any species in the genus *Agrostistachys*.



1 $R_1=R_2=OH$, $R_3=H$
2 $R_1=O$, $R_2=OH$, $R_3=H$
5 $R_1=O$, $R_2=OAc$, $R_3=H$
3 $R_1=R_2=R_3=OH$
6 $R_1=R_2=R_3=OAc$



4 R=OH
7 R=OAc

RESULTS AND DISCUSSION

The molecular formula of **2** was determined as $C_{20}H_{28}O_3$ by hrms. Analysis of the uv, ir, ^{1}H-nmr, ^{13}C-nmr, and ms data of **2** suggested it was a closely related diterpenoid to agrostistachin [**1**]. Thus, when the ^{1}H-nmr spectrum of **2** was compared to

[1]For the previous paper in this series, see Choi *et al.* (1).

that of **1**, there was a downfield shift (from δ 5.11 to δ 5.88) and simplification of a signal (from a doublet to a singlet) that was assignable to the 13-H proton, along with the appearance of an AB quartet centered at δ 3.18 and 2.85, assignable to the 1-H protons. Furthermore, in the ^{13}C-nmr spectrum of **2** there was the appearance of a second carbonyl carbon (δ 197.81), in addition to the C-5 carbonyl apparent in the analogous spectrum of **1**. These preliminary observations suggested that the 14-hydroxyl group of compound **1** was missing in compound **2** and that a keto group at C-14 was present in the latter compound. Unambiguous assignments of the ^{1}H- and ^{13}C-nmr spectra of **2** were obtained after the performance of a ^{1}H-^{13}C heteronuclear chemical shift correlated nmr experiment. Chemical shifts of the quaternary carbons of this new isolate were definitively assigned by use of the selective INEPT nmr technique, which also provided confirmation of the structure proposed for **2**. When the 11-H signal of **2** was irradiated at δ 3.42 (J=6 Hz), C-12 was enhanced (Figure 1b), along with other carbons two or three bonds distant from the irradiation site, namely, C-13 (δ 124.24), C-10 (δ 25.98), and C-18 (δ 27.12). Analogous irradiation of 1-H (δ 3.18) of **2** selectively enhanced carbons C-2, C-3, and C-14 (Figure 1c), as well as carbon C-19 (δ 16.61). In addition, irradiation of 8-H (δ 1.57) resulted in enhancement of C-6 (Figure 1d), and carbons C-15 (δ 26.88), C-16 (δ 15.84), and C-17 (δ 29.28). On acetylation of **1** under normal conditions, the monoacetate **5** was produced. It may be pointed out that the ^{1}H- and ^{13}C-

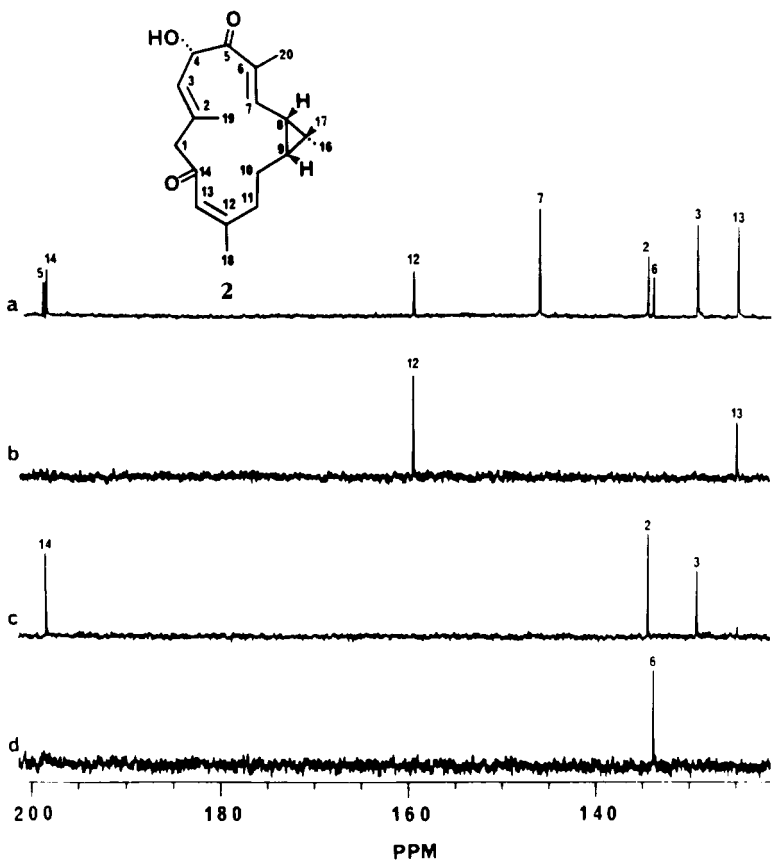


FIGURE 1. Downfield region of the ^{13}C-nmr spectrum of 14-dehydroagrostistachin [**2**]. (a) Proton-noise decoupled spectrum; (b–d) SINEPT spectra obtained by irradiation of 11-H (δ 3.42), 1-H (δ 3.18), and 8-H (δ 1.57), respectively.

nmr spectra of **5** indicated that the compound exists in solution in two favorable conformers (5).

