KIMIKA Volume 20, Number 1/2, pp.1–4 (2004)

ISSN 0115–2130

Triterpenes and sterols from *Achras zapota*

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The freeze-dried unripe fruit of *Achras zapota* afforded a mixture of β-amyrin pentanoate (**1a**) and α-amyrin pentanoate (**1b**) in a 2:1 ratio, a mixture of spinasterol (**2a**) and taraxerol (**2b**) in a 2.4:1 ratio, and a fatty acid ester of oleanolic acid (**3**). Their structures were identified by NMR spectroscopy. **3** and the mixture of **1a** and **1b** were tested for antimicrobial activity by the agar cup method. The mixture of **1a** and **1b** showed moderate activity against the fungus, *Candida albicans* and low activity against the fungi, *Aspergillus niger* and *Trichophyton mentagrophytes*. **3** gave low activity against *C. albicans* and *A. niger*, and inactive against *T. mentagrophytes*. They were inactive against the bacteria: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Bacillus subtilis.*

Keywords: *Achras zapota;* Sapotaceae; chico; triterpenes; sterols; antimicrobial

INTRODUCTION

Achras zapota, commonly known as chico is cultivated in most parts of the Philippines for its fruit. Gum chicle, the principal substance derived from the milky juice of the bark is used in chewing gum manufacture [1]. The astringent fruit is recommended for dysentery and an excellent preventive against biliousness and febrile attacks [1]. The crude leaf extracts of *A. zapota* indicated antiinflammatory, analgesic, and antipyretic activity [2]. The n-butanol extract of chicle from *A. zapota* has antiallergenic affects [3], while the bark contains a water soluble tuberculostatic principle [4]. Chemical studies on the leaves of the plant afforded myricetin and myricetin-3-O-L-rhamnoside, myricetin-3-arabinoside, and quercetin-3-galactoside [5].

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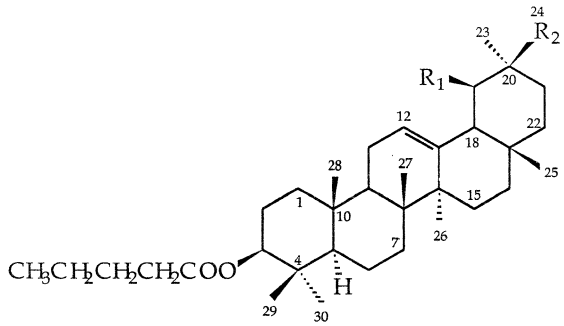
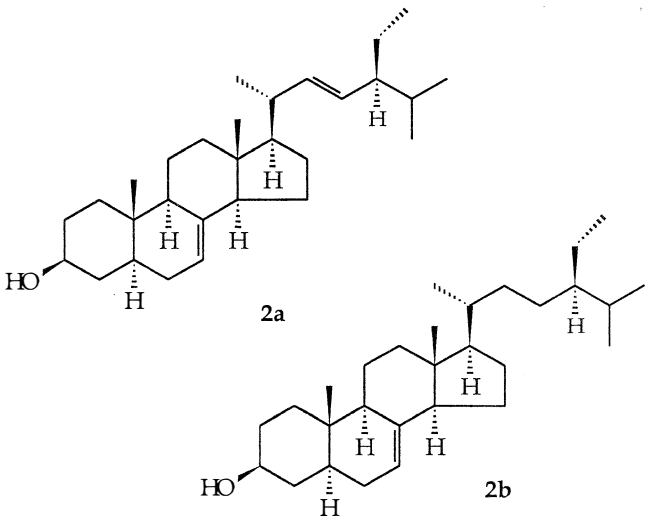
We report here the identification of a mixture of β-amyrin pentanoate (**1a**) and α-amyrin pentanoate (**1b**), a mixture of spinasterol (**2a**) and taraxerol (**2b**), and a fatty acid ester of oleanolic acid (**3**) from the freeze-dried unripe fruit of *A. zapota.* Antimicrobial test results of **1a**, **1b**, and **3** are also reported. To the best of our knowledge this is the first report on the isolation of these compounds from *A. zapota.*

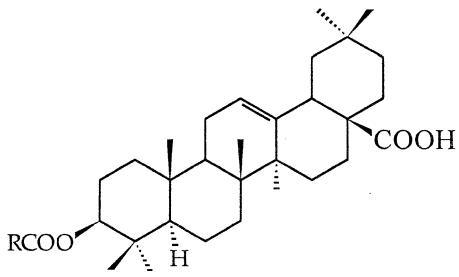
RESULTS AND DISCUSSION

The ^{1}H NMR spectrum of sample **1** indicated resonances for a mixture of two compounds (**1a** and **1b**) in a 2:1 ratio based on resonance intensities. The structures of the major compound (**1a**) and the minor compound (**1b**) were deduced as follows.



