PERGAMON

Phytochemistry 51 (1999) 1013–1015

PHYTOCHEMISTRY

Copaïba oil: isolation and characterization of a new diterpenoid with the dinorlabdane skeleton

Honoré Monti[a,*], Nicolas Tiliacos[a], Robert Faure[b]

[a]*Laboratoire de Réactivité Organique Sélective, URA 1409, Faculté des Sciences d'Aix–Marseille III, Centre de St Jérôme, Avenue Escadrille Normandie–Niemen, 13397 Marseille Cedex 20, France*

[b]*ESA 6009, Faculté des Sciences d'Aix–Marseille III, Centre de St Jéôme, Avenue Escadrille Normandie–Niemen, 13397 Marseille cedex 20, France*

Received 20 April 1998; received in revised form 4 September 1998

Abstract

A new diterpenoid, (−)-15,16-dinorlabd-8(17)-en-3β,13-diol was isolated from copaïba oil and its structure elucidated by NMR spectroscopy. In addition, the two structurally known cleroda-3,13-dien-15-ol (kolavenol) and labda-8(17),13(*E*)-dien-15-ol (9,10-*anti*-copalol) were also identified for the first time in the product.

Keywords: Copaïfera sp.; Leguminosae; Copaïba oil; Diterpenoid; Dinorlabdane; (−)-15,16-dinorlabd-8(17)-en-3β,13-diol; Cleroda-3,13-dien-15-ol; labda-8(17),13(*E*)-dien-15-ol; 1-D and 2-D NMR

1. Introduction

Copaïba oleoresin is obtained from the trunk of various species of the genus *Copaifera* L. (Leguminosae).

In continuation with our chemical studies on the deterpened neutral fraction of this oleoresin (Monti, Tiliacos, & Faure, 1996; Monti, Tiliacos, Faure, & Aubert, 1997), we report here the isolation and characterization of a new diterpenoid, (−)-15,16-dinorlabd-8(17)-en-3β,13-diol **1** and two already known compounds, cleroda-3,13-dien-15-ol (kolavenol) **2** and labda-8(17),13(*E*)-dien-15-ol (9,10-*anti*-copalol) **3**. Their stereostructures were established on the basis of one- and two-dimensional NMR experiments.

2. Results and discussion

(−)-15,16-dinorlabd-8(17)-en-3β,13-diol **1** was isolated as an oil $\{[\alpha]_D^{25}$ −1.70 ($CHCl_3$; $c=0.7$)$\}$. The IR spectrum showed significant bands for hydroxyl groups (3340 cm^{-1}) and exocyclic double bond (3060, 1640 and 890 cm^{-1}). Its E.I. mass spectrum gave a $[M]^+$ peak at m/z 280 consistent with a molecular formula of $C_{18}H_{32}O_2$.

The ^1H NMR spectrum (400 MHz) displayed four methyl protons, three as singlets at δ 0.66, 0.75 and 0.97, and the other one as a doublet at δ 1.16 ($J=6.2$ Hz). Close inspection of the remaining ^1H resonances established the presence of an exocyclic methylene group (two one proton quadruplets at δ 4.48 ($J=1.4$ Hz) and 4.82 ($J=1.4$ Hz)), two protons on two carbons bearing a hydroxyl function [δ 3.23, (dd, $J=11.7$, 4.5) and δ 3.74 (m)] and allylic methylene signals at δ 1.95 (br dt, $J=12.8$, 5.3) and δ 2.38 (ddd, $J=12.8$, 4.2, 2.4).

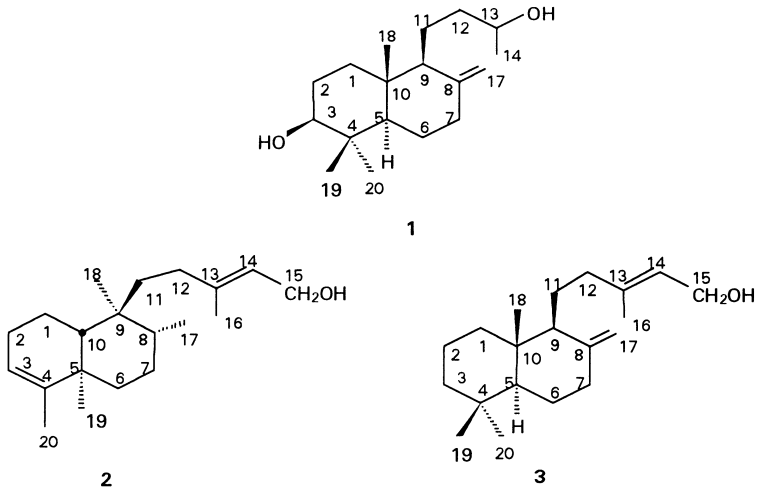
In the ^{13}C NMR spectrum, the presence of an exocyclic double bond ($C{=}CH_2$) and two hydroxyl groups were respectively supported by the resonances at δ 148.19, 106.78, 78.94 and 68.90.

