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TWO SUBSTITUTED BIBENZYLS AND A DIHYDROPHENANTHRENE FROM *CYMBIDIUM ALOIFOLIUM**

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Key Word Index—*Cymbidium aloifolium*, Orchidaceae; roots; bibenzyls; 9,10-dihydrophenanthrene.

Abstract—Two new substituted bibenzyls (1,2-diarylethanes) aloifol I and aloifol II and a new substituted 9,10-dihydrophenanthrene, 6-*O*-methylcoelonin in addition to batatasin III, coelonin and gigantol have been isolated and characterized. The three new compounds are 1-(4′-hydroxy-3′,5′-dimethoxyphenyl)-2-(3″-hydroxyphenyl)ethane, 1-(4′-hydroxy-3′,5′-dimethoxyphenyl)-2-(4″-hydroxy-3″-methoxyphenyl)ethane and 2,7-dihydroxy-4,6-dimethoxy-9,10-dihydrophenanthrene.

INTRODUCTION

In a research programme directed towards the chemical investigation of ethnomedicinal plants, a crude ethanolic extract of *Cymbidium aloifolium* Sw. [1, 2] roots was found to possess antifungal activity. The ethanolic extract further fractionated into hexane, chloroform, *n*-butanol and aqueous parts. The chloroform fraction exhibited antifungal activity against *Trichophyton melangrophytes* at 250 μg/ml. Column chromatography of this active fraction resulted in the isolation and characterization of two new substituted bibenzyls aloifol I (**1**) and aloifol II (**4**) and one new 9,10-dihydrophenanthrene, 6-*O*-methylcoelonin (**7**) along with three known compounds, batatasin III (**9**), coelonin (**11**) and gigantol (**10**).

RESULTS AND DISCUSSION

The chloroform soluble fraction was chromatographed over silica gel and the compounds thus obtained after elution were rechromatographed over polyamide. Aloifol I (**1**), $C_{16}H_{18}O_4$ ($[M]^+$ 274), was obtained as brownish viscous product. It developed an orange spot on TLC plate with a 2% solution of ceric sulphate in 8% sulphuric acid and blue colour with ferric chloride solution indicating the presence of phenolic group. IR bands were at 3450 (–OH) and 1600, 1520, 1470, 850, 820, 810 (aromatic –CH). The UV spectrum of **1** was characteristic of bibenzyls [3]. On acetylation it formed a diacetate, **2**, confirming the presence of two phenolic groups.

The ^{1}H NMR spectrum of **1** exhibited a signal for four equivalent benzylic protons at δ2.75, characteristic of methylene protons in a bibenzyl nucleus [4], a singlet at δ3.72 (6H) for two aromatic methoxyl groups, 6.27 (2H, *s*, H-2, H-6), 6.62 (3H, *dd*, *J* = 9.0, 2.5 Hz, H-2′, H-4′, H-6′) and a 1H *ortho* coupled triplet was present at δ7.03 (H-5′). The MS of **1** had two intense peaks at *m/z* 167 and 107 arising by the cleavage of benzylic linkage due to the formation of tropylium moieties. The ion at *m/z* 167 required two methoxyl and one hydroxyl group in one ring and the remaining fragment at *m/z* 107 required the placement of only one hydroxyl group in the other ring. In the ^{1}H NMR spectrum of aloifol I diacetate (**2**) the 3H double doublet at δ6.62 suffered a downfield shift of 0.33 ppm and 1H triplet also suffered a downfield shift of 0.15 ppm but 2H singlet remain unchanged (δ 6.3). Thus two hydroxyl groups could be placed at C-4′ and C-3″ and two methoxyl groups at C-3′ and C-5′, respectively. Hence aloifol I is 4′,3″-dihydroxy-3′,5′-dimethoxy bibenzyl. On methylation, the tetramethyl ether **3** was obtained. The ^{13}C NMR spectrum (Table 1) of compound **1** is in good agreement with the values of 2-hydroxy-1,3-dimethoxybenzene [5] and phenol, the other segment of the molecule, by using known additivity parameters [6].

Aloifol II (**4**), $C_{17}H_{20}O_5$ ($[M]^+$ 304) obtained as a viscous compound. It gave colour reactions showing a phenolic nature; IR bands were at 3500 (–OH), 1620, 1500, 1460, 920 and 830 (aromatic –CH–) cm^{-1}. The UV spectrum of **4** was characteristic of bibenzyl compounds. The MS had ion peaks at *m/z* 304 ($[M]^+$), 167 (base peak) and 137, formed by the benzylic cleavage of the bibenzyl nucleus resulting in the formation of substituted tropylium moieties. The ion at *m/z* 167 required one hydroxyl and two methoxyl groups in one ring as with **1**, while the ion at *m/z* 137 could be satisfied with one hydroxyl and one methoxyl group. The formation of the diacetate **5** showed the presence of two hydroxyl groups.

