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BIOACTIVE AND OTHER SESQUITERPENOIDS FROM *PORELLA CORDEANA*

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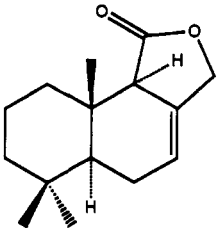
ABSTRACT.—Bioassay-directed fractionation of the MeCOEt extract of *Porella cordeana* yielded drimenin [**1**] and aristolone [**4**], which were moderately toxic towards DNA-repair-deficient mutants of *Saccharomyces cerevisiae*. Three inactive sesquiterpenes, 7-ketoisodrimenin [**2**], 7-ketoisodrimenin-5-ene [**3**], and norpinguisanolide, were also obtained. Compounds **2** and **3** are new.

Bioassays that detect the presence of compounds that act by a specific biochemical mechanism likely to result in selective anticancer activity are becoming more frequently deployed in the biological screening of natural product extracts (1–4). One assay recently developed (4) utilizes DNA repair- or recombination-deficient mutants of the yeast *Saccharomyces cerevisiae* to detect the presence of compounds which induce DNA damage. This assay was recently employed in our laboratory to isolate three bioactive sterols from *Pseudobersama mossambicensis* (5). The present paper documents the further use of such an approach in the isolation of two moderately bioactive sesquiterpenes from the liverwort *Porella cordeana* (Hueb.) Moore (Porellaceae). Two novel but inactive drimane sesquiterpenes were also isolated. Previous phytochemical studies of *Po. cordeana* have afforded the sesquiterpenes pinguisanin, norpinguisone methyl ester, porellapinguisanolide, porellapinguisenone, spiropinguisan, and striatenone, and the diterpene perrottetianal A (6).

RESULTS AND DISCUSSION

Three major DNA repair pathways have been defined in yeast and are known as the RAD3, RAD6, and RAD52 pathways. The RAD3 pathway is associated with excision repair, the RAD6 pathway is the error-prone recombinational pathway, and RAD52 is associated with the repair of double strand breaks and meiotic recombination (7). A plant extract that inhibits yeast mutants lacking one or more of these pathways to a greater extent than it inhibits wild-type strains should contain agents that induce DNA damage. In this mechanism-based bioassay, an MeCOEt extract of *Po. cordeana* showed weak to moderate activity (Table 1).

Bioactivity-directed fractionation of this extract, involving liquid-liquid partition, followed by chromatography on Sephadex LH-20 and Si gel, yielded the two moderately active known sesquiterpenoids drimenin [**1**] and aristolone [**4**]. The two new but



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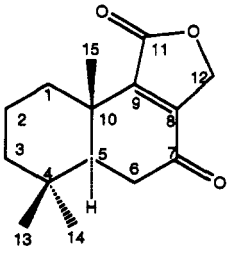

2
3 $\Delta^{5,6}$

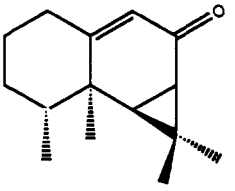

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TABLE 1. Bioactivity Data of *Porella cordeana* Extract and Sesquiterpenoids.[a]

Compound	*Saccharomyces cerevisiae* strains		
	RS322YK rad 52	RS167N rad 6	RS188N RAD$^+$
MeCOEt extract	1641	2438	2475
Drimenin [**1**]	200	>1000	>1000
7-Ketoisodrimenin [**2**]	>1000	>1000	>1000
7-Ketoisodrimenin-5-ene [**3**]	>1000	>1000	>1000
Aristolone [**4**]	390	>1000	>1000
Norpinguisanolide	>1000	>1000	>1000

[a]Results expressed as IC_{12} (μg/ml).

inactive sesquiterpenoids **2** and **3** were also isolated together with the known sesquiterpenoid norpinguisanolide.

The ir, ms, and nmr spectral data of compound **1** correlated well with those reported for drimenin (8,9). ^1H and ^{13}C assignments (see Experimental) were established by ^1H-^1H COSY, HETCOR, and COLOC spectra. This is the first time carbon assignments have been made for drimenin.

