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TWO DITERPENOIDS FROM COPAIBA OIL

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Key Word Index—*Copaifera* sp.; Leguminosae; copaiba oil; (+)-7α-acetoxybacchotricuneatin D; (−)-3β-hydroxy-15,16-dinorlabd-8(17)-ene-13-one; diterpenoid; furanoid clerodane; dinorlabdane.

Abstract—Two new diterpenoids with the furanoid clerodane and dinorlabdane carbon skeletons, termed respectively (+)-7α-acetoxybacchotricuneatin D and (−)-3β-hydroxy-15,16-dinorlabd-8(17)-ene-13-one, were isolated from copaiba oil and their structures were elucidated by NMR spectroscopy.

INTRODUCTION

Commercial copaiba oil is a mixture of oleoresins extracted from *Copaifera* species [1]. Originally, it was used for medicinal purposes as an antiseptic, expectorant, diuretic and antimicrobial [2–7]. It is also used in cosmetics, as a base for soaps and bubble baths [8], diesel-like fuels [9, 10] (i.e. an alternative source of energy [11]), and recently, a French patent presents a natural preservative which contains this oil [12]. Thus, numerous studies [13–17] have been carried out to identify the different compounds which make up this oleoresin.

In the present paper, we report on the isolation and identification of two new diterpenoids, (+)-7α-acetoxybacchotricuneatin D (**1**) and (−)-3β-hydroxy-15,16-dinorlabd-8(17)-ene-13-one (**2**), the structures of which were established on the basis of one- and two-dimensional NMR spectral experiments.

RESULTS AND DISCUSSION

(+)-7α-Acetoxybacchotricuneatin D (**1**) was obtained as an oil {$[\alpha]_D^{25}$ +9.39 ($CHCl_3$; *c* 1.4)}. The IR spectrum showed significant bands for a hydroxyl group (3400 cm^{-1}), an ester group (1740 cm^{-1}) and a furan ring (1460, 880 cm^{-1}).

The ^1H NMR (400 MHz) spectrum of compound **1** displayed three methyl protons singlets at δ 0.96, 1.23 and 2.02 (acetate) and one methyl proton doublet at δ 0.89 ($^3J = 7$ Hz). The spectrum also exhibited the resonances of one ethylenic group (δ 5.52, *br s*), a hydroxymethylene (δ 4.08 and δ 4.03, *br* AB quartet, $J = 13$ Hz) and one proton on a carbon bearing an acetate function (δ 5.11, *q*, $J = 3.3$ Hz). Moreover, a set of ^1H NMR signals at δ 6.21 (*m*), 7.17 (*m*), 7.31 (*t*, $J = 1.5$ Hz) was diagnostic of a β-monosubstituted furan ring.

The molecular ion cannot be determined by either EI- or CI-mass spectrometry because the compound is thermolabile. However, microanalyses suggested a molecular formula corresponding to $C_{22}H_{32}O_4$. This formula is also in agreement with a characteristic fragment ion at *m/z* 279 resulting from the loss of furan ring.

The ^{13}C NMR spectrum confirmed the presence of a tertiary double bond, a furan ring and an acetate function. From the multiplicities of the ^{13}C NMR signals determined from the DEPT pulse sequence [18] and the above results, it was concluded that **1** was a furanoid bicyclic compound.

As previously reported [19], structural determination of any natural product can be accomplished from the concerted use of homonuclear and both direct and long-range heteronuclear chemical shift correlation experiments. One-bond ^1H–^{13}C connectivities were established using the proton-detected C,H correlation technique HMQC [20]. Multibond connectivities were determined from the analysis of long-range correlation response over two or three bonds (^2J or ^3J couplings) using a HMBC [21] diagram. Thus, for compound **1**, the substructure **A1** was deduced from the connectivities observed between the proton methyl shifts and the carbons α and β to these groups.

