Pergamon

Tetrahedron Vol. 50, No. 42, pp. 12109-12112, 1994

0040-4020/94 $7.00+0.00

0040-4020(94)00773-X

New Furanoid Diterpenoidal Constituents of *Tinospora malabarica*

Atta-ur-Rahman*, Sultan Ahmad, S. Safdar Ali, Zahir Shah and M. Iqbal Choudhary*
H. E. J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan
Jon Clardy*
Department of Chemistry, Baker Laboratory, Cornell University
Ithaca, New York 14853-1301, U. S. A.

Abstract: Two new furanoid diterpenes, malabarolide B_1 (1) and menispermacide, have been isolated from the stems of *Tinospora malabarica*. Their structures were established by single crystal X-ray diffraction. The structure of menispermacide (2) was revised on the basis of single crystal X-ray diffraction analysis. Menispermacide provides the only known example of the occurrence of disulfide bearing diterpene from higher plants.

Tinospora malabarica (Meirs) belongs to plant family Menispermaceae and it is cultivated throughout Pakistan. The aqueous extract of the plant is used in the indigenous system of medicine for the treatment of intermittent fever, liver and eye ailments, and is reputed to be a tissue builder and emetic.[1] A number of chemical constituents have been reported from this plant.[2-6] In the present paper we report the isolation of a new furanoid diterpene, malabarolide B_1 (1), from the fresh stems of *T. malabarica*. Malabarolide B_1 (1) is structurally related to malabarolide A_1 (3) (originally reported as malabarolide), the first reported example of this type.[5] The structure of an already reported furanoid, menispermacide[7] (2) is revised on the basis of single crystal X-ray diffraction analysis. Compound 2 is a novel disulfide furanoid diterpene which provides the only known example of the occurrence of disulfide bearing diterpene from higher plants.

Malabarolide B_1 (1) was isolated by combination of column and thin-layer chromatography on silica gel. An absorption at 210 nm in the UV spectrum suggested a furan ring, which was confirmed by Ehrlich's color test.[8] The IR spectrum displayed intense absorptions at 3400 (OH), 1725 (lactone carbonyl) and 1705 (ketone carbonyl) cm^{-1}. Compound 1 showed the M^+ peak at m/z 318.1464 corresponding to the molecular formula $C_{18}H_{22}O_5$ (318.1467). The major peaks in the mass spectrum occurred at m/z 318, 300, 95, and 81. The fragment at m/z 81 resulted from the cleavage of the C-11/C-12 and C-12/ester oxygen bonds.

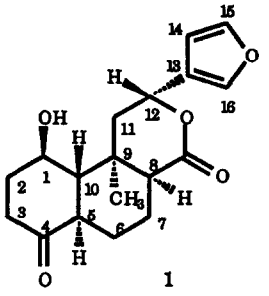
The structure 1 was definitely established by single crystal X-ray diffraction. Malabarolide B_1 (1) was recrystallized from MeOH-$CHCl_3$, and a suitable crystal was selected for further study. Crystals formed in the orthorhombic space group $P2_12_12_1$ with a = 6.2594(8), b = 11.683(2), c= 22.584(3) Å, one molecule of composition $C_{18}H_{22}O_5$ forming the asymmetric unit. A total of 1290 unique reflections were collected with CuKα radiation and θ:2θ scans. Of these 1194 (93%) were judged observed [$|Fo| \geq 3\sigma(Fo)$] and used in further calculations. The structure (Fig. 1) was solved by direct methods and refined by full-matrix least-squares techniques to the final discrepancy index of 0.041 for the observed data[9].

In the ^{1}H-NMR spectrum (Table-1), a double doublet for a methine proton centered at δ 5.69 could be assigned to the oxygen-bearing C-12 proton. The downfield chemical shift of

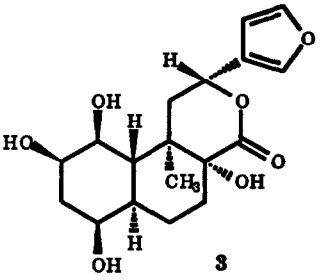
H-1 (δ 4.18) was attributed to the deshielding effect of geminal hydroxyl group. The ^{13}C-NMR spectrum ($CDCl_3$) of malabarolide B_1 (1) (Table-1) was assigned on the basis of DEPT and 2D HETCOR. The direct $^{13}C/^{1}H$ chemical shift correlation (HETCOR)[10,11] spectrum established that the double doublet at δ 1.87 was due to the proton at C-10 (δ 48.1), and that the multiplet at δ 2.30 belonged to the proton at C-8 (δ 50.4). The ^{13}C-NMR chemical shifts for C-8, C-10, C-11 and C-17 were also confirmed by the long range HETCOR (COLOC).[10,11]

Table-1: ^{13}C- and ^{1}H-NMR Assignments for 1 and 2 in $CDCl_3$.

