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RESEARCH NOTE

Sterols from *Cucurbita maxima*

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The flowers of *Cucurbita maxima* Duch. afforded a 4:1 mixture of spinasterol (1a) and 24-ethyl-5α-cholesta-7, 22, 25-trien-3β-ol (1b). Their structures were elucidated by extensive 1D and 2D NMR analyses. Antimicrobial activity tests on sample 1 indicated that it was slightly active against the fungi (*Aspergillus niger* and *Candida albicans*) and the bacteria (*Bacillus subtilis* and *Pseudonomas aeruginosa*). It was inactive against *Escherichia coli*, *Staphylococus aureus*, and *Trichophyton mentagrophytes*.

Key Words: Cucurbitaceae, spinasterol, 24-ethyl-5α-cholesta-7, 22, 25-trien-3β-olt

INTRODUCTION

Cucurbita maxima Duch., commonly known as squash is widely used as vegetable and a source of vitamin A, iron, phosphorus, and calcium. A recent study reported that spinasterol from the flowers of *C. maxima* showed potential anticarcinogenic, antigenotoxic (Villasenor et al. 1996), antimutagenic, and antitumorigenic activity (Villasenor and Domingo 2000). The seeds were used in the treatment of liver and digestive disorders (Prokhvatilova et al. 1998), while the oil from the seeds exhibited anthelmintic property (Basaran et al. 1998). Earlier studies reported the isolation of cucurbitaxanthin (Zechmeister and Tuzson 1934), gibberellin (Inouye et al. 1991), ent-6α,7α,12α-trihydroxykaur-16-en-19-oic acid (Matsuna et al. 1986), α-tocopherol (Beale et al. 1984), and N^5-(4-methoxyphenyl)methyl-1-glutamine (Prokhvatilova et al. 1998) from the plant.

We now report the isolation, structure elucidation, and antimicrobial assay of a 4:1 mixture of spinasterol (**1a**) and 24-ethyl-5α-cholesta-7, 22, 25-trien-3β-ol (**1b**) from the flowers of squash.

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MATERIALS AND METHODS

General Experimental Procedure

NMR spectra were recorded on a Bruker Avance 400 in $CDCl_3$ at 400 MHz for ^1H and 100 MHz for ^{13}C. Column chromatography was performed with silica gel 60 (70-230 mesh). TLC was performed with plastic backed plates coated with silica gel F_{254}. TLC plates were visualized by spraying with vanillin-H_2SO_4, then warming.

Sample Collection

The fresh flowers of the plant material were collected from Sinait, Ilocos Sur in January 2002. It was identified by Alfredo F. Vendivil as *Cucurbita maxima* Duch. at the Philippine National Museum and voucher specimen # 045 is kept at the Chemistry Department, De La Salle University.

Isolation

The air-dried flowers (571.10 g) of *C. maxima* was extracted with dichloromethane. The crude extract (15.60g) was chromatographed with increasing proportions of acetone in dichloromethane (10% increment) as eluents. The fraction eluted with 50% acetone in dichloromethane from the first column was rechromatographed (2×) using Et_2O:CH_3CN:CH_2Cl_2 (1.5:1.5:7) as eluent. Fractions

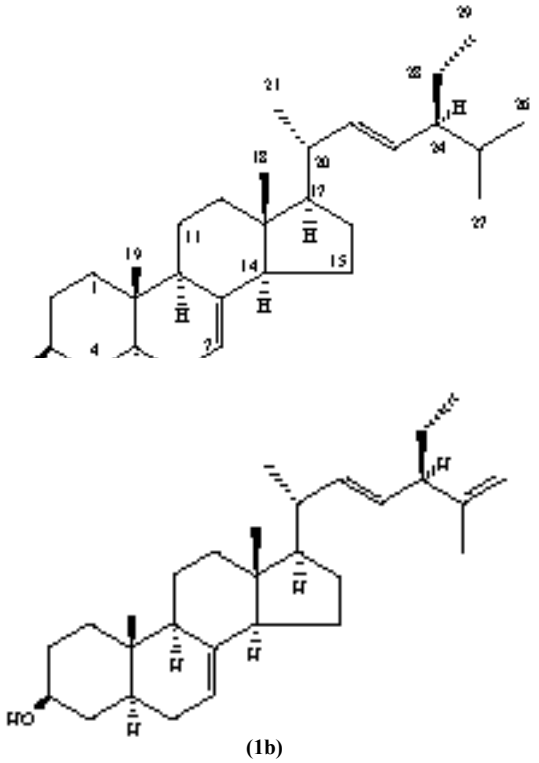
1-2 from this column were again rechromatographed using Et_2O:CH_3CN:CH_2Cl_2 (1:1:8) as eluent to afford sample 1 (colorless solid, m.p.164-166°C, 100 mg) after recrystallization from diethyl ether.

