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A HEMOLYTIC SAPONIN, RANDIANIN, FROM *RANDIA DUMETORUM*

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Key Word Index—*Randia dumetorum*; Rubiaceae; triterpene diglucoside; randianin; hemolysis; acidic saponin.

Abstract—Randianin, a new triterpene diglucoside, was isolated from the methanolic extracts of the fruits of *Randia dumetorum*. Its structure has been established as β-D-glucopyranosyl (1→3)-β-D-glucopyranosyl(1→3)-3β-hydroxyolean-12-en-28-oic acid on the basis of hydrolysis and spectral evidence including COSY NMR and NOE studies.

INTRODUCTION

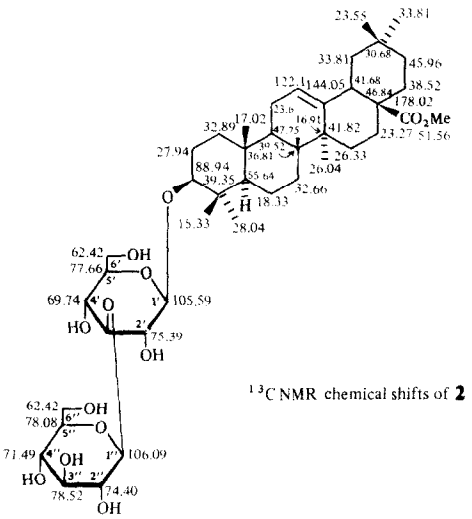
The fruits of the tropical plant *Randia dumetorum* Lam. (Rubiaceae) are used in the traditional system of medicine in Asia. Though several saponins have been isolated from this plant [1–3], structures of most of the saponins are yet to be elucidated [1, 3]. The structure of one of the saponins has been determined [2] as β-D-galactopyranosyl (1→3) oleanolic acid (randioside A) based on hydrolysis and permethylation studies. We now report another saponin, randianin, from the fruit extracts of *Randia dumetorum*, the structure of which has been shown to be β-D-glucopyranosyl (1→3)-β-D-glucopyranosyl (1→3)-3β-hydroxyolean-12-en-28-oic acid (**1**) on the basis of hydrolytic and spectral evidence including COSY NMR and NOE studies.

RESULTS AND DISCUSSION

Randianin (**1**), $C_{42}H_{68}O_{13}$, was obtained as an amorphous powder by methanol extraction of the fruits of *Randia dumetorum*, subsequent partitioning with *n*-butanol–water and final purification by passage through a column of reverse phase silica gel using methanol–water (17:3) as the eluent.

The IR spectrum of randianin exhibited hydroxyl absorption bands. The ^{1}H NMR spectrum of randianin showed the presence of 14 sugar CH–O– protons at δ3–4 ppm. The ^{13}C NMR spectrum of randianin indicated the presence of the following signals for sugar carbon atoms: δ106.28 (CH), 105.89 (CH), 88.91 (CH), 78.63 (CH), 78.19 (CH), 77.85 (CH), 75.45 (CH), 74.38 (CH), 71.38 (CH), 69.81 (CH), 62.53 (CH_2) and 62.43 (CH_2) ppm. The presence of two sugar molecules in randianin could be discerned from (i) two signals for anomeric carbons at δ106.28 and 105.89 ppm and (ii) two signals for CH_2OH at δ62.53 and 62.43 ppm. The ^{13}C NMR spectrum of randianin in pyridine-d_5 also showed signals at δ180.13 (q.c, $-CO_2H$), 144.82 (q.c., [–C =) and 123.11 (CH, =CH) ppm. The ^{1}H NMR data also showed the presence of seven Me groups. These data indicated randianin to be an acidic saponin and to be a triterpenoid diglycoside. Randianin hemolysed red blood cells when tested on a blood coated agar plate. It also gave a positive froth test. Randianin reacted readily with diazomethane giving a methyl ester **2**. The ^{13}C NMR data of **2** which are given in Fig. 1 showed that the aglycone portion of randianin is oleanolic acid.

