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Isolation and Structure of Clematine, A New Flavanone Glycoside from *Clematis armandii* Franch

Yingfong Chen[a], Jian Liu[b*], R. Stephen Davidson[b], Oliver W. Howarth[c]

[a] Department of Pharmacy, Hunan College of Traditional Chinese Medicine, Changsha, Hunan, P. R. of China

[b] The Chemical Laboratory, University of Kent at Canterbury, Canterbury, Kent CT2 7NH, U. K.

[c] Department of Chemistry, University of Warwick, Coventry CV4 7AL, U. K.

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Abstract: A novel flavanone glycoside has been isolated from the Chinese crude drug "*mu tong*" which is used in Hunan province, China. The structure of this new natural product is identified as 5,4′-dihydroxy-3′-methoxyflavanone-7-(6"-O-β-L-rhamnopyranosyl)-β-D-glucopyranoside by ^{1}H, ^{13}C NMR, COSY, ^{1}H,^{13}C HETCOR, LOCOR and 1D NOE-difference spectra.

Introduction

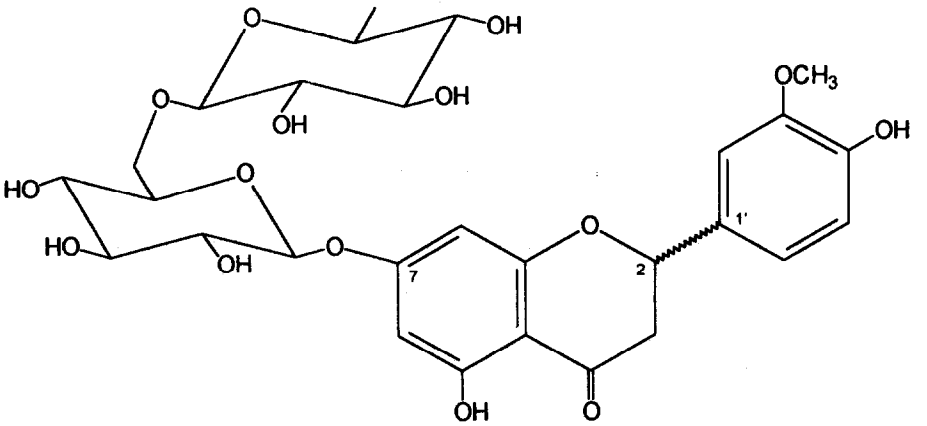
Some plants of the *Clematis* genus are acrid and poisonous, and are used as folk medicines in India and China. In India the leaves of *Clematis montana* are used in skin diseases and the seeds have purgative properties [1, 2]. A new triterpenic bisglycoside has been isolated from this plant [3]. The roots of the *clematis chinese* is a source of the Chinese crude drug "*wei ling xian*" which has been used as an analgesic, diuretic and anti-inflammatory agent [4]. Recently, a new triterpenoid saponin has been isolated from it [5]. Plants of this genus *Clematis armandii* and *Clematis montana* all are used as "*mu tong*" in traditional Chinese medicines [6]. Although some research has been conducted on *C. montana*, and leading to the isolation of a triterpenoid and its glycoside [3], no research has been concerned to *C. armandii* so far.

The Chinese crude drug "*mu tong*" (Ranunculaceae) is widely used in traditional Chinese medicine as a diuretic. But different plants from the Ranunculaceae family are used as "*mu tong*" in different parts of China. In Hunan province, *Clematis armandii* is used as "*mu tong*" in all hospital clinics that prescribe traditional Chinese medicines. As part of a project to clarify the relationship between the two kinds of "*mu tong*", we have studied the chemical constituents of *C. armandii*. In a previous research report [7] concerning the genus *Clematis*, alkaloids, triterpenoids and

flavanones were found to be biologically active constituents. During the course of our research, a new flavanone glycoside was isolated and its structure is here identified as 5,4′-dihydroxy-3′-methoxyflavanone-7-[6"-1"′-rhamnosyl]glucoside. We describe the isolation and structure determination of this new natural product below.

Results and Discussion

Ground *C. armandii* was extracted with methanol and clematine was obtained by direct crystallization, and recrystallization from ethanol.



Clematine

Clematine 1; $C_{28}H_{34}O_{15}$, (found: C; 53.31, H; 5.80, calc.: C; 55.08, H; 5.57); mp: 263-265 °C; FAB/MS, m/z 611 (M^++1). The UV spectrum, λ_{max} (MeOH) 284 and 331 nm, indicate a chromophore of dihydroflavanones. The IR spectrum (KBr) shows the presence of hydroxyl groups ($\tilde{\nu}$ 3578, 3476 and 1000-1100 cm^{-1}), a carbonyl group ($\tilde{\nu}$ 1646 cm^{-1}), benzene rings ($\tilde{\nu}$ 1606, 1518 cm^{-1}).

