Absolute Configuration of some Dinorlabdanes from the Copaiba Oil

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Um novo ent-dinorlabdano (–)-13(R)-14,15-dinorlabd-8(17)-eno-3,13-diol foi isolado a partir do óleo de copaíba comercial juntamente com dois outros dinorditerpenos conhecidos. A configuração absoluta destes dinorditerpenos foi determinada pela primeira vez através de síntese partindo do ácido (–)-3-hidróxi-copalico isolado do mesmo óleo.

A novel ent-dinorlabdane (–)-13(R)-14,15-dinorlabd-8(17)-ene-3,13-diol was isolated from commercial copaiba oil along with two known dinorlabdanes. The absolute configuration of these dinorditerpenes was established for the first time through synthesis starting from known (–)-3-hydroxycopalic acid, which was also isolated from the same oleoresin.

Keywords: Copaiba oil, dinorditerpenes, absolute configuration

Introduction

Copaiba oil is a resin exudate obtained from the Copaifera sp. tree (Fabaceae-Caesalpinioideae) distributed throughout the Amazon basin. This resin is commonly used in folk medicine to treat inflammations and tumors, especially in northern Brazil. In early investigations, diterpenes belonging to the clerodane, ent-labdane, labdane and ent-kaurane skeletons were isolated from copaiba oil and recently, the presence of dinorditerpenes was reported. This paper describes the isolation and structural elucidation of three dinorditerpenes, each bearing a small excess of the levorotatory enantiomer. The new (–)-13(R)-14,15-dinorlabd-8(17)-ene-3,13-diol (1) was isolated together with known (–)-13(S)-14,15-dinorlabd-8(17)-ene-3β,13-diol (2) and (–)-3-hydroxy-14,15-dinorlabd-8(17)-en-13-one (3), and additional diterpenes previously described in the literature (Figure 1). The absolute configurations of diols (–)–1 and (–)–2 and hydroxyl-ketone (–)–3 were elucidated through total synthesis beginning from (–)-3-hydroxycopalic acid (4). The stereochemistry of the carbinolic carbon at C-13 of 1 and 2 was established through analysis of 1H NMR spectra of (S)-α-methoxyphenylacetate derivatives.

Results and Discussion

The commercial copaiba oleoresin was fractionated as described in the Experimental Section. Successive column chromatography on SiO2 of the neutral fraction employing a gradient of petroleum ether and Et2O furnished two known ent-dinorlabdanes. These were characterized as (–)-3-hydroxy-14,15-dinorlabd-8(17)-en-13-one (3) {oil, [α] 20°–1.3° (c 1.6, CHCl3), lit.10 [α] 20°–1.0° (c 1.4, CHCl3)} and (–)-13(S)-14,15-dinorlabd-8(17)-ene-3,13-diol (2) {oil, [α] 20°–1.0° (c 1.7, CHCl3), lit.11 [α] 20°–1.7° (c 0.7, CHCl3)}. All other spectral data for both compounds matched those previously reported in the literature. A third ent-dinorlabdane, identified as the novel (–)-13(R)-14,15-dinorlabd-8(17)-ene-3,13-diol (1), was isolated as colorless crystals, mp 165.0-166.5 °C, [α] 20°–1.3° (c 1.1, CHCl3). The HREIMS spectrum indicated a molecular formula of C 18H32O2 (m/z 281.2484, [M+H]+) and the IR spectrum showed characteristic absorptions of a hydroxyl group at 3333 cm–1 and an exocyclic double bond at 2930, 1642, and 885 cm–1. The contour of the 1H NMR spectrum of 1 was nearly superimposable on that of 2 and displayed three methyl group singlets at δ 0.70, 0.78 and 1.00, and one
methyl group doublet at $\delta$ 1.18 ($J$ 6.2 Hz). The presence of two characteristic exocyclic methylene hydrogens was also confirmed as singlets at $\delta$ 4.56 and 4.85, and two carbinolic hydrogens at $\delta$ 3.25 (dd, $J$ 11.5, 4.6 Hz) and $\delta$ 3.77 (m) were also present. The $^{13}$C NMR spectra displayed resonances for the four methyl groups at $\delta$ 14.4, 15.4, 23.7 and 28.3, for the exocyclic methylene carbons at $\delta$ 147.9 and 106.9, and for the two carbinolic carbons at $\delta$ 68.4 and $\delta$ 78.8. Based on these spectroscopic data and considering their similarity with those of compound 2, structure 1, a C-13 epimer of 2, was proposed. To confirm the structure and subsequently elucidate the absolute configuration of any of the natural dinorlabdanes, the synthesis of the dinorlabdanes 1-3 was undertaken starting from known (–)-3-hydroxycopalic acid (4), isolated from the same oleoresin (Scheme 1).

