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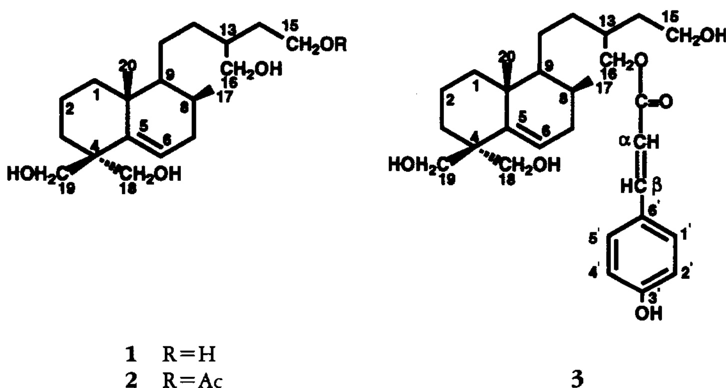
CYTOTOXIC CONSTITUENTS OF *BACCHARIS GAUDICHAUDIANA*

FEKADU FULLAS,[1] RAOUF A. HUSSAIN,[2] HEE-BYUNG CHAI, JOHN M. PEZZUTO, DOEL D. SOEJARTO, and A. DOUGLAS KINGHORN*

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

ABSTRACT.—Three new labdane diterpenes, gaudichaudols A–C [**1–3**], a new clerodane diterpenoid, gaudichaudone [**4**], and the known clerodane, articulin acetate [**5**] have been isolated from the aerial parts of *Baccharis gaudichaudiana*, together with the known compounds, apigenin, hispidulin, spathulenol, and ursolic acid. Through the application of 1D- and 2D nmr spectroscopy, the structures of the new diterpenoids [**1–4**] were, in turn, elucidated as 15,16,18,19-tetrahydroxylabd-5-ene, 15-*O*-acetyl-16,18,19-trihydroxylabd-5-ene, 16-*O*-*p*-*trans*-coumaroyl-15,18,19-trihydroxylabd-5-ene, and 2β-hydroxy-15,16-epoxycleroda-1(10),15,16-trien-18,19-olide. The isolated compounds were evaluated in P-388 lymphocytic leukemia cells as well as a battery of human cancer cell lines. Among the diterpenoids, gaudichaudol C [**3**], gaudichaudone [**4**], and articulin acetate [**5**] exhibited significant cytotoxic activity against certain cancer cells.

Baccharis gaudichaudiana DC. (Compositae) is a shrub that grows in regions of Argentina, Brazil, and Paraguay. It is used as an antidiabetic remedy in Paraguay, where it is known by the local name "chilca melosa." Our interest in this plant commenced when we observed that it tasted sweet-bitter. In earlier reports, we have described the isolation of six new labdane-type diterpene glycosides (gaudichaudiosides A–F) from the aerial parts of this plant (1,2). Gaudichaudioside A was isolated as a prototype member of a new type of potently sweet natural product, whereas gaudichaudiosides B-F exhibited a wide range of taste characteristics ranging from sweet-bitter, entirely bitter to neutral (1,2). As a continuation of our study of the bioactive diterpenoids and other constituents of the aerial parts of *B. gaudichaudiana*, we have isolated three new labdanes [**1–3**], a new clerodane [**4**], the known clerodane, articulin acetate [**5**], and the known compounds, apigenin, hispidulin, spathulenol, and ursolic acid from the cytotoxic EtOAc-soluble extract (P-388, ED_{50} 15.6 μg/ml). This report describes the structure elucidation and cytotoxic activity of compounds **1–4**.

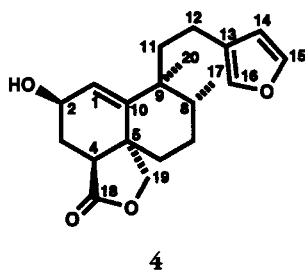


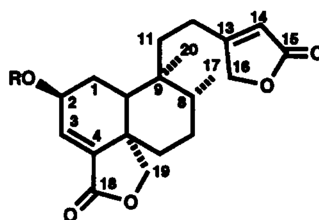
1 R=H
2 R=Ac

3

[1]Current Address: Research Triangle Institute, P.O. Box 12194, Research Triangle Park, North Carolina 27709.

