Porric Acids A–C – New Antifungal Dibenzofurans from the Bulbs of *Allium Porrum* L.

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Three new dibenzofurans, porric acids A (**1**), B (**2**) and C (**3**), have been isolated from the bulbs of *Allium porrum* L. Their structures have been elucidated by a detailed spectroscopic analysis, including 2D HMBC and ROESY correlation experiments. When tested against *Fusarium culmorum*, porric acids A–C (**1**–**3**) were found to exhibit antifungal activity.

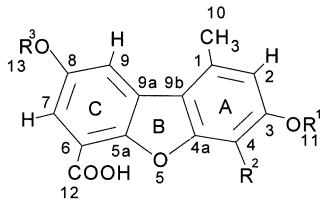
To date, a large number of antifungal metabolites have been characterized in plants[1]. About half of these are constitutive agents, whereas the remainder are induced as phytoalexins, but there is no well-defined chemical division between constitutive and induced antifungal agents. Chemically, the most common antifungal compounds of plant origin are terpenoids or phenols. A subgroup of the latter metabolites are dibenzofurans, which are produced by living wood tissues of the plants as part of their dynamic defense system[2] and are of relatively rare occurrence[3]. They are reported as constitutive agents of lichens[4], as well as phytoalexins produced by sapwood of the woody members of *Rosaceae* in response to fungal inoculation[5].

As part of a systematic screening of the bioactive compounds in the *Allium* species from southern Italy[6][7][8][9], we have now found three novel dibenzofurans in the bulbs of the leek, *Allium porrum* L. We report herein on the isolation and characterization of these compounds, as well as on their activity towards the fungus *Fusarium culmorum*. This is the first report of the presence of dibenzofurans in plants belonging to the *Allium* genus.

Samples of *A. porrum* were collected and extracted first with *n*-hexane, and then with $CHCl_3$, $CHCl_3$/MeOH (9:1), and MeOH. Repeated column chromatography of the $CHCl_3$/MeOH (9:1) extract yielded the pure dibenzofurans, porric acids A (**1**), B (**2**) and C (**3**).

Compound **1**, isolated as pale-yellow microcrystals (m.p. 200–202°C), showed a molecular ion peak at *m/z* 302.0789 in the HREIMS, in accordance with the composition $C_{16}H_{14}O_6$. This was also supported by ^{13}C-NMR data. The molecular formula indicated 10 degrees of unsaturation.

A sharp singlet at δ = 11.89 in the ^{1}H-NMR spectrum, as well as a carbon signal at δ = 164.2, interrelated in the HMBC spectrum, were diagnostic of a carboxylic group. The UV spectrum (bands at λ = 338, 260, 238, 206 nm) was suggestive of a dibenzofuran skeleton[10], which was confirmed by the presence of 12 sp^2 carbon signals, other



	R^1	R^2	R^3
1	CH_3	OH	CH_3
2	CH_3	OH	H
3	H	H	H

than that of the carboxylic group, in the ^{13}C-NMR spectrum (Table 1). Such a structure thus accounted for the unsaturations implied by the molecular formula. From the presence of three aromatic methine groups, as indicated by the ^{1}H-, ^{13}C-, and DEPT-NMR spectra, it was apparent that the dibenzofuran structure was pentasubstituted [^{1}H: δ = 7.25 (br s, 1 H, H-9), 6.95 (s, 1 H, H-2), and 6.64 (br s, 1 H, H-7); ^{13}C: δ = 113.1 (C-2), 99.6 (C-7), and 104.0 (C-9)]. The ^{1}H-NMR spectrum also featured signals attributable to two methoxy groups (singlets at δ = 3.89 and 3.91, H_3-11 and H_3-13, respectively), and one methyl group (singlet at δ = 2.73, H_3-10). An HMQC experiment correlated all these proton signals with the corresponding carbon resonances (see Table 1). Finally, a further D_2O-exchangeable proton (singlet at δ = 9.27) in the ^{1}H-NMR spectrum indicated the presence of one phenolic group.

