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A SESQUITERPENE FROM *GARDENIA SOOTEPENSIS*

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Key Word Index—*Gardenia sootepensis*; Rubiaceae; sesquiterpene; guaiane; flavones; benzoic acid derivatives.

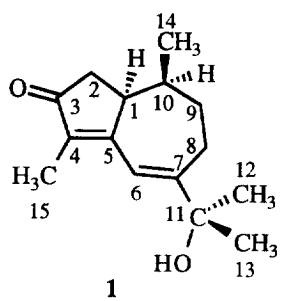
Abstract—From the twigs of *Gardenia sootepensis* a new sesquiterpene with a guaiane skeleton named sootepdienone was isolated, in addition to known flavones and benzoic acid derivatives.

INTRODUCTION

The genus *Gardenia* Ellis consists of more than 80 species spread among the tropical forests of certain regions of the world. Seventeen species of this genus occur in Thailand [1]. One of these is *G. sootepensis*, local name: Kam-mok-luang, which grows only in the northern part of Thailand. Although there are a number of reports on the isolation of triterpenes [2–6], iridoid glycosides [7–13], quinic acid derivatives [8, 14–15], a keto fatty acid [16] and flavones [6, 17–24] from many *Gardenia* species, the chemical constituents of *G. sootepensis* have not been extensively investigated. The flowers were found to contain β-sitosterol, a highly conjugated diterpene carboxylic acid, 7,4′-dihydroxyflavone and long-chain aliphatic compounds [6], while the fruits contain a quinic acid lactone (quinide) [25]. In an examination of twigs, we have now isolated a new sesquiterpene together with benzoic acid, 4-hydroxy-3-methoxybenzoic acid, 4-hydroxy-3,5-dimethoxybenzoic acid, 5,7,4′-trihydroxy-6-methoxyflavone [26–28] and 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone [29–31].

RESULTS AND DISCUSSION

A crude MeOH extract of the ground twigs of *G. sootepensis* was partitioned between 20% aq. NaOH and ether. The ether-soluble material was repeatedly chromatographed on silica gel to give compound **1**.



1

The alkali-soluble fraction was separated into phenolic and acidic fractions which were chromatographed on silica gel. The phenolic fraction yielded 5,7,4′-trihydroxy-6-methoxyflavone and 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone. The acidic fraction afforded benzoic acid, 4-hydroxy-3-methoxybenzoic acid and 4-hydroxy-3,5-dimethoxybenzoic acid.

Compound **1**, sootepdienone, was obtained as an optically active, colourless solid. Strong peaks at 3420 and 1680 cm^{-1} in the IR spectrum indicated the presence of hydroxyl and unsaturated carbonyl functionalities. The UV spectrum (λ_{max} 245, 300 nm) indicated an extended conjugated system. The HRMS and ^1H and ^{13}C NMR data (Table 1) established the molecular formula to be $C_{15}H_{22}O_2$. The ^1H NMR spectrum was assigned by decoupling and 2D ^1H–^1H COSY experiments and the ^{13}C NMR signals were assigned from DEPT, HMQC and GSHMBC spectra. The ^1H–^1H correlations enabled us to establish the following subunits.

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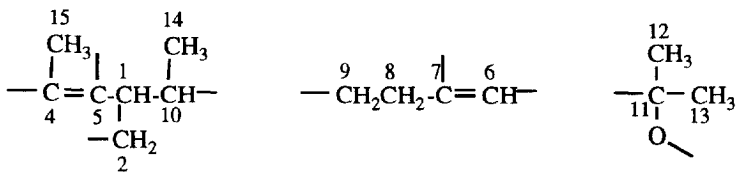

The ^{13}C NMR signals for quaternary carbons at δ_C 74.4 (C-11) and 209.3 (C-3) indicated the presence of a tertiary hydroxy group and an unsaturated keto function, respectively. An olefinic proton at δ_H 6.75 (*brd*, J = 1.7 Hz, H-6) in the ^1H NMR spectrum together with four signals of sp^2 carbons at δ_C 167.1 (C), 160.4 (C), 137.4 (C) and 118.2 (CH) confirmed the presence of trisubstituted and tetrasubstituted double bonds. The ^1H NMR and ^{13}C NMR spectra further showed the presence of four methyl groups [δ_H 0.71 (3H, *d*, J = 6.6 Hz, H-14; δ_C 14.5), 1.41 (6H, *s*, H-12 and H-13; δ_C 28.6 and 28.8) and 1.70 (3H, *d*, J = 1.8 Hz, H-15; δ_C 8.3], two methine groups [δ_H 3.33 (1H, *m*, H-1; δ_C 43.6) and 2.23 (1H, *m*, H-10; δ_C 32.9)] and three methylene groups [(δ_H 2.50, 1H, *dd*, J = 6.6 and 18.6 Hz, H-2α; δ_H 2.24, 1H, *dd*, J = 2.1 and 18.6 Hz, H-2β; δ_C 39.7), (δ_H 2.58, 1H, *m*; H-8α; δ_H 2.35, 1H, *ddd*, J = 2.7, 7.8 and 16.5 Hz, H-8β; δ_C 26.5) and (δ_H 2.15, 1H, *m*; H-9α; δ_H 1.48, 1H, *m*; H-9β; δ_C 35.1)].

