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[CONTRIBUTION FROM THE CANCER RESEARCH LABORATORY, DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, UNIVERSITY OF FLORIDA]

# The Synthesis of 3-Deoxy-D-ribohexose-6-phosphate and 3-Deoxy-D-gluconic Acid-6-phosphate[1]

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3-Deoxy-D-ribohexose-6-phosphate and 3-deoxy-D-gluconic acid-6-phosphate have been prepared from 3-deoxy-D-ribohexose for testing as potential antimetabolites for cancer chemotherapy.

It is believed at the present time that glucose is metabolized in tumor tissue by two pathways, the Embden-Meyerhof glycolytic pathway, which is the main pathway quantitatively, and the pentose phosphate pathway, which apparently serves to supply reduced triphosphopyridine nucleotide (TPNH) for use in reductive syntheses.[4] There is evidence that the enzymes involved in the pentose phosphate pathway are present in greater amount in some tumor tissues than in normal tissues.[5]

Of the carbohydrate analogs which have been tested as glucose antagonists only those substituted in the 2-position have shown activity.[6] 2-Deoxy-D-glucose (2-deoxy-D-arabohexose) and 2-deoxy-D-galactose (2-deoxy-D-lyxohexose) are potent glycolytic inhibitors of human leucocytes, human leukemic cells, and a number of animal tumors.[7] Since the inhibition is competitive and is overcome by glucose-6-phosphate, it is apparently hexokinase, the enzyme which is necessary for the phosphorylation of glucose, which is affected.[7] This block occurs at a very early stage in the glycolytic pathway, before the pentose phosphate pathway begins to operate. It would be of interest, therefore, to prepare compounds having the potential ability to block the metabolic pathway at a later stage. This paper describes the preparation of two compounds which may have this potentiality, 3-deoxy-D-ribohexose-6-phosphate and 3-deoxy-D-gluconic acid-6-phosphate.

Using the procedure described by Reynolds and Evans[8] for the corresponding glucose derivative, 3-deoxy-D-ribohexose[9] was treated with triphenylmethyl chloride and then with acetic anhydride to obtain 1,2,4-tri-*O*-acetyl-6-*O*-triphenylmethyl-3-deoxy-$\beta$-D-ribohexose (I). Upon treatment with hydrobromic acid in acetic acid the triphenylmethyl group was removed, giving 1,2,4-tri-*O*-acetyl-3-deoxy-$\beta$-D-ribohexose (II). Analysis showed this compound to be a monohydrate and the infrared spectrum had an absorption band at 1655 cm.$^{-1}$, indicating the presence of water. Attempts to remove the water azeotropically were only partially successful. To prove it was actually the 1,2,4-triacetate it was converted back to 1,2,4-tri-*O*-acetyl-6-*O*-triphenylmethyl-3-deoxy-$\beta$-D-ribohexose (I).

The triacetate (II) was phosphorylated in the 6-position with diphenylphosphorochloridate using the procedure described by Lardy and Fischer[10] for the preparation of glucose-6-phosphate. The 1,2,4-tri-*O*-acetyl-3-deoxy-$\beta$-D-ribohexose-6-diphenylphosphate (III) was obtained as an oil. Low-pressure hydrogenation of (III) yielded 1,2,4-tri-*O*-acetyl-3-deoxy-$\beta$-D-ribohexose-6-phosphate (IV), also as an oil. Deacetylation in acid solution gave 3-deoxy-D-ribohexose-6-phosphate, which was isolated as the barium salt (V) and further purified as the brucine salt (VI).

Oxidation of the barium salt (V) was accomplished with barium hypoiodite by a modification of the procedure used by Levene and Raymond[11] for

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the preparation of barium D-gluconic acid-6-phosphate. The barium 3-deoxy-D-gluconic acid-6-phosphate (VII) thus obtained was purified by conversion to the brucine salt (VIII). Just as in the case of the corresponding *D*-glucose derivatives,[11] 3-deoxy-D-ribohexose-6-phosphate forms a dibrucine salt and 3-deoxy-D-gluconic acid-6-phosphate a tribrucine salt.

Results of the biological testing of these two compounds will be reported elsewhere.

