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ABIETANE DITERPENOIDS FROM *COLEUS ZEYLANICUS*

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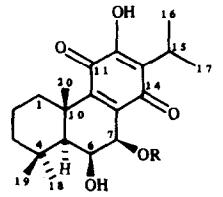
Key Word Index—*Coleus zeylanicus*; Lamiaceae, diterpenoids; abietane; 7β-acetoxy-6β-hydroxyroyleanone; 7β, 6β-dihydroxyroyleanone.

Abstract—An ethanolic extract of the plant *Coleus zeylanicus* afforded two new abietane type diterpenoids characterized as 7β-acetoxy-6β-hydroxyroyleanone and 7β, 6β-dihydroxyroyleanone. The known stereoisomer, 7α-acetoxy-6β-hydroxyroyleanone has also been isolated from the same plant.

INTRODUCTION

The genus *Coleus* Lour. (Lamiaceae, Labiatae) has been investigated as a rich source of highly oxidized abietane and labdane diterpenoids [1]. The isolation of unique labdane diterpenoid forskolin (coleonol) from the Indian plant *Coleus forskohlii* Briq. [3, 4], which is a potent experimental drug [2] for glaucoma, congestive cardiomyopahty and asthma, has generated further interest in the diterpenoids of *Coleus* and related genera. In continuation of our chemical investigation of *Coleus* species [5], we now report the isolation of new abietane diterpenoids from *Coleus zeylanicus* (Benth) cramer., syn. *Plectranthus zeylanicus*, an Ayurvedic drug for diarrhoea grown in South India and Sri Lanka [6, 7]. The diterpenoids isolated from *C. zeylanicus* have been characterized as 7β-acetoxy-6β-hydroxyroyleanone (**1**) and 7β, 6β-dihydroxyroyleanone (**2**). The known stereoisomer of **1**, 7α-acetoxy-6β-hydroxyroyleanone (**6**), has also been isolated from the plant.



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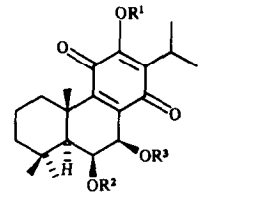
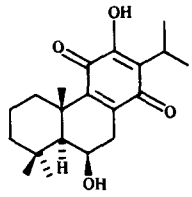
2 R = H



3 R¹ = R² = R³ = Ac
4 R¹ = Me, R² = H, R³ = Ac



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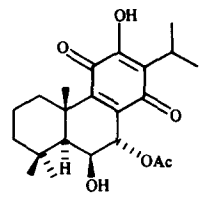

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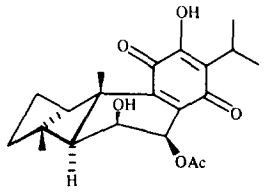

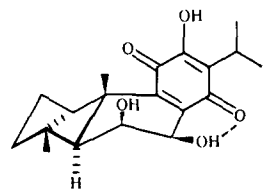

Conformation of **1** and **2**

RESULTS AND DISCUSSION

The abietane diterpenoid **1** isolated as orange crystals, mp 228° showed in its IR spectrum hydroxyl (3580 cm^{-1}) and ester (1735 cm^{-1}) absorptions. The IR bands at 1640 cm^{-1} showed the presence of a *p*-benzoquinone moiety and the absorptions at 1385 and 1375 cm^{-1} indicated the presence of an isopropyl group [8] confirmed by the ^1H NMR spectrum. The absorption maxima in the UV spectrum at 272 and 410 nm supported the presence of a chromophore like that of royleanone [9, 10]. The mass spectrum exhibited a molecular ion (M^+ at *m/z* 390, $C_{22}H_{30}O_6$), loss of MeCO (*m/z* 347) and loss of HOAc (*m/z* 330, base peak). The ^1H NMR spectrum of **1** did not show signals in the low field region so the quinone was fully substituted. The doublets at δ1.15 and 1.20 (3H each, $J=7$ Hz) and a septet, *ca* 3.15 (1H, $J=7$ Hz) were due to the protons of an isopropyl group. The singlets at δ0.90, 1.20 and 1.60 were attributed to the 4α-methyl (H-18), 4β-methyl (H-19) and 10β-methyl (H-20), respectively. The double doublet at δ4.25 ($J=3.5$ and 2.0 Hz) was assigned to H-6 and the doublet at 5.62 ($J=3.5$ Hz) was assigned to H-7, the base proton of the acetate group (2.00). The stereochemistry of C-6 hydroxy group was assigned as β-axial on the basis of the 1, 3-diaxial interaction between the C-6 hydroxyl and the C-10 methyl resulting in a paramagnetic shift in the C-10 methyl which appeared at δ1.60, a deshielded position [11]. This β-axial conformation of the C-6 hydroxyl was further confirmed by the hydrogenolysis of **1** using Pd/C catalyst to afford 6β-hydroxyroyleanone (**5**), mp 187°, identical in all respects (mp, ^1H NMR, UV, IR and MS data) to authentic **5** [11]. The hydrogenolysis of **1** to the 7-deacetoxylated product **5** confirmed the regiochemical