The structure of **3** ($C_{20}H_{30}O_4$) was assigned as 17-hydroxyagrostistachin on the basis of spectral comparison with the parent compound, agrostistachin [**1**]. Thus, in the ^{1}H-nmr and ^{13}C-nmr spectra of **3**, there was a disappearance of the C-17 methyl group signal of **1** (^{1}H nmr, δ 1.14; ^{13}C nmr, δ 28.99), and the appearance of a methylene peak (δ 3.48, 71.57, respectively), which clearly indicated the presence of a C-17 hydroxymethyl group in **3**. On acetylation, compound **3** afforded the triacetate **6**. Hydroxylation at the C-17 rather than the C-16 position was confirmed by the diagnostic shifts of 8-H and 9-H (which have been shown to possess the same stereochemistry as 17-CH_2 in compound **3**) in the ^{1}H- and ^{13}C-nmr spectra of compound **3**.

The final diterpenoid obtained in this investigation, compound **4**, was shown to be structurally related to compounds **1–3** on the basis of its spectral parameters. While the available evidence suggested that this compound possessed two hydroxy groups, one of these appeared to be tertiary, owing to the formation of only a monoacetate [**7**], under

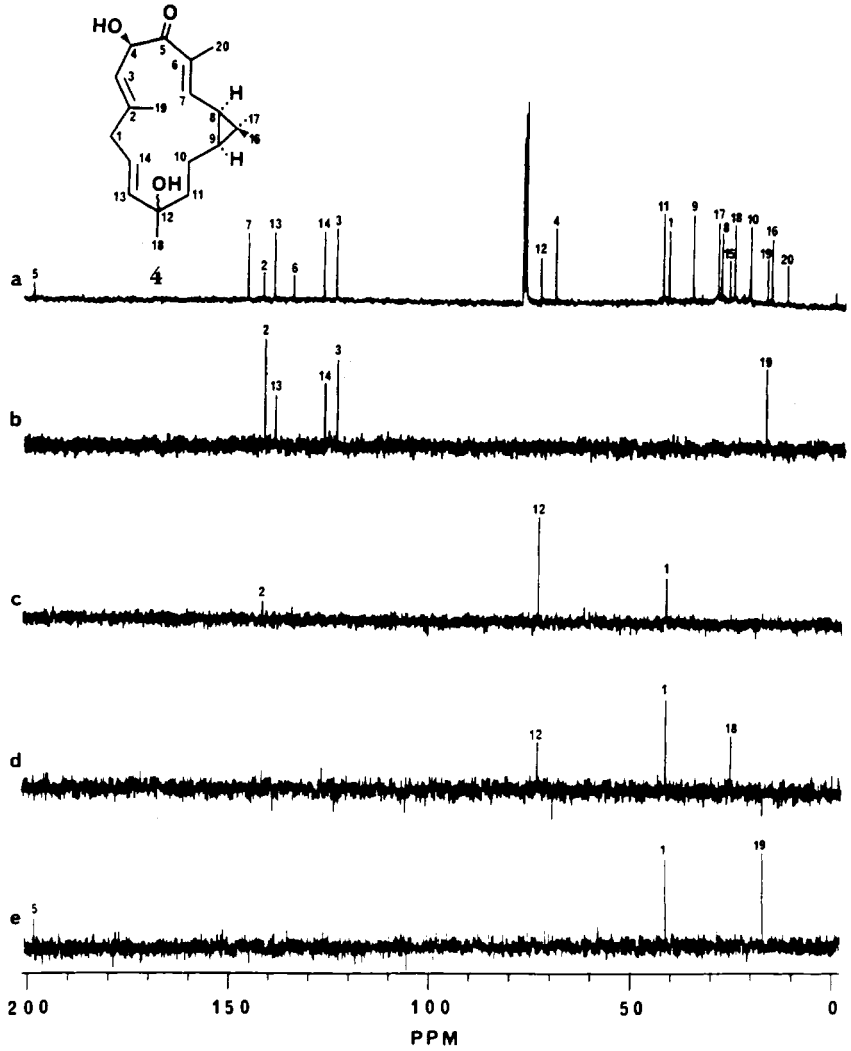


FIGURE 2. ^{13}C-Nmr spectrum of agroskerin [**4**]. (a) Proton-noise decoupled spectrum; (b–e) SINEPT spectra obtained by irradiation of 1-H (δ 2.59), 14-H (δ 5.70), 13-H (δ 5.40), and 3-H (δ 5.08), respectively.