The multiplicities of the other ^{13}C NMR signals, deduced from the DEPT pulse sequence (Doddrell, Pegg, & Bendall, 1982), were indicative of four methyl, seven methylene, four methine and three quaternary aliphatic carbons. Further, the presence of a *gem*-dimethyl group was supported by the number of sp^3-hybridized quaternary carbons. These results suggested a bicyclic dinorditerpenediol skeleton with an exocyclic methylene group.

At this point, compound **1** was identified as (−)-15,16-dinorlabd-8(17)-en-3β,13-diol because its ^{13}C NMR chemical shifts (Table 1) were in good agreement with those of the alcohol yielded by reduction with $LiAlH_4$ of the corresponding 13-oxo compound previously described by us (Monti et al., 1996). However, like for the ketone, the absolute configuration of **1** remains unsolved.

* Corresponding author. Fax: +33-4-9128-8862; e-mail: honore.monti@ros.u-3mrs.fr

0031-9422/99/$ - see front matter
PII: S0031-9422(98)00711-0



1 2 3

Structure 1.

Table 1
^{13}C NMR chemical shifts[a] for (−)-15,16-dinorlabd-8(17)-en-3(*β*),13-diol **1**

Carbon	**1**
1	37.16
2	27.98
3	78.94
4	39.18
5	54.69
6	24.06
7	38.23
8	148.19
9	56.80
10	39.52
11	20.05
12	38.54
13	68.90
14	23.60
17	106.78
18	14.47
19	15.46
20	28.36

[a] δ in ppm from TMS. Assignments obtained by concerted use of 2-D experiments.

Semipreparative high performance liquid chromatography of this deterpened neutral fraction allowed also the isolation of a mixture of two other compounds, **2** and **3**, which we were unable to obtain pure. However, their stereostructures as cleroda-3,13-dien-15-ol **2** (kolavenol) and labda-8(17),13-dien-15-ol **3** (9,10-*anti*-copalol) were unambiguously assigned by comparison of their ^{13}C NMR chemical shifts and the literature values (Kapadi, Soman, Sobti, & Sukh, 1983; Yee & Coates, 1992; Piers & Roberge, 1992; Bloor & Gainsford, 1993; Lu, Menelaou, Vargas, Fronczek, & Fisher, 1993; Ansell, Pegel, & Taylor, 1993; Nagashima, Tanaka, Kan, Huneck, & Asakawa, 1995; Su, Fang, & Cheng, 1996).

3. Experimental

3.1. General

All NMR spectra were recorded on a Bruker AMX-400 spectrometer in $CDCl_3$ solutions; TMS was used as standard in ^1H and ^{13}C measurements. Standard Bruker pulse sequences were used for homonuclear and heteronuclear correlation experiments. For other NMR experimental details, see (Rahariveldmanana, Bianchini, Cambon, Azzaro, & Faure, 1995). EI-MS (70 eV) were recorded on a Hewlett Packard 5987 Spectrometer (temperature 170°C). Specific rotations were determined with a Perkin Elmer 241 polarimeter, at the wavelength 529 nm (Na) and 25°C. CC were made over silica gel 60H and eluted with increasing gradients of Et_2O–pentane under low pressure (air). For TLC, Merck $60F_{254}$ silica gel plates were used. HPLC (lichrosorb L5.25F, 5 μm, 250 mm) were performed using a mixture of ethyl acetate and isooctane (1:4 v/v). IR ν_{max}^{film} were recorded on a Perkin Elmer 257 spectrophotometer.

3.2. Isolation

Commercial copaïba oil (100 g) was chromatographed over a silica gel column using pentane to remove the hydrocarbon sesquiterpenic fr. Elution with methanol gave, after evaporation, the deterpened fraction which was then dissolved in Et_2O and washed with 5% aqueous KOH. The alkaline layer was discarded and the organic phase washed with brine, dried over $MgSO_4$ and concentrated, yielding the deterpened 'neutral' fraction (DNF) (fraction without acid components). CC of 2.5 g of this fr. (gradient of Et_2O:pentane) provided 35 mg of **1**. Semipreparative HPLC (ethylacetate:isooctane, 1:4) of 1 g yielded 31 mg of a mixture containing **2** and **3**.

