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J. Nat. Prod., **1993**, 56 (1), 140-143• DOI:
10.1021/np50091a022 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 56, No. 1, pp. 140-143, January 1993

A NEW ANTIBACTERIAL SESQUITERPENE FROM *PREMNA OLIGOTRICHA*

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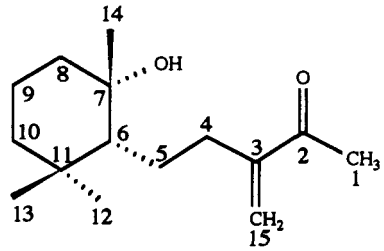
ABSTRACT.—A novel sesquiterpene, 7α-hydroxy-6,11-cyclofarnes-3(15)-en-2-one [**1**], has been isolated from the aerial parts of *Premna oligotricha* (Verbenaceae) using an antimicrobial bioassay-guided isolation procedure. The sesquiterpene was identified on the basis of spectroscopic data and showed weak activity against Gram-positive bacteria *Bacillus pumilus*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Streptococcus faecalis*.

Recent studies on the biological and chemical constituents of *Premna oligotricha* Baker (Verbenaceae) have resulted in the isolation of diterpenes and flavonoids, of which some have pronounced antibacterial activity (1–3). Further investigation on the antibiotic fraction of the EtOH extract of the aerial parts of this plant has resulted in the isolation of a minor constituent, 7α-hydroxy-6,11-cyclofarnes-3(15)-en-2-one [**1**]. This novel sesquiterpene shows weak antibiotic activity against a range of Gram-positive bacteria.

RESULTS AND DISCUSSION

Compound **1** was a minor constituent obtained in a yield of 0.00075% by bioassay-guided separation. Hreims established the molecular formula as $C_{15}H_{26}O_2$, suggesting a dioxygenated sesquiterpene. A tertiary hydroxyl group, which could not be acetylated (pyridine/Ac_2O), was evident by a broad band in the ir spectrum at ν max 3490 cm^{-1}. The single maximum in the uv spectrum at λ max 230 nm and an ir band at ν max 1670 cm^{-1} were typical of an α,β-unsaturated carbonyl functional group. This functional group was further substantiated by the ^{13}C-nmr spectrum (Table 1), which showed signals for a carbonyl carbon at δ 200.5 (C-2) and two olefinic carbons as a quaternary and an exomethylene resonance at δ 150.0 and 125.5, respectively. In the ^1H-nmr spectrum (Table 1) the methylene protons appeared as broad singlets at δ 6.00 and 5.82.

The ^1H- and ^{13}C-nmr spectra further revealed one strongly deshielded methyl singlet (δ 2.34, δ_C 26.1, Me-1) and deshielded aliphatic methylene protons (δ 2.45 and 2.35, δ_C 34.4, H_2-4) thereby allowing placement of a terminal methyl



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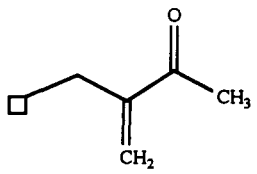

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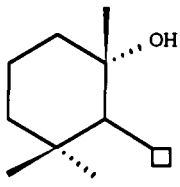

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TABLE 1. ^1H- and ^{13}C-nmr Chemical-Shift Data ($CDCl_3$) for **1**.

Carbon	δ_C	Proton	δ_H	Coupling constant (J, Hz)
C-1	26.1	H-1	2.34 s	
C-2	200.5			
C-3	150.0			
C-4	34.4	H_a-4	2.45 dddd	15.1, 10.1, 5.1, 1.1
		H_b-4	2.35 dddd	15.1, 14.4, 5.7, 0.9
C-5	25.8	H_a-5	1.49 m	
		H_b-5	1.39 m	
C-6	57.1	H-6	1.40 m	
C-7	74.8			
C-8	43.2	H-8ax	1.52 m	
		H-8eq	1.72 m	
C-9	20.6	H-9ax	1.32 m	
		H-9eq	1.36 m	
C-10	41.7	H-10ax	1.22 m	
		H-10eq	1.35 m	
C-11	35.6			
C-12	32.9	H-12	0.95 s	
C-13	21.4	H-13	0.78 s	
C-14	23.6	H-14	1.17 s	
C-15	125.5	H_a-15	6.00 br s	
		H_b-15	5.82 br s	

group α to the carbonyl and a methylene next to the olefinic double bond. These data, together with the eims which showed a fragment at m/z 83 $[C_5H_7O]^+$, established the partial structure **2**.

In addition to the previously mentioned resonances, the ^{13}C-nmr spectrum showed signals for the quaternary carbinol, a methine, three tertiary methyls, four saturated methylenes, and one saturated quaternary carbon. Based on these data and the already established molecular composition, which requires three double-bond equivalents, one ring system was necessary in the structure of **1**. Further assignment of structure **1** and unambiguous ^1H- and ^{13}C-nmr chemical shift values were based on HMBC (4), ^1H-^1H COSY, and NOESY nmr studies.