Compound **2** was assigned the molecular formula $C_{15}H_{20}O_3$ from hreims, and the ir spectrum indicated the presence of a ketone carbonyl (1685 cm^{-1}) and a lactone carbonyl (1762 cm^{-1}). By postulating a drimane skeleton it was possible to assign some of the carbons and protons of ring A by comparison with the nmr spectra of drimenin. This allowed the assumption that the ketone carbonyl (δ 196.0, s) was part of ring B. The signal at δ 1.89 (1H, dd, J=3.5, 13.9 Hz, H-5), which showed a direct connectivity to C-5 (δ 52.0, d), demonstrated coupling to signals at δ 2.51 (1H, dd, J=13.9, 17.4 Hz, H-6) and δ 2.65 (1H, dd, J=3.5, 17.4 Hz, H-6), placing the ketone carbonyl at C-7. The presence of long-range connectivities between both H-6 protons and the ketone carbonyl was established by HMBC as was a connectivity between these protons and a quaternary carbon at δ 36.7 (C-10) confirming their position at C-6 and by implication the position of the ketone carbonyl at C-7. It also follows that a ketone carbonyl at C-7 places the double bond at C-8 (δ 149.0, s) and C-9 (δ 152.5, s).

Again, by assuming a drimane skeleton, the 3H singlet at δ 1.30 was assigned as C-15 (δ_C 18.1) since the signals at δ_H 0.98, δ_C 21.0 and δ_H 0.94, δ_C 32.8 were clearly analogous to C-13 and C-14, respectively, of drimenin. This C-15 methyl showed HMBC connectivities to a signal at δ 152.5 (C-9) supporting the positioning of the double bond at C-8 and C-9.

The lactone carbonyl group (δ 170.9, s) was placed at C-11 rather than C-12 since a carbonyl group at C -12 would cause deshielding of C-9 whereas in compound **2** C-8 and C-9 have almost identical chemical shifts. Furthermore a carbonyl group at C-11 would strongly deshield H-1β (δ 2.67, δ_C 33.2, t). The structure of this compound was therefore established as the novel 7-ketoisodrimenin [**2**].

Compound **3** was assigned the molecular formula $C_{15}H_{18}O_3$ from hreims. The ir spectrum indicated the presence of a ketone carbonyl (1680 cm^{-1}) and a lactone carbonyl group (1768 cm^{-1}). By proposing a drimane skeleton for compound **3** one can assign C-1 (δ 34.4, t), C-2 (δ 17.6, t), C-3 (δ 41.4, t), and C-4 (δ 38.3, s) by comparison with compounds **1** and **2**. Two methyl signals (δ_H 1.35, δ_C 27.8, C-13 and δ_H 1.26, δ_C 32.7, C-14) showed connectivities to each other and to signals at δ_C 38.3 (C-4) and 41.4 (C-3). There is also an HMBC connectivity between these two methyl signals and one at δ 177.0, implying that C-5 is part of the conjugated carbonyl system indicated by the

TABLE 2. ^{1}H- and ^{13}C-nmr Data for 7-Ketoisodrimenin [**2**].

Position	δ_H[a]	δ_C[b]	HMBC[c]
1α	1.64 m	33.2 t	
1β	2.73 m		
2	1.73 m	17.9 t	
3	1.54 m	41.1 t	H-13, H-14
4	—	33.1 s	H-13, H-14
5	1.89 dd (J=3.5, 13.9)	52.0 d	H-13, H-14, H-15
6	2.51 dd (J=13.9, 17.4)		
	2.65 dd (J=3.5, 17.4)	36.1 t	
7	—	196.0 s	H-6
8	—	149.0 s	H-11
9	—	152.5 s	H-15
10	—	36.7 s	H-6, H-15
11	—	170.9 s	
12	4.85 s	67.5 t	
13	0.98 s	21.0 q	H-14
14	0.94 s	32.8 q	
15	1.30 s	18.1 q	

[a]J in Hz.
[b]Multiplicity deduced from a DEPT experiment.
[c]Protons showing multiple-bond coupling to the indicated carbon.

presence of a proton signal at δ 6.42 (δ_C 124.6, C-6). This would place the ketone carbonyl (δ 182.5, s) at C-7. Signals at δ 149.6 and δ 149.7 were assigned to C-8 and C-9, respectively, with the signal at δ 149.7 showing an HMBC connectivity to H-15. The equivalence of the signals for C-8 and C-9 would imply that with a carbonyl at C-7, the lactone carbonyl (δ 170.7, s) is at C-11 rather than C-12. This conclusion is supported by comparison of the chemical shifts for C-11 and C-12 with those of compound **2** (see Tables 2 and 3) and by the ^{1}H signal at δ 2.73 attributable to the

TABLE 3. ^{1}H- and ^{13}C-nmr Data of 7-Ketoisodrimenin-5-ene [**3**].

