g. of diketone III, 1.0 g. of anhydrous sodium sulfate, 0.5 g. of freshly fused zinc chloride and 20 ml. of ethyl mercaptan was stored overnight in a 0° refrigerator. After the excess mercaptan was removed under vacuum, 10 ml. of water and 10 ml. of ether were added. The aqueous layer was separated and extracted three times with 10-ml. portions of ether. The combined ether extracts were dried over sodium sulfate. After the solvent was removed the residual solid was recrystallized from acetonitrile to give 0.164 g. of furan IX, m.p. 220–221°, identical in all respects with an authentic sample.

Isolation of Diethyl Disulfide.—A mixture of 20.6 g. (0.05 mole) of diketone III, 6.8 g. (0.05 mole) of freshly fused zinc chloride and 6.2 g. (0.10 mole) of ethyl mercaptan was dissolved in a mixture of 150 ml. of deaerated methylene chloride and 150 ml. of deaerated ether. After the solution was stored for 24 hr. at room temperature, it was washed twice with water and dried over magnesium sulfate. The solvents were removed by slow careful distillation through a 1-foot glass helices-packed column. The column was replaced with a small Vigreux column and the solid residue vacuum distilled. The fraction collected from 45–47° at 11 mm. was redistilled to give 2.62 g. (43%) of diethyl disulfide, $n^{25}D$ 1.5044 (lit.[24] 1.5046, 1.5047), the infrared spectrum of which was identical with that recorded.[25]

Recrystallization of the solid residues from acetonitrile gave 17.0 g. (86%) of the furan IX.

Other Furanization Conditions.—The furan IX was obtained in similar high yield from 0.1 g. of the diketone III, 2 ml. of 1,2-ethanedithiol and 5 drops of boron trifluoride etherate.[26]

No reaction occurred when ether, ethanol or acetonitrile were substituted for ethyl mercaptan in the presence of zinc chloride or boron trifluoride etherate. Similarly, no reaction occurred in ethyl mercaptan when the zinc chloride was not present.

(24) E. Emmet Reid, "Organic Chemistry of Bivalent Sulfur," Vol. III, Chemical Publishing Co., Inc., New York, N. Y., 1958, p. 396, and references therein.

(25) American Petroleum Institute Catalog of Infrared Spectral Data, Serial No. 1113.

(26) E. E. van Tamelen and C. I. Judd, *J. Am. Chem. Soc.*, **80**, 6305 (1958).

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH BETHESDA 14, MARYLAND]

2-Deoxy-D-ribose. VIII.[1] Synthesis of the Anomeric 2-Deoxy-D-ribofuranose 1-Phosphates[2]

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Condensation of 2-deoxy-3,5-di-*O*-*p*-toluoyl-D-ribosyl chloride with disilver phosphate affords a mixture of the anomeric 2-deoxy-α-D-ribofuranose 1-phosphates which is comparatively rich in the α-anomer. The latter was obtained in a purity approaching that of the natural phosphate. Condensation of the same chloride with tri-*n*-pentylamine phosphate gives a mixture of anomers in which the β-isomer predominates; this was purified. Comparison of the properties of the two 2-deoxy-D-ribofuranose phosphates supports the assumption that the natural anomer has the α-D-configuration.

Chemical synthesis of the two anomeric D-ribofuranose 1-phosphates[3] has established the structure of the ribose phosphate, originally obtained through the cleavage of a ribonucleoside by a nucleoside phosphorylase,[4] as α-D-ribofuranose 1-phosphate. Analogy suggests that the extremely acid-labile 2-deoxy-D-ribose 1-phosphate which is formed through the action of the appropriate nucleoside phosphorylases on deoxynucleosides[5–7] may also be an α-furanose isomer. The optical rotation of the cyclohexylammonium salt of the substance, $[\alpha]^{20}D$ +38.8°,[7] tends to support this assumption. Synthesis of the β-anomer, hitherto unknown, and comparison of its rotation with that of the natural phosphate, should provide further evidence[8] bearing on the anomeric configuration in question. The present paper describes the chemical synthesis of both the natural 2-deoxy-D-ribose 1-phosphate and its anomer.

