Mes), 1.66 (6H, *s*, 2-Me_2), 1.6–2.3 (2H, *m*, H-6), 2.36 (3H, *s*, Ac), 2.6–2.9 (2H, *m*, H-7), 3.03 (2H, *m*, H-5 and iso-propyl CH), 6.95 (1H, *d*, J = 7.2 Hz, H-3), 7.17 (1H, *dd*, J = 7.2, 1.2 Hz, H-4); ^{1}H NMR (90 MHz, C_6D_6, TMS as int. standard): δ1.31 (3H, *d*, J = 7.2 Hz, 5-Me), 1.56 (6H, *s*, 2-Me_2), 1.74 (6H, *d*, J = 7.2 Hz, $CHMe_2$), 1.6–2.1 (2H, *m*, H-6), 2.05 (3H, *s*, Ac), 2.6–3.2 (3H, *m*, H-5 and H-7), 3.47 (1H, *hept*, J = 7.2 Hz, $CHMe_2$), 6.84 (1H, *d*, J = 7.2 Hz, H-3), 7.21 (1H, *dd*, J = 7.2, 1.2 Hz, H-4). High-resolution EIMS (direct inlet) m/z: $[M]^+$ 338.1879 (calc. 338.1882 for $C_{22}H_{26}O_3$).

Compound **3**. UV λ_{max}^{MeOH} nm (log ε): 218 (4.47), 243 (4.71), 278 sh (3.61), 290 (3.65), 304 (3.61), 325 sh (3.36), 340 (3.44); ^{1}H NMR (90 MHz, $CDCl_3$): δ1.18 (3H, *s*, 2-Me), 1.27 (3H, *d*, J = 7.2 Hz, *iso*-propyl Me), 1.33 (3H, *d*, J = 7.2 Hz, isopropyl Me), 2.35 (3H, *s*, 5-Me), 2.44 (3H, *s*, Ac), 2.4–4.6 (4H, *m*, H-2a, H-4, *iso*-propyl CH), 7.04 (1H, *d*, J = 9 Hz, H-6 or H-7), 7.27 (1H, *d*, J = 9 Hz, H-7 or H-6). High-resolution EIMS (direct inlet) m/z: $[M]^+$ 338.1879 (calc. 338.1882 for $C_{22}H_{26}O_3$).

Hydrolysis of **2** *and* **3**, *and subsequent air oxidation to* **1**. A soln of **2** (or **3**) (50 μg) in MeOH–H_2O (1:1) (0.2 ml) was treated with two microdrops of 5 M NaOH, and the mixture was allowed to stand overnight. After acidification, the product was taken up in Et_2O. The Et_2O was evaporated and the residue was dissolved in Me_2CO. Analysis by GC (OV-1, FID, He 15 ml/min, 1 m × 3 mm packed with OV-1) revealed the quantitative formation of salvilenone (**1**).

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24-METHYLENE CYCLOARTANYL *p*-HYDROXYCINNAMATE FROM THE ORCHID *CIRRHOPETALUM ELATUM*

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Key Word Index—*Cirrhopetalum elatum*, Orchidaceae; 24-methylenecycloartanyl *p*-hydroxycinnamate; triterpene.

Abstract—From the orchid *Cirrhopetalum elatum* was isolated a new triterpene of the cycloartane series, which was shown to be 24-methylenecycloartanyl *p*-hydroxycinnamate from spectral and chemical evidence.

INTRODUCTION

Systematic chemical investigations of a series of Himalayan orchids in our laboratory earlier led to the isolation of several 9,10-dihydrophenanthropyrans and pyrones, 9,10-dihydrophenanthrenes, phenanthrenes, bibenzyl derivatives and steroids [1, 2]. We report in this paper the isolation of a new triterpene from yet another Himalayan orchid *Cirrhopetalum elatum*. The triterpene was shown to be 24-methylenecycloartanyl *p*-hydroxycinnamate (**1a**) from the following spectral and chemical evidence.

