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FLAVONOL GLYCOSIDES WITH ACETYL SUBSTITUTION FROM *KALANCHOE GRACILIS*

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Key Word Index.—*Kalanchoe gracilis*; Crassulaceae; flavonoids, mono, di and triacetylated derivatives of patuletin 3,7-di-*O*-rhamnoside, FABMS, ^{1}H and ^{13}C NMR.

Abstract—The aerial parts of *Kalanchoe gracilis* yielded 10 flavonoids, three of which were patuletin, patuletin 3-*O*-rhamnoside and patuletin 3,7-di-*O*-rhamnoside. The other seven flavonoids are new: patuletin-3-*O*-rhamnosyl-7-*O*-[3-*O*-acetylrhamnoside], patuletin-3-*O*-rhamnosyl-7-*O*-[4-*O*-acetylrhamnoside], patuletin-3-*O*-rhamnosyl-7-*O*-[3,4-*O*-diacetylrhamnoside], patuletin-3-*O*[4-*O*-acetylrhamnosyl]-7-*O*-[3-*O*-acetylrhamnoside], patuletin-3-*O*-[3-*O*-acetylrhamnosyl]-7-*O*-[3-*O*-acetylrhamnoside], patuletin-3-*O*-[4-*O*-acetyl rhamnosyl]-7-*O*-[3,4-diacetylrhamnoside] and patuletin-3-*O*-[4-*O*-acetylrhamnosyl]-7-*O*-[2,4-*O*-diacetylrhamnoside].

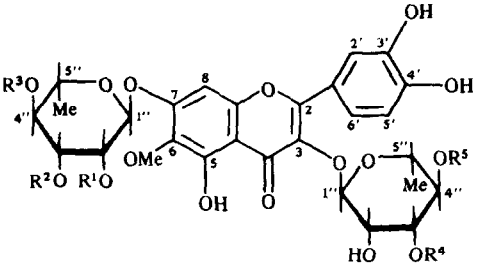
INTRODUCTION

Kalanchoe gracilis Hance, a perennial herb from the mountain area of Taiwan, is used as a herbal remedy for the treatment of tissue inflammation caused by muscle injuries [1, 2]. In a study of the plant to determine the substances responsible for its medicinal use, the major isolatable constituents were found to be flavonoids. Flavonoids are known to be anti-inflammatory and therefore it is plausible that the flavonoid constituents are important in the traditional use of this herb [3, 4]. Flavonoids previously isolated from *Kalanchoe* species include quercetin, kaempferol [5], quercetin 3-diarabinoside and kaempferol 3-glucoside from *K pinnata* [6]. *Kalanchoe blossfeldiana* leaves and flowers have yielded cyanidin 3,5-diglucoside, cyanidin 3-monoglucoside, pelargonidin 3,5-diglucoside and leucocyanidin [7]. *Kalanchoe daigremontiana* yielded kaempferol coumaroylarabinoside of uncertain structure [8]. More recently, patuletin 3,7-di-*O*-rhamnoside has been isolated from *K. spathulata* [9]. Bufadienolides have also been reported to occur in *K diagremontiana* and *K lanceolata* [10, 11]. This paper reports the isolation and identification of three known flavonoids, patuletin, patuletin 3-*O*-rhamnoside, patuletin 3,7-di-*O*-rhamnoside and seven new acetylated patuletin rhamnosides

RESULTS AND DISCUSSION

The ethanolic extract of the dried plant material was reduced to dryness and sequentially extracted with petrol, chloroform and ethyl acetate. The residue from the ethyl acetate was fractionated on a polyamide column and TLC indicated that each fraction contained a complex mixture of flavonoid glycosides. Purification of the individual compounds was achieved by a combination of silica gel and Sephadex LH-20 chromatography. Apart from patuletin **10**, patuletin 3-*O*-rhamnoside **9** [12] and patuletin 3,7-di-*O*-rhamnoside **1** [9], a new series of mono-, di- and triacetylated derivatives of patuletin 3,7-di-*O*-rhamnoside was isolated (**1**–**8**) (Fig. 1).

