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LACTIFLORIN, A MONOTERPENE GLYCOSIDE FROM PAEONY ROOT

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Key Word Index—*Paeonia anomala* var. *intermedia*; Paeoniaceae; roots; monoterpene glycoside; lactiflorin; NMR; herbal medicine.

Abstract—The structure of the unique monoterpene glycoside, lactiflorin, a constituent of several species of *Paeonia* with medicinal properties, has been determined by spectroscopic techniques.

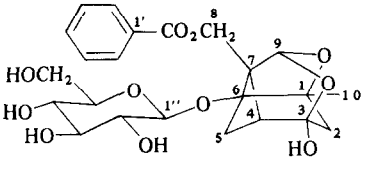
INTRODUCTION

Paeoniae Radix (Shaoyao) is one of the most important herbal drugs used in traditional Chinese medicine and is derived from the roots of several herbaceous species of Paeoniaceae. Significant chemical and pharmalogical investigations have been conducted on the roots of two species of *Paeonia*, *P. lactiflora* Pall and *P. suffruticosa* Andr., which are the main sources of the herbal medicine in China. So far 10 monoterpene compounds have been isolated [1–10] and these compounds have been shown to have anticoagulative [11], sedative [12] and antiinflammatory [13] activities, as well as a blocking effect on the neuromuscular junction [14]. A number of these compounds have a cage-like skeleton [1–10] as exemplified by paeoniflorin (**1**).

Lactiflorin, a new but related monoterpene glycoside, was first isolated from *P. lactiflora* by Lang Huiying *et al.* in 1983 [15]. The structure of this compound was tentatively assigned as **2**, but details of the structural elucidation were not reported. We have now isolated this compound from the related species *P. anomala* var. *intermedia* (C. A. Mey.) O. & B. Fedtsch. and its structure was elucidated as follows.

RESULTS AND DISCUSSION

Lactiflorin (**3**), $C_{23}H_{26}O_{10}\cdot H_2O$, mp 207–209° crystallized from chloroform–methanol as fine needles. A FAB mass spectrum showed it has M_r 462 (*m/z* 485 $[M+Na]^+$ and 463 $[M+1]^+$) and the IR spectrum exhibited the presence of hydroxy groups (3544, 3484 cm^{-1}) and two carbonyl groups (1746, 1728 cm^{-1}) which were assigned to ketonic and ester carbonyl groups, respectively. The presence of these groups was further confirmed by the



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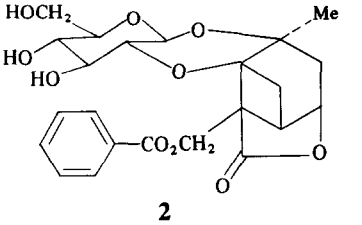

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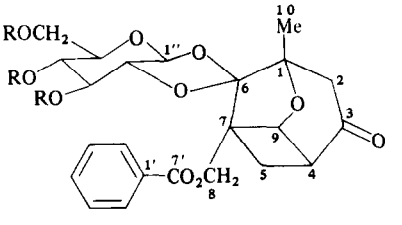
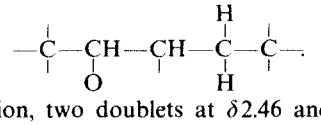


3 R = H
4 R = Ac

^{13}C NMR spectrum (216.4, 166.5 ppm). In the ^{1}H NMR spectrum (300 MHz) the five benzoate protons in the aromatic region and the methyl protons of C-10 at the highest field (1.45 ppm, 3H, *s*) were readily recognized. The assignment of the anomeric proton of the glucose moiety (4.65 ppm, *d*, *J* 8.1 Hz, $H_{1''}$) and the methylene protons at C-8 (4.79, *s*, 2H) could also be made. The homonuclear decoupled ^{1}H NMR spectrum showed that the signals at 2.15, 2.48 and 2.79 ppm consisted of a CH_2CH system. A further methine signal at 4.92 was coupled with the methine proton of the CH_2CH system, and from its chemical shift is likely to be connected to an oxygen functionality. Thus, lactiflorin would appear to contain the following moiety in the aglycone,



In addition, two doublets at δ2.46 and 2.80, which overlapped the signals at 2.48 and 2.79, respectively, comprised an AB system with J_{gem} 18 Hz. These two protons did not show further coupling and were presumed to be connected to two quaternary carbons.

