Kawasaki Med. J. Vol. 3: No. 2, 91-99, 1977

ALLYLIC STEROID ALCOHOLS. II. METHOXYLATION OF THE EPIMERIC 3-HYDROXYCHOLEST-4-ENES IN THE MEDIUM OF METHANOL-HYDROCHLORIC ACID.

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Accepted for Publication on Jan. 13, 1977

Abstract

It was established that 3α-hydroxycholest-4-ene in methanol containing a trace of acid was converted into the corresponding 3α-methyl ether with a great ease and the ether was readily interconverted into the 3β-epimer in the solvent. The same treatment for 3β-hydroxycholest-4-ene was also carried out, yielding 3β-methyl ether as the major component, but the 3α-methyl ether in the reaction mixture enhanced along with increasing reaction time. The evidence of the structures of 3α- and 3β-methyl ethers was obtained from isolation of respective products and their characterization by means of the differences in $[M]_D$, NMR and mass spectrometry.

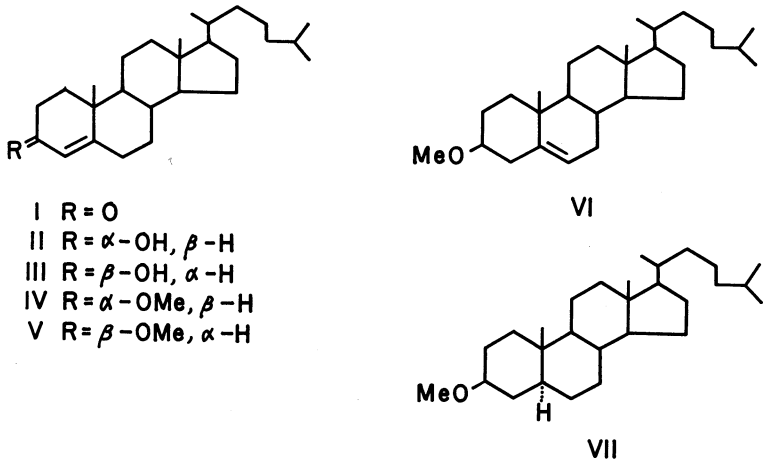
INTRODUCTION

It had been found that in the treatment of 7α-hydroxycholesterol in methanol with a trace of acid a mixture of methoxylated compounds containing 7α-methoxy derivative as the major component had resulted, but along with increasing reaction time the 7β-epimer in it became predominant, causing the interconversion in the solvent[1-3]. However, the acid-catalyzed conversion of the epimeric 3-hydroxycholest-4-enes, a kind of the allylic steroidal alcohols like 7-hydroxycholesterol, into the corresponding 3-methoxy derivatives in the same manner has not yet been reported. This paper deals with the behavior and characterization of the products resulting from the epimeric 3-hydroxycholest-4-enes by the treatment in methanol with a trace of hydrochloric acid. Recently, the authors reported that in the studies on the biogenetic pathway of bile acids in human bile the existences of some unknown compounds were observed and deduced 3α, 7α-dihydroxychol-4-en-24-oic acid and its 3α- and 3β-methoxy derivatives as artifacts in view of several physical data about them[4]. The investigation of the behaviors

of 3-hydroxycholest-4-enes in the acidified solvent will be helpful in the isolation and purification of by-products or metabolic intermediates in bile.

EXPERIMETAL PROCEDURES

All the melting points were measured with a Yanagimoto micro-melting point apparatus but uncorrected. The specific rotations, $[\alpha]_D$, were measured for the solution of chloroform with a Yanagimoto automatic digital polarimeter Model OR-50. The NMR spectra were measured for the solution of deuterochloroform with a Hitachi Perkin-Elmer spectrometer (90 MHz), using tetramethylsilane as an internal standard (δ-values). The mass spectra were recorded on a Hitachi spectrometer Model RMU-6MG, using the direct insertion probe; electron impact energy, 70 eV; temperature, 220°C. The purification of the samples used in this work was conducted by column chromatography on silica gel (E. Merck, Kieselgel type 60, 230 mesh), using chloroform as an eluent and checked on chromatoplate of silica gel (E. Merck, Kieselgel H, 0.25 mm thick) by using isooctane-isopropyl ether (1 : 1, v/v) as a developer and spraying the dried plate with 10% sulfuric acid-acetic acid and on gas liquid chromatography (Shimadzu gas chromatograph Model 4BPTF; column, glass column (4 mm×2 m) packed with 0.75% SE-52; carrier gas, nitrogen (88 ml/min); temperature, 230°C).