Compound **1**. The ^{1}H NMR spectrum (Table 1) showed the expected signals of aromatic protons at δ7.37 (*d*, J = 2 Hz), 7.34 (*dd*, J = 2, J = 9 Hz) and 6.91 (*d*, J = 9 Hz) for H-2′, H-6′ and H-5′, respectively. A singlet signal at δ6.64 was assigned to H-8 and the methoxy protons at 3.85 were assigned to C-6 which was explained by the characteristic mass ion at m/z 317 $[A-15]^+$ [13]. The signal for 5-OH proton was located downfield at δ12.4. Therefore patuletin is the aglycone of **1**. In the ^{1}H NMR spectrum, two doublet signals at δ0.86 and 1.14 were observed



	R¹	R²	R³	R⁴	R⁵
1	H	H	H	H	H
2	H	Ac	H	H	H
3	H	H	Ac	H	H
4	H	Ac	Ac	H	H
5	H	Ac	H	H	Ac
6	H	Ac	H	Ac	H
7	H	Ac	Ac	H	Ac
8	Ac	H	Ac	H	Ac

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Table 1. ^{1}H NMR spectral data of compounds **1**–**8** (250 MHz, TMS as int. standard)

	1*	2*	3*	4*	5*	6†	7†	8†
Patuletin (aglycone)								
OH-5	12.4 *br s*	12.4 *br s*	12.4 *br s*	12.4 *br s*	—	—	—	—
OMe-6	3.85 *s*	3.91 *s*	3.88 *s*	3.92 *s*	3.87 *s*	3.93 *s*	3.93 *s*	3.94 *s*
H-8	6.64 *s*	6.69 *s*	6.69 *s*	6.74 *s*	6.67 *s*	6.65 *s*	6.64 *s*	6.65 *s*
H-2′	7.37 *d* (2)	7.40 *d* (2)	7.39 *d* (2)	7.41 *d* (2)	7.35 *d* (2)	7.43 *d* (2)	7.39 *d* (2)	7.45 *d* (2)
H-5′	6.91 *d* (9)	6.94 *d* (9)	6.91 *d* (9)	6.95 *d* (9)	6.94 *d* (9)	6.96 *d* (9)	6.96 *d* (9)	6.97 *d* (9)
H-6′	7.34 *dd* (2/9)	7.35 *dd* (2/9)	7.34 *dd* (2/9)	7.37 *dd* (2/9)	7.28 *dd* (2/9)	7.44 *dd* (2/9)	7.33 *dd* (2/9)	7.48 *dd* (2/9)
C-3-*O*-Rhamnose								
H-1	5.23 *d* (1)	5.28 *d* (1)	5.26 *d* (1)	5.29 *d* (1)	5.20 *d* (1)	5.40 *d* (1)	5.51 *d* (1)	5.41 *d* (1)
H-2	3.9 *dd* (2/5)	4.05 *dd* (2/5)	3.5–4 *m*	4.05 *dd* (2/5)	4.04 *dd* (2/5)	4.3 *dd* (2/5)	4.31 *dd* (2/5)	4.24 *dd* (2/5)
H-3	3.2–3.8 *m*	3.1–3.9 *m*	3.5–4 *m*	3.6 *dd* (2/10)	3.75 *dd* (2/10)	5.0 *dd* (2/10)	3.90 *dd* (2/10)	3.85 *dd* (2/10)
H-4	3.2–3.8 *m*	3.1–3.9 *m*	3.18 *t* (10)	3.21 *t* (10)	4.73 *t* (10)	3.60 *t* (10)	3.3–4 *m*	4.9 *t* (10)
H-5	3.2–3.8 *m*	3.1–3.9 *m*	3–4 *m*	3–4 *m*	3.3–3.7 *m*	3.3–4 *m*	3.3–4 *m*	1.0 *d* (7)
Me-6	0.86 *d* (7)	0.88 *d* (7)	0.88 *d* (7)	0.86 *d* (7)	0.72 *d* (7)	1.0 *d* (7)	0.82 *d* (7)	1.0 *d* (7)
OAc-2	—	—	—	—	—	—	—	—
OAc-3	—	—	—	—	—	2.14 *s*	—	—
OAc-4	—	—	—	—	2.01 *s*	—	2.10 *s*	2.12 *s*
C-7-*O*-Rhamnose								
H-1	5.57 *d* (1)	5.65 *d* (1)	5.66 *d* (1)	5.75 *d* (1)	5.60 *d* (1)	5.60 *d* (1)	5.65 *d* (1)	5.68 *d* (1)
H-2	4.0 *dd* (2/5)	4.15 *dd* (2/5)	4.0 *dd* (2/5)	4.25 *dd* (2/5)	4.11 *dd* (2/5)	4.40 *dd* (2/5)	4.32 *dd* (2/5)	5.34 *dd* (2/5)
H-3	3.2–3.8 *m*	5.03 *dd* (2/10)	3.5–4 *m*	5.20 *dd* (2/10)	4.97 *dd* (2/10)	5.20 *dd* (2/10)	5.34 *dd* (2/10)	4.41 *dd* (2/10)
H-4	3.2–3.8 *m*	3.1–3.9 *m*	4.93 *t* (10)	5.15 *t* (10)	3.55 *t* (10)	3.80 *t* (10)	5.24 *t* (10)	5.0 *t* (10)
H-5	3.2–3.8 *m*	3.1–3.9 *m*	3–4 *m*	3–4 *m*	3.3–3.7 *m*	3.3–4 *m*	3.3–4 *m*	3.5–4 *m*
Me-6	1.14 *d* (7)	1.20–*d* (7)	1.04 *d* (7)	1.11 *d* (7)	1.17 *d* (7)	1.30 *d* (7)	1.20 *d* (7)	1.17 *d* (7)
OAc-2	—	—	—	—	—	—	—	2.14 *s*
OAc-3	—	2.12 *s*	—	2.08 *s*	2.08 *s*	2.16 *s*	2.058 *s*	—
OAc-4	—	—	2.07 *s*	2.07 *s*	—	—	2.055 *s*	2.20 *s*

