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BIBENZYL DERIVATIVES FROM THE ORCHID *BULBOPHYLLUM PROTRACTUM*

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Key Word Index—*Bulbophyllum protractum*; Orchidaceae; bulbophyllin; bulbophyllidin; bibenzyl derivatives.

Abstract—Two new bibenzyl derivatives were isolated from the orchid *Bulbophyllum protractum* and designated bulbophyllin and bulbophyllidin. In addition batatasin-III (3,3′-dihydroxy-5-methoxy bibenzyl), 3,3′,5-trimethoxybibenzyl, aloifol-I (3′,4-dihydroxy-3,5-dimethoxybibenzyl), 3,3′-dimethoxy-4,5-methylenedioxybibenzyl, flavidin (2,7-dihydroxy-9,10-dihydro-5H-phenanthro[4,5-bcd]pyran), dihydroconiferyl alcohol, stigmasterol and sitosterol were isolated. The structures of bulbophyllin and bulbophyllidin were established as 2,3′-dihydroxy-3-methoxy-4,5-methylenedioxybibenzyl and 2,3′-dihydroxy-3,5-dimethoxybibenzyl, respectively, from spectral and chemical evidence. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

We reported previously the isolation of a fairly large number of compounds of diverse structural types from a series of Indian Orchidaceae plants. They included simple aromatic compounds [1], a wide variety of stilbenoids [2–21], such as bibenzyls [2–5], phenanthrenes [6–8] and 9,10-dihydrophenanthrenes [9–12] and their dimers [13–15], phenanthropyrans and pyrones [16–18] and their 9,10-dihydro derivatives [17, 19–21], a few other polyphenolics [22–24], several triterpenoids [25] and steroids of biogenetic importance [26]. As part of this general programme of research we have investigated another new Indian orchid, *Bulbophyllum protractum*. This has resulted in the isolation of two new bibenzyl derivatives, designated bulbophyllin and bulbophyllidin, besides batatasin-III (**1a**) [2, 27], batatasin-III dimethyl ether (**1b**) [28], aloifol-I (**1d**) [29], 3,3′-dimethoxy-4,5-methylenedioxybibenzyl (**1f**) [30], flavidin (**2**) [20], dihydroconiferyl alcohol (**3**) [22], stigmasterol and sitosterol of previously known structures. While the known compounds were identified by comparison with their respective authentic samples and independent spectral analysis, bulbophyllin and bulbophyllidin were shown to have the structures **1g** and **1k**, respectively, from the following spectral and chemical evidence.

RESULTS AND DISCUSSION

Both bulbophyllin (**1g**), $C_{16}H_{16}O_5$ ($[M]^+$ at m/z 288), mp 110°, and bulbophyllidin (**1k**), $C_{16}H_{18}O_4$ ($[M]^+$ at m/z 274), obtained as a semi-solid mass, showed typical benzenoid UV absorptions [**1g**: λ_{max} 215, 282.5 and 291.5 nm (log ε 4.89, 4.26 and 4.24); **1k**: λ_{max} 208.5 and 282 nm (log ε 4.80 and 4.08)]. The phenolic nature of both **1g** and **1k** was indicated by their characteristic colour reactions ($FeCl_3$: violet; phosphomolybdic acid reagent: intense blue), alkali-induced bathochromic shifts of their UV maxima and by their characteristic IR absorption bands [**1g**: ν_{max} cm^{-1} 3400 (OH); **1k**: ν_{max} cm^{-1} 3540 (OH)]. The presence of two phenolic hydroxyl groups in each of **1g** and **1k** was confirmed by the formation of their respective diacetates with acetic anhydride and pyridine [bulbophyllin diacetate (**1h**), $C_{20}H_{20}O_7$ ($[M]^-$ at m/z 372); bulbophyllidin diacetate (**1l**), $C_{20}H_{22}O_6$ ($[M]^+$ at m/z 358)] and dimethyl ether derivatives with CH_2N_2 [bulbophyllin dimethyl ether (**1j**), $C_{18}H_{20}O_5$ ($[M]^+$ at m/z 316); bulbophyllidin dimethyl ether (**1m**), $C_{18}H_{22}O_4$ ($[M]^+$ at m/z 302)]. The reaction of **1g** with CH_2N_2 also afforded a monomethyl ether derivative **1i**, $C_{17}H_{20}O_4$ ($[M]^+$ at m/z 288).

