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MYCOSPORULONE, A METABOLITE FROM *CONIOTHYRIUM SPORULOSUM*

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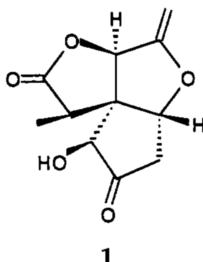
ABSTRACT.—Mycosporulone [**2**], a novel fungal metabolite, was isolated from the organic extract of *Coniothyrium sporulosum* culture fluids. Its purification was achieved by centrifugal tlc on Si gel and its structure was deduced from spectral evidence to be (2-hydroxy-6-methylcyclohex-4-en-3-one)-spiro-(4′-methylen-5′-oxobutanolide).

The antifungal properties exhibited by *Coniothyrium sporulosum* (W. Gams & Domsch) van der Aa (class Coelomycetes, order Sphaeropsidales) (1) prompted us to investigate its chemical constituents. A literature survey revealed that no chemical work is reported on the secondary metabolites of this species. Recently, we described the structure of a trycyclo[6.3.3.0.0]undecane derivative, coniothyriol [**1**], the major exometabolite produced by *Co. sporulosum* (2). However, this compound was inactive against *Candida albicans* and *Candida tropicalis*. Further analysis of the EtOAc-extractable portion of the cell-free culture medium led to the isolation of the bioactive secondary metabolite **2**, another C_{11} compound. Named mycosporulone, this natural product exhibited a less polar chromatographic mobility than **1** on Si gel tlc. The present paper reports on the isolation and the structure of the new compound from *Co. sporulosum.*

RESULTS AND DISCUSSION

Isolation of mycosporulone [**2**] was achieved by two chromatographic steps using silica cc and then centrifugal tlc on Si gel. This procedure was applied to the



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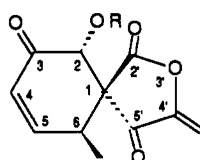
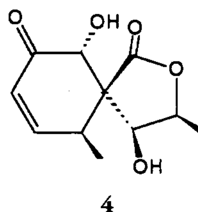


2 R=H
3 R=Ac



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EtOAc extract of culture broth (see Experimental). The eims of the natural product did not reveal the molecular ion but gave only two prominent fragment ions at *m/z* 82 (100%) and *m/z* 54 (85%). In the cims, the quasi-molecular ions were recorded at *m/z* 240 $[M+NH_4]^+$ and *m/z* 223 $[M+H]^+$ in agreement with the molecular formula $C_{11}H_{10}O_5$ requiring seven degrees of unsaturation. Peaks at *m/z* 82 and *m/z* 54 were also present in the last spectrum. The ^1H as well as the ^{13}C nmr (Tables 1 and 2) showed signals for H_{10} and C_{11}. The ^{13}C-nmr spectrum displayed eleven signals related to five quaternary carbons (δ 194.7, 193.4, 168.5, 149.9, and 60.9), four methine groups (δ 150.1, 125.3, 72.7, and 35.8), one methylene (δ 96.3), and one methyl group (δ 15.2). The multiplicity of these carbons was deduced from both the DEPT sequence and the 2D direct heteronuclear chemical shifts correlation spectroscopy (^1H-^{13}C-XHCORR). Mycosporulone exhibited a uv band (λ max 248 nm) typical of cyclohex-2-enone (3) and three intense ir absorptions at 1820, 1760, and 1700 cm^{-1} most probably corresponding to a 5-membered ring lactone, a 5-membered ring ketone, and a 6-membered ring ketone, respectively (3). The ^1H-nmr spectrum of **2** (Table 1) featured a singlet at δ 4.07 assigned to one hydroxyl group (responsible for the ir band at 3500 cm^{-1}) and replaced by an acetyl group (δ 2.07) in the monoacetate **3**. Both the ^1H-nmr spectrum and the corresponding 2D homonuclear chemical shifts correlation spectrum (^1H-^1H-COSY), suggested a Me-CH(6)-CH(5)=CH(4)-chain including two *cis*-ethylenic protons ($J_{4,5}$=10.2 Hz). The chemical shift value for H-5 (δ 6.56 in **2** and δ 6.54 in **3**) corresponded well to that of an olefinic methine conjugated to an electron-withdrawing function (CO group), as typically observed in cinnamic acids, coumarins, chalcones, and isoflavones (3–8). This hypothesis was fully confirmed by the ^{13}C-nmr lowfield chemical shift relative to C-5 (δ 150.1 in **2** and δ 147.9 in **3**) (9–11) and further supported by the presence of prominent ms peaks at *m/z* 82 $[C_5H_6O]^+$ and *m/z* 54 $[C_4H_6]^+$ (Scheme 1). Finally, the remaining three protons of this molecule belong to two isolated groups: a methine appearing as a singlet in the ^1H nmr at δ 4.53 and an exocyclic methylene bearing two non-equivalent protons (J_{gem}=3.1 Hz) recorded as sharp doublets at δ 5.14 and 5.47. As a consequence of the lowfield ^1H-nmr shift for the above-mentioned methine upon acetylation (Δδ +1.22), the OH group was assigned at C-2. Furthermore, the CH-OH must be linked via the CO group to lead to Me-CH(6)-CH(5)=CH(4)-CO-CH(2)-OH according to both the shielding of the α-keto group (Δδ −6.6) after acetylation (9,10,12) and the presence of a cross peak between H-4 (δ 6.18) and C-2 (δ 72.9) in the long-range ^1H- ^{13}C shift correlation

