Grignard Reaction of Testosterone Acetate in the Presence of Cu_2Cl_2——The Grignard reaction of testosterone acetate (3.0 g.) in the presence of Cu_2Cl_2 (600 mg.) and Girard separation were made as described above. From the keto fraction no product was obtained. From the non-keto fraction, 3-methylandrosta-3,5-dien-17β-ol (XII), m.p. 126~132°, was obtained. Yield, 1.6 g. Repeated recrystallization from MeOH gave white needles, m.p. 131~134°, $[\alpha]_D^{26}$ −175° (c=1·02, $CHCl_3$), UV λ_{max}^{MeOH} mμ (log ε): 231~232 (4.28), 239 (4.31). reported[9] m.p.134~136°, $[\alpha]_D^{20}$: −175°. UV λ_{max}^{EtOH} mμ (log ε): 232 (4.46), 240 (4.49).

The author is very grateful to Dr. M. Chuman, President of Tsurumi Research Laboratory of Chemistry, Dr. S. Niinobe, Director of Research Laboratory of this company, Mr. M. Sawai, and Dr. J. Yamada for their valuable advices and to Dr. E. Yamaguchi, President of this company, and to Dr. F. Ueno, Director of the Manufacturing Section of this company, for their encouragement throughout this work. The author is also indebted to Mr. K. Hirama for for his technical help.

Summary

The Grignard reaction of 17β-hydroxy-17α-methylestr-4-en-3-one (VIIa) in the presence of cuprous chloride gave 5β,17α-dimethyl-17β-hydroxyestran-3-one (VIIIa) and 3,17α-dimethylestra-3,5-dien-17β-ol (Xa). Similarly, (VIIIb) and (Xb) were obtained by the Grignard reaction of 17β-hydroxyestr-4-en-3-one (VIIb). The oxidation of (VIIIb) with chromium trioxide gave 5β-methyl-estrane-3,17-dione (XI). Discussion was made on the configuration of C-5 methyl group in (VIIIa) and (VIIIb).

(Received January 21, 1961)

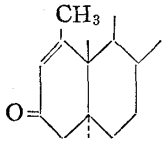
UDC 547.92.07

62. Hiromu Mori: Studies on Steroidal Compounds. VII.[1] Synthesis of 1α-Methyl Steroids.

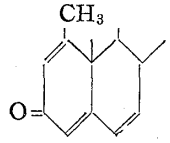
(*Research Laboratory, Teikoku Hormone Mfg. Co., Ltd,*[*1])

Many methylated steroidal hormones have been prepared and some of them show higher hormonal activity than their parent steroidal hormones.[2] 1-Methylated steroids which have been prepared up to date are 1-methyl-1-en-3-oxo (I) and 1-methyl-1,4,6-trien-3-oxo (II) type steroids,[3] other than estrane series. Some observations on 1α-methylated steroids[4] will be described.

In the preceding paper,[1] 1,4-addition of Grignard reagent to 19-nor-4-en-3-oxo-ster-



(I)



(II)

*1 1604, Shimosakunobe, Kawasaki, Kanagawa-ken (森　弘).

1) Part VI: This Bulletin, **10**, 382 (1962).
2) A. Zaffaroni: Acta Endocrinol., Suppl. 50, **34**, 139 (1960).
3) R. Wiechert, E. Kaspar: Chem. Ber., **93**, 1710 (1960).
4) In estrane series, many 1-methylated steroids have been prepared.

oids was described.[1] Grignard reaction of 5α-cholest-1-en-3-one (IIIa) in the presence of cuprous chlorids was carried out and the product obtained was considered to be 1α-methyl 5α-cholestan-3-one (IVa), because it is different from 5α-cholestan-3-one and its infrared spectrum showed it to be a saturated ketone. The configuration of methyl group introduced will be discussed below.

(IVa) was brominated with one equivalent of bromine in acetic acid by the usual method. The crude monobromo compound was not purified but dehydrobrominated with lithium chloride-dimethylformamide system[5] to give an unsaturated ketone having a strong ultraviolet absorption at 241 mμ. The unsaturated ketone was different from known 1-methyl-5α-cholest-1-en-3-one,[3] and it must, therefore, be 1α-methylcholest-4-en-3-one (VIa). Accordingly, bromination product of (IVa) is supposed as a 4-bromo compound. as monobromination of A/B-*trans* 3-oxo-steroid occurs usually at C-2, Fried has recently stated that 5β-methyl-3-oxo-steroid is probably brominated at C-2.[6]

