[CONTRIBUTION FROM THE CHEMICAL LABORATORY, HARVARD UNIVERSITY]

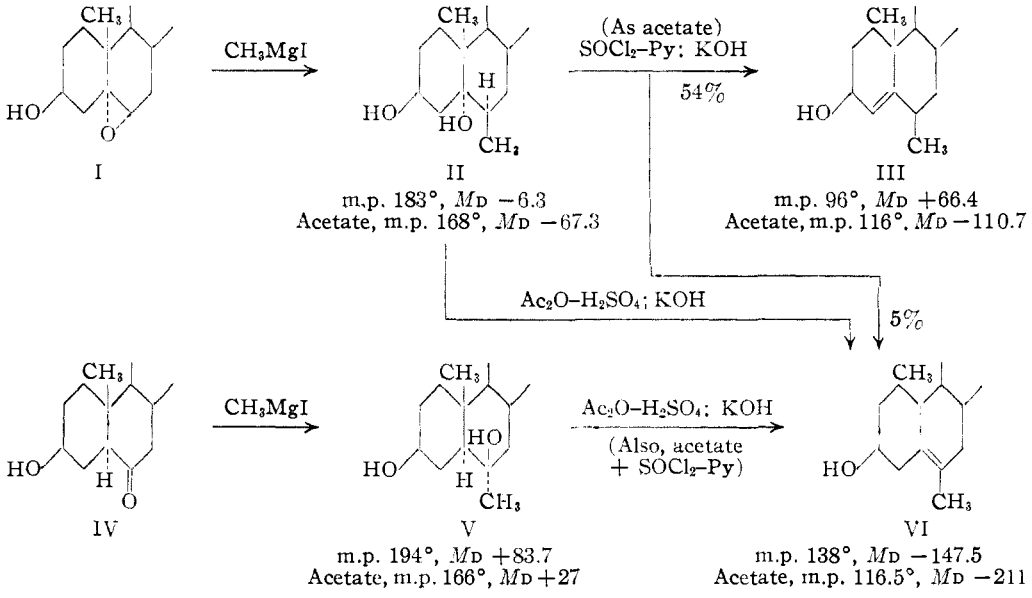
Action of Methylmagnesium Iodide on Cholesterol α-Oxide and 6-Ketocholestanol

BY LOUIS F. FIESER AND JEAN RIGAUDY[1]

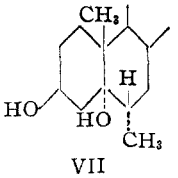
The reaction of cholesterol α-oxide with methylmagnesium iodide proceeds by normal ring fission and affords 6β-methylcholestane-3β,5α-diol (II); 6-ketocholestanol yields the isomeric 6α-methylcholestane-3β,6β-diol (V). Acidic dehydration of II proceeds abnormally by *cis*-elimination to give 6-methylcholesterol (VI), which is the normal product of *trans*-elimination of V. Diol II is dehydrated by thionyl chloride in pyridine in the cold chiefly to the normal product 6β-methyl-Δ⁴-cholestene-3β-ol (III), accompanied by a small amount of the abnormal product VI.

Ushakov and Madaeva[2] found that methylmagnesium iodide reacts slowly in boiling benzene with cholesterol α-oxide (I) to give a diol that forms a monoacetate and a monoketone and that is readily dehydrated with acetic anhydride and sulfuric acid in the cold. They assumed that the diol is formed by normal fission of the oxide ring with inversion at C_6 and has the structure II, and they regarded the dehydration product as 6-methylcholesterol (VI). No rotations were reported. Fieser and Fieser[3] noted that the dehydration postulated would involve a *cis*-elimination and suggested that under the forcing conditions the ionic Grignard reagent may have first caused isomerization of the oxide to 6-ketocholestanol (IV) and that the reaction product may be the 6-methyl-3,6-diol V. Urushibara and Chuman[4] independently suggested that the oxide may have undergone abnormal fission to give 6α-methylcholestane-3β,5α-diol (VII), which could undergo normal *trans*-elimination to 6-methylcholesterol (VI).