Acetylation of **2** gave an acetate (**3**) which had seven acetoxy signals in its ^{1}H NMR spectrum. This showed that the two sugar molecules in randianin were linked together. This observation was further confirmed by the mass spectral fragmentations of **1** which showed a prominent fragment ion at *m/z* 325 (**4**). The mass spectrum of **1**



^{13}C NMR chemical shifts of **2**

Fig. 1.

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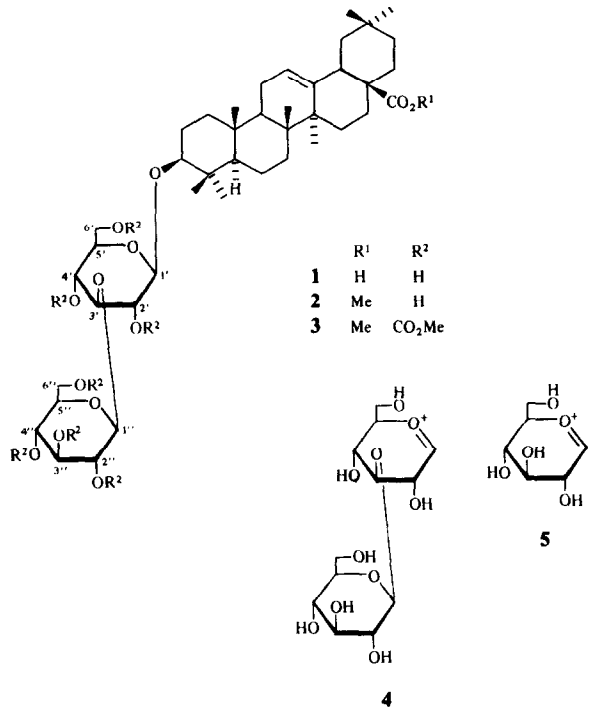

also showed an intense fragment ion at m/z 163 (**5**). The presence of the base peak at m/z 203 and the prominent peak at m/z 248, which arose by the retro-Diels–Alder fragmentation of the ring C of the aglycone molecule, confirmed the Δ^{12} double bond in randianin. Similar fragmentations were also observed in the mass spectrum of **2**.

Randianin on complete hydrolysis using methanol–hydrochloric acid yielded oleanolic acid and glucose. The sugar linkage has been confirmed to be 1″,3′ from the COSY NMR and NOE spectra of the acetate **3**. The chemical shifts of the sugar protons and their coupling constants are given in Table 1. A complete assignment of the sugar protons was possible by a study of the COSY NMR spectrum of **3**. The well separated anomeric hydrogens ($\delta = 4.34$, 1′ and $\delta = 4.54$, 1″) and the 2′-H ($\delta = 4.97$) and 2″-H($\delta = 4.81$) protons were assigned without difficulty. A vicinal coupling constant of 8 Hz between these protons, in each case, proved the configuration at the anomeric centres to be β. The resonances of protons 3″ and 4″ ($\delta = 5.08$ and 5.00) were very close, but identification was possible by the cross peak showing coupling between 5″-H, which is well separated, and 4″-H. The chemical shift of 3′ ($\delta = 3.82$) is typical for protons at the sugar linkage [4]. This proton could be assinged also by the COSY NMR spectrum. The result was confirmed by the NOE-difference experiment [5]. Both the protons, 1″-H and 3′-H, showed dipolar interaction, which is only possible for a 1″-3′ β-glycosidic bond. The disaccharide link with the aglycone was established by an NOE experiment.

Saturation of 1′-H led to an enhancement of 3-H of the aglycone. The disaccharide must, therefore, be connected to the 3-OH of oleanolic acid. Randianin is thus a new saponin and has been identified as β-D-glucopyranosyl (1→3)-β-D-glucopyranosyl (1→3)-3β-hydroxyolean-12-en-28-oic acid (**1**).

