A New 3,4-*seco*-Lupane Derivative from *Lasianthus gardneri*

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A new *seco*-ring A lupane triterpene derivative (**1**), along with lupenone, lupeol, β-sitosterol, ursolic acid, and stigmasterol 3-*O*-β-D-glucoside, were isolated from a methanol extract of mature stems of *Lasianthus gardneri*, a shrub from the family Rubiaceae growing in Sri Lanka. The structure and stereochemistry of the new compound were determined using a combination of ^{13}C and ^{1}H homo- and heteronuclear 2D NMR experiments and from mass spectral data. The structure of **1** was confirmed by partial synthesis from lupeol.

As a part of our studies on Rubiaceae plants endemic to Sri Lanka[1] we have investigated the methanol extract of *Lasianthus gardneri* (Thw.) Hook., a shrub growing in mountain forests. There are about 80 species of the genus *Lasianthus*[2] around the world, and nine of them found in Sri Lanka are considered to be endemic.[1] To the best of our knowledge no phytochemical or bioactivity work has been previously reported on *L. gardneri*.

In this paper we describe the isolation and characterization of a new triterpene derivative (**1**) with a *seco*-lupane skeleton, together with lupenone, lupeol, β-sitosterol, ursolic acid, and stigmasterol 3-*O*-β-D-glucoside. The structure of **1** was elucidated mainly by 1D and 2D NMR experiments and by comparison with an authentic sample prepared starting from lupeol.

Dried ground mature stems of *L. gardneri* were extracted with methanol. Purification of the extract by passage on a series of silica gel columns and by preparative thin-layer chromatography afforded six compounds. Five of them were identified as lupenone, lupeol, β-sitosterol, ursolic acid, and stigmasterol 3-*O*-β-D-glucoside, respectively, from mass spectral and ^{1}H and ^{13}C NMR data and by direct comparison with authentic samples. Moreover, the identification of lupenone was confirmed by the oxidation of lupeol to lupenone with pyridinium chlorochromate (PCC), whereas stigmasterol glucoside was subjected to acid hydrolysis to confirm the presence of stigmasterol as the aglycon and glucose as the sugar.

The molecular formula of compound **1** was determined as $C_{30}H_{50}O$, from high-resolution mass spectrometry (*m*/*z* 426.3834) as well as from its DEPT NMR spectrum. In the ^{1}H NMR spectrum the presence of two methyl groups (δ_H 1.70 and 1.68) located at sp^2 carbons and four protons (δ_H 4.80, 4.68, 4.62, and 4.56), whose chemical shifts and small coupling constants were typical of terminal double bonds, confirmed the presence of two isopropenyl moieties. Using combined ^{1}H and ^{13}C NMR data and DEPT experiments, six singlet methyl groups, 13 methylenes, five methines, and six quaternary carbons, for a total of 30 carbons, were identified (Table 1). No carbonyl or carboxylic carbons were detected. Considering the molecular formula $C_{30}H_{50}O$, two of the implied six degrees of unsaturation were explained by the two isopropenyl groups. The absence of carbon−oxygen double bonds led us to hypothesize that **1** is a tetracyclic triterpene. Furthermore, closer examination of HMQC, HMBC, TOCSY, and NOESY experiments revealed that the compound was a lupeol-type triterpene[3−5] (Table 1).

