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ACYLATED FLAVONOLS FROM *CROCOSMIA CROCOSMIIFLORA*

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Key Word Index—*Crocosmia crocosmiiflora*, Iridaceae, Montbretin A, Montbretin B, acylated myricetin glycosides, ^{13}C NMR

Abstract—Two new acylated flavonols, montbretins A and B were isolated from the corms of *Crocosmia crocosmiiflora* Their structures were established as myricetin 3-*O*-[β-D-glucopyranosyl(1→2)-6-*O*-caffeoyl-β-D-glucopyranosyl(1→2)-α-L-rhamnopyranoside]-4′-*O*-[α-L-rhamnopyranosyl(1→4)-β-D-xylopyranoside] and 3-*O*-[β-D-glucopyranosyl(1→2)-6-*O*-*p*-coumaroyl-β-D-glucopyranosyl(1→2)-α-L-rhamnopyranoside]-4′-*O*-[α-L-rhamnopyranosyl(1→4)-β-D-xylopyranoside] by spectral and chemical methods.

INTRODUCTION

Crocosmia crocosmiiflora N E.Br. (Montbretia) is well known as a garden plant and is used as an antitumour agent in Japanese folk medicine

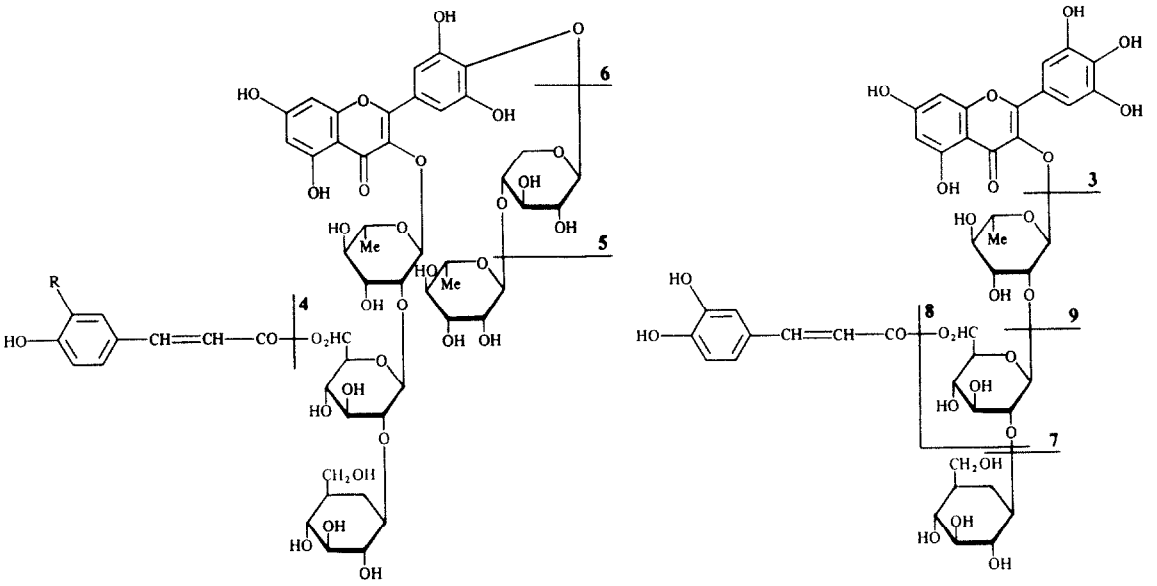
We report here the isolation and characterisation of two new acylated flavonols, named montbretins A(**1**) and B(**2**) from the corms of this plant.

RESULT AND DISCUSSION

Montbretins A(**1**) and B(**2**) were isolated from the butanol-soluble fraction of a methanolic extract of *C crocosmiiflora* corms by a combination of silica gel and Sephadex LH-20 chromatography.