The ^{1}H NMR spectrum of aloifol II showed signals at δ2.76 (4H, *s*, benzylic protons), 3.75 (9H, *s*, 3 × $-OCH_3$), 6.28 (2H, *s*, H-2′, H-6′) 6.54 (1H, *br s*, H-2″), 6.67 (1H, *dd*, *J* = 9.5, 2 Hz, H-6″) and 6.76 (1H, *d*, *J* = 9.5 Hz, H-5″). In the ^{1}H NMR spectrum of the diacetate **5** the 5″-H (*O*-proton to the hydroxyl group) resonated downfield (δ0.11), which suggested the placement of a hydroxyl group at C-4″ and the methoxy group at the C-3″ position in ring B of the molecule. Gibb's test [3] further confirmed the *p*-positions of the hydroxyl groups. Thus aloifol II is 4′,4″-dihydroxy-3′,5′,3″-trimethoxybibenzyl. Structure **4** was also supported by its ^{13}C NMR spectral studies (Table 1) and by SFORD and DEPT techniques.

6-*O*-Methylcoelonin (**7**) $C_{16}H_{17}O_4$ ($[M]^+$ 272), mp

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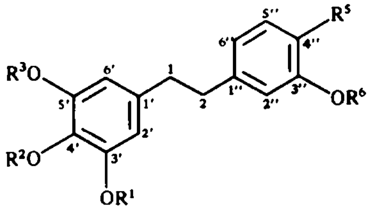
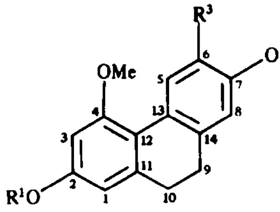
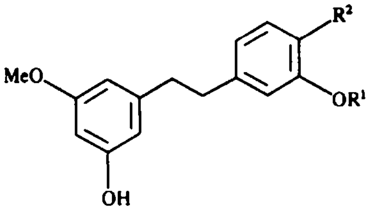
*CDRI Communication no. 3959.



1 R^1 = R^3 = Me, R^2 = R^4 = R^5 = H
2 R^1 = R^3 = Me, R^2 = R^4 = Ac, R^5 = H
3 R^1 = R^2 = R^3 = R^4 = Me, R^5 = H
4 R^1 = R^3 = R^4 = Me, R^2 = H, R^5 = OH
5 R^1 = R^3 = R^4 = Me, R^2 = Ac, R^5 = OAc
6 R^1 = R^2 = R^4 = Me, R^5 = OMe



7 R^1 = R^2 = H, R^3 = OMe
8 R^1 = R^2 = Ac, R^3 = OMe
11 R^1 = R^2 = R^3 = H



9 R^1 = R^2 = H
10 R^1 = Me, R^2 = OH

Table 1. ^{13}C NMR data of bibenzyl compounds* δc (ppm)

Carbon	1	4	9	10
1′	132.8	132.8	145.0	144.5
2′	105.4	105.6	108.8	108.1
3′	146.8	146.8	159.2	156.7
4′	132.9	133.1	99.9	99.2
5′	146.8	147.0	161.9	160.9
6′	105.4	105.6	106.3	106.9
1″	143.3	133.5	144.3	133.7
2″	115.2	114.2	116.2	114.3
3″	155.9	146.5	158.2	146.3
4″	112.9	141.3	113.6	143.8
5″	129.2	111.4	130.0	111.4
6″	120.5	121.1	120.4	121.0
C-1	36.7†	37.8†	38.4†	37.1†
C-2	37.7†	38.3†	38.1†	38.1†
–OMe	56.2 (2 × OMe)	55.9 56.3 (3 × OMe)	55.3	55.2 55.9 (2 × CMe)

*Spectra of **1**, **4**, **9**, **10** were recorded in CDCl$_3$.
†Values are interchangeable within a vertical column.

118° (acetone–hexane): the phenolic nature was evident by IR spectrum 3400 (–OH), 1620, 1480, 1460, 980, 880, 820 (aromatic nucleus) cm^{-1} and colour reactions. The UV spectrum resembled those of 9,10-dihydrophenanthrenes [7].