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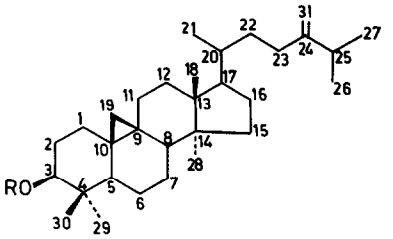
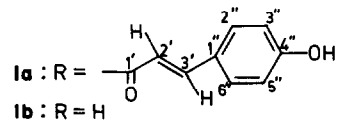
RESULTS AND DISCUSSION

Elemental analysis of the triterpene, mp 255°, $[\alpha]_D$ +28.3° ($CHCl_3$), corresponded to a molecular formula $C_{40}H_{58}O_3$ which was confirmed by its chemical ionization mass spectrum showing a peak at m/z 587 $[M+1]^+$. However, it did not show the molecular ion peak in its electron-impact mass spectrum which, instead, exhibited a peak at m/z 422 $[M - C_9H_8O_3]$ at the highest mass region corresponding to the loss of *p*-hydroxycinnamic acid.

The presence of the *p*-hydroxycinnamate ester moiety in the compound was indicated by its characteristic UV [λ_{max} 213, 228 and 314 nm (log ε3.99, 4.02 and 4.33); large alkali-induced bathochromic shifts, $\lambda_{max}^{0.1\,N\,NaOH}$ 242 and 367 nm (log ε3.82 and 4.45)], IR [ν_{max} 3190 (OH), 1675 (conjugated C=O), 985 (*trans*-CH=CH–) and 830 (1,4-

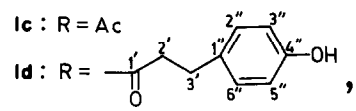




Ia : R =

Ib : R = H

Ic : R = Ac



Id : R =

, 24,31-Dihydro

disubstituted benzene) cm^{-1}] and ^1H NMR [δ7.62 and 6.32 (each 1H, *d*, J = 16 Hz; *trans*-olefinic protons), 7.45 and 6.85 (each 2H, *d*, J = 8 Hz; four aromatic protons of the *p*-disubstituted benzene moiety) and 5.36 (1H, *br s*, disappeared on deuterium exchange; phenolic OH)] spectral data, while the nature of its triterpene moiety was revealed by its other ^1H NMR signals [δ0.67 and 0.37 (each 1H, *d*, J = 4 Hz; methylene protons of cyclopropane [3, 4] as in cycloartenol [5] and related compounds [3, 4, 6–8]), 4.67 and 4.72 (2H, split into two seemingly singlets; terminal olefinic methylene protons), 4.67 (1H, *m*, obscured in the above olefinic protons signal; ester methine proton) and 0.89–1.05 (7C-methyls)], as well as from its characteristic mass spectral fragmentation [9]. That the two fragments are joined in a manner as expressed by **Ia** was clearly indicated by its ^{13}C NMR spectral data. The degree of protonation of each carbon atom was determined by DEPT experiments, and the carbon chemical shifts were assigned by comparison with the δ_c values of structurally related compounds [10–13] taking into consideration simple additive rules. Thus the chemical shifts of the *trans*-cinnamoyl moiety of the triterpene are comparable with those of the *p*-acetoxy-*trans*-cinnamoyl residue of the acetyl derivative of rubicoumaric acid [14], the difference being due to the replacement of the phenolic hydroxyl of the former by an acetoxy group in the latter. Regarding the triterpene moiety, the δ_c values of C-1–C-19 and C-28–C-30 of the compound are almost identical with those of the corresponding carbon atoms of cycloartenyl acetate [11, 13], while the chemical shifts of C-20–C-27 and C-31 constituting its side chain compare well with those of the corresponding carbon atoms of cycloeucalinol acetate [13].

Catalytic hydrogenation of the triterpene over 10% Pd–C afforded a tetrahydro derivative, $C_{40}H_{62}O_3$, mp 62°, which from its spectral data was shown to have structure **Id**. The ^1H NMR signal of H-3 in the latter is shifted upfield (δ4.42, $W_{1/2}$ = 16 Hz) compared to that of the original compound by ~ 0.23 ppm presumably due to reduction of the olefinic double bond of the *p*-hydroxycinnamic ester moiety.