Finally, analysis of the long-range heteronuclear correlation responses for the other ^1H resonances, in conjunction with the one-dimensional NMR data and the proton intercoupling network determined from the homonuclear ^1H–^1H correlation spectrum [22, 23], permitted us to assign the other resonances found in the

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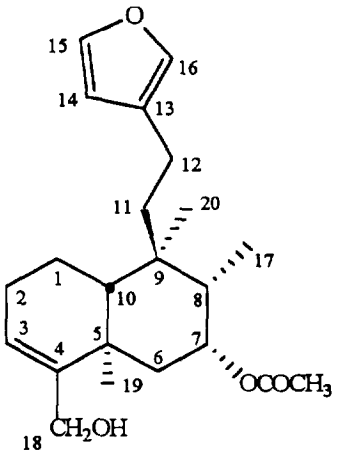

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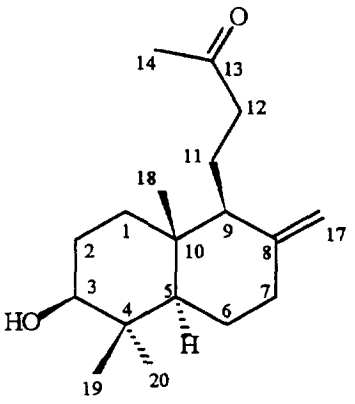


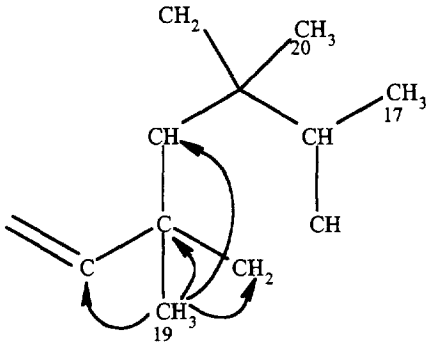
2

previously determined structural fragments. Compound **1** was then identified as (+)-7α-acetoxybacchotricuneatin D, and its ^{13}C NMR chemical shifts (Table 1) were in good agreement with those of the previously reported bacchotricuneatin D [14, 24].

Compound **2** [(oil, $[\alpha]_D^{25}$ −1.00 ($CHCl_3$, *c* 1.4))] displayed hydroxyl and ketone carbonyl bands at 3450 and 1720 cm^{-1}. Its mass spectrum showed a molecular peak at *m/z* 278, which agrees with the molecular formula $C_{18}H_{30}O_2$. The ^{1}H NMR spectrum (400 MHz) indicated four singlets at δ 0.65 (3H), 0.75 (3H), 0.95 (3H) and 2.07 (3H). The last signal was assigned to a carbonyl methyl group. Close inspection of the remaining ^{1}H resonances established the presence of an exocyclic methylene group (two broad one proton singlets at δ 4.41 and δ 4.80) and an oxygen-bearing methine proton (δ 4.12, *dd*, *J* = 11.8 and 4 Hz). In the ^{13} NMR spectrum, the presence of a carbonyl function and a secondary double bond ($C{=}CH_2$) was supported, respectively, by the resonance at δ 209.5, 147.8 and 106.8.

The multiplicities of the other ^{13}C NMR signals, deduced from the DEPT pulse sequence, were indicative of four methyl, six methylene, three methine and two quarternary aliphatic carbons. Moreover, the presence of a gemdimethyl group was further supported by the number of sp^3-hybridized quarternary carbons. These results suggested that compound **2** was a bicyclic dinorditerpenol with a carbonyl function.