mild conditions of acetylation. Compound **4** was isomeric with agrostistachin [**1**] ($C_{20}H_{30}O_3$), and preliminary observations from its ^{1}H- and ^{13}C-nmr spectra indicated that the double bond at position 12,13 in the parent compound had been translocated to position 13,14 in **4**, and that the C-14 hydroxy group of **1** had migrated to C-12. These structural modifications in **4** were further investigated using the selective INEPT nmr technique. Irradiation at the 1-H position ($J=6$ Hz) resulted in selective enhancements of carbons C-2, C-3, C-13, C-14, and C-19 (Figure 2b), which clearly showed the proximity of C-1 to the olefinic carbons C-13 and C-14 and strongly supported the placement of a double bond at the 13,14 position in **4** instead of the 12,13 position. This proposition was substantiated by observing the long-range coupling between 13-H and 1-H in the ^{1}H-^{1}H COSY spectrum of **4**. Furthermore, irradiation at 14-H enhanced C-1 and C-12 (Figure 2c), whereas irradiation at 13-H selectively intensified carbons C-12, C-1, and C-18 (Figure 2d). A correlation between resonances due to C-1 and C-3 was also clearly demonstrated by irradiation of 3-H with $^3J_{CH}$ coupling constant ($J=8$ Hz) (Figure 2e). The geometry of the double bond at the 13,14 position was determined as *trans* by observing the coupling constant at δ 5.40 ($J=16$ Hz). Finally, the C-18 methyl group signal appeared at δ 1.29, and the C-12 signal resonated at δ 72.63 in the ^{1}H-nmr and ^{13}C-nmr spectra of **4**, respectively, thereby suggesting that the 18-methyl group was attached to an oxygen-substituted quaternary carbon instead of a double bond found in compounds **1–3**. Attempts to establish the relative stereochemistry of **4** at C-12 by additional nmr techniques were hampered by the flexible nature of the 14-membered ring system containing this carbon site and by the quaternary nature of C-12. Furthermore, efforts to prepare a suitable crystal for X-ray crystallography to resolve this situation were unsuccessful. Compound **4** has been accorded the trivial name agroskerin and is the $\Delta^{13,14}$-14-deoxy-12,13-dihydro-12-hydroxy derivative of agrostistachin [**1**].

Casbane-type diterpenes are rare in the plant kingdom and have only been found so far in two other plants in the family Euphorbiaceae, namely, *Ricinus communis* L. (6,7) and *Croton nitens* Sw. (8,9). Compounds **1–3** are the first examples of 12,13-*cis*-casbene-type compounds to have been discovered, while agroskerin [**4**] is the first known oxygenated derivative of a (−)-casbane derivative thus far. The unusual oxidation pattern of agrostistachin [**1**], relative to the biogenetically related phorbol esters (10), has been commented on previously (1).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Kofler hot-stage instrument and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. The uv spectra were obtained on a Beckman DU-7 spectrometer and ir spectra measured on a Nicolet MX-1 Ft-ir (AgCl or KBr) interferometer. ^{1}H- and ^{13}C-nmr spectra were recorded in $CDCl_3$, using TMS as internal standard, employing either Nicolet NT-360 or Varian XL-300 instruments. Low resolution mass spectra were obtained with a Varian MAT 112S instrument operating at either 70 or 20 eV. Droplet counter-current chromatography (dccc) was performed on a Model-A instrument (Tokyo Rikakikai, Tokyo, Japan).

PLANT MATERIAL.—The twigs of *A. bookeri* were collected in Sri Lanka in July 1979, by staff members of the Economic Botany Laboratory, Agriculture Research Service, BARC-East, USDA, Beltsville, Maryland, and specimens documenting these collections are deposited in the Herbarium of the National Arboretum, Washington, DC.

EXTRACTION AND FRACTIONATION.—The air-dried, milled plant material (18.9 kg) was defatted with petroleum ether (bp 60-80°). The marc was extracted with MeOH which yielded 639.2 g of a dried MeOH extract on removal of solvent in vacuo. A portion of the MeOH extract (470.2 g) was partitioned between H_2O (3 liters) and $CHCl_3$ (500 ml × 12). The dried $CHCl_3$ extract (50.4 g) was found to exhibit activity against cultured P-388 cells (4) with an ED_{50} of 6.0 μg/ml. A portion of the $CHCl_3$ extract (50.1 g) was dissolved in $CHCl_3$-MeOH (10:1) solution (100 ml) and impregnated into Si gel (50 g) and dried

completely under a N_2 stream. The impregnated $CHCl_3$ extract was subjected to column chromatography over Si gel (2 kg, 230–400 mesh; J.T. Baker Chemical Co., Phillipsburg, New Jersey) using as eluents $CHCl_3$, and $CHCl_3$/EtOAc and $CHCl_3$/MeOH mixtures of increasing polarity. A total of 215 fractions (700 ml each) was collected. Fractions showing similar tlc profiles were pooled to give 10 combined fractions, which were tested for cytotoxicity using cultured P-388 cells in order to guide further fractionation.

