NMR STUDIES AND STURUCTURAL ASSIGNMENT OF PAEDEROSIDE †

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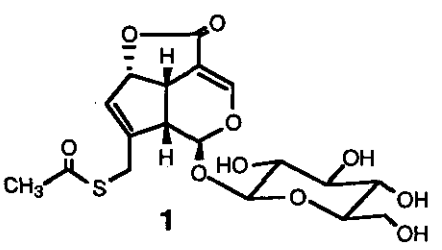
Abstract - Detailed and extensive nmr analyses of paederoside have been carried out resulting to allow complete assignment of all of the ^{1}H and ^{13}C signals. The results provided unambiguous bases to support methyl thiocarbonate structure (**3**) for paederoside, a novel natural sulfur-containing iridoid glucoside of *Paederia scandens*.

Paederoside was first isolated from *Paederia scandens* (Syn. *P. chinensis*, Rubiaceae), and was given a structure (**1**),[1] based mainly on the elemental analysis and its transformation to a derivative of asperuloside (**2**)[2] which differed only in a side chain acyl group. Later, the same glucoside was obtained from *P. foetida*, and another structure (**3**) was proposed to conform its mass fragment ions attributable to a thiomethyl (CH_3S-) group.[3]

Since the molecular formula was inconclusive by the elemental analysis[1] and the mass spectra did not show up the molecular ion but a weak ion peak at *m/z* 464 corresponding to [M + NH_4]$^+$ in a CI technic,[3] the most important disputing point, in the structure determination of paederoside, was the assignment of a characteristic ^{1}H methyl signal at δ 2.34 ppm, which might be compatible both with a thioacetyl methyl group (δ 2.34 ppm for CH_3COS-) as well as a *S*-methyl thiocarbonate group (δ 2.35 ppm for CH_3SCOO-).[3]

† This paper is dedicated to Professor Dr. Edward C. Taylor, on the occasion of his 70th birthday.

This article describes our extensive nmr studies and complete analysis of ^{1}H signals of paederoside. It also presents new ^{13}C nmr spectral data with fully consistent assignment providing conclusive evidences to support the structure (**3**) for paederoside.



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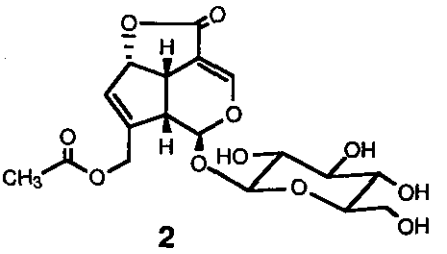

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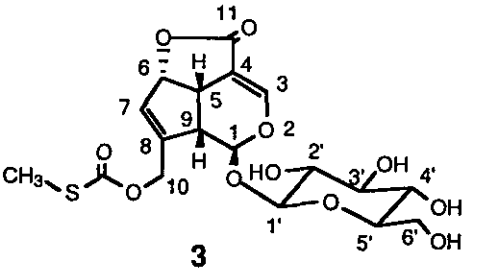

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Paederoside (**3**), mp 122 ℃, [α]$_D$ − 195° (MeOH) was isolated from *Paederia scandens* fruits and exhibited an intense parent ion peak at *m/z* 469 ([M + Na]$^+$) in a FAB-mass spectrum, in addition to an uv absorption maximum at 235 nm (log ε 4.02) and ir bands at ν 1740, 1710 and 1655 cm^{-1}.

Chemical shifts and coupling constants in the ^{1}H nmr spectrum of paederoside (**3**) were very similar to those of the corresponding signals of asperuloside (**2**), except for the peaks attributable to the side chain grouping. Namely, the acetoxymethylene and the acetyl methyl signals of **2** were observed at δ 4.66 and 4.78, and 2.08 ppm, respectively, while **3** exhibited the corresponding peaks in relatively lower field at δ 4.83 and 4.91, and 2.34 ppm (Table I).

