MAGNETIC RESONANCE IN CHEMISTRY, VOL. 33, 923–953 (1995)

REVIEW

NMR Spectroscopy of Steroidal Sapogenins and Steroidal Saponins: an Update†,‡

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The ^{13}C NMR chemical shifts of 126 steroidal sapogenins published between 1983 and 1993 are listed and critical spectral features and advances made in the NMR characterization of these compounds are discussed as a guide for the identification of the parent skeleton and the determination of substitution patterns. The NMR spectroscopic methods applicable to deduce the complete structure of the oligosaccharide moiety and its linkage to the sapogenin residue are also presented to elucidate the structure of steroidal saponins.

KEY WORDS NMR; ^{1}H NMR; ^{13}C NMR; steroidal sapogenins; steroidal saponins; structure elucidation; review

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1. INTRODUCTION

Steroids of the spirostane and furostane series (steroidal sapogenins) and their glycosides (steroidal saponins), widely distributed in various plant families,[1–7] are attracting the attention of research workers not only as economically important raw materials convertible into various steroid hormonal drugs,[4,8–10] but recently also as biologically active materials having independent value.[3,6,8,11] From 1973 to 1983, there was a dramatic increase of interest in the application of ^{13}C NMR spectroscopy to the study of these compounds, which prompted us to publish a general review of the use of ^{13}C NMR in structural analysis of steroidal sapogenins and steroidal saponins.[12] This paper extends that review from 1983 to the present.

Nowadays, ^{13}C NMR spectroscopy has become an integral part of the procedure for establishing the structure of new steroidal sapogenins and saponins. The simplicity of proton-decoupled ^{13}C spectra, with a single line for each carbon atom, and the availability of various pulse sequences for distinguishing between carbon atoms according to the number of attached protons have facilitated the interpretation. Moreover, the structural and substituent effects that control ^{13}C chemical shifts are well documented for steroids,[13–16] allowing good estimates to be made for individual carbon atoms.

The ^{1}H NMR spectra, in contrast, are complicated by extensive interproton coupling, as a result of which only a few signals are readily recognizable, such as singlets for angular methyl groups (18-C$\underline{H}_3$, 19-C$\underline{H}_3$) and doublets for secondary methyl groups (21-C$\underline{H}_3$, 27-C$\underline{H}_3$) whereas the rest of the methylene and methine resonances appear as a series of overlapping multiplets in the so-called 'methylene envelope' which was difficult to resolve with 60, 80 and 100 MHz spectrometers. This is the most probable reason why most of the early publications[17] incorporate mainly only the ^{1}H NMR chemical shifts for the methyl signals and for the more downfield signals ($\delta > 3$ ppm) such as olefinic, oxy-substituted methylene and methine resonances. Signal linewidths and spectral integration identify the kinds of protons and their relative abundance present in various spectral regions.

Full assignments of the ^{1}H NMR resonances for a steroid with a low degree of functionalization has been

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† NMR Spectral Investigations, Part 37, for Part 36, see P. K. Agrawal and A. K. Pathak, *Magn. Reson. Chem.* **32**, 753 (1994). CIMAP Publication No. 94-11J.

‡ Dedicated to Professor H.-J. Schneider, Universität des Saarlandes, Saarbrücken, Germany, on the occasion of his 60th birthday.

CCC 0749–1581/95/120923–31

Received 14 September 1994
Accepted (revised) 22 March 1995

difficult because typically >20 protons will have chemical shifts crowded in the region 1–2 ppm. The advent of high-field spectrometers and advances made in two-dimensional (2D) NMR techniques made such tasks feasible.[18] Despite the fact that homo- and hetero-nuclear correlation 2D NMR experiments were employed for the structure establishment of the sapogenin 25(*S*)-spirost-4-en-3,12-dione (**57**) from *Kallstroemia maxima* in 1985,[19] the assignment of the ^{1}H NMR resonances of hecogenin acetate [3β-acetoxy-(25*R*)-5α-spirostan-12-one] determined at 500 MHz by use of two-dimensional NMR techniques was not recorded until 1990[20] and that of diosgenin not until 1993.[21] Although complete assignments of the ^{1}H NMR spectra of the sugar portion of the steroidal saponins have been reported fairly frequently, this has not been the case for the sapogenin residue.[22–31]

The aim of this paper is not merely to tabulate the ^{13}C NMR chemical shifts of the new steroidal sapogenins which have been characterized since 1983,[19,25,26,32–69] but also to discuss advances made in this field and to provide a basis for the structural assignment of these compounds. The ^{1}H NMR spectral features which may be of importance in the structural analysis of these compounds have also been considered.

1.1. Nomenclature

Normally the steroids, e.g. androstane and cholestane, contain the perhydrocyclopentenophenanthrene nucleus (rings A, B, C and D). The spirostanes contain, in addition, one five-membered ring (E) and one six-membered ring (F), both of which are heterocyclic and fixed in a spiran fashion at C-22 (Fig. 1). All the steroidal sapogenins possess a parent cholestane carbon skeleton (C_{27}), the side-chain of which undergoes cyclization resulting in either a hexacyclic system (four carbocyclic and two heterocyclic rings) as in spirostanes or a pentacyclic system (four carbocyclic and one heterocyclic ring) as in furostanes. Those cholestane derivatives which are formed by the ring opening of both heterocyclic rings are also included because these have been regarded as intermediates in the biosynthesis of steroidal sapogenins[4,70–72] and also because these compounds have been frequently isolated from plants which either produce steroids of the spirostane and furostane type or belong to plant families and genera rich in such compounds.[4,24,73]

2. STEROIDAL SAPOGENINS

In general, steroidal sapogenins have been divided into four groups: spirostane (**1.1**) (16,22;22,26-bisepoxycholestane), furostane (**1.2**) (16,22-epoxycholestane), furospirostane (**1.3**) (16,22;22.25-bisepoxycholestane) and 16,22-dihydroxycholestane (**1.4**) (Fig. 1).

To be of any use, an NMR spectrum must be assigned, which means working out which peaks are associated with which hydrogen or carbon in the structure. No doubt certain resonances such as those of oxy-substituted and olefinic protons and carbons can be identified and assigned on the basis of shift arguments, but a complete and unambiguous assignment is necessary for any extended interpretation of the results of NMR experiments. This information, in most cases, can not be obtained from one-dimensional ^{1}H and ^{13}C NMR spectral data alone but must be derived with the aid of two-dimensional experiments, which simplifies spectral analysis by spreading out information into two frequency dimensions and by revealing interactions between nuclei.

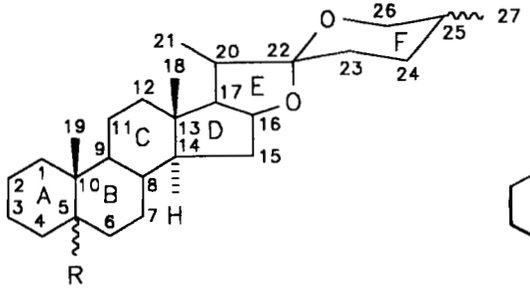
Although a very great number of different two-dimensional NMR experiments have been devised to solve chemical structures and it would be both impossible and unprofitable to describe even a fraction of these in this paper, most of these are variations on a common theme: observation of the transfer of magnetization from one nucleus to another. Comprehensive and critical reviews covering this field have appeared. Some workers approach the topic from the point of view of the chemical information obtainable from such techniques,[74–83] others adopt a more physico-chemical view[74,75,84–88] and finally there are advanced texts which adopt a strict quantum mechanical approach to the subject.[89,90] Although the mechanisms on which the various pulse sequence rely may be intricate, the interpretation of two-dimensional NMR spectra is usually straightforward.

Some of the most often applied and widely used experiments[91–110] are presented, along with their acronyms, in Table 1. It is important to mention that there is no definite sequence for the NMR experiments to be followed to solve chemical structures, but their choice depends, of course, on the complexity of the structural problem to be solved.

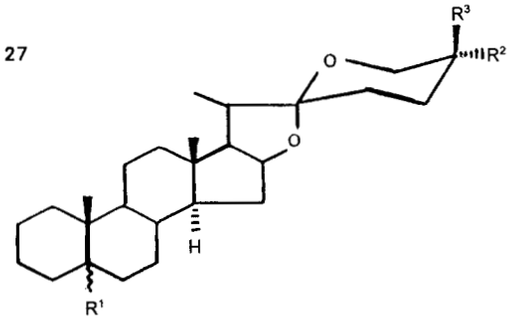
2.1. NMR strategies for structural elucidation of steroidal sapogenins and saponins

2.1.1. Determination of parent skeletal type. Primary information about the parent skeletal types can be obtained from the broadband ^{13}C NMR spectrum followed by determination of the number of directly bonded hydrogen atoms to individual carbon atoms. Thus, after establishing the number of ^{13}C NMR signals and their types, i.e. methyl, methylene, methine and quaternary, the next question is concerned with deducing the subgroup to which the steroidal sapogenin belongs. A consideration of the types of carbon resonances constituting the parent skeleton of various subgroups reveals that the basic skeletons of **1.1** and **1.2** are composed of the same carbon types, whereas those of **1.3** and **1.4** differ from each other, particularly with respect to the number of non-protonated and methylene resonances (Table 2).

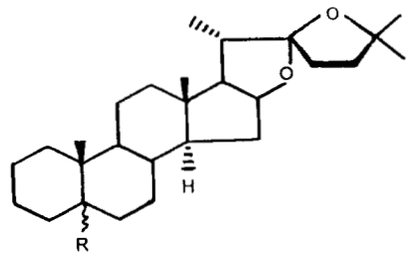
The multiplicity and chemical shift of C-22 are of prime importance in establishing the parent skeleton. It is of the quaternary type for skeletons **1.1**, **1.2**, **1.3** and **1.4.3**, of the methine[26] type in **1.4.1** and of the methylene type in the case of 22-deoxy **1.4.2**.[111] The typical chemical shift ranges are presented in Table 3 from which it is evident that the chemical shift of C-22 is not only sensitive to size and ring opening of ring F, and hence indicative of skeletal type, but also exhibits a remarkable dependence on the presence of hydroxy groups, predominantly in rings D and F. Consideration



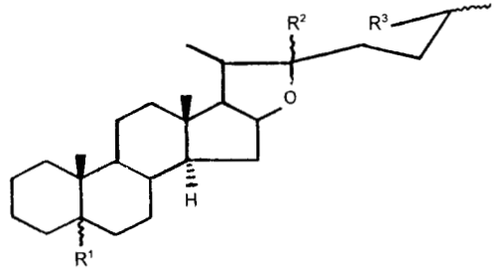
1.1 R = H; Spirostanoids
1.1.1 R = α-H; 5α-Spirostane
1.1.2 R = β-H; 5β-Spirostane
1.1.1 R = $\Delta^{5(6)}$; Δ^{5}-Spirostane



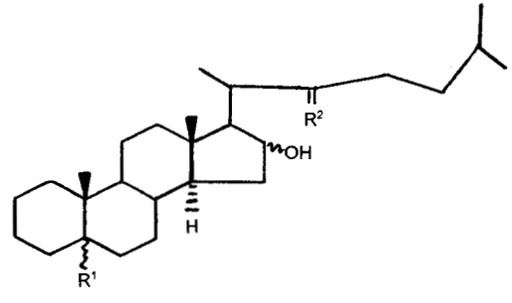
25R 1.1 $R^1 = R^3 = H$, R^2 = Me
25S 1.1 $R^1 = R^2 = H$, R^3 = Me



1.3 R = H; Furospirostane
1.3.1 R = α-H; 5α-Furospirostane
1.3.2 R = β-H; 5β-Furospirostane



1.2 $R^1 = R^2$ = H, OH or OMe, R^3 = H or OH; Furostanoids
1.2.1 $R^1 = R^2 = H$, R^3 = OH; 22-deoxyfurostanol
1.2.2 $R^1 = R^2 = R^3$ = OH; 22-hydroxyfurostanol
1.2.3 R^1 = H, R^2 = OMe, R^3 = OH; 22-methoxyfurostanol
1.2.4 $R^1 = R^2 = R^3$ = H; 22-deoxyfurostane
1.2.5 $R^1 = R^3 = H$, R^2 = OH; 22-hydroxyfurostane
1.2.6 $R^1 = R^3 = H$, R^2 = OMe; 22-methoxyfurostane



1.4 R^1 = H, R^2 = H, OH or H_2 or O; Cholestane
1.4.1 R^1 = H, R^2 = H, OH; 16,22-dihydroxycholestane
1.4.2 R^1 = H, R^2 = H_2; 16-hydroxycholestane
1.4.3 R^1 = H, R^2 = O; 16-hydroxycholest-22-one

Figure 1. Parent skeleton of various subgroups of steroidal sapogenins.

Table 1. Selected NMR approaches[a] used for the structure establishment of steroidal sapogenins and steroidal saponins

NMR experiment	Comments
APT[91] (attached proton test)	Discriminates among carbon types
DEPT[92] (distortionless enhancement by polarization transfer)	Discriminates among carbon types, spectral editing
INEPT[93] (insensitive nuclei enhanced by polarization transfer)	Discriminates among carbon types, spectral editing
INADEQUATE[94] (incredible natural abundance double-quantum transfer experiment)	^{13}C–^{13}C connectivity, establishment of molecular skeleton
^1H,^1H-COSY	Homonuclear shift correlation
(a) Normal[95]	Elucidation of direct couplings
(b) With delay[96]	Detection of small couplings
(c) DQF-COSY[97] (double-quantum filtered-COSY)	Determination of coupling constants
(d) E.COSY[98] (exclusive COSY)	Accurate determination of J
(e) GEM-COSY[99] (geminal COSY)	Identification of geminal spins
(f) TQF-COSY[100] (triple-quantum filtered COSY)	Detection of three mutually coupled spin systems
TOCSY[101a], HOHAHA[101b–d] (total correlation spectroscopy and homonuclear Hartmann–Hahn experiment	Identification of all protons belonging to a single spin system
RCT[102] (relayed coherence transfer spectroscopy)	Stepwise coherence transfer across spin system
Homonuclear nuclear Overhauser and exchange spectroscopy	Identification of protons that are within 5 Å of one another (^1H,^1H correlation through space)
(a) NOESY[104]	Three-dimensional structure, intra- and interresidual (sequence analysis in sugar chain including sugar–aglycone linkage), stereochemical analysis
(b) ROESY[105]	Separation of chemical exchange and NOE in medium-sized molecules without NOE effects, determination of IGL and sequence (interresidual ROE)
Heteronuclear shift correlation	Cross assignments of ^1H and ^{13}C shifts
(a) HETCOR[106]	Assignments of ^1H and ^{13}C shifts
(b) DEPT-COSY[107]	Permits editing
(c) HMQC[108] (^1H-detected heteronuclear multiple-quantum correlation spectroscopy)	Assignments of ^1H and ^{13}C shifts
Multiple bond correlations	Assignment of quaternary C, confirmation of ^1H NMR assignments
(a) Long-range HETCOR[109]	Indirect detection of C-connectivity, Establishment of IGL and sequence
(b) HMBC[110] (^1H-detected heteronuclear multiple-bond correlation spectroscopy)	Correlation of a proton resonance with a carbon resonance by coupling over 2–4 bonds. Intra- and interresidual assignments, conformation of molecular structure

[a] For a most recent and complete review of modern NMR techniques, see W. E. Hull, in Ref. 83, pp. 67–456.

Table 2. Types of carbon resonances constituting the parent skeleton of the steroidal sapogenins

		CH_2		CH		C	
Compound type	CH_3	Non-oxygenated	Oxygenated	Non-oxygenated	Oxygenated	Non-oxygenated	Oxygenated
Spirostane	4	11	1	7	1	2	1
Furostane	4	11	1	7	1	2	1
Furospirostane	5	11		7	1	1	2
16,22-Dihydroxycholestane	5	11		7	2	2	

Table 3. Chemical shift ranges of C-22 for various steroidal sapogenins

Skeletal type	Chemical shift, δ (ppm)
Spirostane (**1.1**)	105.5–117.7
a. General	108.9–110.0
b. 12-OH (and 14-OH/OAc)	105.5–105.7
c. 16α-OH/OMe	110.1–111.1
d. 17α-OH	112.0
e. 23α-OH	117.7
f. 23β-OH	112.6–113.5
g. 24-OH	110.9–111.6
h. 25-OH	108.3
Furostane (**1.2**)	90.2–113.5
a. 22-H	90.2–90.6
b. 22-OH/OMe	110.0–113.5
Furospirostane (**1.3**)	119.6–121.7
16-Hydroxycholestane (**1.4**)	34.8–219.5
a. 22-Deoxy	34.8–35.4
b. 22-Deoxy, 23-oxo	50.4
c. 22-Hydroxy	71.5–73.4
d. 22-Oxo	214.6–219.5

of the chemical shift of C-22 implied the following pattern of chemical shift, in increasing order, 16-hydroxycholestane (**1.4.2**) < 16,22-dihydroxycholestane (**1.4.1**) < furostane (**1.2**) < spirostane (**1.1**) < 22-hydroxy furostane (**1.2.2**) < 22-methoxy furostane (**1.2.3**) < furospirostane (**1.3**) and < 16-hydroxycholestan-22-one (**1.4.3**), as these absorb at *ca.* 35, 72, 90, 109, 110, 112, 119 and 216 ppm, respectively (Table 3).

The naturally occurring steroidal saponins with a furostane skeleton are mostly in the 26-*O*-glycosidic form and possess an OH/OMe group at C-22. The 22-OH and 22-OMe forms of **1.2** are distinguishable on the basis of the chemical shift of C-22 as it resonates about 1–2 ppm at higher field in the case of the former (**1.2.2**) relative to the latter (**1.2.3**). This observation may be complimented by the absence of an OMe resonance at 47.0–47.5 ppm[112–114] in the case of **1.2.2**. The three protons singlet at δ 3.25 ± 0.2 due to OMe can also be utilized in discriminating **1.2.2** from **1.2.3**, as it would only appear in case of **1.2.3**.[22,29,111,112] The appearance of C-25 at 84.0–85.5 ppm[12,26,115] and its non-protonated nature is peculiar for the identification of the **1.3**-type skeleton.

