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Unusual Lactones from *Cananga odorata* (Annonaceae)

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Two lactone compounds have been isolated from the leaves and branches of ylang-ylang (*Cananga odorata* forma *genuina* Hook. f. et Thomson, Annonaceae). One was already known as isosiphonodin **1**. The other, canangone **2**, is a new terpenoid spirolactone with an unusual backbone. Its structure has been established as 6-hydroxy-1-oxo-2-oxaspiro[4.5]dec-7-ene-8-carbaldehyde by using 1-D and 2-D NMR.

KEYWORDS: ***Cananga odorata*; Annonaceae; canangone; spirolactone; isosiphonodin**

INTRODUCTION

Since the isolation of the first acetogenin from *Uvaria accuminata* (*1*) interest in the chemistry of plants belonging to the Annonaceae family has been growing, mostly because of the potent biological activities of these natural polyketides. The Annonaceae are now investigated by many groups. Acetogenins (*2*, *3*), but also alkaloids, carbohydrates, lipids, proteins, polyphenols, terpenes, and flavonoids (*4*, *5*), have been isolated from numerous species. Ylang-ylang (*Cananga odorata* forma *genuina* Hook. f. and Thomson) belongs to the Annonaceae. This species has long been cultivated on a large scale in Madagascar and the Comoro Islands for the production of essential oils from the flowers, which is extensively used in the perfume and soap industries. Despite an outstanding resistance to diseases and insect attacks, this tropical tree had not been investigated for molecules of biological interest. Our search for bioactive constituents from ylang-ylang has led to the isolation and characterization of two lactones: isosiphonodin **1** and a new spirolactone, canangone **2**, the structures of which were established using 1-D and 2-D NMR (Figure 1).

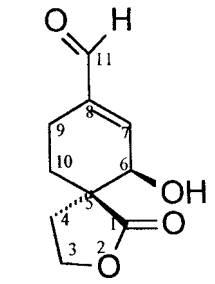

Figure 1. Structure and carbon numbering of isolated compounds from *Cananga odorata*.

MATERIALS AND METHODS

Materials. Plant material was collected in Nosy Be (Madagascar), air-dried on site, and shipped to France. A voucher specimen (Mada no. 001) is kept in the Analytical Chemistry Laboratory for Environment, Faculty of Sciences, University of Marseille, France.

Extraction Procedures. Dried leaves and branches (2.0 kg) were ground and extracted with acetone (20 L) at room temperature for 4 days, with occasional shaking, and then filtered. The solvent was removed under vacuum to give a dark green crude extract (17 g), which was further treated with water (100 mL) and filtered. Water was then evaporated from the filtrate to dryness, giving a yellow waxy residue (2 g).

Compound Purification Steps. CH_2Cl_2 was added to the residue and the soluble part was fractionated by flash chromatography (over silica gel 60H 5−40 μm, eluting with a CH_2Cl_2−EtOAc−MeOH gradient (100:0:0 to 0:100:0 then 0:80:20). Fifteen fractions of 20 mL each were collected; compounds **1** and **2** were present in fractions 8 to 9 and 10 to 12, respectively. Further purification of spirolactone (35 mg) and isosiphonodin (80 mg) was performed by preparative HPLC, using a Nucleosil 100-7 C18 column (125 mm × 10 mm i.d.), elution with MeOH−H_2O (5:95) at 3 mL/min and dual UV detection at 210 and 230 nm.

Brine Shrimp Bioassay (*6*). Brine shrimp eggs were hatched in a commercial two-compartment hatchery filled with artificial seawater. Eggs were sprinkled over the larger darkened compartment, and after

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Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100.6 MHz) Data of Canangone **2**[a]

C	δ ^{13}C (ppm)	H	δ ^{1}H (ppm)	*J* (Hz)
1	178.38			
3	65.45	3 (×2)	4.34 ddd	18.3, 9.2, 7.0
			4.38 ddd	18.1, 9.1, 3.5
4	32.25			
5	46.65	4 (×2)	2.06 ddd	12.9, 6.9, 3.4
			2.46 dt	12.9, 9.0
6	69.38	6	4.36 m	
7	142.12	7	6.68 dt	3.5, 1.8
8	145.83			
9	18.69	9 (×2)	2.33 m A	
			2.39 m B	
10	25.63	10 (×2)	1.76 ddd	13.9, 7.8, 5.9
			2.21 dq	14.0, 5.7
11	193.24	11	9.50 s	

[a] δ OH = 2.99 d, J = 7.3 Hz.

