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JESSIC ACID AND RELATED ACID TRITERPENOIDS FROM *COMBRETUM ELAEAGNOIDES*

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Key Word Index—*Combretum elaeagnoides*; Combretaceae; acid triterpenoids; jessic acid; α-L-arabinopyranoside

Abstract—A novel triterpenoid acid, jessic acid, was extracted from the leaves of *Combretum elaeagnoides*, where it was found together with its methyl ester and its α-L-arabinopyranoside, all three compounds occurring in significantly large amounts. Jessic acid is 1α,3β-dihydroxy-23-oxo-24-methylenecycloartan-30-oic acid.

INTRODUCTION

Previous work has described the isolation of mollic acid (**1**) and its β-D-glucoside from the leaves of *Combretum molle* [1]. As part of a continuing investigation into extractives from the Combretaceae, we now wish to report on a related novel triterpenoid acid, given the trivial name 'jessic acid', and congeneric compounds isolated from the leaves of *Combretum elaeagnoides*.

RESULTS AND DISCUSSION

The major component in the ether extract from air-dried, milled leaves of *C. elaeagnoides* was readily crystallized from petrol–ethyl acetate to give jessic acid (**2**), obtained in a yield of 0.17% from the dry leaf material. UV and IR spectra of **2** indicated the presence of an α,β-unsaturated ketone, while spot tests confirmed unsaturation and gave evidence of triterpenoid character and an acid function. The latter was confirmed by the preparation of a monomethyl ester (**3**) which could be acetylated to give methyl jessate diacetate (**4**). The ^{1}H NMR spectrum ($CDCl_3$) of **4** showed two doublets at δ 0.50 and 0.83 (each J = 4 Hz), characteristic of a methylene group in a cyclopropyl ring system. Additionally, two apparent singlets at 5.90 and 5.63 corresponds to terminal methylene protons while a double doublet at 5.47 and an apparent triplet at 4.62 are acetoxymethyne proton signals. The double doublet at 5.47 was ascribed to a 3α-axial proton in view of the coupling constants ($J_{3ax,2ax}$ = 11 Hz, $J_{3ax,2eq}$ = 5 Hz). This therefore places one of the acetoxy groups at a 3β position, as would be expected on biosynthetic grounds. The upfield position of the remaining acetoxymethyne proton signal (δ 4.62) is the result of its proximity to the cyclopropyl ring system, and thus places this proton at C-1 or C-11, the former position being selected in view of subsequent evidence and relating to the previously published structure of **1** confirmed by direct crystal structure determination of its 3-β-D-glucoside [Laing, M., personal communication].

The ^{13}C NMR spectral signals of **2** and **4** closely coincide with those of **1** and its methyl ester diacetate

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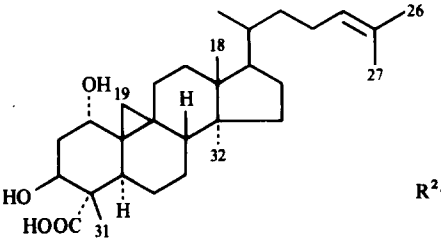

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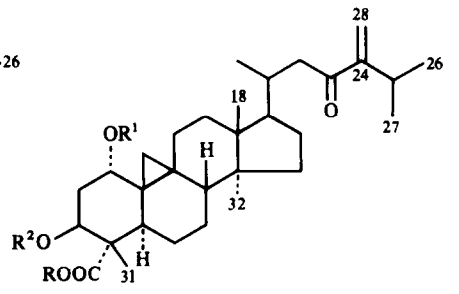