ISOLATION AND CHARACTERIZATION OF AGROSTISTACHIN [**1**].—Fractions 68–84 (1.3 g), eluted from the column with $CHCl_3$-EtOAc (20:3), exhibited an ED_{50} of 2.3 μg/ml in the P-388 cell-culture system. An amorphous precipitate was produced when this fraction was taken up into $CHCl_3$ and was removed by filtration. An initial quantity of agrostistachin [**1**] (150 mg) was obtained when the mother liquor (300 mg) was repeatedly recrystallized from EtOAc. Further quantities of compound **1** were obtained from column chromatographic fractions 55–67 [1.1 g; eluted with $CHCl_3$-EtOAc (20:3); P-388, ED_{50}, 2.3 μg/ml], on removal of the precipitate obtained with $CHCl_3$ (0.8 g), and subsequent low pressure cc of the mother liquor (0.3 g). The solute was dissolved in $CHCl_3$-MeOH (1:1, 5 ml) and fractionated on a C-8 phase-bonded low-pressure column (Lobar®, Size A; Merck, Darmstadt, W. Germany) using mixtures of H_2O/MeOH/MeCN of decreasing polarity as eluent. Pure agrostistachin [**1**] (50 mg) was obtained in fractions eluted by H_2O-MeOH-MeCN (5:4:2), and was recrystallized as colorless prisms from EtOAc. Compound **1** was obtained in a total yield of 0.00145% w/w (200 mg), and its characterization by uv, ir, ^1H nmr, ^{13}C nmr, ms, and X-ray crystallography has been published by us previously (1).

ISOLATION OF 14-DEHYDROAGROSTISTACHIN [**2**].—Combined fraction 8–23 (9.2) from the initial chromatography of the $CHCl_3$ extract, which were eluted with $CHCl_3$-EtOAc (10:1), showed activity against the P-388 cell-culture test system (ED_{50}, 4.2 μg/ml). After removal of a $CHCl_3$-insoluble amorphous precipitate (8.0 g) by filtration, the mother liquor (1.2 g) was chromatographed over Si gel (100 g) by gradient elution. The column was eluted with *n*-hexane, *n*-hexane/EtOAc, and *n*-hexane/EtOAc/Et_2O mixtures of increasing polarity. Fractions which were obtained by elution with *n*-hexane-EtOAc-Et_2O (10:1:1) were combined and recrystallized from Et_2O to afford pure 14-dehydroagrostistachin [**2**] (20 mg, 0.00014% w/w).

ISOLATION OF17-HYDROXYAGROSTISTACHIN [**3**].—Combined fractions 85–116 (5.1 g) from the initial $CHCl_3$ extract, which were eluted with $CHCl_3$-EtOAc (10:2), showed activity against the P-388 cell culture test system (ED_{50}, <1.0 μg/ml). After removal of a $CHCl_3$-insoluble amorphous precipitate (4.1 g) by filtration, the mother liquor (1.0 g) was fractionated by dccc using an equilibrated mixture of cyclohexane-Et_2O-iPrOH-EtOH-H_2O (7:16:6:10:8), with the upper phase employed as mobile phase. The solute (0.5 g) was dissolved in 3.5 ml mobile phase and, on the addition of 3.5 ml of stationary phase, was introduced into a 7-ml sample chamber. Ascending development was employed at a pressure of 2–4 kg/cm^2. Fractions (300 drops each) were collected using an automatic fraction collector. This procedure was repeated once. Dccc fractions exhibiting similar tlc profiles were combined. Fractions 85–110 from the first separation and equivalent fractions in a second dccc run were combined to yield 0.3 g of solute (ED_{50}, 1.2 μg/ml) that was further fractionated by C-8 phase-bonded Si gel low pressure cc (Lobar®, size A; Merck), using H_2O-MeOH-MeCN (6:3:1) as elution solvent. Fractions (300 drops each) were collected using an automatic fraction collector. Fractions 35–70 were combined to give pure 17-hydroxyagrostistachin [**3**] (20 mg, 0.00014% w/w).

ISOLATION OF AGROSKERIN [**4**].—Combined fractions 24–39 (6.7 g) from the initial $CHCl_3$ extract, which were eluted with $CHCl_3$-EtOAc (10:1), showed activity against the P-388 cell-culture test system (ED_{50}, 3.0 μg/ml). After removal of a $CHCl_3$-insoluble amorphous precipitate (4.2 g) by filtration, a portion (0.5 g) of the mother liquor (2.5 g) was chromatographed by C-8 phase-bonded Si gel low pressure cc (Lobar®, size A; Merck) using H_2O-MeOH-MeCN (6:2:1) as elution solvent. Fractions (600 drops each) were collected using an automatic fraction collector. Fractions 85–115 were combined to yield pure agroskerin [**4**] (40 mg, 0.00145% w/w).

