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Mycoepoxydiene represents a novel class of fungal metabolites

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Abstract: A novel epoxycyclooctadiene-δ-lactone, mycoepoxydiene, was isolated from a fungal culture (OS-F66617). Its structure and relative stererochemistry were established using spectroscopic studies, including single-crystal X-ray diffraction analysis.

Our laboratory has a continuing effort to identify new metabolic products from rare fungi [1-3]. As a recent result of those efforts, we have uncovered a novel epoxycyclooctadiene from the solid-state fermentation of a fungus designated as OS-F66617. This communication describes the isolation and structure elucidation of that new compound, mycoepoxydiene. The structure was studied extensively by nmr, and fully elucidated by X-ray diffraction analysis.

The producing fungus, a sterile dematiaceous culture, was isolated from twig litter collected from a deciduous alluvial forest near Curitiba, in the state of Parana, Brazil. The organism was grown on agar-based medium [4] for 11 days, then lyophilized and thoroughly extracted with MeOH. The extract was concentrated *in vacuo* to a slurry and subjected to solvent partitioning between aqueous methanol and hexane, followed by $CHCl_3$. The latter fraction was concentrated *in vacuo* and subsequently fractionated by silica-gel flash column chromatography. Compound **1** was obtained as colorless needle-shaped crystals from methanol ($[\alpha]_D$ + 210, *c* 0.106, CH_3OH, λmax 256 nm, CH_3OH). The molecular formula of $C_{16}H_{18}O_5$ was assigned from the HRFABMS, which gave a protonated molecular ion at *m/z* 291.1230 (-0.7 ppm from calculated value).

Based on chemical-shift analysis and HMQC data (Table 1), the 16 resonances of the ^{13}C-NMR spectrum were assigned to two methyls, six methines, six mono-protonated sp^2-hybridized carbons, and two carbonyls. The ^1H-NMR displayed a total of 18 signals, including two methyl groups, six double-bond protons, four carbinols, and two methines. The acetyl

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group was recognized by the connectivity of a methyl-proton singlet (δ 2.01, H-16) to a carbonyl resonance (δc 170.0, C-15). An α,β–unsaturated δ-lactone substructure was deduced based on the following ^{1}H-^{13}C(HMBC) and ^{1}H-^{1}H(COSY) connectivities. An olefinic-proton doublet (δ 6.23, H-2) correlated to both a carbonyl (δc 162.2, C-1) and a down-field proton (δ 7.02, H-3). The doublet-of-doublets at δ 7.02 (H-3) also coupled to a carbinol proton (δ 5.05, H-4), which in turn coupled to another carbinol proton (δ 4.47, H-5). In addition, H-3 had connectivities to C-2 and C-4. The attachment of the acetyl group to C-4 of the lactone ring was indicated by long-range connectivity of H-4 to the down-field carbonyl (δc 170.0).

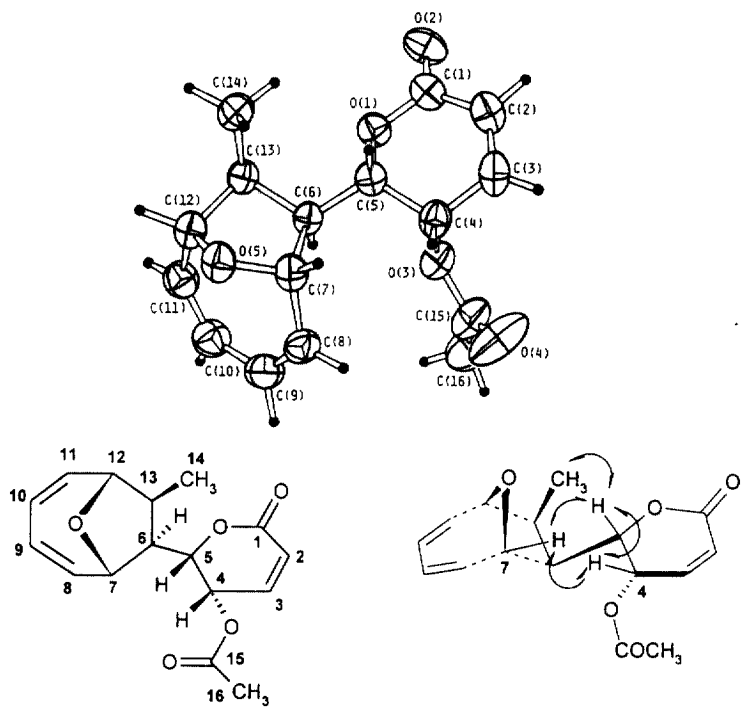
Further analysis of the proton spectra, including the COSY data, uncovered some symmetry in the structure. In particular, eight protons could be grouped in pairs, according to like chemical shifts and multiplicity. Two methine protons, H-6 (δ 3.03) and H-13 (δ 3.01), correlated to the carbinol protons H-7 (δ 4.27) and H-12 (δ 4.31), respectively. The latter also coupled to two olefinic protons (δ 6.02, H-8 and δ 6.08, H-11), and in turn, H-8 and H-11 correlated to the double-bond protons H-9 (δ 5.88) and H-10 (δ 5.90), respectively. The above observations, and the ^{1}H-^{13}C connectivities (Table 1), revealed this system as an eight-membered ring with two conjugated double bonds. The respective attachments of the methyl group (δ 1.12) and the lactone to the C-13 and C-6 positions of the cyclooctadiene, was based on two key ^{1}H-^{1}H connectivities; a methine proton (δ 3.01, H-13) correlated to a methyl-proton doublet (δ 1.12, H-14), and H-6 (δ 3.03) correlated to H-5 (δ 4.47) of the lactone ring. These connections were supported by the results of the HMBC experiment. For example, in addition to the correlation between the methine proton H-13 (δ 3.01) and the C-14 methyl carbon, H-6 correlated to both C-5 (δc 77.8) and C-4 (δc 63.3) of the lactone ring.

