for 1.5 hr. After an addition of 0.7 cc. of AcOH, the mixture was concentrated to remove MeOH under vacuum and the residue was solidified on standing. Filtration and washing with water furnished 1 g. of white powder. On purification of this material from MeOH–Me_2CO, the major portion remained as an oil and only few amounts of crystals were isolated. Recrystallization from MeOH gave colorless needles, m.p. 270°. *Anal.* Calcd. for $C_{21}H_{36}O_4 \cdot \frac{1}{2}H_2O$: C, 69.62; H, 10.31. Found : C, 69.83; H, 10.28.

The authors express their deep gratitude to Dr. S. Kuwada, Director of these Laboratories, for his encouragement and to Drs. T. Matsukawa and Y. Abe for their helpful advices.

Thanks are also due to Mr. M. Kan for elemental analyses and to Mr. H. Nakamachi for optical measurements.

Summary

5α-Pregnane-3β, 5, 16β, 20α-tetrol (VIa) and 5α-pregnane-3β, 5, 16β, 20β-pentol (VIII) were synthesized according to the scheme shown in the Figure.

(Received February 19, 1962)

UDC 547.92.07 : 543.422.4

24. Katsura Morita, Shunsaku Noguchi, Kentaro Hiraga, Toyokazu Kishi, Hayao Nawa, and Takuichi Miki : Synthesis of Polyhydroxysteroids. V.*1 Preparation and Infrared Spectra of 16β,20α-Isopropylidenedioxysteroids.

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In the previous paper[1] authors described a novel method for the side chain cleavage of diosgenin *via* the chlorinated intermediates to give pregn-5-ene-3β,16β,20α-triol (I) in overall yield of 60~70%.

A selective protection of a hydroxyl group or groups was hence desired in order to make use of this triol as a useful starting material. For this purpose an attempt was made on the formation of a cyclic derivative between two hydroxyl groups at C-16β and 20α, for diosgenin involves a furan ring at this site of the molecule.

On treatment with acetone and a few drops of borontrifluoride-ether solution, the triol I furnished, in a quantitative yield, the acetonide IIa, which was readily hydrolyzed by boiling in aqueous acetic acid to the triol (I). The isomeric 16β,20β-diol[2] and 16α,20β-diol,[2] however, gave no cyclic compound under similar conditions. The failure of the acetonide formation would be due to a steric reason.

In pursuing our work concerned with the preparation of polyhydroxysteroids, a number of 16β,20α-isopropylidenedioxysteroids were prepared. These compounds were generally obtained from 16β,20α-diol in the pregnane series by the reaction with acetone in the presence of a small amount of borontrifluoride-ether solution. It is

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*1 This paper constitutes Part XXIX of Takeda Laboratories' series entitled "Steroids;" Part XXVIII : This Bulletin, **11**, 139 (1963).

*2 Juso-nishino-cho, Higashiyodogawa-ku, Osaka (森田 桂, 野口俊作, 平賀謙太郎, 貴志豊和, 那波速男, 三木卓一).

1) Part XXV : This Bulletin, **11**, 90 (1963).

2) Part XXVI : This Bulletin, **11**, 95 (1963).

noteworthy that the 16β,20α-isopropylidenedioxysteroids exhibit the characteristic infrared bands at 9, 10, 11, and 11.8 μ, which served as a useful criterion for the acetonide formation or hydrolysis of the acetonide in the synthetic work.

Authors' interest in this group of derivatives was also arose from the well known fact that some acetonides in the steroid series possess an enhanced biological activity[3] and that these derivatives might be a useful intermediates for various synthetic purposes.

For one instance, 16β,20α-isopropylidenedioxypregn-5-en-3β-ol (IIa) was oxidized by the Oppennauer oxidation to yield 16β,20α-isopropylidenedioxypregen-4-en-3-one (III), which was hydrolyzed by heating with aqueous acetic acid giving the 16β,20α-diol (IV).

In another instance, the acetonide IIa was acetylated to the 3-acetate (IIb), which was hydrolyzed in acidic medium and the resulting 16β,20α-diol V was oxidized with cromium trioxide-sulfuric acid and reagent in acetone*3 to afford the diketone (VI). The diketone structure was evidenced by the ultraviolet absorption spectrum,[4] which solution showed its maximum absorption at 285 mμ ($\varepsilon=4.55\times10^3$) in an ethanol and at 309 mμ ($\varepsilon=1.78\times10^4$) in *N*-potassium hydroxide solution in ethanol. Treatment of the diketone VI with hydrazine hydrate in ethanol on a steam bath furnished the pyrazole[5] VII.

