[CONTRIBUTION FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

Syntheses of Allopregnane Analogs of Cortol and Cortolone[1]

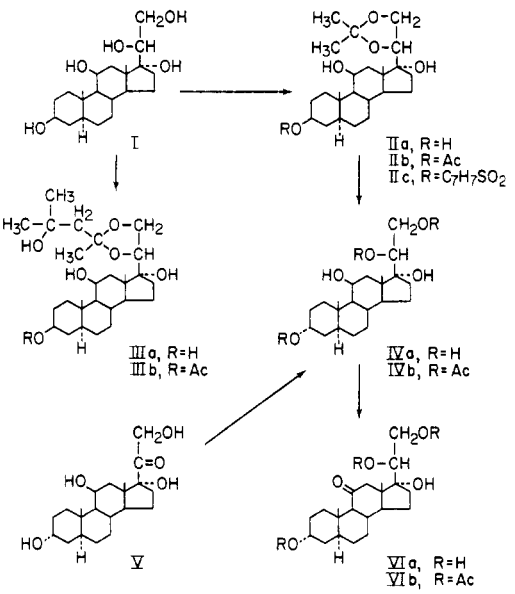
SOL DAUM AND DAVID K. FUKUSHIMA

Received June 6, 1960

The synthesis of allopregnane-3α,11β,17α,20α,21-pentol, its 20β-hydroxy epimer, 3α,17α,20α,21-tetrahydroxyallopregnane-11-one, and its 20β-hydroxy epimer are described. These compounds have been named allocortol, β-allocortol, allocortolone, and β-allocortolone, respectively.

Cortol (pregnane-3α,11β,17α,20α,21-pentol) and cortolone (3α,17α,20α,21-tetrahydroxypregnane-11-one) and their 20β epimers are pregnane derivatives and are distinguished by having a glycerol side-chain, 3α-hydroxyl group and an 11β-hydroxyl or an 11-keto group.[2] They comprise together 20–30% of the radioactivity of the neutral extract of the urine following the administration of labeled hydrocortisone to normal man.[3] The syntheses of the allopregnane analogs of cortol and cortolone were undertaken to determine their importance as metabolites of hydrocortisone. The compounds desired were allopregnane-3α,11β,17α,20α,21-pentol (XVI), named allocortol, and its 20β-hydroxy epimer (IVa), β-allocortol, and 3α,17α,20α,21-tetrahydroxyallopregnane-11-one (XIII), named allocortolone, and its 20β-hydroxy epimer (VIa) β-allocortolone.

β-Allocortol (IVa) was readily prepared from the known allopregnane-3β,11β,17α,20β,21-pentol (I)[4] by isomerization of the 3β-hydroxyl group. The 20β,21-dihydroxy groups of I were protected by the formation of the acetonide II and the 3β-*p*-toluene sulfonate IIc was prepared in the usual way; solvolysis with acetic acid and potassium acetate afforded the epimerized product which was hydrolyzed with 50% acetic acid to remove the ketal and then saponified with base to remove the ester group at C-3. β-Allocortol (IVa, allopregnane-3α, 11β,17α,20β,21-pentol) was readily isolated by chromatography on a partition type column of silica gel and ethanol. The compound was characterized by acetylation to the 3,20,21-triacetate IVb and by oxidation with sodium bismuthate to 3α,11β-dihydroxyandrostane-17-one. β-Allocortol (IVa) was also prepared by the reduction of 3α,11β,-



17α,21-tetrahydroxyallopregnane-20-one(V) with sodium borohydride.

In the preparation of the 20,21-acetonide IIa from allopregnane-3β,11β,17α,20β,21-pentol (I), a product IIIa, more polar than IIa, was obtained in about 10% yield. It yielded a monoacetate (IIIb) and was hydrolyzed to the parent allopregnane-3β,11β,17α,20β,21-pentol (I) with 50% acetic acid. The carbon-hydrogen analysis of IIIa and its monoacetate IIIb, hydrolysis of IIIa to I and chromatographic mobility of IIIa suggested that the product was the ketal of allopregnanepentol I and diacetone alcohol, the latter formed from acetone during the reaction. The monoacetate group was assigned to C-3, as the other hydroxyl groups are either hindered or tertiary.