*DMSO-d_6
†CD_3OD

indicating the presence of two sets of rhamnose methyl protons and the signals for the anomeric protons appeared at δ5.23 and 5.57, respectively. The signals for the carbon atoms of two rhamnose moieties were also observable in the ^{13}C NMR spectrum (Table 2). Comparison of ^{1}H and ^{13}C NMR spectra of **1** before and after hydrolysis confirmed the presence of two rhamnose moieties, and rhamnose was also detected on TLC. The FABMS spectrum revealed significant peaks at *m/z* 479 $[(M+H)-\text{rhamnose}]^+$, 333 $[(M+H)-2\times\text{rhamnose}]^+$ apart from the peak of a molecular ion at *m/z* 625 $[M+H]^+$ and this result further confirmed **1** to be a dirhamnoside.

The UV spectrum of **1** in methanol and the changes observed in this spectrum after the addition of shift reagents [14] suggested the presence of free hydroxyl groups at C-3′, C-4′ and C-5 positions, while the 7-hydroxy group was substituted. The purple coloured spot of **1** under UV_{366} on the TLC plate gave a further purple spot and a yellow spot after hydrolysis, indicated that one rhamnose was attached at C-7 and the other at C-3 This assignment of rhamnoside moieties was further confirmed by the ^{13}C NMR spectrum [15]. Compound **1** is therefore patuletin 3,7-di-*O*-rhamnoside.

Compound **2**. The UV spectral shifts of **2** with standard reagents indicated an identical pattern to **1**. A comparison of the ^{1}H NMR spectrum of **2** with that of **1** (Table 1), indicated that the signals for H-2′, H-6′, H-5′, H-8 and 6-methoxy protons were similar. Thus **2** is also a patuletin diglycoside. In the ^{1}H NMR spectrum of **2**, an extra singlet signal of three protons was exhibited at δ2.12, suggesting that one of the sugar hydroxyl groups was acetylated. The signals for methyl protons of the two rhamnose units appeared at δ0.88, 1.20 and signals for two anomeric protons were located at δ5.28 and 5.65. The characteristic double doublet signal ($J=2$, $J=10$ Hz) for one of the sugar protons at C-3 of a rhamnose moiety [16] was shifted downfield from δ3.0–4.5 to 5.03, indicating it had an acetylated hydroxyl group. In the ^{13}C NMR spectrum of **2**, the presence of signals at δ170.3 for one acetyl carbon (−OCOMe), 21.2 for one methyl carbon (−OCOMe) and signals for carbons of two rhamnose supported the ^{1}H NMR spectral data. In the FABMS spectrum, the molecular ion at *m/z* 667 [M

