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CHRYSANTHONES B AND C, SECONDARY METABOLITES PRODUCED BY THE FUNGUS *ASCOCHYTA CHRYSANTHEMI**

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Key Word Index—*Ascochyta chrysanthemi*; Ascomycetes; heptaketides; structural elucidation; NMR analysis.

Abstract—The structure and absolute configuration of chrysanthones B and C, two new reduced deoxyfusarubins produced by the fungus *Ascochyta chrysanthemi*, have been elucidated on the basis of ^1H, ^{13}C NMR and CD spectra.

INTRODUCTION

During the screening of the genus *Ascochyta* for secondary metabolites, we have investigated *A. chrysanthemi*, the agent of the so called ray blight disease of chrysanthemum. In a preceding paper [1] we reported the isolation of chrysanthone A (**1**) from a toxigenic culture of *A. chrysanthemi*, strain L.I.1. This fungal metabolite, which is structurally related to members of the fusarubin-javanicin family [2], was found to be phytotoxic causing yellowing and necrosis of leaves, and to possess some bacteriostatic and antifungal activity.

Further investigations on the same strain resulted in the isolation of two new metabolites, which we have named chrysanthones B and C (**2** and **3**), belonging to the above class.

RESULTS AND DISCUSSION

The strain of *A. chrysanthemi* was grown on wort agar for two weeks. Ethyl acetate extracts of the mycelium were evaporated and subjected to column chromatography on silica gel to give **2** as pale yellow crystals, mp 182°, $[\alpha]_D$ −37° ($CHCl_3$; *c* 0.13). The molecular formula ($C_{15}H_{16}O_5$) was established by high-resolution mass spectroscopy. Two strong peaks at *m/z* 218 $[C_{12}H_{10}O_4]^+$ and 190 $[C_{11}H_{10}O_3]^+$ were indicative of sequential loss of methylvinyl ether and carbonyl from ring C. The IR (KBr) and the UV (EtOH) spectra revealed a carbonyl band at 1615 cm^{-1} and absorptions at λ_{max}^{EtOH} 218 and 350 nm (ε 18 000 and 6 000).

The ^{13}C NMR spectrum of **2** in chloroform (Table 1) showed one ketonic carbonyl carbon atom resonating at δ199.94 (C-9), two methine (C-4 and C-5) and six quaternary (C-3, C-4a, C-5a, C-9a, C-10 and C-10a) sp^2-hybridized carbon atoms resonating between δ_C 160.27 and 100.92 and six signals due to two methyl (C-11 and C-12), one of which is oxygen-bearing, two methylene (C-1 and C-8), one of which is oxygen-bearing and two oxygen-bearing methine (C-6 and C-7) sp^3-hybridized carbon atoms. The ^1H NMR spectrum of **2** in chloroform (Table 2) confirmed and extended these findings through the appearance of one chelated phenolic hydroxy proton at δ12.49 (OH-10), one aromatic proton at δ6.66 (H-5) benzylically coupled to H-6 (^4J = 1.0 Hz) and one isolated $OC(1)H_2$ group. In addition, it indicated the presence in **2** of a partial structure such as **4** in which the hydroxyl and $OC(12)H_3$ groups are placed at C-6 and C-7, because the hydroxy proton exhibited in DMSO-d_6 a vicinal coupling of 6.4 Hz with H-6 and C-12 presenting a three-bond coupling of 4 Hz with H-7, and of a partial structure such as **6** in which the H_3-11 protons at δ1.94 are allylically coupled to H-4 at δ5.57. The NOE observed between H_3-11 and H-4 (3%) indicates that these protons are *cis*-disposed.