Table 1. ^{13}C and ^{1}H NMR Data ($CDCl_3$) for Compound **1**

position	δ_C	δ_H, *J* (Hz)	HMBC (H → C)
1	35.2 t	1.25−1.14	9, 11
2	25.2 t	1.59	1, 10
3	63.7 t	3.55	1, 2
4	148.3 s		5, 24
5	50.4 d	2.02 dd (13.0, 2.8)	6, 25, 23, 10, 24, 4
6	24.7 t	1.76 m (12.4, 2.8) 1.32	5, 7
7	32.8 t	1.41−1.32	5, 26
8	40.6 s		26, 27
9	40.7 d	1.60 dd (12.6, 3.1)	26, 12, 25[a]
10	39.1 s		25, 9, 5
11	21.5 t	1.33−1.22	
12	29.7 t	1.24	
13	38.2 d	1.66	15, 27, 8
14	43.2 s		27,26
15	27.5 t	1.65−1.02	16,27
16	35.5 t	1.48 ddd (12.9, 2.5, 4.6) 1.35	15, 28, 17
17	43.0 s		18, 28, 22
18	48.2 d	1.37	28, 16, 17, 13, 19
19	48.0 d	2.37 ddd (11.0, 11.0, 5.8)	18, 22, 13, 30, 29, 20
20	151.0 s		19, 30, 18
21	29.8 t	1.91−1.29	22, 30
22	40.0 t	1.37−1.18	21, 19
23	112.8 t	4.80 dq (2.7, 1.5)−4.62 d (2.7)	5, 24
24	23.1 q	1.70 d (1.5)	5, 23
25	20.5 q	0.79 s	5, 1, 10, 9
26	16.0 q	1.06 s	9, 7, 8,14
27	14.5 q	0.95 s	13, 15, 14, 8
28	18.0 q	0.78 s	22, 16, 17, 18
29	109.4 t	4.68 d (2.5)−4.56 dq (2.5, 1.3)	19, 30
30	19.3 q	1.68 d (1.3)	19, 29

[a] Only observed in the spectrum measured in pyridine-d_5.

Prominent differences in the ^{1}H and ^{13}C NMR data between **1** and lupeol were observed only in the A ring region; these were the presence of a CH_2 instead of a CH group linked to an alcoholic oxygen atom (hydrogen exchangeable with D_2O), a further isopropenyl moiety, and the absence of a geminal dimethyl group at C-4. From these considerations we deduced that these differences arose from the cleavage of C-3,C-4 bond (*seco*-lupane A ring). The

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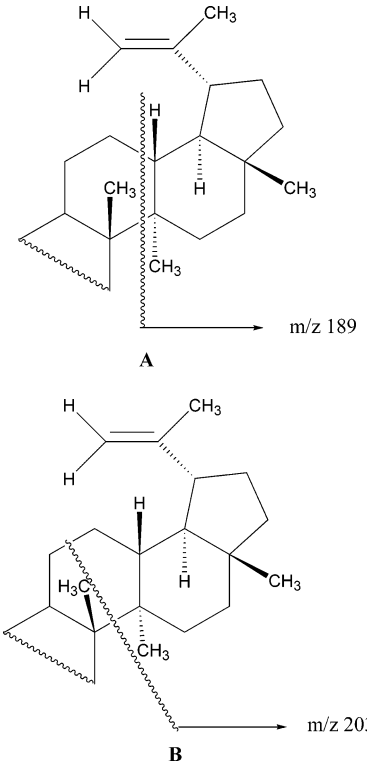


Figure 1. Mass spectral fragment ions of **1** at *m/z* 189 ($C_{14}H_{21}$) and 203 ($C_{15}H_{23}$).

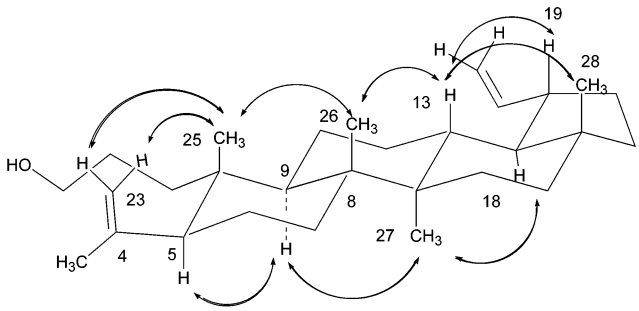
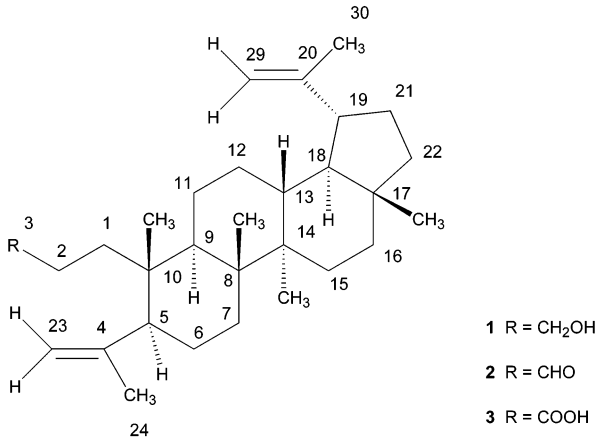


