Pergamon

Tetrahedron Vol. 51, No. 42, pp. 11595-11600, 1995

0040-4020/95 $9.50+0.00

0040-4020(95)00720-2

Cyclodione, an Unsymmetrical Dimeric Diterpene from *Cylicodiscus gabunensis*

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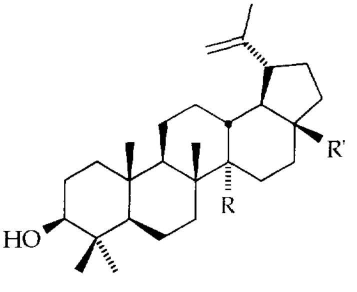
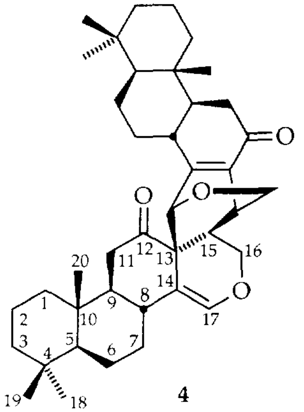
Abstract: The structure of cyclodione (**4**) isolated from the stem bark of the tree *Cylicodiscus gabunensis* has been determined by spectroscopic methods. Cyclodione (**4**) is a nonacyclic dimeric cassane-type diterpenoid.

Cylicodiscus gabunensis (Mimosaceae) Harms is a large tree, reaching 60 m in height and 11 m in girth, common in the tropical rain forest of West and Central Africa.[1] It has many applications in the traditional medicine, a decoction of the leaves is used, for example, to treat migraine while an infusion of the stem bark in water is a reputed remedy for rheumatism and stomach disorders.[2] Previous investigations[2-4] of the secondary metabolites of *Cylicodiscus gabunensis* have yielded a triterpenoid, cylicodiscic acid (**3**), and five saponins. Our interest in the systematic investigation of the chemical constituents of Cameroonian medicinal plants, and particularly those with antiprotozoal activity,[5] has led us to investigate the stem bark of *C. gabunensis*. This paper reports the isolation and structural elucidation of a new unsymmetrical cassane diterpene dimer, cyclodione (**4**).



1 $R = CH_3$; $R' = CH_3$
2 $R = CH_3$; $R' = COOH$
3 $R = CH_2OH$; $R' = COOH$



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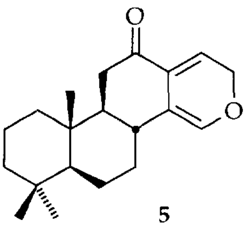

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Table 1. ^1H (500 MHz) NMR data for cyclodione (4) in $CDCl_3$ and C_6D_6. The solvent signals (7.26 and 7.16 ppm) were used as reference. The coupling constants *J* are given in Hz.

H	$CDCl_3$	C_6D_6	H	$CDCl_3$	C_6D_6
1α	0.89; m	0.51; m	1'α	0.89; m	0.46; m
1β	1.59; m	1.13; m	1'β	1.59; m	1.25; m
2α	1.45; m	1.22; m	2'α	1.45; m	1.31; m
2β	1.55; m	1.34; m	2'β	1.55; m	1.36; m
3α	1.14; m	1.01; m	3'α	1.14; m	0.96; m
3β	1.42; m	1.31; m	3'β	1.42; m	1.25; m
5	0.89; m	0.59; m	5'	0.87; m	0.63; m
6α	1.76; m	1.48; m	6'α	1.72; m	1.58; m
6β	1.40; m	1.15; m	6'β	1.39; m	1.14; m
7α	1.29; m	0.99; m	7'α	0.74, m	0.87; m
7β	2.03; m	1.77; m	7'β	2.03; m	1.92; m
8	2.52; m	2.49; m	8'	2.58; ddd; 4, 10, 13	2.31; m
9	1.30; m	1.02; m	9'	1.50; m	1.36; m
11α	2.52; m	2.50; dd; 4.8, 15	11'α	2.47; dd; 3.5, 16	2.46; dd; 3.4, 16
11β	2.32; dd; 13.2, 14.7	2.30; m	11'β	2.10; dd; 14.2, 16.2	1.88; dd; 14.1, 15.9
15	2.50; m	2.76; m	15'	3.32; m	3.53; m
16α	3.52; dd; 4.2, 11.5	3.49; dd; 4.2, 11.5	16'α	2.99; dd; 1.9, 7.7	3.11; dd; 2.0, 7.7
16β	4.10; d; 11.5	3.84; d; 11.5	16'β	4.16; d; 7.7	4.36; d; 7.7
17	6.46; d; 0.9	6.48; d; 1.4	17'	4.78; s	4.92; s
18	0.89; s	0.82; s	18'	0.86; s	0.69; s
19	0.88; s	0.79; s	19'	0.84; s	0.73; s
20	1.02; s	0.78; s	20'	0.90; s	0.60; s