1a $R_1 = CH_3$, $R_2 = H$, $R_3 = CH_3$
1b $R_1 = H$, $R_2 = CH_3$, $R_3 = CH_3$





3 R = long chain fatty acid

Table 1. 400 MHz ^{1}H NMR and 100MHz ^{13}C NMR Correlations of 1a

Position	δ_C	δ_H mult. (J Hz)
1	38.5	1.13, 1.67
2	25.2	1.65
3	80.6	4.51 dd (5.6, 10)
4	38.0	–
5	55.3	0.87
6	18.3	1.43, 1.56
7	32.6	1.35
8	40.2	–
9	47.7	1.57
10	36.9	–
11	23.6	1.90, 1.92
12	124.4	5.13 9 (3.6 Hz)
13	139.6	–
14	41.7	–
15	26.5	1.83
16	34.8	1.32, 1.33
17	41.6	–
18	59.1	1.33
19	39.7	1.45
20	47.6	1.90
21	28.1	2.0
22	23.8	1.33
23	33.1	0.87 (d)
24	17.5	0.80 (d, 6.0)
25	17.5	0.80 (s, Me)
26	23.3	1.07 (s, Me)
27	28.4	0.80 (s, Me)
28	15.7	0.98 (s, Me)
29	16.8	1.10 (s, Me)
30	16.9	0.87 (s, Me)
1'	173.7	–
2'	34.9	2.30 (t, 7.2)
3'	24.8	1.63
4'	20.7	1.30
5'	28.73	0.80

The ^{1}H NMR spectrum of **1a** (Table 1) indicated resonances for a carbinyl proton (δ 4.51), an olefinic proton (δ 5.13), and eight methyl singlets (δ 1.07, 1.01, 0.98, 0.87 (2×), 0.80 (3×). This indicated a triterpene with an olefin and ester functionalities. An α-methylene to a carbonyl at δ 2.30 and a methyl triplet at δ 0.88 supported an ester attached to the triterpene. The ^{13}C NMR spectrum (Table 1) indicated resonances for a carbinyl carbon at δ 80.6, an olefin at δ 124.4 and 140, eight methyls, ten methylenes, three methines, and six quaternary carbons for the triterpene, while the ester attached to the triterpene gave resonances at δ 173.7 (C = O), three methylenes (δ 34.9, 20.7, 25) and a methyl group (28.73). This suggested a pentanoate attached to the triterpene.

The ^{1}H and ^{13}C assignments were verified by HSQC and connectivities were verified by HMBC. The ester was attached to C-3 due to long-range correlation between the carbonyl (δ 173.7) and the carbinyl proton (δ 4.51). The olefinic proton (δ 5.13) was attached to C-12 due to long-range correlation between this proton and the carbons at C-11, C-13, and C-14. All long-range correlations observed were consistent with the structure of **1a**.

The ^{1}H NMR spectrum of **1b** (Table 2) indicated resonances for a carbinyl proton (δ 4.51), an olefinic proton (δ 5.18), and eight methyl singlets (δ 1.14, 0.973, 0.967, 0.93, 0.92, 0.88, 0.87, and

0.83). This indicated a triterpene with an olefin and ester functionalities. An α-methylene to a carbonyl at δ 2.30 and a methyl triplet at δ 0.88 supported an ester attached to the triterpene. The ^{13}C NMR spectrum (Table 2) indicated resonances for a carbinyl carbon at δ 80.6, an olefin at δ 121.7 and 143.6, eight methyls, ten methylenes, three methines, and six quaternary carbons for the triterpene, while the ester attached to the triterpene gave resonances at δ 173.7 (C = O), three methylenes (δ 34.8, 20.7, 25) and a methyl group (28.73). This suggested a pentanoate attached to the triterpene.

The ^{1}H and ^{13}C assignments of **1b** were verified by HSQC and connectivities were verified by HMBC. The ester was attached to C-3 due to long-range correlation between the carbonyl (δ 173.7) and the carbinyl proton (δ 4.51). The olefinic proton (δ 5.18) was attached to C-12 due to long-range correlation between this proton and the carbons at C-11, C-13, and C-14. All long-range correlations observed were consistent with the structure of **1b**.

