TABLE IV

CONVERSION OF GLUCOSE 6-PHOSPHATE TO KETOSE PHOSPHATE

Each vessel contained 0.2 μmoles of glucose 6-phosphate 12 μmoles of sodium borate and 15 μmoles of NaOH in a total volume of 0.2 ml.

Time at 90–100°, sec.	0	30	45	60	90	120
Ketose phosphate present, %	0	30	55	75	80	70

absence and presence of borate is about the same as the maximum conversion at 100°. The rate of formation of ketose in each case is much slower at 37°.

TABLE V

ISOMERIZATION AT 37°

Each vessel contained 2 μmoles of each of the sugars indicated; vessel A contained 50 μmoles of NaOH; vessel B contained 400 μmoles of NaOH and 80 μmoles of $Na_2B_4O_7$; the final volume in each case was 2.0 ml.; aliquots were taken at the indicated times.

	Sugar added					
	A Glucose	B	A Lactose	B	A Xylose	B
Time, hr.	Alkali alone	With borate	Alkali alone	With borate	Alkali alone	With borate
	Ketose formed, μmoles		Ketose, formed, μmoles		Δ 540 mμ	
8	0.7	1.2	0.7	1.3	1.2	2.9
18	.8	1.5	.7	1.6	0	3.9
25	.8	1.6	.7	1.5	0	3.7
42	.4	0.75	.5	1.0	0	1.9

Discussion

The Lobry de Bruyn–Alberda van Ekenstein transformation usually gives mixtures of aldoses and ketoses as well as products from various side reactions. Organic acids are the principal products of the irreversible reactions along with small amounts of saccharinic acids. Another side reaction, the formation of color, is directly related to the time of heating, molarity of base and the aldose concentration.[18] Increasing amounts of alkali cause an increase in the conversion of aldose to ketose but also a corresponding increase in the formation of acids from the ketose. Thus a maximum yield of ketose is obtained when the rate of reactions forming ketose equals the rate of reactions removing ketose.

The present study indicates that by using dilute alkali and sugar concentrations and by the addition of borate to the reaction mixture the yield of ketose is increased. It appears that the additions of borate causes the alkali-catalyzed isomerization reaction to be altered in favor of ketose. Secondary irreversible reactions arising from further transformations of the ketose are greatly reduced in the presence of borate ion.

Cohen[4] observed that the degradation of ribulose and ribulose-5-P at neutral or slightly alkaline *p*H's is greatly inhibited by the addition of borate at *p*H 8. In the present study it would appear that D-fructose and D-xylulose are also protected from degradation in alkali when borate is added to the solution. The reaction was allowed to proceed only one minute. At longer times or at higher sugar concentrations, the yield of ketose decreases and the amount of color formed increases. The percentage of ketose formed also appears to be a function of the sugar to borate ratio. It is probable that this ratio would be different for different aldoses. The ratio of sugar to borate for optimum conversion to the ketose has been shown to vary greatly in enzyme-catalyzed isomerizations.[4,5,7]

When the starting aldose is available in only small quantities or when identification of an aldose by conversion to the corresponding ketose is desired a procedure for bringing about the formation of ketose in high yield by alkali isomerization might prove to be quite useful. Amounts from 0.2 μmole to 3000 μmoles of aldose were used in the experiments described.

Acknowledgments.—The author wishes to express his gratitude to Dr. Luis Leloir and Dr. Enrico Cabib for their inspiring guidance and advice throughout his stay in this Laboratory. The author acknowledges with deep appreciation the assistance and helpful criticism of the members of the "Instituto de Investigaciones Bioquimicas."

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, GEORGETOWN UNIVERSITY, WASHINGTON 7, D. C.]

2-Deoxy Sugars. II. 3β-(2,6-Dideoxy-α-D-*ribo*-hexopyranosyl)-14β-hydroxy-5β-card-20(22)-enolide. A Direct Method of Synthesis of 2-Deoxyglycosides Involving a Crystalline 2-Deoxy-acylglycosyl Halide[1]

BY W. WERNER ZORBACH AND THOMAS A. PAYNE

RECEIVED FEBRUARY 11, 1960

The partial synthesis of a 2'-deoxycardenolide is described. The coupling of a crystalline digitoxosyl chloride with digitoxigenin gave, after saponification, a monodigitoxoside of digitoxigenin. The anomeric configurations of the new monoside and of two crystalline digitoxosyl halides have been assigned. The synthetic cardenolide shows a definite digitalis-like action.

