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CONSTITUENTS OF BROSIMOPSIS OBLONGIFOLIA.3*

STRUCTURE OF A NEW DIELS-ALDER TYPE ADDUCT, BROSIMONE A

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Abstract - From the acetone extract of the roots of Brosimopsis oblongifolia Ducke brosimone A, 1, a new Diels-Alder type adduct, was isolated. The structure was determined on the basis of chemical and physical evidences.

In the previous papers [1,2] we reported the structure determination of a series of isoprene-substituted flavones showing antimicrobial and cytotoxic activity, isolated from the roots of Brosimopsis oblongifolia Ducke (Moraceae), a Brazilian tree known by the trivial name of "manichi".

In this communication we report the isolation and structure elucidation of a new phenolic compound, identified as a Diels-Alder type adduct, and named brosimone A, 1.

The acetone extract of the roots of B. oblongifolia was fractionated sequentially by silica gel column chromatography , and by reversed-phase ch··omatography resulting in the isolation of brosimone A, together with some r phenolic compounds still under investigation.

ɯone A, 1 , amorphous powder, $[\alpha]_D^{20}$ =-711 (MeOH), [M + 1]$^+$ at m/z \B-MS). Its ^{1}H NMR spectrum (table 1) showed signals attributable to a 2,4-disubstituted phenyl ring, a methyl group, three aromatic and/or olefinic singlets, together with four broad signals in the aliphatic region, that could not be analysed satisfactorily. The polyphenolic nature of the compound was indicated by the presence of four singlets, exchangeable with D_2O. The UV spectrum of 1 (MeOH) showed maxima at 279 and 332 nm, with a bathochromic shift at 281, 311 and 374 nm on addition of $AlCl_3$ (not reversible with HCl), that was ascribed to the presence of a chelated hydroxyl group not ortho disubstituted [3].

In the ^{13}C NMR spectrum of compound 1 (table 2) registered at 90°C, because of the broadness of the signals observed in the spectrum registered at room temperature (phenomenon attributed to a conformational equilibrium in solution), the resonances of a carbonyl group, fourteen aromatic and/or olefinic carbons and five aliphatic carbons were present. The ^{13}C NMR data pointed out the presence of a 2,4-dihydroxysubstituted benzoyl and a 2,4-dihydroxyphenyl moiety in the molecule, and also evidentiated the dimeric nature of the

compound, taking account of the mass spectrum of brosimone A.
By prolonged treatment with dimethylsulfate 1 gave the hexamethyl derivative 1a, $C_{46}H_{48}O_{10}$ (M^+ at m/z 760), the heptamethyl derivative 1b, $C_{47}H_{50}O_{10}$ (M^+ at m/z 774), and the octamethyl derivative 1c, $C_{48}H_{52}O_{10}$ (M^+ at m/z 788).
Compound 1b, in agreement with the dimeric structure of 1, showed the splitting of the ^1H and ^{13}C NMR signals, due to the methylation of only one of the two chelated hydroxyl groups. The analysis of the mass spectra of the three methyl derivatives of compound 1 suggested a Diels-Alder adduct type structure for brosimone A (see figure 1). As a matter of fact in the mass spectrum of 1a the fragment ions at m/z 543 (93%), and 217 (29%), arise by a retro Diels-Alder, and subsequent C-8, C-4 bond fragmentation , as already observed in other Diels-Alder type adducts. As expected in the mass spectra of 1b and 1c the corrisponding peaks appeared at m/z 557 (40% in 1b, and 72% in 1c), and 231 (100% in 1b, and 87% in 1c).

