Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters
Tetrahedron Letters 46 (2005) 2615–2618

Sodium paeoniflorin sulfonate, a process derived artefact from paeoniflorin

Patricia Y. Hayes,[a] Reg Lehmann,[b] Kerry Penman,[b] William Kitching[a] and James J. De Voss[a,*]

[a]*SMMS, The University of Queensland, Chemistry Department, Brisbane Qld 4072, Australia*
[b]*MediHerb Research Laboratories, The University of Queensland, Chemistry Department, Brisbane Qld 4072, Australia*

Received 6 December 2004; revised 7 February 2005; accepted 15 February 2005

Abstract—Sodium paeoniflorin sulfonate **2** was isolated from processed, but not unprocessed, *Paeonia lactiflora* roots and characterized by mass spectrometry and NMR spectroscopy. A notable and characteristic downfield shift in the ^{1}H NMR was observed for the hydrogens β to the alkoxysulfonate moiety in **2** and in other model compounds.

Paeonia lactiflora root is one of the most important sources of bioactive materials in traditional Chinese medicine, with claims of antispasmodic, tonic, astringent and analgesic properties.[1] Intensive chemical investigations have been conducted and paeoniflorin **1**, a complex monoterpene glucoside isolated from the root of *P. lactiflora* in 1963[2] is one of the bioactive constituents with many ascribed pharmacological activities such as analgesia,[3] anti-inflammatory,[4] anti-allergic,[4] anti-hyperglycemic,[5] anti-thrombotic effects[6] and neuromuscular blocking.[7] Recent reports address the ability of paeoniflorin to stimulate the release of noradrenaline,[8] and enhance glucose uptake.[9]

Sulfiting agents are commonly used in the preparation of some Chinese herbal medicines to preserve the plant material's moist appearance, as well as its colour and freshness by reduction of enzymatic browning. These agents usually consist of a solution of sodium or potassium metabisulfite, bisulfite or sulfite.[10] Herbs can also be fumigated with sulfur dioxide gas, sometimes generated by burning of bituminous coal.[10] However, such treatments have not previously been reported to alter the phytochemical profiles of herbal extracts. A recent study[11] indicated that the form of processing of *P. lactiflora* roots may have a significant influence on the level of **1** isolated, although the question of material balance was not considered. We now report that processing (sulfiting) of *P. lactiflora* roots can cause a dramatic decrease in the levels of **1** with the concomitant formation of an artefactual derivative, identified here through a combination of ^{1}H, ^{13}C NMR and mass spectrometry.

Extraction and isolation: Commercial, processed *P. lactiflora* roots (5 g) were ground and extracted using boiling water (500 mL). After removal of the solvent under reduced pressure, the residue was diluted with MeOH and the insoluble material removed by filtration.

The major constituent of the extracts **2** was purified by preparative HPLC (Econosil C-18 column, Alltech, 10 μm, 25 cm × 10 mm), eluting with 10% acetonitrile in 50 mM aqueous phosphoric acid and using a photodiode array detector (DAD) set at 230 nm (rt = 10.5 min). Unprocessed roots were treated in the same way and yielded paeoniflorin **1** (rt = 16.2 min).

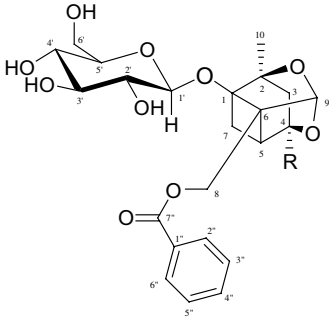
Structure elucidation: HPLC analysis (at 230 nm) of a non-processed sample of *P. lactiflora* root showed mainly one component, paeoniflorin **1**, whilst the processed sample contained a very low level of **1** and a major, more polar compound **2**. This was subsequently isolated by preparative HPLC as described above. Initial analysis of both the ^{13}C and ^{1}H NMR spectra (chemical shifts and coupling constants) confirmed structural

Keywords: *Paeonia lactiflora*; Paeoniflorin; Sodium paeoniflorin sulfonate; Sulfites; NMR.
* Corresponding author. Tel.: +61 7 3365 3825; fax: +61 7 3365 4299; e-mail: j.devoss@uq.edu.au

0040-4039/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2005.02.082

Table 1. ^{13}C NMR and ^{1}H NMR spectral data and HMBC, NOESY correlations of paeoniflorin **1**[13] and **2** (500 MHz, pyridine-d_5)

