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QUERETAROIC (30) CAFFEATE AND OTHER CONSTITUENTS OF *MELIANTHUS MAJOR**

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Key Word Index—*Melianthus major*; Sapindaceae; queretaroic (30) caffeate; cyclolaudenol; sitosterol-β-D-glucoside; oleanolic acid; ursolic acid.

Melianthus is a South African plant and one of the species, *M. comosus*, used by natives for medicinal purposes [1] contains four toxic bufadienolides and hellebrigenin-3-acetate [2–5]. Recently the alcoholic extract of the Indian species, *M. major* L. was reported to show a transient hypotensive activity [6] at a dose of 1 mg/kg (20 mm Hg. 5 min) and hypertensive activity at 2·5–10 mg/kg (20–30 mm. Hg, 5–15 min) when administered intravenously to anaesthetized cats.

The hexane, ethylacetate and butanol fractions of the alcoholic extract were separately subjected to repeated chromatography over alumina and silica gel to obtain substances A, B, C, D, E and F. While the hexane fraction was biologically inactive, the ethyl acetate fraction showed mild hypotension at 2·5 mg/kg (60 mm Hg. 45 min) and the butanol fraction caused transient hypertension at 1 mg/kg (24 mm Hg. 4 min) and hypotension at 2·5 mg/kg (36 mm Hg. 35 min). Both the fractions caused death at 5 mg/kg. No pure product could be isolated from the butanol-soluble fraction.

Substance E, mp 230–2°, $C_{39}H_{54}O_7$ was soluble in alkali and gave a green colour with ferric chloride which indicated its phenolic nature whereas positive Liebermann–Burchard and Noller's reactions showed it to be an unsaturated triterpenoid. The IR spectrum exhibited the presence of a hydroxyl group (3380), a carboxyl group (1710) and a trisubstituted double bond (840 cm^{-1}).

It formed a triacetate, mp 225–8°, $C_{45}H_{60}O_{10}$, whose IR and NMR spectra showed that one of the acetoxyl groups was secondary aliphatic and the other two were phenolic in nature. The triacetate gave a methyl ester, mp 212–7°. However, the methylation of substance E, under similar conditions, led to the formation of a mixture of a monomethoxy methyl ester, mp 114–18° and a dimethoxy methyl ester, mp 107–10°.

Substance E on alkaline hydrolysis yielded two products E-A1 and E-A2. The former product (E-A1), mp 318–20°, $C_{30}H_{48}O_4$, (M^+ at *m/e* 472) formed a diacetate, mp 295–9°. The acetate gave a methyl ester, mp 210° and product E-A1 was characterised (IR, mmp) as queretaroic acid [7].

The second component E-A2, mp 209°, $C_9H_8O_4$, (M^+ at *m/e* 180) yielded a dimethyl ether, mp 179° and a diacetate, mp 198° and was confirmed as caffeic acid.

Thus, substance E was established as the caffeic acid ester of queretaroic acid whose primary hydroxyl group was involved in the esterification and, therefore, must be queretaroic (30) caffeate [3β-hydroxy-30(3′,4′-dihydroxy cinnamoyl)oxy-olean-28-oic acid]. It caused a fall in blood pressure in cat at 1 mg/kg (40 mm. Hg. 15 min) and death at 5 mg/kg.

EXPERIMENTAL

Mp's are uncorrected. R_f values pertain to TLC on Kiesel gel G and IR spectra were recorded in KBr. The alcoholic extract of the plant (aerial parts, 4·5 kg) was successively macerated with hexane and EtOAc. The hexane fraction (68·0 g) was chromatographed on neutral alumina (activity 2·5). Elution with hexane–C_6H_6 (1:1) gave Substance A crystallised from MeOH (1·24 g). The residue (18·90 g) from the C_6H_6–MeOH (98:2) eluate was crystallised from EtOH to give substance B (10·2 g). The C_6H_6–MeOH (95:5) eluate (4·60 g) afforded substance C crystallised from EtOH (3·56 g). EtOAc residue (36·0 g) was chromatographed over Si gel, the C_6H_6–EtOAc (3:1) fraction, on crystallisation from EtOH, gave substance D (3·02 g). The residue from the C_6H_6–EtOAc (1:1) fraction was rechromatographed and the $CHCl_3$–MeOH (96:4) eluate