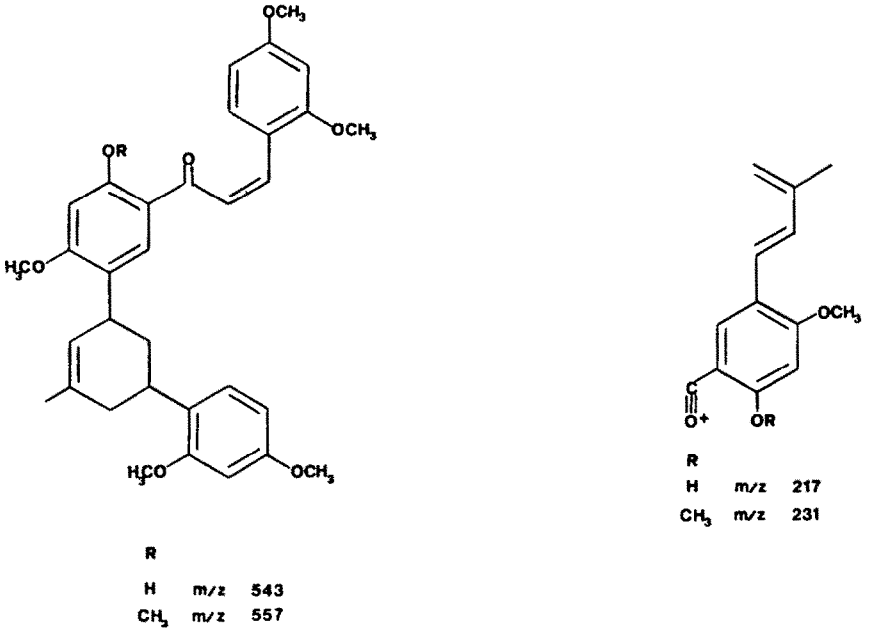
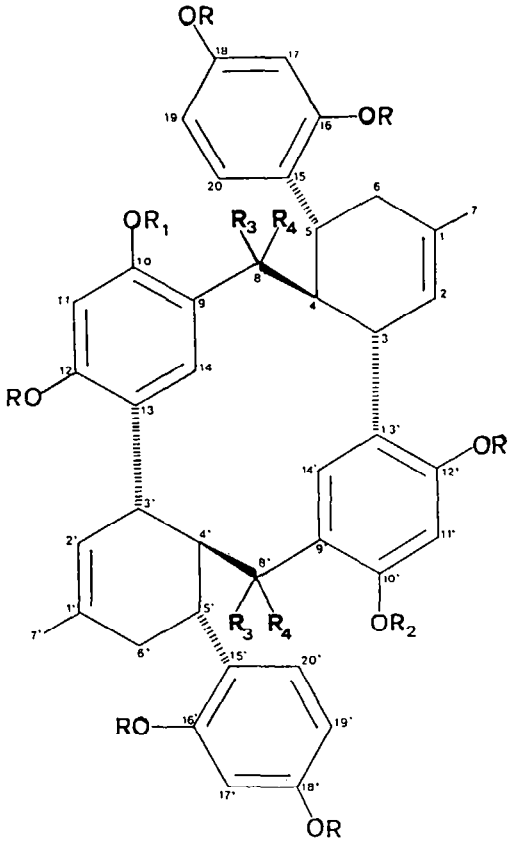


