sugar V as described above. Sirupy V in 50 cc. of xylene was added to an azeotropically dried suspension of 15.1 g. of chloromercuri-5,6-dimethylbenzimidazole[22] on Celite[40] (the mixture contained 10.48 g., 27.5 mmoles, of the chloromercuri-salt) in 200 cc. of xylene. The stirred mixture was kept under reflux for three hours and was then filtered while hot. The precipitate was washed with a little xylene and the filtrate was evaporated *in vacuo*. The dark colored residue was dissolved in 200 cc. of chloroform, and the solution was washed with two 15-cc. portions of 30% potassium iodide solution and then with 30 cc. of water. The chloroform solution was dried over magnesium sulfate and the filtrate was evaporated under reduced pressure. The residue was partially decolorized by solution in 100 cc. of ether and filtration through Norit. Evaporation *in vacuo* left a dark yellow oil which could not be made to crystallize. This was dissolved in 40 cc. of absolute methanol containing 0.4 cc. of a 1 *N* methanolic sodium methoxide solution. The solution was allowed to reflux for a few minutes and another 0.4 cc. of the sodium methoxide solution was added. The mixture was then kept under reflux on the steam-bath for 30 minutes. It remained at *p*H 8 throughout this time. The dark red solution was evaporated *in vacuo* to a small volume until crystallization started. The solid was collected and washed with ethanol to remove the color. A second crop was obtained by concentrating the mother liquors. The combined solids were recrystallized from ethyl acetate–acetone to afford 3.33 g. (47%), m.p. 173–175°. An additional recrystallization gave 2.63 g., m.p. 175–176°. Further recrystallizations did not change the m.p.; $[\alpha]^{25}D$ −43.3° (*c* 1.1 in methanol); $\lambda_{max}^{methanol}$ 277 and 285 mμ (ε 7,830 and 7,380 in acid); 248, 279 and 287 mμ (ε 7,420, 4,760, and 4,900 in methanol); 250, 280 and 288 mμ (ε 7,280, 4,920 and 4,760 in base).

Anal. Calcd. for $C_{14}H_{17}FN_2O_3$: C, 59.99; H, 6.12; F, 6.78; N, 10.00. Found: C, 60.00; H, 6.38; F, 6.47; N, 10.20.

Paper Chromatography.—Circular paper chromatograms were run in the apparatus described by Kawerau.[41] The apparatus (26-cm. diameter) was purchased from the Shandon Scientific Co., London, England. It was found that the plastic holder[41] sold with this apparatus was attacked by solvents; it was replaced by a similarly shaped, glass holder. A special Whatman #1 filter paper was used (KCT-26) which had been slotted for the Kawerau apparatus. Solvents were mixed just before use, and the paper was not equilibrated with the solvent mixture. Unless stated otherwise, spots were detected by inspection under ultraviolet light.

(40) In all probability the Celite could have been left out of this reaction without affecting the results.

(41) E. Kawerau, "Chromatographic Methods," Vol. 1, No. 2, [published by H. Reeve Angel and Co., 52 Duane Street, New York 7, N. Y., 1956, p. 7]. The Kawerau apparatus has been found to be a very useful improvement for running paper chromatograms. The apparatus is not very bulky and it can be used to run 5 chromatograms at the same time. In general, circular paper chromatography takes less time and probably gives better resolution than paper strip or sheet chromatography.

PEARL RIVER, N. Y.

[JOINT CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, GEORGETOWN UNIVERSITY, AND THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE]

2-Deoxy Sugars. I. 3,4-Di-*O*-*p*-nitrobenzoyl-1-chloro (and 1-Bromo)-1,2,6-trideoxy-D-*ribo*-hexose. Two Crystalline 2-Deoxy Acylglycosyl Halides[1]

BY W. WERNER ZORBACH AND THOMAS A. PAYNE, JR.[2]

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Certain acylated derivatives of 2-deoxy-D-*ribo*-hexose (2-deoxy-D-allose) and 2,6-dideoxy-D-*ribo*-hexose (D-digitoxose) were prepared and investigated in an effort to secure corresponding crystalline acylglycosyl halides. 1,3,4-Tri-*O*-*p*-nitrobenzoyl-2,6-dideoxy-D-*ribo*-hexose was converted to 3,4-di-*O*-*p*-nitrobenzoyl-1-chloro (and 1-bromo)-1,2,6-trideoxy-D-*ribo*-hexose, both of which are crystalline, reasonably stable under anhydrous conditions, and display a high reactivity.