Montbretin A(**1**) was shown by elemental analysis and FABMS (*m/z* 1229[M + H]$^+$), to have the constitution $C_{53}H_{64}O_{33}$. It showed UV maxima at 265 and 326 nm and IR bands at 3400, 1695, 1660 and 1600 cm^{-1}. The ^1H NMR spectrum of **1** showed the presence of myricetin, a caffeoyl group and five sugar moieties. Myricetin was indicated by the appearance of three aromatic proton signals at δ6.16 (1H, *d*, *J* = 2 Hz), 6.21 (1H, *d*, *J* = 2 Hz) and 6.91 (2H, *s*) The caffeoyl group was suggested by the appearance of ABC-type aromatic proton signals at δ6 65 (1H, *d*, *J* = 8 Hz), 6.74 (1H, *dd*, *J* = 8, 2 Hz) and 6 85 (1H, *d*, *J* = 2 Hz) and two *trans*-olefinic proton signals at δ6 04 (1H, *d*, *J* = 16 Hz) and 7 38 (1H, *d*, *J* = 16 Hz). The appearance of five anomeric proton signals at δ4 56 (1H, *d*, *J* = 8 Hz), 4.61 (1H, *d*, *J* = 8 Hz), 4.83 (1H, *br s*), 4.84 (1H, *d*, *J* = 7 Hz) and 5.66 (1H, *br s*) showed the presence of five sugar moieties. Furthermore, the appearance of five anomeric carbon signals at δ99.9, 102 6, 105.4, 106 5 and 107.1 in the ^{13}C NMR spectrum of **1** suggested the presence of five monosaccharide units. Acid hydrolysis of **1** with 4% sulphuric acid gave myricetin (**3**) [1], glucose, rhamnose and xylose (2:2·1) by HPLC and TLC Alkaline hydrolysis of **1** with 4% potassium hydroxide yielded descaffeoylmontbretin A(**4**) and caffeic acid. The structure of **4** was confirmed by a comparison of its IR and ^1H NMR spectra with those of **1**, which showed that the band at 1695 cm^{-1} in the IR spectrum and signals for the caffeoyl group in the ^1H NMR spectrum of **4** had disappeared In the ^1H NMR spectrum of **4**, the C_6-proton signals of glucose moiety were shifted to a higher field compared with those of **1** [2, 3]. Furthermore, in the ^{13}C NMR spectra, acylation shifts [4, 5] of glucose carbons for **1**, compared with **4** were observed at C-4 (+1.0 ppm), C-5 (−2.4 ppm) and C-6 (+2.0 ppm) (Table 1) These results indicated that the caffeoyl group may be attached to the C_6-position of a glucose in **1**.

In order to elucidate the linkage positions and glycosidic linkage between the constituents in **1**, enzymatic hydrolyses were performed Thus, hydrolysis of **1** with naringinase [6] for 1 hr yielded the hydrolysate **5** and rhamnose In addition, the assignable methyl proton and carbon signals of rhamnose moiety present in the ^1H and ^{13}C NMR spectra of **1** had disappeared in the correspondent spectra of **5**. The linkage of the rhamnose in **1** to the C_4 position of the xylose moiety was decided on the basis of the ^{13}C NMR spectra of **1** and **5** which showed glycosylation shifts [7, 8] of the xylose carbons for **1**, compared with **5** at C-3(−1.6 ppm), C-4(+4.2 ppm) and C-5 (−2.9 ppm) (Table 1) On enzymatic hydrolysis with naringinase for 4 days, **5** gave a hydrolysate (**6**) and xylose. The hydrolysate (**6**) was also prepared directly from **1**. The ^{13}C NMR spectrum of **6** showed the disappearance of the xylose moiety when compared with that of **5**. The glycosylation shifts [9, 10] of aglycone carbons for **5**, compared with **6** were observed at C-1′ (+6 6 ppm), C-3′,5′(+4.9 ppm) and C-4′(−0 4 ppm) suggesting that the xylose moiety in **5** was linked to the 4′-hydroxyl group of the aglycone (myricetin) (Table 2) Comparison of the ^{13}C NMR spectra of **6** with myricetin (**3**) revealed glycosylation shifts of the aglycone carbons for **6** at C-2(+10 3 ppm), C-3(−0.3 ppm) and C-4 (+2.2 ppm) indicating that the three sugars in **6** were attached to the 3-position of myricetin Mild enzymatic hydrolysis of **6** with hesperidinase [11, 12] for 26 hr. gave caffeic acid, hydrolysates **7** and **8** and myricitrin(**9**) [10, 13] The structure of **7** was confirmed by analysing the ^{13}C NMR spectra of **6** and **7**. Thus, the ^{13}C NMR spectrum of **7** showed the disappearance of a glucose



1,**5**,**6** R = OH
2 R = H

Table 1. ^{13}C chemical shifts and assignments of sugar moieties of montbretins A(**1**), B(**2**) and related compounds (CD_3OD)