In the present investigation, we first confirmed the observations of Ushakov and Madaeva[2] and found that the dehydration product is strongly levoratory, as expected for the Δ⁵-structure VI. A study of the Grignard reaction of 6-ketocholestanol (IV) then established that this substance is not an intermediate in the reaction of the oxide, since it gives a secondary–tertiary diol (monoacetate) different from that (II) resulting from the oxide, the two substances and their acetates have similar melting points, but they show melting point depressions and II is levorotatory whereas V is dextrorotatory. The diol from the 6-ketone is converted by cold acetic anhydride–sulfuric acid in 40–50% yield into an unsaturated acetate identical with that from II, a result consistent with the formulation of the dextrorotatory diol as the 6α-methyl-6β-hydroxy derivative V resulting from rear-bond opening of the β-hindered 6-keto group.[5] The formation of 6-methylcholesterol (VI) from V would thus represent a normal *trans*-elimination.



The hypothesis[4] of an abnormal ring fission of the oxide to give the 6α-methyldiol VII appears very unlikely on the basis of molecular rotation data.



VII

Since, according to Marker's empirical rule[6] a methyl group has an ordinal number comparable

(1) On a fellowship from the Centre National de la Recherche Scientifique of the French Government.

(2) M. I. Ushakov and O. S. Madaeva, *J. Gen. Chem. U.S.S.S.R.*, **9**, 436 (1939) [*C. A.*, **33**, 9309 (1939)].

(3) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd Ed., Reinhold Publishing Corp., New York, N. Y., 1950, p. 224.

(4) Y. Urushibara and M. Chuman, *Bull. Chem. Soc. Japan*, **22**, 69 (1949).

(5) L. F. Fieser, *Experientia*, **6**, 312 (1950).

(6) R. E. Marker, THIS JOURNAL, **58**, 976 (1936).

to that of a hydroxyl group, an approximate estimate of the relative rotational contribution of a 6α- and 6β-methyl group can be made from comparison of the molecular rotations of cholestane-3β,5α,6α-triol, M_D + 89° Di,[7] and of cholestane-3β,5α,6β-triol, M_D 0, Di,[7] with that of cholestane-3β,5α-diol, M_D +81 Chf,[8] which indicates the increments: $\Delta^{6\alpha\text{-OH}} = +8$; $\Delta^{6\beta\text{-OH}} = -81$. The contribution of the 6-methyl group of the Grignard product II is −87.3, which clearly corresponds to the β-orientation of the substituent. A 6α-methyl group would then be expected to make a dextrorotatory contribution, and the diol from the 6-ketone, regarded as 6α-methylcholestane-3β,6β-diol (V), is indeed more dextrorotatory (M_D +83.7 Di) than the parent cholestane-3β,6β-diol (M_D +57 Chf[8]).

In seeking chemical evidence of the configuration of the diol from the oxide, we investigated the dehydration of the substance under non-acidic conditions. The 3-acetate proved resistant to the action of phosphorus oxychloride in pyridine but was dehydrated readily at room temperature by thionyl chloride in pyridine. a reagent known[9] to convert the comparable cholestane-3β,5α,6β-triol 3,6-diacetate into the Δ^4-anhydro derivative and found in the present work to effect smooth dehydration of the diol V to the normal product VI. The chief product of the reaction of II with thionyl chloride and pyridine (54% yield) is an isomer of VI, and since it is strongly dextrorotatory it can be assigned the structure of the acetate of 6β-methyl-Δ^4-cholestene-3β-ol (III), the product of normal *trans*-elimination. 6-Methylcholesteryl acetate was formed in about 5% yield even when all contact with acid during washing was avoided. We thus conclude that 6β-methylcholestane-3β,5α-diol (II) undergoes abnormal *cis*-elimination to some extent even with thionyl chloride–pyridine and that the abnormal reaction predominates in acid dehydration. The abnormal reaction may proceed by an S_N1 mechanism involving transitory formation of a coplanar carbonium ion with the charge at C_5 and expulsion of a proton according to the Saytzeff rule. A similar ion is probably an intermediate in the acid rearrangement of cholestane-3β,5α,6β-triol 3,6-diacetate to Westphalen's diol diacetate,[10] in the present case the tendency of the methyl group to promote introduction of a 5,6-double bond seems to take precedence.

