PERGAMON

Phytochemistry 62 (2003) 239–243

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

5*H*-Furan-2-ones from fungal cultures of *Aporpium caryae*

Laura M. Levy[a], Gabriela M. Cabrera[a,*], Jorge E. Wright[b], Alicia M. Seldes[a]

[a]*Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II, 1428 Buenos Aires, Argentina*
[b]*Departamento de Biología and PRHIDEB-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II, 1428 Buenos Aires, Argentina*

Received 2 July 2002; received in revised form 12 September 2002

Abstract

Four furanones **1**–**4** with an unusual skeleton containing an acetylene unit, named aporpinones, were isolated from the culture of the basidiomycete *Aporpium caryae* and their structures were elucidated by spectroscopic methods. Compounds **3** and **4** showed weak to moderate antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*.

Keywords: *Aporpium caryae*; Basidiomycete; 5*H*-Furan-2-ones

1. Introduction

In a previous study of the culture of the basidiomycete *Aporpium caryae,* two antifungal indole derivatives were reported (Levy et al., 2000). In an attempt to obtain more of these products, four additional metabolites, two of them with antibiotic activity, were detected. As part of our continued interest in the studies on bioactive components from fungi that could be useful in the development of novel drugs (Levy et al., 2000; Cabrera and Seldes, 1997), the isolation and structural elucidation of these additional compounds named aporpinones was undertaken. The structural assignments were based on spectral data.

