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NEW CYCLOARTANOL SULFATES FROM THE ALGA *TYDEMANIA EXPEDITIONIS:* INHIBITORS OF THE PROTEIN TYROSINE KINASE pp60$^{v\text{-}src}$

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ABSTRACT.—Bioactivity-directed fractionation of the extracts of the green alga *Tydemania expeditionis* using the protein tyrosine kinase pp60$^{v\text{-}src}$ led to the isolation of three new cycloartanol disulfates, **1–3**, which show modest inhibition of this enzyme. The structures were deduced by spectroscopic methods.

As part of an ongoing natural product screening program, we have identified three sulfated cycloartanol derivatives from *Tydemania expeditionis* as inhibitors of pp60$^{v\text{-}src}$, the oncogenic protein tyrosine kinase encoded by Rous sarcoma virus. Protein tyrosine kinases comprise a large family of enzymes that regulate cell growth and intracellular signalling pathways (1-4). Inhibitors of these enzymes may have utility in cancer and other hyperproliferative conditions (5); a number of small molecule tyrosine kinase inhibitors have been described, including several natural products (6).

RESULTS AND DISCUSSION

An earlier chemical investigation of the green alga *Tydemania expeditionis* collected in Guam resulted in the isolation of three new norcycloartenes and several conventional sterols (7). We were prompted to investigate *T. expeditionis* Weber van Bosse (Udoteaceae), when our screening program revealed that MeOH and MeOH-$CHCl_3$ extracts of a sample of this alga collected at Weno Island, Chuuk State, Federated States of Micronesia, inhibited pp60$^{v\text{-}src}$ protein tyrosine kinase (88% inhibition at 50 μg/ml).

A larger batch of *T. expeditionis* was collected, frozen for shipment, and later extracted extensively with MeOH and $CHCl_3$-MeOH (1:1). The combined concentrates of these extracts were partitioned between hexane and MeOH-H_2O (9:1); then the aq. MeOH layer was diluted to MeOH-H_2O (7:3) and extracted with $CHCl_3$. Upon partial evaporation, the aq. MeOH layer deposited substantial amounts of crystals which showed the best pp60$^{v\text{-}src}$ protein tyrosine kinase inhibition (IC_{50}=4 μg/ml) of the various fractions. That this mixture consisted of sulfated alcohols was indicated by the strong absorption it exhibited in the ir spectrum at 1224-1247 cm^{-1} (8) and the fact that the mixture could be solvolyzed to a corresponding mixture of alcohols. Resolution of this difficult-to-separate mixture of sulfated cycloartanols was achieved on reversed-phase hplc using MeOH-H_2O (7:3) containing 0.0025 M sodium sulfate.

The formula for the most abundant salt, **2**, was established by negative ion hr-fabms which showed a peak corresponding to M-Na^+ ($C_{30}H_{48}O_9S_2Na$). Several distinctive features of the ^{1}H-nmr spectrum immediately indicated that **2** possessed a cycloartanol skeleton: two mutually coupled one-proton doublet signals at δ 0.40 and 0.60 ppm appropriate for an isolated cyclopropyl methylene group; a double doublet at δ 4.46 (13, 5 Hz) corresponding to H-3 of a 4,4-disubstituted sterol, three methyl doublet signals, and three quaternary methyl resonances (see Table 1). The remaining methyl signal

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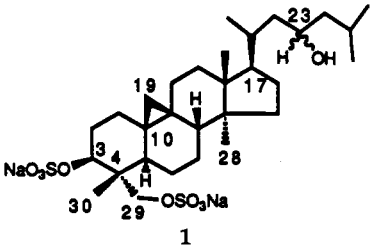

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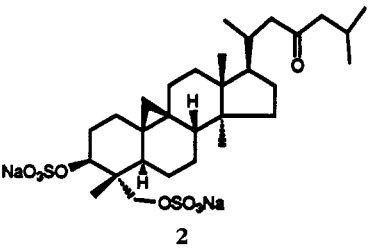

