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CONFUSARIN AND CONFUSARIDIN, TWO PHENANTHRENE DERIVATIVES OF THE ORCHID *ERIA CONFUSA*

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Key Word Index—*Eria confusa*; Orchidaceae; confusarin; confusaridin; phenanthrenes.

Abstract—From the orchid *Eria confusa* were isolated two new polyoxygenated phenanthrene derivatives, confusarin and confusaridin, which were shown to be 2,7-dihydroxy-3,4,8-trimethoxyphenanthrene and 2,6-dihydroxy-3,4,7,8-tetramethoxyphenanthrene, respectively.

INTRODUCTION

As part of our general programme on the chemistry of Indian orchids, we reported earlier 9,10-dihydrophenanthrenes [1], 9,10-dihydrophenanthropyrans and pyrones [2–4], phenanthrenes [5, 6], bibenzyls [7], triterpenoids [8] and steroids [9]. Our continuing search has resulted in the isolation of two new polyoxygenated phenanthrene derivatives, confusarin and confusaridin, from the orchid *Eria confusa*. In this paper we report the evidence leading to the elucidation of their structures.

RESULTS AND DISCUSSION

Confusarin, $C_{17}H_{16}O_5$ ($M^{+\cdot}$ 300), mp 185°, and confusaridin, $C_{18}H_{18}O_6$ ($M^{+\cdot}$ 330), mp 192°, showed UV absorptions, λ_{max} 212, 232, 286, 295, 309 and 350 nm (log ε 4.35, 4.25. 4.12, 4.00, 3.92 and 3.18) and λ_{max} 221, 265, 283 and 316 nm (log ε 4.02, 4.43, 4.09 and 3.64), respectively, exhibiting a general resemblance to those of the substituted phenanthrene derivatives [10]. The phenolic nature of both the compounds was indicated by their characteristic colour reactions, alkali-induced bathochromic shifts of their UV maxima and by their IR spectra, both of which showed bands at 3300 cm^{-1}. The presence of two phenolic hydroxyl groups in both confusarin and confusaridin was established by the formation of their respective diacetyl derivatives, $C_{21}H_{20}O_7$ ($M^{+\cdot}$ 384), mp 155°, and $C_{22}H_{22}O_8$ ($M^{+\cdot}$ 414), mp 122°. This was also corroborated by the ^1H NMR spectra of the compounds. Thus the spectrum of confusarin showed a two-proton singlet at δ5.90 and that of confusaridin exhibited two one-proton singlets at δ5.97 and 6.00 (all these signals disappeared on deuterium exchange). The presence of three aromatic methoxyl groups in confusarin [δ3.89 (6H, *s*) and 4.03 (3H, *s*)] and four such groups in confusaridin [δ3.97 (3H, *s*), 4.00 (3H, *s*) and 4.08 (6H, *s*)] was also indicated by their ^1H NMR spectra. Confusarin contains five aromatic protons, while confusaridin possesses four such protons. Two of these protons in each of these compounds appeared as a pair of one-proton doublets (confusarin: δ7.51 and 7.79, J = 10 Hz; confusaridin: δ7.42 and 7.89, J = 9 Hz) which are typical [11] of H-9 and H-10 of phenanthrene derivatives. One of the remaining aromatic protons of both the compounds resonated at a relatively downfield position (confusarin: δ9.12, *d*, J = 10 Hz; confusaridin: δ8.83, *s*). The chemical shifts of these protons are reminiscent [11] of those of H-4 and H-5 of a phenanthrene derivative. Further, the splitting patterns of the signals corresponding to these protons clearly indicate that in confusarin it must have an *ortho* hydrogen atom, while in confusaridin the carbon atom *ortho* to this hydrogen atom is substituted by an oxygen function. The upfield shift of this proton in confusaridin by 0.29 ppm compared to the corresponding proton of confusarin is in conformity with the above contention. If the above downfield signals are assigned to H-5 of a phenanthrene skeletal structure for both confusarin and confusaridin then C-4 of both the compounds must bear a substituent; but while C-6 of the latter is also substituted, the corresponding carbon atom of the former must contain a hydrogen atom. This is borne out by the appearance of H-6 of confusarin as a clean doublet at δ7.22 (J = 10 Hz), which also implies that C-7 and C-8 of the compound are also substituted. Confusaridin was also assumed to contain substituents at C-7 and C-8. The only other aromatic proton in confusarin and confusaridin appeared as a sharp singlet at δ7.11 and 7.14, respectively, which were assigned to H-1 by comparison with the chemical shift of the corresponding proton of nudol (**1e**) [5]. The foregoing spectral data thus establish the 2,3,4,7,8-pentaoxygenated and 2,3,4,6,7,8-hexaoxygenated phenanthrene formulations for confusarin and confusaridin, respectively.

