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NOVEL ACYLATED SAPONINS, CROCOSMIOSIDES A AND B, FROM MONTBRETIA (*CROCOSMIA CROCOSMIIFLORA*)

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Two novel triterpenoid saponins, named crocosmiosides A (**1**) and B (**2**) were isolated from montbretia, and their structures were elucidated on the basis of spectral and chemical evidence. They are the first examples of saponins containing hydroxylated palmitic acid derivatives as acyl moieties.

KEYWORDS——*Crocosmia crocosmiiflora*; Montbretia; Iridaceae; crocosmioside A; crocosmioside B; acylated saponin; ^{13}C-NMR

Montbretia (*Crocosmia crocosmiiflora* N.E.Br., Iridaceae) is widely cultivated as garden plant. Hot water extract of its corms has recently been found to have antitumor activities. We have already isolated two new flavonol glycosides from the methanolic extract of the corms of montbretia.[1] The present paper deal with the strusture determination of two novel saponins, named crocosmiosides A (**1**) and B (**2**).

Crocosmiosides A (**1**), a white powder, $C_{90}H_{150}O_{43}\cdot 4H_2O$, $[\alpha]_D$ -33.6°(MeOH), revealed a molecular ion peak at *m/z* 1917[M-H]$^-$ in the negative FAB-MS spectrum. The IR spectrum showed absorption at 3420 cm^{-1} (OH), 1735 cm^{-1} (ester) and the ^{13}C-NMR spectrum showed eight anomeric carbon signals (Table I) and two ester carbon signals at δ174.7 and 177.7. Also, methylene carbon signals due to carboxylic acid were observed at δ20-40.

The negative FAB-MS spectrum of crocosmioside B (**2**), a white powder, $C_{84}H_{140}O_{39}\cdot 6H_2O$, $[\alpha]_D$ -31.2°(MeOH) revealed a moleculer ion peak at *m/z* 1771 [M-H]$^-$. The ^{1}H and ^{13}C-NMR spectra of **2** were similar to those of **1** except for the lack of signals due to a rhamnosyl moiety. Results of methylation analysis of **1** and **2** by GC-MS supported the above spectral evidence.

Acidic hydrolysis of **1** yielded polygalacic acid (**3**)[2,3] and component sugars. On hydrolysis with 0.5% $NaHCO_3$, **1** afforded a carboxylic acid glycoside (**4**), $[\alpha]_D$ -46.6°(pyridine), MS *m/z* 605[M+Na]$^+$and desacylcrocosmioside A (**5**). The ^{13}C-NMR spectrum of **4** showed signals due to rhamnoside and xyloside moieties and signals at δ68.8(t), 72.6(d), 79.6(d) which suggested the presence of three OH groups in hexadecanoic acid. The positions of the OH groups were determined to be C-2, -9, and -16 by the ^{1}H and ^{13}C-NMR spectra of **4** and the MS fragmentation of methyl ester trimethylsilyl ether (**6**) derived from **4** (Fig. 1). From the

results of emzymatic hydrolysis of **1** with naringinase which afforded **2** and rhamnose[4] and the ^{1}H-^{13}C-2D-NMR data of **1**, it was suggested that the xyloside and rhamnoside moieties were attached to the 2 and 16 OH groups in the carboxylic acid, respectively. The ^{13}C-NMR spectrum of **5**, $[\alpha]_D$ -31.8°(MeOH), showed six anomeric carbon signals. Signals at δ177.5 and the IR absorption at 1735 cm^{-1} suggested the presence of ester type glycoside-linkage. Treatment of **5** with anhydrous LiI, 2,6-lutidine and anhydrous methanol,[5] afforded a hydrolysate (**7**) and a methyl glycoside (**8**). The positive FAB-MS spectrum of **7**, $[\alpha]_D$ +12.9°(MeOH), revealed a pseudo-molecular ion peak at *m/z* 821[M+Na]$^+$. In the ^{13}C-NMR spectrum of **7**, signals due to the glucoside and arabinoside moieties were observed, with glycosylation shifts at C-3 carbon of sapogenin moiety (δ84.3, downfield shift of 10.4 ppm compared with that of **3**) and at C-6 methylene carbon of the glucoside moiety (δ70.1). Thus, **7** was regarded as polygalacic acid 3-*O*-α-arabinopyranosyl(1→6)-β-glucopuranoside. Methyl glycoside (**8**), an α and β anomeric mixture at the methyl glycoside linkage, revealed two methoxyl and seven anomeric proton (carbon) signals in the $^{1}H(^{13}C)$-NMR spectra. The anomeric ratio was determined by intensities of methoxyl signals in the ^{1}H-NMR

