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Article

Two New Cneorubin Related Diterpenes from the Leaves of *Guarea guidonia* (Meliaceae)

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Dois novos diterpenos do tipo das cneorubinas, além de dois conhecidos, foram isolados das folhas da *Guarea guidonia* (Meliaceae). Um dos novos diterpenos possui o sistema *trans* biciclo [5.1.0] octano, encontrado muito raramente entre os produtos naturais. As estruturas foram determinadas com base na análise de dados espectrais. A análise dos espectros de RMN dos diterpenos conhecidos permitiu uma re-atribuição de dados da literatura.

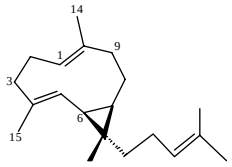
Two new and two known cneorubin related diterpenes were isolated from the leaves of *Guarea guidonia* (Meliaceae). One of the new diterpenes has the unusual *trans* bicyclo[5.1.0]octane ring system. The structures were elucidated by spectral data. The analysis of the NMR spectra of the known diterpenes allowed the reassignment of their NMR data.

Keywords: Guarea guidonia, Meliaceae, diterpenes, cneorubinanes, NMR data

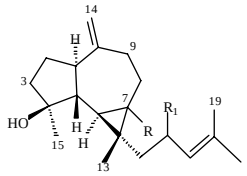
Introduction

Guarea guidonia (L) Sleumer is a brazilian Meliaceae species occurring all over the country. The leaves of *G. guidonia* have already been chemically investigated and different compositions have been found for specimens collected in different country regions[1-3]. We are now investigating the leaves of a specimen collected in Campo Grande-MS, western of Brazil.

This paper describes the isolation and identification of two known (**1** and **2**) and two novel (**3** and **4**) diterpenes besides sitosterol, stigmasterol, β-selinene and a mixture of unidentified hydrocarbon sesquiterpenes obtained from the hexane fraction of the methanolic extract of *G. guidonia* leaves. Despite the report of the known compounds from *Cneorum tricoccom*, Cneoracea, 19 years ago[4], it is particularly surprising that they have not been identified from any other natural source. Furthermore, no structurally related natural products have been isolated from plant sources. There is only a report about the isolation of emmottene, a related diterpene from *Briareum polyanthes*, a Bermudian gorgonian[5] .



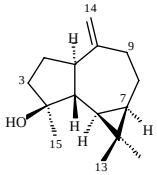
(**1**)



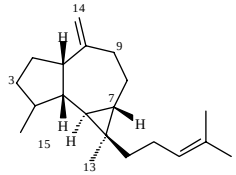
(**2**) R=αH, R_1=H (Cneorubin X)

(**3**) R=αH, R_1=OH (Cneorubin A)

(**4**) R=βH, R_1=OH (Cneorubin B)



Spathulenol



Emmottene

Results and discussion

The ^{13}C NMR data of diterpenes **1** and **2** isolated from *Guarea guidonia* are identical to those reported for cneorubin Y and X, respectively[4]. Comparison of the ^{13}C and ^{1}H NMR data of **2** with those reported for the aromadendrane sesquiterpene, spathulenol,[6] showed that the diterpene and the sesquiterpene have identical ring system, including the stereochemistry (Tables 1 and 2). However, the five and seven membered rings of cneorubin X (**2**) are not *cis*-fused as previously proposed[7] but *trans*-fused. The analysis of the 2D NMR (^{1}H-^{1}H COSY and ^{13}C-^{1}H COSY) spectra allowed the reassignment of the NMR data for **1** and **2** . The former has the same ring system as the sesquiterpene bicyclogermacrene[8].

Table 1. ^{13}C NMR data for **1**, **2**(50 MHz), **3**, **4** (100MHz) and spathulenol. (δ, $CDCl_3$).

^{13}C	**1**	**2**	spathulenol[6]	**3**	**4**
1	124.8	52.9	54.3	51.4	48.3
2	26.0	26.3	26.7	25.6	24.0
3	41.2	41.6	41.7	41.0	39.5
4	127.9	81.0	81.0	80.6	80.0
5	126.4	53.8	53.4	53.3	53.0
6	26.5	29.1	29.9	26.5	25.0[a]
7	29.4	26.8	27.5	27.4	25.2[a]
8	26.9	24.8	24.8	24.8	25.3
9	37.2	38.9	38.8	38.8	39.0
10	140.8	153.5	153.4	153.5	154.5
11	23.8	24.3	20.2	21.7	20.7
12	43.6	43.2	28.6	49.8	49.7
13	12.8	13.6	16.3	14.6	13.2
14	20.8	106.2	106.2	106.5	106.5
15	17.6	25.7[a]	26.1	25.1	23.3
16	25.0	25.2	-	67.2	66.5
17	124.8	124.8	-	128.4	128.2
18	130.9	131.1	-	134.8	134.5
19	16.5	17.6	-	18.1	18.2
20	25.7	25.9[a]	-	25.8	25.7

[a] Values can be interchanged

Table 2. ^{1}H NMR data for **1, 2** (200MHz), **3**, **4** (400MHz) and spathulenol (δ, $CDCl_3$).