Finally, a combination of two-dimensional experiments (COSY, HMQC and HMBC) was used to determine the structural units of compound **2** (Fig. 1). The structure of (−)-3β-hydroxy-15,16-dinorlabd-



A1

Table 1. ^{13}C NMR chemical shifts* for (+)-7α-acetoxy-bacchotricuneatin D (**1**) and (−)-3β-hydroxy-15,16-dinorlabd-8(17)-ene-13-one (**2**)

C	1	2
1	17.9	37.0
2	26.4	27.9
3	121.9	78.8
4	148.0	39.2
5	37.0	54.6
6	39.3	24.0
7	75.1	38.1
8	38.1	147.8
9	38.4	56.0
10	46.1	39.5
11	39.5	17.6
12	18.5	42.8
13	125.4	209.5
14	111.0	30.1
15	142.2	–
16	138.5	–
17	12.2	106.8
18	62.8	14.4
19	23.0	15.5
20	19.6	28.4
CO	170.8	
CH_3	21.5	

*δ in ppm from TMS. Assignments obtained by concerted use of two-dimensional experiments.

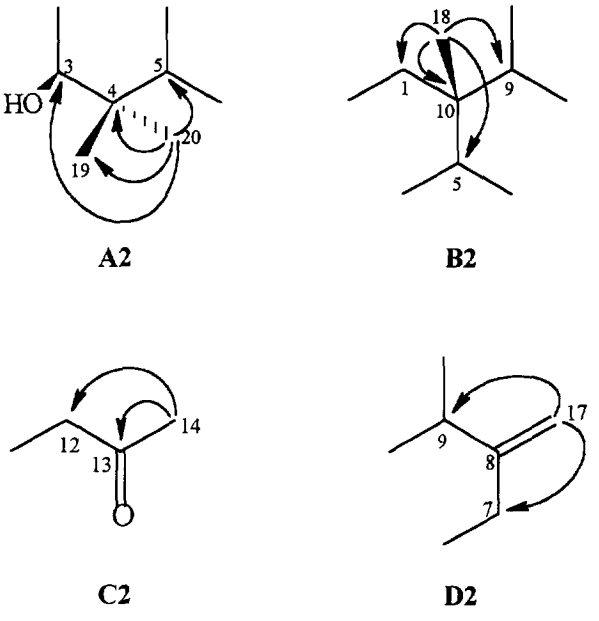


Fig. 1. Structural fragments (**A2** to **D2**) of 3β-hydroxy-15,16-dinorlabd-8(17)-ene-13-one (**2**) determined from the HMBC connectivities observed for the methyl and vinyl protons.

8(17)-ene-13-one was deduced from these observations.

It is noteworthy, that H-3 α resonated as a doublet–doublet at δ 3.21 (J = 12.4 and 2.7 Hz), whereas H-3β resonated as a triplet at δ 3.43 (J = 2.7 Hz) [25].

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR : $CDCl_3$, TMS as int. standard. Standard pulse sequences were used for homonuclear and heteronuclear correlation experiments. For other NMR experimental details, see ref [26]. EI-MS: 20 eV; CI-MS: 200 eV with a CH_4 press. ion source equal to 1 mm Hg and a source temp. of 170°. Specific rotations: 529 nm (Na) and 25°. CC: silica gel 60 H, eluted with increasing gradients of Et_2O–pentane under low press. (air).

Isolation. Commercial copaiba oil (200 g) was rapidly chromatographed on silica gel with pentane to remove the hydrocarbon sesquiterpenoid fr. Elution with MeOH gave after evapn of the solvent, the deterpened fr. (92 g) which was then dissolved in 300 ml Et_2O and washed with 5% aq. KOH (4 × 150 ml). The alkaline layer was removed and the organic phase washed with brine, dried over $MgSO_4$ and concd, yielding 33 g of a neutral de-terpened fraction. CC of 2.3 g this fr. yielded 90 mg of a mixt. containing **1** and **2** (same R_f on TLC in all solvent systems tested. The different reactivities of allylic and secondary alcohols were used to separate these two compounds: Ac_2O (0.1 ml) and pyridine (5 ml) were added, the mixt. stirred at room temp. for 90 min, then poured onto acid ice-water and extracted with Et_2O. The organic phase was dried over molecular sieves (4 Å), concd and chromatographed on silica gel to yield 35 mg of (+)-7α-acetoxybacchotricuneatin D acetate (**1**-Ac) and 30 mg of (−)-3β-hydroxy-15,16-dinorlabd-8(17)-ene-13-one (**2**). Selective deprotection of **1**-Ac with K_2CO_3/MeOH at reflux for 60 min provided the expected alcohol (**1**) in 95% yield.

