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CHALCONE AND DIHYDROCHALCONE DERIVATIVES FROM THE ORCHID *LUSIA VOLUCRIS*

P. L. MAJUMDER*, S. LAHIRI and N. MUKHOTI

Department of Chemistry, University College of Science, 92 Acharya Prafulla Chandra Road, Calcutta 700 009, India

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Key Word Index—*Lusia volucris*; Orchidaceae; chalcone and dihydrochalcone derivatives; 2-propene-1-one-1-(2′,4′-dihydroxy-3′-methoxyphenyl)-3-(4″-methoxyphenyl); 2-propene-1-one-1-(2′-hydroxy-4′-methoxyphenyl)-3-(4″-methoxyphenyl);1-propanone-1-(2′,4′-dihydroxy-3′-methoxyphenyl)-3-(4″-methoxyphenyl).

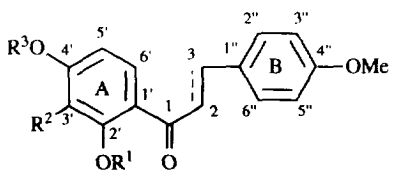
Abstract—A new dihydrochalcone derivative, lusianin, two other known chalcones and a phenanthrene derivative were isolated from the orchid *Lusia volucris*. The two chalcones and the phenanthrene derivative were characterized as 2-propene-1-one-1-(2′,4′-dihydroxy-3′-methoxyphenyl)-3-(4″-methoxyphenyl) and 2-propene-1-one-1-(2′-hydroxy-4′-methoxyphenyl)-3-(4″-methoxyphenyl) and 6-hydroxy-2,4,7-trimethoxyphenanthrene(batatasin-I), respectively, by independent spectral analysis of the compounds and their acetyl derivatives. The structure of lusianin was established as 1-propanone-1-(2′,4′-dihydroxy-3′-methoxyphenyl)-3-(4″-methoxyphenyl) from spectral and chemical evidence.

INTRODUCTION

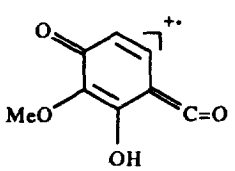
We reported earlier the isolation of a fairly large number of compounds of diverse structural types from a series of Indian orchids. These compounds encompass a wide variety of stilbenoids [1–7], triterpenoids [8] and steroids of biogenetic importance [9], besides several other phenolic compounds. As part of this general programme of research we investigated the orchid, *Lusia volucris*, which earlier afforded eight compounds [7, 10], characterized as volucrin (6,6′,7,7′-tetrahydroxy-2,2′,4,4′-tetramethoxy-8,8′-biphenanthryl), 3,4′-dihydroxy-3′,5-dimethoxy-bibenzyl, the perfumery constituents, methyl 2,4-dihydroxy-6-methylbenzoate and methyl 2,4-dihydroxy-3,6-dimethylbenzoate, *p*-hydroxyphenylpropionic acid, betulinic acid, dihydroconiferyl alcohol and the lignan, (+)-syringaresinol. Further chemical investigation of this orchid has now resulted in the isolation of a new dihydrochalcone derivative, designated lusianin, two other structurally related chalcones and a phenanthrene derivative of previously known structure. The present paper deals with the structural elucidation of lusianin and the characterization of the above chalcones and the phenanthrene derivative.

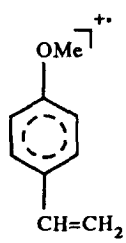
RESULTS AND DISCUSSION

The physical constants and the spectral data of the two chalcones and the phenanthrene derivative compared well with those reported for **1a** [11], **1d** [12, 13] and 6-hydroxy-2,4,7-trimethoxyphenanthrene (batatasin-I) [14, 15], respectively, suggesting their identity. Since direct comparison of these compounds with **1a**, **1d** and batatasin-I could not be made due to unavailability of authentic samples, the identity of these compounds was



1a: $R^1 = R^3 = H$, $R^2 = OMe$, 2, 3–dehydro-
1b: $R^1 = H$, $R^2 = OMe$, $R^3 = Ac$, 2, 3–dehydro-
1c: $R^1 = R^3 = Ac$, $R^2 = OMe$, 2, 3–dehydro-
1d: $R^1 = R^2 = H$, $R^3 = Me$, 2, 3–dehydro-
1e: $R^1 = Ac$, $R^2 = H$, $R^3 = Me$, 2, 3–dehydro-
1f: $R^1 = R^3 = H$, $R^2 = OMe$, 2, 3–dihydro-





