Guieranone A, a Naphthyl Butenone from the Leaves of *Guiera senegalensis* with Antifungal Activity

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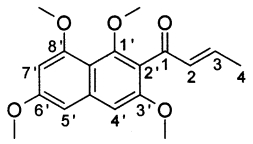
A new methoxylated naphthyl butenone, guieranone A (**1**), was isolated from the leaves of *Guiera senegalensis*. Its structure was elucidated as (2*E*)-1-(1,3,6,8-tetramethoxy-2-naphthyl)but-2-en-1-one, on the basis of spectroscopic data. Also isolated were two known naphthopyrones, 5-methyldihydroflavasperone (**2**) and 5-methylflavasperone (**3**). Guieranone (**1**) exhibited potent antifungal activity against *Cladosporium cucumerinum* and is the first naphthyl ketone derivative to have been isolated from the family Combretaceae.

Guiera senegalensis J. F. Gmel. (Combretaceae) was collected in Guinea-Bissau as part of our ongoing project on chemical and pharmacological studies of the local plants used in folk medicine. A decoction of its leaves has been used by traditional healers in Guinea-Bissau for skin diseases[1] and as an antimicrobial in the treatment of venereal diseases.[2] In our previous work with an ethanol extract of *G. senegalensis* leaves (Gsl) and the liquid−liquid partition fractions obtained from this extract, we reported an in vitro antimicrobial activity profile against *Neisseria gonorrhoeae* (including penicillin- and tetracycline-resistant and susceptible strains) and localized this activity to the diethyl ether fraction (Gsl-2).[3] In this paper, we present the results of the phytochemical investigation on this bioactive fraction, which led to the isolation and structure elucidation of guieranone A (**1**), a new ketone possessing a tetramethoxy-2-naphthyl radical, and to the isolation of two naphthopyrans previously identified in this plant, 5-methyldihydroflavasperone (**2**)[4] and 5-methylflavasperone (**3**).[5] Additionally, results are also presented from an antifungal direct bioautography TLC bioassay conducted with the crude plant extracts and compounds **1**−**3**, against *Cladosporium cucumerinum* Ell. & Artur.



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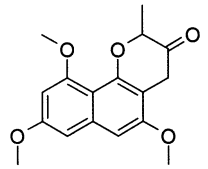

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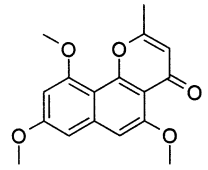

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Compound **1** was isolated as an amorphous yellow-brown solid. The EIMS showed an ion peak at *m/z* 316 $[M]^+$ and two acylium ion fragments at *m/z* 275 $[C_{15}H_{15}O_5]^+$ and *m/z* 69 $[C_4H_5O]^+$. The molecular formula was determined by HRMS as $C_{18}H_{20}O_5$, [HREIMS *m/z* $[M]^+$ 316.1296, calcd 316.1311]. In the IR spectrum, diagnostic peaks were observed for aromatic protons (3001 cm^{-1}), methyl C−H groups (2963, 2937, 2841 cm^{-1}), a conjugated carbonyl group (1656 cm^{-1}), and an alkene conjugation with an aromatic ring (1620 cm^{-1}). The structure of **1** was deduced from detailed analysis of the ^1H NMR and ^{13}C NMR data aided by 2D NMR experiments ($^1H-^1H$ COSY, HMQC, and HMBC). These data, summarized in Table 1, suggested the naphthyl ketone nature of **1**. The ^1H NMR spectrum of **1** showed a doublet at δ 1.90, indicative of a methyl group, and four singlets at δ 3.70, 3.83, 3.88, and 3.91 (3H each), attributed to four methoxyl groups attached to an aromatic ring system. The aromatic region exhibited two doublets (δ 6.47, 6.81) corresponding to H-5′,-7′ and a singlet at δ 7.01 corresponding to H-4′. These two doublets at δ 6.47 and 6.81 correlated with each other (J = 2 Hz), and the coupling constant indicated a *meta*-substitution pattern. The methyl group at δ 1.9 (J = 6.8 Hz) showed a vicinal coupling to a quartet at δ 6.60 (1H) and to a doublet at δ 6.34 (1H). The quartet signal at δ 6.60 (1H) was also correlated with the doublet at δ 6.34, with a coupling constant (J = 15.6 Hz) indicating an alkene diastereomer with *E* stereochemistry. The chemical shifts of the protons at δ 6.60 and 6.34 and the signal at ^{13}C δ 197.9 were consistent with the presence of an α,β-unsaturated ketone functionality. The existence of this group was confirmed by the HMQC observed couplings between the carbon signal at δ 135.2 and the proton signal at δ 6.34 and between the carbon signal at δ 149.3 and the proton signal at δ 6.60.