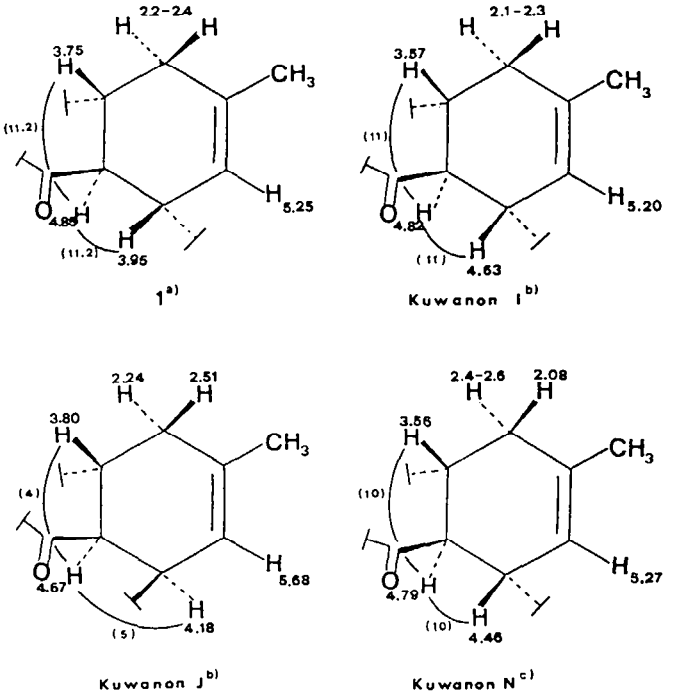
Figure 1

Further ^1H NMR decoupling experiments carried out on 1 confirmed the presence in the molecule of the methylcyclohexene ring on the basis of the chemical shift and coupling constant values observed for H-2/H-6, in comparison with reported data of other Diels-Alder type adducts (fig.2)[4,5]. Moreover the fragment ions at m/z 217 and 231 in the mass spectra of 1a and 1b,1c, respectively, arising from the benzoyl moiety, indicated that the latter should be linked through C-13 at C-3 of the methylcyclohexene ring. ^1H NMR data also suggested that brosimone A has the same arrangement of the 2,4-dihydroxyphenyl and the 2,4-dihydroxy-5-alkylbenzoyl moiety on the cyclohexene ring, as well as the same trans relative configuration of kuwanons I, N and O [5,6].
In order to corroborate these assignments, compound 1c was treated with methyllithium in THF to give the alcohol 1d ($C_{50}H_{60}O_{10}$, M^+ at m/z 820). The ^1H NMR spectrum of 1d, in comparison with that one of 1c (table 1), clearly showed the



	R	R_1	R_2	R_3 R_4
1	H	H	H	=O
1a	CH_3	H	H	=O
1b	CH_3	CH_3	H	=O
1c	CH_3	CH_3	CH_3	=O
1d	CH_3	CH_3	CH_3	CH_3, OH

Fig. 2



a) DMSO-d_6. Coupling constants measured through selective dec. exp.

b) Data from reference 4.

c) Data from reference 5.

TABLE 1. ^{1}H NMR chemical shift assignments of 1, 1a, 1b, 1c, 1d (400 MHz, δ)

H	1 (DMSO-d_6,30°C)	1a ($CDCl_3$,30°C)	1b ($CDCl_3$,30°C)	1c ($CDCl_3$,30°C)	1d ($CDCl_3$,30°C)
2,2'	5.25,2H,bs	5.30,2H,bs	5.15 and 5.39,2H,2s	5.28,2H,bs	5.28,2H,bs
3,3'	3.95,2H,bd	4.02,2H,bd	4.12,2H,bd†	ov.	3.90,2H,ov.*
4,4'	4.85,2H,bt	4.7-4.8,2H,b	4.65 and 4.77,2H,2t,J=10.5	4.31,2H,t,J=11.0	3.48,2H,t,J=9.0
5,5'	3.75,2H,bm	ov.	ov.	3.30,2H,bd,W1/2=22.0	3.73,2H,ov.*
6,6'	2.2-2.4,4H,bm	2.30,2H,bd 2.6-2.8,2H,b	2.20,and 2.34,2H,2bd 2.6-2.8,2H,b	2.1-2.4,4H,m	2.0-2.2,4H,m
7,7'	1.73,6H,bs	1.70,6H,bs	1.65 and 1.75,6H,2bs	1.67,6H,bs	1.52,6H,s
11,11'	5.85,2H,s	5.90,2H,s	5.87,2H,s	5.95,2H,s	5.90,2H,s
14,14'	8.70,2H,bs	7.80,2H,s	7.56 and 7.67,2H,2s	6.56,2H,s	7.17,2H,s
17,17'	6.25,2H,d,J=2.0	6.3-6.4,4H,m	6.35 - 6.45,4H,m	6.42,2H,d,J=2.0	6.47,2H,d,J=2.0
19,19'	6.05,2H,dd, J=2.0,8.0			6.37,2H,dd, J=2.0,8.0	6.52,2H,dd, J=2.0,8.0
20,20'	6.97,2H,d,J=8.0	7.12,2H,d, J=8.0	7.10 and 7.22,2H, 2d,J=8.0	7.32,2H,d,J=8.0	7.44,2H,d,J=8.0
CH_3	-	-	-	-	1.24,6H,s
OH	8.00,8.85,10.10, 12.30,8H	11.90,2H,s	11.80,1H,s	-	1.6-1.7,1H,bs
OCH_3	-	3.82,3.72, 3.62,18H,3s	3.92,3.80,3.72, 3.62,3.52,21H,5s	3.89,3.80,3.77, 3.74,24H,4s	3.88,3.84,3.72, 3.62,24H,4s