Table 2. ^{1}H (400 MHz) and ^{13}C NMR (100.6 MHz) Data of Isosiphonodin **1**

C	δ ^{13}C (ppm)	H	δ ^{1}H (ppm)	*J* (Hz)
2	173.6			
3	133.7			
4	146.2	4	7.37 q	1.8
5	70.9	5 (×2)	4.85 dd	1.8, 2.3
6	56.0	6 (×2)	4.45 dd	1.8, 2.3

48 h, the phototropic nauplii had moved to the lighted second compartment. They were collected by pipet. Artificial seawater (500 μL) was added to each well of microwell EIA plates, and about 10 nauplii were introduced to each well. Then, 5 μL of aqueous plant extract were added in various concentrations. Survivors were counted after 24 h. An extract with an average of less than 50% survivors was considered positive.

Kedde's Reagent. TLC plates were sprayed first with 2% 3,5-dinitrobenzoic acid in methanol, then with 2M methanolic KOH. Faint red-pink spots which faded quickly were indicative of a positive reaction to Kedde's reagent and the establishment of an α,β-unsaturated γ-lactone subunit.

General Experimental Procedures. Optical rotation was determined in H_2O on a Perkin-Elmer 341 digital polarimeter using a 1-cm microcell, at 25 °C. IRTF spectra were carried out on a Nicolet 20SXB spectrometer. EIMS was performed on a Nermag R10-10C spectrometer. NMR spectra were recorded on a Bruker AMX400 spectrometer in $CDCl_3$ solutions. TMS was used as standard in ^{1}H and ^{13}C measurements. Standard Bruker pulse sequences were used for homonuclear correlation experiments (gradient selected COSY and NOESY). HPLC was carried out with a Waters system L-6200 equipped with a Waters PDA 996 spectrophotometer. Data were acquired using Waters Millenium chromatography software.

Isosiphonodin, 1. Colorless oil. UV (MeOH) λ_{max} 207.5 nm. IR ν_{max} (film) cm^{-1}: 3423, 1743. ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100.6 MHz) see Table 2. EIMS (probe) 20 eV *m*/*z*: 114[M]$^+$. Anal. C, 52.17%; H, 4.90%; O, 42.93%. Calcd for $C_5H_6O_3$: C, 52.63%; H, 5.30%; O, 42.07%.

Canangone, 2. Wax. $[\alpha]_D^{25}$ +58.8° (c = 0.68 g/100 mL MeOH). UV (MeOH) λ_{max} (ϵ) 231 (3615) nm. IR ν_{max} (film) cm^{-1}: 3424, 2724, 1758, 1681, 1176. ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100.6 MHz) see Table 1. EIMS (probe) 20 eV *m*/*z*: 196[M]$^+$. Anal. C, 60.95%; H, 6.02; O, 33.03. Calcd for $C_{10}H_{12}O_4$: C, 61,22%; H, 6.16; O, 32.62%.

RESULTS AND DISCUSSION

The aim of our study was to investigate acetogenins in ylang-ylang. Extraction, fractionation, and purification were monitored by Kedde's reagent (*7*) and the brine shrimp test. Kedde's reagent is a specific TLC spray reagent diagnostic for α,β-unsaturated γ-lactone subunits. The brine shrimp lethality assay detects a broad range of bioactivity of natural products. It is a simple and convenient prescreen for antitumor activity (*8*, *9*). Both tests are extensively used for the determination of acetogenins in plant extracts.

Leaves and branches of ylang-ylang were extracted with acetone. This extract was positive to the brine shrimp test ($LC_{50} \leq 150$ μg/mL). The water soluble part of the acetone extract showed toxicity to the brine shrimp ($LC_{50} \leq 75$ μg/mL), whereas the residue did not. HPLC analysis on a reversed-phase column showed the presence of two groups of peaks. Compound **2** (canangone) was found to be the largest peak (λ_{max} = 231 nm) of the second group. **2** was obtained as a white waxy solid after further purification by preparative HPLC. Compound **1** was found to be the largest peak (λ_{max} = 207.5 nm) of the first group and was positive to Kedde's reagent.

