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THUNALBENE, A STILBENE DERIVATIVE FROM THE ORCHID *THUNIA ALBA*

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Key Word Index—*Thunia alba*; Orchidaceae; thunalbene; stilbene derivative.

Abstract—Thunalbene, a new stilbene derivative, was isolated from the orchid *Thunia alba* which also afforded six known stilbenoids: batatasin-III, lusianthridin, 3,7-dihydroxy-2,4-dimethoxyphenanthrene, 3,7-dihydroxy-2,4,8-trimethoxyphenanthrene, cirrhopetalanthrin and flavanthrin. The structure of thunalbene, the first stilbene derivative isolated so far from an Orchidaceae plant, was established as 3,3′-dihydroxy-5-methoxystilbene from spectral and chemical evidence. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

We reported earlier on the isolation of a fairly large number of compounds of diverse structural types from a series of Indian Orchidaceae plants. These compounds encompass a wide variety of stilbenoids, i.e. bibenzyls [1], phenanthrenes [2] and their dimers [3–5], 9,10-dihydrophenanthrenes [6] and their dimers [7], phenanthropyrans and pyrones [8, 9] and their 9,10-dihydro derivatives [10], besides a few other polyphenolics [11, 12], simple aromatic compounds [13], several triterpenoids [14] and steroids of biogenetic importance [15]. Our continued search for new phytochemicals from the same source has now resulted in the isolation of a new stilbene derivative, designated thunalbene, from the orchid *Thunia alba* which also afforded six known stilbenoids, i.e. batatasin-III (3,3′-dihydroxy-5-methoxybibenzyl) [16, 17], lusianthridin (4,7-dihydroxy-2-methoxy-9,10-dihydrophenanthrene) [18], 3,7-dihydroxy-2,4-dimethoxyphenan-threne [19], 3,7-dihydroxy-2,4,8-trimethoxyphenanthrene [2], cirrhopetalanthrin (2,2′,7,7′-tetrahydroxy-4,4′-dimethoxy-1,1′-biphenanthryl) [3] and flavanthrin (2,2′,7,7′-tetrahydroxy-4,4′-dimethoxy-9,9′,10,10′-tetrahydro-1,1′-biphenanthryl) [7]. While the known compounds were identified by direct comparison with their respective authentic samples, thunalbene was shown to have the structure **1a** from the following spectral and chemical evidence.

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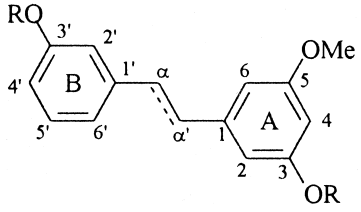
RESULTS AND DISCUSSION

Thunalbene (**1a**), $C_{15}H_{14}O_3$ (M^+ at m/z 242), showed the UV absorptions [λ_{max}^{EtOH} 214 and 303 nm ($\log \varepsilon$ 4.30 and 4.72)] expected of a *trans*-stilbene derivative. The phenolic nature of the compound was indicated by its characteristic colour reactions with $FeCl_3$ (violet) and phosphomolybdic acid reagent (intense blue), alkali-induced bathochromic shifts of its UV maxima and by its IR band at 3360 cm^{-1}. The presence of two phenolic hydroxyl groups in **1a** was confirmed by the formation of a diacetyl derivative (**1b**), $C_{19}H_{18}O_5$ (M^+ at m/z 326), with Ac_2O and pyridine. The IR absorption band at 980 cm^{-1} of **1a** indicated the presence of a *trans*-double bond in the compound.

