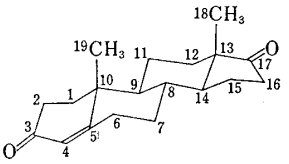
UDC 543.422.25[539.143.43]:547.92[577.176.3/.5]

57. Yutata Kawazoa,*1 Yoshihiro Sato,*2 Toshihiko Okamoto,*1 and Kyosuke Tsuda*2 : Application of Nuclear Magnetic Resonance to Stereochemistry. II.*3 Determination of the Conformation of Hydroxyl Groups by Nuclear Magnetic Resonance; Androst-4-en-3-one and Pregn-4-en-3-one Series.*4

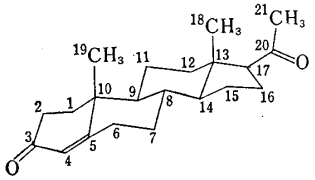
(*Faculty of Pharmaceutical Sciences, University of Tokyo**1 *and Institute of Applied Microbiology, University of Tokyo**2)

A number of proton magnetic resonance studies have been applied to stereochemistry, based on the fact that the magnetic resonance frequencies are very sensitive to changes of the spatial environments in the molecule. Among them, interesting is to apply nuclear magnetic resonance to the axial-equatorial assignment of alicyclic protons and it has already been carried out by many workers.[1~10] They found that there was a certain difference in the resonance frequencies between an axial and an equatorial proton, the resonance signal of the former falling in a higher field than that of the latter. This relationship, regarded as due to the anisotropic effect of C-C bonds, has been established by using simple model compounds, such as halocyclohexanes,[9,10] cyclohexanols[1,3] and decalols.[6,7] This regularity has previously been proved to be true for 3- and 11-positions of hydroxy steroids[2] and further applications have been tried by analogy to other positions of the same series of the compounds.[4,5,8] The limitations in the applications of this rule should, however, be clarified experimentally for various sorts of complex compounds, since the bond-anisotropy effect is very sensitive to even a small change in the geometry of the ring to which the proton under consideration is attached. In such circumstances, it must be very useful for organic chemists to collect the chemical shift data of the protons of this type from the conformational interest. And then, this collection would be of great help for the quantitative elucidation of C-C bond anisotropy.



Androst-4-ene-3,17-dione

Chart 1.



Pregn-4-ene-3,20-dione

Chart 2.

*1 Motofuji-cho, Bunkyo-ku, Tokyo (川添 豊, 岡本敏彦).
*2 Yayoi-cho, Bunkyo-ku, Tokyo (佐藤良博, 津田恭介).
*3 Part I : This Bulletin, **10**, 338 (1962).
*4 This paper constitutes Part II of a series entitled "Nuclear Magnetic Resonance Studies" by T. Okamoto and Y. Kawazoe and also Part XXXVI of a series entitled "Steroid Studies" by K. Tsuda.

1) R.U. Lemieux, R.K. Kullnig, H.J. Bernstein, W.G. Schneider : J. Am. Chem. Soc., **80**, 6098 (1958).
2) J.N. Shoolery, M.T. Rogers : *Ibid.*, **80**, 5121 (1958).
3) L.M. Jackman : "Application of Nuclear Magnetic Resonance in Organic Chemistry" p. 117, Pergamon Press, London (1960).
4) L.F. Fieser, T. Goto : J. Am. Chem, Soc., **82**, 1693 (1960).
5) L.F. Fieser, C. Yuan, T. Goto : *Ibid*,. **82**, 1996 (1960).
6) J.I. Musher : J. Chem. Phys., **35**, 1159 (1961).
7) *Idem* : J. Am. Chem. Soc., **83**, 1146 (1961).
8) R.C. Tweit, A.H. Goldkamp, R.M. Dodson : J. Org. Chem., **26**, 2856 (1961).
9) A.A. Bothner-By, C. Naar-Colin : J. Am. Chem. Soc., **80**, 1728 (1958).
10) E.L. Eliel : Chem. & Ind. (London), **1959**, 568.

In this paper, nuclear magnetic resonace measurements were made on 23 kinds of hydroxyl and acetoxyl derivatives of androst-4-ene-3,17-dione (Chart 1) and pregn-4-ene-3,20-dione (Chart 2), and the chemical shifts of CH protons adjacent to oxygen functions are summarized for the above purpose.

Experimental

The NMR spectra were obtained in dilute $CHCl_3$ solutions (ca. 10 mg. to 25 mg. in 0.5 cc. of the solvent) of the steroids with a Varian Associates DP-60 NMR spectrometer, operating at 60 Mc. p. s. The chemical shifts are given in the c.p.s. unit from the internal cyclohexane, the sign of the shift being chosen as positive when the resonance falls in a lower field than the reference. The details of the compounds used here are given in the experimental part of the reference 11.