Table 2 ^{13}C NMR data of compounds **1**–**4***

C		1	2	3	4
Patuletin (aglycone)					
2		157.6	157.8	157.8	157 9
3		134 5	134 6	134.6	134.7
4		178 0	178 3	178 2	178.3
5		154 5	154 4	154.2	154.0
6		129 2	129 6	129 4	129.7
6-OMe		61 3	61 5	61 5	61 6
7		155 7	155.8	155 8	155 9
8		98 5	98.8	98.6	98 5
9		148 6	148 8	148.8	148 9
10		105 4	105 8	105 8	106 1
1′		121.2	121.4	121 0	121 4
2′		115 5	115 6	115 5	115.7
3′		145.2	145 4	145 3	145 4
4′		148 2	148.3	148.3	148 4
5′		115 5	115 7	115.6	115.7
6′		120 6	120 7	120 6	120.7
C-3-*O*-Rhamnose					
1		102 0	102.1	102.0	102.1
2		70 6	70 7	70 7	70.8
3		70 6	70 5	70 4	70 5
4		71 4	71 3	71 2	71 1
5		70 3	70 4	70 1	70 2
6		17 8	17.9	17.5	17.6
C-7-*O*-Rhamnose					
1		98 8	98.8	98.8	99.1
2		70 0	68 6	69.9	67 3
3		70.0	73 8	68 9	70.0
4		71.1	67 4	73 2	71 3
5		69.9	70 1	67 2	67 7
6		17.4	17 6	17 5	17 4
3	–OCOMe		170.3		170 1
3	–OCOMe		21.2		20.9
4	–OCOMe			169 9	169 8
4	–OCOMe			20 9	20.6

*^{13}C NMR (250 MHz, DMSO-d_6)

$+H]^+$ was consistent with the structure of a monoacetylated dirhamnosyl patuletin. Moreover, the significant fragment at *m/z* 521 due to loss of one rhamnose, and the signal at *m/z* 333 due to further loss of monoacetylrhamnose, indicated the attachment of rhamnose at C-3 and monoacetylrhamnose at C-7. Acid hydrolysis of **2** gave acetylrhamnose (R_f 0.68) in addition to rhamnose (R_f 0.74) on TLC.

Upon partial hydrolysis of **2**, the monoglycoside obtained gave a yellow colour under UV_{366} on TLC, suggesting that the more easily hydrolysable sugar on the C-3 position was liberated. Comparison of ^1H and ^{13}C NMR spectra of **2** and the partially hydrolysed product, confirmed that signals for one rhamnosyl-Me disappeared, whereas signals for the acetyl group remained unchanged. The acetylated hydroxyl group was therefore assigned to C-3 of the rhamnose unit at C-7 of patuletin. The signal of δ73.8 in the ^{13}C NMR spectrum (Table 2) of **2** was 3.8 ppm downfield to the corresponding signal of **1** (δ70.0) confirming that the acetyl substituent was as suggested [15, 17]. Thus **2** is patuletin 3-*O*-rhamnosyl-7-*O*-[3-*O*-acetylrhamnoside].

Compound **3** In the FABMS spectra, the identical molecular ion and fragmentation pattern of **3** and **2** suggested that **3** was also a patuletin diglycoside containing a 3-*O*-rhamnose as well as 7-*O*-acetylrhamnose substituent. The chemical shifts of signals in the ^1H NMR spectrum (Table 1) for the aromatic protons (H-2′, H-6′, H-5′, H-8), the 6-methoxy protons, the anomeric protons of rhamnose, the two rhamnosyl-Me and one acetyl protons were all similar to that of the corresponding protons in **2**, indicating a structure similar to **2**. However, a characteristic triplet signal (16) at δ4.93 (J = 10) rather than a double doublet signal (δ5.03) as in **2** indicated that a hydroxyl group on one of the rhamnose units at C-4 was acetylated. Due to the presence of 7-O-acetylrhamnose as revealed in the FABMS spectrum and with further supportive evidence from the downfield shift of 2.1 ppm of C-4 of the rhamnose unit at C-7 of the aglycone (73.2) as compared to that of **1** (δ71.1) in the ^{13}C NMR spectra, the acetylated hydroxyl group was assigned to C-4 of the rhamnose at C-7 of the aglycone. The ^{13}C NMR spectrum of **3** (Table 2) confirmed carbon signals for patuletin, two rhamnose and an acetyl moiety. Therefore, **3** is patuletin 3-*O*-rhamnosyl-7-*O*[4-*O*-acetylrhamnoside]