CHARACTERIZATION OF 14-DEHYDROAGROSTISTACHIN [**2**].—This isolate exhibited the following data: colorless needle-shaped crystals from Et_2O; mp 132–134°; $[\alpha]D$ +170.0° (*c* 0.13, $CHCl_3$); uv (EtOH) λ max (log ε) 251.5 (4.59), 274.0 (sh, 4.43) nm; ir ν max (KBr) 3436 (OH), 1682 (α,β unsaturated C=O), 1667 (α,β unsaturated C=O), 1642 (C=C), 1622 (C=C), 1615 (C=C), 1266, 1025 cm^{-1}; ^1H nmr (360 MHz, $CDCl_3$) δ 6.19 (1H, d, $J_{7,8}$=11 Hz, 7-H), 5.88 (1H, s, 13-H), 5.28 (1H, d, $J_{3,4}$=10 Hz, 3-H), 5.21 (1H, m, 4-H), 4.30 (1H, d, exchangeable with D_2O, 4-OH), 3.42 (1H, m, 11-H), 3.18 (1H, d, J_{AB}=10 Hz, 1-H), 2.85 (1H, d, J_{AB}=10 Hz, 1-H), 2.11 (1H, m, 10-H), 1.94 (1H, m, 11-H), 1.93 (3H, d, $J_{7,20}$=1.1 Hz, 20-CH_3), 1.91 (3H, d, $J_{3,19}$=1.1 Hz, 19-CH_3), 1.88 (3H, d, $J_{13,18}$=1.2 Hz, 18-CH_3), 1.57 (1H, dd, $J_{7,8}$=11 Hz, $J_{8,9}$=8 Hz, 8-H), 1.18 (3H, s, 17-CH_3), 1.15 (1H, m, 9-H), 0.99 (3H, s, 16-CH_3), 0.91 (1H, m, 10-H); ^{13}C nmr (90.8 MHz, $CDCl_3$) δ 198.19 (s, 5-C), 197.81 (s, 14-C), 158.75 (s, 12-C), 145.29 (d, 7-C), 133.80 (s, 2-C), 133.28 (s, 6-C), 128.58 (d, 3-C), 124.24 (d,

12-C), 69.10 (d, 4-C), 55.56 (t, 1-C), 35.88 (d, 9-C), 32.64 (t, 11-C), 29.28 (d, q, 8-C, 17-C), 27.12 (q, 18-C), 26.88 (s, 15-C), 25.98 (t, 10-C), 16.61 (q, 19-C), 15.84 (q, 16-C), 11.81 (q, 20-C); eims (70 eV) *m/z* $[M]^+$ 316 (4%), 283 (2), 255 (2), 233 (4), 217 (5), 203 (8), 189 (15), 179 (13), 161 (22), 135 (32), 108 (72), 95 (100), 82 (39); hrms, mass measurement, *m/z* $[M]^+$ 316.2043 (calcd for $C_{20}H_{28}O_3$, 316.2039).

ACETYLATION OF COMPOUND **2**.—Compound **2** (5 mg) was acetylated in Ac_2O-pyridine (0.5 ml each) at room temperature for 4 h. Work-up in the usual manner afforded 14-dehydroagrostistachin-4-monoacetate [**5**] (4 mg). The purity of compound **5** was examined with three different solvent systems on tlc, $CHCl_3$-EtOAc (20:1), *n*-hexane-EtOAc (4:1), *n*-hexane-Et_2O-EtOAc (4:1:1), R_f values, 0.56, 0.20, 0.45, respectively. Compound **5** exhibited the following data: resin; $[\alpha]_D + 198.0°$ (*c* 0.07, $CHCl_3$); uv (EtOH) λ max (log ε) 246.5 (4.30), 273.5 (sh, 4.15) nm; ir ν max (AgCl) 1738 (C=O), 1677 (α,β unsaturated C=O), 1620 (C=C), 1235, 1026 cm^{-1}; ^1H nmr (360 MHz, $CDCl_3$) δ 6.34, 6.09 (1H, d, $J_{7,8}$=10 Hz, 7-H), 6.25, 6.15 (1H, d, $J_{3,4}$=11 Hz, 4-H), 6.11, 5.87 (1H, s, 13-H), 5.42 (1H, m, 3-H), 3.45 (1H, m, 11-H), 3.21, 3.12 (1H, d, J_{AB}=10 Hz, 1-H), 2.95, 2.88 (1H, d, J_{AB}=10 Hz, 1-H), 2.16, 2.14 (3H, s, -OAc), 2.11 (1H, m, 10-H), 2.08, 1.88 (3H, s, 18-CH_3), 1.89 (1H, m, 11-H), 1.96, 1.88 (3H, s, 20-CH_3), 1.88, 1.73 (3H, s, 19-CH_3), 1.61, 1.54 (1H, dd, $J_{7,8}$=11 Hz, $J_{8,9}$=8 Hz, 8-H), 1.24, 1.12 (1H, m, 9-H), 1.19, 1.18 (3H, s, 17-CH_3), 1.12, 1.02 (3H, s, 16-CH_3), 0.90 (1H, m, 10-H); ^{13}C nmr (90.8 MHz, $CDCl_3$) δ 198.40, 193.88 (s, 5-C), 197.26, 192.67 (s, 14-C), 170.49, 170.30 (s, -$OCOCH_3$), 159.79, 159.31 (s, 12-C), 142.91, 141.44 (d, 7-C), 141.10, 137.66 (s, 2-C), 136.59, 134.41 (s, 6-C), 123.89, 123.36 (d, 3-C), 123.61, 122.38 (d, 13-C), 72.14, 70.90 (d, 4-C), 56.01, 55.62 (t, 1-C), 35.48, 34.74 (d, 9-C), 41.11, 32.69 (t, 11-C), 29.25, 29.10, 29.01 (d, q, 8, 17-C), 28.05, 27.34 (q, 18-C), 26.56, 26.32 (s, 15-C), 27.45, 26.11 (t, 10-C), 20.88, 20.79 (q, -$COCH_3$), 17.11, 16.94 (q, 19-C), 15.86, 15.76 (q, 16-C), 12.13, 11.67 (q, 20-C); eims (70 eV) *m/z* $[M]^+$ 358 (5%), 315 (5), 298 (15), 255 (4), 217 (6), 189 (18), 175 (11), 161 (34), 148 (45), 136 (28), 108 (71), 95 (66), 43 (100).