In a previous communication[2] we reported the successful coupling of 3β,14β-dihydroxy-5β-card-20(22) - enolide (digitoxigenin) (VI) with 2,6 - dideoxy-D-*ribo*-hexose (digitoxose) (I), which are, respectively, the steroidal and carbohydrate components of the natural cardenolide, digitoxin. The latter is very important clinically as a cardiotonic agent and is the U.S.P. reference standard for related drugs.

At the outset it was hoped that a successful synthesis would yield a monodigitoxoside identical

(1) This work was supported largely by National Science Foundation Grant G-7351.

(2) W. W. Zorbach and T. A. Payne, THIS JOURNAL, **81**, 1519 (1959).

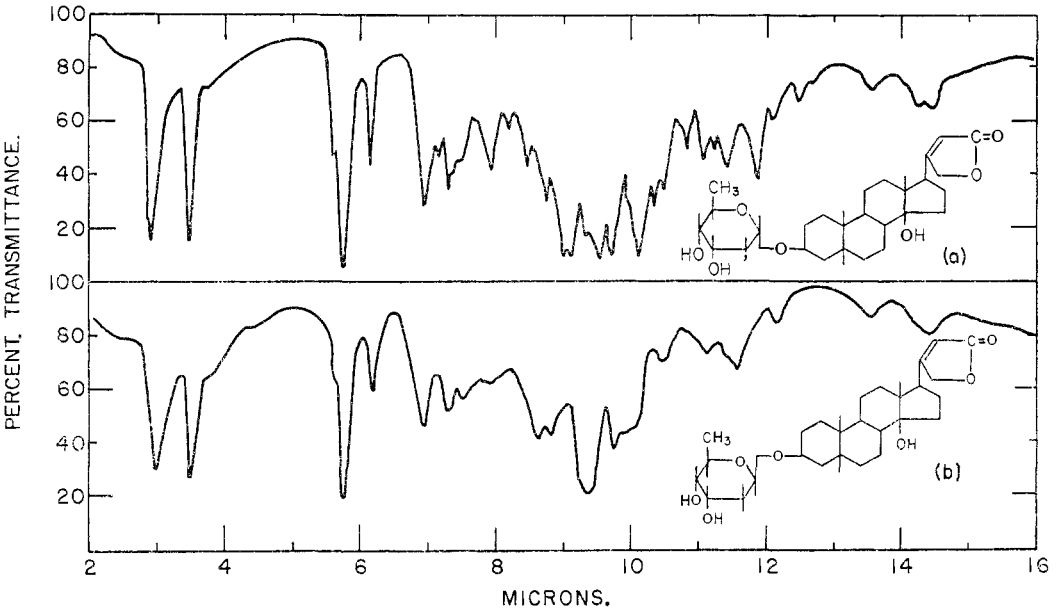


Fig. 1.—Infrared spectra of the anomeric monodigitoxosides of digitoxigenin: (a), digitoxigenin-3-(α-D-digitoxopyranoside); (b), digitoxigenin-3-(β-D-digitoxopyranoside).

with that obtained by Kaiser and co-workers as the result of a controlled, partial hydrolysis of digitoxin.[3] Physical data and paper chromatographic analysis revealed that our monoside VII was, in fact, *not* identical with Kaiser's compound; this has since been verified.[4] On the assumption that the "natural" monoside is a β-glycoside, our synthetic compound was most likely the anomeric α-glycoside VII, but because of the extremely small amount of material at hand, optical rotational measurements were not possible and application of Klyne's rule[5] could not be made.