The major ^2J and ^3J ^1H-^{13}C connectivities in the HMBC studies are shown in Table 2. A ^3J interaction between two methyls (δ_H 0.78, δ_C 21.4; δ_H 0.95, δ_C 32.9) allowed them to be placed as geminal (C-11) substituents. A ^2J interaction between the methyl protons and a quaternary carbon (δ 35.6) and ^3J interaction with a methylene (δ 41.7) and methine carbon (δ 37.1) identified the C-11, C-10, and C-6 positions, respectively. A ^3J coupling of the C-6 carbon with a methyl ^1H resonance (δ 1.17), which in turn showed ^2J and ^3J couplings with the carbinol (δ 74.76) and methylene (δ 43.2) carbons, similarly identified the C-7 position. This required the compound to have partial structure **3**.

TABLE 2. HMBC Correlations for **1**.

^1H	δ ^{13}C	
	^2J	^3J
H_3-1	200.51	
H_3-12	35.6	21.4, 41.7, 57.1
H_3-13	35.6	32.9, 41.7, 57.1
H_3-14	74.8	43.2, 57.1
H_2-4	25.8, 150.0	57.1, 125.5
H_2-5	34.4, 57.1	74.8, 150.0
H_2-15	150.0	34.4, 200.5

Analysis of connectivities also sup-

ported partial structure **2** in the six-carbon side-chain. The carbonyl carbon (δ 200.5, C-2) showed ^2J coupling with the protons of the deshielded methyl group (δ 2.34) and ^3J interaction with the olefinic methylene protons (H-15) and aliphatic methylene protons (δ 2.35, 2.45) which are assigned to H_2-4. The latter protons (H_2-4) showed further ^2J interactions with C-5 (δ 25.8) and C-3 (δ 150.0) and ^3J couplings with the olefinic methylene carbon (δ 125.5, C-15) and the methine carbon of the cyclohexane skeleton (C-6). All the expected connectivities were also observed for the C-5 methylene protons (Table 3). Thus, except for C-9, all carbons could be assigned through this process.

The relative stereochemistry of **1** and assignment of all protons in the ^{1}H-nmr spectrum were supported by the COSY and NOESY nmr studies. In the NOESY studies (Figure 1), interactions between Me-13 (δ 0.78) and Me-14 (δ 1.17) and H-6 (δ 1.40) were observed. This required them to be placed on the same face of the molecule and also supports the relative stereochemistry at C-6 and C-7 as shown in Figure 1. In the COSY studies, an isolated ^{1}H resonance at δ 1.72 (H-8eq) showed strong interaction with multiplets at δ 1.52 and 1.32, which leads to identification of the axial H-8 and H-9 protons. The NOESY further revealed interaction between the H-8eq ^{1}H resonance, and a multiplet at δ 1.36 which thereby identified H-9eq. H-10 protons could be identified as they showed strong interaction with Me-12 in the NOESY study.

TABLE 3. Minimum Inhibitory Concentration (MIC μg ml^{-1}) of **1**.[a]

Test organism	**1**	Streptomycin sulfate
Bacillus pumilus	50	0.94
Bacillus subtilis	100	3.75
Staphylococcus aureus . .	50	3.75
Streptococcus faecalis . . .	150	15

[a]**1** was inactive against *Escherichia coli* and *Pseudomonas aeruginosa* at the level of 200 μg·ml^{-1}.

Compound **1** showed a weak antibiotic activity against Gram-positive bacteria (Table 3). Its mechanism of action has not yet been studied but the compound, like the active *Premna* diterpenes (2,5), contains an α,β-unsaturated moiety which could react with biological nucleophiles (6).

EXPERIMENTAL

PLANT MATERIAL.—The leaves of *P. oligotricha* were collected in September 1989 from beside

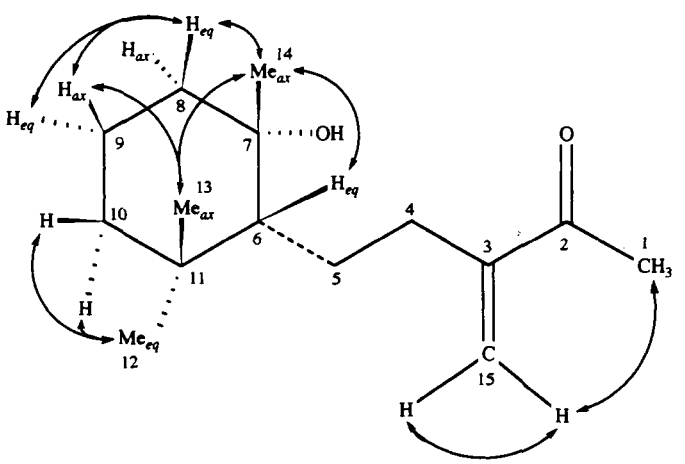

FIGURE 1. Stereochemistry of **1** based on nOe studies.

the Yabellow-Mega Road at ca. 1600 m, Sidamo Province, Ethiopia. A voucher specimen (SHM-12) has been deposited at the National Herbarium of Ethiopia, Addis Ababa University.