The role of 2-deoxy sugars as constituents of important biologically active compounds, *e.g.*, cardenolides, is well known. The synthesis of such compounds, partial or otherwise, is therefore of immediate interest; hence, a method for the preparation of reasonably stable, crystalline, acylated glycosyl halides of 2-deoxy sugars should have considerable import. It was in this connection that we turned to a study of 2,6-dideoxy-β-D-*ribo*-hexopyranose (D-digitoxose) (I), which is the chief carbohydrate constituent of the important cardenolide digitoxin. 2-Deoxy-D-*ribo*-hexopyranose (2-deoxy-D-allose) (II) was simultaneously investigated because of its close structural relationship with I.

The 2,6-dideoxy-D-*ribo*-hexopyranose (I) is tentatively assigned a β-configuration at C_1 based on the knowledge that the β-anomeric forms of D-hexoses as well as 2-deoxy-D-hexoses[3] invariably mutarotate in the direction of more positive values. This is found to be the case with I,[4] but it was necessary to establish that the mutarotation involved only a simple α,β-pyranoside interconversion. Accordingly, a rate study was carried out which disclosed a straight line plot of log $(\alpha_t - \alpha_\infty)$ *vs.* *t*, and which may be regarded as evidence for a simple anomerization.

Further support to this assignment may be gained from a comparison of various hexose and 2-deoxyhexose anomers and their corresponding methyl pyranosides,[5] which reveals that the molecular rotational shift produced in proceeding from a given anomeric form of a hexose to the methyl pyranoside with the same anomeric configuration is

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(2) This paper is taken from a dissertation presented to the Graduate School of Georgetown University, Washington, D. C., in partial fulfillment for the degree of Doctor of Philosophy in Chemistry.

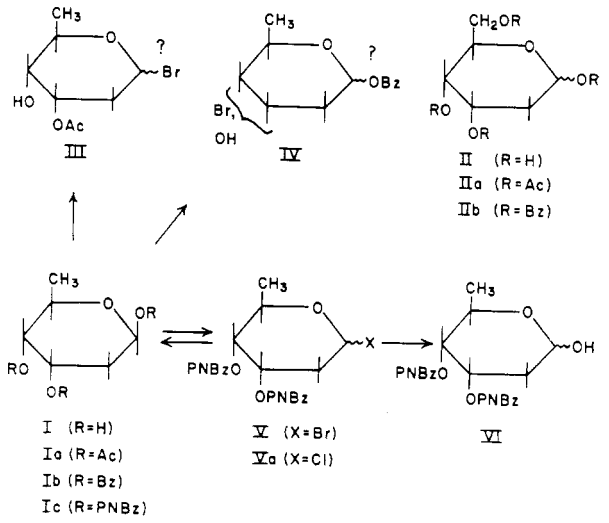
(3) F. J. Bates and Associates, "Polarimetry, Saccharimetry and the Sugars," U. S. Government Printing Office, Washington, D. C., 1942, p. 712.

(4) H. R. Bolliger and P. Ulrich, *Helv. Chim. Acta*, **35**, 97 (1952).

(5) (a) L. F. Fieser and M. Fieser, "Organic Chemistry," Reinhold Publishing Corp., New York. N. Y., 3rd ed., 1956, p. 377; (b) W. G. Overend and M. Stacey, *Adv. in Carbohydrate Chem.*, **8**, 94 (1953).

very nearly constant and averages 99 ± 10 × 10^2 units. The [M] difference between methyl 2,6-dideoxy-α-D-*ribo*-hexopyranoside ([M] = +31100),[4] prepared unequivocally from methyl α-D-glucopyranoside, and I ([M] = +6000) amounts to 251 × 10^2 units, which further suggests that crystalline I cannot be the α-anomeric form. 2-Deoxy-D-*ribo*-hexopyranose (II), unfortunately, does not mutarotate[6]; hence, it is not possible to assign an anomeric configuration.

The 2,6-dideoxy-β-D-*ribo*-hexopyranose (I) is available commercially or may be obtained from the hydrolysis of digitoxin. It was necessary, however, in the case of 2-deoxy-D-*ribo*-hexopyranose (II), to secure this rare sugar by synthetic means. The procedure followed was essentially the same as that described by Gut and Prins,[6] except for two minor modifications. The first of these involved the lithium aluminum hydride reduction of methyl 2,3-anhydro-4,6-O-benzylidine-α-D-alloside. By substituting tetrahydrofuran (in which the latter is soluble) for ethyl ether, the reduction of this compound at reflux temperature was greatly facilitated, giving yields comparable to those described in the original procedure of the expected methyl 4,6-O-benzylidine-2-deoxy-α-D-*ribo*-hexoside.[6] Secondly, the latter compound was hydrolyzed completely in one operation by refluxing with 0.01 *N* sulfuric acid to give the desired sugar II.