2.1.2. Determination of substitution pattern

2.1.2.1. General spectral features. The common feature of all steroidal sapogenins is the presence of mostly four, but occasionaly five, methyl resonances. These are distinguishable into two *tert-* and two *sec*-methyl groups in **1.1**, **1.2.2** and **1.2.3** but into two *tert-* and three *sec*-methyl groups in **1.2.4**–**1.2.6** and **1.4**. The presence of four *tert*-methyl group in addition to one *sec*-methyl group identifies the furospirostane skeleton (**1.3**). Hydroxyl substitution modifies the signal produced by methyl groups. For example, OH substitution at C-25 in spirostanes alters the 27-Me from being *sec* to *tert*. The methyl groups may be oxidized to CH_2OH in some cases, whereas generation of the 25(27) exocyclic methylene system can be considered as a consequence of dehydration of the 27-hydroxylated form. Most of the steroidal sapogenins occur in hydroxylated form, having OH-substitution on any ring (A–D and F) position. So far, hydroxylation in ring E has not been reported, at least in categories belonging to **1.1** and **1.3** skeletal types as F-ring opening of the former (**1.1**) results in an acyclic system, **1.2**, having OH groups at C-22 and C-26. In the cholestane type, the 22-hydroxyl group become further oxidized to form an oxo function (**1.4.3**).

The ^1H NMR resonances appearing in the region 3.2–5.0 ppm are informative in determining the number and type of hydroxyl groups, if the spectrum is well resolved, but requires initially subtraction of resonances belonging to H-16 and 26-C$\underline{H}_2$ from the above region. It is worth mentioning that C-22 is quaternary in **1.1**, **1.2.2**, **1.2.3**, **1.2.5**, **1.2.6** and **1.3**, but it is of the methine type in **1.2.1**, **1.2.4** and **1.4**, thus giving rise to an absorption for H-22 also in the above region. H-16 resonates between δ 4.10 and 4.50 as a quartet, doublet of doublets or a multiplet having $^3J_{16,15} = {}^3J_{16,15} = {}^3J_{16,17} \approx 6.2$–$8.2$ Hz in the case of both spirostanes and furostanes,[17,61] whereas H-16 and H-22 appear at *ca.* 4.80 ppm as a doublet of triplets ($^3J_{16,17} = 8.0$ Hz) and at 4.14 ppm as a multiplet ($^3J_{22,23} = 8.5$, $^3J_{22,23} = 4.5$ Hz), respectively, in the case of 16,22-dihydroxycholestane.[26] The geminal protons of H-26 appear as a triplet ($J = 9.0$–12.0 Hz) and a doublet of doublets ($J = 3.0$–4.9, 9.0–12.0 Hz) between 3.20 and 3.90 ppm.[17] Although the multiplicity pattern of the H-16 and 26-C$\underline{H}_2$ signals is distinctive, these are not easily identifiable if a steroidal saponin is under investigation owing to the overlapping of these signals with those of the sugars, which results in a highly congested region.

Hydroxyl substitution at ring CH_2 groups, at ring junctions and at methyl groups leads to an increase in CH—O—, C—O— and CH_2—O— resonances in the ^{13}C NMR spectrum while reducing the number of CH_2, CH and CH_3 signals, respectively, in comparison with the parent skeleton (Table 2). This implies that the number and type of hydroxyl group can be ascertained by a careful comparative study of the observed multiplicities of the carbon resonances of a steroidal sapogenin with those of the parent skeleton. The oxy-substituted carbon resonances absorb between 64.0 and 118.8 ppm, the chemical shift of an individual oxy-substituted carbon being influenced by various factors such as the site of substitution, the presence of substituents in the neighbouring positions and stereochemical relationships.

Hydroxyl substitution has been reported at ring junctions A/B, C/D and D/E, i.e. at C-5, C-14, C-16 and C-17 and also at C-25, which leads to the non-protonated nature of these carbons and their appearance between δ 65.0–116.1. The chemical shifts of these oxy-substituted carbons follow the sequence C-25 < C-5 < C-14 < C-17 < C-16 in order of increasing downfield shift. The chemical shift ranges are δ 65.1–65.9 (C-25), 73.2–78.3 (C-5), 83.4–86.2 (C-5), 85.5–87.8 (C-14), 90.0–90.5 (C-17) and 114.3–116.1 (C-16) for 25-OH, 5-OH, 5-OH and 6-oxo, 14-OH, 17-OH and 16-OH, respecitvely. The hydroxylated methine absorbs between δ 67.4 and 79.5 and could appear at a further lower field position depending on the vicinal functional groups. For example, C-3 appears at δ 83.0 in 5β-spirostane with hydroxyl groups at the 2β, 3β and 4β positions[12] and C-12 at δ 84.4 in the case of **38** with an oxo group at the 11-position.[32] Concerning the hydroxyl substitution of the CH_3 group, 21- and 27-hydroxylated compounds have so far been characterized in which C-21 and C-27 resonate at δ 61.5[66] and δ 64.4–64.9, respectively.[56,62,116] Owing to oxy substitution, C-16 and C-26 absorb between δ 80.7 and 81.4 and δ 65.1 and 67.1, respectively, and therefore these signals should not be considered if one wishes to determine the hydroxylation pattern.

2.1.2.2. Applications of 1D- and 2D-pulse experiments. One of the reliable methods to obtain information about the hydroxylation pattern is the analysis of homonuclear two-dimensional shift correlated spectroscopy (^1H–^1H COSY), which offers the advantage of requiring a sample of less than 1 mg to obtain a spectrum of high quality. The oxy-substituted methine and methylene provide entry points for the assignment of ring protons.[21,117–119] In Δ^5-spirostane, the vinylic H-6 shows coupling with 7-C$\underline{H}_2$ and H-4 which, in turn, exhibit cross peaks to H-8, and so on, resulting in the ^1H NMR assignments for protons belonging to ring B and subsequently to the other ring resonances.[21,120] The identification of H-4 leads to the assignments of resonances belonging to ring A. The protons on ring F, including 27-C$\underline{H}_3$, can be assigned by following connectivities observed by employing 26-C$\underline{H}_2$ geminal protons as the starting points whereas proton assignments for ring D can be ascertained by following connectivities started with H-16. The connectivities traced out in such a manner lead to continuous correlations and hence to the assignment of proton signals of rings A, B, C, D and F. The E. COSY experiment has also been found to be useful in identifying $^3J_{HH}$ coupling constants and proton–proton coupling networks in steroidal compounds.[118] In the case of severe cross-peak overlapping, the use of the ω_1-decoupled COSY (COSYDEC) method can sometimes facilitate ^1H NMR analysis of steroids,[121] but it is inherently dangerous since if the constant time delay $\Delta \approx 1/2J_{HH}$, cross peaks will disappear.

Delayed COSY,[96] is particularly useful in determining the long-range ^1H–^1H connectivity for, e.g., protons of an angular methyl group at the 18-position, showing characteristic coupling with H-12.[21,117–119] These long-range connectivities help to penetrate into the heart of the convoluted region of the spectrum and to verify

assignments made by the use of H-3 and H-6 as the starting points.

The ^{1}H NMR assignments can be further verified by multi-step RCT[102] or HOHAHA[101b,c] spectroscopy, in which an individual proton resonance displays cross peaks not only to vicinal protons, but also to protons that are not directly spin-coupled.[122] The success of RELAY experiments depends on the correct choice of delays t_1, t_2 and t_3 in the pulse sequence. The presence of strong coupling, however, as found in ^{1}H spectra that are non-first-order, can interfere with the transfer efficiency of the experiment, resulting in the detection of weak cross peaks. HOHAHA spectroscopy is related to TOCSY[101a] and provides an excellent tool to obtain multi-step coherence transfer between all spins within the coupling network. To generate a suitable tailored Hamiltonian during the mixing time for coherence transfer, a spin-locking pulse is usually employed with different degrees of compensation for resonance offset. These techniques remove ambiguities arising from coincident chemical shifts and particularly facilitate the identification and assignment of each constituent residue of the oligosaccharide moiety (see below).

To determine the orientation of the hydroxyl groups, ^3J coupling constants are of significance[17] and the NOESY or ROESY (two-dimensional NOE or ROE spectroscopy) or one-dimensional NOE difference spectroscopy may provide further evidence as these define spatial relationships.[21,38,117,111,123–125] NOESY, although less demanding on spectrometer hardware, has the problem that the NOE cross peaks change from positive to negative as molecular tumbling slows with increasing molecular mass and/or solvent viscosity. Steroidal saponins having molecular masses between 600 and 1200, in particular, run the risk of tumbling at rates which gives zero NOE effects in either NOESY or NOE-difference spectra,[24] thereby limiting the utility of the method. In contrast, the nuclear Overhauser effect that occurs under spin-lock conditions is known as the transverse or rotating frame NOE, which increases monotonically in a positive direction with increasing correlation time and provides a potential solution to the problem. The contributions from scalar coupling (TOCSY artifacts) near the diagonal, however, are a disadvantage in ROESY experiments.[118]

The other approach involves the application of ^{13}C NMR. Since the hydroxyl-bearing carbons usually resonate at a relatively distinct position (δ 60–85), application of the ^{13}C NMR spectral editing techniques such as DEPT primarily lead to the identification of the type of hydroxylated carbon present. The substitution site can then be established in a straightforward manner from the observation of cross peaks in the ^{1}H,^{13}C HETCOR experiment because in such a spectrum, each cross peak arises from connectivity between the ^{13}C nucleus and its directly bonded proton. Thus ^{1}H,^{13}C correlations are useful for resolving overlaps in the ^{1}H dimension and for identifying the origin of ^{1}H signals (based on the chemical shift of the attached ^{13}C). This means that quaternary carbons do not appear in these spectra as they do not have directly attached protons. Therefore, tertiary hydroxyl-bearing carbons will not show up and thus their presence or absence can be established by comparison of the 1D ^{13}C NMR and the ^{1}H,^{13}C HETCOR spectral data. An additional advantage of these experiments lies in the assignment of ^{1}H resonances if the unambiguous ^{13}C NMR assignments are available.[117b,126–129]

Once the chemical shifts of hydroxyl-bearing ^{1}H NMR resonances have been established, then their three-bond ^{1}H–^{1}H correlation observed in a COSY experiment will lead to the identification of ^{1}H NMR chemical shifts of vicinal partners and hence the ^{13}C NMR chemical shifts of C-β can be deduced. The fact that the presence of the hydroxyl group causes an appreciable deshielding of the resonances of accompanying β-positions provides a means for the establishment of the site of substitution of the hydroxyl group. Hence such correlations are valuable in determining the number and locations of secondary and primary OH groups and the results can further be verified against the results of ^{1}H–^{1}H COSY and other related experiments (Table 1).

Recently it has been shown that there are considerable advantages in recording ^{13}C–^{1}H correlation spectra with observation of the proton signal rather than that of carbon-13. These techniques are the ^{1}H-detected heteronuclear multiple-quantum correlation (HMQC) and ^{1}H-detected heteronuclear multiple-bond correlation (HMBC) experiments, exhibiting one-bond and multiple-bond ^{1}H–^{13}C connectivities, respectively. Such experiments are generally known as inverse experiments[108] as they are recorded in the opposite way from the normal practice, that is, ^{1}H instead of ^{13}C detection. The F_1 domain now contains the ^{13}C resonances while the protons are detected in F_2. These inverse experiments benefit from the larger magnetogyric ratio of the proton and the sensitivity of the inverse experiments is an advantage which enables such experiments to be carried out with small quantities of material for which the ^{13}C-detected HETCOR usually is tedious or even fails.

Despite the fact that the ^{1}H-detected experiments have distinct sensitivity advantages, they suffer from limited ^{13}C resolution. By contrast, the ^{13}C-detected HETCOR sequences can have excellent ^{13}C resolution and despite their lower sensitivity they are useful for distinguishing resonances with very similar chemical shifts. In the case of smilagenin [(25*R*)-3β-hydroxy-5β-spirostane], C-6 and C-7 exhibit coincidence at δ 26.5 (δ 26.56 and 26.54) and thus the ^{1}H NMR chemical shifts for these methylene resonances could not be ascertained from the HMQC spectrum. Under these circumstances, the ^{13}C-detected HETCOR spectrum was found to be useful.[117b,c] In a similar manner, LR-HETCOR experiments eventually suffer from sensitivity limitations but if sufficient quantities of material are available and if no solubility problem exists, it is preferable to record ^{13}C-detected long-range connectivity spectra. The ^{1}H,^{13}C multiple bond correlations observed in either LR-HETCOR or HMBC experiments reveal primarily connectivities between protons and carbons separated by two to four bonds and also facilitate the assignment of quaternary carbons. The α- and β-protons have also been distinguished from the analysis of HMBC spectra.[21,117b,118] The angular methyl protons exhibit correlation to the quaternary carbon to which they are bonded; thus 18-C$\underline{H}_3$ and 19-C$\underline{H}_3$ exhibit correlations

to C-13 and C-10, respectively, in addition to three-bond correlations with C-17 and C-14, and C-1, C-5 and C-9, respectively.[21,118] The iterative comparison between ^1H–^{13}C correlation and ^1H–^1H COSY spectra provides valuable information for the differentiation between geminal and vicinal protons. The 21-C$\underline{H}_3$ and H-20 atoms exhibit their three- and two-bond correlations with C-22.[21,69,118]

The unambiguous assignment of the ^{13}C chemical shifts can also be obscured owing to the overlapping of corresponding protons and a short heteronuclear correlation sequence with a relayed transfer as in HMQC-TOCSY[130] can resolve such ambiguities. As a result, an individual ^{13}C resonance correlates not only with its directly bonded proton(s) but also with the neighbouring protons two and three bonds away for a range of mixing times, and are also helpful in resolving some ambiguities concerning the ^{13}C assignments for some pairs of carbon atoms in case of steroidal sapogenins.[117b]

Thus, concerted application of the homo- and heteronuclear spectroscopic techniques can be utilized in the establishment of hydroxylation patterns in a molecule. To clarify further the ^{13}C NMR assignments, and to provide independent proof for the entire carbon skeleton, two-dimensional INADEQUATE experiments are of great importance as these exhibit ^{13}C–^{13}C connectivities[107,131] but, owing to the low sensitivity, they have been not routinely employed.[25]

2.1.2.3. Keto group assignment. The keto group has been recognized at C-3, -6, -7, -11, -12, -22 and -23, which has led to the appearance of carbonyl absorption between δ 200.0 and 218.1. The keto group at C-26, being of the ester type, leads to its appearance at relatively higher field *ca.* 180.5 ppm.[129] The α,β-carbonyl appears 5–10 ppm at higher field relative to the position of the non-conjugated carbonyl.[12,13,132] The carbonyl chemical shift is dependent on structural environment and cannot be reliably employed in determining the location of the group. The best way to achieve this information is to identify the ^1H and ^{13}C NMR chemical shifts for the adjacent methylene/methine resonances, as these absorb at relatively deshielded positions owing to the β-effect of the CO function,[12,16,32,133,134] and then to observe cross peaks between $COCH_2$ and carbonyl resonances in the HMBC spectrum.[135]

2.1.2.4. Unsaturation. Unsaturated steroidal sapogenins are widely distributed. So far, olefinic bonds have been reported at positions 4, 5, 7, 9(11), 11, 17(20) and 25(27). The olefinic carbon atoms absorb between δ 108.1 and 172.1 and the nature of the carbon resonance (C, CH, CH_2) identifies the type of olefinic bond (di-, tri- or tetrasubstituted). The olefinic bond at C-5 is one of the most frequently encountered unsaturation, resulting in the appearance of C-5 and C-6 at δ 139.7–142.1 (C) and δ 120.9–124.3 (CH), respectively, and can be substantiated by the absorption at 5.21–5.62 ppm as a doublet (J = 4–5 Hz) or multiplet for the H-6 signal.[16,37,116] The chemical shift of C-5 is almost unaffected by the hydroxyl substitution in ring A, but a 7-oxo group causes appreciable deshielding, which is reflected by its appearance at 166.5 ppm as in **71**.[37] The olefinic resonances at *ca.* 116.0 (CH) and 139.3 (C), 147.5 (C) and 116.2 (CH) and 124.9 (CH) and 137.6 ppm (CH) identify olefinic bonds at the 7, 9(11) and 11 positions in compounds **1** and **2**, respectively.[22,32] As discussed above, an oxo function at the adjacent position, e.g. C-12 in a 9(11)-unsaturated spirostane (**17**), causes a significant downfield shift and the appearance of C-9 at δ 172.1.[32] The ^1H NMR spectrum is almost uninformative owing to the lack of any olefinic ^1H NMR absorption to determine unsaturation at C-17(20), but the introduction of two quaternary carbons absorbing at 142.5 and 145.6 ppm, respectively, as in 17(20)-dehydrokryptogenin,[53] makes ^{13}C NMR spectroscopy the ideal technique for this problem. The exocyclic methylene absorbing at 108.6 $\pm$ 0.5 ppm (C-27), accompanied by a quaternary carbon at 144.4 $\pm$ 0.5 ppm (C-25), is characteristic of 25(27)-olefinic unsaturation.[50] Both C-20 and C-22 are of the quaternary type, absorbing at δ 103.7–103.9 and δ 151.3–151.9, respectively, in 20(22)-furostane type steroidal sapogenins,[136,137] whereas C-13 and C-14 absorb at δ 176.0 and δ 138.7 in 18-norspirostanol.[138]

2.1.3. Determination of ring fusion. The naturally occurring steroidal sapogenins so far reported possess either *cis* or *trans* fusion between rings A and B but always a *trans* ring fusion between rings B/C and C/D. The D/E ring junction is *cis* in **1.1–1.3**. Thus, structure establishment of these compounds requires the determination of the A/B ring junction. The ^1H NMR chemical shifts, splitting pattern and coupling constants for the resonances belonging to rings A and B and those belonging to H-5 and 19-C$\underline{H}_3$ are particularly informative for acquiring such information. However, as unambiguous ^1H NMR assignments are available for only a few steroidal sapogenins, it is not possible to derive general trends applicable for the determination of stereochemistry at the ring junction based on ^1H NMR data. A comparison of the ^{13}C NMR chemical shifts for the 5α- and 5β-series with analogous substitution patterns in rings A and B led to the conclusion that C-5 (CH), C-7 (CH_2), C-9 (CH) and C-19 (CH_3) exhibit a remarkable dependence on A/B ring fusion, i.e. these absorb, in general, at lower field in the *trans*-fused compounds than in the *cis*. The chemical shift difference for C-9 and C-19 ($\delta_{trans} - \delta_{cis}$) is 9–13 ppm, and 3–6 ppm for C-7 and C-5. This can be illustrated by taking (25*R*)-5α- and -5β-spirostanes with an equatorially oriented OH group at the C-3 position, i.e. tigogenin and epismilagenin ($\delta_{tigogenin} - \delta_{epismilagenin}$: C-5 −2.8, C-7 −5.5, C-9 −13.8, C-19 +11.1 ppm) as representative examples.[12]

As already mentioned, the chemical shift values for C-5 and C-6 are highly characteristic for Δ^5-steroidal sapogenins. However, a comparison of the ^{13}C NMR chemical shifts for 5α-spirostane with Δ^5-spirostane reflects appreciable similarities for most of the signals belonging to rings A and B, except for C-4, C-10 and C-19, as these exhibit deshielding of 2–7 ppm whereas C-8 and C-9 exhibit 3–5 ppm shielding. These ^{13}C NMR spectral features are useful in determining the A/B ring fusion and Δ^5 unsaturation (Table 4). These spectral features are also useful aids for the establishment of ring geometry (*cis* or *trans*) of steroidal sapogenins of other skeletal types (**1.2–1.4**).