b = broad

* = chemical shift values determined and assigned by selective decoupling experiments.

† = H-3 or H-3', overlapped with other signals.

ov. = overlapped signals.

upfield shift of the triplet attributed to H-4 ($\Delta\delta$=0.83), assigning finally the C-4 position to the benzoyl moiety, and thus the C-5 position to the phenyl moiety. Owing the absence of proper models, it was not possible to correlate the CD curve of 1 to its absolute configuration.

From these results the structure of brosimone A was concluded to be represented as 1.

The optically active Brosimone A can be considered to be originated from two enzymatic Diels-Alder cycloaddition between two identical dehydroprenylchalcone units.

TABLE 2. ^{13}C NMR chemical shift assignments of 1, 1a, 1b, 1c.

	1	1a	1b	1c
	DMSO-d_6,90°C	$CDCl_3$,30°C	$CDCl_3$,30°C	$CDCl_3$,30°C
C-1,1'	133.8s	133.8	132.9 134.7	131.2
C-2,2'	125.9d	125.4	125.3 125.4	124.2
C-3,3'	41.9d*	40.2*	39.5* 40.2*	50.2*
C-4,4'	49.4d	50.5	52.2 53.1	53.0*
C-5,5'	37.4d*	36.7*	36.1* 36.9*	34.1
C-6,6'	39.2t	40.2	41.4	38.7
C-7,7'	23.5q	22.9	22.9 23.0	22.9
C-8,8'	208.2s	n.o.	200.7 206.8	206.9
C-9,9'	115.2s	115.2	115.2 124.8	125.0
C-10,10'	162.7s	162.3	160.3# 161.8#	158.5#
C-11,11'	102.0d	98.1#	95.6^ 97.4^	95.2
C-12,12'	162.7s	162.3	161.8# 162.9#	161.4
C-13,13'	122.9s	123.8	123.3@ 123.9@	123.3
C-14,14'	128.7d	131.3	131.5 132.6	128.1
C-15,15'	121.1s	121.3	121.5@ 122.7@	127.0
C-16,16'	156.8s#	159.5@	159.4# 159.6#	158.2#
C-17,17'	104.0d	99.2#	99.2^ 99.3^	98.4
C-18,18'	156.4s#	158.2@	158.1# 158.3#	158.3#
C-19,19'	107.3d	104.2	104.5 104.6	104.3
C-20,20'	136.4d	134.5	134.3 135.1	137.7

*,#,@,^: Assignments bearing the same superscript may be reversed.

1a OMe: 54.9, 55.2, 55.8; 1b OMe: 54.8, 54.9, 55.2, 55.3, 55.5, 55.8, 56.0; 1c OMe: 55.2, 55.3, 55.5, 55.8.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were registered on a Bruker AM 400 spectrometer. Chemical shifts are expressed in ppm downfield from TMS, and coupling constants (J) in Hz. Mass spectra were recorded on a VG Analytical 7070 EQ Mass spectrometer. The circular dichroism curve was registered with a Jasco J-40 apparatus. Kieselgel 60 was used for TLC and column chromatography, LiChroprep RP-8 for reversed-phase chromatography.

Plant material.

See ref 2.

Extraction and purification.