Elucidation of partial structure **5**, as well as its connection to **4** and **6** to give the gross structure of chrysanthone B (**2**), were readily obtained by extensive use of low-power selective ^{13}C–{^1H} decoupling experiments, as corroborated by the above evidence, and chemical-shift criteria. Thus, the $C(1)H_2O$ group must be placed at C-10a as the carbocyclic carbon atoms, C-10 and C-10a, resonating at δ157.33 and 112.24 showed long-range couplings of 4–5 Hz with both the 10-hydroxy and 1-methylene protons typical of two-and three-bond interactions [3, 4] while the C-9 carbonyl group must be located at C-9a because it is responsible for the hydrogen bond with OH-10. In addition to the above, the presence of long-range couplings between C-9 and H-7 and H_2 8 [J(CH) = 5–6.5 Hz] and between the carbon at δ160.27 (attributable to C-3 because it exhibited long-range couplings of 5.5 and 7 Hz with H-4 and H_3-11) and H_2-1 [^3J(CH) = 4 Hz] enabled us to connect C-8 to C-9 and O-2 to C-3. Finally, the fact that C-10a and the carbon at δ_C113.51 (attributable to C-9a because it was long-range coupled, like C-10 and C-10a to OH-10) presented three-bond couplings of 6 and 7 Hz with H-5 indicating that the two remaining substituents of the aromatic ring B must be placed at C-4a and C-5a and, hence, that C-4 must be linked to C-4a and C-6 to C-5a.

The NOEs observed for **2** in chloroform–DMSO-d_6 (*ca* 2:1) between the 8-methylene proton at δ2.78, as-

*Part 30 in the series 'Secondary Mould Metabolites'. For part 29 see ref. [8].

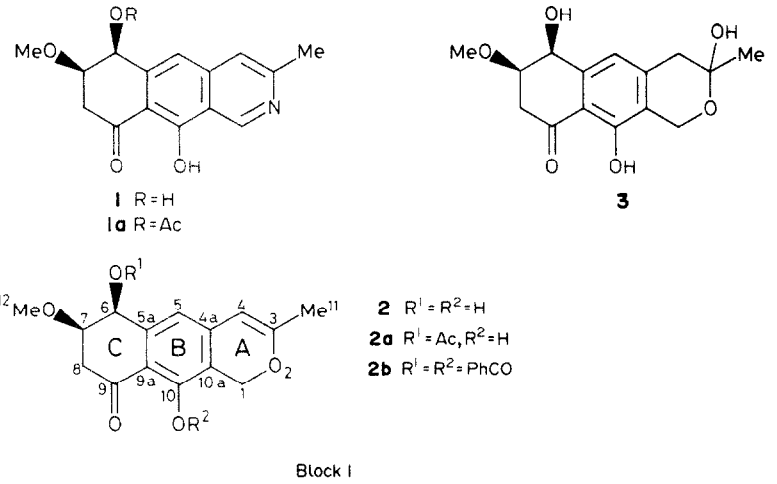


Table 1. ^{13}C NMR data for compound **2** (in chloroform-*d*)

C	δ(ppm)	^1J(CH)(Hz)	$^{>1}J$(CH)(Hz)
1	63.16 *T s*	150	—
3	160.27 *S dtq*	—	5.5 (H-4), 4 (H_2-1), 7 (H_3-11)
4	100.92 *D dq*	165.5	5 (H-5), 4 (H_3-11)
4a	141.06 *S m*	—	*ca* 4 (H_2-1)*
5	113.30 *D dd*	163	3.5 (H-4), 3.5 (H-6)
5a	142.86 *S dd*	—	4 and 3.5 (H-6 and H-7)
6	68.81 *D dddd*	146	4 (H-5), 6.5, 4, and 1.5 (H-7 and H_2-8)
7	78.22 *D m*	146	—
8	38.87 *DD m*	131.5 and 127.5	—
9	199.94 *S dt*	—	5 and 6.5 (H-7 and H_2-8)
9a	113.51 *S m*	—	7 (H-5)*, 4 (OH-10)*
10	157.33 *S m*	—	5 (OH-10)*, *ca.* 4 (H_2-1)*
10a	112.24 *S dddt*	—	6 (H-4), 6 (H-5), 5 (OH-10), 4 (H_2-1)
11	19.97 *Q d*	128	3 (H-4)
12	56.90 *Q d*	142	4 (H-7)

*Determined by selective low-power decoupling experiments.

