Cazolobine, a New Sesquiterpene from *Isolona hexaloba* (Annonaceae)

Hilarion Mathouet[a], Isabelle Cazoulat[a], Hassan Oulyadi[b], Elisabeth Seguin[a], Adam Daïch[c], and Abdelhakim Elomri[a]

[a] Laboratoire de Pharmacognosie, Université de Rouen, Faculté de Médecine et de Pharmacie, 22 Boulevard Gambetta, F-76183 Rouen Cedex, France
[b] Laboratoire de RMN, Institut de Recherche en Chimie Organique Fine, UMR 6014-CNRS, Université de Rouen, F-76821 Mont-St-Aignan Cedex, France
[c] URCOM, EA3221, UFR des Sciences et Techniques de l'Université du Havre, 25 Rue Philippe Lebon, B.P: 540, 76058 Le Havre Cedex, France

Reprint requests to Dr. A. Elomri. Fax: 0033235148592. Hakim.Elomri@univ-rouen.fr

Z. Naturforsch. **59b,** 1118 – 1120 (2004); received April 19, 2004

Methyl 7-(5',5'-dimethyltetrahydrofuran-2'-yl)-3-methylocta-2,6-dieneoate (cazolobine), a new sesquiterpene derivative was isolated from the root of *Isolona hexaloba* (Annonaceae) collected in Gabon. The structure of cazolobine was elucidated on the basis of its spectral data, mainly MS and multiple-pulse NMR.

Key words: Isolona hexaloba, Sesquiterpene

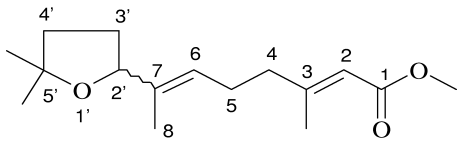
Introduction

The genus *Isolona* Engl. belongs to the family of the Annonaceae, sub family Monodoroideae. It consists in 20 species most originate from tropical Africa and Madagascar. Numerous species are used as medicinal plants. Thus, in the Ivory Coast *Isolona campanulata* Engl. & Diels is used as an aphrodisiac and for increasing fertility in sterile women [1, 2]. Some *Isolona* have been extensively studied from both chemical and pharmacological points of view as *I. cauliflora* [3], *I. ghesquiereina* [4], *I. zenkiri, I. pilosa* [5] and some compounds identified in several Isolona show antimalarial and antitrypanosomial properties [6].

I. hexaloba Engl. & Diels is a tree of 10 – 40 m high, with a trunk of 60 cm diameter. It grows in dense and humid forests of tropical Africa and has soft bark of 1 cm thickness. *I. hexaloba* is characterized by the horizontal positions of its petals of its flowers, and its ovoidal to sub globular fruits with bumps and longitudinal ribs. Leaves are 6 – 30 cm length, 3 – 10 cm large and sub coriaceous [7]. The plant is used in Zaire and Congo as a purgative and in treating sores, and smoke from the bark as a strained muscle relaxant [1]. The previous studies of *I. hexaloba* only concerned the major alkaloids of bark of roots [5]. In contribution to systematic chemical studies of African medicinal plants, we report here the structure determination of the methyl 7-(5',5'-dimethyltetrahydrofuran-2'-yl)-3-methylocta-2,6-dieneoate (cazolobine), a new sesquiterpene isolated from the root of *I. hexaloba* Engl. & Diels.

Results and Discussion

Repeated column chromatography of dichloromethane extract of the root of *I. hexaloba* yielded cazolobine (**1**) as pale yellow viscous oil. The empirical formula was determined by accurate elemental analysis as $C_{16}H_{26}O_3$. This formula suggests four degrees of unsaturation. The IR spectrum showed characteristic absorption bands at 1714, 1649, and 1265 cm^{-1} associated with carbonyl, alkenes and ether groups, respectively.



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The structure of cazolobine was deduced from both ^1H NMR and ^{13}C NMR spectrum.

The ^{13}C NMR and DEPT experiments of **1** showed 16 resonance lines consisting of five methyl carbons including methoxy signal at δ 51.3 ppm, three methines carbons, two of them were olefenic at 115.8

and 124.1 ppm, four methylene and four quaternary carbons including one carbonyl signal at δ 167.8 ppm.

^{1}H NMR spectrum of compound **1** confirmed the presence of five singlet methyl groups including methoxy signal at δ 3.71 ppm, two deshielded methines at δ 5.17 and 5.68 ppm probably olefinic structure and oxygen-bearing carbon.

The analysis of these data and a careful comparison of the ^{13}C NMR signals of **1** with those of methyl geranoate [8] suggest terpenic structure containing a chain with methyl ester, two double bonds not in conjugation positions and one carbocyclic ring. Location of these substitutions was carried out using an array of multi-impulsional experiments. Of particular interest were the following HMQC connectivities: i) H-3'a and H-3'b at 1.42 and 1.59 ppm, and C3', ii) H-4'a and H-4'b at 2.10 and 2.26 ppm, and C4', iii) 2 x CH_2 at 2.21 ppm and C4 and C5. One set of consecutive protons, H-4, H-5 and H-6, and the other set of consecutive protons, H-2', H-3' and H-4' are also revealed from COSY spectrum. These assignment, were checked by correlations observed in the HMBC spectrum and the relative configuration of all substituents in the structure of cazolobine (**1**) were justified from correlations in the NOESY spectrum.