The difficulties inherent in preparing acylglycosyl halides of 2-deoxy sugars are due to their abnormally high reactivity when compared with acylglycosyl halides prepared from normal hexoses. The rate of methanolysis of this class of compounds has been studied carefully by Phillips and coworkers[7] who showed that, for a given hexose, such reactivity is under steric control and is influenced primarily by the degree of shielding around C_1 provided by substitution at C_2.[7c] Thus it was found that, by increasing the bulk of the group attached at C_2, the reactivity could be lowered. It was further shown that the reactivity was also influenced in a like manner by the substituent attached at C_5 but to a lesser degree. Although 2-deoxyacylgylcosyl halides *per se* were not investigated, the studies by the Phillips group clearly infer the unusually reactive character of the latter, in which the only substitution at C_2 is hydrogen and shielding is not possible. The case for 2-deoxyacylglycosyl halides is further strengthened by a consideration of the rate of methanolysis of 2,3-dichlorotetrahydropyran (which may be construed as the prototype of all acylglycosyl halides) which was demonstrated to be 800–1000 times greater than that for 2,3,4,6-tetra-O-acetyl-1-chloro-1-deoxy-α-D-glucose.[7b] Accompanying the methanolysis of the former was some elimination of hydrogen halide, a phenomenon which was observed on several occasions during the course of our investigation.

Preliminary studies were conducted along more or less classical lines. Acetylation of I and II under the usual conditions at 0° gave the crystalline acetates Ia and IIa, respectively. By attenuating considerably the conditions usually employed for the preparation of acylglycosyl bromides, bromidation of Ia yielded a crystalline product which proved to be a 1-halide by virtue of rapid positive tests with alcoholic silver nitrate and Benedict solution. Analysis, however, suggested a mono-O-acetyl-1-bromo-1,2,6-trideoxy-D-*ribo*-hexopyranose (III). It was evident that, during the bromidation, deacetylation had occurred and, in view of the comparative ease with which equatorial acetates may be hydrolyzed, it is likely that the open position is at C_4 assuming, of course, a C1conformation.[8] Bromidation of IIa under similar conditions failed to yield a crystalline product.

A free hydroxyl group present in an acylglycosyl halide renders the latter useless in glycosidation studies where the reaction requires extended periods of time. Consequently, III was investigated no further and attention was diverted to other derivatives of I and II which could better resist deacylation. It is known, for example, that benzoates are considerably more resistant to hydrolysis than the corresponding acetates,[9] and the crystalline benzoates Ib and IIb were accordingly secured. Preparation of an acylglycosyl bromide from Ib was complicated by the separation of solid benzoic acid and, in an attempt to overcome this, the reaction mixture was extracted rapidly at 0°. The crystalline product thus isolated contained halogen but, as in the case of the bromidation of the acetate Ia, its composition was that of a bromomonobenzoate IV. In this case, however, the halogen must be attached at some point other than C_1, as no reaction whatever took place even when the substance was treated with hot alcoholic silver nitrate. The material was no longer reducing, indicating the migration of a benzoyl group to C_1.

The conditions for the bromidation were changed at this point and both Ib and IIb were treated with one mole-equivalent of hydrogen bromide in methylene chloride.[10] On one occasion, the bromidation of Ib was followed polarimetrically and appeared

(6) M. Gut and D. A. Prins, *Helv. Chim. Acta*, **30**, 1223 (1947).

(7) F. H. Newth and G. O. Phillips, *J. Chem. Soc.*, (a) 2896, (b) 2900, (c) 2904 (1953); G. L. Mattok and G. O. Phillips, *ibid.*, (d) 1836 (1956), (e) 268 (1957).

(8) R. E. Reeves, THIS JOURNAL, **72**, 1499 (1950).

(9) R. K. Ness, H. G. Fletcher, Jr., and C. S. Hudson, *ibid.*, **72**, 2200 (1950).

(10) R. K. Ness and H. G. Fletcher, Jr., *ibid.*, **76**, 1663 (1954).

to be complete in 8–9 minutes. Repeated experiments failed to yield crystalline 1-bromides but, instead, gave only sirups which fumed in moist air. Further studies with the benzoates were not carried out.

In an effort to overcome the inability to secure crystalline material before any appreciable decomposition (by hydrolysis or elimination) had occurred, *p*-nitrobenzoyl derivatives were considered next, since the latter are known to be excellent characterizing derivatives for alcohols in general. Thus, by treating I with *p*-nitrobenzoyl chloride in pyridine at 0°, Ic was obtained in 91% yield. Treatment of a suspension of Ic in dry methylene chloride with slightly more than one mole-equivalent of hydrogen bromide in the same solvent caused a rapid dissolution of the material, accompanied by the separation of another substance. Subsequent examination of the latter revealed it to be *p*-nitrobenzoic acid which is the expected by-product in the halogenation and which could, indeed, be removed conveniently by filtration. In a subsequent experiment, in which it was discovered that the reaction was complete in *ca.* 8 minutes, the separating *p*-nitrobenzoic acid was recovered quantitatively and amounted to 99% of theory. Evaporation of the filtrate and recrystallization of the solid residue gave approximately 76% of 3,4-di-*O*-*p*-nitrobenzoyl-1-bromo-1,2,6-trideoxy-D-*ribo*-hexose (V), which gave strong, positive Beilstein, Benedict and alcoholic silver nitrate tests. When exposed even momentarily to moist air, V hydrolyzed rapidly; consequently, elemental analysis was contraindicated.

