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PHENOLIC CONSTITUENTS OF *COELOGYNE OVALIS**

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Key Word Index—*Coelogyne ovalis*; Orchidaceae; 9,10-dihydrophenanthrenes; bibenzyls; 3'-*O*-methylbatatasin III; 3-hydroxy-3',5-dimethoxybibenzyl; spasmolytic activity.

Abstract—The structure of 3'-*O*-methylbatatasin III, a new bibenzyl of the East Himalayan orchid, *Coelogyne ovalis*, has been established on the basis of spectral and chemical data. Known 9,10-dihydrophenanthrene derivatives, a bibenzyl and sterols have also been isolated from the orchid.

INTRODUCTION

The isolation of 2,7-dihydroxy-3,4,6-trimethoxy-9,10-dihydrophenanthrene from *Coelogyne ovalis* Lindl. has been reported by Majumder *et al.* [1]. An alcoholic extract of this plant showed spasmolytic activity. In the present communication, we report the isolation of the active principles coelogin (**6**) and flavidin (**9**) and a new bibenzyl compound, 3'-*O*-methylbatatasin III (**1**) in addition to batatasin III (**4**), flavidinin (**5**), coeloginin (**7**), imbricatin (**8**), β-sitosterol and its glycoside.

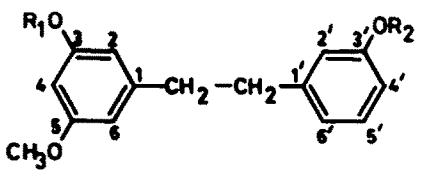
RESULTS AND DISCUSSION

The alcoholic extract of the aerial parts of *C. ovalis* plant was fractionated into *n*-hexane, chloroform, *n*-butanol and water soluble fractions. Activity was found in the butanol and chloroform fractions. CC of the butanol fraction over silica gel and polyamide yielded batatasin III (**4**), coelogin (**7**), coeloginin (**8**), imbricatin (**9**) and flavidin (**10**). In addition, 3'-*O*-methylbatatasin III (**1**) and flavidinin (**6**) were obtained from the chloroform fraction.

3'-*O*-Methylbatatasin III (**1**) was obtained as a viscous solid. Its phenolic nature was evident from specific colour tests, IR absorption bands and alkali shifts of the UV absorption bands. On acetylation it furnished a monoacetate (**2**, $[M]^+$ *m/z* 300) thus revealing the presence of only one hydroxyl group. Mass spectral fragmentation of **1** ($[M]^+$ *m/z* 258) required a bibenzyl nucleus having a hydroxyl group and a methoxyl group on one aromatic ring and a methoxyl group on the other ring, as was evident by the appearance of two very intense peaks at *m/z* 137 (75) and 121 (base peak) corresponding to the tropylium moieties resulting from the benzylic cleavage [2, 3].

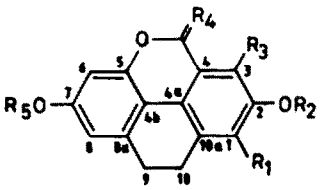
The position of the substituents was ascertained on the basis of the ^{1}H NMR spectrum which displayed a 4H singlet at δ2.8 for the benzylic protons [3, 4]. The presence of two methoxyl groups was evident by two 3H singlets at δ3.67 and 3.69, respectively. A 3H narrow multiplet at δ6.19 indicated the presence of three *meta*-coupled protons. The ^{1}H NMR spectrum also displayed a 1H triplet (J = 9 Hz) resulting from spin interactions with two *ortho* protons at δ7.11 and a 3H multiplet centred at 6.7. The shape of this multiplet suggested that it was composed of two *ortho*, *meta* coupled and a *meta* coupled protons. The multiplicity pattern of the aromatic protons suggested the placement of three oxygen functions at C-3, C-5 and C-3'. Since the mass fragmentation required the presence of a methoxyl and a hydroxyl function in the same aromatic nucleus, 3'-*O*-methylbatatasin III was assigned the structure 3-hydroxy-3',5-dimethoxybibenzyl (**1**).