Antimicrobial Tests

The microorganisms used in these tests were obtained from the University of the Philippines Culture Collection (UPCC). These are *Aspergillus niger* UPCC 4219, *Candida albicans* UPCC 2168, Bacillus subtilis UPCC 1295, *Pseudomonas aeruginosa* UPCC 1244, Escherichia coli UPCC 1195, *Staphylococcus aureus* UPCC 1143, and *Trichophyton mentagrophyte* UPCC 4193. The test compound was dissolved in 95% ethanol. The antimicrobial assay procedure (agar cup diffusion method) reported in the literature (Guevara and Recio 1985) was employed.

RESULTS AND DISCUSSION

The dichloromethane extract of the air-dried flowers of *C. maxima* afforded a 4:1 mixture of **1a** and **1b** (Figure 1). Their structures were elucidated by extensive 1D and 2D NMR spectral analyses as follows.



(1b)

Figure 1. Chemical structures of spinasterol (**1a**) and 24-ethyl-5α-cholesta-7,22,25-trien-3β-01 (**1b**) extracted from *Cucurbita maxima*

The 1H-NMR spectrum of sample **1** indicated resonances for a mixture of two compounds based on integrals and disparity in single hydrogen peaks. Resonances with large intensities and integrals were attributed to **1a**, while small resonance intensities and integrals were assigned to **1b**. The ^{1}H-NMR spectrum of **1a** (Table 1) indicated resonances for three olefinic protons at δ 5.16 (dd, J=8.8, 15.2 Hz), δ 5.15 (br s), and δ 5.02 (dd, J=8.4, 15.2 Hz); a carbinyl proton at δ 3.59; and six methyl protons at δ 1.03 (d, J=6.8 Hz), 0.85 (d, J=6.4 Hz), 0.84 (d, J=6.0 Hz), 0.81 (t, J=7.2 Hz), 0.80 (s), and 0.55 (s). The *J*-mod ^{13}C-NMR spectral data of **1a** (Table 1) indicated resonances for twenty-nine carbons with the following functionalities: four olefinic carbons, a carbinyl carbon, seven methine carbons, two quaternary carbons, nine methylene carbons, and six methyl carbons. These are characteristic resonances of a sterol with an alcohol and two olefinic bonds.

The COSY 2D NMR spectrum of **1a** showed correlations for three spin systems: H2-1/H2-2/H-3/H2-4/H-5/H2-6/H-7, H-9/H2-11/H2-12, and H-14/H2-15/H2-16, H-17/H-20/H3-21, H-22/H-23/H-24/H-25/H3-26/H3-27, H2-28/H3-29 (Figure 2).

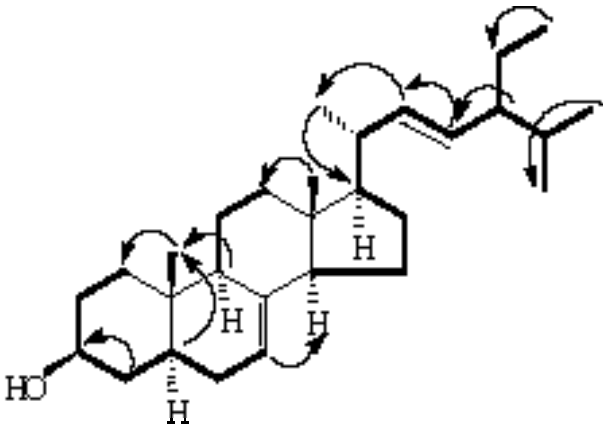


Figure 2. Key COSY (**–**) and HMBC (↶) correlations for spinasterol (**1a**) extracted from *Cucurbita maxima*.

