Journal of Natural Products
Vol. 48, No. 1, pp. 102-107, Jan-Feb 1985

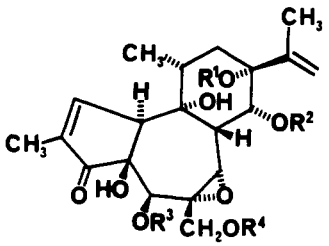
DAPHNANE DITERPENES FROM *DIARTHRON VESICULOSUM*: VESICULOSIN AND ISOVESICULOSIN[1]

RICHARD G. POWELL,* DAVID WEISLEDER, and CECIL R. SMITH, JR.

Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604

ABSTRACT.—An alcoholic extract of *Diarthron vesiculosum* seed has yielded two novel daphnane diterpene esters, vesiculosin (**1**) and isovesiculosin (**2**). Compounds **1** and **2** undergo rapid interconversion to an equilibrium mixture containing approximately 80% **1** and 20% **2** in solution at elevated temperatures. At 25° or below, **1** and **2** may be isolated and stored for extended periods with minimal interconversion. Excoecariatoxin (**3**) and simplexin (**4**) are the major daphnane orthoesters present in *D. vesiculosum*, and **1** and **2** apparently are biosynthetic precursors of **3**. Isolation of these compounds was monitored by bioassay against PS leukemia (in vivo), and structures were determined by ^{1}H nmr, ^{13}C nmr, ms, and comparison with known compounds.

Diarthron vesiculosum (Fisch. & C.A. Mey. ex Kar & Kir.) Mey. (Thymelaeaceae) is an annual native to the Plains near Kabul (Afghanistan) and adjacent areas of Central Asia (1-3). Previous investigations of *D. vesiculosum* have been limited to studies of fatty acid composition during development and the effect of drought on mineral and carbohydrate content. Alcoholic extracts of *D. vesiculosum* seed typically give a 50-70% increase in lifespan in the murine P-388 lymphocytic leukemia (PS) system (4). Our attempts to isolate active principles present in these extracts have culminated in the characterization of two previously unknown daphnane esters, vesiculosin (**1**) and isovesiculosin (**2**). Also isolated in this study were two known daphnane orthoesters, excoecariatoxin (**3**) and simplexin (**4**), as well as daphnoretin (**5**), a well known biscoumarin derivative found principally in the Thymelaeaceae (5). Compounds **3** and **4** account for most of the PS activity, whereas **1** and **2** are only marginally active. Excoecariatoxin (**3**) has been identified previously in *Excoecaria agallocha* (6) and *Wikstroemia monticola* (7), and simplexin is known to occur in various *Pimelia* spp. (8,9), *W. monticola* (6) and *Baliospermum montanum* (10). The pro-inflammatory, tumor-promoting, and antitumor diterpenes of the plant families Euphorbiaceae and Thymelaeaceae have been subjects of intensive investigation for nearly two decades



1 R^1=H; R^2=$CH_3(CH_2)_4(CH{=}CH)_2CO-$; R^3=R^4=H
1a R^1=H; R^2=$CH_3(CH_2)_4(CH{=}CH)_2CO-$; R^3=R^4=Ac
2 R^1=$CH_3(CH_2)_4(CH{=}CH)_2CO-$; R^2=R^3=R^4=H
2a R^1=$CH_3(CH_2)_4(CH{=}CH)_2CO-$; R^2=R^3=R^4=Ac

[1]Presented at the 25th Annual Meeting of the American Society of Pharmacognosy, Austin, TX, August 19-23, 1984. The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

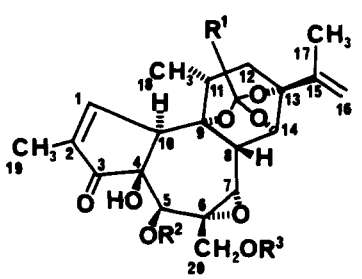
(11). Daphnoretin is not active against PS leukemia but has shown activity against Ehrlich ascites carcinoma in mice (5).