*Author to whom correspondence should be addressed.

established by independent spectral analysis. The ^{1}H NMR spectra of the monoacetyl (**1b**) and diacetyl (**1c**) derivatives of **1a**, and the ^{13}C NMR spectrum of **1c**, recorded for the first time, further affirmed the assigned structure of **1a**. The changes in the chemical shifts of the ring A carbon atoms of **1c** (Table 1) compared to the corresponding carbon atoms of **1a**, in particular, confirmed the relative positions of the methoxyl and hydroxyl groups in ring A of **1a**.

The new compound, lusianin (**1f**), $C_{17}H_{18}O_5$, obtained as a semi-solid mass, showed UV absorptions at 204.5, 215(sh) and 283.5 nm (log ε 4.40, 4.20 and 3.10) resembling those of *p*-hydroxyacetophenone derivatives. The phenolic nature of the compound was indicated by its characteristic colour reactions ($FeCl_3$:purple; phosphomolybdic acid reagent: deep blue), alkali-induced bathochromic shifts of its UV maxima at 212, 256.5 and 340 nm (log ε 4.70, 2.90 and 3.80), and by its IR spectrum showing bands at 3360 cm^{-1}. The IR spectrum of **1f** also showed a band at 1705 cm^{-1} for a carbonyl function. The relatively low IR absorption frequencies of the hydroxyl and carbonyl groups indicated their involvement in intramolecular hydrogen-bonding.

The ^{1}H NMR spectrum of **1f** showed signals for a chelated and normal phenolic hydroxyl function [δ12.86 and 6.21 (each 1H, *s*, disappeared on deuterium exchange)], two aromatic methoxyl groups [δ3.71 and 3.75 (each 3H, *s*)] and six aromatic protons, as in **1a**. Four of these aromatic protons appeared as a pair of doublets [δ7.07 and 6.76 (each 2H, *d*, J = 8.5 Hz)] representing an A_2B_2 system of a *p*-disubstituted benzene derivative. The remaining two aromatic protons of **1f** also appeared as a pair of doublets [δ7.35 and 6.40 (J = 8.9 Hz)] corresponding to two *ortho*-coupled protons, which resembled those of H-5′ and H-6′ of **1a**. This would suggest an identical substitution pattern for rings A and B of both **1a** and **1f**. However, while the spectrum of **1a** showed two one-proton doublets at δ7.80 and 7.38 (J = 15.3 Hz) characteristic of *trans*-olefinic protons (H-2 and H-3) of a chalcone [11, 16], that of **1f** exhibited two two-proton triplets at δ3.11 and 2.93 (J = 7.5 Hz) typical of H_2-2 and H_2-3 of a dihydrochalcone derivative [17]. The above observation, coupled with the relatively high field shifts of the four aromatic protons at δ7.07 and 7.76, compared with those of H-2″, H-6″ and H-3″, H-5″ of **1a**, thus strongly suggest that **1f** is the 2,3-dihydro derivative of **1a**.

The distributions of the hydroxyl and methoxyl groups in the two benzene rings of **1f** were confirmed by the mass spectral fragmentation of the compound. The mass spectrum of **1f** showed an intense peak at *m/z* 166 (ion-fragment α), which is also discernible in the spectrum of **1a**, indicating the presence of a methoxyl and two hydroxyl groups in ring A of both **1f** and **1a**. While the spectrum of **1a** exhibited a base peak at *m/z* 134 (ion-fragment **b**), that of **1f**, instead, showed a base peak at *m/z* 121 (ion-fragment **c**). This also suggests that ring B of both compounds contains one aromatic methoxyl group. The appearance of the peak at *m/z* 121 in the spectrum of **1f**, instead of the peak at *m/z* 134 discernible in the spectrum of **1a**, further corroborated the fact that the 2,3-double bond of **1a** was fully saturated in **1f**. The

