[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

Microbiological Transformations of Steroids. XI. The Transformation of 3-Ketobisnor-4-cholen-22-al to 11α,22-Dihydroxybisnor-4-cholen-3-one and 6β,11α,22-Trihydroxybisnor-4-cholen-3-one by *Rhizopus*[1]

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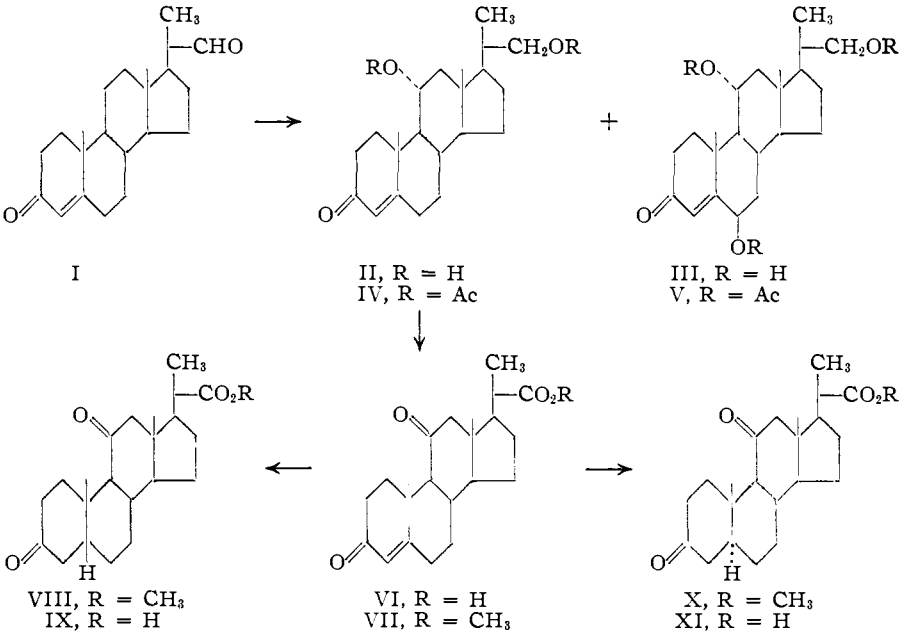
3-Ketobisnor-4-cholen-22-al (I) is converted by *Rhizopus nigricans* (A.T.C.C. 6227b) to 11α,22-dihydroxybisnor-4-cholen-3-one (II) and 6β,11α,22-trihydroxybisnor-4-cholen-3-one (III). The latter compound is formed almost exclusively upon incubation of the aldehyde (I) with *Rhizopus arrhizus* (A.T.C.C.11145).

Results

In preceding communications of this series we reported on the microbiological oxygenation of C_{19}- and C_{21}-steroids by *Rhizopus*. The facile introduction of hydroxyl groups into positions 6 and/or 11 of these steroids within broad limits of structure variation focused our interest on the bioconversion of C_{22}-steroids. 3-Ketobisnor-4-cholen-22-al (I) was found to be a suitable substrate. This compound is readily available since it can be easily prepared from stigmastadienone[2] or from ergostadienone[3] by preferential ozonization of the side chain double bond.

In view of our main interest in the oxygenation of the 11-position, compound I was first incubated with *Rhizopus nigricans* Ehrb. (A.T.C.C. 6227b) which was hitherto known to produce, chiefly and in good yield, the 11-oxygenated metabolite. Transformation periods of 24–48 hours in certain instances left some unchanged starting material and produced two new compounds as indicated by papergram analysis. Chromatography over Florisil or alumina afforded a good fractionation of the three components.



The less polar of the two conversion products was obtained in yields of 20–40%. Its infrared and ultraviolet spectra indicated the retention of the α,β-unsaturated ketone in ring A, the loss of the aldehyde group and the formation of two or more hydroxyl groups. The formula of $C_{22}H_{34}O_3$ and the formation of a diacetate $C_{26}H_{38}O_5$ established the presence of two unhindered hydroxyl groups. On the basis of these data the less polar compound was postulated to be 11α,22-dihydroxybisnor-4-cholen-3-one (II). The absorption maximum of the ultraviolet spectrum of II (λ_{max} 242 mμ) excluded the possibility of one hydroxyl being at the 6β-position.