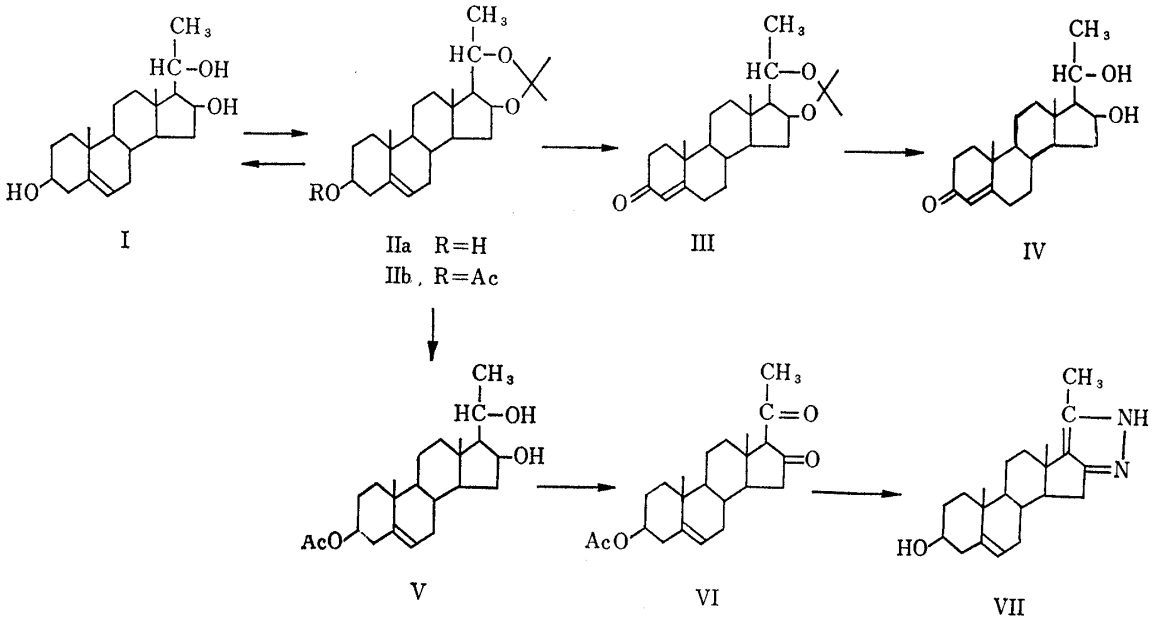

Chart 1.

Similar results were obtained from 5α-pregnane-3β,16β,20α-triol (VIII).[1] The triol was treated with acetone and a small amount of borontrifluoride-ether to yield the acetonide (IXa), which afforded on oxidation the 3-ketone (X). On acetylation, the acetonide (IXa) afforded the 3-acetate (IXb), which was hydrolyzed with boiling aqueous acetic acid giving the 3β,16β,20α-triol 3-acetate (XI). The resulting 16β,20α-diol (XI) was oxidized to afford the diketone (XII).

*3 A solution of 26.72 g. of CrO_3 in 23 cc. of conc. H_2SO_4 was diluted with H_2O to 100 cc. and it was used as a standard reagent. C. Djerassi *et al.* : J. Org. Chem., **21**, 1548 (1956).

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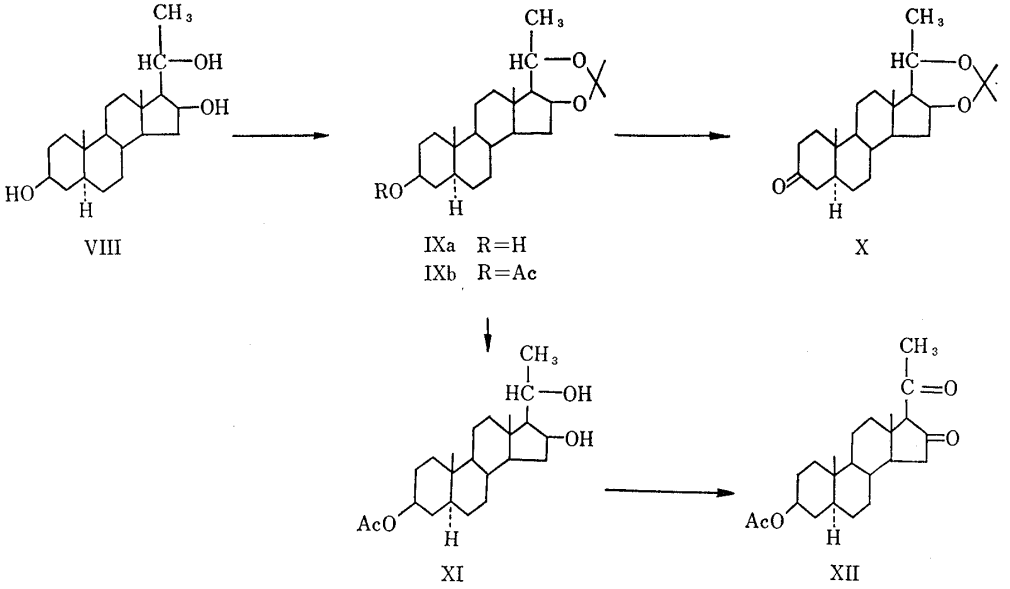

