# Karnatakafurans A and B: Two Dibenzofurans Isolated from the Fungus *Aspergillus karnatakaensis*

Søren Manniche,[†] Kenneth Sprogøe,[†] Petur W. Dalsgaard,[‡] Carsten Christophersen,*[,‡] and Thomas O. Larsen[§]

*Department of Medicinal Chemistry, The Danish University of Pharmaceutical Sciences, Universitetsparken 2, DK-2100 Copenhagen, Denmark, Marine Chemistry Section, Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark, and Center for Microbial Biotechnology, BioCentrum-DTU, Building 221, Technical University of Denmark, DK-2800 Lyngby, Denmark*

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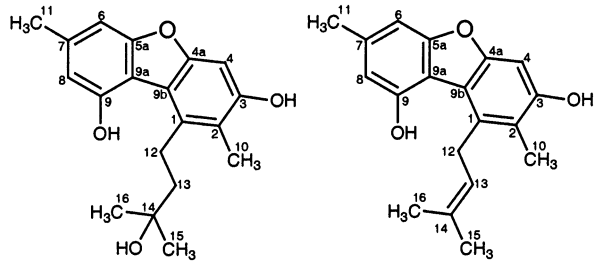
Karnatakafurans A (**1**) and B (**2**), two novel dibenzofurans, have been isolated from the Specie Novum *Aspergillus karnatakaensis* Frisvad. The compounds were the major secondary metabolites and were isolated through UV-guided fractionation of the organic extract. The structures were elucidated by spectroscopic methods including MS and NMR. The compounds were tested for antimicrobial and antimalarial activity and proved to be moderately active against *Plasmodium falciparum*.

The use of UV-guided isolation of compounds from fungal extracts has proven a useful and effective method of discovering novel fungal metabolites.[1] Being presented with a novel *Aspergillus* species, *A. karnatakaensis* Frisvad, this approach was found applicable. The UV spectra of the two main metabolites, eluting at 12.6 and 18.6 min, did not resemble any other frequently encountered metabolites from *Aspergillus* or *Penicillium*, which led to the isolation of karnatakafurans A (**1**) and B (**2**). The structures of the two compounds, both novel dibenzofurans, isolated from *A. karnatakaensis* were elucidated by means of spectroscopic data, including 2D-NMR, EIMS, and elemental analysis.

In this paper we report the isolation, structure elucidation, and preliminary in vitro testing for antimicrobial and antimalarial activity of the two dibenzofurans.

The defatted ethyl acetate extract was lyophilized. The dry extract (5.51 g) was coated on an equal amount of Celite and fractionated by flash chromatography. The fractions eluting with 70% and 80% aqueous methanol were subjected to further purification. The final isolation of **1** and **2** was achieved by preparative reverse-phase HPLC.




**1**




**2**

Karnatakafuran A (**1**) had a UV spectrum very similar to that of other dibenzofurans.[2] The EIMS revealed the molecular ion at *m/z* 314, indicating the molecular formula $C_{19}H_{22}O_4$, substantiated by $^{13}C$ NMR assignments (see Experimental Section). This conclusion was further supported by elemental analysis. The molecular formula requires 9 degrees of unsaturation, as present in the dibenzofuran ring system. The $^{13}C$ spectrum displayed four signals in the range $\delta$ 148−158, characteristic of aromatic carbon atoms attached to oxygen. Since the $^1H$ NMR data (see Experimental Section) indicated only two phenolic hydroxy groups, and since no methoxy signals appeared in either of the spectra, the four signals must originate from two phenolic carbons and two carbons attached to an ether oxygen.

From the NMR spectra it was evident that compound **1** contained four methyls, two methylenes, and one quaternary alcohol. The signals for the methyl protons attached to the carbon appearing at $\delta$ 18.3 and 22.7 resonated at $\delta$ 2.98 and 2.39, respectively, suggesting these two methyl groups to be directly attached to the dibenzofuran ring system. Their locations were established by HMBC and NOESY data. The remaining two methyl groups are part of the aliphatic isopentyl-3-ol side chain. The structure of the side chain was determined through studies of coupling patterns, NOE effects, and the HMBC experiment, as was the position on the dibenzofuran skeleton. Since the two methyl groups appeared as singlets, with identical proton and carbon chemical shifts, it was evident that the hydroxy, as well as the two methyls, was attached to the quaternary carbon resonating at $\delta$ 71.9.

