Phytochemistry, Vol. 24, No. 4, pp. 801–804, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00
Pergamon Press Ltd.

NUDOL, A PHENANTHRENE OF THE ORCHIDS *EULOPHIA NUDA*, *ERIA CARINATA* AND *ERIA STRICTA*

S. R. BHANDARI, A. H. KAPADI, P. L. MUJUMDER*, MUKTA JOARDAR* and J. N. SHOOLERY†

Chemistry Department, M.A.C.S. Research Institute, Law College Road, Pune 411 004, India; *Department of Chemistry, University College of Science, Calcutta-700009, India; †Varian, Palo Alto, CA 94303, U.S.A.

(*Revised received* 15 *September* 1984)

Key Word Index—*Eulophia nuda*; *Eria carinata*; *Eria stricta*; Orchidaceae; 2,7-dihydroxy-3,4-dimethoxyphenanthrene.

Abstract—An amorphous phenanthrene, named nudol has been isolated from *Eulophia nuda*, *Eria carinata* and *E. stricta*. It was identified as 2,7-dihydroxy-3,4-dimethoxyphenanthrene. Synthesis of nudol and its dimethyl ether is described.

INTRODUCTION

We have reported earlier eulophiol, a new 9,10-dihydrophenanthrene from the tubers of *Eulophia nuda* [1]. The chemical investigations of a series of high altitude Himalayan orchids have so far yielded two 9,10-dihydrophenanthrenes [2, 3], nine 9,10-dihydrophenanthropyrans and pyrones and two steroidal ketones [4–10]. We report here the structure and synthesis of a phenanthrene, named nudol, isolated from the tubers of *Eulophia nuda*, and as a minor constituent of *Eria carinata* and *E. stricta*, isolated as the diacetate.

RESULTS AND DISCUSSION

The molecular formula of nudol is $C_{16}H_{14}O_4$ based on elemental analysis and its mass spectrum ($M^{+\cdot}$ 270). The UV spectrum of nudol diacetate and dimethyl ether derivative of nudol showed characteristic pattern of substituted phenanthrene [11–14], further supported by a very much downfield doublet of C-5 proton in ^{1}H NMR spectra of nudol, nudol diacetate and nudol dimethyl ether [11–15] (Table 1). The IR spectrum of nudol showed the absence of a carbonyl function and the presence of phenolic hydroxyl which was confirmed by positive ferric chloride test.

Since nudol gives a diacetate, a dimethyl ether and it has two D_2O exchangeable protons, two oxygen atoms in nudol are accounted for by two hydroxyl groups. The other two oxygen atoms in nudol are accounted for by two methoxyl groups as shown by ^{1}H NMR and further supported by M-15 and M-30 peaks in the mass spectrum. The 80 MHz ^{1}H NMR spectrum of nudol shows six aromatic protons, such as two C-9 and C-10 (AB-quartet centred at δ7.63) protons, one *ortho*-coupled, (*d*, 1H, J = 10 Hz at δ9.33), one *ortho–meta*-coupled (*dd*, 1H, J_1 = 10 Hz; J_2 = 3 Hz, δ7.30), *one-meta*-coupled (*d*, 1H, J = 3 Hz, δ7.35) and one uncoupled (singlet) proton (*s*, 1H, δ7.33). The ^{1}H NMR of nudol, acetate also showed a similar pattern of six aromatic protons. In both nudol and its acetate the *ortho*-coupled proton appeared considerably downfield which indicated that it must be at C-4 or C-