Our synthesis depended on the securing of crystalline and reasonably stable glycosyl halides from 2,6-dideoxy-β-D-*ribo*-hexose (I),[6] and the use of *p*-nitrobenzoyl derivatives *in lieu* of acetates or benzoates permitted a satisfactory resolution of this difficult problem. The only prior report of a crystalline 2-deoxy-acylglycosyl halide is that of 3,4,6-tri-*O*-benzoyl-2-deoxy-D-*arabino*-hexosyl bromide (3,4,6-tri-*O*-benzoyl-2-deoxy-D-glucosyl bromide).[7] No crystalline halides from *O*-acetyl and *O*-benzoyl derivatives of other 2-deoxy sugars have been described and we were equally unsuccessful with the tribenzoate of digitoxose (I).[6] The two crystalline glycosyl halides IIIa and IIIb are very reactive; the chloride IIIa, as expected, was less sensitive than the bromide IIIb and was, therefore, used in this investigation but, considering the ease with which IIIa (as well as IIIb) split out hydrogen halide, heating and the use of an acid acceptor were contraindicated. That the evolved hydrogen chloride might lead to an equilibrium not favoring glycoside formation was ignored because of the bulk of the steroid moiety which should render the glycosidic linkage relatively inaccessible to attack. Further, the reaction was carried out under anhydrous conditions in a non-polar solvent. Also, the possibility that the liberated hydrogen halide might hydrolyze the protecting groups during glycosidation was disregarded since *p*-nitrobenzoates are more stable than acetates.

The coupling of digitoxigenin (VI) and an excess of the crystalline chloride IIIa was carried out in dry dichloromethane. Attempts to obtain the *O*-acylated glycoside in crystalline form failed; the total reaction mixture was, therefore, carefully saponified and extracted with ether to remove the monoside from the troublesome sugar derivatives; in this way it was possible to obtain a 45% yield of the crystalline monoside VII. Its optical rotation was determined and application of Klyne's rule of molecular rotational additivities[5] indicated that VII was an α-glycopyranoside.

TABLE I

Substance	$[\alpha]_D$ (in methanol)	$[M] \times 10^{-2}$
Methyl α-D-digitoxopyranoside[a]	+192°	+311°
Methyl β-D-digitoxopyranoside (V)[b]	− 5.1	− 8.3
Digitoxigenin (VI)	+ 19.1	+ 71
Digitoxigenin β-D-digitoxopyranoside[c]	− 5.5	− 27.7
Digitoxigenin α-D-digitoxopyranoside (VII)	+ 85.1	+429

[a] H. R. Bolliger and P. Ulrich *Helv. Chim Acta*, **35**, 97 (1952). [b] This paper. [c] Private communication from Dr. F. Kaiser. [d] [M](VI) + [M](methyl α-D-digitoxopyranoside) = +382°.

The infrared spectrum of VII is of particular interest in view of its marked similarity to that of Kaiser's monoside.[3] Comparison of the two spectra[8] (Fig. 1) reveals only minor differences

(3) F. Kaiser, E. Haack and H. Spingler, *Ann.*, **603**, 75 (1957).

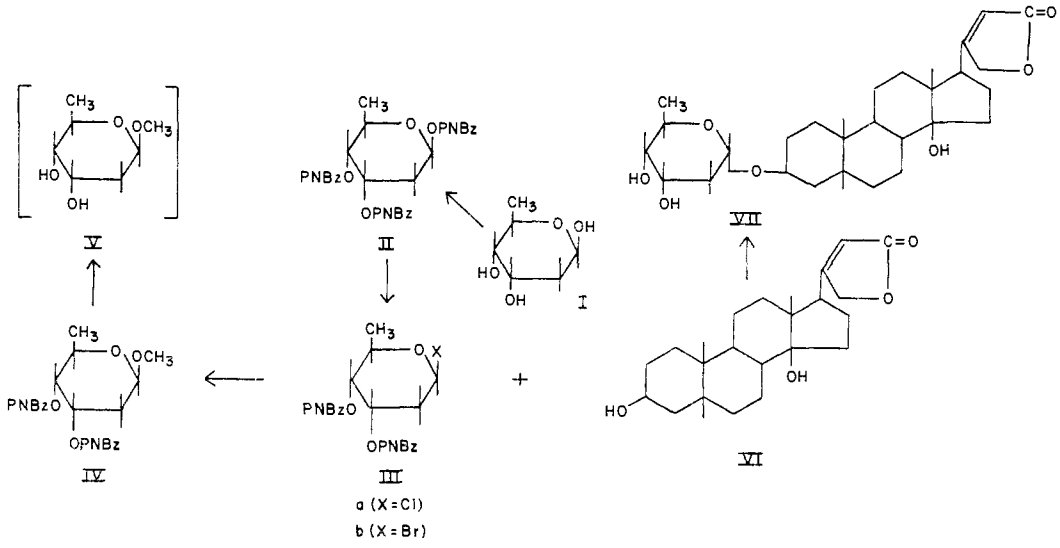
(4) Private communication from Dr. F. Kaiser.

