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Cycloartane triterpenoids from *Guarea macrophylla*

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Abstract

Nine cycloartane triterpenoids, including two new derivatives 22,25-dihydroxy-cycloart-23*E*-en-3-one and 24-methylenecycloartane-3β,22-diol have been isolated from leaves of *Guarea macrophylla*. The structures were elucidated by interpretation of spectral data, mainly ^{1}H and ^{13}C NMR, including bidimensional analysis (HOMOCOSY, HMQC and HMBC). © 2002 Published by Elsevier Science Ltd.

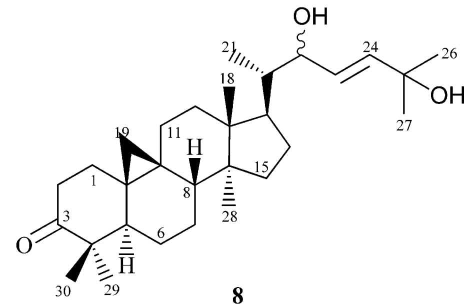
Keywords: Guarea macrophylla; Meliaceae; Cycloartane triterpenoids; NMR; HMQC; HMBC

1. Introduction

Guarea macrophylla ssp. *tuberculata* (Meliaceae) occurs in Brazil from Rio Grande do Sul to Minas Gerais States and in the Amazon region (Correa, 1984). Several terpenoids have already been isolated and characterized from *Guarea* species (Furlan et al., 1993, 1996; Garcez et al., 1998). In a previous paper, we reported the isolation of one monoterpenoid, four sesquiterpenoids, six diterpenoids and one triterpenoid from the CH_2Cl_2 extract from *G. macrophylla* leaves (Lago et al., 2000). In continuation of our chemical studies, the ethanolic extract from the leaves of *G. macrophylla* has been investigated. In this work, several separation methods have been used to isolate nine closely related cycloartane triterpenoids (**1**–**9**), including two new derivatives: 22,25-dihydroxycycloart-23*E*-en-3-one (**8**) and 24-methylenecycloartane-3β,22-diol (**9**).

2. Results and discussion

Column chromatography on silica gel and Sephadex LH-20 of the hexane and CH_2Cl_2 phases from ethanolic extract of the leaves from *G. macrophylla* resulted in the isolation of nine closely related cycloartane triterpenoids. The known compounds cycloarta-23*E*,25-dien-3-one (**1**), cycloart-24-ene-3,23-dione (**2**), 25-hydroxycycloart-23-en-3-one (**3**), 23-hydroxycycloart-24-en-3-one (epimeric mixture **4** and **5**), 3β-hydroxycycloart-24-en-23-one (**6**) and cycloart-23-ene-3β,25-diol (**7**) were identified by comparing their ^{1}H and ^{13}C NMR spectral data with those published in the literature. These compounds were previously reported in *G. macrophylla* (**1**, Lago et al., 2000) and in *G. trichilioides* (**2**–**7**, Furlan et al., 1993).



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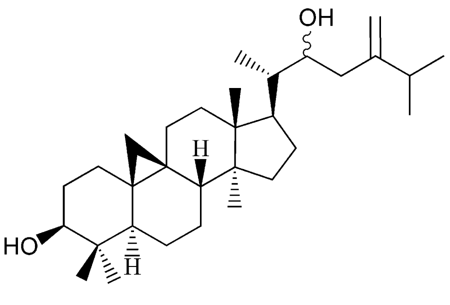

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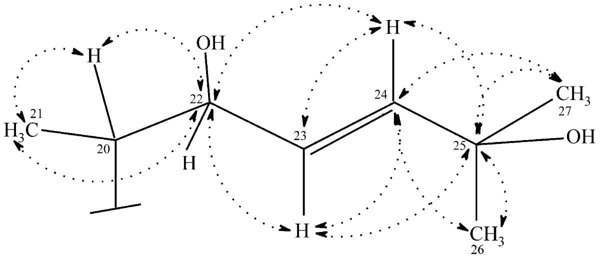
The structures of **3** and **7** have already been reported, but the configuration of the side chain double bond has not been determined because of the overlap of the H-23 and H-24 signals in the ^{1}H NMR spectrum. The resolution of these signals was possible when the ^{1}H NMR spectra were registered in benzene-d_6, where one doublet at δ 5.60 ($J = 15.6$ Hz) was observed for H-24 and one double-doublet at δ 5.66 ($J = 15.6$ and 5.8 Hz) for H-23, for both triterpenoids. These data indicated a *trans* configuration for the double bond of the side chain. The structures of **3** and **7** are thus defined as 25-hydroxycycloart-23*E*-en-3-one and cycloart-23*E*-ene-3β,25-diol, respectively.