Table 1. ^{1}H NMR data of nudol, nudol diacetate and nudol dimethyl ether

Type of proton (*s*)	Type of signal	Nudol (Y, δ-values)	Nudol diacetate (X, δ-values)	Nudol dimethyl (Z, δ-values)	X–Y	X–Z
OMe-3, or	*s* (3H)	4.00	4.00	*s* (6H) 4.00	—	—
OMe-4	*s* (3H)	4.05	4.05	*s* (6H) 4.05	—	—
OH-2, or	*br s* (1H) exch. D_2O	8.00	—	—		
OH-7	*br s* (1H) exch. D_2O	8.65	—	—		
MeCOO-2, or	*s* (3H)	—	2.45	—		
MeCOO-7	*s* (3H)	—	2.38	—		
H-1	*s* (1H)	7.33	7.55	7.30	0.22	0.25
H-5 (*ortho*-coupled)	*d* (^{1}H, J = 10 Hz)	9.33	9.38	9.33	0.05	0.05
H-6 (*ortho–meta*-coupled)	*dd* (1H, J = 10 Hz)	7.30	7.55	7.25	0.25	0.30
	J = 3 Hz	(centre)	(centre)	(centre)		
H-8 (*meta*-coupled)	*d* (1H, J = 3 Hz)	7.35	7.80	7.38	0.45	0.42
H-9 and H-10	AB-quartet (2H, J = 8 Hz)	7.63	*s*, 7.90	*s*, 7.70	0.27	0.20
	A = 7.55, B = 7.77	(centre)				

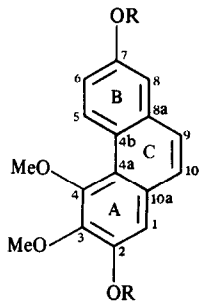
5 [11–15]. If we place this proton at C-5 (ring B) then the *ortho–meta*-coupled proton must be at C-6 and consequently *meta*-coupled proton at C-8 leaving C-7 for one oxygen substituent. Ring A contains only one proton as singlet at normal aromatic proton shift indicating that C-4 is substituted thus leaving C-1, C-2 and C-3 for two oxygen substituents and one proton. On biogenetic considerations, 2,3,4,7-tetrasubstitution pattern appears to be more likely and this was confirmed by showing the identity of nudol dimethyl ether to the synthetic 2,3,4,7-tetramethoxyphenanthrene (**3**) which was obtained by oxidative photocyclisation of stilbene (**6**). The stilbene (**6**) was prepared by Wittig condensation of 3-methoxybenzaldehyde (**5**) with 3,4,5-trimethoxybenzyltripenylphosphonium bromide (**4**) which was obtained from gallic acid (Scheme 1).

The substituent at C-2 in nudol must be a hydroxyl since the proton at C-1 suffers a downfield shift of 0.22 to 0.25 in the acetate and dimethyl ether respectively. Similarly substituent at C-7 must be a hydroxyl as both the adjacent protons (C-6 and C-8) suffer a downfield shift of 0.25 to 0.45 in the acetate and dimethyl ether derivatives. Thus the structure of nudol is established as **1**.

Structure **1** for nudol received confirmation from the ^{13}C NMR spectral data (Table 2) of its diacetate. The degree of protonation of each carbon atom was determined by APT experiments [16]. Except for the ring-A carbon atoms, the carbon chemical shifts of nudol diacetate are in good agreement with the values calculated for **2** using the additive parameters of the functional groups on the reported δ_c values of parent phenanthrene [17]. All the carbon atoms of ring-A show varying degrees of downfield shifts. This may be attributed to the polysubstituted nature of ring-A and this finds analogy with similar downfield shifts of the substituted carbon atoms of the benzene derivatives bearing three consecutive oxygen substituents [18], where a simple additive rule fails. The slight downfield shift of C-5 may be due to the proximity of the C-4 methoxyl group. The value of C-1 also showing a downfield shift of 3.5 ppm, caused by polysubstituted nature of ring-A, provides the strong evidence for the placement of an acetoxy function at C-2 of **2** (and hence OH at C-2 in **1**).

Further confirmation of the hydroxyl position assignment is obtained by studying partial demethylation of 2,3,4,7-tetramethoxyphenanthrene (**3**). An isolated methoxyl (C-7) is known to undergo facile demethylation [19], while angular substituents (C-4 or C-5) are known to react very slowly [13, 20, 21] and C-3 methoxyl is hindered hence partial demethylation should preferentially demethylate C-2 and C-7 methoxyls thus giving **1** (nudol). When **3** was demethylated by anhydrous aluminium chloride/ethanethiol [22] at $-10°$ it showed the formation of nudol in 60% yield on HPLC along with two other minor products, thus confirming the structure of nudol.