TABLE 1. ^1H-nmr Spectra for Mycosporulone [**2**] and its Acetylated Derivative **3** (200 MHz, δ ppm).[a]

Proton	Compound	
	2	**3**
H-2	4.53, s	5.75, s
H-4	6.14, dd, J=10.2 and 3.1 Hz	6.18, dd, J=10.2 and 3.0 Hz
H-5	6.56, dd, J=10.2 and 2.1 Hz	6.54, dd, J=10.2 and 2.2 Hz
H-6	3.26, qdd, J=7.5, 3.1, and 2.1 Hz	3.34, qdd, J=7.4, 3.0, and 2.2 Hz
6-Me	1.06, d, J=7.5 Hz	1.17, d, J=7.4 Hz
CH_{2A}-4′	5.47, d, *J*=3.1 Hz	5.55, d, *J*= 3.1 Hz
CH_{2B}-4′	5.14, d, *J*=3.1 Hz	5.25, d, *J*=3.1 Hz
2-OH	4.07, br s	
2-OAc		2.07, s

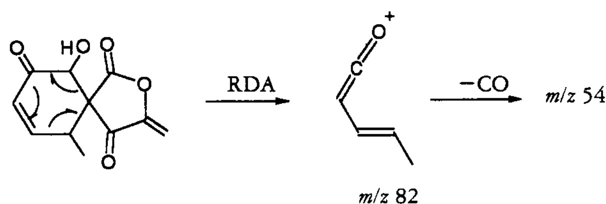
[a]In $CDCl_3$ (δ 7.27).

TABLE 2. ^{13}C-nmr Data for Mycosporulone [2] and the Acetate 3 (50 MHz, δ ppm).[a]

Carbon	Compound 2	Compound 3
C-1	60.9	58.3
C-2	72.7	72.9
C-3	194.7[b]	188.1
C-4	125.3	127.4
C-5	150.1	147.9
C-6	35.8	37.3
C-2′	168.5	167.7
C-4′	149.9	150.2
C-5′	193.4[b]	192.5
6-Me	15.2	15.6
CH_2-4′	96.3	97.2
2-OAc		168.4
		20.0

[a]In $CDCl_3$ (δ 77.0).
[b]Assignments may be interchanged.

2D nmr spectrum (COLOC) of monoacetate **3**. The presence of seven double bonds equivalent in this molecule, as well as that of three CO functions (δ 194.7, 193.4, and 168.5), one quaternary ethylenic C (δ 149.9) linked to one olefinic methylene (δ 96.3), and finally two ethylenic CH (δ 150.1 and 125.3) belonging to the cis unsaturation, supported two rings including the fully substituted C (δ 60.9) in a spiro structure. The first ring is formed by the above-mentioned system, and the aliphatic quaternary carbon gives rise to a 5,5-disubstituted 4-methyl-6-hydroxycyclohex-2-enone. This result was confirmed, in the acetylated derivative, by both the upfield shift of the related aliphatic C (Δδ −2.6), localizing it in the α position to CH(2)-OH, and the three cross peaks that this carbon exhibited with the methyl group (δ 1.17) and H-5 (δ 6.54), both protons being at the β position, as well as with H-2 (δ 5.75) in the long-range ^{1}H- ^{13}C shift correlation 2D nmr spectrum. This α,β-unsaturated 6-membered ring ketone was obviously the starting point for the fragmentation at *m/z* 82, via a retro-Diels-Alder reaction, which was subsequently fragmented into the ion at *m/z* 54 following the loss of a CO molecule as depicted in Scheme 1. The second ring of this molecule bears an ester function (δ 168.5), a keto group (δ 193.4), and the remaining C=CH_2 (δ 149.9 and 96.3). Deshielding of the quarternary ethylenic carbon indicated *O*-binding and consequently its placement between the ester and the ketone, which are both linked to the spiro carbon to lead to a 3,3-disubstituted 4-oxo-5-methylenbutanolide. This result was effectively supported in **3** by the ^{1}H- ^{13}C heteronuclear long-range couplings between the non-equivalent methylene protons (δ 5.55 and 5.25) and both the keto group (δ 192.5) and the quaternary ethylenic C (δ 150.2). Consequently, mycosporulone was identified as (2-hydroxy-6-methylcyclohex-4-en-3-one)-spiro(4′-methylen-5′-oxobutanolide), a new fungal metabolite. According to a Dreiding model and to nOe experiments performed on the acetylated derivative **3**, which showed interaction between the "syn-related" protons at δ 5.75 (H-2) and δ 3.34 (H-6), the relative configuration 2*R*,6*R* was assigned to this molecule. Furthermore, the result was corroborated by a NOESY experiment (homonuclear dipolar-correlated 2D nmr), which showed correlations between the olefinic methylene group (δ 5.55 and 5.25) and protons at δ 1.17 (6-Me) and δ 2.07 (2-OAc).