The ^1H NMR spectrum of **1a** showed signals for an aromatic methoxyl function [δ 3.73 (3H,*s*)], two phenolic hydroxyl groups [δ 5.28 (2H,*s*);



1a : R=H, α,α'-dehydro
1b : R=Ac, α,α'-dehydro
1c : R=H, α,α'-dihydro
1d : R=Ac, α,α'-dihydro

disappeared on deuterium exchange], seven aromatic protons at δ 6.27–7.14, and a two-proton singlet at δ 6.96 which is typical of the vinylic protons of a *trans*-stilbene derivative. The relative positions of the methoxyl and hydroxyl groups in the two phenyl rings of **1a** were ascertained from the chemical shifts and the splitting patterns of the aromatic protons of the compound and its diacetyl derivative **1b**. Thus, the chemical shifts and the splitting patterns of the aromatic protons of **1a** resonating at δ 7.14 (1H, *appt t*; $J_1 = 8.1$ Hz and $J_2 = 7.8$ Hz), 6.97 (1H, *br d*; $J = 7.5$ Hz), 6.96 (1H, *br* signal; obscured in the signal of the olefinic protons), 6.67 (1H, *dd*; $J_1 = 7.8$ Hz and $J_2 = 1.8$ Hz) and 6.55, 6.51 and 6.27 (each 1H, *br* signal) are strikingly similar to those of H-5′, H-6′, H-2′, H-4′, H-6, H-2 and H-4, respectively, of batatasin-III (**1c**) [16], except that the signals corresponding to H-2, H-2′, H-6 and H-6′ of **1a** showed characteristic downfield shifts compared to the corresponding protons of **1c** due to the diamagnetic anisotropic effect of the olefinic double bond between C_α–$C_{\alpha'}$ in **1a**. This was also corroborated by the similarities of the chemical shifts and the splitting patterns of the aromatic proton signals of thunalbene diacetate (**1b**) and batatasin-III diacetate (**1d**) exhibiting the same differences in regard to their H-2, H-2′, H-6 and H-6′ resonances. The above ^{1}H NMR spectral data of **1a** and **1b**, thus, not only indicated identical substitution patterns of the hydroxyl and methoxyl functions in both **1a** and **1c**, but also implied that the former was the corresponding stilbene derivative of the latter.

The structure of thunalbene (**1a**) was further supported by the ^{13}C NMR spectral data of the compound and its diacetyl derivative **1b** (Table 1). The degree of protonation of the carbon atoms of each compound was confirmed by APT experiments and the assignments of the carbon chemical shifts of **1a** and **1b** were made by comparison with the δ_C values of structurally similar compounds like batatasin-III (**1c**) [20] and its diacetate **1d** [17] taking into consideration the alteration in additive parameters caused by the change of the state of hybridization of C-α and C-α' from sp^3 in **1c** and **1d** to sp^2 in **1a** and **1b**. Thus, the δ_C values of C-3, C-3′, C-5 and C-5′ of **1a** and **1c** were virtually identical, while those of C-1 and C-1′ of **1a** showed upfield shifts of *ca.* 4–5 ppm compared to the corresponding carbon atoms of **1c** due to the change in the state of hybridizations of C-α and C-α' from sp^3 in **1c** to sp^2 in **1a**. The observed upfield shifts of C-2, C-2′, C-6 and C-6′ by *ca.* 2–2.5 ppm and the downfield shifts of C-4 and C-4′ by *ca.* 1.5–1.8 ppm of **1a** compared to the corresponding carbon atoms of **1c** may also be attributed to the different states of hybridizations of C-α and C-α' of the two compounds, which, as expected appeared at *ca.* δ_C 129 in **1a** as against *ca.* δ_C 38 in **1c**. The δ_C values of **1b** are also compatible with the placement of the two hydroxyl groups at C-3 and C-3′ and the methoxyl group at C-5 in **1a** and exhibited expected downfield shifts of C-2, C-4, C-6, C-2′, C-4′ and C-6′. The same trend in the changes of C-1, C-1′, C-2, C-2′, C-4, C-4′, C-6 and C-6′ resonances of **1a** compared to the corresponding carbon atoms of **1c** are also discernible in the δ_C values of the above carbon atoms of **1b**, when compared with the corresponding carbon resonances of **1d**.

The structure of **1a** was finally confirmed by the conversion of its diacetyl derivative **1b** to batatasin-III diacetate (**1d**) by hydrogenation of **1b** over PtO_2.