	1			2		
Carbon	^{13}C-NMR (δ, ppm)	^{1}H-NMR (δ, ppm)	J (Hz)	^{13}C-NMR (δ, ppm)	^{1}H-NMR (δ, ppm)	J (Hz)
1	69.7 d	4.18 m	---	19.6 t	1.54 m (H-1α)	---
			---		1.73 m (H-1β)	
2	34.3 t	1.87 m (H-2α)	---	27.4 t	2.07 m (H-2α)	
		2.15 m (H-2β)	---		2.20 m (H-2β)	
3	36.9 t	2.45 m	---	57.5 d	3.20 m	---
4	210.2 s	---	---	81.7 s	---	---
5	45.4 d	2.29 m	J = 8.7	46.9 s	---	---
6	20.9 t	1.63 m (H-6α)	---	75.8 d	4.60 dd	J = 11.9,4.2
		2.40 m (H-6β)				
7	22.1 t	1.90 m (H-7α)	---	25.5 t	2.40 m (H-7α)	---
		1.35 m (H-7β)	---		2.21 m (H-7β)	---
8	50.4 d	2.30 m	---	46.9 d	2.65 dd	J = 13.1, 5.2
9	36.3 s	---	---	35.9 s	---	---
10	48.1 d	1.87 dd	J = 11.2, 5.5	47.5 d	1.90 dd	J = 12.9, 5.4
11	42.3 t	1.73 dd (H-11α)	J= 14.1,12.3	44.2 t	1.97 dd (H-11α)	J = 15.3, 12.6
		2.97 dd (H-11β)	J = 14.1, 3.6		2.32 dd (H-11β)	J = 15.3, 4.7
12	71.4 d	5.69 dd	J = 12.3, 3.6	70.7 d	5.64 dd	J = 12.6, 4.7
13	125.6 s	---	---	124.2 s	---	---
14	108.4 d	6.37 dd	J = 1.7,0.8	108.3 d	6.41 dd	J = 1.8, 0.8
15	143.4 d	7.36 t	J = 1.7	143.9 d	7.42 t	J = 1.8, 1.8
16	139.2 d	7.41 m	J = 3.3	139.7 d	7.46 m	---
17	21.9 q	---	---	20.1 q	1.13 s	---
18	---	---	---	177.6 s	---	---
19	---	---	---	23.5 q	1.21 s	---
20	171.7 s	---	---	172.1 s	---	---
S-CH_3	---	---	---	23.5 s	2.45 s	---



1



3

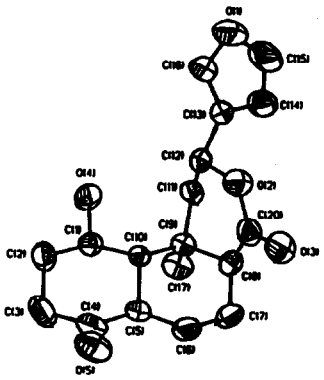
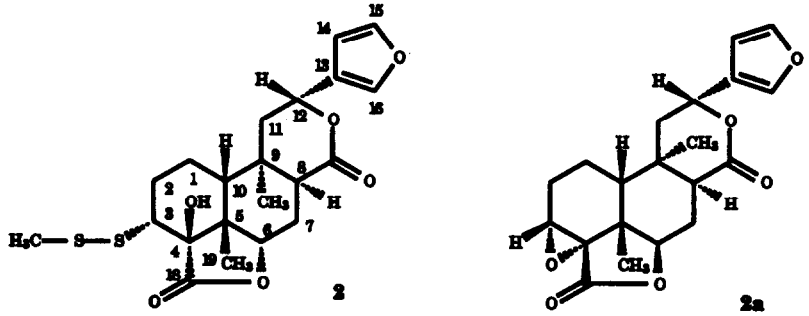


Figure 1: A computer generated ORTEP drawing of the final X-ray model of malabarolide B_1 (1). No absolute configuration is implied.

Malabarolide (1) is the second example of this new class of 18,19-bisnorditerpenes and it may arise in nature by the oxidative removal of the 18-methyl group from a 19-norclerodane or by decarboxylation of a tinophyllol-type compound.[12]



Menispermacide (2) was also isolated by us from the stems of *Tinospora malabarica* by combination of column and thin-layer chromatography on silica gel. The structure 2a was previously proposed on the basis of spectroscopic evidences.[7] The presence of a disulfide substituent was not suspected since the electron-impact mass spectrum showed the highest mass peak at m/z 358.1498 ($C_{20}H_{22}O_6$). Recently we have succeeded in obtaining well-formed crystals of compound 2 and carried out single crystal X-ray diffraction studies.