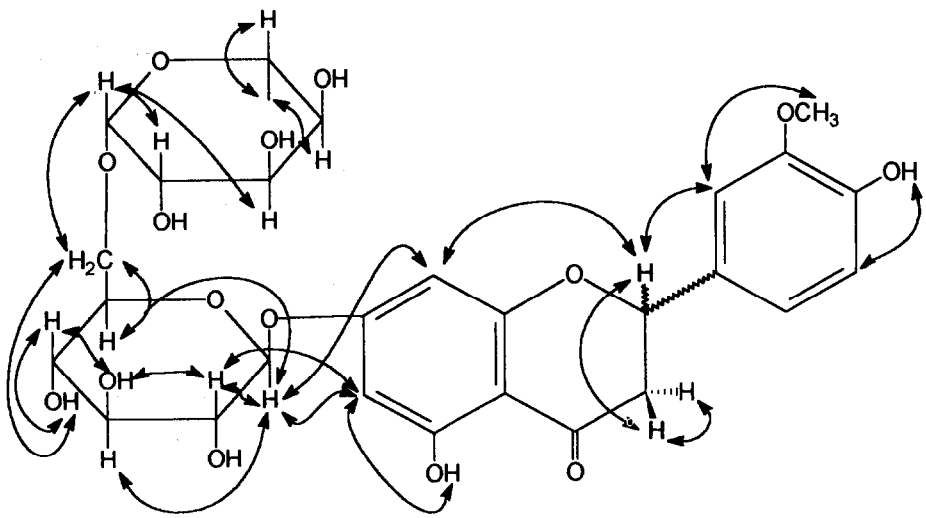
The ^1H NMR of 1 shows a singlet (3H) at δ 3.77 which was assigned to a methoxy group. Two hydroxy protons were observed at δ 12.02 and 9.10 ppm as singlets, and they could be assigned to 5-OH, 4′-OH or 3′-OH, respectively. A doublet and a double doublet δ 6.94 and 6.90 ppm were assigned to 5′- and 6′-H respectively, while 2′-H appears as a doublet (2Hz) at δ 6.93 ppm. Two doublets each with J=2.4 Hz appear upfield at δ 6.14 and 6.13 ppm and are assigned to 8-H and 6-H, respectively. At this stage, a flavanone skeleton seems probable. The ^{13}C NMR spectrum supports this proposal. In particular, it shows a ketone resonance at δ_c 196.95 ppm, a methoxy resonance at 55.77 ppm, and methylene and a methine resonances

at δ_C 42.08 and 78.37 ppm. They were assigned to 3-C and 2-C of the flavanone moiety by means of a $^1H,^{13}C$ HETCOR spectrum. The resonances of twelve aromatic carbons appear at δ_C 165.18, 163.05, 162.51, 148.00, 146.53, 130.98, 117.91, 114.20, 112.21, 103.37, 96.44, 95.59 ppm. They are completely assigned (Table 1) *via* HETCOR and LOCOR spectra. The proton spin systems 2-3-3 and 5′-6′ of clematine could be identified by a $^1H,^1H$ COSY spectrum and by their characteristic shift and coupling patterns. Also, the double doublet at δ 5.51 ppm could also be assigned to 2-H by comparison with other flavanones [8]. In the 2D spectrum it showed cross peaks with methylene protons at δ 3.32 (3Hz) and 2.76 ppm (12Hz), which were therefore assigned to 3-H_{eq} and 3-H_{ax}. The EIMS shows a characteristic fragment ion at m/z 124, suggesting that the B ring has one hydroxyl and one methoxy substitution. A fragment ion at m/z 179 derived from the A ring, possibly formed after loss of the glycosidyl moiety, shows the presence of 5,7-dihydroxyl substitution on the A ring. Thus the skeleton of the aglycone moiety could be set as 5,7,4′-trihydroxy-3′-methoxyflavanone, or 5,7,3′-trihydroxy-4′-methoxyflavanone.

