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CARBON-13 NMR SPECTRAL ASSIGNMENTS OF PAEONIFLORIN HOMOLOGUES WITH THE AID OF SPIN-LATTICE RELAXATION TIME

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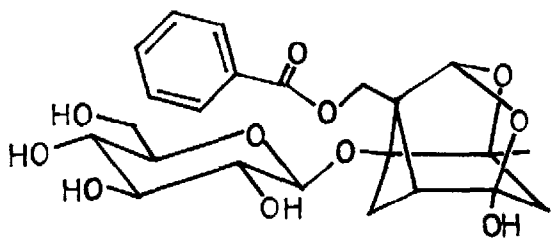
The structural determination of paeoniflorin (I) and albiflorin (II), monoterpene (pinane type) glucosides of the roots of Paeonia albiflora Pallas (Paeoniaceae) have been established.[1,2] Since I is represented by a unique bridge head-O-β-glucoside involving a highly oxygenated cage-like aglycone, the assignment of its ^{13}C NMR spectrum has attracted much attention in continuation of our studies on terpenoid glycosides.[3,4]

Not only the usual ^{13}C NMR experiments and comparison with the spectra of II and desbenzoylpaeoniflorin monomethyl ether (III) (derived from I), but also spin-lattice relaxation time (T_1) measurements were effective for the characterization of the resonances. The spectra were recorded in pyridine-d_5 and as references, the spectra of I were also taken in other solvent systems. The process of the assignments of the signals of I in pyridine-d_5 is as follows and the results were shown in Table I.



(I)

The signals associated with benzoyl carbons (C-1"-C-7") were readily recognized.[5] All of the corresponding signal due to the β-glucosyl carbon except C-1' appeared at almost the same positions as those of the methyl β-glucoside (within 0.2 ppm differences). A triplet signal close to C-6' was identified as C-8 by proton selective decoupling (PSD) technique (C-6'-H_2: δ ca. 4.3 ABX and C-8-H_2: δ 5.1 br.s). The comparison of spectra of I with that of III further secured these assignments of C-6' and C-8; the latter was shifted upfield on desbenzoylation, whereas C-6' remained unaffected. Three resonances

between δ 100 and 106 were attributed to the carbons bearing two oxygen functions. Among them, a singlet (δ 106.0) was assigned to C-4 by the multiplicity, and two doublets (δ 100.5 for C-1', 101.7 for C-9) were differentiated by PSD technique (C-1'-H : δ 5.10 d, C-9-H : δ 5.71 s) as well as T_1 data (vide infra). As already pointed out in our previous paper,[3] the anomeric carbon (C-1') of bridge head-O-β-D-glucoside of I resonates characteristically at higher field than those of the sterically less hindered prim- or sec-OH (eg. Me-O-β-glc: δ 105.5, i-Pr-O-β-glc : δ 102.4).[6]

Of four signals at high field, δ 43.9 (d) and 19.9 (q) were assigned to C-5 and C-10, respectively, by their multiplicities, and two triplets, δ 44.8 and 23.5 were attributed to C-3 and C-7, respectively, by the difference of deshielding effects of β-O-functional groups, of which C-3 has three while C-7 has only one. The assignments of C-3 and C-7 were also supported by comparison of the spectrum with that of III. In the spectrum of III, introduction of methyl group (δ 51.1 q) at 4-C-O caused remarkable downfield shift at C-4 and upfield shift at adjacent C-3 and C-5, while C-7 remained unchanged.

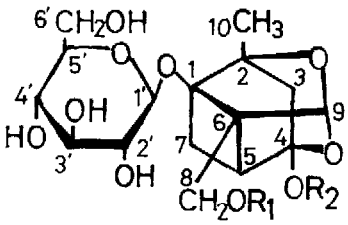
Of three signals between δ 90 and 70, signals at δ 88.9 and 86.1 must be attributed to C-1 and C-2, both of which has one oxygen on it. Unambiguous differentiation between these two signals was achieved by T_1 measurement (vide infra). Subsequently, a remaining singlet at δ 71.7 should be assigned to C-6. This remarkable lowfield resonance of C-6 would be due to the presence of four oxygen function on its adjacent carbons (one on C-1 and C-8, two on C-9) in such a cage-like structure. It should be notable that on going from I to III, the constructional change of cage-skeleton resulted in the unexpected upfield displacement of this carbon signal.

The T_1 value for each carbons of I were determined in pyridine-d_5 by inversion recovery technique and shown in Table II. The relaxation of quaternary carbons, C-1 and C-2 must be dominated proximately α-protons (protons attached to adjacent carbon atoms).[7,8] As the number of α-protons are only two (7-CH_2) for C-1, and five (3-CH_2 and 10-CH_3) for C-2, T_1 value for C-1 is predicted to be larger than that of C-2. It follows that, of two singlets, δ 88.9 and 86.1, the former (T_1 3.2 sec) can be assigned to C-1, the latter (T_1 2.3 sec) as C-2.

