Carbon-13 Nuclear Magnetic Resonance Spectra of Monounsaturated Steroids. Evaluation of Rules for Predicting Their Chemical Shifts

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Received April 24, 1981

^{13}C NMR spectra have been obtained and assigned for 13 monounsaturated androstanes and cholestanes as well as a number of hydroxyl derivatives. Representatives of all possible locations of a nuclear double bond are included. Due to a serious conformational distortion of the skeleton when a double bond is introduced, no overall good correlation between double bond substituent effects and structural features can be expected. Therefore a reliable prediction of ^{13}C NMR spectra of polyfunctional steroid olefins should be based upon experimental values for model systems incorporating the appropriate unsaturated skeleton.

Introduction

For chemists working in the steroid field the determination of the position of a double bond in an unknown steroid is a frequently encountered problem. In this paper we have examined the ^{13}C NMR spectra of monounsaturated steroids in order to apply ^{13}C NMR spectroscopy in structure elucidation of steroid olefins. The steroids studied include representatives of all 14 different locations of a nuclear double bond in the skeletons androstane and cholestane. For most double bond positions spectra of steroid olefins both with and without hydroxyl substituents have been obtained.

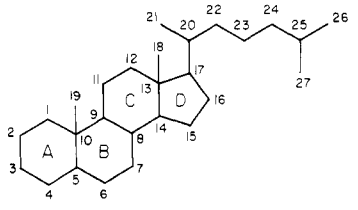
Experimental Section

The steroids examined are all known compounds. They have been prepared following standard methods for introduction of a double bond into the steroid system.[1]

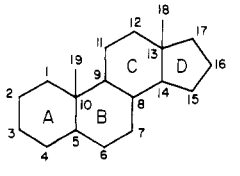
The ^{13}C NMR spectra were recorded as $CDCl_3$ solutions (0.1–0.5 M) under the same conditions and with the same instruments as described previously.[2]

Results

The ^{13}C shielding data for the steroid olefins are collected in Table I and the data for the hydroxyl-substituted analogues in Table II. The assignments as presented in the tables were based upon a combination of techniques, the details of which are given in ref 2 and 4, and upon our earlier assigned spectra of the parent hydrocarbons and monohydroxy steroids.[2] Olefinic carbon atoms in unsymmetrically substituted double bonds (Δ^4, Δ^5, Δ^7, $\Delta^{9(11)}$, and Δ^{14}) were assigned directly from the splittings in the off-resonance decoupled spectra. In order to obtain unambiguous assignments for the olefinic carbon atoms also in symmetrically substituted double bonds, we have obtained the ^{13}C NMR spectra of the following deuterium-labeled steroids: Δ^1-3β-cholestenol-2,4,4-d_3, Δ^2-1α-cholestenol-3-d_1, Δ^3-cholestene-3,5-d_2, Δ^{11}-3α-cholestenol acetate-12-d_1, and Δ^{16}-3β-androstenol-16-d_1.



5α-cholestane



5α-androstane

Olefinic carbon atoms in Δ^6-cholestene were assigned by comparison with the spectrum of Δ^6-androstene and supported by shift reagent experiments on Δ^6-3β-cholestenol. In Δ^8-cholestene the resonances for C-8 and C-9 were assigned by comparison with the corresponding signals of the 3β-hydroxyl-substituted analogues.[3] Shift reagent data for $\Delta^{8(14)}$-3β-cholestenol allowed distinction between the olefinic carbon atoms in the $\Delta^{8(14)}$ double bond, the relative induced shifts for C-8 and C-14 being 2:1.

The ^{13}C NMR data for the monounsaturated steroids show that introduction of a double bond in the steroid skeleton exerts no simple distinctive changes on allylic and homoallylic carbon atoms, thus precluding assignments of these carbon atoms by comparison with saturated analogues. Instead, spectral comparison of the steroid olefins with one or more hydroxyl-substituted analogues was applied. Introduction of the hydroxyl group greatly facilitates the assignments as use can be made of lanthanide shift reagent experiments and acetylation shifts. ^{13}C NMR data for certain monounsaturated steroids have been reported previously. Of these the data[4] for Δ^2, Δ^4, and Δ^5-cholestene were used in the assignments for compounds **2**, **4**, and **5**. Compounds **8**, **9**, **10**, and **13** were assigned by comparison with the reported shifts for hydroxyl-substituted analogues.[3]

