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CARBON-13 NMR SPECTROSCOPY OF STEROIDAL SAPOGENINS AND STEROIDAL SAPONINS*

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Key Word Index—Steroidal sapogenins; steroidal saponins; ^{13}C NMR spectral analysis; structure elucidation.

Abstract—The ^{13}C NMR chemical shifts of 130 naturally occurring steroidal sapogenins and saponin derivatives published up to 1983 are listed and a number of methods for signal assignment are explained. The utility of ^{13}C NMR spectral analysis for the structure elucidation of these compounds is discussed.

INTRODUCTION

Saponins, which exhibit a wide spectrum of biological activities [1] are glycosides and characterized by a number of common properties such as froth formation, haemolytic activity, toxicity to fish and complex formation with cholesterol [2, 3]. Saponins are divided into three major groups, triterpenoid, basic steroid and steroid saponins. Various reviews dealing with the distribution, isolation techniques and characterization of triterpenoid saponins have been published [4, 5]. Similarly, basic steroidal saponins have been reviewed by several authors [6–9] and steroid saponins were the subject of two comprehensive reviews [10, 11].

The characterization of a saponin is not an easy task and conventional methods include acid hydrolysis followed by characterization of the aglycone and sugar moieties. However, the major disadvantage of this method is the cyclization of furostanol to spirostanol aglycone and it can yield several alternative structures to that of the original compound. A decision between various alternative structures can not be achieved by FD-MS [12, 13] and FAB-MS [14, 15]. In such cases, ^{13}C NMR spectral analysis provides a non-destructive way for the characterization of a saponin.

An extensive compilation, with an excellent discussion of the ^{13}C chemical shifts for various categories of natural products has been presented by Wehrli and Nishida [16]. A systematic compilation of the ^{13}C NMR chemical shifts for around 400 steroidal derivatives has been published by Blunt and Stothers [17] but which includes only eight steroidal sapogenins. Tsuda and Schropfer [18] discussed the ^{13}C NMR shielding behaviour for the olefinic carbon in a variety of steroidal olefins but there was no particular emphasis on steroidal sapogenins.

For structure elucidation of a new compound by ^{13}C NMR studies it is always desirable to compare the observed data with the reported data for model and related compounds. As the ^{13}C NMR shielding data is still scattered in the literature and there is no systematic compilation we considered it worthwhile to tabulate the ^{13}C NMR chemical shifts of steroidal sapogenins and saponins published up to 1983 and to present various salient features which could conveniently be utilized for the structure determination of these and other related categories of natural products.

Before discussing the ^{13}C NMR shielding behaviour of sapogenins and saponins, it has been thought appropriate to deal with the techniques used for signal assignment. Thus, the whole discussion has been divided into three main areas, namely methods of signal assignment, ^{13}C NMR spectral analysis of sapogenins, and similarly for saponins.

1. METHODS FOR SIGNAL ASSIGNMENT

In general, ^{13}C NMR spectra are recorded under proton-noise (broad band) decoupling [19] in order to avoid the severe signal overlap due to large one bond ^{13}C–^{1}H coupling constants (*ca* 120–250 Hz). For single frequency off resonance decoupled (SFORD) spectra [20] the decoupler frequency is positioned outside the ^{1}H resonance range and thus all ^{13}C–^{1}H couplings are reduced to give rise to small residual couplings (J_r) from which the number of attached hydrogens can be determined (singlet for quaternary carbon, doublet for methine, triplet for methylene and quartet for methyl groups). However, the reduced coupling pattern still exhibits so much overlap that only a few of the carbon signals can be confidently assigned.

New techniques such as APT (Attached Proton Test) [21, 22], INEPT (Insensitive Nuclei Enhanced By Polarization Transfer) [23, 24], DEPT (Distortionless Enhancement by Polarization Transfer) [25] and *J*-coupled spin echoes [26, 27] have been developed for discriminating carbon types, particularly CH from CH_2 and Me in the cases where their signals are overlapped in

*Part 10 in the series "^{13}C NMR Spectral Investigations". For Part 9 see Agrawal, P. K. and Thakur, R. S. (1985) *Magn. Reson. Chem.* **23** (in press).

the ordinary SFORD spectrum. In these methods, by a single excitation sequence, the information about the number of adjacent hydrogens is reflected in signal phases and intensities. The technique of 2D-spectroscopy (2D-J-NMR) has been introduced recently which allows separation of chemical shifts and spin coupling information in weakly spin coupled systems [28–30]. Carbon–carbon connectivity patterns can also be determined at natural abundance in organic molecules [31, 32]. An excellent discussion of these methods, together with their applications, has been recently presented by Shoolery [33].

Another variant is selective single-frequency proton decoupling in which irradiation of a given proton signal in the ^1H NMR spectrum causes a collapse of the signal splitting for the directly bonded carbon atom only, whereas all other carbon signals remain more or less broadened owing to their couplings. Unambiguous assignments for C-18, C-19, C-21 and C-27 in various spirostane derivatives has been achieved by this method [34].

Coupling constants

Carbon–hydrogen coupling constants are useful aids for peak assignment in ^{13}C NMR spectroscopy [35] and their application for the structure elucidation of aromatic compounds is quite evident [36–40]. However, such information is of limited importance because ^{13}C resonances in sapogenins appear in a narrow range (*vide infra*). Hydroxylated, unsaturated and carbonyl carbon can be assigned unambiguously on the basis of chemical shift values. Most of the carbon atoms, except a hydroxylated one, exhibit one bond ^{13}C–^1H coupling constants in the range 122–130 Hz while a hydroxylated carbon is at about 142 ± 2 Hz [41].

Solvent effect

The ^{13}C NMR shielding, in general, is not very sensitive to solvent variations but as the solute–solvent interaction varies with the change of solvent a change in chemical shift can be observed. Such studies are mainly concerned with the use of chloroform and pyridine [42]. A common trend for the pyridine induced shift is the upfield shift (0.6–0.9 ppm) of C-3 and the downfield shift of up to 0.9 ppm for the other remaining carbon atoms of monohydroxylated sapogenins. However, the presence of a homoallylic unsaturation at C-5 as in diosgenin (**46**) and yamogenin (**60**) leads to reduction in shielding for C-3 and thus C-3 exhibits only 0.4 ppm upfield shift while C-2, C-4 and C-5 shift downfield (1.1–1.2 ppm) with the downfield shift, max. 0.7 ppm, for all the remaining carbon atoms. The above mentioned behaviour has not been exhibited by the vicinally hydroxylated sapogenins, such as yonogenin (**35**) and neoyonogenin (**42**), where all the skeletal carbon signals present downfield shifts of up to 0.9 ppm [42].

Isotopic labelling

This method has proved to be very useful in making unequivocal assignments. The use of ^{13}C labelled precursor compounds in biosynthetic studies not only enhances the signal intensity of a particular specified carbon but also introduces ^{13}C–^{13}C coupling [43]. Such studies have been carried out in cholesterol biosynthetic studies [44].

Deuterium labelling also facilitates the unequivocal assignment of specified deuterated carbons by their characteristic multiplets in the proton-decoupled spectra. When a deuterium atom is bonded to a carbon, the absorption for the carbon becomes triplet because of ^{13}C–^2H coupling and the line exhibits quadrupolar broadening. Thus, there is a significant decrease in signal to noise ratio (S/N). Because of different quantum number ($I_D = 1$) signal multiplicities; triplet for CD, pentate for CD_2 and septate for CD_3. These fully deuterated centers may be difficult to detect because of multiplicity, a possibly reduced Overhauser enhancement and their longer T_1 values; often these are described as missing from the spectra of deuterated steroids [17]. One more feature of deuterium labelling is the isotope effect which leads to upfield shifts of 0.05–0.1 ppm per deuterium atom, on the shielding of carbons, adjacent to deuteration [45–47]. This technique has been used for the straightforward assignment of C-28, C-23 and C-25 in isodiotigenin (**37**) which on treatment with DCl–EtOD yields [20, 23, 23′, 25-$^2H_4$]isodiotigenin in which signals for the above mentioned carbon disappear while those for C-17, C-21, C-24 and C-27 are found to be shifted upfield by upto 0.4 ppm [42].

Shift reagents

Another possibility of producing explicable signal displacements is the addition of complexing reagents such as titanium tetrachloride ($TiCl_4$) and lanthanide shift reagents. Bose *et al.* [48–50] reported that titanium tetrachloride in deuteriochloroform can be used as a shift reagent in ^1H NMR and ^{13}C NMR spectroscopy, especially for carbonyl compounds but this method has not been widely used for steroids.

The use of lanthanide shift reagents (LSR) to simplify NMR spectra is one of the most promising methods for structural analysis of molecules in solution [51, 52]. These studies utilize the addition of LSR to a deuteriochloroform solution of the substrate. Generally, $Eu(Fod)_3$, $Pr(Fod)_3$ and $Yb(Fod)_3$ have been employed. Recently we have observed [53] that due to minimal contribution of complex formation and particularly contact shifts, yt-terbium is the most suitable reagent, at least for ^{13}C NMR studies. Again the use of relative shift (RS) values [bound shift for Cα for 1:1 (substrate: LSR) complex = 100%] provides a definitive method for unambiguous assignments of Cα, Cβ, Cγ etc. To confirm the possibility of the use of $Yb(Fod)_3$ for analysis of spirostanes and to compare the binding ability between the spiroketal oxygen and a hydroxyl group, lanthonide induced shift (LIS) studies have been carried out with tigogenin (**2**) which revealed clearly that the hydroxyl group provides a well suited site for LSR complex formation [Agrawal, P. K. and Schneider, H. J. unpublished results]. In the case of hecogenin acetate (**20**) competition between the 12-carbonyl, 3-acetoxyl and spiroketal functions occurs for the LSR-complexation. Experiments show [54] that acetyl carbonyl provides a better binding site than a C-12 carbonyl perhaps due to steric hindrance imposed by the C-18 and C-21 methyl groups (Fig. 1).

Derivatization

Information for signal assignment can be obtained by comparing the chemical shifts of the original compound

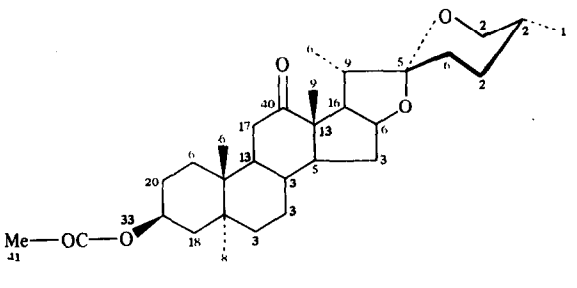


Fig. 1. Yb(Fod)$_3$-induced ^{13}C NMR shifts in hecogenin acetate (**20**) [C_α, ppm, s; other C, %, RS].

with those of its derivatives. Such derivatization can be carried out *in situ* by adding the reagent to the substrate solution in the NMR tube or by a separate chemical reaction prior to the measurements.

Bose and Srinivasan [55] have suggested the use of trichloroacetyl isocyanate as an *in situ* derivatizing reagent which generates urethane derivatives with hydroxyl compounds. Derivatization shifts the carbinol carbon downfield and adjacent carbons (Cβ) shift to upfield positions. It has also been suggested that it is possible to distinguish between primary, secondary and tertiary hydroxy functions from the magnitude of the downfield shift for carbinol carbon and this has been successfully employed for assignment purposes in steroids [56] and related compounds [Agrawal, P. K. and Schneider, H.-J., unpublished work] but in several cases it results in undesirable reactions [57, 58] and it is therefore of limited application.

Acetylation has been successfully used at least for the assignment of the hydroxyl bearing carbon and adjacent carbon atoms as it shifts the α-carbon downfield ($\sim$ 2.3 ppm) with a concurrent upfield shift (2.0–5.0 ppm) of the β-carbon atoms while other signals remain almost unaffected [59, 60]. The β-upfield shifts are reasonably attributed to a γ-effect of the carbonyl carbon, since the free rotation of the acetate group produces conformations where the carbonyl carbon is in a *gauche* position with respect to the β-carbon atoms.

Acetylation of an homoallylic alcoholic function results in a downfield shift (2.2–2.5 ppm), and an upfield shift (3.8–4.2 ppm) of the β-carbon, an upfield shift ($\sim$ 1.5 ppm) of the γ sp^2-carbon and a downfield shift ($\sim$ 1.1 ppm of the δ sp^2 carbon [61, 62].

2. ^{13}C NMR SPECTRAL ANALYSIS OF SAPOGENINS

Sapogenins are formed as a result of acidic or enzymatic hydrolysis of saponins or they occur as such in nature [10, 11]. Depending upon the carbon skeleton, these may be classified as spirostane, furostane, furospirostane and miscellaneous types.

Spirostane type

These have been characterized by the presence of a spiroketal ring system and can be further categorized as 5α-spirostane, 5β-spirostane and Δ^5-spirostane compounds.

5α-Spirostane. In these cases, the fusion between ring A and ring B is *trans* and the C-5 hydrogen possesses the α-orientation and therefore this category is regarded as the 5α-series. As the orientation of the C-27 methyl group may be *R* or *S* which was earlier differentiated by IR [63, 64] and ^{1}H NMR spectral analysis [65, 66] but ^{13}C NMR spectral analysis shows that the carbon signals of ring F are remarkably affected by the disposition of the C-27 methyl group. Hence, these are distinguished into two sub-groups.

