UDC 541.63 : 539.143.4.083.2

53. Yutaka Kawazoe,*1 Yoshihiro Sato,*2 Mitsutaka Natsume,*1 Hiroko Hasegawa,*2 Toshihiko Okamoto,*1 and Kyosuke Tsuda*2 :

Application of NMR to Stereochemistry. (I). The Spatial Interaction Effect of the Hydroxyl Group to Methyl Resonance.*3

(*Faculty of Pharmaceutical Sciences,*1 University of Tokyo and Institute of Applied Microbiology,*2 University of Tokyo*)

Increasing use of NMR spectra for solution of stereochemical problems has been made in the last few years. This paper concerns the position and configuration of hydroxyl groups in steroid molecules by the considerations of spatial interactions between the methyl and hydroxyl groups present in these molecules.

When an extraordinary downward shift of the 18- and 19-methyl resonances of 4-pregnene-3,20-dione going to its 11β-hydroxyl derivative was reported by Shoolery and Rogers,[1] it appeared to be due to the interaction of hydroxyl group with a methyl group located in its vicinity. Accordingly the NMR spectra were examined of 42 steroids having one or two hydroxyl groups located in various positions with both α and β configurations as listed in Table I. Some of these steroids have hydroxyl groups at a position in the vicinity of either the 18- or 19-angular methyl group and therefore can be regarded as suitable model compounds to establish the relationship between the spatial interaction of hydroxyl groups to protons and the chemical shift of the interacted protons. If this downward shift of the methyl resonance is attributable to spatial interaction then this fact should be very useful in determining the position and configuration of hydroxyl groups relative to the methyl group in various types of compounds such as steroids, terpenes, etc. In order to emphasize this spatial relationship of the two substituents on their NMR spectra, the methyl resonance shifts of the acetylated derivatives were also compared with the shifts exhibited by the parent hydroxysteroids. It was expected that acetylation of the hydroxyl groups would give rise to some effect toward the methyl group——aside from the electronic effect through chemical bond——resulting in a certain characteristic acetylation shift for the methyl resonance spatially interacted by the hydroxyl groups. These expectations were borne out by the experimental data shown below.

Experimental

All spectra were obtained by a Varian Associates DP 60 NMR spectrometer, operating at 60 Mcps. with high resolution. The compounds were examined in dilute $CHCl_3$ solutions, the concentrations ranging from 10 mg. to 25 mg. in 0.5 cc. of solvent. The zero reference in each spectrum was taken as the resonance position of cyclohexane dissolved in each solution examined, so that the effect of volume susceptibility can be neglected. Cyclohexane as an internal reference can be regarded as one of the best standard substances because of its negligible capacity toward hydrogen bonding with the solute or solvent; hence, the values of chemical shifts obtained here can be correlated with the values found in the literature providing they were initially correctly calibrated. The sign of the shift is chosen as positive when the resonance falls in a higher field than the reference. For comparison with values obtained at other field strengths, the position of a peak can be represented in a dimensionless unit, δ, by dividing the frequency in cps. units by 60. Further-

*1 Motofuji-cho, Bunkyo-ku, Tokyo (川添 豊, 夏目充隆, 岡本敏彦).
*2 Yayoi-cho, Bunkyo-ku, Tokyo (佐藤良博, 長谷川弘子, 津田恭介).
*3 This paper constitutes Part XXXIV of a series entitled "Steroid Studies" by K. Tsuda and also Part I of a series entitled "Nuclear Magnetic Resonance Studies" by T. Okamoto.
1) J.N. Shoolery, M.T. Rogers : J. Am. Chem. Soc., **80**, 5121 (1958).

