22a,5α-spirostan-3-one (IX) with m.p. 214–216°, $[\alpha]D$ −45°, $\nu_{max}^{CHCl_3}$ 1700 cm.$^{-1}$.

Anal. Calcd. for $C_{27}H_{41}O_3Br$: C, 65.70; H, 8.37; Br, 16.19. Found: C, 65.75; H, 8.42; Br, 16.55.

22a,5α-Spirostan-2β-ol-3-one Acetate (Xb).—23-Bromo-22a,5α-spirostan-3-one (40 g.) dissolved in 1.4 l. of glacial acetic acid was heated on the steam-bath with 49 g. of lead tetraacetate for 5 hours. Addition of water, extraction with chloroform and crystallization from benzene–hexane yielded 20.8 g. of crude 23-bromo-22a,5α-spirostan-2β-ol-3-one acetate (Xa) with m.p. 215–220°. This material was refluxed in 2 l. of ethanol with 200 g. of zinc dust for 24 hours, another 100 g. of zinc having been added after the first 10 hours. The metal was removed, most of the alcohol was evaporated, and the product was extracted with chloroform. Chromatographic purification on 800 g. of neutral alumina, and crystallization of the fractions eluted with benzene–hexane (3:2) from acetone–hexane afforded 5.7 g. of 22a,5α-spirostan-2β-ol-3-one acetate (Xb) with m.p. 225–228°. The analytical sample was crystallized from methanol and showed m.p. 230–231°, $\nu_{max}^{CHCl_3}$ 1736 cm.$^{-1}$.

Anal. Calcd. for $C_{29}H_{44}O_5$: C, 73.69; H, 9.38. Found: C, 73.96; H, 9.67.

The compound differed from the 2α-isomer IV as evidenced by a depression in m.p. on admixture and by differences in the infrared spectra.

22a,5α-Spirostane-2β,3β-diol (XIa).—The ketol acetate Xb (1.30 g.) dissolved in 400 cc. of dry tetrahydrofuran was reduced with 1.30 g. of lithium aluminum hydride in 100 cc. of tetrahydrofuran as described above for the 2α-isomer. The product, obtained by benzene extraction, was purified by chromatography on alumina followed by crystallization from acetone. This procedure yielded 0.76 g. of the 2β,3β-diol XIa with m.p. 266–267°, $\nu_{max}^{CHCl_3}$ free hydroxyl band only.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 75.11; H, 10.45.

The compound was shown to differ from gitogenin as well as from the corresponding 2β,3α- and 2α,3α-diols described previously[6] through depressions in m.p. on admixture and differences in the infrared spectra. The pure diol XIa, as well as the total reduction product before purification, gave only a light yellow color with sulfuric acid.

The diacetate XIb was crystallized from acetone and showed m.p. 237–238°, $\nu_{max}^{CHCl_3}$ 1736 cm.$^{-1}$.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 72.11; H, 9.33.

22a,5α-2;3-Secospirostane-2,3-dioic (Gitogenoic) Acid (V). (a) **From IV.**—The saturated ketol acetate IV (200 mg.) was saponified by refluxing a solution in 40 cc. of methanol with 300 mg. of sodium carbonate for 1 hour. The free ketol was isolated with ether, and without purification was oxidized with 200 mg. of chromic acid in chloroform–acetic acid for 1 hour at room temperature. Crystallization of the acidic product from dilute acetic acid furnished 85 mg. of gitogenoic acid with m.p. 240–242°, $\nu_{max}^{CHCl_3}$ 1700 cm.$^{-1}$. No depression in m.p. was observed on admixture with an authentic sample[6] (m.p. 241–243°), and the infrared spectra were identical.

(b) **From XIa.**—The 2β,3β-diol XIa (200 mg.) was oxidized with chromic acid as described under (a). Crystallization of the acidic product from dilute acetic acid yielded 120 mg. of gitogenoic acid with m.p. 239–242°, identified with authentic material in the usual way. Gitogenin on oxidation under the same conditions yielded the acid in comparable yield.

APARTADO 2679
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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX, S. A.]