2 R = R¹ = R² = H
3 R = Me, R¹ = R² = H
4 R = Me, R¹ = R² = Ac
5 R = R¹ = R³ = H, R² = Ara
6 R = H, R¹ = R³ = Ac, R² = Ara (Ac)$_3$

respectively in corresponding solvents for carbons 1–19, as do their significant ^{1}H NMR signals in these two identical cycloartane nuclear systems. Thus the only structural difference between mollic acid (**1**) and jessic acid (**2**) is in the side chain. The side chain of **2**, **3**, **5** and their acetates therefore contains the terminal methylene group and the carbonyl function in conjugation. Since the accurate mass determination on **3** provides for the formula $C_{32}H_{50}O_5$, the side chain contains one carbon additional to that of the mollic acid compounds. The 500 MHz ^{1}H NMR spectrum of **3** shows a single proton septet at δ 2.88 which exhibits allylic and long range *W* coupling (J = 1.25 Hz) to the terminal methylene proton *cis* to the carbonyl group. This signal must therefore be due to a C-25 isopropyl proton which places the exo-methylene group at the biosynthetically expected C-24 position [2]. Hence the carbonyl oxygen must be at C-23 providing a side chain identical to that of 4α-methyl-3β,8β-dihydroxy-5α-ergost-24(28)-en-23-one, a compound isolated from the soft coral *Litophyton viridis* [3]. To our knowledge such a side chain has not previously been reported in plant triterpenoids. Compound **2** is therefore 1α,3β-dihydroxy-23-oxo-24-methylenecycloartan-30-oic acid, or 'jessic acid', the trivial name being derived from the 'jesse bush' vegetational type to which *C. elaeagnoides* is a major contributor in Zimbabwe.

Apart from the preparation of methyl jessate (**3**) by conventional methylation procedures, **3** was also the major product (TLC and all spectral data) in the petrol extract of the leaf material, being obtained at a level of 0.66%. Compound **3** was hydrolysed back to its parent acid (**2**) by refluxing in 10% ethanolic potassium hydroxide. This indicates an equatorially-orientated carboxy function since the corresponding axial methyl esters require substantially longer for complete hydrolysis under similar conditions [4], and hence a C-30 assignment for the acid group is validated. Both **2** and **4** hydrogenated readily, taking up one equivalent of hydrogen to give the corresponding 24,28-dihydrocompounds with expected spectral characteristics.

The acetone extract of *C. elaeagnoides* leaves afforded jessic acid α-L-arabinopyranoside (**5**) which crystallized from ethanol, obtained in a yield of 0.90% from the dry leaf material. Spot tests indicated the presence of an unsaturated, acidic, triterpenoid glycoside. The aqueous fraction after hydrolysis (HCl) of **5** gave only arabinose (PC and TLC). In a comparison of the molecular rotations of **5** ($[M_D]_{py}$ +332.4°) and **2** ($[M_D]_{py}$ +277.5°), a difference ($\Delta[M_D]$ +54.9°) is obtained which is more consistent with the rotation of an α-L-arabinopyranoside (lit. $[M_D]_{aq}$ of methyl-α-L-arabinopyranoside + 28.4° [5] than with its β-anomer (lit. $[M_D]_{aq}$ of methyl-β-L-arabinopyranoside + 402.6°) or with the corresponding D-glycosides. The ^{1}H NMR spectrum (C_5D_5N) of **5** gave clear evidence of an anomeric proton (δ 4.50, *d*, J = 7 Hz) consistent with an α-equatorial orientation of the L-arabinopyranoside linkage; for example, in a study by Kitagawa *et al.* [6] methyl-3-*O*-α-L-arabinopyranosyl-oleanolate triacetate and its β-anomer gave anomeric proton ^{1}H NMR shift values (C_5D_5N) of δ 4.45 (J = 7 Hz) and 5.23 (J = 3 Hz) respectively. The ^{13}C NMR spectrum (C_5D_5N) of **5** shows five shift signals additional to those of the aglycone (**2**); these are individually ascribed to the C atoms of an α-L-arabinopyranoside moeity in accordance with published data [7, 8]. In the ^{13}C NMR spectrum of **5** apart from the signals arising from C-3, immediately adjacent atoms and the sugar moeity, spectral features of **5** are identical to those of **2**. Acetylation of **5** afforded a tetraacetate (**6**) with spectral characteristics as expected. Thus **5** is 1α,3β-dihydroxy-23-oxo-24-methylenecycloartan-30-oic-acid 3-*O*-(α-L-arabinopyranoside) or jessic acid α-L-arabinopyranoside.