I R=O
II R=α-OH, β-H
III R=β-OH, α-H
IV R=α-OMe, β-H
V R=β-OMe, α-H

VI

VII

Fig. 1. Structures of sterols

Preparation of Cholest-4-en-3-one (I). Oppenauer oxidation of the commercial cholesterol (2 g) was carried out in the same way as reported

in the literature[5]. Recrystallization of the crude product from methanol gave (I) as colorless needles, mp 80-81°C, $[\alpha]_D$ +93.3° (lit.[5] mp 78-80°C, $[\alpha]_D$ +90°); yield, 1.3 g. IR (Nujol, cm^{-1}): 1685 (α, β-unsaturated carbonyl group), 1615 (double bond).

Reduction of Cholest-4-en-3-one (I) with Metallic Hydride.

(1) *With lithium aluminum hydride.* To a solution of (I) (400 mg) in anhydrous ether (50 ml) was added dropwise a solution of lithium aluminum hydride (100 mg) in anhydrous ether (10 ml) and the mixture was allowed to stand at 4°C for 12 hr with occasional stirring. The reaction was terminated by adding a small amount of water. The resulting mixture was condensed under reduced pressure to dryness. The residue was redissolved in ether, washed with water and dried on anhydrous sodium sulfate. The evaporation of the solvent gave a crude product (ca. 460 mg). The product was dissolved in ethanol (20 ml) and added a solution of digitonin (1.5 g) in 80% ethanol (150 ml), and then the mixture was allowed to stand overnight at 4°C. The digitonide was collected and washed with a small amount of hot ether. Digitonide was dissolved in a small amount of pyridine and added a large amount of ether, and then the solution was allowed to stand overnight at 4°C. The precipitates were filtered off, the filtrate was condensed and the residue was crystallized from methanol to give a corresponding 3β-hydroxycholest-4-ene (III) as colorless needles, mp 134-135°C, $[\alpha]_D$ +47.6° (lit.[6] mp 132°C, $[\alpha]_D$ +43.7°); yield, 300 mg. The mother liquor removed digitonide was condensed, extracted with ether, the extract was treated in the routine manner. The evaporation of the solvent gave a corresponding 3α-hydroxycholest-4-ene (II) as amorphous ($[\alpha]_D$ +84.4°); yield, ca. 30 mg. Although it could not be crystallized from methanol, the chromatographic analysis of it showed to be pure and the retention time of it was not in agreement with that of the above compound (III).

(2) *With sodium boron hydride.* The reduction of (I) by using of sodium boron hydride instead of lithium aluminum hydride was also carried out. The separation of II and III was conducted with the same manner as described above. The percentage of the mixture was approximately 10% of II and 90% of III.

Acetylation of II and III. The acetylation was carried out as follows: Acetic anhydride was added to a solution of the sample in pyridine and the mixture was allowed to stand overnight at room temperature. The mixture was poured into ice-water, the precipitates were collected, washed with water and crystallized from methanol to give

acetates. 3α-Acetoxycholest-4-ene: mp 76-78°C (lit.[6] mp 82.5°C), $[\alpha]_D$ +165.5°. MS (m/e) : 428 (M^+), 368 (base ion), 353, 316, 260, 255, 247. 3β-Acetoxycholest-4-ene: mp 86-88°C (lit.[6] mp 85°C), $[\alpha]_D$+16.5°. MS (m/e): 428 (M_+), 368 (base ion), 353, 316, 260, 255, 247.

Treatment of II and III with Acidified Methanol.

(1) II (14 mg) was dissolved in methanol (5 ml), to which a drop of 2N hydrochloric acid was added, and the mixture was left standing at room temperature. The reaction was terminated by adding a saturated sodium bicarbonate solution, extracted with ether, washed well with water and dried. The solvent was evaporated to dryness and the residue chromatographed to separate to two components. 3α-Methoxycholest-4-ene (IV) (amorphous, $[\alpha]_D$+74.6°) and 3β-methoxycholest-4-ene (V) (mp 75-76°C, $[\alpha]_D$+37°; lit. mp 72-73°C[7], mp 71.5-73°C, $[\alpha]_D$ +36.5°[8]) were isolated individually.

(2) III was also treated in the same manner as described above. The products corresponding to IV and V fractions were isolated and their chromatographic behaviors were identical.