			1	**2**	**4**	**5**	**6**	**7**	**8**	**9**
3-*O*-Glycoside	Rha	1	102 6	102 5	102 6	102 7	102 7	102 8	102 8	103 7
		2	84 2	84 3	83 7	84 3	84 5	83 7	83 0	72 0
		3	72 1[a]	72 1[a]	72 3[a]	72 2	72 3	71 9	72 0	72 0
		4	73 9	73 9	74 0	74 0	74 1	73 8	73 8	73 5
		5	71 8	71 8	71 8	71 9	71 8	71 9	71 9	72 1
		6	17 7	17 8	17 8	17 8	17 7	17 7	17 7	17 7
	Glc	1	105 4	105 4	105 6	105 5	105 6	107 3	107 2	
		2	84 6	84 6	85 2	84 7	84 7	75 4	75 4	
		3	77 7[b]	77 7[b]	77 9[b]	77 8[b]	77 7[b]	77 7	77 8	
		4	71 4	71 6	70 4	71 4	71 0	71 6	70 8	
		5	75 3[c]	75 3[c]	77 7	75 4	75 3	75 3	77 9	
		6	64 1	64 3	62.1	64 2	63 9	64 2	62 2	
	Glc	1	106 5	106 5	106 7	106 5	106 5			
		2	75 9	75.9	76 0	76 0	76 0			
		3	79 1	79 1	79 2	79 2	79 2			
		4	70 8	70 9	70 9	70 8	71 0			
		5	77 8[b]	77 8[b]	77 7[b]	77 8[b]	77 9[b]			
		6	62 4	62 4	62 4	62 4	62 4			
4'-*O*-Glycoside	Xyl	1	107 1	107 2	107 2	107 3				
		2	74 8	74 9	74.8	74 7				
		3	75 6	75 6	75 6	77 2				
		4	75 2[c]	75 2[c]	75 3	71 0				
		5	64 5	64 6	64 5	67 4				
	Rha	1	99 9	99 9	99 9					
		2	72 1[a]	72 1[a]	72 2[a]					
		3	72 4[a]	72 4[a]	72 4[a]					
		4	73 9	73 9	74 0					
		5	70 1	70 1	70 2					
		6	18 0	18 0	18 0					

[a–c] Assignments may be reversed in each column
Rha α-L-rhamnopyranosyl, Glc β-D-glucopyranosyl, Xyl β-D-xylopyranosyl

Table 2 ^{13}C chemical shifts and assignments of the aglycone of montbretins A(1) and B(2) and related compounds (CD_3OD)

		1	2	3	4	5	6	7	8	9
Aglycone	2	157 9	157.7	148.3	158 6	157 9	158.6	158.4	159 4	159.7
	3	136 1	136 1	137.2	136.2	136.1	136.9	136.9	136 7	136.5
	4	179.6	179 6	177.6	179.6	179 5	179.8	179.8	179 8	179 9
	5	163.0	163.1	162 7	163 2	163.2	163 1	163.2	163 2	163.3
	6	100.0	100.0	99.5	100 1	100 2	99.9	99.8	100.0	100 0
	7	166.0	165 9	165.8	166.2	166.4	165 9	165 7	166 0	166 0
	8	95.0	95 0	94 6	95.0	95.1	94 9	94.8	94 8	94.9
	9	158.4	158 3	158 5	158.7	158.5	158.7	158 7	158 6	158.7
	10	106 1	106.1	104.7	106.1	106.0	106.0	106.0	106 0	106.1
	1′	128.7	128.6	123.4	128.6	128.7	122 1	122 1	122.0	122 2
	2′	109 8	109 8	108 9	109.8	109.8	109 5	109.6	109 6	109 5
	3′	151 7	151.7	146.9	152 0	151 8	146.9	146.9	147 0	147.0
	4′	137.3	137.4	137.9	137.1	137 4	137 8	137.8	137 9	138 0
	5′	151.7	151.7	146.9	152.0	151 8	146 9	146 9	147.0	147 0
	6′	109.8	109.8	108.9	109.8	109 8	109 5	109.6	109 6	109 5
Caffeic acid and	1	127 6	127.0			127 6	127.7	127 8		
p-coumaric acid	2	115 1	131.0			115 1	115 0	115.1		
	3	149.4	116 7			149 5	149 4	149.4		
	4	146.6	161 0			146.7	146 7	146.7		
	5	116 6	116.7			116.4	116.4	116 4		
	6	122.9	131.0			123.0	123.0	123.0		
	1′	168.9	168.9			168.9	169 0	169 1		
	2′	114 7	114 8			114.8	114 9	114 9		
	3′	147.1	146 7			147 0	147.1	147 1		