Table 2. ^1H NMR chemical shifts (δ/ppm) and coupling constants (*J*/Hz) for compounds **2**, **2a**, **2b** and **3***

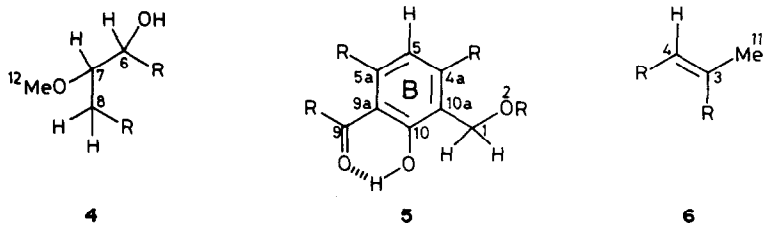
H	2	2a	2b	3	*J*	2	2a	2b	3
1a	5.22 (5.15, 5.10)‡	5.20	5.20	4.63	1a, 1b	13.4 (13.5, n.a.)‡	13.5	13.5	n.a.
1b	5.16 (5.13, 5.10)	5.16	5.03	4.63	4, 11	1.0 (1.0, 1.0)	0.9	1.0	
4	5.57 (5.64, 5.78)	5.57	5.68	†	5, 6α	1.0 (1.0, 1.0)	0.8	0.8	n.a.
5	6.66 (6.66, 6.67)	6.53	7.06	6.82	6α, 7α	2.9 (2.9, 2.7)	2.9	2.8	2.7
6α	4.82 (4.83, 4.84)	6.26	6.58	4.88	6α, 8α	<0.5 (<0.5, n.a.)	1.0	1.0	n.a.
7α	3.88 (3.86, 3.83)	3.87	4.05	3.84	6α, OH-6	n.a. (6.3, 6.4)			6.3
8α	2.76 (2.78, 2.88)	2.85	2.88	2.92	7α, 8α	3.6 (3.6, n.a.)	4.5	4.7	n.a.
8β	3.08 (3.00, 2.88)	3.07	3.09	2.92	7α, 8β	6.8 (6.9, n.a.)	10.0	9.8	n.a.
11	1.94 (1.93, 1.92)	1.95	1.94	1.43	8α, 8β	17.5 (17.3, n.a.)	17.5	17.3	n.a.
12	3.44 (3.40, 3.30)	3.44	3.46	3.32					
OR-6	2.85 (5.42, 5.60)	2.11	7.3–8.3	5.58					
OR-10	12.49 (12.57, 12.62)	12.53	7.3–8.3	12.50					

*The spectra of **2**, **2a** and **2b** were recorded in $CDCl_3$, that of **3** in DMSO-d_6.

†H_2-4 Resonate at δ_H 2.82 and 2.73 ppm (^2J = 17.2 Hz) and OH-3 at δ_H 5.96 ppm.

‡Values in parentheses are chemical shifts and coupling constants both obtained in chloroform-*d*–DMSO-d_6 (*ca* 2:1) and DMSO-d_6, respectively.

n.a., Not assigned.



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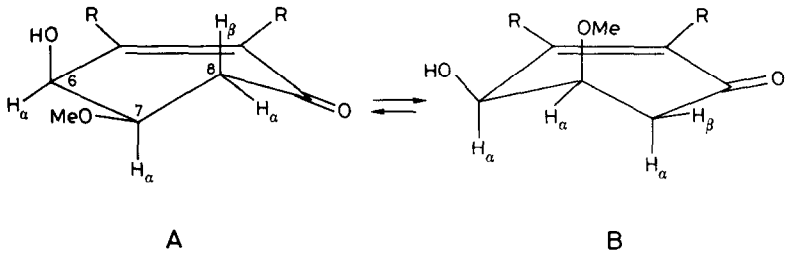

A B

Fig. 1. Preferred conformations of the ring C of compound **2**.

