



Indole alkaloids from a culture of the fungus *Aporpium caryae*

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Abstract

In a screening for antifungal metabolites, two indole compounds of mixed biogenesis, 1H-indole-3-carboxylic acid, 1-(1,1-dimethyl-2-propenyl) methyl ester and 1H-indole-3-carboxylic acid, 1-(2,3-dihydroxy-1,1-dimethylpropyl) methyl ester were isolated from a culture of the basidiomycete *Aporpium caryae*. The structural elucidation of these compounds was accomplished by spectroscopic methods. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Aporpium caryae*; Basidiomycete; Indole alkaloids

1. Introduction

Basidiomycetes are increasingly being investigated for their production of biologically active secondary metabolites, since they are known to produce compounds with a variety of biological activities (Arnone et al., 1998; Büchel et al., 1998; Becker et al., 1997). During our screening for antifungal and antibacterial fungal metabolites, we investigated the wood-inhabiting fungus *Aporpium caryae* (Schw.) Teix. et Rog. No *Aporpium* species have previously been chemically investigated. In this paper, the isolation and structural elucidation of the indole metabolites **1** and **2**, which possess antifungal activity against the phytopathogenic fungus *Cladosporium cucumerinum*, are reported.

2. Results and discussion

A. caryae was cultivated in a malt extract broth for

30 days and filtered. After partitioning with EtOAc, the filtrate supplied a crude organic extract. Fractionation of the extract by silica gel chromatography, followed by prep. TLC afforded compounds **1** and **2**.

HR-MS of compound **1** showed an $[M]^+$ at m/z 243.1257, indicating a molecular formula of $C_{15}H_{17}NO_2$. The 1H -NMR spectrum of **1** revealed the characteristic pattern of an indole nucleus (δ 8.19 *dd*, 6.6 and 1.8 Hz, H-4; δ 8.03 *s*, H-2; δ 7.54 *dd*, 6.9 and 1.8 Hz and δ 7.20 *m*, H-5 and 6) and the presence of a vinylic double bond (δ 6.13 *dd*, 17.5 and 10.6 Hz; δ 5.27 *d*, 10.6 Hz and δ 5.18 *d*, 17.5 Hz), two methyl groups (δ 1.79 *s*) and a methoxy group (δ 3.91 *s*). The absence of a coupling constant for H-2 and the absence of the proton attached to the indole ring nitrogen suggested the existence of substituents at C-3 and the ring nitrogen. EIMS showed two important fragments at m/z 175 ($M - C_5H_8$)⁺ and m/z 144 ($M - C_5H_8 - OCH_3$)⁺. The EIMS and 1H -NMR spectral data suggested the presence of an isoprenyl moiety in the molecule; ^{13}C -NMR spectral data confirmed these assumptions, and showed a carbonyl ester carbon at δ 165.5. This evidence, together with the chemical shift for C-3, indicated that the isoprenyl substituent should be attached to the *N*-position and the CO_2CH_3 to the

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C-3 position. The structure of compound **1** was therefore determined as 1H-indole-3-carboxylic acid, 1-(1,1-dimethyl-2-propenyl) methyl ester. The spectroscopic data of **1** were in good agreement with those of the related compound **3**, which was obtained as a derivative in an oxidative cleavage of asterriquinone, a metabolic product of *Aspergillus terreus* (Yamamoto et al., 1976).

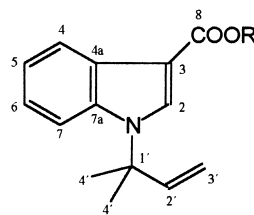
The molecular formula $C_{15}H_{19}NO_4$ was established for **2** on the basis of the HR-EIMS spectrum; the NMR characteristics were very similar to those of **1** (Table 1), except for the absence of the double bond. In the NMR spectra of **2**, signals attributable to a hydroxymethyl group and a hydroxymethine group replaced those corresponding to the double bond of **1**, leading to the structure of **2** as 1H-indole-3-carboxylic acid, 1-(2,3-dihydroxy-1,1-dimethylpropyl) methyl ester. 1H -NMR decoupling spectra and the HETCOR experiment allowed the complete assignment of the molecule, and confirmed the proposed structure. The absolute configuration of C-2' was assigned as *S* based on the very small but consistent differences between the chemical shifts of protons H-3' ($\Delta\delta + 0.25$ Hz) and H-4' ($\Delta\delta - 0.25$ Hz) of the (*S*)- and (*R*)- α -(trifluoromethyl)phenylacetic acid (MTPA) diesters (Ohtani et al., 1991) of **2** (**2b** and **2a**).

Compounds **1** and **2** each gave inhibition zones of 8 mm against *C. cucurmerinum* at a concentration of 50 μg /spot. Benomyl, which was used as a control test compound, showed an inhibition zone of 28 mm at 25 μg /spot.