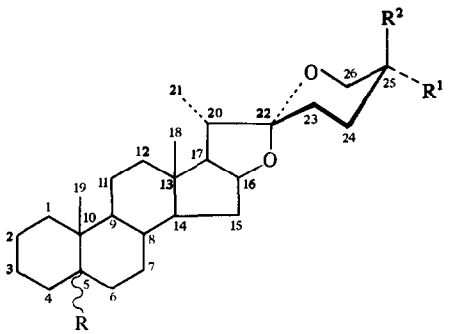
(25R)-*5α-Spirostane.* The equitorial orientation of the C-25 methyl in 22α-*O*-spirostane, and the axial orientation of the C-25 methyl in 22β-*O*-spirostane, constitute this series. The signal of the methyl on C-25 resonates at 17.1 $\pm$ 0.1 ppm in the absence of substituents in the F-ring. Other diagnostic shifts are for C-23, C-24, C-25 and C-26 which normally appear at 31.3 $\pm$ 0.3, 28.8 $\pm$ 0.3, 30.3 $\pm$ 0.3 and 66.9 $\pm$ 0.2 ppm, respectively. In most of the cases, the signal for C-22 appears at 109.5 $\pm$ 0.1 ppm but its position can vary (108.7–110.0 ppm) depending upon the solvent and structural environment. An introduction of a 14β-hydroxyl group shifts it to a higher field position at 105.8 $\pm$ 0.3 ppm [67].

Normally, the chemical shifts of the ring-F carbon atoms remain unaffected by the presence of substituents in rings A, B and C. Therefore, the substituent induced shifts (SIS) reported [17] for a large variety of androstane and cholestane derivatives can be successfully employed for the substituent pattern determination and for the stereochemical assignments. A typical example of the successful utilization of SIS for the location of the substituents is that of a hecogenin derivative isolated from *Cunninghamella elegans*. This compound clearly exhibited a close similarity with the 1β,7β-dihydroxylated pattern instead of the 1β,6β derivative, thus it leads to the characterization of the isolate as 1β,7β-dihydroxyhecogenin (**21**) [68].

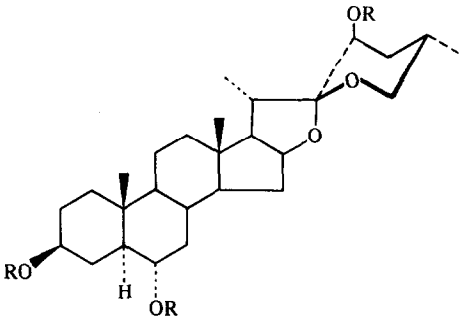
(25S)-*5α-Spirostane.* The 22α-*O*-spirostane with a C-25 axial methyl group and the 22β-*O*-spirostane with an equitorial C-25 methyl constitute this series. In the case of 22α-*O*-spirostane, the characteristic shieldings for C-23, C-24, C-26 and C-27 are at 27.3 $\pm$ 0.3, 26.1 $\pm$ 0.3, 65.1 $\pm$ 0.1 and 16.2 $\pm$ 0.2 ppm, respectively. If the chemical shifts for neotigogenin (**22**) are compared with tigogenin (**2**), this clearly demonstrates the shielding of all ring F carbon atoms due to the axially oriented C-27 methyl group. In particular the shift of C-23 exhibits a dramatic shielding of 5.4 ppm due to the existance of γ-*gauche* interactions. The signal due to C-22 generally absorbs at the same position as for the (25*R*)-series, and for the determination of functional groups in rings A, B and C the SIS reported [17] can be used (*vide supra*).

Recently, several 23-hydroxy spirostanes have been isolated from *Solanum hispidium* and their ^{13}C NMR spectral analysis has been carried out which reveals a clear cut dependence of the chemical shift of C-23 on the hydroxyl group orientation [34]. Interestingly, ^{13}C NMR chemical shift analysis is available [34] for only one 22β-*O*-spirostane type, namely hispigenin (**26**), which shows strong interactions between the C-23 methylene and the C-21 methyl group and it has been analysed by analogy with basic steroids such as tomitidine and solasodine [69].

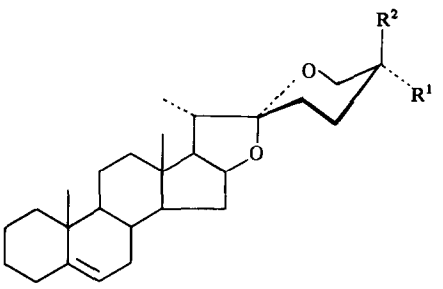
5β-Spirostane. The *cis*-fusion of ring A and ring B generates this series in which the hydrogen at the C-5 position acquires the β-orientation. A detailed analysis for this category has been reported by Tori *et al.* [42] who



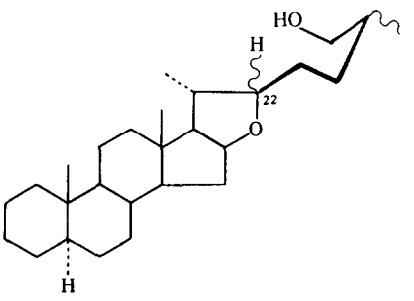
(25*R*) - 5α - Spirostan (**1**) R = α - H, R^1 = Me, R^2 = H
(25*S*) - 5α - Spirostan R = α - H, R^1 = H, R^2 = Me
(25*R*) - 5β - Spirostan (**31**) R = β - H, R^1 = Me, R^2 = H
(25*S*) - 5β - Spirostan R = β - H, R^1 = H, R^2 = Me
$\Delta^{25(27)}$ - 5β - Spirostan R = β - H, $R^1 + R^2 = CH_2$



26 R = H
27 R = Ac



(25*R*) - Δ^5 - Spirostene R^1 = Me, R^2 = H
(25*S*) - Δ^5 - Spirostene R^1 = H, R^2 = Me
$\Delta^{5,25(27)}$ - Spirostene $R^1 + R^2 = CH_2$



5α - Furostan

used INEPT techniques for the signal multiplicity determination and in specific cases by deuterium labelling, for example in the case of isodiotigenin (**37**). The usefulness of the INEPT experiments include the assignment of the signals in $CDCl_3$ (C_5D_5N) at δ_C 27.1 (27.5) to C-25 which was earlier assigned to C-23 by Eggert and Djerassi [70] and to C-24 by Marquardt [71]. These authors carried out selective ^1H NMR decoupling experiments for unambiguous signal assignments in di-, tri- and tetrahydroxylated A-rings as the hydroxy-substitution shift rule for the steroid skeleton [72] is no longer operative for 1,2-dihydroxylation [73] and for 1,3 and 1,2,3-trihydroxylation. For the establishment of the substitution pattern in rings A, B and C, substituent induced shifts reported [17] for 5β-androstane and 5β-cholestane can be employed for calculation of the shifts for a defined substitution pattern. This category can also be sub-grouped in 25(*R*) and 25(*S*) sub-groups on the basis of C-27 methyl group orientation and they exert a similar shielding phenomenon as already discussed for the 5α-spirostane series.

Convallamarogenin (**45**), is a unique example of a C-25(27) unsaturated 5β-spirostane for which ^{13}C NMR chemical shifts for only ring F are available [74] while shifts for the remaining part of the molecule can be regarded as similar to those of isorhodeasapogenin (**34**) and rhodeasapogenin (**41**) [42]. Due to this unsaturation, C-27 appears as a triplet at 108.7 while C-25 is a singlet at 144.5 ppm with 7.5 and 3.0 ppm downfield shifts of C-24 and C-23, respectively, with the negligible β-effect on C-26 [42, 74].

Δ^5-*Spirostane.* A large number of compounds belonging to this series have been reported [10, 11] and all of them possess a 3β-hydroxyl group. The existence of unsaturation between C-5 and C-6 introduces easily recognizable signals at 141.2 ± 0.8 and 121.0 ± 0.4 ppm to be assigned to the carbons C-5 and C-6, respectively, thus causing ~ 96 ppm and ~ 92.7 ppm downfield shifts of the signals for these carbons when compared with the saturated compound [70]. A comparison of the shifts for Δ^5-unsaturated and saturated compounds also reveals downfield shifts of the C-4 and C-10 signals by about 4.0 and 1.1 ppm respectively, while C-8 and C-9 shift to higher field positions by 3.3–4.5 ppm. In isonuatigenin (**55**), the hydroxyl at C-25 shifts the signals belonging to the α, β and neighbouring carbon atoms to expected shift values [75]. In pennogenin (**54**), the C-21 signal appears at 9.7 ppm due to steric interactions with the C-17α hydroxyl, thus both C-16 and C-17 appear around 90.0 ppm [71]. When the shifts for 5,6-epoxy diosgenin acetate (**59**) were compared [76] with diosgenin acetate (**47**), it was observed that a number of signals are affected by epoxidation of the Δ^5-bond which is apparently not due to simple substituent effects but also due to strain effects [77]. The oxirane ring affects the bond angles and to a lesser extent the internuclear distances and hence a slight alteration of the hybridization states of the nearby carbon atoms. An introduction of the 16α-hydroxyl as in compound **52**, causes a downfield shift of 35.3 ppm for C-16, hence it appears at 116.1 ppm in addition to the 7–8 ppm downfield shift of the β-related C-15 and C-17 carbon atoms [78]. Bethogenin (**53**) which is the 16-*O*-methyl ether of compound **52** exhibits a signal at 118.8 ppm corresponding to C-16 and an additional signal at 50.7 ppm due to the methoxyl group [78].

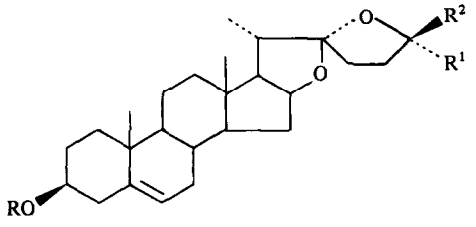
Information regarding the substitution pattern can be obtained as discussed earlier; the *R*- and *S*-configuration of the 27-methyl at C-25 are in accordance with the discussion for the 25(*R*) and 25(*S*) series (*vide supra*).

Furostane type

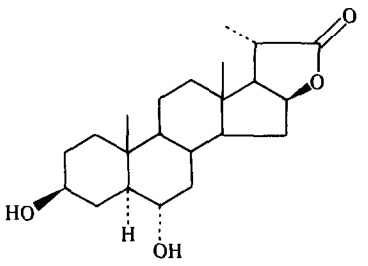
This type of sapogenin has been reported to occur as glycosides but not in the free form and it is regarded as the biogenetic precursor of spirostane saponins. During hydrolysis they produce not only the genuine aglycone but also the corresponding cyclic spirostane product. However, as the ^{13}C NMR spectral data of the furostane skeleton differs significantly from that of the spirostane skeleton, particularly in chemical shifts for the E- and F-ring carbon atoms, this provides an excellent non-degradative way for their characterization. Generally, furostanol sapogenins possess hydroxyl or methoxyl groups at the C-22 position but recently ^{13}C NMR spectral data has been reported for furostane derivatives **62** and **63** which lack the presence of such substituents [79]. In such cases, C-22 appears around 90.3 ppm while it is at ~ 110.8 ppm and ~ 113.5 ppm in the case of C-22 hydroxyl and C-22 methoxyl furostane derivatives, respectively. The C-22 methoxyl signal usually appears at 47.2 ± 0.2 [78, 80] but with some exceptions where it has been found to appear at 56.5 ppm [81]. As there is no report for the occurrence of a free furostanol in nature, most of the reports deal with ^{13}C NMR spectral analysis of the glycosides which will be discussed in detail later.

Furospirostane type

In this category ring F becomes a five membered furan ring instead of a six membered pyran ring as in spirostane. Methyl and hydroxymethyl groups are usually substituted to C-25 which appears at 85.6 ppm. The signal due to the dioxygenated C-22 absorbs at 120.9 ppm which is very characteristic for immediate differentiation of this category from other skeletal types. The only known example of this category for which the ^{13}C NMR chemical shifts are known [82, 83] is nuatigenin (**64**) and its derivatives.



64 R = H, R^1 = CH_2OH, R^2 = Me
66 R = Ac , R^1 = Me, R^2 = CH_2OAc



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Miscellaneous types

Those compounds which are derived from the steroidal skeleton are included in this group. Solanolide (**67**), a steroid lactone isolated from *Solanum hispidum*, is an example of this type, which instead of ring F possess only a keto function at C-22 which appears at 180.9 ppm [84]. A comparison of the chemical shifts of solanolide acetate (**68**) with neochlorogenin acetate (**25**) reveals that C-17 and C-18 are shifted ~ 3 ppm upfield while C-16 and C-17 are shifted ~ 2 ppm downfield in the case of the former (**68**), which is due to the modification of ring F to a lactone.

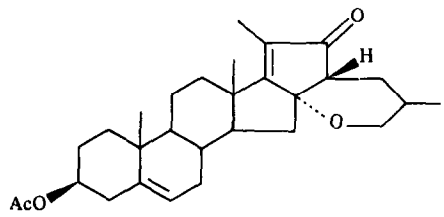
Kryptogenin (**71**) is the only example which is devoid of rings E and F due to the presence of keto functions at C-16 and C-22 which appear at 214.4 and 218.1 ppm, respectively [78].