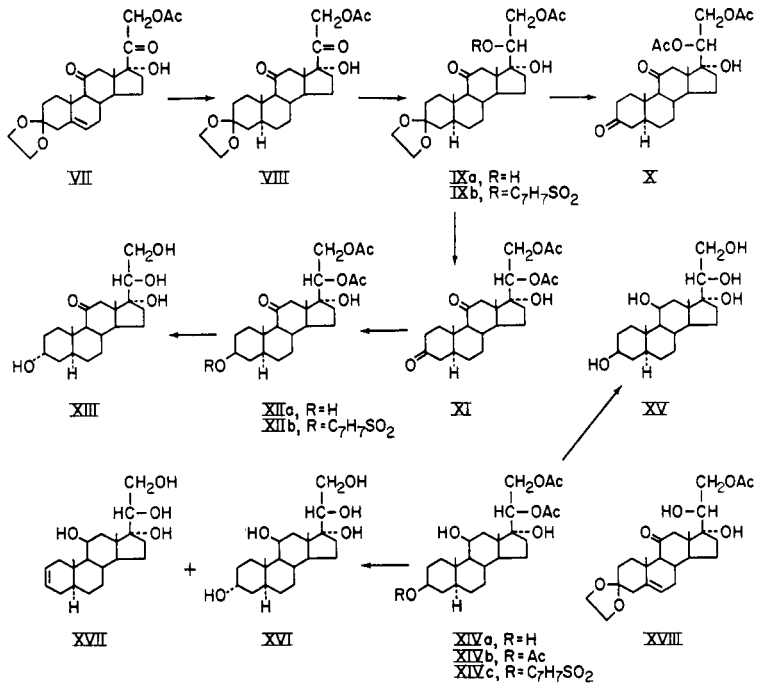
β-Allocortolone [3α,17α,20β,21-tetrahydroxyallopregnane-11-one, (VIa)] was synthesized by the oxidation of β-allocortol 3,20,21-triacetate (IVb) with chromic acid. The triacetate VIb was obtained crystalline but saponification yielded only an oily product which could not be crystallized. The product was homogeneous as judged by movement as a single spot on a paper chromatogram. The infrared spectrum of the saponified product was consistent with the structure of β-allocortolone (VIa). Oxidation of VIa with sodium bismuthate afforded 3α-hydroxyandrostane-11,17-dione.

(1) This investigation was supported in part by a grant from the American Cancer Society and a research grant (CY-3207) from the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

(2) D. K. Fukushima, N. S. Leeds, H. L. Bradlow, T. H. Kritchevsky, M. B. Stokem, and T. F. Gallagher, *J. Biol. Chem.*, **212**, 449 (1955).

(3) D. K. Fukushima, H. L. Bradlow, L. Hellman, B. Zumoff, and T. F. Gallagher, *J. Biol. Chem.*, **235**, 2246 (1960).

(4) (a) T. Reichstein, *Helv. Chim. Acta*, **19**, 29 (1936). (b) H. L. Mason, C. S. Meyers, and E. C. Kendall, *J. Biol. Chem.*, **114**, 613 (1936). (c) O. Wintersteiner and J. J. Pfiffner, *J. Biol. Chem.*, **111**, 599 (1935).



Allocortol (XVI) and allocortolone (XIII) have the hydroxyl group at C-20 of the glycerol side-chain in the α-orientation. Reduction of the 20-keto group of steroids with dihydroxyacetone side-chain by conventional methods yields the 20β-hydroxyl group preponderantly. In order to obtain the C-20α-hydroxysteroid, epimerization of the 20β-hydroxyl group was accomplished by the method used in the preparation of cortolone.[2] For the preparation of allocortolone (XIII), 3,3 - ethylenedioxy - 17α - hydroxy - 21 - acetoxy-Δ^5-pregnene-11,20-dione (VII) was employed. Catalytic hydrogenation of VII with 10% palladium on charcoal in ethanol–ethyl acetate yielded the allopregnane derivative VIII. The steric course of this reduction to the desired product was shown by the hydrolysis of the 3-ketal to the known 17α-hydroxy-21-acetoxyallopregnane-3,11,20-trione. A small amount of the 5β-isomer was also obtained from the catalytic reduction of VII. The catalytic hydrogenation of the 20-keto group in 3,3-ethylene-dioxy-17α-hydroxy-21- acetoxyallopregnane-11, 20-dione (VIII), with platinum in acetic acid afforded the desired 20β-hydroxy derivative IXa as the principal product. Removal of the ketal group at C-3 and acetylation yielded 17α-hydroxy-20β,21-diacetoxyallopregnane-3,11-dione (X). In the catalytic reduction of VIII, a small amount of the 20α-hydroxy epimer was formed; this was isolated after acid hydrolysis and acetylation as 17α-hydroxy-20α,21-diacetoxyallopregnane-3,-11-dione(XI).

Rearrangement of the 20β-hydroxyl group in IXa was achieved by the formation of the tosylate IXb, acetylation of the 17α-hydroxyl group with acetic acid–acetic anhydride and *p*-toluenesulfonic acid, and solvolysis with moist acetic acid and potassium acetate. The latter treatment also hydrolyzed the ethylenedioxy group at C-3 and the product isolated was 17α-hydroxy-20α,21-diacetoxyallopregnane-3,11-dione (XI).

