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Bibenzyl derivatives from the orchid *Dendrobium amoenum*

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Abstract

Reinvestigation of the orchid *Dendrobium amoenum* afforded two new bibenzyl derivatives, amoenylin and isoamoenylin, and their known structural analogues, 3,4′-dihydroxy-5-methoxybibenzyl and 4,4′-dihydroxy-3,3′,5-trimethoxybibenzyl (moscatilin), besides the sesquiterpenoids, amotin, amoenin and flaccidin reported earlier from this orchid. The structures of amoenylin and isoamoenylin were established as 4-hydroxy-3,4′,5-trimethoxybibenzyl and 3′-hydroxy-3,4,5-trimethoxybibenzyl, respectively, mainly from spectral evidence.

Keywords: Dendrobium amoenum; Orchidaceae; Amoenylin; Isoamoenylin; Moscatilin and 3,4′-dihydroxy-5-methoxybibenzyl; Bibenzyl derivatives; Amotin and amoenin; Sesquiterpenes; Flaccidin; 9,10-Dihydrophenanthropyran derivative

1. Introduction

As part of a general programme of research on orchids, we have chemically reinvestigated the orchid *Dendrobium amoenum* which has afforded two new bibenzyl derivatives, designated amoenylin and isoamoenylin, and two of their known structural analogues, moscatilin (**4g**) (Majumder & Sen, 1987) and 3,4′-dihydroxy-5-methoxybibenzyl (**4e**) (Crombie & Crombie, 1982; Crombie & Jamieson, 1982), besides the picrotoxin group of sesquiterpenoids, amotin (**1**) and amoenin (**2**) (Dahmen & Leander, 1978) and the 9,10-dihydrophenanthropyran, flaccidin (**3**) (Veerraju et al., 1989) reported earlier from the same orchid. The above group of authors reported the isolation of flaccidin (**3**) from this orchid giving a new name to it (amoenumin) and claiming it to be a new compound which was, however, isolated from *Coelogyne flaccida* about one year back (Majumder & Maiti, 1988). While the structure of **4e** was further confirmed by its ^{13}C-NMR spectral data, those of amoenylin and isoamoenylin were shown to be **4a** and **4c**, respectively, mainly from the following spectral evidence.

2. Results and discussion

Both amoenylin (**4a**) and isoamoenylin (**4c**) were shown to have the same molecular formula $C_{17}H_{20}O_4$ from elemental analysis and their mass spectrometrically derived molecular weight 288. Both **4a** and **4c** showed typical benzenoid UV absorptions [**4a**: λ_{max} 208.5 and 274 nm (log ε 4.20 and 3.47); **4c**: λ_{max} 217.5 and 280 nm (log ε 4.04 and 3.90)] which are similar to those of bibenzyl derivatives (Majumder & Ghosal, 1994; Majumder, Roychoudhury & Chakraborty, 1997). The phenolic nature of the compounds was indicated by their characteristic colour reactions with $FeCl_3$ (violet) and phosphomolybdic acid reagent (intense blue), alkali induced bathochromic shifts of their UV maxima and their IR absorption bands [**4a**: ν_{max} 3400 cm^{-1} (OH); **4c**: ν_{max} 3410 cm^{-1} (OH)]. The presence of a single phenolic hydroxyl group in each of **4a** and **4c** was confirmed by the formation of their respective monoacetyl derivatives **4b** and **4d**, both having the same molecular formula $C_{19}H_{22}O_5$ (M^+ at m/z 330).

The ^{1}H-NMR spectrum of each of **4a** and **4c** showed signals for one phenolic hydroxyl proton (**4a**: δ 5.30; **4c**: δ 5.03; each disappeared on deuterium exchange), three aromatic methoxyl groups [**4a**: δ 3.71 (3H, s) and

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3.76 (6H, s); **4c**: δ 3.72 (6H, s) and 3.76 (3H, s)], six aromatic protons [**4a**: 6.28 (2H, s), 6.75 (2H, d, J = 9 Hz) and 7.0 (2H, d, J = 9 Hz); **4c**: 6.26 (2H, s), 6.56 (1H, ill-resolved d, J = 1.8 Hz), 6.62 (1H, ill-resolved d, J = 8.0 Hz and J_2 = 1.8 Hz), 6.76 (1H, ill-resolved d, J = 8.7 Hz) and 7.13 (1H appt. t)] and four methylene protons [**4a**: δ 2.75 (4H, s); **4c**: δ 2.76 (4H, s)] which are typical of the four benzylic protons of a bibenzyl derivative (Majumder & Ghosal, 1994; Majumder et al., 1997).