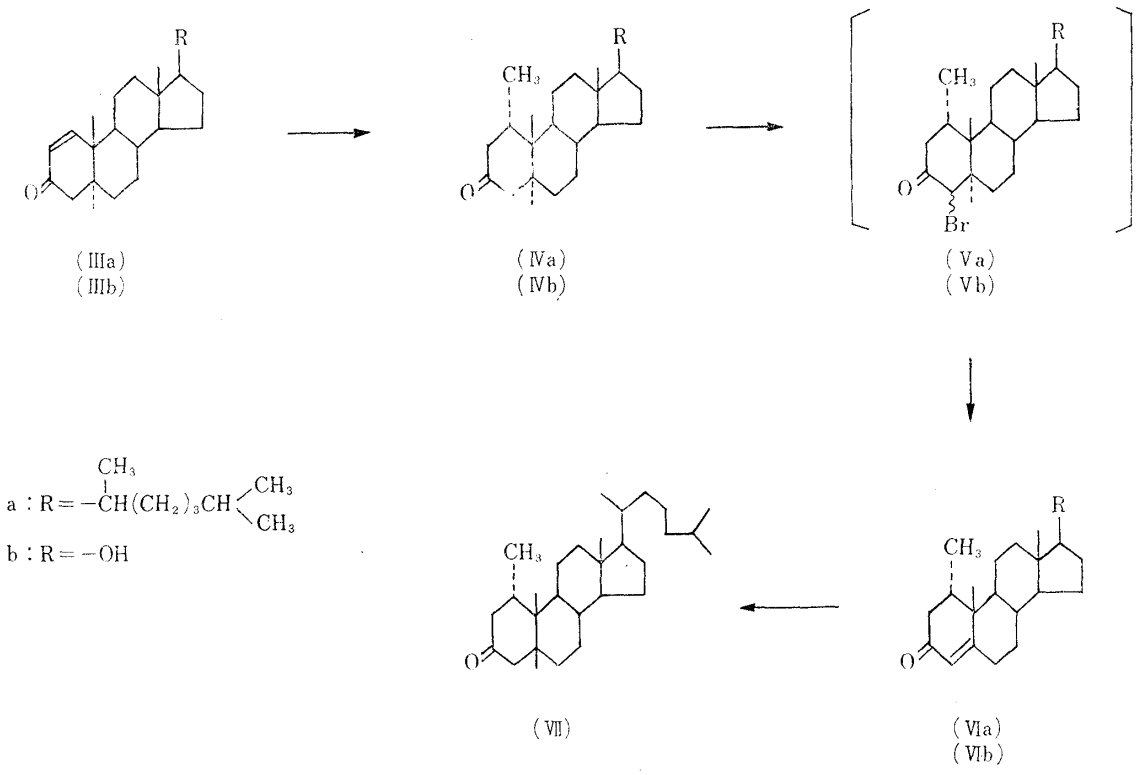


Chart 1.

(VIa) was hydrogenated over palladium-charcoal in ethanol. The hydrogenation product was assumed to be A/B-*cis* 3-oxo compound, because 4-en-3-oxo-steroid is usually transformed into A/B-*cis* 3-oxo compound on hydrogenation.[7] The product was different from (IVa) and it was considered to be 1α-methyl-5β-cholestan-3-one (VII).

The configuration of C-1 methyl group will now be discussed. When the Grignard reagent attacks 5α-cholest-1-en-3-one (IIIa), attack from the rear side is more likely from the stereochemical point of view. Attack from the front side will be disturbed seriously by the interaction with C-11 methylene group. The rotatory dispersion curves for (IVa) and (VII) (Fig. 1) offered effective data for determination of the configuration of C-1 methyl group. (IVa) shows the typical rotatory dispersion curve for A/B-*trans* 3-oxo-steroids,

5) R.P. Holysz : J. Am. Chem. Soc., **75**, 4432 (1953).
6) J.H. Fried, G.E. Arth, L.H. Sarett : J. Am. Chem. Soc., **82**, 1684 (1960).
7) H.J.E. Loewenthal : Tetrahedron, **6**, 269 (1958).

whereas that for (Ⅶ) is an unpredicted positive Cotton effect curve which, however, is of different shape from that of A/B-*trans* 3-oxo-steroid. The molecular model of (Ⅶ, B) (all-chair conformation) shows that 1α-methyl group is interacted seriously with C-11 methylene group. The conformational change will occur in (Ⅶ) due to this interaction

