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STRUCTURAL ANALYSIS OF PHENOLIC GLUCOSIDES FROM SALICACEAE BY NMR SPECTROSCOPY

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Key Word Index—Salicaceae; phenolic glycosides; structure determination; ^{1}H and ^{13}C NMR spectroscopy.

Abstract—Some characteristic features of the NMR spectra of phenolic glycosides are described in order to facilitate the structural determination of these compounds.

INTRODUCTION

A large number of phenolic glucosides have been isolated from the bark and leaves of trees of the family Salicaceae during the last two decades, mainly by the research groups of Pearl [1, 2] and Thieme [3, 4]. The structure elucidation of these compounds was achieved in many cases by time consuming and mostly destructive chemical methods. Tedious two-dimensional PC procedures were also used for identification [5]. A few papers comment on the mass spectral behaviour of phenolic glucosides after

Table 1. ^{1}H NMR chemical shifts* and multiplicities of glucosides 1–11

Compound	Solvent	Carbohydrate protons (7H; H-1′–H-6′)	Aglycone protons
1	DMSO-d_6	3.24–4.99 (*m*)	6.60 (2H; H-2, 6; *d*; $J_{2,3}$ = 7 Hz) 6.88 (2H; H-3, 5; *d*)
2	DMSO-d_6	3.18–5.20 (*m*)	7.06 (2H; H-2, 6; *d*; $J_{2,3}$ = 8 Hz) 7.91 (2H; H-3, 5; *d*)
3	Acetone-d_6	2.94–4.46 (*m*)	5.34 (2H; H-12; *s*) 7.00 (1H; H-5; *dd*; $J_{5,6}$ = 9 Hz; $J_{3,5}$ = 3 Hz) 7.24–7.60 (7H; *m*)
4	Acetone-d_6	3.50–4.89 (*m*) 5.52 (H-1′; *d*; $J_{1',2'}$ = 3 Hz)	6.98 (1H; H-3; *d*; $J_{3,5}$ = 3 Hz) 6.79 (1H; H-5; *dd*; $J_{5,6}$ = 9 Hz) 7.18 (1H; H-6; *d*) 8.13 (2H; H-8, 12; *dd*; $J_{8,9}$ = 8 Hz; $J_{8,10}$ = 2 Hz) 7.50–7.77 (3H; H-10, 11, 12; *m*) 8.23 (1H; OH; *s*)
5	Acetone-d_6	3.40–4.61 (*m*)	6.90–7.43 (4H; *m*)
6	DMSO-d_6	3.23–5.39 (*m*)	6.96–7.40 (4H; H-3–H-6; *m*) 6.12 (1H; H-11; *dt*; $J_{10,11}$ = 7 Hz; $J_{11,12}$ = 4 Hz) 5.96 (1H; H-10; *d*) 2.45–2.90 (4H; H-12, 13; *m*)
7	Acetone-d_6	3.50–4.74 (*m*)	6.97–8.12 (9H; *m*)
8	Acetone-d_6	3.60–5.42 (*m*)	6.88–8.16 (13H; all ^{1}H; *m*)
9	DMSO-d_6	3.32–5.48 (*m*)	6.80–7.39 (4H; H-3–H-6; *m*) 7.40–7.61 (3H; H-10, 11, 12; *m*) 7.98 (2H; H-9, 11; *dd*; $J_{9,10}$ = 8 Hz; $J_{9,11}$ = 2 Hz)
10	Acetone-d_6	3.56–5.50 (*m*)	8.07 (2H; H-9, 11; *dd*; $J_{9,10}$ = 8 Hz; $J_{9,11}$ = 2 Hz) All other ^{1}H; 6.77–7.77 (*m*)
11	DMSO-d_6	3.22–5.23 (*m*) including H-1 and H-2	1.14–1.60 (8H; H-3–H-6; *m*) 6.37 (1H; H-8; *d*; $J_{8,9}$ = 16 Hz) 6.78 (2H; H-12, 14; *d*; $J_{11,12}$ = 7.5 Hz) 7.55 (2H; H-11, 15; *d*) 7.57 (1H; H-9; *d*)

*All chemical shifts are given in δ-values (ppm) referred to internal TMS as reference. The spectra were measured at 99.50 MHz.

derivatization to their peracetylated derivatives [6–9].