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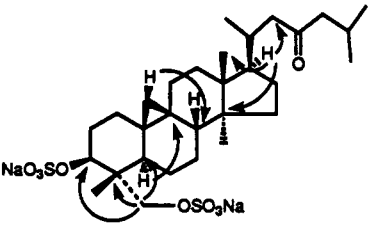
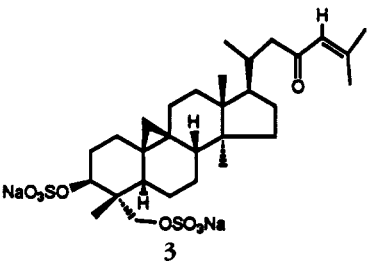


2 Selected HMBC Correlations



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expected of cycloartanol was accounted for by an isolated AB quartet (doublets at δ 3.78, 4.05, $J=11$ Hz) which corresponded to an oxygenated methylene group attached to a quaternary carbon. The previous report (7) describing norcycloartene derivatives from *T. expeditionis* strengthened the supposition that **2** was a cycloartanol derivative. Because only two ^{13}C-nmr signals corresponding to oxygenated sp^3 carbons were observed, eight of the oxygens were assigned to two sulfate groups. The remaining oxygen could be accounted for by a ketone group which was evidenced by a 1705 cm^{-1} absorption in the ir spectrum and a ^{13}C-nmr resonance at δ 214.1. That **2** possessed a cycloartanol skeleton was further supported by the correspondence of many ^{13}C-nmr signals (with multiplicities determined by a DEPT experiment) with those expected for cycloartanol (9). Results of difference decoupling, COSY and RCT nmr (10) experiments confirmed the H-3 to H-1 segment of **2**. More importantly, these data revealed that a sharp, two-proton doublet at δ 2.28 was coupled to a methine proton at δ 2.05, which in turn was coupled to two methyl doublets (δ 0.94, 0.96). This is consistent with a cycloartanol side-chain possessing a ketone at C-23, a feature also present in two of the norcycloartenes reported earlier (7). Through further analysis of the nmr correlation spectra a spin system corresponding to H-22→H-20→H-21 of a cycloartanol side-chain was identified: a pair of methylene protons (δ 2.49 and 2.16) coupled to a methine proton (δ 1.96) which was coupled to the remaining methyl doublet (δ 0.91).

All but two of the protonated carbon resonances could be assigned from the data of an HMQC experiment (11), and when this data was evaluated together with COSY and RCT nmr data many ^{1}H-nmr resonances could be assigned (see Table 1). With these data in hand the correlations from an HMBC experiment (12) led to extensive tracing of the carbon skeleton as shown in **2**. The HMBC data showed conclusively that the oxygenated methylene group was located at C-4.

The stereochemistry at C-4 was deduced from nOe and ^{13}C-nmr shift data. Irradiation of the H-29 proton at δ 3.78 enhanced one quaternary methyl signal (δ 0.89) and H-6 (eq) (δ 1.73). The H-6 (eq) signal was identified via its coupling to H-5 which, in turn, was assigned on the basis of the nOe noted for this signal upon irradiation of H-3 (ax). Due to the relatively high field position, δ 11.7, of the carbon signal correlated with the methyl resonance at δ 0.89, this methyl group was assigned the axial orientation (9). The remaining nOe observed was that of H-8 when the cyclopropyl proton at δ 0.60 was irradiated. Formula **2** is thus assigned to this cycloartanol disulfate.

TABLE 1. Nmr Data for **1–3**.