Catalytic Hydrogenation of V and Cholesterol Methyl Ether (VI). *Hydrogenation of V.* Platinum oxide (50 mg) was added to a solution of V (50 mg) in acetic acid (10 ml) and the mixture was hydrogenated at atmosphere while it was stirred mechanically. When the equivalent molar of hydrogen was absorbed, the reaction ceased. Catalyst was filtered off, the filtrate was condensed under reduced pressure, the residue was crystallized from acetone to give 3β-methoxy-5α-cholestane (VII) as colorless needles, mp 82-83°C, $[\alpha]_D$+24.8° (lit.[9] mp 82.5-83°C, $[\alpha]_D$ +21°); yield, 30 mg.

Preparation and Hydrogenation of VI. To a solution of commercial cholesterol and excess potassium *tert*-butoxide in absolute ether was added excess methyl iodide, and then the mixture was allowed to stand at room temperature for 6 hr. A large amount of water was added to the solution, extracted with ether and treated in the routine manner. The solvent was evaporated, the residue was crystallized from acetone to give VI as colorless needles, mp 83-84°C, $[\alpha]_D$ −46° (lit.[9] mp 84°C, $[\alpha]_D$ −46.8°). The catalytic hydrogenation of VI was carried out in the same manner as described above. The sample resulting from acetone solution was identical in all respects with that derived from V.

RESULTS AND DISCUSSION

After the reduction of cholest-4-en-3-one (I) with metallic hydride[6],

3α- (II) and 3β-hydroxycholest-4-ene (III) were separated from the mixture by treatment with digitonin and purified by column chromatography, whose purity was checked by means of thin layer chromatography (Fig. 2), mass spectrometry, specific rotations and gas liquid chromatography. II in methanol containing a trace of acid was easily converted into nonpolar compounds (Fig. 2), of which the gas liquid chromatogra-

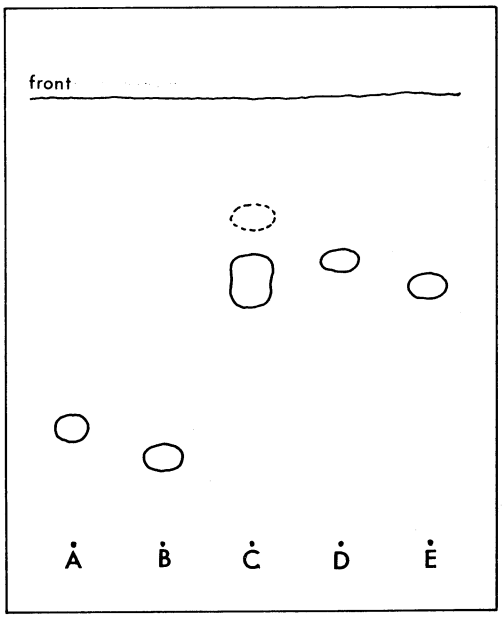


Fig. 2. Thin layer chromatography.
A and B: 3α- (II) and 3β-hydroxycholest-4-ene (III).
C: The mixture resulting from 3α- (II) or 3β-hydroxycholest-4-ene by the treatment with acidified methanol (4 hr).
D and E: Separated 3α- (IV) and 3β-methoxycholest-4-ene (V).
All the spots were colored violet with spraying 10% sulfuric acid-acetic acid.

phic detection after the treatment for 30 min showed a sharp peak at 12.5 min (peak *a*) of the retention time (Fig. 3). In the long time of the treatment the peak *c* (t_R: 13.4 min) because a major peak, showing a shoulder peak within a short period of time and the peak *a*, more nonpolar component, also became more prominent along with prolongation of the reaction time. From the treatment of III in the same manner the peaks *a*, *b* and *c* also appeared, which corresponded in the retention time to that of the peaks *a*, *b* and *c* derived from II. On the chromatogram of the mixture resulting from III the peak *d*, which was