Table 4. Characteristic ^{13}C NMR chemical shifts for the determination of A/B ring fusion and Δ^5-unsaturation in spirostane-type steroidal sapogenins[12]

Skeletal type	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-19
5α-Spirostane(3β-OH)	38.2	44.9	28.6	32.2	35.7	54.4	35.6	12.3
5β-Spirostane(3α-OH)	36.5	42.1	27.1	26.7	35.1	40.6	34.7	23.4
Δ^5-Spirostane(3β-OH)	42.3	140.9	121.3	32.0	31.4	50.1	37.6	19.4

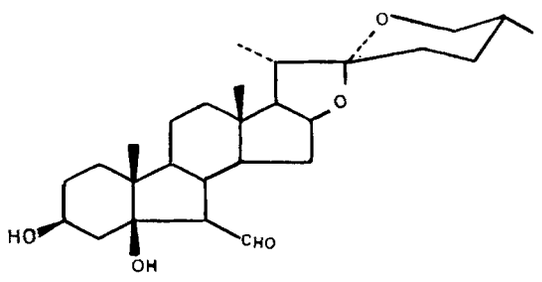
2.2. Individual classes of Compounds

2.2.1. Spirostanes. The 27-methyl group at C-25 in spirostanes can adopt either equatorial or axial orientation resulting in the 25*R* and 25*S* forms of spirostane (**1.1**). These epimeric forms are differentiable on the basis of the chemical shift of 27-$C\underline{H}_3$, which usually absorbs about 0.3 ppm to higher field in the former relative to the latter,[17,139] and also the ^{13}C NMR chemical shifts of the ring-F carbon atoms since C-23, C-24, C-25 and C-26 absorb at 2.5–4.0 ppm and C-27 at 1.5–2.0 ppm at lower field in the 25*R* compounds in comparison with (25*S*)-spirostanes having an unsubstituted ring F.[12,45,140,141] The diagnostic shifts are δ 31.3, 28.8, 30.3, 66.9 and 17.1 for (25*R*)-spirostane and δ 26.6, 26.2, 27.5 and 65.0 and 16.3 for (25*S*)-spirostane with a standard deviation of ±0.4 ppm for C-23, C-24, C-25, C-26 and C-27, respectively. These features have led to the identification of 25-epimeric forms in mixtures of spirostanes, e.g. gitogenin and neogitogenin,[140] samogenin (**45**) and markogenin (**44**)[48] and glycosidic mixtures[141] which were otherwise difficult to separate chromatographically. The above-mentioned epimeric shift difference is, however, not valid for distinguishing between 25*R* and 25*S* forms of 25-hydroxyspirostanes such as pompeygenin (**37**) and chenogenin (**36**); the C-23 and C-24 resonances were noted at 2.6 and 1.6 ppm lower field than in the case of the latter[44] and other ring F-substituted spirostanes.[130] The additional methyl group at the 6-position in the case of 6-methyldiosgenin (**74**) appears at 20.0 ppm.[60] A B-ring contracted spirostane leontogenin (**75**) (Fig. 2) has been isolated from the acid hydrolysate of an extract of leaves of *Tacca leontopetaloides* in which C-6 becomes an exocyclic aldehydic type carbon giving rise to an absorption at 203.4 ppm.[67] Anzurugenin B (**76**) is a unique type of spirostane sapogenin (Fig. 2), isolated from the acid hydrolysis of the saponins from the collective fruit of co-cultivated *Allium suvorovii* and *A. stipitatum*, having an additional 2α,5α-epoxy bridge and thus resulting in the appearance of C-5 at 90.4 ppm.[69]

2.2.2. Furostanes. A comparison of the ^{13}C NMR chemical shifts for spirostane and 22-hydroxylated, 26-*O*-glucosylated furostanoids with identical substitution in the carbocyclic rings reveals a significant resemblance and pronounced effects due to ring opening of the ring F are C-20 −1.3 to 1.6, C-21 +0.9 to 1.6, C-22 +1.3 to 1.5, C-23 +5.1 to 5.4, C-24 −0.8 to 1.0, C-25 +3.5 to 3.7 and C-26 +8.2 to 8.4 ppm, respectively.[31,43,95,127,142] A similar comparison with furostanols reflects a downfield shift of 3.1–3.6 ppm for C-22 and an upfield shift of 5.5–7.0 ppm for C-23. These spectral features can be utilized emperically for predicting chemical shifts for furostanols derived from spirostanols.

Recently, furostane sapogenins lacking a 26-hydroxyl group and having an unsubstituted 22-position have been isolated from the acid hydrolysate of steroidal glycosides of *Cordyline rubra*[61] and *Allium suvorovii*,[26] which can be easily identified by the appearance of C-22 at 90.5 ± 0.1 ppm. The C-26 and C-27 methyl resonances appear between 22.5 and 22.9 ppm,[26,61] but at 67.9 ± 0.2 and 16.7 ± 0.1 ppm, respectively, in 26-hydroxylated furostane.[61,71] The appearance of the C-26 position at *ca.* 2 ppm to lower field (*ca.* 69.3 ppm) is characteristic of 26-*O*-esterified (acetylated) furostanes.[114]

2.2.3. Furospirostanes. Most of the furospirostane-type steroidal sapogenins are 26-hydroxylated and consequently the 27-methyl group which may acquire either an *R* or *S* configuration appears as singlet at *ca.* 1.15 ppm.[115] Despite the fact that the two 25-epimeric forms exhibit marginal ^{13}C shielding variations in ring F resonances, these are still significant as C-25 shows an upfield shift, whereas C-27 shows a downfield shift of about 1.5 ppm. This can be exemplified by strictagenin (**84**) and rubragenin (**83**), where these resonances were observed at δ 85.8 and 23.9 ppm and δ 84.1 and 25.3 ppm, respectively.[46] Both C-26 and C-27 absorb at 65.4 ± 0.5 ppm in taccagenin (**84**) owing to the presence of hydroxyl groups at these positions.[62] A C_{29} furo-



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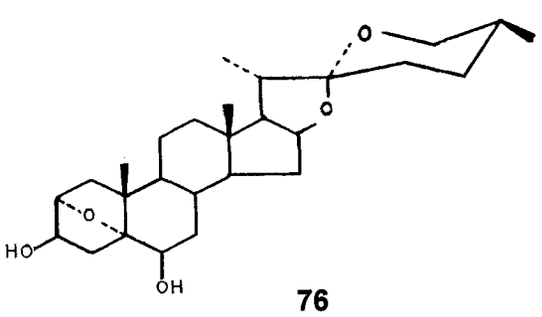

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Figure 2. Structures of miscellaneous steroidal sapogenins

Table 5.1. ^{13}C NMR chemical shifts for 5α-spirostanes[a]

Substituents	25*R*, 3β-(OH), $^{9(11)}$	25*R*, 3β-(OH), $^{11(12)}$	25*R*, 3-oxo	25*R*, 3-oxo, 6α-OH	25*R*, 1β, 3α-$(OH)_2$	25*R*, 1β, 3β-$(OH)_2$	25*R*, 3β, 6β-$(OH)_2$	25*R*, 3β, 7β-$(OH)_2$	25*S*, 3-oxo, 6α-OH	25*R*, 3β-(OH), 6-oxo	25*R*, 3β, 7β-$(OAc)_2$	25*R*, 3,6-$(oxo)_2$	25*R*, 3β, 11α-$(OH)_2$	25*R*, 3β, 12β-$(OH)_2$
Atom	**1**[32]	**2**[32]	**3**[33]	**4**[34]	**5**[35]	**6**[35]	**7**[36]	**8**[37]	**9**[34]	**10**[38]	**11**[37]	**12**[39]	**13**[32]	**14**[32]
C-1	35.7	36.3	38.5	38.6	72.5	78.0	39.2	36.8	38.5	36.7	36.4	38.0	38.8	37.1
C-2	31.6	31.1	38.1	36.6	39.5	42.4	32.7	31.4	37.6	30.7	27.3	37.0	31.7	31.5
C-3	70.9	71.0	211.9	210.9	64.6	68.1	71.0	71.0	210.9	70.6	73.1	211.1	70.2	71.0
C-4	38.1	37.9	44.7	39.5	35.7	38.2	37.2	37.7	39.5	30.1	33.7	37.4	38.8	38.0
C-5	43.5	44.9	46.7	53.1	37.6	41.5	48.5	41.7	53.1	56.8	41.5	57.5	45.2	45.0
C-6	28.7	29.3	28.8	69.7	27.9	28.4	71.4	38.2	69.7	210.2	32.8	208.8	29.3	28.7
C-7	33.5	31.1	31.8	41.8	29.5	32.2	41.0	74.7	41.8	46.8	76.4	46.6	32.3	31.9
C-8	36.1	34.1	35.0	34.0	35.3	35.3	30.7	43.4	34.0	37.4	39.5	37.4	34.7	34.1
C-9	147.5	57.4	53.9	53.5	54.7	54.8	54.8	52.5	53.5	54.0	52.4	53.4	60.3	53.5
C-10	38.1	36.1	35.8	36.5	43.1	—	36.2	35.1	36.5	41.0	34.9	41.2	37.3	35.6
C-11	116.2	124.9	21.3	21.1	23.5	24.3	21.3	21.3	21.1	21.4	21.2	21.5	68.0	30.7
C-12	42.1	137.6	39.9	39.8	40.1	40.0	40.3	40.1	39.8	39.6	39.9	39.4	51.6	80.7
C-13	39.2	43.0	40.6	40.6	41.0	40.4	40.9	41.4	40.6	40.9	41.2	40.9	40.8	46.0
C-14	54.2	53.1	56.1	55.9	55.9	56.3	56.5	55.3	55.9	56.6	54.7	56.3	55.4	54.9
C-15	33.0	30.8	31.8	31.8	31.8	32.1	32.3	34.9	31.8	31.6	33.4	31.6	31.4	31.4
C-16	81.5	81.0	80.8	80.6	79.9	80.7	81.2	81.8	80.7	80.5	81.0	80.3	80.8	79.8
C-17	61.8	58.8	62.3	62.3	62.2	62.3	63.1	61.4	62.3	62.2	61.2	62.0	62.1	61.9
C-18	15.9	20.5	16.4	16.0	15.8	16.4	16.7	16.6	16.0	16.4	16.5	16.4	17.2	10.5
C-19	18.0	12.9	11.5	12.7	5.5	6.8	16.3	12.5	12.7	13.2	12.3	12.6	12.7	12.3
C-20	42.5	41.6	41.6	41.8	41.6	41.7	42.0	42.1	42.3	41.7	41.7	41.6	41.6	42.3
C-21	14.2	14.4	14.5	14.3	14.1	14.6	15.1	14.5	14.1	14.4	14.5	14.5	14.4	13.8
C-22	109.9	109.5	109.2	109.1	107.9	—	109.2	109.2	109.6	109.3	109.2	109.0	108.9	109.4
C-23	31.4	31.4	31.4	31.5	31.4	31.4	31.8	31.4	26.1	31.4	31.3	31.3	31.3	31.4
C-24	28.9	28.8	28.8	28.8	29.3	28.8	29.2	28.2	25.8	28.8	28.8	28.7	28.8	28.9
C-25	30.5	30.3	30.3	30.3	30.8	30.3	30.6	30.3	27.1	30.3	30.3	30.2	30.1	30.3
C-26	67.2	67.0	66.9	66.8	65.8	66.8	66.9	66.9	65.1	66.9	66.8	66.9	66.7	66.9
C-27	17.2	17.1	17.1	16.9	16.6	17.1	17.3	17.0	16.3	17.1	17.1	17.1	17.1	17.1
	C	C	C	C	C	C	P	C	C	C	C	C	C	C
	INEPT	INEPT			DEPT	DEPT				DEPT			INEPT	INEPT

[a] For an explanation of the abbreviations used in this and the following tables see Section 2.3.

Table 5.1 (continued)

Substituents Atom	25*R*, 3β, 12α-$(OH)_2$ **16**[32]	25*R*, 3β-OH, 12-oxo, $^{9(11)}$ **16**[32]	25*S*, 3β-OH, 12-oxo **17**[40]	25*R*, 3,12-$(oxo)_2$ **18**[41]	25*R*, 3β-OAc, 25(OH) **19**[42]	25*R*, 2α, 3β, 6β-$(OH)_3$ **20**[43]	25*R*, 2α, 3β, 5α-$(OH)_3$ **21**[22]	25*R*, 2α, 3β-$(OH)_2$, 12-oxo **22**[44]	25*R*, 2α, 3β, 5α-$(OH)_3$, ^{7} **23**[22]	25*R*, 2α, 3β, 5α, 6β-$(OH)_4$ **24**[25,27]	25*R*, 3β, 7β-$(OH)_2$, 12-oxo **25**[37]	25*R*, 3, 7, 12-$(oxo)_3$ **26**[37]	25*R*, 3β-OH, 7, 12-$(oxo)_2$ **27**[37]	25*R*, 3β-OAc, 7, 12-$(oxo)_2$ **28**[37]
C-1	37.1	34.8	36.6	37.8	36.7	47.9	40.7	49.5	39.8	42.2	36.3	37.9	35.6	35.5
C-2	31.6	31.4	31.2	37.8	27.5	73.1	73.3	72.8	73.1	73.8	31.4	36.5	30.7	26.8
C-3	71.3	70.0	70.9	210.9	73.6	77.2	73.7	76.8	73.1	73.8	70.7	210.7	70.3	72.2
C-4	38.4	37.6	37.9	44.4	34.0	34.9	44.0	37.0	43.4	41.1	37.4	43.9	37.8	31.9
C-5	45.1	42.7	44.7	46.2	44.6	48.4	73.9	45.0	73.3	75.8	41.9	47.0	46.1	45.8
C-6	28.8	27.7	28.3	28.5	28.5	70.2	34.5	28.0	37.3	75.6	38.4	45.4	45.8	45.6
C-7	32.1	32.6	31.3	31.4	32.2	40.8	26.6	31.7	116.0	23.9	73.0	207.6	209.1	208.6
C-8	35.1	37.0	34.5	34.4	35.7	30.0	34.3	33.8	139.3	30.3	42.3	48.7	48.9	48.8
C-9	47.9	172.1	55.7	54.9	54.2	54.7	45.8	55.6	43.7	46.0	53.1	54.7	55.9	55.5
C-10	35.4	39.5	36.2	36.2	35.6	37.6	41.2	37.9	40.5	41.0	35.6	36.4	36.5	36.5
C-11	31.6	119.8	38.0	37.8	21.4	21.4	21.8	38.2	21.8	21.7	37.6	37.2	37.6	37.7
C-12	72.5	205.6	213.5	212.7	39.9	40.2	40.5	212.5	41.1	40.6	213.0	208.5	211.5	211.2
C-13	45.0	51.1	55.2	55.1	40.9	40.8	41.0	55.4	41.8	41.0	55.6	54.7	54.7	54.7
C-14	48.4	53.8	55.9	55.9	56.2	56.3	56.4	55.9	55.1	56.4	54.8	48.0	48.3	48.3
C-15	31.6	31.4	31.6	31.2	31.6	32.2	32.2	31.8	31.5	32.2	34.1	31.9	33.8	33.7
C-16	80.4	79.9	79.4	79.1	81.1	81.1	81.3	79.7	80.9	81.3	79.7	79.2	79.3	79.3
C-17	53.7	52.5	53.5	53.6	62.0	63.0	63.1	54.3	62.8	63.2	52.6	52.8	52.8	52.8
C-18	17.1	15.1	16.1	16.0	16.5	16.6	16.8	16.1	16.5	18.5	16.2	16.1	16.1	16.1
C-19	12.3	18.5	12.0	11.1	12.2	17.4	17.4	13.1	19.3	16.7	12.1	10.7	11.5	11.4
C-20	41.9	42.6	42.8	42.1	41.0	41.9	42.0	42.6	42.5	42.1	42.3	42.2	42.1	42.2
C-21	14.4	13.1	13.1	13.3	14.3	15.0	15.0	13.1	14.9	15.9	13.3	13.3	13.3	13.3
C-22	109.2	109.6	109.1	109.2	108.3	109.2	109.2	109.3	109.3	109.3	109.3	109.2	109.2	109.2
C-23	31.6	31.0	26.1	31.2	23.9	31.7	31.9	31.4	31.8	31.9	31.1	31.4	31.4	31.5
C-24	29.0	28.8	25.8	28.8	34.9	29.2	29.3	29.2	29.3	29.3	28.8	28.8	28.8	28.8
C-25	30.4	30.2	27.2	30.2	67.4	30.5	30.6	30.5	30.6	30.6	30.2	30.2	30.2	30.2
C-26	67.0	67.0	65.2	66.9	69.1	66.8	66.9	66.9	66.9	66.9	66.9	66.9	69.1	66.8
C-27	17.1	17.1	16.1	17.1	29.7	17.3	17.3	17.3	17.3	17.3	—	17.1	24.0	17.1
	C INEPT	C INEPT	C DEPT	C	C	P	P	P INEPT	P DEPT	P DEPT	C	C	C	C