As expected,[9] segment movements of glucosyl and benzoyl moiety, as well as fast rotation of CH_3 group were also reflected to NT_1 values. NT_1 values for glucosyl carbons were higher than that of aglycone carbons due to faster internal reorientation. This fact differentiated the resonance of C-6' from C-8, and C-1' from C-9 definitely. With regard to benzoyl carbons, C-2", 6", 3" and 5" showed higher NT_1 values than C-4" indicating the rotation around symmetry axis (C-1" - C-4"). The signal of C-4 with four α-protons (3-CH_2, 5-CH and 4C-OH) showed T_1 value of 2.2 sec similar to that of C-2 with five α-protons. This fact was consistently explained by the argument that the 10-CH_3 group

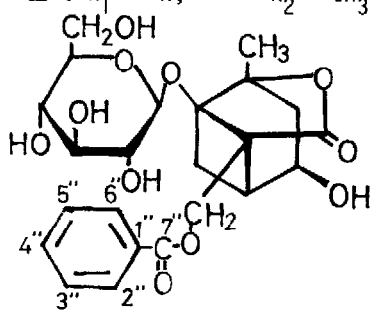
Table I Carbon-13 Chemical Shifts of Paeoniflorin (I), Albiflorin (II), and III [a]

Compound	I			II	III
Solvent [b]	Py	M-W	W	Py	Py
C-1	88.9	88.2	88.8	91.3	88.6
2	86.1	86.8	87.7	86.3	86.0
3	44.8	43.1	43.6	41.8	42.0
4	106.0	105.6	106.3	67.4	108.5
5	43.9	42.6	43.0	41.4	40.2
6	71.7	70.6	71.1	56.1	73.0
7	23.5	22.4	23.3	28.3	23.4
8	61.5	61.2	61.5	61.7	58.5
9	101.7	100.8	101.4	175.7	102.0
10	19.9	18.4	19.0	20.7	19.7
1'	100.5	98.7	99.2	100.3	99.9
2'	74.9	73.5	73.8	74.8	74.7
3'	78.4	76.3	76.6	78.4	78.4
4'	71.7	70.1	70.3	71.6	71.7
5'	78.4	76.3	76.6	78.4	78.1
6'	62.9	61.2	61.5	62.8	62.7
1"	130.6	129.2	129.6	130.8	
2" 6"	129.9	129.5	130.3	130.1	
3" 5"	128.8	128.8	129.6	128.7	
4"	133.3	134.0	134.9	133.2	
7"	166.6	167.9	168.9	166.6	
OMe					51.1



I : R_1 = C_6H_5CO-, R_2 = H

III : R_1 = H, R_2 = CH_3



II

a. δ ppm from internal TMS unless otherwise stated; with a JEOL PFT-100 spectrometer at 25.15 MHz, spectral width: 5 KHz, 4096 data points, 600-3600 accumulations, pulse length: 12 μ sec (45°) at an interval of 1.0 sec. The samples (85-200 mg) were dissolved in 1 ml of solvent at 25°.

b. Py: pyridine-d_5, M-W: CD_3OD / D_2O (1:1), W: D_2O (δ converted from internal dioxane, 67.4)

Table II Spin-Lattice Relaxation Times (T_1 in sec) for I in Pyridine-d_5 [a]

aglycone	T_1	NT_1	glucosyl	T_1	NT_1	benzoyl	T_1	NT_1
C-1	3.2		C-1'	0.21	0.21	C-1"	> 6	
2	2.3		2'	0.25	0.25	2" 6"	0.45	0.45
3	0.08	0.16	3'	0.23 [c]	0.23	3" 5"	0.46	0.46
4	2.2		4'	b		4"	0.22	0.22
5	0.13	0.13	5'	0.23 [c]	0.23	7"	> 6	
6	b		6'	0.11	0.22			
7	0.08	0.15						
8	0.08	0.16						
9	0.16	0.16						
10	0.61	1.83						

N : Number of directly attached protons

a. Measured by inversion recovery method within the reproducitivity of ± 10 %, not degassed.

b. Not measured due to overlapping of signals.

c. Average value of C-3' and C-5' due to overlapping of signals.

contributed the relaxation of C-2 less than predicted based on the numbers of protons due to the fast rotation of the CH_3 group, which was already observed at the NT_1 value of C-10 itself. Thus, T_1 mesurement, or at least PRFT technique, which is a qualitative application of relaxation behavior, was very useful for structural elucidation of plant glycosides.

By analogy of I, with the aid of shielding reguralities, spectra of II and III were readily assigned, part of which was already discussed (Table I).

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