(5) W. Klyne, Proc. Biochem. Soc., 288 Meeting, *Biochem. J.*, **47**, xli (1950).

(6) W. W. Zorbach and T. A. Payne, THIS JOURNAL, **80**, 5564 (1958).

(7) M. Bergmann, H. Schotte and W. Leschinsky, *Ber.*, **56**, 1052 (1923).

(8) Permission to publish these spectra was kindly given by the Sadtler Research Laboratories, Philadelphia, Penna.



which may be ascribed solely to the difference in configuration at the glycosidic linkage of the two cardenolides.

The percentage of digitoxose (I) in the monoside VII was estimated colorimetrically and gave results in good agreement with the calculated value. The cardenolide VII consumed one mole of periodate,[9] indicating a pyranoside ring inasmuch as the furanoside form would consume no periodate. Cleavage of the monodigitoxoside VII by transglycosidation,[10] which prevents the concomitant formation of "anhydrogenin," yielded only digitoxigenin (VI). After removal of the crystalline genin, the filtrate was heated to convert the methyl digitoxoside to the free sugar I, which was likewise isolated in crystalline form.

Halidation of the β-tri-*p*-nitrobenzoate II thus appears to proceed with retention of configuration. 2,6-Dideoxy-β-D-*ribo*-hexopyranose (digitoxose) (I) more closely resembles a pentose than a hexose from a structural standpoint when one considers the substitution of a methyl for the usual hydroxymethyl group at C5. According to the rules of Hassel and Ottar in their extended form,[11] the anomeric configuration of an *O*-acylated digitoxosyl halide should depend not on the orientation of the substituent at C5, but, rather, on that at C3. Thus, the more stable of the two halides must be the β-anomer, in which the C1 halogen and the C3 acyloxy group are *trans*. Support for this contention, *i.e.*, IIIa and IIIb are β-anomeric halides, is given by the observation that the chloride IIIa ([M] = +86500) is more dextrorotatory than the bromide IIIb ([M] = +70800).

It was reported previously[6] that treatment of the chloride IIIa with anhydrous methanol in the presence of silver carbonate failed to yield crystalline material. We have since felt that under these conditions glycal formation (brought about by the removal of elements of hydrogen halide by the silver carbonate)[12] might conceivably take precedence over methanolysis. In order to overcome this, both IIIa and IIIb were treated with a large excess of anhydrous methanol containing *no* acid acceptor; evaporation of the solvent without prior removal of the liberated hydrogen halide gave in each case the same crystalline methyl 2,6-dideoxy-3,4-di-*O*-*p*-nitrobenzoyl-D-*ribo*-hexoside (IV).

In an attempt to secure information regarding anomeric configuration, IV was converted to a methyl 2,6-dideoxy-D-*ribo*-hexopyranoside (V) employing methoxide ion; the transesterification was followed polarimetrically and was complete in 25 minutes, reaching a final specific rotation of −5.1°, indicating a β-anomeric configuration. Calculation of molecular rotational differences (see Table I) gives a Δ[M] of 31930 units which is well within the range of Δ[M] values for α- and β-anomeric methyl hexopyranosides. Although V could not be obtained in crystalline form and was not further characterized, this study strongly suggests that methanolysis of the halides IIIa and IIIb proceeds with retention of configuration. Even treatment of IIIa and IIIb with silver *p*-nitrobenzoate results in retention of configuration, giving the original β-tri-*p*-nitrobenzoate II.[6]

The fact that the coupling of the chloride IIIa with the genin VI gives rise to VII (an α-glycopyranoside) appears anomalous. It may be suggested that, because of the considerable bulk of the steroid moiety, the formation of the di-*p*-nitrobenzoate of VII is not governed by the same equilibrating conditions which lead, for example, to the formation of the β-glycoside IV from the chloride IIIa.