Table 1
 1H - and ^{13}C -NMR spectral data ($CDCl_3$) for compounds **1** and **2**

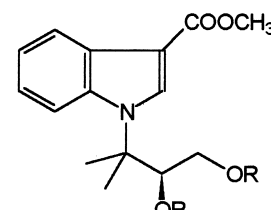
	1			2		
	δ 1H	<i>J</i> (Hz)	δ ^{13}C	δ 1H	<i>J</i> (Hz)	δ ^{13}C
2	8.03 <i>s</i>		132.1	8.00 <i>s</i>		133.2
3			106.4			106.6
4a			128.0			128.3
4	8.19 <i>dd</i>	6.6, 1.8	121.6 ^a	8.21 <i>dd</i>	6.9, 2.6	122.1
5	7.20 <i>m</i>		121.7 ^a	7.26 <i>m</i>		121.9
6			121.9 ^a	7.22 <i>m</i>		122.4
7	7.54 <i>dd</i>	6.9, 1.8	114.4	7.67 <i>dd</i>	6.9, 1.8	113.9
7a			135.9			135.5
8			165.7			165.5
OCH ₃	3.91 <i>s</i>		50.8	3.88 <i>s</i>		51.0
1'			60.1			62.3
2'	6.13 <i>dd</i>	17.5, 10.6	143.0	4.50 <i>dd</i>	6.6, 4.4	75.1
3'a	5.18 <i>d</i>	17.5	114.4	3.49 <i>m</i>		62.4
3'b	5.27 <i>d</i>	10.6				
4'	1.79 <i>s</i>		27.9	1.78 <i>s</i>		24.4
				1.77 <i>s</i>		24.0

^a Assignments may be interchanged.



1 R = CH₃

3 R = H



2 R = H

2a R = (*R*)-MTPA

2b R = (*S*)-MTPA

3. Experimental section

3.1. General

The UV spectrum was recorded on a Hewlett-Packard 8451 A diode-array spectrophotometer. NMR spectra were recorded on a Bruker AC-200 instrument operating at 200.1 MHz for 1H -NMR and on a Bruker AM-500 instrument operating at 125.13 MHz for ^{13}C -NMR. EIMS was obtained on a Trio-2 quadrupole mass spectrometer (VG Biotech). HR-EIMS was performed on a ZAB-SEQ (BEqQ) instrument (VG Analytical, Manchester, UK).

3.2. Fermentation

The strain of *A. caryae* (Schw.) Teix. et Rog. was supplied by Dr J.E. Wright from the BAFC Culture Collection (Cult. No. 1170). An agar slant of the fungus was used to inoculate 250 ml Erlenmeyer flask containing 75 ml of malt extract medium (malt extract, 30 g l⁻¹ and peptone, 5 g l⁻¹). The fermentation was carried out at 25°C for 30 days under static conditions.

3.3. Extraction and isolation

The fermentation broth (2 l) was filtered and the filtrate was partitioned with EtOAc. The crude extract (197 mg) was fractionated by vacuum chromatography using cyclohexane–EtOAc as solvent system. The fraction eluted with cyclohexane–EtOAc 4:6 was subjected to prep. TLC on silica gel employing EtOAc as a solvent, yielding compounds **1** (2 mg) and **2** (4 mg).

3.4. Antifungal assay

Direct bioautography on TLC, as described previously (Homans and Fuchs, 1970), was employed as the method for detecting fungitoxic substances.

3.5. Compound 1

Oil, UV (CH_2Cl_2) λ_{max} ($\log \epsilon$) 240 (3.05), 244 (3.05), 288 (3.03); HR-EIMS, m/z $[\text{M}]^{+}$: found 243.1257, calcd. for $\text{C}_{15}\text{H}_{17}\text{NO}_2$, 243.1259; EIMS 70 eV m/z (rel. int.): 243 $[\text{M}]^{+}$ (40), 175 $[\text{M} - \text{C}_5\text{H}_8]^{+}$ (75), 149 (34), 144 (79), 69 (25), 41 (100); ^1H - and ^{13}C -NMR: spectral data: Table 1.

3.6. Compound 2

Oil, $\alpha_D = -11.8^\circ$ (CH_2Cl_2 , c 0.21). UV (CH_2Cl_2) λ_{max} ($\log \epsilon$) 242 (3.05), 248 (3.05), 284 (3.03); HR-EIMS, m/z $[\text{M}]^{+}$: found 277.1316, calcd. for $\text{C}_{15}\text{H}_{19}\text{NO}_4$, 277.1314; EIMS 70 eV m/z (rel. int.): 277 $[\text{M}]^{+}$ (4), 246 (1), 216 (23), 144 (21), 84 (48), 49 (100); ^1H - and ^{13}C -NMR spectral data: Table 1.

3.7. Preparation of (*R*)- or (*S*)-MTPA diesters of 2

(*R*)- or (*S*)-MTPA chloride (10 μl) was added to a solution of **2** (1 mg) in pyridine (200 μl) and the mixture was stirred at room temperature for 1 h. The mixture was then poured onto HCl/ice, extracted with EtOAc (3 $\times$ 2 ml), and washed with 5% aq. NaHCO_3 and water. The solution was evaporated to dryness and the diester derivatives **2a** (*R*) and **2b** (*S*) were purified by prep. TLC (cyclohexane–EtOAc 7:3).

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