The results obtained showed that the ethanol extract, Gsl, and the *n*-hexane (Gsl-1) and diethyl ether (Gsl-2) extracts of *G. senegalensis* inhibited the growth of *C. cucumerinum* and permitted the localization of a major active compound, **1** (yellow orange spot, R_f 0.66). All of the other extracts (Gsl-3 to Gsl-5) were inactive against this fungus. Guieranone A (**1**) showed antifungal activity against *C. cucumerinum*. The minimal amount of compound required for inhibition of fungal growth on the TLC plate was 1 μg. This value is comparable with those of

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Table 1. ^{1}H NMR, ^{13}C NMR, and HMBC Spectral Data for Guieranone A (**1**)

position	δ_H[a]	δ_C[b]	HMBC[c]
α,β-unsaturated ketone moiety			
1		197.9	3, 4′
2	6.34, d, 1H (J = 15.6 Hz)	135.2	4
3	6.60, dq, 1H (J = 6.8, 15.6 Hz)	149.3	4
4	1.90, d, 3H (J = 6.8 Hz)	18.4	
naphthyl moiety			
1′		155.6	OCH_3-1′
OCH_3-1′	3.70, s	64.2	
2′		122.8	4′
3′		156.5	OCH_3-3′, 4′
OCH_3-3′	3.83, s	56.3	
4′	7.01, s	103.4	5′
4′a		140.8	
5′	6.81, d, 1H (J = 2 Hz)	99.8	4′, 7′
6′		160.9	5′, OCH_3-6′, 7′
OCH_3-6′	3.88, s	56.1	
7′	6.47, d, 1H (J = 2 Hz)	98.4	5′
8′		158.7	7′, OCH_3-8′
OCH_3-8′	3.91, s	55.8	
8′a		111.6	4′, 5′, 7′

[a] 400 MHz; CD_3OD (δ 3.35). [b] 100 MHz; CD_3OD (δ 49.0). [c] Carbons that correlate with the proton resonance.

amphotericin B, nystatin, and miconazole, antifungal agents usually used in medicine.[6] Within the concentration range tested (10−0.01 μg), the other two isolates obtained, 5-methyldihydroflavasperone (**2**) and 5-methylflavasperone (**3**), isolated from the bioactive Gsl-2 extract, were inactive against this filamentous fungus.

Naphthalene ketone derivatives, present on the essential oils of some plants, are examples of aromatic compounds with antimicrobial activity. On the other hand, the naphthopyran derivatives, very rare in higher plants and more common as constituents in fungi,[5] are well-known antifungal,[7] antimicrobial,[8] and antiproliferative[9] agents. Although other classes of compounds have been isolated from this species, namely, alkaloids[10] and polyphenols (flavonoids[11,12] and galloylquinic acid derivatives[13]), compound **1** is the first naphthyl ketone to have been isolated from *G. senegalensis* and also from the family Combretaceae.

Experimental Section

General Experimental Procedures. UV spectra were recorded in MeOH on a Hitachi U2000 UV−visible spectrometer. IR spectra were recorded on a Perkin-Elmer V-FT spectrometer. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), and 2D NMR spectra (COSY, HMQC, and HMBC, delay 0.07000000 s) were taken in MeOD on a Bruker ARX 400 MHz. EI mass spectra were measured with a Micromass Autospeq mass spectrometer. HREIMS were determined using a Micromass Q-TOF mass spectrometer. HPLC analysis was carried out using a Waters Alliance instrument with a photodiode array detector.

