REFERENCES

1. Steglich, W. (1980) in *Pigments in Plants* (Czygan F.-C., ed.) 2nd edn., p. 393. Gustav Fischer, Stuttgart.
2. Gruber, I. (1970) *Z. Pilzkd.* **36**, 95.
3. Keller, G. (1982) *Sydowia* **35**, 110.
4. Keller, G. and Ammirati, J. F. (1983) *Mycotaxon* **18**, 357.
5. Neelakantan, S. and Seshadri, T. R. (1952) *J. Sci. Ind. Res.* **11B**, 126.
6. Steglich, W., Lösel, W. and Reininger, W. (1967) *Tetrahedron Letters* 4719.
7. Ashley, J. N., Raistrick, H. and Richards, T. (1939) *Biochem. J.* **33**, 1291.
8. Anslow, W. K. and Raistrick, H. *Biochem. J.* **34**, 1124.
9. Thomson, R. H. (1971) *Naturally Occurring Quinones*. Academic Press, London.
10. Steglich, W., Lösel, W. and Austel, V. (1969) *Chem. Ber.* **102**, 4104.
11. Steglich, W. and Reininger, W. (1972) *Chem. Ber.* **105**, 2922.
12. Steglich, W. and Oertel, B. (1984) *Sydowia* **37**, 284.
13. Steglich, W., Töpfer-Petersen, E., Reininger, W., Gluchoff, K. and Arpin, N. (1972) *Phytochemistry* **11**, 3299.
14. Archard, M., Gill, M. and Strauch, R. J. (1985) *Phytochemistry* **24**, 2755.
15. Gill, M. and Strauch, R. J. (1985) *Tetrahedron Letters* **26**, 2593.

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MOSCATILIN, A BIBENZYL DERIVATIVE FROM THE ORCHID *DENDROBIUM MOSCATUM*

P. L. MAJUMDER and R. C. SEN

Department of Chemistry, University College of Science, Calcutta 700009, India

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Key Word Index—*Dendrobium moscatum*; Moscatilin; bibenzyl derivative; Orchidaceae.

Abstract—Moscatilin, a new bibenzyl derivative isolated from the orchid *Dendrobium moscatum*, was shown to have the structure 4,4′-dihydroxy-3,3′,5-trimethoxybibenzyl.

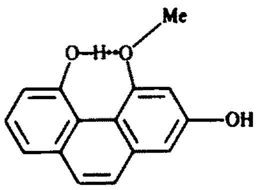
INTRODUCTION

In our previous communications [1–14] we reported the structure elucidations of a number of compounds isolated from a series of Indian orchids. These compounds represent several structural types, *viz.*, 9,10-dihydrophenanthrenes [1], 9,10-dihydrophenanthropyrans [2–6] and pyrones [2–4, 7], phenanthrenes [8, 9], bibenzyl derivatives [10], fluorenones [11], triterpenoids [12, 13] and steroids [14]. One of the above phenanthrene derivatives, moscatin (**1**) was earlier isolated [9] from the orchid *Dendrobium moscatum* which also showed the presence of another compound forming a difficultly separable mixture with **1**. Repeated chromatography of this mixture has now resulted in the isolation of this compound in pure and crystalline state. The structure of this compound, designated as moscatilin, has been established as **2a** from the following spectral and chemical evidence.

RESULTS AND DISCUSSION

Moscatilin, $C_{17}H_{20}O_5$ m/z: 304 $[M]^+$, mp 84°, showed UV absorptions, λ_{max} 211, 234 sh and 281.5 nm (log ε 4.51, 4.10 and 3.61), which are strikingly similar to those of erianin (**2e**) [10], a bibenzyl derivative of the orchid *Eria carinata*. The phenolic nature of the compound was indicated by its characteristic colour reactions, alkali-induced bathochromic shift of the UV maxima [$\lambda_{max}^{0.1N\ NaOH-EtOH}$ 221, 252.5 and 293.5 nm (log ε 4.28, 4.21 and 3.84)] and IR spectrum showing a band at 3428 cm^{-1}. The presence of two phenolic hydroxyl groups in moscatilin was confirmed by the formation of a diacetyl derivative, $C_{21}H_{24}O_7$ m/z: 388 $[M]^+$, mp 112°, with acetic anhydride and pyridine.