Compound **4** Comparison of the UV, ^1H NMR and FABMS spectra of **4** with that of **2** and **3**, indicated that **4** was also a 3,7-di-*O*-rhamnoside of patuletin. In the ^1H NMR spectrum (Table 1), two signals were observed at δ2.08 and 2.07, indicating the presence of two acetyl protons. Two downfield shifted characteristic sugar protons, in which a double doublet signal at δ5.2, (1H, J = 2, J = 10 Hz) for H-3 of one of the rhamnose moieties and a triplet signal at δ5.15 (1H, J = 10 Hz) for H-4 were observed, indicating two of the hydroxyl groups at either C-3 or C-4 of either of the two rhamnose moieties was acetylated. After acid hydrolysis, the hydrolysate of **4** gave a diacetylrhamnose (R_f 0.52) in addition to rhamnose (R_f 0.74) on TLC.

Upon partial hydrolysis of **4**, the monoglycoside obtained gave a yellow colour under UV_{366} on TLC, suggesting that the sugar on the C-3 hydroxyl was released. The ^1H NMR spectrum of this partially hydrolysed compound revealed the loss of one of the two rhamnosyl methyl groups. However proton signals for the two acetyl groups were retained in the spectrum. Thus the hydroxyl groups on C-3 and C-4 of the rhamnose moiety at the C-7 hydroxyl of patuletin were acetylated. In the FABMS spectrum the molecular ion at *m/z* 709 $[M+H]^+$ showed one more acetyl substituent than either **2** or **3**. The mass fragment at *m/z* 563 due to loss of one rhamnose and *m/z* 333 due to further loss of diacetylrhamnose provided good evidence for this assignment. In the ^{13}C NMR spectrum (Table 2), the carbon signals for patuletin and those of 3-O-rhamnose moiety were identical to that in **3**. Chemical shifts assigned for acetylation of rhamnose at C-3 (δ71.37), C-4 (δ70.0) showed the expected lower field than the adjacent C-5 (δ67.7) and C-2 (δ67.3) protons and were therefore assigned to the C-7-*O*-rhamnose of the aglycone. Therefore **4** is patuletin 3-*O*-rhamnosyl-7-*O*-[3,4-*O*-diacetylrhamnoside]

Compound **5** The ^1H NMR spectrum of **5** (Table 1) was very similar to that of **4** except signals around δ5 were observed for downfield shifted sugar protons. Compound **5** was identified as a diacetylated patuletin 3,7-di-*O*-rhamnoside. The two characteristic signals for sugar protons appeared at δ4.97 (*dd*, J = 2, J = 10 Hz) and δ4.73 (*t*, J = 10 Hz), indicating acetylation at C-3 or C-4 of one or other of the rhamnose units. In the FABMS spectrum, a significant fragment mass at *m/z* 521 due to loss of monoacetylrhamnose from the molecular ion of *m/z* 709 $[M+H]^+$, indicated a 3-*O*-rhamnose bearing one acetyl group, the mass at *m/z* 333 due to loss of the second monoacetylrhamnose, was evidence that the other acetyl group was on 7-*O*-rhamnose. Further comparison of the ^1H NMR spectrum of **5** with that of **2**, indicated that the chemical shift of the signal at δ4.97 corresponded with the signal at δ5.03 assigned to H-3 of the acetylated C-3 of the C-7-*O*-rhamnose moiety in **2**, suggesting C-3 of the C-7-*O*-rhamnose moiety of **5** was also acetylated. Thus, the other acetyl group had to be at C-4 of C-3-*O*-rhamnose of patuletin. Thus **5** is patuletin 3-*O*-[4-*O*-acetylrhamnosyl]-7-*O*-[3-*O*-acetylrhamnoside].