[2]Current Address: Bristol-Myers Squibb Company, Pharmaceutical Research Institute, Wallingford, Connecticut 06492.





RESULTS AND DISCUSSION

The molecular formula of gaudichaudol A [**1**] was determined as $C_{20}H_{36}O_4$ from its hrfabms (*m/z* 339.254 $[M-H]^-$). The eims fragment at *m/z* 303 $[M-2H_2O-H]^+$ was suggestive of the presence of two hydroxyl groups in the side-chain of **1**. In the ir spectrum, absorptions occurred at 3377 cm^{-1} (OH) and 1675 cm^{-1} (double bond). The ^{1}H-nmr spectrum (Table 1) showed resonances at δ 0.77 and 0.83 ppm, attributable to unfunctionalized methyl groups at C-10 and C-8, respectively. Two primary alcohol groups were positioned in the side-chain of **1** as indicated by the resonances at δ 3.47 (H_2-16) and 3.59 (H_2-15). In the downfield region an olefinic signal occurred at δ 5.69 (H-6). In the ^{1}H-^{1}H COSY nmr spectrum, two mutually coupled two-proton signals were observed at δ 3.78 and 4.13 (H_2-18), and δ 3.57 and 3.96 (H_2-19). The ^{1}H- and ^{13}C-nmr assignments of these hydroxymethyl groups were supported by comparison with reported values of similarly substituted labdanes (3,4).

The ^{13}C-nmr spectrum of **1** (Table 2) displayed 20 carbon signals (2 CH_3-, 7 CH_2-, 3CH-, 2 quaternary carbons, 4 CH_2OH and 1 double bond). A ^{1}H-^{13}C HETCOR nmr experiment allowed ^{13}C-nmr assignments of the protonated carbons, C-6 (δ 128.8), C-15 (δ 61.1), C-16 (δ 65.9), C-17 (δ 16.3), C-18 (δ 64.5), and C-20 (δ 19.6). The ^{13}C-nmr resonances at δ 128.8 (C-6) and 146.4 (C-5) were ascribed to a Δ^5 double bond, with the placement of the double bond at this position being made after analysis of ^{13}C-nmr data of decalin model compounds (5). Complete ^{13}C-nmr assignments of **1** were achieved by

TABLE 1. ^{1}H-Nmr Chemical Shifts (δ, ppm) of Gaudichaudols A–C (**1–3**) (CD_3OD, 300 MHz).[a]

Position	Compound		
	1	**2**	**3**
H-6	5.69 t (3.3)	5.70 t (3.4)	5.67 t (3.4)
H-15	3.59 t (5.8)	4.11 t (5.9)	3.63 t (5.8)
H-16	3.47 d (4.5)	3.64 m	4.12 d (5.6)
H-17	0.83 d (6.5)	0.84 d (6.5)	0.82 d (6.1)
H-18a	4.13 d (11.8)	4.12 d (11.7)	4.13 d (11.3)
H-18b	3.78 d (11.8)	3.78 d (11.7)	3.78 d (11.3)
H-19a	3.96 d (10.5)	3.95 d (10.7)	3.94 d (10.3)
H-19b	3.57 d (10.5)	3.57 d (10.7)	3.55 d (10.3)
H-20	0.77 s	0.77 s	0.77 s
CH_3CO-15	—	2.01 s	—
H-α	—	—	6.36 d (16.1)
H-β	—	—	7.60 d (16.1)
H-1′,5′	—	—	7.43 d (7.9)
H-2′,4′	—	—	6.80 d (7.9)

[a] *J* (in Hz) in parentheses.