The bibenzylic formulations of both **4a** and **4c** and the distributions of the hydroxyl and the methoxyl groups in their two phenyl rings were established from the characteristic mass spectral fragmentations of the compounds. The mass spectrum of **4a** showed two intense peaks at m/z 167 and 121, which may be attributed to the ion-fragments **a** and **b**, respectively. The mass spectrum of **4c** also exhibited two intense peaks at m/z 181 and 107, which corresponded to the ion-fragments **c** and **d**, respectively. The appearance of the above peaks in the mass spectra of **4a** and **4c** thus not only affirmed the bibenzylic nature of the compounds, but also implied that while **4a** contained two methoxyl groups and the lone hydroxyl function in one of its phenyl rings (ring A) and the remaining methoxyl group in the other phenyl ring (ring B), **4c** had all the three methoxyl groups in one of its phenyl rings (ring A) and its lone hydroxyl group in the other phenyl ring (ring B).

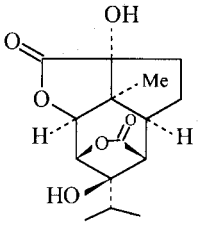
The exact positions of the hydroxyl and methoxyl functions in the two phenyl rings of **4a** and **4c** were ascertained from the chemical shifts and the splitting patterns of the signals of the aromatic protons of the compounds and their respective acetyl derivatives **4b** and **4d**. Thus, the two-proton doublets at δ 6.75 (J = 9 Hz) and 7.00 (J = 9 Hz) in the ^{1}H-NMR spectrum of **4a** exhibiting a typical A_2B_2 splitting pattern of a *p*-disubstituted phenyl moiety may be assigned to H-3′ and H-5′ and H-2′ and H-6′, respectively, with a methoxyl group being placed at C-4′. The remaining two aromatic protons of **4a** appearing as a singlet at δ 6.28 correspond to H-2 and H-6 of a 4-hydroxy-3,5-dimethoxybenzyl moiety present in **4g**. That the six aromatic protons of **4a** remained virtually unchanged in the ^{1}H-NMR spectrum of its acetyl derivative **4b** lent further support for the placement of a methoxyl group at C-4′ and the hydroxyl function at C-4 in **4a**. In an analogous manner, the two-proton singlet at δ 6.26 in the ^{1}H-NMR spectrum of **4c**, which remained almost unchanged in the spectrum of its acetyl derivative **4d** were assigned to H-2 and H-6 of **4c** possessing three methoxyl groups at C-3, C-4 and C-5. The signals at δ 6.56, 6.62, 7.13 and 6.76 in the ^{1}H-NMR spectrum of **4c** and their splitting patterns, on the other hand, showed striking similarities to those of H-2′, H-4′, H-5′ and H-6′, respectively, of batatasin-III (**4k**) (Sachdev & Kulshreshtha, 1986; Majumder & Basak, 1991). This would suggest that the lone hydroxyl group of **4c** must be placed at C-3′ as in **4k**. This was further supported by the fact that while the signals at δ 6.56, 6.62 and 6.76 of **4c** assigned to its H-2′, H-4′ and H-6′ showed the expected downfield shifts in the ^{1}H-NMR spectrum of its acetyl derivative **4d**, that at δ 7.13 corresponding to H-5′ of **4c** remained virtually unchanged in the spectrum of **4d**. In fact the chemical shifts and the splitting patterns of the signals of H-2′, H-4′, H-5′ and H-6′ of **4d** were, as expected, almost identical to those of the corresponding protons of batatasin-III diacetate (**4l**). Based on the foregoing evidence, amoenylin and isoamoenylin were assigned the structures **4a** and **4c**, respectively.

Table 1
^{13}C-NMR spectral data of **4a**, **4g**, **4i**, **4j**, **4e**, **4k** and **4m**

