4‴″-Acetylsagittatin A, a Kaempferol Triglycoside from *Kalanchoe streptantha*

S. S. Costa,† A. Jossang, and B. Bodo*

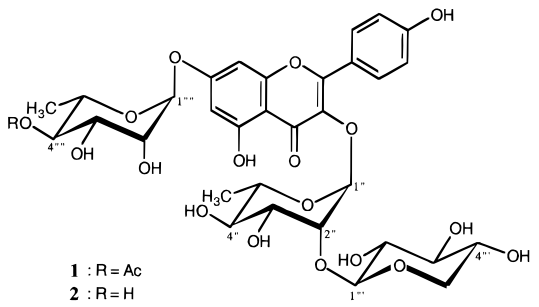
Laboratoire de Chimie, URA CNRS 401, Muséum National d'Histoire Naturelle, 63 rue Buffon, 75005 Paris, France

Received August 30, 1995[⊗]

The methanolic extract from the leaves of *Kalanchoe streptantha* (Crassulaceae) afforded a new kaempferol 3-*O*-*β*-xylopyranosyl-(1→2)-α-rhamnopyranoside 7-*O*-(4‴″-*O*-acetyl-α-rhamnopyranoside), named 4‴″-acetylsagittatin A (**1**), and the known sagittatin A (**2**). The structures were determined by ^{1}H-^{1}H and ^{1}H-^{13}C COSY NMR and FAB mass spectroscopy and confirmed by analysis of the peracetylated derivative.

Kalanchoe streptantha Baker (Crassulaceae) is one of the 59 *Kalanchoe* species reported in Madagascar.[1] Major constituents previously isolated from the genus were cytotoxic bufadienolides,[2,3] flavonoid glycosides,[4−6] and terpenoids.[7] We recently described the isolation from *K. brasiliensis* of five patuletin acetylrhamnosides, three of them showing strong inhibition of induced lymphocyte proliferation.[8]

In continuation of our research on immunomodulating substances from *Kalanchoe* species (syn. *Bryophyllum*), often used in traditional medicine for treatment of infections, tissue injuries, abscesses, and enlarged ganglia,[9,10] two flavonoids were isolated from *K. streptantha*: a new acetylated flavonoid triglycoside, named 4‴″-acetylsagittatin A (**1**), and the known sagittatin A (**2**)[11] which are described here.



Results and Discussion

The methanolic extract of the leaves of *K. streptantha* was chromatographed on a reversed-phase gel column (RP-2). Flavonoid-containing fractions were further purified on a Sephadex LH-20 column to afford compound **1** as the major component of the extract along with a smaller amount of **2**.

Compound **1** was isolated as an amorphous yellow powder and suggested to be a flavonol derivative from its UV spectrum. The molecular formula $C_{34}H_{40}O_{19}$ was deduced from the pseudomolecular ion at *m*/*z* 753.2244 on the HR FAB mass spectrum.

The ^{1}H NMR spectrum showed the presence of a kaempferol aglycon characterized by two doublet signals at *δ* 6.87 and 7.69 assigned to H-3′, -5′ and H-2′, -6′, respectively, and two doublet signals at *δ* 6.30 (H-6) and 6.56 (H-8) (Table 1). The presence of two rhamnopyranosyl units was suggested by two characteristic methyl doublet signals at *δ* 0.90 (*J* = 6.5 Hz) and 1.09 (*J* = 6.5 Hz) and two broad singlets corresponding to the anomeric protons at *δ* 5.38 and 5.53. An acetyl group was depicted from a sharp singlet signal at *δ* 2.11 (3 H). Each carbohydrate spin system was assigned by ^{1}H−^{1}H and ^{1}H−^{13}C COSY NMR experiments (Table 1). ^{1}H−^{13}C long-range correlations were observed in the heteronuclear long-range COSY spectra between H-1″ (*δ* 5.38) and C-3 (*δ* 136.8) and between H-1‴″ (*δ* 5.53) and C-7 (*δ* 162.8) and allowed to link the two rhamnopyranosyl units to the aglycon oxygen atoms at the 3- and 7-positions, respectively. The signal at *δ* 4.98 ppm having two large coupling constants (*J* = 9.0 and 9.0 Hz) was assigned to H-4‴″ of 7-*O*-rhamnopyranoside and was deshielded by the geminal acetoxyl group.

