with Barton's compound[8] obtained from usnic acid phenylhydrazone monoanhydride on treatment with methanolic KOH, by the mixed melting point determination and the comparison of UV and IR spectra.

The authors wish to express their deep gratitude to Dr. Y. Asahina, Emeritus Professor of University of Tokyo, for his encouragement throughout the course of this study and Dr. S. Shibata, Professor of University of Tokyo, for the gift of usnic acid. The authors are indebted to Mr. S. Matsuoka of this university and Japan Electron Optics Laboratory Co. Ltd. for measurement of NMR spectra. Thanks are due to Mr. Y. Itatani for elemental analysis and Miss E. Katarao for co-operation.

Summary

1) The ozonolysis of methyl- and methyldihydrousnic acid was discussed.

2) The structure of the formolysis product of methylusnic acid was elucidated as being II.

3) The structure of monoacetylusnic acid isomethoxide was reinvestigated and established as being V.

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198. Shunsaku Noguchi and Katsura Morita: Aldol-Condensation of Corticoids with Formaldehyde.*1 Syntheses of 21-Hydroxymethyl-corticoids and 21-Methyl-11β,17α-dihydroxypregna-1,4-diene-3,20,21-trione 17-Acetate.

(*Research Laboratories, Takeda Chemical Industries, Ltd.**2)

While many corticoids have been prepared which are modified by the addition of a methyl[1] or a hydroxyl[2] group at various positions in the molecules, and their biological properties noted, no hydroxymethyl[3]-substituted corticoids have been reported. In the present paper we describe the synthesis of 21-hydroxymethyl-prednisolone and -hydrocortisone by the aldol condensation of the parent steroids with formaldehyde.

When prednisolone (I) was treated with aqueous formaldehyde in the presence of sodium acetate as catalyst, the addition of a hydroxymethyl group to the methylene at C-21 resulted and 21-hydroxymethylprednisolone (II) was obtained in about 50% yield as amorphous hydrated crystals, from which the crystalline water was hardly removed by the ordinary drying conditions. Anhydrous crystals of II were obtained by repeating the following procedure: the amorphous hydrated crystals were dissolved in methanol-ethyl acetate and the solvent evaporated under azeotropic conditions.[4] II gave the diacetate (III) by the usual acetylation with acetic anhydride and pyridine. On treatment

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*1 This paper constitutes Part XXX of Takeda Laboratories' series entitled "Steroids"; Part XXIX: This Bulletin, **11**, 144 (1963).

*2 Juso-nishino-cho, Higashiyodogawa-ku, Osaka (野口俊作, 森田 桂).

1) E. Toromanoff: Bull. soc. chim. France, **1960**, 888.

2) S. Bernstein: Recent Progress in Hormone Research, **14**, 1 (1958).

3) cf. A.L. Nussbaum, T.L. Poper, E.P. Oliveto, S. Friedman, I. Wender: J. Am. Chem. Soc., **81**, 1228 (1959); P.F. Beal, M.A. Rebenstore, J.E. Pike: *Ibid.*, **81**, 1231 (1959) (6-hydroxymethylation by oxo reaction).

4) T. Miki, K. Morita, S. Noguchi, T. Kishi, K. Hiraga, H. Nawa: This Bulletin, **11**, 95 (1963).

with acetone in the presence of boron trifluoride, II furnished the acetonide (IV), which regenerated II on heating with aqueous acetic acid. Sodium bismuthate oxidation of II gave 11β-hydroxyandrosta-1,4-diene-3,17-dione (V).

When carbonate or bicarbonate was substituted for sodium acetate as catalyst in the reaction, partial degradation of the side chain was observed, thus, the reaction of I with formaldehyde in the presence of sodium bicarbonate at 60° gave a mixture of the 17-ketone (V) and the 21-hydroxymethylated compound (II). When the reaction was carried out at 100° using the same catalyst, V was the main product and none of II was obtained; instead, a small quantity of a more polar product was formed. The compound was isolated and the structure was assigned to 21,21-bis(hydroxymethyl)-prednisolone (VI) on the basis of the elemental analysis and other chemical and physical properties. VI was also obtained by the treatment of II with formaldehyde in the presence of sodium bicarbonate. Sodium bismuthate oxidation of VI afforded the 17-ketone (V).