Pos. #	**1**				**2**				**3**
	^1H[a]	^{13}C[b,c]			^1H[a]	^{13}C[b,c]			^1H[a]
	δ	δ	Mult.[d]	HMBC Corr.	δ	δ	Mult.[d]	HMBC Corr.	δ
1	1.55 (m). 1.28 (m)	32.6	CH_2		1.55 (m). 1.28 (m)	32.6	CH_2		1.55 (m), 1.28 (m)
2	2.26 (m), 1.75 (m)	27.6	CH_2		2.25 (m), 1.77 (m)	27.5	CH_2		2.28 (m), 1.77 (m)
3	4.46 (dd, 13, 5)	80.8	CH	C-29	4.46 (dd, 13, 5)	80.8	CH		4.46 (m)
4		44.6	C			44.6	C		
5	1.83 (dd, 7, 2)	42.1	CH	C-9	1.83 (dd, 7, 2)	42.1	CH	C-9	-g
6	1.73 (m), 0.85 (m)	21.5	CH_2		1.73 (m), 0.80 (m)	21.5	CH_2		-g
7	1.55 (m), 1.24 (m)	29.2	CH_2	C-14	-g	29.3	CH_2		-g
8	1.52 (dd, 13, 5)[e]	49.6	CH	C-19, C-28	1.52 (dd, 13, 5)[e]	49.4	CH		-g
9		21.2	C			21.1	C		
10		26.6	C			26.6	C		
11	2.02 (m), 1.12 (m)	26.8	CH_2	2.02/C-19	2.05 (m), 1.15 (m)	26.8	CH_2	1.15/C-9	-g
12	1.26 (m.2H)	36.7	CH_2		1.32 (m, 2H)	36.6	CH_2	C-11, C-14, C-18	-g
13		46.7	C			46.6	C		
14		50.1	C			50.2	C		
15	2.04 (m), 1.70 (m)	34.3	CH_2		-g	34.1	CH_2		g
16	1.84 (m), 1.30 (m)	28.2	CH_2		1.95 (m), 1.30 (m)	28.2	CH_2		-g
17	1.53 (m)	54.5	CH	C-12	1.64 (m)	53.6	CH	C-14, C-18, C-20, C-22	-g
18	1.04 (s)	18.7	CH_3	C-14, C-17	1.10 (s)	18.6	CH_3		1.05 (s)
19	0.58 (d, 2.5), 0.39 (d, 2.5)	30.8	CH_2		0.60 (d, 2.5), 0.40 (d, 2.5)	30.7	CH_2	0.60/C-8	0.60 (d, 2.5), 0.40 (d, 2.5)
20	1.7 (m)	33.7	CH		1.96 (m)	34.0	CH		-g
21	0.91 (d, 7)	18.9	CH_3	C-17, C-20, C-22	0.91 (d, 7)	19.8	CH_3		0.91 (d, 7)
22	1.36 (m), 1.13 (m)	49.3	CH_2	C-20, C-23, C-24	2.49 (dd, 17, 2), 2.16 (dd, 17, 10)	51.6	CH_2	C-20, C-21, C-23	2.53 (dd, 9, 3), 2.29 (dd, 9, 3)
23	3.72 (m)	67.7	CH			214.1	C		
24	1.50 (m), 0.95 (m)	46.0	CH_2	C-22, C-23, C-26/27	2.28 (d, 2H, 7)	53.4	CH_2	C-23, C-25, C-26/27	6.18 (m, $w_{1/2}$=5 Hz)
25	1.30 (m)	25.8	CH		2.05 (m)	25.6	CH		
26	0.91 (d, 7)	23.6[f]	CH_3		0.96 (d, 7)	22.9[f]	CH_3		1.90 (s)
27	0.91 (d, 7)	22.7[f]	CH_3	C-24	0.94 (d, 7)	22.8[f]	CH_3		2.15 (s)
28	0.94 (s)	19.8	CH_3	C-14	0.99 (s)	19.9	CH_3		0.92 (s)
29	4.02 (d, 11), 3.76 (d, 11)	69.8	CH_2	C-3, C-4, C-5, C-9	4.05 (d, 11), 3.78 (d, 11)	69.8	CH_2	C-3, C-4, C-5	4.04 (d, 11), 3.79 (d, 11)
30	0.85 (s)	11.7	CH_3	C-4, C-5, C-29	0.89 (s)	11.7	CH_3		0.85 (s)

