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CONSTITUENTS OF BROSIMOPSIS OBLONGIFOLIA. 4 . STRUCTURES OF TWO NEW DIELS-ALDER TYPE ADDUCTS, BROSIMONE B AND BROSIMONE D[1]

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Abstract- Brosimones B and D, two new Diels-Alder type adducts, were isolated from the acetone extract of the roots of Brosimopsis oblongifolia. The structures of brosimones D and B were shown to be 1 and 2, respectively, on the basis of spectral data.

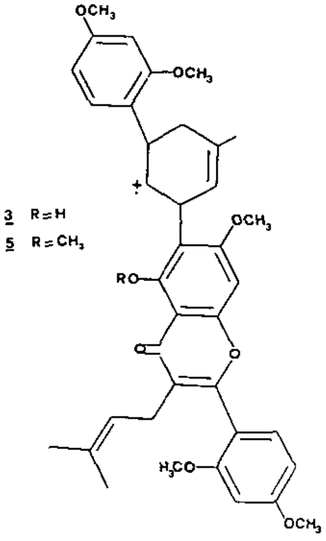
Brosimopsis oblongifolia is a large tree of the Amazonian area, known by the trivial name of "manichi". From the acetone extract of the roots, that showed cytotoxic and antimicrobial activity[2,3], we have already isolated several phenolic compounds, i.e. isoprene substituted flavonoids and a new Diels-Alder type adduct, named brosimone A [4-6].

In this paper we report the structure determination of two new compounds , that were identified as Diels-Alder type adducts, and named brosimone D, 1, and brosimone B, 2.

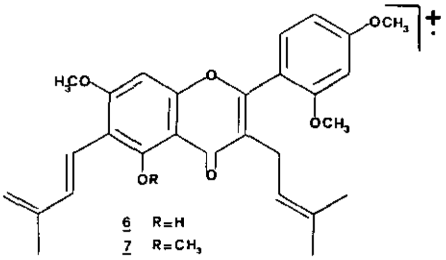
Brosimone D, 1, is an amorphous powder; $[\alpha]_D = -204°$ (MeOH); $[M+1]^+$ at m/z 761 (FAB-ms). The ^1H nmr spectrum of brosimone D registered at 27°C showed a complex pattern of signals, which varied depending on the temperature. The phenomenon, due to a conformational equilibrium in solution, was already observed in the spectra of brosimone A and other Diels-Alder type adducts [7,8]. The ^1H nmr spectrum of 1 registered at 65°C (400 MHz, DMSO-d_6, table 1) appeared not completely intelligible, nevertheless the signals attributable to a 1,2,4-trisubstituted phenyl ring, two prenyl chains, a methyl group, four singlets at δ 5.21, 6.03, 6.19 and 7.48, together with the signals of several OH groups (exchangeable with

D_2O) were evident. The presence of an alkylcyclohexene ring in the molecule was evidenced by the signals in the ^{13}C nmr spectrum of 1 (100 MHz, acetone-d_6, 27°C, table 2) at 38.2 (CH + CH_2), 46.2 (CH), 132.3 (C=), 124.5 (CH=), and 23.1 ppm (CH_3). The signals at 209.5 and 183.0 ppm were assigned to the carbonyl groups of a benzophenone and a flavone moiety , respectively. The uv spectrum of 1 (MeOH, λ: 323, 282, 265, 230sh nm), practically identical to those of Kuwanon H [9], a Diels-Alder adduct having a flavone partial structure, was in agreement with the presence of a flavone residue in the molecule.