The catalytic reduction of XI afforded predominantly 3β,17α - dihydroxy - 20α,21 - diacetoxyallopregnane-11-one (XIIa) together with approximately 25% yield of allopregnane-3β,11β,17α,20α,-21-pentol 20,21-diacetate (XIVa). The epimerization of the 3β-hydroxyl group of XIIa was accomplished by the formation of the tosylate XIIb and solvolysis with potassium acetate and acetic acid. Allocortolone (XIII) thus prepared was further characterized by preparation of its triacetate and by oxidation to 3α-hydroxyandrostane-11,17-dione. Allocortol (XVI) was similarly obtained from XIVa by the epimerization of the 3β-hydroxyl group through the tosylate XIVc. During the solvolysis of XIVc elimination product Δ^2-allopregnene-11β, 17α,20α,21-tetrol (XVII) was also formed. Allocortol was characterized by oxidation with sodium bismuthate to 3α,11β-dihydroxyandrostane-17-one. The triacetate of allocortol could not be crystallized.

An interesting reaction was observed when the 3-ethylenedioxy derivative (VII) of cortisone acetate was hydrogenated with Adam's catalyst in ethyl acetate–ethanol. The 5,6-double bond was unreactive and only the 20-keto group was reduced to the 20β-hydroxy derivative (XVIII). Under these conditions Δ^5-3β-hydroxy- or acetoxysteroids are reduced to give C-5α derivatives. It was unexpected that the dioxolane group at C-3 would hinder the hydrogenation at C-5,6 on the platinum catalyzed reduction and that it would have no effect on the palladium catalyzed reaction (VII→VIII). The reduction product XVIII was characterized by

hydrolysis with acetic acid and acetylation to Reichstein's Substance U diacetate.[5]

EXPERIMENTAL[6]

Allopregnane-3β,11β,17α,20β,21-pentol 20,21-acetonide (IIa). A solution of 4.96 g. of allopregnane-3β,11β,17α,20β,21-pentol (I) and 50 g. of freshly fused zinc chloride in 1 l. of anhydrous acetone was refluxed for 40 hr. The solution was cooled and 200 ml. of water containing 80 g. of potassium carbonate were added. The acetone was decanted and the residue washed three times with 500 ml. of acetone. The combined acetone solutions were concentrated to a small volume which was extracted with ether. The ether solution was washed with water, dried, and the solvent evaporated to give 5.6 g. of product. Chromatography on 125 g. of alumina and elution with ether-benzene afforded 4.26 g. of allopregnane-3β,11β,17α,20β,21-pentol 20,21-acetonide (IIa). Recrystallization from acetone gave two crystalline forms, prisms and needles, with the same m.p. 202–206°; $[\alpha]_D^{27}$ +18.8°; ν_{max}^{KBr} 3574, 3440, and 2640 cm.$^{-1}$

Anal. Calcd. for $C_{24}H_{40}O_5$; $^1/_2H_2O$ (needles): C, 69.03; H, 9.66. Found: C, 68.82; H, 9.90.

Acetylation with pyridine and acetic anhydride gave the *3-monoacetate* IIb, m.p. 184–187°; $[\alpha]_D^{29}$ +16.7°; ν_{max}^{KBr} 3605, 3565, 1737, and 1240 cm.$^{-1}$

Anal. Calcd. for $C_{26}H_{42}O_6$: C, 69.30; H, 9.40. Found: C, 69.34; H, 9.26.

Further elution of the alumina column with 10% methanol in ether gave 600 mg. of a compound more polar than the acetonide IIa. Recrystallization from methanol gave a product which had an analysis corresponding to the *ketal* (IIIa) of allopregnane-3β,11β,17α,20β,21-pentol with diacetone alcohol, m.p. 210–212°.

Anal. Calcd. for $C_{27}H_{46}O_6$: C, 69.49; H, 9.94. Found: C, 69.86; H, 10.12.

Acetylation yielded the *3-monoacetate* IIIb, m.p. 190–192°; $[\alpha]_D^{22}$ +31.8°.

Anal. Calcd. for $C_{29}H_{48}O_7$: C, 68.47; H, 9.51. Found: C, 68.12; H, 9.16.

Hydrolysis of IIIa with 50% acetic acid regenerated the parent allopregnanepentol, I.

3-p-Toluenesulfonate of allopregnane-3β,11β,17α,20β,21-pentol 20,21-acetonide (IIc). A cold solution of 4.26 g. of allopregnane-3β,11β,17α,20β,21-pentol 20,21-acetonide (IIa) in 40 ml. of pyridine was treated with 4.2 g. of *p*-toluenesulfonyl chloride in 10 ml. of pyridine to give 5.69 g. of 3-tosylate IIc. The analytical sample on recrystallization from ethyl acetate melted at 162–166° dec.; $[\alpha]_D^{28}$ +15.7°; ν_{max}^{KBr} 3575, 3495, and 2700 cm.$^{-1}$

Anal. Calcd. for $C_{31}H_{46}O_7S$: C, 66.40; H, 7.91. Found: C, 66.21; H, 8.03.