3. ^{13}C NMR SPECTRAL ANALYSIS OF SAPONINS

Earlier methods for structure determination of saponins are quite tedious and involve a great deal of chemical derivatization or degradation work. Usually, this is carried out by permethylation by Hakomori's method [85] followed by identification of the methylated monosaccharides. These studies, no doubt, provide definite proof for the structure, but at the same time, require sufficient amounts of the substance which in most cases is not available. In this respect ^{13}C NMR spectroscopy offers a convenient and non-destructive method for studying the structure of a saponin as the sugar carbon resonances occur largely in a definite region and they are quite distinct from the resonances of the sapogenin nucleus [86]. The spectra of saponins are often best measured at higher than ambient temperature as this not only sharpens the sugar signals but also eliminates the possibilities of conformational equilibria through hindered rotation in branched oligosaccharides. The structure elucidation of a saponin may be accomplished by the following procedures.

Type and number of monosaccharides

Sugars commonly occurring in saponins are readily distinguishable from one another by ^{13}C NMR spectro-



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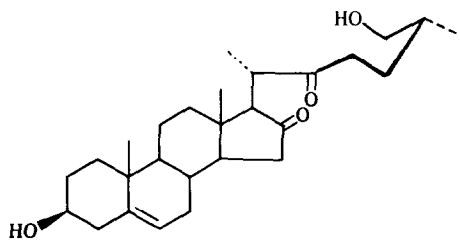

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Table 1. ^{13}C NMR chemical shifts of steroidal sapogenins*

	Trivial name	Substituents	Solvent	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8
Spirostane type: (25*R*)-5α-spirostanes											
1	Spirostane	—	C	38.7	22.2	26.8	29.0	47.1	29.0	32.4	35.2
2	Tigogenin	3β OH	C	37.0	31.4	71.2	38.2	44.9	28.6	32.2	35.1
			P	37.5	32.5	70.6	39.3	45.2	29.1	32.5	35.4
3	Tigogenin acetate	3β OAc	C	37.4	29.2	73.3	36.1	45.3	28.8	32.2	35.2
4	—	15β OH, Δ^2	C	39.7	125.7	125.5	30.3	41.6	28.6	31.4	31.2
5	—	2α, 3α $(OH)_2$	C	40.9	69.0	69.1	34.3	38.1	27.6	32.0	34.4
6	Gitogenin	2α, 3β $(OH)_2$	C	45.1	73.0	76.4	35.6	44.9	27.9	32.1	34.5
7	—	2β, 3α $(OH)_2$	C	40.1	71.7	70.6	31.8	39.0	28.2	32.1	34.6
8	—	3α, 4β $(OH)_2$	C	31.7	24.6	70.2	76.0	43.8	25.0	32.5	35.1
9	—	3α, 4β $(OAc)_2$	C	32.0	22.2	69.4	73.2	44.0	24.6	32.0	34.9
10	Chlorogenin	3β, 6α $(OH)_2$	C+M	37.8	31.9	71.2	32.2	52.1	69.3	42.2	34.4
11	—	3β OAc, 14β OH	C	37.0	27.5	73.6	34.0	44.6	28.9	27.5	40.0
12	—	3β OAc, 12β, 14β $(OH)_2$	C	36.7	27.3	73.4	33.8	44.3	28.4	27.2	40.0
13	—	3β, 12α $(OAc)_2$ 14β OH	C	36.8	27.2	73.1	33.8	44.2	28.3	27.2	39.9
14	—	3β OAc, 12β, 14β $(OH)_2$	C	36.8	27.3	73.2	33.8	44.5	28.3	27.6	39.2
15	—	3β, 12β $(OAc)_2$ 14β OH	C	36.6	27.2	73.1	33.8	44.3	28.3	27.4	39.1
16	—	3β OAc, 12CO, 14β OH	C	36.4	27.2	72.1	33.8	44.5	28.2	27.8	39.0
17	Solaspigenin acetate	3β, 6α, 23β $(OAc)_3$	C	36.8	27.1	73.6	28.4	48.5	72.0	37.7	33.8
18	11-Ketotigogenin	3β OH, 11CO	C	37.3	31.4	70.6	37.4	45.1	28.3	32.9	36.0
19	Hecogenin	3βOH, 12CO	C	36.5	31.2	70.7	37.8	44.6	28.3	31.4	34.4
20	Hecogenin acetate	3βOAc, 12CO	C	36.2	27.2	72.8	33.8	44.6	28.2	31.4	34.4
21	1β, 7β dihydroxyhecogenin	1β, 3β, 7β, $(OH)_3$, 12CO	C	76.2	42.4	67.6	37.5	39.4	37.8	73.4	42.7
(25*S*)-5α-Spirostanes											
22	Neotigogenin	3β OH	C	37.0	31.4	71.2	38.2	44.9	28.6	32.2	35.1
			P	37.5	32.5	70.6	39.3	45.2	29.1	32.5	35.4
23	—	25α OH	C	38.6	22.2	26.8	29.0	47.0	29.0	32.4	35.2
24	Polygenin	1β, 3β $(OH)_2$	C	77.9	42.3	67.9	38.0	42.3	28.4	32.0	35.6
25	Neochlorogenin acetate	3β, 6α $(OAc)_2$	C	36.8	27.1	73.0	28.4	48.5	72.0	37.8	33.7
26	Hispigenin	3β, 6α, 23β $(OH)_3$	M	38.6	31.7	72.1	33.0	53.0	70.1	42.8	35.2
27	Hispigenin acetate	3β, 6α, 23β $(OAc)_3$	C	36.8	27.1	72.9	28.3	48.5	71.9	37.8	33.6
28	Neosolaspigenin acetate	3β, 6α, 23 $(OAc)_3$	C	36.8	27.1	73.0	28.4	48.5	72.0	37.7	33.8
29	Paniculogenin	3β, 6α, 23α $(OH)_3$	M	38.6	31.7	72.0	33.0	52.9	70.0	42.8	35.2
30	Paniculogenin acetate	3β, 6α, 23α $(OAc)_3$	C	36.8	27.1	72.9	28.4	48.5	71.9	37.7	33.8
(25*R*)-5β-Spirostanes											
31	Spirostane	—	C	37.6	21.3	27.0	27.2	43.7	27.4	26.8	35.5
32	Smilagenin	3β OH	C	29.9	27.8	66.9	33.5	36.5	26.6	26.6	35.3
			P	30.6	28.6	66.0	34.4	37.0	27.2	26.9	35.6
33	Epismilagenin	3α OH	C	35.5	30.5	71.8	36.5	42.1	27.1	26.7	35.5
			P	36.0	31.4	71.1	37.2	42.4	27.5	27.0	35.7
34	Isorhodeasapogenin	1β, 3β $(OH)_2$	P	73.4	32.9	68.2	34.4	31.2	26.7	26.7	35.9
35	Yonogenin	2β, 3α $(OH)_2$	C	43.8	71.0	76.2	35.4	41.7	26.4	26.6	35.4
			P	44.7	71.3	77.0	35.5	42.3	26.9	26.9	35.7
36	Tokorogenin	1β, 2β, 3α $(OH)_3$	P	76.6	74.2	71.2	35.3	35.9	26.5	26.5	35.6
37	Isodiotigenin	2β, 3α, 4β $(OH)_3$	P	44.2	68.7	83.0	70.8	49.2	21.5	26.6	35.6
38	Kogagenin	1β, 2β, 3α, 5β $(OH)_4$	P	79.2	73.8	69.0	42.5	76.8	35.6	28.8	34.9
39	Kitigenin	1β, 3β, 4β, 5β $(OH)_4$	P	73.6	33.3	71.1	68.0	78.3	30.4	28.5	35.0
(25*S*)-5β-Spirostanes.											
40	Sarsasapogenin	3β OH	C	29.9	27.8	66.9	33.5	36.5	26.6	26.6	35.3
41	Rhodeasapogenin	1β, 3β $(OH)_2$	C	73.4	32.9	68.2	34.4	31.2	26.7	26.7	35.9
42	Neoyonogenin	2β, 3α $(OH)_2$	C	43.8	71.0	76.2	35.4	41.7	26.4	26.6	35.4
			P	44.7	71.3	77.0	35.5	42.3	26.9	26.9	35.7
43	Neotokorogenin	1β, 2β, 3α $(OH)_3$	P	76.6	74.2	71.2	35.3	35.9	26.5	26.5	35.6
44	Diotigenin	2β, 3α, 4β $(OH)_3$	P	44.2	68.7	83.0	70.8	49.2	21.5	26.6	35.6
$\Delta^{25(27)}$-5β-Spirostane											
45	Convallamarogenin	1β, 3β $(OH)_2$		—	—	—	—	—	—	—	—
45a	$\Delta^{25(27)}$-Pentologenin-tetraacetate	1β, 2β, 3β, 4β $(OAc)_4$, 5β OH	C	65.3	67.3	69.8	74.2	75.6	—	—	—
(25*R*)-Δ^5-Spirostenes											
46	Diosgenin	3β OH	C	37.3	31.4	71.6	42.3	140.9	121.3	32.0	31.4