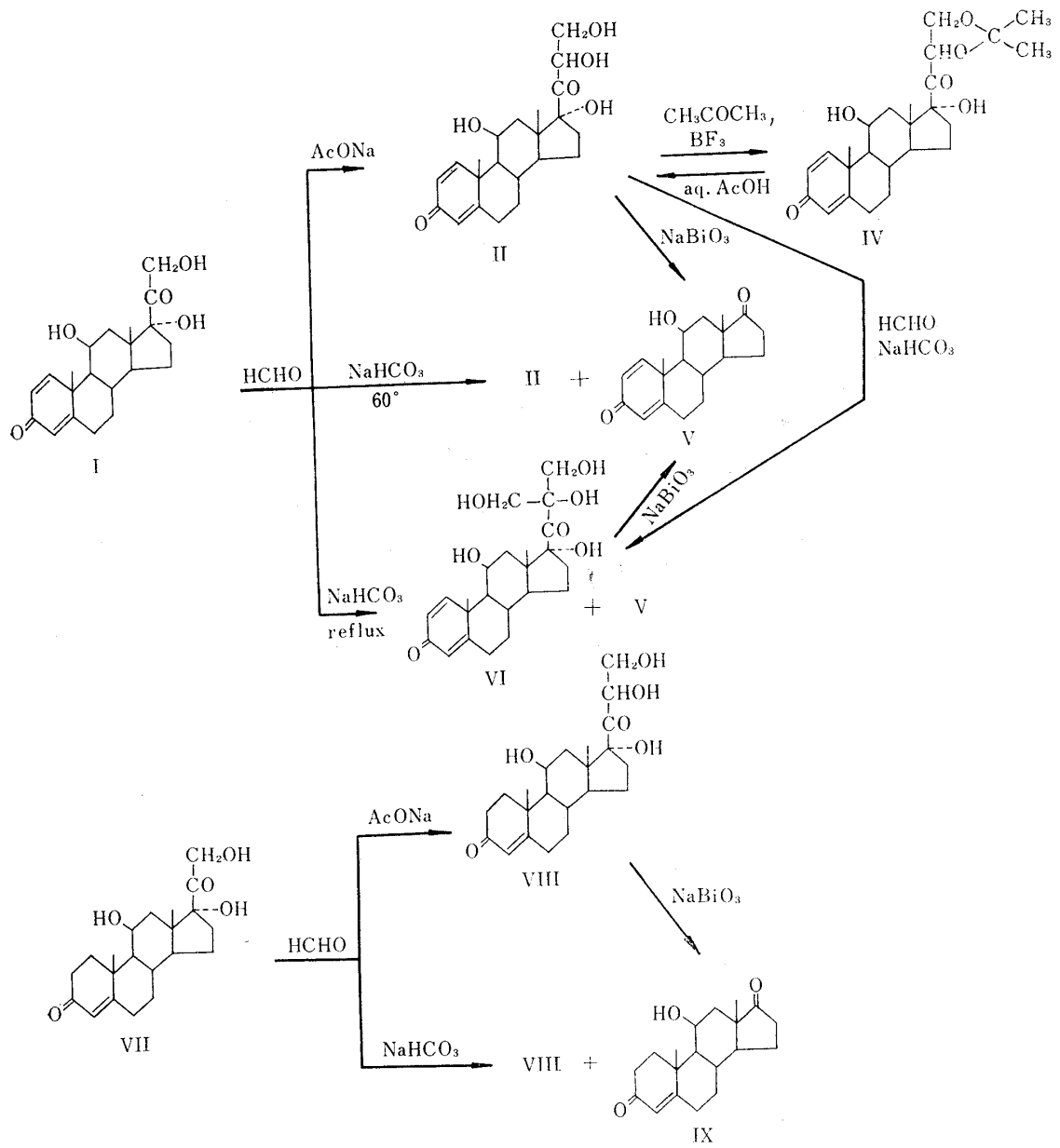

Chart 1.

Similar results as in the case of I were obtained with hydrocortisone (VII). By the use of sodium acetate as catalyst 21-hydroxymethylhydrocortisone (VIII) was obtained as the sole reaction product; however, under the conditions employing sodium bicarbonate at 60°, the 21-hydroxymethylated compound (VIII) and 11β-hydroxyandrost-4-ene-3,17-dione (IX) were obtained. Oxidation of VIII with sodium bismuthate likewise gave the 17-ketone (IX).