moiety when compared with that of **6** and the glycosylation shifts of glucose carbons for **6** were observed at C-1 (−1.7 ppm) and C-2(+9 3 ppm) compared with the spectrum of **7** suggesting that the terminal glucose in **6** is linked to the C-2 position of the inner glucose. The ^1H and ^{13}C NMR spectra of **8** showed the disappearance of the caffeoyl group as compared with those of **7**. The position of the caffeoyl group in **8** was decided from the acylation shift in the ^{13}C NMR spectrum. The acylation shifts of glucose carbons for **7** were observed at C-4 (+0.8 ppm), C-5(−2.6 ppm) and C-6(+2.0 ppm) as compared that of **8** suggesting that the caffeoyl group in **7** was attached at C-6 of the glucose A comparison of the ^{13}C NMR spectra of **8** and **9** showed the glycosylation shifts of rhamnose carbons for **8** at C-1(−0.9 ppm), C-2 (+11.0 ppm) and C-3(−0.2 ppm) indicating the glucose in **8** was attached at C-2 of the rhamnose. On enzymatic hydrolysis with hesperidinase for 2 days, **6** yielded caffeic acid, hydrolysates **8**, myricetin and myricitrin.

With regard to the anomeric configuration of the glycosidic linkage, the β-orientations of the glucopyranoside and xylopyranoside were indicated by the corresponding anomeric proton signal patterns (*d*, $J=7-8$ Hz) in the ^1H NMR spectrum of **1**, **4**–**8** The anomeric configuration of the rhamnose in **1** was assigned as α by the application of Klyne's rule [14]. $[M]_D$ of **1** $-[M]_D$ of **6** $=-355°$. From these results, montbretin A(**1**) was characterised as myricetin 3-*O*-[β-D-glucopyranosyl (1→2)-6-*O*-caffeoyl-β-D-glucopyranosyl (1→2)-α-L-rhamnopyranoside]-4′-*O*-[α-L-rhamnopyranosyl (1→4)-β-D-xylopyranoside]

The molecular formular of montbretin B(**2**) was confirmed as $C_{53}H_{64}O_{32}$ by elemental analysis and FABMS (*m/z* 1213 $[M+H]^+$) It gave UV maxima at 267, 304 and 311 nm and IR bands at 3380, 1685, 1650 and 1600 cm^{-1}. The ^1H NMR spectrum of **2** is similar to that of **1** except for the A_2B_2-type aromatic proton signals at δ6.67 (2H, *d*, $J=8$ Hz) and 7.22 (2H, *d*, $J=8$ Hz) suggesting the replacement of the caffeoyl group in **1** by a *p*-coumaroyl moiety in **2**. In the ^{13}C NMR spectrum of **2**, the resonances due to the aglycone and sugar carbons are quite coincident with that of **1** (Tables 1 and 2), suggesting that **2** has the same sugar components as **1** and that the *p*-coumaroyl group in **2** may be attached to the C-6 position of the inner glucose as the caffeoyl group is in **1**. Acid hydrolysis of **2** with 4% sulphuric acid gave myricetin (**3**), glucose, rhamnose and xylose (2:2:1). Alkaline hydrolysis of **2** with 4% potassium hydroxide yielded *p*-coumaric acid and the hydrolysate (**4**) which was identified as descaffeoylmontbretin A(**4**) obtained from **1**

On the basis of chemical and spectroscopic evidence, montbretin B(**2**) was identified as myricetin 3-*O*-[β-D-glucopyranosyl(1→2)-6-*O*-*p*-coumaroyl-β-D-glucopyranosyl (1→2)-α-L-rhamnopyranoside]-4′-*O*-[α-L-rhamnopyranosyl(1→4)-β-D-xylopyranoside]

Flavonoid pentaglycosides rarely occur in the plant kingdom and this is the first report of a 3,4′-diglycosylmyricetin with either a caffeoyl or *p*-coumaroyl moiety [1, 10, 15].