Paederoside (**3**) also showed similar ^{13}C nmr spectrum with that of **2** , as far as the signals for the A-ring and sugar carbons were concerned, but displayed clearly different chemical shifts for 7, 8 and 10 carbons (Table II and Figure 1).

Further, a very significant discrepancy was recognized in the carbon chemical shifts for the side chain substituent, in that, a methyl carbon was characterized at δ 13.5 ppm in paederoside, while the acetyl methyl group of asperuloside (**2**) was observed at δ 20.6 ppm (Table II).

Table I. ^{1}H Nmr Signals of Paederoside and Asperuloside

	Paederoside (3)		Asperuloside (2)	
H	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)
1	5.94 d	2.0	5.96 d	1.5
3	7.30 d	2.0	7.30 d	2.2
5	3.69 ddd	6.5, 4.0, 2.0	3.68 ddd	6.5, 3.5, 2.2
6	5.56 ddd	6.5, 2.5, 1.8	5.56 ddd	6.0, 3.0, 1.4
7	5.73 ddd	2.5, 1.2, 0.8	5.73 ddd	3.0, 2.2, 1.4
9	3.37 m		3.37 m	
10	4.83 ddd	14.0, 2.5, 1.2	4.66 ddd	14.0, 2.2, 1.4
	4.91 ddd	14.0, 2.5, 1.2	4.78 ddd	14.0, 2.2, 1.4
CH_3CO			2.08 s	
CH_3SCO	2.34 s			
1'	4.68 d	8.0	4.68 d	8.0
2'	3.19 dd	9.0, 8.0	3.19 dd	9.0, 8.0
3'	3.37 dd	9.0, 9.0	3.37 dd	9.0, 9.0
4'	3.28 dd	9.0, 9.0	3.28 dd	9.0, 9.0
5'	3.34 ddd	9.0, 9.0, 2.2	3.34 ddd	9.0, 9.0, 2.2
6'	3.67 d	11.7	3.66 d	11.7
	3.92 dd	11.7, 2.2	3.92 dd	11.7, 2.2

Table II. ^{13}C Nmr Signals of Paederoside and Asperuloside

	Paederoside (3)	Asperuloside (2)
C1	93.2 (d)	93.3 (d)
C3	150.3 (d)	150.3 (d)
C4	106.1 (s)	106.2 (s)
C5	37.5 (d)	37.4 (d)
C6	86.2 (d)	86.3 (d)
C7	129.5 (d)	128.9 (d)
C8	143.8 (s)	144.3 (s)
C9	45.3 (d)	45.3 (d)
C10	64.3 (t)	61.9 (t)
C11	172.7 (s)	172.6 (s)
CH_3	13.5 (q)	20.6 (q)
C=O	172.5 (s)	172.2 (s)
C1'	100.0 (d)	100.0 (d)
C2'	74.6 (d)	74.6 (d)
C3'	77.9 (d)	77.9 (d)
C4'	71.5 (d)	71.6 (d)
C5'	78.4 (d)	78.4 (d)
C6'	62.8 (t)	62.8 (t)

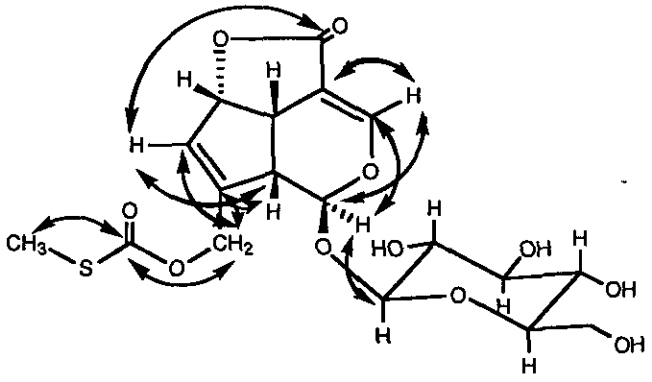
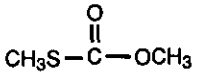


Figure 1 C -H Long range couplings in paederoside

Since the chemical shift alone did not indicate whether it was a thioacetate or a methyl thiocarbonate, some reference compounds (**4** - **6**) had been synthesized and were subjected to nmr studies.

