[Chem. Pharm. Bull. 26(11)3594—3596(1978)]

UDC 547.918.02 : 543.422.25.06

Confirmation of the Structure of Tiliroside, an Acylated Kaempferol Glycoside, by ^{13}C-Nuclear Magnetic Resonance

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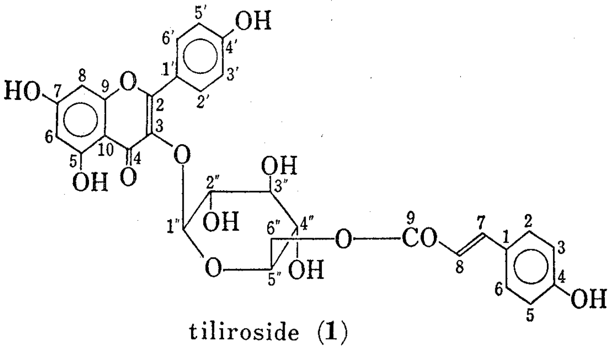
(Received June 13, 1978)

The structure of tiliroside was confirmed to be kaempferol 3-β-D-(6″-*p*-coumaroyl)-glucopyranoside (**1**), by ^{13}C-nuclear magnetic resonance.

Keywords——*Pteridium aquilinum*; flavonoids; tiliroside; tribuloside; ^{13}C-NMR

Tiliroside is an acylated glucoside of kaempferol, first isolated from *Rosa canina*[2] and *Tilia argentea*[3] and then from several Tiliaceae and other plants. The proposed structure,[3] 7-*p*-coumaroylkaempferol 3-β-D-glucoside, was doubted by the spectral data[4] and isolation of a *p*-coumaroylglucose by hydrolysis, showing identical *Rf* value with that produced from the anthocyanins, monardein and hyacinthin, suggested the attachment of the acyl residue on the sugar portion.[4] The acylated anthocyanin, monardein, was then proved to be a 6-*p*-coumaroylglucoside[5] and, accordingly, tiliroside was suggested to be kaempferol 3-β-D-(6″-*p*-coumaroyl)glucoside (**1**). This assumption was recently supported for the compound isolated from *Anaphalis contorta* by periodate titration analysis.[6] Meanwhile Indian workers[7] isolated a flavonoid, named tribuloside, from *Tribulus terrestris* and proposed the same structure, though they noticed the similarity but clear difference of the both compounds in mp and other properties.



tiliroside (**1**)

In our course of studies on the constituents of *Pteridium aquilinum*, a flavone glycoside was isolated and identified with the authentic sample of tiliroside[3] by every means.[8] The same compound was also obtained by Wang, *et al.* from the same source.[9] Since kaempferol derivatives are becoming noticeable by their mutagenicity with *Salmonella*/microsome test,[10–12]

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confirmation of the structure by ^{13}C-nuclear magnetic resonance which had been proved to be the best guide for the structures of acylated glycosides.[13,14]

Experimental conditions and results obtained by kaempferol, astragalin (kaempferol 3-β-D-glucopyranoside), and tiliroside (**1**) are shown in Table I. The assignments were based on the reported data on the related compounds[13–18] and the acylation shifts observed on C_5 and C_6 of the glucose residue clearly indicated the attachment of the acyl group at the 6-position. Thus the ambiguity existed in the structure of tiliroside (**1**) has been excluded. Although the direct comparison with the sample of tribuloside has not been carried out because of nonavailability of the specimen, further study on the compound is assumed to be required. The structure of the same type of an acylated glucoside of quercetin was established by X-ray analysis.[19]

TABLE I. Carbon Chemical Shifts (δ_C) from Internal TMS[a]

Carbon		Kaempferol (K)	Astragalin (A)	Tiliroside ($\Delta\delta_C$)[b] (T)
	2	146.7	156.3	156.3
	3	135.7	133.2	133.1
	4	175.8	177.5	177.4
	5	156.2	156.3	156.3
	6	98.2	98.6	98.7
	7	163.8	164.1	164.1
	8	93.4	93.6	93.6
	9	160.7	161.1	161.1
	10	103.0	104.0	103.9
	1′	121.7	120.9	120.8
	2′,6′	129.5	130.8	130.8
	3′,5′	115.4	115.0	115.0
	4′	159.2	159.8	159.9
Glucosyl	1″		101.0	101.0
	2″		74.2	74.2
	3″		76.4	76.2
	4″		69.9	69.9
	5″		77.4	74.2 (−3.2)
	6″		60.9	63.0 (+2.1)
p-Coumaroyl	1			124.9
	2,6			130.1
	3,5			115.7
	4			159.7
	7			144.5
	8			113.6
	9			166.1

a) Measured in DMSO-d_6 at the concentrations of 80 mg/ml (K), 75 mg/ml (A) and 100 mg/ml (T), at 25°. The spectrometer (JEOL PFT-100, equipped with EC-6 computer) was operated at 25.15 MHz with a 22 μ sec (90°) pulse every 3.0 sec (1000 scans). Computer limited resolution = ±0.1 ppm (4096 data points for 5 KHz).

b) $\Delta\delta_C = \delta_C(T) - \delta_C(A)$; significant shifts only.

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After completion of the work we learned that the same conclusion was obtained for the same compound by the same method.[20)]

Acknowledgement We thank Prof. H. Wagner for the sample of tiliroside and the information, and Prof. S. Rangaswami for his efforts to obtain the sample of tribuloside.

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