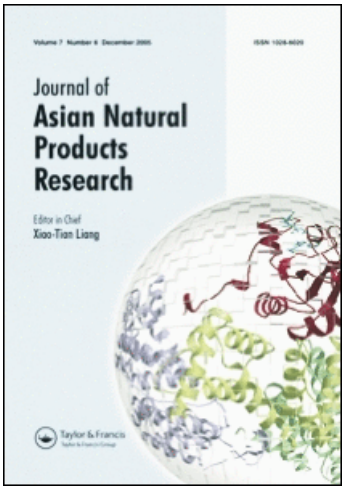

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Terpenoids and sterols from *Lagerstroemia speciosa*

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The leaves of *Lagerstroemia speciosa* afforded a new natural product, 31-norlargerenol acetate (**4**), along with known compounds 24-methylenecycloartanol acetate (**1**), its 31-nor analog (**2**), largerenol acetate (**3**), tinotufolins C (**5**) and D (**6**), lutein, phytol, sitosterol and sitosterol acetate. The structure of **4** was elucidated by 1D and 2D NMR and mass spectroscopy.

Keywords: *Lagerstroemia speciosa*; Lythraceae; 31-Norlargerenol acetate; Terpenoid

1. Introduction

The Philippines is rich in medicinal plants that have been used as therapeutic treatment of human diseases since the early times of our ancestors, and are still used by rural folk in treating ailments. *Lagerstroemia speciosa* is a common medicinal plant that is available in the local market as a tea. A decoction of the leaves is used as a diuretic, a decongestant, and to treat diabetes mellitus. A decoction of the bark is used as a stimulant, febrifuge, and for relief of abdominal pains. A decoction of the roots is employed against ulcers of the mouth, while the seeds have narcotic properties and are employed for mouth ulcer pain relief [1]. Earlier studies on the plant reported the isolation of sitosterol, ellagic acid, campesterol and stigmasterol [2], lagerstannins A, B, and C [3], colosolic acid and maslinic acid [4], lageracetal, lasubine I and lasubine II, flosin A, reginins C and D, pterocarinin [5], 3,3′,4-tri-*O*-methylellagic acid and 3-*O*-methylellagic acid [6]. Colosolic acid was shown to be a glucose transport activator and to have hypoglycemic activity [4].

We now report the identification of a new natural product, 31-norlargerenol acetate, along with known compounds 24-methylenecycloartanol acetate and its 31-nor analog (cycloeucalenol acetate), largerenol acetate, the diterpenes tinotufolins C and D, lutein, phytol, sitosterol, and sitosterol acetate from the chloroform extract of the air-dried leaves of the plant.

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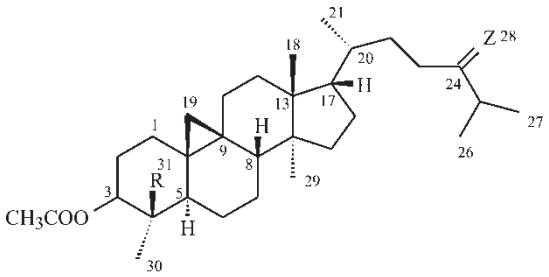
2. Results and discussion

Chloroform extracts of the air-dried leaves of *Lagerstroemia speciosa* afforded chromatographic fractions containing a series of steroids and other terpenoids. Two fractions were characterised by the appearance in the ^{1}H NMR spectra of two doublet signals for an isolated cyclopropane methylene. The first of these fractions was a mixture of 24-methylenecycloartanol acetate (**1**) [7], and cycloeucalenol acetate (**2**) [8], whilst the second fraction contained largerenol acetate (**3**) [9] and the new natural product, 31-norlargerenol acetate (**4**) (figure 1).

The ^{1}H NMR spectra of **3** and **4** were broadly similar, both possessing the same side-chain; however, there were three very significant differences between the compounds. Like **2**, the carbinyl signal of **3** at δ 4.51 appeared as a doublet of triplets, with coupling constants of 10.6, 10.6 and 4.8 Hz. This clearly implied that the carbinyl hydrogen of **3** had two axial-axial type couplings. The ^{1}H NMR also showed four doublet methyl signals ($J = \mathbf{6.5}$ to 6.9 Hz), compared to three methyl doublets in **3**, and only seven methyl signals (including the acetate methyl) in total. Thus, the loss of one methyl group, the extra methyl doublet signal, and the extra axial coupling to H-3 all support a cycloeucalenol-type structure **4** for the minor component, wherein the axial methyl at C-4 of **3** has been replaced by a hydrogen.