![Scheme 1](image_url)

The synthesis began with (–)-3-hydroxycopalic acid (4) [colorless crystals, mp 153-155 °C, $[\alpha]_D^{20}+38.3$° ($c$ 0.8, CHCl$_3$), lit.$^4$ mp 158-160 °C, $[\alpha]_D^{20}+38.7$° ($c$ 3.0, CHCl$_3$)], which was submitted to an oxidative cleavage of the side chain with KMnO$_4$.$^{13}$ After work-up and purification on SiO$_2$ (hexane:EtOAc, 85:15), keto-alcohol 3 was obtained in 80% yield. All spectroscopic data of 3 were identical with those reported for the natural product, except for the optical rotation, for which a higher value was observed for the synthetic product [{[\alpha]_D^{20}+8.8$°$ (c 1.5, CHCl$_3$) and [\alpha]_D^{10}+1.3$°$ (c 1.6, CHCl$_3$) for the natural product}, lit.$^3$ $[\alpha]_D^{10}+1.0$° (c 1.4, CHCl$_3$)]. Next, the synthesis of keto-alcohol 3 with LiAlH$_4$ and purification on SiO$_2$ (petroleum ether : Et$_2$O; 9:1) furnished epimeric diols 1 and 2. The less polar diol was isolated with a 49% yield as colorless crystals, mp 165.0-167.0 °C, $[\alpha]_D^{20}+27.0$° ($c$ 1.1, CHCl$_3$) [natural product proposed as 1: mp 165.0-166.5 °C, $[\alpha]_D^{20}+13.1$° ($c$ 1.1, CHCl$_3$) and the more polar diol (46% yield) was also isolated as colorless crystals, mp 169.5-171.0 °C, $[\alpha]_D^{20}+12.9$° ($c$ 1.7, CHCl$_3$) [natural product identified as 2: oil, $[\alpha]_D^{10}+1.0$° ($c$ 1.7, CHCl$_3$), lit.$^1$ $[\alpha]_D^{10}+1.7$° ($c$ 0.7, CHCl$_3$)]. All spectroscopic data for both synthetic diols (1 and 2) were in agreement with those observed for the natural products, except for the optical rotation for which a higher value was observed for the synthetic products. Finally, in order to establish the absolute configuration of the carbon at C-13 of diols 1 and 2, the C-13 (S)-(+)-$\alpha$-methoxyphenylacetate derivatives 5 and 6 were prepared in 90% and 85% yield, respectively, using Trost’s protocol.$^{14}$ According to the Trost model, the $^1$H NMR chemical shift of the methyl group at C-16 of ester 5 was observed at $\delta$ 1.12 (upfield) and the C-16 methyl group of ester 6 was observed at $\delta$ 1.21 (downfield), indicating the absolute configuration of carbon C-13 for isomer 5 as $R$ and for isomer 6 as $S$ (Figure 2). No signals corresponding to the diastereoisomeric ester prepared from the possible enantiomer of acid 4 were observed.

![Figure 2](image_url)

Reduction of a sample containing natural dinorlabdane 3 with LiAlH$_4$ also yielded the C-13-epimeric diols 1 and 2 with the same absolute value for the optical rotation observed for the isolated natural products. Thus, in the present investigation we observed that dinorditerpenes 1-3 are present in the resin as a mixture of enantiomers. At this point, the (–)-3-hydroxycopalic acid (4)$^{15}$ was considered enantimERICally pure since the optical rotation was comparable with that reported for the enantiomer isolated from the leaves of Metasequoia glyptostroboids.$^{16}$ [mp 157.5-158.5 °C, $[\alpha]_D^{20}+40.7$° ($c$ 2.0, CHCl$_3$)] and for the corresponding methyl ester derivative isolated from the needles of Pinus pumila$^{17}$ [[$\alpha]_D^{20}+36.0$° ($c$ 13.0, CHCl$_3$)] for methyl (–)-3-hydroxycopalate (4a), $[\alpha]_D^{20}+35.0$° ($c$ 2.0, CHCl$_3$)].

