^{13}C NMR SPECTRA OF STEROID GLYCOSIDES.

I. PENNOGENIN GLYCOSIDES

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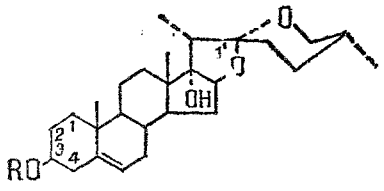
The ^{13}C NMR spectra of five pennogenin glycosides have been measured. The signals have been assigned to individual atoms of the carbon skeleton on the basis of the glycosidation shifts. A mutual influence of the aglycone and of the carbohydrate chain on the chemical shifts of the corresponding carbon atoms has been established.

In recent years, ^{13}C NMR spectroscopy has become one of the main methods for the structural investigation of natural steroid glycosides [1, 2]. The comparative analysis of the ^{13}C NMR spectra of related glycosides permits the solution of practically all structural problems: the determination of the structure of the native aglycone, the position of glycosylation, and the structure of the carbohydrate chain, including the determination of the configurations of the glycosidic bonds.

We have previously shown shifts due to 17α-hydroxyl substitution in the ^{13}C NMR spectra ($CDCl_3$) of pennogenin (I) [3] and have made an assignment of the signals of the C atoms in the ^{13}C NMR spectrum ($CDCl_3$) of pennogenin tetra-O-acetyl-β-D-glucopyranoside [4].



I. R=H
II. R=Ac
III. R=β-*D*-Glc$_p'$
IV. R=α-*L*-Rha$_p''$ (1→4)-β-*D*-Glc$_p'$
V. R=α-*L*-Rha$_p''$ (1→2)-β-*D*-Glc$_p'$
VI. R=α-*L*-Rha$_p''$ (1→2)
α-*L*-Ara$_p'''$ (1→4)
β-*D*-Glc$_p'$
VII. R=α-*L*-Rha$_p''$ (1→2)
α-*L*-Rha$_p'''$ (1→4)
β-D-Glc$_p'$

Characteristic glycosidation shifts, together with other information, have been used in the determination of the structures of pennogenin glycosides (IV-VII) [5, 6]. Here we give complete information on the chemical shifts of the C atoms of pennogenin (I) and the glycosides (III-VII) in the ^{13}C NMR spectra (C_5D_5N).

In these ^{13}C NMR spectrum ($CDCl_3$) of (I), the narrow region with δ 30.9-31.6 ppm, including the signals of the C-2, C-8, C-12, C-15, and C-23 atoms, is difficult for unambiguous assignment. The assignment of the C-2 signals (δ 31.6 ppm) has now been confirmed by a β acetylation shift [7] in the ^{13}C NMR spectrum ($CDCl_3$) of the corresponding 3-O-acetate (II): $\Delta\delta_{C-2}$ −3.8 ppm. A variation of the solvent caused additional shifts of the signals. The ^{13}C NMR spectrum of (I) (C_5D_5N) and the solvent variation ($CDCl_3 \rightarrow C_5D_5N$)

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TABLE 1. ^{13}C NMR Chemical Shifts, δ (ppm), and Glycosidation Shifts (Δδ, ppm) of Ring A of the Aglycone and of the Carbohydrate Moieties of Compounds (III), (IV), and (V) (C_5D_5N)