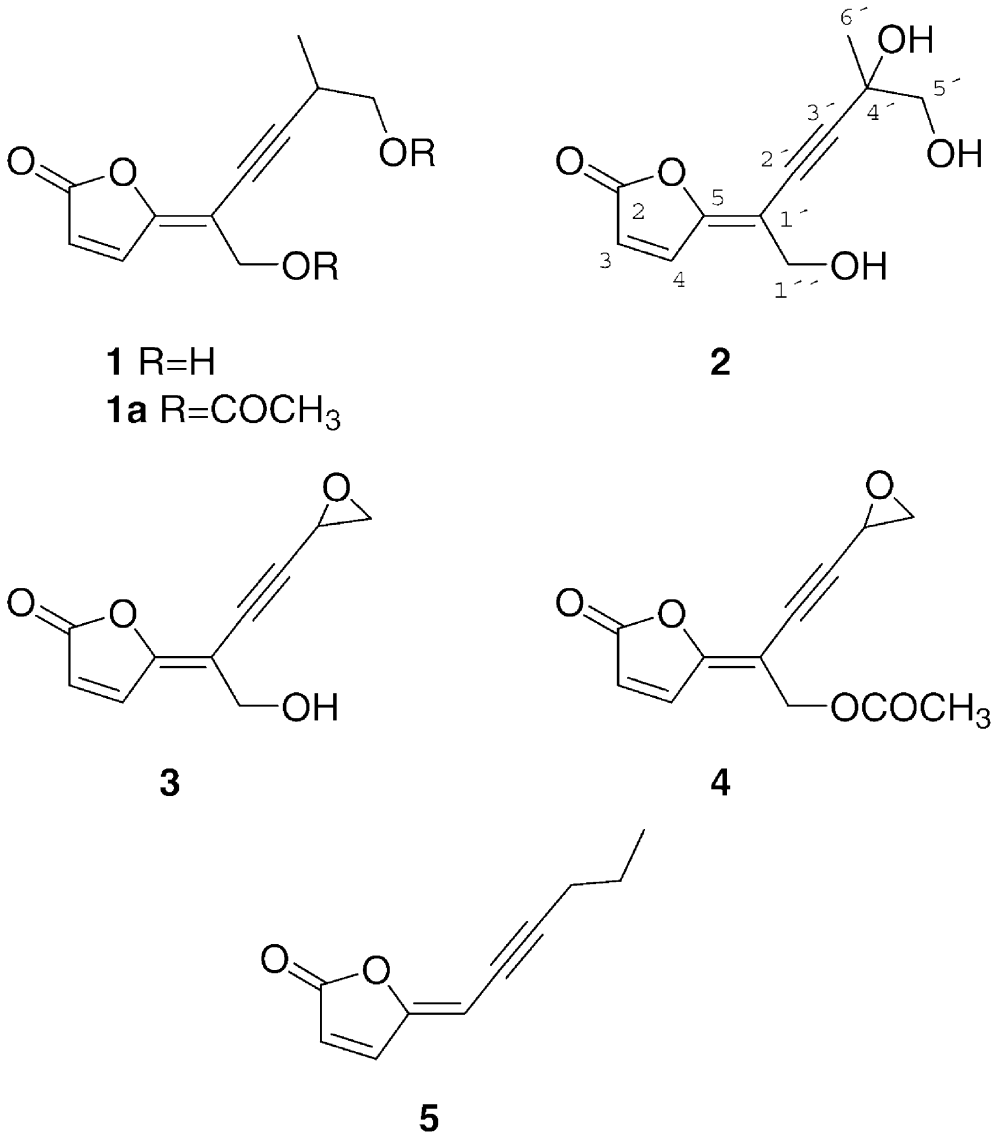
2. Results and discussion

The aporpinones, **1**–**4**, were obtained after successive chromatography of an EtOAc extract from a liquid fermentation of *A. caryae*.

The molecular formula for compound **1** was determined as $C_{11}H_{12}O_4$ by EI-HRMS, indicating six degrees of unsaturation. The ^{13}C NMR spectrum of **1** confirmed the carbon count and showed the presence of a carbonyl at δ 168.9 and two signals at δ 120.0 and 139.6 corresponding to protonated carbons. These observations combined with the presence of two aromatic protons at δ 7.78 and 6.23, both of them doublets with a characteristic coupling constant of 5.7 Hz in the ^1H NMR spectrum, suggested the existence of a 5*H*-furan-2-one with an exo-double bond, as in gomphilactone (Jägers et al., 1986) or protoanemonin (Suga et al., 1977). This exo double bond should be quaternary as no other olefinic protons were observed in the ^1H NMR spectrum. In the IR spectrum, two sharp bands at 1790 and 1754 cm^{-1} confirmed the presence of a butenolide ring (Shimojima et al., 1982), and an important diagnostic weak band at 2218 cm^{-1} indicated the presence of a triple bond. At this point, and assisted by COSY, a furanone with an exo-double bond, CH_3–CH–CH_2O– and CH_2O– fragments, and a triple bond were identified, thereby completing all the requirements for carbons and degrees of unsaturation. Both oxygenated methylene carbons at δ 60.6 and 66.4 were assigned as CH_2OH groups since upon acetylation two acetyl groups were incorporated. A COLOC experiment enabled the relationship of these fragments to be determined and hence to assign a possible gross structure. The methine group at 2.95 ppm correlated with the carbon at δ 106.5, and the methylene protons at δ 4.36 exhibited correlations with carbons at δ 77.2, 109.1 and 153.6. Fig. 1 shows the correlations

* Corresponding author. Tel./fax: +54-11-4576-3346.
E-mail address: gabyc@qo.fcen.uba.ar (G.M. Cabrera).

0031-9422/03/$ - see front matter
PII: S0031-9422(02)00455-7



observed by COLOC. A correlation between the protons at δ 7.78 and 4.36 in the NOESY experiment determined the stereochemistry of the exo-double bond. On this evidence, the structure for compound **1**, named aporpinone A was determined as 5-(5-hydroxy-1-hydroxymethyl-4-methyl-pent-2-ynylidene)-5*H*-furan-2-one. The spectroscopic data of the furane conjugated system in compound **1** were consistent with those reported for *trans*-lachnophyllum lactone **5** isolated from the plant *Conyza bonariensis* (Sanz and Marco, 1991).