[a] ^1H -nmr spectra were obtained in CD_3OD at 500 MHz.
[b] ^{13}C-nmr spectra were obtained in CD_3OD at 125 and 75 MHz.
[c] Assignments made from HMQC data and by comparison with the ^{13}C-nmr spectrum of cycloartanol.
[d] Multiplicities determined from DEPT nmr spectrum at 75 MHz.
[e] From nOe experiment vs. H-19.
[f] Interchangeable.
[g] Not assigned, part of a complex signal.

The next most abundant component of the $pp60^{v\text{-}src}$ inhibitory mixture, **1**, was assigned the formula $C_{30}H_{50}O_9S_2Na$ based on negative-ion hr-fabms data (M-Na observed). The ir spectrum lacked any carbonyl absorption, but sulfate (1224-1247 cm^{-1}) and OH absorptions (3453 cm^{-1}) were present. The ^{1}H-nmr spectrum of **1** was very similar to that of **2** with the same distinctive signals for the cyclopropyl methylene group, the 3α-H, and the AB quartet for an oxygenated quaternary methylene group (see Table 1). The spectrum of **1** lacked the distinctive, sharp two-proton doublet corresponding to the H-24 protons in **2**, but possessed a broad one-proton multiplet at 3.72 ppm. These data all suggested that **1** was the 23-alcohol analog of **2** with stereochemistry at C-23 unspecified. Analysis of COSY, HMQC and HMBC nmr spectra supported this conclusion. In agreement with this proposed structure, oxidation of a 1 mg sample of **1** with Jones reagent yielded a product whose ^{1}H-nmr spectrum was identical to that of **2**. The stereochemistry at C-4 was assigned using the same experiments and arguments as for **2**. The proton and carbon assignments for **1** in Table 1 are based on analysis of a combination of results from COSY, DEPT, HMQC, and HMBC nmr experiments plus analogy to cycloartanol assignments (9).

The sulfated cycloartanol **3** was a minor component of this mixture. The highest mass ion observed in the lr-fabms was 637 mass units, just two mass units lower than that observed for $[M\text{-}Na]^+$ of the ketone **2**. The uv spectrum of **3** showed a maximum at 240 nm consistent with the presence of an α,β-unsaturated ketone. The ^{1}H-nmr spectrum showed the same absorptions as **1** and **2** for the cyclopropyl protons, H-3, and H-29 (see Table 1). In addition, the proton spectrum showed a broad, one-proton, olefinic signal at δ 6.1 (m, $w_{1/2}$ ca. 5 Hz) and two 3-proton singlets at δ 1.90 and 2.15 which corresponded closely with shifts reported for other sterols with a 23-keto-Δ^{24} side-chain (13) and one of the norcycloartenes reported by Paul *et al.* (7). The upfield region of the spectrum showed three singlet signals, δ 1.05, 0.92, 0.85, and only one doublet signal, δ 0.91. Based on these data, structure **3** was assigned.

Cycloartanol sulfates **1**, **2**, and **3** showed IC_{50}'s of 100, 32 and 39 μM in the $pp60^{v\text{-}src}$ assay. A small sample of the cycloartanol sulfate mixture was solvolyzed (14) to give the corresponding alcohol mixture. The mixture of alcohols showed no significant inhibitory activity against $pp60^{v\text{-}src}$.

EXPERIMENTAL

EXTRACTION AND ISOLATION OF CYCLOARTANOL SULFATES.—*Tydemania expeditionis* Weber van Bosse was collected at Weno Island, Chuuk State, Federated States of Micronesia in August of 1991 and 1992. A voucher sample (10-T-91) is stored at the University of Oklahoma.

