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Carbon-13 Nuclear Magnetic Resonance of 24-Substituted Steroids[1)]

NAOYUKI KOIZUMI,[2a)] YOSHINORI FUJIMOTO,[2a)] TORU TAKESHITA,[2b)] and NOBUO IKEKAWA[2a)]

Laboratory of Chemistry for Natural Products, Tokyo Institute of Technology[2a)] and Teijin Institute of Biomedical Research[2b)]

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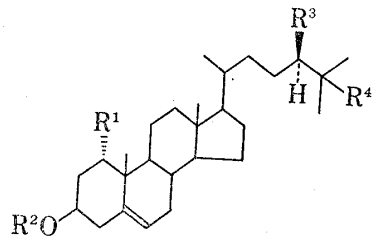
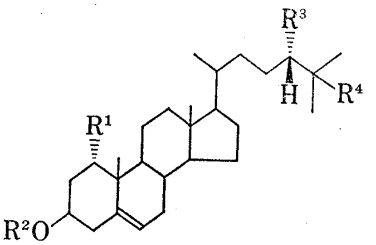
^{13}C-NMR spectra of epimeric pairs of 24-hydroxy-, 24,25-dihydroxy-, 1,24-dihydroxy-, 24-methyl- and 24-ethyl-cholesterols were measured and the signals of all carbons assigned. Most of the side chain carbons in the C-24 epimeric mixtures showed split signals and characteristic differences between 24*R* and 24*S* isomers were recognized for the C-20 and C-24 signals, these resonances in the 24*R* compounds being always at slightly higher field than in the 24*S* isomers.

Keywords——^{13}C-NMR of steroids; configuration at C-24 position of steroids; [24*R*]- and [24*S*]-24-hydroxycholesterols; [24*R*]- and [24*S*]-24-alkylcholesterols; stereochemical assignments

During the course of our studies on the synthesis of [24*R*]- and [24*S*]-24-hydroxy and alkyl substituted cholesterols, it was found that the ^{13}C-nuclear magnetic resonance (NMR) is useful for stereochemical assignment of the C-24 epimers and some of data was already reported.[3)] In this paper we would like to report the assignments of all carbons of epimeric pairs of 24-hydroxy- (**1**, **2**), 24,25-dihydroxy- (**3**), 1,24-dihydroxy- (**4**, **5**), 24-methyl- (**6**), and 24-ethyl- (**7**) cholesterol derivatives. The usefulness of ^{13}C-NMR spectra for determination of the configuration at C-22 of 22-substituted cholesterols have been reported by Letourneux *et al.*[4)]



24*R* series
1a: R¹=H, R²=H, R³=OH, R⁴=H
2a: R¹=H, R²=Bz, R³=OBz, R⁴=H
3a: R¹=H, R²=Bz, R³=OBz, R⁴=OTMS
4a: R¹=OH, R²=Bz, R³=OBz, R⁴=H
5a: R¹=OAc, R²=Bz, R³=OBz, R⁴=H
6a: R¹=H, R²=H, R³=Me, R⁴=H
7a: R¹=H, R²=H, R³=Et, R⁴=H



24*S* series
1b: R¹=H, R²=H, R³=OH, R⁴=H
2b: R¹=H, R²=Bz, R³=OBz, R⁴=H
3b: R¹=H, R²=Bz, R³=OBz, R⁴=OTMS
4b: R¹=OH, R²=Bz, R³=OBz, R⁴=H
5b: R¹=OAc, R²=Bz, R³=OBz, R⁴=H
6b: R¹=H, R²=H, R³=Me, R⁴=H
7b: R¹=H, R²=H, R³=Et, R⁴=H

Chart 1

1) This is Part 49 in the series of "Studies on Steroids." Part 48 see M. Ishiguro, H. Saito, and N. Ikekawa, *J. Chem. Soc., Perkin I*, in press.
2) Location: *a) Nagatsuta, Midori-ku, Yokohama 227, Japan*; *b) Hino-shi, Tokyo 191, Japan.*
3) N. Koizumi, M. Morisaki, N. Ikekawa, A. Suzuki, and T. Takeshita, *Tetrahedron Lett.*, **1975**, 2203.
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TABLE I. Chemical Shifts of ^{13}C-NMR