The fact that our new cardenolide VII contains an α-glycosidic linkage is interesting since natural cardenolides containing D-sugars are invariably

(9) These observations are the result of a separate study in this Laboratory and will be published at a later date.

(10) J. P. Rosselet and A. Hunger, *Helv. Chim. Acta*, **34**, 1036 (1951).

(11) W. Pigman, "The Carbohydrates," Academic Press, Inc., New York, N. Y., 1957, p. 152.

(12) This phenomenon has been clearly demonstrated in this Laboratory during nucleosidation studies. The chloride IIIa failed to couple with mercury derivatives of pyrimidine bases; with dithyminylmercury, the mercury was eliminated and converted to mercuric chloride. A carbohydrate product was isolated and had the composition of a 6-deoxy-3,4-di-*O*-*p*-nitrobenzoyl-D-*ribo*-hexopyranos-1-ene. Details of this conversion will be included in a forthcoming publication.

β-anomers. Biological assay of VII revealed a definite digitalis-like action. In 10 cats, the mean (geometric) lethal dose was found to be 0.5544 ± 0.0475 mg. kg.$^{-1}$.[13]

Acknowledgments.—The authors are indebted to Dr. Nelson K. Richtmyer for his advice and aid during the course of this investigation. They wish to thank also the personnel of the Microanalytical Laboratory, NIAMD, National Institutes of Health, under the direction of Dr. W. C. Alford, for the elemental analyses.

Experimental

All melting points were determined using a Kofler hot-stage.

3β-(2,6-Dideoxy-α-D-*ribo*-hexopyranosyl)-14β-hydroxy-5β-card-20(22)-enolide (VII).—To 37 ml. of dichloromethane containing 0.218 meq. ml.$^{-1}$ of anhydrous hydrogen chloride was added 2380 mg. (4.0 mmoles) of 2,6-dideoxy-1,3,4-tri-*O*-*p*-nitrobenzoyl-β-D-*ribo*-hexopyranose (II) and the suspension stirred magnetically for 0.5 hour with the exclusion of moisture. The material was worked up in a manner previously described[6] and was allowed to crystallize overnight from 20 ml. of ether–dichloromethane (1:1). This procedure provided an excess of the crystalline chloride IIIa of satisfactory quality for the following coupling reaction.

After decantation and rinsing with a small volume of ether–dichloromethane (1:1) the crystalline material thus secured was immediately dissolved in 5 ml. of dry dichloromethane; to this solution were added 523.6 mg. (1.4 mmoles) of digitoxigenin (VI) and an additional 5 ml. of dichloromethane. The resulting mixture was stirred in a stoppered flask for 24 hours without interruption; during the first 6 hours, however, the liberated hydrogen chloride was removed periodically by brief applications of vacuum. The resulting clear solution was transferred quantitatively to a stirring suspension of 1 g. of silver carbonate in 40 ml. of water–acetone (1:7) in order to quench any unreacted chloride IIIa and remaining hydrogen chloride. After 15 minutes, 2 g. of Celite 535 was added, stirring was continued for an additional 10 minutes and, finally, the mixture was filtered by suction. The clear filtrate was evaporated to dryness *in vacuo*, yielding a sirup which was dissolved in 10 ml. of acetone; this was added dropwise to a rapidly stirred solution of 1.6 g. of potassium carbonate and 72 ml. of water in 200 ml. of methanol. Stirring was continued for 10 days at room temperature after which time the solvents were evaporated *in vacuo* at 50° to a final volume of *ca.* 75 ml. The solution thus concentrated was extracted three times with 200-ml. portions of ether, which were combined and back-extracted with 100 ml. of water. The washed ether solution, after drying over magnesium sulfate, was evaporated to dryness, leaving a residue consisting chiefly of product and methyl *p*-nitrobenzoate (the expected by-product formed during the transesterification). The latter material was removed conveniently by sublimation for 4 hours at 100° under a reduced pressure of 10^{-3} mm. The remaining residue was triturated with a small amount of warm ethanol and filtered. One crystallization from 2-propanol gave 225 mg. (45%) of the pure monoside VII, m.p. 251–255°, $[\alpha]^{20}D$ +85.1 ± 0.5° (*c* 0.500, methanol), λ_{max}^{MeOH} 218 mμ (4.20). The compound gave a positive Kedde and a negative tetranitromethane test.