The ^1H NMR spectrum of moscatilin showed a nine-proton singlet at δ3.81 for three aromatic methoxyl groups, a four-portion singlet at 2.79 for the four benzylic methylene protons of a bibenzyl derivative, and two one-proton singlets at 5.30 and 5.39 (both disappeared on deuterium exchange) for two phenolic hydroxyl groups. The spectrum also revealed signals for five aromatic protons, two of which appeared as a two-proton singlet at δ6.30. The relatively upfield position of these protons corresponds to the two *ortho*-protons of a 3,4,5-trioxygenated benzyl moiety [10]. The remaining three aromatic protons of moscatilin resonated at δ6.60 (d, $J = 2$ Hz), 6.77 (d, $J = 8$ Hz) and 6.74 (dd, $J_1 = 8$ Hz, $J_2 = 2$ Hz). The chemical shifts and the splitting patterns of these protons are typical of H-2, H-5 and H-6, respectively, of a 3,4-dioxygenated benzyl system. The foregoing spectral data thus suggest a 3,3′,4,4′,5-pentaoxygenated bibenzyl structure for moscatilin.



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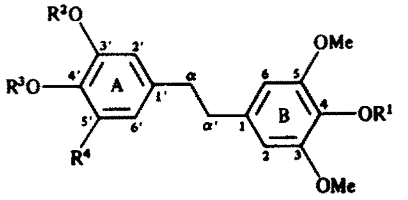
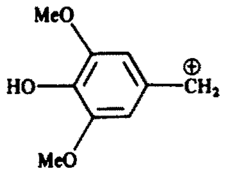
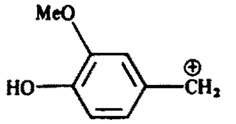


2a $R^1 = R^3 = R^4 = H$, $R^2 = Me$
2b $R^1 = R^3 = Ac$, $R^2 = Me$, $R^4 = H$
2c $R^1 = R^3 = H$, $R^2 = Me$, $R^4 = D$
2d $R^1 = R^3 = Ac$, $R^2 = Me$, $R^4 = D$
2e $R^1 = R^3 = Me$, $R^2 = R^4 = H$
2f $R^1 = R^3 = Me$, $R^2 = Ac$, $R^4 = H$



a (*m/z* 167)



b (*m/z* 137)

The mass spectrum of moscatilin showing intense peaks at *m/z* 167 (fragment **a**) and 137 (fragment **b**) has not only confirmed its bibenzyl formulation but also established the distribution of the five oxygen functions in the two benzene rings, one having a methoxyl and a hydroxyl group and the other bearing one hydroxyl and two methoxyl groups.

The relative positions of the hydroxyl and methoxyl groups in the two benzene rings of moscatilin were indicated by the ^{1}H NMR spectrum of its diacetyl derivative, which is essentially similar to that of moscatilin except that the signal at δ6.77 of the latter corresponding to H-5′ suffered a downfield shift of 0.17 ppm in the latter. This would suggest that H-5′ in moscatilin is the only aromatic proton which is *ortho* and or *para* to a phenolic hydroxyl group. This was further confirmed by the ^{1}H NMR spectrum of the acetyl derivative of the product obtained by base-catalysed deuteration [15] of moscatilin with D_2O in presence of *t*-BuOK, which differed from that of pure moscatilin diacetate only by about 60 % reduction in the integrated intensity of the signal at δ6.94 corresponding to H-5′. Deutero-moscatilin diacetate must therefore have the structure **2d** and the deutero-moscatilin has structure **2c**. Since deuteration has taken place only at C-5′ of moscatilin, it must have one of the hydroxyl groups at C-4′ and the other at C-4. This, therefore, establishes the structure of moscatilin as 4,4′-dihydroxy-3,3′,5-trimethoxybibenzyl (**2a**).