Karnatakafuran B (**2**) was isolated as a white solid. The EIMS revealed the molecular ion at *m/z* 296, indicating the molecular formula $C_{19}H_{20}O_3$, substantiated by 19 signals in the $^{13}C$ NMR spectrum (see Experimental Section). The composition was further supported by elemental analysis. The molecular formula required 10 degrees of unsaturation. This information in conjunction with the difference weights of 18, suggested that one molecule of water had been lost from **1**, with formation of a double bond or a ring. The similarity of the NMR, UV, and MS spectra of **1** and **2** supports this theory. The NOESY and HMBC spectra indicated identical substitution patterns in the dibenzofuran ring system.

Karnatakafurans A (**1**) and B (**2**) showed moderate in vitro activity against a chloroquine-sensitive *Plasmodium falciparum* 3D7 parasite (Table 1) in a modification of the assay specified by Desjardins et al.[3] When tested for antimicrobial effect, karnatakafurans A (**1**) and B (**2**) did not inhibit growth of the bacteria and fungi tested at 100

* To whom correspondence should be addressed. Tel: +45 35320157. Fax: +45 35320212. E-mail: carsten@kiku.dk.
[†] Danish University of Pharmaceutical Sciences.
[‡] University of Copenhagen.
[§] Technical University of Denmark.

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**Table 1.** $IC_{50}$ Values ($\mu$g/mL) against *Plasmodium falciparum*

| compound | *Plasmodium falciparum* (3D7) $IC_{50}$ ($\mu$g/mL) |
|---|---|
| **1** | 3.9 |
| **2** | 3.6 |
| chloroquine | 0.012 |

$\mu$g/disk, except for *Pseudomonas aeruginosa*, which was somewhat inhibited by both compounds.

## Experimental Section

**General Experimental Procedures.** The UV spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer. NMR data were recorded in $CD_3OD$, $CDCl_3$, or DMSO-$d_6$ on a Varian 400 FT-NMR spectrometer operating at 400 and 100.6 MHz for $^1H$ and $^{13}C$ NMR spectra, respectively. EIMS mass spectra were recorded on a JEOL JMS_MX/HX 110A. HPLC was performed on a Waters 600E system with a Waters 996 photodiode array detector using Millennium software and a Waters Delta Pak C18 column (19 mm × 300 mm, 15 $\mu$m 100A).

**Fungal Material and Fermentation.** The isolate of *Aspergillus karnatakaensis* (IBT 22154) was obtained from the IBT Culture Collection at BioCentrum-DTU, The Technical University of Denmark. Isolates were cultured as three-point mass inoculations on 149 yeast extract sucrose (YES) agar plates for 11 days in the dark at 25 °C.

**Extraction and Separations.** Mycelium and agar were harvested and extracted twice overnight with EtOAc. After filtration through a Whatman 1PS phase separation filter, the extract was evaporated in vacuo, leaving the crude extract (11.0 g). This was partitioned between heptane and 90% aqueous MeOH, yielding a defatted extract (5.5 g). After coating onto 5.5 g of Celite, it was placed on top of a column for separation into eight fractions (MeOH/$H_2O$ (4:6, 5:5, 6:4, 7:3, 8:2, 9:1, 10:0) and 100% $CH_2Cl_2$) by flash chromatography (Phenomenex, C-18, 50 $\mu$m, 150 mm × 40 mm). The 70% MeOH fraction was subjected to HPLC (Waters Delta Pak C18 column (19 mm × 300 mm, 15 $\mu$m 100A) using an isocratic solvent of $H_2O$/MeCN, 55:45, with a flow of 30 mL/min. Karnatakafuran A (57 mg) eluted at 12.6 min.

The 80% MeOH fraction was subjected to HPLC using an isocratic solvent of $H_2O$/MeCN, 40:60, with a flow of 20 mL/min, to give further karnatakafuran A (16 mg) at 12.6 min and karnatakafuran B (44 mg) at 18.6 min.

