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A 9,10-DIHYDROPHENANTHRENE FROM TUBERS OF *EULOPHIA NUDA*

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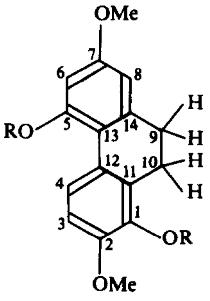
Key Word Index—*Eulophia nuda*; Orchidaceae; tubers; eulophiol; 1,5-dihydroxy-2,7-dimethoxy-9,10-dihydrophenanthrene.

Abstract—A crystalline 9,10-dihydrophenanthrene, named eulophiol, has been isolated from the tubers of *Eulophia nuda*. It was identified as 1,5-dihydroxy-2,7-dimethoxy-9,10-dihydrophenanthrene by ^{1}H and ^{13}C NMR.

INTRODUCTION

Eulophia nuda (Amarkanda) is a small shrub found in the Himalayas, Asam, Burma and the south-west coast of India. The plant extract is used in medicine as an antitumor, anthelmintic, vermifuge and blood purifier [1]. Merchant *et al.* [2] isolated *n*-hexacosyl alcohol and lupeol from the tubers. We report here the structure of a new crystalline compound, eulophiol, isolated from an ethanol extract of the tubers.



Eulophiol, R = H

Eulophiol diacetate, R = COMe

RESULTS AND DISCUSSION

The molecular formula of eulophiol is $C_{16}H_{16}O_4$ based on elemental analysis and the mass spectrum (M^+ 272). The ^{1}H NMR spectrum of eulophiol (Table 1) shows four benzylic protons with a characteristic shift of a 9,10-dihydrophenanthrene, further supported by its UV spectrum [4, 5]. The ^{13}C NMR spectrum of eulophiol diacetate (Table 2) unequivocally establishes the assignment of the skeleton [3]. The IR spectrum shows the absence of a carbonyl group and the presence of a phenolic hydroxyl, (positive ferric chloride test).

Since eulophiol gives a diacetate and it has two D_2O exchangeable protons, two oxygen atoms are accounted for two hydroxyl groups. The other two oxygen atoms are two methoxyl groups as shown by the ^{1}H NMR spectrum and further supported by $[M-15]^+$ and $[M-30]^+$ peaks in the mass spectrum. The ^{1}H NMR shows four aromatic protons, two *ortho* coupled and two *meta* coupled. One of the *ortho* coupled protons is considerably downfield, which indicates that it is either at C-4 or C-5 [6, 7]. If this proton is at C-4, then the other proton must be at C-3. Similarly none of the *meta* coupled protons deviates from the normal aromatic proton shift indicating that C-5 is substituted. Hence, the remaining two protons are placed at C-6 and C-8, and eulophiol is

Table 1. ^{1}H NMR data of eulophiol and its diacetate derivative

Type of proton(s)	Type of signal	Eulophiol (δ-values)	Eulophiol diacetate (δ-values)	Deshielding in diacetate (δ-values)
H-9 and H-10	*br s* (4H)	2.70	2.65	0.05
OMe-2	*s* (3H)	3.800	3.900	0.1
OMe-7	*s* (3H)	3.875	3.875	0
OH-1	*br s* (1H) exch. D_2O	7.25	—	—
OH-5	*br s* (1H) exch. D_2O	8.37	—	—
MeOAc-1	*s* (3H)	—	2.325	—
or				
MeOAc-5	*s* (3H)	—	2.375	—
H-6 } *meta* coupled	*d* (1H, $J = 2$ Hz)	6.47	6.70	0.23
H-8 }	*d* (1H, $J = 2$ Hz)	6.57	6.80	0.23
H-3 } *ortho* coupled	*d* (1H, $J = 8$ Hz)	6.87	7.00	0.13
H-4 }	*d* (1H, $J = 8$Hz)	7.85	8.23	0.38

Table 2. ^{13}C NMR shifts of eulophiol diacetate

Carbon	Shift
1	148.2
2	149.8
3	113.4
4	126.7
5	149.5
6	104.8
7	157.3
8	109.1
9	29.6
10	22.2
11	131.9
12	125.5
13	120.5
14	136.2
$2 \times \mathrm{O\underline{Me}}$	55.6 55.8
$2 \times \mathrm{OCO\underline{Me}}$	21.1 20.4
$2 \times \mathrm{O\underline{C}OMe}$	169.4 168.4

therefore, a 1,2,5,7-tetrasubstituted-9,10-dihydrophenanthrene. Both the methoxyl protons are similar to normal aromatic methoxyl proton positions, hence none of them could be at C-5 [8, 9]. Furthermore, the substituent at C-5 must be a hydroxyl since the proton at C-4 suffers a downfield shift of $\Delta\delta$0.38 in the acetate derivative where it comes in the deshielding zone of the acetate carbonyl at C-5. In the diacetate derivative, all the protons suffer a downfield shift from $\Delta\delta$0.1 to 0.38, hence the two hydroxyl groups must be in two different aromatic rings. The second hydroxyl function could be placed at C-1, which is *meta* to the proton at C-3 causing only a $\Delta\delta$0.13 downfield shift, while this shift is $\Delta\delta$0.23 where protons are *ortho* or *para* with respect to the acetate group, e.g. protons at C-6 and C-8.

Thus, the structure of eulophiol is established as 1,5-dihydroxy-2,7-dimethoxy-9,10-dihydrophenanthrene. Only one of the methoxyl groups suffers a deshielding of 0.1 ppm in the diacetate, which is consistent with the proposed structure, because the acetoxy group at C-1 can cause deshielding of the *ortho* methoxyl at C-2. The assignment of the methoxyl groups is further supported by a nuclear Overhauser effect experiment. When both methoxyl groups are irradiated simultaneously, there is an enhancement of intensity by 12% of the H-6 and H-8 signals and by 18% of the H-3 signal.

EXPERIMENTAL

Mps are uncorr. UV spectra were recorded in MeOH, IR spectra in KBr discs and ^{1}H NMR spectra in CD_3COCD_3 using TMS int. standard at 80 MHz.

Isolation of eulophiol. E. nuda tubers were repeatedly extracted with EtOH. Most of the EtOH was removed *in vacuo*, diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was chromatographed on Si gel. The fraction eluted with C_6H_6–MeOH (19:1) was further purified by prep. TLC on Si gel G using C_6H_6–MeOH (97:3). Eulophiol crystallized from $CHCl_3$ as needles, yield 0.21%, mp 202–203°. (Found: C, 70.36; H, 5.87; $C_{16}H_{16}O_4$ requires: C, 70.60; H, 5.90.) It gave a positive $FeCl_3$ test. UV $\lambda_{\max}$ nm (log ε): 308.4 (4.05), 281.7 (4.31), 214 (4.59). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3500, 2950, 1610, 1480. ^{1}H NMR: Table 1. MS *m/z*: 272 $[M]^+$, 257 $[M-15]^+$, 242 $[M-30]^+$, 239, 229, 214, etc.

The diacetate of eulophiol was prepared by acetylation with Ac_2O–pyridine at ambient temp. for 24 hr. It crystallized as needles, mp 143°. (Found: C, 67.36; H, 5.69; $C_{20}H_{20}O_6$ requires: C, 67.2; H, 5.75%.) UV $\lambda_{\max}$ nm (log ε): 280 (4.34), 210 (4.53). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1720, 1250. ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

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