The formation of the 21-hydroxymethyl derivatives, II and VIII, introduces a new asymmetric center at C-21 and hence should give rise to two stereoisomers. Only one isomer has been isolated at present, and the configuration still remains to be clarified.

It is interesting to note that in the reaction of I or VII with formaldehyde in the presence of sodium bicarbonate the main reaction product was the 17-ketone, V or IX. The mechanism of the formation of the 17-ketones seems to involve the following two steps : i) The isomerization of 21-hyroxymethylated compounds into 21-oxo-20,17-diols, and ii) subsequent splitting of C-17,20 linkage through a reverse aldol-type reaction as shown in Chart 2.

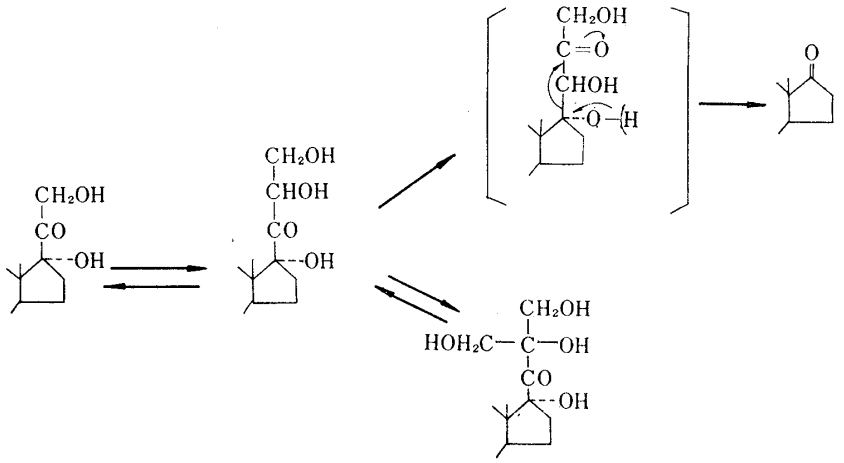

Chart 2.

When the diacetate (III) was passed through a column of aluminum oxide, a light yellow compound, m.p. 254°, was obtained in good yield. The elemental analysis of the compound showed the correct value for $C_{24}H_{30}O_6$, and corresponded to that of the compound which has lost one molecule of acetic acid from III. The structure of the compound was eventually established to be 21-methyl-11β,17α-dihydroxypregna-1,4-diene-3,20,21-trione 17-acetate (X) on the basis of chemical and physical properties. The ultraviolet spectrum of the compound exhibited the absorption maxima at 243 and 397 mμ and the nuclear magnetic resonance spectrum*3 showed four singlets at 0.93 (18-CH_3), 1.47 (19-CH_3), 2.00 (acetyl-CH_3) and 2.31 p.p.m., each equivalent to three protons, respectively. The ultraviolet absorption at 397 mμ and the nuclear magnetic resonance band at 2.31 p.p.m. suggested the presence of a moiety of CH_3-CO-CO- and the evidence for the α-diketone structure was further confirmed by the formation of the quinoxaline derivative (XI). Mild oxidation of X with one equivalent of chromium trioxide gave the 11-ketone (XII), whose infrared spectrum had no absorption maxima in the hydroxyl region. These observations, together with nuclear magnetic resonance spectrum of X, suggested that the tertiary hydroxyl group at C-17 should have been acetylated during the course of the reaction.

*3 NMR spectrum was obtained with a Varian A-60 NMR spectrometer at 60 Mc.p.s. in $CHCl_3$ solution containing tetramethylsilane as an internal reference. s : singlet, b : broad, numerals in parentheses : the number of protons.