Structures of excoecariatoxin (**3**) and simplexin (**4**) were readily deduced from spectral evidence and from comparison with related daphnane orthoesters, such as wikstrotoxin B (**6**), montanin (**7**), and huratoxin (**8**). The ^{1}H nmr of **3** (Table 1) agrees well with literature values (6,7). Although the corresponding ^{13}C nmr (Table 2) has not been reported previously, comparison with the ^{13}C nmr of a closely related homolog, **8** (7), gives excellent support for our assignments. Chemical ionization mass spectrometry (cims) of **3** yields a base peak at *m/z* 529 (MH^+) along with a prominent ion at *m/z* 169; the latter is due to loss of the orthoester group as protonated decadienoic acid. Also evident in the cims of **3** were relatively minor peaks at *m/z* 511 (MH^+-H_2O), 361

TABLE 1. ^{1}H-nmr Assignments for Vesiculosin (**1**), Isovesiculosin (**2**), Excoecariatoxin (**3**) and Their Acetates[a]

Proton assignments	1	1a	2	2a	3	3a
1	7.64 m	7.56 m	7.62 m	7.54 m	7.61 m	7.52 m
5	4.24 bs	5.50 bs	4.25 bs	5.51 bs	4.23 bs	5.52 d (0.9)
7	3.14 bs	3.13 bs	3.34 bs	3.09 bs	3.43 bs	3.34 d (0.9)
8	3.63 bd	3.71 bd (2.5)	3.63	3.67 bd (2.6)	2.92 d (2.5)	2.99 d (2.5)
10	3.86 m	3.95 m	3.87 m	3.99 m	3.79 m	3.93 m
11	——[b]	——[b]	——[b]	——[b]	2.47 m (8.7)	2.39 m
12β	——[b]	——[b]	——[b]	——[b]	2.21 dd (14.5)	2.21 m (14.5)
12α	——[b]	——[b]	——[b]	——[b]	1.66 d (14.5)	1.67 d (14.5)
14	5.65 m	5.67 m	4.44 bs	5.69 bs	4.41 d (2.5)	4.41 d (2.5)
16a	5.06 m	5.08 m	5.18 m	5.25 m	4.88 m	4.89 m
16b	5.11 bs	5.13 bs	5.23 bs	5.34 bs	5.00 m	5.00 m
17	1.87 m	1.89	1.79 m	1.86 m	1.78 m	1.78 m
18	1.03 d (6.6)	1.00 d (6.6)	1.03 d (6.7)	0.97 d (6.5)	1.16 d (7.1)	1.11 d (7.1)
19	1.76 m	1.74 m	1.75 m	1.75 m	1.77 m	1.75 m
20a	3.63 m	3.52 d (12.0)	3.63 m	3.54 d (12.0)	3.79 m	3.59 d (11.9)
20b	3.63 m	4.67 d (12.0)	3.63 m	4.69 d (12.0)	3.79 m	4.65 d (11.9)
2′	5.88 d (15.2)	5.88 d (15.2)	5.75 d (15.2)	5.59 d (15.2)	5.69 d (15.4)	5.68 d (15.4)
3′	7.30 m	7.32 m	7.20 m	7.14 m	6.68 dd (10.6)	6.68 dd (10.6)
4′	6.18 m	6.18 m	6.15 m	6.13 m	6.04 m (15.2)	6.04 m (15.2)
5′	6.18 m	6.18 m	6.15 m	6.13 m	5.81 dt (6.9)	5.83 dt (6.9)
6′	——[b]	——[b]	——[b]	——[b]	2.08 m	2.08 m
7′-9′	1.30 m	1.30 m	1.30 m	1.29 m	1.30 m	1.30 m
10	0.88 m	0.89 m	0.88 m	0.88 m	0.86 m	0.87 m
MeC=O	——	2.18 s	——	2.23 s	——	2.16 s
MeC=O	——	1.99 s	——	2.18 s	——	2.00 s
MeC=O	——	——	——	2.01 s	——	——

[a]Chemical shifts (δ) are expressed in ppm from TMS, and coupling constants (*J*), in parentheses, are expressed in Hz.
[b]Complex signals for these protons appear in the δ 1.50-2.50 range, but definitive assignments have not been made.