Table 2. ^{13}C (125 MHz) NMR data for cyclodione (4) in $CDCl_3$ and C_6D_6. The solvent signals (77.0 and 128.4 ppm) were used as reference.

C	$CDCl_3$	C_6D_6	C	$CDCl_3$	C_6D_6
1	38.5; t	38.8; t	1'	37.9; t	37.9; t
2	18.6; t	19.3; t	2'	18.6; t	19.2; t
3	41.8; t	42.4; t	3'	41.8; t	42.2; t
4	33.2; s	33.5; s	4'	33.1; s	33.5; s
5	54.8; d	55.0; d	5'	54.3; d	54.7; d
6	21.1; t	21.7; t	6'	21.5; t	22.1; t
7	29.8; t	30.3; t	7'	29.8; t	30.7; t
8	33.8; d	34.5; d	8'	35.8; d	36.5; d
9	56.4; d	56.6; d	9'	52.9; d	53.3; d
10	37.6; s	38.0; s	10'	36.7; s	37.1; s
11	39.0; t	39.6; t	11'	37.1; t	37.8; t
12	208.0; s	207.4; s	12'	195.3; s	194.3; s
13	54.3; s	55.2; s	13'	137.7; s	138.8; s
14	118.5; s	119.4; s	14'	159.8; s	159.4; s
15	32.7; d	33.9; d	15'	32.9; d	34.0; d
16	68.4; t	69.1; t	16'	62.8; t	63.6; t
17	143.1; d	143.8; d	17'	70.3; d	71.1; d
18	33.4; q	34.0; q	18'	33.2; q	33.6; q
19	21.9; q	22.4; q	19'	21.6; q	22.1; q
20	13.9; q	14.3; q	20'	14.5; q	14.9; q

A methanol extract of the stem bark of C. *gabunensis* was subjected to sequential partition extraction with chloroform followed by ethyl acetate. Repeated column chromatography of the chloroform soluble fraction on silica gel eluting with mixtures of hexane and ethyl acetate afforded lupeol (**1**),[6] betulinic acid (**2**),[6] cylicodiscic acid (**3**),[2] and the new compound cyclodione (**4**).

Cyclodione (**4**) was obtained as colourless crystals. The EI mass spectrum showed only two major peaks, at *m/z* 300 (base peak) and 600 (M^+), and high resolution measurements indicated that the elemental composition of cyclodione is $C_{40}H_{56}O_4$, and that the composition of the fragment is $C_{20}H_{28}O_2$. This suggested that cyclodione (**4**) is composed of two similar parts, although the NMR spectra clearly demonstrated that cyclodione (**4**) is not a symmetrical dimer. It does not react with (trimethylsilyl)diazomethane in hexane/methanol or with acetic anhydride in pyridine, i.e. it does not contain any carboxylic acid or primary/secondary alcohol functions.

The ^1H NMR spectrum (data given in Table 1) contained six methyl singlets in $CDCl_3$ (δ 1.02; 0.90; 0.89; 0.88; 0.86; 0.84) which were all shifted up-field in C_6D_6. Additional proton signals included those of two distinct allylic methines (δ 2.52, m; 2.58, ddd), one olefinic proton (δ 6.46, d), and several protons attached to oxygenated carbons which apparently are part of ether links. Resonances in the ^{13}C NMR spectrum recorded in $CDCl_3$ (data given in Table 2) confirm the presence of two keto groups (δ 208.0, s; 195.3, s), and revealed in addition two double bonds of which one is tetrasubstituted and conjugated to a carbonyl (δ 159.8, s; 137.7, s) and the other trisubstituted (δ 143.1, d; 118.5, s), as well as three oxygenated carbons (δ 70.3, d; 68.4, t; 62.8, d).