EXPERIMENTAL

Plant material. Fresh leaves from *Combretum elaeagnoides* Klotsch were collected from the National Botanic Gardens, Harare, Zimbabwe, and identification of the species confirmed by Mr. R. B. Drummond, Keeper of the Zimbabwe National Herbarium.

Extraction. Dried, powdered leaf material (500 g) was extracted successively with petrol, ether and acetone (Soxhlet, 2 l. solvent, 48 hr) and the extracts concd under red. pres. to give crude residues (31 g, 25 and 37 g respectively) each of which was subjected to chromatography on silica gel eluting with a petrol–EtOAc gradient (0 → 100% EtOAc), giving methyl jessate (**3**), jessic acid (**2**) and jessic acid α-L-arabinoside (**5**) as respective major components.

Jessic acid (**2**). Needles (850 mg) from petrol–EtOAc (1 : 1), mp 196–202°; $[\alpha]_D^{22}$ + 55.5° (C_5H_5N; *c* 1.0); UV λ_{max}^{EtOH} 219 nm (ε 6625) (calc. for CH_2=C(R)CO −225 nm [9]); IR ν_{max}^{KBr} cm^{-1}: 3460–3400 (OH), 2640 (COOH) and 1680 (H_2C=C–C=O); ^{1}H NMR (60 MHz, C_5D_5N) δ 5.53 and 5.20 (2H, 2 × *s*, =CH_2), 5.0 (1H, *m*, H-3α), 3.37 (1H, apparent *t*, $W_{1/2}$ = 7 Hz, H − 1β), 1.17–0.55 (18H, 6 × Me), 0.33 and 0.03 (2H, 2 × *d*, J = 4 Hz, cyclopropyl); ^{13}C NMR (20 MHz, C_5D_5N) δ 202.4 (*s*, C-23), 180.0 (*s*, C-30), 156.1 (*s*, C-24), 121.1 (*t*, C-28), 72.6 (*d*, C-1), 70.7 (*d*, C-3), 55.7 (*s*, C-4), 52.8 (*d*, C-17), 49.3 (*s*, C-14), 48.1 (*d*, C-8), 45.7 (*s*, C-13), 45.7 (*t*, C-22), 30.3 (*s*, C-10), 22.1 (2*q*, C-26 and C-27), 20.8 (*s*, C-9) and 9.7 (*q*, C-31); positive Liebermann–Burchard test (pale orange colour → dull reddish brown in 30 min); decolourized weak aq. $KMnO_4$; caused effervescence of satd $NaHCO_3$ soln on warming.

Hydrogenation of **2**. Compound **2** (125 mg = 0.25 mmol) in EtOH (20 ml) was stirred under H_2 with a Pd catalyst (65 mg, 2% $Pd(OH)_2$–$CaCO_3$) at room temp. until the uptake of H_2 ceased (90 min, 0.228 mmol H_2) to give 24,28-dihydrojessic acid which crystallized from EtOH as needles (115 mg, 92%), mp 225–227°, UV, IR and ^{1}H NMR showing absence of =CH_2 features.