CHARACTERIZATION OF 17-HYDROXYAGROSTISTACHIN [**3**].—Compound **3** exhibited the following data: resin; $[\alpha]_D + 167°$ (*c* 0.09, $CHCl_3$); uv (EtOH) λ max (log ε) 271.0 (4.01) nm; ir ν max (AgCl) 3400 (OH), 1665 (α,β-unsaturated C=O), 1644 (C=C), 1625 (C=C), 1381, 1268, 1021 cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ 6.32 (1H, d, $J_{7,8}$=11 Hz, 7-H), 5.29 (1H, d, $J_{3,4}$=9 Hz, 4-H), 5.19 (2H, m, 3-H, 13-H), 4.27 (1H, m, 14-H), 3.48 (2H, s, 17-H_2), 2.53 (1H, dd, J_{gem}=12 Hz, $J_{1,14}$=2.9 Hz, 1-H), 2.35 (1H, m, 11-H), 2.10 (2H, m, 10-H, 1-H), 1.94 (3H, s, 20-CH_3), 1.87 (1H, m, 11-H), 1.84 (3H, d, $J_{3,19}$=2.7 Hz, 19-CH_3), 1.78 (3H, br s, 18-CH_3), 1.73 (1H, dd, $J_{7,8}$=11 Hz, $J_{8,9}$=8 Hz, 8-H), 1.31 (1H, m, 9-H), 1.09 (3H, s, 16-CH_3), 0.95 (1H, m, 10-H); ^{13}C nmr (90.8 MHz, $CDCl_3$) δ 199.22 (s, 5-C), 143.20 (d, 7-C), 138.06 (s, 2-C), 137.79 (s, 12-C), 134.48 (s, 6-C), 128.74 (d, 3-C), 127.26 (d, 13-C), 71.57 (t, 17-C), 70.52 (d, 14-C), 68.58 (d, 4-C), 47.63 (t, 1-C), 32.40 (s, 15-C), 31.82 (t, 11-C), 30.17 (d, 9-C), 23.17 (d, 8-C), 22.93 (q, 18-C), 22.75 (t, 10-C), 17.97 (q, 19-C, 16-C), 11.78 (q, 20-C); eims (70 eV) *m/z* $[M-H_2O]^+$ 316 (5%), 187 (15), 173 (19), 149 (31), 145 (52), 119 (29), 107 (69), 43 (100); hrms, mass measurement $[M-H_2O]^+$ *m/z* 316.2031 (calcd for $C_{20}H_{28}O_3$, 316.2039).