Table I(1). 18 and 19 Methyl Resonances of Androstane Series

No.		18-CH_3		19-CH_3	
		18-H (cps.)	Diff. from parent compd.	19-H (cps.)	Diff. from parent compd.
1	4-androstene-3,17-dione	30.0	——	12.2	——
2	6α-OH 〃	29.4	− 0.6	12.6	0.4
3	6α-OAc 〃	29.5	− 0.5	9.8	− 2.4
4	6β-OH 〃	27.7	− 2.3	1.4	−10.8
5	6β-OAc 〃	26.7	− 3.3	5.7	− 6.5
6	11α-OH 〃	27.8	− 2.2	4.8	− 7.4
7	11β-OH 〃	16.0	−14.2	− 3.4	−15.6
8	11β-OAc 〃	22.2	− 7.8	7.1	− 5.1
9	14α-OH 〃	22.9	− 7.1	12.0	− 0.2
10	15α-OAc 〃	25.5	− 4.5	11.9	− 0.3
11	1,4-androstadiene-3,17-dione	28.8	——	9.9	——
12	11β-OH 〃	13.8	−15.0	− 4.1	−14.0
13	11β-OAc 〃	21.4	− 7.4	7.2	− 2.7
14	15α-OH 〃	26.2	− 2.6	9.1	− 0.8

Table I(2). 18 and 19 Methyl Resonances of Pregnane Series

No.		18-CH_3		19-CH_3	
		18-H (cps.)	Diff. from parent compd.	19-H (cps.)	Diff. from parent compd.
15	4-pregnene-3,20-dione	45.0	——	13.7	——
16	7β-OH 〃	43.0	− 2.0	12.6	− 1.1
17	7β-OAc 〃	42.3	− 2.7	10.7	− 3.0
18	11α-OH 〃	43.1	− 1.9	6.3	− 7.4
19	11α-OAc 〃	41.3	− 3.7	9.6	− 4.1
20	14α-OH 〃	38.6	− 6.4	13.3	− 0.4
21	15α-OH 〃	43.5	− 1.5	13.3	− 0.4
22	15α-OAc 〃	41.3	− 3.7	13.9	+ 0.2
23	15β-OH 〃	29.0	−16.0	11.9	− 1.8
24	15β-OAc 〃	31.4	−13.6	11.3	− 2.4
25	6β-OAc, 11α-OAc 〃	37.9	− 7.1	3.4	−10.3
26	6β-OH, 15β-OH 〃	27.2	−17.8	2.1	−11.6
27	6β-OAc, 15β-OAc 〃	30.6	−14.4	5.9	− 7.8
28	6β-OAc, 15β-OH 〃	27.6	−17.4	5.6	− 8.1
29	6α-OAc, 15β-OH 〃	28.4	−16.6	8.9	− 4.8
30	7β-OH, 15β-OH 〃	26.6	−18.4	10.3	− 3.4
31	7β-OAc, 15β-OH 〃	27.0	−18.0	9.7	− 4.0
32	7α-OH, 15β-OH 〃	29.5	−15.5	12.0	− 1.7
33	7α-OAc, 15β-OAc 〃	32.0	−13.0	9.9	− 3.8
34	14α-OH, 15α-OH 〃	37.8	− 7.2	13.2	− 0.5
35	14α-OH, 15β-OH 〃	25.2	−19.8	11.5	− 2.2
36	14β-OH, 15α-OH 〃	26.3	−18.7	16.8	+ 3.1
37	14β-OH, 15β-OH 〃	22.6	−22.4	14.3	+ 0.6
38	5α-pregnane-3,20-dione	47.2	——	24.6	——
39	7α-OH 〃	46.9	− 0.3	25.2	+ 0.6
40	7α-OAc 〃	45.7	− 1.5	22.7	− 1.9
41	7β-OH 〃	44.4	− 2.8	22.8	− 1.8
42	7β-OAc 〃	45.0	− 2.2	22.5	− 2.1

more, for comparison with data in τ-unit recommended by some workers,[2,3] the τ-values of the resonance positions listed in this paper can be calculated by assuming the resonance position of cyclohexane as 8.56, the value calibrated against tetramethylsilane in a separate experiment.

Most of the steroids used in this work were obtained by microbiological oxidations of parent steroids, the preparation and characterization of which have previously been published.[4],*4

Discussion

In Table I are shown the chemical shifts (relative to internal cyclohexane) of 18- and 19-methyl protons of 42 compounds of androstane and pregnane series. The substances measured are mono- and di-substituted compounds derived from 4-androstene-3,17-dione (No. 1), 1,4-androstadiene-3,17-dione (No. 11), 4-pregnene-3,20-dione (No. 15), and 5α-pregnane-3,20-dione (No. 38). Each substituent is either a hydroxyl or an acetoxyl group. In the fourth and sixth columns of Table I are listed the frequency differences of the methyl protons from the 18- or 19-methyl protons in the parent steroids, No. 1, No. 11, No. 15, and No. 38, respectively.