The structure **2a** for moscatilin was further corroborated by its ^{13}C NMR spectral data. The degree of protonation of each carbon atom of moscatilin was determined by off-resonance decoupling techniques, and the assignments of the carbon chemical shifts (Table 1) were made by comparison with the δ_c values of structurally similar compounds [16], particularly with those of erianin acetate (**2f**) [10]. As in the case of **2f**, the carbon chemical shifts of C-3, C-4 and C-5 of moscatilin do not follow the simple additive rules due to consecutive substitution at these carbon atoms [16]. This also holds to a lesser extent for C-3′ and C-4′ for the same reason. The carbon atoms of all the methoxyl groups of moscatilin resonated in the region δ_c 55–56 indicating that all the methoxyl groups of the compound must bear at least one *ortho*-hydrogen atom. An alternative arrangement with one methoxyl flanked by a hydroxyl and a methoxyl group in ring B of moscatilin would have shifted the carbon resonance of such a methoxyl group to about 60–61 ppm similar to that of the 4-methoxyl carbon atom of **2f**. This, therefore, justifies the placement of one of the hydroxyl groups of moscatilin at C-4. The greater shielding of C-1 of moscatilin (**2a**) compared with that of the corresponding carbon atom of **2f** is presumably due to greater electron-donating capacity of a less bulky hydroxyl group in the former than a more bulky methoxyl group in the latter at C-4, both being under the steric influence of two *ortho*-methoxyl groups. The significant shielding of C-2′ and C6′ and a slight deshielding of C-5′ of moscatilin compared with the corresponding carbon atom of **2f**, considered in the light of the additive parameters of the functional groups, confirm the reverse oxygenation patterns in ring A of moscatilin (**2a**) and erianin (**2e**).

Moscatilin (**2a**) is thus a new addition to the growing list of naturally occurring bibenzyl derivatives, and its co-occurrence with moscatin (**1**) in the same orchid presents an interesting biogenetic feature.

Table 1. The carbon chemical shifts of moscatilin (**2a**) and erianin acetate (**2f**)

Carbon atoms	Chemical shifts (δ values)* **2a**	Chemical shifts (δ values)* **2f**	Carbon atoms	Chemical shifts (δ values)* **2a**	Chemical shifts (δ values)* **2f**
C-1	132.84†	139.50	C-4′	143.69	149.30
C-2, C-6	105.19	105.40	C-5′	114.07	112.28
C-3, C-5	146.77‡	153.0	C-6′	120.98	126.68
C-4	133.53†	134.27	C-3, C-5-OMe	56.15¶	56.05‡‡
C-α	38.28§	36.96**	C-4-OMe	—	60.86
C-α′	37.75§	38.82**	C-3′-OMe	55.76¶	—
C-1′	132.76†	137.30††	C-4′-OMe	—	55.99‡‡
C-2′	111.18	122.40	-OCOMe	—	169.12, 20.68
C-3′	146.14‡	136.20††			

*δ values are in ppm downfield from TMS: $\delta_{TMS} = \delta_{CDCl_3} + 76.9$ ppm.
†–‡‡ values are interchangeable.

EXPERIMENTAL

Mps are uncorr. Silica gel (60–120 mesh) was used for CC (unless stated otherwise) and silica gel G for TLC. UV spectra were measured in 95% aldehyde-free EtOH and IR spectra in KBr discs. ^{1}H NMR spectra were measured at 100 and 200 MHz in $CDCl_3$ using TMS as int. standard and chemical shifts are expressed in δ values. ^{13}C NMR spectra were run at 20 MHz in the same solvent with the same int. standard. MS were recorded with a direct inlet system operating at 70 eV. All the analytical samples were routinely dried over P_2O_5 for 24 hr *in vacuo* and were tested for purity by TLC and mass spectrometry, Na_2SO_4 was used for drying organic solvents and the petrol used had bp 60–80°.