In the ^{1}H NMR spectrum of the monoacetate two *meta*-coupled protons shifted downfield by 0.20 and 0.27 ppm which now appeared as triplets (each J = 2 Hz) at δ6.4 and 6.46, respectively. These protons must be located at C-2 and C-4, *ortho* to the hydroxyl group. The remaining *meta* coupled proton signal shifted by only 0.04 ppm and this was assigned to H-6. Protons of the other ring appeared as a 3H triplet centred at δ6.72 comprising two *ortho*, *meta* coupled protons H-4' and H-6' and a *meta* coupled proton H-2'. A triplet (J = 9 Hz) at δ7.08 was due



1 $R_1 = H$; $R_2 = CH_3$
2 $R_1 = Ac$; $R_2 = CH_3$
3 $R_1 = R_2 = CH_3$
4 $R_1 = R_2 = H$

*CDRI Communication No. 3692.



5 $R_1 = R_3 = R_5 = H$; $R_2 = CH_3$; $R_4 = H_2$

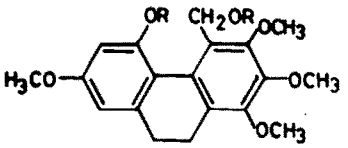
6 $R_1 = OCH_3$; $R_2 = CH_3$; $R_3 = OH$; $R_4 = H_2$; $R_5 = H$

7 $R_1 = OCH_3$; $R_2 = CH_3$; $R_3 = OH$; $R_4 = O$; $R_5 = H$

8 $R_1 = R_2 = R_5 = H$; $R_3 = OCH_3$; $R_4 = H_2$

9 $R_1 = R_2 = R_3 = R_5 = H$; $R_4 = H_2$

10 $R_1 = R_3 = OCH_3$; $R_2 = R_5 = CH_3$; $R_4 = O$



11 R = H

12 R = Ac

to H-5'. Thus, ^{1}H NMR of the acetate also supported structure **1** for 3'-*O*-methylbatatasin and structure **2** for its monoacetate.

On methylation with ethereal diazomethane **1** afforded a monomethyl ether (**3**) whose IR, mass spectral and ^{1}H NMR data (see Experimental) were identical in all respects with the dimethyl ether of batatasin III prepared by methylation of batatasin III (**4**) under similar conditions.

The structure of **1** was further confirmed by ^{13}C NMR spectral data. The substitution at various carbon centres in **1** was determined by the SFORD technique. The chemical shifts of all carbon atoms, as shown in Table 1, were in excellent agreement with the calculated values using known additivity parameters of the functional groups [5]. The ^{13}C NMR spectrum of batatasin III (**4**) was also studied.

Other compounds isolated viz. flavidinin (**5**) [6], coelogin (**6**) [7], coeloginin (**7**) [7], imbricatin (**8**) [8], flavidin (**9**) [9] and batatasin III (**4**) [10] were identified by their IR, UV (Table 2) ^{1}H NMR (Table 3) and mass spectral data.

Conversion of coeloginin (**7**) into coelogin (**6**) was attempted by lithium aluminium hydride reduction of permethylated coeloginin (**10**). Instead, it led to the formation of **11**. The presence of the aforementioned constituents in *C. ovalis* strongly indicated that 3-methoxybibenzyls were the biosynthetic precursors of 9,10-dihydrophenanthrenes containing oxymethylene bridge. Thus flavidinin (**5**) could be derived from 3'-*O*-methylbatatasin III (**1**) and flavidin (**9**) from batatasin III (**4**). The co-occurrence of **1**, **4**, **6** and **10** in the plant lent strong support to this proposition.

Flavidin (**10**) and coelogin (**6**) were found to be the spasmolytic principles of *C. ovalis*. Compound **10** exhibited 50% and 90% inhibition of $BaCl_2$ induced spasm in the guinea pig ileum at 1.0 and 2.0 μg/ml dose levels, respectively, whereas **7** showed 50% and 51% activity at 0.5 and 1.0 μg/ml dose levels, respectively. Papaverine hydrochloride was used as a standard spasmolytic drug for comparison.

Table 1. ^{13}C NMR data of **1** and **4**

Carbons	1		4	
	Calculated values	Observed values	Calculated values	Observed values
1	146.5	143.8 *s*	146.5	143.7 *s*
2	107.7	106.39 *d*	107.7	107.23 *d*
3	156.4	158.0 *s*	156.4	157.46 *s*
4	98.8	97.4 *d*	98.8	98.26 *d*
5	161.3	160.5 *s*	161.3	160.15 *s*
6	106.4	103.87 *d*	106.4	104.6 *d*
1'	145.1	143.0 *s*	145.5	142.56 *s*
2'	113.7	112.49 *d*	115.4	114.59 *d*
3'	159.9	159.4 *s*	155.4	156.42 *s*
4'	111.5	109.12 *d*	113.4	112.04 *d*
5'	129.5	127.50 *d*	129.9	128.34 *d*
6'	120.4	119.03 *d*	120.8	118.74 *d*
2 × OMe	—	58.86 *q*	—	53.70 *q*
Ar–CH_2	—	35.76 *t*	—	36.85 *t*
Ar–CH_2	—	36.0 *t*	—	36.65 *t*