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yielded substance E (1·22 g) as light citron coloured needles from EtOH–$CHCl_3$. The EtOAc eluates were conc and crystallised from EtOH to yield substance F (0·16 g). Substance A, mp 125°, $[\alpha]_D$ + 46°, $C_{31}H_{52}O$. IR ν_{max} cm^{-1}: 3450 (OH), 1658, 889 (C=CH_2), 1000 (cyclopropane). NMR ($CDCl_3$): δ 0·30 and 0·58 (2H, *d*, *J* 5Hz, cyclopropyl H): 0·80–1·05 (18H, 6Me), 1·63 (3H, *s*, Me–C=C), 3·3 (1H, *m*, CHOH) and 4·7 (2H, C=CH_2). MS *m/e*: 440 (M^+) 425, 422, 407, 379, 353, 315, 300, 297, 185. Monoacetate, mp 120°, $C_{33}H_{54}O_2$, M^+ at *m/e* 482; monobenzoate. mp 193°, $C_{38}H_{56}O_2$, M^+ at *m/e* 544. It was oxidised to a keto derivative, mp 114°, $C_{31}H_{50}O$, M^+ at *m/e* 438, having a six membered ring carbonyl function (1710 cm^{-1}) and yielded formaldehyde on ozonolysis. It was identified as cyclolaudenol [8]. Substance B, mp 138°, $[\alpha]_D$-40°, acetyl derivative mp 128°, was confirmed as sitosterol (mmp). Substance C, mp 278°, $[\alpha]_D$-46°, gave a positive Fiegel's test. It was hydrolysed with 6 N HCl which yielded sitosterol and glucose. It was, therefore, established as sitosterol-β-D-glucoside. Substance D, mp 309–10°, $C_{30}H_{48}O_3$, ν_{max} cm^{-1}: 3380 (OH), 2900, 2840, 1700 (COOH), 1390, 833 (–CH=C–). Monoacetate, mp 265°; methyl ester, mp 198–200°; methyl ester acetate, mp 218–20°. It was identified as oleanolic acid (mmp, TLC, IR, NMR).

Substance E (queretaroic (30) caffeate). mp 230–2°, $[\alpha]_D$ + 152°, R_f 0·2 ($CHCl_3$–MeOH 96:4). IR ν_{max} cm^{-1}: 3380 (OH), 2900, 2850, 1710 (COOH), 1380, 840 (C=CH). UV λ_{max} nm: 217, 250, 302 and 333 (log ε 4·279, 4·185, 4·247, 4·346). NMR: δ 0·76, 0·85 (6H each, *s*, 4 Me), 0·9, 1·03 (3H each, *s*, 2 Me), 3·2 (1H, *m*, CH–OH), 4·5 (2H, *s*, CH_2O–), 5·73 (1H, *m*, C=CH), 6·5, 7·85 (2H, *d*, *J* 16Hz, *trans* CH=CH), 7·3–7·45 (3H, Ar). MS *m/e*: 634 (M^+). (Found: C, 73·71; H, 8·46. $C_{39}H_{54}O_7$ requires C, 73·82; H, 8·52%).

Acetyl derivative, mp 225–8°. IR ν_{max} cm^{-1}: 2900, 2850, 1790 (ArOAc), 1735 (OAc), 1710 (COOH), 1380, 840. UV λ_{max} nm: 222, 280 (log ϵ 3·96, 4·09). NMR: δ 0·78–1·0 (18H, *s*, 6 Me), 2·05 (3H, *s*, OCOMe), 2·33 (6H, *s*, 2 ArOCO Me), 4·35 (2H, *m*, CH_2O), 4·5 (1H, *m*, CHOAc), 5·73 (1H, *m*, C=CH), 6·5, 7·82 (2H, *d*, *J* 16Hz, CH=CH), 7·3–7·45 (3H, Ar). (Found: C, 70·89; H, 7·81. $C_{45}H_{60}O_{10}$ requires C, 71·00; H, 7·89%).