Substituent Effect and their Spatial Relations

The substituent effect of hydroxyl or acetoxyl groups toward the resonance frequencies of 18- and 19-methyl protons is represented by the magnitude of the shift of the mono-substituted derivatives from their respective parent compounds. The values of these effects are shown in Table II, the average values being given in the last

Table II. Substituent Effects

Methyl-Signal / Function	18-CH_3			19-CH_3		
O⁄⁄				−24.9(Z)		
17-C=O				− 1.5(Z)		
17-$COCH_3$				± 0.0(Z)		
6α-OH	− 0.6(2)			+ 0.4(2)		
6α-OAc	− 0.5(3)			− 2.4(3)		
6β-OH	− 2.3(4)			−10.8(4)		
6β-OAc	− 3.3(5)			− 6.5(5)		
7α-OH	− 0.3(39)			+ 0.6(39)		
7α-OAc	− 1.5(40)			− 1.9(40)		
7β-OH	− 2.0(16)	− 2.8(41)	− 2.4[a]	− 1.1(16)	− 1.8(41)	− 1.5[a]
7β-OAc	− 2.7(17)	− 2.2(42)	− 2.5	− 3.0(17)	− 2.1(42)	− 2.6
11α-OH	− 2.2(6)	− 1.9(18)	− 2.1	− 7.4(6)	− 7.4(18)	− 7.4
11α-OAc	− 3.7(19)			− 4.1(19)		
11β-OH	−14.0(7)	−15.0(12)	−14.5	−15.6(7)	−14.0(12)	−14.8
11β-OAc	− 7.8(8)	− 7.4(13)	− 7.6	− 5.1(8)	− 2.7(13)	− 3.9
14α-OH	− 7.1(9)	− 6.4(20)	− 6.8	− 0.2(9)	− 0.4(20)	− 0.3
15α-OH	− 1.5(21)	− 2.6(14)	− 2.1	− 0.4(21)	− 0.9(14)	− 0.7
15α-OAc	− 4.5(10)	− 3.7(22)	− 4.1	− 0.3(10)	+ 0.2(22)	− 0.1
15β-OH	−16.0(23)			− 1.8(23)		
15β-OAc	−13.6(24)			− 2.4(24)		

The compound numbers are given in parentheses after each value.
(Z) means the datum reported in the Zürcher's paper.[6] *a*) Mean value.

*4 The structures of some of these compounds used in the present work will be published in a forthcoming paper.

2) G. V. D. Tiers : J. Phys. Chem., **62**, 1151 (1958).
3) L. M. Jackman : "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," 47 (1959), Pergamon Press, New York.
4) K. Tsuda, *et al.* : J. Gen. Appl. Microbiol., **4**, 63, 67, 79 (1958); This Bulletin, **6**, 387 (1958); J. Gen. Appl. Microbiol., **5**, 1, 7 (1959); This Bulletin, **7**, 534 (1959); *ibid.*, 8, 626 (1960); *ibid.*, **9**, 409, 735, 740 (1961).

column, when the two values have been obtained for one substituent; for example, −2.4 for 7β-OH toward 18-methyl. In order to see whether the values (cps.) of each mono-substituted compounds could be applied to the disubstituted compounds, in other words, to see whether the effect of each substituent is additive, the total effect of two substituents was estimated by adding the corresponding substituent effects listed in Table II. These calculated values are compared with the experimental ones, as shown in Table III. Since