EXPERIMENTAL

All mps are uncorr. IR spectra were recorded in KBr discs or $CHCl_3$ soln. and UV spectra in MeOH. ^{1}H NMR spectra were measured at 90 MHz using TMS as int. standard and ^{13}C NMR in Me_2CO-d_6 at 20 MHz. Polyamide and silica gel were used as adsorbents for CC and silica gel coated plates were used for prep. TLC. Solvents (1) C_6H_6–MeOH (49:1) and (2) C_6H_6–MeOH (47:3) were used for TLC. The chromatogram was developed by spraying with 1% ceric sulphate in 2N H_2SO_4.

Isolation. Air dried powdered plant material (7 kg, voucher specimen preserved in the herbarium of the Botany Division, CDRI) was extracted with EtOH. The EtOH concentrate was fractionated into *n*-hexane, $CHCl_3$, *n*-BuOH and H_2O soluble fractions. The *n*-BuOH fraction was subjected to coarse separation by CC over silica gel elution being carried out with (i) EtOAc–C_6H_6 (3:1) satd with H_2O and (ii) EtOAc satd with H_2O containing increasing proportions of MeOH. The resulting fractions when repeatedly chromatographed over polyamide using gradient elution with *n*-hexane–Me_2CO, yielded coelogin (**6**), coeloginin (**7**), imbricatin (**8**), flavidin (**9**) and betatasin III (**4**). All these compounds were obtained from the $CHCl_3$ fraction in addition to flavidinin (**5**) and 3'-*O*-methylbatatasin III (**1**). The resolution of these compounds was achieved by CC of the $CHCl_3$ fraction over silica gel followed by CC over polyamide using mixtures of *n*-hexane–Me_2CO of increasing polarity.

Flavidinin (**5**). Colourless needles, mp 169° (hexane–Me_2CO). TLC solvent 1. MS $[M]^+$ *m/z* 254. UV: Table 2; ^{1}H NMR: Table 3.

3'-*O*-*Methylbatatasin III* (**1**) was obtained as a viscous solid by prep. TLC on silica gel in solvent 1. IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3400, 1610,

Table 2. UV spectral data of compounds **5**–**9** (λ_{max} in nm)

	5	6	7	8	9
MeOH	286, 303	283, 305, 318	251, 288, 363	285, 305	285, 302
NaOMe	299, 314	297, 321, 332	260, 291, 312, 397	300, 318	305

Table 3. ^{1}H NMR spectral data of compounds **5**–**9***

	5	6	7	8	9
H-1	6.55 *br s*	—	—	6.6 *s*	6.45 *d* (2.5)
H-3	6.62 *br s*	—	—	—	6.50 *d* (2.5)
H-6	6.18 *d* (2.5)	6.22 *br s*	6.52 *br s*	6.18 *d* (2.5)	6.19 *br s*
H-8	6.26 *d* (2.5)	†	†	6.26 *d* (2.5)	6.26 *br s*
H-9 and H-10	2.76 *s*	2.69 *s*	2.90 *br s*	2.68 *s*	2.75 *br s*
$-OCH_2-$	4.99 *s*	5.06 *s*	—	5.05 *s*	4.98 *br s*
OMe	3.71 *s*	3.71 and 3.7 each *s*	3.86 and 3.97 each *s*	3.69 *s*	—

*Spectra of **5**, **8** and **9** were recorded in Me_2CO-d_2, **6** in $CDCl_3$ and **7** in $CDCl_3$ + DMSO $-d_6$. Values are given in δ values (ppm) downfield from TMS as internal standard. J values in Hz are given in parentheses.

†H-6 and H-8 resonated at the same frequency.

1590, 1460, 950, 845. UV λ_{max}^{MeOH} nm: 272 and 280. ^{1}H NMR ($CDCl_3$): given in text. MS *m/z* (rel. int.): 258 $[M]^+$ (96.4), 151 $[CH_2CH_2C_6H_3(OH)OMe]^+$ (34), 137 $[CH_2C_6H_3(OH)OMe]^+$ (75), 121 $[CH_2C_6H_4OMe]^+$ (100), 107 $[C_6H_4OMe]^+$ (10), 91 (21).

