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ANTINEOPLASTIC AGENTS, 195. ISOLATION AND STRUCTURE OF ACERATIOSIDE FROM *ACERATIUM MEGALOSPERMUM*[1]

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ABSTRACT.—A new tetralin glucoside [**1**], named aceratioside, has been found to be a weakly cytostatic (PS ED_{50} 9 μg/ml) constituent in leaves produced by the Australian rain forest tree *Aceratium megalospermum*. Structural determination of aceratioside was primarily accomplished with results from a series of acetylation, methylation, and high field nmr (including heteronuclear multiple bond correlation) experiments.

The largest (200 of 350 species) genus, *Elaeocarpus*, of the subtropical to tropical Elaeocarpaceae (2) contains the *Elaeocarpus* alkaloids (3), a series of indolizidines that have received principal phytochemical attention. Chemical constituents of the genus *Aceratium* (4) in this family have remained essentially unexplored. *Aceratium* species occur primarily in the rain forests of Papuasia (5), and their relative inaccessibility probably accounts for the lack of prior chemical investigation.

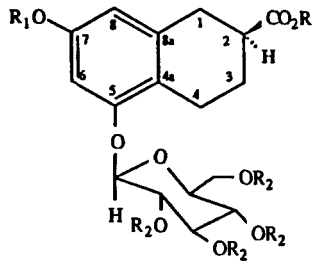
Early (1962) in the U.S. National Cancer Institute's (NCI) world-wide evaluation of plants with potential anticancer constituents, extracts of the Queensland tree (to 15 m high) *Aceratium megalospermum* (F. v. M.) von Balgooy (6) began to be evaluated, and in the period 1964–66 they were confirmed as active against the NCI KB cell line (ED_{50} 0.17 μg/ml) and Walker carcinosarcoma (72% tumor reduction at 22 mg/kg). This lead was pursued with a 1979 re-collection of *Ac. megalospermum* leaves and the NCI P-388 lymphocytic leukemia cell line (PS system).

A CH_2Cl_2-MeOH (1:1) extract of the leaves (55 kg) was successively partitioned between MeOH-H_2O (9:1→3:2) and hexane→CH_2Cl_2. The PS activity was found concentrated in the CH_2Cl_2 (ED_{50} 0.07 μg/ml) and residual MeOH/H_2O (ED_{50} 0.16 μg/ml) fractions. The more pronounced cell growth inhibitory activity of the former fraction was due to the presence of cucurbitacins; cf. Bittner *et al.* (7). The latter fraction was separated by Si gel chromatography to afford the cytostatic constituent, aceratioside [**1**], in 5.0×10^{-3}% yield, as a colorless powder (PS ED_{50} 9 μg/ml) from $Me_2CO/CHCl_3$.

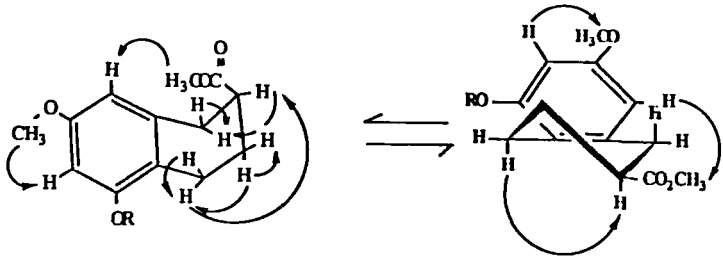
The high resolution fab mass spectrum of aceratioside [**1**] showed a molecular ion at *m/z* 371 $[M+H]^+$ corresponding to the molecular formula $C_{17}H_{22}O_9$ and suggested seven double bond equivalents. Uv and ir spectra indicated the presence of an aromatic ring system bearing a phenolic hydroxyl group (bathochromic shift by addition of NaOMe). The ir spectrum also exhibited absorption bands for hydroxyl groups (3446–3380 cm^{-1}), a carboxyl type carbonyl (1682 cm^{-1}) group, and an aromatic ring. The 400 MHz ^1H-nmr spectrum (Table 1) showed a complex pattern, and the proton spin systems required resolution employing 2D $^1H, ^1H$ COSY (8). The latter 2D data revealed structural segments consisting of meta-coupled aromatic protons, $ArCH_2CH_2CH(X)CH_2$-Ar (subunit A), and -OCH(O)-CH(O)-CH(O)-CH(O)-CH(O)(CH_2O)- (subunit B).