As the result, dimethyl thiocarbonate (**4**) and *O*-ethyl *S*-methyl thiocarbonate (**5**) exhibited the thiomethyl signal at δ 13.5 and 13.4 ppm, respectively, and coincided perfectly well with the methyl signal at δ 13.5 ppm in paederoside (**3**), while thioacetyl methyl signal in **6** was observed in a much lower field of δ 30.2 ppm. Further more, the carbonyl carbon of the thioacetate (**6**) was assigned to a signal at δ 197.8 ppm, but paederoside (**3**) exhibitated a peak at δ 172.5 ppm (asperuloside δ 172.2 ppm) which, in contrast, accorded very well with the data of thiocarbonates (**4** : δ 173.3 ppm) and (**5** : δ 172.7 ppm).



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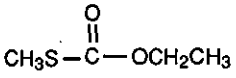

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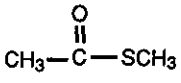

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Ir spectrum of paederoside (**3**) exhibited two carbonyl bands at ν 1740 and 1710 cm^{-1}. The former band is a reasonable position to the α,β-unsaturated γ-lactone, and thus the latter should correspond to the thiocarbonate group. Two synthetic thiocarbonyl esters (**4**) and (**5**) exhibited respective carbonyl bands at ν 1717 and 1711 cm^{-1}, in good accordance to the expected region, while methyl thioacetate (**6**) showed the carbonyl absorption at a considerably lower frequency of ν 1694 cm^{-1}.

Based on these nmr studies in addition to the determination of the molecular ion peak in FAB-mass spectrum and examination of carbonyl stretching bands in the ir spectrum, the structure of

paederoside was assigned rigorously as the thiocarbonate structure (**3**).

Since dimethyl disulfide had also been characterized in the same plant,[4] the sulfur atom in paederoside (**3**) might have been incorporated as a thiomethanol in its biosynthesis.

EXPERIMENTAL

General : Melting points were measured with a BÜCHI 535 melting point apparatus and are reported uncorrected. Ir spectra were recorded on a JASCO FT/IR-500 spectrophotometer. Uv spectra were recorded on a Beckman DU-64 spectrophotometer. Optical rotations were measured on JASCO DIP-360 instrument. Nmr spectra were obtained with a JEOL GSX-400 (400MHz) spectrometer with tetramethylsilane as an internal standard. Chemical shifts are given in ppm (δ), and signals are expressed as s (singlet), d (doublet), dd (double doublet), ddd (double double doublet), t (triplet), q (quartet), m (multiplet) respectively. Mass spectral data (EI, FAB) were obtained on a JEOL JMS DX-303 GC-mass spectrometer.

Paederoside (3) : *Paederia scandens* fruits (1.2 kg) was extracted with methanol (1 *l*) at room temperature for 3 days and the extract was partitioned successively with ethyl acetate and then with butanol. The butanol soluble portion (2 g) was subjected to repeated column chromatography on Sephadex LH-20 (MeOH) and silica gel ($CHCl_3$ -MeOH 2 : 1) to yield paederoside (**3**, 20 mg) : mp 122 ℃ (from water) ; $[\alpha]_D$ −195° (c 0.41, MeOH) ; FAB-ms *m/z* : 469 $[M+Na]^+$; uv λ_{max}^{MeOH} nm (log ε) : 235 (4.02) ; ir ν_{max}^{KBr} cm^{-1} : 1740, 1710, 1655 ; ^1H nmr : Table I ; ^{13}C nmr : Table II.

Asperuloside (2) : Asperuloside (**2**, 19.3 g) was obtained by the same procedure, as above, from methanol extract of *Daphniphyllum macropodum* leaves (1.3 kg) : mp 125-127 ℃ ; ir ν_{max}^{KBr} cm^{-1} : 1740, 1700, 1661; ^1H nmr : Table I ; ^{13}C nmr : Table II.