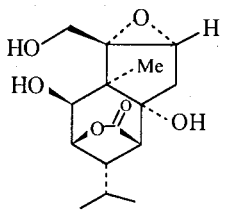
C	Chemical shift (δ, ppm)[a]						
	4a	**4g**	**4i**	**4j**	**4e**[b]	**4k**	**4m**
1	133.4	132.8[c]	137.4	139.5	145.2	145.0[c]	145.0
2	105.0	105.2	105.2	105.4	108.0	108.8	107.1
3	147.0	146.8	152.8	153.0	159.5	159.2	157.9
4	132.4	133.5[c]	135.8	134.3	98.9	99.9	100.7
5	147.0	146.8	152.8	153.0	161.4	161.9	157.9
6	105.0	105.2	105.2	105.4	106.7	106.3	107.7
1′	132.6	132.7	133.4	137.3	133.8	144.3[c]	133.2
2′	129.3	111.2	111.0	122.4	129.4	116.2	129.9
3′	113.6	146.1	146.1	136.2	115.1	158.2	115.8
4′	157.9	143.7	143.6	149.3	154.2	113.6	154.8
5′	113.6	114.1	114.0	112.3	115.1	130.0	115.8
6′	129.3	121.0	120.8	126.7	129.4	120.4	129.9
α	38.3[c]	38.3[d]	38.5[c]	38.8[c]	38.1[c]	38.4[d]	38.8[c]
α′	37.2[c]	37.8[d]	37.5[c]	36.9[c]	36.6[c]	38.1[d]	37.3[c]
OMe	55.2	55.2	55.9	56.0	55.2	55.3	–
	56.2	55.8	60.6	56.1			
				60.9			
	–	–		169.1	–		–
OAc	–	–		20.7	–		–

[a] The spectra were run in $CDCl_3$ and the chemical shifts were measured with $\delta_{(TMS)} = \delta_{(CDCl_3)} + 76.9$ ppm.
[b] Reported for the first time.
[c,d] Values are interchangeable within the same column.

The structure of amoenylin (**4a**) was further confirmed by its ^{13}C-NMR spectral data (Table 1). The ^{13}C-NMR spectrum of **4c**, however, could not be run due to paucity of material. The degree of protonation of each carbon of **4a** was determined by DEPT experiments. The chemical shifts of the carbon atoms of **4a** were assigned by comparison with the δ_C values of structurally related compounds, viz. moscatilin (**4g**) (Majumder & Sen, 1987), crepidatin (**4i**) (Majumder & Chatterjee, 1989) and erianin acetate (**4j**) (Majumder & Joardar, 1984). Thus, the δ_C values of C-α′, C-1, C-2, C-3, C-4, C-5 and C-6 of **4a** are virtually identical with those of the corresponding carbon atoms of moscatilin (**4g**) confirming the presence of a 4-hydroxy-3,5-



1



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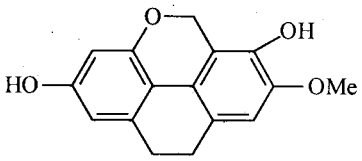

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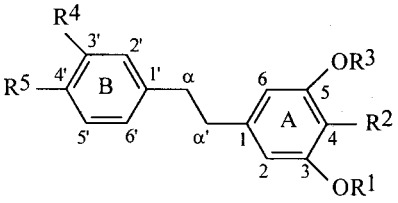
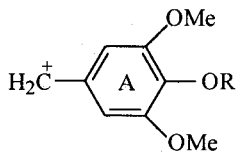
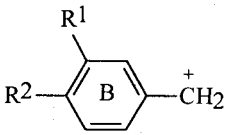


4a : R^1=R^3=Me, R^2=OH, R^4=H, R^5=OMe
4b : R^1=R^3=Me, R^2=OAc, R^4=H, R^5=OMe
4c : R^1=R^3=Me, R^2=OMe, R^4=OH, R^5=H
4d : R^1=R^3=Me, R^2=OMe, R^4=OAc, R^5=H
4e : R^1=R^2=R^4=H, R^3=Me, R^5=OH
4f : R^1=Ac, R^2=R^4=H, R^3=Me, R^5=OAc
4g : R^1=R^3=Me, R^2=R^5=OH, R^4=OMe
4h : R^1=R^3=Me, R^2=R^5=OH, R^4=OMe
4i : R^1=R^3=Me, R^2=R^4=OMe, R^5=OH
4j : R^1=R^3=Me, R^2=R^5=OMe, R^4=OAc
4k : R^1=R^2=R^5=H, R^3=Me, R^4=OH
4l : R^1=Ac, R^2=R^5=H, R^3=Me, R^4=OAc
4m : R^1=R^2=R^3=R^4=H, R^5=OH



a (R = H) : m/z 167
c (R = Me) : m/z 181



b (R^1=H, R^2=OMe) : m/z 121
d R^1=OH, R^2=H) : m/z 107

dimethoxybenzyl moiety in **4a**. The δ_C values of these carbon atoms of **4a** are also comparable with the corresponding carbon atoms of crepidatin (**4i**) and erianin acetate (**4j**), the differences being due to consecutive methoxyl groups at C-3, C-4 and C-5 in the latter compounds. The chemical shifts of C-α, C-1′, C-2′, C-3′, C-4′, C-5′ and C-6′ of **4a** are again comparable to those of the corresponding carbon atoms of dihydroresveratrol (**4m**) (Adesanya, Ogundana & Roberts, 1989; Majumder & Pal, 1993), the marginal differences being due to the presence of a methoxyl group at C-4′ in **4a** in place of a hydroxyl group at the same carbon atom in **4m**. This would further suggest the presence of a 4-methoxybenzyl moiety in **4a** constituting the second benzylic part of the compound.