Freshly thawed sample (2 kg wet wt) was extracted overnight with MeOH (2 liters) and then three times with 2 liters of $CHCl_3$-MeOH (1:1). The extracts were combined and concentrated at reduced pressure at ca. 35°. The aqueous concentrate was diluted with MeOH to make a 9:1 MeOH-H_2O solution (1 liter) and this was extracted three times with 1 liter of hexane. The combined hexane extracts weighed 5.6 g.

The 9:1-MeOH-H_2O solution was diluted with H_2O to 7:3 and this solution was extracted three times with $CHCl_3$ (1 liter). The $CHCl_3$ extract, 5.1 g (IC_{50} for $pp60^{v\text{-}src}$ = 47 μg/ml) was chromatographed over Si gel using gradient elution (hexane→EtOAc→CH_2Cl_2/MeOH→MeOH). Six fractions (ca. 150 ml each) were collected, the last of which (MeOH elution) showed an IC_{50} = 10 μg/ml vs. $pp60^{v\text{-}src}$. This fraction was resolved by vacuum flash chromatography (RP C_{18}) using MeOH-H_2O (7:3) as eluent to give several fractions with IC_{50}'s of 4.5-7.5 μg. These fractions contained the mixture of cycloartanol sulfates.

Upon slow evaporation, the 7:3 MeOH-H_2O solution that remained after $CHCl_3$ extraction deposited white crystals (IC_{50} = 4 μg/ml vs. $pp60^{v\text{-}src}$). These were recrystallized three times from MeOH. The recrystallized solid was comprised of nearly the same cycloartanol sulfate mixture (IC_{50} = 4 μg/ml) as was obtained by chromatography of the $CHCl_3$ extract as judged by nmr analysis. Initial attempts to resolve the cycloartanol mixture using either Si gel or C_{18} reversed-phase hplc and a variety of solvents were unsuccessful. Reversed-phase (C_{18}) hplc using MeOH-H_2O (3:1) containing 0.005 M tetrabutylammonium phosphate resolved the mixture, but difficulties were experienced in removing the buffer from the purified triterpene sulfates.

Most of the triterpenol sulfate mixture was resolved by reversed phase hplc using a 5 μm C_{18} column (300×10 mm; 1.8 ml/min; 3 mg/injection) and MeOH-H_2O (7:3) containing 0.0025 M Na_2SO_4 as eluent. Fractions A, B, and C corresponding to the peaks obtained in order of elution were collected. Using the same hplc system, these fractions were repurified to give, in >95% purity compounds **1** (from fraction A), **2** (from fraction C) and **3** (from fraction B). Sulfate buffer was removed by several triturations of the residue from evaporation of hplc fractions with minimum volumes of MeOH and finally by recrystallization via dissolution in MeOH and dilution with H_2O until the solution became cloudy. Yields were not calculated inasmuch as no effort was made to quantitatively isolate all the sulfate metabolites.

Cycloartan-3,23,29-triol 3,29-disodium sulfate [**1**].—White solid, 8 mg, mp 203–204° (dec); $[\alpha]_D$ +20.5° (*c*=0.002 g/ml); ir 3453 (OH), 1464, 1376, 1224–1247 (-OSO_3-) cm^{-1}. ^1H and ^{13}C nmr, see Table 1; neg. ion hr-fabms *m/z* $[M-Na]^-$ 641.2824, $C_{30}H_{50}O_9S_2Na$ requires 641.2794.

Cycloartan-3,29-diol-23-one 3,29-disodium sulfate [**2**].—White solid, 18 mg, mp 198–199° (dec); $[\alpha]_D$ +23.6° (c=0.005 g/ml); ir cm^{-1} 1705 (CO), 1465, 1374, 1221–1247 (-OSO_3^-); ^1H and ^{13}C nmr, see Table 1; neg. ion hr-fabms *m/z* $[M-Na]^-$ $C_{30}H_{48}O_9S_2Na$ requires 639.2637.