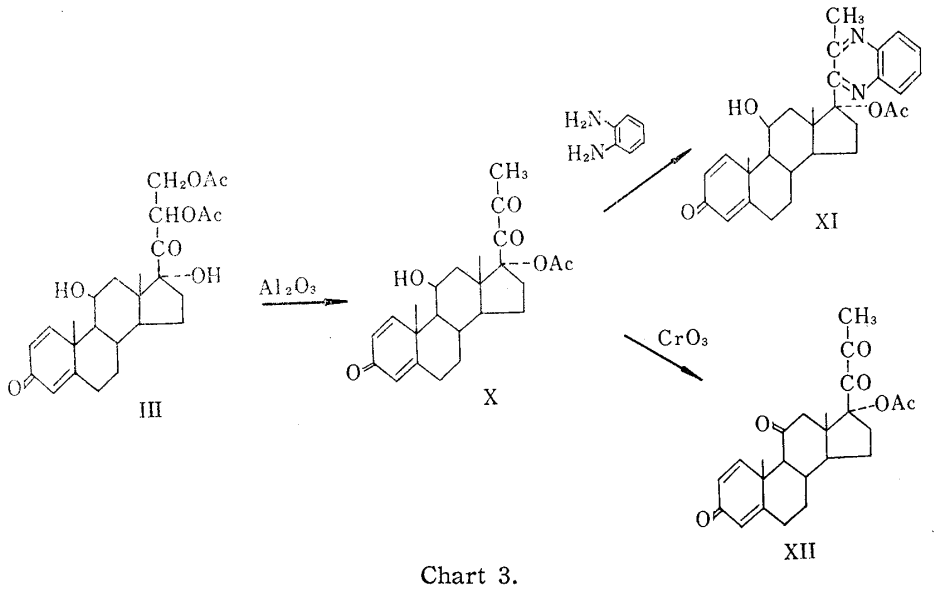

Chart 3.

Recently, Agnello, *et al.*[5] described the synthesis of 21-methyl-11β,17α-dihydroxypregna-1,4-diene-3,20,21-trione (XIII)*4 by the dehydrohalogenative rearrangement of 21-chloromethylprednisolone. Our compound (X), therefore, is the 17-acetate of Agnello's substance (XIII). The mechanism of the formation of X from III by the reaction with aluminum oxide could be illustrated as shown in Chart 4.

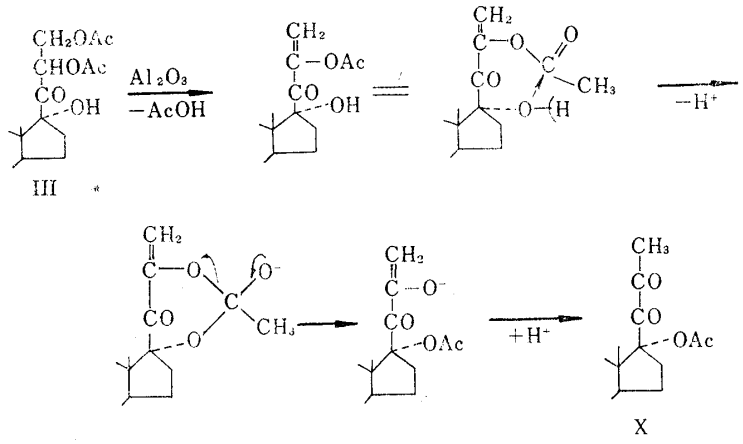

Chart 4.

Experimental*5

21-Hydroxymethylprednisolone (II)——a) To a solution of 1.0 g. of prednisolone (I) in 20 ml. of MeOH and 5 ml. of 37% aqueous formaldehyde was added a solution of 0.75 g. of AcONa in 3 ml. of H_2O and the mixture was heated under reflux on a steam bath for 5 hr. After acidification with AcOH, the solution was concentrated to half the original volume and extracted with AcOEt. The extracts were combined, washed with water, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue (hydrate of II) was dissolved in a small volume of MeOH, diluted with AcOEt and the solution was evaporated slowly on a steam bath at an atmospheric pressure to distil off the water under

*4 Recently, the compound (XIII) was also obtained by the hydrolysis of 21-methyleneprednisolone 17,21-acetonide, which was prepared by the reaction of prednisolone 17,21-acetonide [M. Tanabe, B. Bigley: J. Am. Chem. Soc., 83, 756 (1961)] with formaldehyde: private communication from Dr. M. Tanabe.

*5 All melting points are uncorrected.