Compound II was oxidized with chromic acid to 3,11-diketobisnor-4-cholenic acid (VI) which, upon treatment with diazomethane, was converted to methyl 3,11-diketobisnor-4-cholenate (VII). This ester was then hydrogenated over a palladium–zinc carbonate catalyst.[4] When the resulting mixture of isomers was chromatographed over activated carbon,[5] acetone eluted methyl 3,11-diketobisnorcholanate (VIII) whereas methylene dichloride eluted methyl 3,11-diketobisnorallocholanate (X). The ratio of VIII to X was 4:1. Although no direct comparison of the two samples was made, compound VIII appeared to be identical in melting point (200–203°) and optical rotation ($[\alpha]_D$ +50° in acetone) to methyl 3,11-diketobisnorcholanate as reported by Lardon and Reichstein.[6] Saponifica-

(1) Paper X of this series: S. H. Eppstein, P. D. Meister, H. Marian Leigh, D. H. Peterson, H. C. Murray, L. M. Reineke and A. Weintraub, THIS JOURNAL, **76**, 3174 (1954).

(2) F. W. Heyl and M. E. Herr, *ibid.*, **72**, 2617 (1950).

(3) D. A. Shepherd, R. A. Donia, J. A. Campbell, B. A. Johnson, R. P. Holysz, G. Slomp, Jr., J. E. Stafford, R. L. Pederson and A. C. Orr, Abstract of Papers of the 124th Meeting of the American Chemical Society, Chicago, Illinois, September 6–11, 1953, page 30.

(4) The preparation and the utility of this catalyst will be published in the near future in a communication by Dr. G. S. Fonken of these laboratories.

(5) This type of chromatogram has been described in a previous communication of this series: D. H. Peterson, *et al.*, THIS JOURNAL, **75**, 419 (1953).

(6) A. Lardon and T. Reichstein, *Helv. Chim. Acta*, **27**, 713 (1944), gave the following constants: m.p. 199–201° and $[\alpha]_D$ +47.6° (in acetone).

TABLE I[a]

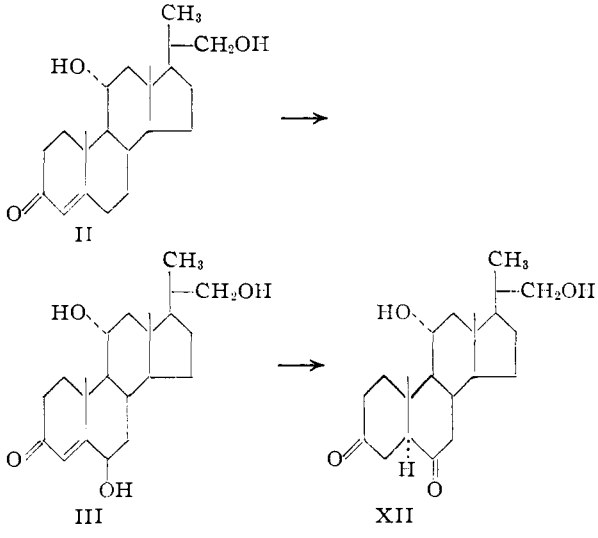
Parent compound	I 6-Desoxy	II 6β-Hydroxy	III 6β-Acetoxy	Δ[M]D II-I	Δ[M]D III-I	Ref.
11α-Hydroxyprogesterone	595	346	.....	−249	...	..
11α-Acetoxyprogesterone	577	.....	349	...	−228	[b]
11-Ketoprogesterone	903	620	626	−283	−277	..
Kendall's Compound F	606(Et)	288	.....	−318	...	[c]
Compound F acetate	667(Di)	.....	352(Me)	...	−315	[c]
11α,22-Dihydroxybisnor-4-cholen-3-one (II)	270	79(Me)	.....	−191	...	..
11α,22-Diacetoxybisnor-4-cholen-3-one (IV)	224	.....	54	...	−170	..

[a] All rotations in chloroform except where otherwise mentioned (Et = ethanol; Me = methanol; Di = dioxane). [b] J. Fried, R. W. Thoma, J. R. Gerke, J. E. Herz, M. N. Donin and D. Perlman, THIS JOURNAL, 74, 3962 (1952). [c] S. Burstein, R. I. Dorfman and E. M. Nadel, *Federation Proc.*, 13, 188 (1954); M. Hayano and R. I. Dorfman, *Arch. Biochem. Biophys.*, 50, 218 (1954).

tion of the two methyl esters (VIII and X) yielded 3,11-diketobisnorcholanic acid (IX) and 3,11-diketobisnorallocholanic acid (XI), respectively.