The proposed structure for **4** was confirmed by additional MS and NMR experimental data. HR-EIMS gave a molecular ion (*m/z* 470.3750) in agreement with the molecular formula $C_{31}H_{50}O_3$, and a base peak at *m/z* 410 for $[M - CH_3COOH]^+$. The DEPT spectrum showed (by comparison with **3**) an additional methine carbon at δ 41.51 for C-4; this carbon was long-range coupled to the new methyl doublet at δ 0.84, which also showed HMBC correlations to the C-3 carbinyl at δ 78.7, and the C-5 methine (δ 43.41). NOESY correlations were observed from the downfield cyclopropane proton (δ 0.41, H-19b) to H-8, H-4, and the upfield H-6 proton, suggesting axial-like orientations for those three protons. The NMR data are summarised in table 1, which also gives comparative reference ^{13}C NMR data (obtained by combining shifts for the ring carbons and methyls of cycloeucalenol acetate (**2**) [8] with the sidechain carbon shifts for cycloartan-3,24-dione [10]). There is excellent agreement between actual ^{13}C data and literature chemical shifts. All other spectral data are consistent with the proposed structure for **4**, which has been previously reported [11,12] as a synthetic intermediate, but not as a natural product.



1 R = CH_3, Z = CH_2
2 R = H, Z = CH_2
3 R = CH_3, Z = O
4 R = H, Z = O

Figure 1. Structures of **1**–**4**.

Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectral data of **4**.

Position	^{13}C, δ	^{1}H, δ	*HMBC* (^{1}H δ)	*NOESY* (^{1}H/^{1}H)	*Lit.* ^{13}C[b]
1	30.48	1.28[a]		1.60	30.54
		1.60		1.28, 2.00	
2	30.93	1.45		2.02	31.00
		1.99		1.45	
3	78.72	4.51 (dt, 10.6, 10.6, 4.8)	0.84		78.79
4	41.51	1.39	0.84	0.84, 0.41	41.59
5	43.41	1.28	0.84		43.49
6	24.64	0.58		0.41, 1.69	24.72
		1.69		0.58, 0.84, 1.32	
7	25.01	1.09		1.32, 0.90	25.08
		1.32		1.09, 0.90	
8	46.84	1.61	0.90	0.41, 0.96	46.91
9	23.63	–			23.75
10	29.34	–			29.44
11	26.94	1.17	1.62	2.00	27.05
		2.00		1.17, 1.28	
12	32.85	1.62	0.96	0.96	32.91
13	45.35	–	0.90, 0.96		45.43
14	48.89	–	0.90, 0.96, 1.62		48.95
15	35.30	1.29	0.90	0.90	35.40
16	28.02	1.32		1.95	28.15
		1.95		0.90, 1.32	
17	52.16	1.59	0.87, 0.96		52.26
18	17.80	0.96 (s)		1.32, 1.61	17.83
19	27.10	0.14 (d, 4.1)		0.41, 1.17, 1.45	27.14
		0.41 (br d, 4.1)		0.14, 0.58, 1.39, 1.61	
20	35.73	1.35	0.87		35.7
21	18.11	0.87 (d, 6.5)		1.62, 2.37	18.1
22	30.14	1.26	0.87	1.78	30.1
		1.78		1.26	
23	37.50	2.37		0.87, 2.48	37.5
		2.48		2.37	
24	215.40	–	1.09		215.4
25	40.82	2.63 (sept, 6.9)		1.09	40.8
26	18.29	1.09 (d, 6.9)		2.63	18.3
27	18.37	1.09 (d, 6.9)		2.63	18.4
29	19.11	0.90 (s)		1.09, 1.29, 1.32, 1.95	19.18
30	14.39	0.84 (d, 6.5)		1.39, 1.69	14.46
OAc	21.31	2.05 (s)			21.36
	170.94	-	2.05		170.94

[a] Multiplets unless otherwise indicated; numbers in brackets are coupling constants (Hz).
[b] Reference data from ring carbons of **2** [8], plus side-chain carbons (C-20 to C-27) of cycloartan-3,24-dione [10].