The ^1H NMR spectrum of **1g** showed signals for an aromatic methoxyl function [δ 4.04 (3H, *s*)], a methylenedioxy group [δ 5.84 (2H, *s*)], two phenolic hydroxyl protons [δ 5.42 and 4.82 (each 1H, *s*); disappeared on deuterium exchange], five aromatic protons at δ 6.29–7.14 and a four-proton singlet at δ 2.81 which is typical of the four benzylic protons of a bibenzyl derivative [2–5]. A similar four-proton singlet at δ 2.80 was also discernible in the ^1H NMR spectrum of **1k**, which, in addition, showed signals for two phenolic hydroxyl protons [δ 5.34 and 5.85 (each 1H, *s*); disappeared on deuterium exchange], two aromatic methoxyl groups [δ 3.72 and 3.82 (each 3H, *s*)] and six

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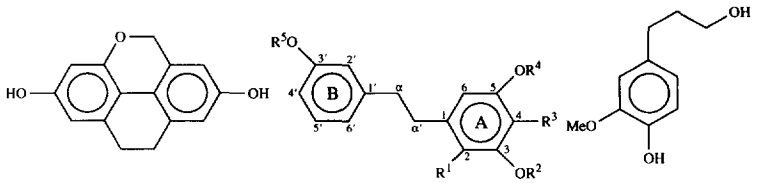

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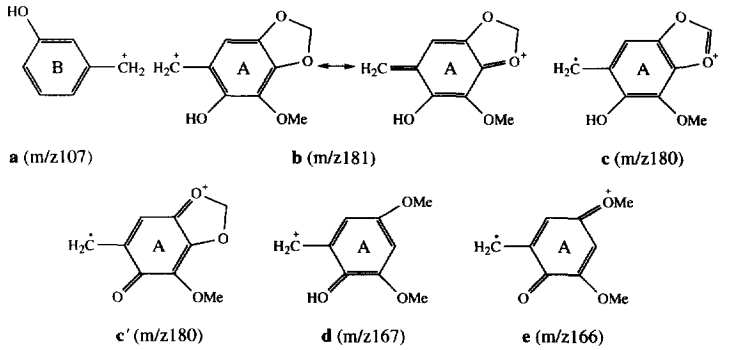
1a : $R^1=R^2=R^3=R^5=H$, $R^4=Me$
1b : $R^1=R^3=H$, $R^2=R^4=R^5=Me$
1c : $R^1=R^3=H$, $R^2=R^5=Ac$, $R^4=Me$
1d : $R^1=R^5=H$, $R^2=R^4=Me$, $R^3=OH$
1e : $R^1=H$, $R^2=R^4=Me$, $R^3=OAc$, $R^5=Ac$
1f : $R^1=H$, $R^2=R^5=Me$, R^3, R4=–O–CH_2–
1g : $R^1=OH$, $R^2=Me$, R^3, R^4=–O–CH_2–, $R^5=H$
1h : $R^1=OAc$, $R^2=Me$, R^3, R^4=–O–CH_2–, $R^5=Ac$
1i : $R^1=OH$, $R^2=R^5=Me$, R^3, R^4=–O–CH_2–
1j : $R^1=OMe$, $R^2=R^5=Me$, R^3, R^4=–O–CH_2–
1k : $R^1=OH$, $R^2=R^4=Me$, $R^3=R^5=H$
1l : $R^1=OAc$, $R^2=R^4=Me$, $R^3=H$, $R^5=Ac$
1m: $R^1=OMe$, $R^2=R^4=R^5=Me$, $R^3=H$
1n : $R^1=R^2=R^5=H$, R^3, R^4=–O–CH_2–
1o : $R^1=H$, $R^2=R^5=Ac$, R^3, R^4=–O–CH_2–



a (m/z107) **b** (m/z181) **c** (m/z180)

c' (m/z180) **d** (m/z167) **e** (m/z166)

aromatic protons at δ 6.24–7.14. The above ^{1}H NMR spectral data of **1g** and **1k** indicated both of them to possess the same bibenzylic skeletal structure, the former containing an aromatic methoxyl, a methylenedioxy and two phenolic hydroxyl groups and the latter bearing two phenolic hydroxyl and two aromatic methoxyl functions.