Table III. 18 and 19 Methyl Resonances of Disubstituted 4-Pregnene-3,20-diones

	18-CH_3				19-CH_3			
		Calcd.	Found	Found − Calcd.		Calcd.	Found	Found − Calcd.
6β-OAc, 11α-OAc	−3.3	−3.7=−7.0	−7.1	−0.1	−6.5	−4.1=−10.6	−10.3	+0.3
6β-OH, 15β-OH	−2.3	−16.0=−18.3	−17.8	+0.5	−10.8	−1.8=−12.6	−11.6	+1.0
6β-OAc, 15β-OAc	−3.3	−13.6=−16.9	−14.4	+2.5	−6.5	−2.4=−8.9	−7.8	+1.1
6β-OAc, 15β-OH	−3.3	−16.0=−19.3	−17.4	+1.9	−6.5	−1.8=−8.3	−8.1	+0.2
6α-OAc, 15β-OH	−0.5	−16.0=−16.5	−16.6	−0.1	−2.4	−1.8=−4.2	−4.8	−0.6
7β-OH, 15β-OH	−2.4	−16.0=−18.4	−18.4	0.0	−1.5	−1.8=−3.3	−3.4	−0.1
7β-OAc, 15β-OH	−2.5	−16.0=−18.5	−18.0	+0.5	−2.6	−1.8=−4.3	−4.0	+0.4
7α-OH, 15β-OH	−0.3	−16.0=−16.3	−15.5	+0.8	+0.6	−1.8=−1.2	−1.7	−0.5
7α-OAc, 15β-OAc	−1.5	−13.6=−15.1	−13.0	+2.1	−1.9	−2.4=−4.3	−3.8	+0.5
14α-OH, 15α-OH	−6.8	−2.1=−8.9	−7.2	+1.7	−0.3	−0.7=−1.0	−0.5	+0.5
14α-OH, 15β-OH	−6.8	−16.0=−22.8	−19.8	+3.0	−0.3	−1.8=−2.1	−2.2	−0.1

the differences between them fall within 1 cps. for most of the compounds, the effect seems to be additive. Some workers[1,5] have already suggested the additive effects of substituents of steroidal compounds and recently, Zürcher[6] proved the additivity of the effects to be true for the 19-methyl resonance in a large number of steroids.*5

The position and configuration of a substituent will now be correlated with a magnitude of the effect shown in Table II. Hydroxyl groups showing effect of more than −10 cps.*6 to 18-methyl group are 11β- and 15β-ols, and that toward 19-methyl group, 6β- and 11β-ols. All others have effect less than −3 cps.*6 to either the 18- or 19-methyl group. Thus, all the effect, with two exceptions, can be classified into two groups, more than −10 cps. and less than −3 cps. It is evident from the above that this downward shift occurs when a methyl group is closely located to a hydroxyl group, as exemplified by a 1,3-diaxial relationship in a cyclohexane ring (chair form) or of a pseudo-1,3-diaxial relationship in a cyclopentane ring, as shown in Chart 1.

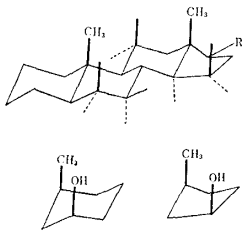


Chart 1.

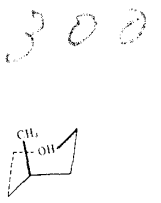


Chart 2.

The 11α-hydroxyl group influences the 19-methyl resonance by −7.4 cps., which is a medium value as far as this effect is concerned. Perhaps this might be explained by the fact that the spatial relation of the 11α-hydroxyl group to 19-methyl group (see

*5 It is doubtful whether this additivity could be applied to any polysubstituted steroids, because the additivity might be disturbed by mutual interaction between substituents, either electronically or spatially.

*6 "More than" or "less than" means that from absolute values.

5) J.S.G. Cox, E.O. Bishop, R.E. Richards: J. Chem. Soc., **1960**, 5118.

6) R.F. Zürcher: Helv. Chim. Acta, **44**, 1380 (1961).

Chart 2) may have some effect on the 19-methyl resonance to some extent. Moreover, the relative position of the 11α-hydroxyl group may be disturbed by the ring strain due to unsaturation. The effect of the 14α-hydroxyl group to the 18-methyl resonance, −6.8 cps., may be explained by the fact that it is located at the β-position of the 18-methyl group, being under its inductive effect.

Comparison of the Effects between α and β Isomers

In order to prove that this substituent effect is dependent on their spatial relationship, namely on their configuration, the difference in the substituent effects between the α- and β-isomers at each position of the substituent was calculated, using the values given in Table II, and are shown in Table IV. First of all, it can be seen that the β-isomers always produced more dominant effect than the corresponding α-isomers.

