11. Kyosuke Tsuda* and Ryoichi Hayatsu**: Transition of N-Cholestenylpyridinium Salts. II[1]. Partial Synthesis of 3(β)-Carboxycholestene-5.

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In the previous report[1], the authors described of their studies on the thermal reaction of cholesteryl *p*-toluenesulfonate with pyridine, β-picoline, γ-picoline, 2,6-lutidine, and 2,4,6-collidine, and clarified that the cholestene cation transited from the nitrogen to carbon at 4-position in the case of pyridine, and from nitrogen to the side-chain methyl in the case of α- and γ-picoline. Such abnormal transition from the nitrogen to methyl was found, by later studies, to be not confined to the cholestene cation and was confirmed to be possible with other simple alkyl group, details of which will be described in later reports.

The present paper describes the C_3-configuration of the cholestene in the compound produced by the transition. The compound obtained in a good yield by heating cholesteryl *p*-toluenesulfonate (I) and γ-picoline is 3(β)-cholestenyl-(4-pyridyl)-methane (II), as described in the previous report[1]. The carbon at 3-position of cholestene in this compound has been proved as taking the β-configuration from the oxidation of the N-methylpyridone derivative (IIA) of this compound to 3(β)-cholest-5-enylacetic acid. Furthermore, the fact that the transition product is identical with the condensation product of lithium γ-picoline and 3(β)-bromocholestene-5 (IV) also endorses the conclusion of Shoppee[2] that the nucleophilic substitution of C_3 in $\Delta^{5(6)}$-steroids does not promote inversion. The transiton product obtained by heating N-cholestenyl-α-picolinium *p*-toluenesulfonate was proved by the synthesis of the product itself from lithium α-picoline and 3(β)-bromocholestene-5 but no mention was made in the previous paper on its C_3-configuration. However, since this synthetic reaction is a nucleophilic substitution of the α-picoline anion to C_3 of cholestene, it leaves no doubt that the β-configuration of C_3 in cholestene is intact, as in the case of the substitution of γ-picoline anion, and therefore, the condensation product should be 3(β)-cholestenyl-(2-pyridyl)-methane (III).

On the other hand, the retention of the β-configuration of the cholestene in the case of the salt formation between cholesteryl *p*-toluenesulfonate and pyridine[3], and in the case of the amination of 3(β)-halocholestene-5[4] has recently been confirmed and, therefore, the C_3-configuration of cholestene in the reaction of cholesteryl *p*-toluenesulfonate with α -and γ-picolines could be represented as shown in Fig. 1.

As described in the previous paper, the transition product obtained by heating N-cholestenylpyridinium *p*-toluenesulfonate is an oil whose oxidation, *per se*, results in the formation of isonicotinic acid, but oxidation after deriving the oil to ammonium hydroxide results in the formation of cholestene-3-carboxylic acid. The purification of the methyl ester of this acid yields 3(α)-carbomethoxycholestene-5, m.p. 101~102°. The purification of its recrystallization mother liquor after saponification and derivation to ethyl ester yield crystals of m.p. 87~90° which was assumed in the previous paper as 3(β)-carbethoxycholestene-5. There are no definite descriptions on 3(β)-carboxycholestene-5 in the existing