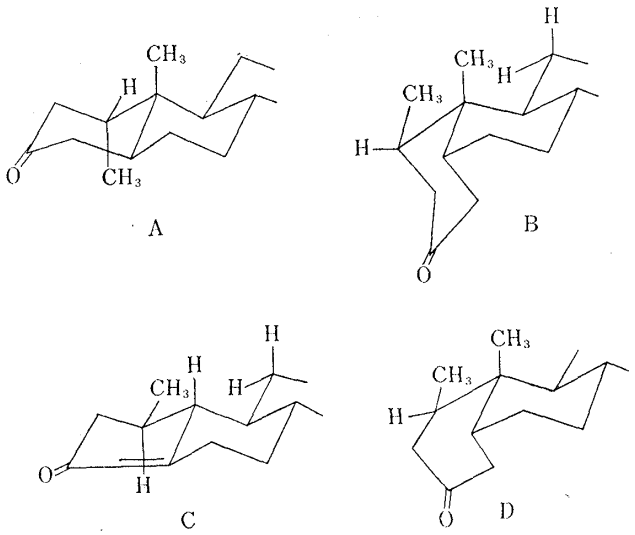


Chart 2.

and the unusual rotatory dispersion curve will be observed. The likely conformation for (Ⅶ), which does not contain such a strong interaction between 1α-methyl group and C-11 methylene group and which will show an unusual rotatory dispersion curve, is ring A-boat conformation (D). On the other hand, such an interaction is absent in the case of 1α-methyl group in (Ⅳa, A). If the methyl group were β-oriented, the rotatory dispersion curve for (Ⅶ) would have a negative Cotton effect characteristic to A/B-*cis* 3-oxo-steroids. The unusual rotatory dispersion curve for the compound supposed to be 1β-methyl-19-norprogesterone (C) has been observed by Djerassi.[8] In this case, there is the same interaction between 1β-methyl group and C-11 methylene group as that in (Ⅶ).

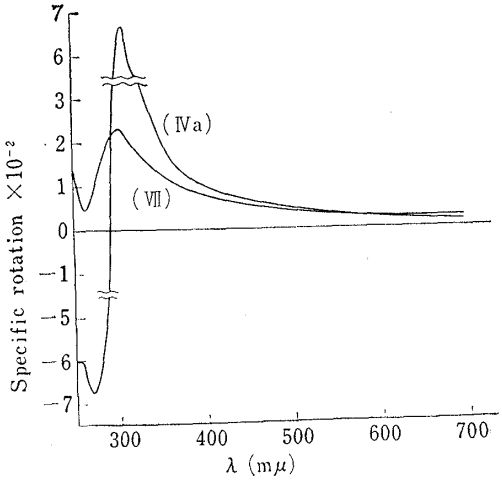


Fig. 1. Rotatory Dispersion Curves of (Ⅳa) and (Ⅶ) (ethanol)

5α-Androst-1-en-3-one (Ⅲb) was converted to (Ⅳb) and (Ⅵb), by the same method as described above. The Grignard reaction of 17β-hydroxyandrosta-1,4-dien-3-one was tried but 1,4-addition product was not obtained and the starting material was recovered. Biological activities of (Ⅳb) and (Ⅵb) will be described elsewhere.

8) C. Djerassi, R. Riniker, B. Riniker : J. Am. Chem. Soc., 78, 6377 (1956).

The molecular rotation differences among (III), (IV), (VI), and (VII) are shown in Table I.

Table I.

No.	Compound	α ($CHCl_3$)	MD	ΔM (III→IV)	ΔM (III→VI)	ΔM (III→VII)
(IIIa)	5α-cholest-1-en-3-one	+ 59[9]	+227			
(IVa)	1α-methyl-5α-cholestan-3-one	+ 32	+127	−100		
(VIa)	1α-methylcholest-4-en-3-one	+121	+482		+255	
(VII)	1α-methyl-5β-cholestan-3-one	+ 28	+110			−117
(IIIb)	17β-hydroxy-5α-androst-1-en-3-one	+ 53[10]	+150			
(IVb)	17β-hydroxy-1α-methyl-5α-androstan-3-one	+ 12	+ 37	−116		
(VIb)	17β-hydroxy-1α-methylandrost-4-en-3-one	+144	+436		+286	