Discussion

sp^2 Carbon Atoms. The chemical shifts of sp^2 carbon atoms in open-chain alkenes can be calculated with good results, using an additivity parameter set.[5] However, large deviations occur when these are applied to alicyclic and especially rigid alkenes. A set of parameters for calculating ^{13}C resonances in alicyclic alkenes (only six-membered rings) has been reported by Beierbeck et al.[6] In order to test the predictive value of this parameter set, we calculated the chemical shifts of the olefinic carbon atoms in the various steroid olefins and compared them to the experimental values of this study. For olefinic carbon atoms in ring A and B, all calculated shifts are within ±3.5 ppm of the experimental values. However, in ring C, condensed to the five-membered ring D, much larger deviations are found. Thus, for C-8 and C-14 in $\Delta^{8(14)}$, C-9 in $\Delta^{9(11)}$, and C-12 in Δ^{11}, deviations between calculated and experimental values are ±5–7 ppm.

In a recent paper,[7] olefinic carbon shieldings in sterols and related cyclic olefins have been analyzed. It was concluded that olefinic shieldings, in the case of symme-

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(3) M. Tsuda and G. J. Schroepfer, Jr., *J. Org. Chem.*, **44**, 1290 (1979).
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(5) D. E. Dorman, M. Jautelat, and J. D. Roberts, *J. Org. Chem.*, **36**, 2757 (1971).
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0022-3263/81/1946-5399$01.25/0

Table I. ^{13}C Chemical Shifts in Unsaturated Steroids[a]

	steroid	1	2	3	4	5	6	7	8	9	10	11	12
	androstane	38.8	22.3	26.9	29.2	47.1	29.2	32.6	36.0	55.1	36.4	20.9	39.0
	cholestane	38.8	22.3	26.9	29.2	47.1	29.2	32.2	35.6	54.9	36.3	20.9	40.2
(1)	Δ^1-cholestene	135.5	124.5	25.4*	26.0*	44.5	28.5	32.0	35.7	51.5	37.3	21.1	40.1
(2)	Δ^2-androstene	39.9	125.7*	125.6*	30.4	41.5	28.9	32.3	36.0	54.4	34.8	21.0	39.0
(3)	Δ^3-cholestene	34.2	23.5	125.1	131.2	45.9	27.5	32.1	35.7	53.5	34.9	21.1	40.1
(4)	Δ^4-androstene	37.9	19.5	25.8	118.8	144.7	32.7	33.7	36.4	54.3	37.1	21.5	38.9
(5)	Δ^5-androstene	40.0	22.7	28.1	33.0	143.4	118.9	32.2	32.2	51.0	37.7	20.9	38.9
(6)	Δ^6-cholestene	36.4	22.0	27.0	27.2	47.7	131.7	128.6	38.2	53.3	35.0	20.9	40.4
(7)	Δ^6-androstene[b]	36.3	22.0	27.0	27.1		131.6	129.0	38.5	53.4	35.0	20.7	39.1
(8)	Δ^7-cholestene	39.1	22.6	26.6	28.9	41.8	30.0	117.5	139.2	50.0	35.0	21.3	39.8
(9)	Δ^8-cholestene	37.0	22.5	26.7	29.3	42.9	25.9	27.2	127.7	135.9	36.5	22.5	37.0
(10)	$\Delta^{8,14}$-cholestene	38.5	22.3	26.7	29.2	46.3	29.4	29.8	126.7	49.8	37.6	19.7	37.5
(11)	$\Delta^{9,11}$-androstene	37.4	22.4	26.8	29.4	45.6	29.0	33.9	37.1	148.8	38.7	115.4	40.6
(12)	Δ^{11}-cholestene	37.8	22.1	26.9	29.4	47.3	29.7	31.0	33.9	57.8	35.8	125.6	138.6
(13)	Δ^{14}-cholestene	38.8	22.2	26.8	29.0*	46.5	28.9*	30.1	35.1	54.1	36.4	21.5	42.6
(14)	Δ^{16}-androstene	38.7	22.3	27.0	29.2	47.4	29.2	32.4	34.3	55.8	36.2	20.9	36.2

[a] In parts per million relative to Me_4Si. Assignment of chemical shifts for close-lying peaks marked with the same symbol.