The proton decoupled ^{13}C NMR spectrum of **3** exhibited signals for all 23 carbons. In particular it showed a ketonic carbonyl carbon (δ216.4) and a methyl carbon (δ16.4). These data confirmed that **3** was an analogue of the other monoterpene glycosides present in Paeoniae Radix because all contained a benzoate moiety, a glucose unit (the presence of which in **3** had been established previously [15]) and a C_{10} monoterpene aglycone.

Acetylation of **3** with acetic anhydride and pyridine afforded the corresponding triacetate (**4**) rather than tetraacetate. This formulation was confirmed by the FAB mass spectrum [*m/z* 589 $[M+1]^+$ (463+126)], the ^{1}H NMR (three acetate methyl groups at δ1.98, 2.03, 2.05) and the ^{13}C NMR spectra. Thus, it was established that there were only three free hydroxy groups present in the sugar moiety. The homonuclear decoupling experiment of **3** showed that the signal due to the glucose 2″-proton (which couples with the anomeric proton) occurred at δ3.15, and did not shift as significantly as other protons of glucose moiety after acetylation. All of these data indicated that the glucose residue had two connections (via C-1″, C-2″) with the aglycone moiety. The ^{1}H NMR spectrum of the triacetate (**4**) exhibited well-separated signals for all the glucose protons and the homonuclear decoupling experiments enabled their full assignment (H-1″–H-6″).

DEPT (distortionless enhancement by polarization transfer) is a technique for multiplet selection of carbons as an aid to spectral interpretation. It is based on polarisation transfer in a heteronuclear *J*-coupled spin system and can be used for assigning multiplicities to all lines in a broadband decoupled ^{13}C NMR spectrum [16–18]. It was applied to lactiflorin triacetate (**4**) and showed that there were nine quaternary carbon atoms, 12 methine carbons, four methylene carbons and four methyl carbon atoms present in this compound. Of the nine quaternary carbons, the signals at δ170.6, 170.0 and 169.7 were due to the three acetyl carbonyl carbons, the δ166.1 signal was due to the benzoyl carbonyl carbon and the δ128.9 signal was due to C-1′ in the benzoate moiety. The remaining four quaternary carbons belonged to the aglycone moiety. The signal at δ215.6 was assigned to the ketonic carbonyl carbon and the signal at δ103.1 was due to a quaternary carbon connected to two oxygen functions and appeared to be due to the carbon atoms bonded to the C-1″ and C-2″ oxygen atoms of the glucose moiety. For the 12 methine carbons, three peaks in the region around δ130 were assigned to aromatic ring carbons (a total of five carbons), while the other five were due to the glucose ring carbons. The signal at δ95.0 was assigned to the glucose C-1″ and occurred at higher field than a normal anomeric carbon of a glucoside [17–20] as a consequence of its being a component of a dioxolane ring. The remaining four methine carbons in the sugar nucleus resonated between δ80.7 and 68.3. The aglycone moiety exhibited two methine carbon signals. The signal at δ37.5 was due to a methine carbon not connected to an oxygen function, but the other methine carbon at δ80.7 appeared to be so. Among the four methylene carbons, two signals were observed at *ca* δ62. One of these was due to the C-6″ of the glucose moiety and the another to the C-8 methyleneoxy group connected to the benzoate unit. The other two such signals at δ47.3 and 30.7, originated from the aglycone and were unlikely to be connected to any oxygen functions. The signal at δ16.1 was due to the C-10 methyl carbon and the other three methyl signals were assigned to the acetate groups.

The HETCOR (heteronuclear correlation) pulse sequence is one of the 2-D NMR techniques which is designed to establish the direct connections between bonded nuclei ($^{1}J_{x,h}$) [16–18]. The 2D NMR spectra of lactiflorin triacetate (**4**) and lactiflorin (**3**) enabled the complete assignment of all ^{1}H and ^{13}C signals in both compounds, and were consistent with the structures assigned. In the spectrum of lactiflorin triacetate (**4**), C-9 gave rise to a signal at δ80.7 in the ^{13}C NMR spectrum and was attached to one proton which appeared as a broad singlet at δ4.97 in the ^{1}H NMR spectrum. The signals at δ73.8, 72.3, 71.3 and 68.3 were assigned to C-5″, C-2″, C-3″ and C-4″ of the glucose unit, respectively, according to their relationships with the ^{1}H NMR spectrum. For the aglycone, C-2 (δ47.3, CH_2) was connected to two protons which gave rise to a singlet at δ2.65; C-4 (δ37.4, CH) was attached to one proton and this proton coupled with the two H-5 protons and appeared as a double doublet at δ2.72. For the two protons on C-5 (δ30.6, CH_2), one appeared as triplet at δ2.66 (5β-H) and the other as double doublet at δ2.25 (5α-H) while of the two quaternary carbons, the one at δ85.6 was assigned to C-1, the other at δ55.2 to C-7. From a comparison of the 2-D HETCOR and the ^{1}H NMR spectra of **3** we could deduce that the two C-2 protons appeared as doublets at δ2.80 and 2.46 (2β-H and 2α-H), respectively, with J_{gem} = 18 Hz. As expected the H-4 proton coupled with the two H-5 protons as well as the H-9 proton, and appeared as a doublet of triplets at δ2.79. The two H-5 protons resonated at δ2.48 and 2.15, the former appearing as a triplet which overlapped with the 2α-H signal and the latter as a double doublet, while the two doublets at δ4.46 and 4.35 were due to the hydroxy protons bonded to C-3″ and C-4″. The ^{13}C NMR data for lactiflorin (**3**), lactiflorin triacetate (**4**) as well as paeoniflorin (**1**) are summarized in Table 1 for comparison and reference.