Chart 2.

Table I. Infrared Absorption Spectra of 16β,20α-Isopropylidenedioxypregnanes

Compound C-3	C-5	C-6	m.p. (°C)	Characteristic bands for 16β,20α-Isopropylidene-dioxypregnane (μ)			
-OH(β)	Δ5		205～206 (IIa)	9.0	10.1	11.1	11.85
-OAc(β)	Δ5		136～137 (IIb)	9.0	10.05	11.0	11.8
-OH(β)	-H(α)		225～226 (IXa)	9.0	10.0[a]	11.05	11.85
-OAc(β)	-H(α)		191～192 (IXb)	9.0	10.05	11.0	11.8
=O	Δ5		182～184 (III)	9.0	10.05	11.0	11.8
=O	-H(α)		215～218 (X)	8.9	10.0	11.0	11.75
-OH(β)	5α,6α-epoxide		241[2]	8.95	10.0	11.0	11.8
-OAc(β)	5α,6α-epoxide		190～191	8.95	10.0	11.0	11.8
$-OOCC_2H_5(\beta)$	-H(α)		180	9.0	10.05	11.0	11.8
$-OOCC_{15}H_{31}(\beta)$	-H(α)		105～106	8.95	10.0	11.0	11.8
$-OOCOC_2H_5(\beta)$	-H(α)		190	9.0	10.0	11.0	11.8
=O	-OH(α)	=O	271～272	9.0	10.0	11.0	11.8
-OH(β)	-H(α)	=O	235[2]	9.0	10.1	11.05	11.9
-OH(β)	-H(α)	-OH(β)	262～264[6]	9.0	10.0	11.0	11.8
-OH(β)	-H(α)	-OH(α)	268～270[6]	9.0	10.0	11.0	11.8
-OH(β)	-OH(α)		270～271[2]	9.0	10.1	11.0	11.8
-OAc(β)	-H(α)	-OAc(α)	167～169[6]	9.0	10.05	11.0	11.8
$-OOCC_6H_5(\beta)$	-OH(α)	=O	286[2]	8.95	10.05	11.0	11.8
-OH(β)	-OH(α)	-OH(β)	269[2]	8.95	10.05	11.05	11.85
$-OOCC_6H_5(\beta)$	-OH(α)	-OH(β)	271[2]	8.95	10.0	11.0	11.8
-OAc(β)	-OH(α)	-OAc(β)	275[2]	8.95	10.0	11.0	11.8
$-OOCC_{15}H_{31}(\beta)$	-OH(α)	-OH(β)	161[2]	8.95	10.05	11.0	11.75

a) Shows a shoulder at the position

Further synthesis of pyrazoles and isoxazoles from 16,20-diketones are still under investigation, and details of the experiment will be published in near future.

Experimental*4

General Procedure for the Preparation of 16β,20α-Isopropylidenedioxy Compound——To a stirred solution or suspension of a 16β,20α-dihydroxysteroid (1 g.) in Me_2CO (50 cc.) was added a BF_3-Et_2O solution (0.05 cc.), and stirring was continued at room temperature for 1～2 hr.

*4 All melting points are uncorrected.

6) Part XXVII: This Bulletin, **11**, 103(1963).

Small quantity (0.1 cc.) of pyridine was added to the solution and the solvent was evaporated to dryness *in vacuo*. The resulting crystal was washed with H_2O and if necessary re-dissolved in CH_2Cl_2, dried, evaporated and recrystallized from MeOH to yield the pure crystal of the acetonide.

The acetonides, which were prepared by the above pracedure, and their IR bands are listed in Table I.

