Tetrahedron Vol. 46, No. 9, pp. 3123-3128, 1990
Printed in Great Britain

0040-4020/90 $3.00+.00

THE STRUCTURE OF LACTIFLORIN, AN ARTEFACT DURING ISOLATION?

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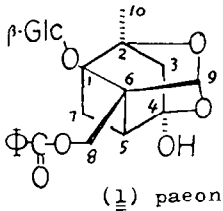
(*Received in Japan* 12 *October* 1989)

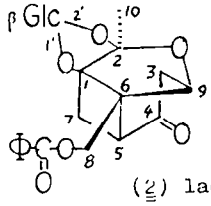
Abstract: The amended structure (2) for lactiflorin was firmly established by chemical correlation with paeoniflorin (1), a known structure.

Both Paeoniflorin (1)[1] and lactiflorin (2), among others, are constituents of Paeonia lactiflora Pall.[2] They were isolated in vastly different amounts, the latter being lower by two to three orders of magnitude.

In this paper, the structure of lactiflorin is amended (the keto-group formerly wrongly assigned as a lactone[3]) and established as 2. The discussion that follows will use 2 and 2a (the triacetate) directly for the sake of clarity, without going through the detailed process of skeletal piecing-together.



(1) paeoniflorin



(2) lactiflorin
(2a) triacetate

The IR spectrum of 2 shows two strong carbonyl bands at 1745 cm^{-1} for the 5-membered cyclic ketone and 1722 cm^{-1} for the benzoyl group. These agree well with the corresponding ^{13}C NMR signals at 215.8 and 166.0, resp.

The ^{13}C NMR data of 2 and 2a are assembled in Table 1 and Table 2, respectively. The ^{13}C NMR of 2 in C_5D_5N at 125 MHz for other purposes (see Table 4) differs little from that in Table 1. Most of the assignments in Table 1 have been checked by selective decoupling experiments.

*Dedicated to Professor Yu WANG on the occasion of his 80th birthday.

The most conspicuous feature of **2** is δ_c 103.1 ppm for 2-C, which is part of a ketal. This also accounts for the ease of glucose release upon acid hydrolysis.

Table 1 ^{13}C NMR of **2**, in C_5D_5N at 22.3 MHz

C-No.	1	2	3	4	5	6	7	8	9	10
δ_c	85.7s	103.1s	47.5t	215.8s	37.8d	55.9s	31.1t	63.0t	80.6d	16.2q
	1'	2'	3'	4'	5'	6'				
	96.0d	76.0d	80.1d	71.3d	74.3d	62.0t				
	1"	2"(6")	3"(5")	4"	7"					
	129.9s	129.7d	128.8d	133.4d	166.0s					

Table 2 ^{13}C NMR of **2a**, in $CDCl_3$ at 100 MHz

C-No.	1	2	3	4	5	6	7	8	9	10
δ_c	85.7s	103.1s	47.3t	215.6s	37.6d	55.3s	30.7t	62.5t	80.7d	16.2q
	1'	2'	3'	4'	5'	6'				
	95.0d	71.3d	73.9d	68.5d	72.4d	61.7t				
	1"	2"(6")	3"(5")	4"	7"					
	129.0s	129.6d	128.9d	133.8d	166.1s					
	20.7q(2×), 20.5q, 170.5s, 170.0s, 169.5s (tri-OAc)									

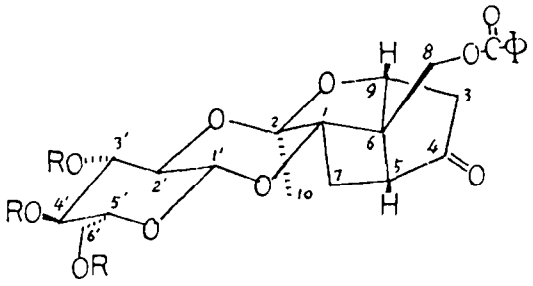
Table 3 ^1H NMR of **2a** in C_6D_6 at 400 MHz