Evidence in support of the relative positions of the hydroxyl and methoxyl groups in the two compounds was provided by the ^1H NMR spectra of the respective diacetate. In the light of the observation [11] that acetylation of a hydroxyl group at C-4 of a phenanthrene causes an upfield shift of its H-5, the placement of a hydroxyl group at C-4 of confusarin and confusaridin is ruled out, since in the ^1H NMR spectra of both diacetates H-5, instead of being shielded, is shifted downfield. Such a downfield shift of H-5 in confusaridin diacetate, in particular, is quite significant (0.27 ppm) to suggest placement of a hydroxyl at its C-6. The other aromatic proton (H-1) of confusaridin also suffered an appreciable downfield shift (0.26 ppm) in its diacetyl derivative suggesting

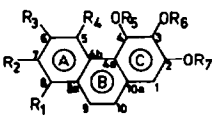
that the remaining hydroxyl group in confusaridin is at C-2. A methoxyl group at C-2 would not have caused such downfield shift of H-1 as has been observed in bulbophyllanthrin diacetate (**1h**). Confusaridin is therefore 2,6-dihydroxy-3,4,7,8-tetramethoxyphenanthrene (**1c**). Similar downfield shifts of H-1 and H-6 of confusarin in the ^{1}H NMR spectrum of its diacetate imply that the two phenolic hydroxyl groups in this compound are at C-2 and C-7. On this basis confusarin was assigned the 2,7-dihydroxy-3,4,8-trimethoxyphenanthrene formulation (**1a**) which was further confirmed by ^{13}C NMR spectral analysis of the compound and its diacetate (**1b**).

The carbon chemical shifts of confusarin (**1a**) and its diacetate (**1b**) are listed in Table 1. The degree of protonation of each carbon atom was confirmed by the DEPT technique, and the δ_c values were assigned by comparison with the carbon chemical shifts of structurally related compounds like nudol diacetate (**1f**) [5] and bulbophyllanthrin (**1g**) [6], and also by a consideration of the additive parameters [12, 13] of the functional groups (OH and OMe) on the reported δ_c values of the parent phenanthrene [12]. Thus the δ_c values of C-1, C-2, C-3, C-4, C-4a and C-10a of confusarin diacetate (**1b**) are essentially the same as those of the corresponding carbon atoms of nudol diacetate (**1f**) indicating an identical substitution pattern of ring C in both compounds. The possibility of an alternative arrangement of the hydroxyl and methoxyl groups in ring C of confusarin similar to that in bulbophyllanthrin (**1g**) has been ruled out by the marked differences in the δ_c values of the ring C carbon atoms of the two compounds. The observed changes in the δ_c values of C-1, C-3, C-4a, C-4b, C-6 and C-8 in particular, of confusarin on acetylation are in conformity with the placement of the two hydroxyl groups at C-2 and C-7. An interesting ^{13}C NMR spectral feature of confusarin and its diacetate is the upfield shift (~8–10 ppm) of one of their C-9 and C-10, which normally appear at δ_c 126–128. This is reminiscent of similar upfield shifts of C-10 of coelogin [2] and coeloginin diacetate [2] caused by the steric effect of a methoxyl group at their C-1 position. A similar upfield shift of the oxymethylene carbon of imbricatin diacetate [3] and coelogin has been attributed to the steric effects of a methoxyl and a hydroxyl group, respectively, at their C-3 position. Since C-1 of confusarin is unsubstituted, the above upfield carbon atom must then be assigned to its C-9, and this, in turn, demands placement of a substituent (OMe or OH) at C-8 of confusarin. That this substituent is a methoxyl rather than a hydroxyl function is borne out by the magnitude of shielding of C-8a and C-5, in particular, of confusarin and its diacetate compared with those of the corresponding carbon atoms of nudol diacetate. The placement of one methoxyl group at C-8 and the other two at C-3 and C-4 in confusarin was further justified by the chemical shifts (δ_c 59–62) of all the methoxyl carbons of the compound and its diacetate indicating that none of these methoxyl groups has an *ortho* hydrogen atom [14]. The carbon atom of an aromatic methoxyl group having at least one *ortho* hydrogen atom normally resonates at ~δ_c 55–56 [14]. An alternative formulation of confusarin with a hydroxyl at C-8 and a methoxyl group at C-7 would have its 7-methoxyl carbon appearing at the normal region instead at the observed downfield position. These observations thus firmly establish the structure **1a** for confusarin.

