(altitude 2200 m.), (FRS-42), was extracted with $CHCl_3$ and worked up in the usual fashion [4]. One half of the crude gum, 73.4 g, was chromatographed over 1.3 kg of silicic acid (Mallinckrodt 100 mesh), 500 ml fractions being collected. Fractions 8, 9 and 10 (C_6H_6–$CHCl_3$ 9:1) solidified. Recrystallization from MeOH and preparative TLC gave 90 mg of a flavone, mp 185° which gave a green color reaction with $FeCl_3$ and a red color with Mg and HCl, NMR signals (DMSO) at 12.59 (C-5 OH), 7.83 *d* and 6.94 *d* (J = 10 Hz, AB *q* of H-2, H-3, H-5 and H-6), 6.66 (H-3), 3.66, 3.66, 3.57 ppm (3 OMe), MS *m/e* 344 (M^+ 72.2%), 329 (base peak), 314, 197, 169, 133: UV in MeOH, MeOH–NaOAc and MeOH–$AlCl_3$, as reported [3]. Acetylation and recrystallization from C_6H_6 afforded a diacetate, mp 165°, lit. mr 170–173 [3], NMR signals as reported [3]. Methylation with Me_2SO_4/K_2CO_3 gave a mixture which was separated by TLC. The pentamethoxyflavone was recrystallized from hexane–C_6H_6 and melted at 149–150°, lit. mp for tangeretin **2c** 152–153.5° [3], mmp undepressed. Direct comparison of the flavone and its diacetate with authentic samples of nevadensin and diacetylnevadensin showed that they were identical. Fractions 19–28 eluted with $CHCl_3$–MeOH (4%) were evaporated and the residue, wt 13 g, was rechromatographed over 560 g of silicic acid. The yield of pure crystalline desacetyleupaserrin, mp 134–135° after recrystallization from EtOAc–hexane, was 1.5 g. Calcd for $C_{20}H_{26}O_6$: C, 66.28: H, 7.23: O, 26.49; MW, 362.1728. Found: C, 66.55; H, 7.24: O, 26.28; MW (MS), 362.1738. Its other properties are presented in the discussion and in Table 1. Acetylation with Ac_2O/K_2CO_3 for 0.5 hr at 40° followed by the usual work-up, separation by preparative TLC and recrystallization from MeOH of the less polar fraction gave eupaserrin (**1b**) mp 159–160°, in whose NMR spectrum the AB quartet of H-4[1] centered 4.21 ppm was shifted downfield to doublets at 4.82 and 4.50 ppm (J = 12). A second more polar monoacetate **1c** was isolated in small quantity only: recrystallization from MeOH afforded needles, mp 174–176°, IR bands at 1755 and 1655 cm^{-1}. The high resolution MS of this substance did not exhibit a peak corresponding to the molecular ion, but strong peaks at 344 ($C_{20}H_{24}O_5$), (M^+-$MeCO_2H$), 326 ($C_{20}H_{22}O_4$, M^+-$MeCO_2H$-H_2O), 288 ($C_{17}H_{20}O_4$, M^+-$C_5H_8O_3$) and 228 ($C_{15}H_{16}O_2$, M^+-$C_5H_8O_3$-$MeCO_2H$) and 99 ($C_5H_7O_2$). These results indicated that the location of the acetate residue was on the germacradiene nucleus as in **1c**, not on the five carbon side chain.

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THE OCCURRENCE OF ATRACTYLOSIDE IN *CALLILEPIS LAUREOLA*

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Key Word Index—*Callilepis laureola*; Compositae; oxeye daisy; diterpene glycoside; atractyloside; hypoglycemic nephrotoxin.

Callilepis laureola has been used as a herbal medicine by the Zulus and other African people [1–3] and is reported to contain a toxic resin [1]. Our attention was drawn to this plant by Prof. J. Wainwright who found that many African patients who had used the enlarged subterranean rootstock as a medicine suffered serious and often fatal results due to severe liver lesions.

The dried, powdered rootstock was extracted in succession with hexane, ether, acetone and methanol. Biological tests showed that the toxic agent(s) were confined to the acetone and particularly to the methanol extracts. On TLC [Si gel EtOAc–MeOH–H_2O–HOAc (25:5:2:1); spray, anisaldehyde, H_2SO_4 and EtOH] the dark hygroscopic methanolic extract separated into a large number of component spots, three of which were contiguous and red in colour (R_f 0.23, 0.38, 0.43). An aqueous solution of the methanol extract on dilution with an equal volume of methanol slowly deposited a dark granular precipitate over a period of five days. Repeated crystallisation of this material from water provided a white powder which, by TLC, was shown to consist of three components, one being predominant. Further recrystallisation from aqueous methanol yielded a pure product of the major constituent as microcrystals, mp 225–226° (dec.), ν_{max}^{KBr} 3400–3600, 2950, 2870, 1720, 1250, 1035, 1000, 800 cm^{-1}. The two minor constituents were not examined further.

Ignition of this material left a white powder which gave a positive reaction for K^+ with sodium cobaltinitrite–silver nitrate solution, and this was confirmed by atomic absorption spectroscopy. Mild base hydrolysis provided isovaleric acid and SO_4^{2-}. Glucose was obtained on drastic base hydrolysis with aqueous KOH (20%, refluxed for 8 hr) producing an acid aglycone, mp 147–149°, M^+ 320, ν_{max}^{KBr} 3415, 2925, 2850, 1705, 1690, 1655, 1445, 1245, 1190, 1035, 995, 905, 775 cm^{-1}.

The identity of the complex glucoside and its aglycone as atractyloside and atractyligenin respectively was established by direct comparison (TLC, mmp, NMR, IR and MS) with authentic samples of each. The hypoglycemic agent, atractyloside, has previously been isolated from the rootstock of a Mediterranean plant, *Atractylis gummifera* L. (Compositae) [4].

Wainwright [5] confirmed the hypoglycemic activity

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

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