TABLE 2. ^{13}C-Nmr Chemical Shifts (δ, ppm) of Compounds **1–5**.[a]

Position	Compound				
	1	**2**	**3**	**4**	**5**
1	36.6	36.6	36.6	128.4	24.7
2	18.4	18.5	18.4	68.2	66.4
3	32.1	32.2	32.2	29.8	128.2
4	44.0	44.1	44.1	39.2	143.9
5	146.4	146.4	146.2	39.9	45.4
6	128.8	128.8	128.8	30.1	33.9
7	27.4	27.4	27.4	28.2	27.4
8	37.5	37.5	37.6	37.6	36.4
9	47.5	47.5	47.6	40.3	37.8
10	39.8	39.8	39.8	153.8	40.3
11	25.0	24.7	24.9	41.1	34.6
12	28.1	28.1	28.0	19.1	21.4
13	39.6	39.5	35.7	126.9	169.6
14	35.4	31.2	35.2	111.9	115.3
15	61.1	64.0	60.7	143.8	173.5
16	65.9	65.6	67.5	139.6	72.8
17	16.3	16.3	16.3	16.1	15.5
18	64.5	64.5	64.5	175.2	168.2
19	65.7	65.8	65.7	69.9	70.5
20	19.6	19.5	16.5	16.8	17.5
CH_3CO	—	20.3	—	—	20.9
CH_3CO	—	172.3	—	—	169.6
CO	—	—	167.0	—	—
αC	—	—	115.0	—	—
βC	—	—	146.3	—	—
1′,5′	—	—	131.0	—	—
2′,4′	—	—	116.8	—	—
3′	—	—	161.2	—	—
6′	—	—	127.0	—	—

[a]Measured at 75.4 MHz in $CDCl_3$ for **1–4** and in CD_3OD for **5** (δ TMS=0).

the selective INEPT technique (6), used in particular to ascertain the position of the double bond. Thus, soft irradiation of H-18a at δ 4.13 ($^3J_{CH}$=4 Hz) led to enhancements of C-5 (δ 146.4) and C-19 (δ 65.7). Analogous irradiation of H-6 at δ 5.69 ($^3J_{CH}$=7 Hz) enhanced C-4 (δ 44.0). The relative stereochemistry of the methyl groups at C-8 and C-10 was determined from a 2D NOESY spectrum, which showed cross-peaks between H-19a and H_3-20. In a 1D nOe nmr experiment, irradiation of H-19a at δ 3.96 gave an nOe for both the H_3-17 (δ 0.83) and H_3-20 (δ 0.77) signals. However, the stereochemistry at neither C-9 nor C-13 could be determined with certainty. The structure of **1** was therefore established as the novel 15,16,18,19-tetrahydroxylabd-5-ene.

Compound **2** differed from **1** in having an acetate group at C-15, as was readily observed from its ^{1}H- and ^{13}C-nmr spectral data (Tables 1 and 2). The ir absorption band at 1725 cm^{-1}, and the ^{1}H-nmr signal at δ 2.01 coupled with the ^{13}C-nmr resonances at δ 20.3 and 172.3 confirmed the presence of an acetate group. Accordingly, in the ^{13}C-nmr spectrum of **2** relative to **1**, C-15 (δ 64.0) was deshielded (−2.9 ppm), while C-14 (δ 31.2) was shielded (+4.2 ppm), which are in close agreement with reported values (7). Therefore, the structure of gaudichaudol B [**2**] was elucidated as 15-*O*-acetyl-16,18,19-trihydroxylabd-5-ene.

The molecular formula of gaudichaudol C [**3**] was determined as $C_{29}H_{42}O_6$ from its fabms (*m/z* 485 [M−H]$^-$). In the ir spectrum, absorptions occurred at 3296 (OH), 1685

(α,β-unsaturated ester), 1637 (double bond) and 1605 and 1438 cm^{-1} (aromatic unit). Inspection of the ^{1}H- and ^{13}C-nmr spectral data of **3** (Tables 1 and 2) indicated that **3** bears an acyl moiety, which was identified as *p-trans*-coumaroyl by comparison of its ^{1}H- and ^{13}C-nmr chemical shifts with those reported in the literature (8). In the ^{1}H-nmr spectrum of **3**, relative to **1**, H_2-16 (δ 4.12) was deshielded by 0.65 ppm, while in the ^{13}C-nmr spectrum C-16 (δ 67.5) was shielded by 1.6 ppm and C-13 (δ 35.7) was shielded by 3.9 ppm. This observation proved that C-16 was the site of acylation. The structure of **3** was thus assigned as 16-*O*-*p*-*trans*-coumaroyl-15,18,19-trihydroxylabd-5-ene.