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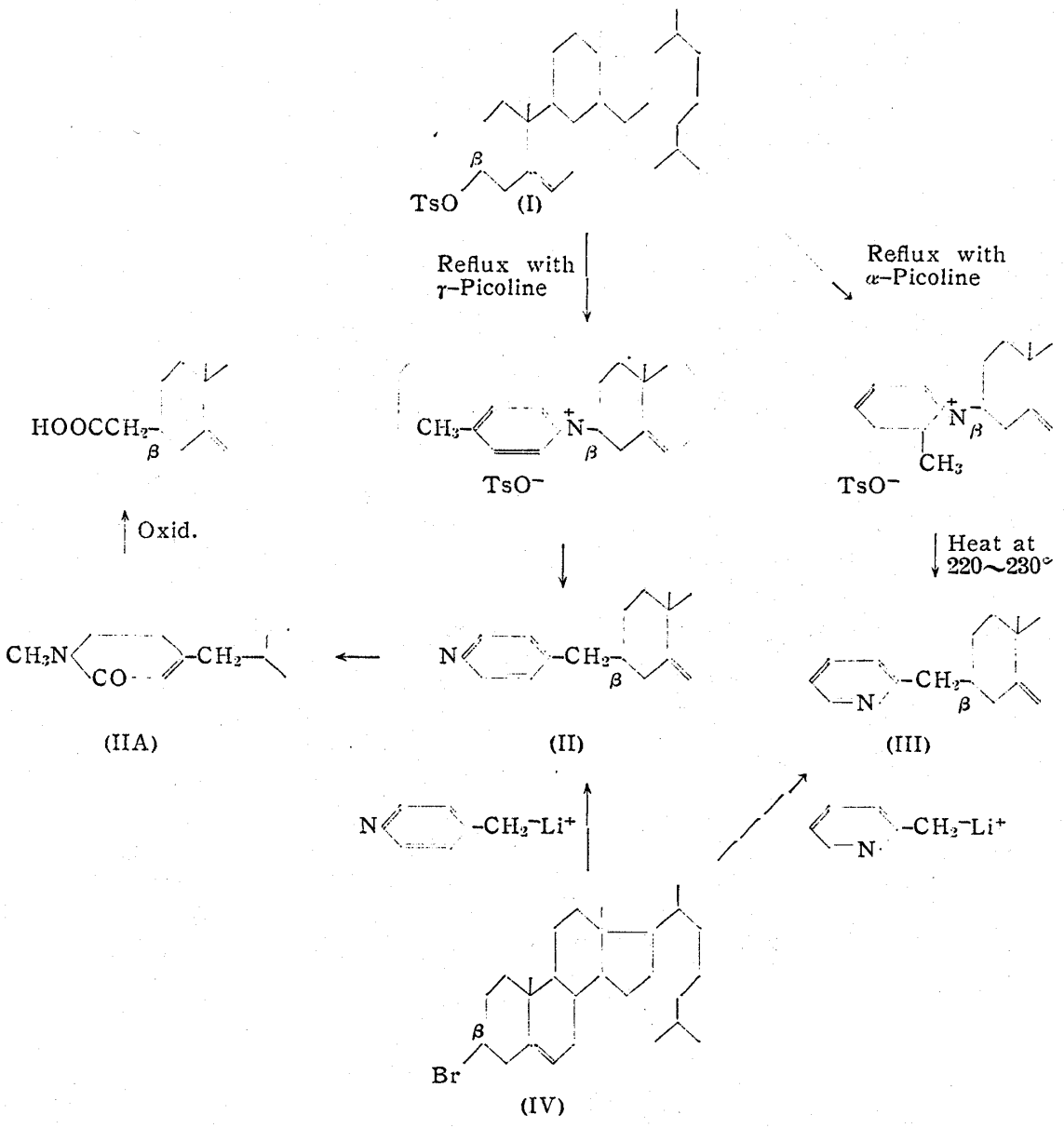

Fig. 1

literature, and 3(α,β)-carboxycholestene is assumed to be formed by the reaction of cholestenylmagnesium halide and carbon dioxide[5]. However, purification of its methyl ester yields only 3(α)-carbomethoxycholestene-5[6] alone, and carboxylic acid of β-configuration has not been purely isolated. 3(β)-Carboxycholestene-5 was, therefore, prepared by the following two processes.

In the first process, 3(β)-cholest-5-enylacetic acid (V) was prepared by Kaiser's method[7] and its methyl ester (VI) was reacted with phenylmagnesium bromide to provide the tertiary alcohol (VII). Dehydration of (VII) with acetic acid yielded the olefine (VIII), m.p. 63~65°, whose chromic acid oxidation finally yielded 3(β)-carboxycholestene-5. Oxidation immediately after bromination, followed by debromination also yielded the same product. The yield by this process was much smaller than that by the second process.