Prolonged treatment of 1 with dimethyl sulfate in acetone gave the following methyl ethers: hexamethyl ether, 1a, $C_{51}H_{56}O_{11}$ (M^+ at m/z 844); heptamethyl ether, 1b, $C_{52}H_{58}O_{11}$ (M^+ at m/z 858) and octamethyl ether, 1c, $C_{53}H_{60}O_{11}$ (M^+ at m/z 872). These findings indicated that 1 has eight hydroxyl groups, and two of them are hydrogen bonded. Further in the ^1H nmr spectrum of 1a (table 1), the resonances attributed to another 2,4-dihydroxyphenyl ring were evident. The mass spectra of 1b and 1c showed peaks at m/z 625 (3), 233 (4), and 639 (5), 233 (4), respectively, arising from the fragmentation of the C4"-C8" bond (see figure 1), as already observed in kuwanon N octamethyl ether [10]. The peak at m/z 233 pointed out that one prenyl chain of brosimone D is located at the benzophenone moiety, whereas the peaks at m/z 462 (6, see mass spectra of 1a and 1b), and at m/z 476 (7, see mass spectrum of 1c) assigned the second prenyl chain at the flavone moiety. Moreover the latter fragments indicated that the C-5 OH chelated hydroxyl group was not methylated in the heptamethyl derivative, 1b.



3 R=H
5 R=CH3



6 R=H
7 R=CH3

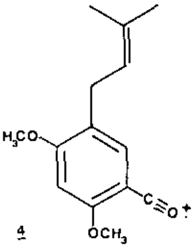


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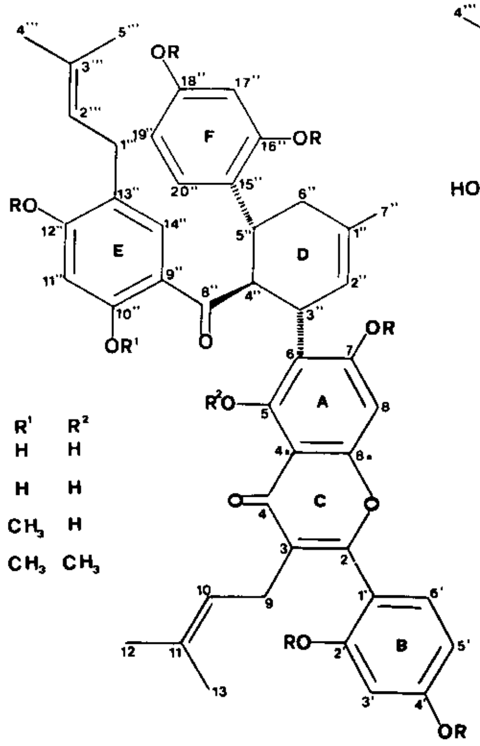
figure 1

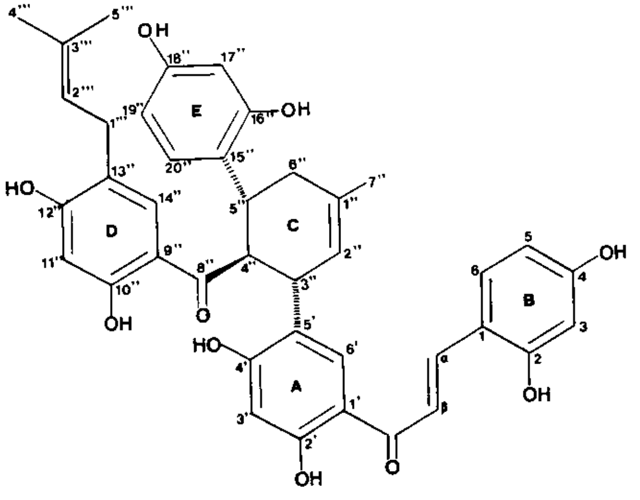
The prenyl chain belonging to the benzophenone moiety was assigned to the C-13" position on the basis of the ^1H and ^{13}C nmr data of 1. Moreover the bathochromic shift observed on addition of $AlCl_3$ in the uv spectrum of 1, and 1a, but not in 1b, indicated that the flavone moiety was substituted at C-6 position [11]. Finally the chemical shift value of the methylene carbon of the second prenyl chain (24.4 ppm) allowed to assign the prenyl chain to C-3 position [12] and consequently the alkylcyclohexene ring to C-6 position. The ^1H nmr chemical shifts and coupling costants of the protons of the methylcyclohexene ring of 1 compared with those of *brosimone* A [6], kuwanon N [10] and kuwanon J [13] (figure 2), allowed us to locate the flavone, the benzophenone, and the phenyl moieties at C-3", C-4", and C-5", respectively ,and also to assign the trans relative configuration to H-3", H-4", and H-5". From these results we propose the formula 1 for brosimone D, that is thus an isomer of kuwanon H (=moracenin A, =albanin G) [14], and kuwanon N [10].

