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CONSTITUENTS OF *GRANGEA MADERASPATANA*. A NEW EUDESMANOLIDE[1]

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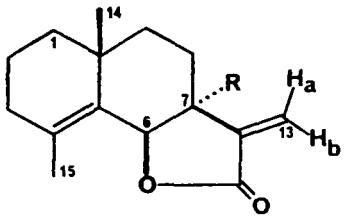
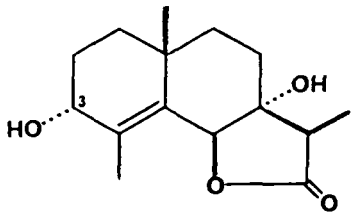
ABSTRACT.—Three components have been isolated from the whole plant of *Grangea maderaspatana*, and their structures have been determined by spectroscopic analysis. Component **1** is the known allergenic eudesmanolide (−)-frullanolide, compound **2** is the recently reported (−)-7α-hydroxyfrullanolide, and the most polar component is a new eudesmanolide (+)-11α,13-dihydro-3α,7α-dihydroxyfrullanolide [**3**], which we have named (+)-grangolide. A crude $CHCl_3$ extract of *G. maderaspatana* exhibits strong cytotoxic activity.

Grangea Adanson, in the subtribe Grangeinae of the tribe Astereae (Compositae), is a genus of suberect or prostate annual herbs (2). Fourteen species of *Grangea* are found in tropical and subtropical Asia and Africa (3,4). *Grangea maderaspatana* Poir., known locally as "Phayaa-mutti," is the only species found widely and used medicinally throughout Thailand (4). This species is utilized in many countries for medicinal purposes. The leaves are used as a stomachic, a sedative, a carminative, an emmenagogue, and an antiflatulent (5). The plant is claimed to facilitate the return of menses after parturition if the delay is accompanied by abdominal and kidney pain (6). In Thailand, the whole plant has been used in folkloric medicine as a bitter tonic or a carminative and for treatment of flatulence and diarrhea (7). Previous reports have indicated the presence of the following compounds in this species: the diterpenes (−)-hardwickiic acid, *ent*-2β-hydroxy-15,16-epoxy-3,13(16),14-clerodatriene-18-oic acid, and strictic acid, the steroids chondrillaterol and chondrillasterone, and the polyacetylene 3-hydroxy-8-acetoxypentadeca-1,9,14-trien-4,6-diyne (8–10). Herein we report the structure elucidation of three sesquiterpene lactones including a new eudesmanolide isolated from *G. maderaspatana*.

The extraction and isolation of three components from the whole plant of *G. maderaspatana* are described in the Experimental, and the structure determination of these compounds will be discussed in the order in which they were eluted from a Si gel column. Compound **1** was a colorless solid which exhibited in its eims a parent ion at *m/z* 232 ($C_{15}H_{20}O_2$) and a base peak at 217, $[M-Me]^+$. An ir absorption at 1767 cm^{-1}



1 R=H
2 R=OH



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[1]Part X in the series of "Studies on Thai Medicinal Plants." For Part IX, see Ruangrungsi *et al.* (1).

TABLE 1. ^{1}H-Nmr Spectra of **1**, **2**, and **3**.[a]

Proton	Compound		
	1	**2**	**3**
1α	1.35 td (13.2,3.3)	1.40 m	1.69 t (14.0)
1β	1.43 dt (13.2,3.5)	1.45 m	1.77 br d (14.0)
2α	1.62 m	1.65 m	2.0 m
2β	1.82 m	1.83 m	2.0 m
3α	2.09 br d	2.12 m	—
3β	2.11 m	2.12 m	4.00 t (3.0)
6	5.27 d (5.9)	5.00 s	4.89 s
7	2.95 dt (10.0,5.9)	—	—
8α	1.70 m	1.68 m	1.36 br d (13.0)
8β	1.65 m	1.99 td (13.2,3.6)	1.54 t (13.0)
9α	1.27 dd (13.2,12.3,4.4)	1.49 td (13.2,3.1)	1.52 m
9β	1.48 dt (13.2,3.9)	1.68 m	1.72 m
11	—	—	2.79 q (7.2)
13a	5.58 d (1.0)	5.81 s	1.21 3H d (7.2)
13b	6.16 d (1.0)	6.27 s	—
14	1.08 s	1.09 s	1.04 s
15	1.76 s	1.78 s	1.96 s
OH	—	2.30 br s	2.65 br s