The α-orientation of the hydroxyl group in position 11 is clearly suggested by the ease of acetylation and the established stereochemical specificity of the enzyme system involved in its formation.

The second, more polar bioconversion product was isolated from fermentations with *Rhizopus nigricans* in only 2% yield. For the preparation of a substantial amount of this compound, 3-ketobisnor-4-cholen-22-al (I) was incubated with *Rhizopus arrhizus* to give a 30% yield of the desired compound for which the formula of 6β,11α,22-trihydroxybisnor-4-cholen-3-one (III) was postulated. This assumption was based on its analysis ($C_{22}H_{34}O_4$), on the ultraviolet spectrum (λ_{max} 238 mμ, E 12,900) which is typical for 6-hydroxy-3-keto-Δ^4-steroids[7-9] and on the formation of a triacetate V. The structure of III was unequivocally established in the following manner.



The presence of the hydroxyl groups in positions 11α and 22 of compound III was proven when 11α,-22-dihydroxybisnor-4-cholen-3-one (II) was exposed to *Cunninghamella blakesleeana* (A.T.C.C. 8688a) to give a compound whose infrared and ultraviolet spectrum was in all details identical to the spectra of the product obtained from bioconversions with *Rhizopus*. The 6-position of the third hydroxyl group in compound III was deduced from the ultraviolet spectrum (*vide supra*) and from the acid rearrangement[8] of compound III to an isomeric compound which is believed to be 11α,22-dihydroxybisnorallocholane-3,6-dione (XII) on the evidence of its analysis, its lack of ultraviolet absorption and its infrared spectrum.

The β-orientation of the hydroxyl group in position 6 of compound III is strongly supported by molecular rotations. The increment in molecular rotation contributed by the 6β-hydroxyl group has been found to average −250 for 3-keto-Δ^4-steroids lacking an oxygen function in position 11. For comparison, the increments of the 6β-hydroxy and 6β-acetoxy groups in steroids with an oxygen function at carbon 11 have been computed. Table I shows that the values for the 6β-hydroxyl group ($\Delta[M]^{II}D - [M]^{I}D$) are distributed over a wide range, but that they give an average value of −260° which is in good agreement with the value cited above for 6β-hydroxy-11-desoxy steroids. It, therefore, appears that there is no noticeable vicinal action with respect to the molecular rotation between oxygen functions at carbon 6 and carbon 11, and that the discrepancy between some of the compounds in Table I is caused by some other factor.

The transformations herein described are additional examples of concomitant oxygenation and reduction of the substrate. From similar cases thus far reported, it is readily apparent that the structure of the substrate has a profound influence on the kinetics of the reduction. The formation of 11α-hydroxyallopregnane-3,20-dione from progesterone by *Rhizopus*[10] and of 16α-hydroxypregnane-3,20-dione from progesterone by an unidentified Proactinomyces[11] proceed at a slow rate with respect to the oxygenation. Likewise, 11α,17α,21-trihydroxypregnane-3,20-dione has been found to be a minor by-product of the conversion of Reichstein's compound S with *Rhizopus*.[12] However, in the formation of 11α-hydroxy-17α-progesterone from 4,16-pregnadien-3,20-dione[13] the reduction of the 16,17-double bond and the oxygenation of the 11-position apparently occur at the same rate. Likewise, in the transformation of I to II the reduction of the aldehyde group proceeds at a rate which is apparently equivalent to the rate of oxygenation.

(7) J. L. Johnson, these Laboratories, unpublished data.

(8) M. Ehrenstein and co-workers, *J. Org. Chem.*, 19, 1331 (1954) and earlier references.

(9) L. Dorfman, *Chem. Revs.*, 53, 47 (1953).

(10) Paper I of this series, THIS JOURNAL, 74, 5933 (1952).

(11) D. Perlman, E. Titus and J. Fried, *ibid.*, 74, 2126 (1952).

(12) Paper IV of this series, *ibid.*, 75, 412 (1953).

(13) Paper II of this series, *ibid.*, 75, 55 (1953).