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assignment of the acetoxy group at C-7 of **1**. Once the 6β-hydroxyl configuration was ascertained, the C-7 acetyl was deduced as β-equatorially oriented on the basis of the coupling constant of H-7 (*d*, $J_{6,7}=3.5$ Hz) [11, 12]. The stereochemical assignment at C-7 was unambiguously assigned by deacetylation of **1** to the 7-deacetylated product **2** on alkali treatment (5% KOH). The ^{1}H NMR spectrum of **2** showed, among other corresponding peaks, a sharp signal as a doublet at δ3.00 due to the C-7 hydroxyl which disappeared on shaking with D_2O clearly indicating that the C-7 hydroxyl was hydrogen bonded to the carbonyl (C-14) of *p*-benzoquinone due to the equatorial orientation of the C-7 hydroxyl. No such H-bonding and the ^{1}H NMR signal at δ3.00 have been observed in the case of 7α,6β-dihydroxyroyleanone reported in the literature [11]. Thus H-bonded deacetylated compound **2**, mp 210–212°, was characterized as 7β,6β-dihydroxyroyleanone. All the physico-chemical data led to the structural assignment of compound **1** as 7β-acetoxy-6β-hydroxyroyleanone. The acetylation of **1** (Ac_2O–pyridine, room temp.) afforded 7β,6β-diacetoxy-12-acetylroyleanone (**3**) and methylation (CH_2N_2) yielded 7β-acetoxy- 6β-hydroxy-12-methylroyleanone (**4**). The sodium borohydride reduction ($NaBH_4$, MeOH, 0–5°) of **1** resulted in a hydrogenolysed product in which the 7-acetoxy group was cleaved resulting in 6β-hydroxyroyleanone (**5**) which was identical to the hydrogenolysis product of the Pd/C-catalysed reaction already discussed. Such unusual cleavage of an acetoxy group by $NaBH_4$ has been reported in the literature [9] for 7-acetoxyroyleanone. All these derivatives were characterised by their ^{1}H NMR, IR, UV and mass spectra (see Experimental).

The second new abietane diterpenoid was isolated as a crystalline product, mp 210–212°, with a molecular formula $C_{20}H_{28}O_5$ (M^+ at *m/z* 348). Its IR spectrum showed free and hydrogen-bonded hydroxyl groups (3520 and 3390 cm^{-1}) and the ^{1}H NMR, UV, IR, mass spectra of this natural product were identical to those of deacetylated product **2** of 7β-acetoxy-6β-hydroxyroyleanone (**1**). The peculiar hydrogen bonded C-7 hydroxyl proton also appeared in the ^{1}H NMR spectrum as a sharp signal (δ3.00, *d*) which disappeared on D_2O shake thus corraborating the stereochemical assignment of the C-7 hydroxyl as β-equatorial. Hence the natural product **2** was characterized as 7β,6β-dihydroxyroyleanone, acetylation of which yielded a triacetate ($[M]^+$ at *m/z* 474) identical to the diacetate of compound **1**. Although 7β,6β-dihydroxyroyleanone (**2**) has been reported in the literature [12] as a synthetic product, this is the first time it has been isolated as a natural product.