Finally, it should be noted that biogenetically compound **1** most probably arises from a farnesyl pyrophosphate precursor of sesquiterpenes series and present original structure; moreover some monoterpenic analogues with a short chain have been isolated in Annonaceae family from *Annona cherimolia*, Mill. Fruits [9].

Experimental Section

General experimental procedures

NMR spectra (^{1}H, 600 MHz; ^{13}C, 150 MHz) were recorded on a Bruker Avance DMX 600 spectrometer. The assignment of ^{1}H and ^{13}C signals was supported by one- and two-dimensional ^{1}H-^{1}H COSY, DEPT, NOESY, ^{1}H-^{13}C HMBC, and HMQC experiments. All the experiments were recorded using $CDCl_3$ as solvent. Optical rotation was measured on a Perkin-Elmer 241 polarimeter. UV spectrum (λ_{max} in nm) was recorded in EtOH spectroscopic grade on a Beckman Model DU-600 spectrometer. The IR spectrum (ν_{max} in cm^{-1}) was obtained in neat film on a Perkin-Elmer FT-IR instrument. Column chromatographies were carried out with silica gel 20 – 45 μm. Flash column chromatographies were conducted using silica gel 60 Merck (35 – 70 μm) with an overpressure of 300 mbar. Mass spectra were recorded with a Hewlett Packard 5973 spectrometer, using electron impact (EI-MS) and desorption-chemical ionization (DCI-MS; reagent gas: NH_3) techniques.

Plant material

The roots of *Isolona hexaloba* (Engl. & Diels) were collected at Sibang (Gabon), in February 2003. A voucher sample (#16967) was deposited in National Herbarium of Gabon.

Extraction and isolation

The pulverized dried roots of *I. hexaloba* (718 g) were extracted successively with cyclohexane, dichloromethane and methanol at room temperature. Extracts were concentrated under reduced pressure on a rotary evaporator to yield the reddish brown sludge of cyclohexane (2.7 g, 0.38% dry wt), pale yellow sludge of dichloromethane (3.6 g, 0.50% dry wt) and reddish brown sludge of methanol (42.1 g, 5.85% dry wt).

The dichloromethane extracts of the roots (3.6 g) was subjected to a silica gel column chromatography with gradient of cyclohexane/CH_2Cl_2 (7/3) to CH_2C_2/MeOH (9/1) to give 73 fractions.

Fractions 47 – 49 (90 mg) were then fractionated by column chromatography on silica gel (20 – 45 μm) using a mixture of cyclohexane/AcOEt (3/2) as eluent to yield 54 mg of compound **1**.

Spectroscopic data

Cazolobine (**1**), $[\alpha]_D^{20} = 0^\circ$ (*c* 0.2, CH_2Cl_2 or EtOH). – UV (EtOH): $\lambda_{max}(\lg\varepsilon) = 217.5$ nm (4.13). – IR (neat): $\nu_{max} = 2984$ and 2952 (CH), 1714 (C=O), 1649 (C=C conj), 1436, 1385 and 1359 ($C(CH_3)_2$), 1265 (C-O), 1150 cm^{-1}. – ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 1.17$ (s, 3H, $(CH_3)_a$-5'), 1.22 (s, 3H, $(CH_3)_b$-5'), 1.42 (m, 1H, CH_a-3'), 1.59 (m, 1H, CH_b-3'), 1.64 (s, 3H, CH_3-8), 2.10 (m, 1H, CH_a-4'), 2.18 (s, 3H, CH_3-3), 2.21 (m, 4H, CH_2-4 and CH_2-5), 2.26 (m, 1H, CH_b-4'), 3.35 (dd, 1H, $J = 10$ Hz, $J = 2$ Hz, CH-2'), 3.71 (s, 3H, CH_3O-1), 5.17 (s, 1H, CH-6), 5.68 (s, 1H, CH-2). – ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 16.3$ (C-8), 19.2 (CH_3-C3), 23.5 ($(CH_3)_a$-C5'), 26.2 (C-5), 26.8 ($(CH_3)_b$-C5'), 30.0 (C-3'), 37.1 (C-4'), 41.2 (C-4), 51.3 (CH_3O-C1), 73.5 (C-5'), 78.4 (C-2'), 115.8 (C-2), 124.1 (C-6), 136.4 (C-7), 160.4 (C-3), 167.8 (C-1). – MS (EI, 70 eV) m/z (%) = 266 (2) [M$^{+\cdot}$], 225 (57), 193 (93), 183 (15), 163 (17), 135 (53), 123 (58), 114 (100). Analysis for $C_{16}H_{26}O_3$ (266.38): calcd. C 72.14, H 9.84; found C 72.25, H 9.78.

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