The structure of the compound **4e** isolated earlier from *Cannabis sativa* (Crombie & Crombie, 1982) was established from its ^{1}H-NMR and mass spectral data and confirmed by its synthesis (Hashimoto & Tajima, 1978; Crombie & Jamieson, 1982). Additional evidence in support of its proposed structure is now provided by its ^{13}C-NMR spectral data (Table 1). The virtually identical δ_C values of C-α, C-1′, C-2′, C-3′, C-4′, C-5′ and C-6′ of **4e** with those of the corresponding carbon atoms of dihydroresveratrol (**4m**) containing a 4-hydroxybenzyl moiety confirmed the presence of the above unit also in **4e**. Again, the striking similarities of the δ_C values of C-α′, C-1, C-2, C-3, C-4, C-5 and C-6 of **4e** with those of the corresponding carbon atoms of batatasin-III (**4k**) established the nature of the second benzylic part in **4e** to be a 3-hydroxy-5-methoxybenzyl moiety as in **4k**.

Amoenylin (**4a**) and isoamoenylin (**4c**) are thus two new additions to the growing list of naturally occurring bibenzyl derivatives. In view of the fact that several bibenzyl derivatives are reported to exhibit pronounced antimitotic property (Pettit, Singh & Schmidt, 1988), while others are known to act as potent endogenous plant growth regulators (Gorham, 1980), it would be interesting to study **4a** and **4c** for similar biological activities.

3. Experimental

Melting points: 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 $CDCl_3$ using TMS as an internal standard. Chemical shifts are expressed in δ (ppm). MS: direct inlet system, 70 eV. All analytical samples were routinely dried over P_2O_5 for 24 h in vacuo and were tested for purity by TLC and MS. The petrol used had bp 60–80°C.

3.1. Plant materials

Dendrobium amoenum Wall was collected from Kalimpong (Darjeeling), India in September, 1996. A voucher specimen was deposited in the Herbarium of the Department of Botany, University of Calcutta (CUH).

3.2. Isolation of amoenylin (**4a**), isoamoenylin (**4c**), 3,4′-dihydroxy-5-methoxybibenzyl (**4e**), moscatilin (**4g**), amotin (**1**), amoenin (**2**) and flaccidin (**3**)

