An Antimutagenic Monoterpene from *Malachra Fasciata* (Malvaceae)

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KEYWORDS
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ABSTRACT

A monoterpene was isolated from the leaves of Malachra fasciata by gravity column chromatography. Its structure was elucidated by extensive 1D and 2D NMR spectroscopy. It was identified as loliolide by comparison of its ^{1}H and ^{13}C NMR spectral data with those found in the literature.

The compound was tested for its antimutagenicity potential by the use of the Micronucleus test. Results of the study indicated a 64.4% reduction in micronucleated polychromatic erythrocytes induced by mitomycin C, when loliolide at a dosage of 14.8 mg/kg was administered to mice of the Swiss strain. Another isolate from the leaves of the plant was stigmasterol which structure was determined by comparison of its ^{1}H NMR spectral data with those found in the literature.

INTRODUCTION

Malachra fasciata or situyo is a shrub found throughout the Philippines at low altitudes. Poultices of the leaves are applied to ulcers and other sores. The roots and leaves may serve as emolients and may be considered specific against haemorrhoids, fevers and impotency and also as a general tonic. A decoction of the leaves is used for treatment of gonorrhoea and rheumatism and as a demulcent and diuretic. The plant was also reported to have antitumor properties (Quisumbing, 1951).

There is no reported chemical study on Malachra fasciata. This is the first report on the isolation, structure elucidation and antimutagenicity studies on loliolide (1) from the leaves of M. fasciata. Loliolide has been identified as a constituent of Lolium perenne (Manske, 1938), Digitalis pupurea (Satoh and Wada, 1956), and Fumaria officinalis)Satoh and Wada, 1964). We also report the isolation of stigmasterol (2) from the leaves of the plant. A previous study showed that stigmasterol at a dosage of 170 mg/kg reduced the number of micronucleated polychromatic erythrocytes induced by Mitomycin C by 79% (Ragasa, et. al., 1995).

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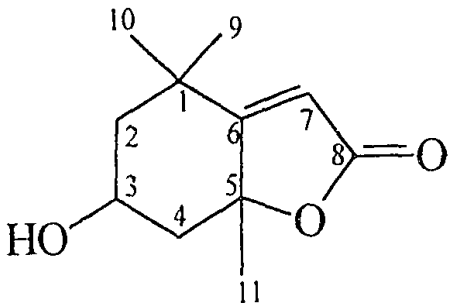

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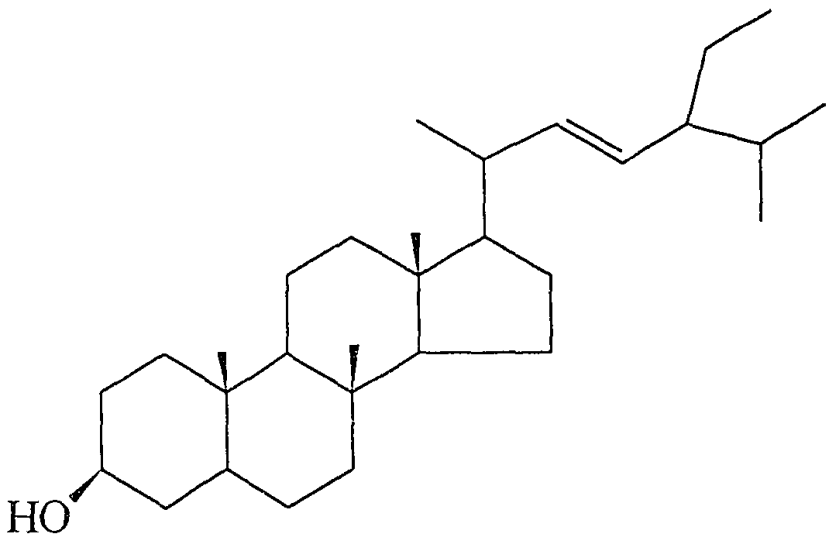

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RESULTS AND DISCUSSION

The chloroform extract of the air-dried leaves of Malachra fasciata afforded loliolide (1) and stigmasterol (2). The structure of loliolide was elucidated by NMR (^{1}H, ^{13}C, COSY, HMQC and HMBC) spectroscopy and the ^{13}C NMR spectral data of 1 was comparable to those of loliolide found in the literature [Ravi, et. al., 1982]. The structure of 2 was determined by comparison of its ^{1}H NMR spectral data with those of an authentic sample of stigmasterol [Zulueta, 1994].