Table 2. 400 MHz ^{1}H NMR and 100MHz ^{13}C NMR of 1b

Position	δ_C	δ_H mult. (J Hz)
1	38.3	1.13, 1.67
2	25.2	1.65
3	80.6	4.51 dd (5.6, 10)
4	38.0	–
5	55.3	0.83
6	18.3	1.43, 1.56
7	32.6	1.35
8	40.0	–
9	47.6	1.55
10	36.8	–
11	23.5	1.88, 1.92
12	121.7	5.18 t (3.6 Hz)
13	145.2	–
14	41.6	–
15	23.6	1.63, 1.65
16	34.9	1.33, 1.57
17	36.5	–
18	49.7	1.34
19	41.6	1.3, 1.45
20	28.7	–
21	28.4	1.35
22	23.7	1.35
23	23.6	0.88 (s, Me)
24	21.4	0.93 (s, Me)
25	13.9	0.92 (s, Me)
26	26.3	1.14 (s, Me)
27	28.7	0.83 (s, Me)
28	15.5	0.967 (s, Me)
29	16.8	0.973 (s, Me)
30	17.5	0.87 (s, Me)
1'	173.7	
2'	34.8	2.30 (t, 7.2)
3'	24.8	1.63
4'	20.7	1.30
5'	28.73	0.80

The ^{1}H NMR spectrum of sample **2** indicated resonances for a mixture of two compounds (**2a** and **2b**) in a 2.4:1 ratio based on resonance intensities. The structures of the major compound (**2a**) and the minor compound (**2b**) were deduced as follows.

The ^{1}H NMR spectrum of **2a** indicated resonances for olefinic protons at δ 5.23 (dd, 8.4, 15.2 Hz), 5.18 (dd, 8.8, 15.2), and 5.16 (s br), a carbinyl proton at δ 3.59, two methyl singlets (δ 0.55, 0.80), three methyl doublets [δ 1.03 (6.8 Hz), 0.85 (6.4 Hz), 0.84 (6.4 Hz)], and a methyl triplet (δ0.80, 6.0 Hz). The ^{13}C NMR spectrum indicated resonances for olefinic carbons at δ 139.63, 138.2, 129.5, and 117.43, and a carbinyl carbon at δ 71.1. Literature search revealed that **2a** is spinasterol as evidenced by similar NMR spectral data [6].

The ^{1}H NMR spectrum of **2b** indicated resonances for olefinic protons at δ 5.16 (s br), a carbinyl proton at δ 3.59, two methyl singlets (δ 0.54, 0.80), three methyl doublets [δ 0.93 (6.4 Hz), 0.84 (6.4 Hz), 0.83 (6.4 Hz)], and a methyl triplet (δ 0.80, 6.0 Hz). The non-appearance of the double bond in the acyclic part of **2b** resulted in the shielding of the methyl protons at C-21 from δ 1.03 to 0.93. The ^{13}C NMR spectrum of **2b** gave resonances at δ 117.43 and 139.57 for the double bond in the ring (C-7 and C-8). Thus, **2b** is taraxerol.

The ^{1}H NMR spectrum of **3** indicated resonances for a carbinyl proton (δ 4.50), an olefinic proton (δ 5.29), and eight methyl singlets (δ 1.14, 0.94, 0.93, 0.91, 0.88, 0.864, 0.857, and 0.77) and a deshielded doublet of doublet at δ 2.80. An α-methylene to a carbonyl at δ 2.30, a large resonance intensity at δ 1.26 for the methylene groups of a long chain fatty acid, and a methyl triplet at δ 0.88 supported a fatty acid ester attached to the triterpene. The ^{13}C NMR spectrum of **3** indicated resonances for a carbinyl carbon at δ 80.6, an olefin at δ 122.6 and 143.6, eight methyls, ten methylenes, three methines, and six quaternary carbons for the triterpene, while the fatty acid ester attached to the triterpene gave resonances at δ 173.7 (C = O), a long chain methylene resonances clustered around δ 29.7 and a methyl group (δ 14.1). Literature search revealed that **3** is a fatty acid ester of oleanolic acid [7].

As part of our continuing studies on potential antimicrobial compounds from Philippine medicinal plants, the antimicrobial potential of **3** and a mixture of **1a** and **1b** were tested by the use of the agar well method. Results of the study (Table 3) indicated that they were inactive against the bacteria: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Bacillus subtilis.* The mixture of **1a** and **1b** showed moderate activity against the fungus, *Candida albicans* and low activity against the fungi, *Aspergillus niger* and *Trichophyton mentagrophytes.* **3** gave low activity against *C. albicans* and *A. niger* and inactive against *T. mentagrophytes.* A previous study reported the antimicrobial activities of **2a** [8].