3 R^1=$CH_3(CH_2)_4(CH{=}CH)_2-$; R^2=R^3=H
3a R^1=$CH_3(CH_2)_4(CH{=}CH)_2-$; R^2=R^3=Ac
4 R^1=$CH_3(CH_2)_8-$; R^2=R^3=H
4a R^1=$CH_3(CH_2)_8-$; R^2=R^3=Ac
6 R^1=$CH_3(CH_2)_6(CH{=}CH)_2-$; R^2=R^3=H
7 R^1=$CH_3(CH_2)_{10}-$; R^2=R^3=H
8 R^1=$CH_3(CH_2)_8(CH{=}CH)_2-$; R^2=R^3=H

(MH^+-168), 343 [MH^+-(168+H_2O)], and 325 [MH^+-(168+2H_2O)]. The ^1H nmr of **4** is in good agreement with previous literature values (7,8,9); the same is true for its ^{13}C nmr (7,8). Cims of **4** gave MH+, *m/z* 533 and loss of protonated decanoic acid, *m/z* 173. Acetylation of **3** and **4** gave diacetates **3a** and **4a** as expected.

Compounds **1** and **2** were isolated by preparative tlc as single, well-separated bands, although analyses of the recovered products by tlc and nmr revealed that mixtures of both were generated during the recovery process. Due to their obvious instability, all subsequent operations involving **1** and **2** were carried out at, or below, room temperature, and spectra were obtained as rapidly as possible after their isolation. Solu-

TABLE 2. ^{13}C-nmr Assignments for Vesiculosin (**1**) and Related Daphnane Diterpenes[a]

Carbon assignments	**1**	**1a**	**2a**	**3**	**3a**	**4**
1	162.2 d	160.1 d	160.3 d	161.0 d	158.7 d	161.2 d
2[b]	134.7 s	135.0 s	134.8 s	136.6 s	136.8 s	136.6 s
3	209.4 s	205.7 s	205.6 s	209.6 s	205.7 s	209.8 s
4[c]	74.1 s	74.1 s	82.1 s	84.4 s	84.4 s	84.2 s
5	77.8 d	77.6 d	77.7 d	82.0 d	81.8 d	82.1 d
6	62.0 s	60.3 s	60.3 s	60.5 s	59.3 s	60.6 s
7	63.6 d	63.7 d	63.9 d	64.1 d	64.2 d	64.3 d
8	39.2 d	39.6 d	38.1 d	36.7 d	36.8 d	36.9 d
9	72.5 s	71.7 s	71.7 s	72.4 s	71.9 s	72.5 s
10	50.0 d	50.6 d	50.4 d	48.2 d	48.6 d	48.4 d
11	37.5 d	37.4 d	38.1 d	34.9 d	34.7 d	34.9 d
12	37.9 t	38.1 t	35.4 t	36.5 t	36.3 t	36.7 t
13[c]	76.7 s	76.4 s	77.2 s	79.6 s	79.3 s	78.8 s
14	70.7 d	68.1 d	68.0 d	71.8 d	68.7 d	72.1 d
15[b]	145.9 s	146.1 s	142.5 s	146.2 s	146.2 s	146.5 s
16	113.9 t	113.9 t	117.7 t	111.2 t	111.2 t	111.2 t
17	19.0 q	19.0 q	19.3 q	20.3 q	20.1 q	20.3 q
18	9.8 q	9.9 q	9.8 q	9.8 q	9.9 q	9.9 q
19	18.2 q	18.0 q	17.8 q	18.9 q	18.9 q	19.0 q
20	65.3 t	65.9 t	65.8 t	65.1 t	66.4 t	65.3 t
1′	167.4 s	167.4 s	165.0 s	116.5 s	116.6 s	119.5 s
2′	117.8 d	117.7 d	119.4 d	122.9 d	122.8 d	32.7 t
3′	147.2 d	147.3 d	145.4 d	138.7 d	138.8 d	——[d]
4′	146.5 d	146.6 d	145.2 d	134.6 d	134.7 d	29.7 t
5′	128.2 d	128.2 d	128.2 d	128.8 d	128.8 d	29.7 t
6′	33.0 t	33.0 t	33.0 t	32.6 t	32.6 t	29.7 t
7′	28.3 t	28.3 t	28.4 t	28.7 t	28.7 t	29.4 t
8′	31.3 t	31.4 t	31.4 t	31.3 t	31.3 t	32.0 t
9′	22.5 t	22.5 t	22.5 t	22.4 t	22.5 t	22.7 t
10′	14.0 q	13.9 q	13.9 q	13.9 q	14.0 q	14.1 q
C=O	——	170.3 s	170.3 s	——	170.4 s	——
C=O	——	168.7 s	169.8 s	——	168.7 s	——
C=O	——	——	168.7 s	——	——	——
CH_3	——	20.7 q	21.3 q	——	20.7 q	——
CH_3	——	20.5 q	20.7 q	——	20.6 q	——
CH_3	——	——	20.6 q	——	——	——