It is important to compare the data with those from various laboratories where different standardizations were employed for spectrum calibrations. As most usual ways for calibrations concerning steroid studies have been used "internal tetramethylsilane,"[8,12,13,15] "external benzene,"[2,4,5,12,14] "external water,"[13,16] and "internal cyclohexane."[11] The chemical shifts reported in the references 2, 4, 5, 8, 11 to 16, and this paper were correlated with each other as possible, and the correlated values were obtained as follows:*5 (Values for 60 Mc.p.s.)

Ext. Benzene—Int. Tetramethylsilane = 384 c.p.s.
Ext. Benzene—Int. Cyclohexane = 298 c.p.s.
Ext. Benzene—Ext. Water = 102 c.p.s.
Int. Cyclohexane—Int. Tetramethylsilane = 86 c.p.s.
Ext. Water—Int. Tetramethylsilane = 282 c.p.s.
Ext. Water—Int. Cyclohexane = 196 c.p.s.
(Solvent $CHCl_3$—Int. Cyclohexane = ca. 349 c.p.s.)

These values seem to be applicable within an error of one c.p.s. to the data in the above literatures. It should, however, be noted that these values have been insured just for the dilute chloroform ($CHCl_3$ or $CDCl_3$) solutions of the steroids which have neither aromatic rings nor carboxylic groups.

Results and Discussions

The substances used for this study are listed in Table I, with their corresponding compound numbers. In Table II are summarized the chemical shifts*6 of CH protons adjacent to a hydroxyl or an acetoxyl group, classified in respect of their positions and configurations.

In Table III are shown the differences of the chemical shifts between the axial protons and their epimeric equatorial protons. Along with our data, those from other laboratories[2,7] are also included in the table. In the cases previously reported, the resonance signals of the axial protons appear at higher fields by more than 30 c.p.s. than that of the corresponding epimeric equatorial ones. But in our cases, although the higher field-appearance of the axial proton is true, the frequency differences between the epimeric protons seem to become decreased when they are located in the complicated environments in the molecule. As an extreme case, no difference was observed for 6-position in 3-keto-Δ^4-steroids where Δ^4-double bonds might magnetically affect 6α- and 6β-protons by different extents.

*5 In some literatures,[12,13,15] the same values were reported as the correlation values between different standardizations.

*6 Since each ring proton is spin-coupled with neighbouring protons, the signal appears sometimes as a low broad hump, so that it is difficult to determine the exact positions of the resonances. The measurement error, therefore, may become over one c.p.s. in some cases. But such an error does not seem to bring a serious mistake into the present discussion.

11) Y. Kawazoe, Y. Sato, M. Natsume, H. Hasegawa, T. Okamoto, K. Tsuda: This Bulletin, **10**, 338 (1962).
12) J. S. G. Cox, E. O. Bishop, R. E. Richards: J. Chem. Soc., **1960**, 5118.
13) G. Slomp, F. A. McKellar: J. Am. Chem. Soc., **84**, 204 (1962).
14) W. E. Rosen, J. B. Ziegler, A. C. Shabica, J. N. Shoolery: *Ibid.*, **81**, 1687 (1959).
15) R. F. Zürcher: Helv. Chim. Acta., **44**, 1380 (1961).
16) G. Slomp, B. R. McGarvey: J. Am. Chem. Soc., **81**, 2200 (1959).

Table I. List of the Compounds Examined

Compd. No.	Compd.	Compd. No.	Compd.
1	6α-OH-Androst-4-ene-3,17-dione	12	15α-OH-Pregn-4-ene-3,20-dione
2	6α-OAc-Androst-4-ene-3,17-dione	13	15α-OAc-Pregn-4-ene-3,20-dione
3	6β-OH-Androst-4-ene-3,17-dione	14	15β-OH-Pregn-4-ene-3,20-dione
4	6β-OAc-Androst-4-ene-3,17-dione	15	15β-OAc-Pregn-4-ene-3,20-dione
5	11α-OH-Androst-4-ene-3,17-dione	16	6β,11α-Di-OAc-pregn-4-ene-3,20-dione
6	11β-OH-Androst-4-ene-3,17-dione	17	6β,15β-Di-OH-pregn-4-ene-3,20-dione
7	11β-OAc-Androst-4-ene-3,17-dione	18	6β,15β-Di-OAc-pregn-4-ene-3,20-dione
8	17β-OH-Androst-4-ene-3-one (Testosterone)	19	6β-OAc-15β-OH-Pregn-4-ene-3,20-dione
		20	6α-OAc-15β-OH-Pregn-4-ene-3,20-dione
9	17β-OAc-Androst-4-ene-3-one	21	7β,15β-Di-OH-pregn-4-ene-3,20-dione
10	11α-OH-Pregn-4-ene-3,20-dione	22	7β-OAc-15β-OH-pregn-4-ene-3,20-dione
11	11α-OAc-Pregn-4-ene-3,20-dione	23	7α,15β-Di-OAc-pregn-4-ene-3,20-dione

