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ASCOCHALASIN, A NEW CYTOCHALASIN FROM *ASCOCHYTA HETEROMORPHA*

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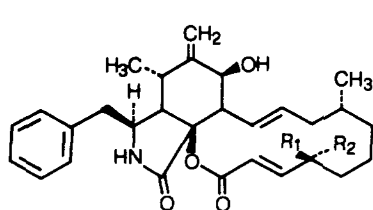
ABSTRACT.—The structure of a new cytochalasin, ascochalasin, isolated together with deoxaphomin from *Ascochyta heteromorpha*, pathogen of oleander (*Nerium oleander*), has been determined mainly by ^{1}H-nmr and ^{13}C-nmr spectroscopy.

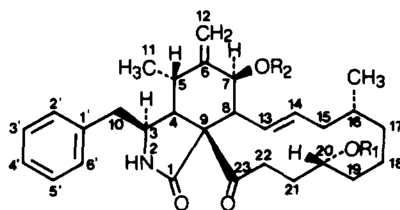
A previous analysis of the organic culture filtrate extracts of *Ascochyta heteromorpha* (Sch. et Sacc.) Curzi, a pathogenic fungus isolated from oleander (*Nerium oleander* L.), led to the isolation of the known cytochalasins A [**1**] and B [**2**] (1). A further investigation of the extracts has revealed the presence of another two cytochalasins: deoxaphomin [**3**], isolated from *Phoma* sp. (strain S 298) (2) and identified as a biosynthetic precursor of cytochalasin B (3), and a new cytochalasin, which we have named ascochalasin [**5**].

In the present note we report the isolation and the structure elucidation of ascochalasin.

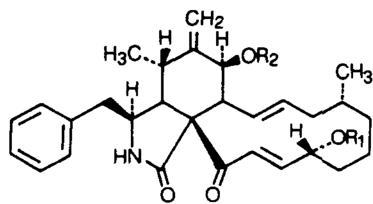
The chromatographic purification of culture filtrate organic extracts was performed using two SiO_2 columns in two successive steps. Cytochalasins A and B were obtained together with a mixture of products with chromatographic behavior very close to that of **2**. The mixture was purified by preparative tlc first on SiO_2 and then on reversed-phase plates, affording pure deoxaphomin and ascochalasin. Deoxaphomin [**3**] was identified by comparing our spectral data, ir, uv, nmr, and ms, with those reported by Binder and Tamm (2). The acetyl derivative **4** of **3** gave a ^{1}H-nmr spectrum in which the appear-



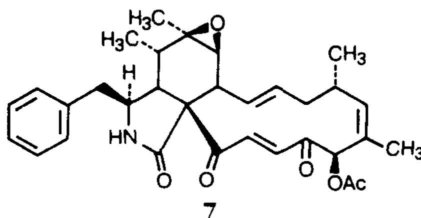
1 $R_1+R_2=O$ **2** $R_1=H, R_2=OH$



5 $R_1=R_2=H$ **6** $R_1=R_2=Ac$



3 $R_1=R_2=H$ **4** $R_1=R_2=Ac$



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ance of two singlets at δ 2.04 and 1.94 due to two acetyl groups was noteworthy. In addition, the cims (isobutane as reagent gas) of **4** showed a pseudomolecular ion at *m/z* $[MH]^+$ 548 (5%); other significant peaks were present at *m/z* $[MH-CH_2CO]^+$ 506, $[MH-HOAc]^+$ 488, $[MH-HOAc-CH_2CO]^+$ 446, $[MH-2\times HOAc]^+$ 428, and $[MH-C_7H_7-HOAc-CH_3CO]^+$ 354. These findings were in full agreement with structure **4** as 7,20-diacetyldeoxaphomin.

Ascochalasin [**5**] was an amorphous solid and had the molecular formula $C_{29}H_{39}NO_4$ from hrms (*m/z* $[M]^+$ 465.2873, calcd *m/z* 465.2869). Other significant peaks were detected at *m/z* 463.2715 ($C_{29}H_{37}NO_4$), likely formed by loss of H_2 in the source from the molecular ion (465.2873); 372.2191 ($C_{22}H_{30}NO_4$) and 354.2075 ($C_{22}H_{28}NO_3$) originated from the ion at *m/z* 463.2715 by loss of a benzyl group and H_2O, respectively; the ion at *m/z* 270.1496 ($C_{17}H_{20}NO_2$) arose from the fragment at *m/z* 354.2075 by losses of H_2O and a cyclopentadienyl moiety. This fragmentation pattern is coincident with that of deoxaphomin [**3**] except for the loss of two hydrogens.