Further 2D HETCOR NMR experiments were carried on **4** to establish the carbon–proton correlations through two and three bond couplings, that is ^{2}J and $^{3}J_{C,H}$ (J_{gem} and J_{vic}) correlations. Minor changes were needed in the experimental conditions for this purpose. Here $J_{1\times h}$ (the

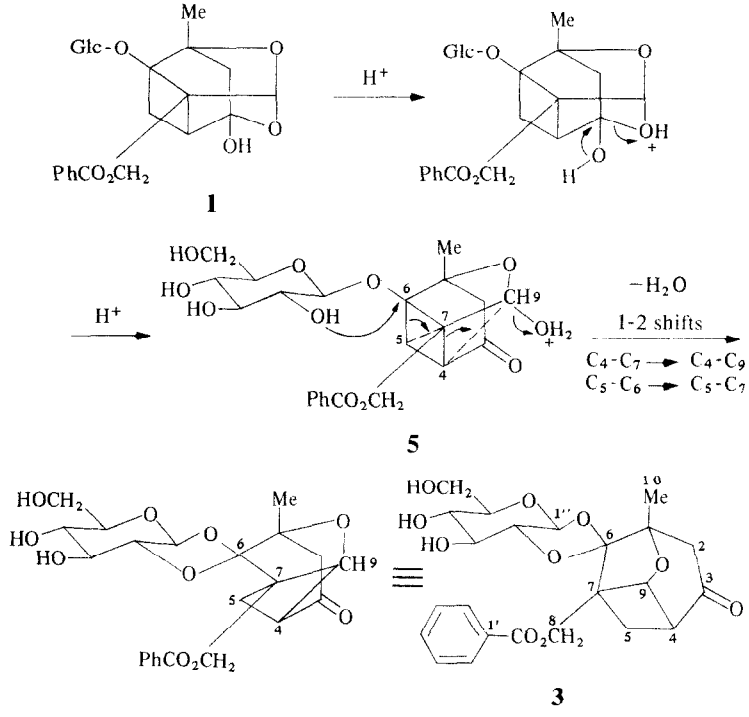
Table 1. ^{13}C NMR data for compounds **1**, **3** and **4**

C	Lactiflorin (3)	Triacetate (4)	Paeoniflorin (1) [20]
1	86.1 *s*	85.6 *s*	86.0 *s*
2	47.9 *t*	47.3 *t*	44.6 *t*
3	216.4 *s*	215.7 *s*	105.8 *s*
4	38.0 *d*	37.5 *d*	43.7 *d*
5	31.3 *t*	30.6 *t*	23.4 *t*
6	103.4 *s*	103.1 *s*	88.8 *s*
7	56.2 *s*	55.2 *s*	71.5 *s*
8	63.6 *t*	62.5 *t*	61.3 *t*
9	81.1 *d*	80.7 *d*	101.5 *d*
10	16.4 *q*	16.1 *q*	19.7 *q*
1′	130.6 *s*	128.9 *s*	130.4 *s*
2′, 6′	130.2 *d*	129.5 *d*	129.8 *d*
3′, 5′	129.6 *d*	128.9 *d*	128.7 *d*
4′	134.2 *d*	133.8 *d*	133.2 *d*
7′	166.5 *s*	166.1 *s*	166.4 *s*
1″	96.0 *d*	95.0 *d*	100.2 *d*
2″	76.0 *d*	72.3 *d*	74.6 *d*
3″	71.7 *d*	71.3 *d*	78.2 *d*
4″	79.5 *d*	68.3 *d*	71.5 *d*
5″	74.5 *d*	73.8 *d*	78.2 *d*
6″	62.4 t	61.7 t	60.7 *t*