Allopregnane-3α,11β,17α,20β,21-pentol (*β-allocortol*, IVa). A solution of 4.1 g. of the above tosylate-acetonide was refluxed with 8 g. of freshly fused potassium acetate in 125 ml. of glacial acetic acid for 3 hr. The solvolysis product (2.68 g.) was heated with 50% acetic acid on the steam bath for 1.5 hr. and then saponified with 5% methanolic potassium hydroxide by reflux for 1 hr. The hydrolysis product was chromatographed on 300 g. of silica gel containing 120 ml. of ethanol. β-Allocortol was eluted with 15% ethanol in chloroform. Recrystallization from ethyl acetate–methanol gave β-allocortol (IVa), m.p. 186–188°; $[\alpha]_D^{30}$ +20.2° (ethanol); ν_{max}^{KBr} 3535 cm.$^{-1}$

Anal. Calcd. for $C_{21}H_{36}O_5$: C, 68.44; H, 9.85. Found: C, 68.34; H, 9.70.

Acetylation with pyridine and acetic anhydride and recrystallization from methanol gave *β-allocortol 3,20,21-triacetate* (IVb), m.p. 210–211°; $[\alpha]_D^{22}$ +62.6°; $\nu_{max}^{CHCl_3}$ 3640 (sh), 3615, 3590, and 1737 cm.$^{-1}$

Anal. Calcd. for $C_{27}H_{42}O_8$: C, 65.56; H, 8.56. Found: C, 65.89; H, 8.77.

A suspension of 19 mg. of β-allocortol, 1.5 ml. of methanol, 15.2 ml. of 50% acetic acid, and 1.9 g. of sodium bismuthate was shaken in the dark for 2 hr. The suspension was filtered through Celite and the filtrate was evaporated to dryness. The residue was dissolved in ethyl acetate, washed with water and the solvent removed. The oxidation product (16 mg.) on recrystallization from ethyl acetate gave 3α,11β-dihydroxyandrostane-17-one, m.p. 197–199°. The infrared spectrum in carbon disulfide and the mobility on paper in the system isooctane:toluene:methanol:water (1:3:3:1) were identical with that of an authentic sample.

β-Allocortol triacetate (IVb). A solution of 200 mg. of sodium borohydride in 25 ml. of methanol was added to 338 mg. of 3α,11β,17α,21-tetrahydroxyallopregnane-20-one (V, Reichstein's Substance C). The reaction mixture was allowed to stand overnight. The excess reagent was destroyed with acetic acid and the steroid isolated by extraction with ethyl acetate. The reduction product melted at 172–180°. The slightly impure β-allocortol was acetylated and recrystallized to give β-allocortol 3,20,21-triacetate (IVb), m.p. 210–211°. Its infrared spectrum was identical with that obtained above.

3α,17α,20β,21-Tetrahydroxyallopregnane-11-one (*β-allocortolone*, VIa). A solution of 287 mg. of β-allocortol 3,20,21-triacetate (IVb) in 2 ml. of acetic acid was treated with 2 ml. of a 2% solution of chromic acid in 90% acetic acid for 2 hr. at room temperature. The excess reagent was destroyed with methanol and the steroid isolated with ethyl acetate. The crystalline oxidation product, 277 mg., was recrystallized from methanol to give β-allocortolone-3,20,21-triacetate (VIb), m.p. 232–234°; $[\alpha]_D^{22}$ +53.5°; ν_{max}^{KBr} 3480, 1746, 1732, 1690, 1255 (sh), 1243 and 1220 (sh) cm.$^{-1}$

Anal. Calcd. for $C_{27}H_{40}O_8$: C, 65.83; H, 8.19. Found: C, 65.76; H, 8.15.

Two hundred fifty milligrams of β-allocortolone triacetate (VIb) was saponified with methanolic potassium hydroxide, but β-allocortolone (VIa) could not be crystallized. The infrared spectrum of the oily product was consistent with the structure for β-allocortolone; ν_{max}^{KBr} 3400, 1725, and 1688 cm.$^{-1}$ The homogeneity of the oily β-allocortolone was shown by paper chromatography in the system benzene:water:methanol (1:1:1) for 3 hr. and in the system benzene:water:methanol:ethyl acetate (1:1:1:0.1) for 20 hr. Oxidation with sodium bismuthate yielded 3α-hydroxyandrostane-11,17-dione as shown by comparison with infrared spectrometry and paper chromatography in the system isooctane:toluene:methanol:water (3:1:3:1).