Experimental[11]

6β-Methylcholestane-3β,5α-diol (II).—Portions of cholesterol α-oxide (2–3 g., m.p. 142–142.5°) were refluxed with methylmagnesium iodide in dry benzene for 5 hr. and for 7 hr. according to Ushakov and Madaeva's[2] first and second procedures. In both cases several recrystallizations of the crude product, m.p. 165–167°, from ethanol afforded pure diol, m.p. 182–183°, $[\alpha]^{25}D$ −1.5° di, in 40% yield. The mother liquor contained a less soluble by-product, probably 6-methylcholesterol. The **3-acetate** was obtained under milder conditions than reported in the Russian paper: 0.4 g. of diol was heated with 3 cc. of pyridine and 2 cc. of acetic anhydride for one hour on the steam-bath and the solution let stand at 25° overnight. One crystallization from methanol gave pure material, m.p. 167–168° (164–165°, uncor., as reported[2]), $[\alpha]^{25}D$ −14.6 Di.

6-Methylcholesterol (VI).—The diol II (0.3 g.) was refluxed with acetic anhydride (35 cc.) for two hours and the solution cooled to 25°, treated with 95% sulfuric acid (3 drops), and let stand for three days, as described.[2] Crystallization from methanol gave 6-methylcholesterol acetate, m.p. 115.5–116.5°, $[\alpha]^{25}D$ −47.7° Di, in 40% yield. Saponification[2] and crystallization from methanol afforded 6-methylcholesterol as needles, m.p. 138.5–140.5°, $[\alpha]^{25}D$ −36.8°.

Dehydration of 6α-Methylcholestane-3β,6β-diol-3-acetate with $SOCl_2$–Pyridine.[12]—To an ice-cooled solution of 47 mg. of the acetate in 1 cc. of pyridine (dried over barium oxide) was added 0.2 cc. of thionyl chloride. On standing at 0° for 20 minutes, the solution turned deep yellow and, terminally, cloudy. The mixture was poured into ice-water and extracted with ether, when the colored material was retained in the aqueous phase. The solution was washed with acid and with bicarbonate solution, dried, and evaporated. Crystallization of the solid residue from methanol afforded 34 mg. (76%) of 6-methylcholesteryl acetate, m.p. 114.4–115.4°, $[\alpha]^{22}D$ −46.5° ± 1° Di; no depression with above sample.

6-Ketocholestanol was prepared by the nitration process[13] by the procedure of Heilbron, *et al.*[14] In the nitration of cholesterol to 6-nitrocholesteryl nitrate the concentration of the nitric acid is highly important, since too strong acid has a destructive action and produces resins. Best results were obtained by nitration of 20 g. of cholesterol in 80 cc. of acetic acid with a mixture of 122.5 cc. of nitric acid of sp. gr. 1.50 and 7.5 cc. of acid of sp. gr. 1.60; the yield of crude product, m.p. 126–127°, was 57–60%, and one crystallization from acetic acid raised the m.p. to 127–129°. Heilbron's procedure of reduction of 6-nitrocholesteryl nitrate with zinc dust and acetic acid followed by hydrolysis of the crude oxime nitrate with ethanol and hydrogen chloride gave 89.5% of crude 6-ketocholestanol; methanol proved more suitable for crystallization than ethanol and afforded needles, m.p. 145–146°.

6α-Methylcholestane-3β,6β-diol (V).—A solution of 2 g. of 6-ketocholestanol in 140 cc. of dry ether was refluxed for ¾ hr. with the reagent from 7 g. of magnesium iodide, 1.2 g. of magnesium, and 100 cc. of ether; two-thirds of the ether was then removed by distillation, 100 cc. of benzene was added, and distillation was continued until the temperature of the exit vapors reached 78°. The mixture was then refluxed for 5 hr., cooled, decomposed with ice and acid, and the product collected in ether. On concentration of the washed and dried extract, the diol separated in shiny plates, m.p. 188–190° (uncor.); yield 91%. The recrystallized material melted at 193–194°, $[\alpha]^{25}D$ +20° Di. The substance depressed the m.p. of the diol II to 155–156°.

Anal. Calcd. for $C_{28}H_{50}O_2$ (418.68): C, 80.31; H, 12.04. Found: C, 80.16; H, 12.08.

The 3-acetate was prepared by allowing a solution of 0.3 g. of the diol in 3 cc. of pyridine and 2 cc. of acetic anhydride to stand at 25° for a few days; crystallization from methanol gave material m.p. 166°, $[\alpha]^{25}D$ +5.9° Di. The substance depressed the m.p. of the acetate of II to 136–145°.

Anal. Calcd. for $C_{30}H_{52}O_3$ (460.72): C, 78.20; H, 11.38. Found: C, 77.94; H, 11.09.