5) E. J. Agnello, R. Pinson, S. K. Figdor, G. M. K. Hughes, H. W. Ordway, B. M. Bloom, G. D. Laubach: Experientia, **16**, 357 (1960).

azeotropic conditions. This procedure for dehydration was repeated to obtain an anhyd. crystals of II, 0.55 g., m.p. 215~219°, $[\alpha]_D^{23}$ +90° (c=1.0, EtOH), UV : λ_{max}^{EtOH} 243 mμ (ε 14,800). *Anal.* Calcd. for $C_{22}H_{30}O_6$: C, 67.67; H, 7.74. Found : C, 67.40; H, 7.80.

b) To a solution of 2.0 g. of I in 40 ml. of MeOH and 10 ml. of 37% aqueous formaldehyde was added a solution of 0.2 g. of $NaHCO_3$ in 6 ml. of water and the mixture was gently warmed at 60~70° on a steam bath. The heating was continued until the starting material (I) had almost disappeared, and the reaction mixture was tracked by thin layer chromatography using micro plates[6] (silica gel G, solvent : AcOEt, Rf : I, 0.25; II, 0.22) or paper partition chromatography (Whatman No. 1, solvent : formamide-$CHCl_3$,[7] Rf : I, 0.3; II, 0.05). After 4 hr., the solution was cooled, diluted with H_2O and extracted with Et_2O. The Et_2O extracts were combined, washed with H_2O, dried over Na_2SO_4 and evaporated. The residue was dissolved in benzene and chromatographed on Florisil. Elution with Et_2O-CH_2Cl_2 (1:1) gave 0.7 g. of crystalline material, m.p. 177~180°. The identity of the substance with 11β-hydroxyandrosta-1,4-diene-3,17-dione (V) was established by mixture melting point and comparison of infrared spectra. The aqueous mother liquor was acidified with AcOH, concentrated to a small volume and extracted with AcOEt. The extracts were combined, washed with water, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue (hydrate of II) was dissolved in a mixture of MeOH and AcOEt and worked up as described in a) to give 0.4 g. of anhyd. crystals of II, m.p. 208~210°

Derivatives of 21-Hydroxymethylprednisolone (II). a) Diacetate (III)——A mixture of 200 mg. of II, 1.5 ml. of pyridine and 1.5 ml. of Ac_2O was warmed on a steam bath to insure a clear solution and then allowed to stand overnight at room temperature. The solution was poured onto ice and the resulting precipitates were separated and washed with water to give the crude diacetate (III), m.p. 80~100° (hydrate). The crude hydrate was dissolved in benzene and chromatographed on Florisil. Elution with benzene-Et_2O (1:1) gave the diacetate (III), which crystallized on treatment with hot hexane, m.p. 160~163°, UV : λ_{max}^{EtOH} 242 mμ (ε 15,000). *Anal.* Calcd. for $C_{26}H_{34}O_8$: C, 65.80; H, 7.22. Found : C, 65.95; H, 7.22.

b) Acetonide (IV)——To a suspension of 0.45 g. of II in 23 ml. of Me_2CO was added 0.25 ml. of 37% BF_3 etherate, and the mixture was stirred at room temperature for 15 min., during which time crystals of II dissolved. After an addition of 0.5 ml. of pyridine, the solution was concentrated under reduced pressure and the residue was mixed with H_2O. The precipitates were collected, washed with H_2O, dissolved in benzene and chromatographed on Florisil. Elution of the column with CH_2Cl_2 gave IV, which was recrystallized from CH_2Cl_2-Et_2O, m.p. 175~177°, UV : λ_{max}^{EtOH} 243 mμ (ε 13,100). *Anal.* Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found : C, 70.11; H, 8.09.

Acid Hydrolysis of the Acetonide (IV) : A solution of 200 mg. of IV in 10 ml. of 80% aqueous AcOH was heated on a steam bath for 45 min. The solution was extracted with AcOEt and the extracts were combined, washed with H_2O, dilute solution of $NaHCO_3$ and H_2O. After drying over Na_2SO_4 and evaporation under reduced pressure, the residue was crystallized from MeOH-AcOEt under azeotropic conditions as described in the purification of II. The crystals of II thus obtained melted at 215~218°.

$NaBiO_3$ Oxidation of 21-Hydroxymethylprednisolone (II)——To a solution of 200 mg. of II in 7 ml. of AcOH and 7 ml. of H_2O was added 2.7 g. of $NaBiO_3$. After stirring of the mixture for 30 min., the reaction mixture was filtered and the filtrate was treated with 30 ml. of H_2O and 30 ml. of 3*N* KOH solution under ice-cooling. The solution was extracted with benzene, and the organic layer was washed with H_2O, dilute solution of $NaHCO_3$, dried over Na_2SO_4 and the solvent evaporated to dryness under reduced pressure. The resulting crystals of V melted at 180°, and its identity with an authentic sample was established by mixture melting point and the comparison of the infrared spectra.