During our continuous search for new chemotherapeutic agents from natural origin, four compounds with antimicrobial activity were isolated by droplet countercurrent chromatography of the crude ethyl acetate extract of the leaves of a Poplar tree (cv. Beaupré) [10]. A ^{1}H and ^{13}C NMR spectral approach was chosen for structure elucidation. The rapidity and non-destructivity of this technique, which lead to a straightforward identification of salicine, salireposide, populine and trichocarpine, prompted us to extend our study to a series of known glucosides **1**–**11** in order to provide a collection of data to facilitate forthcoming structural elucidations within this class of compounds. For this purpose Dr. I. A. Pearl (Wisconsin, U.S.A.) generously sent us samples of the glucosides **4**–**11**.

The obtained 100 MHz ^{1}H NMR spectra alone do not contain enough information to characterize the structures completely, but are nevertheless very useful in confirming the presence of the carbohydrate moiety besides the aromatic part in these molecules and to rule out other glucosides, e.g. flavonoids, iridoids and ethers. The 25 MHz ^{13}C NMR spectra enable the identification of the aglycone, using aromatic substituent shift increments [11] and the data published for some phenolic compounds. The identification of glucose and the determination of its substitution pattern and anomeric configuration can also be obtained from the ^{13}C NMR spectra.

RESULTS AND DISCUSSION

All the spectral data are summarized in Tables 1 and 2 for ^{1}H NMR and ^{13}C NMR, respectively. Some molecular fragments can be readily recognized in the ^{1}H NMR, e.g. the *para*-substituted phenyl ring in arbutine (**1**) and piceine (**2**); the 1,2,4-trisubstitution pattern of the aryl groups in trichocarpine (**3**) and salireposide (**4**); the benzoyl groups present in **4** and **7**–**10** can be assigned separately except for **8** where its peaks coincide with other signals of the aglycone and the typical shifts and coupling constants of the cinnamoyl group in **11**.

