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OCHRONE A, A NOVEL 9,10-DIHYDRO-1,4-PHENANTHRAQUINONE FROM *COELOGYNE OCHRACEA*

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ABSTRACT.—The structures of two new 1,4-phenanthraquinones, ochrones A [**1**] and B [**2**] from the orchid, *Coelogyne ochracea*, have been established from spectroscopic evidence.

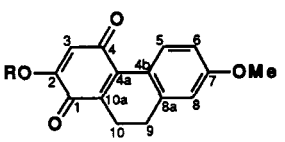
Earlier work on *Coelogyne ochracea* Ldl. (Orchidaceae) led to the isolation of a 9,10-dihydrophenanthrene, coelonin (1), and ochrolide (2). Besides the known batatasin III (3), coelogin (3), and coeloginin (3), we now report the isolation of a novel 2-hydroxy-7-methoxy-9,10-dihydrophenanthraquinone, ochrone A [**1**] and its 9,10-dehydro derivative ochrone B [**2**].

The Me_2CO extract of the whole plant of *C. ochracea* yielded ochrone A [**1**] ($C_{15}H_{12}O_4$, $[M]^+$ *m/z* 256, mp 253°) by chromatographic methods. The formation of a light green with NaOH and pink with MeOH/$Mg(OAc)_2$ suggested a hydroxyquinone structure. It gave a positive $FeCl_3$ reaction [ir (KBr) ν max 3390 cm^{-1}] indicating a free hydroxyl group, and its uv spectrum exhibited absorption bands at (MeOH) λ max 260, 337, and 487 nm, indicating a hydroxyquinone (4). The presence of one hydroxyl group was confirmed by the formation of a monoacetate **3** ($C_{17}H_{14}O_5$, $[M]^+$ *m/z* 298 with Ac_2O and pyridine). The ir spectrum of **1** also exhibited two carbonyl absorption bands at (KBr) ν max 1637 and 1610 cm^{-1}.

The 270 MHz ^{1}H-nmr spectrum (Me_2CO-d_6) of **1** showed the presence of one methoxyl at δ 3.86 (3H, s). The two multiplet signals at δ 2.61 (2H) and 2.73 (2H) indicated a 9,10-dihydrophenanthrene skeleton as in 9,10-dihydro-2,5-dimethoxyphenanthrene-1,7-diol (5). Hence, **1** could be a 9,10-dihydrophenanthraquinone derivative as it gave positive color tests for a quinone moiety. The molecular ion $[M]^+$ 256 supported a 9,10-dihydrophenanthraquinone skeleton with one hydroxyl and methoxyl groups.

The one-proton singlet at δ 5.99 (1H) could be assigned to H-2 or H-3. The hydroxyl group was allocated to C-2 based on earlier biogenetic conclusions (6). This allocation is also supported by the upfield shift of the signal at δ 2.61, due to one of the methylenes in the 9,10-dihydro system, to δ 2.70 in the monoacetate, indicating the close proximity of the acetoxyl group to the 9,10-dihydro system. The signals at δ 2.73 and 2.61 were assigned to the CH_2-9 and CH_2-10 protons, respectively.

The three aromatic signals centered at δ 6.77 [2H, m, J = 9.4, 2.4, 2.4 Hz (values calculated on the expanded spectrum)] and δ 7.96 (1H, d, J = 9.4 Hz) indicated an ABX pattern. The downfield signal at δ 7.96 was assigned to H-5 as in other 1,4-phenanthraquinones (7) and phenanthrenes (1) and the multiplet at δ 6.77 (2H) to H-6 and H-8. Hence, the methoxyl was allocated to C-7.



1 R=H
2 R=H; 9,10-dehydro
3 R=Ac
4 R=Ac; 9,10-dehydro

The acetyl signal in the ^{1}H-nmr spectrum ($CDCl_3$, 80 MHz) of the monoacetate **3** appeared at δ 2.31 (3H), confirming the presence of only one hydroxyl group in ochrone A. The signals at δ 2.70 (2H) and δ 2.75 (2H) were assigned to H-10 and H-9, respectively.