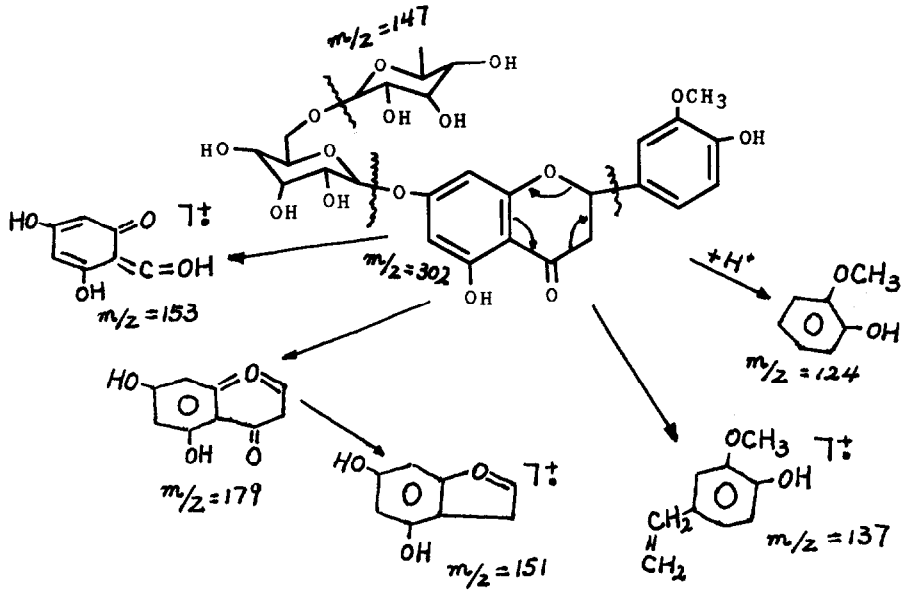
The remaining problem is to identify the position of the hydroxy and methoxy groups of B ring. This required evidence from heteronuclear experiments, notably the LOCOR technique. Apart from carbons 4 and 2, which show characteristic shifts, initial ^{13}C assignments were done by using the HETCOR spectrum in conjunction with the proton shifts already identified. This allowed assignment of carbons 2, 3, 6, 8, 2′ and 5′, 6′. Evidence of long range ^1H–^{13}C coupling from the LOCOR spectrum was then used to identify the position of B ring methoxy group. A cross peak from the methoxy proton to 3′-C in the LOCOR spectrum indicated that the methoxy substituent was at C-3′. Further evidence that the OCH_3 is at C-3′ was provided by NOE enhancements observed for 2′-H, and 5′-H upon irradiation of 3′-OCH_3 or 4′-OH respectively. Thus, the methoxy located at C-3′ was confirmed, and the possibility of a hydroxy located at C-3′ was excluded, even though the partial overlap of the 2′-H and 5′-H resonances made this measurement difficult. Thus the skeleton of the aglycone was unambiguously set as 5,7,4′-trihydroxy-3′-methoxyflavanone.

Six doublets appear respectively at δ 5.41, 5.19, 5.18, 4.69, 4.62 and 4.49 ppm, and disappear following equilibration of the sample with D_2O. They were therefore assigned to six hydroxy groups of the glycosidyl part of clematine molecule. These hydroxy groups were also identified by the

sensitivity of their ^{1}H-NMR shifts to temperature and *via* saturation transfer. Each hydroxy group was assigned by COSY-45° experiments and additional homonuclear decoupling. Analysis of the proton-proton connectivities derived from the COSY plot yielded two sugar structural fragments. The first sugar fragment is glucosyl and second one is rhamnosyl. For the glucosyl fragment, a doublet at δ 4.97 ppm was assigned to 1"-H *via* NOE difference and COSY spectroscopy, as described below. In the ^{1}H,^{1}H COSY spectrum, it gave a coupling cross-peak to 2"-H (δ 3.21). COSY cross peaks also connected the glucosyl protons at δ 5.41 and 3.21 (2"-OH and 2"-H), 5.19 and 3.15 (4"-OH and 4"-H), also 5.18 and 3.25 (3"-OH and 3"-H). Thus three of the six hydroxy groups could be assigned to 2"-, 4"-, and 3"-OH of the glucosyl moiety. Similarly, the other three hydroxy group signals were assigned to 2"′-, 3"′-, and 4"′-OH (δ 4.62, 4.49, and 4.69 respectively) of the rhamnosyl moiety, *via* COSY. The rhamnosyl proton spin system is established by cross peaks between proton signals at δ 4.52 and 3.63, 3.63 and 4.62, 3.43 and 4.49, also between 4.69 and 3.17, 1.08 and 3.41 ppm. Thus the singlet at δ 4.52 could be assigned to 1"′-H of the rhamnosyl part, and the multiplet at δ 3.41 ppm could be assigned to 5"′. The ^{1}H,^{13}C HETCOR spectrum then gave the carbon assignments. An L-rhamnosyl configuration is assigned, by analogy with other plant materials.