The nmr data indicated six oxygen-bonded carbons, including two carbonyl groups. However, the molecular formula required five oxygens. This could be reconciled by the presence of an ether bridge between two carbons. An ether bridge between C-7 and C-12 was corroborated by the long-range ^{1}H-^{1}C correlations of H-7 to C-12, and H-12 to C-7 (Table 1);

The relative stereochemical structure of **1** was assigned based on $^{1}H/^{1}H$-coupling constants, NOE (NOESY), and X-ray diffraction studies. First of all, H-4 and H-5 were determined to occupy the same face of the lactone ring, because there was a clear H-4/H-5 NOE, and the $J_{H\text{-}5/H\text{-}4}$ was <2.5 Hz. Furthermore, that H-4 was the pseudoequatorial proton was revealed by the relatively large $J_{H\text{-}4/H\text{-}3}$ of 6 Hz, which was determined upon irradiation of the H-2 resonance; therefore, the dihedral angle of H-4/H-3 should be approximately 40°. The $J_{H\text{-}5/H\text{-}6}$ ~11 Hz indicated that H-5 and H-6 had a relative orientation of 180°. The NOSEY spectrum also demonstrated connectivities of H-4 with H-7, and H-5 with both H-7 and the methyl protons. The structure and relative stereochemistry, as depicted, were firmly established by X-ray-diffraction analysis [5].

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of Mycoepoxydiene in $CDCl_3$

Assignment	δC (multiplicity)	δH (multiplicity, $J_{H/H}$ Hz)	HMBC H to δC
1	162.2 (s)		
2	125.1 (d)	6.23 (d, 10)	162.2, 63.3
3	140.2 (d)	7.02 (dd, 10, 6.0)	77.8, 63.4
4	63.3 (d)	5.05 (dd, 6.0, 2.4)	170, 125.1, 77.8
5	77.8 (d)	4.47 (dd, 10, 2.4)	63.3, 52.8, 50.2
6	50.2 (d)	3.03 (m)	137.0, 77.8, 76.0, 63.3, 52.8, 14.5
7	76.0 (d)	4.27 (dd, 6, 5.6)	137.0, 126.3, 86.6, 77.8, 52, 50.2
8	137.0 (d)	6.02 (bdd, 11, 6)	126.3, 76.0, 50.2
9	126.3 (d)	5.88 (m)	137.8, 76.0, 50.2
10	124.5 (d)	5.90 (m)	137.0, 86.6
11	137.8 (d)	6.08 (bdd, 11, 6)	86.6
12	86.6 (d)	4.31 (d, 4.4)	137.8,124.5,76.0, 52.8, 50.2, 14.5
13	52.8 (d)	3.01 (m)	137.8, 76.0, 50.2, 14.5
14	14.5 (q)	1.12 (d, 7.0)	86.6, 52.8, 50.2
15	170.0 (s)		
16	21.0 (q)	2.01 (s)	170.2



Compound 1, the observed NOE's used to define relative stereochemistry, and the ORTEP diagram (40% probability ellipsoids) showing crystallographic atom numbering and solid-state conformation.

Conclusion

To the best of our knowledge, the only natural products containing an oxygen-bridged cyclooctadiene are the dibenzocyclooctadiene lignans related to kadsulignan [6,7]. However, there is no precedent for this skeleton existing without the fused biphenyl system. We can speculate that mycoepoxydiene may be of polyketide origin; with C-1 of the attached δ-lactone as a starting point, **1** could be produced from seven acetate units. We conclude that compound **1** is novel and represents a new class of fungal metabolites.

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References and Notes

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[4] *Microbiology and Fermentation:* The fungus OS-F66617 produced moderate growth on 1.8% malt-extract agar. The organism was maintained on an agar slants, and expanded in seed culture according to our standard methods for fungal cultures[1,2]. Production was accomplished on Nunc plates (20 x 20 cm) holding 500 ml of a medium containing 1.8% agar, 0.5% yeast nitrogen base (Sigma), and 1% dextrin (type 1, Sigma) in deionized water. The culture was spread over the entire surface using a glass rod, and then incubated at 22° C for 11 days at which time the culture was well grown over the entire agar surface.

[5] *Crystallographic data* : $C_{16}H_{18}O_5$ (**1**), M = 290.32, monoclinic, space group $P2_1$(no. 4), a = 12.197(1) Å, b = 5.668(1) Å, c = 11.014(1) Å, β = 98.53(1)°, V = 753.0(3) Å^3, Z = 2, $D_{calcd.}$ = 1.280 g cm^{-3}, μ(Cu-$K\alpha$ radiation, λ = 1.5418 Å) = 7.5 cm^{-1}. Intensity data ($+h,+k, \pm l$; 1707 non-equivalent reflections, $\theta_{max.}$ = 75°), recorded at 298 K on an Enraf-Nonius CAD-4 diffractometer [Cu-$K\alpha$ radiation, graphite monochromator; ω-2θ scans, scanwidth (0.90 + 0.14tanθ)°] from a crystal of dimensions 0.12 x 0.14 x 0.54 mm, were corrected for the usual Lorentz and polarization effects. The crystal structure was solved by direct methods. Full-matrix least-squares refinement of atomic positional and thermal parameters (anisotropic C, O; isotropic H) converged at R = 0.047 (R_w = 0.061) over 1305 reflections with $I > 3.0\sigma(I)$. Crystallographic calculations were performed by use of the Enraf-Nonius Structure Determination Package (SDP 3.0). Tables of atomic positional and thermal parameters, bond lengths and angles have been deposited at the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.

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