Although various 3,5-di-*O*-acyl-2-deoxy-D-ribosyl chlorides have been obtained in crystalline form[9,10] (and, therefore, presumably, represent essentially pure anomeric forms) condensation of these with various salts is not, normally, stereospecific, both anomeric 2-deoxy-D-ribofuranose derivatives being obtained.[1,9] Variations in the nature of the salt have, however, a marked effect upon the proportions of anomers formed. This phenomenon was used to advantage in the present research.

Condensation of 2-deoxy-3,5-di-*O*-*p*-toluoyl-D-ribosyl chloride[9,10] in benzene solution with disilver phosphate occurred rapidly at room temperature; after removal of the toluoyl groups with alkali the product was converted to a cyclohexylammonium salt which was isolated in about 40% yield. After removal of inorganic phosphate as magnesium ammonium phosphate, the product (26%) showed a specific rotation of $[\alpha]^{20}D$ *ca.* 24° in water. Chromatography on paper revealed that it was still contaminated with a phosphorus-containing material.[11] Preparative paper chromatography

(1) 2-Deoxy-D-ribose. VII: R. K. Ness, D. L. MacDonald and H. G. Fletcher, Jr., *J. Org. Chem.*, **26**, 2895 (1961).

(2) Preliminary communication: D. L. MacDonald and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, **82**, 1832 (1960).

(3) R. S. Wright and H. G. Khorana, *ibid.*, **78**, 811 (1956); G. M. Tener, R. S. Wright and H. G. Khorana, *ibid.*, **79**, 441 (1957).

(4) H. M. Kalckar, *J. Biol. Chem.*, **167**, 477 (1947).

(5) M. Friedkin and H. M. Kalckar, *ibid.*, **184**, 437 (1950).

(6) M. Friedkin, *ibid.*, **184**, 449 (1950); M. Friedkin and D. Roberts, *ibid.*, **207**, 257 (1954).

(7) H. L. A. Tarr, *Can. J. Biochem. Physiol.*, **36**, 517 (1958).

(8) R. U. Lemieux and M. Hoffer [*Can. J. Chem.*, **39**, 110 (1961)] have recently discovered that two pyrimidine 2-deoxy-D-ribonucleosides and some of their derivatives constitute exceptions to Hudson's rule [C. S. Hudson, *J. Am. Chem. Soc.*, **31**, 66 (1909)]. While there is consistency among the rotations of a wide variety of 2-deoxy-D-ribofuranose esters and glycosides and no evidence currently available to indicate that Hudson's rule is not applicable to such substances. It is obvious that some dubiety regarding the anomeric configuration of all 2-deoxy-D-ribofuranose derivatives must remain in the absence of absolute physical or chemical proof.

(9) M. Hoffer, R. Duschinsky, J. J. Fox and N. Yung, *J. Am. Chem. Soc.*, **81**, 4112 (1959).

(10) M. Hoffer, *Chem. Ber.*, **93**, 2777 (1960).

(11) This impurity did not migrate in the isopropyl alcohol–ammonia–water system. It appears to contain no organic moiety and

removed this impurity; fractional crystallization of the cyclohexylammonium salt then was used to obtain a product which was enriched in the α-anomer. This was purified *via* the barium salt and through further paper chromatography of the cyclohexylammonium salt which, with considerable loss, was obtained with a rotation of $[\alpha]^{20}D$ $+34.5°$ in water. While this rotation falls short of that reported by Tarr[7] for the salt of the natural product, the high lability of the material made further purification impracticable.