Table II. Chemical Shifts of the Protons Adjacent to Oxygen Functions

Position and Configuration	Functional Group on the Same Carbon Atom	Chemical Shifts (c.p.s.) R=H (No.)	Average	R=$COCH_3$ (No.)	Average
6α (equatorial)	6β-OR	179-S[a] (3)		244-S (4)	
		176-S (17)	178±2	240-S (16)	
				240-S (19)	
				236-S (18)	240±4
6β (axial)	6α-OR	178-B[b] (1)		243-B (2)	
				241-B (20)	242±1
7α (axial)	7β-OR	129-B (21)		193-B (22)	
7β (equatorial)	7α-OR			210-S (23)	
11α (equatorial)	11β-OR	180-S (6)		241-S (7)	
11β (axial)	11α-OR	157-B (5)		223-B (11)	
		157-B (10)	157±0	? (16)[c]	
15α (equatorial)	15β-OR	172-S (14)		222-S (15)	
		173-S (20)		222-S (18)	
		171-S (17)		210-S (23)[d]	222±0
		172-S (19)			
		182-S (21)[d]			
		159-S (22)[d]	172±1		
15β (axial)	15α-OR	160-B (12)		211-B (13)	
17α (axial)	17β-OR	132-B (8)		182-B (9)	

a) "S" means that the half-width of the signal is smaller than 12 c.p.s. (0.20 p.p.m.).
b) "B" means that the half-width of the signal is larger than 16 c.p.s. (0.27 p.p.m.).
c) The corresponding signal could not be found in the spectrum because of very low concentration of the solution of this compound.
d) The resonance frequencies of 15α-protons must be strongly affected by 7 (especially, 7β)-oxygen functions since they are closely located to each other.[11] These values were, therefore, excluded out of the averaged values. The detail of this irregularity will be discussed in the forthcoming paper.

Table III. Frequency Differences between the Conformational Isomers

Position	$\Delta\nu_{axial-equatorial}$ (c.p.s.) -CH-OH	-CH-OAc
6	0	2
7	—	17
11	23	18
15	12	11
2-decalols[a]	32	—
3-OH-steroids[b]	33	—

a) data from ref. 7. *b*) data from ref. 2.

Fig. 1 shows the chemical shifts of these protons graphically. These data will be usefully referred in determining the position and conformation of an unknown hydroxyl group in these series of steroids. Thus, the axial protons on 7- and 17-positions may be distinguished from those at other positions by the higher field-apperance of their resonance signals.

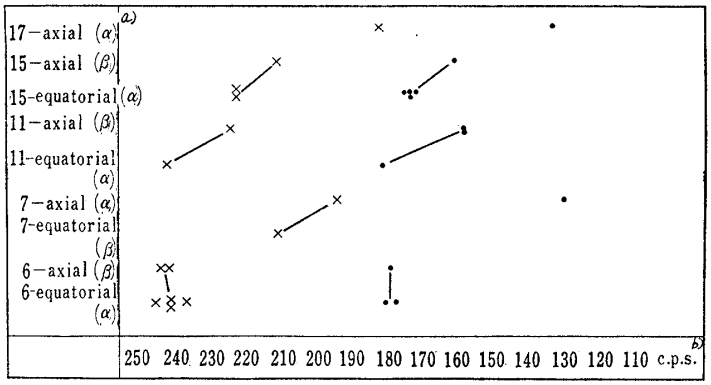


Fig. 1. Chemical Shifts of "-CH-OR" plotted in Respect of their Locations in Androst-4-ene-3,17-diones and Pregn-4-ene-3,20-diones
(•: R=H, ×: R=$COCH_3$)

a) configuration of the proton adjacent to oxygen function
b) c.p.s. from the signal of the internal cyclohexane