Compound **6**. In the FABMS spectrum of **6**, the peak of molecular ion at *m/z* 709 $[M+H]^+$ and two major mass fragments at *m/z* 521, 333, were identical to those obtained with **5**, indicating that **6** is a patuletin glycoside containing a monoacetyl 3-*O*-rhamnose and a monoacetyl-7-*O*-rhamnose. In the ^1H NMR spectrum (Table 1) in the region of δ3.5–4.5, two signals at δ4.3 (1H, *dd*, J = 2, J = 5 Hz) and 4.4 (1H, *dd*, J = 2, J = 5 Hz) for two H-2 and two signals at δ3.6 (1H *t*, J = 10 Hz) 3.8 (1H *t*, J = 10 Hz) for two H-4 associated with rhamnose moieties were observed. In addition to two signals at δ5.6, 5.4 for the anomeric protons, the presence of two double doublet signals, downfield shifted at δ5.0 (1H, *dd*, J = 2, J = 10 Hz), 5.2 (1H, *dd*, J = 2, J = 10 Hz) for two H-3 clearly indicated that C-3 on both the C-3-*O*- and C-7-*O*-rhamnose moieties were acetylated. Thus **6** is patuletin-3-*O*-[3-*O*-acetylrhamnosyl]-7-*O*-[3-*O*-acetylrhamnoside].

Compound **7** In the FABMS spectrum of **7** the molecular ion appeared at *m/z* 751 $[M+H]^+$, indicating a compound with one more acetyl group than either **4**, **5** or **6**. The major fragmentation pattern showed loss of monoacetyl rhamnose from the molecular ion to give an ion at *m/z* 563 and subsequent loss of diacetylrhamnose to give an ion at *m/z* 333, the latter fragmentation being exactly the same as that of **4**. Thus **7** is an acetylated patuletin glycoside with one acetyl group on the 3-*O*-rhamnose and two acetyl groups on the 7-*O*-rhamnose. In the ^1H NMR spectrum (Table 1) signals at δ2.054, 2.458 and 2.10 indicated the presence of three acetyl substituents. In

addition to the signals at δ5.65, 5.51 for the anomeric protons of the sugar moieties, two downfield signals at δ5.34 (1H, *dd*, $J=2$, $J=10$ Hz) and δ5.24 (1H, *t*, $J=10$ Hz) were assigned to H-3 and H-4 of the C-7-*O*-rhamnose moiety, confirming the presence of a diacetyl 7-*O*-rhamnose moiety. The signals at δ4.31, 4.32 are easily identified as the H-2 of the C-3-*O* and C-7-*O*-rhamnosides and 3.9 for H-3 of the C-3-*O*-rhamnoside were easily identified. However the signal for a rhamnose H-4 which was shifted downfield (δ4.8, 4.9) despite some overlapped signals due to solvent had to be associated with the attachment of acetyl group on C-4 of the C-3-*O*-rhamnose moiety. Therefore, **7** is patuletin 3-*O*-[4-*O*-acetylrhamnosyl]-7-*O*-[3,4-diacetylrhamnoside].

Compound **8**. Comparison of the FABMS spectrum of **8** with that of **7** revealed that the molecular ion and fragmentation pattern was practically the same. In the ^{1}H NMR spectrum of **8** (Table 1) signals at δ2.05, 2.12 and 2.14 for three acetyl groups were exhibited. In the normal region of sugar protons (δ3.5–4.5) two double doublet signals at δ3.9 (1H, $J=2$, $J=10$ Hz), 4.41 (1H, $J=2$, $J=10$ Hz) for H-3 of the C-3-*O*- and C-7-*O*-rhamnose moieties and one double doublet signal at δ4.24 (1H, $J=2$, $J=5$ Hz) for H-2 of a rhamnose moiety were observed, whereas in the downfield region, besides the two signals at δ5.41, 5.68 for the rhamnose anomeric protons, two shifted triplet signals at δ4.9, 5.0 for the H-4 of both the C-3-*O*- and C-7-*O* rhamnose moieties were also present, indicating that both these carbons were acetylated. The indication of only one acetyl group on the rhamnose moiety at C-3 provided by MS analysis, suggested the other acetylated carbon had to be on a rhamnose moiety at C-7. The signal at δ5.34 (*dd*, $J=2$, $J=5$ Hz) was then assigned for H-2 of the C-7-*O*-rhamnose moiety. Therefore, **8** is patuletin 3-*O*-[4-*O*-acetylrhamnosyl]-7-*O*-[2,4-*O*-diacetylrhamnoside].