Compound **8** was isolated as a white amorphous powder. The ^{1}H NMR spectrum displayed two doublets at δ 0.58 ($J = 4.3$ Hz) and δ 0.79 ($J = 4.3$ Hz), which were characteristic for a C-9/C-10 cyclopropyl methylene group of a cycloartan-3-one triterpenoid (Furlan et al., 1993). The presence of a carbonyl group was confirmed by the IR spectrum, which showed an absorption at 1706 cm^{-1}, as well as by the ^{13}C NMR spectrum, which showed a peak at δ 216.5. The ^{1}H NMR spectrum showed the presence of a *trans* disubstituted double bond at δ 5.85 (*d*, $J = 15.9$ Hz, 1H) and at δ 5.70 (*d*, $J = 15.7$ and 7.0 Hz, 1H) with a secondary oxygenated carbon in one allylic position at δ 4.22 (*dd*, $J = 6.9$ and 3.3 Hz, 1H). This spectrum also showed two methyl groups at δ 1.34 (*s*) linked to a tertiary carbinolic carbon. The ^{13}C NMR spectra (BBD and DEPT 135°) revealed secondary and tertiary oxygenated carbons at δ 74.4 and δ 70.8, respectively. These data suggested that the side chain had the following structure—$CH(CH_3)CH(OH)CH{=}CHC(OH)(CH_3)_2$—with an *E* configuration on the double bond. On the basis of these data, the structure of **8** was defined as 22,25-dihydroxycycloart-23*E*-en-3-one. This conclusion was supported by the HMBC spectrum (Fig. 1), EIMS and elemental analysis.

Compound **9** was also isolated as a white amorphous powder. The ^{1}H NMR spectrum revealed two doublets in δ 0.56 ($J = 4.3$ Hz) and δ 0.34 ($J = 4.3$ Hz) characteristic of a cycloartan-3β-ol derivative (Furlan et al., 1993). The presence of a double doublet at δ 3.28 ($J = 11.2$ and 4.3 Hz), assigned to H-3α, confirmed this observation. The ^{13}C NMR (BBD and DEPT 135°) exhibited two sp^2 carbon resonances at δ 153.6 (C) and δ 109.7 (CH_2), corresponding to the carbon atoms of an exocyclic methylene group. The comparison of the ^{13}C NMR spectral data of **9** with those of 24-methylenecycloartanol, isolated from *Euphorbia broteri* (Pascual Teresa et al., 1987) in conjunction with the CH signal at δ 70.4, indicated the presence of a secondary hydroxyl group in the side chain. The presence of a double-triplet at δ 3.78 ($J = 9.0$ and 3.0 Hz) positioned this group at C-22. All the data, EIMS, elemental analysis and correlations in the HMBC spectrum (Fig. 1), are consistent with structure **9**, 24-methylenecycloartane-3β,22-diol.



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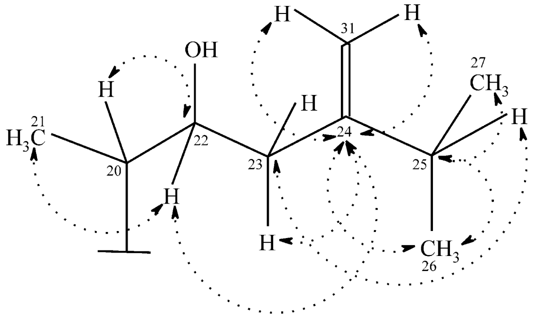

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Fig. 1. Important long-range correlations for the side chain of **8** and **9** observed in the HMBC spectra.

The protonated carbons were assigned from HMQC spectrum (Table 1).