The bibenzylic formulations of **1g** and **1k** and the distributions of the functional groups in their two phenyl rings were established by their characteristic mass spectral fragmentations. The appearance of an intense peak at *m/z* 107 in the mass spectra of **1g** and **1k** (in the case of **1k** it is the base peak) (corresponding to the ion-fragment **a**) indicated the presence of a single phenolic hydroxyl group in one of the benzylic moieties of both **1g** and **1k**. The mass spectrum of **1g** also showed an intense peak at *m/z* 181 and the base peak at *m/z* 180, which were attributed to the ion-fragments **b** and **c** (and or **c'**), respectively. These mass spectral peaks thus established the presence of the second phenolic hydroxyl group, the aromatic methoxyl and the methylenedioxy function in the phenyl ring associated with the other benzylic unit of **1g**. The presence of a hydroxyl and two methoxyl groups in the phenyl ring associated with the second benzylic moiety of **1k** was indicated by the appearance of the intense peaks at *m/z* 167 (ion-fragment **d**) and 166 (ion-fragment **e**) in the mass spectrum of **1k**.

The relative positions of the functional groups in the two phenyl rings of **1g** and **1k** were ascertained from the chemical shifts and the splitting patterns of the aromatic protons of the compounds and their acetyl and methyl ether derivatives. Thus, the chemical shifts and the splitting patterns of four aromatic protons of both **1g** and **1k** [**1g**: δ 6.69 (1H, ill-resolved *meta*-coupled doublet; H-2'), 6.66 (1H, *dd*, $J_1 = 7.8$ Hz and $J_2 = 2.4$ Hz; H-4'), 7.14 (1H, app. *t*, $J = 7.8$ Hz; H-5') and 6.79 (1H, ill-resolved *ortho-meta*-coupled *dd*; H-6'); **1k**: δ 6.71 (1H, ill-resolved *meta*-coupled doublet; H-2'), 6.66 (1H, *dd*, $J_1 = 7.9$ Hz and $J_2 = 2.4$ Hz; H-4'), 7.14 (1H, app.*t*, $J = 7.9$ Hz; H-5') and 6.80 (1H, ill-resolved *ortho-meta*-coupled *dd*; H-6') are strikingly similar to those of H-2', H-4', H-5' and H-6' of batatasin-III indicating the presence of a hydroxyl group at C-3' in all the three compounds. The remaining aromatic proton signal of **1g** appearing as a sharp singlet at δ 6.29 was assigned to H-6 keeping the other hydroxyl group at C-2, the methoxyl function at C-3 and the

methylenedioxy group at C-4 and C-5 positions. The other two aromatic protons of **1k**, on the other hand, appeared as a pair of ill-resolved *meta*-coupled doublets at δ 6.24 and 6.38, which were attributed to H-4 and H-6, the remaining hydroxyl group being placed at C-2 and the two methoxyl groups at C-3 and C-5. The above signals of **1g** and **1k** remained virtually unchanged in the ^{1}H NMR spectra of their respective diacetates **1h** and **1l** indicating that the protons corresponding to these signals of **1g** and **1k** must be *meta* to their second hydroxyl group at C-2. But the signals corresponding to H-2′, H-4′ and H-6′ of **1g** and **1k** showed the expected downfield shifts in the ^{1}H NMR spectra of their diacetates **1h** and **1l**, confirming the placement of the other hydroxyl group at C-3′ in both **1g** and **1k**.