Figure 2. Selected NOESY correlations for **1**.

remaining part of the skeleton of lupeol remained intact, as shown by the two diagnostic fragmentations[6,7] at *m/z* 189 $[M - C_{16}H_{29}O]^+$ and 203 $[M - C_{15}H_{27}O]^+$ in the mass spectrum corresponding to species **A** and **B** (Figure 1). From these data compound **1** was characterized as 3,4-*seco*-lupa-4(23),20(29)-dien-3-ol. Among natural compounds with a *seco*-lupane structure, canaric acid (**3**)[8] shows spectroscopic properties[9] very similar to compound **1**, apart from the presence in **1** of a hydroxymethyl group in position C-3 instead of a carboxylic function. The relative configuration of **1** was assigned on the basis of NOESY data. The trans B/C/D ring junctions were consistent with the strong NOE correlations between the H-9α/Me-27; Me-27/H-18α and between Me-26/H-13; H-13/Me-28. The absence of a NOE between H-5α and Me-25 together with a strong NOE between Me-25/Me-26 and H-5/H-9α was consistent with a *seco*-ring A in a diequatorial conformation. The NOE correlation between H-13 and H-19 allowed the assignment of the configuration at C-19 (Figure 2).

To confirm unambiguously the structure and stereochemistry of compound **1**, we undertook a synthesis of 3,4-*seco*-lupa-4(23),20(29)-dien-3-ol starting from lupeol. According to a fragmentation reaction initiated by an alkoxy radical,[10,11] treatment of lupeol with lead tetraacetate followed by immediate reduction with $LiAlH_4$ of the aldehyde intermediate **2** afforded a product that showed physical and spectroscopic data completely identical to those of compound **1**, thus confirming the assignment of its structure. To the best of our knowledge compound **1** has never been described before.



Experimental Section

General Experimental Procedures. All reagents and solvents were reagent grade or were purified by standard methods before use. Melting points were determined in open capillaries on a Büchi melting point apparatus and are uncorrected. NMR spectra were recorded in $CDCl_3$ (when not otherwise stated) with a Bruker AMX-600 spectrometer, at 600.13 MHz for ^1H and 150.92 MHz for ^{13}C. Chemical shifts (δ values) and coupling constants (J values) are given in ppm and Hz, respectively. EIMS and HREIMS were recorded at an ionizing voltage of 70 eV on a Finnigan TQ70 spectrometer. The relative intensities of mass spectrum peaks are listed in parentheses. Column chromatography (when not otherwise stated) was carried out on flash silica gel (Merck 230−400 mesh). TLC analysis was conducted on silica gel plates (Merck $60F_{254}$). All reactions requiring anhydrous conditions were performed under a positive nitrogen flow, and all glassware was oven-dried and/or flame-dried. Authentic lupeol was purchased from Extrasynthèse, Genay, France.

Plant Material. Aerial parts of *Lasianthus gardneri* were collected from Horton Plains, Central Province of Sri Lanka, in April 1997. A voucher specimen (No. IFS/97/LG1) has been deposited at Institute of Fundamental Studies, Hantana Road, Kandy, Sri Lanka.