C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-16	C-17	C-18	C-19	C-20	C-21	C-22	C-23	C-24	C-25	C-26	C-27	Ref.
54.8	36.3	20.7	40.2	40.6	56.5	31.8	80.8	62.3	16.5	12.3	41.6	14.5	109.0	31.4	28.9	30.3	66.7	17.1	70, 73
54.4	35.6	21.1	40.1	40.6	56.3	31.8	80.8	62.3	16.5	12.3	41.6	14.5	109.2	31.4	28.8	30.3	66.8	17.1	42, 67
54.6	35.9	21.1	40.3	40.8	57.6	32.1	81.1	63.1	16.7	12.5	42.0	15.0	109.2	31.9	29.3	30.6	66.9	17.3	42
54.5	35.8	21.2	40.1	40.6	56.3	31.8	80.9	62.4	16.5	12.7	41.7	14.5	109.3	31.5	28.9	30.4	66.8	17.2	110
54.6	34.9	22.0	42.6	40.6	61.3	69.6	82.1	60.7	19.1	11.7	42.6	14.2	109.0	31.4	28.6	30.2	67.1	17.1	70
54.2	37.0	20.7	40.0	40.6	56.2	31.7	80.7	62.2	16.5	12.4	—	—	—	—	—	—	—	—	72, 73
54.3	37.6	21.2	40.0	40.6	56.1	31.8	80.7	62.2	16.5	13.5	—	—	—	—	—	—	—	—	73
55.2	35.9	20.8	40.1	40.6	56.3	31.8	80.7	62.2	16.5	14.4	—	—	—	—	—	—	—	—	73
55.1	35.9	20.0	39.9	40.5	56.3	31.6	80.7	62.1	16.5	14.4	—	—	—	—	—	—	—	—	73
54.9	35.6	22.2	39.8	40.4	56.2	31.7	80.7	62.2	16.4	13.7	—	—	—	—	—	—	—	—	73
54.4	36.8	21.4	40.3	41.1	56.5	31.6	81.4	62.7	16.6	13.6	41.8	14.6	110.0	31.0	29.1	30.6	67.1	17.2	76
49.6	35.9	20.7	39.7	46.7	86.3	38.9	79.9	62.4	14.4	12.1	46.3	14.7	106.1	31.6	28.7	30.3	67.1	17.1	67
42.6	35.2	28.7	75.7	50.5	85.8	40.6	81.1	57.7	15.2	12.1	46.1	14.8	105.6	31.5	28.7	30.2	66.7	17.1	67
43.4	35.1	25.4	78.2	49.6	85.5	40.4	80.7	57.6	14.6	12.0	46.0	14.9	105.6	31.5	28.7	30.1	66.7	17.0	67
45.9	35.6	29.7	74.4	53.0	86.3	38.6	79.5	57.0	7.4	12.0	45.9	14.4	105.7	31.5	28.7	30.1	66.8	17.0	67
45.8	35.7	25.8	76.8	51.4	86.2	38.4	79.3	57.2	8.7	12.0	45.8	14.3	105.5	31.5	28.7	30.1	66.7	17.0	67
46.9	36.0	37.5	212.3	62.0	87.0	36.9	78.8	51.3	14.6	11.8	45.6	14.6	105.7	31.6	28.7	30.0	66.7	17.0	67
53.8	36.6	20.8	39.3	40.9	56.0	31.7	81.2	63.9	16.1	13.3	40.3	16.1	107.0	72.0	33.8	24.7	66.5	16.7	34
62.8	35.3	211.2	57.8	44.6	55.9	31.1	81.0	61.0	17.0	12.2	42.0	14.2	109.5	31.1	28.8	30.3	67.1	17.2	76
55.5	36.0	37.8	213.0	55.0	55.8	31.5	79.1	53.5	16.0	12.0	42.2	13.2	109.0	31.2	28.8	30.2	66.8	17.1	110
55.3	36.0	37.6	211.7	54.9	55.6	31.4	78.9	53.6	15.9	11.8	42.2	13.2	108.7	31.1	28.9	30.2	66.6	17.1	67, 76
53.5	41.2	40.2	213.7	55.5	54.5	34.4	79.4	52.7	16.2	6.6	42.4	13.3	109.3	31.4	28.8	30.2	67.0	17.1	68
54.4	35.6	21.1	40.1	40.6	56.3	31.8	80.8	62.3	16.5	12.3	42.2	14.3	109.7	27.1	25.8	26.0	65.2	16.1	42
54.6	35.9	21.4	40.3	40.8	56.6	32.1	81.1	63.1	16.7	12.5	42.5	14.9	109.7	27.5	26.2	26.4	65.1	16.3	42
54.8	36.4	20.6	40.1	40.6	56.5	31.7	81.3	62.0	16.5	12.3	41.5	14.4	108.8	24.7	32.7	66.6	68.9	27.0	70
54.9	42.3	24.3	40.0	40.0	56.4	32.0	80.8	62.2	16.4	6.8	41.5	14.3	109.8	27.1	25.8	25.8	65.1	16.0	112
53.6	36.6	20.9	39.8	40.5	55.9	31.6	80.6	61.9	16.4	13.3	42.1	14.3	109.6	27.1	25.7	25.9	65.1	16.0	34
55.4	37.4	22.2	41.3	42.3	56.7	34.7	85.4	64.4	16.5	13.9	43.4	16.6	113.5	70.9	38.6	31.8	69.5	17.1	34
53.7	36.6	20.9	39.9	41.3	55.2	33.4	83.7	63.0	16.2	13.3	42.3	16.0	110.3	73.3	33.6	30.1	68.5	16.5	34
53.6	36.6	20.8	39.9	40.9	56.0	31.7	81.4	63.4	16.1	13.3	40.6	15.6	107.6	71.8	30.6	25.8	64.7	19.3	34
55.3	37.4	22.1	41.3	42.1	57.3	32.5	82.6	63.1	17.0	13.8	37.0	14.3	112.6	64.0	36.0	31.2	65.2	17.6	34
53.5	36.6	20.8	39.6	41.0	55.9	31.5	81.1	61.4	16.0	13.3	36.4	13.9	109.2	65.2	31.5	29.6	64.1	17.2	34
40.6	35.5	20.6	40.3	40.6	56.5	31.7	81.0	62.3	16.4	24.2	41.6	14.5	109.2	31.4	28.8	30.3	66.8	17.1	42
40.3	35.3	20.9	39.9	40.7	56.5	31.7	80.9	62.3	16.4	23.9	41.6	14.5	109.2	31.4	28.8	30.3	66.8	17.1	42, 70
40.4	35.6	21.2	40.2	41.0	56.6	32.2	81.2	63.2	16.6	24.2	42.0	15.0	109.2	31.9	29.3	30.6	66.9	17.3	42
40.6	34.7	20.6	40.3	40.6	56.4	31.8	80.9	62.3	16.5	23.4	41.6	14.5	109.2	31.4	28.8	30.3	66.8	17.1	42
40.8	34.9	20.9	40.2	40.8	56.4	32.1	81.1	63.1	16.6	23.7	42.0	15.0	109.2	31.9	29.3	30.6	66.9	17.3	42
42.2	40.4	21.1	40.4	40.7	56.4	32.2	81.2	63.2	16.6	19.4	41.6	14.6	109.1	31.4	28.8	30.3	66.8	17.1	42
42.2	36.7	20.9	40.1	40.6	56.2	31.8	80.8	62.2	16.5	23.5	41.6	14.5	109.2	31.4	28.8	30.3	66.8	17.1	42, 79
42.3	37.2	21.1	40.2	40.8	56.2	31.8	81.2	63.1	16.6	23.6	42.0	15.0	109.2	31.9	29.3	30.6	66.9	17.3	42
42.0	41.2	21.2	40.1	40.6	56.3	32.1	81.1	63.1	16.6	19.1	41.6	14.5	109.2	31.4	28.8	30.3	66.8	17.1	42, 79
44.2	36.6	21.2	40.1	40.8	56.3	32.0	81.2	63.0	16.6	23.8	41.6	14.4	109.1	31.4	28.8	30.3	66.8	17.1	42
45.3	44.7	21.6	39.9	40.6	56.1	32.2	81.1	63.0	16.5	13.6	41.6	14.4	109.1	31.4	28.8	30.3	66.8	17.1	42
45.4	45.7	21.4	40.1	40.7	56.3	32.2	81.1	63.0	16.6	13.8	41.6	14.4	109.1	31.4	28.8	30.3	66.8	17.1	42
40.3	35.3	20.9	39.9	40.7	56.5	41.7	80.9	62.3	16.4	23.9	42.2	14.3	109.7	27.1	25.8	26.0	65.2	16.1	42, 70
42.2	40.4	21.1	40.4	40.7	56.4	32.2	81.2	63.2	16.6	19.4	42.2	14.3	109.7	26.0	25.8	27.1	65.2	16.1	42
42.2	36.7	20.9	40.1	40.6	56.2	31.8	80.8	62.2	16.5	23.5	42.2	14.3	109.7	26.0	25.8	27.1	65.2	16.1	42
42.3	37.2	21.1	40.2	40.8	56.3	32.1	81.2	63.1	16.6	23.6	42.5	14.9	109.7	26.4	26.2	27.5	65.1	16.3	42
42.0	41.2	21.2	40.1	40.6	56.3	32.1	81.1	63.1	16.6	19.1	42.2	14.3	109.7	26.0	25.8	27.1	65.2	16.1	42, 79
44.2	36.6	21.2	40.1	40.8	56.3	32.0	81.2	63.0	16.6	23.8	42.2	14.3	109.7	26.0	25.8	27.1	65.2	16.1	42
—	—										42.0	15.0	109.4	29.0	33.3	144.5	65.1	108.7	74
—	—								16.3	12.3	41.6	14.4	109.3			143.5	64.9	108.5	80
50.1	37.6	20.9	39.8	40.3	56.8	31.8	80.8	62.1	16.3	19.4	41.6	14.5	109.2	31.4	28.8	30.3	66.8	17.1	42, 62, 70, 71, 107, 111, 113, 114

Table 1 (*Continued*)

	Trivial name	Substituents	Solvent	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8
46	Diosgenin	3β OH	P	37.2	31.6	71.5	42.2	140.8	121.3	32.0	31.4
46	Diosgenin	3β OH	D	37.2	31.6	71.5	42.2	140.8	121.3	32.0	31.4
47	Diosgenin acetate	3β OAc	C	36.9	27.8	73.9	38.1	139.7	122.4	32.1	31.4
48	Chlorodiosgenin	3β Cl	C	39.2	33.5	60.1	43.5	141.0	122.2	32.0	31.5
49	6-Methyl diosgenin acetate	3β OAc, 6 Me	C	37.3	27.8	73.8	28.8	132.2	126.6	39.4	31.4
50	Ruscogenin	1β, 3β $(OH)_2$	P	78.2	44.0	68.3	43.7	140.5	124.3	33.2	32.5
51	Isochiapagenin	3β, 12β $(OH)_2$	C	37.2	31.6	71.6	42.1	140.8	121.3	31.4	30.4
52	16α-Hydroxydiosgenin	3β, 16α $(OH)_2$	C	37.2	31.6	71.6	42.3	141.7	121.4	32.1	31.2
53	Bethogenin	3β OH, 16α OMe	C	37.2	31.6	71.6	42.3	140.9	121.3	31.9	31.0
54	Pennogenin	3β, 17α $(OH)_2$	P	37.9	32.4	71.2	43.4	142.0	121.0	32.2	32.4
55	Isonuatigenin	3β, 25β $(OH)_2$	P	37.8	31.8	71.2	43.4	141.9	120.9	32.6	32.3
56	Bahamagenin	3β, 12β, 15α $(OH)_3$	C	37.2	31.6	71.2	42.2	139.9	121.7	31.2	30.0
57	Botogenin	3β OH, 12 CO	C	36.9	31.3	71.2	42.0	140.8	121.0	31.6	30.9
58	5-Epoxydiosgenin acetate	3β OAc, 5α, 6β, epoxy	C	32.2	27.4	71.4	36.3	65.2	59.0	29.6	29.1
(25*S*)-Δ⁵-Spirostenes											
59	Yamogenin	3β OH	C	37.3	31.4	71.6	42.3	140.9	120.3	32.0	31.4
			P	37.8	32.5	71.2	43.4	142.0	121.0	32.3	31.8
60	Neoruscogenin	1β, 3β $(OH)_2$	P	78.2	44.0	68.2	43.6	140.3	124.3	33.1	32.4
61	Neobotogenin	3β, 12 CO	C	36.9	31.3	71.2	42.0	140.9	121.0	31.6	30.9
Δ⁵, Δ$^{25(27)}$-Spirostenes											
61a	—	1β, 3β $(OH)_2$	P	78.1	43.5	68.1	43.6	140.5	124.2	—	—
5α-Furostanes											
62	—	3β OAc, 26 OH	C	36.8	27.5	73.7	34.1	44.7	28.5	32.2	35.3
63	—	3β, 26 $(OAc)_2$	C	36.8	27.5	73.7	34.0	44.7	28.5	32.2	35.3
Furospirostanes											
64	Nuatigenin	3β, 26 $(OH)_2$	P	37.8	31.7	71.3	43.5	142.0	120.3	32.6	32.2
65	Nuatigenin acetate	3β, 26 $(OAc)_2$	—	37.0	27.8	73.9	38.1	139.7	122.4	31.8	31.4
66	(25*R*)-Nuatigenin acetate	3β, 26β $(OAc)_2$	—	37.0	27.8	73.9	38.1	139.7	122.4	31.8	31.4
Miscellaneous											
67	Solanolide	3β, 6α $(OH)_2$	P	38.2	32.1	70.8	33.2	52.5	68.3	42.5	33.9
68	Solanolide acetate	3β, 6α $(OAc)_2$	P	36.8	27.0	72.8	28.2	48.4	71.6	37.6	33.4
69	—	3, 6 $(CO)_2$	P	33.0	37.1	210.5	36.9	57.4	207.7	46.3	37.2
70	—	—	C	37.0	27.8	73.8	38.1	140.0	121.9	31.8	31.5
71	Kryptogenin	—	C	37.2	31.4	71.3	42.1	141.2	120.8	31.7	31.0

*Other signals: (OCO, Me): **3** 171.60, 21.30; **9** 169.2, 169.3, 20.9, 21.1; **11** 170.6, 21.4; **12** 170.1, 21.3; **13** 179.1, 169.7, 21.2, 21.6; **14** 170.0, 21.3; **28** 170.1, 170.5, 170.7, 21.3; **30** 170.5, 170.8, 21.2, 21.3, 21.4; **47** 170.3, 21.3; **63**, 170.7, 21.4; **64** 170.7, 171.3, 21.0, 21.4; **66** 170.5, 171.1,

scopy. The number of anomeric signals determines the number of monosaccharides, while best-fit matching with appropriate sugars lead to their identification. This is clearly seen from the selection of ^{13}C NMR spectral data [87–89] for monosaccharides which usually constitute the sugar part of saponins (Table 3). Solvents may alter the chemical shifts markedly [90, 91] and therefore comparisons should be carried out in the same solvent.

Furanose sugars are readily distinguished from their pyranose isomers as these differ significantly in the chemical shifts for C-1, C-2 and C-4 which appear 4–14 ppm downfield whereas C-5 is shifted 4–7 ppm upfield in the furanose form compared to the respective pyranose isomers [90–92]. Thus, as a general principle, a count of the anomeric carbon signals determines the number of sugars while the resemblance of the chemical shifts with those of appropriate sugars will establish the ring size and type of each monosaccharide present.

Interglycosidic linkage

The site at which one sugar is attached to another sugar of a saponin can readily be determined by ^{13}C NMR spectroscopy and this is perhaps the most significant information contained in the spectrum which is difficult to obtain by other methods. The sequence of the sugars in a saponin oligoglycoside can be predicted on the basis of chemical shifts as well as by determination of relaxation time (T_1) measurements.