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

	Chemical shift (δ values)*			
C	**1f**	**1a**	**1c**	**1d**
1	204.4	193.3	195.5	191.6
2	39.9	119.0	120.6	117.6
3	29.5	144.8	145.5	144.0
1′	114.3	116.2	122.0	113.9
2′	134.2	136.1	131.8	130.9
3′	156.6[a]	162.9[a]	165.2[a]	107.5
4′	155.1[a]	157.1	157.1	166.3[a]
5′	106.5	108.1	122.9[b]	100.8
6′	126.2	127.3	123.8[b]	165.8[a]
1″	132.7	128.0	127.2	127.3
2″,6″	129.2	131.4	130.2	130.1
3″,5″	114.1	115.2	114.5	114.2
4″	158.2[a]	160.3[a]	161.9[a]	161.6[a]
OMe	60.6 (OMe at C-3′) 55.2 (OMe at C-4″)	60.4 (OMe at C-3′) 55.7 (OMe at C-4″)	61.2 (OMe at C-3′) 55.4 (OMe at C-4″)	55.6 55.2 (OMe at C-4″ and C-4′)
OCOMe	—	—	168.5 168.0 22.5 20.6	—

*Spectra recorded in $CDCl_3$; chemical shifts were measured with $\delta_{(TMS)} = \delta_{(CDCl_3)} + 76.9$ ppm.

[a,b] Values interchangeable within the same column.

placement of one methoxyl group at C-4″ in ring B of **1f** was based on the splitting patterns of the signals of the four aromatic protons associated with its ring B. The presence of a chelated phenolic hydroxyl function in **1f** implies that one of the hydroxyl groups in the compound must be placed at C-2′. By analogy with **1a**, the remaining hydroxyl and methoxyl groups in **1f** were placed at C-4′ and C-3′, respectively.

More convincing evidence in support of the structure of **1f** was provided by ^{13}C NMR spectral data (Table 1). The degree of protonation of each carbon atom was determined by DEPT experiments and assignments of carbon chemical shifts made by comparison with the δ_C values of structurally related compounds, *viz.*, **1a**, **1c** and **1d** (Table 1), taking into consideration the additive parameters of the functional groups. Thus, the replacement of the low-field olefinic carbon resonances (C-2 and C-3) of **1a**, **1c** and **1d** by the signals at δ_C 39.9 and 29.5 in the ^{13}C NMR spectrum of **1f** confirmed its dihydrochalcone structure. This was also in conformity with the low-field shifts of the carbonyl carbon and C-1″ of **1f**, compared with the corresponding carbon atoms of **1a**, **1c** and **1d** caused by the saturation of the 2,3-double bond of the above compounds in **1f**. The carbon atoms of ring B of **1f** appeared essentially at the same positions as the corresponding carbon atoms of **1a**, **1c** and **1d**, indicating the presence of the same *p*-methoxyphenyl group (ring B) in all these compounds. The methoxyl carbon signal in **1f**, appearing in the normal region (δ_C 55.2), thus corresponded to the methoxyl group at C-4″. The placement of the remaining aromatic methoxyl group in **1f** at C-3′ was supported by the relatively low-field shifts of the other methoxyl carbon signal appearing at δ_C 60.6, similar to the C-3′ methoxyl carbon resonances of **1a** and **1c**. Such low-field shifts of the carbon atom of an aromatic methoxyl function is characteristic of such a group having no *ortho*-hydrogen atom. This would imply that the two hydroxyl groups in **1f** must be placed at C-2′ and C-4′.

The structure of **1f** was finally confirmed by its conversion from **1a** by catalytic hydrogenation of the latter over 10% Pd-C.

The isolation of **1f**, **1a** and **1d** from *L. volucris* is the first report of the occurrence of chalcone derivatives in a member of the Orchidaceae and is of considerable chemotaxonomic importance.

EXPERIMENTAL

Mps: Uncorr. Silica gel (100–200 mesh) was used for CC and silica gel G for TLC. UV: 95% aldehyde-free EtOH. IR: KBr discs. ^1H and ^{13}C NMR spectra were measured at 300 and 75 MHz, respectively, in $CDCl_3$ using TMS as int. standard. Chemical shifts are expressed in δ values. MS were recorded with a direct inlet system at 70 eV. All analytical samples were routinely dried over P_2O_5 for 24 hr *in vacuo* and were tested for purity by TLC and MS. Dry Na_2SO_4 was used for drying organic solvents and the petrol used had bp 60–80°.