[a]Coupling constants are in parentheses in Hertz.

suggested the presence of an α-methylene-γ-lactone moiety, and this was confirmed by the ^{1}H-nmr spectrum of the substance. The latter spectrum was the same as that reported previously for (−)-frullanolide (11) and established that **1** is this known eudesmanolide. In Table 1 we report the 400 MHz ^{1}H-nmr spectrum of **1** [previous spectra were recorded at 60 MHz (11)] for comparison with the related components **2** and **3**. The ^{13}C spectrum of **1** has not been reported previously and is recorded in Table 2. Frullanolide is the allergenic component of a liverwort (12), and its levorotatory enantiomer has been isolated previously from *Frullania tamarisci* while its dextrorotatory

TABLE 2. ^{13}C-Nmr Spectra of **1**, **2**, and **3**.[a]

Carbon	Compound		
	1	**2**	**3**
1	39.1 (+)	38.8 (+)	35.6 (+)
2	18.2 (+)	18.2 (+)	25.2 (+)
3	33.1 (+)	33.1 (+)	69.3 (−)
4	138.5 (+)	140.5 (+)	140.5 (+)
5	128.5 (+)	126.8 (+)	131.5 (+)
6	75.9 (−)	81.4 (−)	80.5 (−)
7	41.2 (−)	76.0 (+)	77.3 (+)
8	25.0 (+)	31.5 (+)	28.5 (+)
9	37.9 (+)	34.9 (+)	34.9 (+)
10	32.6 (+)	32.7 (+)	31.6 (+)
11	142.3 (+)	144.7 (+)	48.6 (−)
12	170.9 (+)	169.1 (+)	177.0 (+)
13	120.1 (+)	121.0 (+)	7.3 (−)
14	25.8 (−)	26.1 (−)	24.2 (−)
15	19.3 (−)	19.4 (−)	17.7 (−)

[a]Chemical shifts are in ppm from TMS; solvent for **1** and **2** was $CDCl_3$; solvent for **3** was Me_2CO-d_6.

form has been reported to be present in *Frullania dilatata* (11). The structure of **1** has been confirmed by synthesis (13).

The second component was a solid whose elemental analysis and eims (parent peak at *m/z* 248) were consistent with the molecular formula $C_{15}H_{20}O_3$. The ir spectrum suggested the presence of an α-methylene-γ-lactone (1772, 1655) and a hydroxyl group (3620 cm^{-1}). The ^1H-nmr spectrum of **2** (Table 1) was similar to that of **1** and suggested the compound possessed a eudesmanolide carbon skeleton. A singlet for the H-6 resonance established that the hydroxyl group must be attached to C-7 and suggested that **2** was (−)-7α-hydroxyfrullanolide. The base peak at *m/z* 233 in the eims of **2** indicated loss of a methyl group with formation of the stabilized tertiary allylic fragment shown in Figure 1. The same type of fragment also was produced from **1** (*m/z*

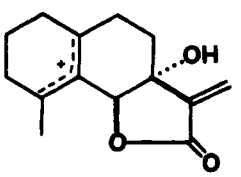

FIGURE 1. Structure of the base peak at *m/z* 233 from eims of **2**.