3α-H	δ 2.28, d, J=17.8 Hz	1'-H	δ 4.56, d, 8.2
3β-H	1.90, dd, 17.8, 4.4	2'-H	3.30, dd, 10.0, 8.2
5-H	2.01, dd, 13.4, 4.0	3'-H	5.35, dd, 10.2, 10.0
7α-H	1.86, dd, 13.4, 4.0	4'-H	5.15, dd, 10.2, 9.2
7β-H	2.11, t, 13.4	5'-H	3.16, ddd, 9.2, 4.2, 2.0
8-H_2	4.19, 4.25, ABq, 12.0	6'-H_2	3.88, dd, 12.5, 2.0
9-H	4.43, d, 4.4		4.08, dd, 12.5, 4.2
10-CH_3	1.06, s	4"-H	7.09, tt, 8.4, 1.5
tri-OAc	1.54, 1.57, 1.58, 3×s	3"(5")-H	7.17, "t", 8.4
		2"(6")-H	8.07, "d", 8.4

C_6D_6 is one of the better solvents for the ^1H NMR of **2a** (Table 3), the ^1H NMR spectrum in $CDCl_3$ being not amenable to meaningful analysis due to serious overlapping of signals from the aglycon moiety. Five consecutive axial protons completely define the β-glucosidic unit. The oxygen bridge between 2'-C and 2-C justifies the formation of only a triacetate, and a high field signal for 2'-H where no geminal acetoxy group is possible. It is further supported by a sizable NOE between 2'-H and 10-CH_3 (see the expanded structural sketch in Fig. 1) in a 1, 3-diaxial relationship. In passing, we might also mention the NOE between 10-CH_3 and 7α-H, which is

also obvious from Fig. 1.

Fig. 1.
Expanded structures for 2 and 2a.



(2) lactiflorin, R = H
(2a) triacetate, R = Ac

In the aglycon part, there are two insulated three-spin systems, -O-$\dot{C}$H-CH_2- and -$\dot{C}$H-CH_2-. Long-range coupling across the carbonyl causes line broading of CH_2 in the former (3-C) and CH in the latter (5-C). No noticeable coupling is detected between 9-H and 3α-H because of the unfavorable dihedral angle ($\sim$90°), while the large coupling (13.4Hz) between 5-H and 7β-H is accounted for by a dihedral angle of $\sim$0°. The carbonyl at 4-C gives rise to a large J_{gem} (17.8 Hz) between 3α- and 3β-H, and a shielding toward higher field for 7α-H. All the other ^{13}C and ^{1}H assignments require no further comment.

Long-range ^{13}C-^{1}H coupling relationships are collected in Table 4.

Table 4 Long-range ^{13}C-^{1}H coupling (COLOC) relationships for 2 in C_5D_5N (500 MHz for ^{1}H)