Steroidal Sapogenins. XXXVII.[1] Experiments in the Hecogenin Series. (Part 6).[2] Conversion to Cortisone

BY CARL DJERASSI,[3] HOWARD J. RINGOLD AND G. ROSENKRANZ

RECEIVED MAY 25, 1954

The conversion of hecogenin to the cortisone intermediate 22a,5α-spirostan-3β-ol-11-one is described. The key reaction involves bismuth oxide oxidation of 22a,5α-spirostane-3β,12β-diol-11-one to the corresponding 11,12-dione followed by removal of the 12-keto function.

The widely distributed[4,5] hecogenin (22a,5α-spirostan-3β-ol-12-one) (I) appears to be the only naturally occurring ring C oxygenated steroidal sapogenin worthy of consideration as a potential raw material for cortical hormone synthesis. In an earlier communication[6] we reported the conversion of hecogenin to 22a,5α-spirostan-3β-ol-11-one (Va), a compound which previously had been transformed[7] into allopregnan-3β-ol-11,20-dione and thence to cortisone.[8] We now wish to report at greater length on the conversion of I into C-11 oxygenated intermediates.

The method of Borgstrom and Gallagher[9] employed in the bile acid series for removal of a 12-hydroxyl function from an 11-keto-12β-hydroxy compound, involving treatment of the ketol with phosphorus tribromide, is inapplicable in the sapogenin series because of reaction of the reagent with the spiroketal side chain. It has now been observed that bismuth oxide, a specific oxidizing agent for acyloins,[10] does not attack tigogenin (22a,5α-spirostan-3β-ol) VII, but reacts smoothly with a ketol such as methyl 3α,12β-dihydroxy-11-ketocholanate (Marker–Lawson acid)[11] to afford, after acetylation, the known[12] enol acetate of methyl 3α-acetoxy-11,12-diketocholanate.

Similarly, treatment of 22a,5α-spirostane-3β,12β-diol-11-one (IIa)[13] for 30 hours in boiling acetic acid

(1) Paper XXXVI, J. Herran, G. Rosenkranz and F. Sondheimer, THIS JOURNAL, **76**, 5531 (1954).

(2) Part 5, C. Djerassi, A. J. Lemin, H. Martinez, G. Rosenkranz and F. Sondheimer, *ibid.*, **75**, 4485 (1953).

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(4) R. E. Marker, R. B. Wagner, P. R. Ulshafer, E. L. Wittbecker, D. P. J. Goldsmith and C. H. Ruof, THIS JOURNAL, **69**, 2167 (1947).

(5) R. K. Callow, J. W. Cornforth and P. C. Spenseley, *Chem. and Ind.*, 699 (1951).

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

(7) (a) E. M. Chamberlin, W. V. Ruyle, A. E. Erickson, J. M. Chemerda, L. M. Aliminosa, R. L. Erickson, G. E. Sita and M. Tishler, *ibid.*, **73**, 2396 (1951); (b) C. Djerassi, E. Batres, J. Romo and G. Rosenkranz, *ibid.*, **74**, 3634 (1952).

(8) G. Rosenkranz, J. Pataki and C. Djerassi, *ibid.*, **73**, 4055 (1951); J. M. Chemerda, E. M. Chamberlin, E. H. Wilson and M. Tishler, *ibid.*, **73**, 4053 (1951).

