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ALLYLIC STEROID ALCOHOLS. I. STRUCTURES OF THE PRODUCTS RESULTING FROM 7-HYDROXYCHOLESTEROL IN THE MEDIUM OF METHANOL-HYDROCHLORIC ACID.

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Abstract

By leaving 7α- and 7β-hydroxy-cholesterol in methanol in the presence of a trace of hydrochloric acid at room temperature, the formation of two compounds was represented by the analysis of gas liquid chromatography. These compounds were isolated by the column chromatography and the chemical structures of them were identified with 7α- and 7β-methoxy-cholesterol, respectively.

INTRODUCTION

In the biosynthetic pathway from cholesterol to bile acids 7α-hydroxycholesterol is an important intermediate. Recently, M. J. Kulig et al.[1] found 7-alkoxy-cholesterol in the study of the sterols in the human aortal tissues, which was considered to be derived from 7-hydroxycholesterol during the treatment of acidification of the hydrolyzate. On the other hand, H. B. Henbest and E. R. Jones once reported that in the replacement reactions of 7-halogeno-cholesterol derivatives the introduction of 7-alkoxyl group by the replacement reaction took place easily, in which it was considered that the formation of 7-hydroxycholesterol and subsequently, the alkylation in alcoholic solution in the presence of acid occurred[2]. In the study of the intermediates and by-products in the metabolism from cholesterol to bile acids, the investigation of the artifacts expected during the experimental procedures might be significant and useful to detect the metabolites in the pathway. This paper deals with the chemical structures of two products resulting from the epimeric 7-hydroxycholesterol in methanolic solution in the presence of a trace of hydrochloric acid.

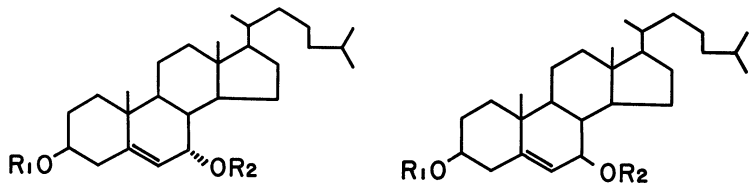
EXPERIMENTAL PROCEDURES

All the melting points were measured with a Yanagimoto micro-melting point apparatus but are uncorrected. The infrared spectra

were measured in Nujol with a Hitachi infrared spectrophotometer model 285.

Specific Rotation $[\alpha]_D$. $[\alpha]_D$ measurements were determined in methanol with a Yanagimoto automatic digital polarimeter model OR-50D.

Thin Layer Chromatography. A thin layer chromatography was conducted on chromatoplate of Silica Gel H (E. Merck, Darmstadt), 0.25 mm thick, irrigated with isooctane-ethyl acetate-acetic acid (20 : 40 : 1, by volume) mixture, using a general technique. Sterols were detected by spraying the dried chromatoplate with 10 % sulfuric acid-acetic acid. Instant blue colors were obtained with all of sterols Ia, Ib, IIa and IIb (Fig. 1) involved in this study. The parent sterols Ia, IIa and



Ia $R_1=R_2=H$

Ib $R_1=H$ $R_2=Me$

Ic $R_1=Ac$ $R_2=Me$

IIa $R_1=R_2=H$

IIb $R_1=H$ $R_2=Me$

IIc $R_1=Ac$ $R_2=Me$

Fig. 1. The structures of sterols.

other exchanged sterols were resolved in the system isooctane-ethyl acetate-acetic acid, using ascending irrigation, giving mobility data (Rf) as follows: Ia, 0.35; Ib, 0.60; IIa, 0.40; IIb, 0.61.

Gas Liquid Chromatography. The analysis of gas liquid chromatography was conducted on silanized glass column (4 mm×2 m) packed with 0.75 % SE-52 on 80-100 mesh chromosorb W, using a Shimadzu gas chromatography model 4BPTF equipped with a hydrogen flame ionization detector. Injection temperature was 250°C, column temperature 230°C and detector temperature 250°C. Nitrogen was used as carrier gas at flow rate of 88 ml/min. The retention time (t_R) data were obtained from the time of injection point.

Mass Spectrometry. The mass spectra of the compounds were determined by the gas chromatography-mass spectrometry and the direct insertion probes with a Hitachi mass spectrometer model RMU-6MG.