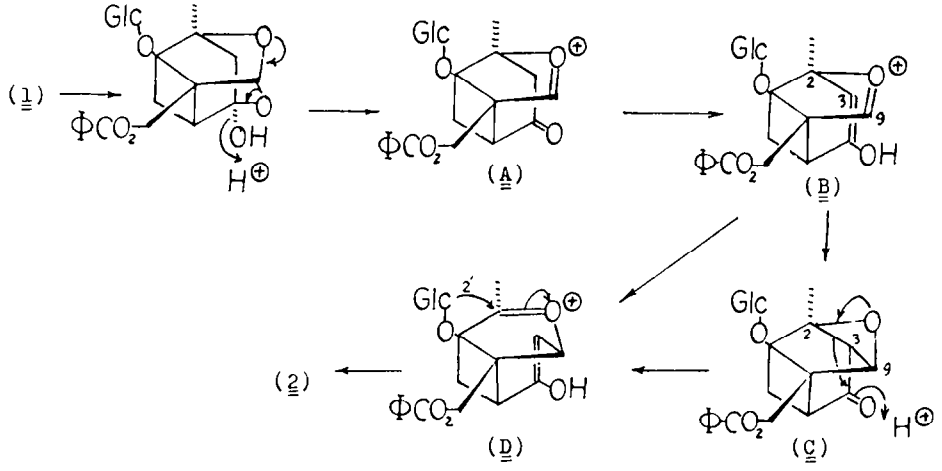
^{13}C	correlated ^{1}H
1-C (86.0 ppm)	5-H (2.96 ppm), 7β-H(2.67), 8-H_2(5.01), 10-H(1.58)
2-C (103.8)	7α-H(2.39), 7β-H(2.67), 10-H(1.58)
4-C (216.1)	3α-H(2.77), 3β-H(2.88), 7α-H(2.39), 9-H(5.14)
5-C(38.1)	3α-H(2.77), 7β-H(2.67), 8-H_2(5.01)
6-C (56.1)	3α-H(2.77), 7α-H(2.39), 8-H_2(5.01)
8-C (63.3)	5-H(2.96), 9-H(5.14)
9-C (80.9)	3α-H(2.77), 5-H(2.96)
3'-C(80.6)	5'-H(3.98)
1"-C(130.0)	3"-H(7.34)
2"-C(129.95)	4"-H(7.46)
4"-C(133.7)	2"-H(8.17)
7"-C(166.2)	8-H_2(5.01), 2"-H(8.17)

Fortunately, conspicuous absences of long-range correlations occur mostly in the glucoside part. Connectivities involving quaternary carbons are especially useful in the tying up of loose ends which allowed us to arrive at 2 as the final solution.

Incorporation of glucose into the polycyclic system makes it a trivial task to assign the absolute configuration of the whole molecule as shown in Fig. 1. This is in accord with all known paeoniflorin congeners.[1,4]

We are now in a position to point out a possible chemical kinship between 1 and 2 as shown in Scheme 1, where A, B, C and D are the postulated intermediates. Here 1 suffers dehydration first to give A, the enolic form (B) of which undergoes aldol condensation to give C with a highly strained oxetane ring. Rupture of the 4-membered ring with the ketonic carbonyl as the electron sink gives D which by intervention with 2'-OH gives 2. Alternatively, it is possible that B can go over to D directly via a 1,3-shift of the electron-rich migrant (3-C) across an electron-deficient allyic system with termini at 2-C and 9-C.

Scheme 1. Acid catalyzed conversion of 1 to 2.



Thus it is highly likely that the unusually "forked" glucoside 2 is an artefact, being formed from 1 during some stage(s) of the isolation process.

Failures of detectable conversion of 1 into 2 under various acidic conditions suggested the possibility of an unfavorably lopsided equilibrium between 1 and 2, in keeping with the relative amounts of isolation, i.e., an unfavorable ratio of *ca.* 100:1 or even worse. A closer look at Scheme 1

indeed shows that the reaction sequence lends itself readily to a reversible mechanism. It was indeed gratifying that a 5 mg sample of 2 in ethanol containing tartaric acid did give, after two hours at reflux (not optimized), a conversion of about 5% into 1, along with some undesired by-products. Similar treatment of 1 would be expected to give a drastically lower conversion into 2 (lower by two to three orders of magnitude), thus escaping detection.

When considered in conjunction with the spectral data, the chemical transformation is regarded as a definitive proof for the newly proposed structure (2) for lactiflorin.

EXPERIMENTAL

Mp's are not corrected. IR spectra were recorded with Nicolet 5DX FT-IR in KBr discs. NMR spectra were recorded with JEOL FX-90Q, JEOL FT-400 and Bruker WM-500 spectrometers. MS were taken on VG ZAB-2F. Optical rotations were recorded with Perkin-Elmer 241 polarimeter.

The roots of P. lactiflora Pall. (56 kg)[4] was extracted three times with 95% EtOH under reflux (2h, 1h and 1h). The combined EtOH extracts were conc'd in vacuo to give a dark brown residue (9.5 kg). Removal of tannin was accomplished by passing through a polyamide column and eluted with H_2O. Evaporation of the solvent gave the total glycosides as a syrup (7.6 kg).