NOE observed for clematine



Scheme I

The position of the interglycosidic linkage could be identified from the ^{13}C NMR spectrum. The glucosyl moiety had very similar chemical shifts as in luteolin-7-O-glucoside [9], except that $C_{(5)}$ and $C_{(6)}$ were shifted upfield by 1.7 ppm and downfield by about 5 ppm, respectively. This indicated the attachment of rhamnose at the 6-hydroxyl of this glucose. The rutinosyl was confirmed by comparison with the ^{13}C-NMR data of pseudobaptisin and hesperidin [11]. The position of the glycosidic linkage was determined by the super-imposition of the ^{13}C NMR chemical shifts for the 2-C and 8-C of the aglycone [10] and ^{1}H NMR data for ring A protons in other flavanone 7-glycosides [11]. Further evidence supporting the 7-O-$C_{(1)}$ glycosidic linkage was provided by 1D NOE experiments. In the NOE spectrum a NOE enhancement was observed at the anomeric proton 1"-H of the glucosyl moiety after pre-irradiation of 6-H and 8-H at δ 6.12 and 6.14 ppm. Thus the structure of clematine was elucidated as 5,4'-dihydroxy-3'-methoxyflavanone-7-O-(β-L-rhamnosyl)-β-D-glucoside.

An earlier report have determined the commercial material hesperidin, which is synthetic compound, as 5,4'-dihydroxy-3'-methoxyflavanone-7-O-rutinoside [11], now it has been proved to 5,3'-dihydroxy-4'-methoxyflavanone-7-O-rutinoside [12]. By comparison the ^{13}C-

NMR data of clematine and hesperidin, it shows some differences between 2′-C and 6′-C, also 7-C and 9-C. For clematine, the resonance of 2′-C is at δ 117.91 ppm while 6′-C signal appears at δ 114.20 ppm. For hesperidin their appear at 114.3 and 117.8 ppm, respectively. These chemical shift differences could be explained by magnetic anisotropy from 3′-OCH_3.

Table 1: NMR spectral data for climatine

No.	δ_C	δ_H		$J_{H,H}$(Hz)	LOCOR
2	78.37	5.51	dd	$^3J_{2,3ax}$ 12, $^3J_{2,3eq}$ 3	1′, 2′, 6′, 3
3	42.08	H_{ax} 3.32	dd	$^3J_{3ax,2}$ 12, $^3J_{3,3}$ 17	1′, 2, 4
3		H_{eq} 2.76	dd	$^3J_{3eq,2}$ 3, $^3J_{3,3}$ 17	4
4	196.95				
5	162.51				
6	96.44	6.14	d	2	7, 5, 10, 8
7	163.00				
8	95.59	6.12	d	2	7, 5, 6, 9
9	165.18				
10	103.37				
1′	130.98				
2′	117.91	6.92	d	2	2, 3′ 4′, 6′
3′	146.53				
4′	148.00				
5′	112.21	6.93	d	10	1′, 3′, 6′
6′	114.20	6.94	dd	10, 2	2, 1′, 4′
3′-OCH_3	55.77	3.77	s		3′
5-OH		12.02	s		7, 10, 6
4′-OH		9.10	s		
1″	99.55	4.97	d	8	
2″	73.02	3.21	dd	9, 9	
3″	76.34	3.25	dd	9, 9	
4″	69.66	3.15	dd	9, 9	
5″	75.59	3.53	t	10	
6″	66.09	H_a 3.80	d	10	
		H_b 3.38	d	10	
2″-OH		5.41	d	5	
3″-OH		5.19	d	5.5	
4″-OH		5.18	d	5.5	
1‴	100.63	4.52	s		3‴, 5‴
2‴	70.77	3.63	t	5	
3‴	70.30	3.43	dd	5, 5	
4‴	72.13	3.17	dd	5, 8	
5‴	68.33	3.41	m		
6‴	17.82	1.08	d	6	5‴
2‴-OH		4.62	d	4	
3‴-OH		4.49	d	6	
4‴-OH		4.69	d	5	

Chemical shifts in ppm from int. ref. TMS, Solvent: DMSO-d_6.

The high resolution EIMS give ions at m/z 303, 302, 285, 179, 165, 153, 151, 150, 147, 137, 124 consistent with the fragmentation shown in scheme I. This evidence confirm the structure.

From these data the structure of clematine 1 was unambiguously identified as 5,4′-dihydroxy-3′-methoxyflavanone-7-O-(1‴-6″-O-β-L-rhamnopyranosyl)-β-D-glucopyranoside.