The structures of **1g** and **1k** were finally confirmed by the ^{13}C NMR spectral data of the compounds and their diacetyl derivatives **1h** and **1l** (Table 1). The degree of protonation of the carbon atoms of each compound was confirmed by DEPT and APT experiments and the assignments of the carbon chemical shifts for each compound were made by comparison with the δ_c values of structurally similar compounds like **1a**, **1c**, **1d**, **1e** and **1o** taking into consideration the known additive parameters of the functional groups. Thus, the virtually identical δ_c values of C-1′, C-2′, C-3′, C-4′, C-5′, C-6′ and C-α of **1g**, **1k**, **1a** [27, 29] and **1d** [29] established the presence of the same 3-hydroxybenzyl moiety in all the four compounds. This was further corroborated by the almost identical δ_c values of the above carbon atoms of **1h**, **1l**, **1c** [2], **1e** and **1o** [2], all having a 3-acetoxybenzyl unit. The upfield shifts of C-α' of both **1g** (δ_c 31.6) and **1k** (δ_c 31.8) compared to their C-α appearing at the normal region [*ca* δ_c 36–37] provided the most convincing evidence in support of the presence of a substituent at C-2 (or C-6) of these compounds. Such upfield shifts of C-α and C-α' have earlier been observed [2, 3, 5] in bibenzyl derivatives having substituents at C-2′ and C-2 or C-6, respectively. That this substituent was a hydroxyl group at C-2 in both **1g** and **1k** was indicated by the observed upfield shifts of their C-1 compared to their C-1′ resonance appearing at the normal region at *ca* δ_c 143–144 with no substituent at C-2′, C-4′ and C-6′. While the C-1 resonance of **1k** (δ_c 127.8) corresponded to the placement of a hydroxyl group at C-2 with no substituent at C-4, the greater shielding of C-1 of **1g** (δ_c 119.3), however, required the placement of an additional oxygen substituent at C-4. The placement of a hydroxyl group at C-2 in both **1g** and **1k** was also affirmed by the expected downfield shifts of their C-1 resonances in the ^{13}C NMR spectra of their respective diacetyl derivatives **1h** and **1l**. In the spectrum of **1h**, while the protonated aromatic carbon at δ_c 102.8 and the oxygenated nonprotonated aromatic carbon at δ_c 134.0 of **1g**, attributed to its C-6 and C-4, respectively, remained practically unchanged, the signals for the oxygenated aromatic carbons at δ_c 131.0 and 141.3 of **1g** assigned to its C-3 and C-4 showed downfield shifts of 4.4 and 5.2 ppm, respectively. This suggests the placement of the methoxy and methylenedioxy groups at C-3, C-4 and C-5 of **1g**, the methylenedioxy carbon resonating at δ_c 100.8. The

Table 1. ^{13}C NMR spectral data of **1g**, **1h**, **1k**, **1l**, **1a**, **1c**, **1d**, **1e** and **1o**

	Chemical shifts (δ values)*								
C	**1g**	**1h**	**1k**	**1l**	**1a**	**1c**	**1d**	**1e**†	**1o**
1	119.3	126.8	127.8	135.0	145.0	143.7	132.8	139.7	137.0
2	139.9	136.1[a]	138.1	135.0	106.3	113.7	105.4	105.0	115.3
3	131.0	135.4[a]	147.0	152.3	159.2	151.5	146.8	151.7	132.8
4	134.0	134.8	97.4	98.9	99.9	105.2	132.9	130.0	135.6
5	141.3	146.5	153.1	158.1	161.9	160.2	146.8	151.7	149.2
6	102.8	102.4	106.6	106.0	108.8	111.8	105.4	105.0	106.5
1′	144.0	143.1	144.2	143.4	144.3	143.0	143.3	143.1	143.0
2′	115.3	121.3	115.6	121.4	116.2	121.3	115.2	121.6	121.3
3′	155.3	150.8	155.7	151.2	158.2	150.7	155.9	150.6	150.7
4′	112.6	119.1	112.8	119.1	113.6	119.1	112.9	119.1	119.0
5′	129.3	129.1	129.3	129.1	130.0	129.1	129.2	129.1	129.1
6′	120.9	125.7	121.0	125.7	120.4	125.8	120.5	125.9	125.8
α	36.0	36.1	35.8	36.0	37.3[a]	37.3[a]	36.7[a]	38.0[a]	37.3[a]
α'	31.6	31.8	31.8	32.2	36.9[a]	37.0[a]	37.7[a]	37.5[a]	37.0[a]
OMe	59.8	59.5	56.1	56.1	55.6	55.2	56.2	55.9	–
			55.9	55.5					
$-OCH_2-O-$	100.8	101.0	–	–	–	–	–	–	101.6
OAc	–	169.2	–	168.7	–	169.3	–	168.8	167.9
		20.9		20.8		21.0		168.2	169.2
		20.2		20.2				20.9	20.9
								20.4	20.4

*The spectra were run in $CDCl_3$ and the chemical shifts measured with $\delta_{(TMS)} = \delta_{(CDCl_3)} + 76.9$ ppm.

†Reported for the first time.

