[CONTRIBUTION FROM ESCUELA DE CIENCIAS QUÍMICAS, UNIVERSIDAD NACIONAL]

Synthesis of Desoxycorticosterone from Pregnenolone

BY FRANCISCO GIRAL

With the discovery of indigenous plants rich in steroidal sapogenins, there has become available in Mexico a ready source of cheap pregnane derivatives. In the course of studies on the preparation of steroidal hormones from these materials, we have carried through an improved synthesis of desoxycorticosterone starting with pregnenolone.

The preparation of Δ^5-pregnendiol-3,21-one-20 diacetate by the action of lead tetraacetate on pregnenolone acetate is described by Reichstein.[1] We have found that the unacetylated pregnenolone reacts in the same way, to give the same final product, the C_3 hydroxyl group becoming acetylated in the process. Treatment of this diacetate with potassium bicarbonate gives partial hydrolysis with formation of the 3-monoacetate of Δ^5-pregnendiol-3,21-one-20. We have not found it possible to carry out the reverse partial hydrolysis, that is, the hydrolysis of the C_3 hydroxyl group with retention of the C_{21} ester grouping, employing either the diacetate or a series of mixed esters described below.

We have found that treatment of the 3-monoacetate of Δ^5-pregnendiol-3,21-one-20 with thionyl chloride produces in good yield the acetate of Δ^5-21-chloropregnenol-3-one-20, providing a new way to this latter substance with pregnenolone as starting material. The acetate of Δ^5-21-chloropregnenol-3-one-20 has been prepared by Reichstein from the diazoketone, either directly[2] or through the 21-tosyl ester,[3] that is, with bile acids as starting materials.

The acid hydrolysis to Δ^5-21-chloropregnenol-3-one-20 and his conversion to the acetate of desoxycorticosterone through the 21-chloroprogesterone is described by Reichstein in different papers.[2,4,5]

The preparation of the C_{21} tosyl ester of Δ^5-pregnendiol-3,21-one-20, 3-monoacetate was not successful, as was to be expected from the fact that desoxycorticosterone itself forms a tosyl derivative with difficulty[3] and loses it easily in a reduced form to form progesterone.[6] All attempts to convert progesterone to desoxycorticosterone with lead tetraacetate, as described by German authors,[7] were unsuccessful, in agreement with the literature.[1]

Several new mixed esters of Δ^5-pregnendiol-3,21-one-20 were prepared in the course of this work and are described in the experimental section, and also the reaction of pregnenolone benzoate with lead tetraacetate.

(1) T. Reichstein and C. Montigel, *Helv. Chim. Acta*, **24**, 360 (1941).

(2) M. Steiger and T. Reichstein, *ibid.*, **20**, 1164 (1937).

(3) T. Reichstein and W. Schindler, *ibid.*, **23**, 669 (1940).

(4) H. Reich and T. Reichstein, *ibid.*, **22**, 1124 (1939).

(5) T. Reichstein and J. v. Euw, *ibid.*, **23**, 136 (1940).

(6) T. Reichstein and H. G. Fuchs, *ibid.*, **23**, 684 (1940).

(7) G. Ehrhart, H. Ruschig and W. Aumüller, *Angew. Chem.*, **52**, 363 (1939).

Experimental Section

3-Monoacetate of Δ^5-Pregnendiol-3,21-one-20.—Five grams of Δ^5-pregnendiol-3,21-one-20 diacetate, prepared according to Reichstein[1] from pregnenolone acetate or from free pregnenolone, is dissolved in 600 cc. of hot methanol to which is added a hot solution of 1.1 g. of potassium bicarbonate in 25 cc. of water and 25 cc. of methanol. The mixture is left to stand at room temperature for twenty-four hours and is then poured into an excess of water and ice and extracted with ether. The ether solution is washed with water, decolorized with carbon, dried, filtered and the ether evaporated. The residuum is crystallized several times in succession in alcohol–water until constant melting point. The yield is 4 g., m. p. 161–162° (Reichstein indicates 149–156° for the product obtained from the diazoketone and crystallized in ether); $[\alpha]^{20}D$ +182° (chloroform), +192° (alcohol). The m. p. of the mixture with the original diacetate (m. p. 158–160°) is 140–145°, which indicates that we are dealing with two different substances. Likewise a change of optical rotation is exhibited and, finally, analysis approximates much more closely the theoretical value of the monoacetate than the diacetate. *Anal.* Found: C, 74.3; H, 9.5. Calcd. as $C_{23}H_{34}O_4$; C, 73.8; H, 9.2. Calcd. as $C_{25}H_{36}O_5$: C, 72.1; H, 8.7. The substance reduces avidly ammoniacal solution of silver and is not precipitated by digitonin. For these reasons it seems unquestionable that the substance is the 3-monoacetate of Δ^5-pregnendiol-3,21-one-20. The same result is obtained by boiling for one hour, instead of twenty-four hours at room temperature.