The substitution pattern of the aromatic rings was elucidated by analysis of ^{1}H-^{1}H coupling constants and 2D COSY, HMBC, and ROESY data. The *meta* relationship of H-7 (δ = 6.64, d) and H-9 (δ = 7.25, d) was established from their small mutual coupling constants (*J* = 2 Hz). In a ROESY experiment, both proton signals correlated via dipolar interactions with the protons of the methoxy group resonating at δ = 3.91 (H_3-13), thus defining the location

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Table 1. ^{1}H- and ^{13}C-NMR assignments ([D$_6$]DMSO) for compounds **1**−**3**

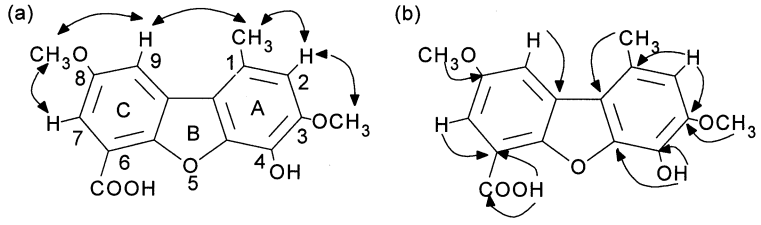
Posn.	**1** δ_C (mult.)	δ_H (mult., *J*)	**2** δ_C (mult.)	δ_H (mult., *J*)	**3** δ_C (mult.)	δ_H (mult., *J*)
1	126.3 (C)		126.2 (C)		117.5 (C)	
2	113.1 (CH)	6.95 (s)	113.1 (CH)	6.95 (s)	109.1 (CH)	6.71 (br s)
3	148.3 (C)		148.2 (C)		152.7 (C)	
4	132.4 (C)		132.4 (C)		101.0 (CH)	6.63 (br s)
4a	140.7 (C)		140.6 (C)		138.1 (C)	
5a	164.6 (C)		164.6 (C)		164.7 (C)	
6	98.6 (C)		98.7 (C)		96.9 (C)	
7	99.6 (CH)	6.64 (d, 2 Hz)	101.3 (CH)	6.40 (d, 2 Hz)	101.6 (CH)	6.33 (d, 2 Hz)
8	166.2 (C)		162.3 (C)		158.5 (C)	
9	104.0 (CH)	7.25 (d, 2 Hz)	104.9 (CH)	7.29 (d, 2 Hz)	104.7 (CH)	7.21 (d, 2 Hz)
9a	138.1 (C)		138.4 (C)		138.3 (C)	
9b	110.6 (C)		110.8 (C)		114.9 (C)	
10	24.8 (CH_3)	2.73 (s)	24.9 (CH_3)	2.71 (s)	25.3 (CH_3)	2.70 (s)
11	56.1 (CH_3)	3.89 (s)	56.1 (CH_3)	3.88 (s)		
12	164.2 (C)		164.1 (C)		164.1 (C)	
13	55.9 (CH_3)	3.91 (s)				
COO*H*		11.89		11.83		11.76
O*H*		9.27		9.25		9.26, 9.25

of the latter between the above protons. A long-range interaction between the aromatic proton resonating at δ = 6.64 (H-7) and the carbon signal of the carboxyl group (δ = 164.2), established their *ortho* relationship. Thus, from the aforementioned data, the substitution pattern of one phenyl ring was established, apart from its position of attachment to the furan ring. This information could be gleaned from the ROESY correlation peak of the aromatic proton signal at δ = 7.25 (H-9) with the methyl signal at δ = 2.73 (H_3-10), which unambiguously located the two substituents at positions 9 and 1, respectively. From the prominent cross-peak in the ROESY spectrum between H_3-10 (δ = 2.73) and H-2 (δ = 6.95), as well as the long-range correlation of C-10 (δ = 24.8) and H-2 (δ = 6.95) observed in the HMBC spectrum, the final aromatic proton was placed at C-2. Furthermore, the existence of a dipolar interaction between H-2 and the methoxy group resonating at δ = 3.89 (H_3-11) suggested that the latter was attached at the 3-position. Thus, the phenolic group was placed at the remaining substitution site C-4. Once the complete substitution pattern of the dibenzofuran had been established, we were able to assign the carbon resonances of all the remaining carbons through accurate analysis of the results of an HMBC experiment (Table 1). Key HMBC and ROESY correlations are shown in Figure 1.