**Experimental**

**General**

$^1$H (500 MHz) and $^{13}$C NMR (125 MHz) spectra were recorded in CDCl$_3$ solution on an INOVA 500 spectrometer, with $\delta$ (ppm), $J$ in Hz, and spectra referred to CDCl$_3$ ($\delta$ 7.27 for $^1$H and 77.0 for $^{13}$C) as an internal standard. IR spectra of neat samples or as a KBr disk were measured using a Perkin-Elmer 1600 series FTIR. The mass spectra of purified compounds were recorded with a Hewlett-Packard 5890 GC equipped with a Model 5970 mass-selective
Isolation

Commercial copaiba oleoresin (Copaifera sp.) (301 g), purchased at “Botica Veado d’ouro”, the market in São Paulo, São Paulo State, was dissolved in Et₂O (600 mL) and extracted with 5% KOH (5 x 100 mL). The aqueous layer was acidified with HCl (pH ca. 2), and extracted with Et₂O (5 x 100 mL). The combined organic layers were washed with brine until neutral, dried over anhydrous Na₂SO₄, and concentrated under vacuum to afford 244 g (81.1%) of the neutral fraction and 55 g (18.3%) of the acidic fraction. Percolation of the neutral fraction (100 g) on silica gel, eluting with hexane followed by hexane-EtOAc (85:15), furnished 1 g of the more polar fraction. Repeated column chromatography of this material (500 mg) eluted with light petroleum ether:Et₂O (9:1) furnished a fraction containing a mixture of (−)-3-hydroxy-14,15-dinorlabd-8(17)-ene-13-one (3) and (+)-7α-acetoxybacchotricuneatin D (300 mg), as previously observed. Continuing the elution with petroleum ether: Et₂O (7:3) furnished fractions containing pure dinorlabdane 1 (20 mg) and dinorlabdane 2 (12 mg). A fraction containing a mixture of dinorlabdane 3 and (+)-7α-acetoxybacchotricuneatin D showed only a slight difference in Rₚ using TLC impregnated with AgNO₃ (15%, hexane-EtOAc, 8:2), and a successive column chromatography using the same conditions as above allowed for the isolation of pure dinorlabdane 3 (7 mg).

(−)-13(S)-14,15-Dinorlabd-8(17)-ene-3,13-diol (2)

Colorless oil, [α]₂⁰D=−1.0° (c 1.7, CHCl₃); IR (KBr) v max/cm⁻¹: 3400, 2934, 2851, 1642, 1033, 885; ¹H NMR (CDCl₃, 500 MHz) δ 0.70 (3H, s, H-18), 0.78 (3H, s, H-19), 1.00 (3H, s, H-20), 1.08 (1H, dd, J 12.5, 2.9 Hz, H-5), 1.20 (3H, d, J 6.2 Hz, H-16), 1.25 (2H, m, H-12), 1.49 (2H, m, H-11), 1.76 (1H, dq, J 10.3, 2.9 Hz, H-6β), 1.81 (1H, dt, J 13.1, 3.6 Hz, H-1β), 1.96 (1H, dd, J 13.0, 2.5, 2.9 Hz, H-7β), 2.40 (1H, dt, J 13.0, 2.9 Hz, H-7α), 3.25 (1H, dd, J 11.5, 4.6 Hz, H-3), 3.77 (1H, m, H-13), 4.56 (1H, brs, H-17'), 4.85 (1H, brs, H-17") ¹³C NMR (CDCl₃, 125 MHz) δ 37.1 (CH₃-C-1), 27.9 (CH₃-C-2), 78.8 (CH, C-3), 39.1 (C, C-4), 54.6 (CH, C-5), 23.9 (CH₃, C-6), 38.2 (CH, C-7), 147.9 (C, C-8), 56.4 (CH, C-9), 39.4 (C, C-10), 19.6 (CH₃, C-11), 38.1 (CH₂, C-12), 68.4 (CH, C-13), 23.7 (CH₃, C-16), 106.9 (CH₂, C-17), 28.3 (CH₃, C-18), 15.4 (CH₃, C-19), 14.4 (CH₃, C-20); HREIMS m/z 281.2484 [M+H]⁺ (calc. for C₁₈H₃₃O₂, 281.2481).

