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DITERPENE ALCOHOLS FROM *CROTON LACCIFERUS*

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Key Word Index—*Croton lacciferus*; Euphorbiaceae; diterpene alcohols; *ent*-kauranoids; antifungal activity.

Abstract—The roots of *Croton lacciferus* afforded a novel *ent*-kauranoid and three other *ent*-kauranoid alcohols, one of which is a new natural product, together with 2,6-dimethoxybenzoquinone. The benzoquinone displayed antifungal activity against *Cladosporium cladosporioides*

INTRODUCTION

The genus *Croton* includes several species which are used in ethnomedical preparations. *C. lacciferus*, a common plant distributed in Sri Lanka and South India, is used in the treatment of fever, colds, dysentery, skin diseases and lung diseases including tuberculosis. In this paper, the isolation and structure elucidation of four diterpene alcohols and a benzoquinone from *C. lacciferus* as well as the examination of their antifungal properties are described.

RESULTS AND DISCUSSION

The root extracts of *C. lacciferus* exhibited antifungal activity against *Cladosporium cladosporioides* and gave **4** [1], **8** [2], a new natural product (**1**), a new compound (**6**) and 2,6-dimethoxybenzoquinone.

The ^{1}H NMR and IR data of **1** showed the presence of three tertiary methyl groups, a hydroxymethylene group and a hydroxymethine group. Acetylation of **1** under mild conditions afforded a diacetate (**3**) still containing a hydroxy group (3570–3300 cm^{-1}). This suggested the presence of a tertiary hydroxy group in **1**. The physical data (mp, IR, ^{1}H NMR) of **1** were identical to those of the lithium aluminium hydride reduction product of abbeokutone (**2**) which had been isolated from *Didymosalpinx abbeokutae* (Rutaceae) [3] and *Hymenopappus newberryi* (Compositae) [4].

The spectral data of **6**, $C_{22}H_{34}O_3$, indicated that the compound contained three tertiary methyl groups, an acetoxy group (1725 cm^{-1}, δ2.05 *s*, $[M - HOAc]^+$), a hydroxymethylene group (3720–3260 cm^{-1}, δ4.16 *br s*) and a trisubstituted olefinic group (δ5.35 *s*). Oxidation (*m*-CPBA) of **7**, the hydrolysis product from **6**, gave **10** which was reduced ($LiAlH_4$) to a mixture of two compounds. The less polar product had physical properties identical to those of **1**.

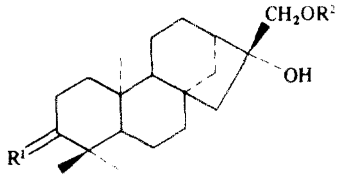
The ^{1}H NMR spectrum of **4**, $C_{20}H_{34}O_2$, was similar to that of **1** but lacked the absorption at δ3.2 (*dd*, $J = 12$, 6 Hz) corresponding to a hydroxymethine proton. The physical data of diol **4**, its monoacetate **5** and diacetate were identical to those reported previously [1]. The diol **4** had been found in *Croton sublyratus* [1] and *Fritillaria thunbergii* (Liliaceae) [2].

The ^{1}H NMR spectrum of compound **8** was similar to those of **6** and **7**, but without an ABX double doublet at δ4.47 (as in **6**) or 3.2 (as in **7**). The monoacetate **5** resulting from the acetylation of **4** yielded a mixture of two allyl acetates, upon dehydration using $POCl_3$–pyridine at $-5°$. The mixture was separated and the products were identified as **9** [2] and the Δ^{16} isomer of **9**. Alkaline hydrolysis of **9** afforded **8**. The allyl alcohol **8** had been isolated previously from *F. thunbergii* [2].

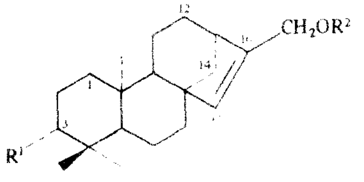
The yellow compound **11**, $C_8H_8O_4$, contained two methoxy groups (δ3.87), two olefinic protons (δ5.89) and carbonyl groups (1680, 1640 cm^{-1}), and was identified as 2,6-dimethoxybenzoquinone by direct comparison with an authentic sample [6]. The benzoquinone **11** showed antifungal activity against *C. cladosporioides* while **1**, **4**, **6** and **8** were found to be inactive.