Treatment of the 3-acetate of 6α-methylcholestane-3β,6β-diol with acetic anhydride and sulfuric acid by the procedure applied to the acetate of II gave, in 40–50% yield, a product, m.p. 115.5–116.5°, that showed no depression when mixed with 6-methylcholesteryl acetate prepared from II.

6β-Methyl-Δ^4-cholestene-3β-ol Acetate.—6β-Methylcholestane-3β,5α-diol-3-acetate (50-mg. samples) was largely recovered unchanged after treatment with phosphorus oxychloride (0.6 cc.) in pyridine (1.8 cc.) for one-half hour on the steam-bath or for 18 hours at 25°; when the mixtures were heated for 3–6 hr. on the steam-bath the reaction product melted over a considerable range but did not depress the starting material.

(7) S. S. Wagle, Dissertation, Harvard University, 1950.

(8) Pl. A. Plattner and W. Long, *Helv. Chim. Acta*, **27**, 1872 (1944).

(9) V. A. Petrow, O. Rosenheim, and W. Sterling, *J. Chem. Soc.*, 679 (1938).

(10) See Fieser and Fieser, Ref. 3, pp. 277–279.

(11) Melting points are corrected. All rotations determined in dioxane. Microanalyses by Mrs. Shirley Golden.

(12) Experiment by Dr. Hans Heymann.

(13) J. Mauthner and W. Suida, *Monatsh.*, **24**, 648 (1903); A. Windaus, *Ber.*, **36**, 3752 (1903).

(14) I. M. Heilbron, H. Jackson, E. R. H. Jones and F. S. Spring, *J. Chem. Soc.*, 102 (1938).

Dehydration was accomplished successfully as follows. A solution of 1 g. of the acetate of II in 5 cc. of dry pyridine was cooled in ice and treated with 0.2 cc. of thionyl chloride, added dropwise with shaking. The solution turned yellow and a white solid separated almost at once. After 5 minutes the solid was collected and triturated with water, and after several hours the material was filtered or extracted with ether. One crystallization from methanol gave two crops of methyl-Δ^4-cholestenyl acetate: 0.40 g., m.p. 113.5–115.5°, and 0.12 g., m.p. 112.5–114.5° (total yield, 54%). Each crop on one recrystallization from methanol afforded pure material in the form of glistening leaflets, m.p. 115–116°, $[\alpha]^{22}D$ −25° Di. A mixture of the substance with 6-methylcholesteryl acetate melted at 91–93°.

Anal. Calcd. for $C_{30}H_{50}O_2$ (442.71): C, 81.39; H, 11.38. Found: C, 81.34; H, 11.42.

The pyridine mother liquor was diluted with water, let stand for a few days, and extracted with ether. Evaporation of the acid-washed and dried extract gave an oil that partially solidified on standing, and six crystallizations of the solid fraction from methanol afforded 0.05 g. (5%) of colorless needles of 6-methylcholesteryl acetate, m.p. 115.5–116.5° (mixed m.p.; depression with the acetate of III). The result was the same when acid-washing of the ethereal extract was omitted and all possible contact with acids thus avoided.

Attempted Isomerization.—A solution of 0.09 g. of 6β-methyl-Δ^4-cholestene-3β-ol acetate in 50 cc. of acetic anhydride was treated with 4 drops of 95% sulfuric acid; a series of transient colors was observed, but after two hours at 25° the reaction mixture was largely soluble in water and ether extraction afforded only a negligible amount of amorphous green material. The Δ^4-isomer is also very sensitive to hydrochloric acid: when a solution of 0.03 g. of acetate in 30 cc. of methanol was treated with 3 drops of 36% acid and let stand overnight the only crystalline product isolated melted at 91–93° and depressed the m.p. of both the Δ^4- and Δ^5-isomers.

6β-Methyl-Δ^4-cholestene-3β-ol (III).—Saponification of the acetate (0.2 g.) by refluxing it for one-half hour with 1 *N* alcoholic potassium hydroxide (30 cc.) gave the free alcohol (0.15 g.), m.p. 95–96°, $[\alpha]^{22}D$ +16.6° Di. The substance tends to combine with methanol, although no definite solvate was isolated. Quick cooling of a methanol solution in an ice-bath gives an oil, whereas slow crystallization affords long prismatic needles. Satisfactory analyses were obtained only when the sample had been dried at 66° for 6–10 hr.