The ^{13}C-nmr spectrum (Table 2) of **1** exhibited signals for seventeen carbons, deduced to be three simple methylenes, an oxymethylene, a shielded methine, five oxymethines, two olefinic methines (one relatively shielded at 102.7 ppm), two olefinic quaternary carbons, two oxygenated quaternary carbons, and a shielded carbonyl car-

[1]For part 194, see Drexler *et al.* (1).



1 R=R_1=R_2=H
2 R=H, R_1=R_2=Ac
3 R=R_1=CH_3, R_2=H



4 **5**

R=glucose

bon. The proton-bearing carbons were correlated to the protons attached to them by interpreting a ^{1}H-^{13}C COSY (9,10) spectrum. For example, the meta-coupled proton appeared at δ 6.95 and was correlated to the carbon at δ 102.7, reminiscent of an aromatic carbon ortho to two oxygen-bearing carbons.

Acetylation of aceratioside yielded a pentaacetate **2**, and methylation (MeI and K_2CO_3 in refluxing Me_2CO) gave the methyl ether **3**. The aromatic methyl ether and methyl ester signals appeared at δ 3.85 and 3.77 and in the ir at 1726 cm^{-1}. These results clearly suggested that subunit A contained a carboxyl group and subunit B a trihydroxypyranose with a hydroxymethyl group. Because the aromatic ring contained only one free hydroxy group, the other aromatic ring bonded oxygen atom must form an ether linkage. These facts suggested a 1,2,3,4-tetrahydronaphthalene-2-carboxylic acid glycoside. Placement of both oxygen atoms in a meta orientation in the aromatic ring was achieved using nOe methods. Irradiation of the isolated proton triplet at δ 3.42 (H-1) in the ^{1}H-nmr spectrum of aceratioside [**1**] significantly enhanced the signal at δ 6.65 (H-8) and placed oxygens at C-5 and C-7 (meta).

Exact position of the glycoside linkage was resolved on the basis of nOe studies (see **4**) and application of 2D nmr heteronuclear multiple bond connectivity (HMBC) (11–13) methods. With the methylation product **3**, nOe irradiation of methoxy singlets at δ 3.85 and 3.77 ppm enhanced signals for the aromatic protons at δ 6.35 and 6.32 ppm, respectively. The observed nOe between the methyl group of the methyl ester and H-8 was explained by a partial boat conformation **4** for the cyclohexene ring, thereby forcing the methyl ester closer to the aromatic ring. In this conformation H-2 and H-4a appear in a quasi 1,3 diaxial relationship or even closer if the molecule resides in a semi chair conformation [**5**]. One likely explanation of the nOe results depends on the two semi boat/chair conformations **4** and **5** existing in equilibrium.

TABLE 1. ^{1}H-nmr Assignments for Aceratioside [**1**], Aceratioside Pentaacetate [**2**] and the Methyl Ether **3**.

Proton	Compound		
	1 (in $C_5D_5N + D_2O$)	**2** (in $CDCl_3$)	**3** (in $CDCl_3$)
H-1a	2.44, dt, 5.4, 12.6	2.30, m	2.39, dt, 4.4, 13
H-1b	3.42, dt, 5.4, 12.6	2.88, dt, 3.5, 10.2	3.02, dt, 4.6, 13
H-2	1.80, m	1.69, m	1.78, m
H-3a	1.75, m	1.78, m	1.87, m
H-3b	1.88, m	1.79, m	1.87, m
H-4a	2.29, dd, 2.3, 14.2	2.44, m	2.27, m
H-4b	2.54, dd, 5.3, 14.6	2.52, m	2.53, td, 4.2, 16
H-6	6.95, d, 2.8	6.78, d, 2.7	6.35, d, 2.8
H-8	6.65, d, 2.8	6.73, d, 2.7	6.32, d, 2.8
H-1′	4.81, d, 8	5.05, d, 6.8	4.30, d, 7.7
H-2′	4.20, dd, 8, 9	5.24, dd, 6.8, 8.6	3.63, t, 8.5
H-3′	4.14, dd, 9, 9	5.18, t, 8.6	3.53, t, 8.6
H-4′	3.84, dd, 9, 9	5.29, t, 10	3.51, t, 8.8
H-5′	4.10, ddd, 3, 9, 11	3.73, dt, 3.5, 10	3.65, m
H-6′a	4.77, dd, 11, 11	3.98, dd, 3.5, 12	4.49, dd, 12, 4
H-6′b	5.00, dd, 3, 11	4.40, dd, 3.5, 12	4.50, dd, 12, 12
Ac	—	2.02, 2.04, 2.07, 2.24, 2.29	—
OMe	—	—	3.77, 3.85
OH	—	—	2.40, 2.67, 4.84