EXPERIMENTAL

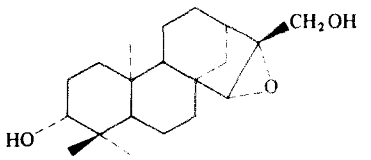
The dried roots (4.5 kg) of *C. lacciferus* collected near Peradeniya, Sri Lanka were sequentially extracted with hot petrol (bp 60–80°) and hot $CHCl_3$ and worked-up in the usual way to yield a brown semisolid (67 g) from petrol and a brown solid (42 g) from $CHCl_3$. The $CHCl_3$ extract (30 g) was chromatographed over silica gel and elution with *n*-hexane–$CHCl_3$ followed by medium pressure chromatography of the major fractions gave:*ent*-kaur-15-en-17-ol (**8**) (56 mg), mp 136–137°, lit [2] 134–136°; $[\alpha]_D$-26° ($CHCl_3$; *c* 0.5); *ent*-kaur-15-en-17-hydroxy-3β-yl acetate (**6**) (32 mg) mp 130–131°; $[\alpha]_D$-50° ($CHCl_3$, *c* 0.4); IR ν_{max}^{KBr} cm^{-1}: 3270–3260, 2970, 1725, 1415, 1375, 1250, 1030; ^{1}H NMR (60 MHz, $CDCl_3$): δ5.35 (1H, *s*, H-15), 4.47 (1H, *dd*, $J = 10$, 6 Hz, H-3), 4.16 (2H, *br s*, H-17), 2.55 (1H, *m*), 2.05 (3H, *s*, Ac), 2.2–2.1 (18H, *m*), 1.08 (3H, *s*, H-18), 0.86 (6H, *s*, H-19 and H-20); EIMS 70 eV *m/z* (rel. int.): 346$[M^+]$ (11), 286$[M - AcOH]^+$ (21), 271 $[M - AcOH - Me]^+$ (77), 253 $[M - AcOH - Me - H_2O]^+$ (13),136 (32). Found: M_r (MS) 346.2506. $C_{22}H_{34}O_3$ requires: 346.2508. *ent*-Kauran-3β,16β,17-triol (**1**) (70 mg), mp 218–220°, lit. [4] 217°; $[\alpha]_D$-39° (MeOH, *c*, 0.59); IR ν_{max}^{KBr} cm^{-1}: 3680–3060, 2920, 2860, 1450, 1040, 1025, 760; ^{1}H NMR ($CDCl_3$–CD_3OD 1:1): δ3.66 (2H, *s*, H-17), 3.20 (1H, *dd*, $J = 12$, 6 Hz, H-3), 2.2–1.1 (20H, *m*), 1.05, 0.97, 077 (3H each, *s*, 3 Me); MS *m/z* (rel. int.): 322 $[M]^+$ (0.6), 291 $[M - CH_2OH]^+$ (94), 286 $[M - 2H_2O]^+$ (20), 273 $[M - CH_2OH - H_2O]^+$ (100), 271 $[M - 2H_2O - Me]^+$ (20), 255 (25). 2,6-Dimethoxybenzoquinone (**11**) (19 mg) mp 242–245°, lit. [6] 254–255°. Found: M_r (MS)



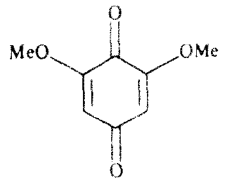
	R¹	R²
1	α-OH, H	H
2	O	H
3	α-OAc, H	Ac
4	H₂	H
5	H₂	Ac



	R¹	R²
6	OAc	H
7	OH	H
8	H	H
9	H	Ac



10



11

168. 0424. Calc. for $C_8H_8O_4$ 168.0428. Identical with authentic sample (mp, IR, ^{1}H NMR, TLC). Chromatographic fractionation of the petrol extract (30 g) on silica gel ($CHCl_3$) gave *ent*-kauran-16*β*,17-diol (**4**) (124 mg) mp 189–191°, lit [1] 186–188°; $[\alpha]_D$ −30.9° ($CHCl_3$, *c* 0.42), lit. [1] −36.2°. Found: $[M-H_2O]^+$ (MS) 288.2449. Calc. for $C_{20}H_{32}O$ 288.2453.