3,3-Ethylenedioxy-17α-hydroxy-21-acetoxyallopregnane-11,20-dione (VIII). 3,3-Ethylenedioxy-17α-hydroxy-21-acetoxy-Δ[5]-pregnene-11,20-dione[7] (VII), 5 g., in 750 ml. of ethyl acetate, and 2250 ml. of ethanol was hydrogenated in the presence of 10% palladium on charcoal until the absorption of hydrogen ceased. A small portion treated with acetic acid showed no absorption at 240 mμ. Five more batches of 5 g. each of VII were reduced in the same manner. The combined reduction product was chromatographed on 1.4 kg. of silica gel containing 560 ml. of ethanol. Elution with 2% ethanol in methylene chloride yielded 23.6 g. of 3,3-ethylenedioxy-17α-hydroxy-21-acetoxyallopregnane-11,20-dione (VIII). The analytical sample from meth-

(5) T. Reichstein and J. von Euw, *Helv. Chim. Acta*, 24, 247E (1941).

(6) Melting points were determined on a micro hot stage and are corrected. Optical rotations were determined in chloroform unless otherwise specified. Infrared spectra were determined in the medium specified on a Model 21 Perkin-Elmer spectrophotometer using sodium chloride and calcium fluoride prisms. Sh = shoulder.

(7) R. Antonucci, S. Bernstein, M. Heller, R. Lenhard, R. Litell, and J. H. Williams, *J. Org. Chem.*, 18, 70 (1953).

(8) R. M. Evans, G. F. H. Green, J. S. Hunt, A. G. Long, B. Mooney, and G. H. Phillips, *J. Chem. Soc.*, 1529 (1958).

ylene chloride–ethyl acetate sintered at 244° and melted at 254° with decomposition; ν_{max}^{KBr} 3420, 1752, 1728, 1707, 1266 (sh), 1259 and 1232 cm.$^{-1}$; reported[8] m.p. 280–281°.

Anal. Calcd. for $C_{25}H_{36}O_7$: C, 66.94; H, 8.09. Found: C, 66.83; H, 8.13.

Hydrolysis with 50% acetic acid afforded 17α-hydroxy-21-acetoxyallopregnane-3,11,20-trione.

Further elution of the column with 5% ethanol in methylene chloride gave 3.86 g. of 3,3-ethylenedioxy-17α-hydroxy-21-acetoxypregnane-11,20-dione, m.p. 194–197°. Hydrolysis with 50% acetic acid afforded 17α-hydroxy-21-acetoxypregnane-3,11,20-trione.

3,3-Ethylenedioxy-17α,20β-dihydroxy-21-acetoxyallopregnane-11-one (IXa). 3,3-Ethylenedioxy-17α-hydroxy-21-acetoxyallopregnane-11,20-dione (VIII, 23.87 g.) in 3150 ml. of ethyl acetate and 350 ml. of ethanol was hydrogenated in the presence of Adam's catalyst until the absorption of hydrogen ceased. The reduction product was recrystallized from methylene chloride–ethyl acetate to give 16.5 g. of IXa, m.p. 190–208°. Further recrystallization from ethanol gave 8.0 g. of IXa, m.p. 206–211°. The analytical sample of 3,3-ethylenedioxy-17α,20β-dihydroxy-21-acetoxyallopregnane-11-one (IXa) melted at 210–213°; $[\alpha]_D^{29}$ +34.2°; $\nu_{max}^{CHCl_3}$ 3575, 1743, and 1707 cm.$^{-1}$

Anal. Calcd. for $C_{25}H_{38}O_7$: C, 66.64; H, 8.50. Found: C, 66.73; H, 8.44.

Hydrolysis with acetic acid followed by acetylation with pyridine and acetic anhydride at room temperature afforded *17α-hydroxy-20β,21-diacetoxyallopregnane-3,11-dione* (X), m. p. 227–230°; $[\alpha]_D^{27}$ +85.8°.

Anal. Calcd. for $C_{25}H_{36}O_7$: C, 66.94; H, 8.09. Found: C, 66.77; H, 7.87.

The mother liquors from the recrystallization of IXa was chromatographed on 800 g. of alumina. Elution with ethanol–ethyl acetate afforded 14.8 g. of a mixture of products. This material was hydrolyzed with 100 ml. of 50% acetic acid for 1.5 hr. The solvent was removed and the residue acetylated with acetic anhydride and pyridine. The acetylation product, 17 g., was chromatographed on 1.4 kg. of silica gel. Elution with benzene–ethyl acetate (1:1) gave 1.66 g. of 17α-hydroxy-20α,21-diacetoxyallopregnane-3,11-dione (XI), m.p. 274–290°. Recrystallization from methylene chloride–acetone gave XI, m.p. 286–290°. Its infrared spectrum was identical with that of XI prepared below.