16β,20α-Isopropylidenedioxypregn-5-en-3β-ol (IIa)——I (500 mg.) was treated with 0.2 cc. of BF_3-Et_2O (35%) in Me_2CO (50 cc.).

After working up as described in the general procedure, IIa was obtained as platelets melting at 205~206°; yield, 500 mg. $[\alpha]_D^{21}$ −69°(c=0.5%, EtOH). *Anal.* Calcd. for $C_{24}H_{38}O_3$: C, 76.96; H, 10.71. Found : C, 76.71; H, 10.51.

16β,20α-Isopropylidenedioxypregn-5-ene-3β-ol Acetate (IIb)——Acetylation of IIa with Ac_2O in pyridine afforded the 3-acetate IIb, which melted at 136~137°. *Anal.* Calcd. for $C_{26}H_{40}O_4$: C, 74.96; H, 9.68. Found : C, 74.52; H, 9.50.

16β,20α-Isopropylidenedioxypregn-4-en-3-one (III)——To a solution of 10 g. of IIa in 500 cc. of cyclohexanone was added 5 g. of Al-isopropoxide and the mixture was refluxed on an oil bath for 2 hr. After cooling, the solution was washed twice with 10% NaOH, twice with H_2O and then the solvent was evaporated *in vacuo*. The resulting residue was dissolved in CH_2Cl_2 and purified through a column of 50 g. of Florisil and finally crystallized from isopropylether to yield 7.0 g. of III melting at 182~184°. $[\alpha]_D^{22}$ +52°(c=1.0, $CHCl_3$). UV λ_{max}^{EtOH} : 240 mμ (ε : 1.59×10^4). *Anal.* Calcd. for $C_{24}H_{36}O_3$: C, 77.38; H, 9.74. Found : C, 77.30; H, 10.04.

16β,20α-Dihydroxypregn-4-en-3-one (IV)——III (1 g.) was heated with 10 cc. of AcOH-H_2O (2:1) on a steam bath for 2 hr. After cooling and careful addition of H_2O, crystals of (IV) deposited were filtered, dried and recrystallized from isopropyl ether to yield 0.7 g. of needles melting at 189°. $[\alpha]_D^{22}$ +113°(c=1.0%, $CHCl_3$). *Anal.* Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found : C, 75.26; H, 9.55.

Pregn-5-ene-3β,16β,20α-triol 3-Acetate (V)——IIb (750 mg.) was heated with 80% AcOH on a steam bath for 1 hr. After evaporation of the solvent *in vacuo*, the residue was recrystallized from aqueous MeOH to yield 500 mg. of V, m.p. 176~177°. $[\alpha]_D^{21}$ −39°(c=1.0%, $CHCl_3$). *Anal.* Calcd. for $C_{23}H_{36}O_4$: C, 73.36; H, 9.64. Found : C, 73.49; H, 9.54.

3β-Hydroxypregn-5-ene-16,20-dione Acetate (VI)——To a stirred solution of V (2 g.) in Me_2CO (200 cc.) was added a CrO_3-H_2SO_4 reagent*3 (3.0 cc.) at 15~20°. After stirring for 15 min., the reaction mixture was diluted with H_2O and extracted with Et_2O. The extract was washed with H_2O, 5% $KHCO_3$ and H_2O, dried over Na_2SO_4 and evaporated. The residue (1.5 g.), m.p. 150~160°, was recrystallized from MeOH to yield the diketone VI melting at 175~178°; yield 1.1 g. UV λ_{max}^{EtOH} : 285 mμ (ε : 4.55×10^3); 309 mμ (ε : 1.78×10^4) in *N* KOH/EtOH.

5'-Methylpyrazolo[3',4'-16,17]androst-5-en-2β-ol (VII)——A mixture of VI (500 mg.), MeOH (3 cc.) and 80% $(H_2N)_2\cdot H_2O$ (0.5 cc.) was refluxed on a steam bath for 2 hr. During this time, crystals of VI gradually dissolved and after being left to stand at room temperature a new crystalline product VII separated.

After an addition of H_2O the crystals deposited were collected, washed with H_2O, dried and recrystallized from MeOH giving leaflets; yield, 300 mg.; m.p. above 300°.*5 UV λ_{max}^{EtOH} : 225 mμ (ε: 6200). VII dissolves in H_2O containing a small amount of HCl giving a clear solution, from which VII is produced by the addition of $NaHCO_3$. *Anal.* Calcd. for $C_{21}H_{30}N_2O$: C, 77.25; H, 9.26; N, 8.58. Found : C, 76.71; H, 9.37; N, 8.78.

