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Secondary Metabolites from *Bauhinia purpurea*

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The leaves of Bauhinia purpurea afforded a mixture of phytol fatty esters (1a, 1b, 1c, 1d, 1e, 1f), lutein, and β-sitosterol. The structure of 1 was elucidated by NMR spectroscopy, while the chain lengths of the esterified fatty acids in 1 were determined by mass spectrometry. The structures of lutein and β-sitosterol were identified by comparison of their NMR spectral data with those reported in the literature. Antimicrobial tests indicated that 1 has low activity against the fungi, A. niger and C. albicans, and inactive against the bacteria, P. aeruginosa, S. aureus, B. subtilis, E. coli, and the fungus, T. mentagrophytes.

Keywords: *Bauhinia purpurea*, Leguminosae, phytyl esters, lutein, b-sitosterol

Bauhinia purpurea, commonly known as alibangbang is a small sized tree found throughout the Philippines. It has a number of reported medicinal uses. The infusion of the fresh flowers and the decoction of the bark are antidysenteric. A decoction of the root bark is a common remedy for liver trouble, while the seeds exhibit hemolytic activity. The leaves are applied to the head during fevers accompanied by headaches (Quisumbing 1978). A number of studies reported the isolation of flavonoids and flavone glycosides (Yavada & Sodhi 2001, Yavada & Tripathi 2000, Kuo et al.1989, Abd-El-Wahab et al.1987), saponin and triterpenoids (Kuo & Yeh 1997, 1998), terpenes (Shah et al. 1977) and fatty acids (Kafuku & Hats 1934).

We report here the isolation, structure elucidation, and antimicrobial assay of phytyl esters (1a-1f) from *B. purpurea*. Lutein and β-sitosterol were also isolated from the plant. This is the first report on the isolation of phytyl cerotate (1f) from a natural source and the first reported isolation of phytyl esters from the *B. purpurea*.

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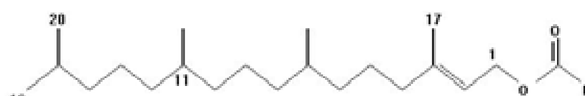


1a R = $CH_3(CH_2)_{14}$–
1b R = $CH_3(CH_2)_{16}$–
1c R = $CH_3(CH_2)_{18}$–
1d R = $CH_3(CH_2)_{20}$–
1e R = $CH_3(CH_2)_{22}$–
1f R = $CH_3(CH_2)_{24}$–

Results and Discussion

The ^{1}H NMR spectrum of 1 (Table 1) indicated resonances for an olefinic proton at δ 5.34; a methylene carbinyl at δ 4.59; an allylic methyl at δ 1.69, four methyl doublets at δ 0.87 (6H), 0.84, and 0.85, and a methyl triplet at δ 0.88; and many overlapping methylene protons at δ 1.26 characteristics of long chain fatty acids.

The ^{13}C NMR spectrum of 1 (Table 1) indicated resonances for twenty-five carbons, excluding many overlapping or almost overlapping carbons at around

Table 1. 400 MHz ^{1}H NMR, 100 MHz ^{13}C NMR, HMBC and NOESY Correlations of 1a-1f in $CDCl_3$.

Position	δC	δH mult. (J Hz)	HMBC Correlations	NOESY Correlations
1	61.2	4.59 d (5.4, 2H)		H-2, H-17
2	118.2	5.34 t (5.4)	H-1, H-4	H-1, H-4
3	142.6	---	H-1, H-4	
4	39.9	2.00 d (6.6, 2H)	H-2	H-2, H-6
5	25.0	1.30	H-4	
6	36.6	1.30	H-4	H-18
7	32.7	1.26		H-18
8	37.4	1.30		H-18
9	24.3	1.30		
10	37.44	1.30		
11	32.8	1.26		
12	37.3	1.30		
13	24.9	1.30		
14	39.4	1.55	H-16	H-16
15	28	1.55		
16	22.7	0.87 d (6.8, Me)	H-14, H-15	H-14
17	16.9	1.69 s (Me)	H-2, H-4	H-1a, H-1b
18	19.74	0.85 d (6.8, Me)	H-7	
19	19.74	0.84 d (6.4, Me)	H-11	
20	22.6	0.87 d (6.8, Me)	H-15	
1’	173.9	---	H-1, H-2’	
2’	34.4	2.29 t (7, 2H)	H-3’	H-3’, H-4’
3’	25.0	1.60 (2H)		H-2’, H-4’, H-5’
4’	29.5	1.26	H-3’, H-5’	H-2’, H-3’
5’-15’	29.3-29.7	1.26	H-16’	
16’-	31.9	1.26	H-15’	
17’	29.5	1.26		
18’	14.1	0.88 t (6.8, Me)		H-17’

δ 29.3-29.7. This supports the assumption from the ^{1}H NMR spectrum that 1 contains long chain fatty acids. The spectrum gave resonances for carbons with the following functionalities: a carbonyl carbon of an ester at δ 173.9; olefinic carbons at δ 118.2 and 142.6; and a carbinyl carbon at δ 61.2. The non fatty acid resonances are similar to those found in the literature for phytol (Goodman et al.1973).