3,3-Ethylenedioxy-17α-hydroxy-20β-p-toluenesulfonoxy-21-acetoxyallopregnane-11-one (IXb). A cold solution of 10.5 g. of *p*-toluenesulfonyl chloride in 45 ml. of pyridine was added to a cold solution of 7.46 g. of IXa in 60 ml. of pyridine. The mixture was kept at room temperature for 3 days. Recrystallization of the reaction product gave 6.89 g. of IXb, m.p. 152–160° dec. The analytical sample melted at 149–161° dec.; $[\alpha]_D^{29}$ +34.2°; $\nu_{max}^{CHCl_3}$ 3575, 1743, and 1707 cm.$^{-1}$

Anal. Calcd. for $C_{32}H_{44}O_9S$: C, 63.55; H, 7.33. Found: C, 63.62; H, 7.36.

17α-Hydroxy-20α,21-diacetoxyallopregnane-3,11-dione (XI). A suspension of 7.79 g. of the tosylate IXb in 155 ml. of acetic acid, 58.5 ml. of acetic anhydride, and 1.4 g. of *p*-toluenesulfonic acid monohydrate was allowed to stand at room temperature for 2 days. The crude acetylated product was then solvolyzed with 1100 ml. of 95% acetic acid and 110 g. of fused potassium acetate under reflux for 2 hr. The product (7.69 g.) was saponified with 250 ml. of 5% potassium hydroxide in methanol by reflux for 45 min. to give 4.58 g. of crude 17α,20α,21-trihydroxyallopregnane-3,11-dione. Acetylation with 40 ml. each of acetic anhydride and pyridine at room temperature gave 5.13 g. of XI. Repeated recrystallization from methylene chloride–acetone gave 946 mg. of 17α-hydroxy-20α,21-diacetoxyallopregnane-3,11-dione (XI), m.p. 287–288° dec. The analytical sample melted at 291–292° dec.; $[\alpha]_D^{25}$ +20.6°; $\nu_{max}^{CHCl_3}$ 3600, 1743, and 1705 cm.$^{-1}$

Anal. Calcd. for $C_{25}H_{36}O_7$: C, 66.94; H, 8.09. Found: C, 66.73; H, 7.92.

The mother liquors were chromatographed on silica gel. Elution with 40 and 50% ethyl acetate in benzene yielded 1.91 g. of XI. Recrystallization afforded 1.13 g. of XI, m.p. 292–294° and 320 mg., m.p. 280–286°.

3β,17α-Dihydroxy-20α,21-diacetoxyallopregnane-11-one (XIIa) *and allopregnane-3β,11β,17α,20α,21-pentol 3,20,21-triacetate* (XIVb). Two grams of 17α-hydroxy-20α,21-diacetoxyallopregnane-3,11-dione (XI) in 200 ml. of acetic acid was shaken in a hydrogen atmosphere with Adam's catalyst until slightly more than 1 molar equivalent was consumed. Recrystallizations of the reduction product from chloroform-methanol gave 1.00 g. of 3β,17α-dihydroxy-20α,21-diacetoxyallopregnane-11-one (XIIa), m.p. 274–278°. The analytical sample melted at 280–282°; $[\alpha]_D^{26}$ +5.2°; $\nu_{max}^{CHCl_3}$ 3600, 1743 and 1704 cm.$^{-1}$

Anal. Calcd. for $C_{25}H_{38}O_7$: C, 66.64; H, 8.50. Found: C, 66.66; H, 8.42

The mother liquors from the recrystallization of the reduction product was chromatographed on 400 g. of silica gel containing 160 ml. of ethanol. Elution with 3% ethanol in methylene chloride afforded an additional 358 mg. of XIIa, m.p. 282–283°. Elution with 4% ethanol in methylene chloride gave, after recrystallization from chloroform-methanol, 520 mg. of *allopregnane-3β,11β,17α,20α,21-pentol 20,21-diacetate* (XIVa), m.p. 267–272°. The analytical sample melted at 270–272°; $[\alpha]_D^{25}$ −20.7°; $\nu_{max}^{CHCl_3}$ 3640 (sh), 3605, and 1740 (broad) cm.$^{-1}$

Anal. Calcd. for $C_{25}H_{40}O_7$: C, 66.34; H, 8.91. Found: C, 66.36; H, 8.99.

The acetylation of the diacetate XIVa gave *allopregnane-3β,11β,17α,20α,21-pentol 3,20,21-triacetate* (XIVb), m.p. 207.5–208.5°; $[\alpha]_D^{26}$ −25.6°; $\nu_{max}^{CHCl_3}$ 3640 (sh), 3610, 1738, and 1725 (sh) cm.$^{-1}$

Anal. Calcd. for $C_{27}H_{42}O_8$: C, 65.56; H, 8.56. Found: C, 65.50; H, 8.50.