Compound **2**, isolated as pale-yellow microcrystals (m.p. 217−219°C), showed a molecular ion peak at *m/z* 288.0636 in the HREIMS, corresponding to the composition $C_{15}H_{12}O_6$, in accordance with the ^{13}C-NMR data. The IR, UV, and ^{1}H- and ^{13}C-NMR spectra of **2** all appeared similar to those of **1**, thus suggesting that this compound also had a pentasubstituted dibenzofuran structure. The difference of 14 a.m.u. between **1** and **2**, together with the absence in the ^{1}H- and ^{13}C-NMR spectra of signals attributable to a methoxy group, strongly suggested that the only difference between **1** and **2** was that a hydroxy group was present in **2** in place of one of the methoxy groups in **1**. Two-dimensional COSY, HMQC, HMBC, and ROESY correlations of **2**, which allowed assignment of all the proton and carbon resonances (see Table 1), fully confirmed this hypothesis. A dipolar interaction between H-2 (δ = 6.95) and the methoxy group (δ = 3.88) placed the latter at C-3. The upfield shift of the C-8 signal (Δδ = −3.9 ppm), and the downfield shifts of the C-7 and C-9 resonances (Δδ = 1.7 and 0.9 ppm, respectively) in the ^{13}C-NMR spectrum of **2**, compared to the corresponding signals of **1**, clearly indicated that the second phenolic group of **2** was located at C-8.

The molecular composition of compound **3** (pale-yellow microcrystals, m.p. 230−232°C) was determined as $C_{14}H_{10}O_5$ from the molecular ion peak at *m/z* 258.0529 in the HREIMS, in accordance with the ^{13}C-NMR data. The IR and UV spectra of **3** appeared similar to those of **1** and **2**, thus suggesting a dibenzofuran structure. Its tetrasubstituted nature was deduced by analysis of the ^{1}H- and ^{13}C-NMR spectra, which showed the presence of four aromatic

Figure 1. (a) ROESY (↔) contacts in compound **1**. (b) HMBC (→) correlations in **1**; HMBC arrows denote correlations from H to C



methine signals [^{1}H: δ = 6.33 (br s, H-7), 6.63 (br s, H-4), 6.71 (br s, H-2) and 7.21 (br s, H-9); ^{13}C: δ = 101.6 (C-7), 101.0 (C-4), 109.1 (C-2) and 158.5 (C-9)]. Further features of the NMR spectra indicated the presence of a methyl group [^{1}H: δ = 2.70 (s); ^{13}C: δ = 25.3], one carboxyl function [^{1}H: δ = 11.76 (s); ^{13}C: δ = 164.1] and two phenolic groups (^{1}H: broad singlets at δ = 9.26 and 9.25). The ^{1}H- and ^{13}C-NMR resonances (Table 1) of ring C in compound **2** were clearly discernible in the corresponding spectra of **3**, indicating the same substitution pattern on this ring in both metabolites. Again, a dipolar correlation in the ROESY spectrum of **3** between H-9 (δ = 7.21) and H_3-10 (δ = 2.70) established that the methyl group was located at C-1. A further NOE between the methyl group protons and the methine signal at δ = 6.71, allowed us to locate a proton at C-2. In a 2D COSY experiment, the H-2 signal showed a correlation via long-range coupling with the proton signal at δ = 6.63, as a result of which this proton could be placed in the 4-position. The final phenolic group was then located at the remaining substitution site C-3.

When tested against the fungus *Fusarium culmorum*, compounds **1**−**3** exhibited ED_{50} values of 20−30 μg/ml. The capability to interfere with fungal growth and development could be due to the presence of a number of methoxy and hydroxy groups, which, as previously hypothesized[5], may give the molecule a suitable degree of lipophilicity. The fact that the dibenzofurans are found in the bulbs of *A. porrum* L. rather than in the aerial parts, might suggest that they play a role in protecting the bulb against invading microorganisms.