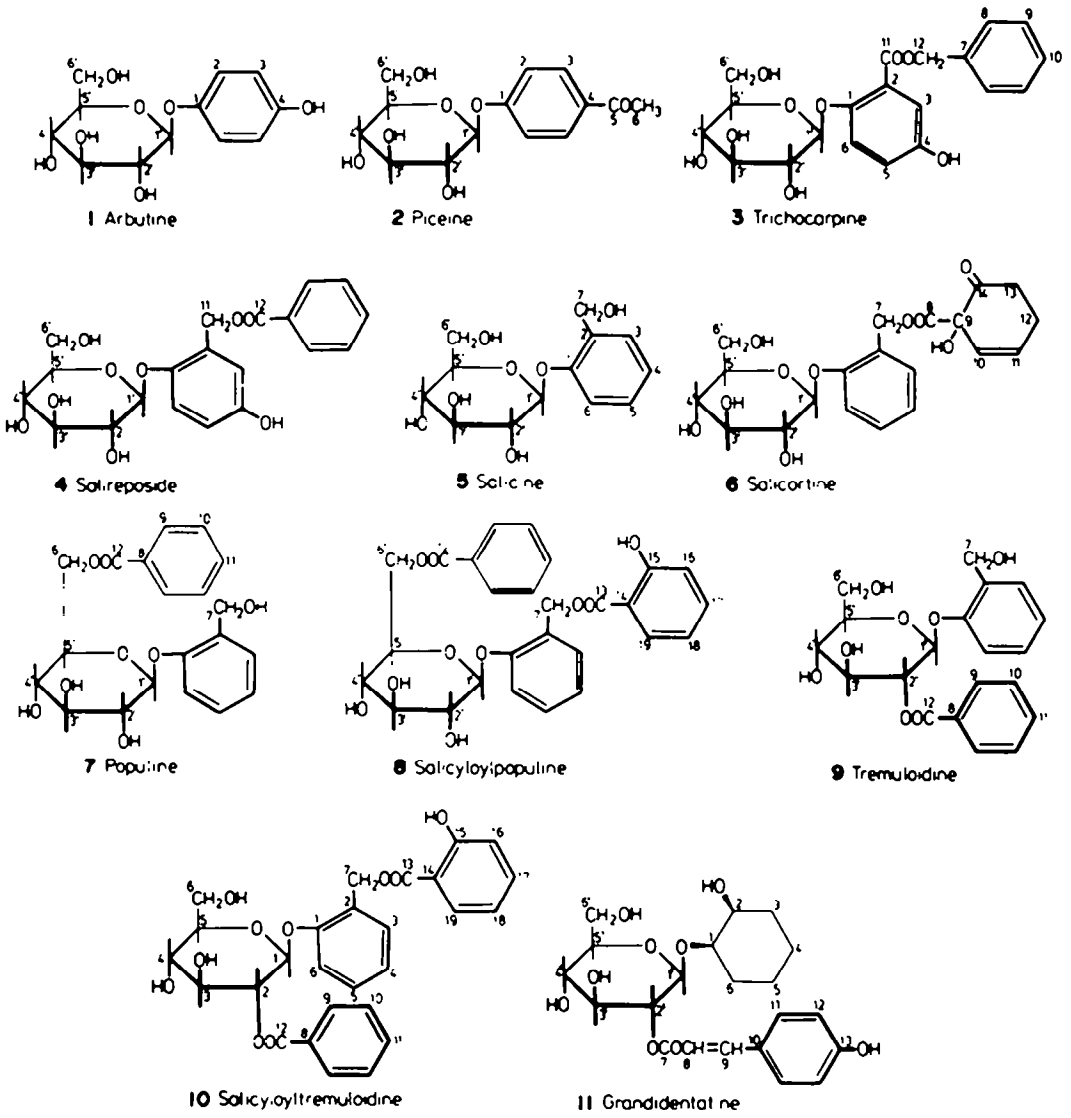
For ^{13}C NMR the identification of the hydroxyl and acetyl groups in **1** and **2** is possible from the shifts of the aromatic ring carbon atoms and those of the acetyl carbon atoms themselves. Salicine (**5**), salicortine (**6**), populine (**7**), salicyloylpopuline (**8**), tremuloidine (**9**) and salicyloyltremuloidine (**10**) all contain a characteristic series of peaks (see Table 2, C-1 to C-6) which corresponds very well to the known chemical shifts of salicyl alcohol [12]. In addition to this part of the aglycone another typical group of five peaks with two double intensity signals due to a benzoyl group is identified in compounds **4**, **7**–**10** [12] (Table 2, C-8 to C-12) and an additional series of six signals due to a salicyloyl group in **8** and **10** (Table 2, C-13 to C-19). The aglycone signals of trichocarpine and salireposide can be rationalized using known chemical shift increments for aromatic substitution [11] and taking