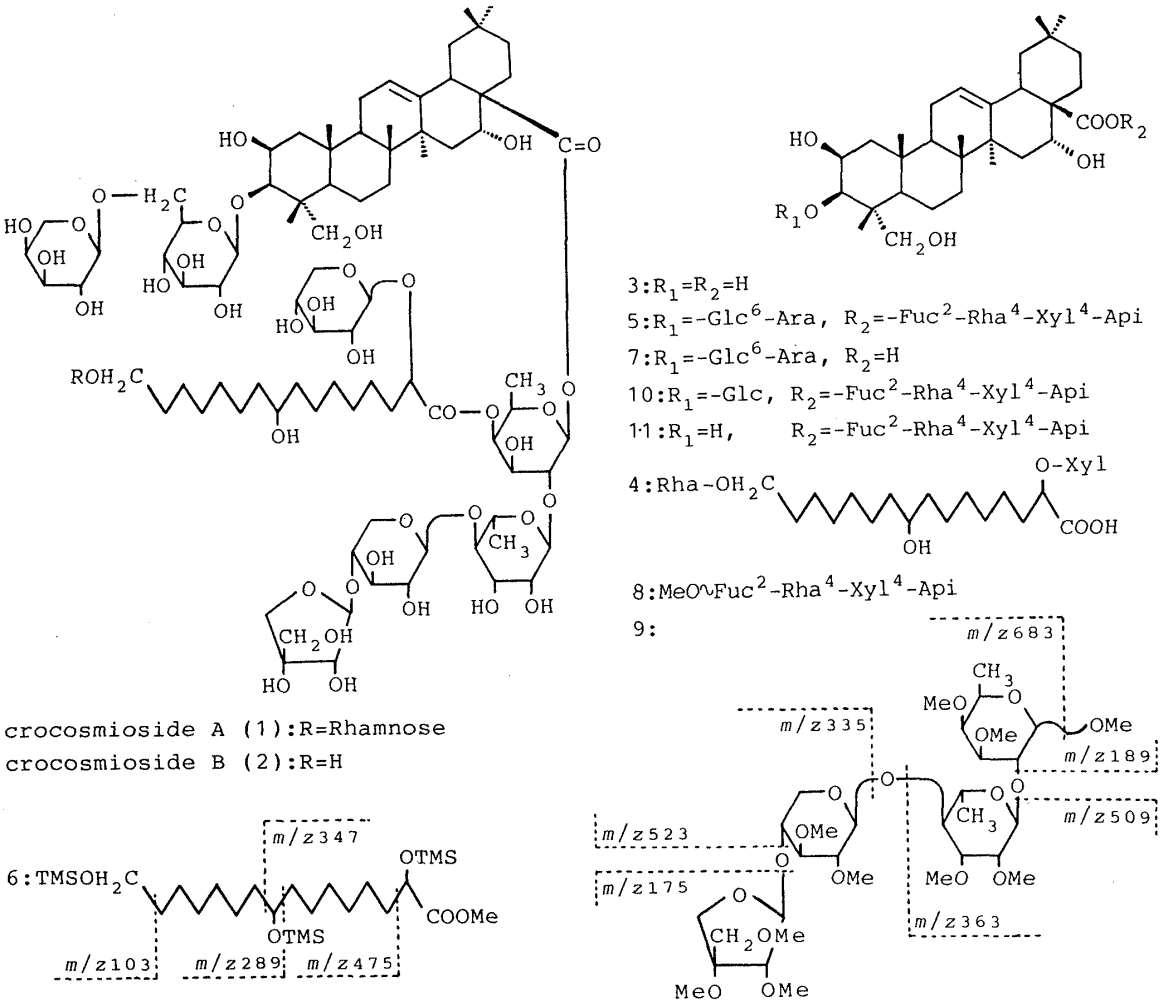

Fig.1. The Structures of Crocosmiosides A, B and Related Compounds

Table I. ^{13}C-NMR Chemical Shifts of Sugar Moieties of Crocosmiosides A, B and Related Compounds[a)]