Interestingly the known stereoisomer of **1**, reported [11] as 7α-acetoxy-6β-hydroxyroyleanone (**6**, lit mp 214° $[\alpha]_D^{24}=0°$) was also isolated as pure crystalline needles, mp 214°. The mp and spectral data (^{1}H NMR, IR, UV and MS) of **6** showed a clear-cut difference between **1** and **6**. The deacetylation product of **6** showed no doublet at δ3.00 for the H-bonded C-7 hydroxyl thus establishing the configuration as α-axial.

EXPERIMENTAL

Mps: uncorr. MS: direct inlet, 70 eV. ^{1}H and ^{13}C NMR: 80 and 20 MHz, respectively, $CDCl_3$, TMS as int. standard. Assignments of ^{13}C NMR chemical shifts were made with the aid of off-resonance (SFORD) and noise decoupled (NDC) spectra. The plant material was collected from Coimbatore, India, a voucher specimen is kept at the Botany Division of the Institute as herbarium specimen.

Isolation of diterpenoids. Dried plant of *C. zeylanicus* was powdered and extracted with EtOH for 6 days. The solvent was evapd *in vacuo* and the residue obtained was fractionated into hexane, $CHCl_3$ and BuOH. The hexane fraction (36 g) was chromatographed over silica gel using hexane–EtOAc mixtures of increasing polarity as eluents. Elution of the column with hexane–EtOAc (9:1) afforded 7α-acetoxy-6β-hydroxyroyleanone (**6**, 200 mg, 0.01%, mp 214°) characterized by comparison with the literature data [11]. Further fractions on rechromatography yielded compound **1** as orange crystals (475 mg, 0.0158%, mp 228°) from hexane–C_6H_6; $[\alpha]_D^{20}=+23°$ ($CHCl_3$; C1), UV λ_{max}^{MeOH} nm: 220, 272 and 410; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 1735, 1640, 1610, 1385, 1375, 1240, 1020, 760; ^{1}H NMR: δ0.90 (3H, *s*, H-18), 1.20 (3H, *s*, H-19), 1.15 and 1.20 (6H, 2*d*, $J=7$ Hz, H-16 and H-17), 1.60 (3H, *s*, H-20), 2.00 (3H, *s*, OCOMe), 3.15 (1H, *sept*, $J=7$ Hz, H-15), 4.25 (1H, *dd*, $J=3.5$ and 2.0 Hz, H-6), 5.62 (1H, *d*, $J=3.5$ Hz, H-7), 7.20 (1H, *s*, C-12 hydroxyl, disappeared on D_2O shake); ^{13}C NMR: δ183.5 (C-11), 179 (C-14), 166 (OCOMe), 151 (C-12), 150.5 (C-9), 137.5 (C-8), 126 (C-13), 70 (C-7), 68 (C-6), 49.5 (C-5), 42.5 (C-3), 39 (C-4), 38.5 (C-1), 34.5 (C-10), 24 (C-2), 23.5 (C-20), 23 (C-15), 22, 22.5 (C-16 and C-17), 21 (C-18), 20.5 (OCOMe), 20 (C-19). MS *m/z*: 390 $[M]^+$, 347, 330, 329 and 314. Further elution of the column with hexane–EtOAc (4:1) afforded compound **2** as yellow crystals (42 mg, 0.0014%, mp 210–212°, hexane–EtOAc): UV λ_{max}^{MeOH} nm: 272, 420; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520, 3390, 1660, 1630, 1460, 1380, 1260, 770; ^{1}H NMR: δ1.05 (3H, *s*, H-18), 1.20 (3H, *s*, H-19), 1.30 (6H, *d*, $J=7$ Hz, H-16 and H-17), 1.65 (3H, *s*, H-20), 3.00 (1H, *d*, $J=3.5$ Hz, C-7 hydroxyl, disappeared on D_2O shake), 3.15 (1H, *sept*, $J=7$ Hz, H-15), 4.50 (2H, *m*, H-6 and H-7). MS *m/z*: 348 $[M]^+$, 330, 315, 312 (base), 207.