Five additional signals observed at *δ* 77.6, 75.0, 71.6, 68.8, and 107.4 indicated the presence of a third carbohydrate which was identified as a xylopyranosyl group by analysis of ^{1}H−^{1}H coupling constants of the peracetate derivative. The large coupling constant (7.5 Hz) observed for the anomeric H-1‴ (*δ* 4.23) indicated a *β*-configuration (Table 1). The long-range correlations observed between H-1‴ (*δ* 4.23) and C-2″ (*δ* 82.4) allowed us to link the *β*-xylopyranosyl moiety to the 2″-position of the 3-*O*-rhamnopyranosyl unit. This substitution pattern agreed with the deshielding effect observed for C-2″ signal (+11 ppm) as compared to the signal for C-2 of an unsubstituted 3-*O*-rhamnosyl.

These results led us to establish for the flavonoid **1** the structure of kaempferol 3-*O*-*β*-xylopyranosyl-(1→2)-α-rhamnopyranoside-7-*O*-(4‴″-*O*-acetyl-α-rhamnopyranoside). The fragmentations observed in the mass spectrum agreed with this structure: the loss of a xylopyranosyl unit led to the ion *m*/*z* 621 and was followed by loss of either the rhamnopyranosyl unit at C-3 to give the ion *m*/*z* 475 or the acetylrhamnopyranosyl unit at C-7 to give the ion *m*/*z* 433. Then, both ions lost the remaining sugar unit to form the protonated aglycon ion at *m*/*z* 287.

Flavonoid **2** was identified by ^{1}H−^{1}H and ^{1}H−^{13}C COSY experiments as kaempferol 3-*O*-*β*-xylopyranosyl-(1→2)-α-rhamnopyranoside 7-*O*- α-rhamnopyranoside, also known as sagittatin A, for which a complete ^{1}H and ^{13}C assignment was obtained (Table 1). This compound was previously isolated from *Epimedium sagittatum* (Berberidaceae).[11] The comparison of the ^{1}H and ^{13}C NMR data of **1** with those of sagittatin A (**2**) afforded further confirmation of the structure (Table 1). Chemical shifts were almost similar, and the acetylation of the hydroxyl group at C-4‴″ resulted in a lower field shift of H-4 (Δ*δ* 1.48 ppm).

† Current address: Nucleo de Pesquisas de Produtos Naturais, UFRJ, 21941 Rio de Janeiro, Brazil.

[⊗] Abstract published in *Advance ACS Abstracts,* March 1, 1996.

0163-3864/96/3259-0327$12.00/0

Table 1. ^{1}H NMR Data for 4′′′′-Acetylsagittatin A (**1**) and Sagittatin A (**2**) (CD_3OD, 300 MHz)

	1				**2**			
	δ_C	δ_H	m	*J* (Hz)	δ_C	δ_H	m	*J* (Hz)
kaempferol								
2	159.2				162.2			
3	136.8				136.8			
4	179.3				179.6			
5	162.5				162.8			
6	100.4	6.30	d	2.1	99.7	6.39	d	2.1
7	162.8				163.3			
8	95.4	6.56	d	2.1	95.5	6.65	d	2.1
9	157.5				157.8			
10	107.3				107.4			
1′	122.0				121.8			
2′	131.8	7.69	d	8.8	131.9	7.73	d	8.8
3′	116.5	6.87	d	8.8	116.7	6.92	d	8.8
4′	161.4				159.6			
5′	116.5	6.87	d	8.8	116.4	6.92	d	8.8
6′	131.8	7.69	d	8.8	131.9	7.73	d	8.8
3-*O*-rhamnosyl								
1′′	102.8	5.38	br s		103.0	5.43	br s	
2′′	82.4	4.17	br d	3.0	82.6	4.23	br d	3.3
3′′	71.7	3.80	dd	3.0; 9.0	72.0	3.86	dd	3.3; 9.5
4′′	73.4	3.28	dd	9.0; 9.0	73.5	3.27	dd	9.5; 9.5
5′′	70.8	3.38	dq	9.0; 6.5	71.8	3.65	dq	9.5; 6.1
6′′	17.6	0.90	d	6.5	17.7	1.01	d	6.1
3-*O*-xylosyl								
1′′′	107.4	4.23	d	7.5	107.6	4.32	d	7.5
2′′′	75.0	3.18	dd	7.5; 9.0	75.0	3.21	dd	7.5; 9.0
3′′′	77.6	3.57	dd	9.0; 9.0	77.7	3.35	dd	9.0; 9.0
4′′′	71.6	3.30	ddd	9.0; 9.0; 3.0	70.9	3.39	ddd	9.0; 9.0; 3.0
5′′′	68.8	3.05	dd	9.0; 9.0	67.0	3.10	dd	9.0; 9.0
5′′′		3.65	dd	9.0; 3.0		3.61	dd	9.0; 3.0
7-*O*-rhamnosyl								
1′′′′	99.4	5.53	br s		99.7	5.56	br s	
2′′′′	71.4	4.02	dd	3.0; 3.0	71.6	4.05	br d	3.3
3′′′′	69.8	3.95	dd	3.0; 9.0	72.0	3.86	dd	3.3; 9.4
4′′′′	74.9	4.98	dd	9.0; 9.0	73.5	3.50	dd	9.4; 9.4
5′′′′	66.9	3.63	dq	9.0; 6.5	71.2	3.57	dq	9.4; 6.0
6′′′′	17.8	1.09	d	6.5	18.1	1.27	d	6.0
4′′′′-OCO*Me*	21.4	2.11	s					
4′′′′-O*CO*Me	172.5							