C atom	III		IV		V	
	δ	Δδ	δ	Δδ	δ	Δδ
1	37.5	−0.4	37.4	−0.5	37.5	−0.4
2	29.8	−2.6	30.0	−2.4	30.0	−2.4
3	78.3*	+7.1	78.3	+7.1	77.9	+6.2
4	39.3	−4.1	39.3	−4.1	38.9	−4.5
5	140.9	−1.1	140.8	−1.2	140.8	−1.2
6	121.7	+0.8	121.7	+0.8	121.6	+0.7
1′	102.6	−2.8	102.5*	−2.9	100.3	−5.1
2′	75.3	+0.3	75.3	+0.5	79.3	+4.5
3′	78.5*	+0.4	76.9*	−1.2	77.9	−0.2
4′	71.8	+0.4	78.3	+6.9	71.7	+0.3
5′	78.3*	+0.2	76.6*	−1.5	77.9	−0.2
6′	62.9	+0.4	61.5	−1.0	62.6	+0.1
1″					101.6	−0.8
2″					72.7*	+0.8
3″					72.3*	−0.2
4″					73.9	+0.3
5″					69.1	−0.3
6″					18.5	+0.1
1‴			102.3*	−0.1		
2‴			72.4	+0.5		
3‴			72.4	−0.1		
4‴			73.8	+0.2		
5‴			70.2	+0.8		
6‴			18.4	0		

*Assignment of the signals not unambiguous within a column.

shifts were (δ, Δδ, ppm): C-1 (37.9, +0.6), C-2 (32.4, +0.8), C-3 (71.2, −0.4), C-4 (43.4, +1.1), C-5 (142.0, +2.0), C-6 (120.9, −0.4), C-7 (32.4, +0.4), C-8 (32.1, +0.5),* C-9 (50.4, +0.6), C-10 (37.0, +0.3), C-11 (21.1, +0.4), C-12 (32.4, +0.8),* C-13 (45.1, +1.2), C-14 (53.1, +0.2), C-15 (32.1, +1.2),* C-16 (90.1, −0.8), C-17 (90.1, 0), C-18 (17.2, +0.1), C-19 (19.6, +0.2), C-20 (44.8, +0.2), C-21 (9.6, +1.5), C-22 (109.8, −0.2), C-23 (31.8, +0.5),* C-24 (28.8, +0.7), C-25 (30.5, +0.4), C-26 (66.7, −0.1), C-27 (17.2, +0.1).

The signals of the C atoms of the aglycone moieties of (III-VII) were identical with those of (I) with the exception of the signals of the C atoms of ring A, which are given in Tables 1 and 2. The assignment of the latter in the spectra of (I) and of (III—VII) was confirmed by the glycosidation shifts [8-10]. The appearance in the spectrum of (III) of a peak with δ 29.8 ppm ($\Delta\delta_{C-2}$ −2.6 ppm) with a simultaneous decrease in the intensity of the δ 32.4 ppm peak confirms the correctness of the assignment of the former in the spectrum of (III) and of the latter in the spectrum of (I) to the C-2 atom. In the spectra of (III—VII), the signals of the C-2 and C-3 atoms of the aglycone show a dependence of their chemical shifts on the structure of the carbohydrate chain. The C-4 signal in the spectra of (I) (δ 43.4 ppm) and of (III) (δ 39.3 ppm) were assigned on the basis of the β glycosidation shift ($\Delta\delta_{C-4}$ −4.1 ppm) in the spectrum of (III). The size of the chemical shift of the C-4 signal also depends on the structure of the carbohydrate chain. Thus, the presence in the carbohydrate chain of the fragment α-L-Rha^{p}''-(1 → 2)-β-D-Glc^{p}' shifts the C-4 signal of the aglycone upward in the spectra of (V), (VI), and (VII) as compared with (III) and (IV). The weakest, narrow, signal in this region, at δ 45.1 ppm in the spectra of (I—VII), is assigned to the unprotonated C-13, and the signal with δ 44.8 ppm [δ 44.7 ppm in the spectrum of IV] to C-20.

The assignment of the signals of the C-atoms of the glycosyl unit in the spectrum of (III) is based on the results for methyl β-D-glycopyranoside [10].

In the spectrum of (IV), the C atoms of the glucosyl and rhamnosyl units resonate in different regions. The chemical shifts of the C atoms (apart from C-5''' of the rhamnosyl

*Assignment of the signals not unambiguous.