ACETYLATION OF COMPOUND **3**.—Compound **3** (5 mg) was acetylated in Ac_2O-pyridine (0.5 ml each) at room temperature overnight. Work-up in the usual manner afforded 17-hydroxyagrostistachin-4,14,17-triacetate [**6**] (4 mg). Compound **6** exhibited the following data: resin; $[\alpha]_D + 106°$ (*c* 0.15, $CHCl_3$); uv (EtOH) λ max (log ε) 266.5 (3.99) nm; ir ν max (AgCl) 1737 (C=O), 1682 (α,β-unsaturated C=O), 1628 (C=C), 1371, 1236, 1026 cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ 6.23 (1H, d, $J_{7,8}$=10 Hz, 7-H), 6.19 (1H, d, $J_{3,4}$=10 Hz, 4-H), 5.42 (1H, d, $J_{3,4}$=10 Hz, 3-H), 5.30 (1H, m, 14-H), 5.10 (1H, d, $J_{13,14}$=10 Hz, 13-H), 4.01, 3.83 (2H, dd, J_{AB}=12 Hz, 17-H_2), 2.51 (1H, m, 1-H), 2.47 (1H, m, 11-H), 2.19 (1H, m, 1-H), 2.15 (3H, s, -OAc), 2.10 (1H, m, 10-H), 2.08 (3H, s, -OAc), 2.00 (3H, s, -OAc), 1.91 (1H, m, 11-H), 1.90 (3H, br s, 20-CH_3), 1.79 (3H, m, 19-CH_3), 1.77 (3H, br s, 18-CH_3), 1.75 (1H, m, 8-H), 1.28 (1H, m, 9-H), 1.10 (3H, s, 16-CH_3), 0.92 (1H, m, 10-H); ^{13}C nmr (90.8 MHz, $CDCl_3$) δ 193.25 (s, 5-C), 171.01 (s, -$OCOCH_3$), 170.35 (s, -$OCOCH_3$), 169.83 (s, -$OCOCH_3$), 141.16 (s, 2-C), 140.17 (s, 12-C), 140.06 (d, 7-C), 136.50 (s, 6-C), 124.25 (d, 3-C), 123.24 (d, 13-C), 73.08 (t, 17-C), 72.43 (d, 14-C), 71.54 (d, 4-C), 44.36 (t, 1-C), 31.60 (t, 11-C), 29.84 (d, 9-C), 28.94 (s, 15-C), 22.35 (d, 8-C), 22.80 (q, 18-C), 22.47 (t, 10-C), 21.30 (q, -$OCOCH_3$), 20.85 (q, -$OCOCH_3$), 20.75 (q, -$OCOCH_3$), 18.67 (q, 19-C), 12.19 (q, 16-C), 11.62 (q, 20-C); eims (20 eV) *m/z* $[M]^+$ 460 (2%), 400 $[M-OAc]^+$ 400 (3), $[M-2\times OAc]^+$ (3), 298 (6), 281 (5), $[M-3\times OAc]^+$ 280 (2), 265 (7), 257 (1), 216 (10), 189 (1), 161 (17), 133 (44), 95 (38), 43 (100).

CHARACTERIZATION OF AGROSKERIN [**4**].—Compound **4** exhibited the following data: resin; $[\alpha]_D - 54°$ (*c* 0.1, $CHCl_3$); uv (EtOH) λ max (log ε) 271.0 (3.76) nm; ir ν max (AgCl) 3430 (OH), 1649 (α,β-unsaturated C=O), 1620 (C=C), 1267, 1029 cm^{-1}; ^1H nmr (360 MHz, $CDCl_3$) δ 6.28 (1H, d, $J_{7,8}$=11 Hz, 7-H), 5.70 (1H, m, 14-H), 5.40 (1H, d, $J_{13,14}$=16 Hz, 13-H), 5.19 (1H, d, $J_{3,4}$=10 Hz,

4-H), 5.08 (1H, d, $J_{3,4}$=10 Hz, 3-H), 2.71 (1H, dd, J_{gem}=13 Hz, $J_{1,14}$=7 Hz, 1-H), 2.59 (1H, dd, J_{gem}=13 Hz, $J_{1,14}$=7 Hz, 1-H), 1.92 (3H, s, 20-CH_3), 1.87 (3H, br s, 19-CH_3), 1.60 (1H, m, 11-H), 1.52 (1H, dd, $J_{7,8}$=11 Hz, $J_{8,9}$=9 Hz, 8-H), 1.43 (1H, m, 10-H), 1.37 (1H, m, 11-H), 1.29 (3H, s, 18-CH_3), 1.15 (3H, s, 17-CH_3), 1.12 (1H, m, 9-H), 1.07 (3H, s, 16-CH_3), 0.90 (1H, m, 10-H); ^{13}C nmr (90.8 MHz, $CDCl_3$) δ 198.58 (s, 5-C), 145.40 (d, 7-C), 141.40 (s, 2-C), 138.78 (d, 13-C), 133.80 (s, 6-C), 126.21 (d, 14-C), 123.21 (d, 3-C), 72.63 (s, 12-C), 69.05 (d, 4-C), 42.39 (t, 11-C), 41.04 (t, 1-C), 35.06 (d, 9-C), 28.77 (q, 17-C), 27.90 (d, 8-C), 25.97 (s, 15-C), 24.64 (q, 18-C), 20.84 (t, 10-C), 16.63 (q, 19-C), 15.61 (q, 16-C), 11.69 (q, 20-C); eims (70 eV) *m/z* $[M-H_2O]^+$ 300 (2%), 257 (9), 175 (14), 149 (25), 109 (53), 81 (37), 43 (100); hrms, mass measurement, *m/z* 300.2090 $[M-H_2O]^+$ (calcd for $C_{20}H_{28}O_2$, 300.2090).