Table 1. Chemical shifts of sugar protons from the COSY NMR and NOE studies on **3**

Proton	δ (ppm)	Coupling constants (J) (Hz)
1′	4.34	$^3J_{1'/2'} = 8$
2′	4.97	$^3J_{1'/2'} = 8$, $^3J_{2'/3'} = 8$
3′	3.82	$^3J_{3'/4'} = 9$, $^3J_{2'/3'} = 8$
4′	4.83	$^3J_{3'/4'} = 9$, $^3J_{4'/5'} = 9$
5′	3.60	$^3J_{5'/6'a} = 5$, $^3J_{4'/5'} = 9$ $^3J_{5'/6'b} = 2$
6′a	4.12	$^3J_{5'/6'a} = 5$, $^2J_{6'a/6'b} = 13$
6′b	4.08	$^3J_{5'/6'b} = 2$, $^2J_{6'a/6'b} = 13$
1″	4.54	$^3J_{1''/2''} = 8$
2″	4.81	$^3J_{1''/2''} = 8$, $^3J_{2''/3''} = 8$
3″	5.08	$^3J_{3''/4''} = 9$, $^3J_{2''/3''} = 8$
4″	5.00	$^3J_{3''/4''} = 9$, $^3J_{4''/5''} = 9$
5″	3.62	$^3J_{5''/6''a} = 4$, $^3J_{4''/5''} = 9$ $^3J_{5''/6''b} = 2$
6″a	4.12	$^3J_{6''a/5''} = 4$, $^2J_{6''a/6''b} = 13$
6″b	3.99	$^3J_{6''b/5''} = 2$, $^2J_{6''a/6''b} = 13$

EXPERIMENTAL

The fruits of *Randia dumetorum* were collected in Sri Lanka for this investigation. The dried fruits (500 g) were defatted with *n*-hexane. The MeOH extract was suspended in 120 ml of H_2O and was extracted with *n*-BuOH (3 × 120 ml). After evapn, the *n*-BuOH extract gave 9 g of a brown solid. The *n*-BuOH extract was sepd on a column of silica gel (*ca* 350 g) which was packed in CH_2Cl_2–MeOH–H_2O (20:3:1; lower layer) and the column was eluted with CH_2Cl_2–MeOH–H_2O (13:7:2, lower layer). Several fractions were collected which gave a complex TLC pattern. One of the fractions (0.75 g) appeared to contain an acidic saponin which had physical properties different to those reported previously. This was investigated further. A portion of this fraction (0.144 g) was purified by a column of reverse phase silica gel (30 g) using MeOH–H_2O (7:3) as the eluent. A pure saponin, randianin, was obtained in a yield of 44 mg (overall 0.05%).

Mps: uncorr. Optical rotations were measured at 25°. ^{1}H and ^{13}C NMR spectra were recorded at 250 MHz, TMS as int. standard. The hemolysis test was conducted as described previously [6]. The COSY NMR spectrum was recorded using the standard Bruker microprogram. Processing of the 2D time domain spectrum was carried out using standard Bruker software. 256 FID's were accumulated with a time-increment in the evolution period of 0.714 msec according to a sweepwidth of ±700.28 Hz. The data matrix of 512 * 256 computer words were transformed after zerofilling to 2048 computer words in F2 and 1024 in F1. A sine-bell function without shifting was used as window function in both domains.

The NOE spectra were measured with the 'frequency cycling' method, again a standard Bruker program was used. Every multiplet line was irradiated for 200 msec, this was done 20 times. Before transformation, a line broadening of 2 Hz was applied to the FID's.

Randianin (**1**). An amorphous powder mp 290–295° (with decomposition); $[\alpha]^{25}$ +0.22° [MeOH; *c* 0.036]. IR ν_{max} cm^{-1} 3300; FDMS m/z 803 $[M^+ + Na]^+$ (100%); EIMS m/z M^+ not recorded, 456 (0.01%), 325 (0.01%), 248 (90), 203 (100), 163 (20).

^{1}H NMR (CD_3OD) δ3.2–3.8 (14H, *m*, sugar protons), 1.4–1.85 (CH_2 envelope), 0.8–1.3 (18H, 5*s*, Me).