Table IV. Dependence on Configuration

$\delta_{\beta\text{-OH}} - \delta_{\alpha\text{-OH}}$

	18-CH_3		19-CH_3	
6	− 1.7(A)		−11.2(A)	
7	− 2.9(P)		− 1.7(P)	
11	−11.8(A)		− 8.2(A)	
15	−14.5(P)	−12.6(P)	− 1.4(P)	− 1.7(P)

A : Androstane derivatives
P : Pregnane derivatives

Of more importance, large difference was found at the 11- and 15-positions for 18-methyl resonance (−11.8, −14.5, −12.6) and at the 6- and 11-positions for 19-methyl resonance (−11.2, −8.2), while the difference between the isomers at other positions was found to be quite small, ranging from −1.4 to −2.9. From these results, it can be predicted that if the configurational isomers, α and β, show a large difference of substituent effect to the methyl resonance, the hydroxyl group in question should be closely located to the methyl group. On the other hand, if the difference between the isomers is not so large as 3 cps., then it may be concluded that the methyl group concerned is too far from the hydroxyl group to be affected in the structure of either α- or β-isomer.

Acetylation Effect

In order to verify that these unusual downward shifts are caused by the spatial interaction between hydroxyl and methyl groups and not electronically, acetylation effect on the methyl resonance was examined. In Table V are shown the differences of the effect between acetoxyl derivatives and their parent hydroxyl compounds. Plus values in the table indicate that acetylation resulted in an upward shift for the methyl resonance. The acetylation shift of methyl resonance caused by a hydroxyl group interacting the methyl group show positive values, ranging from +2.4 to +11.3 cps., while

Table V. Acetylation Shifts

	18-CH_3			19-CH_3		
6α	+ 0.1(A)			− 2.8(A)		
6β	− 1.0(A)	+ 0.4(P)		+ 4.2(A)	+ 3.5(P)	
7α	− 1.2(P)			− 2.5(P)		
7β	− 0.7(P)	+ 0.4(P)	+ 0.6(P)	− 1.9(P)	− 0.6(P)	− 0.3(P)
11α	− 1.8(P)			+ 3.3(P)		
11β	+ 6.2(A)	+ 7.6(D)		+10.5(A)	+11.3(D)	
15α	− 2.2(P)			+ 0.6(P)		
15β	+ 2.4(P)	+ 3.0(P)		− 0.6(P)	+ 0.3(P)	

A : Androstane derivatives
P : Pregnane derivatives
D : 1,4-Diene-3-one derivatives

the shift caused by a non-interacting hydroxyl group show negative values or a value smaller than +0.6, even if positive. It might be suggestive for theoretical treatments of this problem, that, with respect to the methyl resonance spatially affected by neighboring hydroxyl group, the characteristic upward shift occurred in spite of the increased negative inductive effect by acetylation.

Source of this Interaction

With regard to the source of this spatial interaction effect, one might consider several reasons : 1) Weak hydrogen bonding between methyl protons and non-bonded electrons of oxygen atom, 2) electric field produced by C–O–H group,[7] and 3) magnetic anisotropy of a bonded oxygen atom containing both bonded and non-bonded electrons, and so on. Among them, magnetic anisotropy of a bonded oxygen atom might be regarded as playing a main part in this interaction effect, but it is still open to further investigation. Further studies along this line are now being pursued in these laboratories.

Application to Other Systems

The same phenomena can be expected to occur in other types of compounds. As an example, NMR data of pseudo-kobusine derivatives, dihydrodiketo compound (A) and its acetate (B), which were studied by Okamoto, *et al.*,[8] are given in Chart 3 (values are given in τ-unit).

The values show a remarkable downward shift for b-methyl of the compound (A), influenced by the neighboring hydroxyl group. Its acetylation on the other hand produced a strong upward shift (15.6 cps.).