Table 2. ^{13}C NMR shifts of glucosides **1**–**11***

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
C-1′	103.63	99.81	103.05	102.81	100.83	100.97	103.53	102.56	98.31	100.22	101.15
C-2′	74.93	73.13	73.66	73.71	73.30	73.31	74.95	75.13	77.19	78.14	78.05
C-3′	79.41	77.14	76.78	76.68	76.40	77.03	77.93	77.85	74.22	75.61	75.46
C-4′	71.42	69.59	70.06	70.11	69.70	69.78	71.64	71.52	69.93	71.58	71.71†
C-5′	77.80	76.53	76.10	76.44	75.92	76.48	75.30	74.59	73.82	75.13	76.14
C-6′	62.55	60.58	61.38	61.38	60.81	62.16†	65.15	65.08	60.56	62.40†	62.60
C-1	152.37	161.03	152.18	152.37	154.87	154.94	157.12	156.71	153.41	156.22	80.04
C-2	119.38	115.83	116.94	127.52	129.71	124.48	132.84	125.96	131.17	125.52	71.47†
C-3	116.60	130.21	121.38	116.41	129.95	129.35	129.23	130.30	127.32†	130.34	30.87
C-4	153.78	130.82	150.18	148.87	123.62	121.74	123.43	123.23	121.86	123.33	21.63
C-5		196.37	122.60	118.70	129.95	128.75	129.23	130.30	126.40†	130.34	23.70
C-6		26.40	120.26	116.02	115.64	115.04	117.14	116.80	114.01	116.31	28.92
C-7			136.10	62.45	59.53	62.59†	61.00	63.23	57.25	62.70†	168.55
C-8			128.59	129.95		170.05	131.37	131.22	129.86	130.98	115.28
C-9			129.08	129.81		77.33	130.40	130.54	129.27	130.98	146.91
C-10			128.88	129.18		131.49	129.47	129.37	128.64	129.18	127.22
C-11			167.19	134.15		129.20	134.06	133.90	133.27	133.81	131.17
C-12			67.47	167.78		25.85	166.78	166.56	165.02	166.07	116.89
C-13						35.54		170.75		170.46	161.40
C-14						205.87		113.59		113.19	
C-15								162.46		162.37	
C-16								120.16		120.01	
C-17								136.68		136.56	
C-18								118.16		118.06	
C-19								130.98		130.73	

*Spectra of compounds **1** and **11** were recorded in MeOH-d_4; compounds **2**, **6** and **9** in DMSO-d_6; compounds **3**, **4**, **7**, **8** and **10** in Me_2CO-d_6 and compound **5** in D_2O. These solvent changes can result in small solvent induced shifts especially for the easily solvated carbohydrate carbon atoms. All chemical shifts are given in ppm with TMS taken as standard. The spectra were recorded at 25.0 MHz.

†Assignments in the same column could be reversed.



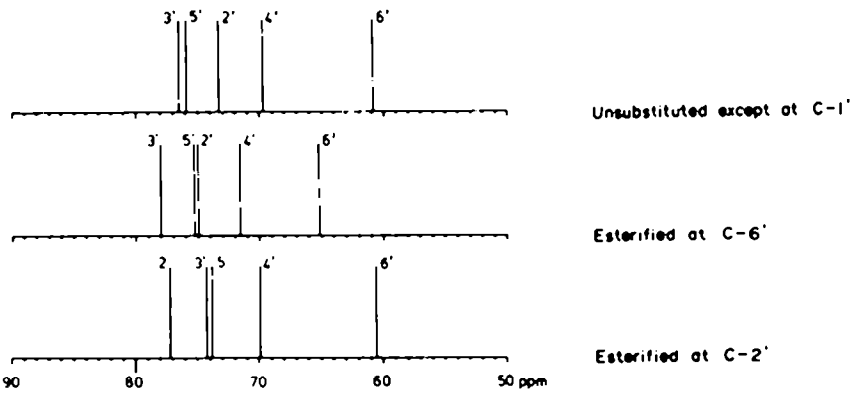


Fig. 1. Typical carbohydrate signal pattern according to the substitution on the glucose molecule.

the 1,2,4-substitution found by ^{1}H NMR into account. The same can be done for the aliphatic ring present in salicortine and grandidentatine by application of the HOSE code increments published by Bremser *et al.* [13]. The esterification of the C-6′ or C-7 hydroxyl functions results in a downfield shift of the corresponding atoms. These appear then at δ 65.1 and 62.7, respectively, instead of at δ 61.6 and 59.3. Esterification in the glucose C-6′ position also causes an upfield shift of *ca* 1–1.5 ppm on C-5′. The same effects are also experienced by C-3′ and C-2′ on benzoylation or cinnamoylation of C-2′. The assignment of other carbohydrate carbon atoms is analogous to reported values for glucose derivatives [12]. Figure 1 illustrates these effects and shows how esterification of a sugar hydroxyl group is readily recognized in the spectra.

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