^{13}H	**1**	**2**	spathulenol[6]	**3**	**4**
1	4.74	2.19		2.23	2.25
2a	2.00			1.65	1.65
2b				1.90	1.95
3a	1.79			1.65	1.70
3b	2.12			1.80	
5	4.28	1.32		1.30	1.30
6	1.20	0.48	0.4 - 0.6	0.54	0.73
7	0.54	0.71	0.4 - 0.6	0.85	0.65
8a	1.15	0.90		0.99	0.93
8b	1.76	2.01			2.00
9a	1.67	2.40		2.05	2.05
9b	2.23			2.40	2.42
12a	1.20	1.03	1.04	1.30	0.90
12b		1.37		1.48	1.70
13	0.94	1.02	1.05	1.08	1.04
14a	1.40	4.65	4.66	4.66	4.69
14b		4.68		4.69	4.71
15	1.53	1.28	1.29	1.28	1.25
16	1.93	2.09		4.58	4.65
17	5.03	5.10		5.17	5.10
19	1.60	1.59		1.67	1.66
20	1.60	1.66		1.69	1.67

The diterpenes **3** and **4** have not been described yet in the literature. Their molecular formula ($C_{20}H_{32}O_2$) have been deduced by the EI/MS and ^{13}C NMR data. Although the ^{1}H NMR spectra of these terpenes reveal significative difference in the region between δ 0.5 and 2.0 (Table 2), the analysis of other spectral data (^{13}C NMR, ^{13}C–^{1}H COSY and HMBC) showed that both have an identical planar structure and differ from that of **2** only due to the presence of an additional hydroxyl group at C-16. Comparison of the ^{13}C NMR data of **3** with those of **2** suggested that both present the same stereochemistry on the ring system. The permanence of the more protected methyl group on the cyclopropyl ring indicated that the lateral chain, which differentiate **2** and **3** from spathulenol, is located at C-12 on the α side of the cyclopropane ring. The methyl group is more protected in **2** (δ 13,6) and **3** (δ 14,6) than in spathulenol (δ 16,3) because of the γ effect of C-16. A NOESY spectrum of **3** (Table 3) confirmed that the relative stereochemistry of the molecule is the same as in spathulenol.

Table 3. Correlation observed in the NOESY spectra for **3** and **4** (400 MHz, δ, $CDCl_3$).

3		**4**	
^{1}H (δ)	^{1}H (δ)	^{1}H (δ)	^{1}H (δ)
H-1 (2.23)	H-6 (0.54)	H-1 (2.25)	H-15 (1.25)
			H-6 (0.70)
	H-15 (1.28)	H-5 (1.30)	H-13 (1.04)
H-6 (0.54)	H-15 (1.28)	H-6 (0.70)	H-15 (1.25)
H-14a (4.66)	H-9β (2.40)	H-14a (4.69)	H-9β (2.42)
H-14b (4.69)	H-2b (1.90)	H-14b (4.71)	H-2b (1.95)
H-16 (4.58)	H-13 (1.08)	H-16 (4.65)	H-13 (1.04)
	H-15 (1.28)		H-19 (1.66)
	H-19 (1.67)	H-17 (5.10)	H-20 (1.67)
H-17 (5.17)	H-20 (1.69)		H-12a (0.90)

The ^{13}C NMR chemical shift of the exocyclic methylene (δ 106.5)[8] as well as the H-6 and H-7 chemical shifts (ca δ 0.7)[8] observed in the ^{1}H NMR of **4** suggested that the five and seven membered rings are also *trans* fused in this diterpene. The NOESY spectrum of **4** (Table 3) showed almost the same spatial correlation as in **3**.The most important of them is the correlation between H-15 and H-1 which is larger in **4** than in **3**, indicating that both compounds have H-1 and the methyl group at C-4 on the same side of the molecule. Considering that the diterpene **4** showed a higher Rf value than **3** on silica gel TLC plate, one can conclude that the hydroxyl groups of **3** have stronger interaction with the silanol groups of the gel. A possible intramolecular hydrogen bonding, between these OH groups in **4**, as consequence of a epimerization at C-16, in relation to **3**, would weaken the interaction of the diterpene with the silica gel. This situation would modify the conformation of the ring system, and also justify the difference in the NMR spectra. Nevertheless the results obtained with molecular modeling experiments showed that an intramolecular hydrogen bonding between the hydroxyl groups in **4** increase the energy of the compound. The side chain in **4** is also at C-12, as shown by the ^{13}C NMR data, so the difference between **3** and **4** must be in the cyclopropyl junction. The

