Pergamon

Tetrahedron Letters 42 (2001) 809–811

TETRAHEDRON LETTERS

Novel lactones from *Aspergillus versicolor*

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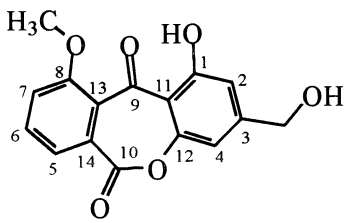
Received 24 July 2000; revised 13 November 2000; accepted 14 November 2000

Abstract—Two novel aromatic lactones were isolated from *Aspergillus versicolor* fermentation broth. The structures were determined mainly by 1D and 2D NMR spectroscopy.

Some species of fungus from the genus Monascus, Aspergillus and Penicillium are known to produce lovastatin (also known as mevinolin or monacolin K), a metabolite that has the property of lowering blood cholesterol level in humans and is a widely prescribed drug known as Mevacor.[1–7] During the course of our screening program for other fungal species that produce this compound, we investigated the fermented material obtained from *Aspergillus versicolor*. A previous work showed that this fungus produces the toxins sterigmatocystin and ochratoxin.[10,11] In this note we report the isolation and structure elucidation of the new lactones (**1**) and (**2**). They are of particular interest because their aromatic lactone ring system has been proposed by Ahmed et al.[12] as the framework of a possible biosynthetic intermediate in the synthesis of flavones by *Aspergillus variecolor*, but to the best of our knowledge has not been isolated until now.†

The fungus was statically cultured in a 2 kg rice medium with 45% humidity, at 28°C for 11 days. To this medium was added ethyl acetate (2 L) and the solid was removed by filtration. The filtrate was concentrated under reduced pressure to afford 13.5 g of a brown residue. The HPLC analysis of this residue, according to the methodology described in the literature,[8,9] revealed the absence of mevinolin. As there is little information on the chemical constituents produced by this fungus, we carried out a chemical study of the residue. This was separated by silica gel column chromatography, eluting with a mixture of hexane and diethyl ether of increasing polarity to produce five fractions. Fraction 1 (0.87 g) was characterized by IR as a mixture of triacylglycerols. Fraction 2 (5.83 g) was similarly characterized as a mixture of saturated and unsaturated fatty acids. Addition of diethyl ether to fractions 3 (2.39 g) and 5 (2.01 g), resulted in precipitation of lactones (**1**) (0.19 g) and (**2**) (0.28 g), respectively.

The molecular formula of compound (**1**) was established as $C_{16}H_{12}O_6$ by HR–MS (m/z 300.0634, calc. 300.0634) and by ^{13}C NMR. This compound was a yellow solid and had a mp of 207.6–207.9°C. The IR spectrum showed characteristic absorption bands from OH (broad, 3422 cm^{-1}), lactone (1736 cm^{-1}), α,β-un-



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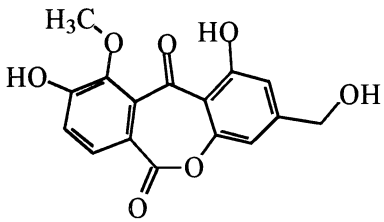

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Keywords: *Aspergillus versicolor*; chemical constituents; aromatic lactones.
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† In the present work no biosynthetic experiments were carried out to test this hypothesis.

0040-4039/01/$ - see front matter
PII: S0040-4039(00)02154-7

Table 1. ^{1}H and ^{13}C NMR data[a] of **1** and **2** in DMSO-d_6, chemical shift (δ), J/Hz

Number	^{1}H NMR		^{13}C NMR	
	1	**2**	**1**	**2**
1	12.06 (brs, OH)	12.25 (brs, OH)	160.5	160.8
2	6.80 (d, 1.1)	6.78 (d, 1.1)	107.7	107.5
3			154.5	154.4
4	7.00 (d, 1.1)	7.00 (d, 1.1)	104.2	104.2
5	7.77 (dd, 8.3, 1.1)	7.62 (d, 8.0)	119.7	120.5
6	7.93 (dd, 8.3, 7.3)	7.48 (d, 8.0)	136.0	125.7
7	7.45 (dd, 7.3, 1.1)	10.5 (brs, OH)	122.9	149.2
8			168.6	167.2
9			180.1	180.1
10			155.5	151.1
11			107.0	106.9
12			155.5	155.8
13			116.6	117.4
14			133.6	117.4
OH	5.40 (t, 5.4)	5.55 (brs)		
CH_2OH	4.60 (d, 5.4)	4.60 (brs)	62.3	62.7
OCH_3	3.90 (s)	3.89 (s)	52.7	52.6

[a] Measured at 75 MHz for carbon-13 and 300 MHz for hydrogen.

saturated ketone (1654 cm^{-1}) and aromatic ring C=C (1618 and 1600 cm^{-1}).