SCHEME 1

Mycosporulone is closely related to rosigenin [**4**], previously isolated from *Mycosphaerella rosigena* (class Ascomycetes, order Dothideales) (13). The two compounds only differ by the oxidative level of the butanolide ring. It may be that during biosynthesis, involving a pentaketide precursor, mycosporulone derives from rosigenin.

This is the second report of a C_{11} component from *Co. sporulosum*. It may be that both compounds coniothyriol [**1**] and mycosporulone [**2**] are linked by the same biosynthetic precursor, probably derived from acetate units, which were further involved in diverse secondary transformations leading to these products by different routes. This hypothesis is supported by the similar elements encountered in the two structures, such as the aliphatic quaternary carbon, the five-ring lactone, and the $-CH{=}CH_2$, Me-CH-, and HO-CH-CO- Partial structures.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Tlc was carried out on pre-coated Si gel 60F-254 plastic sheets (Merck). Separation by centrifugal tlc on Si gel used a Chromatotron apparatus (Harrison Research). Nmr spectra were recorded with an AC200 Bruker spectrometer. The solvent signal was used as reference.

FUNGAL SOURCE AND CULTURE CONDITIONS.—Metabolites were extracted from the culture broth of a strain of micromycetes obtained from our laboratory collection (CMPG: Collection Mycologie Pharmacie Grenoble). The strain was isolated from Swedish crab shells and identified as *Co. sporulosum*. It was recorded as CMPG 741 and maintained on solid malt extract medium (1.5%) at 4°. Subcultures were grown on a solid malt extract medium for a week at 24° to provide sufficient inoculum. Culture plates were scraped and inoculated in 3 liters of liquid malt extract medium (1.5%, pH 5.5) in a 10-liter fermentor. The strain was grown for 4 days at 24° with aeration.

EXTRACTION AND ISOLATION OF MYCOSPORULONE [**2**].—The mycelium of the fungal strain was removed by filtration at the end of the growth period, and the metabolites present in the filtrate were extracted with EtOAc (3×3.5 liters). After concentration, the pooled extracts gave a viscous brown mass (8 g) which was further fractionated on a silica column (Bio-Sil A 200–400 mesh). Successive elution with an *n*-hexane/toluene gradient and then with toluene yielded twelve fractions. Mycosporulone was the main uv-absorbing constituent in the middle fractions, which were pooled. After concentration under reduced pressure, a part (686 mg) was subjected to centrifugal tlc on Si gel (layer thickness 2 mm) eluted $CHCl_3$-Me_2CO (98.5:1.5.) This procedure yielded 225 mg of pure mycosporulone.

Mycosporulone [**2**].—A white amorphous powder: mp 97–98°, R_f 0.57 on tlc Si gel system with toluene-EtOAc (6:4); $[\alpha]_D$ +27° (c=0.1, $CHCl_3$); uv λ max (MeOH) 248 nm; ir ν max (KBr) 3500, 1820, 1760, 1700, 1660, 1270, 1140, 1105, 1065, cm^{-1}; eims m/z (rel. int. %) 82 (100), 54 (85); cims m/z (rel. int.) $[M+NH_4]^+$ 240 (64), $[M+H]^+$ 223 (22), 195 (5), 178 (15), 150 (12), 135 (14), 123 (15), 82 (100), 54 (15); ^1H nmr see Table 1; ^{13}C nmr see Table 2.

Mycosporulone monoacetate [**3**].—Mycosporulone (50 mg) was dissolved in fresh distilled pyridine (0.3 ml) and kept in Ac_2O (5 ml) overnight. After the usual work-up, this procedure yielded 53 mg of acetate **3**: R_f 0.33 on tlc Si gel system with toluene-MeOH (97:3); ^1H nmr see Table 1; ^{13}C nmr see Table 2.

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