Experimental

Isolation of 11α,22-Dihydroxybisnor-4-cholen-3-one (II) and 6β,11α,22-Trihydroxybisnor-4-cholen-3-one (III) from Bioconversion of 3-Ketobisnor-4-cholen-22-al (I) with *Rhizopus nigricans* Ehrb. (A.T.C.C. 6227b).—*Rhizopus nigricans* was grown on 12 l. of medium H[14] for 24 hours. Then a solution of 3 g. of substrate I in 100 ml. of acetone was added. After an incubation period of 48 hours, the extraction with methylene dichloride gave 4.3 g. of material. Papergram analysis showed the formation of two new components which were moving slower than the starting material.

The extract was chromatographed over 240 g. of Florisil. Ethylene dichloride with increasing concentrations of acetone was used as elution solvent. Ethylene dichloride–acetone 15:1 eluted unchanged starting material as shown by papergram analysis and recrystallization of these fractions to give 350 mg. of crystals, m.p. 155–158°,[15] identified by infrared spectrum as starting material. Ethylene dichloride–acetone 10:1 and 8:1 eluted 967 mg. of a crystalline material, shown by papergram to consist largely of one component. This material was recrystallized from 10 ml. of acetone–ether to give 468 mg. of crystals, m.p. 128–131°. A small sample was recrystallized once more to give pure 11α,22-dihydroxybisnor-4-cholen-3-one (II), m.p. 130–133°, $[\alpha]^{23}D$ +78° (*c* 1.02 in chloroform), λ_{max}^{alc} 242 mμ (*E* 12,700).

Anal. Calcd. for $C_{22}H_{34}O_3$: C, 76.26; H, 9.89. Found: C, 75.86; H, 9.77.

11α,22-Diacetoxybisnor-4-cholen-3-one (IV).—The diacetate was prepared by dissolving 11α,22-dihydroxybisnor-4-cholen-3-one (54 mg.) in 4 ml. of acetic anhydride–pyridine (1:1). Fifty-three mg. of crystals, m.p. 125–126°, was obtained and recrystallized once from ether–petroleum ether and once from methanol to give 38.5 mg. of 11α,22-diacetoxybisnor-4-cholen-3-one (IV), m.p. 128–129°, mixture melting point with starting material, 105–115°. The infrared spectrum confirmed the acetylation of both hydroxyl groups; λ_{max}^{alc} 242 mμ (*E* 14,600), $[\alpha]^{23}D$ +52° (*c* 0.837 in chloroform).

Anal. Calcd. for $C_{26}H_{38}O_5$: C, 72.52; H, 8.90. Found: C, 72.64; H, 8.73.

The later fractions of the above chromatogram (ethylene dichloride–acetone 2:1) were shown by papergram to contain a more polar compound. Upon trituration of these fractions with acetone, 105 mg. of crude crystals was recovered. Recrystallization from methanol yielded 55 mg. of compound III, m.p. 232–238°. The infrared spectrum showed this compound to be identical to 6β,11α,22-trihydroxybisnor-4-cholen-3-one (III) as described below.

B. **Transformation of 3-Ketobisnor-4-cholen-22-al (I) to 6β,11α,22-Trihydroxybisnor-4-cholen-3-one (III) by *Rhizopus arrhizus* Fischer (A.T.C.C. 11145).**—*Rhizopus arrhizus* was grown on 12 l. of medium H for 24 hours. Then 3 g. of substrate was added in acetone solution (150 ml.). The fermentation was continued for 48 hours. Extraction with methylene dichloride gave 12.05 g. of solids which was repeatedly triturated with 25-ml. portions of ether. The residue so obtained was recrystallized from 20 ml. of methanol to yield 809 mg. of crystals, m.p. 232–240°. The mother liquors (10.88 g.) were chromatographed in benzene solution over 200 g. of alumina. Acetone eluted 344 mg. of a crystalline material which was recrystallized twice from methanol to give an additional crop (168 mg.) of crystals, m.p. 230–238°. A sample of this compound was recrystallized from acetone–chloroform to a constant melting point of 238–240°, $[\alpha]^{23}D$ +22° (*c* 0.387 in methanol). The infrared and the ultraviolet spectrum, λ_{max}^{alc} 238 mμ (*E* 12,900), supported the structure of 6β,11α.22-trihydroxybisnor-4-cholen-3-one (III).