Gaudichaudone [**4**] was isolated as a colorless gum. Its molecular formula was determined as $C_{20}H_{26}O_4$ by hreims (*m/z* 330.1835 $[M]^+$). A characteristic fragment peak at *m/z* 95 was indicative of an ethyl-furan side-chain (9). In the ir spectrum, diagnostic absorption bands occurred at 887 and 1537 cm^{-1} (furan ring) and 1713 cm^{-1} (lactone). The presence of a β-substituted furan moiety was confirmed by the ^{1}H-nmr resonance pattern at δ 6.23 (H-14), δ 7.22 (H-16), and δ 7.35 (H-15), similar to other furan-ring bearing diterpenoids (9). In the ^{1}H-^{1}H COSY nmr spectrum of **4**, H-14 (δ 6.23) showed a strong vicinal coupling to H-15 (δ 7.35) and a weaker long-range coupling to H-16 (δ 7.22). The ^{13}C-nmr spectrum (Table 2) was consistent with a diterpenoid 20-carbon count (2 CH_3-, 6 CH_2-, 7 CH-, and 5 quaternary carbons). The absence of a $\Delta^{3(4)}$ double bond was clearly indicated by the ^{13}C-nmr chemical shift of C-18 (δ 175.2) as compared with about δ 169 reported for C-18 in the $\alpha\beta$-unsaturated [$\Delta^{3(4)}$] γ-lactone portion of similar clerodanes (10). Further evidence of the position of the double bond was derived from a selective INEPT experiment, wherein irradiation of H-1 at δ 6.44 ($^3J_{CH}$=8 Hz) enhanced C-2 (δ 68.2) and C-5 (δ 39.9). Therefore, the double bond was placed at $\Delta^{1(10)}$ rather than at $\Delta^{3(4)}$.

The stereochemistry at C-2, C-5, C-8, and C-9 was determined by nOe nmr experiments. Thus, nOe interactions between H-19a and H_3-20, H-2 and H_3-20, H_3-17 and H_3-20 indicated that H-2, H_3-17, H_3-20, and the C-19 oxymethylene are all in the α-orientation. The absence of a downfield chemical shift for H-4 in the ^{1}H-nmr spectrum of **4** indicated that this proton is in an opposite orientation to the C-2 hydroxyl group, so H-4 must be in the α-position. The structure of **4** was therefore assigned as 2β-hydroxy-15,16-epoxycleroda-1(10),15,16-trien-18,19-olide.

The known clerodane diterpene, articulin acetate [**5**] was isolated as a colorless crystalline substance. It was identified by comparison of its physical and spectroscopic data (mp, ir, ms, and ^{1}H-nmr) with reported values (11). Comparison of ^{13}C-nmr data reported for deoxyarticulin (12), coupled with extensive selective INEPT and ^{1}H-^{13}C HETCOR experiments permitted a complete ^{13}C-nmr assignment of **5** (Table 2). Compound **5** was converted to the corresponding alcohol, articulin [**6**], which was reported as a natural product from a closely related species, *Baccharis articulata* (11). The other known compounds obtained in the present investigation, apigenin (13), hispidulin (14), spathulenol (15,16), and ursolic acid (17) were identified by comparison of their physical and spectroscopic data with the corresponding literature values.