Table 5.1 (continued)

Substituents / Atom	25*R*, 3-oxo, 7β, 12β-$(OH)_2$ **29**[41]	25*R*, 3β, 7β 12β-$(OH)_3$ **30**[41]	25*R*, 3β, 7α, 12β-$(OH)_3$ **31**[41]	25*R*, 3β-OH 11β-Br. 12-oxo **32**[32]	25*R*, 3β, 12β-$(OH)_2$, 11-oxo **33**[32]	25*R*, 3β, 6α, 23β-$(OH)_3$ **34**[45]	25*S*, 3β, 27-$(OH)_2$, 6-oxo **35**[38]	25*S*, 1β, 3α, 25-$(OH)_3$ **36**[46]	25*R*, 1β, 3α, 25-$(OH)_3$ **37**[46]	25*R*, 3β, 12β-$(OH)_2$, 11-oxo, 23α-Br **38**[32]	25*R*, 3β-OAc, 11α, 23α-$(Br)_2$, 12-oxo **39**[32]	25*R*, 1β, 3β, 7β-$(OH)_3$, 12-oxo **40**[37]	25*S*, 1β, 3β, 7β-$(OH)_3$, 12-oxo **41**[37]
C-1	38.1	36.7	37.5	35.5	35.7	38.0	36.7	74.0	74.1	35.6	38.3	76.2	77.0
C-2	37.9	31.3	31.6	30.8	31.2	32.3	30.7	40.2	40.0	31.0	47.6	42.4	33.5
C-3	211.1	70.9	70.7	70.2	70.7	71.0	70.4	66.4	66.9	70.6	72.7	67.4	68.8
C-4	44.0	37.5	39.1	37.3	37.5	33.6	30.1	36.0	35.9	37.3	34.0	37.5	33.1
C-5	43.9	42.2	37.3	46.6	45.0	52.7	56.8	38.3	38.2	44.9	44.9	39.4	38.8
C-6	38.5	38.4	38.0	27.5	28.1	68.6	210.4	28.2	28.1	28.0	29.0	37.8	33.1
C-7	73.7	74.2	66.8	30.8	32.6	42.8	46.8	32.2	32.2	32.6	31.0	73.8	74.7
C-8	41.9	42.2	39.3	31.0	37.2	34.2	37.3	35.6	35.5	37.0	36.5	42.7	38.9
C-9	50.5	51.1	45.6	50.4	62.8	54.3	53.9	54.8	54.7	62.8	63.9	53.5	53.0
C-10	35.2	35.0	36.2	37.3	35.3	36.6	40.9	42.5	42.5	35.2	38.6	41.2	39.9
C-11	30.7	30.5	31.1	58.8	210.6	21.4	21.4	24.4	24.5	210.5	54.4	40.5	39.7
C-12	79.3	79.6	79.3	205.2	84.5	40.5	39.5	40.4	40.3	84.4	204.3	213.7	211.7
C-13	46.7	46.7	46.4	54.7	50.0	41.4	40.8	40.0	40.0	50.6	55.1	55.2	54.7
C-14	53.7	53.8	49.8	55.5	53.0	56.4	56.5	56.4	56.3	52.9	55.1	54.5	52.6
C-15	34.3	34.3	32.5	31.7	31.0	31.7	31.6	31.8	31.9	31.3	31.9	34.4	33.0
C-16	81.1	81.2	81.4	78.7	80.6	81.6	80.5	81.0	81.3	81.4	79.7	79.4	79.3
C-17	60.9	60.9	63.1	56.7	60.8	62.6	62.1	62.2	62.0	59.5	50.5	52.7	52.4
C-18	10.5	10.5	11.2	20.3	11.3	16.8	16.4	16.4	16.4	11.7	15.8	16.2	16.0
C-19	11.5	12.4	11.5	15.4	12.3	13.7	13.2	5.8	5.6	12.3	12.5	6.6	7.7
C-20	42.2	42.2	43.1	42.1	42.5	38.7	41.7	41.0	41.6	38.9	38.9	42.4	42.4
C-21	13.9	13.9	14.4	13.2	13.3	14.7	14.4	14.4	14.5	12.8	12.7	13.3	13.1
C-22	109.4	109.5	109.5	109.3	109.4	117.7	109.5	109.0	109.1	109.1	108.8	109.3	109.2
C-23	31.4	31.4	31.9	13.3	13.4	67.4	31.6	29.6	27.0	49.6	49.9	31.4	31.3
C-24	28.8	28.7	29.3	28.7	28.8	35.8	23.2	34.9	32.7	40.5	40.5	28.8	28.8
C-25	30.3	30.3	30.6	30.1	30.2	32.1	38.2	67.0	66.7	33.5	33.5	30.2	30.2
C-26	66.9	66.9	66.8	66.8	66.9	66.0	64.8	69.1	69.0	65.9	65.8	67.0	66.9
C-27	17.1	17.1	17.3	17.1	17.1	16.9	64.2	24.0	24.7	16.2	16.2	—	—
	C	C	P	C	C	P	C	C	C	C	C	C	C
	DEPT			INEPT	INEPT		DEPT	INEPT	INEPT	INEPT	INEPT		

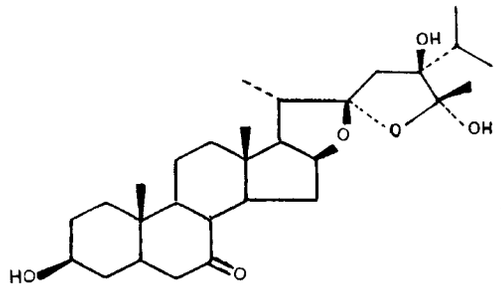
[a] **3** = Yuccagenone; **5** = cannigenin; **6** = brisbagenin; **8** = β-chlorogenin; **12** = neochlorogenone; **17** = neohecogenin; **20** = agigenin; **22** = maxogenin; **24** = alliogenin; **32** = hongguanggenin; **35** = sieboldogenin; **36** = chenogenin; **37** = pompeygenin.

Table 5.2. ^{13}C NMR chemical shifts for 5β-spirostanes[a]

Substituents / Atom	25*S*, 3-oxo **42**[47]	25*S*, 3α-OH **43**[47]	25*S*, 2β, 3β-$(OH)_2$ **44**[48]	25*R*, 2β, 3β-$(OH)_2$ **45**[48]	25*R*, 3β, 12β-$(OH)_2$ **46**[49]	25*R*, 3β-OH, 12-oxo **47**[44]	25*R*, 2β, 3β-$(OH)_2$, 12-oxo **48**[44]	25*R*, 2β, 3β, 12β-$(OH)_3$ **49**[49]	$\Delta^{25(27)}$ 1β, 3β, 4β, 5β-$(OH)_4$ **50**[50]	$\Delta^{25(27)}$ 1β, 3β, 4α, 5β-$(OH)_4$ **51**[50]	$\Delta^{25(27)}$ 1β, 3β, 5β, 6β-$(OH)_4$ **52**[50]	25*S*, 1β, 3β, 4α, 5β-$(OH)_4$ **53**[50]	25*S*, 2α, 3β 5β, 24-$(OH)_4$, 6-oxo **54**[51]	$\Delta^{25(27)}$ 1β, 2β, 3β, 4β, 5β-$(OH)_5$ **55**[50]	$\Delta^{25(27)}$ 1β, 2β, 3β, 4β, 5β, 7α-$(OH)_6$, 6-oxo **56**[50]
C-1	37.1	35.3	38.1	38.1	30.7	30.3	39.1	39.4	73.8	74.2	74.7	74.2	32.5	77.9	76.5
C-2	36.9	30.4	69.2	69.2	28.5	28.3	70.2	70.4	33.5	30.1	35.0	33.7	71.7	67.4	68.9
C-3	213.1	71.7	67.5	67.5	66.0	65.7	67.3	67.4	71.2	71.8	68.1	69.2	71.7	75.6	75.5
C-4	42.3	36.4	32.5	32.5	34.4	34.2	33.5	32.1	68.1	68.1	30.0	66.8	42.8	68.3	71.2
C-5	44.2	42.0	36.8	36.8	36.8	36.6	35.9	36.1	78.4	77.9	77.9	73.2	83.4	78.2	86.2
C-6	26.0	26.7	25.8	25.8	26.6	26.5	26.2	26.5	30.4	36.1	71.9	36.0	212.2	30.3	211.1
C-7	26.5	27.1	26.5	26.5	27.2	27.0	26.5	26.7	28.5	29.0	36.0	28.2	32.0	28.5	75.2
C-8	35.1	35.4	35.5	35.5	34.7	34.8	34.8	34.8	35.1	35.0	30.0	34.5	37.2	34.9	38.0
C-9	40.7	40.5	41.4	41.4	39.2	41.8	42.7	40.6	45.4	45.6	45.6	45.9	45.6	45.1	41.0
C-10	35.0	34.7	35.5	35.5	35.6	36.0	37.5	37.1	45.7	45.6	40.0	45.6	45.2	45.4	50.2
C-11	20.9	20.6	21.1	21.1	31.5	37.8	38.0	31.7	21.5	21.7	21.7	21.5	22.7	21.7	22.0
C-12	40.1	40.2	40.3	40.3	79.5	212.9	212.8	79.4	40.1	39.2	39.2	39.6	39.8	40.0	39.5
C-13	40.6	40.6	40.7	40.7	46.7	55.7	55.7	46.7	40.7	40.1	40.7	40.3	41.3	40.7	40.7
C-14	56.2	56.3	56.4	56.4	55.4	56.2	56.0	55.3	56.3	56.3	56.3	55.9	56.7	56.3	49.3
C-15	31.6	31.7	31.7	31.7	31.9	31.8	31.8	31.9	32.3	32.3	32.2	31.3	33.6	32.1	31.5
C-16	80.6	81.0	81.0	81.0	81.3	79.8	79.8	81.3	81.5	81.4	81.5	80.6	81.4	81.4	81.4
C-17	62.0	62.0	62.2	62.4	63.0	54.3	54.3	63.0	63.1	63.1	63.0	61.9	62.5	63.1	62.9
C-18	16.4	16.5	16.5	16.5	11.2	16.1	16.1	11.2	16.6	16.6	16.6	16.2	16.5	16.6	16.4
C-19	22.6	23.4	23.8	23.8	24.2	23.4	23.4	24.1	13.9	13.8	13.9	12.2	18.1	13.8	13.0
C-20	42.1	42.1	42.2	41.7	43.0	42.7	43.0	43.1	41.9	41.9	41.9	42.2	42.3	41.9	42.1
C-21	14.3	14.3	14.3	14.4	14.4	13.9	14.0	14.3	15.0	15.0	15.0	14.3	15.0	15.0	15.0
C-22	109.7	109.7	109.8	109.4	109.5	109.3	109.3	109.5	109.7	109.7	109.4	109.3	111.9	109.4	109.7
C-23	25.9	25.9	25.8	31.4	31.9	31.5	31.5	32.0	29.0	29.0	29.0	25.9	41.8	29.0	29.0
C-24	25.7	25.7	26.0	28.8	29.3	29.2	29.2	29.3	33.2	33.3	33.2	25.8	70.7	33.2	33.2
C-25	27.0	27.0	27.1	30.3	30.6	30.3	30.6	30.7	144.2	144.4	144.4	27.0	39.9	144.4	144.4
C-26	65.1	65.1	65.2	66.9	66.9	67.0	67.0	66.9	65.1	65.1	65.1	65.1	65.5	65.1	65.1
C-27	16.0	16.0	16.0	17.1	17.3	17.3	17.3	17.4	108.7	108.7	108.7	16.0	13.7	108.7	108.8
			C + M	C + M	P	P INEPT	P INEPT	P	C	C	C	C + P	P	C	P

[a] **42** = Sarsasapogenone; **43** = *epi*-sarsasapogenin; **44** = markogenin; **45** = samogenin; **46** = 12β-hydroxysmilagenin; **47** = gloriogenin; **48** = mexogenin; **49** = 12β-hydroxysamogenin; **50** = ranmogenin A; **51** = ranmogenin B; **52** = ramnogenin C; **53** = ranmogenin D; **54** = anzurogenin C; **55** = $\Delta^{25(27)}$-pentrogenin.

spirostane pogosterol (**87**) has recently been characterized from *Vernonian pogosperma* (Asteraccae) in which the hydroxyl substitution at C-25 lead to its appearance at 108.1 ppm.[69]



87

2.2.4. 16-Hydroxylated cholestanes. This subgroup of steroidal sapogenins contains only a few representatives; all lack ring E but possess either ring F or are devoid of ring F. Polypodogenin (**88**) is an example of the former type whereas **89–91** are examples of the latter type. Owing to the presence of the 22,26-epoxycholestane skeleton and a hydroxyl group at C-26, both C-22 and C-26 are of the methine type and appear at 78.1 and 101.3 ppm, respectively, in polypodogenin (**88**).[63] C-22 absorbs at 75.7 in **90**[65] and at 216.3 and 213.8 ppm in alliosterol (**89**) and leontogenin (**91**), respectively.[64,66]

2.3. Tables of ^{13}C NMR shielding data

The ^{13}C NMR shielding data for the steroidal sapogenins, excluding those which we compiled earlier,[12] are arranged according to skeletal type and according to substitution pattern on the parent skeleton: spirostane [Tables 5: 5α-spirostane (**1–41**) (Table 5.1), 5β-spirostane (**42–56**) (Table 5.2), Δ^4-spirostane (**57**) and Δ^5-spirostane (**58–74**) (Table 5.3); furostane (**77–79**) (Table 6); furospirostane (**80–87**) (Table 7) and cholestane type (**88–91**) (Table 8). Trivial names are given in the footnotes. Solvents in which the chemical shifts were measured are given (C = deuterated chloroform, D = deuterated dimethyl sulphoxide, M = deuterated methanol, P = deuterated pyridine), along with pertinent references. Specific one- or two-dimensional NMR techniques, if utilized, have also been incorporated.

