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ACID METABOLITES FROM *SPIROSTACHYS AFRICANA*

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Key Word Index—*Spirostachys africana*; Euphorbiaceae; heartwood; diterpene acids.

Abstract—Two acid metabolites, *ent*-2,3-seco-beyer-15-ene-2,3-dioic acid and *ent*-2,4-seco-4-oxo-norbeyer-15-en-2-oic acid, have been isolated and identified by spectroscopic methods. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Our previous reports [1, 2] have dealt with the non acidic components of *Spirostachys africana*. We now report the isolation and identification of two acids. Baarschers *et al.* [3] resolved the latex of the plant into acidic and non acidic components by partitioning between hexane and potassium hydroxide solution, but did not report any compounds from the acidic fraction. We carried out a similar partitioning between ethyl acetate and sodium carbonate and found that eleven percent of the latex readily extracted into the carbonate layer.

RESULTS AND DISCUSSION

Two crystalline acids were obtained on subjection of acidic latex to chromatography and recrystallisation. In their IR, both compounds showed broad band hydroxyl absorptions consistent with acidic compounds. Both acids when boiled in acetic anhydride gave dehydration products. In the presence of diazomethane, both gave methyl ester derivatives. The acids and their derivatives had the beyerenic 15,16-ene system (ca 750 cm^{-1}, two doublets between δ 5.5 and 6.0 in the ^{1}H NMR spectrum and two peaks between δ 130 and 140 in the ^{13}C NMR spectrum). Rings B, C, and D were present in all these compounds as deduced from comparing their ^{13}C NMR data in Table 1 to those in the literature [1, 2, 4].

By comparison to the documented melting point, IR spectrum and reactions [3], acid **1** was identified as the previously prepared *ent*-2,3-seco-beyer-15-ene-2,3-dioic acid. It had two carbonyl peaks (1720 and 1690 cm^{-1}; δ 176.3 and 187.9) and readily gave a seven-membered ring anhydride **1a** when boiled in acetic anhydride. Diagnostic in its ^{1}H NMR spectrum were protons at C-1 which appeared as doublets at δ 2.38 and 2.79 with a large coupling constant (J = 19.6 Hz). In the HR mass spectrum acid **1** gave a fragment of molecular mass 316.2038 for $C_{20}H_{34}O_4$-H_2O identical to the HR mass spectrum of the anhydride **1a** (316.2056 for $C_{20}H_{32}O_3$). The dimethyl ester **1b** had data consistent with a dimethyl derivative of acid **1**. Acid **1** is reported as a natural product for the first time.

Table 1. ^{13}C NMR data of acids and derivatives (δ) in $CDCl_3$

Carbon	1	1a	1b	2	2a	2b	2c
1	45.6	46.4	40.3	42.5	43.8	45.4	41.9
2	179.3	176.7	172.0	174.4	168.9	167.8	167.8
3	187.9	165.6	179.9				
4	39.5	39.4	46.2	216.1	140.3	157.9	157.9
5	44.7	46.6	44.6	44.8	118.0	34.8	44.8
6	20.4	19.9	20.6	23.1	22.1	21.5	20.3
7	36.8	36.7	36.4	35.1	34.5	34.5	35.6
8	48.8	49.1	49.0	48.7	48.8	48.4	48.9
9	48.9	53.2	49.2	57.0	49.3	49.0	56.4
10	41.2	41.4	41.5	40.1	36.9	43.8	39.5
11	20.8	20.7	22.1	20.4	21.3	20.7	23.2
12	33.0	32.5	33.2	32.7	32.5	32.3	32.9
13	43.5	43.8	43.5	43.8	44.0	45.2	43.8
14	61.1	60.4	60.7	60.5	60.4	60.5	60.8
15	137.1	137.8	137.0	137.8	138.1	137.8	137.2
16	135.2	133.8	134.4	133.6	132.4	133.1	134.1
17	24.7	24.7	24.8	24.7	24.7	24.7	24.7
18	29.6	30.4	28.1	18.0	20.3	93.1	31.5
19	21.7	19.8	23.9				
20	19.6	18.9	18.8	30.6	15.5	14.9	17.5