Experimental Section

General Experimental Procedures. Column chromatography was carried out on silanized silica 60 gel (RP2) (70-230 mesh) Merck, on Lichroprep RP8 (40−63) Merck, and on Sephadex LH-20 (Pharmacia) using a H_2O/MeOH gradient. Elutions were checked by TLC (silica 60 F_{254}, Merck) using BuOH/AcOH/H_2O 8/1/1, as solvent system. TLC was visualized under UV (254 nm) and by spraying an ethanolic vanillin/sulfuric acid solution followed by heating. The $[\alpha]_D$ value was measured on a Perkin-Elmer 141 polarimeter, and IR spectra were registered on a Nicolet Impact 400 D spectrometer. NMR spectra were recorded on a Bruker AC 300 spectrometer (^{1}H, 300 MHz; ^{13}C, 75 MHz; CD_3OD or $CDCl_3$), with CHD_2OD (δ 3.313) and $CHCl_3$ (δ 7.24) signals as internal reference. Long-range ^{1}H−^{13}C COSY NMR spectra were obtained with J = 7 Hz. Positive FAB mass spectra were obtained on a ZAB-HF mass spectrometer.

Plant Material. *K. streptantha* Baker was cultivated in the tropical housegarden of the Arboretum National de Chèvreloup, Muséum National d'Histoire Naturelle, France and collected in spring 1994. A voucher specimen (*Allorge* 716, C-L 2448) is on deposit in the herbarium of the Muséum National d'Histoire Naturelle, Paris.

Extraction and Isolation. Fresh leaves (1.73 kg) were homogenized and extracted with MeOH exhaustively. The extract was concentrated to dryness under reduced pressure to afford a brownish material (47 g) which was dissolved in distilled water (80 mL) and chromatographed on a RP-2 column. Elution with a H_2O/MeOH gradient, starting with pure water, gave 18 fractions. The flavonoid-enriched fraction (3.93 g) was further purified on a Sephadex LH-20 column eluted with a H_2O/MeOH gradient, from 7/3 to pure MeOH, to yield three fractions (I−III). Fraction I (0.67 g) was rechromatographed on a RP-8 column eluted with a mixture of H_2O/MeOH 6/4 to afford sagittatin A (**2**) (70 mg). Fraction II (0.87 g) yielded crude 4′′′′-acetylsagittatin A (**1**) which was further purified on LH-20 Sephadex column to give pure (**1**) (0.83 g).

4′′′′-Acetylsagittatin A [Kaempferol 3-*O*-*β*-xylopyranosyl-(1→2)-α-rhamnopyranoside 7-*O*-(4′′′-*O*-acetyl-α-rhamnopyranoside)] (1): $C_{34}H_{40}O_{19}$; $[\alpha]^{21}_D$ −148° (c = 2.5, MeOH); IR (KBr) ν cm^{-1} 3434, 2926, 1735, 1659, 1598, 1450, 1376, 1254, 1214, 959, 833; UV (MeOH), λ_{max}, nm (log ϵ) 356 (4.15), 345 (4.21), 345 (4.09), 301 (4.09), 265 (4.37); positive FAB MS m/z (rel int) 753, 711 ([M + H]$^+$) (2), 621 (4), 517 (20), 475 (41), 433 (5), 382 (3), 360 (6), 327 (9), 287 (100), 286 (52), 271 (18), 252 (74), 221 (31), 197 (12), 140 (35); HRFABMS [M + H]$^+$ 753.2244 (calcd for $C_{34}H_{41}O_{19}$ 753.2242).

