quality in about 70% yields. Thus 600 mg. of the bromohydrin in 40 ml. of glacial acetic acid with 1 g. of zinc dust, stirring at 100° for four hours, gave 350 mg. (70%) of product, m.p. 160–170°. Two recrystallizations from alcohol gave a product of high purity, m.p. 174–176°.

2α,3β-Diacetoxy-16-allopregnene-12,20-dione (XIII).—Pseudomanogenin triacetate, m.p. 159–161°, was prepared in 55% yield by the published procedure.[24] In order to record its rotation the material was recrystallized to a constant melting point, 164–165°, $[\alpha]^{24}D$ +26°.

Oxidation of 2.0 g. of the triacetate at 15° for six hours in 50 ml. of glacial acetic acid with 20 ml. of perhydrol yielded 1.9 g. (90%) of colorless, sirupy manone triacetate, isolated by extraction with ether. This was deacylated with potassium carbonate in *t*-butyl alcohol, as described for hecone diacetate, and the glassy residue crystallized from 1:1 ether–petroleum ether; then from 20% methanol in ether. The yield was 0.98 g. (72%) of platelets, m.p. 223–227°. The analytical sample was prepared by further recrystallization from ether; m.p. 240.0–240.5°, $[\alpha]^{25}D$ +68° (chloroform), $\lambda_{max}^{alc.}$ 227.5 mμ (log ε 4.07). *Anal.* Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 69.76; H, 7.94.

2α,3β-Diacetoxyallopregnane-12,20-dione (XIV).—One-tenth gram of the pregnene was hydrogenated in 100 ml. of dry ether over 200 mg. of palladium-black catalyst for three hours at 40 p.s.i. Filtration and concentration gave 90 mg. (89%) of small plates, m.p. 252–256°. Three recrystallizations from ether gave small plates which sublimed as long needles at 215° and melted at 258–260°, $[\alpha]^{29}D$ +51°, $\lambda_{max}^{alc.}$ 288 mμ (log ε 2.14). *Anal.* Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.47; H, 8.22.

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(24) *Cf.* ref. 1, p. 2183. The preparation of pseudomanogenin triacetate herein described is erroneously entitled "pseudomanogenin."

[CONTRIBUTION NO. 122 FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF TENNESSEE]

Ring C Ketols in the Hecogenin and Allopregnane Series

BY GEORGE P. MUELLER, LILBURN L. NORTON, ROBERT E. STOBAUGH, LIN TSAI AND ROBERT S. WINNIFORD

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The 11α,23- and 11β,23-dibromohecogenin acetates have been characterized, dehydrobrominated and hydrolyzed under varying conditions; derivatives of three of the four possible isomeric ketols have been obtained. 3β,12β-Dihydroxy-5α,22a-spirostan-11-one (11-ketorockogenin) has resisted many attempts to remove the hydroxyl group at C-12 but has been degraded to 3β,12β-diacetoxy-16-allopregnene-11,20-dione and related compounds. Two rockogenin derivatives and 23-bromomanogenin diacetate are reported.

The dibromination of hecogenin acetate[1,2] proceeds readily in either acetic acid or chloroform and the yield of crude product is nearly quantitative. Even so, the reaction is erratic, going sometimes with little color development but more often with formation of green, blue or purple side products. Material contaminated with these colored products decomposes on standing, even in the dry state. In addition to the compound already reported, which we regarded as 11β,23-dibromohecogenin acetate IV, we now have obtained the α-isomer III, which is higher melting and more stable. The 11β-bromide was readily dehydrobrominated by treatment with ordinary solvents and by adsorption on alumina. Chromatography or collidine treatment of the dibromo mixture furnished 9(11)-dehydro-23-bromohecogenin acetate[1] which was debrominated to the known 9(11)-dehydrohecogenin acetate.[3] Attempts to extend the conjugation in these unsaturated ketones with the formation of 3β-acetoxy-5α,22a-spirosta-7,9(11)-dien-12-one were made using selenium dioxide and the 23-bromo compound and by treating 9(11)-dehydrohecogenin acetate itself with mercuric acetate under a variety of conditions. These were not successful.

The 11,23-dibromohecogenin acetates are similar to the 3α-hydroxy-11-bromoetiocholane-17β-carboxylic acids[4] in that they are formed in less than two hours by bromination at room temperature, and are almost completely hydrolyzed in six minutes at room temperature in 0.25 *N* alkali. Likewise, hydrolysis and rearrangement to the more stable 12β-hydroxy-11-keto structure may be carried out at room temperature. As Gallagher has indicated, this behavior stands in marked contrast to that of the two 11-bromo-12-ketocholanates,[5] which are far more resistant both to formation and to hydrolysis and rearrangement. Although he has suggested that increased activity in the etianic acids lies with some sort of activation by the carboxyl group at C-17, it appears here as with the sapogenins that this activity must be ascribed rather to the absence of hindering groups in the vicinity of ring C. Thus, the configuration of rings C, D and E[6] is such that the bulky spiroketal side chain is rigidly held away from ring C and even the carbonyl group is not seriously hindered. However, free rotation of the cholanic acid side chain about the C-17,C-20 axis not only permits greater interference between the C-12 carbonyl and C-21 methyl groups but the carboxyl group and remainder of the side chain are capable of shielding large areas near C-11 and C-12 *on either the α- or β-side of the molecule.* Thus bromination, if by Newman's mechanism,[7] may receive interference at both the oxygen and α-carbon atoms, while hydrolysis of 11β-, and more so, 11α-bromine atoms will be similarly hindered. We have not yet studied the pure 11-bromosapogenin isomers sufficiently to be able to assess the relative magnitudes of these effects.