Methyl jessate (**3**). Needles (3.3 g) from MeCN, mp 220–223°, $[\alpha]_D^{20}$ + 60.4% (C_5H_5N; *c* 1.0); (C, 74.54; H, 9.76; $C_{32}H_{50}O_5$ requires C, 74.67; H, 9.79%); UV λ_{max}^{EtOH}: 221 nm (ε 7130); IR ν_{max}^{KBr} cm^{-1}: 3550 (OH), 3060 (*w*, cyclopropyl), 1715 (ester C=O), 1675 (H_2C=C–C=O), 1622 (H_2C=C–C=O) and 1268 (ester C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.88 and 5.62 (2H, 2 × *s*, =CH_2), 4.52 (1H, *dd*, J_1 = 11 Hz and J_2 = 5 Hz, H-3α), 3.68 (3H, *s*, COOMe), 3.58 (1H, apparent *t*, $W_{1/2}$ = 6 Hz, H-1β, 2.88 (1H, septet, $J_{25.2Me}$ = 6.8 Hz, $J_{25.28}$ = 1.25 Hz, H-25) 1.08–0.81 (18H, 3*s* and 3*d*, 6 × Me), 0.70 and 0.47 (2H, 2 × *d*, J = 4 Hz), cyclopropyl); ^{13}C NMR δ(20 MHz; $CDCl_3$): 202.8 (*s*, C-23), 177.5 (*s*, C-30), 156.1 (*s*, C-24), 120.7 (*t*, C-28), 73.0 (*d*, C-1), 70.4 (*d*, C-3). 54.8 (*s*, C-4), 52.6 (*d*, C-17), 51.8 (*q*, COOMe), 48.8 (*s*, C-14), 47.8 (*d*, C-8), 45.3 (*s*, C-13), 45.3 (*t*, C-22), 29.4 (*s*, C-10), 21.9 (2*q*, C-26, C-27), 20.8 (*s*, C-9) and 8.4 (*q*, C-31); MS *m/z* (rel. int.): 514.3657 ($C_{32}H_{50}O_5$ requires 514.3658 $[M]^+$ (7.8), 496 (16.7) 402 (62.0), 384 (36.7), 366 (12.3), 307 (12.4), 201 (26.4) and 175 (59.0); spot tests as per **2** but did not cause effervescence of satd $NaHCO_3$ soln.

Compound **3** also prepared by methylation (CH_2N_2 soln, 150 ml) of **2** (100 mg, 25 cm^3 EtOH, 3 hr at room temp.) affording needles from MeCN (88 mg, 86%), identical in all respects to **3** above.

Methyl jessate diacetate (**4**). Acetylation of **3** (250 mg) in pyridine (10 ml) with Ac_2O (10 ml) (35°, 24 hr) and usual workup gave needles (245 mg, 84%) from MeOH, mp 136–138°, $[\alpha]_D^{20}$ + 38.4° ($CHCl_3$; *c* 1.0); UV λ_{max}^{EtOH} 221 nm (ε 4036); IR ν_{max}^{KBr} cm^{-1}: 3050 (*w*, cyclopropyl) 1755–1740 (2 × acetate C=O), 1725 (ester C=O), 1675 ($H_2C{=}\underline{C}{-}\underline{C}{=}O$), 1630 ($H_2\underline{C}{=}\underline{C}{-}\underline{C}{=}O$) and 1265–1230 (3 × ester C=O); ^{1}H NMR (60 MHz, $CDCl_3$): δ 5.90 and 5.63 (2H, 2 × *s*, $=CH_2$), 5.47 (1H, *dd*, J_1 = 11 Hz and J_2 = 5 Hz, H-3α), 4.62 (1H, apparent *t*, $W_{1/2}$ = 6 Hz, H-1β, 3.67 (3H, *s*, COOMe), 2.12 and 1.95 (6H, 2 × *s*, 2 × acetate Me), 1.28–0.95 (18H, 6 × Me), 0.83 and 0.50 (2H, 2 × *d*, *J* = 4 Hz, cyclopropyl); ^{13}C NMR (20 MHz, $CDCl_3$): δ 202.6 (*s*, C-23), 175.6 (*s*, C-30), 170.1, 169.4 (2 × *s*, 2 × MeCOO), 156.1 (*s*, C-24), 120.6 (*t*, C-28), 74.9 (*d*, C-1), 73.0 (*d*, C-3), 52.6 (*s*, C-4), 52.4 (*d*, C-17), 52.0 (*q*, COOMe), 48.9 (*s*, C-14), 46.1 (*d*, C-8), 45.4 (*t*, C-22), 45.3 (*s*, C-13), 27.5 (*s*, C-10), 21.9 (2*q*, C-26, C-27), 21.1 (*s*, C-9) and 9.6 (*q*, C-31); spot tests as per **2** but did not cause effervescence of satd $NaHCO_3$ soln.