Anal. Calcd. for $C_{22}H_{34}O_4$: C, 72.89; H, 9.45. Found: C, 73.04; H, 9.59.

6β,11α,22-Triacetoxybisnor-4-cholen-3-one (V).—6β,11α,-22-Triacetoxybisnor-4-cholen-3-one (V) was prepared by acetylation of III (100 mg.) with acetic anhydride–pyridine 1:1 at room temperature. Dilution of the reaction mixture with ice-water gave a crystalline precipitate (110 mg.) which was recrystallized twice from aqueous methanol to give 78 mg. of compound V, m.p. 145–146°, $[\alpha]^{23}D$ +11° (*c* 0.966 in chloroform).

Anal. Calcd. for $C_{28}H_{40}O_7$: C, 68.83; H, 8.25. Found: C, 68.48; H, 8.33.

C. **Structure Proof of 11α,22-Dihydroxybisnor-4-cholen-3-one. Oxidation of Compound II to 3,11-Diketobisnor-4-cholenic Acid (VI).**—11α,22-Dihydroxybisnor-4-cholen-3-one (1.23 g.), m.p. 128–133°, was dissolved in 20 ml. of glacial acetic acid. To the chilled and vigorously stirred solution of the steroid there was added dropwise a solution of 804 mg. of chromium trioxide in 7 ml. of 80% acetic acid. The reaction mixture was allowed to stand at room temperature overnight.

The solution was then diluted with 25 ml. of methanol, concentrated *in vacuo* to one tenth of its volume, diluted with 200 ml. of water and brought to *p*H 11 with 5% aqueous sodium hydroxide. The usual procedure of extraction with ether–chloroform 10:1 gave 117.0 mg. of neutral products which were not further investigated.

The alkaline solution was brought to *p*H 2 with 5% hydrochloric acid and extracted with chloroform to give 982 mg. of crystals which were recrystallized from 10 ml. of methanol–ether. After two recrystallizations, the compound had a melting point of 244–246°, $[\alpha]^{23}D$ +137° (*c* 1.024 in methanol), λ_{max}^{alc} 239 mμ (*E* 12,300). The infrared spectrum was in agreement with the proposed structure of 3,11-diketobisnor-4-cholenic acid (VI).

Anal. Calcd. for $C_{22}H_{30}O_4$: C, 73.71; H, 8.44. Found: C, 73.51; H, 8.29.

Methyl 3,11-Diketobisnor-4-cholenate.—The methyl ester VII was prepared by treating a solution of 3,11-diketobisnor-4-cholenic acid (657 mg.) in 20 ml. of methanol and 10 ml. of methylene dichloride with a freshly prepared ether solution of diazomethane until the yellow color persisted. The solvent and the excess diazomethane were evaporated *in vacuo*.

The residue, obtained from this methylation, was recrystallized from acetone–hexane. After two recrystallizations 560 mg. of methyl 3,11-diketobisnor-4-cholenate was obtained, m.p. 177–179°, $[\alpha]^{23}D$ +170° (*c* 0.654 in chloroform), λ_{max}^{alc} 238 mμ (*E* 14,000). The infrared spectrum was in agreement with the proposed structure. *Anal.* Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 74.37; H, 8.89.

Hydrogenation of Methyl 3,11-Diketobisnor-4-cholenate.—Methyl 3,11-diketobisnor-4-cholenate (640 mg.) was dissolved in 75 ml. of methanol and hydrogenated at 10 p.s.i. over 640 mg. of a palladium–cadmium carbonate catalyst which had been prereduced at the same pressure. The suspension was filtered and the residue was washed with 100 ml. of acetone. The combined filtrates were chromatographed over 25 g. of activated carbon (Darco-G60)–diatomaceous earth (Celite) 1:2. The 9 acetone fractions which were eluted first gave 537 mg. of crystalline material (fraction A). Following the acetone, four methylene dichloride fractions eluted 133 mg. of crystals (fraction B).

Methyl 3,11-Diketobisnorcholanate (VIII) and 3,11-Diketobisnorcholanic Acid (IX).—Fraction A was recrystallized from 5 ml. of chloroform–hexane. After two recrystallizations, 441 mg. of crystalline material, m.p. 192–197°, was recovered. A small sample was recrystallized once more to give pure methyl 3,11-diketobisnorcholanate (VIII), m.p. 200–203°, $[\alpha]^{23}D$ +50° (*c* 1.0 in acetone).[6] A comparison of the infrared spectra showed that compounds VIII and X possessed the same functional groups but were definitely different.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.63; H, 9.03.