217) and appears to be diagnostic of the $\Delta^{4,5}$-unsaturated eudesmanes. After we had completed our investigation of **2**, a paper at a conference described the isolation of the same compound from *Sphaeranthus indicus* (14). A recent publication by another group also reported the isolation of **2** from *S. indicus* and confirmed the structure by an X-ray crystallographic study of a derivative (15). These latter investigators reported **2** was an oil, while we have isolated it in crystalline form and our optical rotation is somewhat higher than theirs. The similarity of the ^{13}C-nmr resonances and other spectral data leaves no doubt that the compounds are identical, but as our ^{13}C assignments differ substantially from those reported previously (15), our assignments are presented in Table 2. Comparisons with the ^{13}C spectra of **1** and **3** were of considerable assistance in making these assignments.

The third and most polar component was a higher melting solid whose accurate mass was consistent with the molecular formula $C_{15}H_{22}O_4$. In the eims of **3**, a fragment at *m/z* 251, $[M-Me]^+$, suggested this component was also a $\Delta^{4,5}$-eudesmane (see discussion of **2**) and the *m/z* 248 fragment, $[M-H_2O]^+$, indicated the compound underwent a facile dehydration reaction. In the ir spectrum of **3**, the absorption at 1771 cm^{-1} indicated a γ-lactone function, but the absence of any absorption between 3000–3100 cm^{-1} suggested an α-methyl rather than an α-methylene group was present. Also, the stronger absorption above 3600 cm^{-1} as compared with **2** indicated the additional oxygen atom in **3** was present as a second hydroxyl group. The absorption at 1007 cm^{-1} suggested this hydroxyl group was secondary and allylic (calculated value about 1010 cm^{-1}) (16); thus, it was placed on C-3. The ^1H- and ^{13}C-spectra of **3** (Tables 1 and 2, respectively) confirmed the suggestions above. In particular, the 3-proton doublet at 1.21 in the ^1H spectrum established the presence of the C-13 methyl group, and the triplet at 4.00 ppm was assigned to the carbinyl proton at C-3. With regard to the ^{13}C spectrum, it was established previously (17) that a hydroxyl group produced a pronounced downfield shift (about 40 ppm) of the carbon to which it is attached (α effect), a small downfield shift (5–10 ppm) of the β carbon, and an upfield shift (a few ppm) of the γ carbon. In the ^{13}C spectrum of **3** (Table 2) the C-3 resonance is shifted downfield by 36.2 ppm relative to C-3 in **2**, C-2 (the β carbon) is shifted downfield by 7.0 ppm, and C-1 (the γ carbon) is shifted upfield by 3.2 ppm. Thus, these assign-

ments confirm the placement of the second hydroxyl group in **3** at C-3 and also indicate the ^{13}C assignments for **1**, **2**, and **3** are internally consistent. Finally, it was necessary to establish the stereochemistry of the 13-Me group and the 3-OH function (assuming that the stereochemistry of all other substituents is the same as in **2**). A detailed ^{13}C-nmr study of eudesmanolides showed that a β-methyl group at C-13 in a *cis*-fused γ lactone resonated at 9.6 while an α-methyl group in the same moiety appeared at 14.9 ppm (18). In **3**, the 13-Me appears at 7.3 ppm, which strongly suggests it is in a β configuration, and the additional upfield shift of 2.3 ppm (7.3 vs. 9.6) is caused by the γ effect of the 7-hydroxy substituent. The 3-OH group was assigned the α configuration and the carbinyl proton the β position (pseudo-equatorial) because of the equal couplings (triplet, $J = 3$ Hz) of the latter with the H-2 protons. The chemical shift of H-3 (4.00 ppm) and its coupling constants are very similar to those reported for two eudesmanolides in which the 3-OH function was also assigned the α configuration (19,20). Thus, component **3** is a previously unreported eudesmanolide that we have given the name (+)-grangolide.