A portion of the syrup (1 kg) was placed on a macroreticular resin and washed with H_2O to remove most of the free sugars. Elution with 95% EtOH gave 296 g of yellow powder. This was chromatographed on a silica column with $CHCl_3$-MeOH-EtOAc (4:1:0.5), collecting 500 ml fractions. Fractions 3-8 were combined and rechromatographed on a dry column with EtOAc. Further purification by column, preparative thin-layer and centrifugal chromatography (CH_2Cl_2-MeOH, 9:1) gave 150 mg of lactiflorin which was recrystallized from MeOH (corresponding to 0.002% of the roots).

Lactiflorin had mp 195-198 °C, $[\alpha]_D^{23}$ +37.2 (c, 0.9, EtOH). FD-MS 463 (M⁺+1). EIMS m/z (%): 462 (M⁺, 6), 371(29), 340(13), 300(67), 285(100), 255(21), 214(8), 196(4), 178(37), 163(19), 162(23), 150(21), 135(63), 127(8), 122(15), 105(21), 77(46). Calcd for $C_{23}H_{26}O_{10}$ C, 59.74; H, 5.62. Fd C, 59.05; H, 5.85. IR cm^{-1}. 3505-3460, 1745, 1722, 1450, 1380, 1340, 1275, 1230, 1174, 1110, 1065, 1025, 968, 892, 850.

Acetylation of 2 was effected with Ac_2O in pyridine at 0 °C for 48 h. After the usual work-up, the triacetate had mp 184-187 °C, FD-MS m/z 589 (M⁺ +1). EIMS m/z (%): 528(M-60, 14), 486 (18), 468(486-18, 13), 426(528-102, 11), 413(22), 342(2), 300 (27), 289(24), 284(32), 271(6), 257(8), 229(25), 211(11), 187(8), 178(20), 169(49), 162(26), 150(26), 145(13),

139(42), 134(42), 127(41), 120(14), 109(42), 105(100).

This triacetate gave nice crystalline needles from ethanol, but too thin for X-ray single crystal analysis (We thank the crystallographers for their patient attempts: Profs Q.T. Zheng and C.H. He of our Institute, and Prof J. Clardy of Conell University).

Conversion of 2 into 1. Lactiforin (5 mg) was dissolved in 2.5 ml of 95% EtOH containing 8 mg of tartaric acid and refluxed for 2 h. Tlc showed a new spot with lower Rf, corresponding to that of paeoniflorin. Hplc showed a conversion of about 5%.

Preparative tlc gave a trace amount of paeoniflorin which was definitely characterized by ^1H NMR (in C_5D_5N at 500 MHz, overnight acquisition). Aside from spurious peaks due to unavoidable impurities, the agreement with an authentic sample was flawless in every detail.

Acknowledgments: - Thanks are due to Prof. Z.C. Miao (Instrument Center, Military Acad Med Sci), Prof. H. Seto (Dept Microbiol, Tokyo Univ) and the analytical staff of our Institute for the spectra.

References and notes

1. M. Kaneda, Y. Iitaka and S. Shibata, Tetrahedron, 1972, 28, 4309.
2. H.Y. Lang, S.Z. Li and X.T. Liang, Acta Pharm. Sinica, 1983, 18, 551.
3. The structure of lactiflorin was wrongly assigned in ref 2 as a lactone due to a faulty ^{13}C NMR of its triacetate which gave a misleading signal at 185 ppm (fold-back?) instead of the true value of 216 ppm (in $CDCl_3$ at 75 MHz). Serious overlapping of proton signals of the aglycon moiety (in $CDCl_3$ at 300 MHz) made it difficult to discover discrepancies of the wrong assignment.
4. H.Y. Lang, S.Z. Li, T. McCabe and J. Clardy, Planta Medica, 1984, 50, 501.