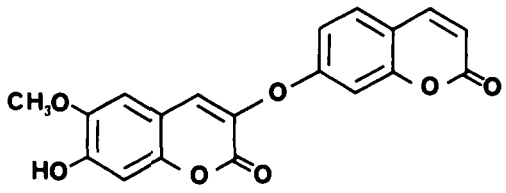
[a]Chemical shifts (δ) are expressed in ppm from internal TMS. Proton decoupled and off-resonance decoupled spectra were recorded in $CDCl_3$ solution on a Bruker WM-300 spectrometer and multiplicities were confirmed in DEPT experiments.

[b,c]Assignments with the same letter designation in any vertical column may be interchanged.

[d]Assignment uncertain.

tions of **1** or **2**, which were either held at 60° for 30 min or were allowed to stand for several days at room temperature, reverted to equilibrium mixtures containing approximately 80% **1** and 20% **2** as determined by ^{1}H nmr.

The cims of both **1** and **2** yielded relatively minor MH^+ ions at *m/z* 547 and intense ions at *m/z* 169. Other significant ions occurred in both spectra at *m/z* 529 (MH^+-H_2O), 379 (MH^+-168), 361 [MH^+-(168+H_2O)], 343 [MH^+-(168+2H_2O)], and 325 [MH^+-(168+3H_2O)]. Thus, **1** and **2** were clearly isomeric, and both differed from **3** by being 18 mass units higher in molecular weight. Although the spectrum of **3** lacked ions at *m/z* 547 and 379, the cims spectra of **1, 2,** and **3** were distinctly similar and exhibited most of the same ions. Both **1** and **2** lost a fragment giving rise to *m/z* 169 (presumably the same protonated decadienoic acid observed in the spectrum of **3**) much more readily than did **3**. These observations were consistent with a hypothesis that **1** and **2** were simple monoesters, rather than orthoesters, of the parent daphnane diterpene.