Isolation of lusianin (**1f**), **1a**, **1d** *and batatasin-I.* Air-dried powdered whole plants of *L. volucris* (3 kg) were soaked in MeOH (10 l) for 3 weeks. The MeOH extract was then drained out, concd under red. pres. to *ca* 100 ml, then diluted with H_2O (500 ml) and extracted with Et_2O. The Et_2O extract thus obtained was fractionated into acidic and neutral frs using aq. 2 M NaOH soln. The aq. alkaline soln was acidified in the cold with conc. HCl and the liberated solid extracted with Et_2O. The Et_2O extract was washed with H_2O, dried and the solvent removed. The residue was then chromatographed. Early fractions of the petrol–EtOAc (80:1) eluate gave methyl 2,4-dihydroxy-3,6-dimethylbenzoate (0.09 g), recrystallized from petrol–EtOAc, mp 139°. Later frs of the same eluate afforded methyl-2,4-dihydroxy-6-methylbenzoate (0.02 g), also recrystallized from the same solvent mixt., mp 138°. Early frs of the petrol–EtOAc eluate (30:1) gave batatasin-I (0.1 g), recrystallized from petrol–EtOAc, mp 135°. ^1H NMR: δ3.94, 4.04 and 4.09 (each 3H, *s*; 3 × OMe), 5.84 (1H, *br s*; OH), 6.73 (1H, *d*, J = 2.5 Hz; H-1), 6.86 (1H, *d*, J = 2.5 Hz; H-3), 7.20 (1H, *s*; H-8), 7.51, 7.61 (each 1H, *d*, J = 8.8 Hz; H-9 and H-10), 9.10 (1H, *s*; H-5). Betulinic acid (0.01 g), mp 310°, was obtained from the later frs of this eluate.

Early frs of the petrol–EtOAc (20:1) eluate gave **1f** as a semi-solid mass (0.02 g). (Found: C, 67.49; H, 5.91. $C_{17}H_{18}O_5$ requires: C, 67.55; H, 5.96%). IR ν_{max} (cm^{-1}): 3360 (OH), 1705 (>C=O), 1630, 1430, 990, 800 (aromatic nucleus). EIMS: (rel. int.) *m/z* 302 ($[M]^+$, 30), 166 (60), 121 (100). Later frs of this eluate afforded **1a** (0.05 g), recrystallized from petrol–EtOAc, mp 174°. IR ν_{max} (cm^{-1}): 3440 (OH), 1640 (>C=O), 1550, 1510, 1230, 820 (aromatic nucleus). EIMS (rel. int.) *m/z* 300 ($[M]^+$, 27), 166 (59), 134 (100). Compound **1a** was acetylated with Ac_2O and pyridine in the usual manner to give the monoacetate **1b** and the diacetate **1c**. Compound **1b**: ^1H NMR: δ2.36 (3H, *s*; OAc at C-4′), 3.87, 3.94 (each 3H, *s*; 2 × OMe), 6.66 (1H, *d*, J = 8.7 Hz; H-5′), 6.96 (2H, *d*, J = 8.7 Hz; H-3″ and H-5″), 7.46 (1H, *d*, J = 15.9 Hz; H-2), 7.62 (2H, *d*, J = 8.7 Hz; H-2″ and H-6″), 7.68 (1H, *d*, J = 8.7 Hz; H-6′), 7.92 (1H, *d*, J = 15.9 Hz; H-3), 13.38 (1H, *s*; OH). Compound **1c**: ^1H NMR: δ2.27, 2.36 (each 3H, *s*; 2 × OAc), 3.84, 3.85 (each 3H, *s*; 2 × OMe), 6.91 (2H, *d*, J = 8.7 Hz; H-3″ and H-5″), 7.01 (1H, *d*, J = 15.3 Hz; H-2), 7.09 (1H, *d*, J = 8.7 Hz; H-5′), 7.41 (1H, *d*, J = 8.7 Hz; H-6′), 7.53 (2H, *d*, J = 8.7 Hz; H-2″ and H-6″), 7.57 (1H, *d*, J = 15.3 Hz; H-3).