Table 3. Antimicrobial Test Results on 1a, 1b, and 3

SAMPLE	Concn. (μg)	*Staphylococcus aureus*		*Escherichia coli*		*Pseudomonas aeruginosa*		*Bacillus subtilis*		*Candida albicans*		*Aspergillus niger*		*Trichophyton mentagrophytes*	
		C.Z.* (mm)	A.I.	C. Z.* (mm)	A.I.	C. Z.* (mm)	A.I.	C.Z.* (mm)	A.I.	C.Z.* (mm)	A.I.	C.Z.* (mm)	A.I.	C.Z.* (mm)	A.I.
1a and 1b	30	-	0	-	0	-	0	-	0	13	0.3	12	0.2	13	0.3
3	30	-	0	-	0	-	0	-	0	-	0	12	0.2	-	0
Standard	30	25	3.2	23	2.8	8	0.3	20	2.3	10	0.7	10	0.7	50	7.3
Antibiotic		Chloramphenicol[a]		Chloramphenicol[a]		Chloramphenicol[a]		Chloramphenicol[a]		Chlortrimazole[b]		Chlortrimazole[b]		Chlortrimazole[b]	

CZ—clear zone, *Average of three trials, AI—activity index, [a]chloramphenicol disc—6 mm diameter, [b]chlortrimazole conc. 50 μg, disc—6 mm diameter

EXPERIMENTAL

General experimental procedures. 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), while the TLC was performed with plastic backed plates coated with silica gel F_{254}. The plates were visualized with vanillin-H_2SO_4 and warming.

Sample collection. Fruits of *A. zapota* were collected from Pilar, Las Pinas in September. It was identified as *Achras zapota* at the Philippine National Museum.

Isolation. The freeze-dried fruits (282 g) of *A. zapota* were ground in an osterizer, soaked in dichloromethane for three days, then filtered. The filtrate was concentrated under vacuum to afford a crude extract. The extract was chromatographed in increasing proportions of acetone in dichloromethane (10% increments). The 30–40% acetone in dichloromethane fractions were rechromatographed in 10% ethyl acetate in petroleum ether, then washed with petroleum ether (2×) to afford **1a** and **1b** (11.1 mg). The 10%-20% acetone in dichloromethane fractions were rechromatographed (2×) in 2.5% ethyl acetate in petroleum ether to afford **3** (6.3 mg). The 30–40% acetone in dichloromethane fractions were rechromatographed (4×) in dichloromethane: diethylether:acetonitrile (1:1:8) to afford **2a** and **2b** (4.5 mg).

Antimicrobial tests. The microorganisms used in these tests were obtained from the University of the Philippines Culture Collection (UPCC). These are *A. niger* UPCC 4219, *C. albicans* UPCC 2168, *B. subtilis* UPCC 1295, *P. aeruginosa* UPCC 1244, *E. coli* UPCC 1195, *S. aureus* UPCC 1143, and *T. mentagrophyte* UPCC 4193. The test compound was dissolved in 95% ethanol. The antimicrobial assay procedure reported in the literature was employed [9].

ACKNOWLEDGMENT

The antimicrobial tests were conducted at the University of the Philippines-Natural Sciences Research Institute (UP-NSRI). Research grants from the Science Foundation and the University Research Coordination Office of De La Salle University is gratefully acknowledged.

REFERENCES

1. Quisumbing, E. *Medicinal Plants of the Philippines,* p. 697. (Manila: Bureau of Printing, 1978).

2. Pal, S. C., and Nandy, A. *Indian Drugs.* 36 (2), 106 (1999).

3. Arakawa, T., Shibata, M., Hosomi, K., Watanabe, T., Honma, Y., Kawasumi, K., and Takeuchi, Y. *Shokuhin Eiseigaku Zasshi.* 33 (6), 569 (1992).

4. Mirimanof, A., and Ihanez, M. L. *Pharm. Acta. Helv.* 36, 97 (1961).

5. Subramanian, S., and Nair, A. G. R. *Phytochemistry.* 11 (10), 3090 (1972).

6. Villaseñor, I. M., and Domingo, A. P. *Teratogenesis, Carcinogenesis, and Mutagenesis.* 20, 99 (2000).

7. Huang, K. F. and Hsu, C. J. *J. Chin.* 12 (1), 61 (2001).

8. Lim, K. M. S. Thesis. De La Salle University-Manila, 2003.

9. Guevara, B. Q. and Recio, B. V. *Acta Manilana Supplement.* (1985).