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A New *ent*-Clerodane Diterpene from *Hymenaea courbaril* var. *altissima*

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Da casca do tronco de *Hymenaea courbaril* var. *altissima* (Ducke) foi isolado um novo *ent*-clerodano na forma de éster metílico, após esterificação com diazometano, o (-)-(5R,8S,9S,10R)-clerod-3-en-15-oato de metila. Foram isolados também dois *ent*-labdanos conhecidos: o (-)-copalato de metila e (-)-eperuato de metila. Do extrato AcOEt do epicarpo foi isolado, após esterificação com diazometano, o (-)-zanzibarato de metila. As estruturas dos compostos foram elucidadas com base na análise de dados espectroscópicos.

A new *ent*-clerodane and two known *ent*-labdanes were isolated from the barks of *Hymenaea courbaril* var. *altissima* (Ducke). The new diterpene was identified, after esterification with diazomethane, as methyl (-)-(5R,8S,9S,10R)-clerod-3-en-15-oate and known diterpenes were identified as methyl (-)-copalate and methyl (-)-eperuate. From the EtOAc extract of seed pods a known methyl (-)-zanzibarate was isolated. The structures of isolated compounds were elucidated based on spectroscopic studies.

Keywords: *Hymenaea courbaril* var. *altissima*, *ent*-clerodane, *ent*-labdane

Introduction

Hymenaea is a genus widely distributed from Central to South America and is known popularly as "jatobá", "jataí" or "jutaí".[1] The wood is used for woodwork and the resin extract is locally used as a remedy for many purposes.[2] The chemical composition of the resin obtained from some species of *Hymenaea* is already known in the literature[3] and recently we reported the isolation of two new *ent*-clerodane derivatives from the seed pods resin of *H. courbaril* var. *stilbocarpa*.[4] We now describe the isolation of two known diterpenes (**1**, **2**) and a novel *ent*-clerodane (**3**) from the ethyl acetate extract of *H. courbaril* var. *altissima* (Ducke) barks, which grows mainly in a subtropical forest on the Atlantic Coast,[5] and the isolation of a known diterpene (**9**) from the ethyl acetate extract of its seed pods. Another five compounds (**4**-**8**) were also identified by GC/MS analyses.

Results and Discussion

The ethyl acetate extract of *H. courbaril* var. *altissima* barks was esterified with diazomethane and submitted to a combination of column chromatography (silica gel) and preparative TLC on $SiO_2/AgNO_3$ (5%, w/w), to afford a new diterpene, methyl (-)-(5R,8S,9S,10R)-clerod-3-en-15-oate (**3**), along with the known methyl (-)-copalate (**1**)[6] and methyl (-)-eperuate (**2**).[7] Through GC/MS analysis of the methylated extract, besides compounds **1**-**3**, five known diterpenes were also identified by their retention indices and by co-injection with authentic samples as: methyl isoozate (**4**),[4] methyl ozate (**5**),[4] methyl kovalenate (**6**),[4] methyl (5S*,8S*,9S*,10R*)-clero-3,13-dien-15-oate (**7**)[4] and methyl (5R*,8S*,9S*,10R*)-clero-3,13-dien-15-oate (**8**).[4]

The ethyl acetate extract of seed pods of *H. courbaril* var. *altissima* was esterified with diazomethane and submitted to a combination of column chromatography (silica gel), preparative TLC on $SiO_2/AgNO_3$ (5%, w/w) and RP-HPLC to give a known methyl (-)-zanzibarate (**9**).[8] Through GC/MS analysis of the methylated extract, three known diterpenes: methyl isoozate (**4**), methyl ozate (**5**) and methyl kovalenate (**6**) were also identified by retention indices and by co-injection with authentic samples.[4]

Methyl (-)-(5R,8S,9S,10R)-clerod-3-en-15-oate (**3**) was obtained as colorless oil, $[\alpha]_D^{20}$ – 54.0 (*c* 0.6, $CHCl_3$) and HRMS data (*m/z* 320.27156) suggested the molecular formula $C_{21}H_{36}O_2$. The ^{1}H NMR spectrum of **3** showed two signals at δ 0.89 (*s*, 3H) and 1.05 (*s*, 3H), corresponding to