ACETYLATION OF COMPOUND [**4**].—Compound **4** (5 mg) was acetylated in Ac_2O-pyridine (0.5 ml each) at room temperature overnight. Work-up in the usual manner afforded agroskerin 4-monoacetate [**7**] (4 mg). Compound **7** exhibited the following data: resin; $[\alpha]_D$ −86° (*c* 0.13, $CHCl_3$); uv (EtOH) λ max (log ε) 271.5 (4.32) nm; ir ν max (AgCl) 3480 (OH), 1740 (C=O), 1675 (α,β-unsaturated C=O), 1621 (C=C), 1375, 1241, 1024 cm^{-1}; ^{1}H nmr (360 MHz, $CDCl_3$) δ 6.19 (1H, d, $J_{3,4}$=10 Hz, 4-H), 6.16 (1H, d, $J_{7,8}$=10 Hz, 7-H), 5.71 (1H, m, 14-H), 5.43 (1H, d, $J_{13,14}$=16 Hz, 13-H), 5.23 (1H, d, $J_{3,4}$=10 Hz, 3-H), 2.75 (1H, dd, J_{gem}=13 Hz, $J_{1,14}$=7 Hz, 1-H), 2.62 (1H, dd, J_{gem}=13 Hz, $J_{1,14}$=7 Hz, 1-H), 2.16 (3H, s, -OAc), 1.88 (3H, d, $J_{7,20}$=0.9 Hz, 20-CH_3), 1.84 (3H, d, $J_{3,19}$=1.2 Hz, 19-CH_3), 1.60 (1H, m, 11-H), 1.49 (1H, m, 8-H), 1.40 (2H, m, 10-H, 11-H), 1.29 (3H, s, 18-CH_3), 1.14 (3H, s, 17-CH_3), 1.10 (1H, m, 9-H), 1.09 (3H, s, 16-CH_3), 0.85 (1H, m, 10-H); ^{13}C nmr (75.4 MHz, $CDCl_3$) δ 193.51 (s, 5-C), 170.55 (s, -$OCOCH_3$), 145.18 (s, 2-C), 142.87 (d, 7-C), 139.53 (d, 13-C), 135.54 (s, 6-C), 126.04 (d, 14-C), 118.80 (d, 3-C), 72.92 (s, 12-C), 72.59 (d, 4-C), 42.75 (t, 11-C), 41.00 (t, 1-C), 34.57 (d, 9-C), 28.96 (q, 17-C), 27.78 (d, 8-C), 25.36 (s, 15-C), 24.85 (q, 18-C), 21.21 (t, 10-C), 20.87 (q, -$OCOCH_3$), 17.31 (q, 19-C), 15.80 (q, 16-C), 11.79 (q, 20-C); eims (70 eV) *m/z* $[M-OAc]^+$ 300 (17%), 285 (12), 257 (23), 239 (12), 207 (11), 177 (28), 151 (35), 123 (100).

CYTOTOXIC ACTIVITY.—The isolates were evaluated for cytotoxic activity against P-388 lymphocytic leukemia cell culture system (4). Compounds **1** to **4** were found to exhibit ED_{50} values of 1.4, 2.8, 0.8, and 1.4 μg/ml, respectively. An isolate is considered active in this system if it shows an ED_{50} of ≤4.0 μg/ml (11).

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LITERATURE CITED

1. Y.-H. Choi, J. Kim, J.M. Pezzuto, A.D. Kinghorn, N.R. Farnsworth, H. Lotter, and H. Wagner, *Tetrahedron Lett.*, **27**, 5795 (1986).
2. J.C. Willis, in: "A Dictionary of the Flowering Plants and Ferns," 8th ed., revised by H.K. Airy-Shaw, Cambridge University Press, Cambridge, UK, 1973, p. 34.
3. H. Trimen and J.D. Hooker, in: "A Hand-book to the Flora of Ceylon," Part IV, M/S. Bishen Singh Mahendra Pal Singh and M/S. Periodical Experts, Delhi, India, 1974, pp. 55–56.
4. M. Arisawa, J.M. Pezzuto, C. Bevelle, and G.A. Cordell, *J. Nat. Prod.*, **47**, 453 (1984).
5. M. Ōki, "Applications of Dynamic NMR Spectroscopy to Organic Chemistry," VCH, Deerfield Beach, FL, 1985, pp. 1–40.
6. D.R. Robinson and C.A. West, *Biochemistry*, **9**, 70 (1970).
7. D.R. Robinson and C.A. West, *Biochemistry*, **9**, 80 (1970).
8. B.A. Burke, B.A. Chan, K.O. Pascoe, J.F. Blount, and P.S. Manchard, *J. Chem. Soc., Perkin Trans. 1*, 2666 (1981).
9. M.A. Commisiong and K. Pascoe, *Tetrahedron Lett.*, **25**, 711 (1984).
10. W. Adolf and E. Hecker, *Isr. J. Chem.*, **16**, 75 (1977).
11. R.I. Geran, N.H. Greenberg, M.M. MacDonald, A.M. Schumacher, and B.J. Abbott, *Cancer Chemother. Rep.*, **3** (3), 1 (1972).

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