(+)-7α-*Acetoxybacchotricuneatin D* (**1**). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (3H, *s*, H-17), 0.96 (3H, *s*, H-20), 1.23 (3H, *s*, H-19), 2.02 (3H, *s*, $MeCO_2$), 4.03 (1H, *br d*, J = 13.0 Hz, H-18A), 4.08 (1H, *br d*, J = 13.0 Hz, H-18B), 5.11 (1H, *q*, J = 3.3 Hz, H-7), 5.52 (1H, *m*, H-3), 6.21 (1H, *m*, H-14), 7.17 (1H, *m*, H-16), 7.31 (1H, *t*, J = 1.5 Hz, H-15); ^{13}C NMR (100 MHz, $CDCl_3$): Table 1; IR ν_{max}^{film} (cm^{-1}): 3400, 2920, 2850, 1740, 1460, 1400, 1380, 880; EI-MS (probe) 20 eV, *mz*: 279 [furan]$^+$, 220 [furan − CH_3CO_2]$^+$, 205 [furan − CH_3CO_2 − CH_3]$^+$, 187 [furan − CH_3CO_2 − CH_3 − H_2O]$^+$, 95 [C_6H_7O]$^+$, 81 [C_5H_5O]$^+$; Analysis (%): Found: C, 73.38; H, 8.91. $C_{22}H_{32}O_4$ requires: C, 73.29; H, 8.95.

(−)-3β-*Hydroxy*-15,16-*dinorlabd*-8(17)-*ene*-13-*one* (**2**). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.65 (3H, *s*, H-18), 0.73 (3H, *s*, H-19), 0.95 (3H, *s*, H-20), 1.03 (1H, *dd*, J = 12.5, 2.7 Hz, H-5), 2.07 (3H, *s*, H-14), 2.27 (1H, *dd*, J = 17.8, 7.6 Hz, H-12A), 2.36 (1H, *ddd*, J = 12.7, 4.3, 2.3 Hz, H-7), 2.54 (1H, *ddd*, J = 18.0, 9.0, 3.8 Hz, H-12B), 3.21 (1H, *dd*, J = 12.5, 2.7 Hz, H-3), 4.41 (1H, *br s*, H-17A), 4.80 (1H, *br s*, H-17B); ^{13}C NMR (100 MHz, $CDCl_3$): Table 1; IR ν_{max}^{film} (cm^{-1}): 3450, 2950, 2840, 1720, 1640, 1460, 1380; EI-MS (probe) 20 eV, *m/z* (rel. int.): 278 [M]$^+$ (2), 260 [M − H_2O]$^+$ (7), 245 [M − H_2O − CH_3]$^+$ (5), 220 [M − C_3H_5O]$^+$ (10), 202 {[M − H_2O − CH_3 − CH_3CO]$^+$ and [M − C_3H_5O − H_2O]$^+$ (11)}, 187 [M − C_3H_5O − H_2O − CH_3]$^+$ (15); CI-MS (CH_4, probe) 200 eV, *m/z* (rel. int.): 279 [M + 1]$^+$ (43), 261 [M +

$1 - H_2O]^+$ (100), 243 $[M + 1 - H_2O - H_2O]^+$ (33), 221 $[M + 1 - CH_3CO - CH_3]^+$ (52), 203 $[M + 1 - CH_3CO - CH_3 - H_2O]^+$ (33); Analysis (%): Found: C, 77.73; H, 10.93. $C_{18}H_{30}O_2$ requires: C, 77.64; H, 10.87.

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