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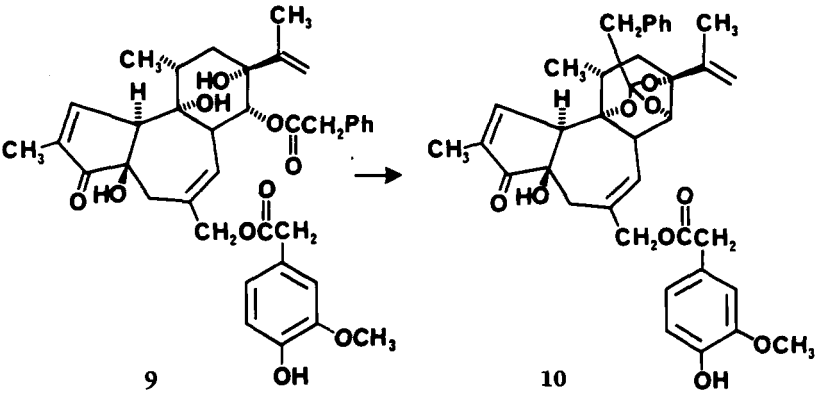
Comparison of the ^{13}C-nmr spectra of vesiculosin (**1**) and excoecariatoxin (**3**) confirmed that both are C-30 compounds and further demonstrated their close structural relationship. Most notable was the shift of the C-1′ (orthoester) signal from δ 116.5 in **3** to δ 167.4 in **1**; the latter shift is typical of an ordinary ester carbonyl. All other ^{13}C-nmr shift differences in the spectra of **1** and **3** may be rationalized by assuming that the 2,4-decadienoate ester moiety of **1** is attached at C-14.

The ^{1}H-nmr spectra of esters **1** and **2** and orthoester **3** (Table 1) are all similar, and shift differences of signals observed for H-8, H-2′, and H-3′ may be readily explained by the proximity of these protons to an ester carbonyl group in **1** and **2** and by the absence of a similar carbonyl group in **3**. Attachment of an ester group at C-14 in **1** is established by the lowfield H-14 signal at δ 5.65. In contrast, the spectrum of **2** shows the H-14 signal more upfield at δ 4.44, indicating that this position is not esterified. The only other likely position for attachment of the ester moiety of **2** is C-13, a tertiary carbon allylic to the 15,16-double bond.

Acetylation of **1** provided a mixture of the 5,20-diacetate (**1a**), MH^+, *m/z* 631, and the 5,14,20-triacetate (**2a**), MH^+, *m/z* 673. Acetylation of **2** also gave a mixture of **1a** and **2a**. ^{1}H- and ^{13}C-nmr data for **1a** and **2a** (Tables 1 and 2) fully support our conclusions concerning the structures of these compounds.

Vesiculosin (**1**) and isovesiculosin (**2**) may be biosynthetic precursors of excoecariatoxin inasmuch as a related ester, pro-resiniferatoxin (**9**), is readily converted to resiniferatoxin (**10**) at elevated temperatures or under acid catalysis (12). Adolf *et al.* (13) have studied similar conversions in greater detail. Conversion of either **1** or **2** to **3** at temperatures below 60° has not been observed by us, and we have been unable to study the problem further due to lack of material. However, some difference in reactivity of **1** and **9** might be expected because **9** contains a 6,7-double bond rather than a 6,7-epoxide group. It is tempting to presume that the interconversion of **1** and **2** proceeds through an intermediate hemiorthoester.



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EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Analytical and preparative tlc were carried out on silica gel 60 F-254 plates (E. Merck) developed with CH_2Cl_2-MeOH (19:1) unless indicated otherwise. Silica gel (60-200 mesh, Baker) was used for column chromatography. Hplc was carried out on a Waters Model ALC/PC-201 instrument equipped with an RI detector and Whatman Partisil 10, M-9 (50 cm) or Whatman Partisil 10 ODS-2, M-9 (50 cm) columns. The ir spectra were recorded on a Perkin-Elmer Model 700 instrument with 1% CH_2Cl_2 solutions; uv spectra were determined on a Beckman DK-2A spectrophotometer and optical rotations were obtained using a Perkin-Elmer Model 241 polarimeter. ^{1}H- (300 MHz) and ^{13}C- (22.63 MHz) nmr spectra were determined with a Bruker WM-300 instrument using $CDCl_3$ solutions with TMS as an internal standard. Extensive decoupling was used to verify assignments. Mass spectra, both electron impact and chemical ionization (isobutane), were obtained using a Finnegan MAT 4535-TSQ instrument equipped with a DEP probe.