Brosimone B, 2, is an amorphous powder, $[\alpha]_D = -447°$ (MeOH). The molecular formula of 2 was determined to be $C_{40}H_{38}O_{10}$ from FAB-ms ($[M+1]^+$ at m/z 679) and ^{13}C nmr data (25.2 MHz, 27°C, table 2). The uv spectrum of 2 exhibited maxima at 268, 281, 340 and 390 nm. The ^1H nmr spectrum of brosimone B registered at 27°C showed a complex pattern, with broad signals like brosimone D. The sharpness of the signals observed in the ^1H nmr spectrum recorded at higher temperature (60°C, 400 MHz, acetone-d_6,table 1) led us to suppose that brosimone B was a Diels-Alder type adduct, existing in solution as an equilibrium mixture of different conformers. The comparison of the ^1H and ^{13}C nmr data of 2 with that ones of 1 and brosimone A, confirmed the presence of a methylcyclohexene, a 2,4-dihydroxyphenyl, and a 2,4-dihydroxy-5-prenylbenzoyl units in the molecule. The remaining resonances of the ^1H nmr spectrum, and in particular the two doublets at δ 7.68, and 8.04 (J=15.0 Hz), taking also account of the uv data of 2, were assigned to a 2,2',4,4'-tetrahydroxy substituted chalcone unit, that should be linked through C-5' at the cyclohexene ring on the basis of the coupling pattern of the protons of the A ring. The red shift observed in the uv spectrum of 2 on addition of $AlCl_3$ (see experimental), imputable to the C-2' and C-10" chelated hydroxyl groups with a free ortho position confirmed the latter assignment.

The location of the substituents on the methylcyclohexene ring and their trans relative configuration were assigned on the basis of the chemical shift and coupling constant values of H-3", H-4", and H-5", in comparison with reported data of brosimone A, kuwanon I and J. From these results we propose the formula 2 for



	R	R1	R2
1	H	H	H
1a	CH_3	H	H
1b	CH_3	CH_3	H
1c	CH_3	CH_3	CH_3



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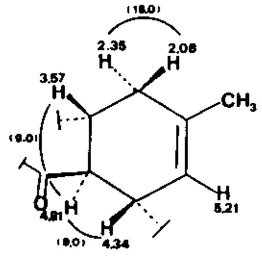
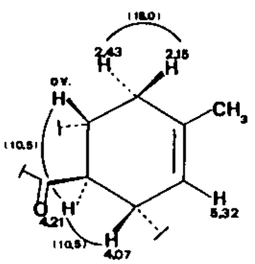


1



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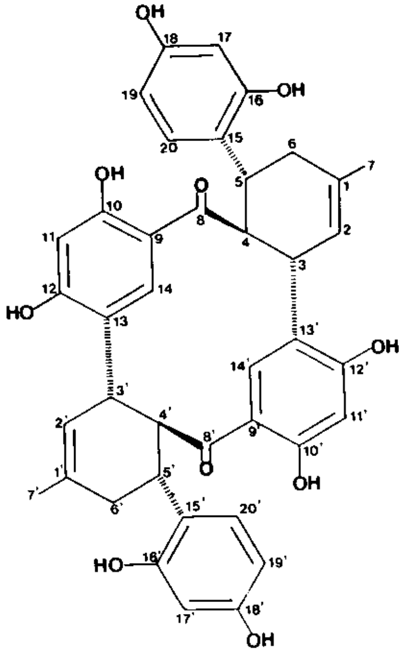
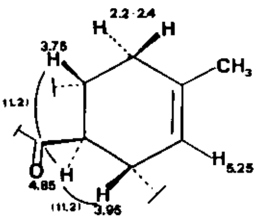
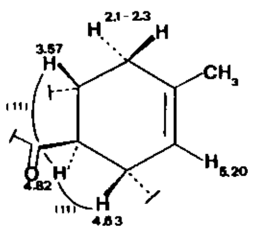