Structure **Ia** for the triterpene was finally confirmed by its alkaline hydrolysis to an acidic and a neutral compound. The acid, $C_9H_8O_3$, mp 212° (dec.) was shown to be identical in all respects with an authentic sample of *p*-hydroxycinnamic acid. The neutral compound, $C_{31}H_{52}O$ ($[M]^+$ 440), mp 115°, $[\alpha]_D$ +44° ($CHCl_3$), on acetylation gave an acetyl derivative, $C_{33}H_{54}O_2$ ($[M]^+$ 482), mp 110°, $[\alpha]_D$ +55° ($CHCl_3$). The physical constants and the spectral data of the two compounds compare excellently with those of 24-methylenecycloartanol (**Ib**) and its acetate (**Ic**) [15–18], respectively, indicating identity in each case, although direct comparison could not be made due to nonavailability of authentic samples. The equatorial orientation of the ester moiety in **Ia** is in accord with the large $W_{1/2}$ value of H-3 in **Id**.

In the array of compounds elaborated by orchids, which comprise bibenzyl, phenanthrene and 9,10-dihydrophenanthrene derivatives derived from *p*-hydroxycinnamic acid and three acetate units [19] on the one hand, and triterpenoids and steroids originating from squalene via lanosterol [19, 20] on the other, **Ia** represents a biogenetically interesting compound which utilises the precursors of both groups of compound.

EXPERIMENTAL

Mps are uncorr. Silica gel (60–100 mesh) was used for CC and silica gel G for TLC. UV spectra were measured in 95% aldehyde-free EtOH and IR spectra in KBr discs. In all cases of NMR measurements $CDCl_3$ was used as the solvent and TMS as the internal standard. Chemical shifts are expressed in δ values. Carbon chemical shifts are in ppm downfield from TMS: $\delta_{(TMS)} = \delta_{(CDCl_3)} + 76.9$ ppm. MS were recorded in an instrument equipped with a direct inlet system and operating at 70 eV. NH_3 was used as the carrier gas in chemical ionization MS. The figures in the first bracket attached to *m/z* values represent rel. int. of peaks. All analytical samples were routinely dried over P_2O_5 for 24 hr *in vacuo*. Na_2SO_4 was used for drying and petrol used had bp 60–80°.

Isolation of **Ia**. Air-dried powdered whole plant of *C. elatum* (1 kg) was kept soaked in EtOH (5 l.) for 3 weeks. The EtOH extract was concd (100 ml) under red. pres., diluted with water (500 ml) and extracted with Et_2O. The ether extract was treated with 2 N aq. NaOH. The aq. alkaline extract was acidified with conc HCl in the cold and the liberated solid was extracted with Et_2O, washed with H_2O, dried and the solvent removed. The residue was chromatographed. The petrol–EtOAc (15:1) eluate gave **Ia** (80 mg), crystallized from petrol–EtOAc, mp 255° (Found: C, 81.89; H, 9.87. $C_{50}H_{58}O_3$ requires: C, 81.91; H, 9.89%). ^{13}C NMR: δ_c 167.37 (C-1'), 157.56 (C-4''), 157.10 (C-24), 144.00 (C-3'), 130.06 (C-2'', C-6''), 127.72 (C-1''), 116.69 (C-2'), 115.98 (C-3'', C-5''), 106.10 (C-31), 80.77 (C-3), 52.40 (C-17), 48.99 (C-14), 48.01 (C-5), 47.37 (C-8), 45.47 (C-13), 39.86 (C-4), 36.28 (C-20), 35.69 (C-15), 35.16 (C-22), 33.97 (C-25), 33.03 (C-12), 31.80 (C-1), 31.48 (C-23), 29.94 (C-19), 28.30 (C-7), 27.09 (C-2), 26.67 (C-16), 26.16 (C-10), 25.99 (C-11), 25.64 (C-30), 22.15 (C-26 or C-27), 22.02 (C-27 or C-26), 21.09 (C-6), 20.31 (C-9), 19.46 (C-18), 18.46 (C-28 or C-21), 18.13 (C-21 or C-28), 15.47 (C-29); MS (EI) *m/z* (rel. int.): 422 $[M - C_9H_8O_3]^+$ (15), 407 (17), 297 (12); MS (CI) *m/z* (rel. int.): 587 $[M + 1]^+$ (0.4), 439 (2.2), 423 (100), 203 (15), 147 (35) and 121 (39).