Experimental*2

1α-Methyl-5α-cholestan-3-one (IVa)——To a solution of 5α-cholest-1-en-3-one (IIIa) (500 mg.) in dehyd. Et_2O (100 cc.) CuCl (100 mg.) was added and stirred vigorously. The Grignard reagent prepared from Mg (550 mg.), MeI (1.5 cc.), and dehyd. Et_2O (25 cc.) was added dropwise to the above suspension with vigorous stirring at 0°. Stirring was continued for additional 3 hr. at room temperature. After water was carefully added to decompose excess Grignard reagent, 10% NH_4Cl was added and the product was extracted with Et_2O. After washing with 10% NH_4Cl, 5% Na_2CO_3, and water, and drying over Na_2SO_4, Et_2O was evaporated. The residue was dissolved in EtOH (50 cc.) and AcOH (5 cc.), and the solution was refluxed with the Girard-T reagent (500 mg.) for 1 hr. The solution was poured into water containing Na_2CO_3 (4.7 g.). After non-keto substance was removed by Et_2O extraction, aqueous layer was acidified with 10% HCl and stored at room temperature overnight. The resulting keto substance was extracted with Et_2O. After washing with 5% Na_2CO_3 and water, and drying over Na_2SO_4, Et_2O was evaporated. The residue was recrystallized from MeOH to 1α-methyl-5α-cholestan-3-one (IVa), m.p. 127~130°. Yield, 243 mg. Further recrystallization from MeOH gave white needles, m.p. 128~130°, $[\alpha]_D^{20}$ +32° (c=1.15, $CHCl_3$). IR : $\nu_{max}^{CS_2}$ 1713 cm^{-1} (3-oxo). RD (c=0.11, EtOH); $[\alpha]_{700}$ +16°, $[\alpha]_{589}$ +37°. $[\alpha]_{310}$ +666° (peak), $[\alpha]_{267.5}$ −667 (trough), $[\alpha]_{260}$ −590°. *Anal.* Calcd. for $C_{28}H_{48}O$: C, 83.93; H, 12.08. Found : C, 84.07; H, 11.98.

1α-Methycholest-4-en-3-one (VIa)——To a solution of 1α-methyl-5α-cholestan-3-one (IVa) (321 mg.) in AcOH (10 cc.), a solution of Br_2 (130 mg.) in AcOH (3 cc.) containing one drop of 47% HBr was added dropwise at room temperature with vigorous stirring. The Br_2 was consumed after 20 min. The solution was poured into water and the resulting precipitate was collected, washed well with water, and dried *in vacuo*; yield, 373 mg.

A solution of the crude bromo compound obtained as above in dimethylformamide (10 cc.) was stirred with dried LiCl (700 mg.) at 140~150°. After cool, the solution was poured into water and the resulting oily product was extracted with Et_2O. After washing with 10% HCl and water, and drying over Na_2SO_4, Et_2O was evaporated. The residue was chromatographed on Florisil and the material eluated with benzene was recrystallized from MeOH to give 1α-methylcholest-4-en-3-one (VIa), m.p. 85~88°. Yield, 132 mg. Further recrystallization from MeOH gave white needles, m.p. 88~90°, $[\alpha]_D^{22}$ +121° (c=0.634, $CHCl_3$). UV : λ_{max}^{EtOH} 241 mμ (log ε 4.12). IR $\nu_{max}^{CS_2}$ cm^{-1} : 1670, 1612 (4-en-3-oxo). *Anal.* Calcd. for $C_{28}H_{46}O$: C, 84.35; H, 11.63. Found : C, 84.52; H, 11.58.

1α-Methyl-5β-cholestan-3-one (VII)——A mixture of 1α-methylcholest-4-en-3-one (VIa) (202 mg.), 5% Pd-C (200 mg.), and EtOH (20 cc.) was shaken in a H_2 atmosphere (atmospheric pressure). H_2 uptake was completed in 30 min. Catalyst was removed by filtration and the filtrate was evaporated. The residue was recrystallized from MeOH to give 1α-methyl-5β-cholestan-3-one (VII), m.p. 86~88°. Yield, 149 mg. Further recrystallization from MeOH gave white needles, m.p. 87~89°, $[\alpha]_D^{18}$ +28° (c=0.967, $CHCl_3$). IR $\nu_{max}^{CS_2}$ 1715 cm^{-1} (3-oxo). RD (c=0.11, EtOH); $[\alpha]_{700}$ +23°, $[\alpha]_{589}$ +31°, $[\alpha]_{302.5}$ +245° (peak), $[\alpha]_{267.5}$ +46°, $[\alpha]_{250}$ +148°. *Anal.* Calcd. for $C_{28}H_{48}O$: C, 83.93; H, 12.08. Found : C, 83.72; H, 12.17.