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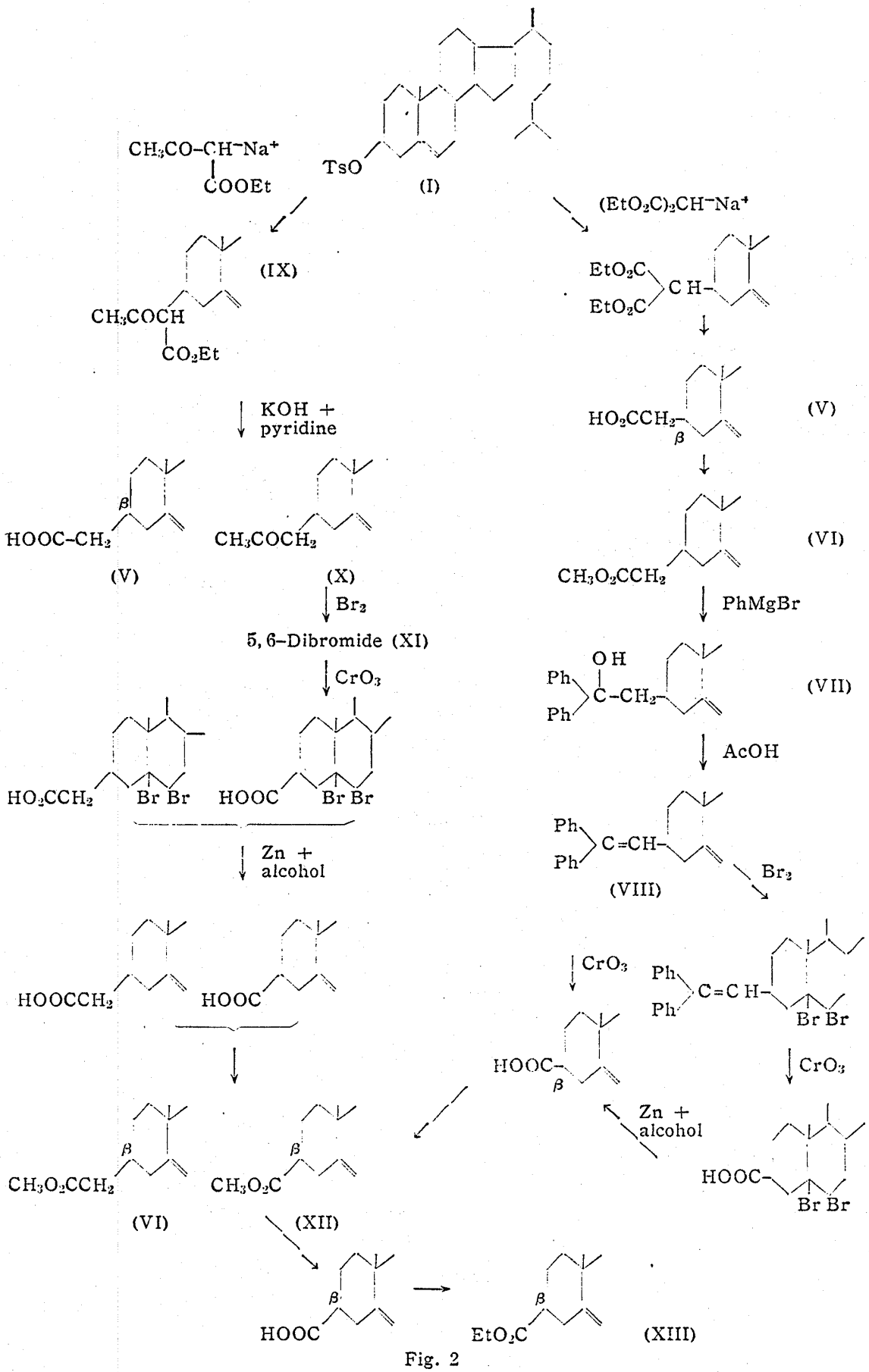