During the revision of this manuscript we came across the report of Stermitz *et al.* [23] on the isolation from *Oncidium cebolleta* (Orchidaceae) and structure elucidation of the same compound, which was isolated as the diacetate (mp 159°) and its structure was established by X-ray crystallography.



1 Nudol, R = H

2 Nudol diacetate, R = Ac

3 Nudol dimethyl ether, R = Me

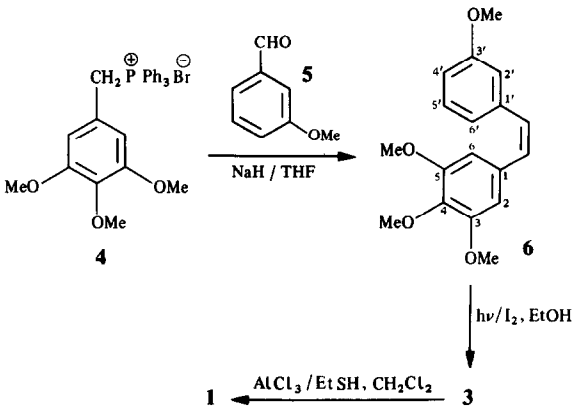
Table 2. ^{13}C NMR data* of nudol in diacetate (**2**)

Carbon atoms	Chemical shifts† in ppm	Carbon atoms	Chemical shifts†
C-1	117.12	C-8	120.87
C-2	144.89	C-8a	133.71
C-3	143.18	C-9	128.86‡
C-4	152.45	C-10	127.38‡
C-4a	123.01	C-10a	127.42
C-4b	129.42	3-OMe	61.18
C-5	126.87	4-OMe	60.05
C-6	119.59	–O–CO Me	169.22 169.55
C-7	148.72	–O–CO Me	20.77 21.27

*The spectra were run on a Varian XL-300 (75 MHz) instrument in $CDCl_3$.

†Chemical shifts were measured with $\delta_{TMS} = \delta_{CDCl_3} + 76.9$ ppm.

‡Values are interchangeable.



Scheme 1. Synthesis of nudol.

EXPERIMENTAL

Mps are uncorr. UV spectra were recorded in MeOH or 95% aldehyde-free EtOH, IR spectra in KBr discs. ^{1}H NMR spectra were recorded in $CDCl_3$ or CD_3COCD_3 using TMS as internal

standard at 80 MHz. ^{13}C NMR of **2** was recorded on Varian XL-300 instrument using TMS as int. standard at 75 MHz in $CDCl_3$. MS were recorded with a direct inlet system at 70 eV.

Isolation of nudol. 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 silica gel. The fraction eluted with C_6H_6–MeOH (19:1) was further purified by preparative TLC on silica gel GF-254 using C_6H_6–MeOH (93:7). Nudol was obtained as a buff coloured amorphous solid, yield 0.033%, mp 253° (Found: C, 70.92; H, 5.28; $C_{16}H_{14}O_4$ requires: C, 71.1; H, 5.22%). It gave positive $FeCl_3$ test, and turns brown on longer exposure to air. IR ν_{max} cm^{-1}: 3460, 2940, 1620, 1580, 1510. ^1H NMR: Table 1. MS *m/z*: 270 $[M]^+$, 255 $[M-15]^+$, 240 $[M-30]^+$, 223, 212, etc.

The diacetate crystallized as needless from MeOH, mp 151° (Found: C, 76.76; H, 5.12; $C_{20}H_{18}O_6$ requires: C, 67.79; H, 5.12%). UV λ_{max} nm (log ε): 300 (3.85), 289 (4.04), 280 (4.13), 258 (4.84). IR ν_{max} cm^{-1}: 1750. MS *m/z*: 354 $[M]^+$, 312 $[M-42]^+$, 270 $[M-84]^+$, 255, etc. ^1H NMR: Table 1.

The dimethyl ether was purified by prep. TLC on silica gel using $CHCl_3$ (R_f, 0.8) and obtained as colourless needles, mp 148° (Found: C, 72.41: H, 6.11. $C_{18}H_{18}O_4$ requires: C, 72.47; H, 6.08). UV λ_{max} nm (log ε): 292 (4.11), 280 (4.21), 259 (4.88). ^1H NMR: Table 1.