The HMBC technique was used to assign (Table 2) all the carbon resonances. Strong and multiple correlation peaks were observed for all the protons two or three bonds away from carbon atoms. The H-1′ (δ 4.81) proton was correlated to aromatic ring C-4a (δ 138.8), C-3′ (74.6), and C-5′ (δ 78.0). Relationship of the carboxyl group (δ 173.3) to H-3 (δ 1.75, 1.88 ppm), H-2 (δ 1.80), and H-4 (δ 2.29, 2.54 ppm) was also established. Proton H-6 (δ 6.95) was related to C-4a, -5, -7, and -8, and H-8 with C-1, -4a, -6, and -7. For other connectivities refer to Table 2. Interestingly, whenever a proton was arranged in a W-type spatial relationship with a carbon, a four-bond-apart HMBC correlation was observed. The HMBC experiments allowed placement of the glycoside at C-5 and confirmed the substitution pattern of the aromatic ring and carboxylic acid at position C-2. The glycoside linkage was assigned β on the basis of the anomeric proton coupling constants for the doublet H-1′ in aceratioside [**1**] ($J = 8$ Hz), pentaacetate **2** ($J = 6.8$ Hz), and methyl ester **3** ($J = 7.7$ Hz). Acid hydrolysis of aceratioside led to the isolation of glucose and assignment of structure **1** to aceratioside.

The cd spectrum of methyl ester **3** in MeOH exhibited two relatively weak negative Cotton effect curves at λ max 230 and 270 nm due to the phenethyl chromophore (14). On the basis of direct comparison of the cd spectrum with that of the 1,2,3,4-tetrahydronaphthalene-(2*S* and 2*R*)-carboxylic acids (15), the absolute configuration of aceratioside [**1**] at C-2 was assigned *S*. [Cd spectra of 1,2,3,4-tetrahydronaphthalene-(2*S* and 2*R*)-carboxylic acids in MeOH are: 2*S* $[\alpha]_D$ −55°; ε (nm) 0 (280), −1.2 (270), 0 (250), 0 (225), −33.3 (210); 2*R*: $[\alpha]_D$ +28.5°; ε (nm) 0 (280), +0.7 (270), 0 (250), +34.4 (210).]

Certain tetralins have shown a variety of biological activities. For example, 6-substituted 1,2,3,4-tetrahydro-1-naphthoic acids have exhibited moderate anti-inflammatory activity (16) in mice. Other 1,2,3,4-tetrahydro-1-naphthoic acids have shown plant-growth-regulating activities (17), and 5,7-dihydroxy-2-aminotetralins have

TABLE 2. ^{13}C-nmr Assignments for Aceratioside [1] and Aceratioside Pentaacetate [2] and HMBC Correlations for Glucoside 1.

Carbon	Compound		
	1 (in C_5D_5N)	2 (in $CDCl_3$)	HMBC of 1 (in C_5D_5N)
C-1	31.2	29.1	H-1↦C-2, -3, -4a, -8, -8a
C-2	31.5	28.7	H-2↦C-3
C-3	25.8	25.6	H-3↦C-1, -4, -9
C-4	34.8	34.1	H-4↦C-2, -3, -9
C-4a	138.8	138.6	
C-5	152.4	146.8	
C-6	102.7	114.7	H-6↦C-4a, -5, -7, -8
C-7	156.7	143.1	
C-8	107.8	120.5	H-8↦C-1, -4a, -6, -7
C-8a	138.0	142.9	
C-9	173.3	173.5	
C-1′	108.4	100.5	H-1′↦C-4a, -3′, -5′
C-2′	75.4	72.2	H-2′↦C-1′, -5′
C-3′	74.6	71.0	H-3′↦C-1′, -2′, -4′
C-4′	73.7	68.7	H-4′↦C-3′, -5′, -6′
C-5′	78.0	73.1	H-5′↦C-6′, -2′, -4′
C-6′	64.7	60.6	H-6′↦C-5′
Ac	—	20.6(×3), 20.8 21.1, 168.2 169.0, 169.2 169.4, 170.3	