Fig. 2

In the second process, cholesteryl *p*-toluenesulfonate was reacted with the sodium salt of ethyl acetoacetate in xylene, and its condensation product (IX) was heated with potassium hydroxide in pyridine, from which the ketone compound (X) was obtained as the chief product and 3(β)-cholest-5-enylacetic acid as the by-product. After the purification of the ketone compound as its semicarbazone, m.p. 178~180°, treatment with sulfuric acid gave the ketone as m.p. 138~140°. Its derivation to the dibromide (XI), m.p. 172°, and oxidation with chromic acid provided the carboxylic acid whose debromination with zinc and alcohol yielded a mixture of carboxylic acids. The methyl ester of this acid was purified by chromatography with alumina from which crystals of m.p. 95~96° and of m.p. 106~108° were isolated.

Recrystallization of the crystals of lower melting point gave crystals of m.p. 107~109° which were found to be identical with the methyl ester of 3(β)-carboxycholestene-5 (XII) obtained from the first process.

The crystals of m.p. 106~108° were found to be identical with methyl 3(β)-cholest-5-enylacetate. The course of these reactions is shown in Fig. 2.

The saponification of 3(β)-carbomethoxycholestene-5 obtained by these two processes yielded 3(β)-carboxycholestene-5, m.p. 230~231°. Derivation of this compound to ethyl ester provided 3(β)-carbethoxycholestene-5 (XIII), m.p. 87~89°. The melting points of the respective esters of 3(α)- and 3(β)-carboxycholestene-5 and -cholest-5-enylacetic acids are given in Table I.

TABLE I

Compound	m.p.	
	α	β
3-Carboxycholestene-5	222~225°	230~231°
3-Carbomethoxycholestene-5	101~102°[5]	107~109°
3-Carbethoxycholestene-5	82~83°[1]	87~89°
3-Cholest-5-enylacetic Acid	175°[6]	211~213°[7]
Methyl 3-Cholest-5-enylacetate	73°[6]	106~108°[7]

The mixed fusion of 3(β)-carbomethoxycholestene-5, m.p. 107~109°, with the 3(α) isomer, m.p. 101~102°, indicated m.p. 94~101°, and that with methyl 3(β)-cholest-5-enylacetate, m.p. 106~108°, indicated m.p. 97~102°, showing depression in both cases.

The transition product (XV), obtained by the heating of N-cholestenylpyridinium salt (XIV), yielded upon oxidation, carbethoxycholestene (XVIB), m.p. 88~90°, together with 3 (α)-carbomethoxycholestene-5 (XVIA). Since no depression of the melting point occured on fusion of (XVIB) with 3(β)-carbethoxycholestene-5, m.p. 87~90°, prepared in the present experiments, the two substances must be identical and this shows that the oxidation product is a mixture of 3(α)- and 3(β)-carbethoxycholestene-5, and consequently, the transition product is a mixture of 3(α)- (XVA) and 3(β)-(4-pyridyl)-cholestene-5 (XVB). It follows, therefore, that the thermal transition is accompanied by the inversion of C_3-configuration. This process is shown in Fig. 3.

The infrared absorption spectra of 3(β)-carbomethoxycholestene-5 prepared by the synthetic method elaborated by the present authors and of 3(α)-carbomethoxycholestene-5, prepared by the method given in existing literature, are shown in Fig. 4, from which it is seen that both curves are identical and there are no specific absorption bands for the respective compounds.

The authors take this opportunity to express their gratitude to Mr. Masao Maruyama of the Takamine Research Laboratory, Sankyo Co., Ltd. for the infrared absorption spectral data.