## EXPERIMENTAL[12]

The 3-deoxy-D-ribohexose (3-deoxy-D-glucose) used in these experiments was an oil having $[\alpha]_D^{24}$ +23.6° (*c*, 0.8; water). Černý and Pacák give $[\alpha]_D^{18}$ +24.9° (*c*, 0.563; water).[9,13]

*1,2,4-Tri-O-acetyl-6-O-triphenylmethyl-3-deoxy-β-D-ribohexose* (I). A mixture of 3.4 g. (0.021 mole) of 3-deoxy-D-ribohexose, 17 ml. of pyridine, and 5.5 g. (0.024 mole) of triphenylmethyl chloride was warmed to 50°, shaken until homogeneous, and then kept at room temperature for 24 hr. Acetic anhydride (11 ml., 0.12 mole) was added and after standing overnight the mixture was stirred into 300 ml. of ice water containing 17 ml. of acetic acid. After allowing the precipitate to settle under refrigeration, the solid was removed by filtration and dried rapidly onfilter paperbefore reaching room temperature. It was taken up in 12 ml. of ethanol, then heated until the oil which formed had dissolved and crystals began to form. Cooling and filtering gave 4.0 g. (36%) of crude 1,2,4-tri-*O*-acetyl-6-*O*-triphenylmethyl-3-deoxy-β-D-ribohexose. Recrystallization from a mixture of ethanol and acetone (3:1) gave 2.2 g. of white crystals, m.p. 196°. $[\alpha]_D^{25}$ +8.3° (*c*, 2.0; chloroform).

*Anal.* Calcd. for $C_{31}H_{32}O_8$: C, 69.9; H, 6.01. Found: C, 70.36, 70.27; H, 6.00, 6.02.

*1,2,4-Tri-O-acetyl-3-deoxy-β-D-ribohexose* (II). A mixture of 7 g. (0.013 mole) of 1,2,4-tri-*O*-acetyl-6-*O*-triphenylmethyl-3-deoxy-β-D-ribohexose and 50 ml. of acetic acid was heated on a steam bath to effect solution, then cooled in an ice bath to 5–10°. Hydrobromic acid (5 ml. of a 30–32% solution in acetic acid) was added at once and the mixture was shaken for 1 min. The precipitate of triphenylmethyl bromide which formed was removed by filtration and the filtrate was poured into 200 ml. of ice water. The precipitate which formed was removed by extraction of the aqueous mixture with chloroform, the chloroform extract was washed three times with ice water and then dried over anhydrous sodium sulfate. The chloroform was removed at reduced pressure, keeping the temperature below 40°. The partly crystalline residue was dissolved in warm chloroform and treated with hexane to turbidity. Upon cooling crystals were obtained. Repeated recrystallization from chloroform-hexane gave 2.8 g. (71.8%) of 1,2,4-tri-*O*-acetyl-3-deoxy-β-D-ribohexose, m.p. 95–96°. Analysis showed this compound to be a monohydrate. $[\alpha]_D^{25}$ −6.8° (*c*, 2; chloroform).

*Anal.* Calcd. for $C_{12}H_{18}O_3.H_2O$: C, 46.75; H, 6.49. Found: C, 46.78, 47.04; H, 6.45, 6.23.

Drying with organic solvents or *in vacuo* did not completely remove the water of hydration. To prove that this was the desired 1,2,4-triacetate, compound II (1 g.) was dissolved in 10 ml. of pyridine and 1 g. of triphenylmethyl chloride was added with ice cooling. After 12 hr. the mixture was poured into ice water, and extracted with chloroform. The chloroform extract was washed with dilute hydrochloric acid, then with water, and evaporated at reduced pressure. Recrystallization from ethanol gave a compound, m.p. 195°, which showed no depression in melting point when mixed with compound I.