PLANT MATERIAL.—Two collections of *D. vesiculosum* seed were supplied for this study. The first (9 kg, BE-3089) was obtained in 1980 as a gift from the Pakistan Forest Institute, Peshawar, arranged by Dr. Anwar A. Khan. In 1981, a second collection (11.6 kg, PR-55598) was arranged in Pakistan and authenticated by Dr. James Duke, USDA, Beltsville, MD, in accordance with the program developed by the National Cancer Institute. The two collections were combined for fractionation after preliminary analysis and bioassay showed them to be identical.

EXTRACTION AND FRACTIONATION.—*D. vesiculosum* seed (20.6 kg) was ground in a Wiley mill and defatted with hexane in a pilot-plant-scale Soxhlet extractor, yielding 3.5 kg of oil. Further extraction of the seed material with 95% EtOH provided 800 g of EtOH-soluble material. EtOH solubles were then partitioned between CH_2Cl_2 and H_2O; evaporation of the CH_2Cl_2 layer to dryness yielded 412 g of activity-enriched material. The CH_2Cl_2-soluble material was divided into nine portions of approximately 45 g each and subjected to chromatography on columns packed with 300 g of silica. Eluting solvents for each of the nine runs consisted of a stepwise gradient of increasing MeOH in CH_2Cl_2. Similar fractions were combined on the basis of tlc analysis, and materials of interest were further concentrated by hplc on silica using CH_2Cl_2-CH_3CN (9:1).[2] Daphnoretin (**5**), 1.08 g, was isolated during this procedure. Further concentration by preparative tlc, CH_2Cl_2-MeOH (9:1), gave 334 mg of marginally active material, mostly **1** (Rf 0.5) and **2** (Rf 0.6), along with 490 mg of highly active material, primarily a mixture of **3** (Rf 0.7) and **4** (Rf 0.7). Final separation using a Whatman M9, 10/50 ODS-2 C_{18} column and MeOH-H_2O (88:12) gave **3** (119 mg) and **4** (85 mg).

VESICULOSIN (**1**).—Final purification by preparative tlc gave 77 mg of **1** as a colorless glass (3.7×10^{-4}% yield); ir (CH_2Cl_2) ν max 3590, 3530, 2920, 1695, 1640 cm^{-1}; uv λ max (MeOH) 260 nm (ϵ 20,600); $[\alpha]^{23}D$ +17° (*c* 0.086, $CHCl_3$); ^{1}H nmr and ^{13}C nmr (Tables 1 and 2); cims *m/z* (rel. int.) 547 (MH^+, 4), 529 (11), 511 (3), 379 (56), 361 (100), 343 (27), 325 (12), 169 (79).

VESICULOSIN ACETATE (**1a**).—A portion of **1** (14 mg) was acetylated in 2 ml of Ac_2O-pyridine (1:1), 18 h at 26°, and the resulting solution was evaporated to dryness under N_2. The residue was subjected to preparative tlc on silica using CH_2Cl_2-MeOH (97:3) yielding **1a** (6.3 mg) and **2a** (2.6 mg). Diacetate **1a** was a colorless glass; ir (CH_2Cl_2) ν max 3580, 2925, 1755, 1740, 1700, 1640 cm^{-1}; ^{1}H nmr and ^{13}C nmr (Tables 1 and 2); cims *m/z* (rel. int.) 631 (MH^+, 52), 613 (60), 595 (12), 463 (19), 445 (39), 427 (23), 403 (8), 385 (16), 325 (8), 169 (100), 151 (4).

[2]Each stage of fractionation was monitored by bioassay against PS leukemia. Separation methods were not optimized, and samples at each step were consumed in biological testing.

ISOVESICULOSIN (**2**).—Final purification by preparative tlc gave 17 mg of **2** as a colorless glass (8.0×10^{-5}% yield); ir (CH_2Cl_2) ν max 3590, 3520, 2920, 1690, 1640 cm^{-1}; uv λ max (MeOH) 260 nm (ϵ 15,300); $[\alpha]^{23}D$ +9.4° (*c* 0.013, $CHCl_3$); ^1H nmr (Table 1); cims *m/z* (rel. int.) 547 (MH^+, 1), 529 (6), 511 (2), 379 (14), 361 (13), 343 (14), 325 (12), 279 (26), 169 (100).