Methylation of randianin. A solution of randianin (**1**, 20 mg) in MeOH was treated with CH_2N_2 at room temp. The product was isolated and was purified by prep TLC [silica gel, MeOH–$CHCl_3$ (v/v 17:100)] to give pure methyl ester of randianin **2**, mp 200–202°. ^{1}H NMR (pyridine-d_5) δ4.89 (2H, *d*, C-1′ and C-1″), 4.5 (2H, *m*, C-6′), 3.8–4.3 (8H, *m*, sugar protons), 3.69 (3H, *s*, $-CO_2Me$), 3.35 (1H, *m*), 3.05 (1H, *m*), 1.2–2.2 (CH_2 envelope), 1.30 (3H, *s*, Me), 1.23 (3H, *s*, Me), 0.99 (3H, *s*, Me), 0.92 (3H, *s*, Me), 0.91 (3H, *s*, Me), 0.82 (3H, *s*, Me), 0.80 (3H, *s*, Me); ^{13}C NMR data see Fig. 1; EIMS *m/z* M^+ was not recorded, 325 (0.01%), 262 (53), 203 (100), 163 (8).

Acetylation of compound 2. **2** (20 mg) was dissolved in pyridine (1 ml) and acetic anhydride (1 ml). The mixture was heated in an oil bath at 70° for 6 hr. The product was worked-up in the usual manner and was purified by prep TLC (silica gel, $CHCl_3$) to yield 20 mg of pure acetate **3**, mp 145. ^{1}H NMR ($CDCl_3$) δ5.28 (1H, *t*, $J = 6.6$ and 7.3 Hz, C-12), sugar protons and coupling constants (See Table 1), 3.62 (3H, *s*, Me), 3.03 (1H, *m*, C-3), 2.13 (3H, *s*, –OAc), 2.08 (3H, *s*, OAc), 2.06 (3H, *s*, OAc), 2.02 (3H, *s*, OAc), 2.01 (6H, *s*, 2 × –OAc), 1.90 (3H, *s*, OAc), 1.11 (3H, *s*, Me), 0.92 (3H, *s*, Me), 0.89 (9H, *s*, 3 × Me), 0.72 (3H, *s*, Me), 0.71 (3H, *s*, Me).

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A CHROMANE DERIVATIVE RELATED TO STELLATIN, AND AN α-PYRONE DERIVATIVE FROM *EMERICELLA HETEROTHALLICA**

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Key Word Index—*Emericella heterothallica*; Eurotiaceae; chromane; emehetin; α-pyrone; stellatin; 6-ethyl-4-hydroxy-3,5-dimethyl-2-pyrone.

Abstract—Emehetin, a novel chromane derivative related to stellatin, and 6-ethyl-4-hydroxy-3,5-dimethyl-2-pyrone isolated along with emeheterone and stellatin from the culture filtrate of the fungus *Emericella heterothallica*. Their molecular structures were investigated by spectroscopic means and chemical correlations.

INTRODUCTION

In the previous paper [2], we reported the isolation of a new pyrazinone derivative designated emeheterone (**1**) and a dihydroisocoumarin, stellatin (**2**), from the dichloromethane extract of the culture filtrate of *Emericella heterothallica* (Kwon, Fennell & Raper) Malloch and Cain (mating type a), strain ATCC 16824. Further investigation of this extract led us to isolate a novel chromane derivative, emehetin (**3**), and a new α-pyrone derivative (**4**).

* Part 27 in the series 'Studies on Fungal Products'. For Part 26 see ref. [1].

RESULTS AND DISCUSSION

Emehetin (**3**), mp 109–110°, gave a molecular ion at *m/z* 252 in EIMS, and its elemental analysis confirmed the molecular formula $C_{13}H_{16}O_5$. The ^{1}H NMR signals at δ3.86 (3H), 6.12 (1H), 10.24 (1H), and 12.39 (1H) were assigned to a methoxy group, an aromatic proton, an aldehyde, and a hydroxy group, respectively, in the benzene ring. The partial structure CH_2CH_2O in **3** was confirmed from the decoupling experiments for the four protons at δ2.56, 2.99, 3.83, and 4.23. Emehetin (**3**) was easily oxidized with manganese dioxide to give compound **5**, which was identified with the compound derived from stellatin (**2**) by oxidation with manganese dioxide. The structure of **5** was determined by its ^{1}H NMR