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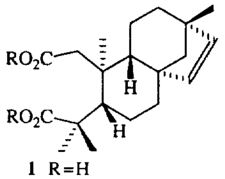
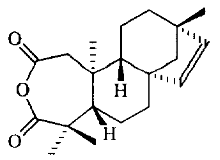
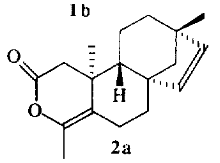


1 R=H

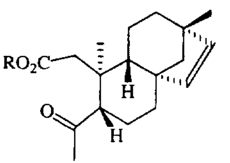
1b R= Me



1b

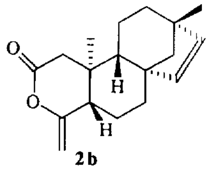


2a



2 R=H

2c R=Me



2b

The HR mass spectrum of acid **2** gave an ion at *m/z* 290.1861 consistent with the formula $C_{18}H_{26}O_3$. ^1H NMR signals at δ 2.44 and 2.45 were assigned to the C-1 protons. The three proton signal at δ 2.25 was attributed to the methyl ketone. The peaks at δ 216.1 and 174.4 in the ^{13}C NMR spectrum were assigned to ketonic and acidic carbonyls, respectively. The rest of the data bore close resemblance to those of acid **1**. On boiling acid **2** in acetic anhydride, two dehydration products in the ratio 7.5 : 1 were obtained with formula $C_{18}H_{24}O_2$ (M^+ at *m/z* 272). The major product, **2a**, showed an enolic methyl at δ 1.89 with C-1 protons at δ 2.22 and 2.66. Additional olefinic carbons were observed at δ 118 and 140.3. Compound **2b** had a terminal methylene as confirmed by narrowly split signals at δ 4.30 and 4.74 in the ^1H NMR spectrum and the peaks at δ 157.9 and 93.1 in the ^{13}C NMR spectrum. Acid **2** was a monoacid as further deduced from the methyl ester **2c**. Acid **2** is *ent*-2,4-seco-4-oxo-norbeyer-15-en-2-oic acid.

EXPERIMENTAL

^1H and ^{13}C NMR: 200 and 50 MHz, 300 and 75 MHz, respectively; COSY and NOESY at 500 MHz; $CDCl_3$ sols with TMS as int. standard. VLC: silica gel 60 G_{254}, visualisation by UV at 254 nm and vanillin–H_2SO_4. Optical rotations were measured in $CHCl_3$, unless otherwise stated.

The acid extract (15.6 g) was chromatographed using vacuum liquid chromatography (VLC) on silica gel (150 g) eluting with a mixture of hexane and EtOAc (4 : 1) and collecting 200 ml frs. Further flash column chromatography (FCC) of pooled frs 1 to 5 followed by recrystallisation gave acids **1** and **2**. Acid **1** recrystallised more readily.

ent-2,3-Seco-beyer-15-ene-2,3-dioic acid (**1**). Recrystallised from hexane, mp 211.212° (lit. 212° [3]), $[\alpha]_D$ 21° (c 1.66) (lit. −25 [3]). IR ν_{max}^{KBr} cm^{-1} 2925 *br* (OH), 1720, 1691 (CO) 753. HRMS *m/z*: 316.2066 $[M-H_2O]^+$ (calcd $C_{20}H_{30}O_4$–H_2O, 316.2038). ^1H NMR: δ 0.85 (3H, *s*, H-20), 0.99 (3H, *s*, H-17), 1.06–1.81 (9H, *m*, others), 1.09 (1H, *d*, J = 9.4 Hz, H-14a), 1.15 (3H, *s*, H-19), 1.22 (3H, *s*, H-18) 2.06 (1H, *d*, J = 12.4 Hz, H-9), 2.38 (1H, *d*, J = 19.6 Hz, H-1′), 2.60 (1H, *d*, J = 9.4 Hz, H-5), 2.79 (1H, *d*, J = 19.6 Hz, H-1), 5.48 (1H, *d*, J = 5.6 Hz, H-16), 5.64 (1H, *d*, J = 5.6 Hz, H-15) and 12.37 (2H, *br*, OH).