Most of the papers about *Guarea* species deal with the isolation of meliacins (limonoids). In *G. trichilioides* (=*G. guidonia*) several cycloartane triterpenoids were isolated, similar to those found in *G. macrophylla*, but only one meliacin has been detected (Garcez et al., 1998). However, in *G. macrophylla* no meliacins were detected in the intermediate polar fractions.

3. Experimental

3.1. Plant material

The leaves of *Guarea macrophylla* (550 g) were collected in November, 1999, in São Paulo city, São Paulo State, Brazil. Identification of the botanical material has been reported previously (Lago et al., 2000).

3.2. General

NMR spectra were recorded at 300 and 500 MHz for ^{1}H and 75 and 125 MHz for ^{13}C on Bruker DPX-300 and DRX-500 spectrometers using $CDCl_3$ as solvent and internal standard. Optical rotations were measured in a digital polarimeter JASCO DIP-370 (Na filter, $\lambda = 588$ nm) in $CHCl_3$. IR spectra were obtained as KBr pellets in a Perkin-Elmer Infrared Spectrometer model 1750; EIMS were obtained at 70 eV on an INCOS 50

Table 1
^{13}C (125 MHz) and ^{1}H (500 MHz) NMR spectral data for **8** and **9** ($CDCl_3$)[a]

	8		**9**	
Position	^{13}C	^{1}H	^{13}C	^{1}H
1	33.4	1.53 (*m*), 1.87 (*m*)	32.0	1.75 (*m*), 1.25 (*m*)
2	37.4	2.70 (*dt*, *J* = 13.9, 6.3 Hz), 2.32 (*ddd*, *J* = 13.9, 4.3, 2.3 Hz)	30.4	1.53 (*m*), 1.73 (*m*)
3	216.5	–	78.8	3.28 (*dd*, *J* = 11.2, 4.3 Hz)
4	50.2	–	40.8	–
5	48.4	1.68 (*m*)	47.1	1.41 (*m*)
6	21.5	0.97 (*m*)	21.1	1.56 (*m*), 0.82 (*m*)
7	27.4	1.93 (*m*), 1.34 (*m*)	27.2	1.97 (*m*), 1.36 (*m*)
8	47.8	1.58 (*m*)	48.0	1.55 (*m*)
9	21.1	–	20.0	–
10	26.0	–	26.1	–
11	25.8	1.40 (*m*), 1.18 (*m*)	26.1	1.41 (*m*), 1.17 (*m*)
12	35.7	1.36 (*m*)	35.7	1.93 (*m*)
13	45.7	–	45.8	–
14	48.4	–	48.4	–
15	32.8	1.62 (*m*)	32.9	1.56 (*m*)
16	26.7	2.05 (*m*)	26.4	1.98 (*m*)
17	49.2	1.63 (*m*)	49.0	1.64 (*m*)
18	18.2	1.03 (*m*)	18.0	1.01 (*m*)
19	29.5	0.79 (*d*, *J* = 4.3 Hz), 0.58 (*d*, *J* = 4.3 Hz)	29.9	0.56 (*d*, *J* = 4.3 Hz), 0.34 (*d*, *J* = 4.3 Hz)
20	42.4	1.45 (*m*)	40.5	1.90 (*m*)
21	12.0	0.91 (*d*, *J* = 6.7 Hz)	12.0	0.90 (*d*, *J* = 9.0 Hz)
22	74.4	4.22 (*dd*, *J* = 6.9, 3.3 Hz)	70.4	3.78 (*dt*, *J* = 9.0, 3.0 Hz)
23	125.3	5.70 (*dd*, *J* = 15.7, 7.0 Hz)	36.1	2.23 (*m*), 1.33 (*m*)
24	140.5	5.85 (*d*, *J* = 15.9 Hz)	153.6	–
25	70.8	–	33.2	1.63 (*m*)
26	30.1	1.34 (*s*)	22.3	1.05 (*d*, *J* = 6.8 Hz)
27	29.9	1.34 (*s*)	21.6	1.08 (*d*, *J* = 6.8 Hz)
28	19.3	0.86 (*s*)	19.5	0.91 (*s*)
29	22.2	1.05 (*s*)	14.0	0.80 (*s*)
30	20.8	1.10 (*s*)	25.4	0.97 (*s*)
31	–	–	109.7	4.93 (*s*), 4.83 (*s*)

[a] Assignments based on 2D experiments (HOMOCOSY, HMQC and HMBC).