TEST ORGANISMS AND ANITMICROBIAL ASSAY.—*Bacillus pumilus* (NCTC 10327), *Bacillus subtilis* (NCTC 10452), *Escherichia coli* (NCTC 9001), *Pseudomonas aeruginosa* (NCTC 6750), *Staphylococcus aureus* (NCTC 6571), and *Streptococcus faecalis* (NCTC 775) were used. The disc diffusion and minimum inhibitory concentration assays were performed as described previously (5).

INSTRUMENTATION AND CHROMATOGRAPHIC MATERIAL.—The ir and uv spectra were recorded on Perkin-Elmer 781 and Perkin-Elmer 552 spectrophotometers, respectively. The ms was recorded on AEI MS 902 spectrometer. ^1H, ^{13}C, ^1H-^1H COSY, NOESY, and heteronuclear multiple bond coherence (HMBC) nmr spectra were recorded on a Bruker AMX-400 instrument (d_6 set for J = ca. 7 Hz). Chemical shifts were reported in ppm relative to solvent ($CDCl_3$). Specific rotation [α]D was obtained using Perkin-Elmer 241 polarimeter. The following Si gel and sephadex supplies were used: Si gel 60 for cc, Si gel (Merck 7749) for vlc, and Si gel 60-PF_{254} for tlc and Sephadex LH-20.

EXTRACTION AND ISOLATION.—Powdered aerial parts (2 kg) of *P. oligotricha* were extracted with cold EtOH for 5 days. Concentration of the solvent yielded a gum (400 g) which was tested for antibacterial activity by the disc diffusion method. The gum was then subjected to vlc over Si gel (Merck 7749), eluting with solvents of increasing polarity; i.e., petroleum ether, petroleum ether/EtOAc, EtOAc, and finally MeOH. The most active fraction [petroleum ether-EtOAc (7:3)] was passed through a small Sephadex LH-20 column to remove chlorophyll and then fractionated by cc over Si gel, again eluting with petroleum ether/EtOAc. The 8:2 fraction was further subjected to repetitive preparative tlc [Si gel, solvent hexane-$CHCl_3$-EtOAc (6:2:1)] to give **1**.

7α-Hydroxy-6,11-cyclofarnes-3(15)-en-2-one [**1**].—Compound **1** (15 mg): oil; [α]D −17 (c = 0.1, $CHCl_3$); uv λ max (EtOH) 230 nm; ir ν max (KBr) 3490 br, 2910, 1670, 1460, 1360, 1100 cm^{-1}; eims *m/z* (rel. int. %) $[M]^+$ 238.1926 ($C_{15}H_{26}O_2$ requires 238.1933) 223 (10.5), 220 (21.6), 205 (14.4), 181 (19.0), 177 (21.6), 137 (24.7), 136 (52.8), 135 (22.4), 123 (31.9), 121 (27.2), 111 (84.4), 109 (88.8), 107 (31.9), 198 (24.1), 97 (24.6), 96 (63.6), 95 (61.7), 94 (23.5), 93 (31.8), 91 (21.3), 85 (61.0), 71 (91.8), 69 (100), 67 (39.1), 59 (21.5), 58 (21.5), 55 (75.2); ^1H and ^{13}C nmr ($CDCl_3$) see Table 1.

ACKNOWLEDGMENTS

Nmr spectra were obtained at the Strathclyde University nmr Laboratory. One of the authors (SH) thanks the University of Strathclyde for the award of a John Anderson Scholarship.

LITERATURE CITED

1. S. Habtemariam, A.I. Gray, C. Lavaud, G. Massiot, B.W. Skelton, P.G. Waterman, and A.H. White, *J. Chem. Soc., Perkin Trans. 1*, 893 (1991).
2. S. Habtemariam, A.I. Gray, and P.G. Waterman, *Planta Med.*, **58**, 109 (1992).
3. S. Habtemariam, A.I. Gray and P.G. Waterman, *Z. Naturforsch.*, **47b**, 144 (1992).
4. A. Bax and S. Subramaniam, *J. Magn. Reson.*, **67**, 566 (1986).
5. S. Habtemariam, A.I. Gray, G.W. Halbert, and P.G. Waterman, *Planta Med.*, **56**, 187 (1990).
6. P.J. Stang and W.L. Trepton, *J. Med. Chem.*, **24**, 468 (1981).

Received 10 June 1992