Compounds **9** and **10** were assigned to patuletin 3-*O*-rhamnoside and patuletin by comparison of the UV and ^{1}H NMR spectra with the published data (9, 12). These patuletin acetylated rhamnosides, found in conjunction with a similar range of kaempferol acetylated rhamnosides (currently under investigation), were the major flavonoids of this plant. The fact that the flavonoid glycosides isolated are almost exclusively rhamnosides with acetylation at C-3″ or C-4″ or both is of particular interest as they constitute a new group of flavonoids.

EXPERIMENTAL

Plant material. Stems and leaves of *Kalanchoe gracilis* Hance were collected in 1987 from the medicinal plant garden of the School of Pharmacy, National Taiwan University, Taipei, Taiwan and identified by Y. H. Tsen. Herbarium specimens are kept in the laboratory of Pharmacognosy, School of Pharmacy, Taiwan University.

General. TLC, which was routinely used to monitor each fraction, was carried out on silica gel 60 F_{254} (Merck) plates with the solvent systems: $CHCl_3$–MeOH–butanone–Me_2CO (12:4:2:1); $CHCl_3$–MeOH–butanone–H_2O (40:20:10:1). The spots were visualized under UV and exposed to NH_3 vapour. ^{1}H and ^{13}C NMR spectra were recorded with TMS as int. standard. UV spectra were recorded following standard procedures of ref. [14].

Extraction and isolation. 2.5 kg dried and powdered plant material was extracted with EtOH at room temp. After removal of the solvent under red pres H_2O was added and the crude extract was extracted successively with petrol, $CHCl_3$, EtOAc and *n*-butanol. The EtOAc extract (15 g) was chromatographed on a polyamide (400 g) column, eluting with $CHCl_3$, followed by increasing concentrations of MeOH. Fractions eluted with $CHCl_3$–MeOH 9 1 (Fr. A); 3:1 (Fr. B) and 1·1 (Fr. C) were rechromatographed on silica gel (230 mesh) column with $CHCl_3$–MeOH–butanone mixtures of increasing polarity. Fractions collected were analysed by TLC and further chromatographed over Sephadex LH-20 column with MeOH. Through repeated separation, Fr. A gave compounds **7** and **8**; Fr B gave compounds **2–6** and Fr. C gave compounds **1**, **9** and **10**.

Acid hydrolysis of **1**, **2** *and* **4**. A solution of each compound (5 mg) in 10% HOAc (5 ml) was heated on a water bath at 100° for 2 hr. During hydrolysis, the reaction was monitored at 10 min intervals by TLC of the hydrolysates on cellulose plates (pyridine–EtOAc–HOAc–H_2O = 36.36:7 31) using aniline phthalate as spraying reagent, followed by heating at 110° for 10 min. The aglycone and its monoglycoside were detected on silica gel plate ($CHCl_3$–MeOH–butanone–Me_2CO = 12·4·2 1) under UV_{366} and fumed with NH_3 vapour.

Compound **1**, *patuletin* 3,7-*di*-O-*rhamnoside*. UV λ_{max}^{MeOH} nm: 260, 268 sh, 352; (NaOMe) 267, 395; ($AlCl_3$) 278, 450, ($AlCl_3$ + HCl) 275, 305 sh, 360; (NaOAc) 267, 390; (NaOAc + H_3BO_3) 266, 390. FABMS *m/z* (rel. int.): 625 $(M+H)^+$ (30), 479 $[(M+H)-146]^+$ (46), 333 $[(M+H)-292]^+$ $(AH)^+$ (100), 317 $[A-15]^+$ (35). 189 $[AH-144]^+$ (15), 147 (30).