Based on the knowledge that chlorides are, generally, less reactive than corresponding bromides, an attempt to prepare the corresponding 1-chloride Va was undertaken. To effect complete reaction, it was necessary to treat Ic with an excess of hydrogen chloride in methylene chloride and extend the reaction time considerably. This resulted, as in the case of V, in a crystalline 3,4-di-*O*-*p*-nitrobenzoyl-1-chloro-1,2,6-trideoxy-D-*ribo*-hexose (Va). Although somewhat less reactive, exposure of the substance to moist air caused rapid hydrolysis; hence, analysis of the material was not practicable.

The structures of V and Va were, accordingly, given support by indirect means. On treating the chloride Va with silver *p*-nitrobenzoate in dry benzene,[11] the tri-*p*-nitrobenzoate Ic was obtained. Treatment of both V and Va with aqueous acetone in the presence of silver carbonate[12] gave the same product, 3,4-di-*O*-*p*-nitrobenzoyl-2,6-dideoxy-D-*ribo*-hexose (VI), which gave immediate positive tests with Benedict solution and ammoniacal silver oxide, but a Beilstein flame test was negative. Final support to the structure of VI was given by the formation of the corresponding anilide.

Experiments also were carried out in an effort to secure a methyl 2,6-dideoxy-D-*ribo*-hexopyranoside by treating Va with anhydrous methanol in the presence of silver carbonate. This treatment resulted in sirups which could not be crystallized. Hydrolysis of the latter by means of barium methoxide likewise gave sirups which resisted attempts to obtain in crystalline form.

(11) R. K. Ness, H. G. Fletcher, Jr., and C. S. Hudson, THIS JOURNAL, **73**, 959 (1951).

(12) R. Jeanloz, H. G. Fletcher, Jr., and C. S. Hudson, *ibid.*, **70**, 4055 (1951).

Acknowledgments.—The authors are indebted to Drs. Robert K. Ness, Hewitt G. Fletcher, Jr., and James W. Pratt for their many helpful suggestions and advice during the course of this investigation. They wish also to thank the personnel of this Institute's Microanalytical Laboratory, under the direction of Dr. W. C. Alford, for the elemental analyses and some of the rotational measurements.

Experimental

1,3,4-Tri-*O*-acetyl-2,6-dideoxy-β-D-*ribo*-hexose (Ia).—To a solution of 15 ml. of anhydrous pyridine and 12 ml. of acetic anhydride at 0° was added 2.15 g. (0.0145 mole) of finely divided 2,6-dideoxy-D-*ribo*-hexopyranose (I). The mixture was maintained for two hours at this temperature with constant stirring and set aside in the refrigerator for 5 days. The mixture was then added, with efficient stirring, to *ca.* one liter of ice-water; it was, however, impossible to detect any solid particles. Examination by means of a Tyndall beam disclosed the presence of a sol, which was subsequently discharged by the portionwise addition of solid ammonium sulfate. Stirring was continued for an additional hour, after which time the crystalline material was filtered by suction and washed carefully with small portions of ice-water. The material, after drying, amounted to 2.85 g. (69%) and melted at 86.5–87.5°. The filtrate was refrigerated overnight and gave an additional 0.23 g. of product, bringing the total yield to 75%. Two recrystallizations from ether–*n*-pentane gave pure, dimorphic Ia, m.p. 75.5–76.5°, 86.5–87.5°; $[\alpha]^{25}D$ +36.2° (*c* 2.06, $CHCl_3$).

Anal. Calcd. for $C_{12}H_{18}O_7$: C, 52.70; H, 6.58. Found: C, 52.67; H, 6.37.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-D-*ribo*-hexose (IIa).—2-Deoxy-D-*ribo*-hexopyranose (II) (500 mg., 3.04 mmoles) was acetylated in a manner identical to that described in the preceding preparation. After refrigerating for four days, the reaction mixture was dissolved in ether and extracted with cold, dilute solutions of sulfuric acid and sodium bicarbonate and, finally, with water. The extract thus purified was dried over sodium sulfate and evaporated to dryness, yielding a sirup which was dissolved in 5 ml. of anhydrous ether and an equal volume of *n*-hexane added. The flask was well stoppered and set aside at room temperature. After two days, no crystalline material having appeared, the stopper was removed, 2 ml. of *n*-hexane added and the flask restoppered. This procedure was repeated every two or three days for six weeks, after which time crystallization still had not taken place. The flask was then unstoppered and the solvent allowed to evaporate partially overnight. By the following morning, the material had crystallized. Three crystallizations from ether–*n*-hexane (1–1) gave 159 mg. (39%) of pure 1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-*ribo*-hexose (IIa), m.p. 73–75°, $[\alpha]^{23}D$ +12.5° (*c* 1.07, $CHCl_3$). Isopropyl ether serves equally well as a crystallizing solvent.