*1,2,4-Tri-O-acetyl-3-deoxy-β-D-ribohexose-6-diphenylphosphate* (III). To an ice-cold solution of 1.5 g. (0.005 mole) of 1,2,4-tri-*O*-acetyl-3-deoxy-β-D-ribohexose (dried in a drying pistol at 65° for 5 hr.) in 7 ml. of pyridine was added dropwise 1.7 g. (0.006 mole) of diphenylphosphorochloridate.[14] After standing in the refrigerator overnight, during which time a precipitate of pyridine hydrochloride had formed, the reaction mixture was poured into ice water, extracted with chloroform, washed with dilute ice-cold hydrochloric acid until all the pyridine had been removed, then with ice water. After drying over sodium sulfate, the chloroform was removed by evaporation at reduced pressure, leaving 2.2 g. (84%) of 1,2,4-tri-*O*-acetyl-3-deoxy-β-D-ribohexose-6-diphenylphosphate as an oil.

*1,2,4-Tri-O-acetyl-3-deoxy-β-D-ribohexose-6-phosphate* (IV). A solution of 5.0 g. (0.01 mole) of III in 25 ml. of absolute ethanol containing 0.4 g. Adams' platinum oxide catalyst was hydrogenated at low pressure. Removal of the catalyst by filtration and evaporation of the solvent gave 3.1 g. (83.7%) of 1,2,4-tri-*O*-acetyl-3-deoxy-β-D-ribohexose-6-phosphate as an oil.

*Barium salt of 3-deoxy-D-ribohexose-6-phosphate* (V). 1,2,4-Tri-*O*-acetyl-3-deoxy-β-D-ribohexose-6-phosphate (3.0 g.; 0.008 mole) was added to a mixture of 3 ml. of conc. hydrobromic acid in 50 ml. of water (*ca.* 0.6*N* acid) and then the mixture was heated on the steam bath for 3 hr. with frequent shaking. After cooling and treating the solution with barium hydroxide to *p*H 8, the mixture was filtered, the residue was washed with cold water, and the clear filtrate was added to four times its volume of ethanol. The precipitate was collected by centrifugation, then suspended successively in 80% ethanol, absolute ethanol, ethanol-ether (3:1), ethanol:ether (1:3), and ether, each time being collected by centrifugation. The solid was again dissolved in 100 ml. of cold water, filtered to remove insoluble material, and then added to 400 ml. ethanol. The precipitate was again collected and washed as described above, giving 1.3 g. (43%) of the barium salt of 3-deoxy-D-ribohexose-6-phosphate. $[\alpha]_D^{28}$ +3.8° (*c*, 2; water).

*Brucine salt of 3-deoxy-D-ribohexose-6-phosphate* (VI). The barium salt of 3-deoxy-D-ribohexose-6-phosphate (0.4 g.) was dissolved in 4 ml. of water, passed through a column of Amberlite IR-120 ion exchange resin, the column was washed with water, and the eluate was treated with methanolic solution of brucine to *p*H 7.5. Evaporation of the solution left a white powder which was recrystallized several times from 2 ml. of acetone-water (2:1); after drying over phosphorus pentoxide at 3 mm. and 60°, $[\alpha]_D^{28}$ −24.2° (*c*, 0.784; water). Analysis corresponded to the dibrucine salt of 3-deoxy-D-ribohexose-6-phosphate.

*Anal.* Calcd. for $C_{52}H_{65}O_{16}N_4P$: N, 5.33; P, 3.0. Found: N, 5.33, 5.46; P, 2.73, 2.80.

*Barium salt of 3-deoxy-D-gluconic acid-6-phosphate* (VII). The barium salt of 3-deoxy-D-ribohexose-6-phosphate (V) (0.8 g.; 0.002 mole) was dissolved in 7.0 ml. of water, 0.5 g. of iodine and 1.0 g. of barium iodide were dissolved in 3 ml. of water, and the two solutions were mixed. With stirring, a solution of 0.5*N* barium hydroxide (16 ml.) was added dropwise at room temperature over a period of 20 min. After stirring for another 20 min. the iodine color had disappeared. Ethanol (50 ml.) was added and the barium salt was isolated by centrifugation, then washed by suspension in water, ethanol, and ether, giving 0.9 g. of a white powder.

*Brucine salt of 3-deoxy-D-gluconic acid-6-phosphate* (VIII). The barium salt of 3-deoxy-D-gluconic acid-6-phosphate

(12) Melting points are uncorrected. Microanalyses were performed by Galbraith Microanalytical Laboratories, Knoxville, Tenn. Rotations were determined with a Keston Polarimeter standardized against sucrose (National Bureau of Standards Standard Sample 17).