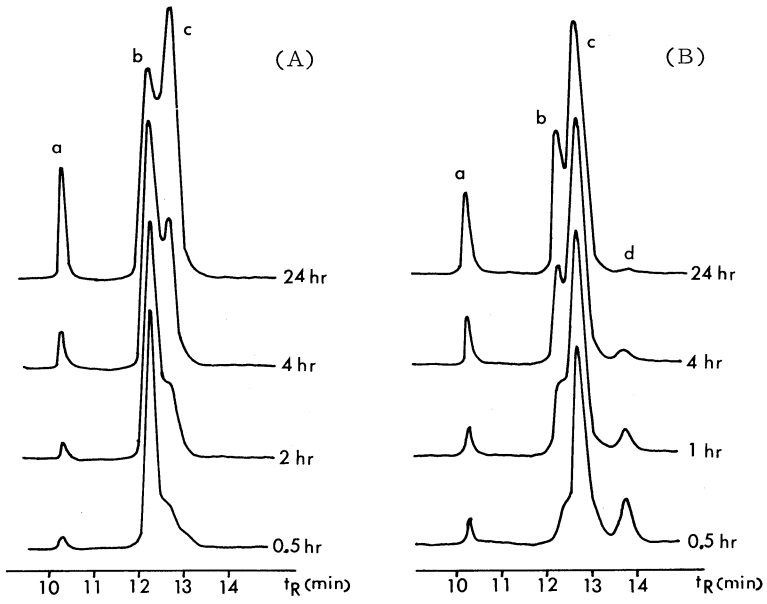


Fig. 3. Gas liquid chromatograms of the resulting mixture for various reaction time.
A: Used 3α-hydroxycholest-4 ene (II) as starting material.
B: Used 3β-hydroxycholest-4-ene (III) as starting material.

identical with the starting material (III), was observed, but on that of II where peak was not observable, it indicated that the II tended to be converted rapidly into other compounds (peaks *a*, *b* and *c*). The mixture formed by the treatment for a short period of time was repeatedly chromatographed on silica gel column to yield the pure components *b* and *c*, respectively. The melting points and specific rotations of the component of the peak *c* were identical with those of 3β-methoxycholest-4-ene (V) in the literature[7,8]. On the contrary, the other component (peak *b*) was isolated as amorphous, having +74.6° of specific rotations. The difference in their molecular rotations was estimated to be 150.4°, elucidating the component *b* to 3α-methoxycholest-4-ene (IV)[10]. The mass spectra of IV and V were of a fragment pattern very similar to each other (Fig. 4). The molecular ions of IV and V appeared at m/e 400 and the fragment ions at m/e 385 (M^+-Me), 368 (M^+-MeOH; base ion), 353 (M^+-MeOH-Me), 316 (M^+-MeOH-C_4H_4), 260 (M^+-MeOH-C_8H_{12}), 255 (M^+-MeOH-side chain) and 247 (M^+-MeOH-C_9H_{13}), respectively whose ions were characteristic in the compounds of cholesterol type[11]. From

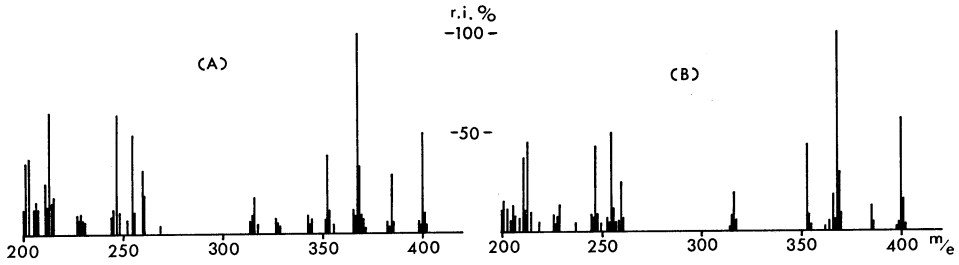


Fig. 4. Mass spectra of 3α-methoxycholest-4-ene (IV): A and 3β-methoxycholest-4-ene (V): B.

the molecular ion their molecules should possess $C_{28}H_{48}O$, enhancing 14 mass units (corresponding to CH_2 grouping) in comparison with 3-hydroxycholestenes. This fact seems to indicate the resulting products to be 3-methoxylated compounds from which the hydroxyl group in allylic steroid alcohols is alkoxylated with a great ease in the acidified alcohol[1-3]. The NMR spectra (Table) of IV and V exhibited the methoxyl signals as

TABLE

Chemical shifts of 3-H, 3-substitutents, 4-H and C-18 and C-19 methyls in the cholest-4-ene derivatives (δ-values)

	3α-				3β-				4-H	C-18	C-19
	H	OH	OAc	OMe	H	OH	OAc	OMe			
II		1.53			4.06				5.48(J=6Hz)	0.68	0.97
Ac* of II			2.00		5.11				5.49(J=6Hz)	0.68	0.98
IV				3.32	3.56				6.49(J=6Hz)	0.68	0.97
III	4.11					1.49			5.29**	0.68	1.03
Ac* of III	5.20						2.00		5.21**	0.67	1.04
V	3.71							3.33	5.34**	0.68	1.03