(−)-3-Hydroxy-14,15-dinorlabd-8(17)-en-13-one (3)

Colorless oil, [α]₂⁰D=−1.3° (c 1.6, CHCl₃); IR (KBr) v max/cm⁻¹: 3436, 2937, 2873, 1713, 1460, 1308, 735; ¹H NMR (CDCl₃, 500 MHz) δ 0.70 (3H, s, H-18), 0.78 (3H, s, H-19), 1.00 (3H, s, H-20), 1.08 (1H, dd, J 12.5, 2.9 Hz, H-5), 1.78 (1H, dq, J 13.0, 2.9 Hz, H-6β), 1.94 (1H, ddd, J 13.0, 12.5, 2.9 Hz, H-7β), 2.40 (1H, dt, J 13.0, 2.9 Hz, H-7α), 2.58 (1H, ddd, J 17.8, 9.0, 4.0 Hz, H-12"), 3.24 (1H, dd, J 11.5, 4.6 Hz, H-3), 4.46 (1H, brs, H-17'), 4.85 (1H, brs, H-17") ¹³C NMR (CDCl₃, 125 MHz) δ 37.0 (CH₃-C-1), 28.0 (CH₃-C-2), 78.8 (CH, C-3), 39.2 (C, C-4), 54.6 (CH, C-5), 24.0 (CH₂, C-6), 38.1 (CH₂, C-7), 147.6 (C, C-8), 56.0 (CH, C-9), 39.5 (C, C-10), 17.6 (CH₃, C-11), 42.7 (CH₃, C-12), 209.0 (C, C-13), 30.1 (CH₂, C-16), 106.6 (CH₂, C-17), 28.2 (CH₃, C-18), 15.3 (CH₃, C-19), 14.3 (CH₃, C-20).

Synthesis of (−)-3-hydroxy-14,15-dinorlabd-8(17)-en-13-one (3)

(−)-3-Hydroxycopalic acid (4) (300 mg), isolated from the same copaiba oleoresin as previously described, was dissolved in acetone (5 mL). KMnO₄ (200 mg) was then added in small portions over a period of 7 h at 0 °C. The excess of KMnO₄ was destroyed by adding isopropanol and the solvent was removed under reduced pressure. The residue was suspended in EtOAc (60 mL), washed with brine (3 x 30 mL) and dried over anhydrous MgSO₄, and the solvent was removed under reduced pressure. Purification of the crude product on SiO₂ (hexane-EtOAc, 9:1) provided ketone 3 (209.1 mg, 80%) of as an oil, [α]₂⁰D=−8.8° (c 1.7, CHCl₃).
Syntheses of dinorlabdane alcohols 1 and 2

To a suspension of LiAlH₄ (50 mg, 1.32 mmol) in anhydrous Et₂O (3 mL) was added a solution of hydroxylketone 3 (150 mg, 0.54 mmol) in Et₂O (5 mL). The reaction mixture was refluxed for 2 h and then the excess of LiAlH₄ was destroyed by adding an aqueous solution of 0.1 mol L⁻¹ NaOH. The solution was filtered and dried over anhydrous MgSO₄ and the solvent was removed under reduced pressure. Purification of the crude product on SiO₂ (petroleum ether : Et₂O, 7:3) furnished (−)-13(20)−14,15-dinorlabd-8(17)-ene-3,13-diol (1) (74 mg, 49%) as colorless crystals, mp 165.0−167.0 ºC, [α]D²⁰−12.0° (c 1.7, CHCl₃).

