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A New Euphane Triterpene from the Brazilian *Melia azedarach*

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O cinamodiol (1), um novo triterpeno da série do eufano, foi isolado das sementes da Meliaceae *Melia azedarach* L. A estrutura foi estabelecida a partir dos dados de RMN. Essa substância não é responsável pela atividade anti-ecdise encontrada no extrato bruto.

A new euphane triterpene cinamodiol (1) was isolated from the seeds of the Meliaceae *Melia azedarach* L. The structure was deduced from the interpretation of NMR data. The compound is not responsible for the anti-ecdysis activity found in the crude extract.

Keywords: Melia azedarach, *Meliaceae, triterpene, euphane, protolimonoid*

Introduction

The Meliaceae *Melia azedarach* L. is a tree of African origin, and is widely cultivated in Brazil where it is known as "cinamomo". The crude methanolic extract of the seeds shows both phagoinhibitory and anti-moulting activities against the haematophagous insect, *Rhodnius prolixus*, one of the vectors of Chagas disease[1]. Partial purification of the cited extract furnishes an active fraction that contains the classical phytosterols and four lignans related to pinoresinol, all of which are devoid of biological activities[1]. As part of our continuing interest in the constituents of this active fraction, we have isolated a new euphane triterpene, the structural determination of which is the object of the present report.

Experimental

Most of the equipment used for this study has been previously described[2]. In addition, the NMR spectra were recorded on a Bruker WM-250 or AC-200 instrument and the HPLC fractionations were performed on a Gynkotek Mod. 480 apparatus coupled a Chromatopac C-R6A integrator (Shimadzu) and a UV-Vis SBD-6 AV detector.

Extraction and fractionation

The seeds of *M. azedarach* (1 300 g), collected in the neighborhood of Niterói (State of Rio de Janeiro, Brazil), were exhaustively extracted with MeOH (2 x 5 l). Filtration and evaporation of the solvent under reduced pressure furnished a gummy residue (245 g). Partition between hexane and 5% aq. MeOH, followed by the evaporation of the methanolic phase and treatment with EtOAc afforded an active fraction (71 g). Bioassay-guided fractionation of a 1 g aliquot by conventional chromatographic processes using silica gel and mixtures of $CHCl_3$-MeOH, furnished the active fraction (21 mg). HPLC purification on a RP-18 column (eluent: MeOH-H_2O 45:55) allowed the isolation of phytosterols, four lignans and the gummy metabolite, cinamodiol **1**. The ^1H and ^{13}C-NMR spectra of 1 appear in Tables 1 and 2.

Bioassays

Fourth instar nymphs of *Rhodnius prolixus* were used. The test material was dissolved in EtOH-saline (1:4). Aliquots were added to blood in order to obtain the desired final concentrations (10 to 100 μg/μl). The test blood was placed in specially designed feeders[3], and the insects were allowed to feed. After feeding, the insects were weighed, incubated at 28 °C and observed every two days over a 1 month period. Only fully fed insects were used; partially fed ones were discarded. Death and ecdysis were counted.

Results and Discussion

Solvent-solvent partition of the crude methanolic seed extract of *Melia azedarach*, followed by conventional column chromatography on silica gel and final purification

by HPLC on a RP-18 column afforded an amorphous colorless material, homogenous in tlc, which was given the name cinamodiol (1).

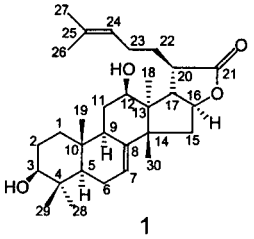
The ^{13}C NMR data (BBD and DEPT) of 1 established the empirical formula $C_{30}H_{46}O_4$, including the two OH hydrogens deduced from the data detailed below. Hence, compound 1 contains eight degrees of unsaturation. The ^1H and ^{13}C NMR data (Tables 1 and 2) also indicated the presence of two trisubstituted double bonds (δ 142.71 s, 132.56 s, 123.64 d and 119.21 d; δ 5.34 m and 5.11 brt; J = 6.8 Hz), two secondary OH groups (δ 78.91 d and 72.03 d; δ 4.01 m and 3.24 m), one γ-lactone function (δ 180.86 s and 82.19 d; 4.18 m; 1760 cm^{-1}), and seven methyl groups, five of which on saturated quaternary carbons (δ 1.34 s, 0.98 s, 0.86 s, 0.83 s and 0.77 s) and two on sp^2 carbons (δ 1.69 brs and 1.62 brs). The remaining 15 carbons were identified as 7 CH_2, 4 CH and 4 quaternary carbon atoms. Compound **1** was thus a tetracyclic triterpene, probably of the euphane series, which is very common in the genus *Melia*[4,5]. In such a skeleton, there is only one way to accomodate a double bond bearing two methyl groups, being in the side chain at the Δ^{24} position. Identification of the olefinic hydrogen H-24 as the signal at δ 5.11 resulted from the correlations observed in ^1H-^1H 2D-NMR with Me-26 and Me-27. The triplet nature of this signal indicated the presence of an allylic methylene group at C-23 which resonates at *c.a.* δ 2.2, and which showed no correlations with any other deshielded hydrogen signal (2D NMR), indicating the absence of an oxygenated function at C-22. The second trisubstituted double bond could be placed *a priori* in either the Δ^5, Δ^7 or $\Delta^{9(11)}$ position. The latter could be discarded from the chemical shift, and the general aspect of the olefinic hydrogen (δ 5.34 m $W_{1/2}$ = 12 Hz) was different from literature data for an H-11 of a $\Delta^{9(11)}$ double bond (δ 5.20 brm)[6]. Similarly, the carbon chemical shifts did not fit a Δ^5 position but were found in agreement with a Δ^7 one (Table 2)[7].

