

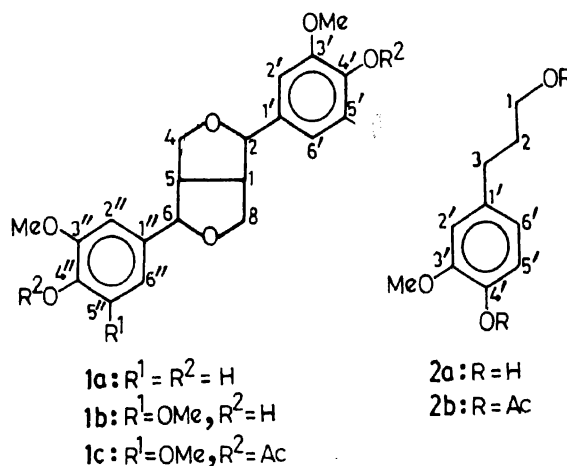
Occurrence of Lignans in the Orchidaceae Plants *Lusia volucris* and *Bulbophyllum triste*

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Manuscript received 17 March 1993, accepted 19 July 1993

We reported¹ earlier a fairly large number of compounds of diverse structural types from a series of Indian orchids, which include a wide variety of stilbenoids, triterpenoids and steroids of biogenetic importance. Included in this general programme of work were also the orchids *Lusia volucris* and *Bulbophyllum triste*. From the first orchid we already reported² the isolation of six compounds which were characterised as the dimeric phenanthrene derivative, volucrin (6,6',7,7'-tetrahydroxy-2,2',4,4'-tetramethoxy-8,8'-biphenanthryl), 3,4'-dihydroxy-3',5-dimethoxybibenzyl, the perfumery constituents methyl 2,4-dihydroxy-6-methyl benzoate and methyl 2,4-dihydroxy-3,6-dimethyl benzoate, *p*-hydroxyphenylpropionic acid and betulinic acid, while the second orchid afforded³ dihydroresveratrol (3,4',5-trihydroxybibenzyl), 3,4'-dihydroxy-5-methoxybibenzyl and tristin (3,4',5-trihydroxy-3'-methoxy-bibenzyl). Further chemical investigation of these orchids has now resulted in the isolation of the lignan, (+)-pinoresinol^{4,5} (**1a**) from *B. triste* and (+)-syringaresinol⁵ (**1b**), and a close metabolite of lignan precursor, dihydroconiferyl alcohol⁶ (**2a**) from *L. volucris*. Since direct comparison of the above compounds with **1a**, **1b** and **2a** respectively, could not be made due to unavailability of authentic samples, the characterisation of the above compounds was confirmed by independent spectral analysis. In some cases additional spectral data [¹³C nmr of syringaresinol diacetate (**1c**), ¹H nmr of **2a**, and ¹H and ¹³C nmr of **2b** (diacetyl derivative of **2a**)] were recorded for the first time for the confirmation of the structural assignments. It is interesting to note that this is the first



report of the occurrence of lignans in Orchidaceae plants, which is of considerable chemotaxonomic significance.

Experimental

Air-dried whole plants of *L. volucris* and *B. triste* (each 3 kg) were separately kept soaked in MeOH (10 l) for 3 weeks. The methanolic extract in each case was drained out and concentrated under reduced pressure to ca 100 ml, then diluted with water (500 ml) and extracted with Et₂O. The Et₂O extracts thus obtained from the two orchids were separately fractionated into acidic and neutral fractions using aqueous 2 N NaOH. The aq. alkaline extract in each case was acidified in the cold with concentrated HCl and the liberated solid was extracted with Et₂O, washed with water, dried over anhydrous Na₂SO₄ and the solvent removed. The residue in each case was separately chromatographed.