Conclusion: From this work it can be seen although Plants *C. montana* and *C. armandii* are used as "*mu tong*" in traditional Chinese medicines, they contain different chemical components. For *C. montana* the triterpenoid and its glycoside are major components, but *C. armandii* contains flavanone glycoside as its major component. Why two plants, which contain respectively different components, are used as same crude drug in clinical practices at China and what is the biologically active constituent still remain unknown.

Experimental

The melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ACP-400 spectrometer at 400 MHz in a 5 mm 4-nuclus probe at 297 K, using standard Bruker software. Carbon-atom types were established in the ^{13}C NMR spectrum by using a combination of broad band proton-decoupling and distortionless enhancement by polarisation transfer (DEPT) experiments. UV spectrum was recorded on a Philips PU 3740 UV/Vis Scanning spectrophotometer, and the IR spectrum was recorded on a Perkin-Elmer 983 spectrometer. FAB-MS was recorded with the negative ion source at 8.3 KeV, and with 3-nitrobenzyl alcohol as matrix, EI-MS; 70 eV.

Plant material was purchased from Hunan Material Medicine Ltd.

Isolation of clematine: The air-dried aerial parts of *Clematis armandii* were extracted by ethanol (95%). The alcohol solution was then treated by using charcoal and concentrated, and kept overnight at room temperature. Crude clematine could be obtained by direct crystallisation after filtration and drying. This solid was recrystallised from methanol to yield clematine 1 as white powder, yield; 1.5%. mp: 263-5 °C. UV: λ_{max} (MeOH), 284, 331 nm; IR: $\tilde{\nu}_{max}$ (KBr), 3548, 3476, 2918, 1646, 1606, 1518, 1444, 1340, 1300, 1277, 1206, 1133, 1096, 848, 816, 798, 744 cm^{-1}. ^{1}H and ^{13}C

NMR: Table I. FAB-MS: m/z (rel. int.) 611 (M^++1, 16), 399 (45), 303 (42), 289 (33), 157 (100), 137 (43). HREI-MS: m/z (rel. int.) 303 ($C_{16}H_{14}O_6$+1, 18), 302 ($C_{16}H_{14}O_6$, 86), 300 (33), 285 ($C_{16}H_{13}O_5$, 7), 191 (7), 180 (6), 179 ($C_9H_7O_4$, 25), 165 (12), 153 ($C_7H_5O_4$, 61), 150 ($C_9H_{10}O_2$, 100), 137 (96), 135 (63), 124 ($C_7H_7O_2$+1, 24), 73 (48).

References

[1], Hooker, J. D; Flora of British India, Vol. 1, L-Reave C., Kent; **1975**, pp. 2-3

[2], Kirtikar, K. R.; Basu, B. D.; Indian Medicinal Plants, Vol. 1, M/S Periodical Experts, New Delhi; **1975**, pp. 3

[3], Bahuguna, R. P.; Jangwan, J. S.; Kaiya, T.; Sakakibara, J.; Phytochemistry, **1989**, 28, 2511-2513

[4], The Pharmaceutical Institute Chinese Academy of Medical Sciences ed.; Zhong Yao Zhi, Vol. 1, Renming Weishen publishing House, Peking, **1973**, pp. 328

[5], Sati, O. P.; Uniyal, S. K.; Bahuguna, S.; Kikuchi, T.; Phytochemistry, **1990**, *29*, 3676-3678

[6], a, Xie M. C. ed.; Chinese Material Medicines, Cheng Chung Book Co. Ltd., **1987**, pp. 216

b, The Pharmaceutical Dictionary of China, Colour Pictures of Chinese Crude Drugs; Gungdong Sciences & Technology Publishing House, **1990**, pp. 33

[7], a, Slavik, J.; Bochorakova, J.; Slavikova, L.; Collect. Czech. Chem. Commun. **1987**, *52*, 804-812

b, Lebreton, P.; Plant Med. Phytother. **1986**, *20*, 275-286

[8], Iinuma, M.; Ohyama, M.; Tanaka, T.; Mizuno, M.; Hong, S.-K.; Phytochemistry; **1992**, *31*, 2855-2858

[9], Markham, K. R.; Ternai, B.; Stanley, R.; Geiger, H.; Mabry, T. J.; Tetrahedron **1978**, *34*, 1389-1397

[10], Aquino, R.; Ciavatta, M. L.; De Simone, F.; Pizza, C.; Phytochemistry; **1990**, *29*, 2358-2360

[11], Markham, K. R.; Ternai, B.; Tetrahedron; **1976**, *32*, 2607-2612

[12], a, Aldrich Chemical Co. Ltd. Aldrich Catalogue, **1992**, pp. 692

b, Fluca Chemical Ltd. Fluca Catalogue, **1993**, pp. 704