The diterpenoids **1–5**, the flavonoids, apigenin and hispidulin, and the sesquiterpene, spathulenol, were evaluated for cytotoxic potential against a number of cell lines, including seven human cancer cell lines (BC-1 breast cancer, Col-2 colon cancer, HT-1080 fibrosarcoma, KB oral epidermoid carcinoma, KB-V1 multi-drug resistant KB, Lu-1 lung cancer, and Mel-2 melanoma) and P-388 murine lymphocytic leukemia. As shown in Table 3, of the gaudichaudol series [**1–3**], only gaudichaudol C [**3**] demonstrated significant activity against P-388 (ED_{50} 2.4 μg/ml). Interestingly, the compound was inactive against KB cells but active against multi-drug resistant KB-V1 cells (ED_{50} 4.7 μg/ml). Gaudichaudone [**4**] was found to be inactive with all the epithelial cell

TABLE 3. In Vitro Cytotoxic Activity of Isolates from *B. gaudichaudiana*.

Cell Line[a]	Compound Tested[b] (ED_{50} μg/ml)					
	3	**4**	**5**	Apigenin	Hispidulin	Spathulenol
BC-1	19.0	>20	>20	>20	19.0	>20
Col-2	19.3	>20	>20	19.3	>20	>20
HT-1080 ..	12.4	>20	>20	12.2	5.2	>20
KB	>20	>20	>20	>20	5.6	>20
KB-V1	4.7	>20	>20	16.8	2.8	>20
Lu-1	13.1	>20	>20	14.0	17.6	>20
Mel-2	11.2	>20	>20	13.6	11.5	6.3
P-388	2.4	11.7	1.7	12.2	0.8	>20

[a]BC-1=human breast cancer, Col-2=human colon cancer, HT-1080=human fibrosarcoma, KB=human oral epidermoid carcinoma, KB-V1=vinblastine resistant KB, Lu-1=human lung cancer, Mel-2=human melanoma, P-388=murine lymphoid neoplasm.

[b]Compounds **1** and **2** showed ED_{50} values of >20 mg/ml with all cell lines.

types tested and weakly active only against P-388 cells (ED_{50} 11.7 μg/ml). A similar pattern was noted with articulin acetate [**5**], although greater activity was observed with P-388 cells (ED_{50} 1.7 μg/ml). Apigenin, hispidulin and spathulenol were weakly to moderately active against certain cell lines, as summarized in Table 3. Ursolic acid has previously been shown to exhibit broad cytotoxic activity (17), and consequently was not evaluated against the cancer cell line panel in this investigation.

The accumulation of the additional labdane diterpenoids [**1**–**3**] in *B. gaudichaudiana* is of chemotaxonomic interest. Although *B. gaudichaudiana* was once treated as a variety of the closely related species, *B. articulata* Pers., labdane diterpenoids have never been detected in the latter plant (18). The present study therefore provides further phytochemical evidence for treating these two taxa as separate species.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Kofler hot-stage instrument and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Uv spectra were obtained on a Beckman DU-7 spectrophotometer and ir spectra were measured with a Nicolet MX-1 Ft-ir interferometer. ^{1}H-Nmr spectra were obtained on a Varian XL-300 instrument operating at 300 MHz. ^{13}C-Nmr spectra were recorded with a Varian XL-300 (75.4 MHz) or a Nicolet NT-360 (90.8 MHz) spectrometer. Lrms and hrms were obtained on a Varian MAT-112S (ei) or a Finnigan MAT-90 spectrometer (negative fab).

PLANT MATERIAL.—The aerial parts of *B. gaudichaudiana* were collected near Pedro Juan Caballero, Amambay Province, Paraguay in August 1987. A voucher specimen (Soejarto *et al.* 6071) representing this collection has been deposited in the John G. Searle Herbarium, Field Museum of Natural History, Chicago, Illinois.