3. STEROIDAL SAPONINS

Most of the steroidal sapogenins exist in the glycosidic form; they are referred to as monodesmosidic when a sugar moiety or sugar chain is bonded to one position of the aglycone and bisdesmosidic when sugar moieties are bonded to two positions of the aglycone. Naturally occurring 22,26-hydroxyfurostanols exist in the bisdesmosidic form, bearing sugar at both the C-3 and

Table 5.3. ^{13}C NMR chemical shifts for Δ^4 (57), Δ^5 (58–74) unsaturated land related spirostanes[75,76] a

Substituents / Atom	25S, 3, 12-(oxo)$_2$, [4] **57**[19]	25S, 3β, 14α-(OH)$_2$ **58**[52]	25R 3β, 14α-(OH)$_2$ **59**[52]	25R, 3β, 12β-(OH)$_2$ **60**[53]	25R, 1β, 3β-(OH)$_2$ **61**[50]	25R, 3β, 25-(OH)$_2$ **62**[54]	Δ$^{25(27)}$ 1β, 3α-(OH)$_2$ **63**[50]	25R, 3β, 14α, 17α-(OH)$_3$ **64**[55]	25R, 3β, 15α, 23α-(OH)$_3$ **65**[56]	23S, 25R 3β, 14α, 23-(OH)$_3$ **66**[253]
C-1	35.3	38.1	38.1	37.7	75.6	37.1	75.3	37.3	37.9	38.0
C-2	32.9	32.7	32.7	32.6	39.4	31.6	40.2	31.6	30.2	32.6
C-3	198.6	71.3	71.3	71.3	66.9	71.3	66.5	71.6	78.1	71.3
C-4	124.7	43.5	43.5	43.5	40.0	42.3	41.0	42.3	39.1	43.5
C-5	168.5	141.6	141.6	142.1	137.9	141.5	140.0	140.2	140.1	141.5
C-6	33.7	121.6	121.6	121.2	126.3	121.5	124.5	121.6	122.5	121.6
C-7	31.1	26.3	26.3	32.3	32.4	32.9	32.9	25.8	32.9	26.7
C-8	34.4	35.7	35.7	32.1	32.0	31.8	32.3	35.5	52.2	35.6
C-9	54.6	43.7	43.7	48.4	50.4	50.6	51.2	42.8	50.2	43.7
C-10	38.7	37.4	37.4	36.8	44.0	37.0	44.5	36.8	36.9	37.4
C-11	37.1	20.5	20.5	29.3	24.0	21.2	24.3	19.6	20.9	20.5
C-12	211.9	32.1	31.1	71.5	39.9	40.1	40.7	25.7	40.7	32.3
C-13	54.8	45.1	45.1	45.1	40.2	40.6	40.7	47.3	40.7	45.7
C-14	54.8	86.5	86.4	44.5	56.6	56.9	57.0	87.7	59.9	86.6
C-15	31.1	39.9	39.9	32.4	32.0	31.9	32.3	39.2	79.0	39.8
C-16	79.1	82.0	81.9	81.0	80.9	81.6	81.3	90.5	91.2	82.5
C-17	53.3	59.8	59.9	53.9	62.4	62.4	63.1	91.0	60.7	59.6
C-18	15.9	20.1	20.1	17.3	16.3	16.4	16.6	20.8	14.7	20.3
C-19	16.9	19.5	19.5	19.5	12.3	19.5	13.2	19.3	19.4	19.4
C-20	42.7	42.6	42.1	42.3	41.7	41.4	41.8	44.6	35.7	35.8
C-21	13.0	15.2	15.4	14.9	14.5	14.3	15.0	8.1	16.8	15.0
C-22	109.7	110.2	109.6	109.3	109.5	109.5	109.4	109.9	111.4	112.0
C-23	25.8	26.7	30.7	31.9	31.4	29.9	28.9	30.8	67.3	67.6
C-24	26.0	26.6	29.4	29.4	28.8	34.6	33.2	28.1	38.6	38.9
C-25	27.0	27.6	30.0	30.7	30.3	81.6	144.5	30.0	31.6	31.7
C-26	65.2	65.1	66.9	66.9	66.6	69.2	65.0	66.9	65.9	65.9
C-27	16.0	16.4	17.3	17.3	17.1	23.8	108.6	17.0	17.8	16.9
	C	C	C	P	C	C	C		P	P
										DEPT

Table 5.3 (continued)

Substituents / Atom	25*R*, 3β, 16α, 17α-$(OH)_3$ **67**[58]	25*R*, 3β, 16α-$(OH)_2$ **68**[58]	25*R*, 1β, 3β, 24β-$(OH)_3$ **69**[59]	25*R*, 3β, 7β, 12β-$(OH)_3$ **70**[37]	25*R*, 3β, 12β-$(OH)_2$, 7-oxo **71**[37]	25*R*, 1β, 3α, 25β-$(OH)_3$ **72**[50]	25*R*, 3β, 17α, 23β, 27-$(OH)_4$ **73**[57]	25*R*, 3β-OH, 6-Me **74**[b 60]	**75**[67]	**76**[68]
C-1	37.2	37.2	78.2	36.9	36.8	75.3	37.1	36.1	26.8	41.4
C-2	31.6	31.6	43.9	31.4	32.0	40.2	32.5	32.1	28.2	80.4
C-3	71.6	71.6	68.3	71.3	70.1	66.5	71.3	71.9	65.8	72.1
C-4	42.3	42.3	43.5	41.5	42.8	40.7	43.5	32.6	43.9	38.3
C-5	140.7	141.7	140.4	143.9	166.5	139.9	142.0	134.5	83.7	90.4
C-6	121.3	121.4	124.5	125.6	125.0	124.5	121.0	126.3	203.4	70.9
C-7	31.8	32.1	32.5	72.8	201.0	32.8	32.4	38.8	63.7	37.3
C-8	31.4	31.2	33.1	39.6	44.3	32.4	32.9	32.2	37.7	30.2
C-9	49.6	50.0	51.5	47.4	49.6	51.2	50.4	51.8	50.4	50.2
C-10	36.6	36.7	43.7	36.7	38.8	44.5	37.1	38.1	45.2	45.7
C-11	20.4	20.8	24.4	30.1	31.4	24.3	21.1	22.1	20.9	21.7
C-12	31.8	39.7	40.7	79.2	77.9	40.7	32.5	40.4	39.2	40.6
C-13	45.6	42.1	40.3	46.2	46.9	41.0	45.8	41.4	42.2	41.2
C-14	51.9	55.5	57.1	54.1	49.3	57.0	51.3	57.8	55.3	56.6
C-15	38.5	38.8	32.5	33.7	34.1	32.4	31.9	32.7	31.8	32.2
C-16	114.3	116.1	81.6	81.0	81.4	81.3	90.1	82.2	80.3	81.3
C-17	88.6	70.8	62.6	61.4	61.8	63.2	90.0	63.8	61.3	63.2
C-18	16.7	15.2	16.7	10.3	11.0	16.6	17.5	16.6	16.4	16.7
C-19	19.4	19.4	14.1	19.1	17.2	13.2	19.6	19.8	18.3	17.2
C-20	44.3	40.0	42.7	42.1	43.1	42.0	40.5	41.0	40.8	42.1
C-21	9.2	14.8	15.0	14.0	14.4	15.0	9.3	15.0	14.0	15.0
C-22	110.1	110.9	111.6	109.5	109.5	109.5	112.7	110.5	108.7	109.3
C-23	31.6	31.9	36.1	30.4	31.9	27.8	68.9	32.6	30.9	31.9
C-24	28.0	28.5	66.6	28.7	29.3	33.7	32.3	29.9	27.5	29.3
C-25	29.9	30.2	35.9	30.3	30.6	65.9	38.9	31.5	29.7	30.7
C-26	68.1	68.0	64.7	66.8	66.8	69.7	63.2	67.9	66.2	67.0
C-27	17.0	17.1	9.9	17.2	17.3	26.8	64.4	17.5	16.6	17.2
	P DEPT APT	C DEPT APT	C Gated decoupling	C	C	C	C	C	P	P Gated decoupling ^1H-^1H NOE

[a] **58** = Neoprazerigenin A; **59** = prazerigenin A; **60** = heloniogenin; **61** = 3-*epi*-ruscogenin; **62** = (25*R*)-isonuatigenin; **63** = 3-*epi*-neoruscogenin; **64** = ophiogenin; **65** = scopologenin; **66** = sibiricogenin; **67** = denfigenin; **68** = 16α-hydroxydiosgenin; **69** = cepagenin; **72** = aurantigenin; **74** = 6-methyldiosgenin; **76** = anzurogenin B.
[b] 6-Me: 20.0.

C-26 hydroxyl positions and having two forms, 22-hydroxy and 22-methoxy, depending on the experimental conditions of extraction and isolation. The former can be converted into the latter on refluxing with methanol and, in contrast, the latter can be transformed back into the former on boiling with aqueous acetone or water. In most cases, such a procedure has been used to obtain the homogeneous saponin.[142,143] It has also been suggested that the 22-methoxyfurostanol saponins are secondary products formed from the corresponding 22-hydroxy form during isolation procedures; 22-methoxyfurostanols are therefore artefacts. On enzymatic hydrolysis with β-glucosidase or during partial hydrolysis, the sugar residue bound to the 26-hydroxyl position is cleaved, which leads to the transformation of the furostanol-26-*O*-glucoside to the respective cyclized spirostane saponin. Acid hydrolysis occasionally affords genuine furostane sapogenin,[144] but mostly results in the formation of spirostane aglycone. The cyclization of ring F (furostanol → spirostanol) can be inferred from the chemical shift of the ring F resonances (C-22 to C-26), as discussed above. Recently, furostanol saponins carrying an acyl substituent at C-26 in place of a sugar have also been identified,[114] and a few furostanol glycosides are reported to be tridesmosidic.[26,145] Esterified steroidal saponins have also been reported; their acyl group, in most cases, is localized in the genin part[25,146] but in some cases these may be in the carbohydrate part of the molecule.[147,148]

3.1. Identification of the oligosaccharide unit

A serious difficulty in assigning the structures of saponins is in identifying the oligosaccharide unit owing to the diversity of monosaccharide residues and the type of substitution. It requires the identification of the number and structure of individual monosaccharide units, the anomeric configurations, interglycosidic linkages, sequence and the site of attachment of the oligosaccharide moiety to the sapogenin residue. All of this information can be deduced from NMR spectroscopic data without degradation.[122,149–153] The anomeric carbon resonances of the sugar occur largely in a well defined region (δ 92–108)[154,155] and are usually distinct from the resonances of the sapogenin moiety. However,

Table 6. ^{13}C NMR chemical shifts for furostanes

Substituents	1β, 3α, 26-(OH)$_3$, 5α	1β, 3α-(OH)$_2$, 5	1β, 3β-(OH)$_2$, 5
Atom	**77**[61]	**78**[61]	**79**[26]
C-1	74.0	77.9	78.2
C-2	38.8	35.9	44.0
C-3	66.7	68.0	68.2
C-4	35.8	42.0	43.7
C-5	39.2	138.1	140.4
C-6	28.1	125.5	124.4
C-7	32.3	32.4	32.4
C-8	35.6	32.5	33.3
C-9	54.6	50.3	51.5
C-10	42.5	42.8	43.6
C-11	24.3	23.6	24.1
C-12	40.0	39.7	40.4
C-13	40.4	40.2	40.7
C-14	56.7	56.9	57.4
C-15	32.1	31.9	33.0
C-16	83.1	83.0	83.4
C-17	65.3	65.3	65.9
C-18	16.5	16.5	16.9
C-19	5.6	13.0	14.0
C-20	37.9	37.9	38.4
C-21	19.0	19.1	19.3
C-22	90.6	90.5	90.5
C-23	29.7	29.7	31.9
C-24	30.0	31.4	36.5
C-25	36.0	28.3	28.4
C-26	67.7	22.5	22.8
C-27	16.8	22.6	22.9
	C	C	P
			APT, COSY HETCOR

spectral analysis of this region may not always be straightforward since a dioxy-substituted carbon such as C-26 of the 26-methoxyspirost-5-en-3β-ol (**92**), which is also of the methine type, resonates at δ 103.1.[28,156] In order to achieve differentiation between the hydroxylated methine belonging to sapogenin and sugar residues, convincing evidence for the ^1H assignments can be secured from COSY and HOHAHA spectra.[149–152,157]

As mentioned already, the C-22 resonances of the spirostanes and furostanes in most cases (Table 3) absorb in the characteristic chemical shift range (δ 108–113.5) and the quaternary nature of C-22 leads to its identification (i.e. no cross peak in the HETCOR/HMQC spectrum), since anomeric carbon resonances of aldopyranoses of the methine type show correlation with the ^1H NMR anomeric resonances in the region δ 4.2–6.4. The ^1H NMR spectrum is equally important for determining the number of monosaccharide residues as anomeric proton resonances usually appear either as broad singlets or doublets ($^3J_{HH}$ = 7–8 or 2–3 Hz) in the region δ 4.2–6.4. The broad singlet and doublets with a small coupling constant ($^3J_{1,2}$ = 1–3 Hz) are usually due to an α-aomer, whereas doublets with large coupling constants ($^3J_{1,2}$ = 7–8 Hz) are due to β-anomers of the pyranose sugars with *gluco* and *galacto* configurations.[149–151] However, both α- and β-anomeric forms of monosaccharides with a *manno* configuration possess $^3J_{1,2}$ = 1–3 Hz. These methods become less significant owing to the absence of the anomeric-H and the quaternary nature of the anomeric-C in the case of ketosidic sugars.[158]

The majority of the proton resonances derived from the bulk of the nonanomeric sugar methine and methylene protons appear in a very small spectral window (δ 3.2–4.5). The subsequent overlap of these with the oxy-substituted methine and methylene resonances of sapogenin make it difficult to assign these resonances to specific monosaccharides and to sapogenin residues. In order to minimize signal overlap in the ^1H NMR spectrum, it is desirable to exchange hydroxyl protons with deuterium[150–152,159] prior to recording the ^1H and homonuclear spectral data in any deuteriated solvent.

The determination of structure of individual monosaccharide residues and interglycosidic linkages requires initially unambiguous assignments of the ^1H and ^{13}C resonances. The first task for the identification of the sugar component is to perform a through-bond connectivity analysis in order to determine the number of different spin systems corresponding to individual sugar residues by the concerted use of two-dimensional NMR techniques involving ^1H–^1H COSY, triple-quantum filtered (TQF) and geminal (GEM) COSY spectra. However, as the number of monosaccharide residues increases, the ^1H NMR spectrum becomes severely overlapped and it becomes difficult to delineate all the protons of each individual sugar by the above variants of COSY techniques. In such instances, the DQF-COSY spectrum maps the vicinal coupling constants [$^3J(H_{ax},H_{ax})$ = 8 Hz; $^3J(H_{ax},H_{eq}) = {}^3J(H_{eq},H_{eq})$ = 3 Hz], thus delineating the spin systems and relative configuration of the carbon atom for each sugar residue beginning from the anomeric proton.[149–152,160] The spin system corresponding to individual sugar residues provides information about the type of sugar residues.

The HOHAHA and multi-step RCT experiments provide valuable information for the assignments, as proton resonances belonging to the same monosaccharide residue are observed clearly and undisturbed and no signal of the other sugar/sapogenin residue appears. However, it is important to mention that the propagation of the magnetization through the coupling network depends on the duration of the mixing time and one must use a long mixing time to be sure that all correlation signals of the corresponding residue are detected.[161] To identify the spin system of an individual monosaccharide, selective TOCSY and 1D HOHAHA experiments[162] with increasing mixing times are worthwhile as subspectra of a carbohydrate moiety can be extracted from crowded overlapping regions. The only prerequisite is that at least one of the proton signals should be sufficiently separated from all the other resonances. In saponins of medium complexity, this is often valid for anomeric protons. From all these experiments, chemical shifts, coupling constants, configurations and the nature of the sugar moieties can be derived.

The anomeric configuration particularly for pyranose sugars, can be inferred from the $^3J_{1,2}$ coupling constants,[163] NOE correlations,[150,151] one-bond ^{13}C–^1H coupling constants ($^1J_{CH}$)[154,164,165] and long-range $^1H\{^{13}C\}$MBC correlations.[163] Once the proton reso-

Table 7. ^{13}C NMR chemical shifts for furospirostanes[a]

Substituents / Atom	25*R*, 3β, 26-$(OAc)_2$, 5α **80**[42]	25*S*, 3β-OAc, 26-OH, 5α **81**[42]	22*R*, 25*R*, 1β, 3α, 26-$(OH)_3$, 5α **82**[46]	22*S*, 25*R*, 1β, 3α, 26-$(OH)_3$, 5α **83**[46]	25*S*, 1β, 3α, 26-$(OH)_3$, 5α **84**[46]	1β, 3α, 26-$(OH)_3$, 5 **85**[50]	3β, 26, 27 $(OH)_3$, 5 **86**[62]	**87**[69]
C-1	36.6	36.9	74.0	74.0	73.9	75.2	36.8	36.8
C-2	27.4	28.1	40.4	40.3	40.4	40.3	31.2	31.0
C-3	73.6	73.8	66.7	66.7	66.2	66.4	70.3	70.5
C-4	33.3	33.5	35.8	35.8	36.1	40.7	41.9	37.8
C-5	44.5	44.6	38.2	38.2	37.4	139.9	140.8	46.6
C-6	28.4	28.1	28.1	28.1	28.3	124.5	120.2	45.8
C-7	31.7	32.1	32.2	32.1	32.0	32.8	31.4	210.9
C-8	35.0	34.9	35.4	35.5	35.6	32.3	30.8	49.3
C-9	51.1	54.2	54.7	54.6	55.0	51.2	49.4	55.0
C-10	35.5	35.8	42.5	42.5	42.6	44.5	36.0	36.1
C-11	21.4	21.4	24.4	24.4	24.4	24.3	20.2	21.6
C-12	38.2	38.4	39.8	39.8	40.4	40.7	39.1	38.6
C-13	39.9	40.1	40.7	40.1	40.3	41.0	39.8	40.7
C-14	56.1	56.2	55.6	56.2	56.3	56.9	55.5	48.5
C-15	32.1	32.4	29.7	31.9	30.7	32.3	31.1	32.2
C-16	80.6	80.6	79.2	80.6	81.1	81.0	80.5	81.7
C-17	61.9	61.9	62.1	62.0	62.1	62.7	61.2	60.2
C-18	16.3	16.4	16.6	16.3	16.2	16.5	18.8	16.3
C-19	12.2	12.3	5.6	5.6	5.9	13.2	15.5	11.9
C-20	40.6	40.6	37.5	38.5	38.3	38.4	37.4	38.6
C-21	14.6	14.8	16.7	14.8	14.7	15.0	14.2	18.5
C-22	120.0	119.9	120.4	120.1	120.2	120.2	119.6	115.3
C-23	33.3	32.5	30.0	32.8	32.2	32.5	36.8	43.5
C-24	33.9	34.1	32.8	34.1	33.8	33.7	32.9	81.3
C-25	81.9	83.9	84.7	84.1	85.8	85.5	87.2	108.1
C-26	69.6	69.6	68.0	69.5	68.9	70.0	65.8	23.9
C-27	25.9	25.0	24.0	25.3	23.9	24.1	64.9	—
	C	C	C	C	C	C	C	C LR-HETCOR

[a] **82** = Wallogenin; **83** = rubragenin; **84** = strictagenin; **85** = tupisgenin; **86** = taccagenin; **87** = pogosterol.
[b] 31.3, 15.8, 14.6.

nances of each sugar have been assigned, assignment of the carbon resonances can be accomplished by tracing multiple-bond ^1H–^{13}C connectivities observed in the $^1H\{^{13}C\}$HMBC spectrum. Comparison of the carbon assignments ascertained in such a way with those of reference methyl glycosides[12,151,154] can further verify the identification of monosaccharides and the attachment positions in the sugar chains through the consideration of glycosidation-induced shifts.[12,16,132,153,154,166] This relies on the fact that glycosidation modifies the ^{13}C NMR chemical shifts for internal sugar moieties, but not for the terminal sugar residues, as these exhibit a remarkable resemblance with the respective methyl glycoside.