Finnigan-Mat-quadrupole spectrometer. Silica gel 60 (Merck) was used for CC and Sephadex LH-20 (Sigma) was used for molecular exclusion chromatography. Elemental analyses were obtained on a Perkin-Elmer Elemental Analyser model 2400 CHN.

3.3. Extraction and isolation

The leaves were air-dried and extracted with cold EtOH to give a crude material (50.1 g) which was partitioned in hexane, CH_2Cl_2, EtOAc and *n*-BuOH. After evaporation, the hexane phase (21.8 g) was subjected to silica gel chromatography using a gradient mixture of hexane–EtOAc–MeOH as eluent, to give seventeen fractions. Fraction 1 was composed of waxy material. Fraction 2 was applied to a silica gel colomn using a gradient mixture of hexane–CH_2Cl_2–EtOAc as eluent, yielding 29 mg of **2**. Fraction 4 was separated by CC on silica gel with a gradient mixture of hexane–CH_2Cl_2–EtOAc to give nine subfractions. Subfraction 3 was submitted to prep. TLC on silica gel (hexane–EtOAc 9:1) to give 12 mg of **4**. The triterpenoid **1** (14 mg) was purified by prep. TLC on silica gel (hexane–EtOAc 8:2) from the subfraction 4. Subfraction 5 was subjected to CC on silica gel eluting with hexane–EtOAc to give 6 mg of **3** and 31 mg of **9**. Fraction 5 was subjected to Sephadex LH-20 using a gradient solvent system [hexane–CH_2Cl_2 (1:4) followed by CH_2Cl_2–Me_2CO (3:2) and (1:4)] to give three subfractions. The triterpenes **5** (24 mg) and **6** (16 mg) were purified after CC on silica gel of subfraction 2, using CH_2Cl_2 with increasing amount of EtOAc as eluent. Fraction 11 was methylated and fractionated on silica gel using CH_2Cl_2 with increasing amount of EtOAc as mobile phase to yield 27 mg of **8**. The CH_2Cl_2 phase (11.3 g), after evaporation of the solvent, was submitted to CC on silica gel using a gradient mixture of hexane–CH_2Cl_2–MeOH as eluent to give 15 fractions. Fraction 6, after separation on Sephadex LH-20 using CH_2Cl_2–Me_2CO (3:2) as eluent, yielding 12 mg of **7**.

3.4. 22,25-Dihydroxycycloart-23*E*-en-3-one **8**

White amorphous powder; $[\alpha]_D$ +38.8 (*c* 0.15, $CHCl_3$); anal. C 78.98%, H 10.68%, calcd for $C_{30}H_{48}O_3$, C

78.90%, H 10.59%; IR (KBr) ν_{max}: 3394, 2931, 2870, 1706, 1459, 1378, 1237, 1155, 1113, 1086, 977, 917, 841, 757 cm^{-1}; ^{1}H and ^{13}C NMR (detected via HMQC and HMBC correlations): see Table 1; EIMS m/z (rel. int.): 456 $[M]^+$(no), 413 (2), 299 (7), 257 (14), 239 (19), 135 (53), 98 (71), 93 (25), 87 (33), 83 (62), 74 (61), 69 (55), 59 (60), 43 (56).

3.5. 24-Methylenecycloartane-3β,22-diol ***9***

White amorphous powder; $[\alpha]_D$ +16.5 (*c* 0.63, $CHCl_3$); anal. C 81.58%, H 11.56%, calcd for $C_{31}H_{52}O_2$, C 81.52%, H 11.47%; IR (KBr) ν_{max}: 3524, 2943, 2867, 1703, 1632, 1457, 1377, 1101, 1041, 895, 755, 554 cm^{-1}; ^{1}H and ^{13}C NMR (detected via HMQC and HMBC correlations): see Table 1; EIMS *m*/*z* (rel. int.): 456 $[M]^+$(no), 438 ($M-H_2O$, 5), 395 (6), 339 (15), 271 (6), 232 (20), 215 (10), 203 (18), 189 (13), 175 (29), 161 (26), 159 (24), 147 (41), 133 (38), 121 (49), 109 (51), 107 (60), 95 (97), 81 (64), 69 (90), 43 (100).

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