ent-2,3-Seco-beyer-15-ene-2,3-dioic anhydride (**1a**). Compound **1** was boiled in Ac_2O for 2 h. Usual work-up and recrystallisation from hexane gave **1a** mp 133–134° (lit. 123–124° [3]), $[\alpha]_D$ −26.70 (c 0.75). IR ν_{max}^{KBr} cm^{-1}: 1800, 1757 (CO), 752. HRMS *m/z*: 316.2056 $[M]^+$ (calcd $C_{20}H_{30}O_4$, 316.2038). ^1H NMR: δ 0.95 (3H, *s*, H-20), 1.03 (3H, *s*, H-17), 1.14 (1H, *d*, J = 9.6 Hz, H-14a), 1.28 (3H, *s*, H-19), 1.37 (3H, *s*, H-18), 1.37–1.75 (*m*, others), 2.21 (1H, *d*, J = 13.6 Hz, H-1′), 2.76 (1H, *d*, J = 13.6 Hz, H-1), 5.53 (1H, *d*, J = 5.7 Hz, H-16) and 5.63 (1H, *d*, J = 5.7 Hz, H-15).

Dimethyl ent-2,3-seco-beyer-15-ene-2,3-dioate (**1b**). Syrup, $[\alpha]_D$ +27 (c 2.96). IR ν_{max}^{KBr} cm^{-1}: 1744, 1728 (ester CO), and 753. EIMS (probe) 70 eV, *m/z* (rel. int.): 362 (63) $[M]^+$, 331 (24) $[M-OMe]^+$, 289 (58) $[M-73]^+$, and 187 (100) $[M-175]^+$. ^1H NMR: δ 0.99 (3H, *s*, H-17), 1.08 (1H, *d*, J = 9.4 Hz, H-14a), 1.2–1.7 (*m*, others), 1.21 (3H, *s*, H-18), 1.23 (3H, *s*, H-19), 2.02 (1H, *dd*, J = 4.7 and 10.9 Hz, H-9), 2.25 (1H, *d*, J = 18.7 Hz, H-1′), 2.37 (1H, *d*, J = 18.7 Hz, H-1), 2.44 (1H, *dd*, J = 3.9 and 10.8 Hz, H-5), 3.61 (3H, *s*, OMe), 3.65 (3H, *s*, OMe), 5.47 (1H, *d*, J = 5.6 Hz, H-16), and 5.66 (1H, *d*, J = 5.6 Hz, H-15).

ent-2,4-Seco-4-oxo-3,19-norbeyer-15-en-2-oic acid (**2**). Recrystallised from hexane (2.6 g), mp 61–62°, $[\alpha]_D$ +2.80 (c 2.12). IR ν_{max}^{KBr} cm^{-1}: 3428 (OH), 1729, 1701 (CO), 750. HRMS *m/z*: 290.1861 $[M]^+$ (calcd $C_{18}H_{26}O_3$, 290.1882). ^1H NMR: δ 0.93 (3H, *s*, H-20), 1.02 (3H, *s*, H-17), 1.12 (1H, *d*, J = 9.7 Hz, H-14a), 1.33–1.97 (6H, *m*, H-6, H-7, H-9, H-11, H-12, and H-14e), 2.24 (1H, *d*, J = 13.9 Hz, H-1′), 2.25 (3H, *s*, H-18), 2.45 (1H, *d*, J = 13.9 Hz, H-1), 2.77 (1H, *dd*, J = 3.3 Hz, 11.9 Hz, H-5), 5.55 (1H, *d*, J = 5.7 Hz, H-16) and 5.71 (1H, *d*, J = 5.7 Hz, H-15).