Anal. Calcd. for $C_{29}H_{44}O_7$: C, 69.00; H, 8.79. Found: C, 68.90; H, 8.50.

Colorimetric Estimation of Digitoxose Content of the Monoside VII Employing Xanthydrol Reagent.—The procedure followed was identical to that described by Kaiser and co-workers[3] except that the optical densities were determined at a wave length of 546 μ in a Beckman DU spectrophotometer.

Micro Cleavage of VII and Paper Chromatographic Detection of the Products. (a) Steroid Component.—To a warm solution of 0.7 mg. of VII in 5 ml. of methanol was added 5 ml. of 0.1 *N* sulfuric acid and the mixture refluxed for 25 minutes. After cooling, 100 mg. of barium carbonate was added and the mixture stirred for 0.5 hour. The suspension was carefully concentrated at room temperature to a volume of 5 ml. and an additional 50 mg. of barium carbonate added. After stirring for 10 minutes, the suspension was evaporated to dryness at 35°, the residue warmed with 5 ml. of methanol, filtered through a bed of barium carbonate and the clear filtrate evaporated to dryness at 25°. The sirupy residue was dissolved in 0.7 ml. of ethanol, giving a solution containing approximately 1 μg. of material per μl. Solutions of pure VII and of authentic digitoxigenin (VI) of comparable concentrations were prepared at the same time. Volumes of 50 μl. of each of the three solutions were spotted on Whatman No. 1 filter paper partially saturated with formamide (accomplished by immersing the paper in a 35% (v./v.) solution of formamide in acetone, then removing and allowing to dry in air for 20 minutes). After spotting, the paper was developed in an ascending system employing isopropyl ether–tetrahydrofuran (3:2) saturated with formamide. After 15 hours, the paper was removed, dried in an oven at 80° for 2 hours, and sprayed with Kedde reagent. R_f-values were: digitoxigenin (VI), 0.58; digitoxigenin-3-(α-D-digitoxopyranoside) (VII), 0.50; products of hydrolysis of VII, 0.58.

TABLE II

Substance	Concn., mg.	O.D.	$E_{1\ cm.}^{1\%}$	Found	Calcd.
Digitoxose (I)	5.1	0.1860	1095	(100)	100
Digitoxin	5.0	.1000	603	55.1	58.0
Digitoxigenin-3-(α-D-digitoxopyranoside) (VII)	5.3	.0555	314	28.7	29.3

(b) **Carbohydrate Component.**—To a solution of 0.5 mg. of VII in 2 ml. of acetone was added 5 ml. of 0.1 *N* sulfuric acid and the mixture heated for 40 minutes at 75°. To this was added 200 mg. of barium carbonate, and the resulting suspension was stirred for 15 minutes and evaporated to dryness at 30°. The residue was extracted three times with 10-ml. portions of acetone, and the acetone extracts were treated with 500 mg. of Celite 535 and filtered by suction. The clear filtrate was evaporated to dryness at 30° and the sirupy residue dissolved in 0.4 ml. of ethanol, giving a solution having a concentration of *ca.* 1 μg. per μl. A Whatman No. 1 filter paper was spotted with 50 μl. of this solution along with 50 μl. of an 0.1% solution of pure digitoxose (I). The paper thus prepared was developed in an ascending system employing ethyl acetate–pyridine–water (2:1:2). After 4 hours, the paper was removed, dried in air at room temperature, and sprayed with a boric acid reagent.[14,15] Only two spots appeared, exactly coincident in position (R_f 0.65).

A duplicate chromatogram was prepared and developed simultaneously. After drying, the paper was sprayed with Kedde reagent. The only Kedde-positive material was a single spot arising from the hydrolysis mixture, and followed the solvent front very closely (R_f 0.95).