Comparison of the T_1 relaxation times of the anomeric carbons also identifies the terminal and interior sugars since the correlation times decrease as the freedom of movement increases, i.e. as the sugars become increasingly distant from the aglycone. As a consequence, the T_1 values increase. Sometimes it is desirable to measure T_1 relaxation times at different temperatures for saponins differing in the number of sugar residues. This is simply because saponin solutions become more viscous as the number of sugars increases, even if the overall concentration of sugars remains unchanged, and the ^{13}C T_1 times of all sugar units are similar and thus information from T_1 measurements is limited if not completely useless.[23]

Final confirmation about the sequence of the oligosaccharide chain can be obtained by measuring dipolar interactions of neighbouring anomeric protons and the linkage site protons of different residues by detecting inter-residual NOE or ROE cross peaks, since nuclear Overhauser enhancements transversing the glycosidic linkages are invariably observed.[23,24,121,167,168] The advantages of ROESY over NOESY have already been mentioned but in several cases, NOEs between each anomeric proton signal and the crucial methine or methylene proton signal of other substituted saccharide or the aglycone residue are severely overlapped. Such studies then become ambiguous and under such situations ^1H–^{13}C long-range coupling traversing the glycosidic bond can be employed to resolve ambiguities of this type. The observation of scalar coupling between a carbon and a proton of the neighbouring residues by means of HMBC experiments can also be employed for the establishment of glycosidic connectivity and for achieving sequence information.[27,30,31,127] In several cases, the ^{13}C resonance assignments could be aided considerably by having simpler saponins or prosapogenins having fewer monosaccharide residues for spectral comparison.[12,29,43,173,174]

Table 8. ^{13}C NMR chemical shifts for cholestane-type steroidal sapogenins[a]

Substituents	*22R, 25S, 26R* $3\beta, 26\beta$-$(OH)_2$, 6-oxo, Δ^7, 5α	*25R,* $3\beta, 16\alpha$-$(OAc)_2$, 17α, 26-$(OH)_2$, Δ^5	$1\beta, 3\beta, 16\beta$, 22β-$(OH)_4$, Δ^5	3β, 26-$(OH)_2$, 6, 22-$(oxo)_2$, 5α
Atom	**88**[63]	**89**[64]	**90**[65]	**91**[66]
C-1	36.8	37.0	78.3	39.6
C-2	30.4	27.9	44.0	31.3
C-3	70.7	74.0	68.3	70.0
C-4	30.2	38.2	43.6	31.8
C-5	53.3	140.0	140.5	52.5
C-6	199.6	122.3	124.6	210.1
C-7	123.1	32.0	32.3	46.8
C-8	163.3	31.9	33.4	37.9
C-9	50.0	49.6	51.6	53.9
C-10	38.2	36.7	43.6	41.0
C-11	21.8	20.4	24.3	21.7
C-12	38.8	31.7	41.3	27.7
C-13	44.7	48.3	42.5	43.2
C-14	55.2	49.0	55.1	57.0
C-15	22.6	33.4	32.1	24.3
C-16	26.9	78.5	71.6	39.9
C-17	52.8	82.6	58.5	56.1
C-18	12.2	14.5	13.8	13.2
C-19	13.2	19.3	14.0	16.7
C-20	40.0	48.6	36.2	49.4
C-21	13.7	12.7	15.3	12.3
C-22	78.1	216.3	75.7	213.8
C-23	23.9	40.9	36.8	37.1
C-24	31.0	26.2	37.5	27.8
C-25	37.8	35.1	28.5	36.2
C-26	101.3	67.6	22.8	67.4
C-27	16.7	16.5	23.0	17.2
	C	C	P	—

[a] **88** = Polypodogenin; **89** = alliosterol; **91** = leontogenin.

3.2. Identification of the sugar–aglycone linkage

Once the structure of the sugar chain is evident, the next question is to establish the sugar–aglycone linkage, i.e. at which position(s) of the sapogenin residue the sugar residue(s) are linked. The effect of *O*-glycosylation on the ^1H NMR spectra of saponins has been studied much less than that on ^{13}C spectra, probably because fully resolved ^1H spectra of sugars have only recently become available. Characteristic downfield (3–10 ppm) and upfield shifts (1–5 ppm) of the carbon resonances of carbon atoms in the adjacent positions have been found to be the consequence of glycosidic bond formation.

The interresidual NOE between the anomeric H of the sugar and the sapogenin observed in either NOESY, ROESY or one-dimensional NOE difference experiments identifies the sugar which is directly glycosylated to the aglycone.[24,80,169,170–172] The anomeric H of the sugar directly bonded to the aglycone moiety exhibits a $^1H\{^{13}C\}$HMBC cross peak to the aglycone carbon or vice versa, i.e. the anomeric H of that particular monosaccharide which is directly glycosidically linked to the sapogenin residue exhibits its long-range connectivity to aglycone-C, thereby identifying the sugar–aglycone linkage and sequence.[30,170,172]

Table 9. Glycosidation-induced shifts in steroidal saponins

Site	C-α	C-β		C-γ
1β-OH[a]	+(5.7–6.4)	−(5.1–7.8)	−(0.6–0.7)	−(0.9–1.1)
	C-1	C-2	C-10	C-19
1β-OH[b]	+12.3	+0.5	+0.5	−0.2
	C-1	C-2	C-10	C-19
2α-OH	+(11.1–11.4)	−(1.8–2.7)	−(1.4–2.3)	
	C-2	C-1	C-3	
3β-OH	+(6.3–7.9)	−(0.8–2.4)	−(2.9–4.8)	
	C-3	C-2	C-4	
5β-OH[c]	+(9.4–9.7)	−0.7	−5.4, −1.6	
	C-5	C-4	C-6, C-10	
6α-OH	+(11.1–12.1)	−(0.5–1.6)	−(1.4–2.3)	
	C-6	C-5	C-7	
12β-OH	+11.1	−(1.7–1.8)	−(0.3–0.4)	
	C-12	C-11	C-13	
24-OH	+(8.8–10.9)	−(0.9–2.4)	−(0.8–1.7)	
	C-24	C-25	C-23	
27-OH	+7.9	−2.6		−(0.2–0.7)
	C-26	C-25		C-24, C-27

[a] In 3β-hydroxylated Δ^5 series.
[b] In 2β, 3α-dihydroxylated 5β series.
[c] In 1β, 2β, 3β, 4β-tetrahydroxylated 5β series.

Table 10.1. ^{13}C NMR chemical shifts for the steroidal part of spirostane-type saponins[a]

Atom	**92**[28,156]	**93**[129]	**94**[129]	**95**[29]	**96**[22]	**97**[138]
C-1	37.5	37.4	37.2	35.7	39.2	84.1
C-2	30.2	30.0	29.9	30.1	70.8	37.2
C-3	78.2	76.8	76.9	100.8	83.0	68.1
C-4	39.0	34.4	34.4	28.9	38.6	42.5
C-5	140.9	44.6	44.6	48.9	73.0	139.4
C-6	121.7	29.2	28.9	79.8	37.6	128.8
C-7	32.2	32.8	32.4	41.2	117.5	29.7
C-8	31.8	35.9	35.0	34.1	135.6	31.7
C-9	50.4	54.6	54.3	53.6	142.6	47.5
C-10	37.2	36.1	35.8	36.8	43.0	42.1
C-11	21.1	21.3	21.2	21.2	121.2	25.2
C-12	39.8	40.9	40.1	40.1	42.4	28.0
C-13	40.5	41.4	41.4	40.8	40.7	176.0
C-14	56.7	60.7	56.3	56.4	51.7	138.7
C-15	31.4	79.0	32.4	32.0	31.6	204.3
C-16	81.4	92.0	82.1	81.0	81.4	81.7
C-17	62.9	60.3	62.9	63.0	62.3	85.9
C-18	16.3	18.1	16.7	16.7	16.0	—
C-19	19.4	12.5	12.5	12.9	26.3	13.6
C-20	42.0	37.3	37.2	42.0	42.6	48.6
C-21	15.0	15.6	15.6	15.0	14.6	61.5
C-22	111.8	109.9	110.0	109.1	109.3	111.6
C-23	32.4	77.7	77.2	31.8	31.8	71.7
C-24	28.4	31.1	31.1	29.3	29.2	67.7
C-25	35.5	34.1	34.1	30.6	30.6	34.1
C-26	103.1	180.6	180.7	66.9	67.0	63.8
C-27	16.7	16.3	16.3	17.4	17.3	12.1
OMe	55.6	—	—	47.5	—	—
	—	—	—	47.4	—	—
	P	P	P	P	P	C
	DEPT	COSY HETCOR 1D-NOE		DEPT	DEPT COSY	

[a] **93** = Soladulcoside B; **94** = soladulcoside A.

Table 10.2. ^{13}C NMR chemical shifts for the steroidal part of furostane-type saponins[a]

Atom	**98**[105]	**99**[31]	**100**[36]	**101**[43]	**102**[114]	**103**[114]	**104**[111]	**105**[36]	**106**[112]	**107**[112]	**108**[112]
C-1	37.2	36.7	38.9	47.1	37.3	37.6	37.8	38.1	73.4	78.3	75.0
C-2	29.9	29.5	30.0	70.4	29.9	30.0	32.2	30.0	34.0	68.1	73.2
C-3	77.6	76.8	77.9	85.0	77.0	77.0	70.7	77.9	71.5	76.0	73.9
C-4	34.8	27.0	32.8	31.7	34.4	34.5	33.3	32.8	68.0	67.6	67.2
C-5	44.7	56.4	47.9	47.8	44.6	44.6	51.4	47.9	87.8	87.3	78.2
C-6	29.0	209.7	70.8	69.9	29.0	29.1	79.6	70.8	24.9	24.9	30.3
C-7	32.4	46.8	40.5	40.7	32.5	27.1	41.3	40.5	28.3	28.1	27.9
C-8	35.3	37.3	30.6	29.8	35.9	31.8	34.1	30.6	34.8	34.6	34.2
C-9	54.5	53.7	54.6	54.5	54.3	46.6	54.0	54.6	46.8	46.2	45.2
C-10	35.8	40.8	36.1	36.9	35.8	36.1	36.7	36.1	47.1	46.5	45.2
C-11	21.3	21.5	21.2	21.3	21.1	20.4	20.2	21.2	21.6	21.7	21.2
C-12	40.2	39.6	40.8	40.1	32.2	27.0	40.0	40.8	40.1	39.9	40.0
C-13	41.1	41.4	41.2	41.1	45.7	48.9	41.1	41.2	40.8	40.8	40.3
C-14	56.4	56.3	56.2	56.0	52.7	88.6	56.3	56.2	56.0	55.8	55.4
C-15	32.4	32.0	32.2	32.4	32.5	40.2	32.0	32.2	32.4	32.2	32.2
C-16	81.1	80.8	81.3	81.0	90.3	90.9	81.3	81.3	81.0	81.3	81.2
C-17	64.0	63.8	64.3	63.8	90.4	91.3	64.2	64.3	64.2	64.2	63.9
C-18	16.7	16.5	16.5	17.1	17.3	21.0	16.2	16.5	16.3	16.3	16.3
C-19	12.3	13.1	16.3	16.3	12.4	12.3	13.6	16.3	13.6	13.6	13.5
C-20	40.7	40.5	40.1	40.5	43.0	43.6	40.5	40.1	40.4	40.6	40.7
C-21	16.4	16.4	16.0	16.6	10.4	10.6	16.6	16.0	16.5	16.5	16.1
C-22	110.6	110.6	112.6	110.6	113.2	113.0	112.6	112.6	112.3	112.6	112.5
C-23	37.2	37.1	30.7	36.9	30.5	30.9	30.7	30.7	31.0	30.7	30.8
C-24	28.4	28.3	28.2	28.3	27.9	28.0	28.3	28.2	28.3	28.2	28.8
C-25	34.3	34.2	34.2	34.1	33.2	33.3	34.2	34.2	34.4	34.3	34.2
C-26	75.3	75.2	75.3	75.2	69.2	69.3	75.2	75.3	75.0	74.9	75.0
C-27	17.5	17.4	17.2	17.4	16.7	16.9	17.2	17.2	17.4	17.3	17.3
OMe	—	—	47.3	—	47.0	47.2	47.3	47.3	47.3	47.4	47.3
	P	P DEPT	P	P	P DEPT	P DEPT	P	P	P	P	P

[a] **101** = Ampeloside-Bf_2; **102** = pardarinoside B; **103** = pardarinoside A.

Table 10.3. ^{13}C NMR chemical shifts for the steroidal part of Δ^5-furostane-type saponins[a]

Atom	**109**[142]	**110**[127]	**111**[141]	**112**[253]	**113**[145]	**114**[145]	**115**[142]	**116**[252]	**117**[185]	**118**[235]	**119**[249]
C-1	37.0	37.5	77.8	83.8	83.9	83.8	37.0	37.9	37.5	37.6	37.7
C-2	30.0	30.1	41.1	35.5	38.1	35.9	30.0	30.4	30.3	29.9	30.3
C-3	77.8	77.8	74.9	68.4	68.2	73.6	77.8	78.8	78.2	77.0	78.2
C-4	39.1	38.7	39.8	44.0	43.8	39.8	39.1	40.2	39.3	39.0	39.1
C-5	140.8	140.8	139.3	139.7	139.7	138.5	140.9	140.5	141.0	140.9	141.0
C-6	121.5	121.8	124.9	124.8	124.9	125.7	121.4	122.4	121.7	121.8	121.8
C-7	31.8	32.2	32.8	33.3	32.1	32.1	31.8	26.8	32.4	31.7	32.6
C-8	30.9	31.7	32.2	32.2	33.1	33.1	30.9	35.7	32.3	32.3	31.6
C-9	52.4	50.3	51.2	50.6	50.6	50.7	52.3	43.7	50.3	50.3	50.6
C-10	37.6	37.2	43.6	43.1	43.0	43.0	37.6	37.6	37.1	37.1	37.3
C-11	37.6	21.1	24.1	24.2	23.9	23.9	37.5	20.2	21.0	20.9	21.3
C-12	212.8	39.8	40.8	40.4	40.6	40.6	212.6	37.2	32.1	37.1	39.9
C-13	55.3	40.8	40.5	40.7	40.1	40.4	55.4	45.6	45.1	45.4	43.6
C-14	55.9	56.6	56.8	56.9	57.0	57.0	56.0	86.9	53.0	53.0	55.2
C-15	31.8	32.3	32.2	32.6	32.8	32.5	31.5	39.5	31.9	32.4	34.6
C-16	79.7	81.3	81.5	81.5	81.3	81.5	80.0	82.3	90.5	90.3	84.6
C-17	54.8	64.2	62.8	64.4	64.0	64.5	55.3	61.3	90.7	90.5	64.7
C-18	16.0	16.3	16.5	16.8	17.0	16.8	15.9	21.1	17.3	17.1	14.3
C-19	18.8	19.4	13.7	15.2	14.9	14.6	18.8	19.5	19.4	19.4	19.5
C-20	41.3	40.7	42.1	40.7	40.8	40.6	41.1	40.6	43.6	43.0	103.6
C-21	15.2	16.5	14.8	17.7	16.4	16.2	14.9	16.8	10.5	10.3	11.8
C-22	110.8	112.7	111.6	112.9	110.8	112.8	112.8	113.5	111.4	113.5	152.6
C-23	37.1	30.8	32.2	31.2	37.2	31.1	30.7	31.2	36.9	30.8	33.6
C-24	28.4	28.2	28.2	28.3	28.4	28.3	28.2	28.4	28.0	28.1	23.8
C-25	34.3	34.2	34.5	34.6	34.5	34.6	34.3	34.7	34.3	34.2	31.6
C-26	75.2	75.2	76.2	75.1	75.4	75.0	75.2	75.2	75.1	75.1	75.0
C-27	17.4	17.2	17.6	17.7	17.5	17.5	17.1	17.7	17.5	17.4	17.4
OMe	—	47.3	—	47.5	—	47.4	47.4	47.5	—	47.1	—
	P INEPT	P	P	P	P	P	P INEPT	P DEPT	P DEPT INEPT	P	P

[a] **109** = Kingianoside C; **112** = alliofuroside A; **113** = nolinofuroside D; **114** = nolinofuroside F; **117** = pratioside B.

Table 10.4. ^{13}C NMR chemical shifts for the steroidal part of cholestane-type and miscellaneous saponins[a]

Atom	120[172]	121[172]	122[172]	123[111]	124[111]	125[238]	126[53]
C-1	35.8	37.1	37.1	37.2	37.6	37.2	37.1
C-2	34.4	32.1	30.3	30.3	32.7	30.1	30.0
C-3	198.4	71.3	78.2	78.6	71.2	77.8	77.8
C-4	121.1	43.5	39.4	38.9	43.0	38.6	38.8
C-5	170.7	142.0	140.9	141.6	142.6	140.7	140.9
C-6	32.9	121.3	121.9	128.6	128.0	128.4	121.3
C-7	32.2	32.7	32.1	72.6	72.7	31.9	31.7
C-8	35.2	31.9	31.8	40.5	40.6	31.0	30.8
C-9	53.9	50.6	50.5	48.0	49.0	50.0	49.9
C-10	38.7	36.9	37.0	36.9	36.9	37.1	37.0
C-11	21.0	21.2	21.2	21.3	21.4	20.7	20.9
C-12	39.8	40.1	40.0	39.9	40.0	40.4	38.7
C-13	42.5	42.5	42.5	43.1	43.2	41.7	43.4
C-14	54.4	55.3	55.3	54.6	54.7	51.1	50.5
C-15	36.8	37.8	37.5	38.9	38.9	38.9	36.0
C-16	82.4	82.5	82.5	82.9	82.9	217.7	210.4
C-17	57.9	58.0	57.9	60.9	61.0	66.4	142.5
C-18	12.7	12.8	12.8	13.3	13.3	15.6	15.7
C-19	17.1	19.7	19.5	19.0	19.2	19.4	19.3
C-20	35.9	36.0	35.9	27.5	27.5	43.7	145.6
C-21	13.5	13.5	13.4	19.6	19.6	12.8	16.7
C-22	72.1	72.1	72.1	50.4	50.4	213.3	205.6
C-23	33.4	33.4	33.4	211.6	211.6	37.4	37.9
C-24	36.9	36.8	36.8	52.4	52.4	27.7	27.9
C-25	28.7	28.8	28.8	24.5	24.5	36.1	33.3
C-26	23.1	23.1	23.1	22.8	22.8	67.4	75.0
C-27	23.0	23.1	23.1	22.8	22.8	17.3	17.4
	P	P	P	P DEPT	P DEPT	P	P

[a] **120** = Schubertoside A; **121** = schubertoside B; **122** = schubertoside C; **123** = camassioside; **125** = kryptogenin-3-*O*-β-chacotrioside; **126** = osladin.