16β,20α-Isopropylidenedioxy-5α-pregnan-3β-ol (IXa)——5α-Pregnane-3β,16β,20α-triol (VIII) was acetonized by the general procedure giving IXa in quantitative yield; m.p. 225~226°; $[\alpha]_D^{22}$ −11°(c=0.5%, EtOH). *Anal.* Calcd. for $C_{24}H_{40}O_3$: C, 76.55; H, 10.71. Found : C, 76.35; H, 10.76.

16β,20α-Isopropylidenedioxy-5α-pregnan-3β-ol Acetate (IXb)——Acetylation of IXa (5 g.) with Ac_2O in pyridine afforded the 3-acetate IXb melting at 191~192°; yield 5 g. *Anal.* Calcd. for $C_{26}H_{42}O_4$: C, 74.64; H, 10.05. Found : C, 74.42; H, 10.35.

5α-Pregnane-3β,16β,20α-triol 3-Acetate (XI)——Heating of IXb (5 g.) in AcOH-H_2O (2:1) on a steam bath for 2 hr. and chilling of the solution afforded crystals (3.5 g.) of XI, which melted at 200°. *Anal.* Calcd. for $C_{23}H_{38}O_4$: C, 73.02; H, 10.05. Found : C, 72.81; H, 10.02.

16β,20α-Isopropylidenedioxy-5α-pregnan-3-one (X)——To a solution of 10 g. of IXa in 500 cc. of cyclohexanone was added 5 g. of Al-isopropoxide and the mixture was refluxed in an oil bath for 2 hr. After similar working up to the case of III, the reaction product was crystallized from isopropylether to yield 7.2 g. of X, which melted at 215~218°. *Anal.* Calcd. for $C_{24}H_{40}O_3$: C, 76.55; H, 10.71. Found : C, 76.78; H, 10.22.

3β-Hydroxy-5α-pregnane-16,20-dione Acetate (XII)——XI (2 g.) was oxidized with CrO_3-H_2SO_4 reagent*3 in Me_2CO. After similar working up to the case of VIb, XII was obtained as needles melting at 199~200°; yield, 1.1 g. UV λ_{max}^{EtOH} : 286 mμ (ε : 4780).

*5 Reported m.p. 305~307°.[5]

Summary

It was found that 16β,20α-dihydroxysteroids react with acetone in the presence of a small quantity of boron-trifluoride etherate giving the 16β,20α-isopropylidenedioxy compounds in good yield, the method being useful for a selective protection of the 16β,20α-dihydroxyl groups.

By this method a number of 16β,20α-isopropylidenedioxysteroids, which exhibit a characteristic infrared bands at 9, 10, 11 and 11.8 μ, were prepared.

(Received February 19, 1962)

UDC 615.398.19

25. Tsutomu Unemoto, Keiko Ikeda, Makoto Hayashi, and Komei Miyaki : Studies on Polyamines. I. A New Fluorometric Determination of Spermine and Spermidine.

(*Laboratory of Prof. K. Miyaki, Institute of Food Microbiology, Chiba University*[*1])

There are several methods to determine various amines in biological materials. They involve separation of amines by paper chromatography,[1,2] by paper electrophoresis[1,2] or by cation exchange resin,[3,4] followed by determination of each amine by Ninhydrin[1,2] or dinitrofluorobenzene[3,4] procedure. In these cases, colorimetric assays are non-specific and specificity of these methods depends on separation procedure. In general, satisfactory separation of amines demands a complicated and time-consuming technique.

In our study on the oxidation of spermine or spermidine by beef plasma amine oxidase,[5,6] it was found that one of the oxidation products reacts with resorcinol to produce a fluorescent compound that has an absorption maximum at 520 mμ (Fig. 1), the intensity of which was proportional to the amount of the amine oxidized. As the formation of the fluorescent compound was quite specific to spermine and spermidine, it was attempted to devise a specific fluorometric method for the estimation of these amines.

Reaction of resorcinol with the oxidation products proceeded fast at a neutral or slightly alkaline pH and the fluorescent compound formed was stable at an acidic pH. We oxidized spermine or spermidine in the presence of resorcinol at pH 7.0, since it did not affect the activity of the amine oxidase. After that the reaction mixture was heated at the same pH to enhance the formation of the fluorescent compound and acetic acid was added to stabilize its intensity. Whether or not the fluorescent compound obtained from spermine or spermidine is the same compound is under investigation.

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