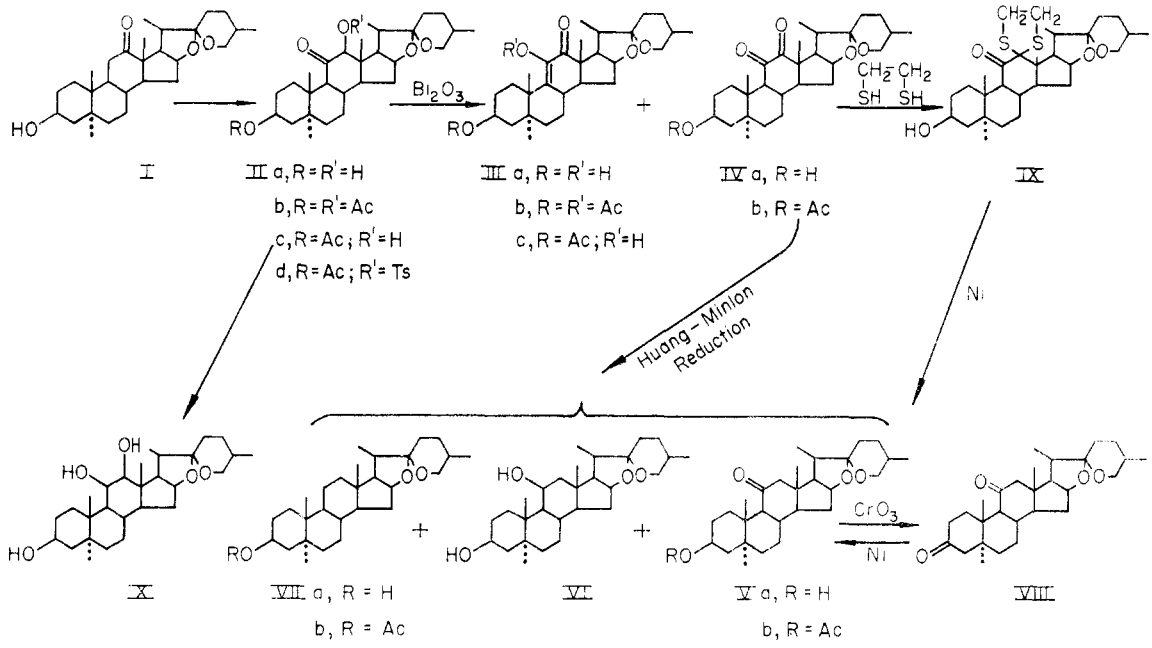
(9) E. Borgstrom and T. F. Gallagher, *J. Biol. Chem.*, **177**, 951 (1949).

(10) W. Rigby, *J. Chem. Soc.*, 793 (1951).

(11) *Cf.* T. F. Gallagher, *J. Biol. Chem.*, **162**, 539 (1946).

(12) O. Wintersteiner and M. Moore, *ibid.*, **162**, 725 (1946).

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



with bismuth oxide, resulted in the formation of the 11,12-diketone as a mixture of the keto IVb and enol IIIc forms. The crude oxidation product was most satisfactorily worked up by conversion to the enol diacetate IIIb; thus, heating of the total crude product with acetic anhydride–pyridine, followed by direct crystallization, afforded a 70% yield of IIIb and a 13% recovery of starting material as the 3,12-diacetate IIb. The extended reaction time for bismuth oxide oxidation of a ring C ketol, even in the presence of a large molar excess of oxidizing agent (24–30 hr. as contrasted to a few minutes for a simple ketol[10]) is worthy of note as is the finding that the oxidation proceeds at a markedly diminished rate in a nitrogen atmosphere.

Alkaline saponification of the crystallized enol diacetate IIIb again gave a mixture of keto and enol compounds (IIIa and IVa), as evidenced by the ultraviolet maximum of the product at 282 mμ (log ε 3.28) and by the dark green coloration with ferric chloride. This mixture could be utilized directly for the subsequent reactions and in some cases the total crude oxidation product was used. Treatment of mixed IIIa and IVa by the Huang-Minlon reduction procedure yielded a mixture of substances containing 22a,5α-spirostan-3β-ol-11-one (Va),[14] 22a,5α-spirostan-3β,11β-diol (VI)[14] and 22a,5α-spirostan-3β-ol (tigogenin) (VIIa). A similar observation has already been recorded by Wintersteiner and Moore[12] in the bile acid series. Purification was most easily effected by chromic acid oxidation of the total crude reduction product, whereupon, by crystallization and alumina chromatography, 22a,5α-spirostane-3,11-dione (VIII)[14] was obtained in 13% over-all yield from IIa. By direct chromatography of the crude Huang-Minlon reduction mixture, small quantities of somewhat impure 11β-hydroxy compound (VI) could be isolated. An alternate purification procedure consisted of mild acetylation at C-3 of the crude reduction product, chromic acid oxidation at C-11, and finally chromatography of the total product to yield 11% of 22a,5α-spirostan-3β-ol-11-one acetate (Vb).[7,14] In this particular experiment tigogenin acetate (VIIb) was also isolated as a by-product.