The roots were extracted as reported in ref 2. The fraction eluted with $CHCl_3$ containing 10% MeOH (1.8 g) was rechromatographed on LiChroprep RP-8 (MeOH-H_2O, 75:25) giving pure 1 (1.0 g).

Brosimone A, 1 .

Amorphous powder . $C_{40}H_{36}O_{10}$ from FAB-MS ([M + 1]$^+$ at m/z 677). $[\alpha]_D^{20}$ =- 711 (c 0.5 , MeOH). UV(MeOH), λ_{max} nm (log ε): 279 (3.98), and 332 (4.32); (MeOH+$AlCl_3$): 281 (4.28), 311 (4.40), and 374 (4.00). IR (KBr), ν_{max}: 3500 (broad), 1630 (broad), 1500 , 1380 , 1250 , 1180 cm^{-1} . ^1H NMR : see table 1 , and figure 2. ^{13}C NMR: see table 2 . CD (MeOH), [θ] (λ_{max}, nm): -1.01 x 10^5 (350), -1.68 x 10^5 (280) , +2.12 x 10^5 (245).

Methylation of brosimone A : 1b, 1c, 1d.

To Brosimone A (120 mg), dissolved in anhydrous acetone (75 ml), 5 g of K_2CO_3 and 3 ml of dimethylsulfate were added. The reaction mixture, refluxed overnight, gave a residue of 100 mg. By column chromatography of the residue on silica gel (n-hexane-EtOAc , 1:1) pure 1a (10 mg), 1b (28 mg), and 1c (35 mg) were obtained.

Hexamethylbrosimone A, 1a.

Crystals from n-hexane-EtOAc, m.p. 152-54 °C . ^1H and ^{13}C NMR : see tables 1 and 2,respectively. EI-MS, m/z (%): 760 (M$^+$, 100); 596 (22); 543 (93); 380 (20); 218 (28); 217 (29); 191 (31); 164 (12); 151 (31).

Eptamethylbrosimone A , 1b.

Crystals from n-hexane-EtOAc , m.p. 215-17 °C . $[\alpha]_D^{20}$ = -450 (c 0.5, $CHCl_3$). IR ($CHCl_3$), ν_{max}: 1670 , 1630 (chelated C=O), 1610 , 1500 , 1460 , 1280, 1160 , 1120 , 1030 cm^{-1} . ^1H and ^{13}C NMR : see tables 1 and 2 , respectively. EI-MS, m/z (%): 774 (M$^+$, 81); 610 (7); 557 (40); 394 (11); 378 (20); 231 (100); 217 (42); 191 (43); 164 (11); 151 (77).

Octamethylbrosimone A, 1c.

Crystals from n-hexane-$CHCl_3$, m.p. 169-72 °C (with dec.). $[\alpha]_D^{20}$ =- 209 (c 0.7, $CHCl_3$). IR ($CHCl_3$), ν_{max}: 1660 , 1600 , 1500 , 1460 , 1270 , 1160 , 1130 , 1030 cm^{-1}. ^1H and ^{13}C NMR : see tables 1 and 2 , respectively. EI-MS , m/z (%): 788 (M$^+$, 100); 573 (12); 557 (72); 393 (40); 231 (87); 217 (43); 191 (38); 151 (70) .

Reaction of octamethylbrosimone A with methyllithium:1d.

Compound 1c (20 mg) was dissolved in anhydrous THF (30 ml), and 0.5 ml of methyllithium were added (at room temperature, under nitrogen). After 1 h the reaction mixture was evaporated. The residue was dissolved in water, neutralized and extracted with $CHCl_3$. The chloroform extract (16 mg) was chromatographed on SiO_2, eluant n-hexane-EtOAc (3:1), giving pure 1d (8 mg), as main product.

1d. Amorphous powder. IR ($CHCl_3$), ν_{max}: 1610, 1500, 1460, 1440, 1290, 1200, 1160, 1120, 1040 cm^{-1} . ^1H NMR : see table 1 . EI-MS , m/z (%): 820 (M$^+$, 1); 802 (5); 784 (55); 392 (100); 179 (70); 151 (88).

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