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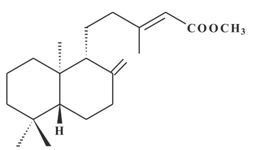

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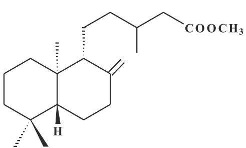

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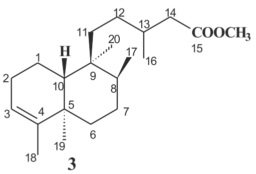

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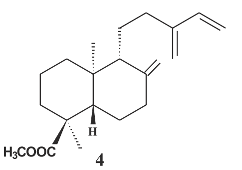

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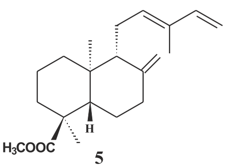

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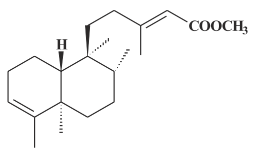

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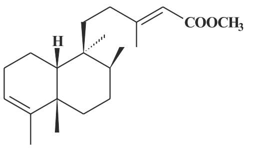

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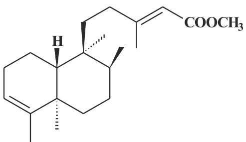

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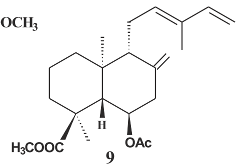

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the methyl groups attached to a quaternary carbon and two signals at δ 0.90 (d, J 7.0 Hz, 3H) and 0.96 (d, J 6.6 Hz, 3H), corresponding to the methyl groups attached to a methine carbon. Signal for an additional methyl group, attached to a sp^2 carbon at δ 1.59 (brd, J 1.5 Hz, 3H) coupled to a vinylic hydrogen at δ 5.20 (brs, 1H), was observed.

The full ^{1}H and ^{13}C NMR signals assignment was based on homonuclear and heteronuclear 2D experiments (COSY-90, HSQC) and by comparison with the ^{13}C NMR spectral data of the previously isolated compound **8**,[4] which showed a good agreement for the chemical shifts of carbons belonging to the decalin moiety.

The ^{1}H NMR spectrum also revealed signals for methylene hydrogens (δ 2.13, dd, J 9.0 and 4.4 Hz; 2.35 dd, J 9.0 and 4.4 Hz) adjacent to a carbomethoxyl group and cross peaks in the COSY spectrum showing their coupling to a methinic hydrogen at δ 1.88 (m). This hydrogen was, in turn, connected to the methyl group at δ 0.96 and methylene hydrogens at δ 1.33 (m). The ^{13}C NMR data connectivity analyses and comparison of ^{13}C NMR data of the side chain of **3** with those of **2** were in good agreement. The chemical shift of carbon C-19, that appeared at δ 20.6, confirms the *trans* relationship for A/B ring.[4] The assignments for all signals were fully confirmed by HSQC and HMBC experiments.

Experimental

General

^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ solution at 300 MHz and 75 MHz, respectively, with a Bruker AC 300/P spectrometer (internal standard TMS). IR spectra of neat samples were obtained on a Perkin-Elmer 1600 series FTIR. Mass spectra of purified compounds were obtained at 70 eV on an Hewlett-Packard 5990/5970 system equipped with a J&W Scientific DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 mm). High-resolution mass spectra (HRMS) were performed with a VG 7070E spectrometer by electron impact with beam energy of 70 eV. Optical rotations were measured with a Carl Zeiss photoelectric polarimeter.