Nuclear Magnetic Resonances. Ths NMR spectra were measured in deuterochloroform with a Hitachi NMR spectrometer model R20, using tetramethylsilane as internal standard (δ-values). The determination of the existence and chemical shifts of the hydroxyl group was carried out by adding deuterium oxides.

Preparation of Epimeric 7-Hydroxycholesterol.

a) *7α-Hydroxycholesterol* (Ia). N-Bromosuccinimide (200 mg) was added to a solution of cholesteryl acetate (400 mg) in refreshed carbon tetrachloride (40 ml). The mixture was refluxed under ultraviolet irradiation for 8 min, while the reaction mixture was being stirred. After cooling, the separated succinimide was filtered off and washed with a small amount of carbon tetrachloride. Alumina (inactivated with ethyl acetate) (10 g) was added to the filtrate and kept stirring at room temperature for 2 hr. The filtrate which was filtered off alumina was condensed under reduced pressure to dryness to give a crude 7-bromocholesteryl acetate. The crude compound was employed to the next reaction without any purification. 4N Potassium hydroxide solution (15 ml) was added to a solution of crude bromo-compound in methanol (15 ml) and then the mixture was allowed to stand overnight at room temperature. Methanol was removed under reduced pressure as much as possible. The residual solution was acidified with 2N hydrochloric acid and extracted with ether. The ethereal extract was sufficiently washed with water and dried on anhydrous sodium sulfate. The solvent was evaporated and the residual solid was crystallized from methanol to give 7α-hydroxycholesterol (Ia). The further purification of Ia was carried out by the column chromatography on Silica Gel (E. Merck, Darmstadt, Kieselgel type 60, 230 mesh) in chloroform. Recrystallization from methanol gave crystals as colorless needles with mp 180–182°C, $[\alpha]_D$ −89° (lit. mp 182–183°C, $[\alpha]_D$ −92° (Methanol)[3,4], mp 188–189°C, $[\alpha]_D$ −91°[5]); yield, 60 mg.

b) *7β-Hydroxycholesterol* (IIa).7 β-Hydroxycholesterol was provided by the reduction of 7-keto-cholesteryl acetate[6], which was prepared from cholesteryl acetate by the oxidation in *tert*-butanol with *tert*-butyl chromate, in dioxane-ethanol with sodium borohydride. The further purification was carried out by the column chromatography on Silica Gel (described above) in chloroform. The recrystallization from methanol gave crystals as colorless needles with mp 176°C, $[\alpha]_D$ +13° (lit.[7] mp 175–176°C, $[\alpha]_D$ +7.6° (Methanol)).

Treatment of 7-Hydroxycholesterol in Methanol with Hydrochloric Acid and Isolation of 7-Methoxycholesterols.

a) 7α-Hydroxycholesterol (100 mg) was dissolved in methanol (10 ml) and one drop of 2N hydrochloric acid was added to the solution, and then the mixture was allowed to stand at room temperature for 2 hr. The reaction mixture was diluted with water, neutralized or alkalized with saturated sodium bicarbonate solution and extracted with ether, successively. The ethereal extract was sufficiently washed with water and dried on anhydrous sodium sulfate. The solvent was evaporated to dryness and the residual solid was chromatographed on 0.25 mm thick chromatoplate using the solvent system described above. The new spot appeared at Rf 0.60, exhibiting two peaks by the analysis of gas liquid chromatography. The solid was chromatographed on Silica Gel to fractionate 7α-methoxycholesterol (Ib, colorless needles: yield, 20 mg. mp 162-163°C, $[\alpha]_D$ −100° (lit. mp 158-160°C[1], mp 158-159°C, $[\alpha]_D$ −127°[2], mp 158-160°C, $[\alpha]_D$ −127°[6]). IR cm^{-1}: 3430 (OH); Acetate (Ic), mp 110-111°C, $[\alpha]_D$ −120° (lit. mp 111°C $[\alpha]_D$ −124°)) and 7β-methoxycholesterol (IIb, colorless needles; yield, 15 mg. mp 149-150°C, $[\alpha]_D$ +8.8° (lit. mp 144-146°C[1], mp 146-147°C, $[\alpha]_D$ +22.9°[2]). IR cm^{-1}: 3200 (OH); Acetate (IIc), mp 90-92°C, $[\alpha]_D$ +15° (lit.[2] mp 93-94°C, $[\alpha]_D$ +18.2°)), respectively.

b) 7β-Hydroxycholesterol was treated by the similar method for 4 hr. The reaction mixture was fractionated to give the corresponding compounds to the sterols Ib and IIb.