While the above-described heterogeneous condensation gave dextrorotatory products comparatively rich in the 2-deoxy-α-D-ribofuranose 1-phosphate, a homogeneous condensation, using a phosphoric acid salt of tri-*n*-pentylamine,[12] afforded material of slightly negative rotation. Following removal of inorganic phosphate, the cyclohexylammonium salt was fractionated from aqueous acetone and the most levorotatory fractions further purified as the barium salt. Reconversion to the cyclohexylammonium salt and paper chromatography then gave a product showing $[\alpha]^{20}D$ $-15.8°$ in water; this substance may, therefore, be designated as 2-deoxy-β-D-ribofuranose 1-phosphate and it is evident that the natural phosphate is, indeed, the α-anomer.

A number of chromatographic techniques were employed in efforts to separate mixtures of the two anomers. Paper chromatography in a wide variety of solvent systems as well as chromatography on ion-exchange paper and paper electrophoresis using a variety of buffers all failed to produce a satisfactory resolution of the two anomers, although slight differences in migration rate were frequently observed.[13] The choice of solvents and of buffers in these experiments was, of course, severely limited by the acid lability of the two anomers.

Enzymatic assays of samples showing $[\alpha]^{20}D$ $+31°$ and $[\alpha]^{20}D$ $+4°$ were kindly carried out by Dr. H. L. A. Tarr using fish nucleoside phosphorylase.[7] The more dextrorotatory material showed an activity 80% of that of the natural product while the less dextrorotatory product had an activity corresponding to 37%. Assuming that the pure α-anomer has $[\alpha]^{20}D$ $+38.8°$ and the pure β-anomer $[\alpha]^{20}D$ $-15.8°$, one may calculate that these samples contained 86 and 36%, respectively, of the α-anomer.

The 2-deoxy-D-ribofuranose 1-phosphates are among the most acid-labile of all sugar phosphates. Heating of concentrated aqueous solutions of the cyclohexylamine salts at 50°, as in vacuum evaporations, may cause partial liberation of inorganic phosphate, while air drying at room temperature of preparative paper chromatograms, where the materials are present as the ammonium salts, invariably brings about partial hydrolysis. Finally, numerous samples of the crystalline cyclohexylammonium salts which appeared to be chromatographically homogeneous gave inexplicably discordant elementary analyses.

behaves as inorganic phosphate in the acid solvent system of J. P. Ebel [*Mikrochim. Acta*, 679 (1954)]; it does not migrate in the alkaline system described by this author. These observations suggest that the material may be some condensation product of phosphoric acid of unknown structure.

(12) M. Smith and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 1141 (1958), described the use of pyridine solutions of tri-*n*-butylammonium salts of nucleoside 5′-monophosphates and of orthophosphoric acid for the synthesis of nucleoside 5′-triphosphates.

(13) For instance, in *n*-propyl alcohol–ammonia (85:15), migration is extremely slow; in one experiment, after 34 days, using Whatman #31 paper, slight separation of the two anomers was observed, the α-isomer migrating 24 cm. and the β-isomer 27 cm. Separations such as this were observed in several similar solvent systems, but none appeared promising for preparative purposes. In all instances, the β-anomer migrated more rapidly.

Experimental

Paper chromatography was carried out on Whatman #31 or #41H paper (the latter giving more compact spots) using isopropyl alcohol–ammonia–water (7:1:2)[14] and phosphates were detected using the molybdate spray.[15] All evaporations were conducted *in vacuo* at a bath temperature below 40°. Except where otherwise noted, aqueous solutions of salts of 2-deoxy-D-ribofuranose 1-phosphate were not concentrated to dryness, but just to a small volume, in order to avoid the danger of undesired hydrolysis.