In this report we have described the structure determination of three eudesmanolides isolated from the whole plant of *G. maderaspatana*: the allergenic compound (−)-frullanolide [**1**], the very recently reported derivative **2** of frullanolide with a hydroxyl function at the unusual 7-position and the previously unreported diol **3** which we have named (+)-grangolide. It should be noted that a crude $CHCl_3$ extract of this species exhibited strong cytotoxic activity ($ED_{50} = 2$ μg/ml) in the KB cell culture as determined by NCI (USA) protocols (21).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^1H- and ^{13}C-nmr spectra were recorded on a Bruker WH-400 spectrometer with TMS ($\delta = 0$) as internal standard and $CDCl_3$ as solvent except where noted. The multiplicities for the ^{13}C spectra were determined by the attached proton test which produced positive (+) quaternary C and CH_2 signals and negative (−) CH and CH_3 signals. Ir spectra were obtained on a Nicolet Model 20 SX/C Ft-ir spectrometer as solutions and mass spectra on a VG Micromass 7070F or a ZAB-E spectrometer. Optical rotations were performed on a Jasco DIP 360 polarimeter in $CHCl_3$ solutions.

PLANT MATERIAL.—The whole plant of *G. maderaspatana* was collected from Suphan Buri province, Thailand, in October 1986. The plant material was authenticated by comparison with the herbarium specimen at the Botany Section, Technical Division, Ministry of Agriculture and Cooperative, Bangkok. A voucher specimen of plant material was deposited in the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok.

EXTRACTION AND ISOLATION.—Dried, powdered plant material (2 kg) from *G. maderaspatana* was macerated twice with EtOH (2 × 5 liters) for 3-day periods and then filtered. The combined filtrate was evaporated in vacuo to give a syrupy mass (250 g). This residue was suspended in H_2O (150 ml), extracted with $CHCl_3$ (6 × 300 ml), and the combined extract was dried (anhydrous Na_2SO_4) and the solvent removed to yield 45 g of a crude $CHCl_3$ extract. A portion of this extract (3 g) was chromatographed on a Si gel column (5 × 5 cm) using $CHCl_3$-Me_2CO (5:1) as eluent and collecting 20-ml fractions. Fractions 3–4 gave 24 mg of **1**, fractions 7–11 afforded 155 mg of **2**, and fractions 21–28 yielded 117 mg of **3**.

(−)-FRULLANOLIDE [**1**].—Mp 70–72° [lit. (12) mp 77°]; $[\alpha]^{24}D$ −110° ($CHCl_3$) [lit. (12) $[\alpha]D$ −113° ($CHCl_3$)]; ir ν max (CCl_4) 1767, 1264, 1142, 940 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (rel. int.) $[M]^+$ 232 (16), 217 (100), 171 (21).

(−)-7α-HYDROXYFRULLANOLIDE [**2**].—Mp 69–71° [lit. (14) 59–60°, (15) oil]; $[\alpha]^{24}D$ −76° ($c = 1.09$, $CHCl_3$) [lit. (15) $[\alpha]^{24}D$ −57° ($c = 0.43$, $CHCl_3$)]; ir ν max (CCl_4) 3620, 3010, 2933, 1772, 1655, 1142, 956 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (rel. int.) $[M]^+$ 248 (19), 233 (100), 230 (8), 178 (18). Calcd for $C_{15}H_{20}O_3$, C 72.55, H 8.12; found C 72.33, H 8.30.

(+)-11α,13-DIHYDRO-3α,7α-DIHYDROXYFRULLANOLIDE [(+)-GRANGOLIDE] [**3**].—Mp 135–139°; $[\alpha]^{24}D$ +12° ($c = 2.76$, $CHCl_3$); ir ν max ($CHCl_3$) 3620, 2940, 1771, 1007, 970 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (rel. int.) $[M]^+$ 266 (58), 251 (27), 248 (53), 164 (76), 123 (100); hrms *m/z* $[M]^+$ 266.1516 (calcd for $C_{15}H_{22}O_4$, 266.1518).

CYTOTOXICITY ASSAY.—The KB cell culture assay was performed at the National Cancer Institute in Bangkok using a previously reported protocol (21). The crude $CHCl_3$ extract from *G. maderaspatana* exhibited strong cytotoxic activity ($ED_{50} = 2$ μg/ml) in this assay based on the NCI (USA) standards (synthetics, $ED_{50} \leqq 4$ μg/ml; plant and animal extracts, $ED_{50} \leqq 20$ μg/ml).

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