The allocation of the methoxyl to C-7 and the hydroxyl to the quinone moiety were also supported by the fragment ions at *m/z* 241 (38%) and 171 (20%) in the mass spectrum (8). The other significant peaks at *m/z* 225 $[M-OMe]^+$ (12%), 213 $[M-Me-CO]^+$ (13%), 197 $[M-OMe-CO]^+$ (19%), 185 $[M-Me-2CO]^+$ (5%), 169 $[M-OMe-2CO]^+$ (9%), 157 $[M-Me-3CO]^+$ or $[M-OMe-68]^+$ (8%), 144 $[M-Me-CO-69]^+$ (6%), and *m/z* 69 (9%) strongly supported the allocation of the methoxyl to C-7 and the hydroxyl to the quinone moiety (4).

Hence, the structure of ochrone A was assigned as 2-hydroxy-7-methoxy-9,10-dihydro-1,4-phenanthraquinone [**1**] and is supported by ^{13}C-nmr spectral analysis.

Ochrone B [**2**] was separated from ochrone A by acetylation and preparative tlc of the acetate.

The ^{1}H-nmr spectrum ($CDCl_3$) of ochrone B acetate [**4**] was found to be similar to that of the ochrone A acetate [**3**], except for the signals at δ 8.08 (1H, d, $J = 8.5$ Hz) and δ 8.23 (1H, d, $J = 8.5$ Hz) for H-10 and H-9, respectively (Table 1), instead of the multiplets at δ 2.70 (2H) and 2.75 (2H) as in **3**, indicating a cis 9,10 double bond. The large downfield shift ($\Delta\ \delta = 1.57$) for H-5 in **4**, when compared to **3**, is similar to the corresponding protons (H-4 and H-5) in phenanthrenes (9).

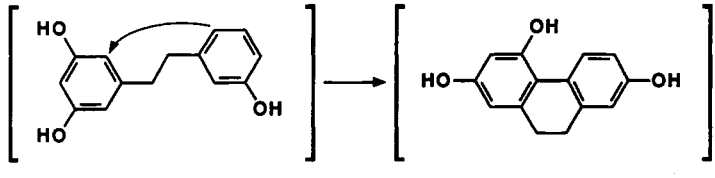
TABLE 1. ^{1}H nmr ($CDCl_3$, 270 MHz) of Ochrone A [**1**], Ochrone A Acetate [**3**] and Ochrone B Acetate [**4**] (values in parentheses are *J* values in Hz).

Proton	Compound		
	1 (Me_2CO-d_6)	**3** ($CDCl_3$)	**4** ($CDCl_3$)
H-3	5.99 s	5.95 s	6.17 s
H-5	7.96 d (9.4)	8.10 d (9.0)	9.67 d (9.5)
H-6	6.77 q (9.4,2.4)	7.00 q (9.0,3.0)	7.46 q (9.5,2.4)
H-8	6.77 d (2.4)	6.95 d (3.0)	7.65 d (2.4)
H-9	2.73 m	2.75 m	8.23 d (8.5)
H-10	2.61 m	2.70 m	8.08 d (8.5)
OMe	3.86 s	3.83 s	3.93 s
OAc		2.31 s	2.38 s

The structure of ochrone B acetate [**4**] was assigned as 2-acetoxy-7-methoxy-1,4-phenanthraquinone, and that of ochrone B [**2**] was assigned as 2-hydroxy-7-methoxy-1,4-phenanthraquinone. The molecular ion $[M]^+$ at *m/z* 296 and other fragment ions at *m/z* 254 $[M-Ac]^+$ and 239 $[M-Ac-OMe]^+$ in the mass spectrum of **4** are consistent with the proposed structure.

Hence, the structures for ochrone A and ochrone B are proposed as **1** and **2**. Ochrone A is the first natural 9,10-dihydro-1,4-phenanthraquinone, and ochrone B is a new 1,4-phenanthraquinone. The possibility of ochrone B being an artifact, due to aerial oxidation, could not be ruled out, as ochrone B was found in pure ochrone A after several days of standing.