TABLE 2. ^{13}C NMR Chemical Shifts (δ, ppm), and Glycosidation Shifts (Δδ, ppm) of the C-Atoms of Ring A of the Aglycone and of the Carbohydrate Moieties of Compounds (VI) and (VII) (C_5D_5N).

C atom	VI		VII	
	δ	Δδ	δ	Δδ
1	37,5	—0,4	37,5	—0,4
2	30,3	—2,1	30,3	—2,1
3	77,7*	+6,5	77,8*	+6,6
4	38,9	—4,5	38,9	—4,5
5	140,9	—1,1	140,8	—1,2
6	121,7	+0,8	121,7	+0,8
1′	100,2	—5,2	100,2	—5,2
2′	78,2*	+3,4	78,8	+4,0
3′	77,5*	—0,6	77,8*	—0,3
4′	77,9*	+6,5	78,1*	+6,7
5′	76,7	—1,4	76,75	—1,35
6′	61,5	—1,0	61,3	—1,2
1″	101,9	—0,5	101,8	—0,6
2″	72,8*	+0,9	72,7*	+0,8
3″	72,4*	+0,1	72,3*	—0,2
4″	74,2	+0,6	73,9	+0,3
5″	69,4	0	69,3	—0,1
6″	18,65	+0,25	18,5	+0,1
1‴			102,8	+0,4
2‴			72,3	+0,4
3‴			72,3	—0,2
4‴			73,8	+0,2
5‴			70,3	+0,9
6‴			18,5	+0,1
1⁗	109,7	+0,2		
2⁗	82,6	+0,6		
3⁗	77,15	—0,75		
4⁗	86,7	+1,9		
5⁗	62,5	0		

*Assignment of the signals not unambiguous within a column.

unit) are close to those of methyl α-L-rhamnopyranoside [10]. The considerable downfield shift of the C-5‴ signal ($\Delta\delta_{C-5'''}$ +0.8 ppm) due to steric factors has been reported previously [14, 15]. The assignment of the signals of the C atoms of the glycosyl unit was made with the use of correlations of the glycosidation shift reported in the literature [11-13]. The chemical shifts of the C-1' and C-2' signals of the glycosyl unit in the spectrum of (IV) are equal to those in the spectrum of (III), and the C-6' signal is shifted upfield ($\Delta\delta_{C-6'}$ —1.0 ppm). Taking the value of the α glycosidation shift into account, the signal with δ 78.3 ppm can be assigned only to C-4' — the most highly screened in the C-2'-C-5' series in the spectrum of methyl β-D-glucopyranoside [10]. The same peak (δ 78.3 ppm) includes the signal of the C-3 atom of the aglycone. The assignment of the peak with δ 78.3 ppm to the C-3 atom in the spectra of (III) and (IV) is based on an assertion of the insignificance, because of its remoteness, of the influence of the formation of a (1 → 4) bond on the degree of screening of the C-3 atom of the aglycone. The assignment of the C-3' and C-5' signals in the spectrum of (IV) is based on results for 4-O-methyl-β-D-glucopyranose and for methyl β-maltoside [12].

The chemical shifts of the C-4' and C-6' signals in the spectrum of (V) are close to those in the spectrum of (III). The signal with δ 79.3 ppm in the spectrum of (V) can be ascribed only to C-2', which follows C-4' in the degree of screening of the C atom in the spectrum of methyl β-D-glucopyranoside [10]. The C-3', C-5', and C-3 signals of the aglycone overlap one another (peak with δ 77.9 ppm). The small upfield shift of the C-3 signal of the aglycone in the spectrum of (V) as compared with the spectrum of (III) is apparently due to steric hindrance in the formation of the (1 → 2) bond. On taking into account the value of the β glycosidation shifts found for (IV), the signal with δ 100.3 ppm was assigned to C-1' of the glucosyl unit and that with δ 101.6 ppm, correspondingly, to C-1" of the rhamnosyl unit. A similar sequence of assignments has been recorded previously for the