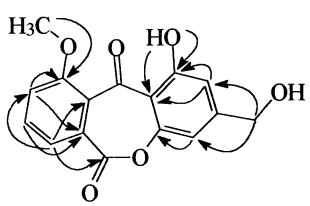
The ^{13}C NMR spectrum of (**1**) gave rise to 16 carbon signals: 2 carbonyls (155.5 and 180.1), seven non-hydrogenated carbons, five aromatic methines, one aliphatic methylene and one methoxy group (Table 1) identified via DEPT.

The ^{1}H NMR spectrum (Table 1) showed a singlet at 12.06, from a hydroxyl group H-bonded to a carbonyl, and two *meta*-coupled aromatic hydrogens, H-2 (6.80) and H-4 (7.00). The presence of the CH_2OH group was confirmed by a doublet at 4.60 (J 5.4 Hz) and a triplet at 5.40 (J 5.4 Hz). The presence of a 1,2,3-trisubstituted aromatic ring was evident from the double doublet at 7.93 (J 8.3 and 7.3 Hz) from H-6, and two double doublets at 7.45 (J 7.3, 1.1 Hz) and 7.77 (J 8.3, 1.1 Hz) from H-7 and H-6, respectively. A NOE difference experiment with irradiation at 3.90 (OCH_3) resulted in a small enhancement at H-7, and also at the phenolic OH, indicating a non-planar central ring. Strong NOEs were also observed at H-2 and H-4 following irradiation at 4.60 (CH_2). Further confirmation of the positioning of the substituents on the rings was obtained from HMBC data shown in Fig. 1. The HMBC experiment was optimized for couplings of 7.7 Hz, so that the anticipated ^{4}J W-couplings of ca. 2 Hz should give rise to peaks having about one half to one third the height of ^{2}J or ^{3}J peaks involving the same hydrogens. The actual HMBC spectrum confirms this. It shows that all the ^{4}J peaks have either one or two less contours than the others, where each contour represents a doubling of the (power mode) peak intensity.

Compound (**2**) was also a yellow solid, mp 250.5–251.6°C. A molecular formula $C_{16}H_{12}O_7$ was deduced from HR–MS (m/z 316.0584, calc. 316.0583) and ^{13}C NMR. The IR spectrum showed absorption bands at 3286 and 3163 cm^{-1} (broad, OH), 1703 cm^{-1} (α,β-unsaturated lactone), 1654 cm^{-1} (α,α′,β,β′-doubly unsaturated ketone), 1611 and 1600 cm^{-1} (C=C).

The ^{1}H and ^{13}C NMR spectra were similar to those of (**1**), except for the signals from the left hand ring. Two ^{1}H doublets (AB type, J 8.0 Hz) were seen at 7.48 and 7.62, and also a broad signal at 10.5, from the extra hydroxyl, with the corresponding loss of a CH resonance. From this information, the hydroxyl could be placed at C-5 or C-7. However, the HMBC correlations (Fig. 1) were only consistent with the proposed structure, having the OH at C-7. All assignments were confirmed by COSY and HMQC. Also, no interaction with any ring hydrogen was observed by NOE-difference spectroscopy with pre-irradiation at the methoxy group, nor the reverse. This also ruled out hydroxylation at C-5.

The insecticidal properties of compounds (**1**) and (**2**) were evaluated using the methodology described by Paula et al.[13] The study was carried out with the following insect species: *Hypothenemus hampei* (Ferr.) (Coleoptera: Scolitidae), Coleoptera: Staphylinidae, *Diaphania hyalinata* (L.), *Diaphania nitidalis* (Cr.) (Lepidoptera: Pyralidae) at the dose of 6.76, 7.55, 2.12 and 2.12 μg of substance/mg of insect, respectively. Compound (**1**) had no activity on the insects tested and compound (**2**) was toxic only to Coleoptera: Staphylinidae (72.5±12% mortality against the control).



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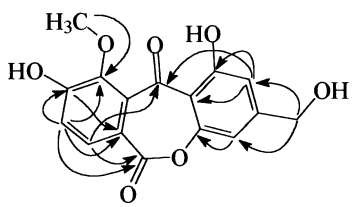

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Figure 1. Long-range ^{13}C-^{1}H correlations (from HMBC) of **1** and **2**.

Acknowledgements

We thank the Brazilian Agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support and a research fellowship (LCAB) and a MSc studentship (MCR); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for financial support. LCAB thanks the Royal Society of Chemistry for a J.W.T. Jones Fellowship. We are also grateful to Professor Marcelo C. Picanço and Mr. Márcio Dionísio for carrying out the biological assays.

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