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Experimental Section

General Methods: High-resolution mass spectra (HREIMS) were obtained by electron impact at 55eV on a Fisons VG Prospec mass spectrometer. − ^{1}H- and ^{13}C-NMR spectra were recorded in [D_6]DMSO solution at 500.13 and 125.795 MHz, respectively, on a Bruker AMX-500 spectrometer. Proton chemical shifts were referenced to the residual solvent signal (δ = 2.50). ^{13}C-NMR spectra were referenced to the center peak of the septet at δ = 39.5. The multiplicities of ^{13}C resonances were determined by DEPT experiments. ^{1}H connectivities were determined by means of COSY experiments. ^{1}H-^{13}C connectivities were determined by 2D HMQC experiments[11], with a BIRD pulse 0.5 sec. before each scan to suppress the signal from protons not directly bonded to ^{13}C. The interpulse delays were adjusted for an average $^1J_{CH}$ of 135 Hz. Two- and three-bond heteronuclear ^{1}H-^{13}C connectivities were determined by means of 2D HMBC experiments[12], optimized for $^{2-3}J_{CH}$ values of 8 Hz. − Nuclear Overhauser effect (NOE) measurements were performed by 2D ROESY experiments. − Medium-pressure liquid chromatography (MPLC) was performed on a Buchi 861 apparatus using SiO_2 (230−400 mesh) and RP-8 columns. − High-performance liquid chromatography (HPLC) in isocratic mode was performed on a Varian apparatus equipped with an RI-3 refractive index detector. Hibar LiChrospher Si60 columns were used.

Extraction and Isolation: Allium porrum L. was collected in May 1995 near Salerno (Campania, Italy). A reference specimen has been deposited at the Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli, Italy. The plants were air-dried immediately after collection (820 g, dry weight) and extracted at room temp. with the following solvents: *n*-hexane, $CHCl_3$, $CHCl_3$/MeOH (9:1), MeOH. The $CHCl_3$/MeOH (9:1) extract (81 g) was concentrated in vacuo to afford 8.54 g of a crude organic extract, which was chromatographed by MPLC on an RP-8 column using a gradient solvent system from H_2O to MeOH. Fractions eluted with MeOH/H_2O (8:2) were further chromatographed by MPLC on an SiO_2 column using sequential mixtures of increasing polarity from 100% $CHCl_3$ to $CHCl_3$/MeOH/H_2O (70:26:4). Fractions were collected and analyzed by TLC on SiO_2 with $CHCl_3$/MeOH (9:1). Fractions eluted with 100% $CHCl_3$ (20 mg) were purified by HPLC on a Hibar LiChrospher Si60 column, mobile phase *n*-hexane/EtOAc (1:1), to give pure compounds **1** (10.0 mg), **2** (2.0 mg) and **3** (2.5 mg).

Porric Acid A (**1**): Yield 10.0 mg, m.p. 200−202°C. − UV (MeOH): λ = 338, 260, 238, 206 nm. − ^{1}H- and ^{13}C-NMR spectra: See Table 1. − HREIMS (70 eV); *m/z*: 302.0789 (obsd.), calcd. for $C_{16}H_{14}O_6$: 302.0786.

Porric Acid B (**2**): Yield 2.0 mg, m.p. 217−219°C. − UV (MeOH): λ = 334, 258, 238, 214, 210 nm. − ^{1}H- and ^{13}C-NMR spectra: See Table 1. − HREIMS (70 eV); *m/z*: 288.0636 (obsd.), calcd. for $C_{15}H_{12}O_6$: 288.0630.

Porric Acid C (**3**): Yield 2.5 mg, m.p. 230−232°C. − UV (MeOH): λ = 334, 298, 288, 256, 238 nm. − ^{1}H- and ^{13}C-NMR spectra: See Table 1. − HREIMS (70 eV); *m/z*: 258.0529 (obsd.), calcd. for $C_{14}H_{10}O_5$: 258.0525.

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