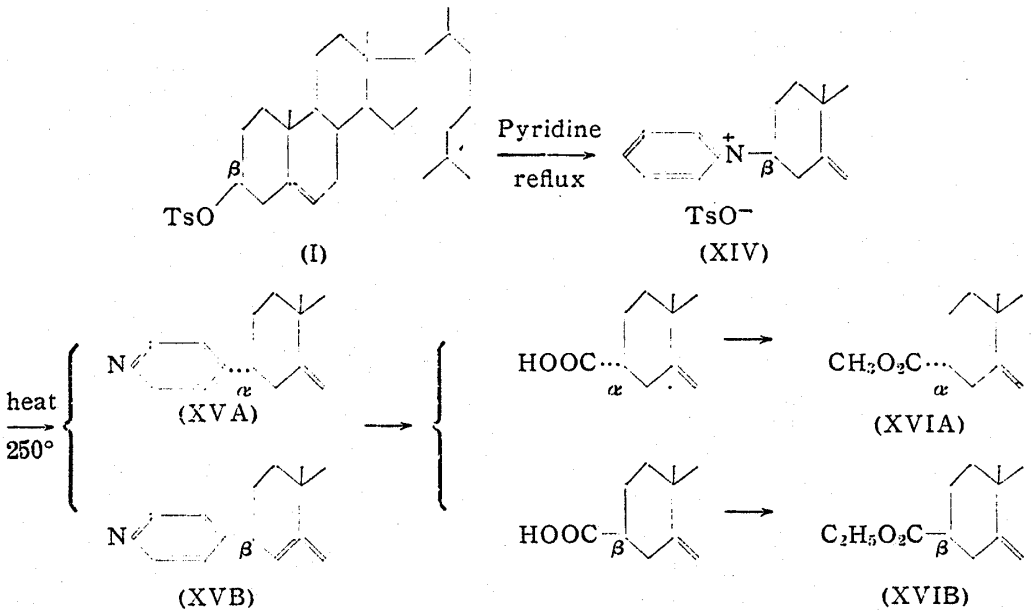

Fig. 3

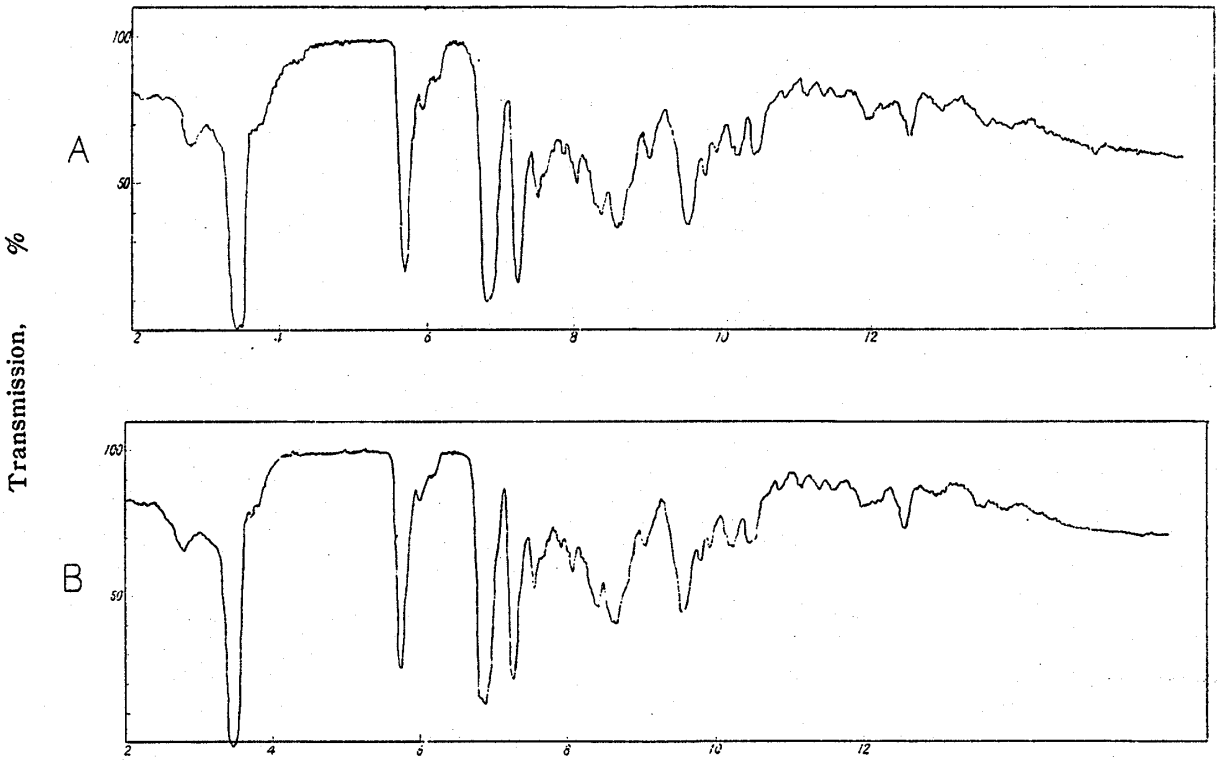

Fig. 4 Infrared Absorption Curves in Nujol Suspension

A: 3(β)-Carbomethoxycholestene-5
B: 3(α)-Carbomethoxycholestene-5

Experimental

3(β)-Cholest-5-enylacetoacetic ester (IX)—A mixture of 2 g. sodium and 40 cc. of xylene was heated to 95~100°, to which was added a solution of 16 g. of ethyl acetoacetate dissolved in 20 cc. of xylene, with stirring. The mixture was heated for 30 minutes at this temperature, then the temperature was raised to 105~110°, and a solution of 40 g. of cholesteryl tosylate dissolved in 80 cc. of xylene was added dropwise. About 0.5~1.0 hour after the addition, sodium tosylate precipitated

out. The heating and stirring were continued for further 10 hours, the mixture was filtered, and the filtrate distilled under a diminished pressure to remove the solvent, from which 31 g. of an oily substance was obtained. The semicarbazone of the oil was obtained as crystals of m.p. 201~202°. *Anal.* Calcd. for $C_{34}H_{57}O_3N_3$ (Semicarbazone): C, 73.51; H, 10.27; N. 7.56. Found: C, 73.29; H, 10.30; N, 7.12.

3(β)-Acetonylcholestene-5 (X)—To a mixture of 30 g. of ethyl 3(β)-cholest-5-enylacetoacetate (IX) and 80 cc. of pyridine, a solution of 5 g. of potassium hydroxide in 15 cc. of water was added and the mixture was refluxed for 4 hours. This mixture was poured into water, acidified with hydrochloric acid, and extracted with