RESULTS AND DISCUSSION

The reaction mixtures formed by the treatment of 7α- (Ia) and 7β-hydroxy-cholesterol (IIa) in methanol containing a trace of hydrochloric acid at the temperature in the room at various time were checked by the development on the thin layer chromatoplate (Fig. 2.) In the treatment, Ia was completely diminished by being converted into the new compound for 1 hr, showing one spot (Rf=0.60) on the plate, but the complete conversion of IIa took 4 hr. However, although the new product showed one spot on the chromatoplate, the analysis of the product by the gas liquid chromatographic means, after acetylation with pyridine-acetic anhydride method, showed two peaks (Fig. 3-1, peak a t_R=20 min and peak b, t_R=24 min) and the area of the latter peak (b) being enhanced along with increasing reaction time. The mass spectra of the acetates corresponding to the peaks a and b obiained by a combined gas

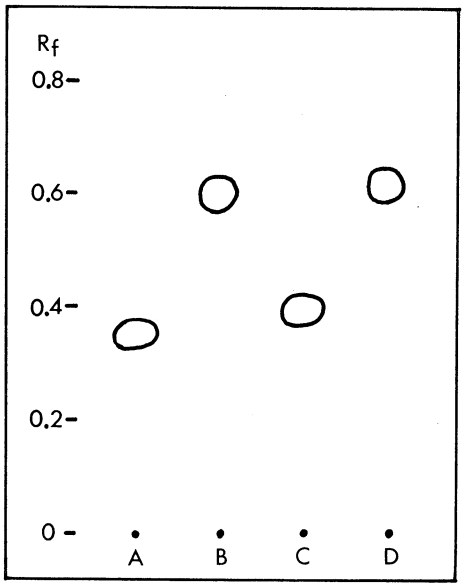


Fig. 2. Thin layer chromatogram of 7-hydroxy- and 7-methoxy-cholesterols, colored by spraying of 10 % sulfuric acid-acetic acid.
A: 7α-Hydroxycholesterol (Ia). B: 7α-Methoxycholesterol (Ib).
C: 7β-Hydroxycholesterol (IIa). D: 7β-Methoxycholesterol (IIb).

chromatography and mass spectrometry are shown in Figs. 3-2 and 3-3, exhibiting the fragment ions at m/e 398, 366, 253 and 247, respectively. The mass spectral patterns of them are very similar and the ion at m/e 398 in the upper field corresponds to the ion eliminated acetic acid (mass units, 60) from the molecular ion (expected at m/e 458), since in the acetates of Δ^5-steroid acetic acid is easily eliminated from the molecular ion in the condition[8,9]. The structures of the other fragment ions are shown in the figure. The reaction mixture was chromatographed on Silica Gel in chloroform to isolate Ib, mp 162-163°C, $[\alpha]_D$ −100° and IIb, mp 149-150°C, $[\alpha]_D$ +8.8°, respectively, and among them the former corresponds to the peak a and the latter to the peak b in the gas liquid chromatogram. The infrared spectra of Ib and IIb showed the absorption band due to the hydroxyl group in the range of 3200-3500 cm^{-1}. The mass spectra of them at 100°C in the ionization chamber with the direct insertion probe are shown in Fig. 4. In the spectra, the molecular ion was observed at m/e 416 (M^+) and the other fragment ions at m/e 398 (M^+-H_2O), m/e 384 (M^+-CH_3OH) and m/e 366 ($M^+-H_2O-CH_3OH$), respectively. Furthermore, the NMR spectra of Ib, IIb and their acetates (Ic,