Isolation of nudol (**1**) *as its diacetate* (**2**) *from* Eria carinata *and* E. stricta. Air-dried powdered whole plants of *E. carinata* and *E. stricta* (1 kg of each) were separately kept soaked in MeOH for 3 weeks. The MeOH extract (3 l.) in each case was concentrated under reduced pressure and diluted with water, extracted with ether. The ether extracts were separately fractionated into neutral and acidic fractions by 2 N aq. alkali. The aq. alkaline extract in each case was acidified in the cold by conc. HCl and the liberated solid was extracted with ether, washed with H_2O, dried and the solvent removed. The residue in each case was chromatographed separately. The petrol–EtOAc (5:1) eluate afforded a gummy phenolic mixture which on repeated CC gave solid which mostly contained **1** (0.03 g from *E. carinata* and 0.08 g from *E. stricta*). The combined crude **1** was acetylated with Ac_2O–pyridine in the usual manner. The crude diacetate was chromatographed in petrol–EtOAc (10:1) to afford pure **2**, mp 156° (Found: C, 67.71; H, 5.10. $C_{20}H_{18}O_6$ requires: C, 67.80; H, 5.08%). UV λ_{max} nm (log ε): 257 (4.73), 280 sh (4.12), 301 (3.94). IR ν_{max} cm^{-1}: 1220, 1760, 1772 (OAc), 1625, 890 (aromatic nucleus).

Compound **2** (from above, 0.03 g) was heated under reflux with 20% methanolic KOH (25 ml) for 3 hr under N_2. MeOH was removed under red. pres., the residue was diluted with water, acidified with conc. HCl in the cold, extracted with ether, washed and dried. The residue after removal of ether was chromatographed. The petrol–EtOAc (5:1) eluate gave a solid (0.025 g) which on repeated crystallizations from petrol–EtOAc gave **1**, mp 138° (Found: C, 70.92; H, 5.21, $C_{16}H_{14}O_4$ requires: C, 71.11; H, 5.19%). UV λ_{max} nm (log ε): 261 (4.21), 283 (4.14): $\lambda_{max}^{0.1\,N\,NaOH}$ nm (log ε): 261 (4.21), 305 (4.23). IR ν_{max} cm^{-1}: 3380 (OH), 1620, 1470, 920 (aromatic nucleus). ^1H NMRs of **1** and **2** isolated from *E. carinata* and *E. stricta* are essentially the same as described in Table 1.

Synthesis of 2,3,4,7-*tetramethoxyphenanthrene.* A mixture of 3,4,5-trimethoxybenzyl–triphenylphosphonium bromide (**4**) [24, 25], 1.35 g and NaH, 0.15 g were stirred as a suspension in dry THF, 30 ml, under N_2 for 90 min. To this a soln of 3-methoxybenzaldehyde (**5**), 0.35 g was added in 3 ml dry THF and the mixture was stirred for 18 hr at ambient temp. The mixture was acidified with dil. HCl and extracted with Et_2O (2 × 50 ml). The Et_2O extract on evaporation gave a pale yellow solid, 2.0 g, which was purified by silica gel CC to give a mixture of *cis*- and *trans*-3,4,5,3′-tetramethoxystilbene (**6**), 0.53 g, yield 69%. ^1H NMR: δ7.25 to 6.7 (*m*, 8H, Ar–H and CH=CH), 3.92, 3.87 and 3.85 (singlets, total 12H, 4 × OMe). UV λ_{max} nm: 298, IR ν_{max} cm^{-1}: 3000, 2940, 1580.

The stilbene (**6**), 0.3 g was dissolved in 600 ml EtOH containing 0.03 g I_2 and irradiated for 8 hr with 450 W UV lamp in immersion well type quartz reactor. The reaction mixture after usual work-up followed by purification with prep. TLC on silica gel gave needles, mp 148°, yield 68%; it was identical with dimethyl ether of nudol (**3**) (mp, mmp, IR and cochromatography).