It is interesting to note that although several stilbene derivatives were reported from a number of plant species [21], i.e. *Gnetum ula* [22], *Alnus virides* [23], *Virola elongata* [24], *Cassia roxburghii* [25], *Diphysia robinioides* [26], *Phoenix dactylifera* [27] and *Combretum caffrum* [28], all belonging to botanical families other than Orchidaceae, the isolation of thunalbene (**1a**) from the orchid *Thunia alba* constitutes the first report of the occurrence of a stilbene derivative in an orchid. This is despite the fact that the large number of orchids so far chemically investigated were shown to elaborate preponderantly a wide range of stilbenoids including a fairly large number of bibenzyl derivatives. In the light of the above observations, the isolation of thunalbene is of considerable biogenetic and chemotaxonomical importance.

Table 1. ^{13}C NMR spectral data of compounds **1a**, **1b**, **1c** and **1d**

C	**1a**†	**1b**‡	**1c**‡	**1d**‡	C	**1a**†	**1b**‡	**1c**‡	**1d**‡
1	139.5[a]	138.5[e]	144.3[i]	143.7[l]	4′	115.4[c]	121.0[g]	113.6	119.1[n]
2	106.8	111.9	108.8	113.7	5′	130.2	128.9[h]	130.0	129.1
3	159.4[b]	151.8[f]	159.2[j]	151.5[m]	6′	118.1	124.2	120.4	125.8
4	101.6	107.0	99.9	105.2	α	129.3[d]	129.5[h]	38.4[k]	37.3[o]
5	161.7	160.5	161.9	160.2	α′	129.4[d]	128.8[h]	38.1[k]	37.0[o]
6	104.1	109.9	106.3	111.8	OMe	55.3	55.4	55.3	55.2
1′	140.1[a]	139.1[e]	145.0[i]	143.0[l]	OAc	–	169.3	–	169.3
2′	113.7[c]	119.3[g]	116.2	121.4[n]		–	169.2	–	
3′	158.4[b]	151.0[f]	158.2[j]	150.7[m]		–	21.0	–	21.0

†Spectra were run in d_6-acetone and chemical shifts were measured with δ (TMS) = δ (d_6-acetone) + 29.6 ppm.
‡Spectra were run in $CDCl_3$ and chemical shifts were measured with δ (TMS) = δ ($CDCl_3$) + 76.9 ppm.
[a–o]Values are interchangeable within each column.

EXPERIMENTAL

M.p.'s: Uncorr.; CC: silica gel (100–200 mesh); MPLC: silica gel (230–400 mesh); TLC: silica gel G; UV: 95% aldehyde-free EtOH; IR: KBr discs; ^{1}H and ^{13}C NMR: 300 and 75 MHz, respectively, in $CDCl_3$ and d_6-acetone using TMS as an int. standard. Chemical shifts are expressed in δ (ppm). MS: direct inlet system, 70 eV. All analyt. samples were routinely dried over P_2O_5 for 24 h *in vacuo* and were tested for purity by TLC and MS. Na_2SO_4 was used for drying organic solvents and the petrol used had b.p. 60–80°. Plant materials were collected from Darjeeling, India in September, 1993. A voucher specimen is deposited in the Herbarium of the Department of Botany, University of Calcutta (CUH).

*Isolation of thunalbene (**1a**), batatasin-III (**1c**), lusianthridin, cirrhopetalanthrin, flavanthrin,* 3,7-*dihydroxy*-2,4-*dimethoxyphenanthrene and* 3,7-*dihyhydroxy*-2,4,8-*trimethoxyphenanthrene from Thunia alba*