The Methyl 2-Deoxy-3,5-di-*O*-*p*-toluoyl-D-ribosides.—In a typical preparation, material prepared by the procedure of Hoffer[10] was crystallized from about 10 parts of hexane to give, in 78% yield, a product melting at 60–78° and showing $[\alpha]^{20}D$ $+63°$ in chloroform. Fractional crystallization from the same solvent afforded **methyl 2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-riboside** as prisms, melting at 76.5–78° and rotating $[\alpha]^{20}D$ $-8.1°$ in chloroform (*c* 2.5). Hoffer[10] reported m.p. 76.5° and $[\alpha]^{20}D$ $-6.2°$ ($CHCl_3$).

Anal. Calcd. for $C_{22}H_{24}O_6$ (384.41): C, 68.74; H, 6.29. Found: C, 68.63; H, 6.55.

Repeated recrystallization from hexane of another sample of the same mixture gave stout needles of **methyl 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-riboside** which melted at 82–83° (after sintering at 81°) and showed $[\alpha]^{20}D$ $+130°$ in chloroform (*c* 0.67).

Anal. Calcd. for $C_{22}H_{24}O_6$ (384.41): C, 68.74; H, 6.29. Found: C, 68.65; H, 6.54.

Disilver Phosphate.—The following details are based on the method of Flatt and Brunisholz.[16] Fifty grams of trisilver phosphate was thoroughly mixed with 50 ml. of sirupy 85% phosphoric acid in a 250-ml. centrifuge bottle and the mixture placed in an oven at 85° for 1.5 hr., the contents of the bottle being mixed occasionally during this period. The brownish solution was cooled, diluted with 100 ml. of ether, and the gray precipitate collected by centrifugation. The product was washed in the same manner with nine 100-ml. portions of ether and then dried *in vacuo* over P_2O_5: 54.4 g. (97%) of light gray powder.

Anal. Calcd. for Ag_2HPO_4 (311.75): Ag, 69.21. Found: Ag, 68.85.

The Anomeric 2-Deoxy-D-ribofuranose 1-phosphates. (a) *Via* **Disilver Phosphate.**—A sample of 2-deoxy-3,5-di-*O*-*p*-toluoyl-D-ribosyl chloride, prepared from a mixture of the anomeric methyl 2-deoxy-3,5-di-*O*-*p*-toluoyl-D-ribosides by the method of Hoffer[10] (7.76 g., 20 mmoles), was dissolved at room temperature in 350 ml. of sodium-dried benzene and 31.2 g. (100 mmoles) of disilver phosphate was added in one batch. The suspension, in a stoppered flask, was stirred vigorously with a magnetic stirrer for 0.5 hr. and then treated with 250 ml. of 1 *N* lithium hydroxide. After 20 min. of further stirring, the insoluble salts were removed by filtration through Hyflo Super-cel and washed thoroughly with benzene and water. The filtrate was concentrated to remove the benzene, then made homogeneous by the addition of *ca.* 500 ml. of ethanol and allowed to stand at room temperature for 18 hr. The solution was concentrated to a volume of *ca.* 150 ml. and a trace of insoluble material removed by centrifugation. Redistilled cyclohexylamine (2 ml.) was added and the solution passed slowly through a column (2.8 × 50 cm.) of Amberlite IR 120 in the cyclohexylammonium form. The resin was washed with *ca.* 300 ml. of water until the effluent was free

(14) D. M. Brown and A. R. Todd, *J. Chem. Soc.*, 2040 (1953).

(15) C. S. Hanes and F. A. Isherwood, *Nature*, **164**, 1107 (1949); R. S. Bandurski and B. Axelrod, *J. Biol. Chem.*, **193**, 405 (1951).