EXTRACTION AND ISOLATION.—The air-dried aerial parts of *B. gaudichaudiana* were extracted with 80% MeOH to afford a residue (380 g) that was suspended in MeOH-H_2O (1:1) and successively partitioned with petroleum ether, EtOAc, and 1-BuOH, as described previously (2). The EtOAc extract (60 g) was found to possess cytotoxic activity against P-388 lymphocytic leukemia cells. This extract was therefore chromatographed over a Si gel column, which was eluted with a $CHCl_3$/MeOH gradient to afford 13 combined fractions (F1–F13). Purification of fraction F8 by Si gel flash cc [MeOH-$CHCl_3$ (3:22)] yielded gaudichaudol A [**1**] (370 mg), while the same procedure applied to F6 gave two major fractions, F6-II and F6-III. Fraction F6-II on further flash cc [MeOH-EtOAc (1:12)] led to the isolation of gaudichaudol C [**3**] (200 mg). Gaudichaudol B [**2**] (30 mg) was purified by prep. tlc of fraction F6-III in MeOH-EtOAc (1:12). Pure gaudichaudone [**4**] (60 mg) was obtained by Si gel flash cc of F-9, followed by prep. tlc in MeOH-$CHCl_3$ (3:7). Articulin acetate [**5**] (182 mg) was crystallized from a solution of fraction F2 in MeOH. Compound **5** (40 mg) was treated with methanolic HCl at room temperature for 8 h. The resulting product

was purified by prep. tlc to afford articulin [6] (20 mg). Ursolic acid (30 mg) was obtained as white crystals from a solution of F1 in MeOH. Si gel flash cc of fraction F3 with MeOH-$CHCl_3$ (1:99) afforded spathulenol (250 mg). Apigenin (5 mg) and hispidulin (20 mg) were purified by Si gel flash cc of fraction F5, followed by prep. tlc [MeOH-$CHCl_3$ (1:12)].

Gaudichaudol A [1].—Light yellow gum: $[\alpha]^{25}D$ −42° (c=0.1, MeOH); uv (MeOH) λ max (log ε) 229 (2.1), 270 nm (2.4); ir (film) ν max 3400, 2925, 1675, 1475, 1375, 1050, 800 cm^{-1}; ^1H nmr, see Table 1; ^{13}C-nmr, see Table 2; eims (70 eV) *m/z* $[M-2H_2O-H]^+$ 303 (2), 271 (7), 255 (11), 176 (14), 173 (60), 149 (14), 145 (41), 133 (20), 105 (83), 95 (57), 55 (92), 41 (100); hrfabms *m/z* $[M-H]^-$ 339.2541 ($C_{20}H_{35}O_4$ requires 339.2535).

Gaudichaudol B [2].—Colorless gum: $[\alpha]^{25}D$ −42° (c=0.1, MeOH); uv (MeOH) λ max (log ε) 229 (3.3), 312 nm (3.2); ir (film) ν max 3400, 2927, 1725, 1675, 1475, 1375, 1237, 1050 cm^{-1}; ^1H nmr, see Table 1; ^{13}C nmr, see Table 2; eims (70 eV) *m/z* $[M-\text{acetate}]^+$ 339 (1), 334 (12), 273 (6), 255 (24), 227 (10), 219 (19), 190 (56), 175 (100), 161 (51), 147 (67), 107 (83), 95 (68).

Gaudichaudol C [3].—White amorphous powder: mp 70–73°; $[\alpha]^{25}D$ −42° (c=0.1, MeOH); uv (MeOH) λ max (log ε) 231 (4.5), 313 nm (4.5); ir (KBr) ν max 3296, 1685, 1637, 1605, 1515, 1438, 1375, 1262, 1200, 1163, 1025, 988, 825 cm^{-1}; ^1H nmr, see Table 1; ^{13}C nmr, see Table 2; eims (70 eV) *m/z* $[M-H_2O]^+$ 468 (0.4), 450 (0.1), 255 (5), 175 (21), 173 (17), 164 (13), 147 (100), 105 (26), 79 (18), 41 (35); fabms *m/z* $[M-H]^-$ 485.