The saponification of XIVa with 5% methanolic potassium hydroxide at reflux for 30 min. afforded *allopregnane-3β,11β,17α,20α,21-pentol* (XV), m.p. 254–251°; $[\alpha]_D^{25}$ +6.6° (ethanol); ν_{max}^{KBr} 3535 and 3425 cm.$^{-1}$

Anal. Calcd. for $C_{21}H_{36}O_5.H_2O$: C, 65.25; H, 9.91. Found: C, 65.04; H, 9.73.

3β-Toluenesulfonoxy-17α-hydroxy-20α,21-diacetoxyallopregnane-11-one (XIIb). One gram of XIIa was treated with 1.1 g. of *p*-toluenesulfonyl chloride in 15 ml. of pyridine at room temperature overnight gave 1.29 g. of product. The analytical sample of the tosylate XIIb melted at 156–158° (dec.); $[\alpha]_D^{26}$ −13.4°; $\nu_{max}^{CHCl_3}$ 3600, 1742, and 1703 cm.$^{-1}$

Anal. Calcd. for $C_{32}H_{44}O_9S$: C, 63.55; H, 7.33. Found: C, 63.57; H, 7.53.

3α,17α,20α,21-Tetrahydroxyallopregnane-11-one (*allocortolone* XIII). The crude tosylate (XIIb, 1.29 g.) was refluxed with 2.6 g. of fused potassium acetate and 40 ml. of glacial acetic acid for 3 hr. The solvolysis product was hydrolyzed with 60 ml. of 5% methanolic potassium hydroxide by reflux for 45 min. The saponified material (756 mg.) was chromatographed on 100 g. of silica gel containing 40 ml. of ethanol. Elution with 9% ethanol in chloroform gave 126 mg. of XIII. Recrystallization from methanol–ethyl acetate yielded allocortolone (XIII), ν_{max}^{KBr} 3630, 3540, and 1698 cm.$^{-1}$; m.p. 263–267°; $[\alpha]_D^{30}$ 32.7°.

Anal. Calcd. for $C_{21}H_{34}O_5$: C, 68.82; H, 9.35. Found: C, 68.64; H, 9.44.

Acetylation with acetic anhydride and pyridine afforded the triacetate which had a double m.p. 171–174° and 183–186°. Oxidation of allocortolone (6 mg.) with 800 mg. of sodium bismuthate in 1 ml. of methanol and 6.4 ml. of 50% acetic acid by shaking in the dark for 2.5 hr. gave 3α-hydroxyandrostane-11,17-dione. Its infrared spectrum in carbon disulfide and mobility on paper chromatography in the system isooctane:toluene:methanol:water (3:1:3:1) were identical with that of an authentic sample.

Allopregnane-3α,11β,17α,20α,21-pentol (*allocortol*, XVI). Tosylation of 950 mg. of allopregnane-3β,11β,17α,20α,21-

pentol 20,21-diacetate (XIVa) was accomplished in the usual manner with 1.2 g. of *p*-toluenesulfonyl chloride in 14 ml. of pyridine. Recrystallization from acetone afforded 1.20 g. of the 3β-tosylate XIVc, m.p. 136–154° dec.; $[\alpha]_D^{26}$ −30.7°; $\nu_{max}^{CHCl_3}$ 3640 (sh), 3605 and 1740 (broad) cm.$^{-1}$

Anal. Calcd. for $C_{32}H_{46}O_8$: C, 63.34; H, 7.64. Found: C, 63.23; H, 7.66.

The 3β-tosylate (1.2 g., XIVc) was solvolyzed as above for the 11-keto analog (XIIb) with 2.4 g. of freshly fused potassium acetate in 38 ml. of glacial acetic acid for 3 hr. The epimerized product (903 mg.) was saponified with methanolic potassium hydroxide to give 690 mg. of oily material. The saponification product was chromatographed on 100 g. of silica gel containing 40 ml. of ethanol. Elution with 9% ethanol in chloroform afforded 152 mg. of allocortol. Recrystallization from methanol–ethyl acetate gave *allocortol* (XVI); m.p. 226–228°; $[\alpha]_D^{30}$ +19.5 (ethanol); ν_{max}^{KBr} 3440 (broad).

Anal. Calcd. for $C_{21}H_{36}O_5$: C, 68.44; H, 9.85. Found: C, 68.61; H, 10.01.

The triacetate was prepared in the usual manner but could not be crystallized. Its infrared spectrum was consistent with the structure of the compound. Oxidation of allocortol (5 mg.) with sodium bismuthate gave 3α,11β-dihydroxyandrostane-17-one, m.p. 180–190°. The infrared spectrum in carbon disulfide and mobility on paper in the system *isooctane:toluene:methanol:water* (1:3:3:1) were identical to that of an authentic sample.