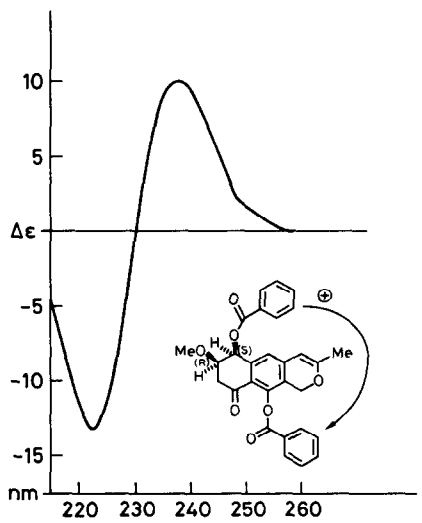

Fig. 2. The CD spectrum of compound **2b**.

sumed as α, and H-6 (2%) and the geminal H-8β at δ3.00 and OH-6 (1%) indicate that ring C exists in rapid equilibrium between two conformers similar to those shown in the Fig. 1. Moreover, the magnitude of the coupling constants of the protons associated with ring C ($^3J_{6\alpha,7}=2.9$, $^3J_{7,8\alpha}=3.6$ and $^3J_{7,8\beta}=6.9$ Hz) suggests that H-7 is in the α-position. In fact, the former two couplings account for the presence in both the conformers of gauche-like relationships between the 6α,7α- and 7α,8α-protons while the latter coupling is consistent with the 7α,8β-protons ranging between diaxial- and diequatorial-like dispositions in a *ca* 1:1 ratio.

The absolute configuration of C-6 was deduced as (*S*) from the CD spectrum carried out on the dibenzoate **2b**. According to the literature data [5] for shinanolone, an analogous tetralone, the CD curve of **2b** exhibited the positive Cotton effect at *ca* 238 nm, this fact indicating the right-handedness of the orientation of the two benzoate groups. Chrysanthone B (**2**) must, therefore, have the 6*S*,7*R* absolute configuration as shown in Fig. 2.

In the case of the monoacetate **2a**, the coupling constants are in agreement with its ring C preferentially adopting the A conformation, probably to relieve non-bonded interactions between H-5 and the OAc group. The presence of a long-range coupling of 1 Hz between 6α- and 8α-protons (which possess a *W*-conformation) and the magnitude of the vicinal couplings observed between 6α,7α-, 7α,8α- and 7α,8β-protons ($^3J=2.9$, 4.5 and 10.0 Hz) were in support of this interpretation.

The same strain of *A. chrysanthemi* when grown on potato-dextrose broth gave **3** and the known **1**. Compound **3** crystallized as yellow crystals, mp 164° and analysed for $C_{15}H_{18}O_6$ ($[M]^+$, *m/z* 294). A comparison of the ^1H and ^{13}C NMR data of **2** and **3** (Tables 1 and 2, and Experimental), coupled to the quantitative transformation of **3** into **2** by heating **3** with acetic anhydride, indicates that the two compounds differ only in the nature of ring A, that is the C(4)H=C(3)Me fragment must be replaced in **3** by C(4)H_2–C(3)MeOH. In fact, the ^1H NMR spectrum of **3** in DMSO-d_6 showed the presence of an isolated two-proton signal at δ4.63 (H_2-4) and of a hydroxy proton at δ5.96 (OH-3) instead of the 4-olefinic proton while the 11-methyl protons exhibited an upfield shift of 0.49 ppm. Accordingly, the olefinic carbon resonances at δ160.27 and 100.92 (C-3 and C-4) have been replaced in **3** by two signals at δ93.31 and *ca* 40 characteristic of a quaternary hemiketal carbon (C-3) and of an aliphatic methylene carbon (C-4).

Chrysanthones B and C are structurally similar to the 5-deoxyfusarubin and anhydro-5-deoxyfusarubin [6], this fact suggesting also that the biosynthetic pathways leading to the two pairs of metabolites are probably related [7].

EXPERIMENTAL

Mps: uncorr. Flash CC was performed with Merck silica gel (0.04–0.06 mm) and TLC with Merck HF_{254} silica gel. MS were recorded at 60 eV. ^1H NMR spectra were recorded at 300 MHz, ^{13}C NMR spectra at 63 MHz. Chemical shifts are in ppm (δ) from TMS as int. standard. NOE difference spectra were obtained by subtracting alternatively right-off resonance-free induction decays (FIDS) from right-on resonance-induced FIDS. Selectively ^{13}C–$\{^1H\}$ decoupled spectra were obtained using low decoupling power of 35 dB below 0.2 W.