The molecular formula for canangone was found to be $C_{10}H_{12}O_4$ by EI mass spectrometry ([M]$^+$ *m*/*z* 196) and elemental analysis. The IR spectrum of **2** displayed characteristic bands for a hydroxyl group (3424 cm^{-1}), conjugated aldehyde (1681 cm^{-1}), and γ-lactone carbonyl function (1758 cm^{-1}). Structural analysis was carried out by NMR. Typical low-field signals at δ 9.50 (1H, s) and 6.68 ppm (1H, dt, J = 3.5, 1.8 Hz) in the ^{1}H NMR spectrum (400 MHz) suggested the presence of aldehydic and olefinic protons, respectively. Other relevant features in the ^{1}H NMR spectrum were the resonance for methylene [δ 4.34 (1H, ddd, J = 18.3, 9.2, 7.0 Hz)] and 4.38 ppm [1H, ddd, J = 18.1, 9.1, 3.5 Hz] and methine [δ 4.36 ppm (1H, m)] groups bearing an oxygen atom. The ^{1}H NMR further showed the presence of an exchangeable hydroxyl proton at δ 2.99 ppm (1H, d, J = 7.3 Hz) which disappeared upon addition of D_2O. Finally, complete ^{1}H−^{1}H correlations were established from the analysis of a gradient-selected COSY diagram (*10*). The hydroxyl position was further supported by the correlation peak observed with the methine signal located at δ 4.36 ppm.

The ^{13}C NMR data together with the DEPT spectrum for compound **2** confirmed the presence of previously determined functions and indicated an aliphatic quaternary carbon at δ 46.65 ppm. Finally, the location of the ethylenic proton stems from cross-peaks observed between H-6 and H-7 in the phase-sensitive NOESY diagram (*11*). It can be concluded from these data that **2** possesses a *spiro* carbon.

The structure of compound **2** was determined as 6-hydroxy-1-oxo-2-oxaspiro[4.5]dec-7-ene-8-carbaldehyde. All the carbon atoms were assigned with the help of the gradient-selected HMQC experiment (*12*). It should be noted that this unusual terpenoid skeleton was found in the *Juniperus communis* L. essential oil (*13*).

The molecular formula of compound **1** was found to be $C_5H_6O_3$ by EI mass spectrography ([M]$^+$ *m*/*z* 114) and elemental analysis. The presence in **1** of an α,β-unsaturated δ-lactone was first suggested by a positive reaction to Kedde's reagent and by an IR carbonyl absorption at 1743 cm^{-1}. It was confirmed by the typical low-field signals at δ 173.6 (C=O) and δ 7.37 (C=C), respectively, in the ^{13}C and ^{1}H NMR spectrum. The existence of an −OH group was indicated by an IR absorption at 3423 cm^{-1}and a resonance at δ 56.0 in the ^{13}C NMR spectrum. The ^{13}C NMR spectrum showed peaks corresponding to 5 carbon atoms.

The ^{1}H NMR spectrum exhibited two double doublets of two protons, at δ 4.45 and 4.85, which could be assigned to two methylene groups respectively at C6 and C5. These data allowed the unambiguous assignment of all carbon signals and confirmed

the structure of **1** as 3-hydroxymethyl-2(5*H*)-furanone, derived from the α-form of angelica lactone (5-methyl-2-furanone). Isosiphonodin has been isolated from two plants, *Euonymus europaeus* (Celastraceae) (*14*) and *Sedum telephium* (*15*), but it is also a major lactone in the small ermine moth *Yponomeuta vigintipunctatus* (*14*), the larvae of which feed on aerial parts of *Sedum telephium*. Lorimer et al. *(1*6) described the isolation of *β*-miroside, an antifungal furanone glucoside of which isosiphonodin is the aglycon. This compound showed antibacterial and cytotoxic activities, whereas the aglycon did not (*15*).

During this investigation, we did not find any unsaturated acetogenins in ylang-ylang extract. This work has led to the isolation of a new spirolactone with an unusual monoterpenoid structure. However, the aqueous extract showed toxicity, and canangone was not positive to the brine shrimp assay. Our study will be carried on until the structure−activity relationship is established.

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