(16) R. Flatt and G. Brunisholz, *Helv. Chim. Acta*, **34**, 692 (1951).

of phosphate (spot test on paper). Cyclohexylamine (5 ml.) was added to the total effluent which was then concentrated to a solid which was dried thoroughly *in vacuo* over P_2O_5. Contaminating cyclohexylammonium *p*-toluate was removed by several extractions with *n*-propyl alcohol (60 ml. plus 4 × 30 ml.) at room temperature and the crude cyclohexylammonium salt of 2-deoxy-D-ribofuranose 1-phosphate which remained was washed with ether; dried in the air it amounted to 3.36 g. (41%). Chromatography of this material showed only traces of *p*-toluic acid (ultraviolet) and inorganic phosphate. Heavy loading of the paper revealed some phosphorus-containing material of R_f 0. Inorganic phosphate was removed in the following manner. The salt was dissolved in 50 ml. of water, magnesium acetate tetrahydrate (200 mg.) and concentrated aqueous ammonia (6 ml.) were added, and the solution left several hours (or overnight) at 0°. The precipitated magnesium ammonium phosphate was removed by centrifugation and washed with cold 1.5 *N* ammonium hydroxide. Excess magnesium was removed by passage of the solution through a column (1.5 × 13 cm.) of Amberlite IR 120 in the cyclohexylammonium form, the effluent treated with 4 ml. of cyclohexylamine and concentrated to a volume of *ca.* 45 ml. Gradual addition of acetone (*ca.* 8 vol.) gave 2.13 g. (26%) of the crystalline cyclohexylammonium salt, free of toluic acid and of inorganic phosphate, which showed $[\alpha]^{20}D$ +23.6° (*c* 2, in water). However, the mixture of anomers thus obtained usually was not analytically pure; sometimes, but not always, pure material could be obtained by preparative paper chromatography on Whatman #31ET paper (*ca.* 0.4 g. of phosphate per 46 × 57 cm. sheet) in the isopropyl alcohol–ammonia–water system. This procedure removed varying quantities of the unidentified material having an R_f of 0 in this system and can be used to remove inorganic phosphate when only a small proportion of it is present. However, precipitation as the barium salt, reconversion to the cyclohexylammonium salt and further purification of the latter by paper chromatography as described below proved to be a more reliable procedure.

Purification of the α-Anomer.—A sample of the cyclohexylammonium salt (1.75 g.) was dissolved in 350 ml. of methanol by prolonged stirring at room temperature. Traces of insoluble material were removed by gravity filtration and ether (125 ml.) added carefully to the filtrate. After a week at room temperature, the crystals were removed by filtration, washed carefully with methanol–ether (3:1) and then with ether. The product (0.647 g.) showed $[\alpha]^{20}D$ +33.2° in water (*c* 2.5); recrystallized again in the same manner from a mixture of 125 ml. of methanol and 45 ml. of ether, the material (0.240 g.) showed $[\alpha]^{20}D$ +34.6° in water. A second crop was obtained by removing the solvent from the second mother liquor and crystallizing the residue from aqueous acetone. The combined crops (0.48 g., 1.16 mmoles) were dissolved in water (8 ml.), 0.31 g. of barium chloride dihydrate added, and the resulting precipitate removed by centrifugation, the precipitate being washed with 2 ml. of cold water. To the combined solution and washing 50 ml. of absolute ethanol was added and, after the solution had stood for several hours at 0°, the solid was collected by centrifugation. It was redissolved in 4 ml. of water, reprecipitated by the addition of 20 ml. of ethanol and, after standing at +5°, collected by centrifugation. The barium salt was then dissolved in 10 ml. of water and the solution passed slowly through a column of Dowex 50W (1.5 × 15 cm.) in the cyclohexylammonium form. The column was washed with water (100 ml.) until free of phosphate, 2 ml. of cyclohexylamine was added to the combined effluent and the latter was then concentrated to a volume of about 20 ml. The cyclohexylammonium salt was then crystallized through the addition, in portions, of 400 ml. of acetone. Dried *in vacuo* over P_2O_5, the product (0.415 g.) was found to be still contaminated with the impurity of R_f 0. It was, therefore, further purified by paper chromatography. Whatman #31ET paper (46 × 57 cm.) was washed chromatographically with isopropyl alcohol–ammonia–water and then with 1% aqueous ammonia. The phosphate (0.415 g.) was dissolved in *ca.* 3 ml. of water and applied as a streak at the origin and, after thorough drying in the air, the chromatogram was run for 10–12 hr. in the isopropyl alcohol–ammonia–water system. While the chromatogram was still wet with solvent, side strips were removed, and these were subsequently sprayed with molybdate to locate the sugar phosphate (at. *ca.* 24–36 cm. from the origin).[17] Inorganic phosphate moves more slowly and, if present, it was removed by careful cutting, and the sugar phosphate was eluted from the paper by chromatography using water containing *ca.* 1% ammonium hydroxide. Five ml. of cyclohexylamine was added to the eluate which was then concentrated to a volume of about 20 ml., treated with Darco X and filtered. Acetone (*ca.* 20 parts) was added portionwise to crystallize the product. After drying *in vacuo* over P_2O_5 and KOH the product weighed 0.35 g. and showed $[\alpha]^{20}D$ +34.5° (*c* 1.3, H_2O). Paper chromatography in the usual solvent system revealed only one component, R_f 0.19 on Whatman #41H and R_f 0.25 on Whatman #31.