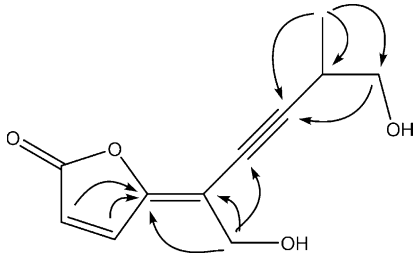


Fig. 1. Relevant COLOC correlations observed for compound **1**.

Compounds **2**–**4** showed in the ^{1}H NMR, ^{13}C NMR, and FTIR spectra the characteristic signals of a furanone with an exo-double bond and the alkyne functionality noted previously in aporpinone A (**1**). Compound **2**, determined as $C_{11}H_{12}O_5$ by HR EIMS, differed from **1** in the presence of an extra hydroxyl group, the absence of a methine, and the downfield shift of the methyl group from δ 1.26 (*d*) in **1** to δ 1.50 (*s*) in **2**, in the ^{1}H NMR spectrum. These data suggested that the hydroxyl group was attached to the vicinal carbon of the methyl group, and this was supported by the presence in the ^{13}C NMR spectrum of a quaternary carbon at δ 68.8 and the downfield shifted methyl carbon at δ 25.0. A COLOC experiment sustained this conclusion allowing the structure 4′-hydroxyaporpinone A to be deduced for compound **2**.

Compound **3** showed a molecular ion of 192.0413 by HR-EIMS, indicating a molecular formula $C_{10}H_8O_4$, bearing an extra degree of unsaturation compared with aporpinone A (**1**). The absence of the methyl group at δ 1.26, the deshielding of the methine group appearing at δ 3.61 and the shielding of the AB methylene protons were the main differences in the ^{1}H NMR spectra of **1** and **3**. These differences, and the presence of two carbons at δ 40.1 and 49.3, suggested the replacement of the hydroxylated isopropyl fragment in **1** by an epoxide in **3**. In this way, the structure of compound **3**, named aporpinone B, was determined to be 5-(1-hydroxymethyl-3-oxiranyl-prop-2-ynylidene)-5*H*-furan-2-one.

Aporpinone B (**3**) and compound **4** were very similar by NMR spectral analysis, the only difference being the presence of an acetyl group at C-1″ in compound **4**, as established by the downfield chemical shift of δ 4.80 observed for this signal in the ^{1}H NMR spectrum. The MS spectrum supported this assumption and allowed the structure of compound **4** to be deduced as 1″-acetylaporpinone B.

Although there are many reports about the isolation of furanones with an exo methylene moiety from microorganisms, there are only a few unsubstituted at positions 3 and 4. These examples are protoanemonin isolated from several *Ranunculaceae* (Suga et al., 1977), gomphilactone from *Gomphidius maculatus* (Jägers et al., 1986), and xerulin, dihydroxerulin, and xerulinic acid from *Xerula melanotricha* (Kuhnt et al., 1990). In all these cases, the biosynthesis of these compounds proceeded via the shikimate–chorismate or acetate–malonate pathways (Gill and Steglich, 1987). Taking into account that the previously isolated indole derivatives from this fungus could be produced by a mixed biogenesis (Levy et al., 2000), a similar mechanism can be proposed for aporpinones. In this case a precursor produced by a general acetate-malonate pathway may be prenylated in the 1′ position yielding aporpinones.

Aporpinone B and 1″-acetylaporpinone B showed weak to moderate antibacterial activity by the agar diffusion method against *Bacillus subtilis*, *Staphylococcus aureus,* and *Escherichia coli*.

3. Experimental

3.1. General

FTIR spectra were recorded on a Nicolet Magna-IR 550 instrument. The UV spectra were taken on a Hewlett-Packard 8451A diode-array spectrophotometer. Optical rotations were measured on a Perkin Elmer 343 polarimeter. NMR spectra were recorded on a Bruker AC-200 instrument at 200.1 MHz for ^{1}H and on a Bruker AM-500 instrument at 125.13 MHz for ^{13}C NMR. EIMS were obtained on a Trio-2 quadrupole mass spectrometer (VG Biotech). HREIMS of aporpinone A was performed on a ZAB-SEQ (BEqQ) instrument (VG Analytical, Manchester, UK). HREIMS of compounds **2**–**4** were recorded at the Washington University Resource for Biomedical and Bio-organic Mass Spectrometry.