Table 1. Carbon chemical shifts of confusarin (**1a**), confusarin diacetate (**1b**), nudol diacetate (**1f**) and bulbophyllanthrin (**1g**)

Carbon atoms	Chemical shifts (δ values)*			
	1a	**1b**	**1f**	**1g**
C-1	107.61	117.23	117.12	106.00
C-2	147.12	145.09	144.89	146.90
C-3	140.40	143.49	143.18	138.90
C-4	150.16	152.69	152.45	140.71
C-4a	118.45	123.21	123.01	117.11
C-4b	124.27	129.56	129.42	117.69
C-5	123.38	123.95	126.87	154.24
C-6	115.56	122.06	119.59	115.74
C-7	144.92	140.74	148.72	127.43
C-8	140.40	146.84	120.87	120.32
C-8a	125.73	128.41†	133.71	134.53
C-9	118.83	120.53	128.86‡	126.85
C-10	126.83	127.36	127.38‡	126.14
C-10a	128.74	128.97†	127.42	127.34
OMe	59.20	60.20	61.18	62.28
	60.76	61.20	60.05	56.27
	61.42	62.08		
OCOMe		169.29	169.22	—
			169.55	—
OCOMe		20.88	20.77	—
		21.07	21.27	—

*δ values are in ppm downfield from TMS: $\delta_{(TMS)} = \delta_{(CDCl_3)} + 76.9$ ppm.
†,‡Values are interchangeable.



1a: R_1=OMe, R_2=OH, R_3=R_4=R_7=H, R_5=R_6=Me
1b: R_1=OMe, R_2=OAc, R_3=R_4=H, R_5=R_6=Me, R_7=Ac
1c: R_1=R_2=OMe, R_3=OH, R_4=R_7=H, R_5=R_6=Me
1d: R_1=R_2=OMe, R_3=OAc, R_4=H, R_5=R_6=Me, R_7=Ac
1e: R_1=R_3=R_4=R_7=H, R_2=OH, R_5=R_6=Me
1f: R_1=R_3=R_4=H, R_2=OAc, R_5=R_6=Me, R_7=Ac
1g: R_1=R_2=R_3=R_6=H, R_4=OH, R_5=R_7=Me
1h: R_1=R_2=R_3=H, R_4=OAc, R_5=R_7=Me, R_6=Ac

EXPERIMENTAL

Mps are uncorr. UV spectra were measured in 95% aldehyde-free EtOH and IR spectra in KBr discs. ^{1}H NMR spectra were recorded in a 80 MHz Varian CFT-20 instrument in $CDCl_3$ soln using TMS as the int. standard. ^{13}C NMR spectra were measured at 62.5 MHz in a Brüker instrument in the same solvent and using the same int. standard. Chemical shifts were measured in δ ppm and for ^{13}C NMR $\delta_{TMS} = \delta_{CDCl_3} + 76.9$ ppm. MS were recorded in an instrument equipped with a direct inlet system and operating at 70 eV. Values in parentheses after *m/z* values represent rel. int. of the peaks. Silica gel (60–100 mesh) were used for CC and silica gel G for TLC. All analytical samples were

routinely dried over P_2O_5 for 24 hr *in vacuo* and were tested for purity by TLC and MS. Anhydrous Na_2SO_4 was used for drying organic solvents and petrol used had bp 60–80°.