3-Acetate-21-(2,4-dichloro)-benzoate of Δ^5-Pregnendiol-3,21-one-20.—Four grams of the 3-monoacetate above is dissolved in 100 cc. of anhydrous pyridine, to which is added 4 g. of 2,4-dichlorobenzoyl chloride and the mixture allowed to stand for twenty-four hours at room temperature. After treatment and crystallization in alcohol 4 g. is obtained, m. p. 157–158°; $[\alpha]^{20}D$ +252° (chloroform). *Anal.* Found: C, 66.3; H, 6.7. Calcd. as $C_{30}H_{36}O_5Cl_2$: C, 65.8; H, 6.6. The substance contains chlorine and depresses the m. p. of the diacetate and of the 3-monoacetate of pregnendiolone. When subjected to partial saponification under conditions described above (2.5 g. in 300 cc. of methanol and 300 cc. alcohol + 0.4 g. $KHCO_3$ in 25 cc. water, room temperature, 24 hr.) a substance free of chlorine is produced, m. p. 155–158°, that does not precipitate with digitonin and that does not depress the m. p. of the 3-monoacetate of pregnendiolone. In the same manner, other mixed esters were prepared, for instance, with *p*-nitrobenzoyl chloride (3-acetate-21-*p*-nitrobenzoate). The crude product contains nitrogen (m. p. 105–110°) and was not purified for analysis, but when partially saponified regenerates the 3-monoacetate of pregnendiolone.

Acetate of Δ^5-21-Chloropregnenol-3-one-20.—To 11 g. of the 3-monoacetate of pregnendiolone dissolved in 500 cc. of anhydrous benzene, 50 cc. of thionyl chloride, purified with linseed oil and freshly distilled over quinoline, is added. The mixture is gently refluxed for two hours in a water-bath and in the absence of moisture. It is then cooled with ice, washed with diluted, cold, sodium hydroxide solution, and with water; decolorized with a little carbon, filtered and the benzene and the excess of thionyl chloride distilled off in vacuum. The oily residue is dissolved in methanol with a little water, decolorized twice

with carbon and cooled with ice. By direct crystallization 8 g. is obtained which recrystallized in absolute alcohol; m. p. 153–155° (according to Reichstein[2] 157–158°). Ammoniacal silver solution is reduced, there is no precipitate with digitonin, and it contains chlorine. *Anal.* Found: C, 70.6; H, 8.6. Calcd. as $C_{23}H_{33}O_3Cl$: C, 70.3; H, 8.5. The acid hydrolysis of this product followed by oxidation of the hydroxyl group and replacement of the chlorine by an acetoxy group according to the methods of Reichstein gave desoxycorticosterone acetate.

Benzoate of Δ⁵-Pregnenol-3-one-20.—To a solution of 10 g. of pregnenolone in 150 cc. of anhydrous pyridine and cooled with ice, 10 g. of benzoyl chloride is added and the mixture is left at room temperature for twenty-four hours. After isolation and separation of the excess benzoic acid, the moist product is crystallized in a large volume (about one liter) of ethyl acetate or of acetone–water. The yield is 11 g., m. p. 192–193°. It forms pretty rectangular strips, $[\alpha]^{20}D$ +56° (chloroform). *Anal.* Found: C, 80.2; H, 8.9. Calcd. as $C_{28}H_{36}O_3$: C, 80.0; H, 8.6. It has the same m. p. as free pregnenolone (191–193°) but the mixture drops to 165–175°. Furthermore from the differences in crystalline form and solubility, pregnenolone benzoate is distinguished clearly from pregnenolone in that it does not precipitate with digitonin. Pregnenolone benzoate is only slightly soluble in alcohol, ether and petroleum ether. It is moderately soluble in acetone and ethyl acetate and very soluble in benzene, carbon tetrachloride and in chloroform.

3-Benzoate-21-acetate of Δ⁵-Pregnendiol-3,21-one-20.—To a solution of 6 g. of pregnenolone benzoate in 300 cc. of glacial acetic acid is added 30 g. of lead tetraacetate and 50 cc. of acetic anhydride. The mixture is heated in water-bath, absolutely water free, for twenty-four hours. It is poured over one and one-half liters of water containing a small amount of ice and extracted with isopropyl ether. The ether solution is washed with dilute sodium hydroxide solution, then with water, dried and the ether evaporated. Reacting differently from the original benzoate, the new substance is much more soluble in ether. The residue after evaporation of the solvent is recrystallized several times in alcohol–water. The yield is 1.5 g., m. p. 175–176°, $[\alpha]^{20}D$ +161° (chloroform). *Anal.* Found: C, 75.7; H, 8.3. Calcd. as $C_{30}H_{38}O_5$: C, 75.3; H, 8.0.