3.2. Fermentation

The strain of *A. caryae* (Schw.) Teix. et Rog. was supplied by Dr. J.E. Wright from the BAFC Culture Collection (No. 1170). A well-grown agar slant of the fungus was used to inoculate 250 ml Erlenmeyer flasks containing 75 ml of malt extract medium containing 30 g malt extract and 5 g peptone per liter. After 10 days, each flask was employed to seed 4 × 4 l Erlemeyers flasks containing 1 l of the above medium. Fermentation was carried out at 25 °C for 30 days under static conditions.

3.3. Extraction and isolation

The fermentation broth (4 l) was filtered and the filtrate was partitioned with EtOAc. The crude organic extract (550 mg) was subjected to Sephadex LH-20 column chromatography with MeOH as eluant. Two fractions (160 and 40 mg) from the column were then applied to prep. TLC on silica gel yielding compounds **1** (40 mg) and **2** (5 mg) from one, and **3** (9 mg), and **4** (2 mg) from the other. The eluants used on the prep. TLC were EtOAc and EtOAc:cyclohexane 8:2, respectively.

3.4. Antibiotic assay

The antibiotic activity was determined by agar diffusion method using 100 μg of sample/disk against *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, and *E. coli* ATCC 25922. Inhibition zones were as follows: *B. subtilis*: comp. **3**, 15 mm (MIC 10 μg/disk), comp. **4**, 8 mm;

Table 1
^{13}C NMR spectral data for compounds **1**–**4** ($CDCl_3$, except for **2** $CDCl_3$–CD_3OD 20%)

	1	2	3	4
2	168.9	169.1	168.2	167.9
3	120.0	120.0	121.1	121.8
4	139.6	139.9	139.6	139.6
5	153.6	153.6	155.1	156.9
1′	109.1	108.9	108.5	101.9
2′	77.2	78.6	78.0	78.0
3′	106.5	104.6	98.8	98.3
4′	30.7	68.8	49.3	49.2
5′	66.4	70.0	40.1	40.1
6′	16.6	25.0		
1″	60.6	59.7	60.6	60.5
$COCH_3$				170.5
$COCH_3$				20.8

Table 2
^{1}H NMR spectral data for compounds **1**–**4** ($CDCl_3$, except for **2** $CDCl_3$–CD_3OD 20%). Coupling constants (*Hz*) are in parentheses

	1	**2**	**3**	**4**
3	6.23 *d* (5.7)	6.27 *d* (5.7)	6.30 *d* (5.6)	6.33 *d* (5.7)
4	7.78 *d* (5.7)	7.86 *d* (5.7)	7.81 *d* (5.6)	7.83 *d* (5.7)
4′	2.95 *m*		3.61 *dd* (3.9, 2.8)	3.60 *dd* (3.9, 2.8)
5′	3.61 dd_{AB} (10.5, 5.6)	3.56 d_{AB} (11.2)	3.04 dd_{AB} (5.9, 3.9)	3.04 dd_{AB} (5.9, 3.9)
	3.69 dd_{AB} (10.5, 5.2)	3.64 d_{AB} (11.2)	3.02 dd_{AB} (5.9, 2.8)	3.02 dd_{AB} (5.9, 2.8)
6′	1.26 *d* (7.1)	1.50 *s*		
1″	4.36 *s*	4.32 *s*	4.39 *s*	4.80 *s*
OH	2.50 *br s*			
$COCH_3$				2.01 *s*

S. aureus: comp. **4**, 7 mm; *E. coli*: comp. **3**, 8 mm. Gentamicin, which was used as a test compound at 20 μg/disk, showed inhibition zones of 23, 24 and 19 mm against *B. subtilis*, *S. aureus* and *E. coli* respectively.