No.	Paeoniflorin **1**		**2**			
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	HMBC	NOESY
1	89.0		89.2		C1/H10,8,7,3	
2	86.1		88.0		C2/H10,9,8,7,3	
3	44.8	2.33 (1H, d, 12.5 Hz) 2.50 (1H, d, 12.5 Hz)	40.5	2.82 (1H, d, 13.0 Hz) 3.01 (1H, d, 13.0 Hz)	C3/H10,5	H3/H10
4	106.0		95.5		C4/H9,5,3	
5	44.0	3.09 (1H, dd, 6.9, 1.6 Hz)	43.3	4.04 (1H, br d, 7.0 Hz)	C5/H8,3	H5/H7
6	71.8		72.6		C6/H9,8,5	
7	23.6	2.31 (1H, dd, 11.0, 1.6 Hz) 2.92 (1H, dd, 11.0, 6.8 Hz)	24.8	2.54 (1H, d, 11.0 Hz) 3.02 (1H, dd, 11.0, 7.0 Hz)		H7/H10,5
8	61.6	5.13 (1H, d, 12.0 Hz) 5.25 (1H, d, 12.0 Hz)	61.6	5.13 (1H, d, 12.1 Hz) 5.18 (1H, d, 12.1 Hz)	C8/H9,5	H8/H9,7
9	101.7	5.95 (1H, s)	105.1	5.99 (1H, s)	C9/H5	H9/H8
10	19.9	1.68 (3H, s)	20.3	1.65 (3H, s)		H10/H7,3
1′	100.6	5.18 (1H, d, 7.8 Hz)	100.7	5.19 (1H, d, 7.8 Hz)	C1′/H2′	H1′/H8,5′,2″,6″
2′	75.1	4.05 (1H, br t, 7.8 Hz)	75.2	4.00 (1H, dd, 8.8, 7.8 Hz)	C2′/H3′	
3′	78.6	4.20–4.22 (1H, m)	78.7	4.11 (1H, t, 8.8 Hz)	C3′/H4′,3′	H3′/H5′
4′	71.7	4.20–4.22 (1H, m)	72.0	4.19 (1H, t, 9.0 Hz)	C4′/H3′	H4′/H6′
5′	78.5	3.95 (1H, ddd, 8.7, 5.6, 2.6 Hz)	78.6	3.88 (1H, ddd, 9.5, 5.3, 2.6 Hz)		H5′/H3′,6″
6′	62.9	4.35 (1H, m) 4.56 (1H, br d, 11.8 Hz)	63.1	4.34 (1H, dd, 11.8, 5.3 Hz) 4.48 (1H, dd, 11.8, 2.6 Hz)	C6′/H4′	H6′/H5′
1″	130.0		131.0		C1″/H5″,4″	
2″	130.1	8.13 (1H, br dd, 7.8, 0.7 Hz)	130.2	8.19 (1H, br dd, 7.8, 0.7 Hz)	C2″/H6″,4″	
3″	128.8	7.29 (1H, br t, 7.8 Hz)	129.0	7.29 (1H, br t, 7.8 Hz)	C3″/H5″	
4″	133.3	7.44–7.48 (1H, m)	133.5	7.41 (1H, br t, 7.6 Hz)	C4″/H6″,2″	
5″	128.8	7.29 (1H, br t, 7.8 Hz)	129.0	7.29 (1H, br t, 7.8 Hz)	C5″/H3″	
6″	130.1	8.13 (1H, br dd, 7.8, 0.7 Hz)	130.2	8.19 (1H, br dd, 7.8, 0.7 Hz)	C6″/H2″,4″	
7″	166.6		166.8		C7″/H8,6″,2″	



R = OH Paeoniflorin **1**

Figure 1. Paeoniflorin **1** from *P. lactiflora*.

similarity between this and paeoniflorin **1**, with no additional hydrogens or carbons being present. The chemical shifts observed were quasi-identical with those of paeoniflorin **1** (see Table 1 and Fig. 2), with the exception being the signals for H-5, which moved dramatically downfield by 0.95 ppm (from 3.09 ppm in **1** to 4.04 ppm in **2**), H-3a and H-3b, which both moved downfield by 0.5 ppm, and C-4, which moved upfield by 10.5 ppm (from 106.0 ppm in **1** to 95.5 ppm in **2**). This suggested some significant structural perturbation in the region of C3–C4–C5 reminiscent of the skeletal rearrangements previously reported for **1**.[12] However, surprisingly, further 2D experiments (COSY, NOESY, HSQC and HMBC, see Fig. 3) confirmed that **2** possessed a carbon connectivity identical to that of paeoniflorin **1**,[13] with both exhibiting the same spatial and coupling correlations.