Since the publication of our last review, ^{13}C NMR spectral data have been reported for a large number of steroidal saponins which could be classified according to skeletal type: (a) spirostane;[7,19,22,23,27–31,36,38,40,43,44,49,51,53,59,62,63,67,71,111,112,114,116,120,127,129,130,134,139,141,142,146–148,167–169,173–233] (b) furostane;[31,36,43,53,63,68,71,111,112,114,127,141,142,145,148,171–173,185,189,194,227,234–253] (c) furospirostane;[167,173] (d) cholestane;[26,111,172,206,238,254–256] and (e) miscellaneous glycosides.[257,258] However, we prefer not to tabulate ^{13}C NMR chemical shift as the glycosylation causes recognizable alterations in the ^{13}C NMR chemical shifts, especially for those carbon atoms involved in the glycosidic linkage and those in adjacent positions. Because of this, we prefer to present changes associated with glycosidation in the ^{13}C NMR spectrum (Table 9) at various positions as a ready reference for their utilization in determining the linkage site. The effect of glycosylation is the difference between the chemical shift of a given carbon atom in a steroidal saponin and that in a free sapogenin ($\delta_{saponin} - \delta_{sapogenin}$). The spectra of the glycosides are usually obtained by dissolving them in pyridine, therefore in those cases where the spectrum of the aglycone was taken in chloroform, these values need to be treated with caution because of the influence of the solvent on the chemical shift.

However, in several instances, the genuine aglycone could not be isolated, e.g. in the case of spirostanol (**92–96**) and most of the furostanol sapogenins (**97–118**). For such cases, ^{13}C NMR shielding data reported for the sapogenin part are given, spirostane (**92–96**) (Table 10.1); 5α-furostane (**97–104**) and 5β-furostane (**105–107**) (Table 10.2); Δ^5-furostane (**108–118**) (Table 10.3) and cholestane type (Table 10.4). C-26 is mostly glycosylated in furostanol saponins, but in a few cases it is acylated. The characteristic feature is the appearance of C-26 methylene resonance at around δ 75 in the case of the former but at around δ69 in the case of the latter. Structures are shown in Figs. 3–5.

3.3. Three-dimensional NMR spectroscopy

Complete assignments can usually be obtained with conventional two-dimensional methods, but severe ^1H NMR signal overlap can be removed by extending the experiments to three dimensions, i.e. three-dimensional (3D) NMR spectroscopy,[259] which gives rise to better resolution. 3D NMR spectroscopy has also been applied to structural problems with oligosaccharides.[260–264] In such a spectrum, there are three combinations possible to take 2D slices. In a 3D TOCSY–ROESY experiment, each of these slices taken at a constant frequency of an anomeric or a linkage site proton contains information about the sequence and position of glycosidation.[161,263] The measurement time for 3D spectra is necessarily longer than that for 2D spectra, and can require up to several weeks of instrument time. However, the large amount of information present in such spectra may make this approach the method of choice for problems too complicated to be handled by conventional 2D NMR experiments. Thus, 3D NMR techniques are advantageous if experiment time, storage capacity and sensitivity are not limiting factors.

Shaped pulses have been used to improve selectivity and have allowed the development of 1D equivalents of the 2D experiments;[265] the 2D equivalents of 3D experiments offer further spectral simplification. The application of selective pulses to 3D NMR techniques leads to a 2D method which shortens the experiment time enormously but retains the dispersion of a third frequency axis. The full sequence information can be gained in few minutes by two semi-soft 2D TOCSY–ROESY experiments.[263,266]

4. CONCLUSION

The structural elucidation of a steroidal sapogenin based on the appearance of characteristic IR absorption bands,[267,268] the fragmentation pattern observed in the mass spectrum[269,270] and by the analysis of the one-dimensional ^1H NMR spectrum[17,271] may not always be straightforward and may lead to several alternative structures. The ^1H NMR spectral region between 1.0 and 2.5 ppm is generally extensively crowded, whereas oxymethine and oxymethylene resonances of steroidal

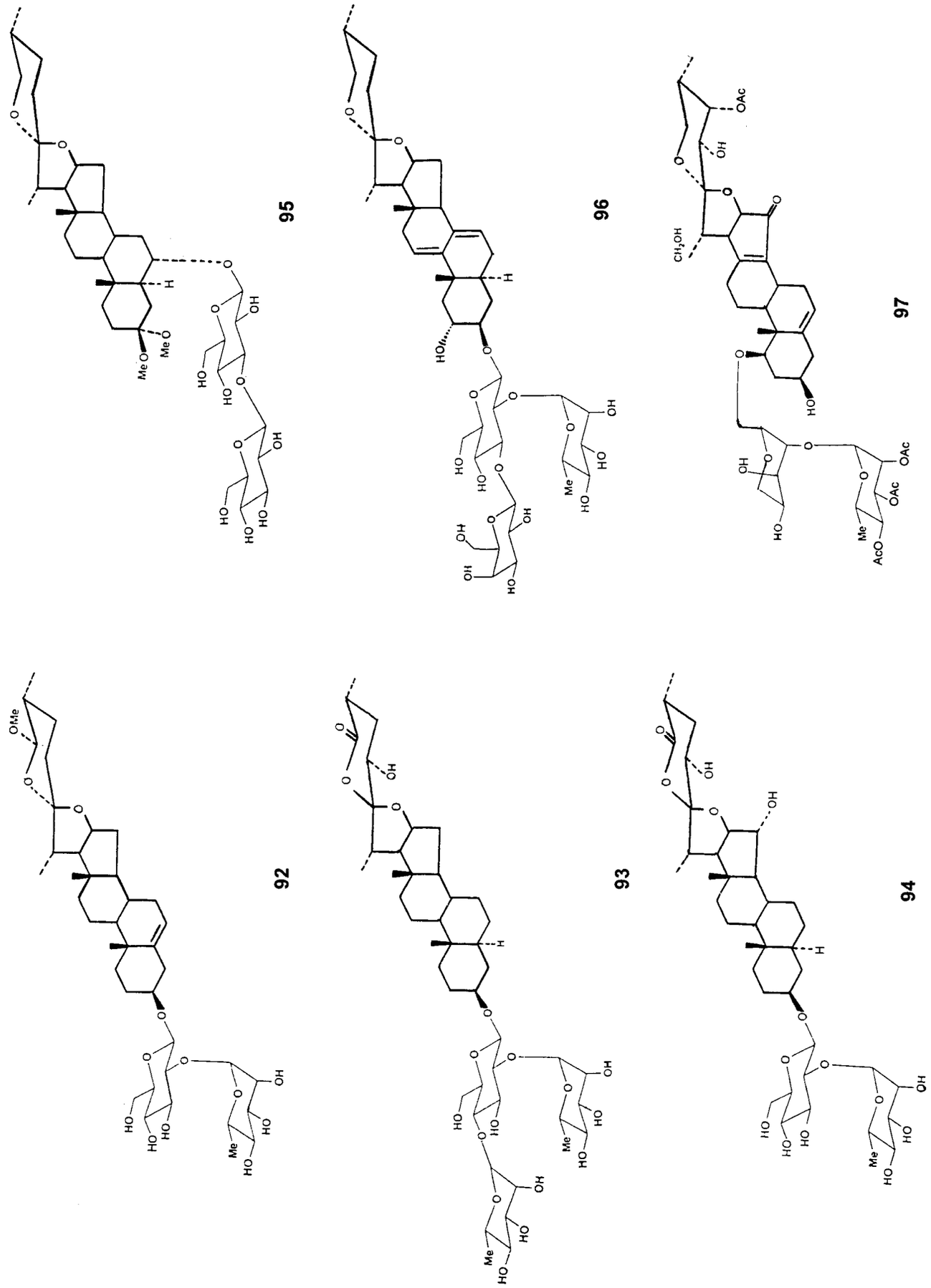


Figure 3. Structures of steroidal saponins having a spirostane skeleton.

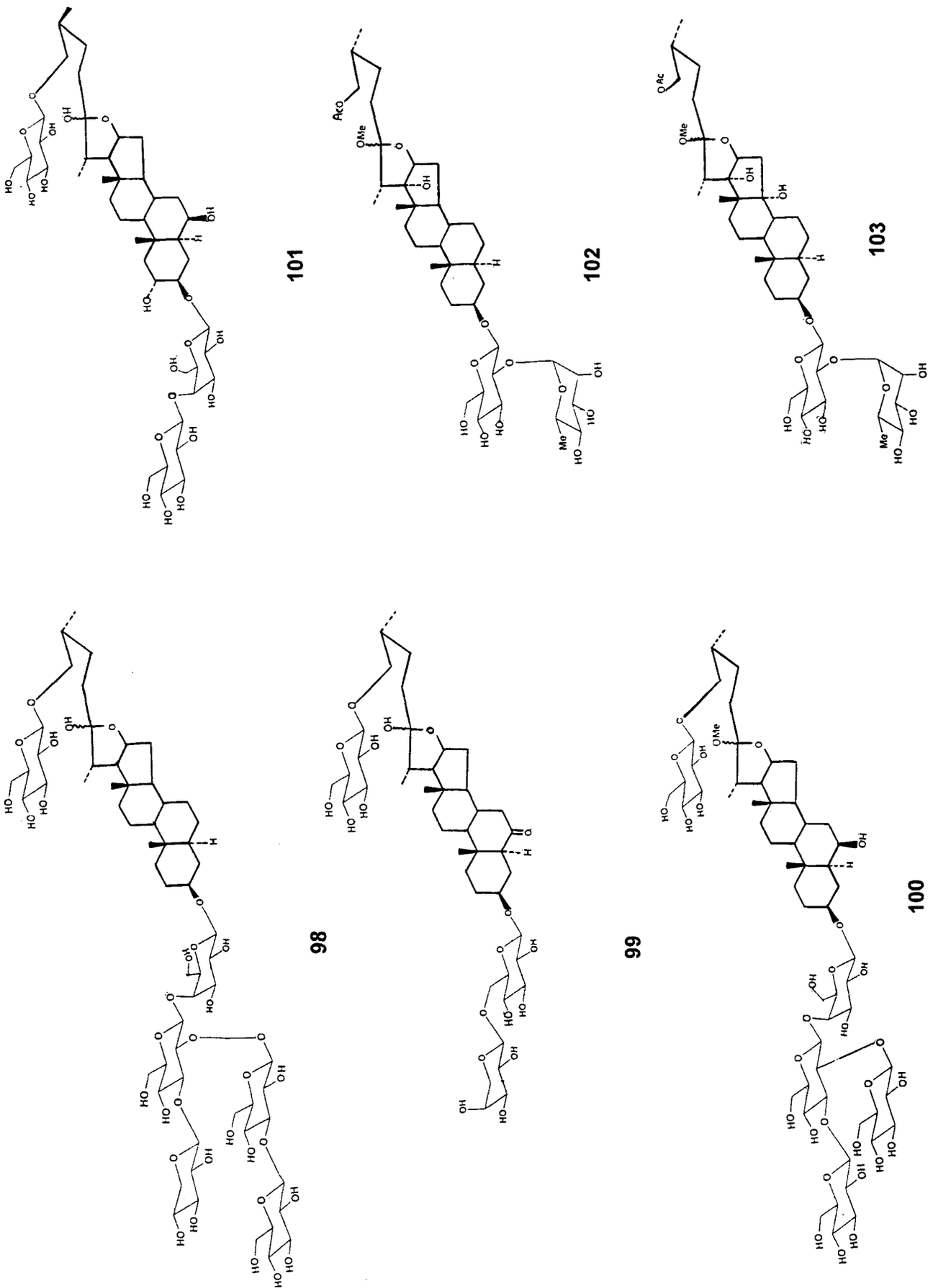


Figure 4. Structures of steroidal saponins having a furostane skeleton.

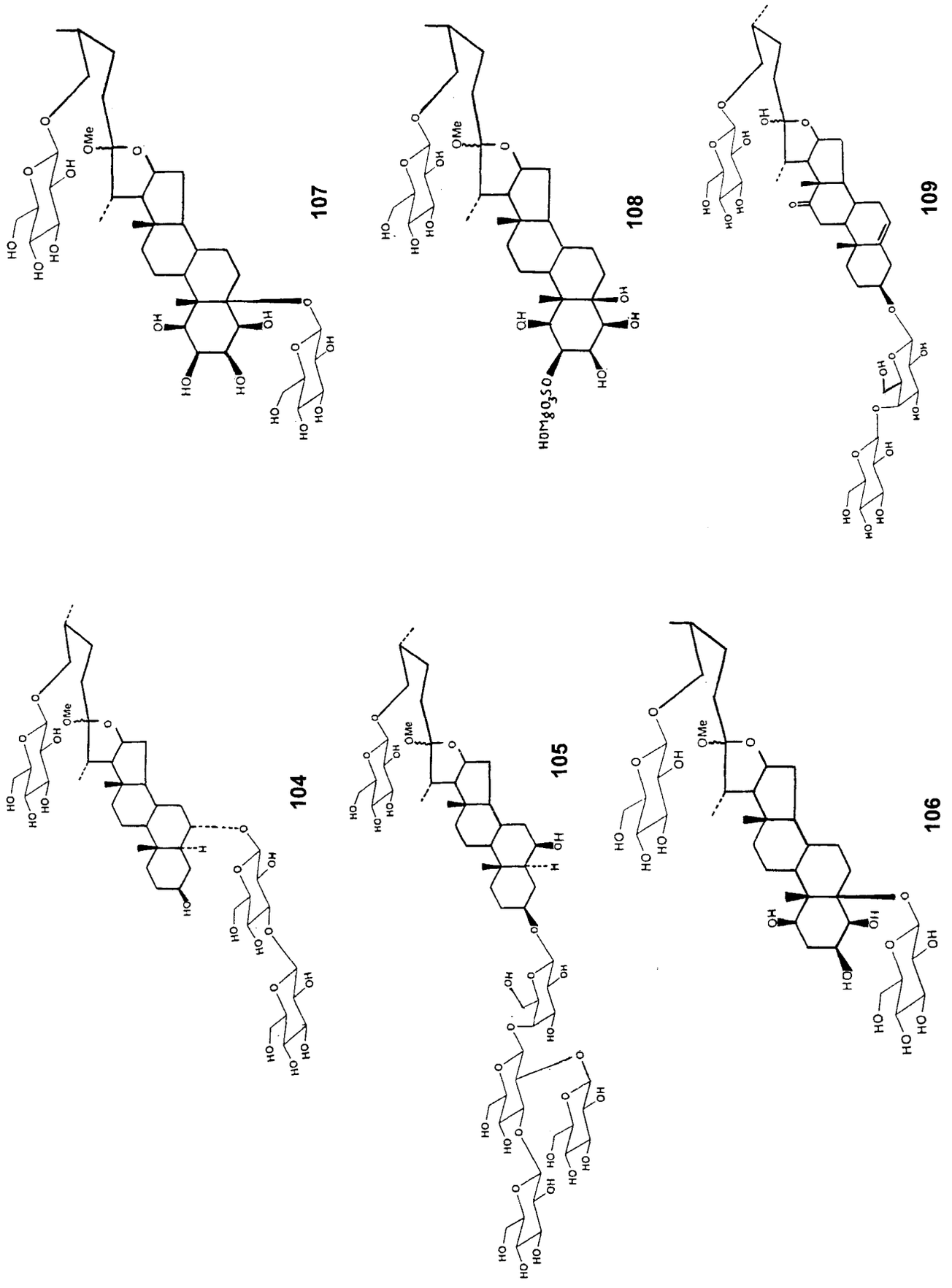


Figure 4. (continued)