Position	δ_H[a]	δ_C[b]	HMBC[c]
1α	1.35 m		
1β	2.73 m	34.4 t	H-15
2	1.70 m, 1.96 m	17.6 t	
3	1.98 m, 1.35 m	41.4 t	H-13, H-14
4	—	38.3 s	H-13, H-14
5	—	177.0 s	H-13, H-14, H-15
6	6.42 s	124.8 d	
7	—	182.5 s	
8	—	149.6 s	H-6, H-12
9	—	149.7 s	H-12, H-15
10	—	40.9 s	H-6
11	—	170.7 s	
12	5.01 ABq (J=17.6)	67.5 t	
13	1.35 s	27.8 q	H-14
14	1.26 s	32.7 q	H-13
15	1.56 s	24.0 q	

[a]Deduced from ^{1}H-^{1}H COSY analysis, J in Hz.
[b]Deduced from DEPT experiment.
[c]Protons showing multiple-bond coupling to the indicated carbon.

deshielded H-1β. The structure of **3** was therefore established as the novel 7-ketoisodrimenin-5-ene [**3**].

Aristolone [**4**] was identified by comparison of uv, ms, and ^{1}H-nmr spectra with literature values (10), although there is a discrepancy in the melting points. ^{1}H and ^{13}C assignments (see Experimental) were established by ^{1}H-^{1}H COSY, HETCOR, and COLOC spectra. This is the first time carbon assignments have been made for aristolone.

The ir, ms, uv, and ^{1}H- and ^{13}C-nmr spectra of norpinguisanolide were consistent with those reported for this compound previously isolated from *Porella elegantula* (11).

The bioactivity observed for the two sesquiterpenoids **1** and **4** is moderate but reproducible. Both compounds showed selective activity against the RAD52 strain RS322YK of *Saccharomyces cerevisiae* and were inactive against the wild-type strain RS188N (RAD$^+$). Drimenin [**1**] was, however, inactive in P388 (murine leukemia) and CHO (Chinese hamster ovary) cytotoxicity assays.

EXPERIMENTAL

PLANT MATERIAL.—The plant material used in this study was collected in Oregon in June 1990 under the auspices of the World Botanical Associates, Laurel, Maryland (WBA# 1189). A voucher specimen is deposited at the U.S. National Herbarium.

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were taken in $CHCl_3$ solution with a Perkin-Elmer Model 241 polarimeter. Uv spectra were obtained in MeOH on a Beckman DU-50 spectrophotometer. Ir spectra were obtained on a Perkin-Elmer Model 1600 spectrometer. The ^{1}H- and ^{13}C-nmr spectra were recorded on a Varian Unity 400 spectrometer at 400 and 100.57 MHz, respectively, with TMS as internal standard. ^{1}H-^{1}H COSY, DEPT, ^{1}H-^{13}C HETCOR, COLOC, HMQC, and HMBC nmr experiments were performed on the same spectrometer using standard Varian pulse sequences. Where only small amounts of material were available (i.e., <4 mg) HMQC and HMBC were used in preference to HETCOR and COLOC experiments, respectively. Hplc separations were performed on a Partisil 5μM column (Whatman, 25.0 cm×0.48 cm) using 1% iPrOH in CH_2Cl_2 at 1 ml/min as the mobile phase. The detector used was a Waters 990 Series photodiode array spectrophotometer.

BIOLOGICAL SCREENING PROCEDURES.—Experimental methods utilized in the screening procedure have been described elsewhere (5). The IC_{12} values refer to the concentration in μg/ml required to produce a zone of inhibition of 12 mm diameter around a 100 μl well during a 48 h incubation period at 37°.

EXTRACTION AND ISOLATION.—*Po. cordeana* (2 kg) was extracted sequentially with hexane followed by MeCOEt and MeOH. The bioactive MeCOEt extract was partitioned between hexane and 80% aqueous MeOH. The aqueous MeOH fraction (1.6 g) was subjected to adsorption chromatography on Sephadex LH-20 (12) eluting initially with hexane-CH_2Cl_2 (1:4), followed by CH_2Cl_2, CH_2Cl_2-Me_2CO (1:1), Me_2CO, and finally MeOH. The hexane/CH_2Cl_2 fraction was further subjected to flash chromatography on Si gel 60 (230–400 mesh, EM Science), eluting initially with hexane-CH_2Cl_2 (1:1) followed by hexane-CH_2Cl_2 (1:4), CH_2Cl_2, and finally EtOAc.

Drimenin [**1**] (31.1 mg) crystallized from the hexane-CH_2Cl_2 (1:1) fraction after standing in MeOH. Preparative tlc of the mother liquor on Si gel GF_{254} (Analtech, 0.5 mm thickness) using CH_2Cl_2-iPrOH (19:1) as mobile phase yielded a further 5.1 mg of drimenin [**1**] (R_f 0.42).