[a] Values interchangeable within the same column.

placement of the methoxy group at C-3 flanked by two *ortho* substituents at C-2 (OH) and C-4 (one oxygen atom of the methylenedioxy group) was confirmed by the downfield shift of the methoxy carbon (δ_c 59.8) of **1g**, which would have resonated at the normal region (*ca* δ_c 55–56), had there been at least one hydrogen atom *ortho* to the methoxyl group. The δ_c values of C-6 of both **1g** and **1h** also corresponded to their being *ortho* and *para* to two oxygenated functions. The above ^{13}C NMR spectral data of **1g** and **1h** thus firmly established the presence of 2-hydroxy-3-methoxy-4,5-methyl-
enedioxy benzyl moiety in **1g**. The structure of the second benzylic unit of **1k** was also confirmed by a comparison of the δ_c values of the ring-A carbon atoms of the compound with those of the corresponding carbon atoms of **1l**. Thus, while the protonated aromatic carbon resonances at δ_c 106.6 and 97.4 of **1k** attributed to its C-6 and C-4, remained practically unchanged in the ^{13}C NMR spectrum of **1l**, the two methoxy bearing carbon atoms of **1k** at δ_c 147.0 (C-3) and 153.1 (C-5) were shifted downfield by 5.3 and 5.0 ppm, respectively, in the spectrum of **1l**, affirming the placement of the two methoxyl groups in **1k** at C-3 and C-5 with one of the hydroxyl groups at C-2. This was also supported by the normal carbon resonances (δ_c 56.1 and 55.9) of the methoxyl groups of **1k** having at least one hydrogen atom *ortho* to the methoxyl groups. The structures of bulbophyllin and bulbophyllidin were thus established as 2,3′-dihydroxy-3-methoxy-4,5-methylenedioxy-bibenzyl (**1g**) and 2,3′-dihydroxy-3,5-dimethoxybib-enzyl (**1k**), respectively.

Bulbophyllin (**1g**) and bulbophyllidin (**1k**) 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 [31], while others are known to act as potent endogenous plant growth regulators [32], it would be interesting to study **1g** and **1k** for similar biological activities.

EXPERIMENTAL

Mps: 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. ^1H and ^{13}C NMR: 300 and 75 MHz, respectively, in $CDCl_3$ 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 hr *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 bp 60–80°.

Isolation of bulbophyllin (**1g**), *bulbophyllidin* (**1k**) *and compounds* **1a**, **1b**, **1f**, **1d**, **2** *and* **3** *from* B. protractum. Air-dried powdered whole plants (2 kg) were kept soaked in MeOH (7 l) for 3 weeks. The MeOH extract was then drained, 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 nonacidic frs with 2 M aq. NaOH. The 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 petrol–EtOAc (10:1) eluate afforded a mixture of **1g** and **1k**, which on rechromatography using petrol–EtOAc (20:1) as the eluent gave in the early frs pure **1g** (0.1 g), recrystallized from petrol–EtOAc mixture, mp 110°. (Found: C, 66.59; H, 5.49. $C_{16}H_{16}O_5$ requires: C, 66.66; H, 5.55%). UV $\lambda_{max}^{EtOH-0.1\ M\ NaOH}$ nm: 218.5 and 293.5 (log ε 4.86 and 4.41); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1605, 1590, 875, 850, 805, 790, 760 and 735 (phenyl nucleus); MS *m/z* (rel. int.): 288 $[M]^+$ (20), 287 (60), 181 (60), 180 (100), 165 (5), 108 (4), 107 (60) and 77 (60).

The later frs in the above chromatography afforded pure **1k** (0.05 g) as a semi-solid mass. (Found: C, 69.99; H, 6.49. $C_{16}H_{18}O_4$ requires: C, 70.07; H, 6.57%). UV $\lambda_{max}^{EtOH-0.1\ M\ NaOH}$ nm: 211.5 and 289.5 (log ε 4.87 and 4.16); IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1610, 1510, 950, 860 and 740 (phenyl nucleus); MS *m/z* (rel. int.): 274 $[M]^+$ (5), 273 (55), 167 (70), 166 (72), 139 (35), 137 (6), 124 (10), 109 (10), 108 (8), 107 (100) and 77 (90).