Methylester acetate. Reaction of the acetyl derivative with ethereal CH_2N_2 gave colourless needles from MeOH, mp 212–7°. IR ν_{max} cm^{-1}: 2970, 2860, 1785 (ArOAc), 1730 (OAc), 1735 (COOMe), 1380, 1685, 1250, 1205, 1176, 1111, 903, 845. NMR: δ 0·72–1·05 (18H, *s*, 6 Me), 2·05 (3H, *s*, OCO Me), 2·25 (6H, *s*, 2 × ArOCO Me), 3·60 (3H, *s*, COOMe), 4·3 (2H, *d*, *J* 7Hz, CH_2O–), 4·5 (1H, *m*, CHOAc), 5·6 (1H, *m*, C=CH), 6·5, 7·82 (2H, *d*, *J* 16Hz, CH=CH), 7·25, 7·55 (3H, Ar). MS *m/e*: 774 (M^+).

Alkaline hydrolysis of substance E. A soln of substance E (500 mg) in 10% alcoholic NaOH (30 ml) was kept for 48 hr at room temp. The reaction mixture was acidified and extracted with $CHCl_3$ followed by EtOAc which yielded two products, E-A1 and E-A2, respectively. E-A1 crystallised from MeOH–$CHCl_3$, mp 318–20°. It gave a violet colour with $SOCl_2$ and a yellow colour with tetranitromethane. IR ν_{max} cm^{-1}: 3480, 2950, 2860, 1710 (COOH), 1395, 833, 1475, 1050. NMR: δ 0·8–1·08 (18H, 6 Me), 3·3 (1H, *m*, CHOH), 3·86 (2H, *d*, *J* 7Hz, CH_2O), 5·78 (1H, *m*, C=CH), MS *m/e*: 472 (M^+).

EA1 acetate. mp 295–9°. IR ν_{max} cm^{-1}: 2950, 2870, 1750, 1710, 1377, 1480, 1250, 835. NMR: δ 0·73–0·95 (18H, 6 Me), 2·05 (6H, *s*, 2 OCOMe), 4·15 (2H, CH_2OAc), 4·53 (1H, *m*, CHOAc), 5·61 (1H, *m*, C=CH). MS *m/e*: 496 (M^+ – 60).

EA1 methylester acetate. mp 210°. IR ν_{max} cm^{-1}: 2970, 2850, 1735 (OCOMe), 1730 (COOMe). 1375, 1450, 1250, 908, 835. NMR: δ 2·02, 2·05 (3H each, *s*, OCOMe), 3·65 (3H, *s*, COOMe), 4·14 (2H, –CH_2OAc), 4·5 (1H, *m*, CHOAc), 5·62 (1H, *m*, C=CH). MS *m/e*: 510 (M^+ – 60). Component E-A2 mp 209° decomp., pale yellow, gave green colour with ferric chloride. IR ν_{max} cm^{-1}: 3400, 1655 (unsaturated COOH), 1610, 1535, 1450 (conjugated phenyl), 860, 822 (1,2,4-trisubstituted phenyl), 1215, 980 and 785. UV λ_{max} nm: 298 (*Sh*), 326, *NMR* (acetone d_6): δ 6·23, 7·50 (1H each, *d*, *J* 16Hz, –CH=CH), 6·67–7·20 (3H, *m*, Ar). MS *m/e*: 180 (M^+), 173, 162, 77, 63 and 51. *Acetyl derivative,* mp 198°. IR ν_{max} cm^{-1}: 1754, 1205 (ArOAc).

Methyl ester. mp 179°. IR ν_{max} cm^{-1}: 2835, 1695, 1639, 1613, 1525, 845, 822, 1460, 1429, 980 and 774. MS *m/e*: 208 (M^+), 193, 161, 133, 119, 91, 77. Substance F, mp 281°, $C_{30}H_{48}O_3$. Monoacetate, mp 244–5°; methyl ester, mp 170°. It was confirmed as ursolic acid (TLC, IR, NMR and MS).

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THE ISOLATION FROM *SARRACENIA FLAVA* AND PARTIAL SYNTHESIS OF BETULINALDEHYDE

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Key Word Index—*Sarracenia flava*; Sarraceniaceae; betulinaldehyde; partial synthesis.

We recently reported the isolation of a new iridoid, sarracenin, from the roots of the insectivorous plant *Sarracenia flava*. We also confirmed the antitumor activity in the roots of this plant and isolated the known antitumor agents lupeol and betulin along with sitosterol, α-amyrin and large quantities of betulinic acid. The

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