Cultivation of fungus and isolation of chrysanthones B and C (**2** and **3**). *Asochyta chrysanthemi* was kindly supplied by Prof. D. L. Schadler (Cornell University), as strain L.I.1. Fifty Roux flasks in stationary culture of potato-dextrose-broth were inoculated with a mycelium suspension [1]. After two weeks the fungal felt was sepd from the culture filtrates and both extracted separately. Mycelium dried under *vacuum* at 40° was ground and extracted with hexane and successively with EtOAc in a Soxhlet apparatus. The EtOAc extracts were evapd and chromatographed on a silica gel column using CH_2Cl_2–MeOH (15:1) to give **1** and **3**. Working with the above conditions but with wort-agar as culture medium, after CC sepn using hexane–EtOAc (1:1), **2** was obtained.

Chrysanthone B (**2**). Yield 200 mg. MS *m/z* 276 $[M]^+$ (100), 218 (11), 190 (46), and 161 (14); (found: *m/z* 276.0982; $C_{15}H_{16}O_5$ requires 276.0997); (found, C, 65.9; H, 6.0%; $C_{15}H_{16}O_5$ requires C, 65.2; H, 5.8%); ^{13}C and ^1H NMR data are reported in Tables 1 and 2.

Chrysanthone C **3**. Yield 500 mg. $[\alpha]_D$ −29.4° (pyridine; *c* 0.1). MS *m/z* 294 $[M]^+$ (10%), 276 (100), and 190 (60). UV $\lambda_{max}^{95\%\ EtOH}$ nm: 267 and 338 (ε: 16 700 and 5 600). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH) and 1650 (ketone CO). ^{13}C NMR (DMSO-d_6): δ28.38 (*q*, C-11), 39.08 and 40.38 (*t*, C-4 and/or C-8), 56.61 (*q*, C-12), 57.14 (*t*, C-1), 67.87 (*d*, C-6), 78.88 (*d*, C-7), 93.31 (*s*, C-3), 112.48 and 120.49 (*s*, C-9a and/or C-10a), 118.51 (*d*, C-5), 142.36 and 142.48 (*s*, C-4a and/or C-5a), 156.47 (*s*, C-10), and 203.08 (*s*, C-9); ^1H NMR data are reported in Table 2.

Acetylation of **2**. Compound **2** (20 mg) was dissolved in Ac_2O (2 ml), KOAc (5 mg) added and the resulting mixt. heated at 65° for 3 hr. After work-up, the monoacetate of chrysanthone B **2a** (15 mg) was obtained as a solid, mp 210°; $[\alpha]_D$ +85.5° ($CHCl_3$; *c* 0.17). MS *m/z* 318 $[M]^+$ (100%), 276 $[M-42]^+$ (10), 259 (29), and 244 (41). IR ν_{max}^{KBr} cm^{-1}: 3460 (OH), 1735 (acetate CO) and 1620 (ketone CO); ^1H NMR data are reported in Table 2.

Benzoylation of **2**. Compound **2** (50 mg) was treated with pyridine (0.5 ml) and benzoyl chloride (0.05 ml). After 1 hr at 0°, H_2O (5 ml) was added and the soln extracted with EtOAc. Evapn of solvent and prep. TLC in hexane–EtOAc (2:1) gave the dibenzoate **2b** as a solid (hexane), mp 98°. MS *m/z* 484 $[M]^+$, 379 $[M-105]^+$, 351 and 257. UV $\lambda_{max}^{95\%\ MeOH}$ nm: 238, 270 and 340, (ε 33 700, 11 900 and 7 000). CD (MeOH, 8×10^{-2} gl) 222 and 127 nm ($\Delta\varepsilon$ −13.20 and +10.27). ^1H NMR: Table 2.

Reactions of **3** *with* Ac_2O. (a) Compound **3** (20 mg) was dissolved in Ac_2O (2 ml) and heated at 80° for 1 hr. Work-up and prep. TLC [silica gel; EtOAc–hexane (1:1)] of the crude reaction mixt. gave a compound (15 mg) identical with an authentic sample of **2**. (b) By heating **3** (20 mg) at 80° with Ac_2O for 4 hr, a compound (12 mg) identical with an authentic sample of **2a** was obtained.

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