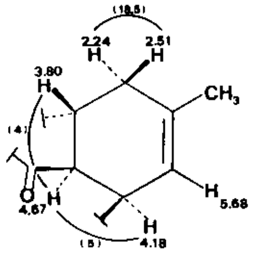
Brosimone A



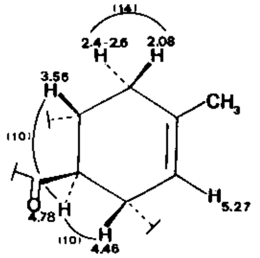
Brosimone A



Kuwanon I



Kuwanon J



Kuwanon N

figure 2

TABLE 1. ^{1}H nmr chemical shift assignments of 1, 1a, 1b, 1c, and 2.

H	1 (DMSO-d_6, 65°C)	1a ($CDCl_3$, 45°C)	1b ($CDCl_3$, 45°C)	1c ($CDCl_3$, 45°C)		2 (acetone-d_6, 60°C)
8	6.03,1H,s	6.06,1H,s	5.9-6.3,m	5.9-6.3,m	3	6.45,1H,d,J=2.3
9,1'''	2.9-3.0,4H,m	3.0-3.1,4H,b	2.9-3.1,4H,2bd	2.95,2H,d,J=7.0 3.02,2H,b	5	6.40,1H,dd,J=8.3,2.3
10,2'''	5.04,1H,m 5.11,1H,t,J=7.0	5.0-5.1,2H,m	5.0-5.2,m	5.0-5.2,m	6	7.44,1H,d,J=8.3
12,13, 4''',5'''	1.38,1.53,1.65, 1.69,12H,4s	1.30,1.45,1.62, 1,72,12H,4s	1.38,1.52,1.60, 1.62,12H,4s	1.35,1.56,1.59, 1.61,12H,4s	α	7.68,1H,d,J=15.0
3'	6.48,1H,d,J=2.0	6.53,1H,d,J=2.0	6.50,1H,d,J=2.0	6.5-6.6,b	β	8.04,1H,d,J=15.0
5'	6.39,1H,dd, J=8.0,2.0	6.55,1H,dd, J=8.0,2.0	6.55,1H,dd, J=8.0,2.0	6.5-6.6,b	3'	6.10,1H,bs
6'	7.06,1H,d,J=8.0	7.22,1H,d,J=8.0	7.18,1H,d,J=8.0	7.18,1H,d,J=8.0	6'	7.79,1H,bs
2"	5.21,1H,bs	5.32,1H,bs	5.24,1H,bs	5.25,1H,bs	2"	5.32,1H,bs
3"	4.34,1H,b	4.3-4.4,1H,b	4.3-4.4,1H,b	4.3-4.4,1H,b	3"	4.07,1H,bd,J=10.5
4"	4.81,1H,bt,J=9.0	4.8-4.9,1H,b	5.0-5.2,m	5.0-5.2,m	4"	4.21,1H,bt,J=10.5
5"	3.57,1H,bt,J=9.0	3.60,1H,b	ov.	ov.	5"	ov.
6"	2.08,1H,dd,J=18.0, 3.0 ; 2.35,1H,m	2.5-2.7,1H,b 2.25,1H,b	2.1-2.2,2H,b	2.1-2.2,2H,b	6"	2.15,1H,dd,J=18.0,4.5 2.43,1H,m
7"	1.71,3H,s	1.80,3H,s	1.70,3H,s	1.72,3H,s	7"	1.75,3H,s
11"	6.19,1H,s	6.01,1H,b	5.9-6.3,m	5.9-6.3,m	11"	6.01,1H,s
14"	7.48,1H,s	7.35,1H,s	7.26,1H,s	7.1-7.2,1H,b	14"	7.24,1H,bs
17"	6.14,1H,bs	6.27,1H,d,J=2.0	5.9-6.3,m	5.9-6.3,m	17"	6.18,1H,d,J=2.3
19"	5.98,1H,dd, J=8.0,2.0	6.26,1H,dd, J=8.0,2.0	5.9-6.3,m	5.9-6.3,m	19"	6.02,1H,dd,J=8.3,2.3
20"	6.79,1H,b	7.07,1H,d,J=8.0	6.88,1H,b	6.78,1H,b	20"	6.80,1H,d,J=8.3
					1'''	2.85,1H,dd,J=18.1,7.5 2.92,1H,dd,J=18.1,6.8
OH	8.70,8.89,9.64,9.69, 10.20,10.44,12.94, 7H,7bs;13.39,13.92, 1H,2s	13.00,13.55,2H,2s	13.25,1H,s	-	2'''	4.98,1H,bt,J=7.0
OMe	-	3.69,3.70,3.75, 3.76,3.85,18H, 5s	3.61,3,68,3.77, 3.82,21H,4s	3.62,3.65,3,75, 3.78,3.85,24H, 5s	4''' 5'''	1.50 and 1.60,6H,2s
					OH	13.53 and 12.95,2H,2s