Isolation

The seed pods and barks of *Hymenaea courbaril* var. *altissima* (Ducke) were collected in March 1994 at Fazenda Santa Elisa (IAC- Campinas), São Paulo State, Brazil. A voucher specimen was deposited in the Herbarium of IB-UEC (# no. GS 01/03). Dry barks (360 g) were powdered and extracted with EtOAc (3L) for 5 days at room temperature. After filtration and evaporation of solvent at reduced pressure a crude extract (34 g) was obtained as an oily resin. A sample of resin (789 mg) was esterified with diazomethane and submitted successively for purification using silica gel column chromatography ($CHCl_3$: MeOH 1% to 5%, v/v), prep TLC on $SiO_2/AgNO_3$ (5%, w/w) eluting with a mixture of different solvents and polarity (petroleum ether/EtOAc/CH_2Cl_2) to yield (-)-methyl copalate (**1**) {11 mg, $[\alpha]_D^{20}$ –19.0 $CHCl_3$, *c* 0.7; lit.,[6] $[\alpha]_D^{20}$ –12.9}, methyl eperuate (**2**) {15 mg, $[\alpha]_D^{20}$ –24.0 $CHCl_3$, *c* 1.1; lit.[7], $[\alpha]_D^{20}$ –26.0} and methyl (-)-(5R,8R,9R,10S) clerod-3-en-15-oate (**3**) {12 mg, $[\alpha]_D^{20}$ –54.0 $CHCl_3$, *c* 0.6}. GC/MS analysis of the barks methylated extract indicated: methyl isoozate (**4**) (3.7%), methyl ozate (**5**) (3.9%), methyl kovalenate (**6**) (3.2%), methyl (5S*,8S*,9S*,10R*)-cleroda-3,13-dien-15-oate (**7**) (2.3%) and methyl (5R*,8S*,9S*,10R*)-cleroda-3,13-dien-15-oate (**8**) (0.8%).

Table 1. ^{1}H and ^{13}C NMR spectral data of **3** (300 MHz, 75 MHz) and ^{13}C NMR spectral data of **2** and **8** in $CDCl_3$ (75 MHz)

	3		**2**	**8**[4]
Position	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_C (ppm)
1	17.8	1.40 m	39.1	17.9
2	26.8	1.97 m, 2.08 m	19.4	26.8
3	120.2	5.20 brs	42.2	120.2
4	144.6		33.6	144.4
5	37.4		55.6	37.6
6	30.2	1.45 m	24.5	30.1
7	25.6	1.25 m	38.4	25.5
8	35.1	1.60 m	148.8	35.1
9	38.3		56.9	38.8
10	45.2	1.45 m	39.7	45.1
11	37.4	1.33 m	20.8	37.5
12	36.6	1.05 dt (*J* 9.2 and 3.5 Hz) 1.40 m	35.8	34.8
13	31.5	1.88 m	30.9	164.0
14	41.7	2.13 dd (*J* 9.0 and 4.4 Hz) 2.35 dd (*J* 9.0 and 4.4 Hz)	41.9	114.3
15	173.8		173.8	170.7
16	20.0	0.96 d (*J* 6.6 Hz)	19.6	19.5
17	14.8	0.90 d (*J* 7.0 Hz)	106.2	14.8
18	18.0	1.59 bd (*J* 1.5 Hz)	33.6	18.0
19	20.6	1.05 s	21.7	20.6
20	20.4	0.89 s	14.5	20.4
OCH_3	51.4	3.50 s	51.4	50.8

Dry seed pods (2.5 kg) were powdered and extracted with *n*-hexane (6L) for 5 days at room temperature. After filtration and evaporation of solvent at reduced pressure a crude extract (110 g) was obtained as an oily resin. A sample of resin (5 g) was submitted to acid and base extraction to furnish acidic fraction (3.3 g). Treatment with excess diazomethane gave esterified extract (3.4 g) which was submitted to successive purification by silica gel column chromatography (petroleum ether : EtOAc, 95:5, v/v) to yield (-)-methyl zanzibarate (**6**) as colorless crystals {150 mg, mp 132-134 °C, $[\alpha]_D^{20}$ –37.5 $CHCl_3$, *c* 2.0; lit.,[8] $[\alpha]_D$ –37.0, $CHCl_3$, *c* 1.0}. GC/MS analysis of the esterified extract indicated the presence of methyl isoozate (**4**) (5.7%), methyl ozate (**5**) (46.5%) and methyl kovalenate (**6**) (10.5%).

Methyl (-)-(5R,8S,9S,10R)-clerod-3-en-15-oate (3)

Viscous oil, $[\alpha]_D^{20}$ –54.0 ($CHCl_3$, c 0.6); HREIMS *m/z*: 320.27156 $[M]^+$ (calcd 320.27153 for $C_{21}H_{34}O_2$); IR ν_{max} / cm^{-1}: 1741, 1458, 1436, 1381, 1162, 1096, 1012 (film); ^{1}H NMR see Table 1; ^{13}C NMR see Table 1.

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