Biogenetic considerations suggested to place one OH group at C-3. The β-orientation was deduced from the chemical shift of H-3, a multiplet at 3.24, identical to the data on melianol **2** (δ 3.24)[7] and quite different from the equatorial 3β-H of kulolactone **3** (δ 3.47, Table 1)[8]. Furthermore, the absence of the Me-21 doublet in the ^1H NMR spectrum of **1** suggested that this carbon participated in the γ-lactone group which must necessarily close at C-16 since C-23 is not functionalized (*vide supra*). The stereochemistry at C-16 was determined by comparison with the ^1H-NMR data of kulolactone **3**[11], sendanolactone **4**[9] and kulactone **5**[8], in which the H-16α appears respectively as a multiplet at δ 4.15, 4.18 and 4.13, in complete agreement with our observed data (Table 1). In addition, direct comparison of the ^{13}C-NMR data of **1** with those of compounds

Table 1: ^1H-NMR data (δ) of cinamodiol (1) in $CDCl_3$ at 200 MHz, compared with 3 ($CDCl_3$)[11] and 5 ($CDCl_3$)[8].

H	1	3	5
H-3α	3.24 m	3,47 t	-
H-7	5.34 m	5.30 m	5.33 ddd
H-12α	4.01 m	n.o.	1.48 m
H-16α	4.18 m	4.15 m	4.13 ddd
H-24	5.11 brt J = 6.8 Hz	5.10 m	5.10 qt
Me-18	0.77 s	0.80 s	1.01 s
Me-19	0.86 s	0.95 s	0.95 s
Me-26	1.62 brs	1.63 brs	1.60 d
Me-27	1.69 brs	1.73 brs	1.68 d
Me-28	0.83 s	0.98 s	1.04 s
Me-29	0.98 s	0.98 s	1.11 s
Me-30	1.34 s	1.23 s	1.24 s

n.o. = not observed



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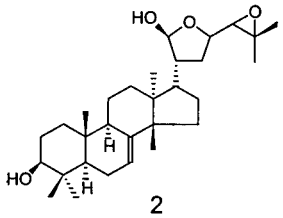

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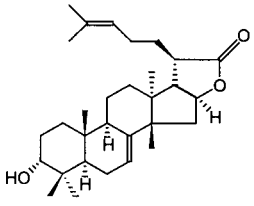

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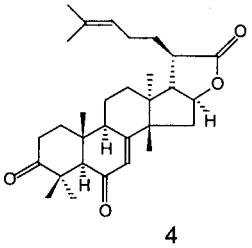

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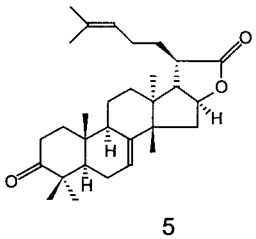

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Table 2: ^{13}C-NMR data (δ) of cinamodiol (**1**) in $CDCl_3$ at 50 MHz compared with 2 ($CDCl_3$)[7] and 5 ($CDCl_3$)[8].

C	1	5	2
1	36.92	38.40	37.28
2	27.43	34.92	27.70
3	78.91	215.10	79.24
4	38.91	47.90	39.03
5	50.62	52.71	50.85
6	23.91	26.00	23.27
7	119.21	118.75	118.28
8	142.71	143.60	145.71
9	48.38	48.00	49.67
10	35.19	35.09	35.11
11	29.12	24.33	17.64
12	72.03	29.25	35.23
13	44.32	39.71	43.87
14	54.77	55.26	50.79
15	36.10	35.81	34.32
16	82.19	82.50	27.40
17	53.17	58.15	47.17
18	12.85	12.50	13.15
19	19.75	21.42	24.06
20	45.49	45.52	33.90
21	180.86	180.30	101.83
22	30.02	29.85	31.61
23	25.88	26.11	78.50
24	123.64	123.50	67.85
25	132.56	132.69	57.97
26	17.85	17.75	19.51
27	25.66	25.75	25.05
28	27.34	24.30	27.55
29	14.59	21.46	14.80
30	33.58	32.15	22.64

2 and **5** (Table 2) confirmed the postulated euphane skeleton with a Δ^7 double bond and an OH group at C-3 (comparison of **1** and **2**). Finally, the remaining secondary OH group could be unequivocally placed at the 12β position by the effects observed in the ^{13}C-NMR spectrum (Table 2) on the β-carbons C-11 (δ +4.79) and C-13 (δ +4.5), by the γ-gauche effect on C-17 (δ -4.98), and by the 1,3-diaxial interaction on Me-30 (δ +1.43), when compared with 5.

All these considerations lead to the proposal for the relative cinamodiol structure 3β,12β-dihydroxy-7,24-euphadiene(21→16)olide (**1**). Although a number of euphane triterpenes have already been described for the genus *Melia*[4,5], cinamodiol **1** is one of the very few protolimonoids which possess a lactone ring between C-21 and C-16. Cinamodiol is also remarkable for its 12-OH group, very rare among triterpenes, except in the damarane series[10].

When tested against *Rhodnius prolixus* for anti-moulting activity, cinamodiol **1** showed almost no activity at 10 μg/μl: it induced some moulting delay and inhibited ecdysis in only 27%. This proved that another minor metabolite must be responsible for the bioactivities observed in the crude extract and in the active fraction.

Conclusion

The methanolic crude extract from the seeds of *Melia azedarach* shows anti-moulting activity on the haematophagous insect *Rhodnius prolixus*. The new euphane triterpene, cinamodiol (**1**), was isolated from the active fraction. Its structure is remarkable mainly for its oxygenated function at C-12. This compound is not responsible for the observed bioactivity.

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