A close resemblance of the chemical shifts due to a terminal sugar with respect to a methyl-*O*-glycoside lead to its immediate characterization whereas chemical shifts of other (inner) sugars differ significantly in comparison to methyl-*O*-glycosides (Table 2) due to α- and β-effects of glycosylation. In oligoglycosides, the glycosylation causes a downfield shift of 4.2–8.5 ppm of the α-carbon, the hydroxyl of which has been directly involved in the glycosylation while neighbouring β-carbon atoms show an upfield shift of 0.5–2.0 ppm. These α- and β-shifts are independent of the nature of the monosaccharide and provide a conclusive method for the establishment of interglycosidic linkages. The shifts for the remaining part of the sugar remain almost unaffected and can be compared with the standard values for appropriate methyl-*O*-glycosides. The reported assignments for C-3 of xylose and galactose have been mutually reversed in

C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-16	C-17	C-18	C-19	C-20	C-21	C-22	C-23	C-24	C-25	C-26	C-27	Ref.
50.1	36.6	20.9	39.8	40.2	56.5	31.8	80.7	62.1	16.3	19.7	41.6	14.5	109.9	31.4	28.8	30.3	66.7	17.1	42
50.1	36.6	20.9	39.8	40.2	56.5	31.8	80.7	62.1	16.3	19.4	41.6	14.5	109.1	31.4	28.4	30.3	66.7	17.1	86, 115
49.9	36.7	20.8	39.7	40.2	56.4	31.9	80.8	62.2	16.3	19.3	41.6	14.5	109.2	31.4	28.8	30.3	66.8	17.1	62
50.2	36.6	20.9	39.8	40.3	56.6	31.9	80.8	62.3	16.3	19.3	41.7	14.6	109.3	31.5	28.9	30.4	66.9	17.2	76
50.2	37.1	21.0	39.9	40.3	56.5	31.9	81.7	62.2	16.3	19.4	41.7	14.6	109.4	31.4	28.8	30.4	66.9	17.2	76
51.6	43.7	24.4	40.8	40.4	57.2	32.6	81.2	63.4	16.7	14.0	42.2	15.1	109.3	32.1	29.5	30.7	66.1	17.4	105
49.7	36.7	30.4	79.6	45.7	55.1	31.8	80.7	61.9	10.4	19.3	42.1	13.9	109.5	31.3	28.8	30.3	66.9	17.1	75
50.0	36.7	20.8	39.7	42.1	55.5	38.8	116.1	70.8	15.2	19.4	40.0	14.8	110.9	31.9	28.5	30.2	68.0	17.1	78
50.0	36.7	20.8	39.7	40.5	55.2	33.8	118.8	69.1	15.5	19.4	42.5	14.8	111.0	33.4	29.1	30.2	68.6	17.3	78
50.4	37.0	21.0	32.4	44.8	53.2	31.8	90.0	90.0	17.2	19.6	45.2	9.7	109.9	32.1	28.8	30.4	66.7	17.3	71, 107
50.4	37.0	21.2	40.0	40.5	56.8	32.2	81.3	63.0	16.4	19.6	42.0	15.1	109.5	27.8	33.8	65.1	69.7	26.9	82
49.2	36.5	30.6	78.9	45.2	58.4	78.5	89.6	58.8	11.3	19.2	41.7	13.3	109.4	31.2	28.6	30.3	66.9	17.0	75
52.2	37.2	37.4	213.4	54.8	56.0	31.3	79.9	53.3	15.5	19.0	42.3	13.2	109.7	31.3	28.8	30.2	66.9	17.1	76
41.7	35.2	20.5	39.5	40.4	56.7	31.5	80.7	62.1	16.3	16.0	42.6	14.5	109.3	31.8	29.1	30.4	66.7	17.2	76
50.1	36.6	20.9	39.8	40.3	56.5	31.8	80.8	62.1	16.3	19.4	42.2	14.3	109.7	26.0	25.8	27.1	65.2	16.1	42, 71, 79
50.5	37.0	21.2	40.0	40.5	46.8	32.2	81.1	62.8	16.4	19.6	42.5	14.9	109.7	27.6	26.2	26.4	65.1	14.9	107
51.4	43.6	24.3	40.7	40.3	57.0	42.5	81.2	63.1	16.7	14.0	42.6	15.0	109.8	26.5	26.3	27.6	65.2	16.4	105
52.2	37.2	37.4	213.4	54.8	56.0	31.3	79.4	53.2	15.9	19.0	42.8	13.1	109.7	26.1	25.8	27.1	65.2	16.0	76
51.6	53.8	24.2	40.4	40.8	57.2	—	81.5	63.5	16.5	13.7	42.2	14.8	109.5	—	—	144.0	65.1	108.1	111
54.3	35.6	20.9	39.7	41.1	56.7	32.2	83.3	65.3	16.6	12.3	38.0	18.9	90.3	30.5	30.2	35.8	68.1	16.6	79
54.3	35.6	20.9	39.7	41.0	56.7	32.2	83.3	65.3	16.6	12.3	38.0	18.9	90.2	30.8	30.5	32.8	69.4	16.8	79
50.5	37.0	21.2	40.0	40.6	56.6	32.3	81.1	62.6	16.2	19.6	38.5	15.2	120.9	32.6	33.8	85.6	70.1	24.1	82
50.0	36.8	21.4	39.7	40.4	56.4	32.0	80.8	61.7	16.2	19.3	38.3	14.6	120.1	32.8	33.1	82.4	70.4	23.9	83
50.0	36.8	21.4	39.7	40.4	56.4	32.0	80.7	62.0	16.2	19.3	38.4	14.6	120.2	33.0	33.8	82.1	69.8	26.0	83
54.2	36.5	20.8	37.9	41.7	54.4	33.5	82.6	58.9	13.8	13.6	36.2	17.9	180.9	—	—	—	—	—	84
53.4	36.6	20.4	38.0	41.7	54.1	32.9	82.3	58.8	13.7	13.2	36.0	17.8	180.8	—	—	—	—	—	84
53.4	41.0	21.0	37.7	42.1	54.6	32.8	82.1	58.8	13.8	12.6	36.0	17.9	180.7	—	—	—	—	—	84
50.2	36.8	20.4	35.5	44.2	53.8	43.9	84.7	180.3	15.3	19.3	130.2	8.4	211.1	51.5	25.7	26.7	68.0	18.1	78
49.7	36.6	20.6	39.6	41.7	51.2	38.6	214.4	66.2	15.4	19.4	43.3	12.9	218.1	37.0	26.3	35.2	67.3	16.7	78

21.3; **15** 169.8, 170.1, 21.0, 21.2; **16** 169.5, 21.2; **17** 170.2, 170.5, 170.8, 21.2; **20** 169.4, 21.2; **25** 170.5, 170.7, 21.3, 21.4; **27** 169.7, 170.5, 170.7, 20.8, 21.0; **67** 170.5, 171.0, 20.8, 20.9; **69** 170.3, 170.5, 21.1, 21.3. OMe: **53** 50.7.

compound **86** [93]. Based on ^{13}C NMR spectral analysis, it is possible to distinguish various sapogenin diglycosides which are not easily distinguishable by other methods (Table 2). The upfield shifts of the β-carbon atoms are quite informative but less consistent whereas the downfield shift of the α-carbon is characteristic enough for the establishment of the interglycosidic linkage.

The use of relaxation time (T_1) data for sugar sequencing in saponins has recently been reported by Hirai *et al.* [80]. This method is based on the principle [94] that the average NT_1 values for sugar carbons increase with increasing distance from the aglycone moiety.

Anomeric configuration

As is evident from Table 2, the chemical shifts of the anomeric carbons are quite dependent upon their configuration and hence they provide an easy means of determining anomeric carbon configurations. The examples of smilagenin-3-*O*-α-D-glucopyranoside (**79**) and smilagenin-3-*O*-β-D-glucopyranoside (**81**) can be cited here which show the appearance of the anomeric C-1 signal at 98.7 ppm in **79** while it is at 103.1 ppm in **81**. Other carbon signals of the glucose and the aglycone are affected to various degrees depending upon the anomeric configuration [95].

Another important feature which can be successfully utilized in hexapyranosides is the one bond ^{13}C–^{1}H coupling constant for the anomeric carbon which strictly depends upon the orientation of the anomeric hydrogen. The one-bond coupling constants for the C-2 to C-6 carbon atoms of sugars vary in the range of 142–148 Hz while the anomeric carbon exhibits a larger value of 160–175 Hz [96, 97]. For pyranose with an axial H-1 the value is *ca* 10 Hz lower than the corresponding value in compounds with an equatorial H-1. This has been found at *ca* 160 Hz in the β-anomer and *ca* 170 Hz in the α-anomer [96, 97]. The two bond ^{13}C–^{1}H coupling constant has an opposite sign for the α (+) than for β (−) anomer [98, 99]. The coupling constant information is of general applicability for evaluating the anomeric configuration. Peracetylation leads to somewhat higher values than in underivatized carbohydrates (Table 3) but the difference between anomers is usually maintained at 10 Hz [35].

Table 2. ^{13}C NMR chemical shifts of steroidal saponins* [(i) refers to the aglycone, (ii)–(iv) to the sugar moieties]

	Glycoside	Solvent	C-1	C-2	C-3	C-4	C-5	C-6	C-7
(i)									
72	To, 1-*O*-ar.	P	88.9	75.0	71.6	35.0	36.5	26.2	26.5
73	Nto, 1-*O*-ar.	P	88.9	75.0	71.6	35.0	36.5	26.2	26.5
73a	**61a**, 1-*O*-ar.	P	83.1	43.6	67.9	42.7	139.5	124.5	—
74	Di, 3-*O*-gl.	P	37.5	30.2	78.5	39.3	141.0	121.7	32.2
75	Di, 3-*O*-gl(Ac)$_4$	P	37.4	30.0	79.7	39.3	140.6	122.1	32.3
76	Di, 3-*O*-ga.	P	37.7	30.5	78.4	39.6	141.3	121.7	32.5
77	Ru, 3-*O*-rh.	P	78.0	41.1	73.9	39.8	139.4	125.1	33.1
78	Ru, 3-*O*-rh; 1-sulphate	P	83.9	37.2	73.5	39.5	138.1	125.9	33.2
79	Sm, 3-*O*-gl.	P	31.2	24.9	73.7	32.8	37.9	27.1	27.1
80	Sm, 3-*O*-gl(Ac)$_4$.	C	30.4	24.0	73.5	32.0	37.4	26.8	26.8
81	Sm, 3-*O*-gl.	P	31.0	27.0	74.7	31.0	37.2	27.1	27.1
82	Sm, 3-*O*-gl(Ac)$_4$.	C	30.5	26.7	74.9	30.5	37.1	26.7	26.7
83	Nru, 1-*O*-rh(1 → 2)Fu.	P	84.1	38.0	68.4	44.0	139.9	124.8	33.4
84	Ru, 1-*O*-rh-sulphate(1 → 2) ar.	P	—	—	—	—	—	—	—
84a	**61a**, 1-*O*-rh(1 → 2)ar.	P	83.5	43.7	68.3	43.0	139.7	124.6	—
85	Di, 3-*O*-rh(1 → 2)gl.	P	—	—	—	—	—	—	—
86	Sa, 3-*O*-xy(1 → 2)ga.	P	—	—	—	—	—	—	—
87	Sa, 3-*O*-gl(1 → 2)ga.	P	—	—	—	—	—	—	—
88	Ru, 3-*O*-gl(1 → 3)rh.	P	77.9	41.0	73.9	39.6	139.3	123.1	33.1
89	Ya, 3-*O*-gl(1 → 3)gl.	P	37.4	30.1	78.5	39.4	140.8	121.6	32.3
90	Di, 3-*O*-rh(1 → 4)gl.	—	37.4	30.2	78.4	39.2	140.9	121.7	32.2
91	Ya, 3-*O*-gl(1 → 4)gl.	P	37.5	30.0	78.5	39.3	140.9	121.7	32.3
92	Di, 3-*O*-gl(1 → 4)ga.	P	37.6	30.4	78.4	39.4	141.2	121.6	32.4
93	Di, 3-*O*-ar(1 → 4)gl. peracetate	C	37.2	30.7	76.6	39.0	140.5	121.8	32.0
94	Pe, 3-*O*-ar(1 → 4)gl. peracetate	C	37.2	29.5	76.6	39.0	140.5	121.7	32.0
95	Nru, 1-*O*-fu; 3-*O*-rh.	P	83.7	35.9	73.7	39.6	138.5	125.6	33.2
96	Di, 3-*O*-rh(1 → 2)[xy(1 → 3)]gl.	P	37.5	30.2	78.1	39.0	140.4	121.7	32.2
96a	Di, 3-*O*-rh(1 → 2)[rh(1 → 3)]gl.	P	37.5	30.0	77.9	38.7	140.8	121.7	32.2
97	Ya, 3-*O*-rh(1 → 2)[xy(1 → 3)]gl.	P	—	—	—	—	—	—	—
98	Di, 3-*O*-gl(1 → 2)[rh(1 → 3)]gl.	P	37.4	30.4	77.4	38.7	140.8	121.4	32.0
99	Di, 3-*O*-rh(1 → 4)[rh(1 → 2)]gl.	P	37.4	30.2	78.7	39.2	140.9	121.8	32.2
100	Di, 3-*O*-rh(1 → 4)[rh(1 → 2)]gl. peracetate	P	37.4	29.9	78.4	38.5	140.4	122.5	32.2
101	Ya, 3-*O*-rh(1 → 2)[rh(1 → 4)]gl.	P	—	—	—	—	—	—	—
102	Di, 3-*O*-rh(1 → 2)[ar(1 → 4)]gl.	P	37.4	30.0	77.6	40.4	140.8	121.6	32.2
103	Di, 3-*O*-gl(1 → 2)[gl(1 → 4)]ga.	P	37.5	30.2	78.4	39.3	141.1	121.5	32.3
104	Di, 3-*O*-rh(1 → 2)[gl(1 → 3)]gl.	P	37.5	30.0	78.4	38.6	140.8	121.7	32.2
105	Di, 3-*O*-rh(1 → 2)[gl(1 → 4)]gl.	P	—	—	—	—	—	—	—
106	Zingiberenin B$^+$	P	37.3	30.3	77.5	38.8	140.8	121.1	32.0
107	Di, 3-*O*-rh(1 → 2)[rh(1 → 4)]gl.	P	—	—	—	—	—	—	—
108	Ya, 3-*O*-rh(1 → 2)[rh(1 → 4)]gl.	P	—	—	—	—	—	—	—
109	Pe, 3-*O*-rh(1 → 2)[ar(1 → 4)]gl.	P	37.4	30.0	77.6	38.8	140.7	121.6	32.2
110	Di, 3-*O*-rh(1 → 3)[ar(1 → 4)]gl.	P	37.4	29.3	77.3	39.2	141.0	121.9	32.5
111	Ya, 3-*O*-rh(1 → 2)[gl(1 → 4)]gl.	P	37.4	30.2	78.2	39.4	141.2	121.6	32.3
112	Ya, 3-*O*-xy(1 → 6)-gl[(1 → 3)[rh(1 → 2)]gl.	P	37.4	29.9	78.2	39.0	141.2	121.5	32.1
113	Ya, 3-*O*-rh(1 → 2)-gl(1 → 4)[rh(1 → 2)]gl.	P	37.2	29.9	78.1	38.7	140.6	121.2	32.0
114	Di, 3-*O*-rh(1 → 4)-rh(1 → 4) [rh(1 → 2)]gl.	P	37.4	30.0	77.2	38.8	140.7	121.6	32.2
115	Ti, 3-*O*-xy(1 → 3)[gl(1 → 4)]gl(1 → 3)ga.	P	37.2	30.6	77.5	35.8	44.8	28.9	32.4
116	Di, 3-*O*-gl(1 → 2)gl(1 → 4)[xy(1 → 3)]ga.	P	37.6	30.2	78.5	39.4	141.2	121.6	32.4
117	Nti, 3-*O*-xy(1 → 2)[xy(1 → 3)]gl(1 → 4)[rh(1 → 2)]ga.	P	37.3	29.9	78.5	34.5	44.8	29.0	32.5
5β-Furostanes									
118	Yo, 26-*O*-gl	P	44.8	71.3	77.0	35.6	42.4	26.9	26.9
119	To, 1-*O*-ar; 26-*O*-gl	P	88.8	74.9	71.6	34.9	36.4	26.1	26.4
Furost-5-enes									
120	3β,22(OH)$_2$, 1-*O*-rh-4-sulphate (1 → 2)ar; 26-*O*-gl	P	—	—	—	—	—	—	—
121	22OH,3-*O*-rh(1 → 2)gl; 26-*O*-gl.	P	—	—	—	—	—	—	—
122	17α, OH, 22 OMe, 3-*O*-rh(1 → 2)gl; 26-*O*-gl.	P	37.6	30.2	77.8	39.0	140.9	121.7	31.7
123	22OH, 3-*O*-gl(1 → 4)[rh(1 → 2)]gl; 26-*O*-gl.	P	—	—	—	—	—	—	—
124	22OMe, 3-*O*-rh(1 → 4)[rh(1 → 2)]gl; 26-*O*-gl.	P	37.4	29.6	77.4	39.2	141.0	122.0	32.3
125	17α, OH, 22OMe, 3-*O*-rh(1 → 4)[rh(1 → 2)]gl; 26-*O*-gl.	P	37.6	30.2	77.9	39.0	140.9	121.8	31.7
126	22OH, 3-*O*-gl(1 → 2)-xy(1 → 3)[gl(1 → 4)]ga; 26-*O*-gl.	P	37.4	29.9	78.5	39.1	141.0	121.4	32.2
127	22OMe, 3-*O*-gl(1 → 2)[xy(1 → 3)]gl(1 → 4)ga; 26-*O*-gl.	P	37.6	30.2	78.6	39.4	141.2	121.6	32.4
128	17α OH, 22OMe, 3-*O*-rh(1 → 4)rh(1 → 4)[rh(1 → 2)]gl; 26-*O*-gl.	P	37.6	29.9	77.7	39.0	140.9	121.8	31.7
129	Nu, 3-*O*-rh(1 → 4) [rh(1 → 2)]gl; 26-*O*-gl.	P	37.5	30.1	78.1	40.5	140.9	120.2	32.2
130	Nu, 3-*O*-gl(1 → 3) [rh(1 → 2)]ga; 26-*O*-gl.	P	37.5	30.1	78.1	40.5	140.7	120.1	32.2