Anal. Calcd. for $C_{14}H_{20}O_9$: C, 50.60; H, 6.03. Found: C, 50.60; H, 6.21.

1,3,4-Tri-*O*-benzoyl-2,6-dideoxy-β-D-*ribo*-hexose (Ib).—To a mixture of 15 ml. of anhydrous pyridine and 20 ml. of benzoyl chloride at 0° was added 2.5 g. (0.017 mole) of 2,6-dideoxy-β-D-*ribo*-hexopyranose (I). The mixture was stirred for one hour at this temperature, set aside in the refrigerator for two days, then treated carefully with an excess of saturated aqueous sodium bicarbonate. This was added rapidly to *ca.* one liter of ice-water, stirred for one hour and the separating material filtered by suction and dried. This gave 6.37 g. (82%) of material which, when recrystallized twice from ether, gave pure 1,3,4-tri-*O*-benzoyl-2,6-dideoxy-β-D-*ribo*-hexose (Ib), m.p. 176–177°, $[\alpha]^{20}D$ +40.7° (*c* 1.05, $CHCl_3$).

Anal. Calcd. for $C_{27}H_{24}O_7$: C, 70.42; H, 5.25. Found: C, 70.46; H, 5.19.

1,3,4,6-Tetra-*O*-benzoyl-2-deoxy-D-*ribo*-hexose (IIb).—2-Deoxy-D-*ribo*-hexose (II) (450 mg., 2.74 mmoles) was benzoylated in a manner identical to that described in the preceding preparation. When the neutralized reaction mixture, however, was added to the ice-water, a colloid formed which was subsequently discharged by the addition of solid ammonium sulfate. The separating material was filtered, dried and recrystallized twice from absolute ethanol, giving 1335 mg. (84%) of pure 1,3,4,6-tetra-*O*-benzoyl-2-deoxy-D-*ribo*-hexose (IIb), m.p. 210–211.5°, $[\alpha]^{20}D$ +88.4° (*c* 0.65, $CHCl_3$).

Anal. Calcd. for $C_{34}H_{28}O_9$: C, 70.34; H, 4.86. Found: C, 70.09; H, 4.88.

3(or 4)-O-Acetyl-1-bromo-1,2,6-trideoxy-D-*ribo*-hexose (III).—To a mixture of 2.5 ml. of acetic acid saturated with hydrogen bromide, 0.2 ml. of acetic anhydride and 1.0 ml. of acetic acid at 0° was added 1000 mg. (3.64 mmoles) of the triacetate Ia. The mixture was allowed to stand at room temperature for 15 minutes then cooled to 0° and maintained at this temperature for one hour. A small amount of crushed ice was added to the mixture, all of which was quickly transferred to a separatory funnel containing 250 ml. of ether at 0°. This was rapidly extracted, in turn, with dilute potassium bicarbonate (0°) and water (0°). The dried extract was evaporated *in vacuo* at 25° to a volume of *ca.* 20 ml., treated with Norit A, and immediately filtered. The filtrate was concentrated to a volume of 5 ml. and 5 ml. of *n*-hexane added. Refrigeration for 48 hours gave crystalline material which, when recrystallized from the ether–*n*-hexane, yielded 250 mg. (27%) of III, m.p. 75–77.5° dec., $[\alpha]^{25}D$ +59.0° (*c* 1.02, $CHCl_3$).

Anal. Calcd. for $C_8H_{13}O_4Br$: C, 37.92; H, 5.18; Br, 31.60. Found: C, 37.51; H, 5.12; Br, 31.64.

The Conversion of Ib to a Bromomonobenzoate IV.—To a stirring suspension of 1000 mg. (2.18 mmoles) of the tribenzoate Ib in 5 ml. of acetic acid was added 15 ml. of acetic acid saturated with hydrogen bromide. Stirring was continued for two hours, after which time the solvent was evaporated *in vacuo* at 40°. The oily residue was dissolved in 10 ml. of anhydrous toluene which likewise was evaporated. This procedure was repeated until all traces of acetic acid and hydrogen bromide were removed and the resulting sirup triturated with dry *n*-hexane. After refrigerating overnight, the crystalline material (which proved to be benzoic acid) was filtered and subsequently discarded. The filtrate and washings were diluted with methylene chloride and successively extracted at 5° with dilute sodium bicarbonate and water. The extract was dried over sodium sulfate and evaporated to dryness *in vacuo* at 30°, giving material which, when repeatedly recrystallized from ether–*n*-hexane, yielded 217 mg. (31%) of the bromide IV, m.p. 110–111°.