Demethylation of 2,3,4,7-*tetramethoxyphenanthrene.* A solution of ethanethiol, 4 ml and anhydrous $AlCl_3$, 48 mg in CH_2Cl_2 (20 ml) was cooled to −10°. To this solution **3** (18 mg) in 2 ml CH_2Cl_2 was added. The mixture was stirred at −10° for 30 min. The reaction mixture was stirred with H_2O (10 ml) for 30 min. The CH_2Cl_2 layer was separated, and H_2O layer was extracted with CH_2Cl_2, 10 ml. The combined CH_2Cl_2 extract yielded a pale yellow solid, 16 mg which on HPLC using microporosil column and cyclohexane–EtOAc–AcOH (40:60:1) as a solvent system at 0.6 ml/min flow rate showed nudol as major peak (60%), confirmed by cochromatography.

Acknowledgements—Thanks are due to Prof. D. S. Datar and Dr. M. M. Mahendru for their keen interest and helpful discussions. Thanks are also due to Dr. G. F. Smith, the University of Manchester, U.K. for the mass spectra. The authors, PLM and MJ wish to acknowledge the support of this work by UGC, New Delhi, India.

REFERENCES

1. Bhandari, S. R. and Kapadi, A. H. (1983) *Phytochemistry* **22**, 747.
2. Majumder, P. L. and Laha, S. (1981). *J. Indian Chem. Soc.* **58**, 928.
3. Majumder, P. L., Laha, S. and Datta, N. (1982) *Phytochemistry* **21**, 478.
4. Majumder, P. L., Bandyopadhyay, D. and Joardar, S. (1982). *J. Chem. Soc. Perkin Trans.* 1, 1131.
5. Majumder, P. L. and Datta, N. (1982) *Indian J. Chem.* **21B**, 534.
6. Majumder, P. L., Sarkar, A. K. and Chakraborti, J. (1982) *Phytochemistry* **21**, 2713.
7. Majumder, P. L. and Sarkar, A. K. (1982) *Indian J. Chem.* **21B**, 29.
8. Majumder, P. L., Datta, N., Sarkar, A. K. and Chakraborti, J. (1982) *J. Nat. Prod.* (*Lloydia*) **45**, 730.
9. Majumder, P. L. and Datta, N. (1984) *Phytochemistry* **23**, 671.
10. Majumder, P. L. and Chakraborti, J. *Tetrahedron* (in press).
11. Letcher, R. M. and Nhamo, L. R. M. (1971). *J. Chem. Soc.* (*C*) 3070.
12. Letcher, R. M. and Nhamo, L. R. M. (1972) *Tetrahedron Letters* 4889.
13. Reisch, J., Bathorg, M., Szedrci, K., Navikand, I. and Minker, E. (1973) *Phytochemistry* **12**, 228.
14. Govindachari, T. R., Laxmikantam, M. V., Nagarajan, K. and Pai, B. R. (1958) *Tetrahedron* **4**, 854.
15. Letcher, R. M. and Wong, K. M. (1978). *J. Chem. Soc. Perkin Trans.* 1, 739.
16. Patt, S. L. and Shoolery, J. N. (1982) *J. Magn. Reson.* **46**, 535.
17. Stothers, J. B. (1972) C-13 *NMR Spectroscopy*. Academic Press, New York.
18. Wenkert, E., Gottlieb, H. E., Gottlieb, O. R., Pereira Das,

M. O. and Formiga, M. D. (1976) *Phytochemistry* **15**, 1547.
19. Erdtman, H. and Ronlan, A. (1969) *Acta Chem. Scand.* **23**, 249.
20. Hardegger, E., Schellenbeaum, M. and Corrodi, H. (1963) *Helv. Chim. Acta* 1171.
21. Rajaram, K. and Rangaswami, S. (1975) *Ind. J. Chem.* **13**, 1137.
22. Fuji, K., Lichikawa, K. and Fujita, F. (1980) *J. Chem. Soc. Perkin Trans.* 1, 1066.
23. Stermitz, F. R., Suess, T. R., Schaur, C. K. and Anderson, O. P. (1983) *J. Nat. Prod.* (*Lloydia*) **46** (3), 417.
24. Patra, A., Mukhopadhyay, P. and Ghosh, G. (1982) *Indian J. Chem.* **21B**, 173.
25. Merric, M. and Ghera, E. (1957) *J. Am. Chem. Soc.* **79**, 7675.