3.5. Aporpinone A

Oil. $[\alpha]_D = -33^\circ$ (CH_2Cl_2, *c* 0.18). HR-EIMS (70 eV), *m/z* $[M]^{+\bullet}$ found 208.0735, calc. for $C_{11}H_{12}O_4$ 208.0734. EIMS (70 eV) *m/z* (rel. int.): 208 $[M]^{+\bullet}$ (17), 191 (16), 178 (49), 160 (100), 154 (31), 147 (25), 132 (31), 77 (32), 54 (67). FAB$^+$MS (glycerol) *m/z* (rel. int.): 209 $[M+Na]^+$ (100). UV (CH_2Cl_2) λ_{max} (log ϵ) 236 nm (3.24), 320 nm (3.86). FT–IR (KBr) ν_{max} 3415 (OH), 2923 (CH), 2880 (CH), 2852 (CH), 2218 (C≡C), 1790 (CO), 1754 (CO) cm^{-1}. ^{13}C and ^{1}H NMR: see Tables 1 and 2. Compound **1** was treated with Ac_2O–Py (1:1) overnight, the product was poured on HCl(c)/ice, extracted with EtOAc, and washed with $NaHCO_3$ and H_2O. The crude product was purified by prep. TLC using CH_2Cl_2–EtOAc 3:7 as solvent yielding **1a**. Compound **1a**. Oil. ^{1}H NMR ($CDCl_3$): δ 7.80 (*d*, 5.5 Hz, H-4), 6.27 (*d*, 5.5 Hz, H-3), 4.78 (*s*, H-1″), 4.16 (*dd*, 10.6 and 6.6 Hz, H-5′), 4.07 (*dd*, 10.6 and 6.6 Hz, H-5′), 3.07 (*sextet*, 6.9 Hz, H-4′), 1.29 (*d*, 6.9 Hz, H-6′), 2.11 (*s*, *Me*COO) and 2.10 (*s*, *Me*COO). ^{13}C NMR ($CDCl_3$): 170.8 (CO), 170.5 (CO), 168.3 (C-2), 155.7 (C-5), 139.5 (C-4), 121.0 (C-3), 104.3 (C-3′*), 103.4 (C-1′*), 66.8 (C-5′), 61.0 (C-1″), 27.3 (C-4′), 20.8 (*Me*COO), 17.1 (C-6′). The signal of C-2′ is overlapped with one of $CDCl_3$. *Signals may be interchanged.

3.6. 4′-Hydroxyaporpinone A

Oil. $[\alpha]_D = -129^\circ$ (MeOH, *c* 0.11). HR-EIMS (70 eV), *m/z* $[M\text{-}30]^{+\bullet}$ found 194.0589, calc. for $C_{10}H_{10}O_4$ 194.0579. EIMS (70 eV) *m/z* (rel. int.): 194 $[M-CH_2O]^{+\bullet}$ (17), 177 (62), 176 (80), 133 (48), 82 (51), 69 (66), 55 (80), 43 (100). UV (CH_2Cl_2) λ_{max} (log ϵ) 203 nm (3.34), 316 nm (3.63). FT–IR (KBr) ν_{max} 3399 (OH), 2942 (CH), 2877 (CH), 2215 (C≡C), 1795 (CO), 1755 (CO), 896, 816 cm^{-1}. For ^{13}C and ^{1}H NMR: spectra, see Tables 1 and 2.