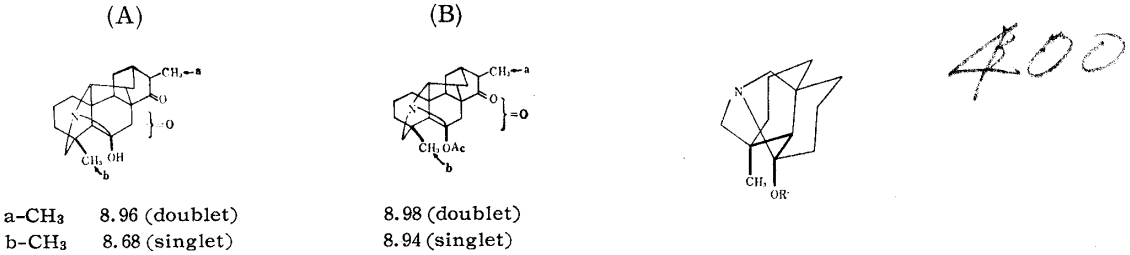
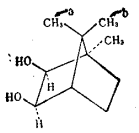


Chart 3.

In another example, the NMR spectra of four diastereoisomers of camphane-2,3-diols, which was reported by Anet,[9] seem to support the present observations. Among the three methyl signals given by each of the isomers, the highest methyl signal appears approximately at the same position in each spectrum, so that it may be attributed to a methyl group not interacted by any hydroxyls, designated as b-methyl in Chart 4. The lowest methyl signal in each spectrum can be seen to depend on the configurations of the two hydroxyl groups. Comparison of these lowest signals with each other leads to the conclusion that these methyl signals should belong to the a-methyl as designated in Chart 4. This signal of the compound A, which has two exo-hydroxyls close to the a-methyl, appears at the lowest field of others and that of the compound D, which has two endo-hydroxyls, occurred at the highest field compared with other three cases.

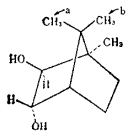
7) A. D. Buckingham : Canad. J. Chem., **38**, 300 (1960).
8) T. Okamoto, M. Natsume, H. Zenda, S. Kamata : Paper presented at the 5th Symposium on Natural Products of Chemistry, Sendai (1961).
9) F. A. C. Anet : Canad. J. Chem., **39**, 789 (1961).

(A)



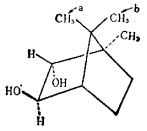
2-exo, 3-exo-isomer

(B)



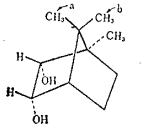
2-exo, 3-endo-isomer

(C)



2-endo, 3-exo-isomer

(D)



2-endo, 3-endo-isomer

Chart 4.

Conclusion

An application of NMR to stereochemistry has been found by considering the spatial interaction of functional groups to the proton resonance in a molecule. The spatial interaction effect of hydroxyl groups to methyl resonance has been considered in the present work. The dependence of this effect on the spatial vicinity of the two groups has been proved by comparison of the effect of hydroxyl groups located in various positions to a methyl group in a number of streoidal compounds. It has been found, moreover, that the acetylation effect on this interaction is useful for ascertaining the said effect. This interaction effect will be observed when a hydroxyl group is at or near the 1,3-diaxial position to the methyl group. More experimental data, however, will be needed for discussions of this effect in more detail, for instance, discussion of its dependence on the angle between the C-C bond of $C-CH_3$ and the C-O bond of C-OH, etc.

The authors express their deep gratitudes to Dr. Kenkichi Nukada and Mr. Akio Suzuki of the Government Chemical Industrial Research Institute of Tokyo, for making an NMR spectrometer available for the present work. They are also indebted to Dr. Shigenobu Okuda and Mr. Hiromichi Hotta for their helpful discussions. Thanks are also due to Dr. Yoshio Sato of National Institutes of Health, Bethesda, Md., U. S. A., for reviewing of this manuscript before publication. The compound No. 20 was kindly provided for this study by Dr. Ryozo Hayashi, Takamine Research Laboratory, Sankyo Co., Ltd.

Summary

The spatial vicinity of hydroxyl and methyl groups caused a remarkable downward shift for the methyl signal and this relationship was acertained by the acetylation of the hydroxyl group, causing a characteristic upward shift. This relationship might be applied to various ring systems, e. g. steroids, diterpenes, etc., predicting the spatial relation between hydroxyl and methyl groups.

(Received November 13, 1961)