Another nuclear magnetic resonance criterion is provided for determining the conformation of a hydroxyl group by the half-width of the signal of the proton adjacent to the hydroxyl group in question. The acetoxyl derivatives can take the place of the hydroxyl compounds for this purpose. The signals of these protons are classified to "S (sharp)" and "B (broad)." These symbols are given in Table II along with the chemical shifts. All of what have half-widths smaller than 12 c.p.s. (0.20 p.p.m.) are represented by "S," while all of what have those larger than 16 c.p.s. (0.27 p.p.m.) are denoted by "B."*7 It is shown to be true even in complex molecules that the signals of axial protons are always much broader than those of equatorial ones, as shown with mono and bicyclic system by Lemieux[1] and others. This relationship might be generalized for extensive applications, provided that the proton may be situated so as to be spin-coupled with more than three spins, such as $-CH_2-\underset{|}{\overset{OR}{C}}H-CH-$, $-CH=\underset{|}{\overset{OR}{C}}-\underset{|}{\overset{OR}{C}}H-CH_2-$ and so on.*8,*9 This fact seems to be reasonable in view of the result obtained by Karplus,[17] using the valence bond method. This regularity must be also useful to assign the proton to be axial or equatorial.

In Table IV are shown the downward shifts of CH protons adjacent to a hydroxyl group caused by its acetylation. It is shown that the magnitudes of the acetylation shifts seem to be characteristic with respect to the position and configuration of the hydroxyl group. The position and configuration-dependence of resonance frequencies are very strict, the deviations from the averaged values being only a half c.p.s. as far

*7 As the signals were usually very weak because of low concentration of the solutions examined, it was difficult to measure the exact half-width of each signal. Therefore, the values for the ranges, 12 c.p.s. and 16 c.p.s., are selected as the upper and lower bounds, respectively, regarding only sufficiency. They may be reduced to more precise ones.

*8 In most cases, where only two of neighbouring protons are taking part of the spin coupling, the resonance signal seems to be splitted into separate lines and the half-width of the whole bunch of signals appears rather sharp whichever the protons concerned may be axial or equatorial.[18]

*9 An exception toward this regularity was reported by Musher[7] in a case of decalol derivative, although no irregularity were observed so far as our available compounds concern.

17) M. Karplus: J. Chem. Phys., **30**, 11 (1959); J. Phys. Chem., **64**, 1793 (1960).

18) Our unpublished data.

Table IV. Acetylation Shifts

Position and Configuration of Oxygen Function	Acetylation Shift of Proton on the Same Carbon Atom (c.p.s.)
6α	65 (No. 2—No. 1)
6β	65 (No. 4—No. 3), 64 (No. 19—No. 17), 64 (No. 16—No. 17)
7β	64 (No. 22—No. 21)
11α	66 (No. 11—No. 10)
11β	61 (No. 7—No. 6)
15α	51 (No. 13—No. 12)
15β	50 (No. 15—No. 14), 50 (No. 18—No. 19), 51 (No. 18—No. 17)
17β	50 (No. 9—No. 8)

as our present samples concern. To be interesting, moreover, the acetylation shift seems to depend on the size of the ring, five or six member, on which the hydroxyl group is located. All the acetylation shifts which were observed for six membered ring protons adjacent to secondary hydroxyl groups, i.e., 6α, 6β, 7β, 11α, and 11β hydroxyls fall between 61 c.p.s. and 66 c.p.s., while those of five membered ring protons adjacent to 15α, 15β, and 17β hydroxyls fall between 50 c.p.s. and 51 c.p.s. The fact that there is a remarkable difference in the acetylation shifts between five and six membered hydroxyls suggests that this relationship may be applied to determine the size of the ring, five or six, on which an unknown hydroxyl group is located. But it is still open to further investigation to make clear whether this result could be applied to other ring systems than the steroid.

Conclusion

The chemical shifts of the protons adjacent to a hydroxyl or an acetoxyl group are characteristic for the position and configuration in which the said protons are situated. The frequency differences between axial protons and the epimeric equatorial ones are also characteristic for the position at which the said hydroxyl groups are located. The acetylation shifts of the protons adjacent to hydroxyl groups seem to depend on the size of the ring, which the oxygen functions are bonded to. The above results might be applied, along with the broadness of the signals depending on the conformations of the protons, for characterization of unknown hydroxyl groups of the steroidal molecules.

The authors express their deep gratitudes to Mr. Hiromichi Hotta of University of Tokyo for his helpful discussion. Thanks are also due 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.

Summary

It is shown from the nuclear magnetic resonance data of 23 kinds of androst-4-ene and pregn-4-ene derivatives that nuclear magnetic resonance can be applied to determination of the position and the configuration of unknown hydroxyl groups in steroidal molecules.

(Received April 10, 1962)