Isolation of moscatilin (**2a**). Air-dried, powdered whole plant of *D. moscatum* (2 kg) was kept soaked in MeOH for 3 weeks. The methanolic extract was drained out and concd under red. pres. to about 75 ml, diluted with H_2O (500 ml) and extracted with Et_2O. The Et_2O extract was fractionated into neutral and acidic fractions with 2 N aq. NaOH soln. The aq. alkaline soln was acidified in the cold with conc HCl and the liberated solids were extracted with Et_2O, washed, dried and the solvent removed. The residue was chromatographed. The earlier fractions of the petrol–EtOAc (7:1) gave **1** (0.05 g), crystallised from petrol–EtOAc, mp 163–64°. The latter fractions of the same eluate on evaporation afforded a mixture of **1** and **2a**, which could not be separated even on repeated chromatography over silica gel (60–120 mesh) because of their close polarity. The above mixture of **1** and **2a** was then chromatographed over silica gel H (TLC grade). The later fractions of petrol–EtOAc (7:1) eluate afforded pure **2a** (0.08 g), crystallised from petrol–EtOAc, mp 84°. It gave a violet colouration with neutral $FeCl_3$ and a blue colouration with ammonium phosphomolybdate reagent. (Found: C, 67.08; H, 6.55. $C_{17}H_{20}O_5$ requires: C, 67.10; H, 6.58%). IR ν_{max}^{KBr} cm^{-1}: 3428 (OH), 2918, 1614, 900, 856, 811, 797 and 627 (phenyl nucleus); MS *m/z* (rel. int.): 304 $[M]^+$ (39), 168 (14), 167 (100), 138 (6), 137 (35) and 122 (7).

Moscatilin (**2a**) was acetylated with Ac_2O-pyridine in the usual manner to give **2b**, crystallised from petrol–EtOAc, mp 112°. (Found: C, 64.93; H, 6.20. $C_{21}H_{24}O_7$ requires: C, 64.95; H, 6.18%). UV λ_{max} nm: 213 and 273 nm (log ε 4.37 and 3.51); IR ν_{max}^{KBr} cm^{-1}: 1759 and 1262 (OAc), 1604, 903, 868, 836, 829 and 640 (phenyl nucleus); ^{1}H NMR: δ2.30 and 2.33 (each 3H, *s*, -OCOMe), 2.89 (4H, *s*, H_2-α and H_2-α′), 3.77 (9H, *s*, Ar-OMe), 6.36 (2H, *s*, H-2 and H-6), 6.65 (1H, *d*, $J = 2$ Hz, H-2′), 6.75 (1H, *dd*, $J_1 = 8$ Hz and $J_2 = 2$ Hz, H-6′) and 6.94 (1H, *d*, $J = 8$ Hz, H-5′); MS *m/z* (rel. int.): 388 $[M]^+$ (1), 347 (6), 346 (29), 304 (17), 168 (13), 167 (100), 138 (5), 137 (34) and 43 (15).

Base-catalysed deuteration of moscatilin (**2a**). A mixture of **2a** (0.03 g), *t*-BuOK (0.01 g) and D_2O (0.5 ml) was heated in a N_2-filled sealed tube at 100° for 72 hr. The reaction mixture was then neutralised with conc. HCl in the cold and the liberated solid was extracted with Et_2O, washed, dried and the solvent removed. The ^{1}H NMR spectrum of the residue indicated it to be a mixture of **2c** and **2a** in the ratio of about 60:40. The above reaction product was then acetylated with Ac_2O and pyridine in the usual manner to give a mixture of **2d** and **2b** in the same ratio as indicated by its ^{1}H NMR spectrum.

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REFERENCES

1. Majumder, P. L. and Joardar, M. (1985) *Indian J. Chem.* **24B**, 1192.
2. Majumder, P. L., Bandyopadhyay, D. and Joardar, S. (1982) *J. Chem. Soc. Perkin Trans. I*, 1131.
3. Majumder, P. L. and Datta, N. (1982) *Indian J. Chem.* **21B**, 534.
4. Majumder, P. L., Sarkar, A. K. and Chakraborti, J. (1982) *Phytochemistry* **21**, 2713.
5. Majumder, P. L. and Sarkar, A. K. (1982) *Indian J. Chem.* **21B**, 29.
6. Majumder, P. L., Datta, N., Sarkar, A. K. and Chakraborti, J. (1982) *J. Nat. Prod.* **45**, 730.
7. Majumder, P. L. and Datta, N. (1984) *Phytochemistry* **23**, 671.
8. Majumder, P. L., Kar, A. and Shoolery, J. N. (1985) *Phytochemistry* **24**, 2083.
9. Majumder, P. L. and Sen, R. C., *Indian J. Chem.* in press.
10. Majumder, P. L. and Joardar, M. (1984) *Indian J. Chem.* **23B**, 1040.
11. Majumder, P. L. and Banerjee, S. *Indian J. Chem.* in press.
12. Majumder, P. L. and Pal, A. (1985) *Phytochemistry* **24**, 2120.
13. Majumder, P. L., Pal, A. and Lahiri, S., *Indian J. Chem.* in press.