EXPERIMENTAL

Mps. uncorr. ^1H and ^{13}C NMR spectra were taken on JEOL FX-270 and Varian XL-300 spectrometers. EI and FABMS were taken with JEOL DX-300 instruments equipped with a direct inlet system.

Isolation of flavonoids The fresh corms (4 5 kg) of C crocosmuflora N E Br were extracted with MeOH The concd methanolic extract was extracted successively with *n*-hexane, C_6H_6, EtOAc and *n*-BuOH The *n*-BuOH extract was repeatedly chromatographed on silica gel with the upper layer of *n*-BuOH–EtOAc–H_2O (5 1 4) to give the fraction containing **1** and **2** (Fr 1). The repeated separation of Fr 1 by Sephadex LH-20 chromatography gave **1** (634 mg) and **2** (155 mg). Montbretin A(**1**) Yellow powder $[\alpha]_D^{21}$ −42 1° (H_2O, *c* 1 23) Anal calcd for $C_{53}H_{64}O_{33}$ C, 51 78, H, 5 23 Found C, 51 76, H, 5 35 UV λ_{max}^{MeOH} nm 265, 326 FABMS *m/z* 1229 (M + 1)$^+$ ^{1}H NMR (CD_3OD) δ 1 07 (3H, *d*, *J* = 6 Hz), 1 28 (3H, *d*, *J* = 6 Hz), 4 20 (1H, *dd*, *J* = 11, 5 Hz), 4 48 (1H, *d*, *J* = 11 Hz), 4 56 (1H, *d*, *J* = 8 Hz), 4 61 (1H, *d*, *J* = 8 Hz) 4 81 (1H, *br* s), 4 86 (1H, *d*, *J* = 7 Hz), 5 66 (1H, *br* s), 6 04 (1H, *d*, *J* = 16 Hz), 6 16 (1H, *d*, *J* = 2 Hz), 6 21 (1H, *d*, *J* = 2 Hz), 6 65 (1H, *d*, *J* = 8 Hz), 6 74 (1H, *dd*, *J* = 8, 2 Hz), 6 85 (1H, *d*, *J* = 2 Hz), 6 91 (2H, s), 7 38 (1H, *d*, *J* = 16 Hz)

Montbretin B(**2**) Yellow powder $[\alpha]_D^{21}$ −71 1° (EtOH, *c* 1 37) Anal calcd for $C_{53}H_{64}O_{32}$ C, 52 46, H, 5 32 Found C, 52 12, H, 5 38 UV λ_{max}^{MeOH} nm 267, 304(sh), 326 FABMS *m/z* 1213(M + 1)$^+$ ^{1}H NMR (CD_3OD) δ 1 08 (3H, *d*, *J* = 6 Hz), 1 29 (3H, *d*, *J* = 6 Hz), 4 20 (1H, *dd*, *J* = 11, 5 Hz), 4 48 (1H, *d*, *J* = 11 Hz), 4 59 (1H, *d*, *J* = 8 Hz), 4 61 (1H, *d*, *J* = 8 Hz), 4 83 (1H, *br* s), 4 83 (1H, *d*, *J* = 7 Hz), 5 69 (1H, *br* s), 6 05 (1H, *d*, *J* = 16 Hz), 6 16 (2H, *s*), 6 67 (2H, *d*, *J* = 8 Hz), 6 89 (2H s), 7 22 (2H, *d*, *J* = 8 Hz), 7 42 (1H, *d*, *J* = 16 Hz)

Acid hydrolysis of **1**. A soln of **1** (51 8 mg) in 4% H_2SO_4 (2 ml) was heated at 100° for 3 5 hr giving a ppt of myricetin (**3**, 4 5 mg), ident by comparison with an authentic marker The filtrate was passed through Amberlite IRA-45 (OH form) and concd to give the sugar fraction Sugars were identified by TLC [silica gel, solvent $CHCl_3$–MeOH–H_2O (6 4 1)] and HPLC [column ERC-NH-1171 (6 × 200 mm), solvent 80% CH_3CN, flow rate 1 5 ml, detecter RI, R_t (min) rhamnose (4 7), xylose (5 4), glucose (7 4)]