The early fractions of the petrol-EtOAc (80 : 1) eluate in the chromatography of the acidic fraction derived from *L. volucris* gave methyl 2,4-dihydroxy-3,6-dimethyl benzoate (0.09 g), crystallised from petrol-EtOAc, m.p. 139°. The later fractions of the same eluate afforded methyl 2,4-dihydroxy-6-methyl benzoate (0.02 g), also crystallised from the same solvent mixture, m.p. 138°. Betulinic acid (0.01 g), m.p. 310°, migrated out in the petrol-EtOAc (30 : 1) eluate. The petrol-EtOAc (10 : 1) eluate on evaporation afforded a solid which was found to be a mixture of three compounds. Repeated chromatography of this mixture gave pure *p*-hydroxyphenylpropionic acid (2 g), m.p. 128°, and 3,4'-dihydroxy-3',5'-dimethoxybibenzyl (2 g), as semi-solid mass. Dihydroconiferyl alcohol (**2a**; 0.01 g) was obtained by preparative tlc of the residue left after removal of most of the above two compounds : ¹H nmr δ 1.84 (2H, m, H₂-2), 2.62 (2H, t, *J* 8 Hz, H₂-3), 3.66 (2H, t, *J* 8 Hz, H₂-1), 3.84 (3H, s, ArOMe), 5.52 (2H, br s, 2 × OH) and 6.64–6.88 (3H, m, H-2', H-5' and H-6'); MS (EI) *m/z* (rel. int.) 182 (*M*⁺, 40) and 137 (100). **2a** was acetylated with Ac₂O and pyridine in the usual manner to give the diacetyl derivative (**2b**) as a viscous liquid : ¹H nmr δ 1.93–2.03 (2H, m, H₂-2), 2.02 (3H, s, OAc at C-1), 2.26 (3H, s, OAc at C-4'), 2.64 (2H, t, *J* 6.4 Hz, H₂-3), 3.79 (3H, s, ArOMe), 4.08 (2H, t, *J* 6.4 Hz, H₂-1), 6.71 (1H, d, *J* 3 Hz, H-2'), 6.75 (1H, dd, *J*₁ 7.9 Hz and *J*₂ 3 Hz, H-6') and 6.91 (1H, d, *J* 7.9 Hz, H-5'); ¹³C nmr* δ 170.75, 168.81, 20.29 and 20.58 (2 × OAc), 150.65 (C-3'), 139.92 (C-4' or C-1'), 137.74 (C-1' or C-4'), 122.27 (C-5'), 120.15 (C-6'), 112.35 (C-2'), 63.46 (C-1), 55.51 (OMe), 31.86 (C-2) and 29.86 (C-3).

Further elution of the main column with petrol-EtOAc (2 : 1) afforded a mixture of volucrin and **1b**, which on repeated chromatography gave pure volucrin (1 g), crystallised from petrol EtOAc, m.p. 280°. Compound **1b** could not be separated