7-Ketoisodrimenin [**2**] (2.2 mg), 7-ketoisodrimenin-5-ene [**3**] (3.7 mg), and norpinguisanolide (1.7 mg) were obtained from the hexane/CH_2Cl_2 fraction by hplc (see above conditions) and had retention times of 6.0, 7.0, and 7.2 min respectively.

Aristolone [**4**] (28.3 mg) was obtained by repeated preparative tlc of the CH_2Cl_2 fraction on Si gel GF_{254} (Analtech, 0.5 mm thickness) using CH_2Cl_2 (triple elution) or CH_2Cl_2-iPrOH (19:1) (R_f 0.54) as the mobile phase.

Drimenin [**1**].—Colorless needles (MeOH): mp 129° [lit. (8) 132–133°]; $[\alpha]_D$ −30.3 (c=1.62, $CHCl_3$); ^{13}C nmr ($CDCl_3$, 100 MHz) δ 175.3 (s, C-11), 129.8 (s, C-8), 121.1 (s, C-7), 69.8 (t, C-12), 53.6 (d, C-9), 49.6 (d, C-5), 42.3 (t, C-3), 38.4 (t, C-1), 34.3 (s, C-4), 33.1 (s, C-10), 33.0 (q, C-14), 23.3 (t, C-6), 21.4 (q, C-13), 18.2 (t, C-2), 13.9 (q, C-15).

7-Ketoisodrimenin [**2**].—White amorphous powder: $[\alpha]_D$ +40.8 (c=0.6, $CHCl_3$); uv (MeOH) λ max 217, 242 nm; ir ν max 2950, 2900, 2825, 1762, 1685, 1438, 1390, 1375, 1330, 1265, 1250, 1155, 1138, 1105, 1050, 1020, 1000, 970, 788 cm^{-1}; eims *m/z* (rel. int.) $[M]^+$ 248.1401 ($C_{15}H_{20}O_3$ requires 248.1412)

(57), 233 (44), 203 (72), 207 (50), 191 (30), 177 (32), 166 (34), 109 (64), 95 (92), 77 (64), 69 (100), 55 (78); ^{1}H nmr ($CDCl_3$, 400 MHz) see Table 2; ^{13}C nmr ($CDCl_3$, 100 MHz) see Table 2.

7-Ketoisodrimenin-5-ene [**3**].—White amorphous powder: [α]D +1.5 (*c*=0.84, $CHCl_3$); uv (MeOH) λ max 250, 275 sh nm; ir ν max 2912, 2875, 1768, 1680, 1650, 1581, 1568, 1462, 1445, 1362, 1330, 1270, 1162, 1145, 1088, 1025, 1000, 988, 975, 888, 788 cm^{-1}; eims *m/z* (rel. int.) $[M]^+$ 246.1258 (67) ($C_{15}H_{18}O_3$ requires 246.1256), 231 (30), 190 (45), 177 (100), 159 (30), 149 (28), 115 (28), 91 (43), 77 (32), 69 (70); ^{1}H nmr ($CDCl_3$, 270 MHz) see Table 3; ^{13}C nmr ($CDCl_3$, 100 MHz) see Table 3.

Aristolone [**4**].—Colorless needles (MeOH): mp 95–97° [lit. (10) 62–63°]; [α]D +252.0 (*c*= 2.32, $CHCl_3$); ^{13}C nmr ($CDCl_3$, 100 MHz) δ 196.3 (s, C-8), 167.6 (s, C-10), 124.2 (d, C-9), 39.5 (s, C-5), 39.1 (d, C-6), 38.6 (d, C-4), 35.5 (d, C-7), 33.1 (t, C-1), 30.5 (t, C-2), 29.7 (q, C-13), 26.1 (t, C-3), 24.3 (s, C-11), 22.5 (q, C-14), 16.7 (q, C-12), 16.3 (q, C-15).

Norpinguisanolide.—Clear oil: [α]D −132.1 (*c*=0.84, $CHCl_3$); ^{13}C nmr ($CDCl_3$, 100 MHz) δ 195.4 (s, C-4), 175.4 (s, C-12), 161.5 (s, C-6), 144.1 (d, C-11), 118.2 (s, C-5), 107.4 (d, C-10), 82.6 (d, C-2), 60.0 (s, C-8), 50.5 (s, C-9), 47.3 (d, C-1), 39.4 (t, C-3), 22.0 (q, C-14), 19.7 (t, C-7), 10.1 (q, C-13).

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