Table II. ^{13}C Chemical Shifts in Hydroxyl-Substituted Unsaturated Steroids[a]

	steroid	1	2	3	4	5	6	7	8	9	10	11	12
(15)	Δ^1-3β-cholestenol	137.9	128.8	68.8	35.7	43.6	28.3	32.0	35.9	51.5	38.1	21.3	40.0
(16)	Δ^2-1α-cholestenol	69.7	127.9	130.4	30.8	34.6	28.7	31.5	35.6	46.1	38.9	20.7	39.8
(17)	Δ^6-3β-cholestenol	34.7	31.5	71.5	36.3	45.1	129.3	130.6	38.2	52.7	34.3	21.2	40.2
(18)	Δ^8-3β-6α-cholestenediol	35.7	31.1	71.0	32.2	47.8	67.5	37.8	126.7	135.2	36.6	22.8	36.8
(19)	$\Delta^{9,11}$-3β-androstenol[b]	35.7	31.7	71.2	38.5	43.3	28.7	33.7	37.1	147.9	38.0	116.0	
(20)	Δ^{11}-3α-cholestenol	31.7	29.0	66.6	36.0	39.1	29.2	30.9	33.9	56.9	36.4	125.3	138.8
(21)	Δ^{15}-17β-androstenol	38.6	22.2	26.8	28.9*	47.3	29.0*	31.9	33.2	55.4	36.5	20.4	34.8
(22)	Δ^{16}-3β-androstenol	36.8	31.3	71.0	38.1	45.0	28.7	31.9	34.1	55.2	35.9	21.2	35.9

[a] See footnote a, Table I. [b] 12,12-d_2 labeled; C-12 resonance not observed.

trically substituted double bonds, strongly depend upon the number of carbon atoms two bonds removed from the olefinic carbon atom. A correlation between chemical shift ranges and number of such substituents was given. The ^{13}C NMR data of the present study do not support the general value of this correlation. For example, C-11 in Δ^{11}-cholestene and C-2 in Δ^1-cholestene have very close lying resonances in spite of their different number of substituents two bonds away. Also, C-6 in Δ^6-cholestene absorbs in the range assumed to be characteristic for tertiary olefinic carbon atoms with one less carbon substituent two bonds away.

sp^3 Carbon Atoms. The experimental double bond substituent shifts at allylic (β) carbon atoms vary ±5 ppm, though most of the shifts are less than ±2 ppm. Large downfield shifts (4.5–6.6 ppm) are observed in the two examples available (Δ^{14} and Δ^{16}) of the ring D double bonds. Allylic substituent shifts in the six-membered rings can also be derived from the substituent parameters of Beierbeck et al.[6] The predicted shifts for the different types of allylic carbon atoms reproduce the trends observed except for the shifts at C-9 in both the Δ^7 and $\Delta^{8(14)}$ unsaturated steroid and at C-7 and C-14 in the Δ^8 unsaturated steroid. These are all found ~4 ppm more upfield than predicted.

It has been reported[7] that the double bond substituent effect on allylic carbon atoms correlates with the degree of substitution of this atom. However, with more experimental data available this dependence is not distinctive. Thus, substituent shifts for allylic methine and methylene carbon atoms in the six-membered rings cover essentially the same range.

The substituent shifts for homoallylic (γ) carbon atoms cover a somewhat wider range (−6 to +7 ppm) than found for the β-carbon atoms, but again most shifts at γ-carbon atoms are small (<±2 ppm). Predicted shifts, using the substituent parameters of Beierbeck et al.,[6] followed the pattern of the experimental γ-carbon substituent shifts. Calculated values were, except for one value (C-9 in the Δ^1 unsaturated steroid), all within ±2.7 ppm of the experimental data; 50% of the values were within ±1 ppm.