Isolation of confusarin (**1a**) *and confusaridin* (**1c**). Air-dried powdered whole plant of *E. confusa* was kept soaked in MeOH for 3 weeks. The MeOH extract was then drained out and concd under red. press. to 150 ml, dil. with H_2O (750 ml) and exhaustively extracted with Et_2O. The Et_2O layer was then extracted with 2 N aq. NaOH soln. The aq. alkaline soln was acidified with conc. HCl in the cold and the liberated solid extracted with Et_2O, washed with H_2O, dried and the solvent removed. The residue was chromatographed. The earlier fractions of petrol–EtOAc (10:1) eluate gave **1a** (0.2 g), crystallized from the same solvent mixture, mp 185° (found: C, 67.89; H, 5.43; $C_{17}H_{16}O_5$ requires: C, 68.00; H, 5.33%). $\lambda_{max}^{EtOH-0.1\ N\ NaOH}$ 214, 274, 310 nm (log ε 4.30, 5.40 and 4.16); IR ν_{max} cm^{-1}: 3300 (OH), 1610, 1580, 850, 825, 795 and 780 (aromatic nucleus); MS *m/z* (rel. int.): 300 $[M]^{+\cdot}$ (100), 285 (69), 271 (11), 253 (49), 241 (9), 227 (29), 213 (7), 199 (19), 170 (7), 143 (8), 69 (8) and 63 (8). The later fractions of the same eluate in the above chromatography afforded **1c**, also crystallized from the same solvent mixture, mp 192° (found: C, 65.38; H, 5.40; $C_{18}H_{18}O_6$ requires: C, 65.45; H, 5.45%). $\lambda_{max}^{EtOH-0.1\ N\ NaOH}$ 214, 247, 275, 286 and 305 nm (log ε 4.10, 4.07, 4.30, 4.31 and 3.98); IR ν_{max} cm^{-1}: 3300–3400 (OH), 1615, 1570, 880, 860, 825, 815, 750, 690 and 650 (aromatic nucleus); MS *m/z* (rel. int.): 330 $[M]^{+\cdot}$ (100), 315 (30), 300 (10), 287 (8), 272 (16), 257 (12), 229 (8), 214 (5), 157 (5), 127 (4), 102 (6), 63 (6) and 43 (4).

Confusarin (**1a**) was acetylated with Ac_2O–pyridine in the usual manner to give **1b**, crystallized from petrol–EtOAc, mp 155° (found: C, 65.54; H, 5.15; $C_{21}H_{20}O_7$ requires: C, 65.63; H, 5.21%). λ_{max} 216, 259, 294, 306 and 340 nm (log ε 4.46, 4.89, 4.13, 4.18 and 2.60); IR ν_{max} cm^{-1}: 1240 and 1770 (OAc); ^1H NMR: δ 9.37 (1H, *d*, *J* = 9.2 Hz, H-5), 8.05 and 7.65 (each 1H, *d*, *J* = 9.1 Hz, H-9 and H-10), 7.40 (1H, *s*, H-1), 7.36 (1H, *d*, *J* = 9.2 Hz, H-6), 4.01, 3.99 and 3.98 (each 3H, *s*, ArOMe), 2.43 and 2.41 (each 3H, *s*, OCOMe); MS *m/z* (rel. int.): 384 $[M]^{+\cdot}$ (32), 342 (29), 300 (100), 285 (32), 253 (14), 241 (7), 227 (6), 213 (5) and 43 (34).

Confusaridin (**1c**) was acetylated in the same manner as above to give **1d**, crystallized from petrol–EtOAc, mp 122° (found: C, 63.69; H, 5.29; $C_{22}H_{22}O_8$ requires: C, 63.77; H, 5.31%). λ_{max} 219, 262, 295 and 308 nm (log ε 4.38, 4.78, 4.05 and 4.07). IR ν_{max} cm^{-1}: 1225 and 1770 (OAc); ^1H NMR: δ 9.1 (1H, *s*, H-5), 8.04 and 7.62 (each 1H, *d*, *J* = 10 Hz, H-9 and H-10), 7.40 (1H, *s*, H-1), 4.04, 4.02, 4.01 and 3.97 (each 3H, *s*, Ar-OMe), 2.43 and 2.41 (each 3H, *s*, –OCOMe); MS *m/z* (rel. int.): 414 $[M]^{+\cdot}$ (30), 372 (28) and 330 (100).

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