Catalytic hydrogenation of **Ia**. To a soln of **Ia** (15 mg) in MeOH–EtOAc (1:1) (15 ml) was added 10% Pd–C (10 mg) and the mixture was stirred in an atmosphere of H_2 for 4 hr. The catalyst was then filtered off and the filtrate on evaporation gave **Id** (13 mg), crystallized from petrol–EtOAc, mp 62° (Found: C, 81.30; H, 10.53. $C_{40}H_{62}O_3$ requires: C, 81.34; H, 10.51%). UV λ_{max} nm: 225 and 279 (log ε 3.62 and 2.98); $\lambda_{max}^{0.1N\ NaOH-EtOH}$ nm: 244 and 297 (log ε 3.51 and 2.09);

IR ν_{max} cm^{-1}: 3445 (OH), 1705 (ester C=O), 1610 and 1515 (aromatic nucleus), 1020 (cyclopropane ring) and 800 (1,4-disubstituted benzene); ^1H NMR: δ7.19 (1H, *br s*, obscured in the signal of $CHCl_3$; Ar–OH), 6.92 (2H, *d*, J = 8 Hz, H-2″ and H-6″), 6.60 (2H, *d*, J = 8 Hz, H-3″ and H-5″), 4.42 (1H, *m*, $W_{1/2}$ = 16 Hz, H-3), 2.2–3.1 (4H, ill-resolved multiplet; H_2-2′ and H_2-3′), 0.70–0.85 (24H, 8C-methyls), 0.42 and 0.21 (each 1H, *d*, J = 4 Hz, H_2-19); MS (EI) *m/z* (rel. int.): 424 $[M - C_9H_{10}O_3]^+$ (20), 409 (15), 381 (5), 355 (8), 302 (10), 297 (11), 203 (15), 175 (20), 147 (14), 135 (10), 123 (28), 107 (100) and 95 (43).

Alkaline hydrolysis of **1a**. A soln of **1a** (60 mg) in 5% MeOH–KOH (15 ml) was refluxed for 4 hr. MeOH was removed under red. pres. The residue was diluted with water, extracted with Et_2O, dried and the solvent evaporated. The residue was chromatographed. The petrol–EtOAc (30:1) eluate gave **1b** (35 mg), crystallized from petrol–EtOAc, mp 115°. IR ν_{max} cm^{-1}: 3420 (OH), 1640 and 885 (C=CH_2) and 1020 (cyclopropane ring); ^1H NMR: δ4.64 (2H, *br*, H_2-31), 3.30 (1H, *m*, $W_{1/2}$ = 16 Hz, H-3), 0.7–1.0 (21H, 7C-methyls), 0.50 and 0.25 (each 1H, *d*, J = 4 Hz, H_2-19); MS *m/z* (rel. int.): 440 $[M]^{+\cdot}$ (13), 425 (25), 422 (35), 408 (15), 379 (13), 353 (8), 315 (5), 300 (30), 285 (10), 273 (8), 259 (9), 257 (9), 245 (10), 227 (10), 217 (20), 216 (24), 203 (40), 201 (29), 175 (58), 173 (34), 163 (34), 149 (41), 147 (50), 135 (65), 133 (48), 123 (49), 121 (64), 119 (46), 109 (69), 107 (71), 105 (41), 95 (100), 81 (66), 69 (84) and 55 (79); compound **1b** was acetylated in the usual manner to give **1c**, crystallized from petrol–EtOAc, mp 110°. IR ν_{max} cm^{-1}: 1240 and 1730 (OAc), 1640 and 890 (C=CH_2) and 1020 (cyclopropane ring); ^1H NMR: δ4.62 (2H, ill-resolved *br* signal, H_2-31), 4.56 (1H, *m*, partly obscured in the signal of H_2-31; H-3), 1.97 (3H, *s*, OAc), 0.78–0.99 (21H, 7C-methyls), 0.50 and 0.25 (each 1H, *d*, J = 4 Hz, H_2-19).

The aq. alkaline soln after removal of **1b** was acidified in the cold with conc. HCl and the liberated solid was extracted with Et_2O, washed, dried and the solvent removed. The gummy residue was chromatographed. The petrol–EtOAc (3:1) eluate gave *p*-hydroxy-*trans*-cinnamic acid (10 mg), crystallized from petrol–EtOAc, mp 212° (dec.). It was identified by comparison (mmp and superimposable IR spectra) with an authentic sample.

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