The ^{1}H NMR spectrum of 1 indicated resonances for olefinic hydrogen on a conjugated double bond at δ 5.70 (1H, s), a carbinyl hydrogen of an

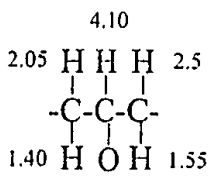
alcohol at δ 4.10 (1H, m) and three methyl singlets at δ 1.26 (3H, s), δ 1.32 (3H, s) and δ 1.66 (3H, s). The ^{1}H NMR spectral data of **1** is presented in Table 1.

Table 1. 300 MHz ^{1}H, ^{13}C and HMQC spectral data of 1 in $CDCl_3$.

Carbon	^{13}C shift, d	1H shift, d
C-1	35	-
C-2	50	1.40, 2.05
C-3	65	4.10
C-4	48	1.55, 2.55
C-5	86	-
C-6	181	-
C-7	114	5.70
C-8	172	-
C-9	30	1.37
C-10	25	1.32
C-11	26	1.67

The ^{13}C NMR spectrum gave evidence to the presence of eleven carbon atoms in **1**. The resonance at δ 172 was attributed to the carbonyl carbon of a lactone, while those at δ 181 and 114 indicated one carbon-carbon double bond. Probably the quaternary carbon at δ 181 is further deshielded due to the steric effect of neighboring methyl groups. Two carbons singly bonded to oxygen were deduced from the resonances at δ 87 and 65, while three methyl groups were assigned to the resonances at δ 30, 26 and 25. The ^{13}C NMR spectral data of **1** is summarized in Table 1.

The COSY spectrum of **1** indicated only one isolated spin system. The hydrogen at δ 2.05 is coupled to the protons at δ 1.40 and 4.10. The latter proton is further coupled to the hydrogens at δ 2.55 and 1.55. Thus, the following is an isolated spin system deduced from COSY.



The HMQC spectrum indicates the hydrogens that are directly bonded to carbons as follows. The olefinic hydrogen at δ 5.70 is bonded to the carbon at δ 114, while the carbinyl hydrogen at δ 4.10 is attached to the carbon at δ 65. The methylene protons at δ 2.05 and 1.40 are bonded to the carbon at δ 50, while those at δ 2.55 and 1.55 are attached to the carbon at δ 48. The methyl protons at δ 1.58, 1.37 and 1.32 are bonded

to the carbons at δ 26, 30 and 25, respectively. These data are summarized in Table 1.

The structure of 1 was deduced from the HMBC spectrum summarized in Table 2. This spectrum indicates long-range correlations between carbons and protons two to three bonds away. The carbon at δ 181 is long-range correlated to the hydrogens at δ 5.70, 2.55, 1.55, 1.67, 1.32, 1.37, 2.04 and 1.40. Long-range correlations were observed between the carbon at δ 172 and the olefinic proton at δ 5.70. Further correlations were deduced from the carbon at 86 and the hydrogens at δ 2.55, 1.67 and 1.55. The carbon at δ 64 is long-range correlated to the protons at δ 2.55, 2.05, 1.67 and 1.40. Additional correlations were attributed to the carbon at δ 50 and the hydrogens at δ 2.55, 1.55, 1.37 and 1.32. The carbon at δ 47 is long-range correlated to the protons at δ 2.05 and 1.40, while the one at δ 35 is correlated to the hydrogens at δ 1.37, 1.32, 1.40, 2.05 and 5.70. The methyl carbon at δ 30 is long-range correlated to the protons at δ 1.32 and 1.40, while another methyl carbon at δ 25 is correlated to the hydrogens at δ 1.37 and 1.40. The third methyl carbon at δ 26 is long-range correlated to the hydrogen at δ 1.55. All long-range correlations observed were consistent with the structure of 1.

Table 2. 300 MHz ^{13}C-^{1}H long-range (HMBC) spectral data of 1.

Carbon	Hydrogens
C-1	H-3a, H-2b, H-7, H-9, H-10
C-2	H-4a, H-4b, H-9, H-10
C-3	H-2a, H-2b, H-4a, H-4b
C-4	H-2a, H-2b
C-5	H-4a, H-4b, H-7, H-11
C-6	H-2a, H-2b, H-4a, H-4b, H-7, H-9, H-10, H-11
C-7	
C-8	H-7
C-9	H-2a, H-10
C-10	H-2a, H-9
C-11	H-4

Literature search revealed that 1 is loliolide. Confirmatory evidence was the ^{13}C NMR spectral data of 1 and loliolide (Ravi, et. al., 1982). The data matched in all essential respects.