Plant Material. The plant material was collected in December 1995 at the Contuboel region of Guinea-Bissau and authenticated by Dr. Adélia Diniz, "Centro de Botânica do Instituto de Investigação Científica Tropical", Lisbon, Portugal. Voucher specimen No. 639 is preserved in the Botanical Centre Herbarium of Lisbon (LISC), Lisbon, Portugal.

Extraction and Isolation. Air-dried, pulverized leaves of *G. senegalensis* (723 g) were exhaustively extracted with EtOH (80% v/v) at room temperature. After filtration, the extract was concentrated under reduced pressure. A portion (51 g) of the total (197.5 g) dried ethanol extract (Gsl) was fractionated by sequential liquid−liquid partition with *n*-hexane (Gsl-1) (1.9 g), diethyl ether (Gsl-2) (12.1 g), ethyl acetate (Gsl-3) (7.3 g), *n*-butanol (Gsl-4) (11.5 g), and water (Gsl-5) (17.5 g). After drying, a portion of extract Gsl-2 (9.3 g) was chromatographed over a silica gel 60 column (Merck art. 7734, 60 × 5.5 cm, 596.5 g) using a 100% *n*-hexane to 100% MeOH gradient as eluent. The resulting 58 fractions were concentrated under reduced pressure. Fractions 10 and 11, eluted with *n*-hexane, were submitted to low-pressure liquid chromatography on a Lobar Gröbe B (310-25) LiChroprep RP-18, 40−63 μm column (Merck) eluted with MeOH−water (70:30). Compound **1**, the major constituent of fractions 21−27, was purified by HPLC on a Nova-Pak 4 μm C_{18} column (150 × 3.9 mm) from Waters, equipped with a C_{18} Nova-Pak Sentry-Pak precolumn and using a MeOH−water linear gradient (50−100 over 15 min, flow rate 1 mL/min) as eluent. The chromatograms were monitored at 370 nm. Guieranone A (**1**) (10.4 mg) eluted at t_R 6.2 min. See Supporting Information for details of the isolation and structure elucidation of compounds **2** and **3**.

Guieranone (1): amorphous yellow brown solid; UV (MeOH) λ_{max} (log ϵ) 241 (5.32), 274.5 (4.45) nm; IR (film) ν_{max} 3001, 2963, 2937, 2841, 1656, 1620 cm^{-1}; ^{1}H NMR, ^{13}C NMR, and HMBC data, see Table 1; EIMS (70 eV) m/z 316 $[M]^{\bullet+}$ (100), 299 $[M]^{\bullet+}$ − OH (17), 275 $[C_{15}H_{15}O_5]^+$ (46), 69 $[C_4H_5O]^+$ (47); HREIMS m/z $[M]^{\bullet+}$ 316.1296 (calcd for $C_{18}H_{20}O_5$, 316.1311); TLC R_f 0.66 (toluene−ethyl acetate, 70:30, v/v); yellow orange spots; HPLC t_R 6.2 min; eluent, linear gradient, MeOH−water for 15 min; flow rate, 1 mL/min.

Biological Testing. The in vitro antifungal activity assay against *C. cucumerinum* were carried out following the bioautographic method of Rahalison et al.[14] Stock solutions of pure compounds were prepared at 1 mg/mL in MeOH and were diluted to 1, 0.1, and 0.01 mg/mL. The ethanol extract (Gsl) and the further solvent partitions were dissolved at a concentration of 10 mg/mL in MeOH (Gsl-1 to Gsl-3) or MeOH−water (50:50, v/v, Gsl-4, Gsl-5). An aliquot (10 μL) of each solution, corresponding to 10, 1, and 0.1 μg of each pure compound, and 100 μg of each extract and of the liquid−liquid partition fractions were, respectively, applied to silica gel 60 F_{254} TLC sheets (Merck, art. 1.5554) by means of a graduated micropipet. TLC plates were developed in toluene−ethyl acetate (70:30, v/v) and thoroughly dried. The chromatograms were sprayed with a spore suspension of *C. cucumerinum* (Ciba-Geigy) in Luria-Bertani agar medium (Ciba-Geigy) and incubated for 2 days at room temperature in Pyrex boxes with moist atmosphere. Inhibition zones appeared against a dark background.

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Supporting Information Available: Details of the isolation and identification of compounds **2** and **3**. This information is available via the Internet, free of charge, at http://pubs.acs.org.

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