Raney nickel reduction of the 3,11-dione VIII in ethanol solution smoothly effected reduction at C-3 to afford the 3β-hydroxy-11-keto compound (Va) and upon acetylation the known cortisone intermediate Vb.

The second method devised for removal of the C-12 ketone function from the 11,12-diketone IVa consisted of formation of a C-12 cycloethylene mercaptal followed by reductive removal of sulfur. Thus, the 11,12-dione, as its mixture of enol and keto forms IIIa and IVa, was treated with ethanedithiol in the presence of anhydrous hydrogen chloride gas[15] as catalyst, to furnish 22a,5α-spirostan-3β-ol-11,12-dione 12-cycloethylene mercaptal (IX). Raney nickel desulfurization of this compound in ethanol solution led directly to Va.

An alternate but unsuccessful reaction scheme to remove the C-12 oxygen function of the 11-keto-12-hydroxy compound (IIa), consisted of blocking the C-3 hydroxyl group by ester formation followed by tosylation at C-12 and C-12 carbon–oxygen cleavage or iodide displacement. Treatment of 22a,5α-spirostane-3β,12β-diol-11-one (IIa) with boiling acetic acid (17 hr.) or with acetic acid–concentrated hydrochloric acid at room temperature (20 hr.) resulted in good yields of the 3-monoacetate (IIc). Tosylation of IIc proceeded smoothly, but reduction of the 3-acetate-12-tosylate (IId) with either Raney nickel[16] or with lithium aluminum hydride[17] resulted in the formation of the ketol IIc and 22a,5α-spirostane-3β,11β,12β-

(14) C. Djerassi, E. Batres, M. Velasco and G. Rosenkranz, THIS JOURNAL, **74**, 1712 (1952).

(15) It originally was reported erroneously (ref. 6) that this condensation may be effected with zinc chloride.

(16) G. W. Kenner and M. A. Murray, *J. Chem. Soc.*, Suppl. Issue, No. 1, S178 (1949).

(17) H. Schmid and P. Karrer, *Helv. Chim. Acta*, **32**, 1371 (1949); P. Karrer, H. Asmis, K. N. Sareen and R. Schwyzer, *ibid.*, **34**, 1922 (1951).

triol (X)[18] (S–O cleavage), respectively. All attempts to effect displacement of the tosylate group by iodide failed.[19]

Following publication of our original communication[6] concerning the transformation of hecogenin to cortisone, Hirschmann, Snoddy and Wendler[20] described the transformation of hecogenin to $\Delta^{7,9(11)}$-22a,5α-spirostadien-3β-ol and since the latter had been converted[7,14] to the 11-ketone Va, this represents a route from hecogenin to cortisone. Subsequently Cornforth and Osborn[21] as well as Schmidlin and Wettstein[21] recorded a novel and elegant synthesis of 22a,5α-spirostan-3β-ol-11-one (V) from hecogenin.

Experimental[22]

$\Delta^{9(11)}$-22a,5α-Spirostene-3β,11-diol-12-one 3,11-Diacetate (IIIb).—22a,5α-Spirostane-3β,12β-diol-11-one (IIa)[13] (10.0 g.) in glacial acetic acid (90 ml.) was refluxed with bismuth oxide (15.0 g.) for 30 hours. To the cooled mixture was added benzene (400 ml.), the free bismuth and bismuth salts were filtered and the solution was concentrated to dryness *in vacuo*. The semi-crystalline residue, dissolved in 20 ml. of pyridine and 30 ml. of acetic anhydride, was heated for 4 hours on the steam-bath and then precipitated by the addition of water to yield 11.0 g. of material, m.p. 140–150°, λ_{max} 244 mμ, log ε 3.90. Fractional crystallization from benzene–hexane gave, after removal of the first fractions consisting of 1.54 g. (13%) of unoxidized starting material (as the 3,12-diacetate IIb), m.p. 215–217°, the enol diacetate IIIb, 8.25 g. (70%), m.p. 175–190°, $\lambda_{max.}$ 244 mμ, log ε 4.02. Recrystallization from hexane gave the analytical sample of IIIb, m.p. 188–192°, $[\alpha]^{20}D$ +14°, λ_{max} 244 mμ, log ε 4.05; $\nu_{max}^{CHCl_3}$ 1736, 1728 and 1700 cm.$^{-1}$.