Anal. Calcd. for $C_{13}H_{15}O_4Br$: C, 49.55; H, 4.79; Br, 25.36. Found: C, 49.47; H, 4.70; Br, 25.39.

1,3,4-Tri-*O*-*p*-nitrobenzoyl-2,6-dideoxy-β-D-*ribo*-hexose (Ic).—To a mixture of 50 ml. of anhydrous pyridine and 5.1 g. of *p*-nitrobenzoyl chloride at 0° was added 810 mg. (5.5 mmoles) of 2,6-dideoxy-β-D-*ribo*-hexopyranose (I). This was stirred for one hour at this temperature and then set aside in the refrigerator for three days. After this time, the mixture was allowed to stand at room temperature for one hour and then shaken carefully with a slight excess of saturated aqueous sodium bicarbonate. The neutralized mixture was added rapidly, under stirring, to *ca.* 500 ml. of ice-water and stirring was continued for another hour. The separating material was filtered, dried and recrystallized from acetone. By carefully working up the mother liquors there was obtained a total of 2976 mg. (91%) of pure dimorphic 1,3,4-tri-*O*-*p*-nitrobenzoyl-2,6-dideoxy-β-D-*ribo*-hexose (Ic), m.p. 181–182°, 182–205° dec.; $[\alpha]^{20}D$ +55.6° (*c* 0.317, $CHCl_3$), $[\alpha]^{20}D$ +42.8° (*c* 0.50, acetone).

Anal. Calcd. for $C_{27}H_{21}O_{13}N_3$: C, 54.44; H, 3.57; N, 7.06. Found: C, 54.50; H, 3.79; N, 6.89.

3,4-Di-*O*-*p*-nitrobenzoyl-1-bromo-1,2,6-trideoxy-D-*ribo*-hexose (V).—To a suspension of 192 mg. (0.323 mmole) of the tri-*p*-nitrobenzoate Ic in 4 ml. of anhydrous methylene chloride was added 1.05 ml. of methylene chloride containing 0.323 meq. $ml.^{-1}$ of hydrogen bromide and the mixture stirred rapidly under the exclusion of moisture for 8 minutes; it then was filtered rapidly by suction through a sintered glass funnel into a receiver which could be attached directly to a rotary evaporation apparatus. The filtrate thus was evaporated without delay at 25° and the solid residue dissolved (do not warm!) in 2 ml. of dry methylene chloride, followed by 2 ml. of anhydrous ether. Crystallization began in a few minutes and, when nearly complete, an additional 2 ml. of ether was put in and the well-stoppered flask set aside in the refrigerator overnight. The material next was allowed to warm to room temperature and the liquors decanted. The crystalline material was rinsed quickly with 2 ml. of ether–methylene chloride (1–1) which was likewise quickly decanted and by maintaining the flask in a partially inverted position, the volatile solvents evaporated in a few seconds, whereupon the flask was stoppered and weighed, yielding 125 mg. (76%) of 3,4-di-*O*-*p*-nitrobenzoyl-1-bromo-1,2,6-trideoxy-D-*ribo*-hexose (V), m.p. 91–101° (with bubbling). The material was transferred rapidly to a small vial and, by storage in the freezing tray of a refrigerator, could be kept indefinitely without decomposition.

The Hydrolysis of 3,4-Di-*O*-*p*-nitrobenzoyl-1-bromo-1,2,6-trideoxy-D-*ribo*-hexose (V).—The tri-*p*-nitrobenzoate Ic (357 mg., 0.6 mmole) was treated with 0.645 mmole of hydrogen bromide in methylene chloride as described in the preceding preparation. After evaporating the filtrate, however, the crude bromide V was not recrystallized but immediately dissolved in 5 ml. of anhydrous acetone. To this was added a slurry of 500 mg. of silver carbonate in 10 ml. of acetone–water (1–1) and the mixture stirred for 15 minutes. This was then treated with a small amount of Darco, filtered, and the filtrate evaporated *in vacuo* at 40°. The sirupy residue was dissolved in 1 ml. of benzene and 5 ml. of ether added. The separating material was recrystallized three times from ether alone, yielding 3,4-di-*O*-*p*-nitrobenzoyl-2,6-dideoxy-D-*ribo*-hexose (VI), which contained one mole of ether of crystallization. After drying, the material amounted to 123 mg. (46% based on Ic) and melted at 140–141°, $[\alpha]^{22}D$ +165° (*c* 0.53, acetone).

Anal. Calcd. for $C_{20}H_{18}O_{10}N_2$: C, 53.81; H, 4.06. Found: C, 54.03; H, 4.31.