Abbreviations: s=singlet; d=doublet; t=triplet; q=quartet; m=multiplet; b=broad; ov.=overlapped .

TABLE 2. ^{13}C nmr chemical shift assignments of 1, and 2 (acetone-d6)

C	1	C	1	C	2^{a}	C	2
2	n.o.	4"	46.2d	1	115.3^{o}	6"	38.7
3	120.0s	5"	38.2d	2	159.8$^{\#}$	7"	23.3
4	183.0s	6"	38.2t	3	103.5*	8"	208.7
4a	105.0s	7"	23.1q	4	162.0^{+}	9"	114.6^{o}
5	156.1s*	8"	209.5s	5	107.3	10"	165.4^{+}
6	105.0s	9"	115.4s	6	132.0	11"	103.3*
7	163.9s$^{\Delta}$	10"	162.6s$^{\Delta}$	α	117.7	12"	162.6^{+}
8	93.1d	11"	103.7d^{+}	β	141.0	13"	123.4
8a	163.4s$^{\Delta}$	12"	161.7s$^{\Delta}$	C=O	193.0	14"	129.7
9	24.4t	13"	123.3s	1'	115.1^{o}	15"	121.8
10	122.4d	14"	129.4d	2'	164.1^{+}	16"	157.0$^{\#}$
11	131.9s	15"	121.3s	3'	103.5*	17"	102.4
12	25.7q^{o}	16"	156.9s*	4'	162.7^{+}	18"	156.2$^{\#}$
13	17.7q§	17"	103.7d^{+}	5'	120.1	19"	108.9
1'	112.8s	18"	156.8s*	6'	132.0	20"	133.1
2'	161.1s$^{\Delta}$	19"	107.4d^{x}	1"	134.8	1"'	28.6
3'	102.4d^{+}	20"	132.8d	2"	125.2	2"'	123.8
4'	161.8s$^{\Delta}$	1"'	28.1t	3"	42.8	3"'	131.7
5'	107.8d^{x}	2"'	123.2d	4"	50.7	4"'	25.6
6'	131.9d	3"'	131.9s	5"	37.8	5"'	17.7
1"	132.3s	4"'	25.5q^{o}				
2"	124.5d	5"'	17.4q§				
3"	38.2d						

Multiplicity of the compound 1 estimated by off-resonance decoupling experiment.

o,*,#,+,§,Δ,x, These values are interchangeable in the same column.