Cycloart-24-en-3,29-diol-23-one 3,29-disodium sulfate [**3**].—White solid, 2 mg, mp 230–232° (dec); uv λ max (MeOH), 240 nm (ε=8700); ^1H nmr, see Table 1; neg. ion lr-fabms, *m/z* $[M-Na]^+$ 637.2 $C_{30}H_{46}O_9S_2Na$ requires 637.

OXIDATION OF **1** TO **2**.—One drop of Jones reagent was added to a solution of 1 mg of **1** in 1 ml of Me_2CO containing 5 drops of H_2O at room temperature. After 5 min the reaction was quenched with several drops of MeOH, H_2O was added and the resulting mixture was extracted with EtOAc. The residue obtained by evaporation of the dried (Na_2SO_4) organic layer was purified by chromatography over a Si gel Sep-pack using MeOH as eluent. The white solid obtained was identical to **2** by ^1H-nmr analysis.

SOLVOLYSIS OF CYCLOARTANOL SULFATE MIXTURE.—Cycloartanol sulfate mixture (20 mg) was solvolyzed in 0.5 ml dioxane-pyridine (1:1) at 120° for 6 h (14). After cooling, H_2O (2.5 ml) was added and the solution was extracted with *n*-BuOH. The organic layer was washed with H_2O and evaporated under vacuum to give a mixture of cyloartanols (10 mg) which was characterized only by ^1H nmr (300 MHz, $CDCl_3$); δ 0.39, 0.60 (ea. d, cyclopropyl H's), 0.7-1.1 (Me signals), 2.22 (d, H-24), 3.53, 3.75 (AB quart., H-29), 3.7-3.9 (br m, H-3, H-24), 6.05 (v. sm. br s, H-24 olefinic H).

PROTEIN TYROSINE KINASE ASSAY.—The $pp60^{v\text{-}src}$ was purified from Rous sarcoma virus-transformed cells and used to screen extracts, fractions, and pure compounds as described previously (15).

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LITERATURE CITED

1. A. Veillette and J.B. Bolen, in: "Oncogenes." Ed. by C. Benz and E. Liu, Kluwer Academic Publishers, Boston, 1989, p. 121-142.
2. D.L. Cadena and G.N. Gill, *FASEB J.*, **6**, 2332 (1992).
3. A. Ullrich and J. Schlessinger, *Cell*, **61**, 203 (1990).
4. M.J. Pazin and L.T. Williams, *Trends Biochem. Sci.*, **17**, 374 (1992).
5. E.M. Dobrusin and D.W. Fry, *Ann. Rep. Med. Chem.*, **27**, 169 (1992).
6. T.R. Burke, *Drugs of the Future*, **17**, 119 (1992).
7. V.J. Paul, W. Fenical, S. Raffii, and J. Clardy, *Tetrahedron Lett.*, **23**, 3459 (1982).
8. J.R. Turvey, *Adv. Carbohydr. Chem.*, **20**, 183 (1965).
9. F.W. Wehrli and T. Nishida, in "Prog. Chem. Org. Nat. Prod." Ed. by W. Herz, H. Grisebach, and G.W. Kirby, Springer-Verlag, New York, 1979, vol. 36, p. 1.
10. P.H. Bolton *J. Magn. Reson.*, **48**, 336 (1982).
11. A. Bax and S. Subramanian, *J. Magn. Reson.*, **67**, 565 (1986).
12. A. Bax and M.F. Summers, *J. Am Chem. Soc.*, **108**, 209 (1986).
13. F.J. Schmitz, M.B. Kasebati, S.P. Gunasekera, and S. Agarwal, *J. Org. Chem.*, **53**, 5941 (1988).
14. R. Riccio, M.V. D'Auria, and L. Minale, *Tetrahedron*, **24**, 6041 (1985).
15. R.H. Lee, D.L. Slate, R. Moretti, K.A. Alvi, and P. Crews, *Biochem. Biophys. Res. Commun.*, **184**, 765 (1992).

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