Anal. Calcd. for $C_{17}H_{37}N_2O_7P$ (412.47); C, 49.50; H, 9.04; N, 6.79; P, 7.51. Found: C, 49.67; H, 9.46; N, 6.87; P, 7.42.

(b) *Via* (Tri-*n*-pentylammonium) **Phosphate.**—2-Deoxy-3,5-di-*O*-*p*-toluoyl-D-ribosyl chloride (5.83 g., 15 mmoles) was dissolved at room temperature in 120 ml. of tetrahydrofuran (distilled from lithium aluminum hydride and stored over fine sodium wire) and to this solution was added a solution of 1.54 g. (15.7 mmoles) of anhydrous, crystalline orthophosphoric acid[18] in 15 ml. of tetrahydrofuran, followed immediately by 13.6 ml. (47.3 mmoles) of tri-*n*-pentylamine (Eastman Kodak Co., white label). In a 0.5-dm. polarimeter tube the solution showed $\alpha^{20}D$ +1.07° (2 min.) and $\alpha^{20}D$ +0.77° (20 min., constant). After a total of 30 min., anhydrous methanol (120 ml.), followed by 45 ml. of 1.1 *N* sodium methoxide,[19] was added and the solution left overnight at room temperature. The mixture, which contained some precipitate, was concentrated to remove the organic solvents; 75 ml. of water was added and the mixture extracted with methylene chloride (100 ml. plus 2 × 25 ml.) to remove methyl *p*-toluate. The *p*H (10.8) was lowered to 7.5 by cautious addition, with stirring, of Amberlite IRC-50 resin, the resin then being removed by filtration and washed with a little water. (At this stage, a sample was treated with excess of cyclohexylamine and IRC-50 resin; chromatography revealed that the inorganic phosphate–deoxyribose phosphate ratio was about 1:1).

Magnesium acetate tetrahydrate (2.1 g., 10 mmoles) was added to the solution (90 ml.), followed by 10 ml. of concentrated ammonium hydroxide, and the mixture was left at 0° for several hours or overnight. The copious precipitate of magnesium ammonium phosphate was removed by centrifugation and washed with a little cold, dilute ammonium hydroxide (*ca.* 3%); the supernatant layer was then passed slowly through a column of Dowex 50W (2.4 × 27 cm.) in the cyclohexylammonium form, the column being washed with water (300 ml.) until no further phosphate was eluted. After the addition of 10 ml. of cyclohexylamine the combined effluent was concentrated to about 40 ml., the salt then being crystallized through the portionwise addition of 10 parts of acetone. Washed with acetone and dried *in vacuo* over P_2O_5 it weighed 3.52 g. (57%). Paper chromatography revealed some of the R_f 0 material and a trace of acetate ion (brom phenol blue[20]) along with the desired 2-deoxy-D-ribose 1-phosphate. Recrystallization in the same manner from 35 ml. of water afforded 3.24 g. (52%) of material showing $[\alpha]^{20}D$ −0.4° in water (*c* 5). In different runs, the rotation varied from −5.8° to about 0°.

