of atractyloside in rats, and also established that this compound possessed renal necrotising activity when given by interperitoneal or subcutaneous injection. He found that although the pure compound did not give rise to any toxic liver activity, it was clearly evident when the crude methanol extract was used. Further investigations in this direction are in progress.

Plant material. Callilepis laureola DC., voucher specimen lodged with Natal Herbarium, Durban, under Collector's No. 11278. *Source*: Uvongo district, South Coast, Natal, South Africa.

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TRITERPENOIDS AND STEROLS FROM THE STEMS OF *HEDYOTIS ACUTANGULA**

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Key Word Index—*Hedyotis acutangula*; Rubiaceae; triterpenoids; aborinone; isoarborinol and its acetate; germanicol; taraxerol; erythrodiol; olean-12-ene-3β,28,29-triol; oleanolic and ursolic acids; phytosterols; stigmasterol; sitosterol.

INTRODUCTION

Previous work on *Hedyotis acutangula* champ. has yielded from the leaves arborinone, isoarborinol, stigmasterol and sitosterol [1], ursolic acid and a new triterpene acid [2]. From *H. auricularia* the alkaloid hedyotine was obtained from roots [3] and auricularine from roots and stems [4] while oleanolic and ursolic acids, stigmasterol and sitosterol were isolated from leaves and stems [5]. Examination of *H. diffusa* (*Oldenlandia diffusa*) gave stigmasterol, sitosterol and ursolic acid from the whole plants [6, 7].

RESULTS

The petrol extract of the stems of *H. acutangula*, on concentration, deposited crystals of a mixture of polyhydroxy compounds. The filtrate on column chromatography yielded in succession isoarborinyl acetate, arborinone, germanicol, taraxerol, isoarborinol, sitosterol, stigmasterol and erythrodiol. Isoarborinyl acetate has only been isolated once from *Quercus championi* (Fagaceae) [8]. The mixture of hydroxy compounds was acetylated, and the product was separated by PLC into erythrodiol diacetate, and a compound, **1**, $C_{36}H_{56}O_6$, a pentacyclic triterpenoid triacetate with one double bond, probably of the olean-12-ene series as shown by signals in the NMR spectrum at δ 2.03 (9H, *s*), and δ 5.23 (1H, *q*, J = 3 and 4 Hz) [9], which also revealed a 3β-OAc group at δ 4.35 (1H, *q*, $J_{ax/eq}$ = 7 Hz and $J_{ax/ax}$ = 9 Hz), a non-hindered equatorial $C\underline{H}_2OAc$ group at δ 3.73 (2H, *s*) and a hindered axial $C\underline{H}_2OAc$ group at δ 3.70 and 4.03 (1H ea, *d*, J = 11 Hz). These CH_2OAc groups were possibly at the C-29 [10, 11] and C-28 positions respectively. That compound **1** was olean-12-ene-3β,28,29-triol triacetate was also indicated in its MS by characteristic fragmentations at *m/e* 189, 249, 276, 289, 349 and 511 [12].

This structure was finally confirmed when **1** on hydrolysis gave a triol, $C_{30}H_{50}O_3$, identical with olean-12-ene-3β,28,29-triol (**2**) obtained by reduction of methyl mesembryanthemoidegenate (**3**) [10]. Compound **2** on acetylation, yielded a triacetate identical with compound **1**.

The NMR spectrum of compound **1** also revealed six tertiary $C\underline{H}_3$ singlets. These could be assigned as follows: δ 0.87 (6H, C-23 and C-24), 0.93 (3H, C-25), 0.96 (6H, C-26 and C-30) and 1.15 (3H, C-27).

Here we report the first isolation of olean-12-ene-3β,28,29-triol (**2**) as a natural product, although it has been prepared from serratagenic acid [13] and compound **3** [10]. Compound **2** also represents the second example of naturally occurring olean-12-ene derivatives with a hydroxy group at C-29, the first being mesembryanthemoidigenic acid (**4**) which has been obtained from *Rhipsalis mesembryanthemoides* [11] and *Randia sinensis* [14] as sapogenins.

Further extraction of the stems with ethanol gave a

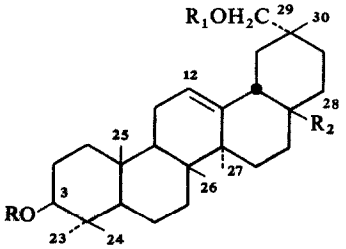
* Part 9 in the series 'An Examination of the Rubiaceae of Hong Kong.' For Part 8, see Aplin, R. T., Hui, W. H., Ho, C. T. and Yee, C. W. (1971) *J. Chem. Soc.* (C) 1067.

mixture from which oleanolic and ursolic acids were isolated.