The 3,11-diketobisnorcholanic acid (IX) was prepared by refluxing 182 mg. of compound VIII in 20 ml. of methanolic 1 *N* potassium hydroxide for 2 hours. Separation into neutral and acidic products as described above yielded 38 mg. of starting material and 126 mg. of an acid which was twice recrystallized from ether to give 85 mg. of 3,11-diketobisnorcholanic acid (IX), m.p. 195–200°, $[\alpha]^{23}D$ +47° (*c* 0.785 in acetone). The infrared spectrum was consistent with the proposed structure.

Anal. Calcd. for $C_{22}H_{32}O_4$: C, 73.30; H, 8.95. Found: C, 73.25; H, 8.89.

Methyl 3,11-Diketobisnorallocholanate (X) and 3,11-Diketobisnorallocholanic Acid (XI).—Fraction B obtained

(14) For the composition of this medium as well as details of fermentation see reference 10.

(15) All melting points were determined on a Fisher-Johns block and are uncorrected.

from the above chromatogram of the hydrogenation product was recrystallized three times from chloroform–hexane to yield 32 mg. of pure methyl 3,11-diketobisnorallocholanate, m.p. 202–203.5°, $[\alpha]^{23}D$ +55° (*c* 0.762 in acetone). The infrared spectrum was in agreement with the structure suggested above.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 74.08; H, 9.38.

3,11-Diketobisnorallocholanic acid (XI) was formed when compound X (60.5 mg.) was refluxed in 10 ml. of methanolic 1 *N* potassium hydroxide for 4 hours. Separation of the product into acidic and neutral components yielded 10 mg. of starting material and 49.2 mg. of the desired acid which was recrystallized twice from ether, m.p. 256–258°, $[\alpha]^{23}D$ +60° (*c* 0.391 in acetone).

Anal. Calcd. for $C_{22}H_{32}O_4$: C, 73.30; H, 8.95. Found: C, 73.03; H, 8.95.

D. Structure Proof of 6β,11α,22-Trihydroxybisnor-4-cholen-3-one. Bioconversion of 11α,22-Dihydroxybisnor-4-cholen-3-one (II) to 6β,11α,22-Trihydroxybisnor-4-cholen-3-one (III) by *Cunninghamella blakesleeana* (A.T.C.C. 8688a).—To 12 l. of medium H was added a vegetative inoculum of *Cunninghamella blakesleeana* and the acetone solution (50 ml.) of 1 g. of substrate (II). After 72 hours of incubation, extraction with methylene dichloride gave 10.32 g. of solids. Papergram analysis indicated that, besides a multitude of very minor components, 6β,11α,22-trihydroxybisnor-4-cholen-3-one was the preponderant metabolite. The extract was dissolved in 100 ml. of benzene and chromatographed over 250 g. of alumina. Acetone–5% methanol and acetone–10% methanol eluted 466.6 mg. of an oil which was shown by papergram to contain about 20% of compound III. This fraction was allowed to crystallize from acetone–ether 1:1 by slow evaporation of the solvents at room temperature to give 38.5 mg. of crystals, m.p. 222–228°. The ultraviolet spectrum [λ_{max}^{alc} 239 mμ (*E* 12,400)] and the infrared spectrum established the identity of this compound with 6β,11α,22-trihydroxybisnor-4-cholen-3-one (III) as isolated from bioconversions with *Rhizopus*.

Rearrangement of III to 11α,22-Dihydroxybisnorallocholane-3,6-dione (XII).—Compound III (185 mg.) was suspended in 35 ml. of freshly distilled *t*-butyl alcohol and 10 ml. of 10% sulfuric acid. The suspension was heated on the steam-bath to complete solution. The reaction mixture was allowed to stand at room temperature overnight and then heated under reflux for 1.5 hours. After dilution with water, the steroids were extracted with ether–chloroform 5:1. The extracts were washed twice with 5% sodium carbonate and twice with water. Evaporation of the solvents yielded 224 mg. of an oily residue which was chromatographed in benzene solution (10 ml.) over 11 g. of alumina. Chloroform–10% acetone, chloroform–30% acetone, chloroform–50% acetone and acetone eluted 145 mg. of a saturated compound which was recrystallized twice from methanol–ether to give 71.5 mg. of crystals, m.p. 191–193°, $[\alpha]^{23}D$ −27° (*c* 0.363 in methanol). The infrared spectrum showed absorption bands for hydroxyl groups (3500 $cm.^{-1}$) and for non-conjugated ketones (1704 $cm.^{-1}$).