The ^{1}H and ^{13}C assignments of **1a** (Table 1) were verified by 2D HSQC experiments, while connectivities were verified by HMBC (Table 1). All long-range correlations observed are consistent with the structure of **1a**.

The relative stereochemistry of **1a** was deduced from NOESY (Table 1 and Figure 2), and is as drawn in the structure of **1a**. Thus, H-3 is close in space to H-5, which is in turn close to H-9, which is also close to H-14, in turn close to H-17, which is finally close to H_3-21. On the opposite face of the molecule are H_3-18 and H_3-19. Literature search revealed that **1a** is spinasterol, as evidenced by similar ^{13}C NMR spectral data (Villasenor and Domingo 2000).

Table 1. The 400 MHz ^{1}H NMR and 100 MHz ^{13}C NMR Spectral Data of spinasterol (1a) in $CDCl_3$ from *Cucurbita maxima*

Position	δ_C	δ_H mult. (J Hz)	HMBC Correlations	NOESY Correlations
1	37.2	1.09, 1.82	H_2-9, H_3-19	
2	31.5	1.39, 1.77	H-1b, H_2-4	
3	71.1	3.59	H-1b, H-2b, H-4	H-5
4	38.0	1.27 1.70	H-5, H-2b	
5	40.3	1.40	H-1b, H_2-4, H-7, H_3-19	H-3, H-9, H-14
6	29.7	1.22, 1.74	H-7	
7	117.5	5.15 br s	H-a, H-14	
8	139.6	-----	H-9, H-14	
9	49.5	1.65	H-7, H_2-12, H-14, H_3-19	H-5, H-14
10	34.2	-----	H-1b, H-4b, H-2b, H_3-19	
11	21.6	1.48 (2H)	H-9, H_2-12	
12	39.6	1.23 2.02	H-9, H_3-18	
13	43.3	-----	H_2-11, H-12a, H-15b, H_2-16, H_3-18	
14	55.1	1.81	H-12b, H-15b, H_3-18	H-9, H-17
15	23.0	1.40, 1.52	H_2-16, H-17	
16	28.5	1.25 (2H)	H-12b, H-15a, H-17	
17	55.9	1.25	H_2-12, H-15a, H_2-16, H_3-21, H-22	H-14
18	12.0	0.55 s (Me)	H-12a, H-14, H-17	H_3-19
19	13.0	0.80 s (Me)	H-1a	H_3-18
20	40.8	2.05	H_3-21, H-22, H-23	H_3-18
21	21.4	1.03 d (6.8, Me)	H-17, H-20, H-22	H-17
22	138.1	5.16 dd (8.8, 15.2)	H-20, H_3-21, H-23	
23	129.5	5.02 dd (8.4, 15.2)	H-20, H-22, H-24, H-25, H_2-28a	H_3-27, H_3-29, H-24
24	51.2	1.55	H-22, H-23, H-25, H-28, H_3-26, H_3-27, H_3-29	
25	31.9	1.55	H-23, H_3-26, H_3-27, H-28a	
26	21.1	085 d (6.4, Me)	H_3-27	
27	19.0	0.84 d (6.0, Me)	H_3-26	
28	25.4	1.18 1.42	H-23, H_3-29	
29	12.2	0.81 t (7.2, Me)	H_2-28	

Many of the resonances in the ^{1}H and ^{13}C NMR spectra of **1a** and **1b** are overlapping, indicating similarity in structures. Their differences are the presence of an additional double bond in **1b** as indicated by the geminal vinylic protons at δ 4.69 (2H), which are coupled to the allylic methyl at δ 1.65, which are in turn coupled to the allylic methine at δ 2.42. The coupling chain was confirmed by the COSY spectrum. The methyl protons at δ 0.84 (H-27) in **1a** were shifted to δ 1.65 (H-27) in **1b**, while the methyl group at δ 0.85 (H-26) in **1a** was converted to vinylic protons at δ 4.69 (H-26) in **1b**. The ^{13}C NMR spectrum of **1b** (Table 2) indicated that it differs in the side chain of 1a, notably, additional olefinic carbons at δ 148.6 (C-25) and 109.5 (C-26) in **1b**, while the methyl

carbon at δ 21.1 (C-26) and the methine carbon at δ 31.9 (C-25) in 1a did not appear in 1b.