21,21-Bis(hydroxymethyl)prednisolone (VI)——a) From prednisolone (I) : To a solution of 1.0 g. of I in 20 ml. of MeOH and 5 ml. of 37% aqueous formaldehyde was added a solution of 0.1 g. of $NaHCO_3$ in 3 ml. of H_2O. After heating of the solution under reflux on a steam bath for 4 hr., the solution worked up as described in the procedure a) for the preparation of II from I. The Et_2O extract of the solution gave V. 0.35 g., m.p. 177~180° and the aqueous mother liquor gave the hydrate of VI, which was crystallized from MeOH-AcOEt under azeotropic conditions to give anhyd. crystals of VI, 0.2 g., m.p. 220~221°, $[\alpha]_D^{23}$ +100° (c=1.0, EtOH), UV : λ_{max}^{EtOH} 243 mμ (ε 14,500). *Anal.* Calcd. for $C_{23}H_{32}O_7$: C, 65.69; H, 7.67. Found : C, 65.64; H, 7.79.

b) From 21-hydroxymethylprednisolone (II) : To a solution of 200 mg. of II in 6 ml. of MeOH and 1 ml. of 37% aqueous formaldehyde was added a solution of 0.02 g. of $NaHCO_3$ in 0.6 ml. of H_2O and the solution was gently warmed on a steam bath at 60~70°. The heating was continued until the starting material (II) had almost disappeared and the reaction mixture was tracked by paper partition chromatography (Whatman No. 1, solvent : formamide-$CHCl_3$,[7] Rf : II, 0.05; VI, 0.01). After heating for 5~10 hr., the solution was worked up as described in a) giving V from the Et_2O extract and VI from

6) *a*) J. J. Peifer : Mikrochim. Acta, 529 (1962). *b*) K. Morita, F. Haruta : J. Chromatography, in press.

7) A. Zaffaroni : Recent Progress in Hormone Research, 8, 51 (1953).

the aqueous mother liquor. VI thus obtained was not depressed on admixture with a sample obtained by method a) and their infrared spectra were superimposable.

$NaBiO_3$ Oxidation of 21,21-Bis(hydroxymethyl)prednisolone (VI)——The oxidation of VI with $NaBiO_3$ was carried out by the same procedure as in the case of II. The product, m.p. 180°, was identical with V.

21-Hydroxymethylhydrocortisone (VIII)——a) Hydrocortisone (VII) (2.0 g.) was treated with aqueous formaldehyde in the presence of AcONa according to the procedure a) for the preparation of II from I. The resulting product was crystallized from MeOH-AcOEt under azeotropic conditions to give 1.0 g. of VIII, m.p. 173~177°, UV : λ_{max}^{EtOH} 241 mμ (ε 15,500), $[\alpha]_D^{21}$ +122°(c=1.0, EtOH). *Anal.* Calcd. for $C_{22}H_{32}O_6$: C, 67.32; H, 8.22. Found : C, 67.46; H, 8.18.

b) VII (2.0 g.) was treated with aqueous formaldehyde in the presence of $NaHCO_3$ according to the procedure b) for the preparation of II from I. The Et_2O extract of the reaction mixture gave 0.8 g. of 11β-hydroxyandrost-4-ene-3,17-dione (IX), m.p. 191~192°, which was identical in all respects (mixture melting point and infrared spectra) with an authentic sample of IX. The aqueous mother liquor gave VIII, which was difficult to be crystallized although the dehydration procedure under azeotropic conditions was repeated.

$NaBiO_3$ Oxidation of 21-Hydroxymethylhydrocortisone (VIII)——The oxidation of VIII with $NaBiO_3$ was carried out by the same procedure as in the case of II. The product, m.p. 191~193°, was identical with IX.