C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-16	C-17	C-18	C-19	C-20	C-21	C-22	C-23	C-24	C-25	C-26	C-27
35.8	42.4	41.7	21.2	40.2	40.7	56.4	32.2	81.1	63.3	16.5	19.1	42.1	14.9	109.2	32.0	29.3	30.6	67.0	17.2
35.8	42.4	41.7	21.2	40.2	40.7	56.4	32.1	81.1	63.1	16.5	19.1	42.7	14.7	109.7	26.6	26.3	27.6	65.3	16.3
—	50.2	—	23.7	—	—	56.7	—	81.3	62.9	16.6	14.7	—	14.9	108.6	—	—	144.3	64.9	109.3
31.7	50.3	37.1	21.1	39.9	40.5	56.7	31.8	81.1	62.9	16.4	19.4	42.0	15.0	109.3	32.2	29.3	30.6	66.9	17.3
31.8	50.3	37.0	21.1	39.9	40.5	56.7	31.7	81.1	63.0	16.4	19.4	42.0	15.0	109.3	32.3	29.3	30.6	66.9	17.3
31.9	50.6	37.3	21.3	40.1	40.7	56.9	32.4	81.2	63.2	16.5	19.5	42.2	19.1	109.4	32.1	29.5	30.7	67.1	17.4
32.5	51.5	43.9	24.3	40.7	40.4	57.1	32.6	81.2	63.4	16.7	13.8	22.2	15.1	109.4	32.1	29.5	30.8	67.1	17.4
32.1	50.0	43.4	23.7	40.6	40.3	56.8	32.5	81.2	63.3	16.7	14.7	42.1	15.0	109.3	32.1	29.4	30.7	67.0	17.3
36.0	40.8	36.0	21.5	40.8	41.2	57.0	32.4	81.4	63.6	16.6	24.1	42.3	14.8	109.3	32.2	29.5	30.8	67.2	17.2
35.7	40.5	35.7	21.1	40.5	41.0	56.8	32.0	81.1	62.9	16.5	24.0	41.9	14.5	109.3	31.8	29.1	30.5	67.1	17.1
35.9	40.6	35.4	21.3	40.8	41.1	56.8	32.3	81.3	63.5	16.5	23.9	42.2	14.8	109.2	32.1	29.4	30.7	67.1	17.2
35.7	40.6	35.3	21.2	40.6	41.0	56.9	32.0	81.1	63.0	16.5	24.0	42.0	14.5	109.3	31.8	29.1	30.5	67.0	17.1
32.3	50.9	43.1	24.1	40.6	40.4	57.0	32.6	81.4	63.3	16.9	14.9	42.7	15.2	109.8	26.6	26.4	27.8	65.2	16.4
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	50.7	—	24.0	—	—	57.1	—	81.5	63.3	16.6	14.7	—	14.9	108.3	—	—	144.7	65.1	109.5
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
32.4	51.4	43.8	24.3	40.7	40.4	47.0	32.5	81.2	63.4	16.7	13.8	42.2	15.0	109.4	32.0	29.4	30.7	67.0	17.4
31.7	50.3	37.1	21.2	39.9	40.4	56.7	32.2	81.2	62.0	16.2	19.3	42.5	14.8	109.8	27.5	26.2	26.4	65.2	16.2
31.6	50.2	37.0	21.1	39.9	40.4	56.6	32.2	81.1	62.8	16.3	19.4	41.9	15.0	109.4	32.2	29.2	30.5	66.9	17.3
31.7	50.3	37.1	21.2	39.9	40.5	56.7	32.2	81.2	62.2	16.3	19.4	42.5	14.8	109.7	27.6	26.3	26.4	65.1	16.4
31.9	50.5	37.2	21.3	40.1	40.6	56.8	32.3	81.2	63.2	16.4	19.5	42.1	15.0	109.3	32.0	29.4	30.7	67.0	17.3
31.5	52.2	36.9	21.0	39.8	40.3	56.6	32.0	82.8	62.3	16.3	19.4	41.7	14.5	109.2	31.5	28.9	29.7	66.9	17.1
31.6	49.8	36.8	20.6	36.8	43.8	52.9	31.6	91.0	90.1	17.1	19.3	44.6	8.0	110.0	31.2	28.1	30.1	66.8	17.2
32.2	50.8	43.2	24.0	40.5	40.4	57.2	32.6	81.3	63.2	16.9	14.7	42.7	14.8	109.8	26.6	26.4	27.7	65.2	16.4
31.7	50.4	37.2	21.2	39.9	40.5	56.7	31.7	81.2	62.9	16.3	19.4	42.0	15.0	109.2	31.8	29.2	30.6	66.9	17.4
31.7	50.3	37.1	21.1	39.8	40.5	56.7	32.2	81.1	62.9	16.3	19.3	42.0	15.0	109.2	31.7	29.3	30.5	66.9	17.3
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
31.7	80.3	36.9	21.1	39.7	40.3	56.6	31.9	81.1	62.6	16.2	19.2	41.9	14.8	109.3	31.6	29.8	30.1	66.8	17.1
31.6	50.2	37.0	21.1	39.9	40.4	56.6	31.8	81.1	62.9	16.3	19.4	42.0	15.0	109.3	32.2	29.3	30.5	66.9	17.3
31.7	50.3	37.1	21.5	39.9	40.5	56.6	31.7	81.1	62.9	16.4	19.4	42.0	15.0	109.3	32.2	29.3	30.6	66.9	17.2
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
31.6	50.3	37.0	21.0	39.8	40.4	56.6	32.1	81.1	62.4	16.3	19.3	41.9	14.9	109.3	31.6	29.1	30.4	66.8	17.2
31.7	50.4	37.1	21.1	39.9	40.5	56.9	32.2	81.1	63.0	16.3	19.4	42.0	14.9	109.2	31.8	29.2	30.5	66.9	17.3
31.7	50.3	37.1	21.1	39.9	40.5	56.7	31.7	81.1	62.9	16.3	19.3	41.9	14.9	109.2	31.7	29.3	30.6	66.9	17.3
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
31.7	50.2	36.9	21.1	39.6	40.3	56.6	31.9	81.0	62.6	16.1	19.2	41.9	14.7	109.2	31.7	29.0	30.3	66.8	17.1
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
31.6	50.1	37.0	20.8	32.4	45.0	52.9	32.2	89.9	90.1	17.1	19.3	44.7	—	109.8	32.0	28.6	30.3	66.7	17.2
31.9	50.4	37.1	21.1	40.1	40.5	56.6	32.4	80.9	62.6	16.7	19.6	42.0	15.3	109.1	31.4	28.4	30.5	66.6	17.8
31.7	50.3	37.0	21.1	40.0	40.5	56.7	32.2	81.2	61.8	16.3	19.3	42.5	14.8	109.8	27.5	26.2	26.4	65.2	16.3
31.6	50.3	37.1	21.0	39.8	40.4	56.6	32.3	81.1	61.5	16.2	19.3	42.4	14.8	109.6	27.5	26.1	26.4	65.0	16.2
31.5	50.3	36.8	20.8	39.2	40.2	56.5	31.9	80.9	62.6	15.9	19.0	42.3	14.3	109.3	27.2	25.9	26.2	64.9	15.9
31.6	50.2	37.0	21.0	39.8	40.4	56.6	32.0	81.0	62.6	16.3	19.3	41.8	14.9	109.3	31.6	29.0	30.4	66.8	17.2
35.3	54.5	35.8	21.3	40.1	40.8	56.8	32.1	81.1	62.5	16.6	12.4	42.0	14.9	109.2	31.8	29.2	30.0	66.9	17.3
32.0	50.5	37.0	21.2	40.0	40.6	56.8	32.3	80.2	63.1	16.4	19.5	42.1	15.0	109.3	31.8	89.4	30.7	67.0	17.3
35.4	54.6	36.0	21.3	40.2	40.8	56.5	32.1	81.1	60.4	16.5	12.4	42.5	14.7	109.4	27.6	26.2	26.5	65.1	16.3
35.7	42.4	37.2	21.1	40.2	41.2	56.2	32.3	81.3	63.9	16.7	23.6	40.6	16.4	110.7	37.1	28.3	34.2	75.3	17.4
35.7	42.3	41.0	21.1	40.2	41.0	56.2	32.3	81.1	63.8	16.6	19.0	40.6	16.1	110.8	36.9	28.3	34.2	75.2	17.3
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
32.3	50.3	37.1	20.9	37.1	45.4	53.0	32.4	90.3	90.5	17.1	19.4	43.0	10.3	113.5	30.8	28.1	34.2	75.1	17.1
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
31.6	50.3	37.0	21.0	40.1	40.8	56.5	31.4	81.0	63.8	16.5	19.6	42.0	16.5	112.5	30.3	28.0	33.6	74.6	17.4
32.3	50.3	37.2	21.0	37.2	45.4	53.1	32.5	90.3	90.5	17.2	19.4	43.1	10.4	113.5	30.8	28.1	34.3	75.2	17.2
31.6	50.3	36.9	21.1	39.8	40.7	56.5	32.2	80.7	63.4	16.3	19.2	40.4	16.0	110.7	36.8	28.1	34.0	75.1	17.2
31.8	50.5	37.2	21.2	39.9	40.9	56.8	32.4	81.2	64.2	16.3	19.5	40.6	16.2	112.8	30.9	28.3	34.3	75.3	17.2
32.3	50.3	37.1	20.9	37.1	45.4	53.0	32.4	90.3	90.5	17.1	19.4	43.0	10.3	113.5	30.8	28.1	34.3	75.1	17.4
31.7	50.3	37.1	21.1	38.6	39.8	56.5	32.2	80.9	62.7	16.2	19.4	38.6	15.1	121.6	33.1	33.8	83.8	77.3	24.3
31.6	50.2	37.0	21.0	38.9	39.8	56.4	32.2	80.9	62.6	16.1	19.3	38.6	15.0	121.7	33.7	33.8	83.8	77.2	24.3