Extraction and Isolation. Ground mature stems (1.3 kg) were extracted with methanol (5 L) to give a brown solid (180 g), which was partitioned between *n*-butanol (1.2 L) and water. Evaporation of *n*-butanol gave a crude solid (11.2 g). Chromatography of this residue on Merck 60 silica gel (0.040−0.063 mm) with hexane, then hexane with increasing (from 10% to 100%) percentage of dichloromethane, then dichloromethane−methanol, 95:5 (total amount of eluent 1.2 L), followed by preparative thick-layer chromatography afforded six compounds. Five of them were identified as lupenone, lupeol, β-sitosterol, ursolic acid, and stigmasterol 3-*O*-β-D-glucoside, from mass spectral and ^1H and ^{13}C NMR data, and by direct comparison with authentic samples. Compound **1** (65 mg) was eluted with 60%−100% dichloromethane from the column and purified by preparative TLC with dichloromethane.

Oxidation of Lupeol.[12] Lupeol (10 mg, 0.02 mmol) was dissolved in 10 mL of dichloromethane. After addition of 10 mg of pyridinium chlorochromate, the solution was stirred 2 h at room temperature. The reaction mixture was then filtered through a Celite pad. Evaporation of the solvent afforded a light yellow solid, which appeared identical to lupenone by comparison with an authentic sample.[13]

Acid Hydrolysis of Stigmasterol Glucoside.[14] Stigmasterol glucoside (5 mg) was refluxed in 2 mL of 4 N HCl for 2

h. Extraction with EtOAc (5 mL × 2) led to the identification in the organic layer of stigmasterol[14] as the aglycon by TLC analysis. The aqueous phase was then adjusted to pH 6 with $NaHCO_3$ and evaporated to dryness. The residue was redissolved in pyridine. TLC analysis showed the presence of glucose (eluent: $CHCl_3$−MeOH, 2:1).

Compound 1: sticky oil, $[\alpha]^{25}_D$ +42.6° (*c* 0.5, $CHCl_3$); ^{13}C and ^1H NMR data, see Table 1; EIMS *m*/*z* 426 $[M]^+$ (54) 367 $[M - CH_2CH_2CH_2OH]$ (29), 345 (100), 315 (13), 245 (11), 231 (15), 217 (14), 203 (15), 191 (11), 189 (17), 181 (17), 163 (14), 149 (18), 135 (13), 129 (13), 109 (20), 85 (42); HREIMS *m*/*z* 426.383 (calcd for $C_{30}H_{50}O$, 426.3862).

Synthesis of 1 from Lupeol. Lupeol (50 mg, 0.12 mmol), 212 mg (0.48 mmol) of lead tetraacetate, and $CaCO_3$ (2 mg) in 8 mL of anhydrous toluene were heated under reflux for 2.5 h. The mixture was filtered, and the filtrate was washed with a 5% aqueous solution of KI, a 10% solution of $Na_2S_2O_3$, and water. Then the toluene solution was evaporated to dryness. The crude product (56 mg) was purified by flash chromatography (hexane−Et_2O, 97:3; 500 mL) to obtain 20 mg of the aldehyde **2**, as an oil: ^1H NMR ($CDCl_3$, 300 MHz) δ 0.70 (3H, s, Me-25), 0.77 (3H, s, Me-28), 0.87 (3H, s, Me-27), 0.99 (3H, s, Me-26), 1.59 (3H, dd, J = 0.8, 1.5 Hz, Me-24), 1.61 (3H, dd, J = 0.7, 1.35 Hz, Me-30), 2.3 (2H, m, H-2), 4.48−4.60 (2H, m, H-29), 4.52−4.73 (2H, m, H-23), 9.62 (1H, t, J = 2.05 Hz, −CHO) that was immediately dissolved in 1 mL of anhydrous THF and treated with 4.8 mL of a solution of 1 M $LiAlH_4$ in THF. The solution was kept 1.5 h at room temperature, then cooled at 0 °C. After addition of 4 mL of water, the mixture was filtered and the filtrate was evaporated to dryness. Purification by flash chromatography (hexane−Et_2O, 85:15; 300 mL) afforded 19 mg (yield 89%) of 3,4-*seco*-lupa-4(23),20(29)-dien-3-ol, which showed physical and spectroscopic characteristics identical to those of compound **1**.

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