(1) G. P. Mueller, R. E. Stobaugh and R. S. Winniford, THIS JOURNAL, **73**, 2400 (1951).

(2) C. Djerassi, H. Martinez and G. Rosenkranz, *J. Org. Chem.*, **16**, 303, 1278 (1951).

(3) R. B. Wagner, R. F. Forker and P. F. Spitzer, THIS JOURNAL, **73**, 2494 (1951).

(4) T. F. Gallagher, *J. Biol. Chem.*, **165**, 211 (1946).

(5) T. F. Gallagher, *et al.*, *ibid.*, **162**, 522, 533 (1946).

(6) *Cf.* G. P. Mueller, R. E. Stobaugh and R. S. Winniford, THIS JOURNAL, **75**, 4888 (1953).

(7) M. S. Newman, *ibid.*, **73**, 4993 (1951).

Brief hydrolysis of the mixed dibromohecogenin acetates followed by acetylation and debromination gave a complex mixture containing bromo derivatives from which the halogen could not be removed by repeated treatments in zinc and acetic acid. From this mixture we isolated small amounts of 3β-acetoxy-11β-hydroxy-5α,22a-spirostan-12-one (IX) characterized by its resistance to acetylation and formation of an oxime X and 3β,11α-diacetoxy-5α,22a-spirostan-12-one (XI) also characterized as a C-12 ketone by oxime formation.

3β,11β-Dihydroxy-23-bromo-5α,22a-spirostan-12-one (VIII) was formed by hydrolysis of III at room temperature. Its isomerization to VI could be followed polarimetrically. The ketol VI was obtained directly from III either by prolonged hydrolysis at room temperature or shorter treatment at elevated temperatures. By analogy with bile acid studies both III and IV form this ketol, and we usually used the mixture of bromo isomers to prepare it. A third bromoketol whose structure requires further study was isolated and is indicated as VII. It was recovered unchanged from hot alcoholic alkali and formed a diacetate with acetic anhydride. Like VI it could be reduced to XIII,[8] which suggests that the compound belongs to another series and is isomeric with VI in the location or configuration of the halogen.

In preparation for the removal of the C-12 hydroxyl group from XIII the mono- and bis-hemisuccinates, XIV, XV and XVI and the tosylate XVII were prepared. However, as has previously been noted[9] we found the 12β-hydroxyl group resistant to replacement by phosphorus tribromide and the only halogen-containing product obtained from XVI was an oil. About 50% of an acidic solid, m.p. 235–238°, was extracted with difficulty from the aqueous washings; this was probably a phosphite ester. The attempt to cleave it with hydrogen bromide[10] gave a bromide which was reduced to a product, m.p. 147–150°, $[\alpha]^{30}D$ +33°, entirely different from the expected 11-ketotigogenin acetate. Among other attempts to remove the C-12 hydroxyl the sodium amalgam reagent, successful in the reduction of 3-hydroxycamphor,[11] and stringent Clemmensen conditions were tried. The use of sodium iodide[12,13] followed by zinc and acetic acid did not affect XVII. Finally several reductions were attempted with zinc and acetic anhydride[14] on the diacetate of XIII but each time the starting material was recovered in good yield.

For entry into the allopregnane series the diacetate of XIII was pseudomerized, oxidized and hydrolyzed to form 3β,12β-diacetoxy-16-allopregnen-11,20-dione (XVIII). Upon equilibration with methanolic potassium hydroxide this gave rise to a mixture from which the 16α-methoxypregnane XIX was isolated through chromatography. Hydrogenation of XVIII led to the pregnane, which was easily hydrolyzed to 3β,12β-dihydroxyallopregnane-11,20-dione (XXI).

The equilibration of XVIII in methanolic alkali has been compared with that of related C-12 ketopregnenes which reacted about ten times as fast.[6] The reaction rate for XIX under the same conditions is the counterpart of XVIII, reaching equilibrium in about 40 minutes with 22% of XVIII. The rate curves for the C-12 acetoxypregnene system are nearly identical with those published for 3β-acetoxy-5,16-pregnadien-20-one.[15]

In connection with other work we have repeated the hydrogenation of hecogenin acetate and wish to report physical constants for rockogenin 3-acetate XXII and the corresponding benzoate. The preparation of 23-bromomanogenin is also included.

Acknowledgment.—We thank the Research Corporation, New York, for their encouragement and financial support.

Experimental

Rotations were determined at a concentration of 10–20 mg. in 1.10 ml. of purified dioxane, unless otherwise specified. The melting points were observed at fifty magnifications on the Kofler block and are corrected.

11α,23-Dibromohecogenin Acetate (III).—23-Bromohecogenin acetate, 1.4 g., in 15 ml. of purified chloroform was treated with a few drops of a solution containing 0.47 g. of dry bromine in 5 ml. of pure chloroform. Evolution of hydrogen bromide was noted after six minutes when the remainder of the bromine solution was added. After an additional 30 minutes the mixture was diluted with 100 ml. of ether and washed several times with sodium sulfite solution, 1% potassium hydroxide and finally water. Concentration to 10 ml., dilution with 25 ml. of alcohol and further concentration in an air stream resulted in the deposition of crystals. These were collected after a period of cooling and washed with cold alcohol, m.p. 185–194° dec.; the yield was 0.6 g. (37%). Recrystallization three times from ether gave flat needles, m.p. 198–199° dec., $[\alpha]^{23}D$ −40.6° (chloroform).