^{1}H and ^{13}C assignments of **1b** (Table 2) were verified by HSQC, while ^{1}H and ^{13}C connectivities were deduced from HMBC (Table 2). The third double bond was placed at C-25 due to long-range correlations between this carbon, H-24, H-27, and H-28b. Correlations were also observed between C-26, H-24 and H-27, which were consistent with the structure of **1b**. Compounds **1a** and **1b** have the same relative stereochemistries since their differences in structure do not involve the chiral carbons.

As part of our continuing research on potential antimicrobial constituents of Philippine medicinal plants, sample 1 was tested for possible activity against seven microorganisms using the agar cup method. Results of the study (Table 3) indicated that it is slightly active against the fungi *A. niger* and *C. albicans* with activity index (AI) of 0.3 at a concentration of 30 μg. It was slightly active against the gram-positive bacterium *B. subtilis* and gram-negative bacterium *P. aeruginosa* with AI of 0.2. It was inactive against the gram-positive bacterium *E. coli*, the gram-negative bacterium *S. aureus*, and the fungus *T. mentagrophytes*.

Table 2. The 400 MHz ^{1}H and ^{13}C Spectral Data of 24-ethyl-5α-cholesta-7,22,25-trien-3β-ol **1b** in $CDCl_3$ from *Cucurbita maxima*

Position	δC	δH mult. (J Hz)	HMBC Correlations
1	37.2	1.09, 1.82	H-9, H_3-19
2	31.5	1.39, 1.77	H-1b, H_2-4
3	71.1	3.59	H-1, H-2, H-4
4	38.0	1.27, 1.70	H-5
5	40.3	1.40	H_3-19
6	29.7	1.22, 1.74	H-7
7	117.5	5.15 br s	H-6, H-14
8	139.5	-----	H-6, H-9
9	49.5	1.65	H-1b, H_2-11, H-12b, H_3-19
10	34.2	-----	H-1, H_3-19
11	21.6	1.48 (2H)	H-9, H_2-12
12	39.6	1.23, 2.02	H-9, H_3-18
13	43.4	-----	H_3-18
14	55.01	1.81	H_3-18
15	23.02	1.40, 1.52	H-15, H_2-16
16	28.3	1.25 (2H)	H-17
17	55.87	1.25	H_2-16, H-20, H-22
18	12.0	0.545 s (Me)	
19	13.0	0.80 s (Me)	H_2-1
20	40.5	2.07	H-17, H_3-21, H-22
21	21.4	1.01 d (6.8, Me)	H-17, H-20
22	137.0	5.25	H-20, H_3-21, H-23
23	130.2	5.16 dd (8.8, 15.2)	H-24
24	52.01	2.42	H-22, H_2-26, H-27, H_3-29
25	148.6	-----	H-24, H-27, H-28b
26	109.5	4.69 (2H)	H-24, H-27
27	20.2	1.65 dd (1.2, 1.6)	H-24, H_2-26
28	25.7	1.18, 1.42	H-24, H_3-29
29	12.12	0.81 t (7.2, Me)	H-24, H_2-28

Table 3. Antimicrobial Test Results on spinasterol (**1a**) extracted from *Cucurbita maxima*

Organism	Sample 1 (30 µg)	Clearing Zone, mm			A.I.
		Replicate 1	Replicate 2	Replicate 3	
Escherichia coli		---	---	---	0
Standard antibiotic	Chloramphenicol	30	---	---	4.0
Pseudomonas aeruginosa		12	12	12	0.2
Standard antibiotic	Chloramphenicol	10	---	---	0.7
Staphylococcus aureus		---	---	---	0
Standard antibiotic	Chloramphenicol	28	---	---	3.7
Bacillus subtilis		12	12	12	0.2
Standard antibiotic	Chloramphenicol	35	---	---	4.8
Candida albicans		12	12	14	0.3
Standard antibiotic	Chlortrimazole	4	---	---	3.0
Trichophyton mentagrophytes		---	---	---	0
Standard antibiotic	Chlortrimazole	45	---	---	6.5
Aspergillus niger		13	13	13	0.3
Standard antibiotic	Chlortrimazole	16	---	---	1.7

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