**Karnatakafuran A (1):** grayish-white solid ($H_2O$/MeCN, 55:45); mp 182−185 °C; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 314.1 (4.07), 301.8 (3.92), 286.9 (4.03), 267.1 (4.07), 227.2 (4.45); $^1H$ NMR ($CD_3OD$, 400 MHz) $\delta$ 6.79 (1H, s, H-6), 6.77 (1H, s, H-4), 6.54 (1H, s, H-8), 2.98 (3H, s, H-10), 2.87 (2H, m, H-12), 2.40 (3H, s, H-11), 1.71 (2H, m, H-13), 1.34 (3H, s, H-15), 1.34 (3H, s, H-16); $^{13}C$ NMR ($CD_3OD$, 100.6 MHz) $\delta$ 159.5 (s, C-5a), 155.9 (s, C-4a), 155.5 (s, C-3), 152.4 (s, C-9), 137.4 (s, C-7), 132.9 (s, C-1), 122.3 (s, C-2), 117.4 (s, C-9b), 112.1 (s, C-9a), 111.1 (d, C-8), 104.0 (d, C-6), 95.9 (d, C-4), 71.5 (s, C-14), 44.3 (t, C-13), 29.1 (q, C-15), 29.1 (q, C-16), 22.7 (q, C-11), 21.6 (t, C-12), 18.3 (q, C-10); EIMS *m/z* 314 $[M]^+$ (32), 296 (35), 281 (5), 241 (100), 211 (8); *anal*. C 70.6%, H 6.99%, calcd for $C_{19}H_{22}O_4$, C 72.6%, H 7.0%.

**Karnatakafuran B (2):** white solid ($H_2O$/MeCN, 40:60); mp 134−136 °C; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 364.5 (2.93), 353.0 (2.94), 346.9 (2.94), 313.9 (3.99), 303.4 (3.83), 286.5 (3.98), 267.0 (4.04), 227.7 (4.45); $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 6.79 (1H, s, H-6), 6.77 (1H, s, H-4), 6.35 (1H, s, H-8), 5.09 (1H, m, $J$ = 6.8, 1.3 Hz, H-13), 3.43 (2H, d, $J$ = 6.8 Hz, H-12), 2.85 (3H, s, H-10), 2.31 (3H, s, H-11), 1.77 (3H, s, H-16), 1.66 (3H, d, $J$ = 1.3 Hz, H-15); $^{13}C$ NMR ($CDCl_3$, 100.6 MHz) $\delta$ 158.2 (s, C-5a), 154.6 (s, C-3), 153.3 (s, C-4a), 148.9 (s, C-9), 136.6 (s, C-7), 133.4 (s, C-14), 131.8 (s, C-1), 122.3 (d, C-13), 121.5 (s, C-2), 117.0 (s, C-9b), 110.5 (s, C-9a), 110.4 (d, C-8), 104.6 (d, C-6), 96.1 (d, C-4), 25.8 (q, C-15), 25.7 (t, C-12), 21.9 (q, C-11), 18.3 (q, C-10), 17.9 (q, C-16); EIMS *m/z* 296 $[M]^+$ (83), 281 (9), 241 (100), 211 (9); *anal*. C 77.1%, H 6.8%, calcd for $C_{19}H_{20}O_3$, C 77.0%, H 6.8%.

**Antimalarial Assay.** The activity of **1** and **2** against a chloroquine-sensitive *Plasmodium falciparum* 3D7 parasite was investigated following a previously established procedure.[3] Radio-labeled phenylalanine was used instead of [G-$^3$H]hypoxanthine.

**Antimicrobial Tests.** **1** and **2** were tested for antimicrobial activities against Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria by an agar overlay method. Test samples, 100, 50, and 25 $\mu$g, of **1** and **2** were applied to a TLC plate, covered with agar, and inoculated at 37 °C for 24 h. Compounds **1** and **2** did not show antibacterial activity at 100 $\mu$g/spot, except against *P. aeruginosa*, which was somewhat inhibited. Compounds **1** and **2** were also tested against various fungi (*Alternaria infectoria, Cladosporium* sp., *Penicillium italicum, P. digitatum, P. expansum, Aspergillus fumigatus, Fusarium avenaceum, F. culmorum, F. solani, F. sporotrichioides, F. oxysporum,* and *Botrytis cinera*) by disk diffusion method. No antifungal activity was evident at 100 $\mu$g/disk after 4 days at 25 °C.

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**Supporting Information Available:** A table of NOESY and HMBC data for **1** and **2** is available. This material is available free of charge via the Internet at http://pubs.acs.org.

## References and Notes

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