3'-O-Methylbatatasin III monoacetate (**2**). Compound **1** (15 mg) was allowed to stand overnight with Ac_2O (0.25 ml) in dry pyridine (0.25 ml) at room temp. After usual work up the product (**2**) was obtained as a semi-solid which did not crystallize. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765. ^{1}H NMR ($CDCl_3$): δ 2.06 (3H, *s*, OAc), 2.5 (4H, *s*, benzylic protons), 3.65, 3.68 (each 3H, *s*, OMe), 6.23 (1H, *t*, J = 2 Hz, H-6), 6.4 (1H, *t*, J = 2 Hz, H-2 or H-4), 6.46 (1H, *t*, J = 2 Hz, H-4 or H-2), 6.72 (3H, *m*, H-2', H-4', H-6') and 7.08 (1H, *t*, J = 9 Hz, H-5').

Methyl ether of 3'-O-*methylbatatasin III* (**3**). Compound **1** (20 mg) in Et_2O–CH_2N_2 (10 ml) was kept overnight at 5°. After evapn it furnished a Me ether (**3**) as viscous material. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1610, 1350, 1060, 930. ^{1}H NMR ($CDCl_3$): δ 2.8 (4H, *s*, benzylic protons), 3.64 (6H, *s*, 2 × OMe), 3.67 (3H, *s*, OMe), 6.24 (3H, *br s*, H-2, H-4 and H-6), 6.69 (3H, *m*, H-2', H-4' and H-6'), 7.1 (1H, *dd*, J = 8, 9 Hz, H-5'). MS *m/z* (rel. int.): 272 $[M]^+$ (56), 165 $[M-C_6H_4OMe]^+$ (15), 151 $[M-CH_2C_6H_3(OMe)_2]^+$ (100), 137 $[M-C_6H_3(OMe)_2]^+$ (3), 135 $[M-CH_2CH_2C_6H_4OMe]^+$ (4), 121 $[M-CH_2C_6H_4OMe]^+$ (49), 91 (21).

Coelogin (**6**) crystallized from *n*-hexane–EtOAc as fine needles, mp 150–152°. TLC in solvent 2. MS *m/z*: 300 $[M]^+$. UV: Table 2; ^{1}H NMR: Table 3.

Coeloginin (**7**). Yellowish, heavy needles, mp 195° (*n*-hexane–EtOAc). TLC in solvent 2. MS *m/z*: 314 $[M]^+$ UV: Table 2; ^{1}H NMR: Table 3.

Coeloginin dimethyl ether (**10**) was prepared by treatment of **7** (50 mg) with Me_2SO_4 (0.5 ml) and K_2CO_3 (50 mg) in dry Me_2CO. It crystallized from *n*-hexane–Me_2CO as colourless flakes, mp 138°. MS $[M]^+$ *m/z* 342.

Reduction of **10**. Compound **10** (50 mg) in THF (10 ml) was refluxed with $LiAlH_4$ (5 mg) for 4 hr. Excess hydride was decomposed with EtOAc. The organic layer was washed free of acid, dried and evapd. The reduced product (**11**) was obtained as a colourless gum. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 2980, 1570, 1025, 820. MS *m/z*: 346 $[M-H_2O]^+$. ^{1}H NMR ($CDCl_3$): δ 2.88 (4H, *s*, 9 and 10-CH_2), 3.05 (2H, *m*, CH_2OH), 3.76, 3.79 (each 3H, *s*, OMe), 4.0 (6H, *s*, 2 × OMe) and 6.39 (2H, *br s*, H-6 and H-8). On acetylation it furnished a diacetate as a gummy solid. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, ^{1}H NMR ($CDCl_3$): δ 1.94 (3H, *s*, OAc), 2.12 (3H, *s*, OAc).

Imbricatin (**8**). Needles, mp 148° (EtOH). TLC solvent 2. MS *m/z*: 270 $[M]^+$, UV: Table 2; ^{1}H NMR: Table 3.

Flavidin (**9**). Fine needles, mp 215° (*n*-hexane–Me_2CO). TLC solvent 2. MS *m/z*: 240 $[M]^+$. UV: Table 2; ^{1}H NMR: Table 3.

Batatasin III (**4**). Colourless crystals, mp 94° ($CHCl_3$–CCl_4). TLC solvent 2. MS *m/z* (rel. int.): 244 $[M]^+$ (48.5), 137 (100), 107 (50).

Dimethylbatatasin III (**3**). Preparation method and spectral data identical with monoMe ether of **1**.

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