[a] Registered on a Varian XL 100 .

brosimone B, that is an isomer of kuwanon I [7], and J [13], isolated from the root bark of the japanese cultivated mulberry tree, and from callus tissue of Morus alba, respectively. *It is interesting to notice the co-occurrence in the roots* of B. oblongifolia of the optically active brosimones B and A. As a matter of fact brosimone A could be considered originated from brosimone B, through an enzymatic Diels-Alder cycloaddition between the dehydroprenyl chain of the D ring and the chalcone unit.

ACKNOWLEDGEMENTS

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EXPERIMENTAL

The ^1H and ^{13}C nmr spectra were registered on a Bruker AM 400 spectrometer, unless otherwise reported; chemical shifts are expressed in ppm downfield from TMS and coupling contants (J) in Hz. Mass spectra were recorded on AEI MS 902,70 eV.

Plant material and extraction. See reference 4.

Purification. Brosimone D (250 mg), and brosimone B (40 mg) were purified sequentially by silica gel column chromatography (MeOH:$CHCl_3$, 9:1), and LiChroprep RP-8 column chromatography (MeOH:H_2O, 8:2).

Brosimone D, 1. $C_{45}H_{44}O_{11}$. Amorphous powder . FAB-ms $[M + 1]^+$ at m/z 761. $[\alpha]_D$= -204° (c 0.3, MeOH) . Uv (MeOH), λ_{max} nm (log ε): 323 (4.13), 282 (4.26), 265 (4.32), 230 sh (4.43); (MeOH + $AlCl_3$): 361, 311, 270 sh; not reversible upon addition of HCl; ^1H and ^{13}C nmr: see tables 1 and 2, respectively.

Methylation of brosimone D. A mixture of brosimone D, 1, (120 mg) , dimethyl sulphate (1 ml), and K_2CO_3 (5 g) in acetone (30 ml) was refluxed overnight. The usual work-up gave a residue, that was purified on silica gel (*$CHCl_3$:MeOH,* 99.5:0.5) to give pure 1a (12 mg), 1b (10 mg), and 1c (17 mg).

Hexamethylbrosimone D, 1a. Amorphous powder. Uv (MeOH), λ_{max} nm: 323, 270 (sh), 265, 230; (MeOH+$AlCl_3$): 360, 305, 276. EI-ms, m/z (%): 844 (M^+, 13), 843 (17), 625 (3, 13), 462 (6, 100), 447 (28), 431 (30), 419 (63), 382 (63), 164 (30). ^1H nmr: see table 1.

Heptamethylbrosimone D, 1b. Amorphous powder. Uv (MeOH), λ_{max} nm: 308, 266, 230; (MeOH+$AlCl_3$): 308, 267, 230. EI-ms, m/z (%): 858 (M^+, 7), 625 (3, 6), 462 (6, 67), 419 (50), 365 (100), 233 (4, 52). ^1H nmr: see table 1 .

Octamethylbrosimone D, 1c. Amorphous powder. EI-ms, m/z (%): 872 (M^+, 3), 871 (5), 640 (7), 639 (5, 16), 616 (9), 476 (7, 32), 462 (41), 445 (26), 433 (26), 419 (33), 365 (90), 233 (4, 100). ^1H nmr: see table 1.

Brosimone B, 2. $C_{40}H_{38}O_{10}$. Amorphous powder . FAB-ms $[M + 1]^+$ at m/z 679. $[\alpha]_D$= -447° (c 0.1, MeOH). Uv (MeOH), λ_{max} nm (log ε): 390 (4.15), 340 (3.98), 281 (4.11), 268 (4.09); (MeOH + $AlCl_3$): 455, 368, 312, 270, not reversible upon addition of HCl. ^1H and ^{13}C nmr: see tables 1 and 2, respectively .

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