Anal. Calcd. for $C_{29}H_{42}O_5Br_2$: C, 55.24; H, 6.72; Br, 25.34. Found: C, 55.30; H, 6.80; Br, 25.18.

11β,23-Dibromohecogenin Acetate (IV).—Hecogenin acetate, 2.5 g., in 25 ml. of chloroform was treated as described above with 1.73 g. of bromine in 10 ml. of chloroform. There appeared to be unreacted bromine present at the end. The entire mixture was evaporated to dryness *in vacuo*, the mass crystallizing as the last of the solvent was removed. The yellow residue was dissolved at room temperature in alcohol, treated with charcoal and crystallized; this process was repeated in hexane. The product was still gray-green. Washing with ether removed most of the color and recrystallization from ether afforded octagonal rods, m.p. 173–183°, $[\alpha]^{26}D$ −21.4° (alcohol).

Anal. Calcd. for $C_{29}H_{42}O_5Br_2$: C, 55.24; H, 6.72; Br, 25.34. Found: C, 55.52; H, 6.79; Br, 25.37.

The melting of this substance occurs with decomposition to a greenish-black oil and gas evolution beginning at a point and rapidly spreading throughout the crystal. Samples obtained from several different brominations both in chloroform and acetic acid, melted at 173–176° after crystallization from ether. Observation with the microscope indicated that there was present a small amount of the material melting usually at 183–185° which was probably the other isomer.

In two attempts to obtain a better sample, one by chromatographic purification on alumina and the other by repeated crystallization from ether, methanol and acetone, 9(11)-dehydro-23-bromohecogenin acetate was the product ob-

(8) This ketol and its 23-bromo precursor were reported as the 11-hydroxyhecogenin derivatives (ref. 1) before we recognized the reactivity of ring C in the series.

(9) C. Djerassi, H. J. Ringold and G. Rosenkranz, THIS JOURNAL, **73**, 5513 (1951).

(10) W. Gerrard, *J. Chem. Soc.*, 848 (1945).

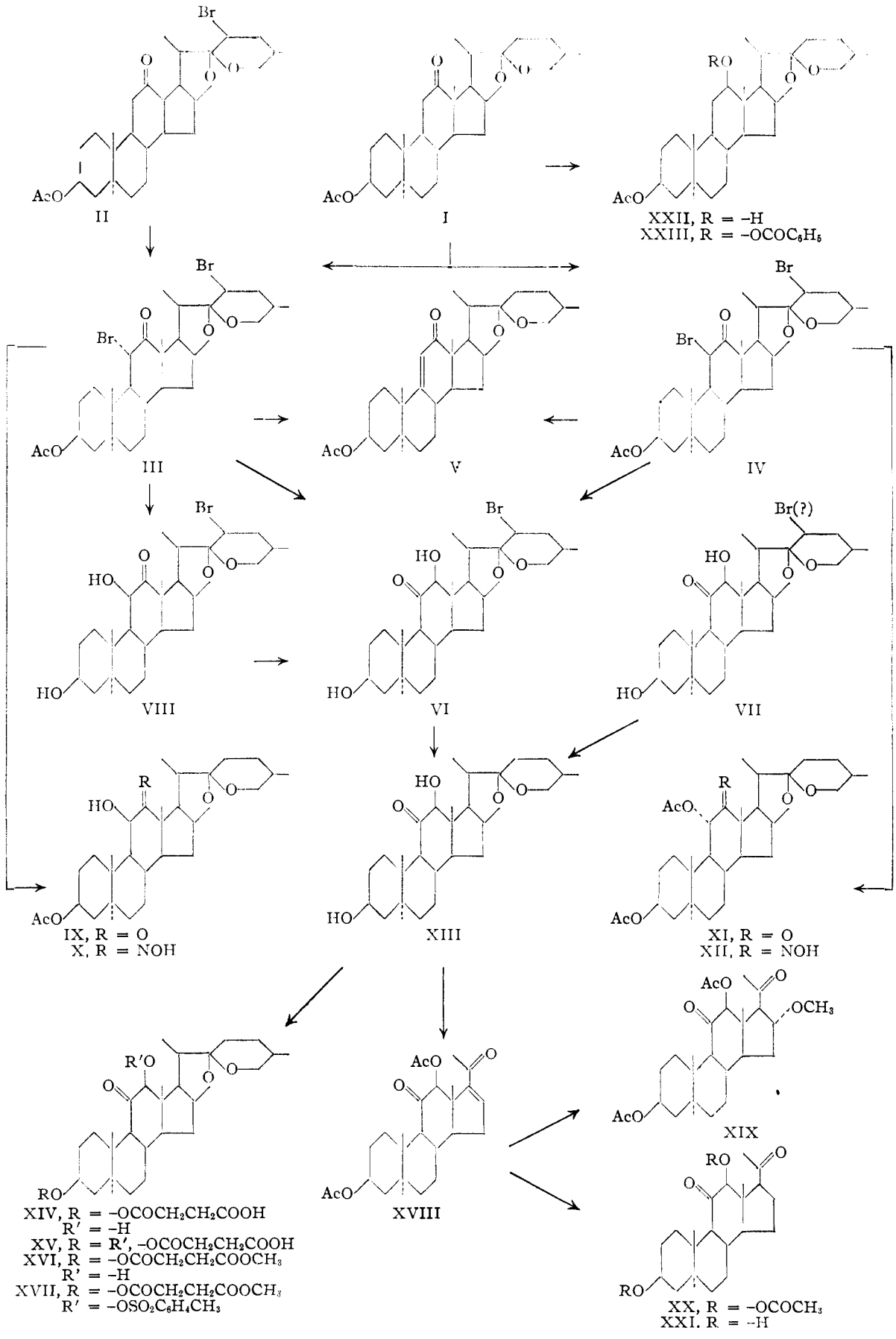
(11) M. Ishidate, *Chem. Zentr.*, **99**, II, 654 (1928).