25 mg **1** was treated with 0.5 ml Ac_2O in 1 ml py at room temp. overnight to give **3** (21 mg), mp 185–186°, lit [3] 186°; $[\alpha]_D$ −45.2° ($CHCl_3$, *c*, 0.84); IR ν_{max}^{KBr} cm^{-1}: 3570–3300, 2920, 1720; ^{1}H NMR ($CDCl_3$) δ4.43 (1H, *dd*, *J* = 10, 6 Hz, H-3), 4.20 (2H, *s*, H-17), 2.08, 2.05 (3H each, *s*, Ac), 2.2–1.1 (18H, *m*), 1.05, 0.83 (3H, 6H, *s*, H-18, H-19 and H-20).

7 was obtained from **6** with 5% KOH in MeOH at room temp. for 24 hr, mp 174–175°; $[\alpha]_D$ −22.7° ($CHCl_3$, *c* 0.44); IR ν_{max}^{KBr} cm^{-1}: 3540–3080, 1680, 1460, 1445, 1385, 1370, 1115, 830; ^{1}H NMR ($CDCl_3$): δ5.35 (1H, *s*, H-15), 4.16 (2H, *br s*, H-17), 3.20 (1H, *dd*, *J* = 10, 6 Hz, H-3), 2.55 (1H, *m*) 2.3–1.1 (18H, *m*), 1.06, 0.97, 0.80 (3H each, *s*, H-18, H-19 and H-20). **10** was prepared from **7** with *m*-CPBA in CH_2Cl_2 at 0° for 15 min, mp 188–190°; $[\alpha]_D$ + 1.1° ($CHCl_3$, *c* 0.62); IR ν_{max}^{KBr} cm^{-1}: 3660–3040, 2920, 1440, 1055, 1000, 930, 840; ^{1}H NMR ($CDCl_3$): δ4.05, 3.75 (1H, each, *d*, *J* = 12 Hz, H-17), 3.18 (1H, *dd*, *J* = 10, 6 Hz, H-3), 2.90 (1H, *s*, H-15), 2.28 (1H, *m*), 2.1–1.05 (18H, *m*), 1.02, 0.76 (6H, 3H, *s*, H-18, H-19, and H-20). **1** was obtained as the less mobile (TLC, 3% MeOH–CH_2Cl_2) product from the reaction of **10** with excess $LiAlH_4$ in Et_2O under reflux for 48 hr.

52 mg **4** was treated with 0.5 ml Ac_2O in 1 ml pyridine at room temp. overnight to give **5** (45 mg), mp 153–154°, lit. [5] 153.5–154°; 10 mg **5** was treated with Ac_2O in pyridine at 60° overnight to give the diacetate (8.6 mg) mp 134–135°, lit. [1] 135–135.5°.

35 mg **5** was stirred with 1 ml $POCl_3$ in 0.5 ml pyridine at −5° for 3 hr. Usual work-up and TLC (20% C_6H_6–petrol) afforded less mobile **9** (8.5 mg) mp 64–66°; IR ν_{max}^{KBr} cm^{-1}: 2915, 1750, 1365; ^{1}H NMR ($CDCl_3$): δ5.43 (1H, *s*, H-15), 4.65 (2H, *br s*, H-17), 2.35 (1H, *m*), 2.05 (3H, *s*, Ac), 2.3–1.1 (20H, *m*), 1.03, 0.85, 0.80 (3H each, *s*, H-18, H-19 and H-20) and the $\Delta^{16(17)}$ isomer of **9** (16.5 mg) mp 112–114°; IR ν_{max}^{KBr} cm^{-1}: 2910, 1750, 1360; ^{1}H NMR ($CDCl_3$): δ6.92 (1H, *s*, H-17), 3.07 (1H, *m*, H-13), 2.18 (3H, *s*, Ac), 2.2–1.1 (20H, *m*), 1.03, 0.86, 0.81 (3H each, *s*, H-18, H-19 and H-20). 8 mg **9** was stirred with 5% KOH in MeOH at room temp. for 3 hr to give a white solid (6.5 mg) identical with **8** (mp, mmp, $[\alpha]_D$, TLC, IR).

1, 4, 6, 8 and **11** (0.1 mg each) were subjected to TLC (silica gel, 0.5 mm), and the developed plate sprayed with *Cladosporium cladosporioides* in Czapex-Dox nutrient solution and incubated in a moist chamber at 26 ± 2° for 48 hr [7]. A light coloured region of diameter 23 mm against a background of green mycelia was observed for **11**.

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