exhibited dopaminergic and adrenergic action in dogs (18). Further biological evaluation of aceratioside is under way.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Analtech Si gel GF (0.25 mm) plates were used for tlc and developed with either 3% ceric sulfate in 3 N H_2SO_4 spray and/or iodine vapor. Stationary phases used for gravity or flash cc were E. Merck (Darmstadt) Si gel (70–230 mesh for gravity and 40–63 mesh for flash), Whatman Si gel LPS-1 (13–24 mesh), or Sephadex LH-20 (Pharmacia Fine Chemicals AB, Uppsala, Sweden).

Melting points were observed with a Kofler-type hot stage apparatus. Optical rotation values were recorded using a Perkin-Elmer 241 polarimeter. The uv spectra were obtained in MeOH solution with a Hewlett-Packard 8450A UV/vis spectrophotometer. A Nicolett MX-1 FT spectrophotometer was employed for ir measurements. TMS, residual $CHCl_3$ (7.256 ppm), or CH_2Cl_2 (5.32 ppm) was used as an internal reference in all nmr experiments, which were determined with Bruker AM 400 (^1H, ^{13}C), or WH 90 (^1H) instruments. Chemical shifts are recorded in ppm. $CDCl_3$ was used as nmr solvent unless otherwise mentioned. The hreims and sp-sims (fabms) were recorded with a Kratos MS 50 instrument in the NSF regional mass spectrometry facility at the University of Nebraska.

PLANT MATERIAL.—The leaves of *Ac. megalospermum*, earlier (1875) classified as *Aristotelia megalosperma*, were collected in the rain forests of Queensland, Australia in 1979 as part of the NCI-USDA programs directed by Drs. John L. Hartwell, Matthew Suffness, and J. Duke (USDA). The plant was assigned NCI B631963, and a herbarium specimen is maintained by the USDA, Beltsville, Maryland.

ISOLATION OF ACERATIOSIDE [1] (7-HYDROXY-1,2,3,4-TETRAHYDRONAPHTHALENE-2-CARBOXYLIC ACID-5-β-D-GLUCOSIDE].—Dried leaves (50 kg) of *Ac. megalospermum* were extracted at ambient temperature with 400 liters of CH_2Cl_2-MeOH (1:1), and the extract was converted to a CH_2Cl_2 fraction (2.07 kg, PS ED_{50} 0.3 μg/ml) by addition of H_2O (100 liters). The CH_2Cl_2 fraction (1.55 kg) was dissolved in 8 liters of MeOH-H_2O (9:1) and extracted with hexane (4 × 4 liters). The MeOH-H_2O phase was adjusted to 3:2 by addition of H_2O and extracted with CH_2Cl_2 (3 × 4 liters) to give CH_2Cl_2 (358 g, PS

ED_{50} 0.07 μg/ml) and residual aqueous fractions (93 g, PS ED_{50} 0.16 μg/ml, PS T/C 121 at 25 mg/kg). An 11.8-g aliquot of the latter fraction was chromatographed on a column of Si gel employing gradient elution with CH_2Cl_2-MeOH (49: 1→17:3) to give an active fraction that crystallized from $Me_2CO/CHCl_3$ to furnish aceratioside [**1**] (0.32 g, 0.005% yield) as a buff colored powder: mp 273–275°, R_f 0.35 [Si gel, CH_2Cl-MeOH (9:1)]; $[\alpha]_D + 10°$ ($c = 1.2$, MeOH); hr fabms *m/z* $[M+H]^+$ 371.1366 (100%) for $C_{17}H_{23}O_9$ (calcd 371.1341); eims *m/z* $[M]^+$ 370 (8%), $[M-H_2O]^+$ 352 (5%), $[M-2H_2O]^+$ 334 (10), $[M-\text{glucose}+H]^+$ 208 (73), $[M-\text{glucose}-CO]^+$ 180 (100), $[M-\text{aglycone}]^+$ 163 (24), $[M-\text{glucose}-2\times CO]^+$ 152 (30); uv (MeOH) λ max 226 (ε 5124), 281 (2032); uv (MeOH + $NaOCH_3$) λ max 230 (ε 5442), 235 (5255), 290 (3097); ir (KBr) ν max 3446, 3380, 1682, 1607, 1492, 1343, 1165, 1147, 1084, 1071, 1054, 1037 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2. *Anal.* calcd for $C_{17}H_{22}O_9 \cdot 1.5\ H_2O$: C 51.38, H 6.09; found C 50.88, H 5.83.