Elution of the main column with petrol–EtOAc (7:1) eluate gave pure **1a** (0.05 g) in the early frs and pure **2** (0.1 g) in the later frs, crystallized from petrol–EtOAc mixture, mp 210°. Washing the main column with petrol–EtOAc (5:1) eluent afforded a mixture of **1d** and **3**, which on rechromatography using petrol–EtOAc (7:1) as the eluent gave pure **3** (0.03 g) in the early frs and pure **1d** (0.05 g) in the later frs; ^1H NMR: δ 2.85 (4H, *s*, H_2-α and H_2-α'), 3.79 (6H, *s*, 2×OMe), 6.25 (2H, *s*, H-2 and H-6), 6.64–6.74 (2H, *m*, H-2′ and H-4′), 6.71 and 6.76 (each 1H, *s*, disappeared on deuterium exchange; 2×ArOH), 7.05 (1H, ill-resolved *ortho-meta* coupled *dd*, H-6′) and 7.13 (1H, app.*t*, J=7.8 Hz, H-5′). (Although the chemical shift of H-6′ of **1d** was found to differ from that reported earlier [29], the ^1H NMR spectral data of the diacetate **1e** were found to be virtually identical with those of reported values. The structure of **1d** was confirmed by the ^{13}C NMR spectral data of the diacetate **1e** recorded for the first time.)

Acetylation and methylation of **1g** *and* **1k**. Both **1g** and **1k** were acetylated with Ac_2O and pyridine in the usual manner. The reaction mixts were separately worked up to give pure **1h** and **1l**, both as semi-solid masses. Compound **1h** (Found: C, 64.46; H, 5.31. $C_{20}H_{20}O_7$ requires: C, 64.51; H, 5.37%). UV λ_{max}^{EtOH} nm: 215.5 and 279 (log ε 5.01 and 4.21); IR ν_{max}^{KBr} cm^{-1}: 1760 and 1280 (OAc), 1630, 1590, 1460, 980, 930, 830 and 790 (phenyl nucleus); ^1H NMR: δ 2.33 and 2.34 (each 3H, *s*, 2×OAc), 2.69–2.83 (4H, *m*, H_2-α and H_2-α'), 3.96 (3H, *s*, ArOMe), 5.91 (2H, *s*, $-OCH_2O-$), 6.34 (1H, *s*, H-6), 6.90 (1H, *d*, J=1.5 Hz, H-2′), 6.91 (1H, *dd*, J_1=9 Hz and J_2=1.5 Hz, H-4′), 7.02 (1H, *br.d*, J=9 Hz, H-6′) and 7.28 (1H, app.*t*, J_1=7.2 Hz and J_2=7.8 Hz, H-5′); MS *m/z* (rel. int.):

372 $[M]^+$ (20), 330 (15), 288 (50), 287 (55), 181 (58), 180 (100) and 107 (55). Compound **1l** (Found: C, 66.97; H, 6.08. $C_{20}H_{22}O_6$ requires: C, 67.03; H, 6.14%), UV λ_{max}^{EtOH} nm: 217.5 and 277 (log ε 4.00 and 4.42); IR ν_{max}^{KBr} cm^{-1}: 1760 and 1270 (OAc), 1600, 1490, 950, 900, 830, 810 and 790 (phenyl nucleus); ^1H NMR: δ 2.24 and 2.26 (each 3H, *s*, 2×OAc), 2.80 (4H, *s*, H_2-α and H_2-α), 3.80 and 3.90 (each 3H, *s*, 2× ArOMe), 6.16 (1H, *d*, J=2.7 Hz, H-4), 6.33 (1H, *d*, J=2.7 Hz, H-6), 6.83 (1H, *d*, J=3 Hz, H-2′), 6.84 (1H, *dd*, J_1=9 Hz and J_2=3 Hz, H-4′), 6.94 (1H, *br.d*, J=9 Hz, H-6′) and 7.20 (1H, app.*t*, J_1=7.8 Hz and J_2=8.4 Hz, H-5′); MS *m/z* (rel. int.): 358 $[M]^+$ (25), 316 (20), 274 (40), 273 (50), 167 (65), 166 (68) and 107 (100).