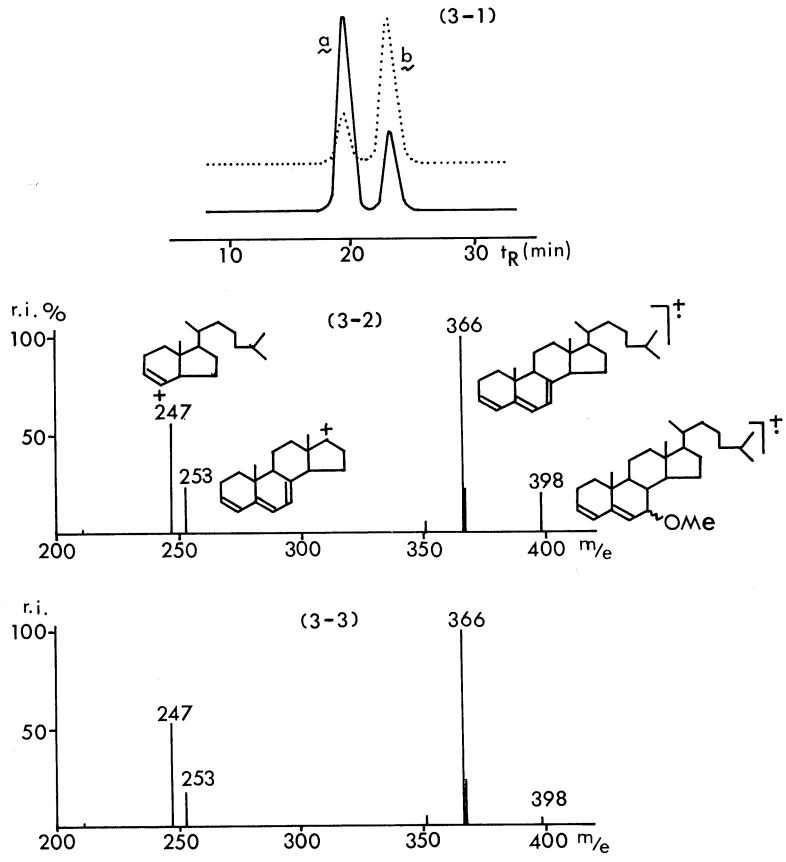


Fig. 3-1. Gas liquid chromatogram of acetates of the reaction mixture formed by the treatment of 7-hydroxycholesterol (Ia) in methanol containing a trace of hydrochloric acid at various time, 1 hr (———) and 2 hr (----------).

Fig. 3-2. Mass spectrogram corresponded to the peak b.

Fig. 3-3. Mass spectrogram corresponded to the peak a.
These spectra were recorded with a combined gas chromatography-mass spectrometry, using a column (4 mm×2 m, glass) packed with 0.75% SE-52; inlet pressure of helium as carrier gas, 1.2 kg/cm^2; column temperature, 220°C; electron impact energy, 20 eV; temperature of ionization chamber, 220°C.

IIc) were measured in deuterochloroform with a 60 MHz spectrometer (Fig. 5 and Table). The proton signals due to the hydroxyl group of

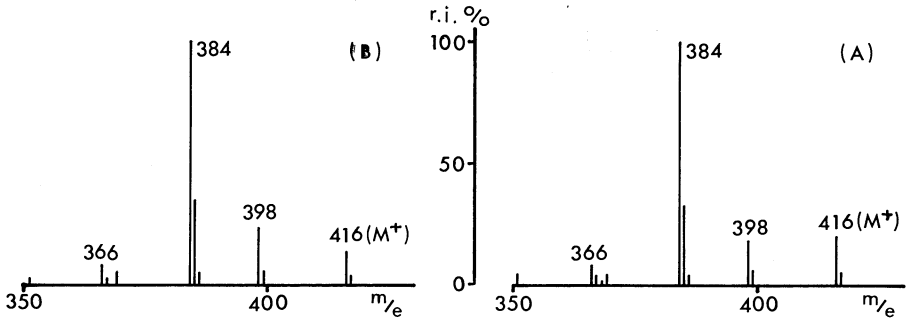


Fig. 4. Mass spectra of 7-methoxycholesterols, described above m/e 350, recorded by a direct insertion probe; electron impact energy, 70; temperature of ionization chamber, 100°C.
A: 7α-Methoxycholesterol (Ib). B: 7β-Methoxycholesterol (IIb).