ether. The ethereal layer was washed with 10 % potassium hydroxide, and the alkaline layer was acidified, by which crystals separated out. This mixture was allowed to stand overnight at 0°. The crystals collected by filtration were recrystallized from absolute alcohol to needle crystals of m.p. 210~213°. Yield, 1.5 g. Its methyl ester, needle crystals, m.p. 107~108°. This acid is 3(β)-cholest-5-enylacetic acid.

The ethereal layer was dried and the ether removed by which 16 g. of neutral oil was obtained. The solution of 1 g. of this oil dissolved in 10 cc. of pyridine, with semicarbazide hydrochloride, was refluxed for 1 hour, poured into water, and extracted with ether. Crystals of m.p. 172~176° were obtained from the ethereal layer, and were recrystallized from acetone to needle crystals, m.p. 178~180°. *Anal.* Calcd. for $C_{31}H_{53}ON_3$ (Semicarbazone): C, 77.18; H, 10.99; N, 8.77. Found: C, 76.79; H, 10.63; N, 8.40.

A mixture of 400 mg. of this semicarbazone dissolved in 40 cc. of alcohol, with 2 cc. of conc. sulfuric acid added, was refluxed for 2 hours, cooled, and the alcohol was removed by distillation under a diminished pressure. Water was added to the residue and extracted with ether. The crystals obtained from the ethereal layer were recrystallized from acetone to 120 mg. of colorless prisms, m.p. 138~140°. *Anal.* Calcd. for $C_{30}H_{50}O$ (3(β)-acetonylcholestene-5): C, 84.50; H, 11.73. Found: C, 84.59; H, 11.99.