The structure of **5** was assigned mainly on the basis of spectral evidence arising from ^{1}H nmr, including a series of decoupling experiments, and ^{13}C-nmr data. Tables 1 and 2 list the proton and carbon shifts, respectively. The ^{1}H-nmr spectrum of **5** resulted in very close approximation to that of deoxaphomin, recorded under the same experimen-

TABLE 1. ^{1}H-nmr Data of Ascochalasin [**5**] and Deoxaphomin [**3**].[a,b]

Proton	Compounds	
	5	**3**
H-3	3.30 ddd	3.30 ddd
H-4	3.14 dd	3.13 dd
H-5	2.88 dq	2.84 dq
H-7	3.94 d	3.97 d
H-8	2.48 dd	2.47 dd
H-10	2.74 dd	2.69 dd
H-10′	2.50 dd	2.47 dd
3H-11	1.06 d	1.03 d
H-12	5.32 br s	5.29 br s
H-12′	5.12 br s	5.13 br s
H-13	6.20 ddd	6.18 ddd
H-14	5.42 ddd	5.37 ddd
H-15	2.15 ddd	2.08 ddd
H-15′		
H-16		
2H-17	0.85–2.00	0.85–2.00
2H-18		
2H-19		
H-20	3.30 m	4.26 m
H-21	0.85–2.00	6.70 dd
H-22	0.85–2.00	7.12 dd
Me-C(16)	0.94 d	0.90 d
2′,6′		
3′,5′	7.20	7.20
4′		

[a]*J* (Hz) **3**, **5**: $J_{3,4}=3.3, J_{3,10}=5.5, J_{3,10'}=3.3, J_{4,5}=5.9, J_{5,11}=6.6, J_{7,8}=10.3, J_{8,13}=9.6, J_{10,10'}=13.6, J_{13,14}=15.4, J_{13,15}=1.8, J_{14,15}=9.6, J_{14,15'}=3.3, J_{15,15'}=12.3, J_{16,Me}=6.6$, **3**: $J_{20,21}=8.8, J_{20,22}=1.8, J_{21,22}=15.4$.

[b]Chemical shifts are in δ values (ppm) from TMS.

TABLE 2. ^{13}C-nmr Spectral Data of Ascochalasin [5].[a,b]

Carbon	Compound 5	Carbon	Compound 5
C-1	164.1 s	C-17	34.7 t
C-3	53.2 d	C-18	25.2 t
C-4	45.8 d	C-19	41.5 t
C-5	31.9 d	C-20	70.3 d
C-6	143.9 s	C-21	39.2 t
C-7	70.6 d	C-22	29.6 t
C-8	50.7 d	C-23	191.6 s
C-9	65.7 s	Me-16	22.2 q
C-10	44.3 t	C-1′	129.3 s
C-11	13.6 q	C-2′	129.1 d
C-12	114.1 t	C-3′	128.9 d
C-13	127.4 d	C-4′	127.0 d
C-14	137.4 d	C-5′	128.9 d
C-15	29.6 t	C-6′	129.1 d
C-16	33.8 d		

[a]Assignment made also by the comparison with the spectral data of cytochalasin B [2] (4) and cytochalasin K [7] (5).

[b]Chemical shifts are in δ values (ppm) from TMS.

tal conditions, although several differences were observed due to the lack of a double bond in **5** in comparison with **3**. In fact, an accurate examination of the ^{1}H-nmr spectrum of **5** revealed the absence of two double doublets at δ 7.12 and 6.70, corresponding to the H-22 and H-21 of **3**. Therefore, **5** has only two double bonds located between C-13 and C-14 ($J_{13,14}$ = 15.4 Hz, *trans*-oriented) and C-6 and C-12 (exocyclic double bond). The abovementioned structural difference is supported also from the upfield shift (Δ δ 0.96) in **5** at δ 3.30 of H-20 which appeared as a more complex system. In addition, the region of the aliphatic protons (δ 0.85–2.00) showed an increase of multiplets, which should be attributed to the C-21 and C-22 methylene groups.

The ^{13}C-nmr data listed in Table 2 further support the structure assigned to ascochalasin. In particular, as compared with the data published for cytochalasin B [**2**] (4), the spectrum of **5** showed the absence of the carbon signals present in **2** at δ 154.2 and 119.2, attributed to C-21 and C-22, respectively, while two signals appeared in **5** at δ 39.2 and 29.6, due to C-21 and C-22, respectively, in addition to the other signals of aliphatic methylene groups. These latter signals were recorded at δ 41.5, 34.7, and 25.2 corresponding to C-19, C-17, and C-18, respectively. The C-15, resonating at the same frequency of C-22, gave rise to a single intense signal at δ 29.6. Moreover, the examination of the chemical shifts of carbonyl groups corroborated the structure of **5**. In fact, the amidic carbonyl group (O=C-1) afforded a signal at δ 164.1, while the other carbonyl group (O=C-23) resonated at δ 191.6, which is a typical chemical shift value for ketonic groups and consistent with the shift reported for the same carbon (δ 196.9) in cytochalasin K [**7**] (5). The ketonic nature of C-23 in **5** determined, in comparison with **2**, the upfield shift of C-9 (Δ δ 18.7) which appeared at δ 65.7. A similar chemical shift value is observed for the C-9 (δ 63.0) of **7** (5).