ISOVESICULOSIN ACETATE (**2a**).—A portion of **2** (9.5 mg) was acetylated and purified by preparative tlc, by the procedure described for **1a**, yielding **2a** (4.4 mg) and **1a** (2.1 mg). Triacetate **2a** was a colorless glass; ^1H nmr and ^{13}C nmr (Tables 1 and 2); cims *m/z* (rel. int.) 673 (MH^+, 20), 655 (16), 613 (13), 505 (100), 487 (19), 445 (18), 427 (18), 385 (8), 367 (13), 169 (72), 151 (9).

EXCOECARIATOXIN (**3**).—Excoecariatoxin (**3**), 119 mg (5.7×10^{-4}% yield) was a colorless glass; ir (CH_2Cl_2) ν max 3540, 2925, 1695, 1670, 1630 cm^{-1}; uv λ max 231 nm (ϵ 34,200); $[\alpha]^{23}D$ +55° (*c* 0.73, $CHCl_3$); ^1H nmr and ^{13}C nmr (Tables 1 and 2); cims *m/z* (rel. int.) 529 (MH^+, 100), 511 (14), 169 (43).

EXCOECARIATOXIN ACETATE (**3a**).—A portion of **3** (25 mg) was acetylated and purified by preparative tlc, by the procedure described for **1a**, yielding **3a** (24 mg) as a colorless glass; uv λ max (MeOH) 231 nm (ϵ 32,200); $[\alpha]^{23}D$ +59° (*c* 0.83, $CHCl_3$); ^1H nmr and ^{13}C nmr (Tables 1 and 2); cims *m/z* (rel. int.) 613 (MH^+, 100), 599 (12), 169 (7).

SIMPLEXIN (**4**).—Simplexin (**4**), 85 mg (4.1×10^{-4}% yield) was a colorless glass; ir (CH_2Cl_2) ν max 3560, 2920, 1695 cm^{-1}; uv λ max (MeOH) 240 nm (ϵ 11,200); $[\alpha]^{23}D$ +57° (*c* 0.54, $CHCl_3$); ^1H nmr and ^{13}C nmr (Table 2) correlate well with literature values (7,8); cims *m/z* (rel. int.) 533 (MH^+, 100), 515 (16), 173 (17).

SIMPLEXIN ACETATE (**4a**).—A portion of **4** (42 mg) was acetylated and purified by preparative tlc, by the procedure described for **1a**, yielding **4a** (41 mg) as a colorless glass; ir (CH_2Cl_2) ν max 3550, 2925, 1755, 1745, 1700 cm^{-1}; uv λ max (MeOH) 244 nm (ϵ 7,560); $[\alpha]^{23}D$ +69° (*c* 0.47, $CHCl_3$); ^1H nmr and ^{13}C nmr correlate well with literature values (9); cims *m/z* (rel. int.) 617 (MH^+, 100), 599 (52), 557 (8), 173 (11).

DAPHNORETIN (**5**).—Daphnoretin, 1.08 g, mp 244-245°; ^1H nmr and ^{13}C nmr were identical to those reported earlier (5); eims *m/z* 352 (M^+, 100%); cims *m/z* 353 (MH^+, 100%).

ACKNOWLEDGMENTS

We thank R. Plattner for mass spectra and Barry Jones and Tim Mueser for technical assistance. We also thank Dr. Geoffrey Cordell, University of Illinois at the Medical Center, Chicago, IL, for a comparison sample of montanin; Dr. G.R. Pettit, Arizona State University, for a comparison sample of simplexin; and Dr. George White, USDA, Beltsville, for expediting shipment of seed from the American Embassy, Islamabad, Pakistan. Bioassays were conducted at Battelle Laboratories, Columbus, OH, under contract with the National Cancer Institute.

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Received 13 August 1984