Anilide of VI.—To a solution of 100 mg. (0.22 mmole) of 3,4-di-*O*-*p*-nitrobenzoyl-2,6-dideoxy-D-*ribo*-hexose (VI) in 20 ml. of absolute ethanol was added 100 mg. of aniline, followed by 1 ml. of water. The solution was refluxed for two hours, decreased in volume to *ca.* 10 ml. and set aside in the refrigerator for three days. The separating material was recrystallized three times from absolute ethanol, giving pure anilide of VI, m.p. 143–143.5° dec.

Anal. Calcd. for $C_{26}H_{23}O_9N_3$: N, 8.06. Found: N, 8.47.

3,4-Di-*O*-*p*-nitrobenzoyl-1-chloro-1,2,6-trideoxy-D-*ribo*-hexose (Va).—To a suspension of 1190 mg. (2.0 mmoles) of 1,3,4-tri-*O*-*p*-nitrobenzoyl-2,6-dideoxy-β-D-*ribo*-hexose (Ic) in 10 ml. of anhydrous methylene chloride was added 20 ml. of a solution of methylene chloride containing 0.191 meq. $ml.^{-1}$ of hydrogen chloride. The mixture was stirred at room temperature for one hour under the exclusion of moisture after which time the separating *p*-nitrobenzoic acid was filtered and the filtrate rapidly evaporated *in vacuo* at 25°. The solid residue was dissolved in 4 ml. of dry methylene chloride (by warming for a few seconds at 35°) and 6 ml. of anhydrous ether added. Crystallization began in a few minutes, whereupon the stoppered flask was set aside in the refrigerator for two hours. After allowing the contents to warm to room temperature, the solvent was decanted quickly and the crystalline material redissolved in a small quantity of ether-methylene chloride (1–1). After refrigerating overnight, 620 mg. (66.5%) of crystalline 3,4-di-*O*-*p*-nitrobenzoyl-1-chloro-1,2,6-trideoxy-D-*ribo*-hexose (Va) was secured, m.p. 96–103° (with bubbling).

The *p*-Nitrobenzoylation and Hydrolysis of the Chloride Va.—The tri-*p*-nitrobenzoate Ic (595 mg., 1.0 mmole) was suspended in 12 ml. of a solution of methylene chloride containing 0.166 meq. $ml.^{-1}$ of hydrogen chloride. The suspension was stirred, however, for only 0.5 hour and the material worked up as described in the preceding preparation. The crude Va was recrystallized once from ether–methylene chloride and after decantation of the solvent the material was dissolved immediately in 20 ml. of dry methylene chloride. The solution was divided into two equal parts, each of which was evaporated *in vacuo* at 25°.

One portion was redissolved in anhydrous benzene, 191 mg. (0.7 mmole) of dry silver *p*-nitrobenzoate added and the resulting suspension stirred magnetically for 4 hours. Ace-

tone (50 ml.) was then added and the mixture filtered with the aid of Celite. The filtrate, which still contained colloidal silver *p*-nitrobenzoate, was repeatedly treated with Darco until a clear solution was obtained. This was evaporated to dryness *in vacuo* at 40° and the solid residue crystallized three times from acetone–ether, giving 47.2 mg. (16% based on 0.5 × 1.0 mmole of Ic) of 1,3,4-tri-*O*-*p*-nitrobenzoyl-2,6-dideoxy-β-D-*ribo*-hexose (Ic), m.p. 178–179°, and giving no depression in the melting point when admixed with an authentic specimen of Ic, $[\alpha]^{22}D$ +37° (*c* 0.43, acetone).

The second portion of the chloro compound Va was hydrolyzed (*cf.* hydrolysis of V) by dissolving in 17.5 ml. of acetone, followed by the addition of 200 mg. of silver carbonate and 2.5 ml. of water. Two crystallizations of the purified filtrate gave material which, on drying, yielded 120 mg. (54% based on 0.5 × 1.0 mmole of Ic) of pure 3,4-di-*O*-*p*-nitrobenzoyl-2,6-dideoxy-D-*ribo*-hexose (VI), m.p. 141–142°, and which did not depress the melting point when admixed with a sample prepared *via* the bromide V.

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Steroidal Sapogenins. XLVII. Preparation of 16α,17α-Epoxy-11α-hydroxypregnane-3,20-dione from 5β-Spirostanes[2]

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Sarsasapogenin (Ia) or smilagenin (Ib) acetates have been converted to a useful cortisone intermediate by the following route. Standard degradation of Ia or Ib gave 3β-acetoxy-16-pregnen-20-one (II) which on epoxidation with alkaline hydrogen peroxide yielded 3β-acetoxy-16α,17α-epoxypregnan-20-one (IIIa). Alkaline hydrolysis of IIIa gave the corresponding 3β-hydroxy compound IIIb. Oxidation of the latter with chromic oxide gave 16α,17α-epoxypregnane-3,20-dione (IV). Microbiological hydroxylation of IV with *Rhizopus nigricans* gave 16α,17α-epoxy-11α-hydroxypregnane-3,20-dione (Va). Oxidation of Va with chromium trioxide gave 16α,17α-epoxypregnane-3,11,20-trione (VI). Treatment of VI with hydrobromic acid followed by reduction in the presence of a palladium catalyst gave 17α-hydroxypregnane-3,11,20-trione (VIII), a known cortisone precursor.