(13) For data on crystalline 3-deoxy-α-D-*ribo*hexose, see J. W. Pratt and N. K. Richtmyer, *J. Am. Chem. Soc.*, **79**, 2597 (1957).

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(0.8 g.) was dissolved in 5 ml. of water and passed through a column of Amberlite IR-120 ion exchange resin. The eluate was treated with a methanolic solution of brucine of *p*H 7.5–8. After evaporation at reduced pressure the residue was recrystallized several times from methanol, giving 0.5 g. of the brucine salt. $[\alpha]_D^{25}$ −20.2° (*c*, 0.97; water). The analysis showed the salt to be the tribrucine derivative of 3-deoxy-D-gluconic acid-6-phosphate.

*Anal.* Calcd. for $C_{76}H_{90}O_{21}N_6P$: N, 5.83; P, 2.15. Found: N, 5.74, 5.61; P, 1.92, 1.61.

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# The Chemistry of the Spiroaminoketal Side Chain of Solasodine and Tomatidine. I.[1] Improved Preparation of 3β-Acetoxy-5,16-pregnadien-20-one and 3β-Acetoxy-5α-pregn-16-en-20-one from Solasodine and Tomatidine.

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The steroidal alkaloids solasodine and tomatidine have been degraded in excellent over-all yields (65–68%) to 3β-acetoxy-5,16-pregnadien-20-one and 3β-acetoxy-5α-pregn-16-en-20-one by conversion of the *O,N*-diacetates of the alkaloids into the respective pseudoacetylamino derivatives, chromic anhydride oxidation of the latter and final hydrolysis with acetic acid.

The announcement from this laboratory[3] concerning the degradation of solasodine (IA) (via VA) to 3β-acetoxy-5,16-pregnadien-20-one (IVA) has spurred several laboratories[4,5,6] to effect an increase in the yields originally obtained by us (10–20%) in this process. We wish to describe in this paper a modified and greatly improved conversion of solasodine (IA), dihydrosolasodine (IC) and tomatidine (IB) to their respective pregnenolone derivatives IVA and IVB.

When a solution of *O,N*-diacetylsolasodine[7] (IIA) in glacial acetic acid (or propionic acid) was refluxed for 15 minutes, a crystalline 3β-acetoxy-26-acetylamino-5,20(22)-furostadiene (IIIA) was obtained in a yield of 95–98%. *O,N*-Diacetyltomatidine[8] (IIB) similarly gave a 95% yield of crystalline 3β-acetoxy-26-acetylamino-5α-furost-20(22)-ene (IIIB) by this procedure. IIIA and IIIB can also be readily obtained, but not as pure and in as good yields, by treating a solution of IIA and IIB respectively in acetic acid with mineral acids (perchloric or hydrochloric) at room temperature.[9] IIIA has previously been obtained by chromatography on alumina of the crude reaction mixture resulting from the treatment of solasodine with acetic anhydride.[10] The 3-hydroxy compound of IIIB has likewise been obtained by the alkaline hydrolysis of the so-called unsaturated triacetyltomatidine (VB).[11]

By chromic acid oxidation of the pseudo compounds IIIA and IIIB in aqueous acetic acid (80%) and subsequent hydrolysis of the acyloxy side chain with acetic acid according to the method of Cameron *et al.*[12] the respective pregnenolone acetates IVA and IVB were obtained in high yields. We have found that optimal results were obtained in the oxidation when two molar equivalents of chromium trioxide were used. Although these products are crystalline and can be readily purified by recrystallization, it has been found expedient to resort to chromatography at this stage. The yields of IVA and IVB (from IIA and IIB) ranged from about 75–80%. In a continuous operation, *i.e.* without the isolation and purification of IIA and IIIA, solasodine (IA) gave 65% of the pregnenolone derivative IVA. Similarly, tomatidine

(1) A preliminary account of this work was published in *J. Org. Chem.*, **24**, 893 (1959).

(2) Visiting Scientist, National Institutes of Health.

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