The COSY 2D NMR spectrum (Figure 1) showed correlations from which the following couplings could be detected: H2-1/H-2/H3-17; H-15/H3-16/H3-20; H2-2' to H3-18' (Figure 1).

The HMQC 2D NMR data enabled assignments of protons attached to carbons (Table 1) and connectivities were deduced from HMBC 2D NMR data (Table 1). Thus, the fatty ester was attached to C-1 due to long-range correlations between the carbonyl carbon at δ 173.9 (C-1') and the methylene carbinyl protons at δ 4.59 (H-1) and the α-methylene protons at δ 2.29 (H-2'). The double bond was placed on C-2 since long-range correlations were observed between the olefinic carbons at δ 118.2 (C-2) and 142.6 (C-3) and the methylene carbinyl protons at δ 4.59 (H-1). All long-range correlations observed were consistent with the structure of 1.

The relative stereochemistry of 1 was deduced from NOESY (Table 1 and Figure 2) spectral data. The methylene carbinyl proton (H-1) is close to the olefinic proton (H-2) and the allylic methyl group (H-17), thus they are close in space. On the other hand, the olefinic proton (H-2) and the methylene protons (H-4) are close

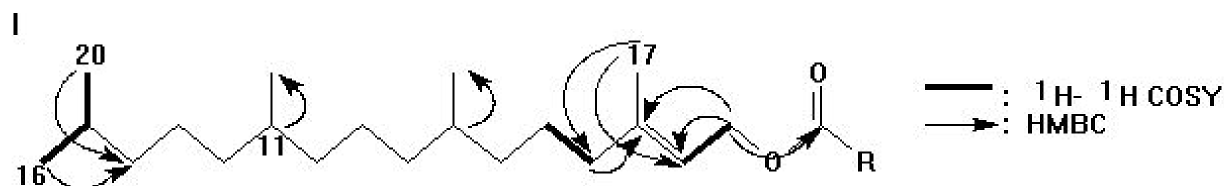


Figure 1. ^{1}H-1H COSY and key ^{1}H-^{13}C long-range correlations for 1.

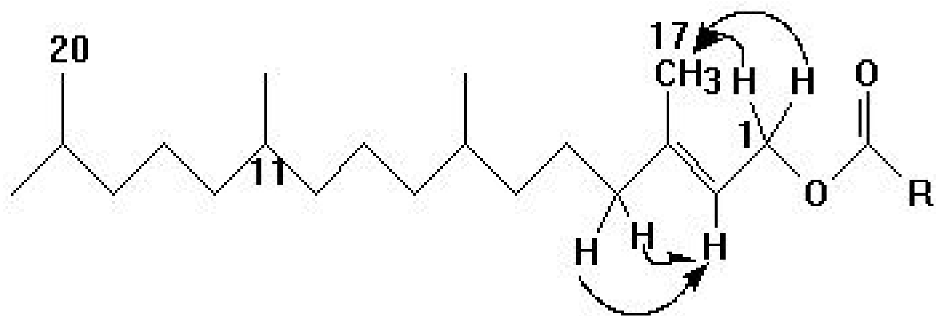
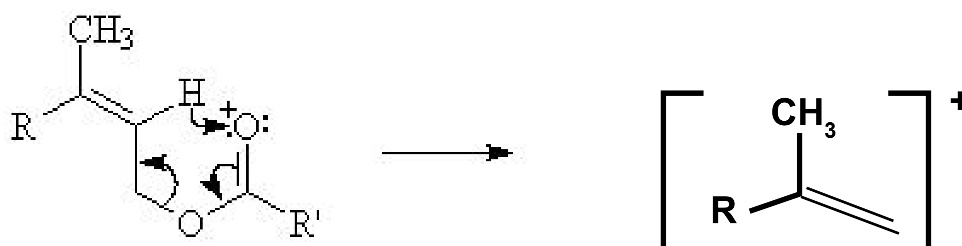


Figure 2. Key NOESY correlation for 1

to each other. This signified an E configuration for the double bond which is the same as in the literature (Chapman and Hall, 1994).