The bibenzyls, phenanthrenes, and 1,4-phenanthraquinones **1–4** are derived from one phenylalanine unit and three malonyl units (6,10). Ochrone A [**1**] and ochrone B [**2**] might have come from the same intermediate **5** supporting the allocation of the hydroxyl to C-2 (10) (Scheme 1). The biogenetic formation of 9,10-dihydrophenanthrenes is established as a radical coupling reaction from dihydrostilbene (6). Further in vivo enzymatic oxidation at C-1 to the 1,4-dihydroxyphenanthrene **6** and subsequent oxidation would lead to the corresponding quinone. The isolation of ochrone A as a major product indicates the predominance of the initial oxidative formation of 9,10-dihydrophenanthraquinone in the plant, compared to dehydrogenation to phenanthrene and subsequent oxidation to the quinone. The in vitro conversion of ochrone A to ochrone B is consistent with the notion that the dehydrogenation step takes place at a later stage.



5 **6**

SCHEME 1

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were uncorrected. Si gel (100–200 mesh) was used for cc and Si gel G for tlc. Uv and ir spectra were determined on Shimadzu-240 and Perkin-Elmer-283b instruments, respectively. ^{1}H- (270 MHz) and ^{13}C-nmr spectra were recorded on a WH-270 Bruker FT instrument. The voucher specimen (No. 82) was deposited in the department of Botany, Nagarjuna University.

EXTRACTION AND ISOLATION.—Plant material (2.4 kg) of *C. ochracea* was collected near Sikkim, India. Air-dried and powdered whole plant of *C. ochracea* was extracted successively with hexane, Me_2CO, and MeOH. The Me_2CO fraction was chromatographed on Si gel using C_6H_6 and C_6H_6/Me_2CO mixtures. Coelogin, mp 150–152° [lit. (3) mp 151°] was isolated from the C_6H_6 fraction. Ochrone A [**1**] and ochrone B [**2**] were obtained from the C_6H_6-Me_2CO (19:1) fraction. Ochrone A [**1**] crystallized from C_6H_6 as dark red crystals; mp 253° (found C 70.35%, H 4.65%; $C_{15}H_{12}O_4$ requires C 70.31%, H 4.68%); ms *m/z* $[M]^+$ 256 (100%), 241 (38), 225 (12), 213 (13), 197 (19), 185 (5), 171 (20), 169 (9), 157 (8), 155 (13), 145 (6), 144 (6), 143 (6), 128 (6), 69 (9); uv (MeOH) λ max 260, 337, 486 nm; ir (KBr) ν max 3390, 3100, 1637, 1610, 1545, 1355, 1240, 1200, 850 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr (DMSO-d_6) 187.3 (C-4), 180.7 (C-1), 159.1 (C-2), 158.2 (C-7), 141.2 (C-8a), 135.8 (C-10a), 135.6 (C-4a), 131.6 (C-5), 120.2 (C-4b), 114.8 (C-6), 113.5 (C-8), 107.5 (C-3), 56.3 (OMe), 26.8 (C-9), 19.8 (C-10).

Compound **1** yielded ochrone A acetate [**3**] with Ac_2O and pyridine at room temperature for 24 h. Found C 68.40%, H 4.80%; $C_{17}H_{14}O_5$ requires C 68.45%, H 4.73%. ^{1}H nmr see Table 1.

Ochrone B [**2**] was running close to ochrone A on tlc and was separated as the acetate. The fraction containing ochrones A and B was acetylated using Ac_2O and pyridine for 24 h at room temperature. After removal of the excess reagents, ochrone B acetate [**4**] was separated by preparative tlc on Si gel G but refused to crystallize (found C 68.88%, H 4.10%; $C_{17}H_{12}O_5$ requires C 68.92%, H 4.08%); uv ($CHCl_3$) λ max 228, 282, 313 nm; ir (KBr) ν max 2900, 2840, 1750, 1730, 1676, 1620, 1460, 1240, 1200, 1070 cm^{-1}; ^{1}H nmr see Table 1; ms *m/z* $[M]^+$ 296 (2%), 254 (25), 239 (2), 225 (5), 197 (2), 185 (3), 167 (7), 155 (11), 149 (18), 111 (15), 97 (25), 83 (33), 71 (40), 69 (50). The C_6H_6-Me_2CO (9:1) fraction on preparative tlc yielded coeloginin, mp 200° [lit. (3) mp 198°] and batatasin III, mp 94° [lit. (3) mp 94°].

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