



TERPENOIDS FROM *COPAIBA CEARENSIS*

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Key Word Index—*Copaifera cearensis*; Leguminosae; copaiba oil; anti-inflammatory; KOH-impregnated silica gel flash column chromatography; sesquiterpenes.

Abstract—Crude oil of *Copaifera cearensis* was subjected to flash chromatography on KOH-impregnated silica gel from which three fractions were obtained: hexane, dichloromethane and methanol fractions. From the MeOH fraction a new sesquiterpenoid acid was identified. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Copaiba oils, also described as Copaiba balsams, are produced by extraction of the trunk of the trees belonging to the genera *Copaifera*. These trees are largely distributed in northern South America, mainly in the Brazilian Amazonas rain forest, in the states of Pará and Amazonas [1]. The main constituents of these oils are sesquiterpene hydrocarbons [2] and, because of the very high commercial value of these compounds, copaiba oils are largely exported from Brazil to be used in the flavour industry.

The anti-inflammatory properties of Copaiba oils have been known in Brazil for centuries [3]. There are descriptions of its use by Indian tribes, which applied these balsams to a baby's navel after cutting the umbilical cord in order to avoid infections. At the present time in Brazil, these oils are used in popular medicines, due to the belief that they have anti-inflammatory action. This has been supported by *in vitro* assays [4, 5].

RESULTS AND DISCUSSION

Chromatography of *Copaifera cearensis* L. oil on a KOH-impregnated silica gel column [6] afforded three fractions. GC-FTIR analysis of the first (eluted with hexane) and second (eluted with dichloromethane) fractions showed the presence of sesquiterpene hydrocarbons and a mixture of sesquiterpenoid alcohols and ketones, respectively. The last fraction was eluted from the column with methanol. Acidification with HCl solution, followed by extraction with dichloromethane, yielded a mixture of carboxylic acid diter-

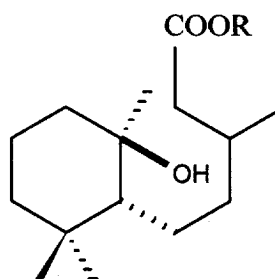
penes. This mixture was esterified with diazomethane and subjected to HPLC to give the methyl esters of the diterpenoid acids, eperuic [7], cativic [7], copalic [8, 9], kolavenic [10], chrolechinic [11], hardwickiic [11,12], patagonic [13], 8-hydroxylabdanoic [14], 3-cleroden-15,18-dioic [15], 3-cleroden-15,16-olide-18-oic [16] and a new sesquiterpenoid acid **1**. Known compounds were identified by comparison of their spectroscopic properties with those reported in the literature.

Compound **1** was identified as its methyl ester **1a**, which has the molecular formula $C_{16}H_{30}O_3$. The IR spectrum of **1a** showed the presence of a hydroxyl group (3636 cm^{-1}), and a carboxymethyl ester (1757 and 1165 cm^{-1}). The ^1H and ^{13}C NMR spectra (CDCl_3) showed signals attributed to four methyl groups, three of which were on quaternary saturated carbons (δ 0.77, 0.89 and 1.14; δ_C 21.3, 32.8 and 23.0) and one on a tertiary carbon (δ 0.95 *d*, $J = 6.5\text{ Hz}/30.8$), as well as a tertiary alcohol function (δ 74.0). The ^1H NMR spectrum also revealed a methylene group, adjacent to the carboxymethyl ester. This methylene group, in turn, was adjacent to a methine group which is coupled with a methyl group. The remaining carbons were identified as five methylene groups and one quaternary carbon. The assignments of all signals were fully confirmed by COSY and HETCOR experiments.

The axial configuration of the methyl group at C-10 was assigned by analogy from the δ 23.0 chemical shift, which was in agreement with the A-ring of velloziolone, a seco-diterpene isolated from *Vellozia caput-ardeae* [17].

Further support for the suggested structure was obtained from the EI mass spectrum of **1a**, which showed fragments at m/z 101(52) and at m/z 112(75). The first fragment results from the cleavage of the

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1: R=H

1a: R=CH₃

C(7)—C(8) bond. The second fragment can be explained by a McLafferty rearrangement with loss of methyl acetate, followed by loss of part of the A-ring affording hepten-6-one. The same ion arising from a McLafferty rearrangement at m/z 196(8) can lose a methyl group to give a fragment at m/z 181(15) and also lose water to give m/z 178(12), thus giving m/z 163(18) by loss of a methyl group.

EXPERIMENTAL

General

Copaifera cearensis crude oil obtained from the trunk of the tree was collected at the state of Minas Gerais, Brazil. The oil was subjected to McCarthy and Duthie KOH-impregnated silica gel pressurized cc. Silica gel (100 g, 63–200 μ m) was impregnated with 200 ml of a sat soln of KOH (250 g) in isopropanol (2 l) and CHCl₃ (500 ml), and the resulting suspension dried in rotary evaporator. 5 g of the oil was then submitted to flash chromatography using the KOH-impregnated silica gel. Elution with hexane (1.2 l), CH₂Cl₂ (1.2 l) and MeOH (1.0 l) yielded three distinct fractions containing, respectively, sesquiterpene hydrocarbons (2.6 g), sesquiterpenoid alcohols and ketones (0.5 g) and sesquiterpenoid carboxylic acids (1.7 g) after neutralization with diluted HCl and CH₂Cl₂ extraction.

HPLC. Semipreparative μ -Bondapak C-18 column (300 mm \times 78 mm i.d. \times 10 μ m, Waters) was used to separate the methyl esters of the carboxylic acids obtained after the esterification with CH₂N₂. MeOH-H₂O (17:3) at pH 5 with H₃PO₄ was used as mobile phase, UV detector (HP-1050) at 230 nm.

3-Methyl-5-(2,2,6-trimethyl-6-hydroxy-1-cyclohexyl)-pentanoic acid (1). GC-FTIR_{max} cm⁻¹: 3636, 1757 and 1165; ¹H NMR (300 MHz, CDCl₃): δ 0.77(3H, s), 0.89(3H, s), 0.95(3H, d, J = 6.5 Hz), 1.07 (H-5, t, J = 4.5 Hz), 1.14 (3H, s), 1.93 (H-8, m), 2.10(H-9, dd, J = 15 and 7.5 Hz) and 2.34(H-9, dd, J = 15 and 7.5 Hz); ¹³C NMR (75.25 MHz, CDCl₃): δ 43.1(C-1), 20.4(C-2), 39.6(C-3), 35.5(C-4), 57.0(C-

5), 23.1(C-6), 41.4(C-7), 30.8(C-8), 41.3(C-9), 74.9(C-10), 178.4(C-11), 19.8(C-12), 21.3(C-13), 32.8(C-14) and 23.0(C-15); HRGC-MS, 70 eV, m/z (rel.int) of **1a**: 270[M]⁺, 255(5), 252(10), 237(16), 223(14), 195(10), 181(15), 178(12), 163(15), 153(20), 123(30), 112(75), 110(50), 109(72), 101(52), 95(35), 82(35), 69(70), 45(52).

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