Macro Cleavage of VII and Isolation of Digitoxigenin (VI) and Digitoxose (I).—To a hot solution of 75 mg. of the monoside VII in 25 ml. of methanol was added 25 ml. of 0.1 *N* sulfuric acid. The resulting solution was brought quickly to boiling and was maintained under gentle reflux for exactly 25 minutes. The methanol was evaporated *in vacuo* at 30° until turbidity appeared. The solution was seeded with crystalline digitoxigenin (VI) and refrigerated for 2 hours, then filtered by suction, and the crystalline residue washed with water. After drying, the material was dissolved in hot 2-propanol, treated with Norit A and filtered again. The filtrate was concentrated to a volume of 1 ml. and set aside for 2 days. The separated material was recrystallized from 2-propanol, giving 13 mg. (23%) of pure digitoxigenin (VI), m.p. 250.5–254°, giving no depression when admixed with an authentic specimen. A tetranitromethane test was negative.

(13) The authors are most grateful to Dr. K. K. Chen of the Eli Lilly and Co., Indianapolis, Ind., for carrying out this assay.

(14) M. Pöhm and R. Weiser, *Naturwiss.*, **24**, 582 (1956).

(15) The prescribed method for heating the paper in an oven to develop the color was found unsatisfactory as charring frequently occurred; this was overcome by placing the sprayed papergram around the outside of a large beaker containing water heated to 95°. Under these conditions, the gray-green coloration appeared in *ca.* 15 seconds, leaving the remainder of the paper unchanged.

The above aqueous filtrate, from which the genin VI was separated, was combined with the water washes and extracted three times with 10-ml. portions of chloroform. The latter, which contained a small quantity of the genin VI not originally separating, was discarded. The aqueous solution was evaporated *in vacuo* at 20° to remove the remainder of the methanol and the resulting aqueous solution heated at 65° for 30 minutes. To this was added 375 mg. of barium carbonate and the resulting suspension was stirred for 1 hour, filtered through a layer of barium carbonate, the filtrate evaporated to dryness at 25° and the resulting sirupy residue dissolved in 5 ml. of methanol. After treating with a small amount of Norit A, the methanolic solution was filtered, evaporated to dryness and the residue redissolved in a small volume of ethyl acetate. After the addition of a little *n*-pentane, the solution was seeded with a small quantity of digitoxose (I). After standing overnight, the crude, crystalline material was separated and recrystallized from ethyl acetate–*n*-pentane, giving 6.5 mg. (30%) of pure I, m.p. 100–103°, and giving no depression when admixed with an authentic specimen.

Determination of the Specific Rotations of the Chloride IIIa and the Bromide IIIb.—For the preparation of IIIa and IIIb for this study, 357 mg. (0.6 mmole) of the tri-*p*-nitrobenzoate II was weighed out in each case and treated according to the original directions.[6] After one crystallization from ether–dichloromethane (1:1), both halides were separated from the solvent by decantation and dried in high vacuum at room temperature. Dry nitrogen was admitted and the flasks were immediately stoppered. The flasks were carefully weighed, the halides dissolved in a minimum quantity of dry dichloromethane and diluted to 10 ml. accurately. Rotational measurements were made without delay. The chloride IIIa gave an $[\alpha]^{25}D$ + 185.9 ± 0.3° (*c* 2.10, $CHCl_3$) and the bromide IIIb an $[\alpha]^{25}D$ + 139.1 ± 0.7° (*c* 1.75, $CHCl_3$). The readings were not altered after 1 hour. It was possible to recover both halides in part.

Methyl 2,6-Dideoxy-3,4-di-O-*p*-nitrobenzoyl-β-D-*ribo*-hexoside (IV).—Crystalline bromide IIIb prepared as in the preceding experiment from 1190 mg. (2.0 mmoles) of the tri-*p*-nitrobenzoate II was dissolved in a minimum amount of dry dichloromethane and added rapidly to 100 ml. of absolute methanol. After 15 minutes, the methanol was evaporated *in vacuo* at 30°. The sirupy residue was dissolved in 5 ml. of methanol and the methanol evaporated again. This procedure was repeated three times more and the sirup which still contained traces of hydrogen bromide was dried at room temperature under a reduced pressure of 10^{-3} mm. The sirup thus dried was dissolved in methanol, treated with Norit A and filtered. The filtrate was evaporated *in vacuo* to a small volume and allowed to crystallize for 20 hours. The material, which amounted to 342 mg. (37% based on II), melted at 124–134°. Four recrystallizations from absolute ethanol gave pure IV, m.p. 132.5–135.5°, $[\alpha]^{25}D$ + 128.0 ± 0.3° (*c* 0.30 methanol). *Anal.* Calcd. for $C_{21}H_{20}O_{10}N_2$: C, 54.77; H, 4.38; N, 6.08. Found: C, 54.56; H, 4.58; N, 6.20.