Hydrogenation of **4**. Compound **4** (500 mg = 0.836 mmol) in EtOH (25 ml) was stirred under H_2 with Adams catalyst (35 mg, PtO_2) at room temp. until the uptake of H_2 ceased (3.5 hr, 0.995 mmol H_2) to give methyl-24,28-dihydrojessate diacetate which gave a glassy solid from MeOH (446 mg, 89%), mp 65°, UV, IR and ^{1}H NMR showing absence of $=CH_2$ features; ^{13}C NMR (20 MHz, $CDCl_3$) δ 214.2 (*s*, C-23), 52.6 (*d*, C-24), 49.2 (*t*, C-22) and 12.3 (*q*, C-28).

Jessic acid α-L-*arabinopyranoside* (**5**). Needles (4.5 g) from EtOH, mp 225–227° (dec); $[\alpha]_D^{21}$ + 52.6° (C_5H_5N; *c* 1.0); C, 67.85; H, 8.99 ($C_{36}H_{56}O_9$ requires C, 68.33; H, 8.92%); UV λ_{max}^{EtOH} 220 nm (ε 5952); IR ν_{max}^{KBr} cm^{-1}: 3580–3340 (OH), 2650 (COOH), 1710 (acid C=O), 1675 ($H_2C{=}\underline{C}{-}\underline{C}{=}O$), 1620 ($H_2\underline{C}{=}\underline{C}{-}\underline{C}{=}O$) and 1260–1240 (acid C=O); ^{1}H NMR (60 MHz, C_5D_5N) δ 5.60 and 5.26 (2H, 2 × *s*, $=CH_2$), 4.98 (1H, H-3α), 4.50 (1H, *d*, *J* = 7 Hz, anomeric H), 3.43 (1H, apparent *t*, $W_{1/2}$ = 7 Hz, H-1β), 4.00–3.17 (various arabinose H), 1.21–0.60 (18H, 6 × Me), 0.32 and 0.33 (2H, 2 × *d*, *J* = 4 Hz, cyclopropyl); ^{13}C NMR (20 MHz, C_5D_5N) δ 202.5 (*s*, C-23), 179.8 (*s*, C-30), 156.6 (*s*, C-24), 120.7 (*t*, C-28), 105.7 (*d*, C-1′), 81.3 (*d*, C-3), 74.0 (*d*, C-3′), 72.9 (*d*, C-2′), 72.6 (*d*, C-1), 68.8 (*d*, C-4′), 65.9 (*t*, C-5′), 54.8 (*s*, C-4), 52.9 (*d*, C-17), 49.4 (*s*, C-14), 48.0 (*d*, C-8), 46.0 (*s*, C-13), 46.0 (*t*, C-22), 30.4 (*s*, C-10), 22.3 (2*q*, C-26, 27), 21.1 (*s*, C-9) and 10.2 (*q*, C-31). Lit. [7] gives 105.9 (C-1′), 72.2 (C-2′), 74.4 (C-3′), 69.1 (C-4′) and 66.6 (C-5′) for methyl-α-L-arabinopyranoside; positive Molisch's and Feigl's tests for carbohydrates.

Hydrolysis of **5**. Compound **5** (300 mg) in EtOH (50 ml) was acidified with HCl (2 ml) and left at 35° for 4 days, after which the mixture was neutralized ($NaHCO_3$) and extracted with Et_2O, the aq. fraction giving only L-(+)-arabinose by descending PC and TLC.

Jessic acid α-L-*arabinopyranoside tetraacetate* (**6**). Acetylation of **5** (200 mg) in pyridine (5 ml) with Ac_2O (5 ml) (35°, 24 hr) and usual workup gave needles (245 mg, 94%) from MeCN, mp 184–187°, $[\alpha]_D^{19}$ + 34.1° ($CHCl_3$; *c* 1.0); ^{13}C NMR (20 MHz, C_5D_5N): δ 202.5 (*s*, C-23), 178.5 (*s*, C-30), 170.4, 170.1, 170.1, 170.0 (4 × *s*, 4 × Me$\underline{C}$OO), 156.6 (*s*, C-24), 120.8 (*t*, C-28), 103.3 (*d*, C-1′), 81.4 (*d*, C-3), 76.3 (*d*, C-1), 71.2 (*d*, C-3′), 70.1 (*d*, C-2′), 69.0 (*d*, C-4′), 63.9 (*t*, C-5′) and 54.3 (*s*, C-4).

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