Compounds		1	2	4	5	7	10	11
C-3 Sugars								
Glc	1	105.0	105.0		105.6	105.7	105.8	
	2	75.6	75.6		75.5	75.5	75.6	
	3	78.6	78.6		78.7	78.7	78.4[c)]	
	4	72.3	72.3		72.2	72.2	71.7	
	5	76.8	76.8		76.6	76.7	78.7[c)]	
	6	70.1	70.1		69.8	69.7	62.8	
Ara	1	105.2	105.2		105.1	105.1		
	2	72.8	72.8		72.6	72.6		
	3	74.4[b)]	74.4		74.3	74.4		
	4	69.8	69.8		69.2	69.2		
	5	67.1	67.0		66.6	66.5		
C-28 Sugars								
Fuc	1	95.2	95.2		95.1		95.0	95.0
	2	75.4	75.3		77.7		73.5	73.7
	3	75.2	75.2		76.9		76.9	76.9
	4	76.2	76.2		73.4		73.4	73.4
	5	71.3	71.3		73.6		72.7	72.7
	6	17.0	17.0		17.1		17.0	17.1
Rha	1	101.8	101.7		101.2		101.2	101.3
	2	72.3	72.3		72.1		72.1	72.1
	3	72.6	72.5		72.6		72.6	72.7
	4	84.7	84.7		83.5		83.4	83.8
	5	69.2	69.2		68.3		68.3	68.3
	6	18.8	18.8		18.6		18.6	18.6
Xyl	1	107.2	107.2		106.5		106.4	106.6
	2	76.3	76.3		76.1		76.1	76.1
	3	76.8	76.8		76.4		76.4	76.4
	4	77.6	77.6		76.7		76.6	76.6
	5	65.1	65.0		64.5		64.5	64.5
Api	1	109.6	109.6		109.1		109.1	109.2
	2	78.3	78.3		77.7		77.7	77.7
	3	80.7	80.7		80.4		80.4	80.4
	4	65.4	65.3		65.3		65.3	65.3
	5	75.4	75.4		75.4		75.4	75.4
Sugars of carboxylic acid								
Xyl	1	105.6	105.6	105.6				
	2	75.0	75.0	75.0				
	3	77.9	77.9	77.9				
	4	71.4	71.4	71.4				
	5	67.4	67.3	67.4				
Rha								
	1	101.9		101.9				
	2	72.7		72.7				
	3	72.8		72.8				
	4	74.3[b)]		74.3				
	5	70.1		70.1				
	6	18.4		18.4				

a) The spectra of **1**, **2** and **4** were measured in CD_3OD, those of **5**, **7**, **10** and **11** in C_5D_5N.
b-c) Assignments may be reversed in each column.
Glc,β-D-glucopyranosyl; Ara,α-L-arabinopyranosyl; Fuc,β-D-fucopyranosyl; Rha,α-L-rhamnopyranosyl; Xyl,β-D-xylopyranosyl; Api, β-D-apiofranosyl.

spectrum (α:β=ca. 2:1). In the ^{13}C-NMR spectrum,[6] the differences of chemical shifts between each carbons, induced by α and β methoxyl groups, decreased in the order of fucoside, rhamnoside and xyloside moieties, but no difference was observed in the apioside moiety. The data suggested that tetrasaccharide in **5** attaches to the C-28 position of aglycone in that order. This was further supported by methylation analysis of **8** and MS fragmentation of permethylate **9** (Fig. 1). On the other hand, enzymatic hydrolysis of **5** with hesperidinase gave hydrolysate (**10**) and (**11**), and their structures were elucidated by ^{1}H and ^{13}C-NMR spectra. The result of this hydrolysis supported the sequence of 3-*O*-glycosidic linkage, described previously in **7**. The linkage site of carboxylic acid in **1** was determined to be the C-4 position of fucose in view of the acylation shift, by comparing the ^{1}H and ^{13}C-NMR spectra of **1** with those of **5**.[7]

Based on the above evidence, the structures of crocosmiosides A and B were concluded to be polygalacic acid(3)-α-L-arabinopyranosyl(1→6)-β-D-glucopyranosido-(28)-2-*O*-[β-D-apiofuranosyl(1→4)-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl]-4-*O*-(9-hydroxy-16-α-L-rhamnopyranosyloxy-2-β-D-xylopyranosyloxyhexadecanoyl)-β-D-fucopyranoside (**1**) and polygalacic acid (3)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosido-(28)-2-*O*-[β-D-apiofuranosyl(1→4)-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl]-4-*O*-(9,16-dihydroxy-2-β-D-xylopyranosyloxyhexadecanoyl)-β-D-fucopyranoside (**2**), respectively.[8]

The sapogenin, polygalacic acid, was previously characterized as the aglycone part of polygalacins D and D_2,[9] while the sugar moieties are similar to those of onjisaponins[10] and senegins.[11] As most saponins so far obtained from monocotyledons are steroid saponins, it is interesting that triterpenoid saponins were obtained from montbretia, a monocotyledon. The biological activities of crocosmiosides will be reported elsewhere.

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4) The glycosylation shifts of carboxylic acid for **1**, compared with **2** were observed at C-16'(+5.6ppm) and C-15'(-3.0ppm).
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6) ^{13}C-NMR data of **8** δ: α-anomer;55.1(OMe),100.7(Fuc),104.4(Rha),107.0(Xyl),109.2(Api), β-anomer;56.3(OMe),103.9(Fuc),102.3(Rha),107.2(Xyl),109.2(Api).
7) A signal at δ5.09(1H,d,J=3.5Hz) where the acylation shift was observed, was assigned to the C-4 proton of fucose by the ^{1}H-^{1}H and ^{1}H-^{13}C-2D-NMR spectra of **1**.
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