ACERATIOSIDE PENTAACETATE [**2**].—A solution of glycoside **1** (50 mg) in pyridine/Ac_2O (1 ml each) was acetylated at room temperature (overnight). After addition of EtOH, the solvent was evaporated at reduced pressure. The pentaacetate (70 mg) crystallized from EtOH as an amorphous powder: mp 189–191°; R_f 0.2 or 0.72 [Si gel, hexane-EtOAc (9:5 or 1:1)]; $[\alpha]_D - 10°$ ($c = 1.2$, MeOH); hreims *m/z* $[M]^+$ 580.1886 (2%) (calcd for $C_{27}H_{32}O_{14}$, 580.1792), $[M-2\times Ac-OHAc]^+$ 436.1356 (6%) (calcd for $C_{21}H_{24}O_{10}$, 436.1370), $[C_{21}H_{22}O_9]^+$ 418.1281 (14), $[M-2\times OHAc-2\times Ac]^+$ 376.1144 (16), $[C_{17}H_{18}O_7]^+$ 334.1046 (7), $[C_{15}H_{16}O_6]^+$ 292.0948 (20), $[C_{13}H_{14}O_5]^+$ 250.0833 (80), $[C_{11}H_{12}O_4]^+$ 208.0739 (100), $[C_{10}H_{12}O_3]^+$ 180.0789 (61), $[C_8H_8O_3]^+$ 152.0474 (34); ir (NaCl) 1757, 1476, 1370, 1214, 1199, 1069, 1035 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2.

METHYLATION OF ACERATIOSIDE.—To a solution of aceratioside [**1**] (11.3 mg) in anhydrous Me_2CO (3 ml) was added anhydrous K_2CO_3 (50 mg) and MeI (1 ml). The mixture was heated at reflux for 3 h. The K_2CO_3 was removed by filtering the solution, and the filtrate was purified by chromatographic separation on a small Si gel column. Elution with EtOAc-hexane-MeOH (9:1:1) provided methyl ester **3** (8 mg) as an amorphous powder: mp 208–210°, R_f 0.32 [Si gel, hexane-CH_2Cl_2-MeOH-H_2O (10:80:10:1)], hreims *m/z* $[M]^+$ 398.1575 (3%) (calcd for $C_{19}H_{26}O_9$, 398.1577), $[C_{13}H_{16}O_4]^+$ 236.1047 (21); cd (MeOH) ● (nm) 0 (283), −1.0 (270), 0 (256), 0 (240), −7.6 (230), 0 (220); ir (NaCl) 3500, 1726, 1598, 1492, 1450, 1350, 1210, 1095, 1075, 1055, 1040 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2.

HYDROLYSIS OF ACERATIOSIDE.—The glucoside **1** (14.3 mg) was heated in a refluxing solution of 1 N HCl-MeOH (1:1) (5 ml) for 24 h. EtOAc (15 ml) was added, and the EtOAc phase was washed with H_2O (2 × 5 ml) and dried (anhydrous Na_2SO_4). Solvent was removed from both phases. The EtOAc extract was found to be a complex mixture, whereas the aqueous phase yielded glucose [identified by direct comparison with an authentic specimen using tlc on a cellulose plate with pyridine-EtOAc-HOAc-H_2O (5:5:1:3) as mobile phase and by ^1H-nmr comparison]. The pentaacetate derivative was also found to be identical with an authentic sample of glucose pentaacetate by the same procedures.

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