Anal. Calcd. for $C_{31}H_{44}O_7$: C, 70.46; H, 8.33. Found: C, 70.90; H, 8.31.

22a,5α-Spirostan-3β-ol-11,12-dione (IVa) and Enol Form IIIa.—The enol diacetate IIIb, m.p. 175–190° (1.0 g.), was heated for 1 hour in boiling 1.5% alcoholic potassium hydroxide (25 ml.), the cooled mixture poured into cold dilute hydrochloric acid solution and the resulting precipitate filtered to afford 0.83 g. (98%) of mixed IIIa and IVa, m.p. 182–193°. The analytical sample of IVa which still contained some IIIa was obtained from benzene–hexane, and exhibited m.p. 196–197°, $[\alpha]^{20}D$ −21°, λ_{max} 282 mμ, log ε 3.28 (dark green color with ferric chloride), $\nu_{max}^{CHCl_3}$ 1700 cm.$^{-1}$ and free hydroxyl band.

Anal. Calcd. for $C_{27}H_{40}O_5$: C, 72.94; H, 9.07. Found: C, 73.45; H, 9.40.

Enol Acetate of Methyl-3α-Acetoxy-11,12-Diketocholanate.—3α,12β-Dihydroxy-11-ketocholanate[11] (2.0 g.) in acetic acid (20 ml.) was treated with bismuth oxide (2.0 g.) and the mixture was boiled for 24 hours. The product, after work-up (including acetylation) as described for IIIb, gave after crystallization from hexane, 1.4 g. (58%) of enol acetate, m.p. 128–130°. The analytical sample from the same solvent melted at 130–132°, λ_{max} 244 mμ, log ε 3.97, $\nu_{max}^{CHCl_3}$ 1736, 1726 and 1702 cm.$^{-1}$; reported[12] m.p. 123–128°, λ_{max} 243 mμ, log ε 3.89.

Anal. Calcd. for $C_{29}H_{42}O_7$: C, 69.29; H, 8.42. Found: C, 69.31; H, 8.70.

A. Huang-Minlon Reduction[23] of 22a,5α-Spirostan-3β-ol-11,12-dione (IIIa + IVa). Preparation of 22a,5α-Spirostane-3,11-dione (VIII).—The total crude product from the bismuth oxide oxidation of 6.0 g. of ketol IIa (a small sample of the oxidation product showed λ_{max} 244 mμ, log ε 3.90 after acetylation) after separation of free bismuth and bismuth salts was treated with ethylene glycol (100 ml.), hydrazine hydrate (10 ml.) and the mixture boiled for 5 hours. Aqueous potassium hydroxide (4.0 g. in 5 ml.) was added, the solution was heated slowly to 190° allowing water and excess hydrazine to be driven off and the mixture was then refluxed for 3 hours. The cooled mixture, on pouring into water, yielded 4.4 g. of solid, m.p. 130–140°. The 4.4 g. of material in acetic acid (120 ml.) was treated with chromic anhydride (2.0 g.) in water (15 ml.) and after standing at room temperature for 16 hr., the solution was poured into excess water and the resulting precipitate was collected. Crystallization of the dried product from ether yielded two crops of crystalline material; 1.6 g., m.p. 207–210°, and 0.36 g., m.p. 188–191°. These two fractions were combined and chromatographed on unwashed alumina (60 g.), whereupon the fractions eluted by 75% hexane–25% benzene furnished, after recrystallization from benzene–hexane, 750 mg. (13%) of VIII, m.p. 227–230°, $[\alpha]^{20}D$ −16°. Further crystallization raised the m.p. to 236–237°, $\nu_{max}^{CHCl_3}$ 1704 cm.$^{-1}$.[24]