* Acetate ** Broad singlet

singlet peak at δ 3.32 ppm and 3.33 ppm, respectively. Although in the NMR spectrum of V the olefinic proton signal at C_4-position was observable as a broad singlet, in that of IV the signal appeared obviously as a doublet, splitting with the coupling constants J=6 Hz. In addition, the proton signal for V on the secondary carbon at C_3-position had scarcely shifted to a higher field in comparison with that for IV. From these facts the configuration of the substituent for IV at C_3-position

should be α-position, quasi-axial, and that for V β-position, quasi-equatrial[12]. Furthermore, the signals of C-18 methyl groups of IV and V resonate at the same position, but that of C-19 methyl group of V slightly shifted to a lower field (ca. 0.05 ppm) in comparison with that of IV, effecting by the electronegative oxygen substituent oriented to β-configuration at C_3-position. The structure of V was further confirmed by the additional evidences. The catalytic hydrogenation of V led easily to 3β-methoxy-5α-cholestane (VII, mp 82-83°C, $[\alpha]_D$ +24.8°), which was identical in gas liquid chromatographic behavior, melting points and specific rotations with the sample derived from cholesterol methyl ether[9].

More nonpolar component, peak *a*, may be the dehydration product, cholest-2, 4-diene, resulting from 3-hydroxycholest-4-enes, since in the studies of dehydration products of 3-hydroxycholestenes Shoenheimer and Evans[6], and Okerholm et al.[13] had reported that the product formed from those in the acidified solution was cholest-2, 4-diene. In this study the component (peak *a*) was purely isolated in a small amount and the UV spectrum of it in ethanol showed the absorption peak at ca. 270 nm, indicating the 2, 4-diene type, but the further investigation was not carried out.

Thus, 3α- (VI) and 3β-methoxycholest-4-ene (V) were prepared from the corresponding 3-hydroxy compounds by the treatment with acidified methanol for a short period of time. However, for a long reaction time the respective compounds (IV and V) resulted as a mixture, undergoing the interconversion during the treatment in the solvent.

Acknowledgment

The authors should like to express here sincere thanks to Prof. Masami Makita, Department of Pharmacology of Okayama University, for the NMR measurements.

REFERENCES

1) Henbest, H. B. and Jones, E. R. H.: Studies in the Sterol Group. Part XLIX. 7-Substituied Cholesterol Derivatives and Their Stereochemistry (Part III). 7-Alkoxy-cholesterol Derivatives. J. Chem. Soc. 1798-1803, 1948
2) Kulig, M. J., Teng, J. I. and Smith, L. L.: Sterol Metabolism: XXXIII. On Derivation of Cholesterol 7-Alkoxy Ethers. Lipids. 10: 93-98, 1975
3) Harano, T. and Harano, K.: Allylic Steroid Alcohols I. Structures of the Products Resulting from 7-Hydroxycholesterol in the Medium of Methanol-Hydrochloric Acid. This Journal. 2: 175-184, 1976
4) Harano, K., Harano, T., Yamasaki, K. and Yoshioka, D.: Isolation of 3β, 7α-Dihydroxychol-5-en-24-oic and 3β, 7α-Dihydroxychol-4-en-24-oic Acids from Human Bile. Proc. Japan Acad. 52: 453-456, 1976
5) Org. Syn., Coll. Vol. 3: 207-209

6) Shoenheimer, R. and Evans, E. A. Jr.: Allocholesterol and Epiallocholesterol. J. Biol. Chem. 114: 567-582, 1936
7) Campion, T. H. and Morrison, G. A.: 3α-Methoxy-5α-cholestane-4β, 5-diol and Related Compounds. Tetrahedron, 29: 239-243, 1973
8) Henbest, H. B. and Wilson, R. A. L.: Aspects of Stereochemistry. Part I. Stereospecificity in Formation of Epoxides from Cyclic Allylic Alcohols. J. Chem. Soc. 1958-1965, 1957
9) These melting points and specific rotations are refered to "The Merck Index".
10) Mills, J. A.: Correlations between Monocyclic and Polycyclic Unsaturated Compounds from Molecular Rotation Differences. J. Chem. Soc. 4976-4985, 1952
11) Knights, B. A.: Identification of Plant Sterols Using Combined GLC/Mass Spectrometry. J. Gas Chromatog. 5: 273-282, 1967
12) Bhacca, N. S. and Williams, D. H.: Application of NMR Spectroscopy in Organic Chemistry. Illustrations from Steroid Field. Holden-Day Inc. pp 13-29, 42-54, 1964
13) Okerholm, R. H., Brecher, P. I. and Wotiz, H. H.: Thermal Dehydration of Steroidal Allylic Alcohols. Steroids. 12: 435-443, 1968
14) Fieser, L. F. and Fieser, M.: Steroids. Van Nostrand Reinhold Company. pp 15-24, 1959