Table 2(ii)

C-1	C-2	C-3	C-4	C-5	C-6	C-1	C-2	C-3	C-4	C-5	C-6	Ref.
107.7	73.9	75.0	69.6	67.3	—	—	—	—	—	—	—	90, 91, 104
107.7	73.9	75.0	69.6	67.3	—	—	—	—	—	—	—	79
102.3	72.5	74.6	69.5	67.4	—	—	—	—	—	—	—	111
102.6	75.3	78.4	71.7	78.1	62.9	—	—	—	—	—	—	107
99.8	72.2	72.3	69.3	73.6	62.5	—	—	—	—	—	—	107
103.4	72.9	75.5	70.4	76.9	62.7	—	—	—	—	—	—	80
100.1	72.9	72.9	74.3	69.9	18.6	—	—	—	—	—	—	105
99.6	72.7	72.7	74.1	69.9	18.2	—	—	—	—	—	—	105
98.7	73.9	75.7	72.9	74.1	63.5	—	—	—	—	—	—	95
93.8	71.5	70.9	69.4	67.7	62.5	—	—	—	—	—	—	95
103.1	75.2	78.5	72.3	77.7	63.3	—	—	—	—	—	—	95
99.3	72.2	73.4	69.5	72.2	62.6	—	—	—	—	—	—	95
100.4	76.8	74.9	73.4	71.2	17.1	101.6	72.6	72.7	74.5	69.3	19.0	105
100.2	75.9	74.5	76.0	65.5	—	101.2	72.1	72.1	74.0	69.3	18.6	116
101.3	75.7	75.2	69.6	68.3	—	100.1	72.5	72.2	74.2	69.3	18.6	111
100.6	79.6	78.0	72.1	78.0	62.9	101.9	72.5	72.9	74.2	69.4	18.6	116
101.6	81.4	74.6	69.7	76.5	62.5	106.7	72.3	76.8	71.1	66.3	—	93
102.4	81.3	76.8	69.9	76.4	62.1	106.0	75.4	77.9	71.6	78.2	62.9	
100.0	71.7	83.7	73.0	69.7	18.4	106.4	75.9	78.4	72.1	78.3	62.7	
102.8	73.8	88.7	69.9	77.7	62.5	104.9	75.2	78.3	71.9	78.3	62.8	
102.5	75.2	76.5	78.5	76.8	61.4	102.3	72.5	72.3	73.7	70.2	18.3	
102.3	74.8	76.5	81.2	76.9	62.5	105.0	74.9	78.3	71.6	78.3	62.8	
103.0	73.5	75.4	79.8	75.9	61.0	107.0	75.2	78.4	72.4	78.7	63.1	
99.5	72.1	72.8	76.0	73.5	62.3	107.4	81.2	79.9	81.7	63.2	—	
99.5	72.1	72.8	76.0	73.5	62.3	107.4	81.1	78.8	81.6	63.2	—	
102.3	72.3	75.4	72.6	71.2	17.3	99.9	72.9	72.9	74.2	70.0	18.6	
100.2	77.2	82.9	70.7	78.5	62.1	101.8	72.3	72.7	74.1	69.4	18.5	
99.9	78.2	87.5	70.5	77.9	62.2	102.4	72.3	72.6	73.4	69.7	18.5	
100.2	77.2	82.9	70.7	78.5	62.1	101.8	72.3	72.7	74.1	69.4	18.5	
99.9	77.1	87.7	69.3	77.9	62.8	104.9	74.9	78.3	71.6	78.2	62.1	
102.9	78.8	76.9	78.1	77.9	61.3	100.3	72.5	72.7	73.9	69.6	18.5	
99.9	78.0	75.9	78.0	75.9	62.9	98.0	70.5	69.4	61.4	67.1	70.5	
100.4	79.5	76.7	78.5	77.9	81.6	101.5	72.2	72.7	72.9	69.4	80.4	
100.0	78.2	77.2	76.2	77.6	62.7	101.6	72.4	71.9	73.6	69.2	18.3	
102.6	73.1	75.4	80.5	76.4	60.5	104.8	80.5	78.1	71.7	77.8	61.7	
100.0	77.7	89.3	69.5	77.7	62.4	102.1	72.6	72.3	74.0	69.5	18.5	
99.9	77.1	88.4	69.5	77.6	62.6	101.6	71.9	72.1	73.3	69.5	18.2	
99.9	79.4	91.5	70.8	77.1	62.6	101.4	72.2	72.2	73.3	70.9	18.2	
100.4	79.5	76.7	78.5	77.9	61.6	101.9	72.2	72.7	73.9	69.4	18.4	
100.4	79.5	76.7	78.5	77.9	61.6	101.9	72.2	72.7	73.9	69.4	18.4	
100.0	78.2	77.2	76.2	77.6	62.4	101.6	72.4	71.9	73.6	69.2	18.3	
100.9	71.3	82.1	77.3	72.7	62.1	99.0	72.7	61.3	75.6	68.6	18.4	
102.9	75.0	76.4	80.9	76.6	62.3	102.3	79.3	77.5	71.8	78.5	62.8	
101.7	81.4	87.3	69.0	77.5	62.7	100.0	72.3	72.6	74.0	69.4	18.5	
101.3	77.7	76.7	80.4	77.1	61.3	101.2	71.3	72.4	74.0	69.0	18.1	
100.1	80.0	76.4	77.6	78.1	61.1	102.0	72.4	71.9	73.6	69.3	18.4	
104.8	73.1	75.0	79.9	76.0	60.6	104.8	75.2	78.5	71.1	77.8	63.1	
102.8	73.1	75.3	79.7	76.0	60.7	104.9	75.1	78.6	70.5	77.5	63.1	
100.2	81.1	70.5	76.8	75.5	62.9	101.6	72.5	72.1	73.9	69.1	18.3	
—	—	—	—	—	—	—	—	—	—	—	—	—
107.5	73.8	75.0	69.5	67.3	—	—	—	—	—	—	—	—
100.1	75.9	74.5	76.0	65.5	—	101.2	72.1	72.1	73.9	69.4	18.7	
100.5	79.5	78.1	72.1	77.9	62.9	101.8	72.4	72.8	74.2	69.3	18.5	
100.3	79.5	77.8	71.9	78.1	62.7	101.9	72.8	72.5	74.1	69.4	18.5	
100.3	78.4	76.2	72.1	77.6	62.2	101.7	72.4	72.8	74.2	69.4	18.6	
101.2	77.9	74.6	77.4	75.9	61.8	99.0	72.6	71.3	75.9	69.3	18.4	
100.3	77.7	74.6	79.4	75.9	61.8	99.0	72.6	71.3	75.9	69.3	18.4	
102.5	72.8	75.1	79.3	75.7	60.6	104.4	80.7	87.0	70.5	77.6	62.3	
102.8	73.1	75.3	79.7	76.0	60.7	104.9	80.2	87.2	70.7	77.9	62.7	
100.3	80.3	76.9	78.0	77.7	61.3	102.1	72.8	72.5	74.0	69.4	18.6	
100.4	79.7	85.0	69.9	74.9	62.2	102.0	72.6	72.2	73.9	69.3	18.5	
100.2	79.0	76.6	77.8	78.1	61.4	101.8	72.5	71.6	73.6	67.3	18.3	

Table 2(iii)

C-1	C-2	C-3	C-4	C-5	C-6	C-1	C-2	C-3	C-4	C-5	C-6	Ref.
—	—	—	—	—	—	—	—	—	—	—	—	93
—	—	—	—	—	—	—	—	—	—	—	—	105
—	—	—	—	—	—	—	—	—	—	—	—	119
—	—	—	—	—	—	—	—	—	—	—	—	107
—	—	—	—	—	—	—	—	—	—	—	—	119
—	—	—	—	—	—	—	—	—	—	—	—	80
—	—	—	—	—	—	—	—	—	—	—	—	108
—	—	—	—	—	—	—	—	—	—	—	—	108
—	—	—	—	—	—	—	—	—	—	—	—	105
105.5	74.8	77.2	70.8	67.2	—	—	—	—	—	—	—	105
103.7	72.3	72.6	73.4	69.9	18.3	—	—	—	—	—	—	114
105.5	74.8	77.2	70.8	67.2	—	—	—	—	—	—	—	105
103.7	72.1	72.2	73.5	69.2	18.2	—	—	—	—	—	—	113, 114
102.0	74.1	72.8	73.7	70.4	18.6	—	—	—	—	—	—	107
99.2	72.7	70.9	71.6	68.4	17.7	—	—	—	—	—	—	107
103.0	72.4	72.9	74.2	70.6	18.6	—	—	—	—	—	—	105
109.4	82.5	77.6	86.1	61.2	—	—	—	—	—	—	—	108
106.5	75.0	78.6	70.4	77.4	62.9	—	—	—	—	—	—	80
104.4	74.8	77.0	71.3	77.0	62.4	—	—	—	—	—	—	115
103.9	74.4	78.0	71.6	78.0	61.9	—	—	—	—	—	—	116
104.4	74.4	78.3	71.6	78.3	61.7	—	—	—	—	—	—	113
103.0	72.4	72.9	74.2	70.6	18.6	—	—	—	—	—	—	105
103.0	72.4	72.9	74.2	70.6	18.6	—	—	—	—	—	—	105
109.4	82.4	77.6	86.1	76.3	—	—	—	—	—	—	—	108
108.5	82.1	77.3	85.3	62.1	—	—	—	—	—	—	—	86
101.9	72.1	72.5	74.4	69.5	18.4	—	—	—	—	—	—	119
104.4	73.8	77.3	70.8	76.8	68.2	106.1	75.1	78.0	70.7	67.3	—	119
102.4	78.8	76.9	71.9	77.5	61.8	99.8	71.5	72.1	73.3	68.9	18.0	119
102.7	72.4	70.0	78.1	68.2	18.3	102.0	72.4	71.9	73.6	69.3	18.1	108
102.4	87.0	81.1	70.6	78.5	63.1	104.8	75.5	77.5	70.4	62.2	—	117
104.9	81.2	87.2	70.7	77.9	62.7	104.7	75.6	78.6	71.3	67.2	—	80
104.7	78.4	87.6	72.1	76.3	62.9	105.1	74.9	77.3	70.2	67.0	—	
105.1	75.2	78.4	71.5	77.6	62.3	—	—	—	—	—	—	
103.6	72.6	71.3	75.9	68.6	18.4	—	—	—	—	—	—	
103.6	72.6	71.3	75.9	68.6	18.4	—	—	—	—	—	—	
104.4	74.8	78.1	70.1	77.1	62.6	104.4	75.2	78.1	71.1	66.9	—	
104.9	75.1	78.6	70.5	77.5	63.0	104.9	75.6	78.6	71.3	67.2	—	
102.2	73.1	72.8	78.3	68.4	18.6	103.1	72.8	72.4	73.9	70.3	18.8	
105.5	76.0	78.1	71.6	77.7	61.7	—	—	—	—	—	—	
102.3	72.5	72.2	73.9	70.3	18.4	—	—	—	—	—	—	

Site of sugar linkage with the aglycone

This information can be obtained by a comparison of the chemical shifts of the sapogenin with those of the saponin as glycosylation of a hydroxyl aglycone causes a change in chemical shift due to the oxy-group modification. In a general way, this leads to the downfield shift of the α-carbon atom and upfield shift of the adjacent carbon atoms [100–102]. The magnitude of these effects depends upon the location of the hydroxyl group on the aglycone nucleus. In most of the cases, saponins possess a sugar moiety linked at C-3 which shows a 6.6 ± 1.0 ppm downfield shift with unsymmetrical effects on vicinal carbons, i.e. higher shielding of C-4 (1.8–4.6 ppm) than that for C-2 (1.1–3.0 ppm). In all the reported cases, shielding experienced by C-4 is about twice that suffered by C-2. This has been explained on the basis of the conformation of the sugar molecule around the glycosidic bond. A greater effect on C-4 has been considered to be due to the pro-*S* relationship with the sugar as compared with the C-2 pro-*R* relationship [103].