ent-2,4-Seco-3,19-norbeyer-4(5), 15-diene-2, 4-lactone (**2a**) *and ent-2,4-Seco-3,19-norbeyer-4 (5), 15-diene-2, 4-lactone* (**2b**). Compound **2** (485 mg) was boiled in Ac_2O (10 ml) for 2 h. Usual work-up followed by CC eluting with a mixture of hexane and EtOAc gave lactones **2a** (345 mg) and **2b** (46 mg). *Lactone* **2a** was recrystallised from MeOH and H_2O, mp 69–70°, $[\alpha]_D$ +4.5 (c 5.5). IR ν_{max}^{KBr} cm^{-1}: 1745 (CO), 754. HRMS *m/z*: 272.1781 $[M]^+$ (calcd $C_{18}H_{24}O_2$, 272.1776). ^1H NMR: δ 0.91 (3H, *d*, J = 0.6 Hz, H-20), 1.04 (3H, *s*, H-17), 1.07 (1H, *d*, J = 9.8 Hz, H-14a), 1.21 (1H, *dd*, J = 5.1 Hz, 11.4 Hz, H-9), 1.25–1.40 (2H, *m*, H-12e and H-12a), 1.38 (1H, *dt*, J = 3.8 Hz, 3.8 Hz, 13.1 Hz, H-7a), 1.47 (1H, *m*, H-11e), 1.56 (1H, *dd*, J = 2.5 Hz, 9.8 Hz, H-14e), 1.65 (1H, *dt*, J = 3.8 Hz, 3.8 Hz, 13.2 Hz, H-7e), 2.15 (1H, *br tq*, J = 1.9 Hz, 13.2 Hz, 15.1 Hz, H-6a), 2.24 (1H, *bd*, J = 13.9 Hz, H-1a), 2.25 (3H, *s*, H-18), 2.37 (1H, *dt*,

$J = 33.9$ Hz, 3.9 Hz, 14.7 Hz, H-6e), 2.45 (1H, *d*, $J = 13.9$ Hz, H-1e), 2.76 (1H, *dd*, $J = 3.5$ Hz, 12.0 Hz, H-5), 5.55 (1H, *d*, $J = 5.8$ Hz, H-16), and 5.71 (1H, *d*, $J = 5.8$ Hz, H-15). *Lactone* **2b** was recrystallised from hexane, mp 114–115°, $[\alpha]_D$ $-65°$ (c 0.65). IR ν_{max}^{KBr} cm^{-1}: 1757 (CO), 1647 (C=C) 833, 756. HRMS *m/z*: 272.1813 $[M]^+$ (calcd $C_{18}H_{24}O_2$, 272.1776). ^{1}H NMR: 0.77 (3H, *d*, $J = 0.9$ Hz, H-20), 1.04 (3H, *s*, H-17), 1.09 (1H, *d*, $J = 10.5$ Hz, H-14a), 1.12–1.82 (9H, *m*, others), 2.14 (1H, *bd*, $J = 10$ Hz, H-5), 2.22 (1H, *d*, $J = 17.7$ Hz, H-1′), 2.66 (1H, *d*, $J = 17.7$ Hz, H-1), 4.30 (1H, *t*, $J = 1.8$ Hz and 1.5 Hz, H-18′), 4.74 (1H, *t*, $J = 1.8$ Hz and 1.5 Hz, H-18), 5.56 (1H, *d*, $J = 5.6$ Hz, H-16) and 5.68 (1H, *d*, $J = 5.6$ Hz, H-15).

Methyl ent-2,4-seco-4-oxo-3,19-norbeyer-15-en-2-oate (**2c**). Syrup, $[\alpha]_D$ -2.6 (c 0.0232). IR ν_{max}^{KBr} cm^{-1}: 1732 (ester CO), 1708 (ketone CO) and 754. EIMS (probe) 70 eV, *m/z* (rel. int.): 288 (21) $[M]^+$, 272 (24) $[M-16]^+$, 231 (100) $[M-57]^+$, 187 (89) $[M-101]^+$ and 159 (50) $[M-129]^+$. ^{1}H NMR: δ 0.97 (3H, *s*, H-20), 1.01 (3H, *s*, H-17), 1.08 (1H, *d*, $J = 10.3$ Hz, H-14a), 1.24–1.82 (*m*, others), 2.21 (3H, *s*, H-18), 2.28 (1H, *d*, $J = 14.1$ Hz, H-1′), 2.38 (1H, *d*, $J = 14.1$ Hz, H-1), 2.96 (1H, *dd*, $J = 2.8$ and 12.2 Hz, H-5), 3.68 (3H, *s*, OMe), 5.50 (1H, *d*, $J = 5.5$ Hz, H-16) and 5.69 (1H, *d*, $J = 5.5$ Hz, H-15).

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