B. Huang-Minlon Reduction of Diketone IIIa + IVa. Preparation of 22a,5α-Spirostan-3β-ol-11-one Acetate (Vb).—The ketol IIa (10.0 g.) was subjected to bismuth oxide oxidation followed by Huang-Minlon reduction as described above. The total reduction product (8.0 g.) was acetylated overnight at room temperature with acetic anhydride (15 ml.) and pyridine (15 ml.) and the acetylated product was oxidized for 2 hours at room temperature in acetic acid (180 ml.) containing chromic anhydride (2.0 g. in 20 ml. water). Water precipitation yielded 7.1 g. of material of m.p. 155–162°. Chromatography of this product on ethyl acetate-washed alumina (300 g.) yielded from the 80% hexane–20% benzene elutions, tigogenin acetate (VIIb), 1.22 g., m.p. 202–204°, identified by direct comparison with an authentic sample, while the 50% hexane–50% benzene washings yielded, after crystallization from acetone–hexane, Vb, 1.17 g. (11%), m.p. 220–225°, $[\alpha]^{20}D$ −30°; reported[7] m.p. 224–229°, $[\alpha]^{20}D$ −39.4°.

22a,5α-Spirostan-3β-ol-11-one (Va) by Reduction of 22a,-5α-Spirostane-3,11-dione (VIII).—The 3,11-diketone (VIII) (300 mg.) was added to 96% ethanol (50 ml.) containing prehydrogenated W-4 Raney nickel catalyst[25] (3 g.) and the compound was hydrogenated for 2 hours at room temperature under atmosphere hydrogen pressure. Hydrogen uptake ceased with the absorption of 21 ml. (calcd. for 1 equiv. 21.9 ml.) whereupon the mixture was heated to boiling, filtered, the alcohol solution concentrated to dryness and the crude product crystallized from acetone to yield 230 mg. (77%) of 3β-hydroxy-11-keto compound (Va), m.p. 223–226°, $[\alpha]^{20}D$ −29°, $\nu_{max}^{CHCl_3}$ 1702 cm.$^{-1}$ and free hydroxyl band, identical with an authentic sample.[24]

Acetylation furnished the 3-acetate Vb, analytical sample from acetone–hexane, m.p. 222–224°, $[\alpha]^{20}D$ −31°, $\nu_{max}^{CHCl_3}$ 1726 and 1702 cm.$^{-1}$. *Anal.* Calcd. for $C_{29}H_{44}O_5$: C, 73.69; H, 9.38. Found: C, 73.92; H, 9.60. This sample was identical in all respects with the product described above in B.

22a,5α-Spirostan-3β-ol-11,12-dione 12-Cycloethylenemercaptal (IX) from IVa.—22a,5α-Spirostan-3β-ol-11,12-dione (IVa) (1.0 g.), m.p. 180–190° (prepared as described above) was dissolved in ethanedithiol (4 ml.), the solution cooled in ice and a slow stream of anhydrous hydrogen chloride was bubbled through the solution for 3 hours while ice-cooling was maintained. The mixture was then stoppered, stored overnight in a refrigerator at 0–5° and finally poured into ether containing solid sodium carbonate in suspension. Water was added, the layers separated, and the ether solution washed with cold 5% sodium hydroxide solution, then

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

(19) After completion of our work, it was reported that tosylate displacement by iodide could not be effected in the similar case of 22a,5α-spirostane-3β,12β-diol-11-one 3-hemisuccinate methyl ester 12-tosylate (G. P. Mueller, L. L. Norton, R. E. Stobaugh, L. Tsai and R. S. Winniford, THIS JOURNAL, **75**, 4892 (1953)).

(20) R. Hirschmann, C. S. Snoddy, Jr., and N. L. Wendler, *ibid.*, **75**, 3252 (1953).