Air-dried powdered whole plants (2 kg) of *T. alba* were soaked in MeOH (7 l) for 3 weeks. The MeOH extract was then drained off, concd under red. pres. to *ca.* 100 ml, diluted with H_2O (500 ml) and the liberated solids exhaustively extracted with Et_2O. The Et_2O extract was fractionated into acidic and non-acidic frs with 2 M NaOH. The aq. alkaline soln was acidified in the cold with conc. HCl and the liberated solids extracted with Et_2O, washed with H_2O, dried and the solvent removed. The residue was chromatographed. The early frs of the petrol–EtOAc (10:1) eluate afforded a mixture of lusianthridin and **1c** which on rechromatography using petrol–EtOAc (20:1) as the eluent gave in the early frs pure lusianthridin (0.05 g), crystallized from petrol–EtOAc, m.p. 162°, and **1c** (0.03 g) as a semisolid mass in the later frs. Elution of the main column with petrol–EtOAc (5:1) afforded a mixture of 3,7-dihydroxy-2,4-dimethoxyphenanthrene, 3,7-dihydroxy-2,4,8-trimethoxyphenanthrene and **1a**, which was subjected to MPLC using petrol–EtOAc (1:1) as the solvent. The early frs afforded pure **1a** (0.04 g) as a semisolid mass (found: C, 74.32; H, 5.73. $C_{15}H_{14}O_3$ requires: C, 74.38; H, 5.78%). UV $\lambda_{max}^{EtOH-0.1\ M\ NaOH}$ nm: 209.0 and 301.5 (log ε, 4.71 and 4.34); IR ν_{max} cm^{-1}: 3360 (OH), 980 (*trans*-double bond), 1595, 1500, 960, 860, 830 and 780 (phenyl nucleus); MS *m/z* (rel. int.): 242 [M$^+$], (82), 225 (3), 211 (5), 210 (5), 198 (2), 182 (2), 181 (36), 169 (3), 165 (6), 153 (9), 152 (15), 149 (21), 115 (15), 97 (16) and 83 (20).

Compound **1a** was acetylated with Ac_2O and pyridine in the usual manner to give **1b**, crystallized from petrol–EtOAc, m.p. 110° (found: C, 69.90; H, 5.49. $C_{19}H_{18}O_5$ requires: C, 69.94; H, 5.52%). UV λ_{max} nm: 211 and 296.5 (log ε 4.79 and 4.78); IR ν_{max} cm^{-1}: 1230 and 1780 (OAc), 1635, 1390, 930, 915, 890 and 810 (phenyl nucleus) and 990 (*trans*-double bond); ^{1}H NMR: δ 7.24–7.30 (2H, *m*; H-5′ and H-6′), 7.14 (1H, *br* signal; H-2′), 6.90–6.99 (3H, *m*; H-4′, H-α and H-α′), 6.80, 6.77 and 6.48 (each 1H, *br* signal; H-2, H-6 and H-4), 3.78 (3H, *s*; ArOMe), 2.22 and 2.23 (each 3H, *s*; 2 × OAc); MS *m/z* (rel. int.): 326 [M$^+$], (23), 284 (24), 242 (82), 226 (2), 225 (2), 198 (2), 181 (9), 152 (6) and 115 (6).

The later frs in the above MPLC afforded a mixture of 3,7-dihydroxy-2,4-dimethoxyphenanthrene and 3,7-dihydroxy-2,4,8-trimethoxyphenanthrene. Further MPLC of this mixture using petrol–EtOAc (1:1) as the solvent finally gave pure 3,7-dihydroxy-2,4-dimethoxyphenanthrene (0.025 g) as a semisolid mass in the early frs and pure 3,7-dihydroxy-2,4,8-trimethoxyphenanthrene (0.015 g) in the later frs. Further elution of the main column with petrol–EtOAc (1:1) eluate gave pure cirrhopetalanthrin (0.015 g), crystallized from petrol–EtOAc mixture, m.p. 296°, in the early frs and pure flavanthrin (0.03 g), crystallized from petrol–EtOAc mixture, m.p. 285°, in the later frs.

*Catalytic hydrogenation of **1b***

A soln of **1b** (0.02 g) in EtOH (20 ml) containing PtO_2 (0.005 g) was stirred under H_2 atmosphere for 4 h. The catalyst was filtered off and the filtrate on evaporation gave a semisolid residue (0.018 g) which was identical in all respects to batatasin-III diacetate (**1d**).

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