By an identical procedure, 1190 mg. of II was converted to the chloride IIIa which, in turn, was subjected to methanolysis. Recrystallization of the resulting material likewise gave pure IV, m.p. 132.5–136°, undepressed when admixed with a specimen from the preceding preparation.

Deacylation of IV.—A solution of 74.8 mg. of methyl 2,6-dideoxy-3,4-di-*O*-*p*-nitrobenzoyl-β-D-*ribo*-hexoside (IV) in 25 ml. of absolute methanol was prepared and 20 ml. of this solution was transferred to a 1-dm. "reaction type" polarimeter tube. An initial reading was made, giving an $[\alpha]^{25}D$ + 128°. To this was added 1 ml. of 0.01 *N* methanolic sodium methoxide and, after 25 minutes, no further change in the optical rotation could be observed. After standing overnight, the reading was redetermined; this gave an $[\alpha]^{25}D$ −5.12°, based on the molecular weight of a methyl 2,6-dideoxy-D-*ribo*-hexopyranoside (V). The contents of the tube were evaporated *in vacuo* to a small volume, and diluted with ether and water. After separating, the ether phase was dried over magnesium sulfate and evaporated, leaving a crystalline residue which, when recrystallized from a little ethanol, gave pure methyl *p*-nitrobenzoate. The water layer was neutralized with dilute sulfuric acid and treated with 2 g. of Amberlite MB-1 ion-exchange resin. After filtering and evaporating to dryness, a clear sirup was obtained, which could not be brought to a crystalline state. The sirup did not reduce ammoniacal silver oxide.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, JOHN CURTIN SCHOOL OF MEDICAL RESEARCH, THE AUSTRALIAN NATIONAL UNIVERSITY, CANBERRA, A.C.T., AUSTRALIA]

The Synthesis of D-, L- and DL-2-amino-2-carboxyethyl 2-Guanidinoethyl Hydrogen Phosphate (Lombricine) and the Identity of the Natural Compound with the D Isomer

BY IVA M. BEATTY[1] AND DAVID I. MAGRATH

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The D, L and DL forms of 2-amino-2-carboxyethyl 2-guanidinoethyl hydrogen phosphate have been synthesized and the D isomer has been shown to be identical with the natural product, lombricine. The route involved the selective guanidination of D-, L- and DL-2-amino-2-carboxyethyl 2-aminoethyl hydrogen phosphate, respectively. The characterization of the products and their metal-catalyzed degradation in alkaline solution are discussed.

Thoai and Robin[2] in 1954 reported the isolation from earthworms (*Lumbricus terrestris*) of a new phosphorus-containing guanidine derivative, lombricine, to which, from evidence based on elementary analysis, a study of the functional groups present and paper chromatographic identification of the products of acid hydrolysis, they allocated the structure, 2-amino-2-carboxyethyl 2-guanidinoethyl hydrogen phosphate (I). The amount of material isolated was small and the configuration of the 2-amino-2-carboxyethyl, *i.e.* the serine, moiety was not investigated.

The corresponding phosphagen, 2-amino-2-carboxyethyl 2-N-phosphorylguanidinoethyl hydrogen phosphate (phosphoryllombricine, II) was also shown[2,3] to be present in the muscle. On mild acid hydrolysis it yielded phosphoric acid and a guanidine derivative, shown by paper chromatography to be identical with lombricine.

Synthesis of lombricine was, from our point of view, desirable for two reasons: first, to provide final confirmation of the structure allocated by Thoai and Robin[2] and to extend further the identification to the configuration of the serine moiety; secondly, to provide substrate material for a study of the enzymes associated with its biochemical function in muscle. The latter requirement has

(1) Australian National University Scholar.

(2) N. v. Thoai and Y. Robin, *Biochim. Biophys. Acta* **14**, 76 (1954).

(3) N. v. Thoai, J. Roche, Y. Robin and N. v. Thiem, *Compt. rend. soc. biol.*, **147**, 1670 (1953).