The saturated nature of the ketone in **5** was supported by its ir data analyzed in comparison to those of **3**. In fact the band corresponding to the double bond conjugated with the ketone which appeared at 1622 cm^{-1} in **3** was absent in **5**. Moreover, a broad intense band at 1715 cm^{-1} due to both ketonic and lactam carbonyl groups appeared in the ir spectrum of **5**, while these groups showed in **3** as two absorptions in the 1710–1670 cm^{-1} region.

On standing in pyridine and Ac_2O, **5** formed a diacetyl derivative **6**. Its cims

showed a peak at m/z 548 formed from the pseudomolecular ion $[MH]^+$ by loss of two hydrogen atoms. Significant peaks, formed from the ion at m/z 548, were observed at m/z $[MH - H_2 - HOAc]^+$ 488, $[MH - H_2 - HOAc - CH_2CO]^+$ 446, and $[MH - H_2 - 2 \times HOAc]^+$ 428.

The ^{1}H-nmr spectrum of **6** showed, in comparison to that of **5**, the downfield shift of H-7 and H-20 ($\Delta\ \delta$ 0.89 and 0.39, respectively) which appeared at δ 4.83 and 3.69, respectively, in **6**. In addition, the two singlets corresponding to the two acetyl groups appeared at δ 2.06 and 1.94.

In conclusion, the structure of ascochalasin [**5**] can be formulated as (7*S*,13*E*,16*R*,20*R*)-7,20-dihydroxy-16-methyl-10-phenyl-[13] cytochalasan-6(12),13-diene-1,23-dione.

The very interesting biological properties of cytochalasins (6,7) suggest the use of ascochalasin in a number of bioassays (phytotoxic, mycotoxic, and antibacterial activity) in comparison with other cytochalasins and some of their derivatives.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on Perkin-Elmer 399 instrument in CH_2Cl_2 solutions; uv spectra were measured on a Varian-Cary 210 spectrophotometer in MeOH solutions; ^{1}H-nmr and ^{13}C-nmr spectra were recorded in $CDCl_3$ at 270 and 67.88 MHz, respectively, on a Bruker spectrometer; ei and ci (isobutane as reagent gas) mass spectra were recorded at 70 and 250 eV, respectively, on a Kratos Ltd. 80 mass spectrometer. Analytical and preparative tlc were performed on SiO_2 (20 × 20 cm, Merck Kieselgel 60 F_{254}, 0.25 mm) and on reversed-phase plates (20 × 20 cm, Whatman Stratocrom C-18 0.2 mm) plates; the spots were visualized by exposure to uv light and/or by spraying the plates first with 10% H_2SO_4 in MeOH and then with 3% phosphomolybdic acid in MeOH, followed by heating for 5 min at 105°. Cc was carried out on SiO_2 (Merck, Kieselgel 60 0.063–0.2 mm).

FUNGUS SPECIES.—*A. heteromorpha* was isolated in 1985 from oleander (*Nerium oleander*) grown in a nursery near Bari, and deposited in the fungus collection of the Istituto Tossine e Micotossine da Parassiti Vegetali del CNR, Bari, Italy.

GROWTH OF FUNGUS.—Single spore cultures of *A. heteromorpha*, freshly reisolated from infected oleander plants and maintained on slants of potato-dextrose-agar, were used. The fungus was cultured in 1000-ml Roux flasks containing 200 ml of a semisynthetic liquid medium (8). The cultures were incubated at 25° for 21 days and then filtered.

EXTRACTION AND ISOLATION OF CYTOCHALASINS.—The culture filtrates (9.0 liters, pH 7) were lyophilized, resuspended in distilled H_2O (650 ml), and extracted with CH_2Cl_2 (5 × 600 ml). The organic extracts were combined, dried (Na_2SO_4), and evaporated under reduced pressure. The residue (600 mg) was chromatographed on a SiO_2 column eluting with $CHCl_3$-iPrOH (9:1). The first fraction yielded crude cytochalasin A [**1**] (57 mg) the successive eluate contained small amounts of cytochalasin A, cytochalasin B [**2**], and a mixture of products (324 mg) with a chromatographic behavior very similar to that of **2**. This mixture was further purified on a SiO_2 column eluting with $CHCl_3$-iPrOH (93:7). Crude cytochalasin B (70 mg) was obtained and a fraction consisting of small amounts of cytochalasin B and a mixture of two products (82 mg) with R_f lower than **2**. The latter was further purified on SiO_2 plates ($CHCl_3$-iPrOH, 93:7) affording crude cytochalasin B (38 mg) and a crude mixture of two other products (20 mg). The mixture was finally purified on reversed-phase plates (MeCN-H_2O, 6:4), giving two homogeneous products with R_f 0.50 (ascochalasin, 5 mg) and R_f 0.45 (deoxaphomin, 7 mg).