This Laboratory has been investigating sources of steroidal sapogenins with emphasis on plants that can be grown in the United States.[3a,b,c] In the course of these surveys we have found some promising sources of 5β-sapogenins, in particular *Yucca schidigera*, a source of sarsasapogenin and *Agave lecheguilla*, a source of smilagenin. The latter species occurs in very high concentrations in the "Big Bend" region of southwestern Texas. Since the pioneer investigations of Marker and his associates,[4] there has been relatively little attention paid to hormonal derivatives which can be prepared from 5β-sapogenins. The present report deals with the preparation of 16α,17α-epoxy-11α-hydroxypregnane-3,20-dione. The route is shown in Fig. 1.

Sarsasapogenin (Ia) or smilagenin acetates (Ib) were converted to 3β-acetoxy-16-pregnen-20-one (II) by previously published procedures.[5] Treatment of II with alkaline hydrogen peroxide at 4° by Julian's procedure,[6,7] gave 3β-acetoxy-16α,17α-epoxy-pregnan-20-one (IIIa) which on alkaline hydrolysis gave the corresponding free hydroxy compound IIIb. Chromium trioxide oxidation of IIIb gave 16α,17α-epoxypregnane-3,20-dione (IV).[8] The over-all yield of IV from II was approximately 80%. Microbiological hydroxylation of IV with *Rhizopus nigricans*[9] or *Aspergillus ochraceus* gave 16α,17α-epoxy-11α-hydroxypregnane-3,20-dione (Va) in 30–40% yield. Unreacted IV accounted for most of the residual steroid and could be separated easily from V by chromatography.[10]

The structure of Va was proved as follows. Mild acetylation with acetic anhydride–pyridine gave 11α-acetoxy-16α,17α-epoxypregnane-3,20-dione (Vb). Oxidation of Va with chromium trioxide in acetic acid gave 16α,17α-epoxypregnane-3,11,20-trione (VI). Treatment of VI with hydrogen bromide in acetic acid gave the bromohydrin VII which was not isolated. Hydrogenation of crude VII with palladium–calcium carbonate catalyst[11] gave 17α-hydroxypregnane-3,11,20-trione (VIII) with melting point and optical rotation almost identical with that of VIII prepared from 3α-hydroxypregnane-11,20-dione[12] and infrared spectrum identical to that published by Dobriner, Katzenellenbogen and Jones.[13] After our re-

(1) A laboratory of the Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture. Article not copyrighted. Paper presented at the 134th National Meeting of the American Chemical Society, Chicago, Ill., September 7–12, 1958.

(2) Paper XLVI, Wall and Walens, THIS JOURNAL, **80**, 1984 (1958).

(3) (a) M. E. Wall, *et al.*, *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 1 (1954); (b) **43**, 503 (1954); (c) **44**, 438 (1955).

(4) R. E. Marker, *et al.*, THIS JOURNAL, **69**, 2167 (1947).

(5) M. E. Wall, H. E. Kenney and E. S. Rothman, *ibid.*, **77**, 5665 (1955).

(6) P. L. Julian, *et al.*, *ibid.*, **71**, 756 (1949); **71**, 3574 (1949); **72**, 5145 (1950).

(7) Under our experimental conditions there was only slight hydrolysis of the axial 3 β-acetate group.

(8) Rather surprisingly we have been unable to find prior references to compounds IIIa or IIIb.

(9) *R. nigricans* has been utilized for 11α-hydroxylation of a large number of steroids; *cf.* S. H. Eppstein, P. D. Meister, H. C. Murray and D. H. Peterson, *Vitamins and Hormones*, **14**, 359 (1956), but has not been recorded as having been tested on IV.

(10) We have not attempted to develop further the microbiological conversion of IV to Va. It would seem from the excellent review of Eppstein, *et al.*, ref. 9, that a detailed study of this conversion would almost certainly lead to higher yields.

(11) F. B. Colton, W. R. Nes, D. A. Van Dorp, H. L. Mason and E. C. Kendall, *J. Biol. Chem.*, **194**, 235 (1952).

(12) T. H. Kritchevsky, D. L. Garmaise and T. F. Gallagher, THIS JOURNAL, **74**, 483 (1952).

(13) K. Dobriner, E. R. Katzenellenbogen and R. N. Jones, "In-