17β-Hydroxy-1α-methyl-5α-androstan-3-one (IVb)——The Grignard reaction of 17β-hydroxy-5α-androst-1-en-3-one (IIIb) (500 mg.) and Girard separtion were carried out as described above. The keto material was recrystallized from Me_2CO-hexane mixture to give 17β-hydroxy-1α-methyl-5α-androstan-3-one (IVb), m.p. 195~201°. Yield, 210 mg. Further recrystallization gave white needles,

*2 All melting points are uncorrected.

9) P. Striebel, Ch. Tamm : Helv. Chim. Acta, **37**, 1094 (1954).

10) A. Butenandt, H. Dannenberg : Ber., **73**, 206 (1940).

m.p. 203~205.5°. $[\alpha]_D^{20}$ +12°(c=1.03, $CHCl_3$), IR $\nu_{max}^{CS_2}$ cm^{-1} : 3640 (-OH), 1717 (3-oxo). *Anal.* Calcd. for $C_{20}H_{32}O_2$: C, 78.89; H, 10.59. Found : C, 78.62; H, 10.48.

17β-Hydroxy-1α-methylandrost-4-en-3-one (VIb)——17β-Hydroxy-1α-methyl-5α-androstan-3-one (IVb) (205 mg.) was brominated and dehydrobrominated as described above. The product was chromatographed on alumina and the material eluted with benzene-Et_2O was recrystallized from Me_2CO-hexane to give 17β-hydroxy-1α-methylandrost-4-en-3-one (VIb), m.p. 183~187.5°. Yield, 93 mg. Further recrystallization gave white needles, m.p. 188~190°, $[\alpha]_D^{17}$ +144°(c=0.624, $CHCl_3$). UV:λ_{max}^{EtOH} 241 mμ (log ε 4.13). IR $\nu_{max}^{CS_2}$ cm^{-1} : 3610 (-OH), 1672, 1615 (4-en-3-oxo). *Anal.* Calcd. for $C_{20}H_{30}O_2$:C, 79.42; H, 10.00. Found : C, 79.51; H, 9.96.

The author is very grateful to Dr. M. Chuman, President of Tsurumi Research Laboratory of Chemistry, Dr. S. Niinobe, Director of Research Laboratory of this company, and Mr. M. Sawai for their valuable advices, and to Dr. E. Yamaguchi, President of this company, and Dr. F. Ueno, Director of Manufacturing Section of this company, for their encouragement throughout this work. The author is also indebted to Mr. K. Tsuneda for his technical help.

Summary

The Grignard reaction of 5α-cholest-1-en-3-one (IIIa) in the presence of cuprous chloride gave 1α-methyl-5α-cholestan-3-one (IVa). (IVa) was brominated and dehydrobrominated to give 1α-methylcholest-4-en-3-one (VIa). (VIa) was hydrogenated over palladium-charcoal to give 1α-methyl-5β-cholestan-3-one (VII). Discussions were made on configuration of C-1 methyl group.

Similarly, 17β-hydroxy-5α-androst-1-en-3-one (IIIb) was transformed into (IVb) and (VIb).

(Received January 21, 1961)

UDC 615.771.7 : 547.233'222

63. Kenichi Sawatari : Studies on Carcinostatic Substances. XXXIX.*1 Antitumor Effect of Derivatives of Nitrogen Mustard containing only one 2-Chloroethyl Group.

(*Yoshitomi Pharmaceutical Industries, Ltd.**2)

In the preceding paper of this series,[1] it was reported that N-(2-chloroethyl)-N-methyl-3-chloropropylamine unexpectedly exhibited a strong antitumor effect on Yoshida sarcoma and several strains of rat ascites hepatomas.

From these observations, it was deduced that 3-chloropropyl group of this compound might also play a role in biological alkylating reaction on the cell constituents of a tumor, in spite of its high inertness in reactivity estimated from chemical reactions in an aqueous solution *in vitro*.

The fact seems to show that even a 2-chloroalkyl group, which does not transform into any active intermediate like aziridinium ion of 2-chloroethyl group of the amine, is able to manifest alkylation activity *in vivo*, if the remaining one functional group of the compound is active enough as a 2-chloroethyl group.

---

*1 This paper constitutes a part of series entitled "Studies on Carcinostatic Substances" by M. Ishidate and Y. Sakurai. Part XXXVIII : This Bulletin, **9**, 996 (1961).

*2 Yoshitomi-machi, Chikujo-gun, Fukuoka-ken (猿渡健市).

1) K. Sawatari : This Bulletin, **9**, 996 (1961).