Table 2. ^{2}J and $^{3}J_{C,H}$ correlations of compound **4**

C	H	Assignment	Related C		$J_{n\times h}$(Hz)
16.1	1.50	10-Me	85.6	C-1	9, 6
			103.1	C-6	
20.5	1.98		169.6		
20.7	2.03	Me CO × 3	170.0	Ac × 3	9, 6
	2.05		170.6		
	2.25	H-5α	55.2	C-7	9
	2.65	H-2	85.6	C-1	9
	2.66	H-5β	55.2	C-7	6
	2.72	H-4	55.2	C-7	9
			80.7	C-4	
	4.73	H-8	55.2	C-7	6
			80.7	C-9	
			166.1	C-7′	
	4.92	H-9	215.7	C-3	9
	3.56	H-2″	95.0	C-1″	6
61.7	4.15	6″-CH_2			6
68.3	5.07	4″-CH	61.7	C-6″	9, 6
			73.8	C-5″	
	5.25	H-3″	72.3	C-2″	6
128.9	7.50	3′, 5′-CH			9, 6
	7.61	4′-H	128.9	C-3′, 5′	9
			129.5	C-2′, 6′	6
129.5	8.00	2′, 6′-CH	133.8	C-4′	9, 6
			166.1	C-7′	6

coupling constant for one bond coupling) was set at 140 Hz as in the normal HETCOR experiment while $J_{n\times h}$ (the coupling constant for two or three bond couplings) were set at 9 and 6 Hz, respectively, in two separate experiments, in order to match the different coupling constants. Because the values of $^{2}J_{C,H}$ extend from *ca*- 20 Hz to +66 Hz and $^{3}J_{C,H}$ have the values up to 16 Hz [17], it was not possible to detect all the ^{13}C–^{1}H correlations in these two experiments. Furthermore, because we were now observing the carbon–proton correlation through two or three bonds, the connections also included the quaternary carbons. The correlations observed in these experiments are summarized in Table 2 and those of the aglycone skeleton are illustrated in Fig. 1.



Scheme 1. Possible biosynthetic sequence for lactiflorin (3) from paeoniflorin (1).

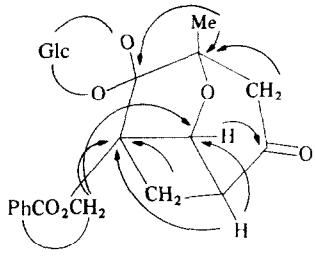


Fig. 1. ^2J and $^3J_{C,H}$ correlations of 4.

In summary, the carbon and proton connections established by these combined NMR experiments could only be accomodated in the unique carbon skeleton depicted in 3 and 4. It would appear that 3 may arise biosynthetically from the co-occurring paeoniflorin (1) as illustrated in Scheme 1. Thus, protonation of the ketal oxygen of paeoniflorin may be followed by ring cleavage to generate an incipient carbocation at C-9 (in 5). The geometry of this intermediate is such that two 1-2 shifts (C-4/C-7→C-4/C-9, C-5/C-6→C-5/C-7) may result in an incipient carbocationic centre at C-6, which could be satisfied by the adjacent nucleophilic hydroxy group on the 2″-position of the glucose moiety. Subsequent deprotonation would then give lactiflorin.

EXPERIMENTAL

Chemical shifts are expressed in ppm (δ) relative to TMS as int. standard. MS were recorded at 70 eV linked on line to a data system. Mp: uncorr. Microanalyses were carried out by the A.N.U. Microanalytical Service Unit.

Isolation. Lactiflorin was isolated from *P. anomala* var. *intermedia* growing in Xinjiang Province in the northwest of China. A ref. specimen is stored in the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing, China. Milled, air-dried root material was extracted with EtOH in a Soxhlet for 56 hr. Evapn of this extract under red. pres. yielded a residue which was digested in MeOH and then filtered. The filtrate obtained was evapd, the residue suspended in H_2O and extracted with $CHCl_3$. The combined $CHCl_3$ extracts were concd and chromatographed on a dry silica gel column using MeOH–$CHCl_3$ (2:23–1:9) as eluant. The faster band contained benzoic acid, while the slower band yielded crude lactiflorin. Crude lactiflorin was rechromatographed on a similar silica gel column using Et_2O–petrol (1:1) and lactiflorin isolated from the slower moving band.