A less polar material (300 mg.) was eluted from the partition column with 5% ethanol in chloroform. Recrystallization from methanol–ethyl acetate gave a product which had an analysis corresponding to *Δ⁵-allopregnene-11β,17α,-20α,21-tetrol* (XVII), m.p. 217–219°.

Anal. Calcd. for $C_{21}H_{34}O_4$: C, 71.97; H, 9.78. Found: C, 71.67; H, 9.88.

Reichstein's Substance U diacetate. 3,3-Ethylenedioxy-17α-hydroxy-21-acetoxy-Δ⁵-pregnene-11,20-dione (VII, 460 mg.) in 100 ml. of ethyl acetate and 100 ml. of ethyl alcohol was hydrogenated in the presence of Adam's catalyst until the absorption of hydrogen ceased. The reduction product XVIII was hydrolyzed with 50% acetic acid at 90° for 1 hr. and then acetylated with pyridine and acetic anhydride to give 428 mg. of crude Reichstein's Substance U diacetate, $\lambda_{max}^{C_2H_5OH}$ 237 mμ, $\epsilon_{1cm}^{1\%}$ 342. Chromatography on silica gel and elution with 50% ethyl acetate in benzene gave 50 mg. of the 20α-acetoxy epimer as judged by its infrared spectrum and mobility on paper. Further elution with the same solvent gave 330 mg. of Reichstein's Substance U diacetate. Recrystallizations from acetone gave 160 mg., m.p. 234–237°. Its infrared spectrum was identical with that of an authentic sample.[9]

Acknowledgment. The interest and support of Dr. T. F. Gallagher is gratefully acknowledged. The authors also express their appreciation to Mrs. Beatrice S. Gallagher for the determination and interpretation of the infrared spectra.

NEW YORK, N. Y.

(9) The authors thank Professor T. Reichstein for this compound.

[CONTRIBUTION FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

Synthesis of Reichstein's Substance C and Related Compounds[1]

DAVID K. FUKUSHIMA AND SOL DAUM

Received June 6, 1960

The synthesis of Reichstein's Substance C, 3α,11β,17α,21-tetrahydroxyallopregnane-20-one, its 3β-hydroxy epimer and 11β,17α,21-trihydroxyallopregnane-3,20-dione from bismethylenedioxyhydrocortisone are described. A side product formed in the preparation of bismethylenedioxyhydrocortisone from hydrocortisone and formalin has been characterized as the 11-methoxymethyl ether of bismethylenedioxyhydrocortisone.

3α,11β,17α,21 - Tetrahydroxyallopregnane - 20-one, Reichstein's Substance C (Va), was first isolated from beef adrenal glands[2–4] and has recently been isolated from human urine.[5,6] This metabolite was found to represent 7–13% of the radioactivity present in the neutral steroid extract of the urine following administration of hydrocortisone-4-C^{14} to normal men.[7] The synthesis of Reichstein's Substance C was desirable in order to obtain sufficient amounts for carrier purposes in radioactive tracer studies and for investigation of its biological properties.

Reichstein's Substance C, commonly called "allotetrahydro F," was recently synthesized by hydrogenation of hydrocortisone with rhodium on alumina as the catalyst.[8] Although obtained in fair yield it was one of four isomeric reduction products with attendant separation problem. A novel method of protecting the dihydroxyacteone side-chain of hydrocortisone and similar steroids by the formation of bismethylenedioxy (BMD) derivatives was recently reported by Beyler and co-workers.[9] This blocking group made the reduction of the α,β-unsaturated ketone in ring A to the 3β-hydroxyallopregnane derivative feasible with lithium, ammonia, and ethanol without destruction of the

(1) This investigation was supported in part by a grant from the American Cancer Society and a research grant (CY-3207) from the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

(2) O. Winstersteiner and J. J. Pfiffner, *J. Biol. Chem.*, 111, 599 (1935).

(3) T. Reichstein, *Helv. Chim. Acta*, 19, 29 (1936).

(4) H. L. Mason, C. S. Meyers, and E. C. Kendall, *J. Biol. Chem.*, 114, 613 (1936).

(5) I. E. Bush and M. Willoughby, *Biochem. J.*, 67, 689 (1957).

(6) L. P. Romanoff, R. M. Rodriguez, J. M. Seeleye, C. Parent, and G. Pincus, *J. Clin. Endocrinol. and Metab.*, 18, 1285 (1958).

(7) D. K. Fukushima, H. L. Bradlow, L. Hellman, B. Zumoff, and T. F. Gallagher, *J. Biol. Chem.*, 235, 2246 (1960).

(8) E. Caspi, *J. Org. Chem.*, 24, 669 (1959).

(9) R. E. Beyler, R. M. Moriarty, F. Hoffman, and L. H. Sarett, *J. Am. Chem. Soc.*, 80, 1517 (1958).