14. Majumder, P. L. and Chakraborti, J. (1985) *Tetrahedron* **41**, 4973.
15. Miles, D. H., Pelletier, S. W., Bhattacharya, J. Mody, N. V. and Heiden, P. A. (1978) *J. Org. Chem.* **43**, 4371.
16. Wenkert, E., Gottlieb, H. E., Gottlieb, O. R., Pereira Das, M. O. and Formiga, M. D. (1976) *Phytochemistry* **15**, 1547.

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SPECTRAL DIFFERENTIATION OF 3,3′-DI-*O*-METHYLELLAGIC ACID FROM 4,4′-DI-*O*-METHYLELLAGIC ACID

TSUGUO SATŌ

Tohoku College of Pharmacy, Komatsushima-4, Sendai 983, Miyagi, Japan

(*Revised received* 21 *November* 1986)

Key Word Index—*Lagerstroemia fauriei*; Lythraceae; 3,3′-di-*O*-methylellagic acid; 4,4′-di-*O*-methylellagic acid; NMR spectral differentiation.

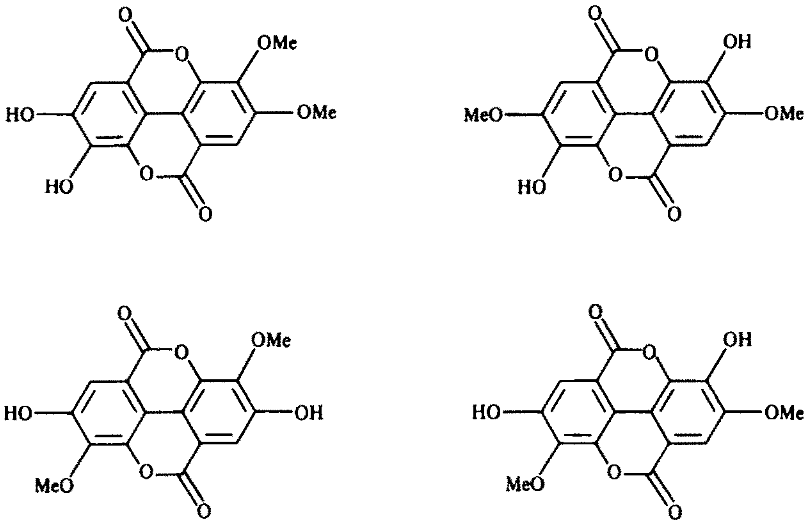
Abstract—3,3′- and 4,4′-di-*O*-methylellagic acid were synthesized, and their spectra (IR, UV, ^{13}C FT-NMR) were compared with each other. UV and ^{13}C FT-NMR spectra were the most useful for distinguishing them.

INTRODUCTION

The isolation of ellagic acid, and the presence of di-*O*-methylellagic acids has been reported from *Lagerstroemia fauriei* Koehne [1]. In the case of natural di-*O*-methylellagic acid, four structural isomers are possible (Scheme 1), but nothing is known of their spectral properties. So 3,3′- and 4,4′-di-*O*-methylellagic acid were synthesized and characterized by their UV, IR, ^{1}H and ^{13}C FT-NMR spectra.

RESULTS AND DISCUSSION

3,3′- and 4,4′-di-*O*-methylellagic acid are difficult to differentiate by using PC, TLC and ^{1}H NMR. The IR spectra show only small differences in the fingerprint region, which are insufficient for separating them. But the UV spectra are very different from each other (Table 1). The addition of alkali or $AlCl_3$ cause large bathochromic shifts in the long wavelength band, and change absorption patterns. The ^{1}H FT-NMR spectra were very similar,



Scheme 1.