(12) E. Borgstrom and T. F. Gallagher, *J. Biol. Chem.*, **117**, 951 (1949).

(13) T. Reichstein and H. G. Fuchs, *Helv. Chim. Acta*, **23**, 684 (1940).

(14) R. B. Woodward, *et al.*, THIS JOURNAL, **73**, 2403 (1951).

(15) D. K. Fukushima and T. F. Gallagher, *ibid.*, **73**, 196 (1951).


II
I
XXII, R = -H
XXIII, R = -OCOC6H5
III
V
IV
VIII
VI
VII
IX, R = O
X, R = NOH
XIII
XI, R = O
XII, R = NOH
XIV, R = -OCOCH2CH2COOH
R' = -H
XV, R = R', -OCOCH2CH2COOH
XVI, R = -OCOCH2CH2COOCH3
R' = -H
XVII, R = -OCOCH2CH2COOCH3
R' = -OSO2C6H4CH3
XVIII
XIX
XX, R = -OCOCH3
XXI, R = -H

tained, along with the 11α-isomer in the case where a crude mixture of the two was chromatographed (see below).

9(11)-Dehydro-23-bromohecogenin Acetate (V).—A solution of 4.0 g. of hecogenin acetate in 250 ml. of glacial acetic acid was treated with 3.07 g. of bromine in small portions over a period of ten minutes and allowed to stand for 20 minutes. It was poured into 1 l. of cold water, the product extracted with ether and the ethereal solution washed free of acetic acid, dried and evaporated. A 200-mg. sample of the residue in 20% benzene in petroleum ether was adsorbed on acid-washed alumina. Elution with 1% ether in benzene yielded 108 mg. of 11α,23-dibromohecogenin acetate, m.p. 197–198°, $[\alpha]^{23}D$ −40° (chloroform), after recrystallization once from ether. A 14-mg. fraction eluted with 50% ether in benzene was recrystallized twice from ether to give 9(11)-dehydro-23-bromohecogenin, m.p. 228–230° dec., $[\alpha]^{26}D$ −24.4° (chloroform), λ_{max}^{alc} 240 mμ (log ε 4.06).

Anal. Calcd. for $C_{29}H_{41}O_5Br$: C, 63.38; H, 7.52; Br, 14.54. Found: C, 63.79; H, 7.92; Br, 14.38.

Crude dibromohecogenin acetate, 2.5 g., in 50 ml. of collidine and 50 ml. of xylene was allowed to stand overnight, refluxed for an hour, cooled and filtered. The filtrate was diluted with ether, washed free of collidine with dilute hydrochloric acid, dried and evaporated to dryness *in vacuo*. The residue was crystallized by evaporating an ethereal solution until crystallization commenced. The first crop was recrystallized twice more in this manner and yielded 0.59 g. (27%) of the 9(11)-dehydrocompound, m.p. 228–229°.

Debromination of this product by treating 0.42 g. with 2.5 g. of zinc dust in 30 ml. of glacial acetic acid at 100° for five hours, followed by precipitation of the product with water and recrystallization from methanol and from ether gave 9(11)-dehydrohecogenin acetate, m.p. 218–220° (not sharp), 0.27 g. (75%), crystallizing as fine needles, $[\alpha]^{25}D$ −8.3°. A sample from an earlier preparation, m.p. 217–219°, $[\alpha]^{30}D$ −8.4°, λ_{max}^{alc} 238 mμ (log ε 4.12), was analyzed.

Anal. Calcd. for $C_{29}H_{42}O_5$: C, 74.01; H, 9.00. Found: C, 73.86; H, 8.89.

Our melting point is somewhat lower than the published value of 224–227°.[16]

3β,12β-Dihydroxy-23-bromo-5α,22a-spirostan-11-one (VI).—A recrystallized dibromohecogenin acetate mixture, m.p. 180° dec., 3.9 g. was hydrolyzed with 30 g. of potassium hydroxide in 1 l. of 90% alcohol at 28° for 70 hours. Neutralization with acetic acid, concentration to 250 ml. and dilution with water precipitated 2.5 g. (78%) of product, m.p. 225° dec. Several recrystallizations from acetone–hexane and alcohol–hexane mixtures gave the pure ketol, m.p. 233–234° dec., $[\alpha]^{26}D$ −23.7°.

Anal. Calcd. for $C_{27}H_{41}O_5Br$: C, 61.70; H, 7.86; Br, 15.21. Found: C, 61.77; H, 7.65; Br, 14.83.

A 50-mg. sample of this ketol was refluxed two hours with 20% methanolic potassium hydroxide and 48 mg. of unchanged material, $[\alpha]^{29}D$ −25.7°, recovered.