Gaudichaudone [4].—Colorless gum: $[\alpha]^{25}D$ +38° (c=0.1, MeOH); uv λ max (MeOH) (log ε) 231 nm (3.7); ir (film) ν max 3400, 2975, 1713, 1690, 1537, 1400, 1375, 1025, 887, 850, 800 cm^{-1}; ^1H nmr (CD_3OD, 300 MHz) δ 7.35 (1H, t, J=1.2 Hz, H-15), 7.22 (1H, br s, H-16), 6.44 (1H, d, J=5.6 Hz, H-1), 6.23 (1H, br s, H-14), 4.13 (1H, d, J=7.8 Hz, H-19a), 4.11 (1H, m, H-2), 3.03 (1H, dd, J=7.8 and 1.0 Hz, H-19b), 0.98 (3H, s, H_3-20), 0.89 (3H, d, J=6.8 Hz, H_3-17); ^{13}C nmr, see Table 2; eims (70 eV) *m/z* $[M]^+$ 330 (2), 282 (7), 205 (12), 183 (12), 152 (12), 149 (27), 137 (18), 105 (22), 95 (53), 91 (51), 81 (100); hreims *m/z* $[M]^+$ 330.1835 ($C_{20}H_{26}O_4$ requires 330.1831).

Articulin acetate [5].—Colorless crystals: $[\alpha]^{25}D$ −276° (c=0.1, $CHCl_3$); uv (MeOH) (log ε) 220 nm (3.9); physical and spectral data (mp, ir, ms ^1H-nmr) comparable to literature values (11); ^{13}C nmr, see Table 2.

Articulin [6].—White amorphous powder: mp 171–173° [lit. (11) 182–186°]; $[\alpha]^{25}D$ −140° (c=0.1, $CHCl_3$); uv λ max (MeOH) (log ε) 220 nm (3.9); ^1H nmr (300 MHz, $CDCl_3$) δ 6.71 (1H, d, J=6.3 Hz, H-3), 5.84 (1H, d, J=1.0 Hz, H-14), 4.76 (2H, d, J=1.6 Hz, H_2-16), 4.53 (1H, m, H-2α), 4.30 (1H, d, J=8.0 Hz, H-19a), 3.91 (1H, d, J=7.7 Hz, H-19b), 0.87 (3H, d, J=6.2 Hz, H_3-17), 0.64 (3H, s, H_3-20); ^{13}C nmr (75.4 MHz, $CDCl_3$) δ 174.4 (C-15), 171.4 (C-13), 169.2 (C-18), 142.3 (C-4), 131.9 (C-3), 114.4 (C-14), 73.3 (C-16), 70.9 (C-19), 63.6 (C-2), 45.7 (C-5), 39.2 (C-10), 37.8 (C-9), 36.5 (C-8), 34.4 (C-11), 34.1 (C-6), 29.5 (C-1), 27.6 (C-7), 21.5 (C-12), 17.7 (C-20), 15.6 (C-17); eims (70 eV) *m/z* $[M]^+$ 346 (12), 328 (8), 298 (39), 159 (23), 111 (100).

Apigenin.—White amorphous powder: mp 325–328; identified by uv, ^1H and ^{13}C nmr (13).

Hispidulin.—Yellow amorphous powder: mp 294–297 [lit. (14) 291–292°]; spectral data (uv, ^1H-nmr, and ms) identical to literature values (14).

Spathulenol.—Light yellow oil: $[\alpha]^{25}D$ +12° (c=0.1, $CHCl_3$); spectral data (nmr and ir) identical with literature values (15,16); eims (70 eV) *m/z* $[M]^+$ 220 (18), 205 (40), 187 (15), 162 (23), 159 (27), 149 (16), 119 (43), 79 (42).

Ursolic acid.—White crystals: physical and spectral data (mp, [α]D, ^1H-nmr) comparable to those reported in the literature (17). Ursolic acid was converted to methyl ursolate: ^{13}C-nmr data identical with reported values (19).

CYTOTOXICITY ASSAYS.—The culture cell lines P-388, HT-1080, and KB were purchased from the American Type Culture Collection. The multi-drug resistant cell line KB-V1, developed by treatment of KB cells with sublethal doses of vinblastine over an extended period of time (20), was supplied by Dr. I.B. Roninson, Department of Genetics, University of Illinois at Chicago. Other human cancer cell types, including BC-1, Col-2, Lu-1, and Mel-2 were established from primary tumors in the Specialized Cancer Center, University of Illinois at Chicago College of Medicine. Cytotoxicity assays were carried out in 96-well microtiter plates as described previously (21).

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