Compound **2** was poorly ionized in the positive ESI mode[14] but showed a weak molecular ion at *m/z* 589 $[M+Na]^+$. In the negative ion mode[14] of ESI-MS, a molecular ion at *m/z* 543 $[M-Na]^-$ and a significant ion at *m/z* 381 $[M-Na-glucose]^-$ were observed. This showed a mass increase of 64 for **2** relative to **1**, suggesting 'SO_2' addition and a molecular formula of $C_{23}H_{27}NaO_{13}S$. These changes are consistent with the occasional use of sulfiting in the medicinal herb industry for cosmetic purposes, such as the inhibition of oxidative or enzymatic browning. Although poor ionization of **2** in HRFABMS made determination of the exact molecular formula difficult, the MS data suggested replacement of the C-4 hydroxyl moiety in paeoniflorin **1** by a sulfonate group in **2** (R = SO_3Na in Fig. 1). The similarity of the NMR data of **1** and **2** was also consistent with such a small structural change. However, it was unclear if sulfonate formation at C-4 would account for the approximately 1 ppm downfield shift observed for H-5 in **2** relative to **1**, as no NMR data was available for such compounds.

Bisulfite addition to aldehydes and non-hindered ketones to give the corresponding hydroxysulfonate is a well-established and reversible reaction that may be used for purification of the starting material as the derivative is generally water soluble and crystalline.[15] To the best of our knowledge, no example of bisulfite addition

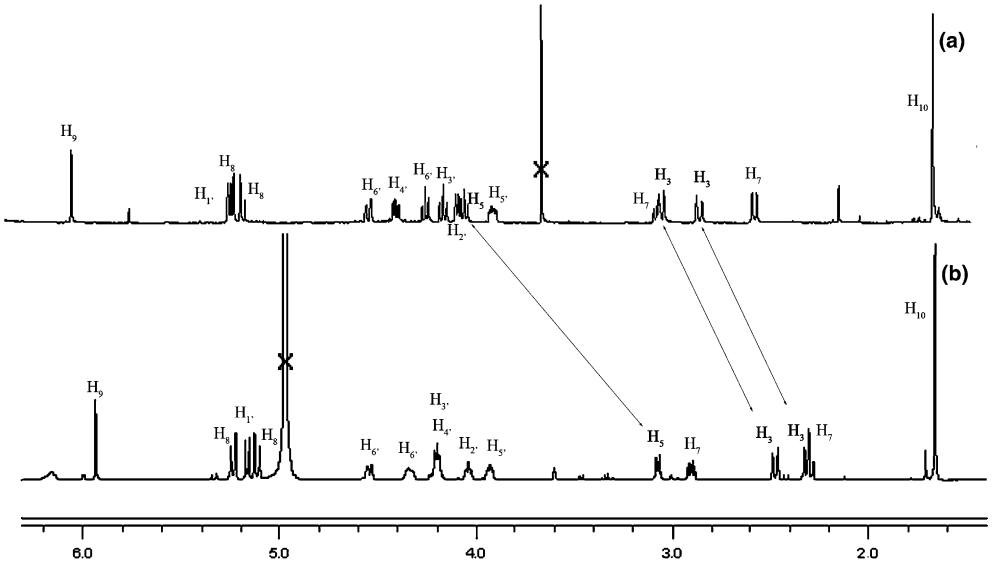


Figure 2. Comparison of the upfield portion of the 500 MHz ^{1}H NMR spectra (d_5-pyridine solvent) of (a) **2** and (b) paeoniflorin **1**.