3.7. Aporpinone B

Oil. $[\alpha]_D = -29^\circ$ (CH_2Cl_2, *c* 0.15). HR-EIMS (70 eV), *m/z* $[M]^{+\bullet}$ found 192.0413, calc. for $C_{10}H_8O_4$ 192.0422. EIMS (70 eV) *m/z* (rel. int.): 192 $[M]^{+\bullet}$ (69), 162 (29), 132 (100), 82 (95), 77 (50), 54 (90). UV (CH_2Cl_2) λ_{max} (log ϵ) 229 nm (3.60), 319 nm (3.99). FT-IR (KBr) ν_{max} 3439 (OH), 2926 (CH), 2853 (CH), 2228 (C≡C), 1795 (CO), 1755 (CO), 889, 857, 817 cm^{-1}. For ^{13}C and ^{1}H NMR spectra, see Tables 1 and 2.

3.8. 1″-Acetylaporpinone B

Oil. $[\alpha]_D = -47^\circ$ (CH_2Cl_2, *c* 0.08). HR-EIMS (70 eV), *m/z* $[M]^{+\bullet}$ found 234.0518, calc. for $C_{12}H_{10}O_5$ 234.0528. EIMS (70 eV) *m/z* (rel. int.): 234 $[M]^{+\bullet}$ (14), 192 (12), 174 (91), 162 (90), 147 (22), 146 (22), 134 (20), 109 (26), 82 (87), 77 (36), 54 (100). UV (CH_2Cl_2) λ_{max} (log ϵ) 231 nm (3.75), 319 nm (3.30). FT–IR (KBr) ν_{max} 3430 (OH), 2927 (CH), 2854 (CH), 2216 (C≡C), 1790 (CO), 1751 (CO) cm^{-1}. For ^{13}C and ^{1}H NMR spectra, see Tables 1 and 2.

Acknowledgements

We thank Dr Jorge A. Palermo and LANAIS-NMR (CONICET-FCEN, UBA) for the NMR spectra, LANAIS-EMAR (CONICET-FCEN, UBA) and the Washington University Resource for Biomedical and Bio-organic Mass Spectrometry for the mass spectra, UMYMFOR (CONICET-FCEN, UBA), CONICET, ANPCYT and Universidad de Buenos Aires for partial financial support.

References

Cabrera, G.M., Seldes, A.M., 1997. Citrinin derivatives from an intertidal marine *Penicillium*. Ann. Asoc. Quím. Arg. 85, 193.

Gill, M., Steglich, W., 1987. Pigments of Fungi. In: Herz, W., Grisebach, H., Kirby, G.W., Tamm, Ch. (Eds.), Progress in the Chemistry of Organic Natural Products, Vol. 51. Springer Verlag, Wien, New York, pp. 1–317.

Jägers, E., Steffan, B., von Ardenne, R., Steglich, W., 1986. Stoffwechselprodukte des 1.2.4-trihydroxybenzols aus fruchtkörpern von *Gomphidius maculatus* und *G. glutinosus* (Boletales). Z. Natrurforsch. 41b, 645.

Kuhnt, D., Anke, T., Besl, H., Bross, M., Hermann, R., Mocek, U., Steffan, B., Steglich, W., 1990. Antibiotics from basidiomycetes, 37. New inhibitors of cholesterol biosynthesis from cultures of *Xerula melanotricha* Dörfelt. J. Antibiotics 43, 1413.

Levy, L.M., Cabrera, G.M., Wright, J.E., Seldes, A.M., 2000. Indole alkaloids from a culture of the fungus *Aporpium caryae*. Phytochemistry 54, 941.

Sanz, J.F., Marco, J.A., 1991. Ein neues Butenolid aus *Conyza bonariensis*. Liebigs Ann. Chem., 339.

Shimojima, Y., Hayashi, H., Ooka, T., Shibukawa, M., 1982. (Studies on AI-77s, microbial products with pharmacological activity) Structures and the chemical nature of AI-77s. Tetrahedron Lett. 23, 5435.

Suga, T., Hirata, T., Okamoto, E., Kataoka, M., 1977. Biosynthesis of protoanemonin in *Ranunculus glaber*. The stereochemistry of the hydrogen elimination in the formation of the double bond of protoanemonin. Chem. Lett., 709–710.