Air-dried powdered whole plant of *D. amoenum* (2 kg) was kept soaked in MeOH (6 l) for 3 weeks. The MeOH extract was concentrated to ca. 100 ml, diluted with H_2O (750 ml) and exhaustively extracted with Et_2O. The Et_2O extract was fractionated into acidic and nonacidic fractions with 2 M NaOH. The aqueous alkaline solution was acidified in the cold with conc. HCl and the liberated solids were extracted with Et_2O, washed with H_2O, dried and the solvent removed. The residue was chromatographed. The petrol–EtOAc (50 : 1) eluate gave **4c** as a semisolid mass (0.015 g) (found: C, 70.74; H, 6.94; $C_{17}H_{20}O_4$ requires: C, 70.79; H, 6.99%). UV $\lambda_{max}^{EtOH-0.1M\ NaOH}$ nm: 221 and 280 (log ε 3.80 and 3.90); IR ν_{max} cm^{-1}: 3410 (OH), 1610, 1565, 890, 760, 750, 740, 675 and 650 (aromatic nucleus); MS *m*/*z* (rel. int.): 288 [M$^+$] (30), 181 (100), 166 (25), 138 (10) and 107 (35). Compound **4c** was acetylated with Ac_2O and pyridine in the usual manner to give **4d** also as a semisolid mass. (Found: C, 69.0; H, 6.63; $C_{19}H_{22}O_5$ requires: C, 69.05; H, 6.71%). ν_{max} cm^{-1}: 1765 and 1245 (OAc); ^{1}H-NMR: δ 2.23 (3H, s; OAc), 2.80 (4H, s, H_2-α and H_2-α'), 3.75 (6H, s, 2xOMe), 3.78 (3H, s; OMe); 6.91 (2H, m; H-2′ and H-4′), 7.20 (1H, appt. t; H-5′), 6.88 (1H, dd, J_1 = 8.0 Hz and J_2 = 2.8 Hz) and 6.23 (2H, s; H-2′ and H-6′); MS *m*/*z* (rel. int): 330 [M$^+$] (15), 288 (25), 181 (100), 166 (20), 138 (8) and 107 (30). Washing the main column with petrol–EtOAc (30 : 1) gave a gummy residue comprising mainly **4a** and **4e**. Repeated MPLC of this mixture using petrol–EtOAc (2 : 1) as the solvent system finally gave pure **4a** (0.09 g) as a semisolid mass and **4e** (0.08 g), crystallized from petrol–EtOAc, mp 112°C. **4a** (found: C, 70.75; H, 6.93; $C_{17}H_{20}O_4$ requires: C, 70.79; H, 6.99%). UV $\lambda_{max}^{ETOH-0.1M\ NaOH}$ nm: 213.5 and 280 (log ε 4.45 and 3.50); IR: ν_{max} cm^{-1} 3400 (OH), 1600, 1520, 1440–1470, 840, 790, 710 and 680 (aromatic nucleus); MS *m*/*z* (rel. int.) 288 [M$^+$] (29), 287 (29), 286 (44), 273 (6), 265 (11), 264 (11), 257 (13), 239 (38), 211 (25), 183 (69), 181 (30), 171 (29), 167 (100), 137 (44), 135 (76), 129 (56), 127 (38), 125 (41), 123 (53), 122 (49) and 121 (98). Acetylation of **4a** with Ac_2O and pyridine in the usual manner gave **4b** also as a semisolid mass. (found: C, 69.01; H, 6.65; $C_{19}H_{22}O_5$ requires: C, 69.05; H, 6.71%). UV λ_{max} nm: 210.5 and 269 (log ε 4.81 and 3.35); IR ν_{max} cm^{-1}: 1760 and 1250 (OAc), 1610, 1515, 1465, 830, 730 and 600 (aromatic nucleus); ^{1}H-NMR: δ 2.26 (3H, s; OAc), 2.78 (4H, s, H_2-α and H_2-α'), 3.71 (6H, s; 2xOMe), 3.72 (3H, s; OMe), 6.32 (2H, s; H-2 and H-6), 7.02 (2H, d, J = 9 Hz; H-2′ and H-6′) and 6.75 (2H, d, J = 9 Hz; H-3′ and H-5′); MS: *m*/*z* (rel. int.): 330 [M$^+$] (15), 288 (25), 167 (100) and 121 (95). Compound **4e**: ν_{max} cm^{-1} 3310 (OH), 1620, 1592, 832, 819 and 687 (aromatic nucleus); ^{1}H-NMR: δ 2.78 (4H, s; H_2-α and H_2-α'), 3.73 (3H, s; OMe), 6.21-6.34 (3H, m; H-2, H-4 and H-6), 6.68 (2H, d, J = 8.3 Hz; H-3′ and H-5′) and 6.97 (2H, d, J = 8.3 Hz; H-2′ and H-6′); MS *m*/*z* (rel. int.): 244 [M$^+$] (25), 138 (20), 137 (10) and 107 (100). Acetylation of **4e** with Ac_2O and pyridine gave the diacetate **4f** as a semisolid mass. ^{1}H-NMR: δ 2.28 (6H, s; 2xOAc), 2.88 (4H, s; H_2-α and H_2-α'), 3.75 (3H, s; OMe), 6.41, 6.45 and 6.50 (each ill-resolved metacoupled dd; H-4, H-2 and H-6), 6.99 (2H, d, J = 9 Hz; H-3′ and H-5′) and 7.16 (2H, d, J = 9 Hz; H-2′ and H-6′). Elution of the main column with petrol–EtOAc (15 : 1) afforded pure **4g** (0.05 g), crystallized from petrol–EtOAc mixture, mp, 84°C. Washing the column with petrol–EtOAc (10 : 1) gave **3** (0.015 g), crystallized from petrol–EtOAc, mp, 200°C. Both **4g** and **3** were identified by direct comparison (superimposable IR spectra) with their respective authentic samples.

Further elution of the main column with petrol–EtOAc (5 : 1) afforded a mixture of **1** and **2**. MPLC of this mixture using petrol–EtOAc (1 : 1) as the solvent system finally gave pure **1** (0.08 g), crystallized from petrol–EtOAc mixture, mp 256°C and **2** (0.02 g), also crystallized from the same solvent mixture, mp 198°C (d). The IR, ^{1}H-, ^{13}C-NMR and mass spectral data of these compounds compared excellently with those reported for amotin (**1**) and amoenin (**2**), respectively.

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