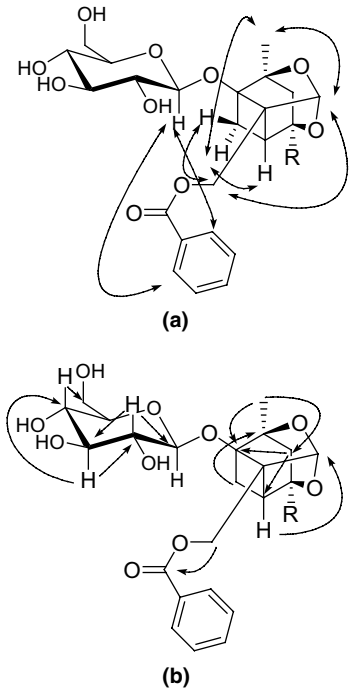


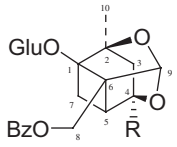
Figure 3. (a) NOESY and (b) HMBC (H to C) correlations for **2**.

involving a hemiketal has been reported. The structure of these bisulfite addition products was confirmed unambiguously in 1967[16] as the α-hydroxysulfonic acid via the crystal structure of the benzaldehyde–potassium bisulfite addition product. We decided to synthesize the sodium sulfonate derivative of glucose[17] for characterization by NMR spectroscopy to provide comparison data for the chemical shifts of **1** and **2**. Contrary to Braverman's original suggestions,[17,18] the ^{1}H NMR spectrum clearly showed the presence of both α and β forms for the glucose sulfonate and no open chain derivative (Fig. 4).

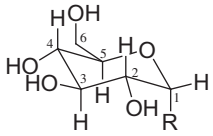
Comparisons of ^{13}C and ^{1}H NMR chemical shifts for paeoniflorin **1** and its sodium sulfonate **2** with those for α and β-glucose (**3** and **5**) and their corresponding sodium sulfonates (**4** and **6**) as models, revealed consistent trends in chemical shift differences for the carbon C-1 bearing these substituents, and the β-protons. In all cases, C-1 was observed to be shifted upfield by about ~10 ppm while the β-hydrogens were shifted downfield by about ~0.5–1.0 ppm for the sodium sulfonate derivatives, relative to hydroxyl (Table 2, Fig. 4).

In order to confirm our finding, a solution of paeoniflorin **1** was stirred at room temperature for 3 h in the presence of an aqueous solution of sodium bisulfite. Paeoniflorin **1** was converted (about 50% conversion, HPLC analysis) to its sodium bisulfite addition product **2**, which was purified by preparative HPLC. The proton and carbon NMR spectra of this product were identical with those of **2**, obtained from the processed dried roots of *P. lactiflora* (Scheme 1).

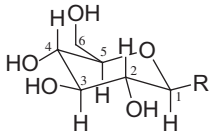
Although the effect of handling on the yield of bioactive compounds from plant material is receiving increasing attention, this work provides a concrete example of treatment resulting in a significant change in the phytochemical profile of the extract from a widely used herbal medicine. The formation of sulfonate **2** from paeoniflorin **1** is the first report to our knowledge of the production of this type of artefact from herbal processing. However, given the ease with which sulfonates can be formed from hemiketals and the prevalence of this functionality in bioactive compounds such as steroidal saponins, for example, protodioscin, it might be expected that such derivatives would commonly be found in plant material treated with 'sulfiting' agents. The resultant structural perturbation would surely alter significantly the bioactivity/bioavailability of the extracted compounds and thus have important implications for both the processing of this herb and subsequent constituent



Paeoniflorin **1** (R = OH) and
Sodium paeoniflorin sulfonate **2** (R = SO_3Na)



α-glucose **3** (R = OH) and
α-glucose sulfonate **4** (R = SO_3Na)

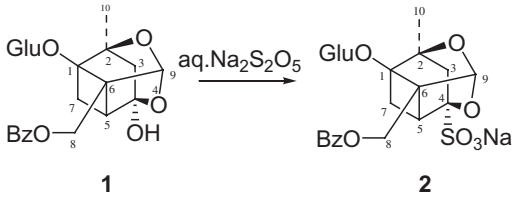


β-glucose **5** (R = OH) and
β-glucose sulfonate **6** (R = SO_3Na)

Figure 4. Structures of paeoniflorin **1**, α- and β-glucose **3** and **5**, and their corresponding sodium sulfonates, **2**, **4** and **6**.