Compounds **1g** (0.02 g) and **1k** (0.02 g), each in 15 ml MeOH, were separately treated with excess of CH_2N_2 in Et_2O and the reaction mixts were kept overnight in an icebath. MeOH was removed under red. pres. and the residue in each case was extracted with Et_2O, dried and the solvent removed. The residues were chromatographed separately. The early frs of the petrol–EtOAc (30:1) eluate in the chromatography of the methylated products of **1g** afforded pure **1j** (0.012 g) as a semi-solid mass. (Found: C, 68.28; H, 6.27. $C_{18}H_{20}O_5$ requires: C, 68.35; H, 6.33%). IR ν_{max}^{KBr} cm^{-1}: 1600, 1585, 780 and 695 (phenyl nucleus); ^1H NMR: δ 2.75 (4H, *s*, H_2-α and H_2-α'), 3.60 (3H, *s*, ArOMe), 3.65 (6H, *s*, 2×ArOMe), 5.81 (2H, *s*, $-OCH_2O-$), 6.29 (1H, *s*, H-6), 6.68 (1H, ill-resolved *dd*, J=9 Hz, H-4′), 6.69 (1H, ill-resolved *meta*-coupled doublet, H-2′), 6.74 (1H, ill-resolved *dd*, J=7.5 Hz, H-6′) and 7.13 (1H, app.*t*, J=9 Hz, H-5′); MS *m/z* (rel. int.): 316 $[M]^+$ (50), 315 (60), 195 (90), 194 (100) and 121 (60). The later frs of the same eluate in the above chromatography gave pure **1i** (0.008 g), also as a semi-solid mass. (Found: C, 67.48; H, 5.87. $C_{17}H_{18}O_5$ requires: C, 67.55; H, 5.96%). IR ν_{max}^{KBr} cm^{-1}: 3400–3500 (OH), 1610, 1595, 780 and 695 (phenyl nucleus); ^1H NMR: δ 2.72 (4H, *s*, H_2-α and H_2-α'), 3.77 and 3.78 (each 3H, *s*, 2×ArOMe), 5.37 (1H, *s*, disappeared on deuterium exchange, ArOH), 5.77 (2H, *s*, $-OCH_2O-$), 6.24 (1H, *s*, H-6), 6.68 (1H, ill-resolved *dd*, J=9 Hz; H-4′), 6.69 (1H, ill-resolved *meta*-coupled doublet; H-2′), 6.75 (1H, ill-resolved *dd*, J=7.5 Hz, H-6′) and 7.14 (1H, app.*t*., J=9 Hz; H-5′); MS *m/z* (rel. int.): 302 $[M]^+$ (45), 301 (50), 181 (70), 180 (100) and 121 (45).

The petrol–EtOAc (30:1) eluate in the chromatography of the methylated product of **1k** afforded pure **1m** (0.019 g) as a semi-solid mass. (Found: C, 71.45; H, 7.22. $C_{18}H_{22}O_4$ requires: C, 71.52; H, 7.28%). IR ν_{max}^{KBr} cm^{-1}: 1610, 1590, 1500, 800 and 695 (phenyl nucleus); ^1H NMR: δ 2.81 (4H, *s*, H_2-α and H_2-α'), 3.65, 3.67, 3.72 and 3.74 (each 3H, *s*, 4×ArOMe), 6.17 (1H, *d*, J=2.7 Hz, H-4), 6.30 (1H, *d*, J=2.7 Hz, H-6), 6.67 (1H, ill-resolved *dd*, J=9 Hz, H-4′), 6.71 (1H, ill-resolved *meta*-coupled doublet, H-2′), 6.76 (1H, ill-resolved *dd*, J=9 Hz, H-6′) and 7.13 (1H, app.*t*, J=9 Hz, H-5′); MS *m/z* (rel. int.): 302 $[M]^+$ (50), 181 (80) and 121 (100).

The neutral fr. after removal of the acidic constituents of *B. protractum* was also chromatographed. The early frs of the petrol–EtOAc (20:1) eluate gave sitosterol (0.05 g), crystallized from the same solvent mixture, mp 136°. The later frs of the same eluate afforded stigmasterol (0.06 g), also crystallized from the same solvent mixt., mp 168°. The petrol–EtOAc (10:1) eluate gave a mixt. of **1b** and **1f**, which was subjected to MPLC using petrol–EtOAc (2:1) as the solvent. The early frs afforded pure **1b** (0.1 g) as a semi-solid mass. It was characterized by direct comparison with an authentic sample obtained by methylation of **1a** with CH_2N_2. The latter frs in the above MPLC gave pure **1f** (0.1 g), also obtained as a semi-solid mass, and characterized by comparison with an authentic sample obtained by methylation of **1n** [2] with CH_2N_2.

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