EXPERIMENTAL

IR spectra were recorded for KBr discs, NMR spectra in $CDCl_3$ were determined at 60 MHz using TMS as internal standard, and optical rotations in $CHCl_3$ solns. Petrol had bp 60–80°. Known compounds were identified by mmp, TLC and IR comparisons with authentic samples.

Neutral compounds from stems. Milled air-dried stems (10 kg) were extracted 2× at room temp. with petrol for 7 days. The combined extracts on concentration deposited a mixture of hydroxy compounds (0.07 g), IR ν_{max}^{KBr} cm^{-1}: 3300, which was filtered, and the filtrate was chromatographed on Al_2O_3 (500 g). Elution with petrol first gave plates of isoarborinyl acetate (0.01 g), mp 288–290° (from $CHCl_3$), IR ν_{max}^{KBr} cm^{-1}: 1730, 1650, 1240, 820, then prisms of arborinone (0.02 g), mp 218–220° (from petrol), IR ν_{max}^{KBr} cm^{-1}: 1700, 1650, 820. Elution with petrol–C_6H_6 (1:1) yielded in succession prisms of germanicol (0.03 g), mp 181–182° (from $CHCl_3$), IR ν_{max}^{KBr} cm^{-1}: 3500, 1650, 820: prisms of taraxerol (0.05 g), mp 284–285° (from $CHCl_3$), IR ν_{max}^{KBr} cm^{-1}: 3500, 3080, 1650, 840; plates of isoarborinol (0.02 g), mp 304–305° (from $CHCl_3$) IR ν_{max}^{KBr} cm^{-1}: 3650, 1650, 812; and a sterol mixture, mp 130–150°, separated by PLC ($AgNO_3$–SiO_2, $CHCl_3$) into sitosterol (0.02 g), mp 139–140°, and stigmasterol (2 mg), mp 167–168°. Further elution with C_6H_6 gave erythrodiol (0.02 g), mp 239–240° (from MeOH–$CHCl_3$), IR ν_{max}^{KBr} cm^{-1} 3300, 1650, 840.



(1) R = R_1 = Ac; R_2 = CH_2OAc
(2) R = R_1 = H; R_2 = CH_2OH
(3) R = R_1 = H; R_2 = COOMe
(4) R = R_1 = H; R_2 = COOH

Separation of mixture of polyhydroxy compounds as acetates. The mixture was acetylated with Ac_2O and C_5H_5N at room temp. and the product was separated by PLC [C_6H_6–$CHCl_3$ (1:1)] into plates of erythrodiol diacetate (0.02 g), mp 185–186° (from petrol), IR ν_{max}^{KBr} cm^{-1}: 1750, 1745, 1650, 1250, 812, and needles of compound 1 (0.04 g), mp 192–194° (from petrol), $[\alpha]_D$ + 87.1° (Found: M^+ 584. $C_{36}H_{56}O_6$ requires: M 584), IR ν_{max}^{KBr} cm^{-1}: 1750, 1740, 1250 (OAc), 1650, 812 (>C=CH—).

Hydrolysis of compound 1. Compound 1 (0.03 g) was refluxed with 5% methanolic KOH (25 ml) for 4 hr to give prisms (0.02 g), mp 289–290° (from MeOH), $[\alpha]_D$ + 45.7°, M^+ at *m/e* 458, IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1655, 820 (>C=CH—), identical with olean-12-ene-3β,28,29-triol (2) [10], which on acetylation yielded a triacetate identical with 1.

Acidic compounds from stems. The stems after extraction with petrol, were further extracted with 95% EtOH. The acid mixture from the extract was isolated through the Na salts and methylated with CH_2N_2. The methylated mixture (4 g) was chromatographed on Al_2O_3 (100 g). Elution with petrol–C_6H_6 yielded methyl oleanolate (0.02 g), mp 198–201° (from C_6H_6), IR ν_{max}^{KBr} cm^{-1}: 3380, 1730, 1650, 1160, 850 and methyl ursolate (0.05 g), mp 168–170°, IR ν_{max}^{KBr} cm^{-1}: 3350, 1720, 1640, 1200, 820.

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