The petrol–EtOAc (10:1) eluate on evapn afforded a solid which was found to be a mixt. of 3 compounds. Repeated chromatography of this mixt. gave pure *p*-hydroxyphenylpropionic acid (2 g), recrystallized from petrol–EtOAc, mp 128°, and 3,4′-dihydroxy-3′,5-dimethoxybibenzyl (2 g), as a semi-solid mass. Dihydroconiferyl alcohol (0.01 g) was obtained by prep. TLC of the residue left after removal of most of the above 2 compounds. Further elution of the main column with petrol–EtOAc (2:1) afforded a mixt. of volucrin and (+)-syringaresinol, which on repeated chromatography gave pure volucrin (1 g), recrystallized from petrol–EtOAc, mp 280°. (+)-Syringaresinol could not be sepd from the remaining volucrin even on repeated chromatography. It was isolated as its diacetate by chromatography of the

acetylated mixt. of the two compounds. Hydrolysis of (+)-syringaresinol diacetate afforded pure (+)-syringaresinol (0.045 g), recrystallized from petrol–EtOAc, mp 183°. $[\alpha]_D$ + 43° ($CHCl_3$) [10].

The petrol–EtOAc (80:1) eluate from chromatography of the neutral fr. gave β-sitosterol (0.08 g), recrystallized from petrol–EtOAc, mp 139°. The petrol–EtOAc (50:1) eluate afforded **1d** (0.5 g), recrystallized from petrol–EtOAc, mp 162°. IR ν_{max} (cm^{-1}): 3070 (OH), 1625 (>C=O), 1505, 1460, 1360, 1210, 905 and 850 (aromatic nucleus). Compound **1d** was acetylated with Ac_2O and pyridine in the usual manner to give **1e**. ^1H NMR: δ2.20 (3H, *s*; OAc), 3.80, 3.82 (each 3H, *s*; 2 × OMe), 6.62 (1H, *d*, J = 3 Hz; H-3′), 6.80 (1H, *dd*, J_1 = 8 Hz and J_2 = 3 Hz; H-5′), 6.88 (2H, *d*, J = 8 Hz; H-3″ and H-5″), 7.08 (1H, *d*, J = 15.9 Hz; H-2), 7.48 (2H, *d*, J = 8 Hz; H-2″ and H-6″), 7.56 (1H, *d*, J = 15.9 Hz; H-3), 7.73 (1H, *d*, J = 8 Hz; H-6′).

Catalytic hydrogenation of **1a**. A soln of **1a** (0.01 g) in 20 ml MeOH containing 10% Pd-C (0.005 g) was stirred under H_2 for 4 hr. The catalyst was filtered off and the filtrate, on evapn, gave **1f** (0.009 g) as a glassy solid.

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REFERENCES

1. Majumder, P. L. and Ghosal, S. (1994) *Phytochemistry* **33**, 205.
2. Majumder, P. L. and Pal, S. (1993) *Phytochemistry* **32**, 1561.
3. Majumder, P. L. and Sen, R. C. (1991) *Phytochemistry* **30**, 2432.
4. Majumder, P. L. and Maiti, D. C. (1991) *Phytochemistry* **30**, 971.
5. Majumder, P. L. and Basak, M. (1991) *Phytochemistry* **30**, 3429.
6. Majumder, P. L. and Banerjee, S. (1988) *Tetrahedron* **44**, 7303.
7. Majumder, P. L. and Lahiri, S. (1990) *Tetrahedron* **46**, 3621.
8. Majumder, P. L. and Ghosal, S. (1991) *J. Ind. Chem. Soc.* **68**, 88.
9. Majumder, P. L. and Pal, S. (1990) *Phytochemistry* **29**, 2717.
10. Majumder, P. L., Lahiri, S. and Pal, S. (1994) *J. Ind. Chem. Soc.* **71**, 645.
11. Dominguez, X. A., Garcia, G. S., Howard, W. J., Ortiz, O., Scott, A. I. and Joseph, H. R. (1989) *J. Nat. Prod.* **52**, 864.
12. Patra, A., Ghosh, G., Sengupta, P. K. and Nath, S. (1987) *Magn. Reson. Chem.* **25**, 734.
13. Lars, P. C., Jorgen, L. and Tove, T. (1990) *Phytochemistry* **29**, 3155.
14. Takasugi, M., Kawashina, S., Monde, K., Katsui, N., Masamune, T. and Shirata, A. (1987) *Phytochemistry* **26**, 371.
15. Coxon, D. T., Ogundana, S. K. and Dennis, C. (1982) *Phytochemistry* **21**, 1389.
16. Harborne, J. B. and Mabry, T. J. (1982) *The Flavonoids: Advances in Research*, Chapman and Hall.
17. Kawanishi, K., Takagaki, T. and Hasimoto, V. (1990) *Phytochemistry* **29**, 2735.