3β,12β-Dihydroxy-23(?)-bromo-5α,22a-spirostan-11-one (VII).—This ketol, isomeric with the above, was isolated on two different occasions. The product from dibromination of 10 g. of hecogenin acetate stood for six hours in 400 ml. of methanol and 22 g. of potassium hydroxide. After addition of 55 g. of potassium hydroxide and refluxing for 2.5 hours, 10 ml. of water was added and the solution chilled. Recrystallization once from ethyl acetate–methanol and three times from aqueous methanol yielded 0.4 g. of needles, m.p. 230.0–230.5° dec., $[\alpha]^{28}D$ −74.1°.

Anal. Calcd. for $C_{27}H_{41}O_5Br \cdot H_2O$: C, 59.66; H, 7.97; Br, 14.70. Found: C, 59.77; H, 7.85; Br, 14.75.

This product was stable toward further heating with alcoholic alkali and like the preceding ketol was reduced with zinc and acetic acid to 11-ketorockogenin (see below). Pyridine and acetic anhydride transformed this to the diacetate crystallizing as needles from methanol, m.p. 190.0–191.5°, $[\alpha]^{27}D$ −93.8°.

Anal. Calcd. for $C_{31}H_{45}O_7Br$: C, 61.08; H, 7.44; Br, 13.11. Found: C, 61.31; H, 7.51; Br, 13.45.

3β,11β-Dihydroxy-23-bromo-5α,22a-spirostan-12-one (VIII).—A 500-mg. sample of crude 11α,23-dibromohecogenin acetate, m.p. 191–193° dec., was hydrolyzed for two hours at 27° in 100 ml. of 0.25 *N* potassium hydroxide in 90% alcohol; the yield was 261 mg. of needles, $[\alpha]^{27}D$ +1.6°. This product was difficult to purify as it formed gels with the usual solvents unless the solutions were cooled slowly. Four recrystallizations from acetone–hexane and from methanol gave the pure ketol crystallizing as solvated needles, m.p. (sinters at 195°) 212–213°, $[\alpha]^{32}D$ +10.1°.

Anal. Calcd. for $C_{27}H_{41}O_5Br \cdot CH_3OH$: C, 60.31; H, 8.14; Br, 14.33. Found: C, 60.29; H, 8.21; Br, 14.91.

A 20-mg. sample in 8 ml. of 1 *N* alcoholic potassium hydroxide gave $[\alpha]^{30}D$ +13.6° upon mixing, but the solution became more levorotatory until after 32 hours the value was constant at −36.0°. Isolation and purification of the isomerized material yielded 10 mg. of 3β,12β-dihydroxy-23-bromo-5α,22a-spirostan-11-one, m.p. 225–229°, $[\alpha]^{30}D$ −24.2°; when mixed with the preparation described above the melting point was 227–230°.

3β-Acetoxy-11β-hydroxy-5α,22a-spirostan-12-one (IX).—Five grams of hecogenin acetate was brominated by a procedure similar to that described by Djerassi, *et al.*[2] The entire amount of well-washed precipitate was immediately placed in 1400 ml. of alcohol and stirred for two hours at room temperature. All of the material had dissolved by this time except for about 0.4 g. Titration of a 10.00-ml. aliquot in 5 ml. of water, 1 ml. of 6 *N* nitric acid and 10.00 ml. of 0.020 *N* silver nitrate with 0.02 *N* potassium thiocyanate showed no detectable concentration of bromine ions. A solution containing 21.0 g. of potassium hydroxide in 100 ml. of alcohol was added to the stirred bromide solution; the small amount of suspended material dissolved instantly. Aliquots were titrated at two-minute intervals. The bromine was liberated rapidly, 95% being in solution in six minutes. The reaction was quenched with 80 ml. of 6 *N* nitric acid after hydrolysis had proceeded 20 minutes. Of the oily solid remaining from the ethereal extract, 4.84 g. was acetylated catalytically[17] to give 4.84 g. of semi-solid product. This mixture, 4.24 g., was debrominated with zinc dust in acetic acid and 3.0 g. of the product (3.1 g.) chromatographed on 100 g. of acid-washed, activated alumina. The material, 608 mg., from 400 ml. of 40% ether in benzene and 200 ml. of 50% ether eluates was combined; this melted diffusely at 234–236°. Recrystallization three times from hexane yielded 3β-acetoxy-11β-hydroxy-5α,22a-spirostan-12-one as fine, silky needles, m.p. 240–241°, $[\alpha]^{33}D$ −7.7°.

Anal. Calcd. for $C_{29}H_{44}O_6$: C, 71.28; H, 9.07. Found: C, 71.33; H, 9.14.

A further attempt was made to acetylate the pure hydroxyketone, this time with boiling acetic anhydride. The product, m.p. 239–241°, proved to be identical with the starting material, showing that no reaction had occurred.

3β-Acetoxy-11β-hydroxy-5α,22a-spirostan-12-one Oxime (X).—The oxime was formed by refluxing 7.5 mg. of hydroxyketone overnight with 68 mg. of sodium acetate trihydrate and 35 mg. of hydroxylamine hydrochloride. The precipitated product was recrystallized twice from alcohol, coming down as hexagonal plates, m.p. 267–274°.

Anal. Calcd. for $C_{29}H_{45}O_6N$: N, 2.78. Found: N, 2.88.