Compound **2** *patuletin*-3-O-*rhamnosyl*-7-O-[3-O-*acetylrhamnoside*]. UV λ_{max}^{MeOH} nm: 259, 268 sh, 360, (NaOMe) 266, 398; ($AlCl_3$) 280, 310, 450, ($AlCl_3$ + HCl) 278, 310, 360; (NaOAc) 268, 396; (NaOAc + H_3BO_3) 268, 396. FABMS *m/z* (rel. int.) 667 $[M+H]^+$ (26), 521 $[(M+H)-146]^+$ (44), 333 $[(M+H)-334]^+$ $(AH)^+$ (100), 317 $[A-15]^+$ (34), 189 (34), 147 (11).

Compound **3** *patuletin* 3-O-*rhamnosyl*-7-O-[4-O-*acetylrhamnoside*]. UV λ_{max}^{MeOH} nm: 258, 268 sh, 356, (NaOMe) 266, 400, ($AlCl_3$) 276, 446; ($AlCl_3$ + HCl) 278, 305, 358; (NaOAc) 266, 400; (NaOAc + H_3BO_3) 261, 400 FABMS *m/z* (rel. int.): 667 $[M+H]^+$ (60), 521 $[(M+H)-146]^+$ (45), 333 $[(M+H)-334]^+$ $[AH]^+$ (100), 317 $[A-15]^+$ (29), 189 (35), 147 (18).

Compound **4**, *patuletin* 3-O-*rhamnosyl*-7-O [3,4-O-*diacetylrhamnoside*]. UV λ_{max}^{MeOH} nm 260, 268, 358, (NaOMe) 266, 398; ($AlCl_3$) 280, 450; ($AlCl_3$ + HCl) 280, 360, (NaOAc) 266, 390; (NaOAc + H_3BO_3) 266, 390 FABMS *m/z* (rel. int.): 709 $[M+H]^+$ (9), 563 $[(M+H)-146]^+$ (26), 333 $[(M+H)-230]^+$ $[AH]^+$ (100), 317 $[A-15]^+$ (26), 189 $[AH-6]$, 147 (7).

Compound **5**, *patuletin*-3-O [4-O-*rhamnosyl*]-7-O-[3-O-*acetylrhamnoside*]. FABMS *m/z* (rel. int.). 709 $[M+H]^+$ (19), 521 $[(M+H)-188]^+$ (21), 333 $[(M+H)-376]^+$ $[AH]^+$ (100), 317 $[A-15]^+$ (21), 189 (100).

Compound **6** *patuletin*-3-O-[3-O-*acetylrhamnosyl*]-7-O-[3-O-*acetylrhamnoside*]. FABMS *m/z* (rel. int.) 709 $[M+H]^+$, 521 $[(M+H)-188]^+$ (11), 333 $[(M+H)-376]^+$ $[AH]^+$ (19), 317 $[A-15]^+$ (11), 189 (100).

Compound **7**, *patuletin*-3-O [4-O-*acetylrhamnosyl*]-7-O-[3,4-*diacetylrhamnoside*]. UV λ_{max}^{MeOH} nm: 258, 268 sh, 353; (NaOMe) 267, 400; ($AlCl_3$) 278, 308 sh, 448, ($AlCl_3$ + HCl) 275, 308 sh, 356; (NaOAc) 268, 394; (NaOAc + H_3BO_3) 268, 394. FABMS *m/z* (rel. int.): 751 $[M+H]^+$ (3), 563 $[(M+H)-188]^+$ (14) 333 $[(M+H)-418]^+$ $(AH)^+$ (100), 317 $[A-15]^+$ (29) 189 (49).

Compound **8**, *patuletin*-3-O-[4-O-*acetylrhamnosyl*]-7-O-[2,4-O-*diacetylrhamnoside*]. UV λ_{max}^{MeOH} nm 259, 268 sh, 356; (NaOMe) 268, 400; ($AlCl_3$) 278, 305 sh, 450, ($AlCl_3$ + HCl) 275, 308 sh, 356; (NaOAc) 268, 396; (NaOAc + H_3BO_3) 268, 396 FABMS *m/z* (rel. int.): 751 $[M+H]^+$ (10), 563 $[(M+H)-188]^+$ (11), 333 $[(M+H)-418]^+$ $(AH)^+$ (74), 317 $[A-15]^+$ (33), 189 (100).

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