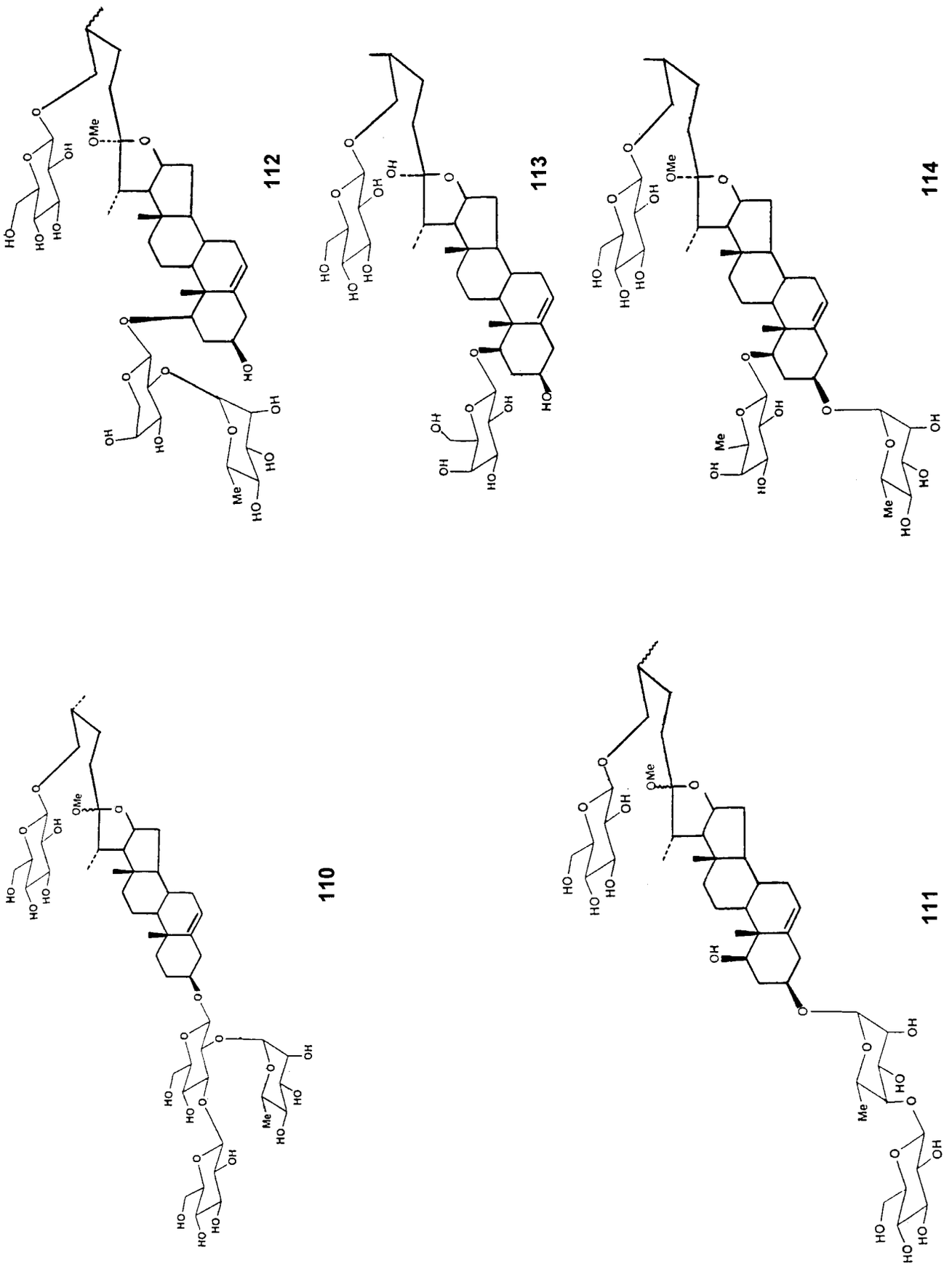
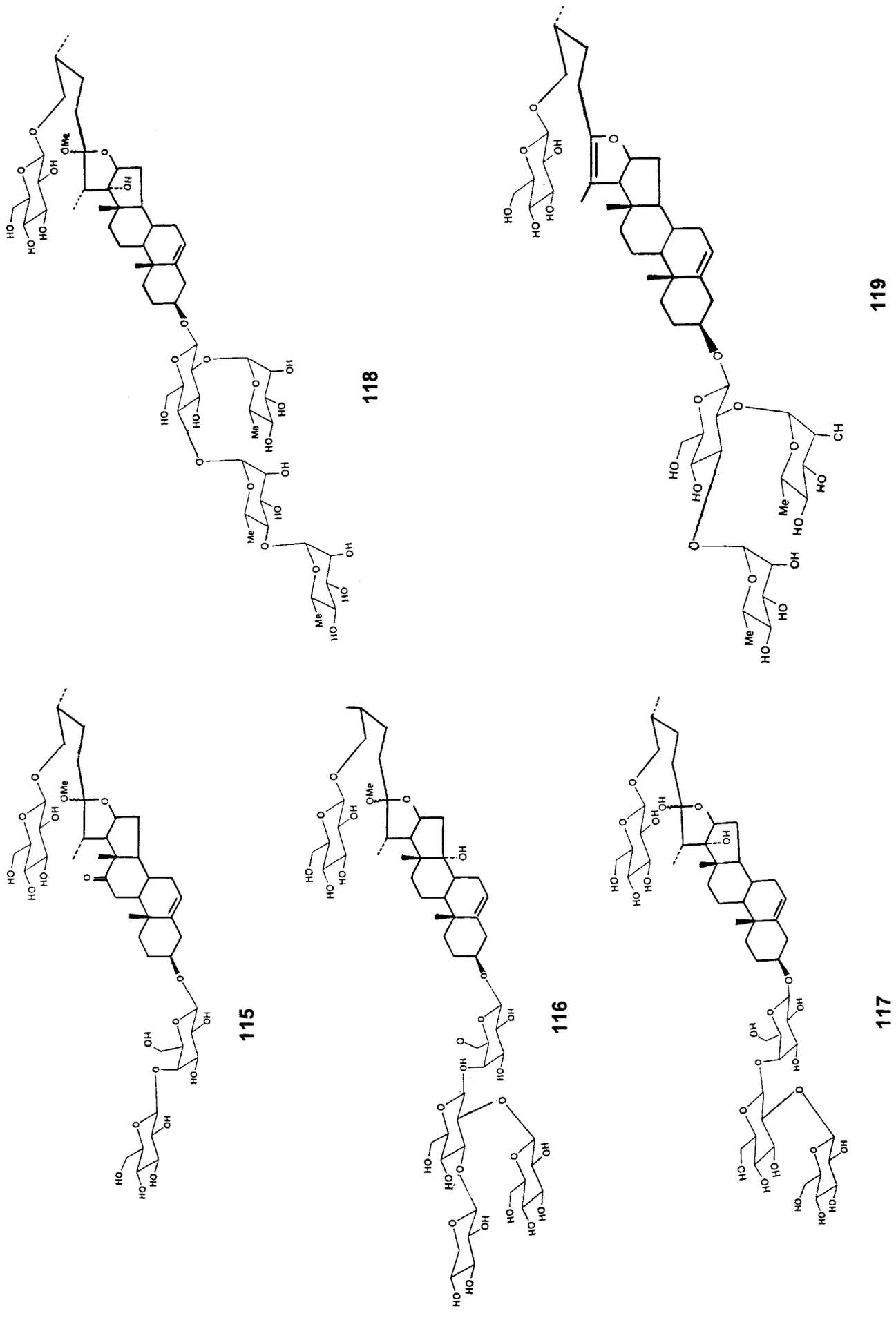
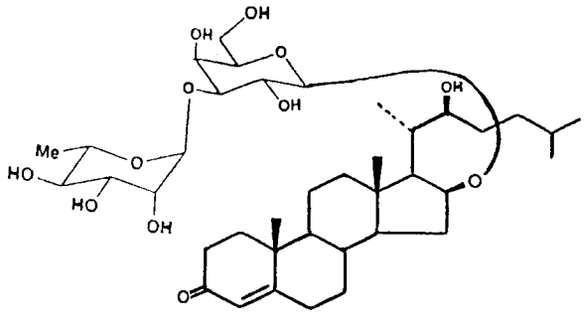


Figure 4. (continued)

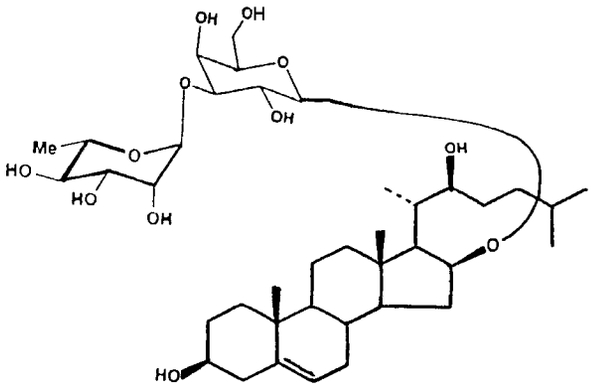


115 116 117 118 119

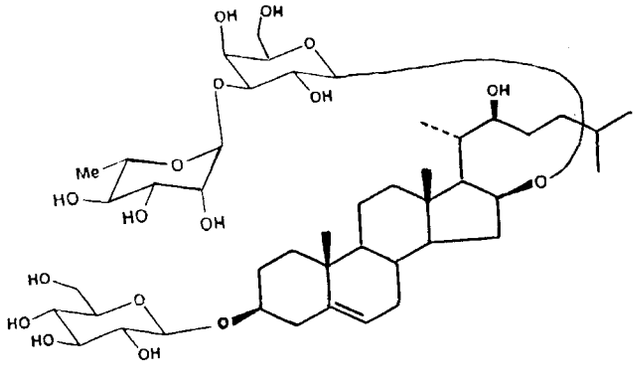
Figure 4. (continued)



120



121



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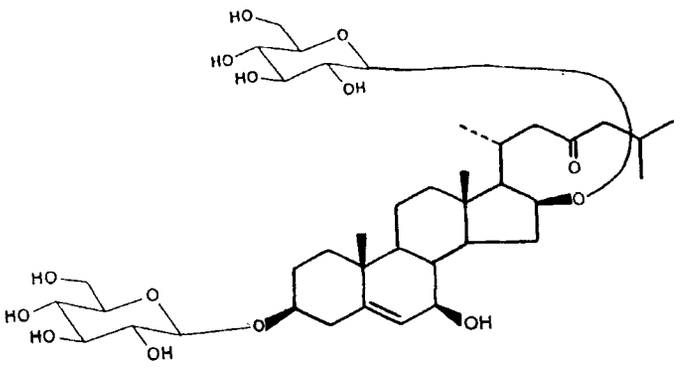

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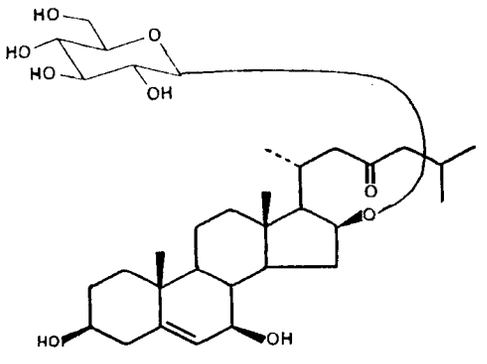
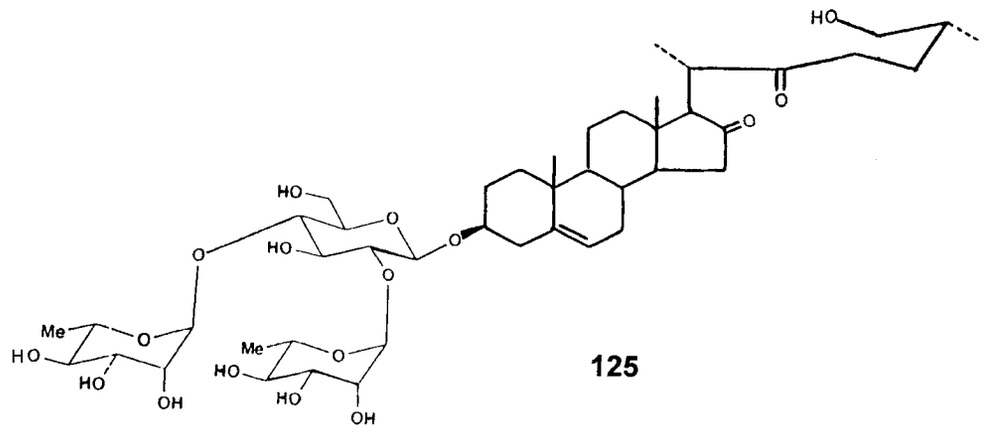
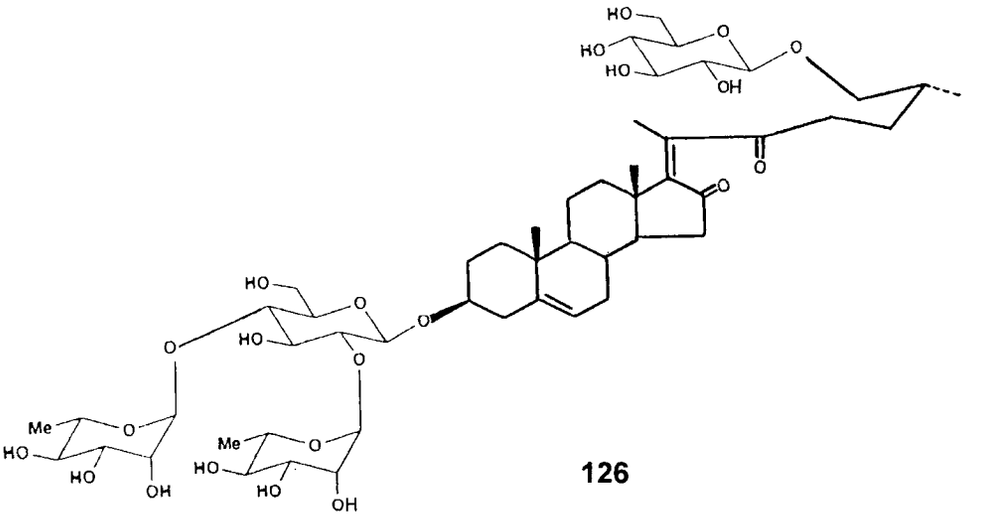


124

Figure 5. Structures of steroidal saponins having a cholestane skeleton.



125



126

Figure 5. (continued)

sapogenins are not readily identifiable in saponins owing to overlapping of these with sugar resonances in the region 3.0–4.5 ppm. To solve the complete structure of a steroidal saponin, the following questions must be addressed: (a) what is the parent skeleton of the steroidal sapogenin (aglycone); (b) what are the sites of substitutions of hydroxyl (including orientation) and oxo groups?; and (c) does the sapogenin possess one or more olefinic bonds? However, if the structure of saponin is under consideration, then one has to establish the monosaccharide composition, the anomeric configuration, the interglycosidic linkage and the sequence. Finally, the site for the sugar–aglycone linkage has to be determined.

All these questions can be resolved by the concerted use of various variants of one- and two-dimensional homo- and heteronuclear NMR correlation spectroscopy. The absorption of C-22 within a characteristic chemical shift range (Table 3) and the determination of its nature (C, CH and CH_2) identifies the skeletal type. A comparison of the observed multiplicities of the ^{13}C NMR resonances with those for the parent skeleton (Table 2) can be initially utilized in determining the number and type of the functional group present, which can further be verified by the ^1H–^1H COSY spectrum, at least in the case of a *sec*-hydroxyl group. This relies on the fact that a hydroxymethine resonance exhibits cross-peak connectivities with the proton resonances occupying vicinal positions, but such connectivity cannot be seen in case of a *tert*-hydroxyl group owing to its substitution to a quaternary carbon.

The oxy-substituted methine (H-3, H-16) and methylene resonances (H_2-26) provide entry points for the assignment of the ring proton resonances belonging to various ring systems. For this purpose, sometimes iterative comparisons of ^1H–^1H COSY and one-bond ^1H–^{13}C correlation spectra may be of significance as geminal and vicinal protons can be differentiated if they are overlapped. Since in the latter spectra cross-peak connectivities between ^{13}C and directly bonded ^1H resonances are observed, no cross peak appears for non-protonated carbons in such a spectrum. The best way to assign quaternary carbon resonances is by the observation of long-range ^1H–^{13}C connectivities in $^1H\{^{13}C\}$ multiple bond experiments (LR-HETCOR or HMBC). The orientation of the hydroxyl group can be established by consideration of the ^{13}C NMR chemical shifts, analysis of the multiplicities in view of coupling constants of the hydroxyl-bearing methine or methylene resonances in a resolution-enhanced one-dimensional ^1H NMR spectrum, one-dimensional NOE difference spectroscopy and/or two-dimensional NOE/ROE spec-

troscopy. The determination of the multiplicity of the olefinic carbon and their ^{13}C and ^{1}H NMR chemical shift values identifies the nature of olefinic bond(s) (endocyclic or exocyclic) and their position(s). Assignment of ^{13}C resonances can be achieved by considering literature values (Tables 5–8 and 10) and can also be accomplished independently via $^{1}H\{^{13}C\}$ single- and multiple-bond correlation experiments. In this way the structure of the sapogenin can be deduced.

However, if the structural analysis of a steroidal saponin is under consideration, then in addition to the identification of the aglycone, one needs to identify anomerically defined individual monosaccharides, the interglycosidic linkage and the sequence. The careful analysis of various homonuclear experiments such as DQF-COSY, multiple-step-relay or TOCSY, NOESY or ROESY experiments combined with one- and multiple-bond ^{1}H–^{13}C correlation spectroscopy and/or of 1D version of multi-dimensional experiments, provides information about the above characteristics which lead to the identification of individual monosaccharides in the sugar chain.[149–153,163,263,264] After identifying the structure of the sugar moiety, the next step is the determination of the aglycone–sugar linkage, i.e. to establish which monosaccharide is directly bonded to the non-sugar moiety. This can be inferred either by identifying carbon resonances involved in glycosylation, as these exhibit glycosylation-induced shifts, as seen from a comparison of ^{13}C NMR chemical shifts of saponin and sapogenin (Table 9), by detection of scalar coupling between the anomeric H with the aglycone C by means of a HMBC experiment or by the investigation of the spatial neighbourhood of the anomeric H and the linkage site proton by detecting NOE or ROE cross peaks. Continuing rapid developments in NMR methodology and equipment are extending the sample requirement, but the most realistic structural problems, of course, require the combined application of various homo- and heteronuclear shift correlation techniques in a systematic manner for deducing the complete structure of a saponin and the unambiguous assignments of ^{1}H and ^{13}C resonances of the natural product under investigation.

Acknowledgements

We are grateful to Dr R. P. Sharma, Head, Phytochemical Technology Division, and Professor Sushil Kumar, Director, CIMAP, Lucknow, for constant encouragement. We are indebted to Professor G. Blunden, University of Portsmouth Polytechnic, UK, Professor J. K. Snyder, University of Boston, USA, for critical reading of the manuscript and invaluable suggestions, and Dr G. A. Morris, University of Manchester, UK, for providing NMR data for saponin (Ref. 24).

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