Compd.	Cholesterol	Cholesterol 3-Bz	1α-OH-Cholesterol 3-Bz	24(*R*)-OH **1a**	24(*S*)-OH **1b**	24(*R*)-OBz 3-Bz **2a**	24(*S*)-OBz 3-Bz **2b**	24(*R*)-OBz 25-OTMS 3-Bz **3a**	24(*S*)-OBz 25-OTMS 3-Bz **3b**	24(*R*)-OBz 1α-OH 3-Bz **4a**	24(*S*)-OBz 1α-OH 3-Bz **4b**	24(*R*)-OBz 1α-OAc 3-Bz **5a**	24(*S*)-OBz 1α-OAc 3-Bz **5b**	24(*R*)-Me **6a**	24(*S*)-Me **6b**	24(*R*)-Et **7a**	24(*S*)-Et **7b**
C-1	37.8	38.1	72.5	37.1	37.1	38.2	38.2	38.4	38.4	72.4	72.4	74.3	74.3	37.3	37.3	37.1	37.1
C-2	31.9	28.1	34.6	31.5	31.5	27.9	27.9	28.0	28.0	34.7	34.7	31.9	31.9	31.7	31.7	31.5	31.5
C-3	71.6	74.3	70.1	71.5	71.5	74.4	74.5	74.8	74.9	70.0	70.1	69.8	69.8	71.8	71.8	71.5	71.4
C-4	42.7	37.0	37.3	42.2	42.1	37.0	37.0	37.1	37.2	37.3	37.3	37.3	37.3	42.4	42.4	42.1	42.1
C-5	141.5	139.3	136.0	140.1	140.1	139.3	139.4	140.1	140.2	136.0	136.0	135.4	135.4	140.7	140.7	140.1	140.1
C-6	121.6	122.4	126.3	121.1	121.0	122.4	122.5	123.1	123.2	126.3	126.3	124.7	124.7	121.7	121.7	121.1	121.1
C-7	32.3	31.8	31.8	31.8	31.8	31.8	31.8	32.0	32.0	31.7	31.7	31.6	31.6	31.9	31.9	31.8	31.7
C-8	32.3	31.8	31.8	31.8	31.8	31.8	31.8	32.0	32.0	31.7	31.7	31.6	31.6	31.9	31.9	31.8	31.7
C-9	50.8	49.9	41.5	49.9	49.9	49.9	50.0	50.2	50.3	41.5	41.5	41.9	41.9	50.2	50.1	49.9	49.9
C-10	36.8	36.6	41.8	36.3	36.3	36.6	36.6	36.7	36.8	41.8	41.7	40.3	40.3	36.5	36.5	36.3	36.3
C-11	21.5	21.0	20.3	21.0	21.0	21.0	21.1	21.1	21.2	20.2	20.2	20.3	20.3	21.1	21.1	21.0	20.0
C-12	40.3	39.7	39.5	39.6	39.6	39.6	39.7	39.8	39.9	39.4	39.3	39.4	39.4	39.8	39.8	39.6	39.6
C-13	42.7	42.2	42.3	42.2	42.1	42.2	42.2	42.5	42.5	42.2	42.2	42.1	42.1	42.4	42.4	42.1	42.1
C-14	57.2	56.5	56.5	56.5	56.5	56.5	56.6	56.8	56.9	56.4	56.4	56.4	56.4	56.8	56.8	56.5	56.5
C-15	24.6	24.2	24.3	24.2	24.2	24.2	24.3	24.3	24.4	24.3	24.3	24.1	24.1	24.3	24.3	24.2	24.2
C-16	28.6	28.1	28.0	28.1	28.1	28.1	28.1	28.0	28.1	28.0	28.1	27.9	27.9	28.2	28.2	28.1	28.1
C-17	56.8	56.0	56.1	55.8	55.7	55.6	55.6	56.1	56.1	55.6	55.6	55.4	55.4	56.2	56.0	55.8	55.8
C-18	12.3	11.8	11.9	11.8	11.8	11.8	11.8	11.9	11.9	11.8	11.8	11.8	11.8	11.9	11.9	11.8	11.8
C-19	19.7	19.3	19.4	19.3	19.3	19.3	19.3	19.4	19.5	19.4	19.4	19.4	19.4	19.4	19.4	19.3	19.3
C-20	36.1	35.7	35.7	35.6	35.8	35.3	35.6	35.3	36.0	35.3	35.6	35.2	35.5	35.9	36.1	36.0	36.1
C-21	19.1	18.6	18.7	18.8	19.0	18.6	18.7	18.6	19.0	18.6	18.7	18.5[a]	18.6[a]	18.7	18.9	18.7	18.7
C-22	36.7	36.1	36.1	31.9	32.1	31.6	31.2	32.3	32.9	31.5	31.5	31.1	31.1	33.8	33.8	33.8	33.6
C-23	24.4	23.8	23.8	30.4	30.6	27.3	27.3	25.6[a]	24.7[a]	27.6	27.8	27.3	27.4	30.3	30.6	26.0	26.3
C-24	39.9	39.6	39.5	76.6	77.0	79.2	79.6	80.7	81.7	79.2	79.6	79.0	79.4	38.9	39.1	45.6	45.8
C-25	28.3	27.9	28.0	33.4	33.0	31.6	31.1	75.6	75.7	31.3	31.3	31.1	31.1	32.4	31.5	29.0	28.8
C-26	22.8	22.5	22.5	17.2	16.7	17.7	17.3	25.9[a]	25.0[a]	17.8	17.3	17.6	17.3	18.2	17.6	19.0	18.9
C-27	23.1	22.8	22.7	18.6	18.8	18.6	18.9	27.3	27.3	18.6	18.7	18.6[a]	18.8[a]	20.2	20.5	19.7	19.5
C-28														15.4	15.5	23.0	22.9
C-29																11.9	12.2