(21) J. W. Cornforth and J. M. Osborn, *Chem. and Ind.*, 919 (1953); with G. H. Phillipps, *J. Chem. Soc.*, 907 (1954); essentially the same procedure was published almost simultaneously by J. Schmidlin and A. Wettstein, *Helv. Chim. Acta*, **36**, 1231 (1953).

(22) Melting points are uncorrected. Rotations were measured in chloroform and ultraviolet absorption spectra in 95% ethanol solution. Unless noted otherwise infrared spectra were determined in chloroform solution. We are indebted to Mrs. A. Gonzalez for the microanalyses and to Mrs. P. Lopez for the rotations and spectral determinations.

(23) Huang-Minlon, THIS JOURNAL, **68**, 2487 (1946).

(24) The 3,11-dione VIII as well as Va proved to be identical with samples prepared from diosgenin by the performic acid method (*cf.* ref. 14).

(25) A. A. Pavlic and H. Adkins, THIS JOURNAL, **68**, 1471 (1946).

with water to neutrality and finally evaporated after drying over sodium sulfate. Crystallization of the residue from acetone gave 375 mg. (32%) of 12-cycloethylenemercaptal of m.p. 270–280°. The analytical sample from acetone melted at 289–291° (Kofler).

Anal. Calcd. for $C_{29}H_{44}S_2O_4$: S, 12.31. Found: S, 11.98.

22a,5α-Spirostan-3β-ol-11-one (Va) from the 12-Cycloethylenemercaptal (IX).—IX (320 mg.) in 96% ethanol (55 ml.) was treated with Raney nickel (5 g., W-4), the mixture boiled for 3.5 hr., filtered hot and the nickel was washed with hot benzene. The combined solutions after concentration to dryness and crystallization of the residue from acetone–hexane yielded 160 mg. of crude Va, m.p. 207–214°. Recrystallization from the same solvent gave 100 mg. (38%) of Va, m.p. 219–224°, $[\alpha]^{20}D$ −30°. Acetylation of the mother liquors yielded 50 mg. (17%) of the 3-acetate Vb, m.p. 220–222°, or an over-all yield of 55%.

22a,5α-Spirostane-3β,12β-diol-11-one 3-Monoacetate (IIc). **A.**—A solution of IIa (2.0 g.) in glacial acetic acid (50 ml.) was boiled for 17 hours, cooled and the mixture poured into water, yielding 2.1 g. of crude product of m.p. 205–214°. Crystallization from methanol furnished 1.25 g. (57%) of IIc, m.p. 217–220°. The analytical sample derived from the same solvent exhibited m.p. 217–221° (Kofler 212–215°), $[\alpha]^{20}D$ −30°, $\nu_{max}^{CS_2}$ 1736 and 1708 cm.$^{-1}$ and hydroxyl group.

Anal. Calcd. for $C_{29}H_{44}O_6$: C, 71.28; H, 9.08. Found: C, 71.46; H, 8.99.

B.—A solution of IIa (2.0 g.) in glacial acetic acid (50 ml.) was treated with concentrated hydrochloric acid (0.5 ml.) and the mixture water precipitated after standing for 20 hours at room temperature. The crude solid (2.1 g., m.p. 208–218°) was crystallized from methanol to yield 1.35 g. (62%) of IIc, m.p. 216–221°, identical with the product obtained in Part A.

22a,5α-Spirostane-3β,12β-diol-11-one 3-Acetate 12-Tosylate (IId).—To a cooled solution of tosyl chloride (0.5 g.) in pyridine (3 ml.) was added 0.5 g. of IIc and the mixture was allowed to stand at room temperature for 22 hours, whereupon it was poured into ice-water and filtered, yielding 0.61 g. of crude tosylate of m.p. 123–129°. Crystallization from ether gave 0.45 g. (69%) of IId, m.p. 134–137°. The analytical sample, from ether, showed m.p. 140–145°, λ_{max} 226 mμ, log ε 4.22.

Anal. Calcd. for $C_{35}H_{48}SO_8$: C, 67.30; H, 7.84; S, 4.98. Found: C, 67.11; H, 7.89; S, 4.99.