The ^{1}H NMR spectrum of 6-*O*-methylcoelonin (**7**) exhibited the signals at δ2.52 (*s*, 4H) characteristic of 9,10-methylene protons of a 9,10-dihydrophenanthrene [8] unsubstituted at C-1 and C-8 positions, 3.72, 3.75 (each 3H, *s*, 2 × OCH$_3$), 5.83 (2H, *br s*, 2 × OH) disappeared on D$_2$O exchange, 7.74 (1H, *s*) due to the C-4 or C-5 proton in a 9,10-dihydrophenanthrene molecule, δ6.24, 6.34 (each 1H, *d*, J = 2.5 Hz, H-1 and H-3) and at 6.55 (1H, *s*, H-8). On acetylation **7** gave the diacetate **8**. The ^{1}H NMR spectrum of **8** had signals at δ2.67 (4H, *s*), 3.78 and 3.82 (each 3H, *s*, 2 × OCH$_3$). Acetylation of **7** caused downfield shift of signals at δ6.24, 6.34 and 6.55 by 0.36, 0.5 and 0.65 ppm, respectively. This observation suggested the placement of two hydroxyl groups at C-2 and C-7, respectively. The signal at δ7.74 (H-5) suffered a downfield shift of 0.2 ppm but according to a well observed study that acetylation of a C-4 hydroxyl function in a 9,10-dihydrophenanthrene causes an upfield shift [7] of its C-5 proton so it requires the placement of a methoxyl group at C-4, instead of a hydroxyl group. As H-5 appeared as a singlet, the remaining methoxyl group must be present at C-6. Thus **7** is 2,7-dihydroxy-4,6-dimethoxy-9,10-dihydrophenanthrene. This structure was confirmed by its ^{13}C NMR spectrum (Table 2). The degree of protonation of each carbon was obtained using DEPT technique. The observed δc values were in good agreement with the values of structurally similar compound 4,7-dihydroxy-2,6-dimethoxy-9,10-dihydrophenanthrene [8]. The observed values for carbons except those at 11–14 were also in good agreement with the values obtained on a unsubstituted 9,10-dihydrophenanthrene using standard additivity parameters [9].

Further evidence on the oxygenation patterns of all the isolated compounds was obtained by NOE experiments in their ^{1}H NMR spectra. In the case of **1**, irradiation of signal at δ3.72 led to the enhancement of two proton singlet at δ6.27 attributed to C-2′ and C-6′ protons without affecting the other signals. This confirms the relationship between these protons while in **4** upon saturation of –OCH$_3$ at C-3′, C-5′ and C-3″ (δ3.75), a NOE difference for protons at C-2′, C-6′ and C-2″ was observed. The NOE difference experiments in **7** also supported the assigned structure. Irradiation at δ2.52 resulted the enhancement of H-1 (δ6.24) and H-8 (δ6.55) signal indicating relationship of methylene protons with these protons. When–OCH$_3$ at C-4 (δ3.75) was saturated a NOE was observed for C-3 and C-5 proton, while saturation of –OCH$_3$ at C-6 (δ3.72) caused the enhancement of only the C-5 proton.

Table 2. ^{13}C NMR data of (7) and (11)*

Carbon	7	11
1	108.5	108.5
2	157.4	156.0
3	99.4	99.4
4	158.7	158.8
5	113.4	129.8
6	146.1	115.0
7	145.3	157.3
8	114.9	113.6
9	31.6†	30.8†
10	30.1†	31.4†
11	141.4	141.3
12	115.3	116.5
13	131.7	125.8
14	125.6	139.9
$-OCH_3$ at C-4	55.9‡	55.8
$-OCH_3$ at C-6	56.6‡	–

*Spectra were recorded in acetone-d_6; chemical shifts are expressed on the TMS scale.

†, ‡ Values are interchangeable within a vertical column.

Other compounds isolated, *viz.* batatasin III (9) [10] coelonin (11) [11] and gigantol (10) [3], were identified by their IR, UV, MS, PMR and ^{13}C NMR spectral data. Batatasin III and gigantol were also compared with authentic samples (co-TLC and superimposable IR and NMR).

EXPERIMENTAL

All mps are uncorr. UV spectra were taken in MeOH. ^{1}H NMR and ^{13}C NMR spectra were recorded either at 90 or 400 MHz using TMS as int. standard. Silica gel and polyamide were used as adsorbents for CC. Plant material was collected from Bastar Dist. (M.P.). A herbarium specimen has been deposited in the ethnobotanical museum of the National Botanical Research Institute, Lucknow.