3(β)-Carboxycholestene-5 (XII)—To the solution of 10 g. of 3(β)-acetonylcholestene-5 (X), m.p. 138~140°, dissolved in 60 cc. of ether and cooled to 0°, a mixture of 5 g. of bromine, 10 cc. of glacial acetic acid, and 20 cc. of ether was added, allowed to stand for 3 hours, and the solvent removed under diminished pressure at 30°. The oily residue was recrystallized from a mixture of ether and methanol to 12.5 g. of crystals, m.p. 160~172° (decomp.). To 12 g. of this bromide (X), 150 cc. of glacial acetic acid was added, warmed to 70°, and, while stirring, a solution of 20 g. of chromic acid in 30 cc. of water and 100 cc. of glacial acetic acid was added. After stirring for 2 hours, the mixture was poured into water, methanol added to remove chromic acid, and the mixture allowed to stand at 0° for 4~5 hours. Filtration yielded 8 g. of a solid. To 8 g. of this oxidation product dissolved in hot alcohol, 8 g. of zinc dust was added and refluxed for 2 hours. Zinc was removed by filtration, and the filtrate concentrated under a diminished pressure to a syrupy consistency. Yield, 4.2 g. This syrupy residue was dissolved in ether, shaken with 10% solution of potassium hydroxide, and the alkaline layer was acidified with hydrochloric acid by which crystals separated out. The crystals were collected by filtration at 0°, and 1.2 g. of an acid substance thereby obtained was refluxed for 18 hours with 30 cc. of absolute methanol and 0.5 cc. of conc. sulfuric acid. Methanol was removed by distillation, water added to the residue and the mixture was extracted with ether. The ether layer was washed with 10 % solution of sodium carbonate, dried, and the ether removed by which 1 g. of oil was obtained. This oil was dissolved in petroleum ether, passed through an alumina column, and developed with petroleum ether. The effluent was fractionally collected every 25 cc. Fractions 1~3 yielded 110 mg. of crystals (A), m.p. 95~96°, and Fractions 7~9, 40 mg. of crystals (B), m.p. 106~108°. The fractions 4~6 contained a mixture of these crystals, and the effluent of Fraction 10 and later, only yielded oily residue.

The recrystallization of the crystals (A) of m.p. 95~96° from alcohol yielded colorless needles, m.p. 107~109°, which were found identical with 3(β)-carbomethoxycholestene-5 (XII). *Anal.* Calcd. for $C_{29}H_{48}O_2$ (3(β)-Carbomethoxycholestene-5): C, 81.30; H, 11.21. Found: C, 81.57; H, 11.00.

A mixture of 80 mg. of this methyl ester, 20 cc. of alcohol, and 0.5 g of potassium hydroxide was refluxed for 6 hours, cooled, poured into water, and extracted with ether. Acidification of the aqueous layer separated crystals which were recrystallized from benzene to 3(β)-carboxycholestene-5, m.p. 230~231°. *Anal.* Calcd. for $C_{28}H_{46}O_2$ (3(β)-Carboxycholestene-5): C, 81.15; H, 11.11. Found: C, 81.44; H, 11.20.

The crystals (B) of m.p. 106~108° obtained by chromatography showed no depression of the melting point when fused with methyl 3(β)-cholest-5-enylacetate (VI), m.p. 106~108°.

(3(β)-Cholest-5-enyl)-methyl-(diphenyl) carbinol (VII)—Methyl 3(β)-cholest-5-enylacetate was prepared by the method of Kaiser[7]. To the Grignard reagent prepared from 16 g. of bromobenzene, 2.5 g. of magnesium, and 50 cc. of ether, 5 g. of this ester dissolved in 30 cc. of ether was added and refluxed for 4 hours. The mixture was then cooled with ice, acidified with 10 % solution of sulfuric acid, and extracted with ether. The ethereal residue yielded 5.4 g. of oil.

1-(3(β)-Cholestenyl)-2,2-diphenylethylene (VIII)—A mixture of 5.4 g. of the foregoing oil

and 50 cc. of glacial acetic acid was refluxed for 3 hours, poured into water after cool, and alkalized with 10 % solution of potassium hydroxide. This was extracted with ether, and the ethereal layer, after drying and distillation, yielded some oil which crystallized upon standing. Recrystallization from a mixture of ether and methanol yielded platelets, m.p. 63~65°. Yield, 4 g. *Anal.* Calcd. for $C_{41}H_{58}$ (1-(3(β)-Cholestenyl)-2,2-diphenylethylene): C, 89.74; H, 10.22. Found: C, 89.33; H, 10.49.