3β,11α-Diacetoxy-5α,22a-spirostan-12-one (XI).—The mother liquors from isolation and purification of the 11β-hydroxyketone (above) were evaporated and the residue recrystallized carefully four times from hexane. The product was obtained as short needles, m.p. 230–231°, $[\alpha]^{31}D$ −24°, which depressed the melting points of the 11β-hydroxyketone and of 11-ketorockogenin diacetate (below).

Anal. Calcd. for $C_{31}H_{46}O_7$: C, 70.16; H, 8.74. Found: C, 70.50; H, 9.06.

3β,11α-Diacetoxy-5α,22a-spirostan-12-one Oxime (XII).—This oxime was prepared as described above from 3.8 mg. of the ketone. After recrystallization from alcohol the product, 1.9 mg., appeared as micro plates, m.p. 269–273°.

Anal. Calcd. for $C_{31}H_{47}O_7N$: N, 2.57. Found: N, 2.58.

3β,12β-Dihydroxy-5α,22a-spirostan-11-one (XIII).—A solution of 0.5 g. of 3β,12β-dihydroxy-23-bromo-5α,22a-spirostan-11-one in 50 ml. of glacial acetic acid was stirred at 100° with 3 g. of zinc dust for six hours. Filtration and dilution with water followed by extraction with ether and evaporation of the extract gave a residue which was treated

(16) R. B. Wagner, J. A. Moore and R. F. Forker, THIS JOURNAL, **72**, 1856 (1950).

(17) B. Whitman and E. Schwenk, *ibid.*, **68**, 1865 (1946).

at room temperature with 0.5 *N* alcoholic potassium hydroxide. After twelve hours the product was precipitated with water. Recrystallization from acetone yielded 260 mg. of rhombic plates, m.p. 216.0–218.5°, $[\alpha]^{26}D$ −37.1°.

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found: C, 72.36; H, 9.68.

Recrystallization from aqueous methanol yielded massive rhombic plates, m.p. 217–218°, $[\alpha]^{28}D$ −40.4°.

Anal. Calcd. for $C_{27}H_{42}O_5 \cdot H_2O$: C, 69.79; H, 9.55. Found: C, 69.70; H, 9.10.

Recrystallization from ether gave an etherate crystallizing as long needles, m.p. 215–216°, $[\alpha]^{30}D$ −33.0°.

Anal. Calcd. for $C_{27}H_{42}O_5 \cdot (C_2H_5)_2O$: C, 71.50; H, 10.07. Found: C, 71.56; H, 9.78.

Repetition of the procedure described by Djerassi, Martinez and Rosenkranz[2] gave results identical with theirs except that the rotation of the product agreed with the figures above.

3β,12β-Diacetoxy-5α,22a-spirostan-11-one was prepared by acetylation of the diol with boiling acetic anhydride, pyridine and acetic anhydride and acetic anhydride–perchloric acid according to Whitman and Schwenk.[17] The rotation, $[\alpha]^{30}D$ −71.8°, and melting point agree with previously published values.[1,2]

3β,12β-Dihydroxy-5α,22a-spirostan-11-one 3-Hemisuccinate (XIV).—Freshly distilled pyridine was redistilled from barium oxide, under nitrogen, directly into a flask containing 163 mg. of the diolone and 367 mg. of succinic anhydride until 6 ml. had been collected. The flask was stoppered, warmed briefly to dissolve the reactants and set aside. After 37 hours the mixture was poured onto 100 g. of ice and 10 ml. of concentrated hydrochloric acid and the whole extracted with ether as usual. Three recrystallizations of the crude product from aqueous alcohol gave 139 mg. (70%) of the 3-hemisuccinate, crystallizing as a hydrate, m.p. 190–192°, $[\alpha]^{31}D$ −39.7°.

Anal. Calcd. for $C_{31}H_{46}O_8 \cdot H_2O$: C, 65.93; H, 8.57. Found: C, 66.00; H, 8.46.

3β,12β-Dihydroxy-5α,22a-spirostan-11-one 3,12-Bis-hemisuccinate (XV).—The neutral equivalent of the crude monohemisuccinate showed the presence of the bis-hemisuccinate. Therefore, the mother liquors from purification of the former were evaporated to dryness and the residue triturated five times with boiling ether. The ether-insoluble fraction, m.p. 242–247°, corresponded to 11% of the diolone. Recrystallization from ethyl acetate gave the pure bis-hemisuccinate, m.p. 247–248°, $[\alpha]^{27}D$ −51.8°.

Anal. Calcd. for $C_{35}H_{50}O_{11}$: C, 64.99; H, 7.79. Found: C, 65.11; H, 7.73.

Due to its insolubility this compound did not react with ethereal diazomethane in 30 minutes. Hydrolysis in alcoholic alkali yielded the original diolone.

Methyl Ester of 3β,12β-Dihydroxy-5α,22a-spirostan-11-one 3-Hemisuccinate (XVI).—Treatment of the acid with ethereal diazomethane, and two recrystallizations of the product from alcohol gave beautiful rhombic plates melting sharply at 172–173°, $[\alpha]^{31}D$ −39.7°. The rotation is somewhat at variance with the reported value.[2]

Anal. Calcd. for $C_{32}H_{48}O_8$: C, 68.54; H, 8.63. Found C, 68.64; H, 8.64.