Other known compounds isolated from *Lagerstroemia speciosa* include the diterpenes tinotufolin C [13] and tinotufolin D, which we previously isolated from *Tinospora rumphii* [14]. Also isolated were lutein [15], phytol [16], sitosterol [17], and sitosterol acetate [17].

3. Experimental

3.1 General experimental procedures

1D and 2D NMR experiments were acquired in $CDCl_3$ at 400 MHz (^{1}H) and 100 MHz (^{13}C) on a Bruker Avance spectrometer. HR-EIMS were recorded on a Micromass Autospec mass

spectrometer. IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR. Column chromatography was performed on silica gel 60 (70–230 mesh). TLC was performed on plastic backed plates coated with silica gel F_{254}; plates were visualized by spraying with vanillin–H_2SO_4 and warming.

3.2 Plant material

The sample was obtained from Sinait, Ilocos Sur, Philippines in May 2002. It was identified as *Lagerstroemia speciosa* at the Philippine National Museum and a voucher specimen # 65 has been deposited at the Chemistry Department of De La Salle University.

3.3 Extraction and isolation

Air-dried leaves (1.2 kg) of *L. speciosa* were extracted with chloroform to afford a crude extract (95 g) that was dissolved in ethanol then placed on an ice bath. To the solution was added aqueous $Pb(OAc)_2$ (45 mL) to precipitate the more polar components [18]. The mixture was then filtered and the filtrate was concentrated under vacuum until a mixture of water and oily residue remained. The concentrate was then extracted with chloroform, and the extract dried with anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under vacuum to afford the treated extract (15 g), which was fractionated by column chromatography using increasing proportions of acetone in dichloromethane (10% increment) as eluent. The 10–20% acetone in dichloromethane fractions were combined and rechromatographed on silica gel with increasing proportions of ethyl acetate in light petroleum (5% increments) to give fractions F5, F10 and F15 from 5, 10 and 15% ethyl acetate elution, respectively.

Fraction F5 was rechromatographed (2 ×) in 5% ethyl acetate in light petroleum to afford sitosterol acetate (10 mg). Fraction F10 was rechromatographed in 10% ethyl acetate in light petroleum. The less polar fractions (F10-1) afforded lutein (5 mg) and sitosterol (50 mg) after rechromatography (5 ×) in 10% ethyl acetate in light petroleum. The more polar fractions (F10-2) afforded phytol (10 mg) after rechromatography (3 ×) in 10% ethyl acetate in light petroleum.

Fraction F15 was rechromatographed in 15% ethyl acetate in light petroleum. After further purification by rechromatography as above, the following compounds (in order of polarity) were identified: **1** and **2** (7 mg); fraction F15-2, containing **3** and **4** (4 mg); tinotufolins C and D (12 mg). Fraction F15-2 was separated by HPLC chromatography on an Astec Chirobiotic T column (250 × 4.6 mm) using light petroleum–ethanol (197:3) at 0.5 mL min^{-1}, to give **3** (2 mg) and **4** (< 1 mg).

3.3.1 24-Methylenecycloartanol acetate (1). ^{13}C NMR δ (ppm): 15.1 (C-30), 17.9 (C-18), 18.3 (C-21), 19.3 (C-29), 20.1 (C-9), 20.9 (C-6), 21.3 (OAc), 21.8 (C-26), 22.0 (C-27), 25.4 (C-31), 25.8 (C-7), 26.0 (C-10), 26.5 (C-11), 26.8 (C-2), 28.1 (C-16), 29.7 (C-19), 31.6 (C-23), 31.6 (C-1), 32.9 (C-12), 33.8 (C-25), 35.0 (C-22), 35.5 (C-15), 36.1 (C-20), 39.4 (C-4), 45.3 (C-13), 47.2 (C-5), 47.8 (C-8), 48.8, (C-14), 52.2 (C-17), 80.6 (C-3), 105.9 (C-28),

156.8 (C-24), 171.0 (OAc); EIMS *m/z* $[M]^+$ 482.4120 (24), calcd for $C_{33}H_{54}O_2$ 482.4124, 422 (100).