Based on ESI mass spectral data, 1 is a mixture of phytyl esters of long chain fatty acids as follows: phytyl palmitate m/z 557 [M + Na]+(C36H70O2), phytyl stearate m/z 585 [M + Na]+ (C38H74O2), phytyl arachidate m/z 613 [M + Na]+ (C40H78O2), phytyl behenate m/z 641 [M + Na]+ (C42H82O2), phytyl lignocerate m/z 669 [M + Na]+ (C44H86O2), and phytyl cerotate m/z 697 [M + Na]+ (C46H90O2) in the ratio of 84:47:64:100:71:42. This pattern was also seen in EI measurements. The most characteristic peak was the base peak at m/z 278 which could have been generated by an E→Z isomerization of the double bond followed by McLafferty rearrangement to an allene and an acid. Only one HR-EIMS was



m/z **278**

$C_{20}H_{38}$

1a $R = C_{16},H_{33}$, $R' = C_{15}H_{32}$

1b $R = C_{16}H_{33}$, $R' = C_{17}H_{36}$

1c $R = C_{16}H_{33}$, $R' = C_{19}H_{40}$

1d $R = C_{16}H_{33}$, $R' = C_{21}H_{44}$

1e $R = C_{16}H_{33}$, $R' = C_{23}H_{48}$

1f $R = C_{16}H_{33}$, $R' = C_{25}H_{52}$

measured for the 563 ion which gave a mass of 562.5677, with the calculated value for phytyl stearate, formula C38H74O2, being 562.5689 (error = 2.1 ppm).

Lutein (Largo et al.1997) and β-sitosterol (Rubistein et al.1976) were identified by comparison of their ^{1}H NMR spectral data with those found in the literature.

Results of the antimicrobial tests indicated that 1 has low activity against the fungi, A. niger and C. albicans, and inactive against the bacteria, *P. aeruginosa*, *S. aureus*, *B. subtilis*, *E. coli,* and the fungus, T. mentagrophytes. The activity index (AI) of 1 against *C. albicans* and *A. niger* is 0.2, while the standard antibiotic chlortrimazole gave an AI of 0.7.

Experimental

General Experimental Procedures. NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer in $CDCl_3$ (400 MHz for 1H NMR and 100 MHz for ^{13}C NMR). The ESI and EIMS were recorded on a Micromass Autospec mass spectrometer. Column chromatography was performed with silica gel 60 (70-230 mesh). TLC was performed with plastic backed plates coated with silica gel F_{254}; plates were visualized by spraying with vanillin-H_2SO_4 and warming.

Sample Collection

The samples were obtained from San Pedro, Laguna in August 2000. They were identified as *Bauhinia purpurea* L. Swartz at the Philippine National Museum and voucher specimens # 051 were deposited at the Chemistry Department of De La Salle University.

Isolation

The air-dried leaves (1.0 kg) of *B. purpurea* were extracted with ethyl acetate to afford a crude extract (43 g) which was fractionated by silica gel chromatography using increasing proportions of ethyl acetate in petroleum ether at 10% increment as eluent. The 10% ethyl acetate in petroleum ether fraction was rechromatographed using dichloromethane and washed with petroleum ether to afford lutein (orange crystals, 45 mg, m.p. 190-192°C; lit. m.p. 196°C). The 20% ethyl acetate in petroleum ether fraction was rechromatographed in petroleum ether (10×) to afford 1 (oil, 15 mg). The 30 % ethyl acetate in petroleum ether fraction was rechromatographed using 10%-30% ethyl acetate in petroleum ether to afford fraction 10. This fraction was rechromatographed using 10% ethyl acetate in petroleum ether to afford β-sitosterol (colorless crystal, 25 mg, m.p. 134-136°C; lit. m.p.: 140°C).

Antimicrobial Tests

The microorganisms used in these tests were obtained from the University of the Philippines Culture Collection (UPCC). These are *Pseudomonas aeruginosa* UPCC 1244, *Bacillus subtilis* UPCC 1149, *Escherichia coli* UPCC 1195, *Staphylococcus aureus* UPCC 1143, *Candida albicans* UPCC 2168, *Trichophyton mentagrophytes* UPCC 4193 and *Aspergillus niger* UPCC 3701.

Microbial suspension containing approximately 6 × 108 cells/mL was prepared from each test organism for 24-hour culture of *S. aureus*, *E. coli*, *P. aeruginosa*, *B. subtilis* and *C. albicans* and from a 5 day old *A. niger* and *T. mentagrophytes*. The suspending medium used for each microbial suspension was 0.1% peptone water. One-tenth (0.1) mL of the bacteria, yeast and molds were transferred into pre-poured nutrient agar (NA, DISCO Laboratories, Detroit, Michigan), glucose yeast peptone agar (GYP) (Kreger-van Rij 1984) and potato dextrose agar (PDA, DISCO Laboratories, Detroit, Michigan), respectively. About 5 mL of corresponding medium, autoclaved and cooled to about 45°C was poured into the 90 mm petri dish. The plate was swirled to distribute the microbial cells evenly on the plate and the agar overlay was allowed to solidify. Three 10 mm wells were cut from equidistant points of the seeded agar plates using sterile cork borer. Thirty (30) μg of samples dissolved in 95 % EtOH were transferred in each well. For the standard agent, 30 μg were used.

The NA, GYP and PDA-based cultures were incubated at 30 ± 1°C for 24, 48 and 72 hours, respectively. Antimicrobial effects were determined by measuring the zone of the growth inhibition represented by a clear zone in mm. The average diameter of the clear zones was used to calculate an antimicrobial index.

Acknowledgments

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