Methyl Ester of 3β,12β-Dihydroxy-5α,22a-spirostan-11-one 3-Hemisuccinate 12-Tosylate (XVII).—A solution of 500 mg. of the methyl 3-hemisuccinate in 2 ml. of anhydrous pyridine was treated with 680 mg. of *p*-toluenesulfonyl chloride in 3 ml. of pyridine. The solution was cooled in an ice–salt-bath at first and then set aside for 30 hours.[18] Hydrolysis with ice and hydrochloric acid and extraction with ether gave 457 mg. (71%) of product, m.p. 187–194°. Recrystallization, once from ether and once from chloroform–ethanol, produced the tosylate in the form of short needles, m.p. 197.0–197.5°, $[\alpha]$ +4.8°.

Anal. Calcd. for $C_{39}H_{54}O_{10}S$: C, 65.52; H, 7.61. Found: C, 65.55; H, 7.77.

3β,12β-Diacetoxy-16-allopregnene-11,20-dione (XVIII).—Two grams of 3β,12β-diacetoxy-5α,22a-spirostan-11-one was treated with 15 ml. of acetic anhydride at 200° for 10 hours. The pseudosapogenin triacetate[2] was not purified but was oxidized directly in 30 ml. of glacial acetic acid with a solution of 1.5 g. of chromic anhydride in 12 ml. of 80% acetic acid, at 15°. After standing at 25° for 90 minutes the solution was diluted and extracted with ether as usual. The residue from the washed and dried ether solution was dissolved in 20 ml. of hot methanol and treated with 12 ml. of water containing 1.2 g. of potassium carbonate. The reaction time was just ten minutes at reflux temperature, whereupon the solution was diluted and extracted. The residue was oily and could not be crystallized, even after acetylating. It was adsorbed onto 30 g. of acid-washed alumina from which elution with benzene–ether mixtures yielded 700 mg. (43%) of crystalline product, m.p. 213–216°. Three recrystallizations from ether–petroleum ether gave the pure pregnene as rectangular prisms, m.p. 217–219°, $[\alpha]^{27}D$ −8.0°, λ_{max}^{alc} 232 mμ (log ε 3.95).

Anal. Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 69.93; H, 7.82.

3β,12β-Diacetoxy-16α-methoxyallopregnane-11,20-dione (XIX).—A crude preparation of 3β,12β-diacetoxy-16-allopregnene-11,20-dione was allowed to stand in 5% methanolic potassium hydroxide for 10 hours. The product seemed incapable of purification and was reacetylated with acetic anhydride and pyridine. One gram of the resulting oil was chromatographed on 60 g. of acid-washed alumina, 2% methanol in ether eluting a 200-mg. fraction which was recrystallized five times from ether. The 16α-methoxy compound showed no maximal absorption in the 225–250 mμ region; m.p. 205–206°, $[\alpha]D$ +29°.

Anal. Calcd. for $C_{26}H_{38}O_7$: C, 67.51; H, 8.28; OCH_3, 6.71. Found: C, 67.62; H, 8.41; OCH_3, 6.60.

3β,12β-Diacetoxyallopregnane-11,20-dione (XX).—Hydrogenation of 200 mg. of the pregnene in absolute ether over 3% palladium-on-barium sulfate catalyst gave 170 mg. of an oil. This crystallized on standing and was recrystallized twice from 50% ether in petroleum ether. The pregnane crystallized as clusters of needles, m.p. 149–150°, $[\alpha]^{25}D$ +29°, λ_{max}^{alc} 288 mμ (log ε 1.94).

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.44; H, 8.02.

3β,12β-Dihydroxyallopregnane-11,20-dione (XXI).—One hundred milligrams of the diacetate was hydrolyzed for 12 hours at room temperature in 10 ml. of 3% alcoholic potassium hydroxide. After dilution, extraction with ether and recrystallization of the extract three times from 50% ether–petroleum ether, the pure diolone was obtained, m.p. 170–171°, $[\alpha]^{25}D$ +90°.

Anal. Calcd. for $C_{21}H_{32}O_4$: C, 72.40; H, 9.25. Found: C, 72.27; H, 9.21.

23-Bromomanogenin Diacetate.—A solution of 198 mg. of manogenin diacetate, containing 8% of 9(11)-dehydromanogenin diacetate, in 3 ml. of pure, dry chloroform was treated with 0.023 ml. of dry bromine in 0.23 ml. of chloroform at 26°. The bromine color was discharged quickly and the solution was poured into water and shaken with dilute sodium bicarbonate solution. The mixture was extracted; the chloroform–ether extract was concentrated to a small volume, and on dilution with 10 ml. of alcohol 143 mg. (65%) of the product crystallized as flat needles, m.p. 233–239°. By recrystallization once from ether and twice from methanol the pure 23-bromomanogenin diacetate was obtained, m.p. 256.0–256.5° dec., $[\alpha]D$ −54.0°.

Anal. Calcd. for $C_{31}H_{45}O_7Br$: C, 61.07; H, 7.44; Br, 13.11. Found: C, 61.11; H, 7.21; Br, 13.23.

This bromosapogenin was unchanged in silver nitrate and anhydrous pyridine at room temperature after two days and was unaffected by boiling for five hours with pyridine.

Rockogenin 3-Acetate (XXII).—Hecogenin acetate, 1.98 g., was hydrogenated over 1 g. of platinum oxide in 100 ml. of ethyl acetate containing 1 ml. of acetic acid. The product, rockogenin 3-acetate,[19] was purified from methanol, m.p. 218–220°, $[\alpha]^{24}D$ −58°. On acetylation this formed the diacetate,[3,19] m.p. 199–202°, $[\alpha]^{20}D$ −61°.