The connections of the subunits were determined from GSHMBC data. The carbonyl carbon at δ_C 209.3 showed cross peaks with Me-15 and H-2 indicating the connection of the carbonyl carbon to C-2 and C-4. The $^3J_{C,H}$ connection between the sp^3 secondary carbon at δ_C 35.1 (C-9) and Me-14 revealed the link between C-9 and C-10. The quaternary hydroxylated carbon at δ_C 74.4 (C-11) showed correlations with Me-12, Me-13 and H-6 whereas the quaternary carbon at δ_C 160.4 (C-7) showed $^3J_{C,H}$ connection with Me-12 and Me-13, and H-9α and $^2J_{C,H}$ connection with H-8α, H-8β. Thus, the hydroxylated carbon C-11 carries two methyl groups and links with C-7 of the trisubstituted double bond. The relative stereochemistry of **1** was determined by ^1H NMR, NOE difference and NOESY spectra. Irradiation of the H-1 signal caused a strong enhancement of the H-10 signal (as well as H-2α and H-8α), and irradiation of Me-14 gave enhancements of H-2β and H-8β, suggesting that Me-14 was *trans* to H-1. H-1α had homoallylic coupling (J = 1.8 Hz) with Me-15 and weak $^4J_{H,H}$ coupling with H-6; H-8α (quasi-axial) had strong (J = 1.7 Hz) allylic coupling with H-6, whereas H-8β (quasi-equatorial) showed no coupling. These results are consistent with the assigned structure, 11-hydroxy-4,6-guaiadien-3-one. A number of hydroxyguaiadienones have been reported from natural sources, including three with the $\Delta^{4,5}$-guaian-3-one structure [32–34]. Nevertheless, sootepdienone represents a new member of the class.

EXPERIMENTAL

General

^1H NMR: 400 and 600 MHz, $CDCl_3$ solns with TMS as int. standard; ^{13}C NMR: 100 and 150 MHz. Multiplicities were determined by DEPT experiments. Known compounds were identified by comparison with their reported spectroscopic data, and the pos-

Table 1. ^1H NMR and ^{13}C NMR data of compound **1**

H	δ	J (Hz)	C	δ	DEPT
1	3.33 *m*		1	43.6	CH
2α	2.50 *dd*	18.6, 6.6	2	39.7	CH_2
2β	2.24 *dd*	18.6, 2.1	3	209.3	C
6	6.75 *brd*	1.7	4	137.4	C
8α	2.58 *m*		5	167.1	C
8β	2.35 *ddd*	16.5, 7.8, 2.7	6	118.2	CH
9α	2.15 *m*		7	160.4	C
9β	1.48 *m*		8	26.5	CH_2
10	2.23 *m*		9	35.1	CH_2
12	1.41 *s*		10	32.9	CH
13	1.41 *s*		11	74.4	C
14	0.71 *d*	6.6	12	28.6	CH_3
15	1.70 *d*	1.8	13	28.8	CH_3
			14	14.5	CH_3
			15	8.3	CH_3

ition of OMe groups confirmed by NOE difference spectra.

Plant material

Twigs of *Gardenia sootepensis* were collected from Doi Sootep, Chiang Mai, Thailand. The voucher specimen (Max 93-1139) has been deposited at the herbarium of the Department of Biology, Faculty of Science, Chiang Mai University.

Extraction and isolation

The ground dried twigs (1.45 kg) were consecutively extracted with hexane, CH_2Cl_2 and MeOH. The concentrated MeOH extract (2 g) was partitioned between 20% aq. NaOH and Et_2O. The Et_2O-soluble fraction was evaporated to dryness to give a yellow viscous oil, which was fractioned by quick CC using gradient elution: CH_2Cl_2–hexane, EtOAc–CH_2Cl_2 and MeOH–EtOAc. Frs 4 and 5, upon prep. TLC using EtOAc–CH_2Cl_2 (1:9), afforded compound **1**. The aq. layer was neutralized with 20% aq. HCl and further extracted with EtOAc (3 × 100 ml). The combined organic extracts were concd to dryness *in vacuo* to afford a residue (331 mg) which was chromatographed on a column of silica gel and eluted with CH_2Cl_2–hexane, EtOAc–CH_2Cl_2 and MeOH–EtOAc. The third eluate (38 mg) was then subjected to prep. TLC on silica gel using MeOH–CH_2Cl_2 (1:19) to give two bands. After eluting with EtOAc, 5,7,4′-trihydroxy-6-methoxyflavone (7 mg) was obtained from the lower band. The upper band afforded 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone (10 mg). The neutral aq. layer was further acidified with 20% aq. HCl and then extracted with EtOAc. Upon evapn, the crude extract was obtained as a brown viscous oil. The oil was partially soluble in Et_2O. Chromatographic sepn of the Et_2O-soluble fraction afforded benzoic acid, 4-hydroxy-3-methoxybenzoic acid and 4-hydroxy-3,5-dimethoxybenzoic acid.

Compound **1**

Colourless solid, mp 47–50°. $[\alpha]_D^{20}$ +12° (*c*. 0.1 $CHCl_3$). IR ($CHCl_3$) ν_{max} 3240, 1680 cm^{-1}; UV λ_{max} EtOH nm (ε) 245 (5900), 296 (10,500); ^1H NMR and ^{13}C NMR ($CDCl_3$): Table 1; MS, *m/z* (rel. int.): 234 [M^+] (11.5), 219 (13.1), 216 (26.2), 173 (15.5), 43 (100): HRMS Calc. for $C_{15}H_{22}O_2$ 234.1620; Found: 234.1612.

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