3.3. (−)-15,16-Dinorlabd-8(17)-en-3(β),13-diol **1**

Oil, $\{[\alpha]_D^{25} -1.70$ ($CHCl_3$; $c=0.7$)$\}$. IR ν_{max}^{film} (cm^{-1}) 3340, 3060, 1640, 890; EI-MS 70 eV, m/z (rel. int. %): 280 $[M]^+$ (2), 262 (7), 247 (5), 229 (10), 207, 189 (15); Anal. (%): Found: C, 77.09; H, 11.46 $C_{18}H_{32}O_2$ requires: C,77.14; H, 11.43. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.66 (3H, s, H-18), 0.75 (3H, s, H-19), 0.97 (3H, s, H-20), 1.06 (1H, dd, $J=12.5$, 2.8 Hz, H-5), 1.16 (3H, d, $J=6.2$ Hz, H-14), 1.95 (1H, br dt, J = 5.3, 12.8 Hz, H-7α), 2.38 (1H, ddd, $J=2.4$, 4.2, 12.8 Hz, H-7β), 3.23 (1H, dd, $J=11.7$, 4.5 Hz, H-3), 3.74 (1H, m, H-13), 4.48 (1H, q, 1.4 Hz, H-17 A), 4.82 (1H, q, 1.4 Hz, H-17 B); ^{13}C NMR (100.61 MHz, $CDCl_3$): Table 1.

3.4. Cleroda-3,13-dien-15-ol **2** (kolavenol)

^{13}C NMR (100.61 MHz, $CDCl_3$): δ 18.37 (C-1), 26.98 (C-2), 120.52 (C-3), 144.60 (C-4), 38.28 (C-5), 36.94 (C-6), 27.61 (C-7), 36.36 (C-8), 38.72 (C-9), 46.53 (C-10), 36.63 (C-11), 32.95 (C-12), 140.93 (C-13), 123.13 (C-14), 59.51 (C-15), 16.52 (C-16), 16.07 (C-17), 18.45 (C-18), 20.03 (C-19), 18.07 (C-20).

3.5. labda-8(17),13-Dien-15-ol **3** (9,10-anti-copalol)

^{13}C NMR (100.61 MHz, $CDCl_3$): δ 39.19 (C-1), 21.75 (C-2), 42.29 (C-3), 33.68 (C-4), 55.67 (C-5), 24.55 (C-6), 38.45 (C-7), 148.67 (C-8), 56.34 (C-9), 39.74 (C-10), 19.49 (C-11), 38.49 (C-12), 140.82 (C-13), 124.00 (C-14), 59.55 (C-15), 16.48 (C-16), 106.34 (C-17), 33.71 (C-18), 21.81 (C-19), 14.60 (C-20).

Acknowledgements

This work was performed with financial support of Technico Flor (Allauch, France) under research grant (Cifre Convention No. 361-94, ANRT). The authors acknowledge Dr. Aubert C. (Faculté de Pharmacie, Marseille, France) for running MS experiments.

References

Ansell, S. M., Pegel, K. H., & Taylor, D. A. H. (1993). *Phytochemistry*, *32*, 945–953.

Bloor, S. J., & Gainsford, G. J. (1993). *Australian Journal of Chemistry*, *46*, 1099.

Doddrell, D. M., Pegg, D. T., & Bendall, M. R. (1982). *Journal of Magnetic Resonance*, *48*, 323.

Kapadi, A. H., Soman, R., Sobti, R. R., & Sukh, D. (1983). *Indian Journal of Chemistry B*, *22*, 964.

Lu, T., Menelaou, M. A., Vargas, D., Fronczek, F. R., & Fisher, N. H. (1993). *Phytochemistry*, *32*, 1483.

Monti, H., Tiliacos, N., & Faure, R. (1996). *Phytochemistry*, *42*, 1653.

Monti, H., Tiliacos, N., Faure, R., & Aubert, C. (1997). *Pharmaceutical Pharmacological Letters*, *7*, 41.

Nagashima, F., Tanaka, H., Kan, Y., Huneck, S., & Asakawa, Y. (1995). *Phytochemistry*, *40*, 209.

Piers, E., & Roberge, J. Y. (1992). *Tetrahedron Letters*, *33*, 6923.

Rahariveldmanana, P., Bianchini, J. -P., Cambon, A., Azzaro, M., & Faure, R. (1995). *Magnetic Resonance in Chemistry*, *33*, 233.

Su, W. -C., Fang, J. -M., & Cheng, Y. -S. (1996). *Phytochemistry*, *41*, 255.

Yee, N. K. N., & Coates, R. M. (1992). *Journal of Organic Chemistry*, *57*, 4598.