Anal. Calcd. for $C_{28}H_{48}O$ (400.66): C, 83.93; H, 12.08. Found: C, 84.20, 84.12; H, 11.94, 12.07.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY]

The Condensation of Nitromethane with D-Mannose: Synthesis of D-Manno-D-*gala*-heptose and D-Manno-D-*talo*-heptose

BY JOHN C. SOWDEN AND ROBERT SCHAFFER

D-Mannose has been condensed with nitromethane under the influence of alkali to give 37% of 1-nitro-1-desoxy-D-manno-D-*gala*-heptitol and 10% of 1-nitro-1-desoxy-D-manno-D-*talo*-heptitol. The nitroalcohols were converted to the corresponding aldoheptoses, D-manno-D-*gala*-heptose and D-manno-D-*talo*-heptose, in good yield by treatment of their sodium salts with aqueous sulfuric acid (Nef reaction). The preparation of D-manno-D-*talo*-heptose by this method is a considerable improvement over its preparation by the cyanohydrin synthesis from D-mannose.

The addition of hydrocyanic acid to the D-mannose molecule proceeds almost exclusively to D-manno-D-*gala*-heptonic nitrile and hydrolysis of the latter readily gives 85–90% of D-manno-D-*gala*-heptonic acid, isolated as the barium salt.[1] The epimeric D-manno-D-*talo*-heptonic acid also has been isolated from the reaction in low yield (4–6%) both as the phenylhydrazide[2] and the lead salt.[3] Thus, the yield of D-manno-D-*talo*-heptose obtainable by the Kiliani–Fischer cyanohydrin synthesis from D-mannose is only about 2%. The difficulty of preparing this aldoheptose apparently has discouraged any detailed study of its chemistry and any attempts to prepare from it the related higher-carbon sugars.

In a continuation of our study of the nitromethane synthesis of higher-carbon aldose sugars[4] and in the hope that this method might make D-manno-D-*talo*-heptose more readily available, we have now studied the products of the alkali-induced condensation of D-mannose with nitromethane. Powdered D-mannose is relatively insoluble in a mixture of methanol and nitromethane containing sodium methoxide but, on shaking the suspension, the sugar gradually disappears from the solid phase and is replaced by a precipitate of the amorphous seven-carbon sodium *aci*-nitroalcohols. Removal of the sodium from the latter then gives the mixed nitrodesoxyheptitols in 45–50% combined yield. Subsequent fractional crystallization, which is greatly aided by a marked difference in the solubility of the epimers in water, shows that the ratio of 1-nitro-1-desoxy-D-manno-D-*gala*-heptitol formed to the epimeric 1-nitro-1-desoxy-D-manno-D-*talo*-heptitol is approximately 3.5:1. Since the latter nitroalcohol can be transformed to the corresponding aldoheptose *via* the Nef reaction in excellent yield (80%), D-manno-D-*talo*-heptose is much more easily available by this method than by the previously-known cyanohydrin synthesis. Although the epimeric D-manno-D-*gala*-heptose is obtained in very high yield by the cyanohydrin synthesis, the relative simplicity of the nitromethane synthesis makes it attractive also for the laboratory preparation of this aldoheptose.

D-Manno-D-*talo*-heptose was first obtained in crystalline condition by Peirce,[2] after regeneration from its *p*-nitrophenylhydrazone. He was unable to find a suitable recrystallization solvent and, consequently, did not report any physical constants for the sugar. Later, Ettel[5] reported the preparation of the heptose by the reduction of epimerized D-manno-D-*gala*-heptonic acid. After several re-

(1) Fischer and Hirschberger, *Ber.*, **22**, 365 (1889).

(2) Peirce, *J. Biol. Chem.*, **23**, 327 (1915).

(3) Isbell, *Bur. Standards J. Research*, **20**, 97 (1938).

(4) Sowden and Fischer, THIS JOURNAL, **66**, 1312 (1944); **67**, 1713 (1945); **68**, 1511 (1946); **69**, 1048, 1963 (1947); Sowden, *ibid.*, **71**, 1897 (1949); **72**, 808 (1950); *Science*, **109**, 229 (1949); *J. Biol. Chem.*, **180**, 55 (1949).

(5) Ettel, *Coll. Czech. Chem. Comm.*, **4**, 504 (1932).