3.3.2 Cycloeucalenol acetate (2). ^{13}C NMR δ (ppm): 14.4 (C-30), 17.8 (C-18), 18.3 (C-21), 19.1 (C-29), 21.3 (OAc), 21.8 (C-26), 22.0 (C-27), 23.7 (C-9), 24.6 (C-6), 25.0 (C-7), 27.0 (C-11), 27.1 (C-19), 28.1 (C-16), 29.3 (C-10), 30.5 (C-1), 30.9 (C-2), 31.3 (C-23), 32.9 (C-12), 33.9 (C-25), 35.0 (C-22), 35.3 (C-15), 36.1 (C-20), 41.5 (C-4), 43.4 (C-5), 45.3 (C-13), 46.8 (C-8), 48.9 (C-14), 52.2 (C-17), 78.7 (C-3), 105.9 (C-28), 156.8 (C-24), 170.9 (OAc); EIMS *m/z* $[M]^+$ 468.3955 (14), calcd for $C_{32}H_{52}O_2$ 468.3967, 408 (100).

3.3.3 Largerenol acetate (3). ^{13}C NMR δ (ppm): 15.1 (C-30), 18.0 (C-18), 18.1 (C-21), 18.3 (C-26), 18.4 (C-27), 19.3 (C-29), 20.1 (C-9), 20.9 (C-6), 21.3 (OAc), 25.4 (C-31), 25.8 (C-7), 26.0 (C-10), 26.5 (C-11), 26.8 (C-2), 28.0 (C-16), 29.7 (C-19), 30.1 (C-22), 31.6 (C-1), 32.9 (C-12), 35.5 (C-15), 35.7 (C-20), 37.5 (C-23), 39.4 (C-4), 40.8 (C-25), 45.3 (C-13), 47.2 (C-5), 47.8 (C-8), 48.8, (C-14), 52.2 (C-17), 80.7 (C-3), 170.9 (OAc), 215.4 (C-24); EIMS *m/z* $[M]^+$ 484.3918 (18), calcd for $C_{32}H_{52}O_3$ 484.3916, 469 (8), 455 (7), 424 (100), 409 (59), 395 (16), 381 (22), 355 (20), 302 (36), 297 (25).

3.3.4 31-Norlargerenol acetate (4). White amorphous solid; IR ($CHCl_3$) ν_{max} (cm^{-1}): 3062 (cyclopropane C—H), 2970, 2932, 2872 (CH), 1720 (ester C═O), 1714 (C═O), 1258, 1025 (C—O); ^1H and ^{13}C NMR data, see table 1; EIMS *m/z* $[M]^+$ 470.3750 (15), calcd for $C_{31}H_{50}O_3$ 470.3760, 455 (10), 424 (10), 410.3547 (100), calcd for $C_{29}H_{48}O$ 410.3549, 395 (62), 355 (12), 302 (15), 283 (21).

3.3.5 Tinotufolin C. ^{13}C NMR δ (ppm): 18.1 (C-17), 20.0 (C-12), 20.7 (C-1), 23.5 (C-20), 25.0 (C-19), 25.5 (C-3), 26.2 (C-2), 28.0 (C-7), 36.6 (C-8), 39.3 (C-9), 42.2 (C-10), 42.4 (C-5), 44.7 (C-11), 52.8, (OCH_3), 72.2 (C-6), 81.4 (C-4), 111.0 (C-14), 125.5 (C-13), 138.4 (C-15), 142.8 (C-16), 175.7 (C-18).

3.3.6 Tinotufolin D. ^{13}C NMR δ (ppm): 17.3 (C-17), 17.8 (C-19), 19.3 (C-12), 20.2 (C-20), 23.4 (C-1), 28.5 (C-7), 32.4 (C-8), 39.8 (C-10), 40.3 (C-9), 41.7 (C-11), 46.2 (C-5), 76.9 (C-4), 77.2 (C-6), 110.8 (C-14), 125.5 (C-13), 125.6 (C-3), 134.7 (C-2), 138.5 (C-15), 142.9 (C-16), 179.1 (C-18).

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