The elucidation of the structure of cyclodione (**4**) was facilitated by 2D NMR experiments. The structure of the central tricyclic moiety containing the two oxygen bridges was determined essentially by long-range ^1H-^{13}C scalar couplings, established by gradient enhanced HMBC NMR experiments and summarised in Table 3. The ^1H-^1H scalar coupling between 15-H and 15'-H is too small to be detected in the COSY spectrum, and two isolated ^1H spin systems were observed for 15-H/16-H_2 and 15'-H/16'-H_2 respectively. However, the long-range ^1H-^{13}C couplings observed between 15-H and 13'-H, 15'-H and 16'-H, as well as between 17'-H and 12-H, 13-H, 14-H and 15-H establish this connection. The connectivities in the hydrocarbon parts could to a large degree be established by ^1H-^1H correlations, confirmed by pertinent ^1H-^{13}C correlations (the same correlations were observed for both unprimed and primed atoms). For example, 18-H_3 and 19-H_3 show strong correlations with C-4 as well as C-3 and C-5, while 20-H_3 correlates with C-10 as well as C-1 and C-9. The connection between the two hydrocarbon part and the oxidised part of the molecule was shown by the HMBC correlations between 11-H_2 and C-12 (and 11'-H_2 and C-12'), and between 8-H and C-14 (and 8'-H and C-14').

The relative stereochemistry of central tricyclic moiety of cyclodione (**4**) was determined by the relative size of NOE interactions, as determined from volume integration of NOESY correlations (see Table 4). Critical NOE enhancements in the hydrocarbon parts were observed between 8-H and 11-Hβ (and 8'-H and 11'-Hβ), and between 20-H_3 and 11-Hβ, 8-H as well as 19-H_3 (and 20'-H_3 and 11'-Hβ, 8'-H as well as 19'-H_3), indicating that both C-19 and C-20 (and C-19' and C-20') have axial positions and that the two saturated six-membered ring systems occur in an all-trans fused chair conformation. In addition, NOESY correlations between the protons 5-H and 7-Hα (and 5'-H and 7'-Hα), between 5-H and 9-H (and 5'-H and 9'-H), and between 7-Hα and 9-H (and 7'-Hα and 9'-H) were observed. The results show that 17'-H is close to 8-H and 11-Hβ, which means that C'-17 is axial, and this determined the relative configuration of C-15. The absence of NOESY correlations between 15-H and 16'-H_2 as well as the weak correlation between 17-H and 16'-Hβ confirm the suggested configuration of C'-15 and C'-17.

Table 3. Long-range correlations observed for 15-H, 16-H_2, 17-H, 15'-H, 16'-H_2 and 17-H in the HMBC NMR spectrum of cyclodione (4) in C_6D_6.

From:	To:								
15-H	C-12	C-13	C-13'	C-15'	C-16'				
16-Hα	C-13	C-15	C-17						
16-Hβ	C-13	C-15	C-17						
17-H	C-8	C-13	C-14	C-16					
15'-H	C-13	C-15	C-16	C-12'	C-13'	C-14'			
16'-Hα	C-13'	C-15'	C-17'						
16'-Hβ	C-13	C-13'	C-15'	C-17'					
17'-H	C-12	C-13	C-14	C-15	C-8'	C-12'	C-13'	C-14'	C-16'

Table 4. Volume integrated NOESY correlations observed for 15-H, 16-H_2, 17-H, 15'-H, 16'-H_2 and 17-H in cyclodione (4) in C_6D_6.

From:	To:			
15-H	16-Hα (6.7 %)	16-Hβ (2.3 %)	15'-H (6.1 %)	
16-Hα	15-H (6.7 %)	16-Hβ (17.9 %)	17-H (0.6 %)	
16-Hβ	15-H (2.3 %)	16-Hα (18.7 %)	15'-H (5.2 %)	16'-Hβ (0.4 %)
17-H	7-Hα (5.4 %)	7-Hβ (4.3 %)	16-Hα (0.7 %)	16'-Hβ (0.8 %)
15'-H	15-H (6.1 %)	16-Hβ (5.2 %)	16'-Hα (3.8 %)	16'-Hβ (3.1 %)
16'-Hα	15'-H (3.8 %)	16'-Hβ (20.0)		
16'-Hβ	16-Hβ (0.5 %)	17-H (0.7 %)	15'-H (3.0 %)	16'-Hα (20.0 %)
17'-H	8-H (8.0 %)	11-Hβ (3.4 %)	15-H (0.3 %)	7'-Hβ (3.4 %)