from the remaining volucrin even on repeated chromatography. The residual mixture was then acetylated with Ac₂O and pyridine in the usual manner and the mixture of the acetyl derivatives was chromatographed. The petrol-EtOAc (7 : 1) eluate gave pure volucrin tetraacetate (0.05 g), crystallised from the same solvent mixture, m.p. 192°. The petrol-EtOAc (5 : 1) eluate in the above chromatography afforded pure **1c** (0.12 g), crystallised from petrol-EtOAc, m.p. 160°; *v*_{max} 1250 and 1775 (OAc), 1615, 1520, 1480, 1430, 835, 765 and 700 cm⁻¹ (phenyl nucleus); ¹H nmr δ 6.59 (4H, s, H-2' H-2'', H-6' and H-6''), 4.76 (2H, d, *J* 3.8 Hz, H-2 and H-6), 4.30 (2H, ill-resolved dd, two protons of H₂-4 and H₂-8), 3.94 (2H, dd, *J*₁ 9.2 Hz and *J*₂ 2.9 Hz, two protons of H₂-4 and H₂-8), 3.82 (12H, s, 4 × ArOMe), 3.09 (2H, br s, H-1 and H-5), 2.33 (6H, s, 2 × OAc); ¹³C nmr δ_c 168.72 and 20.38 (2 × OAc), 152.31 (C-3', C-3'', C-5' and C-5''), 139.63 (C-1', C-1'', C-4' and C-4''), 102.33 (C-2', C-2'', C-6' and C-6''), 85.82 (C-2 and C-6), 72.07 (C-4 and C-8), 56.20 (4 × OMe), 54.38 (C-1 and C-5); MS (EI) *m/z* (rel. int.) 502 (*M*⁺, 0.5), 460 (18), 418 (42), 236 (5.2), 226 (7.4), 221 (6.3), 210 (22), 209 (28.4), 205 (8.4), 194 (12.4), 193 (38), 192 (10.9), 191 (14.7), 184 (8.2), 183 (18.9), 182 (51.7), 181 (98.4), 180 (31.8), 168 (24.2), 167 (100), 161 (28.9), 154 (20.2), 153 (11.7), 151 (10.8), 123 (13.4), 95 (12.1), 91 (11.1), 81 (11.6) and 55 (17.4); MS (CI) *m/z* (rel. int.) 503 [(*M*⁺ + 1), 28.4]. Compound **1c** (0.05 g) was heated under reflux with aqueous 10% methanolic solution (20 ml) of Na₂CO₃ for 2 h. MeOH was then removed under reduced pressure and the residue was diluted with water, acidified with concentrated HCl in the cold and extracted with Et₂O, washed with water, dried and the solvent removed. The residue was chromatographed. The petrol-EtOAc (2 : 1) eluate gave **1b** (0.045 g), crystallised from petrol-EtOAc, m.p. 183° [α]_D + 43° (CHCl₃).

Chromatography of the acidic fraction of the methanolic extract of *B. triste* afforded in the

*The degree of protonation of the carbon atoms in this and all other cases was determined by DEPT experiments.

early fractions of the petrol-EtOAc (7 : 1) eluate a gummy residue which on repeated chromatography gave 3,4'-dihydroxy-5-methoxybibenzyl (0.3 g) as a semi-solid mass. The later fractions of the petrol-EtOAc (3 : 1) eluate gave a mixture of tristin and dihydroresveratrol. Repeated chromatography of the mixture finally yielded pure tristin (0.12 g) and dihydroresveratrol (0.135 g), both as semi-solid mass. Further elution of the main column with petrol-EtOAc (2 : 1) afforded pure **1a** (0.08 g) as a viscous liquid, $[\alpha]_D^{+50}$ (CHCl₃); ν_{\max} 3 400 (OH), 1 580, 1 500, 1 460 and 785 cm⁻¹ (phenyl nucleus); ¹H nmr (d₆-acetone) δ 6.97 (2H, d, *J* 1.6 Hz, H-2' and H-2''), 6.81 (2H, dd, *J*₁ 8 Hz and *J*₂ 1.6 Hz, H-6' and H-6''), 6.76 (2H, d, *J* 8 Hz, H-5' and H-5''), 4.66 (2H, d, *J* 4 Hz, H-2 and H-6), 4.16–4.21 and 3.76–3.78 (each 2H, m, H₂-4 and H₂-8), 3.80 (6H, s, 2 × ArOMe) and 3.05 (2H, br s, H-1 and H-5); ¹³C nmr δ_c (CDCl₃) 146.63 (C-3' and C-3''), 145.42 (C-4' and C-4''), 133.10 (C-1' and C-1''), 119.01 (C-6' and C-6''), 114.41 (C-5' and C-5''), 108.86 (C-2' and C-2''), 85.93 (C-2 and C-6), 71.72 (C-4 and C-8), 56.04 (ArOMe), 54.26 (C-1 and C-5); MS (EI) *m/z*

(rel. int.) 358 (*M*⁺, 20), 163 (20), 151 (80), 137 (20), 69 (60) and 57 (100).

Acknowledgement

The authors thank Dr. J. M. Wilson, University of Manchester, U.K. and Dr. B. C. Maiti, Indian Institute of Chemical Biology, Calcutta, for the mass spectra. The work was supported by C.S.I.R. and U.G.C., New Delhi.

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