3(β)-Carbomethoxycholestene-5 (XII)—To a solution of 4 g. of the ethylene compound (VIII) dissolved in 20 cc. of chloroform, 30 cc. of glacial acetic acid was added, cooled to 10° to 15°, and a solution of 8 g. of chromic acid dissolved in a mixture of 8 cc. of water and 50 cc. of glacial acetic acid was added with stirring. After stirring for further 6 hours, the mixture was poured into water, and was allowed to stand for 2 hours at 0°. The solid thereby obtained was collected by filtration, dissolved in ether, and shaken with 10% solution of potassium hydroxide. The alkaline layer was separated, filtered, and acidified with hydrochloric acid by which some crystals separated out. These crystals were purified through alumina chromatography and 30 mg. of 3(β)-carbomethoxycholestene-5, m.p. 107~109°, were obtained. This substance showed no depression of the melting point when fused with the same substance obtained by the method described above.

Oxidation of the dibromide—To a solution of 5 g. of the ethylene compound (VIII) dissolved in 30 cc. of ether, cooled to 0°, a solution of 1.5 g. of bromine in 20 cc. of ether was added. After a few minutes, ether was removed by low-pressure distillation, and 6 g. of a crude product, m.p. 150~165°(decomp.), was obtained.

This crude bromide was dissolved in 100 cc. of glacial acetic acid, and a solution of 20 g. of chromic acid dissolved in a mixture of 20 cc. of water and 70 cc. of glacial acetic acid was added at 55~60°, with stirring. The stirring was continued for further 4 hours, and the mixture was poured into cold water. This mixture was allowed to stand for 3~4 hours at 0°, and filtered. The pale yellow solid thereby obtained was refluxed in 40 cc. of alcohol with 6 g. of zinc for 2 hours. After the removal of zinc by filtration, the alcohol was removed by distillation, and the residue was treated as in the previous case. The methyl ester of m.p. 106~107° was obtained. Yield, 120 mg.

3(β)-Carbethoxycholestene-5 (XIII)—3(β)-Carboxycholestene-5, m.p. 230~231°, obtained by the saponification of 3(β)-carbomethoxycholestene-5, was esterified with alcohol and sulfuric acid, and recrystallized from alcohol to needles, m.p. 87~89°. *Anal.* Calcd. for $C_{30}H_{50}O_2$ (3(β)-Carbethoxycholestene-5): C, 81.44; H, 11.31. Found: C, 81.38; H, 11.62.

No depression of the melting point was observed when this substance was fused with the ethyl ester, m.p. 87~89°, obtained on the oxidation of the transition product of N-cholestenylpyridinium tosylate, together with 3(α)-carbomethoxycholestene-5.

Summary

3(β)-Carboxycholestene-5 was prepared by two different processes starting with 3(β)-cholest-5-enylacetic acid and ethyl 3(β)-cholest-5-enylacetoacetate. Its ethyl ester, m.p. 87~89°, was found to be identical with the ethyl ester, m.p. 87~89°, obtained together with 3(α)-carbomethoxycholestene-5 by the oxidation of 3-(4-pyridyl)-cholestene-5 as described in the previous report[1]. It follows, therefore, that the substance obtained by the thermal transition of the N-cholestenylpyridium salt is a mixture of 3(α)- and 3(β)-(4-pyridyl)-cholestene-5. This shows that the transition of cholestene group from nitrogen to the methyl side-chain by the thermal reaction of N-cholestenylpicolinium salt is not accompained by the inversion of the β-configuration of C_3 in cholestene, while the inversion partially occurs in the transition of cholestene group from nitrogen to the 4-position of pyridine in the case of similar reaction in N-cholestenylpyridinium salt.

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