***O,S*-Dimethyl thiocarbonate (4) and *O*-ethyl *S*-methyl thiocarbonate (5)** : Methyl chloroformate (5.0 g, 53 mmol) or ethyl chloroformate (5.8 g, 53 mmol) was treated with aqueous solution (15 %) of sodium methyl sulfide (25 ml, 53 mmol) at 60–70 ℃ for 2 h, followed by usual working up and fractional distillation to yield respective esters, **4** (1.5 g, 27 %) and **5** (1.8 g, 28 %).

4 : bp 119-120 ℃ / 760 mmHg ; EI-ms *m/z* : 106 $[M]^+$, 75 $[CH_3SC{=}O]^+$, 59 $[M-SCH_3]^+$ 47 $[CH_3S]^+$, 32 ; ir ν_{max}^{neat} cm^{-1} : 1717 (-SCOO-); ^1H nmr (CD_3OD) δ : 2.37 (3H, s, SCH_3), 3.85 (3H, s, OCH_3) ; ^{13}C nmr δ : 13.5 (SCH_3), 54.5 (OCH_3), 173.3 (C=O).

5 : bp 136-138 ℃ / 760 mmHg ; EI-ms *m/z* : 120 [M]$^+$, 75 [$CH_3SC{=}O$]$^+$, 47 [CH_3S]$^+$, 32 ; ir ν_{max}^{neat} cm^{-1} : 1711 (-SCOO-) ; ^{1}H nmr δ : 1.37 (3H, t , *J* 7.0 Hz, $CH_2\underline{CH_3}$), 2.41 (3H, s, SCH_3), 4.35 (2H, q, *J* 7.0 Hz, $\underline{CH_2}CH_3$) ; ^{13}C nmr (CD_3OD) δ : 13.4 (SCH_3), 14.6 ($CH_2\underline{C}H_3$), 64.4 ($\underline{C}H_2CH_3$), 172.7 (C=O).

Methyl thioacetate (6) : Aqueous solution (15 %) of sodium methyl sulfide (25 ml, 53 mmol) was treated with acetic anhydride (5.5 g, 53 mmol) at 60-70 ℃ for 2 h, followed by usual working up and purification as above to yield **6** (2.01 g, 42 %) : bp 97-98 ℃ / 760 mmHg ; EI-ms *m/z* : 90 [M]$^+$, 75 [M – CH_3]$^+$, 47 [CH_3S]$^+$, 43 [$CH_3C{=}O$]$^+$; ir ν_{max}^{neat} cm^{-1} : 1694 (-$COSCH_3$) ; ^{1}H nmr (CD_3OD) δ : 2.27 (3H, s, CH_3CO), 2.31 (3H, s, SCH_3) ; ^{13}C nmr (CD_3OD) δ : 11.8 (SCH_3), 30.2 (CH_3CO), 197.8 (C=O).

REFERENCES

1. H. Inouye, S. Inouye, N. Shimokawa, and M. Okigawa, *Tetrahedron Lett.*, **1968**, 683; *idem*, *Chem. Pharm. Bull.*, **1969**, *17*, 1942.
2. A. R. Trim, *Nature*, **1951**, *167*, 485; L. H. Briggs, and B. F. Cain, *J. Chem. Soc.*, **1954**, 4182. L. H. Briggs, B. F. Cain, P. W. Le Quesne, and J. N. Shoolery, *Tetrahedron Lett* ., **1963**, 69.
3. G. J. Kapadia, Y. N. Shukla, A. K. Bose, H. Fujiwara, and H. A. Lloyed, *Tetrahedron Lett.*, **1979**, 1937.
4. T. Kurihara, M. Kikuchi, and S. Suzuki, *Yakugaku Zasshi* , **1975**, *95*, 1380.

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