The homoallylic substituent shifts observed at the angulary methyl groups C-18 and C-19 should be of diagnostic importance with respect to the location of a double bond in the steroid skeleton. Thus, the C-19 resonance is shifted downfield by 4–7 ppm upon introduction of a Δ^1, Δ^4, Δ^5, Δ^8, or $\Delta^{9(11)}$ double bond and the C-18 resonance is shifted in the same way upon introduction of a $\Delta^{8(14)}$, Δ^{11}, or Δ^{14} double bond. As the only exception, the Δ^{16} unsaturated steroid does not show a downfield double bond substituent shift at the homoallylic methyl group C-18, but instead a small upfield shift is observed. This finding is reproduced in the predictions.[6] Thus, introduction of the Δ^{16} double bond causes, as the only case, elimination of a 1,3-diaxial hydrogen interaction of the homoallylic angular methyl group. The large deshielding substituent shift is predicted for exocyclic γ-carbon atoms (not only methyl groups) only in cases where no such elimination takes place.

Large effects on sp^3 carbon chemical shifts by introducing a double bond are restricted to allylic and homoallylic positions. The effects at carbon atoms further removed from the double bond are in most cases found to be less than ±1 ppm. Somewhat larger shifts, up to ±2 ppm, are measured in some examples for carbon atoms three bonds away. However, as the major part of the substituent shifts at both allylic and homoallylic carbon atoms are minor shifts, the long-range substituent effects of the double bond easily exceed the effects at allylic and homoallylic carbon atoms.

Allylic Alcohols. Introduction of a hydroxyl group allylic to the double bond usually produces downfield shifts at both unsaturated carbon atoms.[8] While in acyclic

(8) E. Breitmaier, G. Haas, and W. Voelter, "Atlas of Carbon-13 NMR Data", Heyden, London, 1979.

13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
40.8	54.7	25.5	20.5	40.5	17.6	12.3								
42.6	56.7	24.2	28.3	56.4	12.2	12.2	35.9	18.7	36.3	24.0	39.6	28.0	22.5	22.8
42.7	56.5	24.1	28.2	56.2	12.2	15.7	35.7	18.6	36.1	23.9	39.5	28.0	22.5	22.7
40.7	54.6	25.5	20.5	40.5	17.5	11.7								
42.7	56.5	24.2	28.2	56.3	12.2	11.9	35.8	18.7	36.2	23.9	39.5	28.0	22.5	22.8
40.8	54.7	25.6	20.5	40.4	17.5	19.4								
40.6	55.0	25.7	20.6	40.4	17.3	19.5								
43.7	54.8	24.0	28.5	56.4	12.3	11.3	35.9	18.8	36.4	24.0	39.7	28.1	22.6	22.8
41.8	52.8	25.3	20.6	40.4	17.7	11.3								
43.5	55.2	23.0	28.0	56.3	11.9	13.0	36.2	18.9	36.2	24.0	39.6	28.0	22.5	22.7
42.2	52.0	23.8*	28.8	54.9	11.2	17.7	36.2*	18.7	36.3*	23.9*	39.6	28.0	22.5	22.8
42.7	141.9	25.8	27.0	57.0	18.3	12.7	34.5	19.1	36.0	23.8	39.6	28.0	22.5	22.7
39.4	52.3	26.8	21.2	40.2	17.5	18.0								
45.0	53.6	23.0	28.6	52.3	16.6	12.7	36.1	18.8	36.4	23.8	39.5	28.0	22.5	22.8
47.1	155.6	116.6	34.0	58.8	16.9	11.9	35.6	19.0	36.1	23.8	39.6	28.0	22.6	22.8
45.7	56.4	32.1	129.3	144.1	17.2	12.3								

may be reversed. [b] 5-*d*, labeled; C-5 resonance not observed.

13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
42.8	56.6	24.2	28.3	56.4	12.2	15.7	35.9	18.7	36.2	23.9	39.6	28.0	22.6	22.8
42.6	56.4	24.3	28.3	56.4	12.0	11.4	35.8	18.7	36.2	24.0	39.6	28.0	22.6	22.8
43.5	54.5	24.0	28.4	56.2	12.2	11.4	35.8	18.7	36.3	24.0	39.5	28.0	22.6	22.8
42.2	51.6	24.0	28.8	55.0	11.3	19.0	36.3	18.8	36.3	24.0	39.6	28.0	22.6	22.8
39.3	52.3	26.8	21.1	40.1	17.5	18.1								
45.0	53.5	23.0	28.5	52.2	16.6	11.6	36.0	18.8	36.4	23.8	39.6	28.1	22.6	22.8
51.0	57.7	134.2*	132.0*	85.7	12.3	12.3								
45.5	56.0	31.9	129.2	143.8	17.0	12.3								