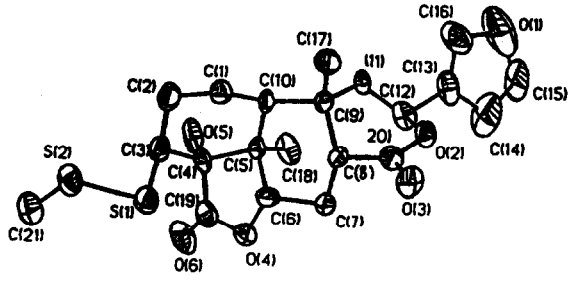


Figure 2: A computer generated ORTEP drawing of the final X-ray model of menispermacide (2). No absolute configuration is implied.

Compound **2** was recrystallized from MeOH-CH_2Cl_2. Crystals formed in the orthorhombic space group $P2_12_12_1$ with a = 10.155(3), b = 11.214(4), c= 18.762(8) Å and one molecule of composition $C_{21}H_{26}O_6S_2$ forming the asymmetric unit. A total of 1462 unique reflections were collected with CuKα radiation and θ:2θ scans. Of these 1375 (94%) were judged observed [$|Fo| \geq 6\sigma(Fo)$] and used in further calculations. The structure was solved by direct methods and refined by full-matrix least-squares techniques to final discrepancy index of 0.062 for the observed data. Structure of **2** (Fig. 2) was unambiguously established by this method.[9]

Acknowledgment: The work at Cornell was partially supported by NIH CA24487 to J.C. Authors are indebted to Mr. Tahir Ali, Plant Taxonomist, Department of Botany, University of Karachi for identification of the plant material.

Isolation and Purification of Malabarolide B_1 (1): Fresh stems of *Tinospora malabarica* (120 kg) were crushed, extracted with EtOH (120 L) and evaporated to a crude gum (400 g). The basic materials were removed by extraction with HCl. The neutral fraction (45 g) was subjected to column chromatography on silica gel (1.35 kg). A step gradient mixture of MeOH in $CHCl_3$ was used as eluent. The fraction obtained on elution with MeOH-$CHCl_3$ (3:97) was evaporated to dryness. This fraction was crystallized from ether and recrystallized from MeOH-$CHCl_3$ to afford light yellow needles of malabarolide B_1 (**1**) (40 mg), mp 201 °C.

REFERENCES AND NOTES

1. Perry, L.M. *Medicinal Plants of East and South East Asia,* MIT Press, Cambridge, Massachusetts, **1980**, p. 268.

2. Mehta, R.; Arora, O.P.; Mehra, M. *Ind. J. Chem.,* Sect. B, **1981**, *20,* 834.

3. Brown, I.H.; Motawe, H.M. *Planta Medica,* **1985**, No. 6, 529.

4. Atta-ur-Rahman; Ahmad, S. *Fitoterapia,* **1987**,*V. LVIII. N. 4,* 266.

5. Atta-ur-Rahman; Ahmad, S.; Rycroft, D. S.; Parkanyi, L.; Choudhary, M.I.; Clardy, J. *Tetrahedron Lett.,* **1988**, *29*, 4241.

6. Atta-ur- Rahman; Ahmad, S. *Phytochemistry,* **1988**, *27*, 1882.

7. Atta-ur-Rahman; Ali, S.S.; Ahmad, S.; Choudhary, M.I. *Phytochemistry*, **1992**, *31*, 3155.

8. Reichstein, T. *Helv. Chim. Acta,* **1932**,*15*,1110.

9. Tables of crystal data, data collections, solutions and refinements, fractional coordinates, bond distances, bond angles, and thermal factors for **1** and **2** have been deposited with the Cambridge Crystallographic Data Center, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1 EW, UK. Please give a complete literature citation when ordering.

10. Atta-ur-Rahman, *One- and Two-Dimensional NMR Spectroscopy,* Elsevier Science Publishers, New York, **1989**.

11. Bax, A. *J. Magn. Reson.,* **1984**, *3*, 517.

12. Kuni, T.; Kagei, K.; Kawakami, Y.; Nagei, Y.; Nezu, Y.; Sato, T. *Chem. Pharm. Bull.* (Japan), **1985**, *33*, 479.

(*Received in USA* 15 *June* 1994; *revised* 26 *August* 1994; *accepted* 29 *August* 1994)