Alkaline hydrolysis of **1** A soln of **1** (143 mg) in 4% KOH (14 ml) was stood for 2 hr at room temp and then acidified with 0 2 N HCl The acidic soln was adsorbed by Diaion HP-20 and eluted successively with H_2O, 60% MeOH and 80% MeOH The 60% MeOH soln was evapd and the residue chromatographed on a silica gel with $CHCl_3$–MeOH–H_2O (7 3 0 5) to give caffeic acid (9 7 mg) and descaffeoylmontbretin A(**4**, 86 0 mg) **4** Yellow powder $[\alpha]_D^{21}$ −73 3 (H_2O, *c* 1 29) UV λ_{max}^{MeOH} nm 269, 311, 337 (sh) IR ν_{max}^{KBr} cm^{-1} 3400, 1655, 1600 ^{1}H NMR (CD_3OD) δ 0 98 (3H, *d* *J* = 6 Hz), 1 28 (3H, *d*, *J* = 6 Hz), 4 49 (1H, *d*, *J* = 8 Hz), 4 58 (1H, *d*, *J* = 8 Hz), 4 83 (1H, *br* s), 4 84 (1H, *d*, *J* = 8 Hz), 5 62 (1H, *br* s), 6 20 (1H, *d*, J = 2 Hz), 6 32 (1H, *d*, *J* = 2 Hz), 6 92 (2H, s)

Enzymatic hydrolysis of **1** *with naringinase* A soln of **1** (21 mg) and naringinase (22 mg) in 10% EtOH (4 ml) was incubated at 37° for 1 hr The reaction mixture was diluted with H_2O (25 ml) and extracted with *n*-BuOH The concd BuOH soln was separated by CC on Sephadex LH-20 (MeOH), affording **5** (10 mg) **5** Yellow powder $[\alpha]_D^{19}$ −150 (H_2O, *c* 0 71) UV λ_{max}^{MeOH} nm 256 (sh), 265, 305 (sh), 326 IR ν_{max}^{KBr} cm^{-1} 3350, 1685, 1650, 1600 ^{1}H NMR (CD_3OD) δ 1 07 (3H, *d*, *J* = 6 5 Hz), 4 55 (1H, *d*, *J* = 8 Hz), 4 58 (1H, *d*, *J* = 8 Hz), 4 82 (1H, *d*, *J* = 7 Hz), 5 65 (1H, *br* s), 6 05 (1H, *d*, *J* = 16 Hz), 6 17 (1H, *d*, *J* = 2 Hz), 6 21 (1H, *d*, *J* = 2 Hz), 6 66 (1H, *d*, *J* = 8 Hz), 6 75 (1H, *dd*, *J* = 8, 2 Hz), 6 85 (1H, *d*, *J* = 2 Hz), 6 91 (2H, s), 7 39 (1H, *d* *J* = 16 Hz) The residual soln was evapd to give rhamnose ident by TLC and HPLC with an authentic sample

Enzymatic hydrolysis of **1** *with naringinase* A soln of **1** (26 mg) and naringinase (56 mg) in 10% EtOH (4 ml) was incubated at 37° for 4 days The hydrolysate was treated just in the same manner as in the case of **5**, affording **6** (12 8 mg) **6** Yellow powder $[\alpha]_D^{19}$ +41 4 (H_2O, *c* 1 06) Anal calcd for $C_{42}H_{46}O_{25}$ C, 53 04, H, 4 88 Found C, 52 51, H, 5 07 UV λ_{max}^{MeOH} nm 249, 263 (sh), 304 (sh), 332 IR ν_{max}^{KBr} cm^{-1} 3340 1695, 1660, 1600 FABMS *m/z* 951 (M + 1)$^+$ ^{1}H NMR (CD_3OD) δ 1 09 (3H, *d*, *J* = 6 Hz), 4 34 (1H, *d*, *J* = 8 Hz), 4 61 (1H, *d*, *J* = 8 Hz), 5 48 (1H, *br* s), 6 10 (1H, *d*, *J* = 1 6 Hz), 6 17 (1H, *d*, *J* = 2 Hz), 6 24 (1H, *d* *J* = 2 Hz), 6 68 (1H, *d*, *J* = 8 Hz), 6 79 (1H, *dd*, *J* = 8, 2 Hz), 6 90 (1H, *d* *J* = 2 Hz), 6 99 (2H, s), 7 41 (1H, *d*, *J* = 16 Hz)

Enzymatic hydrolysis of **5** *with naringinase* A soln of **5** (60 mg) and naringinase (124 mg) in 10% EtOH (10 ml) was incubated at 37° for 4 days, affording **6** (33 mg)