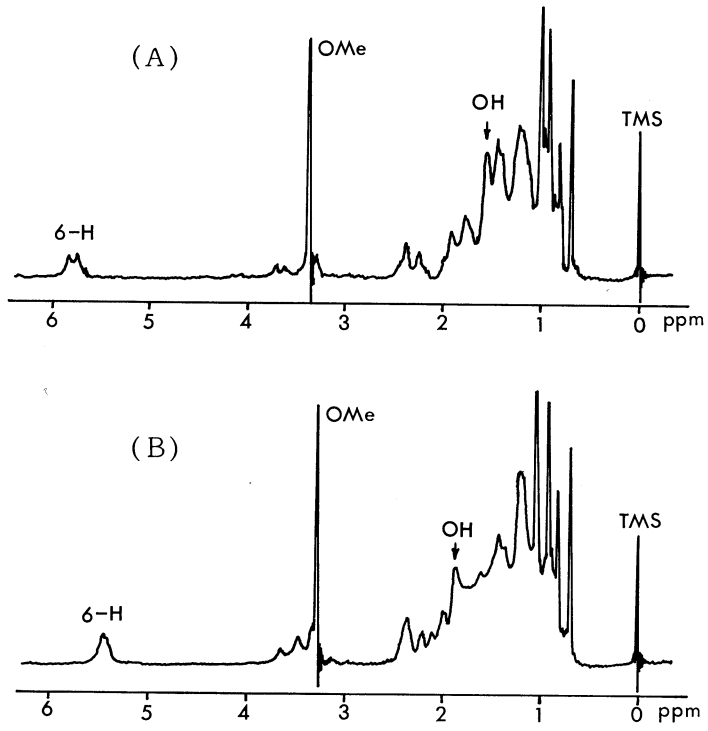


Fig. 5. NMR spectra of 7-methoxycholesterols, using tetramethylsilane (TMS) as internal standard (δ-value, $CDCl_3$).
A: 7α-methoxycholesterol (Ib). B: 7β-Methoxycholesterol (IIb).

TABLE

Chemical shifts of methoxyl, hydroxyl and acetoxyl groups and olefinic proton (δ-values).

Compounds	7α-OMe	7β-OMe	3β-OH	3β-OAc	6-H
Ib	3.38	—	1.57	—	5.78*
IIb	—	3.28	1.75	—	5.47**
Ic	3.35	—	—	2.04	5.72*
IIc	—	3.27	—	2.04	5.45**

* Broad doublet, J~6.0 Hz.
** Broad singlet, J~0 Hz.

Ib and IIb were observed at δ 1.57 and 1.75 ppm and the methyl signals due to the methoxyl group at δ 3.38 and 3.28 ppm, indicating the methyl etherification of the hydroxyl group of allylic alcohol. The olefinic C-6 proton signals are observed at δ 5.78 and 5.47 ppm and the former signal causes the splitting with ca. 6.0 Hz of coupling constant. In addition, the differences of the chemical shifts of the olefinic proton signals between hydroxyl-compound (Ib or IIb) and its acetate (Ic or IIc) were not observed and then the configuration of the oxgen group at C-3 is equatrial, β-configuration[10]. Although the configuration at C-7 in the 7-methoxy-compounds can be assigned by considering the signals due to the 7-proton, these are not clearly visible in the range of δ 3.0-4.0 ppm in the 60 MHz spectra. The configuration at C-7 in I and II appears to be reflected in the values of the coupling constant $J_{6,7}$ ($J_{6,7\beta} > J_{6,7\alpha}$), which are consistent with the dihedral angles 6-H/7-H of ca. 25° and ca. 85° derived from the measurements of the model[11] (Fig. 6). The structures of the products formed by the treatment of Ia and/or IIa in methanolic solution with hydrochloric acid are elucidated to be

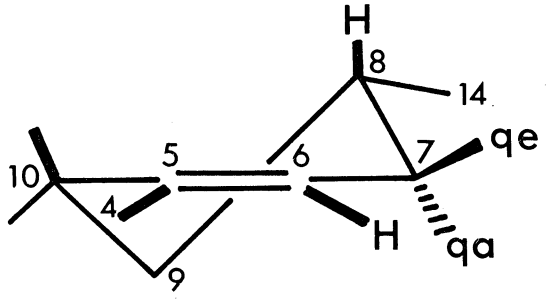


Fig. 6. The configuration of B-ring in the skeleton of sterol.

7α- (Ib) and 7β-methoxycholesterol (IIb), respectively. This conclusion is also supported by the measurements of the molecular rotation, $[M]_D$, of these isolated compounds[12].

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