Anal. Calcd. for $C_{22}H_{34}O_4$: C, 72.89; H, 9.45. Found: C, 73.14; H, 9.42.

Acknowledgment.—We are grateful to the following members of The Upjohn Research Division for their coöperation and assistance on various aspects of our problems: Dr. J. L. Johnson and his associates for infrared and ultraviolet spectra; Mr. W. A. Struck and his associates for rotations and microanalyses; the Misses Jennie I. Mejeur, Henrietta Triemstra, Irene N. Pratt and Mr. G. Staffen for technical assistance.

KALAMAZOO, MICHIGAN

[CONTRIBUTION FROM THE ROBERT W. LOVETT MEMORIAL LABORATORIES FOR THE STUDY OF CRIPPLING DISEASES, MASSACHUSETTS GENERAL HOSPITAL, AND THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

4-*O*-Methyl-D-galactosamine Hydrochloride (2-Amino-2-deoxy-4-*O*-methyl-D-galactose Hydrochloride)[1,2]

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4-*O*-Methyl-D-galactosamine hydrochloride (2-amino-2-deoxy-4-*O*-methyl-D-galactose hydrochloride) has been prepared in crystalline form from 1,6:2,3-dianhydro-β-D-talopyranose and has been characterized through the following crystalline derivatives: *N*-(2′-hydroxynaphthylidene), methyl *N*-acetyl-α-D-glycoside and methyl *N*-acetyl-3,6-di-*O*-acetyl-α-D-glycoside.

In recent papers from this Laboratory,[4] syntheses of methylated galactosamines and methods for their identification and separation have been reported. The present paper describes the preparation of a new monomethylgalactosamine, 4-*O*-methyl-α-D-galactosamine hydrochloride (2-amino-2-deoxy-4-*O*-methyl-α-D-galactose hydrochloride) (VI) by the method shown in the accompanying diagram.

It is inconvenient to use D-galactosamine as a starting material because of the length of time involved in its preparation, and also because the route leading to the 4-methyl derivative requires a large number of intermediates as is analogously shown in the synthesis of 4-*O*-methyl-D-glucosamine.[5]

Hann and Hudson[6] and James, *et al.*,[7] describe a convenient route for the synthesis in quantity of 1,6:2,3-dianhydro-β-D-talose (I), from lactose.

The course of the reaction of alkaline reagents on epoxy sugars is well known and the results can be predicted with a high degree of certainty.[8] James, *et al.*,[7] treating I with ammonia were able to obtain the 2-amino-2-deoxy-D-galactose derivative in a 56% yield, whereas the 3-amino-3-deoxy-D-idose

(1) Studies on hyaluronic acid and related substances XIII. This is publication No. 165 of the Robert W. Lovett Memorial Foundation for the Study of Crippling Diseases, Department of Medicine, Harvard Medical School, Boston, and the Massachusetts General Hospital. This investigation has been supported by research grants from Eli Lilly and Company and from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service (Grant A-148-C1).

(2) Presented before the Division of Carbohydrate Chemistry at the 126th Meeting of the American Chemical Society, New York, N. Y., September 1954.

(3) Present address: Service de Chimie Organique, Université Libre de Bruxelles, Bruxelles, Belgique.

(4) P. J. Stoffyn and R. W. Jeanloz, THIS JOURNAL, **76**, 561, 563 (1954).

(5) C. T. Bothner-By and R. W. Jeanloz, unpublished.

(6) R. M. Hann and C. S. Hudson, THIS JOURNAL, **64**, 2435 (1942).

(7) S. P. James, F. Smith, M. Stacey and L. F. Wiggins, *J. Chem. Soc.*, 625 (1946).

(8) A. K. Bose, D. K. R. Chaudhuri and A. K. Bhattacharyya, *Chem. Ind.*, 869 (1953); F. H. Newth, *ibid.*, 1257 (1953).