21-Methyl-11β,17α-dihydroxypregna-1,4-diene-3,20,21-trione 17-Acetate (X)——21-Hydroxymethylprednisolone diacetate (III) (hydrate, m.p. 80~100°) (3.0 g.) was dissolved in benzene and passed through a column of 300 g. of alumina. The column was eluated with benzene, benzene-Et_2O (1:1) and Et_2O. Elution with Et_2O gave 1.4 g. of a light yellow product (X), which was recrystallized from benzene or MeOH and showed m.p. 254°, UV : λ_{max}^{EtOH} 243 mμ (ε 15,000), $\lambda_{max}^{CHCl_3}$ 397 mμ (ε 34), $[\alpha]_D^{24}$ +23°(c=1.0, $CHCl_3$), IR λ_{max}^{KBr} μ : 2.96, 5.78, 6.01, 6.17, 6.22, NMR*3 δ (p.p.m.)^{CHCl_3} : 0.93/s (3) (18CH_3), 1.47/s (3) (19-CH_3), 2.00/s(3)(17-Ac-CH_3), 2.31/s(3)(21a-CH_3), 4.54/b(1)(11-H). *Anal.* Calcd. for $C_{24}H_{30}O_6$: C, 69.54; H, 7.30. Found : C, 69.61; H, 7.29.

17β-(3-Methyl-2-quinoxalinyl)-11β,17α-dihydroxyandrosta-1,4-dien-3-one 17-Acetate (XI)——To a solution of 400 mg. of X in 12 ml. of MeOH and 4 ml. of AcOH was added 250 mg. of *o*-phenylenediamine. After reflux for 5 hr., the reaction mixture was diluted with H_2O and neutralized with $NaHCO_3$. The resulting yellow solid was collected and washed with H_2O. The solid, 500 mg., was dissolved in $CHCl_3$-Me_2CO-EtOH (90:10:3) and chromatographed on 600 g. of silica gel (Mallinckrodt Chemical Works, for Chromatographic analysis, 100 mesh), which was impregnated with 30% (w/w) of H_2O. Elution with the same solvent gave firstly 180 mg. of the desired quinoxaline derivative (XI). The quinoxaline derivative (XI) was recrystallized from CH_2Cl_2-MeOH, m.p. 244°, UV λ_{max}^{EtOH} mμ (ε) : 240 (48,000), 320 (9,200). *Anal.* Calcd. for $C_{30}H_{34}O_4N_2$: C, 74.05; H, 7.04; N, 5.76. Found : C, 74.32; H, 7.01; N, 5.93.

Continued elution with the same solvent gave 180 mg. of a yellowish product, which after recrystallization from CH_2Cl_2-MeOH showed m.p. 234°, whose structure has not been made clear.

17α-Acetoxy-21-methylpregna-1,4-diene-3,11,20,21-tetrone (XII)——To a solution of 240 mg. of X in 24 ml. of Me_2CO was added 2.4 ml. of CrO_3-H_2SO_4 solution.*6 After one min., the excess of CrO_3 was decomposed with MeOH. The resulting green solution was diluted with H_2O and concentrated under reduced pressure until crystals deposit. The crude XII was collected, washed with H_2O and recrystallized from CH_2Cl_2-Et_2O, 200 mg., m.p. 215~218°, UV : λ_{max}^{EtOH} 240 mμ (ε 15,200), $\lambda_{max}^{CHCl_3}$ 395 mμ (ε 35). *Anal.* Calcd. for $C_{24}H_{28}O_6$: C, 69.88; H, 6.84. Found : C, 69.72; H, 6.79.

The authors express their deep gratitude to Dr. S. Tatsuoka, Director of these Laboratories, for his kind encouragement, to Drs. Y. Abe and M. Nishikawa for their guidance and to Dr. T. Miki for his helpful advice. Thanks are also due to Miss F. Haruta for her technical assistance, to Mr. H. Kamio for spectral determinations and to Mr. M. Kan for elemental analyses.

Summary

21-Hydroxymethylprednisolone (II), 21-hydroxymethylhydrocortisone (VIII) and 21,21-bis(hydroxymethyl)prednisolone (VI) were synthesized by the aldol condensation of the parent steroids with formaldehyde. When 21-hydroxymethylprednisolone diacetate (III) was passed through a column of aluminum oxide, a light yellow compound was obtained and the structure was assigned to 21-methyl-11β,17α-dihydroxypregna-1,4-diene-3,20,21-trione 17-acetate (X). The mechanism of the formation of X from III was also proposed.

(Received May 29, 1963)

*6 A solution of 26.72 g. of CrO_3 in 23 ml. of conc. H_2SO_4 diluted with H_2O to a volume of 100 ml. [C. Djerassi, R.R. Engle, A. Bowers : J. Org. Chem., **21**, 1548 (1956). And see also K. Bowden, I.M. Heilbron, E.R.H. Jones, B.C.L. Weedon : J. Chem. Soc., **1946**, 39].