Acetylation of 1 by Ac_2O/Pyr and usual treatment yielded sagittatin A decaacetate: ^{1}H NMR ($CDCl_3$) δ 7.08 (1H, H-6), 6.75 (1H, H-8), 7.85 (2H, H-2′,

H-6′), 7.21 (2H, H-3′, H-5′), 5.53 (1H, H-1‴′, br s), 5.38 (1H, H-2‴′, dd, J = 2, 3.5 Hz), 5.42 (1H, H-3‴′, dd, J = 3, 10 Hz), 5.13 (1H, H-4‴′, dd, J = 10, 10 Hz), 3.89 (1H, H-5‴′, dq, J = 10, 6.5 Hz), 1.22 (3H, H-6‴′, d, J = 6.5 Hz), 5.41(1H, H-1″, s br), 4.37 (1H, H-2″, dd, J = 2, 3 Hz), 5.18 (1H, H-3″, dd, J = 3, 10 Hz), 4.84 (1H, H-4″, dd, J = 10, 10 Hz), 3.43 (1H, H- 5″, dq, J = 10, 6.5 Hz), 0.87 (3H, H-6″, d, J = 6.5 Hz), 4.56 (1H, H-1‴, d, J = 6.5 Hz), 4.88 (1H, H-2‴, dd), 5.10 (1H, H-3‴, dd, J = 9, 9 Hz), 4.85 (1H, H-4‴), 3.31 (1H, H-5‴, dd, J = 12, 8 Hz), 4.04 (1H, H-5‴, dd, J = 12, 5 Hz), 2.39 (3H, −OAc), 2.28 (3H, −OAc), 2.16 (3H, −OAc), 2.06 (3H, −OAc), 2.03 (6H, 2 × −OAc), 2.01 (6H, 2 × −OAc), 2.00 (3H, −OAc) and 1.94 (3H, −OAc).

Sagittatin A (2): ^{1}H and ^{13}C NMR (CD_3OD) (Table 1).

Acknowledgment. We gratefully acknowledge Mrs. L. Allorge-Boiteau for plant identification, Mr. J. Touche let for collecting plant material, and the “Centre d’Analyses du CNRS, Lyon” for the FABMS.

References and Notes

(1) Boiteau, P.; Allorge-Boiteau, L. *“Kalanchoe (Crassulaceae) de Madagascar*; Karthala: Paris, 1995.
(2) Wagner, H.; Lotter, H.; Fischer, M. *Helv. Chim. Acta* **1986**, *69*, 359 (1986).
(3) Yamagishi, T.; Haruna, M.; Yan, X. Z.; Chang, J. J.; Lee, K. H. *J. Nat. Prod.* **1989**, *52*, 1071.
(4) Gaind, K. N.; Singla, A. K.; Wallace, J. W. *Phytochemistry*, **1981**, *20*, 530.
(5) Liu, K. C. S.; Yang, S. L.; Roberts, M. F.; Phillipson, J. D. *J. Nat. Prod*., **1989**, *52*, 970.
(6) Liu, K. C. S.; Lin, Y. S.; Roberts, M. F.; Phillipson, J. D. *Phytochemistry* **1989**, *28*, 2813.
(7) Siddiqui, S.; Faizi, S.; Siddiqui, B. S.; Sultana, N. *Phytochemistry*, **1989**, *28*, 2433.
(8) Costa, S. S.; Jossang, A.; Souza, M. L. M.; Moraes, V. L. G.; Bodo, B. *J. Nat. Prod*., **1994**, *57*, 1503.
(9) Lucas, V.; Machado, O. *Rev. Flora Med.* **1946**, 77.
(10) Morton, J. F. *J. Ethnopharmacol.* **1990**, *29*, 245.
(11) Oshima, Y.; Okamoto, M.; Hikino, H. *Planta Med.* **1989**, *55*, 309.

NP960203U