Synthesis of (S)-(+)−methoxyphenylacetate ester 5

DMAP (17.7 mg, 0.143 mmol) was added in one portion to a solution of 4 (40 mg, 0.143 mmol), (S)-(+)−methoxyphenylacetic acid (24.1 mg, 0.143 mmol), and of DCC (40 mg, 0.143 mmol) in CH₂Cl₂ (5 mL). After stirring for 2 h at room temperature, the dicyclohexylurea was heated to reflux for 2 h. Work-up and purification on SiO₂ (petroleum ether : Et₂O, 7:3) afforded alcohol 3 (20 mg, 0.143 mmol), and of DCC (30.3 mg, 0.143 mmol) in CH₂Cl₂ (5 mL). Following the same work-up and purification procedure as described previously, ester 6 (62.9 mg, 90%) was obtained as a colorless oil. IR (KBr) ν max/cm⁻¹: 3330, 2930, 2850, 1744, 1623, 1454, 1177, 1100, 737, 696; ¹H NMR (CDCl₃, 500 MHz) δ 0.45 (3H, s, H-20), 0.73 (3H, s, H-19), 0.96 (3H, s, H-18), 1.21 (3H, d, J 6.2 Hz, H-16), 3.18 (1H, dd, J 11.5, 4.6 Hz, H-3), 4.20 and 4.58 (each 1H, bs, H-17), 3.41 (3H, s, OCH₃), 4.73 (1H, s, ArCH(OCH₃)CO), 7.29-7.39 (3H, m, Ar), 7.43–7.48 (2H, m, Ar); EIMS 70 eV, m/z (rel. int. %): 262 [M−C₆H₄O₂]²⁺ (2), (5), 244 (5), 220 (7), 201 (7), 159 (8), 135 (15), 121 (100), 105 (14), 91 (20).

Reduction of natural (−)-3-hydroxy-14,15-dinorlabd-8(17)-en-13-one (3)

To a suspension of LiAlH₄ (40 mg, 1.06 mmol) in anhydrous Et₂O (10 mL) was added a solution of 3 (20 mg) in Et₂O (3 mL). The reaction mixture was heated to reflux for 2 h. Work-up and purification on SiO₂ (petroleum ether : Et₂O, 7:3) afforded alcohol 1 (5 mg, 25%) [(α)D²⁰−1.2° (c 0.5, CHCl₃)] and 2 (5 mg, 25%) [(α)D²⁰−1.0° (c 0.5, CHCl₃)].

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Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br, as PDF file.

References


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Figure S1. IR spectrum of (–)-13(R)-14,15-dinorlabd-8(17)-ene-3,13-diol (1).
Figure S2. $^1$H NMR spectrum of $(-)-13(R)$-14,15-dinorlabd-8(17)-ene-3,13-diol (I) (300 MHz, CDCl$_3$).

Figure S3. $^{13}$C NMR spectrum of $(-)-13(R)$-14,15-dinorlabd-8(17)-ene-3,13-diol (I) (75.5 MHz, CDCl$_3$).
Figure S4. $^{13}$C NMR spectrum (DEPT 135 and 90) of (−)-13(R)-14,15-dinorlabd-8(17)-ene-3,13-diol (1), (75.5 MHz, CDCl$_3$).

Figure S5. IR spectrum of (−)-13(S)-14,15-dinorlabd-8(17)-ene-3,13-diol (2).
Figure S6. $^1$H NMR spectrum of ($\neg$)-13(S)-14,15-dinorlabd-8(17)-ene-3,13-diol (2) (300 MHz, CDCl$_3$/TMS).

Figure S7. $^{13}$C NMR spectrum of ($\neg$)-13(S)-14,15-dinorlabd-8(17)-ene-3,13-diol (2) (75.5 MHz, CDCl$_3$).
Figure S8. $^{13}$C NMR spectrum (DEPT 135 and 90) of (–)-13(S)-14,15-dinorlabd-8(17)-ene-3,13-diol (2), (75.5 MHz, CDCl$_3$).

Figure S9. $^1$H NMR spectrum of (–)-3-hydroxy-14,15-dinorlabd-8(17)-en-13-one (3) (300 MHz, CDCl$_3$/TMS).
Figure S10. $^{13}$C NMR spectrum of (−)-3-hydroxy-14,15-dinorlabd-8(17)-en-13-one (3) (75.5 MHz, CDCl$_3$).

Figure S11. $^{13}$C NMR spectrum (DEPT 135 and 90) of (−)-3-hydroxy-14,15-dinorlabd-8(17)-en-13-one (3) (75.5 MHz, CDCl$_3$).