carbohydrate chain of solanine, which contains the fragment α-L-Rha$_p$-(1 → 2)-α-D-Gal$_p$- [16]. In the spectrum of (V), in contrast to that of (IV), the C-2" and C-3" atoms give separate signals, which is in agreement with the results for the terminal rhamnosyl unit of the oligosaccharide α-L-Rha$_p$-(1 → 2)-α-L-Rha$_p$ [17]. Apparently, the steric hindrance and the restriction to free rotation around the (1 → 2)-glycosidic bond connected with it are responsible for the upfield shift of the C-5" signal ($\Delta\delta_{C-5"}$ −0.3 ppm) of the rhamnosyl unit in contrast to (IV) ($\Delta\delta_{C-5'''}$ +0.8 ppm).

It is obvious from a comparison of the spectra of (IV) and (V) that for an α-L-Rha$_p$-(1 → 2) unit, C-1" and C-5" are more screened, and C-2" is less screened, than the corresponding C atoms of an α-L-Rha$_p$-(1 → 4) unit. This difference is also preserved in the spectrum of (VII), which contains both types of bonds and is apparently characteristic for the corresponding glycosidation positions.

The assignment of the C-1"", C-2"", C-4"", and C-5"" signals of the arabinofuranosyl unit in the spectrum of (VI) is based on result for methyl α-L-arabinofuranoside [18]. A considerable downfield shift is observed for the C-4"" signal ($\Delta\delta_{C-4''''}$ +1.9 ppm). The chemical shifts of the C-atoms of the rhamnosyl unit in the spectrum of (VI) are close to those in the spectrum of (V). The small upfield shifts of the C-2' and C-4' signals in the spectrum of (VI) as compared with those of (V) and (IV), respectively, are apparently due to the mutual influence of two glycosidic bonds located in the ortho position with respect to one another. The assignment of the C-3' and C-5' signals of the glycosyl unit in the spectrum of (VI) was made in a comparison of the spectra of (VI) and (IV) and (V) taking the β glycosidation shifts into account [10-12]. On passing from (V) to (VI), the upfield β shift of the C-5' signal should be close to that in the spectrum of (IV).

Similar considerations have been applied in the assignment of the signals of the C atoms of the glycosyl unit of (VII). The assignments in the δ 76.7-78.8 ppm in the spectra of (VI) and (VII) are the preferred ones. Because of the close values of the chemical shifts of the corresponding protons, the use of the method of selective decoupling is difficult in this region. The chemical shifts of the signals of the C atoms of the rhamnosyl units in the spectrum of (VII) are close to those for (IV) and (V). The signal with δ 77.15 ppm in the spectrum of (VI) has been ascribed to the C-3"" atom of the arabinosyl unit on the basis of the results of a comparison of the spectra of (VI) and (VII). The chemical shifts of the signals of the C atoms of the glucosyl unit in the spectrum of (VII) are identical with (C-1', C-5'), or close to (C-6'), those for (VI). In the spectrum of (VI), the C-2', C-3', and C-4' signals are present in a stronger field than those in the spectrum of (VII), which must be ascribed to the influence of the arabinofuranosyl unit.

EXPERIMENTAL

The ^{13}C NMR spectra were taken on a Bruker HX-90 E instrument in $CDCl_3$ and C_5D_5N at 30-32°C using concentrations of ∿5%. The glycosides (VI) and (VII) were isolated from the roots of the plant *Polygonatum stenophyllum* Maxim. [19], and (I) and (III)-(V) were obtained by the acid and enzymatic hydrolysis of a mixture of (VI) and (VII) [4]. The acetate (II) was obtained by the usual method.

SUMMARY

1. An assignment of the ^{13}C signals in the NMR spectra of five pennogenin glycosides has been made and the mutual influence of the aglycone and of the carbohydrate chains on their chemical shifts has been determined.

2. It has been shown that the glycosidation shifts for the C atoms of the terminal monosaccharide may have diagnostic value for determining the position of its attachment.

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