Anal. Calcd. for $C_{29}H_{46}O_5$: C, 73.36; H, 9.77. Found: C, 73.42; H, 9.70.

Hydrolysis of the monoacetate gave rockogenin,[19] m.p. 210–213°, $[\alpha]^{23}D$ −53°.

(18) *Cf.* E. Borgstrom and T. F. Gallagher, *J. Biol. Chem.*, **177**, 951 (1949).

(19) R. E. Marker, *et al.*, THIS JOURNAL, **69**, 2175 (1947).

Rockogenin 3-Acetate 12-Benzoate (XXIII).—Two hundred milligrams of rockogenin 3-acetate was treated at room temperature for five hours with 0.2 ml. of purified benzoyl chloride in 2 ml. of pyridine and 5 ml. of benzene. The solvents were removed *in vacuo* and the residue treated for a time with methanol and pyridine, which were removed likewise. The product was dissolved in ether, washed with dilute hydrochloric acid, then sodium bicarbonate and re-isolated. It was finally purified in methanol from which it crystallized as fine needles or large prisms, m.p. 204–205°, $[\alpha]^{28}D$ −59.7°.

Anal. Calcd. for $C_{36}H_{50}O_6$: C, 74.71; H, 8.71. Found: C, 74.70; H, 8.48.

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[CONTRIBUTION FROM THE STARCH AND DEXTROSE DIVISION, NORTHERN REGIONAL RESEARCH LABORATORY[1a]]

Polysaccharide Aryl Carbamates. III. Tricarbanilates of Polyglucosans with Various Glucosidic Linkages[1b]

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Tricarbanilates were prepared from water-soluble sweet corn polysaccharides no. 1 and no. 2, glycogen β-amylase limit dextrin, the polysaccharide formed by *Phytomonas tumefaciens*, laminarin, yeast polyglucosan, cellulose, lichenin and several selected types of dextrans. The various classes of polysaccharide carbanilates prepared had optical rotations in pyridine and morpholine which were dependent on the position and anomeric type of the predominant glucosidic linkages. This observation may prove useful in studies of the chemical structures of various polyglucosans.

In our previous publications[2] a relation was shown to exist between the structure of an amylaceous polysaccharide and the optical rotation of its tricarbanilate in pyridine. This paper reports the extension of this work to a number of other polyglucosans differing in their glucosidic linkages. One group of polysaccharides, comprising water-soluble sweet corn polysaccharides and glycogen β-amylase limit dextrin, had predominantly α-1,4′-linkages with some α-1,6′-branch points. A second set, having β-linkages, included 1,2′-, 1,3′-, 1,4′- and 1,6′-linked materials. Several different dextran samples (chiefly α-1,6′-linkages) were also studied. The optical rotation data obtained are of value in furnishing preliminary or confirmatory evidence of the structure of polysaccharides composed exclusively of anhydroglucose units.

Experimental

Most of the polysaccharide samples were available only in very small amounts and were used as obtained without further purification. The dextrans were isolated at this Laboratory by published procedures[3] and had very low nitrogen, phosphorus and ash contents. Those samples in Table I designated as "partially hydrolyzed" were acid degraded, alcohol-fractionated materials with a weight average molecular weight of approximately 75,000,[4] and meeting current specifications for dextran of clinical-injection type.

Each polysaccharide was dried by azeotropic distillation from pyridine dispersion, and was treated in that medium with phenyl isocyanate at 100°.[2] At the end of the reaction period the mixture was filtered through fritted glass and poured into absolute ethanol to precipitate the ester. In some cases addition of an equal volume of water to the ethanol-containing mixture was necessary to cause precipitation of the carbanilate. One treatment, for time periods varying from 5 to 24 hours, was sufficient to give trisubstitution with all of the polysaccharides except luteose, which resisted complete carbanilation after two successive reaction periods of 23 and 22 hours, each in the presence of excess reagent.

Results

In Table I are listed pertinent data on the polysaccharides used and the optical rotations of their carbanilates.

Amylaceous Polysaccharides.—Since it was found[2] previously that those predominantly 1,4′-linked polysaccharides having larger percentages of branch linkages yielded carbanilates with smaller negative optical rotations in pyridine (*e.g.*, amylose, −82.5°; amylopectin, −62°; glycogen, −31.5°) further application of this principle to available samples of this type appeared to be of interest. The water-soluble polysaccharides of sweet corn have been separated by most recent investigators into two fractions based on their solubility in 67% acetic acid.[5–7] The soluble fraction (called polysaccharide no. 2, corn glycogen or phytoglycogen) has been assigned a repeating chain length of 11–12 while the insoluble fraction (polysaccharide no. 1, glycoamylose, starch) has been found to have an average chain length of 12 by periodate end-group assay of material passed through a cotton column,[5] or of 25 by methylation study of a fraction not so treated.[6] Glycogen from animal sources is usually considered to have an average branch length of 11–13 glucose units,[8,10] and is therefore indistinguishable by end-group methods from the soluble sweet corn polysaccharides.[9,10] However, it would appear from our data (Table I and reference 2) that these sweet corn polysaccharides may have on the average a slightly lower extent of branching than animal glycogen and are intermediate in this respect between glycogen and amylopectin. The data are in agreement with those of Dvonch and

(1) (a) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted. (b) Presented before the Division of Sugar Chemistry at the 124th National Meeting of the American Chemical Society, Chicago, Ill., September, 1953.

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