Purification of the β-Anomer.—Crude product from several such preparations (7.98 g., $[\alpha]^{20}D$ −3.4°), containing only a small proportion of inorganic phosphate, was treated in the following fashion to obtain the β-anomer. It was dissolved in 50 ml. of water and acetone added portionwise: 100 ml. of acetone gave 3.52 g. of material of $[\alpha]^{20}D$ +2.4°; a further 100 ml. of acetone gave 2.15 g. of material of $[\alpha]^{20}D$ −5.3°; 150 ml. more acetone afforded 0.88 g. showing $[\alpha]^{20}D$ −12.2° and a final 300 ml. of acetone led to the crystallization of 0.70 g. which showed $[\alpha]^{20}D$ −13.6°.

(17) The main portion of the chromatogram should be returned to the basic atmosphere of the chromatography cabinet during this operation, and it should be maintained in a dampened condition throughout the subsequent steps. If the paper is allowed to dry in air in the ordinary fashion, subsequent examination of the eluted sugar phosphate reveals that some hydrolysis to inorganic phosphate has occurred.

(18) Available from Fluka AG, Buchs, S. G., Switzerland.

(19) Use of lithium methoxide at this point allowed the concomitant removal of the inorganic phosphate but resulted in variable but consistently lowered yields (13–42%) of product.

(20) E. P. Kennedy and H. A. Barker, *Anal. Chem.*, **23**, 1033 (1951).

This final fraction was converted to the barium salt, reconverted to the cyclohexylammonium salt, and then subjected to preparative paper chromatography to remove R_f 0 material, as described for the α-anomer. The **cyclohexylammonium β-D-ribofuranose 1-phosphate** thus purified showed $[\alpha]^{23}D$ −15.8° in water (*c* 1.2) and was chromatographically pure although it could not be distinguished from its α-anomer using isopropyl alcohol–ammonia–water.

Anal. Calcd. for $C_{17}H_{37}N_2O_7P$ (412.47): C, 49.50; H, 9.04; N, 6.79; P, 7.51. Found: C, 49.13; H, 9.30; N, 6.79; P, 7.11.

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[CONTRIBUTION FROM THE CHEMICAL PROCESS IMPROVEMENT DEPARTMENT, LEDERLE LABORATORIES, AMERICAN CYANAMID CO., PEARL RIVER, N. Y.]

16α-Hydroxy Steroids. XII.[1a] 21-Amino Derivatives of 9α-Fluorohydrocortisone

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Fermentations of 9α-fluorohydrocortisone with *Streptomyces roseochromogenus* afford as a minor product a steroidal amide, 21-acetylamino-9α-fluoro-11β,17α-dihydroxy-4-pregnene-3,20-dione, whose isolation, characterization, structural elucidation, and synthesis from 9α-fluorohydrocortisone are described. A synthesis of the 21-amino analog of 9α-fluorohydrocortisone is also described.

The complex alteration of 9α-fluorohydrocortisone (I) by *Streptomyces roseochromogenus* includes 16α-hydroxylation,[2] 2β-hydroxylation,[3] 20-carbonyl reduction,[4] together with D-homoannulation of the 16α-hydroxylated products[5] and conversion to other non-reducing products. In a continuing study of the bioconversions of this microörganism on 9α-fluorohydrocortisone, we have noted the regular occurrence in a variety of fermentation samples of one non-reducing component II, located midway between the substrate I and the major fermentation product 16α-hydroxy-9α-fluorohydrocortisone (III) on standard paper chromatograms. Instrumental evaluation of the fluorescence of the isonicotinic acid hydrazones on paper chromatograms indicated that about 4–5% of the substrate could be accounted for as the non-reducing component II.