The plant was reported to have antitumor property. Because of a strong correlation between antitumor and antimutagenic activities, the antimutagenicity potential of 1 was determined by the Micronucleus test. Results of the study presented in Table 3 indicated that at a dosage of 14.8 mg/kg, 1 reduced the number of micronucleated polychromatic erythrocytes (MPCE) induced by mitomycin C by 64.4%. Statistical

analysis using the T-test indicated a significant reduction of MPCE at α = 0.01. Thus, 1 is an antimutagen.

Table 3. Effect of 1 on the number of micronucleated polychromatic erythrocytes induced by mitomycin C.

Sample	Ave. MPCE/1000 PCE	% Reduction in MPCE
exp'l mouse 1	2.3	65.2
exp'l mouse 2	2.3	65.2
exp'l mouse 3	2.0	69.7
exp'l mouse 4	2.3	65.2
exp'l mouse 5	2.7	59.1
Average	**2.3 ± 0.13***	**64.9**
Control mouse 1	6.0	
Control mouse 2	6.7	
Control mouse 3	7.0	
Control mouse 4	6.7	
Control mouse 5	6.7	
Average	**6.6 ± 0.20***	

*Average of 15 slides

The structure of 2 was deduced by comparison of its ^{1}H NMR spectral data with those of an authentic sample of stigmasterol (Table 4) (Zulueta, 1994). The spectra matched in all essential respects, and hence, confirming that 2 is stigmasterol. Antimutagenicity test was not conducted on 2 since a previous study already reported its antimutagenic activity (Ragasa, et. al., 1995).

Table 4. A comparison of 1H NMR spectral data of 2 and stigmasterol from an authentic sample (Zulueta, 1994).

Protons	^{1}H Shift of 2, d	^{1}H Shift, d from an Authentic Sample [Zulueta, 1994]
H-3	3.51	3.52
H-6	5.08	5.09
H-18	0.69 (3H)	0.70
H-19	1.00 (3H)	1.00
H-21	1.02 (3H)	1.02
H-22, H-23	5.33	5.33
H-26, H-27	0.85 (6H)	0.85
H-29	0.82 (3H)	0.82

EXPERIMENTAL

NMR spectra were recorded in $CHCl_3$ on a 300 MHz Bruker AMX spectrometer. Silica gel 60 (70-230 mesh) was used for column chromatography and plastic backed plates coated with Si gel F_{254} were used for TLC. Plates were visualized by spraying with vanillin:H_2SO_4 and warming. The number of MPCE was counted with the use of a Zeiss microscope.

The plant sample was collected from Sta. Barbara, Pangasinan, Philippines in August 1995. It was identified by the botanists at the National Museum as Malachra fasciata.

Air-dried leaves of the plant samples (500 g) were soaked in 2 L of $CHCl_3$ for 3 days, then filtered. The filtrate was concentrated under vacuum to afford a crude extract (20 g) which was treated with 4% aqueous $Pb(OAc)_2$ to precipitate the pigments (Padolina, et. al., 1974). The treated extract (1 g) was subjected to gravity column chromatography (dry packing). The solvent system was based on step gradient technique, starting with $CHCl_3$, then Me_2CO in $CHCl_3$ (10% increments). The 30% Me_2CO in $CHCl_3$ fraction was rechromatographed in 20% Me_2CO in $CHCl_3$ to afford 1 (colorless oil, 3.85 mg). The $CHCl_3$ fraction was rechromatographed in $CHCl_3$ to afford 2 (20 mg, colorless crystals, m. pt. 170 °C).

Antimutagenicity Test

The test compounds (14.8 mg/kg mouse) dissolved in DMSO (7.5 mL/kg mouse, solvent control) were administered simultaneously with mitomycin C (2.75 mg/kg mouse, positive control) to mice of the Swiss strain (source: DOST). For the control, only mitomycin C and DMSO were administered orally to the mice. Five mice replicates were tested for each compound and control. The second administration was carried out after 24 h. The mice were killed by dislocation of the neck, 6 h after the second administration. Blood from the bone marrow was smeared on slides (three per mouse). The slides were stained with May Grunwald and Giemsa solutions [Schmid, 1972]. The numbers of MPCE/1000 PCE were counted by the use of a high power Zeiss microscope.

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