Lactiflorin (3). Crystallized (0.002%) from $CHCl_3$–MeOH as fine needles, mp 207–209° (Found: C, 56.9; H, 6.1%. $C_{23}H_{26}O_{10}\cdot H_2O$ requires C, 57.0; H, 6.0%). IR: ν_{max}^{KBr} 3544–3484 cm^{-1} (OH), 2932 (C–H), 1746, 1728 (C=O), 1270, 1114 (C–O). ^1H NMR [$(CD_3)_2CO$] δ1.45 (3H, *s*, Me), 2.15 (1H, *dd*, J = 4.5, 13.5 Hz, H-5α), 2.46 (1H, *d*, J = 18 Hz, H-2α), 2.48 (1H, *t*, J = 13.2, 13.5 Hz, H-5β), 2.79 (1H, *dt*, J = 4.5, 4.5, 13.2 Hz, H-4), 2.80 (1H, *d*, J = 18 Hz, H-2β), 3.15 (1H, *dd*, J = 8.1, 9.3 Hz, H-2″), 3.41 (1H, *td*, J = 4.35, 7.4, 9.6 Hz, H-4″), 3.57 (1H, *td*, J = 4.45, 7.4, 9.3 Hz, H-3″), 3.70–3.64 (1H, *m*, H-5″), 3.81 (2H, *dq*, J = 2.1, 8.4, 14 Hz, H-6″), 4.35 (1H, *d*, J = 4.35 Hz, OH-4″), 4.46 (1H, *d*, J = 4.45 Hz, OH-3″), 4.65 (1H, *d*, J = 8.1 Hz, H-1″); 4.79 (2H, *s*, H-8), 4.92 (1H, *d*, J = 4.5 Hz, H-9), 7.35 (2H, *t*, J = 7.2, 7.5 Hz, H-3′, H-5′), 7.65 (1H, *t*, J = 7.5 Hz, H-4′), 8.03 (2H, *d*, J = 7.2 Hz, H-2′, H-6′). FAB MS *m/z* 485 $[M+Na]^+$, 463 $[M+1]^+$, 329, 301, 285, 219, 207. EIMS *m/z* 122, 105, 77.

Lactiflorin triacetate (4). Lactiflorin (3) (20 mg, 0.043 mmol), Ac_2O (0.8 ml) and pyridine (1.2 ml) were stirred at room temp.

for 3.5 hr. The soln was then poured into dil. HCl and extracted with Et_2O. The Et_2O extract was washed with H_2O, satd brine and dried ($MgSO_4$). The triacetate (**4**) (23 mg, 90%) was crystallized from CH_2Cl_2–petrol as needles, mp 185° (Found: C, 58.8; H, 5.5%. $C_{29}H_{32}O_{13}$ requires C, 59.2; H, 5.5%). ^{1}H NMR ($CDCl_3$) δ1.50 (3H, *s*, CMe), 1.96, 2.03, 2.05 (each 3H, *s*, MeCO), 2.25 (1H, *dd*, J=4.4, 13.4 Hz, H-5α), 2.66 (1H, *t*, J=13.4 Hz, H-5β), 2.65 (2H, *s*, H-2), 2.72 (1H, *dd*, J=4, 13.4 Hz, H-4), 3.56 (1H, *dd*, J=8.2, 10 Hz, H-2″), 3.78 (1H, *dq*, J=2.1, 4.8, 10 Hz, H-5″), 4.25, 4.15 (*dq*, J=2.1, 4.8, 12.5 Hz, H-6″), 4.68 (1H, *d*, J=8.2 Hz, H-1″), 4.75, 4.70 (2H, ABq, J=12 Hz, H-8), 4.97 (1H, *br s*, H-9), 5.07 (1H, *t*, J=9.2, 10 Hz, H-4″), 5.25 (1H, *dd*, J=9.2, 10 Hz, H-3″), 7.5 (2H, *t*, J=6, 9 Hz, H-3′, H-5′), 7.61 (1H, *t*, J=6 Hz, H-4′), 8.00 (2H, *d*, J=9 Hz, H-2′, H-6′). FAB MS *m/z* 589 $[M+1]^+$. EIMS *m/z* 588 ($[M]^+$, 0.1%), 528 (0.1), 413 (0.2), 358 (0.1), 331 (0.4), 304 (0.04), 301 (0.3), 300 (0.3), 287 (0.1), 284 (1.7), 257 (0.1), 238 (0.1), 230 (0.1), 229 (0.4), 211 (0.2), 195 (0.1), 179 (0.8), 178 (0.8), 169 (9.3), 163 (1.0), 150 (1.5), 135 (1.5), 127 (3.6), 105 (47.3), 97 (6.4), 77 (7.2).

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