Ascochalasin [**5**].—Ascochalasin was obtained as an amorphous solid: ir ν max 3580–3500, 3400, 1715, 1605 cm^{-1}; uv λ max nm (log ϵ) <220; ^{1}H nmr and ^{13}C nmr see Tables 1 and 2, respectively; ms m/z (rel. int.) 465 (3), 463 (5), 372 (10), 354 (22), 270 (14), 211 (70), 91 (100).

Deoxaphomin [**3**].—Deoxaphomin was obtained as an amorphous solid: ir ν max 3590–3540, 3410, 1710–1670, 1622, 1600 cm^{-1}; uv λ max nm (log ϵ) 215 (4.03) and 237 (3.87); ^{1}H nmr see Table 1; ms m/z (rel. int.) 463 (8), 445 (15), 372 (30), 354 (20), 174 (18), 91 (100).

Diacetylascochalasin [**6**].—Ascochalasin (6 mg) was acetylated with Ac_2O/pyridine. The usual workup of the reaction afforded an oil, which was purified on SiO_2 plates (eluent $CHCl_3$-MeOH, 98:2) giving pure **6** (5.6 mg, 80%): uv λ max nm (log ϵ) <220; ^{1}H-nmr spectrum was very similar to that of **5** except for the downfield shifts of H-7 and H-20 ($\Delta\ \delta$ 0.89 and 0.39, respectively) which appeared at δ 4.83 and 3.69, respectively, in **6**, and the presence of two new signals (both singlets) at δ 2.06 and 1.94, corre-

sponding to the two acetyl groups on C-7 and C-20; ms *m/z* (rel. int.) $[MH-H_2]^+$ 548 (3), $[MH-H_2-HOAc]^+$ 488 (8), $[MH-H_2-HOAc-CH_2CO]^+$ 446 (11), $[MH-H_2-2\times HOAc]^+$ 428 (100).

Diacetyldeoxaphomin [**4**].—Deoxaphomin (4 mg) was acetylated using the same method to convert **5** to **6**. The pure product (4.6 mg, 82%) showed uv λ max (log ϵ) 220 (4.2), 240 (3.5); ^{1}H nmr was very similar to that of **3** except for the presence of the two new signals (both singlets at δ 2.04 and 1.94) corresponding to two acetyl groups on C-7 and C-20; ms *m/z* (rel. int.) $[MH]^+$ 548 (5), $[MH-CH_2CO]^+$ 506 (10), $[MH-HOAc]^+$ 488 (65), $[MH-HOAc-CH_2CO]^+$ 446 (55), $[MH-2\times HOAc]^+$ 428 (100), $[MH-C_7H_7-HOAc-CH_3CO]^+$ 354 (10), 336 (8), 174 (5), 91 (10).

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LITERATURE CITED

1. R. Capasso, A. Evidente, G. Randazzo, A. Ritieni, A. Bottalico, M. Vurro, and A. Lo Grieco, *J. Nat. Prod.*, **50**, 989 (1987).
2. M. Binder and C. Tamm, *Helv. Chim. Acta,* **56**, 966 (1973).
3. J.L. Robert and C. Tamm, *Helv. Chim. Acta,* **58**, 2501 (1975).
4. W. Graf, J.L. Robert, J.C. Vederas, C. Tamm, P.H. Solomon, I. Miura, and K. Nakanishi, *Helv. Chim. Acta,* **57**, 1801 (1974).
5. T. Fex, *Tetrahedron Lett.*, **22**, 2703 (1981).
6. D.D.S. Thomas, in: "Cytochalasins Biochemical and Cell Biological Aspects." Ed. by S.W. Tanenbaum. North Holland Publishing, Amsterdam, 1978, pp. 257–275.
7. S. Natori, in: "Mycotoxins in Human and Animal Health." Ed. by J.V. Rodricks, C.W. Hesseltine, and M.A. Mehlman. Pathotox, Park Forest South, Illinois, 1977, pp. 559–581.
8. A. Nachmias, I. Barash, Z. Solel, and G.A. Strobel, *Physiol. Plant Pathol.*, **10**, 147 (1977).

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