Isolation. Air dried powdered roots of *Cymbidium aloifolium* (1 kg) were exhaustively extracted with 95 % EtOH (3 × 5 l.). The residue obtained was defatted with hexane and then extracted with $CHCl_3$. The $CHCl_3$ soluble fraction (9.5 g) was chromatographed over silica gel. The resulting fraction on repeated chromatography over polyamide using an *n*-hexane–MeOH solvent system with increasing polarity yielded aloifol I (**1**), aloifol II (**4**), 6-*O*-methylcoelonin (**7**), batatasin III (**9**), coelonin (**11**) and gigantol (**10**). Aloifol I (**1**) was obtained as a viscous mass which was further purified by prep. TLC on silica gel (C_6H_6–MeOH, 23:2), UV: $\lambda_{\max}^{\mathrm{MeOH}}$ nm(log ε) 218 (4.5), 274 (3.8) and 280 (3.7).

Aloifol I diacetate (**2**). UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm (log ε) 218 (4.6), 268 (3.6) and 276 (3.5); ^{1}H NMR ($CDCl_3$): δ2.23, 2.26 (each 3H, *s*, OAc), 2.86 (4H, *s*, benzylic protons), 3.71 (6H, *s*, 2 × OMe), 6.3 (2H, *s*, H-2′, H-6′), 6.95 (3H, *dd*, *J* = 9, 2.5 Hz, H-2″, H-4″ and H-6″) and 7.18 (1H, *t*, *J* = 9 Hz, H-5″); MS *m/z*: 358 ([M]$^+$), 316, 274, 167 (base peak).

Dimethyl ether of aloifol I (**3**). UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm (log ε) 204 (4.4), 266 (3.4) and 280 (3.3); ^{1}H NMR ($CDCl_3$) δ2.87 (4H, *s*, benzylic protons), 3.79 (3H, *s*, OMe), 3.82 (9H, *s*, 3 × OMe), 6.37 (2H, *s*, H-2′, H-6′), 6.76 (3H, *dd*, *J* = 8.5, 2 Hz, H-2″, H-4″, H-6″) and 7.21 (1H, *t*, *J* = 8.5 Hz, H-5″) and MS *m/z*: 302 ([M]$^+$), 181 (base peak).

Aloifol II (**4**) was obtained as viscous material purified by prep. TLC (C_6H_6–MeOH, 23:2); UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm (log ε) 218 (3.99), 278 (3.58) and 312 (3.32).

Aloifol II diacetate (**5**). UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm (log ε) 218 (4.7), 267 (4.3), 278 (4.27); ^{1}H NMR ($CDCl_3$): δ2.23, 2.25 (each 3H, *s*, OAc), 2.8 (4H, *s*, benzylic protons), 3.79 (9H, *s*, 3 × OMe), 6.25 (2H, *s*, H-2′, H-6′), 6.55 (1H, *d*, *J* = 2 *Hz*, *H*-2″), 6.74 (1*H*, *m*, *J* = 8, 2 Hz, H-6″) and 6.87 (1H, *d*, *J* = 8 Hz, H-5″); and MS *m/z* 388 ([M]$^+$) 346, 304 and 167.

Dimethyl ether of aloifol II (**6**). UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm (log ε) 204 (4.3), 276 (3.4) and 304 (3.2); ^{1}H NMR ($CDCl_3$): δ2.65 (4H, *s*, benzylic protons), 3.83 (9H, *s*, 3 × OMe), 3.86 (6H, *s*, 2 × OMe), 6.39 (2H, *s*, H-2′, H-6′), 6.68 (1H, *d*, *J* = 2 Hz, H-2″), 6.79 (1H, *dd*, *J* = 9, 2 Hz, H-6″) and 6.84 (1H, *d*, *J* = 9 Hz, H-5″) and MS *m/z* 332 ([M]$^+$), 270 and 181.

6-O-*methylcoelonin* (**7**) was obtained as a gummy mass which was purified by prep. TLC (C_6H_6–MeOH, 47:3), mp 118–120° (Me_2CO–hexane); UV $\lambda_{\max}^{\mathrm{MeOH}}$ (log ε) 268 (4.51), 276 (4.33), 303 (4.39) and 312 (4.23).

6-O-*Methylcoelonin diacetate* (**8**). UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm 264 (3.9), 270 (3.9), 298 (3.9), 310 (3.8); PMR ($CDCl_3$): δ2.25 (6H, *s*, 2 × OAc), 2.67 (4H, *s*, 9,10-methylene protons), 3.78 (3H, *s*, OMe), 3.82 (3H, *s*, OMe), 6.6 (1H, *d*, *J* = 2 Hz, H-1), 6.84 (1H, *d*, *J* = 2 Hz, H-3), 7.20 (1H, *s*, H-8) and 7.94 (1H, *s*, H-5); MS *m/z* 356 ([M]$^+$), 314, 272, 257, 225.

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