The arabinosylation of the C-1β hydroxyl in tokorogenin-1-*O*-α-L-arabinopyranoside (**72**) as expected, shows significantly higher deshielding (12.3 ppm) of C-1 and no β-upfield shift of the vicinal carbon atoms but a downfield shift of upto 1 ppm has been observed [104]. A

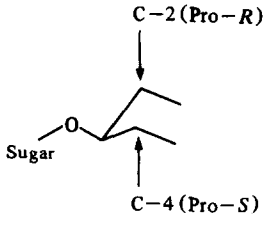

Table 2(iv)

C-1	C-2	C-3	C-4	C-5	C-6	Ref.
105.0	74.9	77.4	70.5	67.0	—	118
104.8	76.1	78.5	71.7	78.4	62.8	104
104.5	74.9	78.3	71.9	77.8	62.9	79, 104
104.5	75.0	78.1	71.7	77.9	62.7	116
104.7	75.1	78.5	71.9	78.1	63.0	116
104.9	75.1	78.5	71.8	78.3	62.9	78
104.9	75.0	78.5	71.9	78.3	63.0	116
103.6	74.1	77.4	71.3	77.4	63.8	86
103.6	74.1	77.4	71.3	77.4	63.8	78
104.4	74.8	78.1	71.6	77.8	62.6	80
104.9	75.1	78.6	71.9	78.2	63.1	80
104.9	75.1	78.5	71.8	78.3	62.9	78
105.1	75.2	78.1	71.6	78.6	62.4	82
105.1	75.1	78.1	72.2	78.1	62.4	82

*Abbreviations: To, tokorogenin; Nto, neotokorogenin; Di, diosgenin; Ru, ruscogenin; Nru, neoruscogenin; Sa, Sarsasapogenin; Pe, pennogenin; Ya, Yamogenin; Ti, tigogenin; Nti, neotigogenin; Nu, nuatigenin; Ac, acetyl; +, the exact structure is not known; ar, α-L-arabinopyranoside except **91** and **92** where ar, α-L-arabinofuranoside; gl, β-D-glucopyranoside except **79** and **80** where gl, α-D-glucopyranoside; rh, α-L-rhamnopyranoside; ga, β-D-galactopyranoside; xy, β-D-xylopyranoside; Fu, β-D-fucopyranoside.

comparison of the shifts for ruscogenin-3-*O*-α-L-rhamnopyranoside (**77**) with its sulphate derivative (**78**) reveals the 5.9 ppm downfield shift of C-1 and upfield shift of the neighbouring carbon atoms (sulphate induced shifts) [105].

In most of the furostanol glycosides reported so far, glucose is the glycosidating sugar at the C-26 hydroxyl, causing a 6.8 ± 0.3 ppm downfield shift along with the usual 1.8 ± 0.4 ppm upfield shift of C-25. Similar α- and β-effects have also been observed for nuatigenin glycosides (**129** and **130**) [82].

Thus, a comparison of the ^{13}C NMR chemical shifts for a sapogenin with the saponin and sometimes with its prosapogenin reveal unambiguously the complete structure.

4. TABULATION OF THE ^{13}C NMR SHIELDING DATA

All of the ^{13}C NMR shielding data which appeared upto 1983 for sapogenins (Table 1) and saponins (Table 2), except for only one reference [106] have been classified according to the foregoing discussion (*vide supra*) and arranged serially according to the increasing substitution pattern on the parent skeleton.

In a few cases, reported assignments have been revised. This includes reversal of the assignments reported by Espijo *et al.* [107] for C-2 and C-3 in compounds **74**, **90**, **99** and **100** while C-2 and C-15 are reversed in **75**. The assignment of the C-2 signal at 34.6 ppm reported by Hirai *et al.* [80] in the case of diosgenin (**46**) seems to be less reliable in view of the large amount of published data (Table 1). There are two reports which deal with signal assignments for pennogenin (**54**) but these two differ significantly for the chemical shift of the C-12 carbon atom. Marquardt [71] reports the appearance of this carbon atom at 32.4 ppm while Miyamura *et al.* [108] report it at 37.0 ppm. The consideration of SIS due to a C-17α hydroxyl group as in 17α-hydroxyandrostane leads to the prediction that the chemical shifts reported by Marquardt [71] are more reliable. The assignments of the signals at 23.9 and 26.0 ppm due to the acetyl methyl in the case of nuatigenin acetate (**66**) and isonuatigenin acetate (**67**) as reported by Tschesche and Fuehrer [83] seems to be less satisfactory as the acetyl methyl usually resonates in the region 18–21 ppm [54, 109, 110]. Therefore, assignments for the acetyl methyl and C-27 have been reversed. The data for the sugars [108] has been analysed and included in Table 2.

Trivial names are included in column 2 while the substitution pattern is listed in column 3 except for compound **61a** which has been given the name neoruscogenin [111]. However, neoruscogenin was previously, [3, 4] identified as 1β,3β-dihydroxy-5β spirostane (**60**) [105]. Therefore we have not used this trivial name for **61a**, or its glycosides **73a** and **84a** [111]. Solvents in which the chemical shifts were measured are given in column 4 (C = deuterated chloroform, D = deuterated dimethyl sulphoxide, M = deuterated methanol, P = deuterated pyridine).

Table 3 deals with the ^{13}C NMR chemical shifts for the commonly encountering monosaccharides which usually constitute the sugar moiety of the saponins.

Finally the chemical shifts for the parent steroidal skeleton, either reported or calculated by additivity considerations of the substituent induced shifts for steroids [17], are included in Table 4. These values make it evident that the chemical shifts for the rings A and B carbon atoms are affected quite markedly and this is helpful for the differentiation of 5α-, 5β- and Δ^5-steroids. The signals due to C-5 and C-19 are of special significance as these exhibit extreme variation of their chemical shifts. Thus, these carbons are observed at 47.1, 12.3 ± 0.1; 43.7, 24.0 ± 0.2 and 144.2 ± 0.5, 19.6 ± 0.1 ppm in 5α-, 5β- and Δ^5 series, respectively. Hence ^{13}C NMR spectroscopy can

Table 3. ^{13}C NMR chemical shifts and $^{1}J_{CH}$ values (in parentheses) of methylglycopyranoside/furanoside pairs*

Methyl glycoside	C-1	C-2	C-3	C-4	C-5	C-6
β-D-Glucopyranoside	103.7	73.7	75.5	70.3	75.5	61.7
	(160)	(145)	(143)	(141)	(143)	(144)
β-D-Glucopyranoside tetraacetate	101.1	70.9	71.4	68.1	72.5	61.6
	(161)	(151)	(140)	(153)	(149)	(148)
α-D-Glucopyranoside	99.9	72.2	73.9	70.4	71.9	61.5
	(170)	(148)	(147)	(145)	(146)	(145)
α-D-glucopyranoside tetraacetate	96.3	68.2	70.4	66.8	69.7	61.6
	(172)	(151)	(151)	(145)	(151)	(148)
β-D-Glucofuranoside	110.0	80.6	75.8	82.3	70.7	64.7
α-D-Glucofuranoside	104.7	—	—	80.4	—	62.6
β-D-Galactopyranoside	104.1	71.2	73.3	69.1	75.3	61.4
	(160)	(146)	(141)	(146)	(141)	(144)
β-D-Galactopyranoside tetraacetate	101.5	68.5	70.2	66.8	70.6	61.0
	(161)	(157)	(142)	(153)	(146)	(150)
α-D-Galactopyranoside	99.8	69.9	70.2	68.9	71.2	61.8
	(170)	(146)	(145)	(146)	(143)	(143)
α-D-Galactopyranoside tetraacetate	96.5	67.0	67.6	65.7	67.6	61.2
	(171)	(145)	(150)	(143)	(150)	(150)
β-L-Rhamnopyranoside	102.7	72.2	75.4	73.8	73.5	18.5
α-L-Rhamnopyranoside	102.4	71.9	72.5	73.6	69.4	18.4
β-D-Fucopyranoside	105.9	72.0	75.2	72.6	71.3	17.2
α-D-Fucopyranoside	101.6	70.0	71.5	73.1	66.9	17.1
α-D-Xylopyranoside	100.3	72.3	74.2	70.3	61.9	
	(170)	(146)	(145)	(146)	(148)	
α-D-Xylopyranoside triacetate	96.4	70.5	69.1	68.8	57.7	
	(177)	(153)	(153)	(152)	(148)	
β-D-Xylopyranoside	104.8	73.9	76.7	70.1	66.0	
	(159)	(144)	(144)	(147)	(50)	
β-D-Xylopyranoside triacetate	100.9	70.2	71.0	68.3	61.3	
	(161)	(153)	(152)	(153)	(151)	
α-D-Arabinopyranoside	104.7	71.6	73.3	69.1	66.9	
	(160)	(148)	(143)	(146)	(150)	
α-D-Arabinopyranoside triacetate	101.9	69.3	70.4	67.9	63.2	
	(159)	(153)	(148)	(152)	(150)	
β-D-Arabinopyranoside	100.7	69.8	69.8	69.1	63.4	
	(169)	(145)	(145)	(145)	(149)	
β-D-Arabinopyranoside triacetate	97.6	68.4	69.3	67.2	60.2	
	(171)	(153)	(152)	(152)	(151)	
α-D-Arabinofuranoside	109.1	81.5	77.2	84.7	62.0	
β-D-Arabinofuranoside	103.0	77.2	75.3	82.8	63.9	
β-L-Arabinofuranoside	103.3	77.9	76.3	83.1	64.3	
	(174)	(148)			(143)	
α-L-Arabinofuranoside	109.5	82.0	77.9	84.8	62.5	
	(173)	(150)	(148)	(149)	(143)	

*Methylglycosides were measured in D_2O while their peracetates were measured in $CDCl_3$.

be efficiently employed for the establishment of the structure of steroidal sapogenins and saponins.

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Table 4. ^{13}C NMR chemical shifts of basic spirostane, furostane and furospirostane skeletons

Carbon No.	Spirostane							Furostane				Furospirostane
	5α		5β			Δ⁵		5α		Δ⁵		
	25R	25S	25R	25S	Δ^25(27)	25R	25S	22OH	22OMe	22OH	22OMe	Δ⁵
C-1	38.7	38.7	37.6	37.6		39.9	40.5	38.5	38.5	39.9	39.9	40.5
C-2	22.2	22.2	21.3	21.3		22.4	23.3	22.2	22.2	22.4	22.4	22.5
C-3	26.8	26.8	27.0	27.0		28.0	27.7	27.1	27.1	28.0	28.0	27.8
C-4	29.0	29.0	27.2	27.2		33.0	34.2	29.2	29.2	33.0	33.0	34.3
C-5	47.1	47.1	43.7	43.7		143.7	144.7	47.1	47.1	143.7	143.7	144.9
C-6	29.0	29.0	27.4	27.4		119.0	118.7	29.2	29.2	119.0	119.0	118.0
C-7	32.4	32.4	26.8	26.8		32.0	32.3	32.4	32.4	32.0	32.0	32.6
C-8	35.2	35.2	35.5	35.2		31.4	31.8	35.4	35.4	31.4	31.4	32.2
C-9	54.8	54.8	40.6	40.6		50.1	50.5	54.8	54.8	50.1	50.1	50.4
C-10	36.3	36.3	35.5	35.5		36.6	37.0	36.4	36.4	36.6	36.6	37.0
C-11	20.7	20.7	20.6	20.6		20.9	21.2	21.1	21.1	20.9	20.9	21.2
C-12	40.2	40.0	40.3	39.9		39.8	40.0	40.2	40.2	39.8	39.8	40.0
C-13	40.6	40.5	40.6	40.7		40.2	40.5	41.2	41.2	41.2	41.2	40.6
C-14	56.5	56.2	56.5	56.5		56.5	56.8	56.2	56.5	56.2	56.5	56.5
C-15	31.8	31.7	31.7	31.7		31.8	32.2	32.3	31.4	32.3	31.4	32.3
C-16	80.8	80.8	81.0	80.9		80.7	81.7	81.3	81.0	81.3	81.0	81.1
C-17	62.3	61.9	62.3	62.3		62.1	62.8	63.9	63.8	63.9	63.8	62.6
C-18	16.5	16.5	16.4	16.4		16.3	16.4	16.7	16.5	16.7	16.5	16.2
C-19	12.3	12.4	24.2	23.9		19.7	19.6	23.6	19.6	23.6	19.6	19.6
C-20	41.6	42.1	41.6	42.2	42.0	41.6	42.5	40.6	42.0	40.6	42.0	38.5
C-21	14.5	14.3	14.5	14.3	15.0	14.5	14.9	16.4	16.5	16.4	16.5	15.2
C-22	109.0	109.5	109.2	109.7	109.4	109.9	109.7	110.7	112.5	110.7	112.5	120.9
C-23	31.4	27.0	31.4	27.1	29.0	31.4	27.6	30.2	30.3	30.2	30.3	32.6
C-24	28.9	25.9	28.8	25.8	33.3	28.8	26.2	29.9	28.0	29.9	28.0	33.8
C-25	30.3	25.8	30.3	26.0	144.5	30.3	26.4	29.9	35.2	29.9	35.2	85.6
C-26	66.7	65.0	66.8	65.2	65.1	66.7	65.1	68.1	67.6	68.1	67.6	70.1
C-27	17.1	16.0	17.1	16.1	108.7	17.1	16.0	17.4	17.4	17.4	17.4	24.1
OMe	—	—	—	—	—	—	—	—	48.9	—	48.9	—

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