Attempted Displacement of Tosylate IId with Iodide.—Treatment of IId with sodium iodide in boiling anhydrous acetone for 24 hours resulted in slight decompositon of IId, but the product so obtained was halogen free. Operation in a sealed tube at 100°, while resulting in extensive decomposition of the tosylate, gave a product containing only a few per cent of halogen.

Treatment of Tosylate IId with Raney Nickel.—IId (0.5 g.) in 96% ethanol (25 ml.) was treated with W-4 Raney nickel catalyst (8 g.) and the mixture boiled for 19 hours. Removal of catalyst and concentration of the solvent gave a product with λ_{max} 226 mμ, log ε 3.39 (some unchanged tosylate present), which on crystallization from methanol furnished 120 mg. of pure 22a,5α-spirostane-3β,12β-diol-11-one 3-monoacetate (IIc), m.p. 217–220°.

Treatment of Tosylate IId with Lithium Aluminum Hydride.—IId (0.5 g.) in anhydrous tetrahydrofuran (25 ml.) was treated with lithium aluminum hydride (0.5 g.) and the mixture boiled for 8 hours. After acetone decomposition of excess hydride and the usual work-up the crude product was crystallized from methanol yielding 0.2 g. of 22a,5α-spirostane-3β,11β,12β-triol, m.p. and mixture m.p. with an authentic sample[18] 261–263°.

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[CONTRIBUTION FROM THE DEPT. OF PEDIATRICS, SCHOOL OF MEDICINE, UNIVERSITY OF PENNSYLVANIA AND THE CHILDREN'S HOSPITAL OF PHILADELPHIA]

The Interaction of Optically Isomeric Dyes with Human Serum Albumin[1]

BY FRED KARUSH

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A study has been made of the interaction of human serum albumin with the optically isomeric forms of an anionic azo dye. By measurement of the competitive effects of structurally related colorless anions it is demonstrated that the combination for both forms of the dye involves an attractive three-point interaction. Since the same combining regions of the protein are utilized by the isomeric dyes it is concluded that these regions possess a high degree of configurational adaptability. Some selectivity is evident however as shown by the competitive effects of the isomeric forms of phenyl-(benzoylamino)-acetate. To account for the distinctive binding features of serum albumins it is assumed that for a considerable portion of the albumin molecule the interhelical attraction is relatively weak compared to other proteins such as γ-globulin. Substantial support for this view is found in the distinctive behavior of albumin in the denaturation studies of Kauzmann, *et al.*, and in the effect of low *p*H on this protein as reported by Yang and Foster. Low interhelical attraction permits the R substituents of the amino acid residues to assume various orientations relative to each other. These arrangements provide combining regions which can take on a variety of configurations. The binding of small molecules to these regions takes place within the protein molecule and causes further separation of the helices, a process which facilitates the binding of additional small molecules.

The combination of serum albumins with organic anions exhibits three distinctive features which serve to define the structural problems involved in this phenomenon. Firstly, the affinity between the protein and small molecule appears, in general, to be at least as great as that found, for comparable molecules, in the interaction between haptens and homologous antibodies and between enzymes and structural inhibitors. Secondly, organic anions of diverse molecular configurations are bound to the same combining regions of the protein as shown by their competitive relation. Thirdly, a single albumin molecule can bind many anions, in fact, as many as twenty, without any detectable irreversible alteration.

To account for the first two features of the complexing behavior of albumins we have utilized the concept of the configurational adaptability of the combining regions of the protein.[2] This means that these regions can assume a large number of configurations, arising from the variation of the relative positions of the amino acid side chains, and, therefore, can present more or less complementary configurations to a wide structural variety of small molecules. A recent investigation[3] of the interac-

(1) Presented in part before the Division of Biological Chemistry at the Los Angeles meeting of the American Chemical Society, March, 1953.

(2) F. Karush, THIS JOURNAL, **72**, 2705 (1950).

(3) F. Karush, Twenty-Fifth National Colloid Symposium, American Chemical Society, Ithaca, New York, June 18–20, 1951; *J. Phys. Chem.*, **56**, 70 (1952).