a) These assignments may be interchangeable.
Carbon resonances of benzoate: 128.3±0.5, 129.6±0.5, 130.8±0.5, 132.7±0.5 and 165.5±0.5.
Carbon resonances of acetate: 21.0 and 169.5±0.03.
Carbon resonances of trimethylsilyl group: 2.5.

We have recently synthesized the epimeric pairs of 24-hydroxy-,[5] 24,25-dihydroxy-,[6] 1,24-dihydroxy-[7] and 1,24,25-trihydroxy-vitamin D_3[8] and demonstrated stereochemical importance of C-24 position for eliciting biological activities: the 24*R* isomers are more active than the 24*S* isomers.[9–11] Separation of those epimers and determination of their configuration on C-24 position were achieved with various 24-hydroxycholesterol derivatives which were the synthetic precursors of the corresponding vitamin D_3. ^{13}C-NMR spectra of those 24-hydroxycholesterols were measured for application to stereochemical determination. Although, C-24 epimers of 24,25-dihydroxy derivatives were distinguished by ^{1}H-NMR,[6] this technique could not differentiate the C-24 epimers of 24-hydroxy derivatives.[5] On the other hand, the configuration on C-24 of natural phytosterols having methyl or ethyl group at C-24 can be determined by ^{1}H-NMR,[12–14] but the ^{13}C-NMR should be also useful for this purpose. Recently we have developed a facile method for synthesis of 24*R*- and 24*S*-alkylcholesterols[15] and a diagnostic difference was observed in the ^{13}C chemical shifts of the side chain carbons in the epimeric pairs. Though the difference involved is small, it may be quite useful for stereochemical assignment when the epimers can be compared.

Chemical shift data examined are listed in Table I. Assignments were based on proton decoupled and off resonance spectra and on comparison with data of the cholesterol derivatives.[16] The assignments of carbon resonances of 24-alkylcholesterols were also confirmed by application of Lindeman and Adams' parameter.[17] ^{13}C-Resonances of ergostane have been reported,[18] but the assignments have been revised by making use of the shieldings for 2,3-dimethylhexane.[16,17]

Most of the side chain carbons in the C-24 epimeric mixtures showed split signals. For examples, in the spectra of [24*R*]- and [24*S*]-24-methylcholesterols chemical shifts of C-20, 21, 23, 24, 25, 26 and 27 as well as C-17 were slightly different as shown in Fig. 1. Similar

Table II. Chemical Shift Difference at C-20 and C-24 between the Two Series

Compound	**1a**	**1b**	**2a**	**2b**	**3a**	**3b**	**4a**	**4b**	**5a**	**5b**	**6a**	**6b**	**7a**	**7b**
Configuration on C-24	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*
C-20 resonance	35.6	35.8	35.3	35.6	35.3	36.0	35.3	35.6	35.2	35.5	35.9	36.1	36.0	36.1
$\Delta(\delta_S-\delta_R)$	0.2		0.3		0.7		0.3		0.3		0.2		0.1	
C-24 resonance	76.6	77.0	79.2	79.6	80.7	81.7	79.2	79.6	79.0	79.4	38.9	39.1	45.6	45.8
$\Delta(\delta_S-\delta_R)$	0.4		0.4		1.0		0.4		0.4		0.2		0.2	

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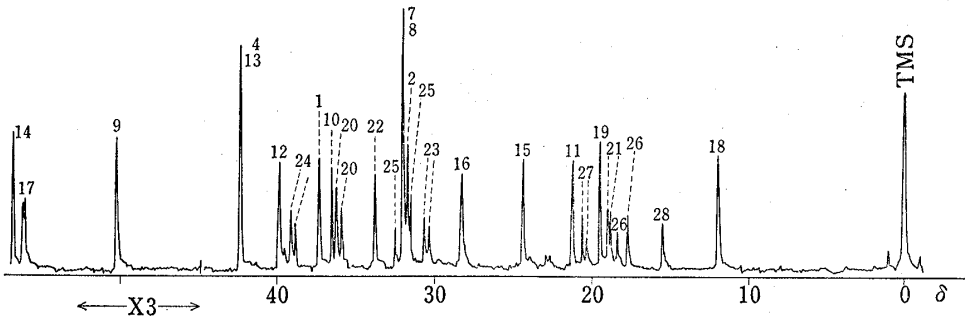