The fragmentation pattern in the EI mass spectrum of cyclodione (**4**) (discussed above) is unexpected for such a complex molecule, and apparently the division of cyclodione (**4**) into two parts with the same composition is facile in the ion source. By performing a hypothetical retro-Diels-Alder reaction (breaking the bonds between C-13 and C'-17 as well as C-15 and C'-15), the cassane diterpene **5** containing both a diene and a dienophilic functionality would be obtained, and it is possible that such a diterpene is the precursor of cyclodione (**4**). A similar example is the sesquiterpene artabsin containing a cyclopentadiene moiety, which through a Diels-Alder reaction can dimerize to absinthin, the well-known constituent of *Artemisia absinthium*.[7] However, so far the presence of the monomer **5** has not been established in the plant studied here, and attempts to prepare it by heating cyclodione (**4**) to 180 °C for several hours, both neat and in toluene,[8] failed.

EXPERIMENTAL

General: ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded at room temperature with a Bruker ARX 500 spectrometer with an inverse 5 mm probe equipped with a shielded gradient coil. COSY, HMQC and HMBC experiments were performed with gradient enhancements using sine shaped gradient pulses, and for the 2D heteronuclear correlation spectroscopy the refocusing delays were optimised for $^1J_{CH}$=145 Hz and $^2J_{CH}$=10 Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker UXNMR software (rev. 930901). The chemical shifts are given in ppm (with the solvent peaks serving as reference) and the coupling constants *J* in Hz. EI mass spectra were recorded by a JEOL SX102 spectrometer at 70 eV. Column chromatography was performed on silica gel 60, and thin-layer chromatography was carried out on 0.25 μm silica gel coated glass plates (Merck 60 F_{254}) using UV and/or 10 % H_2SO_4 and heat as the developing agents. Melting points, which are uncorrected, were determined using a Reichert microscope.

Collection, extraction and isolation: The stem bark of *Cylicodiscus gabunensis* Harms was collected in February 1994 from Mvam-Yetom, south Province of Cameroon. Voucher specimens for this collection are maintained for botanical reference at the National Herbarium, Yaounde, Cameroon. The air-dried and powdered plant material was macerated in methanol. Removal of the solvent under reduced pressure provided an organic extract which was subjected to partition extraction with chloroform followed by ethyl acetate. The chloroform-soluble fraction after concentration *in vacuo* furnished a dark brown residue (21 g). This extract was dissolved in chloroform (20 ml) and chromatographed on a column of silica gel (500 g). Elution started with hexane and continued stepwise through hexane/ethyl acetate mixtures, ethyl acetate, and ethyl acetate/methanol mixtures. The eluate was collected in 250 ml fractions, which were analysed by TLC in appropriate solvent systems. From this chromatographic separation, lupeol (**1**) (1.5 g), betulinic acid (**2**) (280 mg), and a mixture of cylicodiscic acid (**3**) and cyclodione (**4**) were obtained. Repeated column chromatographic purification of the mixture afforded pure cylicodiscic acid (**3**) (80 mg) and cyclodione (**4**) (112 mg). The three triterpenes were identified by comparison of their physical and spectral data with published data.

Cyclodione (4): Colourless crystals, m.p. 272-273 °C (ethanol). $[\alpha]_D$ +174° (c 1.0 in $CHCl_3$). UV (methanol) λ_{max} (ε): 250 nm (3500). IR (KBr): 2920, 2850, 1690, 1670, 1380, 1360, 1180, 1050 and 920 cm^{-1}. The NMR data are presented in Tables 1 and 2. MS: *m/z*: 600.4186 (M$^+$, 17 %, $C_{40}H_{56}O_4$ requires 600.4178), 396 (2 %), 300 (100 %), 161 (4 %), 133 (8 %), 69 (12 %).

Acknowledgements

Financial support from the International Programs in the Chemical Sciences (IPICS) and the Swedish Science Research Council is gratefully acknowledged.

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8. Cyclodione (**4**) was heated in D_8-toluene in a sealed tube at 180 °C for 5 hours, whereafter the solution was analysed directly by ^1H NMR. No transformation had occured.

(*Received in UK* 8 *August* 1995; *revised* 31 *August* 1995; *accepted* 1 *September* 1995)