Table 2. Chemical shifts comparisons for paeoniflorin **1**, α- and β-glucose **3** and **5**, and their corresponding sodium sulfonates, **2**, **4** and **6**

Product	Position	δ (ppm) R = OH	δ (ppm) R = SO_3Na	Δ (ppm)
3 and **4**	C_1	92.0	82.1	−9.9
	C_2	71.4	70.2	−1.2
	H_2	3.52	4.20	+0.68
5 and **6**	C_1	95.8	83.1	−12.7
	C_2	74.04	72.6	−1.44
	H_2	3.23	4.00	+0.77
1 and **2**	C_4	106.0	95.5	−10.5
	C_5	44.0	43.3	−0.7
	H_5	3.09	4.04	+0.95
	H_3	2.33	2.82	+0.5
	H_3	2.50	3.01	+0.5



1 **2**

Scheme 1. Conversion of paeoniflorin **1** to sodium paeoniflorin sulfonate **2**.

analysis. The significant downfield shift reported here for the hydrogens β to the hydroxysulfonate moiety should facilitate identification of this class of molecules. Studies to determine the pharmacokinetics of sodium paeoniflorin sulfonate **2** are in progress.

Acknowledgements

The authors are grateful to the Australian Research Council for support of this work.

References and notes

1. WHO monographs on selected medicinal plants, **1999**, 195.
2. Shibata, S.; Nakahara, M.; Aimi, N. *Chem. Pharm. Bull.* **1963**, *11*, 372.
3. Sugishita, E.; Amagaya, S.; Ogihara, Y. *J. Pharmacobiodyn.* **1984**, *7*, 427.
4. Yamahara, J.; Yamada, T.; Kimura, H.; Sawada, T.; Fujimura, H. *J. Pharmacobiodyn.* **1982**, *5*, 921.
5. Hsu, F. L.; Lai, C. W.; Cheng, J. T. *Planta Med.* **1997**, *63*, 323.
6. Kimura, M.; Kimura, I.; Muroi, M.; Nakamura, T.; Shibata, S. *Jpn. J. Pharmacol.* **1995**, *41*, 263.
7. Dezaki, K.; Kimura, I.; Miyahara, K.; Kimura, M. *Jpn. J. Pharmacol.* **1995**, *69*, 281.
8. Liu, T. P.; Liu, M.; Tsai, C. C.; Lai, T. Y.; Hsu, F. L.; Cheng, J. T. *J. Pharm. Pharmacol.* **2002**, *54*, 681.
9. Tang, L. M.; Liu, I. M.; Cheng, J. T. *Planta Med.* **2003**, *69*, 332.
10. Kim, Y.-K.; Koh, E.; Park, S.-Y.; Chang, S.-Y.; Park, S.-J.; Na, W. I.; Kim, H.-J. *J. AOAC Int.* **2000**, *83*, 1149.
11. Sheu, S.-J.; Jan, Y.-J. *Chin. Pharm. J.* **1994**, *46*, 565.
12. (a) Yu, J.; Elix, J. A.; Iskander, M. N. *Phytochemistry* **1990**, *29*, 3859; (b) Lang, H. Y.; Li, S. Z.; Wang, H. B.; Yu, D. Q.; Liang, X. T. *Tetrahedron* **1990**, *46*, 3123.
13. (a) See Yamasaki, K.; Kaneda, M.; Tanaka, O. *Tetrahedron Lett.* **1976**, *44*, 3965, for a report of the ^{13}C NMR chemical shifts of paeoniflorin in d_5-pyridine; (b) Mitova, M.; Handjieva, N. *Fitoterapia* **1999**, *70*, 109, for a report of the ^{13}C and ^{1}H NMR chemical shifts of paeoniflorin in D_2O; (c) See Zhang, X.-Y.; Wang, J.-H.; Li, X. *J. Shenyang Pharm. Univ.* **2001**, *18*, 27, for a report of the ^{13}C and ^{1}H NMR chemical shifts of paeoniflorin in d_4-methanol.
14. Zhao, X.; Sun, Y. *Anal. Sci.* **2003**, *19*, 1313.
15. Kjell, D. P.; Slattery, B. J.; Semo, M. J. *J. Org. Chem.* **1999**, *64*, 5722, and references cited therein.
16. Kuroda, T.; Ashida, T.; Sasada, Y.; Kakudo, M. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 1377.
17. Braverman, J. B. S.; Kopelman, J. *J. Food Sci.* **1961**, *26*, 249.
18. Braverman, J. B. S. *J. Sci. Food Agric.* **1953**, *4*, 540.