compounds shifts are larger for the β than for the γ olefinic carbon atom, the reverse has been reported for $\Delta^{8(14)}$-cholestene.[9] The present study includes two allylic hydroxyl-substituted steroid olefins, Δ^1-3β- and Δ^2-1α-cholestenol, while ^{13}C NMR data for Δ^4-3β-cholestenol are reported in ref 3. Examination of the data available shows that the variation in allylic hydroxyl substituent shifts is not a consequence of a cyclic vs. an acyclic structure. Rather, it appears that the substituent pattern is related to the orientation of the hydroxyl group. Thus, when the hydroxyl group is fixed at an antiperiplanar[10] position with respect to the double bond (Δ^1-3β- and Δ^4-3β-cholestenols), the hydroxyl substituent effects are similar to the pattern found for acyclic olefins. Hydroxyl groups in synclinal[10] ($\Delta^{8(14)}$-15β- and $\Delta^{8(14)}$-15α-cholestenols[9]) and anticlinal[10] positions (Δ^2-1α-cholestenol and $\Delta^{8(14)}$-7α-cholestenol[9]), however, give rise to substituent shifts which are larger for the γ sp^2 carbon atom. For a hydroxyl group placed synperiplanar[10] to the double bond the same downfield shifts are observed for both sp^2 carbon atoms ($\Delta^{8(14)}$-7β-cholestenol[9]). This variation in allylic hydroxyl substituent effect is also found when comparing the ^{13}C NMR data for $\Delta^{9(11)}$-podocarpane with the 12α- (anticlinal) and the 12β- (antiperiplanar) hydroxyl-substituted analogue.[11]

Summary and Conclusion

In this paper we have examined the effects on ^{13}C shieldings of introducing a double bond at the different positions in the steroid skeleton. The observed ^{13}C chemical shifts and double bond substituent shifts have been compared to predicted values, using the parameter set of Beierbeck et al.[6] Furthermore, the applicability of some chemical shift rules reported for unsaturated steroids[7] has been tested. It is apparent that the predicted chemical shifts in both cases are not sufficiently reliable to be used for determination of double bond positions in unknown steroids. This result is not surprising as attempts to provide such general correlations in the case of cyclic olefins raise obvious conformational problems. Introduction of a double bond in a cyclic system produces substantial conformational distortion of the system. In a polycyclic molecule, the geometric states of condensed rings are interdependent and the deformation is propagated through all rings. The degree of such conformational transmission depends upon the rigidity of the ring containing the double bond as well as nearby condensed rings. In the steroid skeleton the flexibility of the four rings is very different. Therefore, some double bond positions will show substantial transmission, while the transmitted distortion for other positions will be much less. For this reason, no overall good correlation between ^{13}C double bond substituent effects and structural features is expected for polycyclic structures like the steroids.

Due to these restrictions, a reliable prediction of ^{13}C NMR spectra of polyfunctional steroid olefins should be based upon experimental values for model systems incorporating the appropriate unsaturated skeleton. The present study of monounsaturated steroids which includes representatives of all different locations of a nuclear double bond in the steroid skeleton should provide a valuable basis for the use of ^{13}C NMR spectroscopy in structure elucidation of unknown steroid olefins.

Acknowledgment. Financial support by NATO Grant No. RG 939 is greatly acknowledged.

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Registry No. **1**, 604-18-2; **2**, 20796-45-6; **3**, 28338-69-4; **4**, 38544-66-0; **5**, 4369-59-9; **6**, 28338-70-7; **7**, 54498-15-6; **8**, 40071-65-6; **9**, 74365-07-4; **10**, 54725-42-7; **11**, 5217-23-2; **12**, 79632-10-3; **13**, 54725-04-1; **14**, 6618-43-5; **15**, 17808-78-5; **16**, 20230-10-8; **17**, 22420-06-0; **18**, 570-92-3; **19**, 79632-11-4; **20**, 79632-12-5; **21**, 79632-13-6; **22**, 7148-51-8; androstane, 438-22-2; cholestane, 481-21-0.