Fig. 1. Carbon Resonances of Epimeric Mixture of 24-Methylcholesterol (**6a** and **6b**)

results for sitosteryl and clionasteryl acetates were reported recently.[19] Characteristic differences between 24*R* and 24*S* isomers were recognized on both C-20 and C-24 resonance (Table II). Those resonance of the 24*R* compounds were always observed in higher field than that of 24*S* isomers. It is interesting to note that this phenomenon was observed in spite of existence to two methylenes between asymmetric centers. The different range between each epimers was at most 1.0 ppm at C-24 and 0.7 ppm at C-20 for 24,25-dihydroxycholesterol 24-benzoate 25-TMS ether (**3**), and at least 0.2 ppm at C-24 and 0.1 ppm at C-20 for 24-ethylcholesterol (**7**). These diagnostic effects might be thought to be due to the gaushe interaction (γ-effect)[20] between C-20 and C-24, caused by the different population of the conformers in the 24-epimeric side chain. All-anti zigzag conformer is considered to be most favorable (I and II). Concerning on the C_{22}–C_{23} bond, conformers (III) and (IV) represent the next favorable for 24*R* and 24*S* compounds, respectively. One of the possible explanations of the upfield shift of 24*R*-series may be obtained by the assumption that the population of the conformer (IV) of *S*-series might be diminished by 1,3-interaction between the C-24 substituent and C-20 in comparison with the conformer (III) for *R*-series. In view of a new interpretation of γ-effect,[21] the conformer (III) may also cause upfield shift of the C-20 and C-24 signals by the effect. It has been explained clearly that chemical shift deference on C-23 between 22-substituted epimers was dependent on the conformation of steroidal side chain.[22]

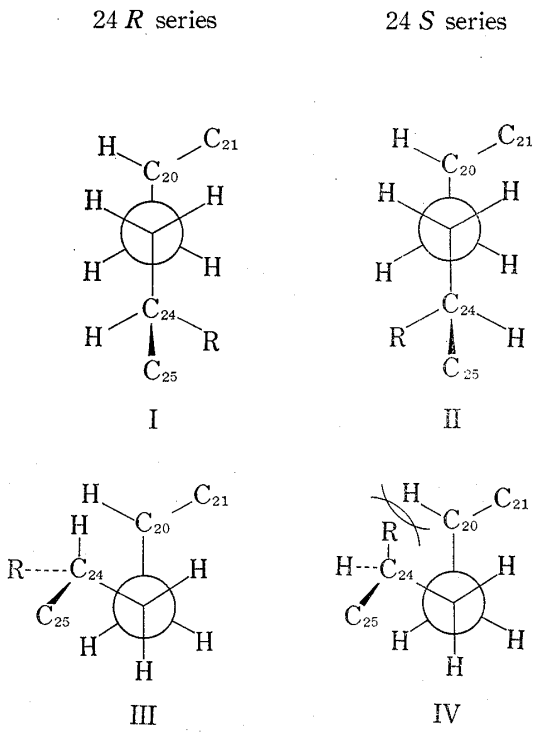

Chart 2

Experimental

Material——Epimeric pairs of 24-hydroxycholesterol (**1a** and **1b**),[5] 24-hydroxycholesterol dibenzoate (**2a** and **2b**),[5] 24,25-dihydroxycholesterol 3,24-dibenzoate 25-trimethylsilyl ether (**3a** and **3b**),[6] 1,24-dihy-

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droxycholesterol 3,24-dibenzoate (**4a** and **4b**),[7] 1,24-dihydroxycholesterol 1-acetate 3,24-dibenzoate (**5a** and **5b**),[7] 24-methylcholesterol (campesterol (**6a**) and dihydrobrassicasterol (**6b**)),[15] 24-ethylcholesterol (sitosterol (**7a**) and clionasterol (**7b**))[15] were all chemically synthesized. 1α-Hydroxycholesterol 3-benzoate was prepared from 1α-hydroxy-cholesterol;[23] mp 172—173.5°.

^{13}C-NMR Measurements——^{13}C-NMR spectra were recorded with a JEOL PS/PFT-100 spectrometer at 25.2 MHz in $CDCl_3$ using 10 mm spinning tubes at 30° with tetramethylsilane as internal standard. Data were accumulated with a maximum of 0.61 Hz per data point. The spectra were determined as 0.1—0.05 M solutions and sample concentrations of each epimeric pair were almost same.

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