Acetylation of **1**. A soln of **1** (100 mg) in pyridine (1 ml) was treated with Ac_2O (2 ml) for 24 hr at room temp. Usual work-up of the reaction mixture afforded **3** (112 mg), UV λ_{max}^{MeOH} nm: 264, 408; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2950, 1757, 1660, 1615, 1560, 1390; ^{1}H NMR: 1.00 (3H, *s*, H-18), 1.20 (3H, *s*, H-19), 1.30 (6H, *d*, H-16 and H-17), 1.65 (3H, *s*, H-20), 2.10 (3H, *s*, C-7 -OCOMe), 2.25 (3H, *s*, C-6-OCOMe), 2.40 (3H, *s*, C-12-OCOMe), 3.15 (1H, *sept*, H-15), 5.50 (1H, *br*, H-6), 5.70 (1H, *d*, $J=3.5$ Hz, H-7).

Deacetylation of **1**. A soln of **1** (100 mg) in MeOH (5 ml) was treated with 5% KOH soln (20 ml) at room temp. The reaction mixture was warmed at 70–80° for 10 min. and neutralized with 5% HCl and extracted with $CHCl_3$. The organic layer was washed with H_2O, dried (Na_2SO_4) and evapd, the residue was crystallized (hexane–EtOAc) to afford **2**, mp 210–212° (35 mg). The mp, IR, UV, ^{1}H NMR, ^{13}C NMR and MS data were identical to those of the natural product, 7β,6β-dihydroxyroyleanone (**2**), isolated from the plant.

Methylation of **1**. Compound **1** (50 mg) in Et_2O (15 ml) was treated with ethereal soln of CH_2N_2 (20 ml at 0–5°). After completion of the reaction the Et_2O was evaporated and **4** (40 mg) was obtained as a viscous solid which was purified by chromatography. UV λ_{max}^{MeOH} nm: 232, 408, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 3300, 1740, 1630, 1600, 1460, 1420, 1375, 1200, 980, 840; ^{1}H NMR: 0.90 (3H, *s*, H-18), 1.18 (3H, *s*, H-19), 1.12 (6H, 2*d*, $J=7$ Hz, H-16 and H-17), 1.60 (3H, *s*, H-20), 1.95 (3H, *s*, OCOMe), 3.05–3.25 (1H, *sept*, H-15), 3.80 (3H, *s*, OMe), 4.20 (1H, *br*, H-6), 5.55 (1H, *d*, $J=3.5$ Hz, H-7); MS *m/z*: 404 $[M]^+$, 390, 348.

Hydrogenolysis of **1**. Compound **1** (25 mg) in MeOH (2 ml) was hydrogenated using Pd/C (10%, 20 mg) as catalyst for 10 hr. After usual work-up 20 mg of **5** was obtained, mp: 187°; UV λ_{max}^{MeOH} nm: 276, 410; IR $\nu_{max}^{CHCl_3}$: 3400, 2950, 1640, 1605, 1560, 1380; ^{1}H NMR: δ1.00 (3H, *s*, H-18), 1.30 (3H, *s*, H-19), 1.20 (6H, *d*, H-16 and H-17), 1.70 (3H, *s*, H-20), 2.75 (2H, *dd*, $J=3.5$ Hz, H-7),

3.0–3.25 (1H, *sept*, H-15), 4.70 (1H, *br*, H-6); MS *m/z*: 332 $[M]^+$, 315, 300, 299, 271, 261 and 245.

$NaBH_4$ *reduction of* **1**. A soln. of **1** (25 mg) in MeOH (20 ml) was cooled to 0–5° and treated with $NaBH_4$ (25 mg) with stirring in an ice bath for 2 hr. After neutralization with AcOH, excess cold water (40 ml) was added, the mixture extracted with Et_2O, dried (Na_2SO_4) and the Et_2O evapd affording compound **5**. The spectral data (UV, IR, ^1H NMR and MS) were identical to those of the hydrogenolysed product obtained using Pd/C catalyst.

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