Enzymatic hydrolysis of **6** *with hesperidinase* A soln of **6** (153 mg) and hesperidinase (76 mg) in H_2O (30 ml) was incubated at 37° for 26 hr The reaction mixture was filtered through a column of Diaion HP-20 and eluted with 80% MeOH The concd MeOH soln was separated by CC on Sephadex LH-20 (MeOH) and silica gel ($CHCl_3$–MeOH–H_2O, 7 3 0 5) to give caffeic acid (14 mg), **7** (10 mg) **8** (30 mg), and myricitrin (**9**, 8 mg) **7** Yellow powder $[\alpha]_D^{19}$ −98 8 (EtOH, *c* 0 58) UV λ_{max}^{MeOH} nm 249, 262 (sh), 303 (sh), 332 IR ν_{max}^{KBr} cm^{-1} 3400 1695, 1660, 1605 ^{1}H NMR (CD_3OD) δ 1 08 (3H *d*, *J* = 6 Hz), 4 36 (1H, *d*, *J* = 8 Hz), 5 58 (1H *br* s), 6 08 (1H *d*, *J* = 16 Hz), 6 15 (1H, *J* = 2 Hz), 6 22 (1H, *d*, *J* = 2 Hz), 6 67 (1H, *d*, *J* = 8 Hz) 6 78 (1H, *dd*, *J* = 8, 2 Hz), 6 89 (1H, *d*, *J* = 2 Hz) 6 97 (2H, s) 7 34 (1H, *d*, *J* = 16 Hz) **8** Yellow powder $[\alpha]_D^{19}$ −130 7 (EtOH, *c* 1 17) UV λ_{max}^{MeOH} nm 256, 306 (sh), 353 IR ν_{max}^{KBr} cm^{-1} 3380, 1655, 1605 ^{1}H NMR (CD_3OD) δ 1 02 (3H *d*, *J* = 6 Hz), 4 34 (1H, *d*, *J* = 7 Hz), 5 56 (1H, *br* s), 6 20 (1H *d*, *J* = 2 Hz), 6 37 (1H, *d*, *J* = 2 Hz) 6 97 (2H, s) Compound **9** was identical with an authentic sample of myricitrin on the basis of mixed fusion and spectral data

Enzymatic hydrolysis of **6** *with hesperidinase* A soln of **6** (153 mg) and hesperidinase (158 mg) in H_2O (30 ml) was incubated at 37° for 2 days and was filtered through a Diaion HP-20 column From the concd filtrate glucose and rhamnose were ident by TLC and HPLC comparison with authentic samples Further elution of the HP-20 column with MeOH and separation of the concd eluate by CC on Sephadex LH-20 (MeOH) and silica gel ($CHCl_3$–MeOH–H_2O, 5 5 1) gave myricetin (**3**, 13 mg), caffeic acid (9 mg), **8** (25 mg), myricitrin (**9**, 7 mg)

Acid hydrolysis of **2** A soln of **2** (10 3 mg) in 2% H_2SO_4 (2 ml) heated at 100° for 2 hr gave **3** (1 7 mg) The filtrate was passed through Amberlite IRA-45 (OH form) and concentrated to give a sugar fraction Sugars were identified by TLC and HPLC as the same method used for **1**

Alkaline hydrolysis of **2** A soln of **2** (21 4 mg) in 4% KOH (3 ml) was stood for 2 5 hr at room temp and then acidified with 0 2 N HCl The acidic soln was adsorbed on Diaion HP-20 and successively eluted with H_2O, 20% MeOH and 60% MeOH The concd 60% MeOH soln was separated by CC on silica gel ($CHCl_3$–MeOH–H_2O = 7 3 1 lower phase and $CHCl_3$–MeOH–H_2O = 6 4 1) to give **4** (8 5 mg) and *p*-coumaric acid (0 4 mg, ident by IR, MS ^{1}H NMR and mp) Compound **4** was identified as descaffeoylmontbretin A (**4**) by direct comparison with **4** obtained from **1**

Acknowledgements The authors thank Professor N Morita (Toyama Medical and Pharmaceutical University) for authentic myricitrin and myricetin samples Thanks are also due to Dr T Mizoguchi (Tanabe Seiyaku Co, Ltd) for supplying the crude hesperidinase

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