overlaping of the signals of H-6 and H-7 in the ^{1}H NMR spectrum of **4** made that analysis difficult. The observation of a spatial correlation between H-5 and H-13 in **4** and not in **3,** as well as between H-15 and H-16 in **3** but not in **4** agrees with the proposed modification. No spatial correlation is observed between H-5 and H-6 or H-7 but a small interaction is observed between H-1 and H-6 or H-7 in **4**. As in **3** we could see a correlation between H-1 and H-6, but not between H-1 and H-7, we can suggest that the correlation in **4** is also between H-1 and H-6. By these observations we proposed that **3** and **4** are epimers at C-7, so the last one has a *trans* bicyclo[5.1.0] system ring as in emmottene[5]. Inspection of molecular model showed that in **4** the methyl group at C-4 is closer to C-1 than in **3**, justifying a strong nOe between H-1 and H-15 observed in the NOESY spectrum. This observation also agrees with the shielding of the cyclopentane ring carbons in the ^{13}C NMR spectrum of the diterpene **4,** in relation to those of **3**. The structures of **4** and of emmottene[5] suggest that a revision in the stereochemistry of aromadendrane like natural products is necessary. We named the skeleton of diterpenes **2-4** as cneorubinane.

It is interesting to mention that species of Cneoraceae family also produce meliacins, tetranortriterpenes that characterise the chemical constitution of Meliaceae species.

Experimental

General Procedures

The IR spectra were registered as KBr pellets in a Perkin Elmer FT 1750 spectrometer. The low resolution mass spectra were obtained in a INCOS 50 Finnigan-Mat instrument operating at 70 eV. The ^{1}H and ^{13}C NMR spectra, using $CDCl_3$ as solvent and TMS as internal reference, were run in a Bruker AC 200 and a Varian U-400. For the column separations Merck silica gel, 63-200 mm and 40-63 mm was used. For preparative TLC Merck silica gel 60 GF_{254} was employed.

Plant material

Leaves of *Guarea guidonia* (L.) Sleumer were collected in February 1994, in Campo Grande, Mato Grosso do Sul, Brazil. A voucher was deposited under the number 1870 in the Herbarium of the Universidade Federal do Mato Grosso do Sul.

Extraction and isolation of the compounds

Dried leaves of *G. guidonia* (1100 g) were extracted at room temperature with methanol. After distillation of the solvent, 72 g of the extract were obtained. This material was submitted to a partition between hexane and MeOH/H_2O (95:5).The hexane phase (47 g) was dissolved in hot MeOH and kept at 4 ºC during 24 h. After filtration, the soluble part was concentrated affording 25 g of a solid residue. This residue was submitted to chromatography on a silica gel column eluted with gradient mixtures of hexane and ethyl acetate. The first fractions were submitted to further purification on silica gel column, eluted with gradient mixtures of hexane and CH_2Cl_2, and to preparative TLC using silica gel impregnated with $AgNO_3$. β-Selinene (40 mg), an unidentified sesquiterpene mixture (60 mg) and **1** (60 mg) and **2** (20 mg) were isolated. The intermediary fractions afforded a mixture of sitosterol and stigmasterol (50 mg). The more polar fractions were chromatographed over silica gel column eluted with step gradient mixtures of hexane and isopropanol yielding **4** (25 mg) and **3** (20 mg).

Cneorubin-A: (1*R*-*4*S**)-16-dihydroxy-(5*R**-6*R**-7*R**)-cneorubin-10 (14),17-diene (**3**): colourless gum. IR υ_{max} (KBr, cm^{-1}) 3371, 3082, 2924, 2866, 1635, 1447, 1378, 1150, 1032. EIMS, m/z (relative intensity in %) 303 (2, $M^{\cdot+}$- H·), 289 (16, $M^{\cdot+}$– Me·), 287 (56, $M^{\cdot+}$-OH·), 269 (100, $M^{\cdot+}$ - HO·-H_2O), 231 (23), 213 (11), 202 (13).^{1}H and ^{13}C NMR see Tables 1-3.

Cneorubin-B:(1*R**-4*S**)-16-dihydroxy-(5**R**-6*R**-7*S**) -16-cneorubin-10(14),17-diene (**4**): colourless gum. IR: υ_{max}(KBr, cm^{-1}) 3343, 3083, 2967, 2924, 2862, 1633, 1451, 1379, 1149, 1121, 1051. EIMS: m/z (relative intensity in %) 303 (2, $M^{\cdot+}$ – H·), 289 (6, $M^{\cdot+}$–Me·), 287 (65, $M^{\cdot+}$– OH ·), 269 (100, $M^{\cdot+}$ – OH ·– H_2O), 231 (14), 213 (6), 202 (13). H^1 and C^{13} NMR see Tables 1-3.

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