The steroidal nature of II was supported by infrared spectra of papergram eluates. However, major concern for the structure of the compound was provoked by strong bands at 6.5 μ, implying that the steroid contained nitrogen.

Isolation of II from very complex extract concentrates from which 16α-hydroxy-9α-fluorohydrocortisone had been removed by crystallization and from which other 16α,17α-diols were removed as their water-soluble 16α,17α-cyclo borates has been described.[3] The pure II was recognized as a neutral polyhydroxy-Δ⁴-3-ketosteroidal amide, $C_{23}H_{32}O_5NF$, from which a $\Delta^{1,4}$-3-ketone analog IV, $C_{23}H_{30}O_5NF$, was prepared microbiologically. Physical properties of the amides II and IV differentiated them from naturally occurring steroidal amides such as the toad poisons, bile acid conjugates, etc., and from the nitrogenous steroid preparations of Voigt and Schroeder,[6] steroid-nucleotide/purine complexes, steroid-polypeptide/protein complexes, and from various synthetic and derived steroid amine and amide compounds.[7]

From the characteristic infrared absorption near 3, 6 and 6.5 μ exhibited by both amides II and IV, a non-cyclic secondary amide was suggested. Absorption in these regions is characteristic for non-cyclic secondary amides in general[8] and for such steroidal amides in particular.[9] Whereas some non-cyclic steroidal secondary amides have been reported without the amide II bands near 6.5 μ, no steroidal secondary amide (cyclic) or tertiary amide is known to us with absorption near 6.5 μ.

Neither amide II nor IV was acetylated by acetic anhydride and pyridine at room temperature,[10] nor was a cyclic acetonide derivative formed between II or IV with acetone–perchloric acid. Mild acid hydrolysis of IV afforded a 16-dehydro amide V, recognized as such by loss of absorption at 5.8 μ and increased absorption at 6.0 μ and at 238 mμ. No hydrolysis occurred with base (as strong as 10 *N*) at room temperature, and no steroids could be isolated when hot alkali was used. Further experiments aimed at amide hydrolysis were not fruitful.[11]

(1) (a) Paper XI, J. J. Goodman and L. L. Smith, *App. Microbiol.*, **9**, 372 (1961); (b) Wyeth Laboratories, Philadelphia, Pa.

(2) R. W. Thoma, J. Fried, S. Bonanno and P. Grabowich, *J. Am. Chem. Soc.*, **79**, 4818 (1957).

(3) L. L. Smith, H. Mendelsohn, T. Foell and J. J. Goodman, *J. Org. Chem.*, **26**, 2859 (1961).

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(5) L. L. Smith, M. Marx, J. J. Garbarini, T. Foell, V. E. Origoni and J. J. Goodman, *J. Am. Chem. Soc.*, **82**, 4616 (1960).

(6) K. D. Voigt and W. Schroeder, *Nature*, **176**, 599 (1955); W. Schroeder and K. D. Voigt, *Acta Endocrinol.*, **21**, 343 (1956); **27**, 110 (1958).

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(10) Forced acetylation with acid catalysis gave complex products which did not absorb at 6.5 μ, but exhibited O-acetate bands.

(11) 6 *N* hydrochloric acid–acetic acid gave complex products without 6.5 μ absorption, which on reacetylation absorbed in the region of O-acetates rather than near 6.5 μ. Hydrolysis of steroidal amides has generally been a problem; *cf.* L. Lábler and F. Šorm, *Coll. Czech. Chem. Commun.*, **25**, 265 (1960); F. Ramirez and S. Stafiej, *J. Am. Chem. Soc.*, **77**, 134 (1955); R. H. Mazur, *ibid.*, **81**, 1454 (1959); J. Schmidt-Thomé, *Chem. Ber.*, **88**, 895 (1955); ref. 12.