Acknowledgment.—The microanalyses were carried out in the Instituto Politécnico Nacional of Mexico by Mrs. Leontina Norymberska to whom I am pleased to express my gratitude.

Summary

Partial hydrolysis of the diacetate of Δ⁵-pregnendiol-3,21-one-20 removes the 21-acetoxy group. Treatment of this product with thionyl chloride gave 21-chloropregnenol-3-one-20 acetate, an intermediate in the preparation of desoxycorticosterone acetate by known methods. Several different esters on C_{21} of the 3-monoacetate of Δ⁵-pregnendiol-3,21-one-20 have been prepared. The partial saponification of all these products invariably regenerates the 3-monoacetate. The preparation of pregnenolone benzoate and its reaction with lead tetraacetate are described.

MÉXICO, D. F. RECEIVED JUNE 5, 1949

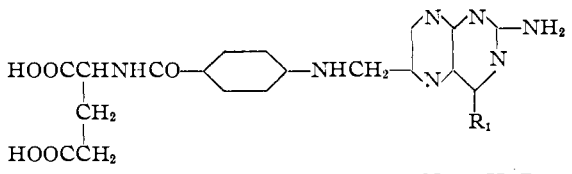
[CONTRIBUTION FROM THE RESEARCH DEPARTMENT, CALCO CHEMICAL DIVISION, AMERICAN CYANAMID COMPANY]

Analogs of Pteroylglutamic Acid. V. 4-Alkylamino Derivatives

BY BARBARA ROTH, JAMES M. SMITH, JR., AND MARTIN E. HULTQUIST

The synthesis of N-[4-(2,4-diamino-6-pteridylmethyl)-aminobenzoyl]-glutamic acid (I) ("Aminopterin," "4-aminopteroylglutamic acid") has been described in earlier communications from this Laboratory.[1] This substance has proved to be a powerful antagonist for pteroylglutamic acid. The use of pteroylglutamic acid antagonists in the treatment of blood dyscrasias such as leukemia was proposed by Franklin, *et al.*[2] Meyer[3] reported the treatment of leukemic patients with the "antifolic compounds" pteroylaspartic acid[4a] and N^{10}-methylpteroic acid,[4b] and observed short-lived remissions in a few cases. These two substances were also employed by Farber[5] with similar observations. Farber[5] and subsequently others[6] have obtained temporary remissions in acute leukemia by treatment with 4-aminopteroylglutamic acid (I). Toxic effects are a serious disadvantage in continued use of the drug. In more recent papers Farber[7] and Dameshek[8] have described the use of 4-amino-N^{10}-methylpteroylglutamic acid,[1b] 4-aminopteroylaspartic acid,[9] and 4-amino-9-methylpteroylglutamic acid,[10] with similar observations.

Since I differs from pteroylglutamic acid only in having an amino group in the 4-position in place of the hydroxyl, modification of the amino group



Pteroylglutamic acid, R_1 = OH; I, R_1 = NH_2; II, R_1 = —$NHCH_3$; III, R_1 = —$N(CH_3)_2$; IV, R_1 = —NC_5H_{10}.

(1) (a) Seeger, Smith and Hultquist, THIS JOURNAL, **69**, 2567 (1947); (b) Seeger, Cosulich, Smith and Hultquist, *ibid.*, **71**, 1753 (1949).

(2) Franklin, Stokstad, Belt and Jukes, *J. Biol. Chem.*, **169**, 427 (1947).

(3) Meyer, *Trans. N. Y. Academy of Science*, II, **10**, 99 (1948).

(4) (a) Hutchings, *et al.*, *J. Biol. Chem.*, **170**, 323 (1947); (b) Cosulich and Smith, THIS JOURNAL, **70**, 1922 (1948).

(5) Farber, *et al.*, *New England Journal of Medicine*, **238**, 787–793 (1948).

(6) (a) Editorial, *Blood*, **3**, 1057 (1948); (b) Jacobson, Levin, and Holt, *J. Lab. Clin. Med.*, **33**, 1641 (1948); (c) Pierce and Alt, *ibid.*, **33**, 1642 (1948); (d) Berman, *et al.*, *ibid.*, **33**, 1643 (1948); (e) Taylor, *et al.*, *ibid.*, **33**, 1645 (1948); (f) Bethell, Meyers and Neligh, *ibid.*, **33**, 1477 (1948).

(7) Farber, *Blood*, **4**, 160 (1949).

(8) Dameshek, *ibid.*, **4**, 168 (1949).

(9) Hutchings, *et al.*, *J. Biol. Chem.*, **180**, 857 (1949).

(10) Seeger, Hultquist and Smith, unpublished.