Since the riboflavin-C^{14} molecule is labeled in but one position, only the degradation of the urea-containing moiety can be followed. The metabolite found in this experiment, although as yet unidentified, can be characterized to some degree from the information obtained through the elimination of several ureides and the compounds resulting from the degradation of the ribityl group of riboflavin and from the cleavage of the pyrimidine ring. In further studies methods for obtaining larger quantities of the metabolite must be found to enable characterization by other means.

An amount of radioactive riboflavin in excess of that required for optimal growth was added to the culture medium in order to prolong the growth period and, hence, increase the total amount of riboflavin destroyed by the bacteria. As a result 290 μg. of riboflavin per liter was destroyed, whereas in experiments in which the culture medium contained 182 μg. per liter, 54 μg. per liter was destroyed during the same incubation period.[23]

Acknowledgment.—The authors wish to express their appreciation to Dr. Ralph W. Helmkamp of the Department of Chemistry for making available to them the method and facilities for the conversion of $BaC^{14}O_3$ to $KC^{14}N$, and to Doctors Leon H. Miller and Charles L. Yuile for gas ionization measurements of radioactivity, to Dr. Louis H. Hemplemann for the generous use of the Geiger–Müller counter and to Mr. Karl Sutter for the processing of the X-ray films.

ROCHESTER, NEW YORK

[CONTRIBUTION FROM THE BIOLOGY DIVISION, OAK RIDGE NATIONAL LABORATORY]

The Action of Acid on 2,7-Anhydro-β-D-*altro*-heptulopyranose[1]

BY L. P. ZILL AND N. E. TOLBERT

RECEIVED JANUARY 21, 1954

The acid treatment of 2,7-anhydro-β-D-*altro*-heptulopyranose has been shown to give rise to two compounds other than D-*altro*-heptulose which have been tentatively identified as 2,7-anhydro-β-D-*altro*-heptulofuranose and 5-(1,2-dihydroxyethyl)-2-furfuraldehyde. A method has been developed for the isolation of small amounts of these two compounds by the use of thick paper chromatography. An alternative method of preparing 2,7-anhydro-β-D-*altro*-heptulofuranose is based on the ion-exchange chromatography of the borate complexes of the components formed by the acid treatment of the pyranose anhydride. The orcinol method has been applied to the quantitative determination of sedoheptulosan and the limitations of the method discussed. The compound responsible for the orcinol reaction has been tentatively identified as 5-(1,2-dihydroxyethyl)-2-furfuraldehyde.

Introduction

During the experimental development of a method for the separation of the borate complexes of D-*altro*-heptulose (compound IV) and 2,7-anhydro-β-D-*altro*-heptulopyranose (compound I) by ion-exchange chromatography[2] it was noted that a third orcinol-reacting component was formed by the acid treatment of I. It was further reported by Noggle[3] that chromatography of an acid-treated sample of I, using phenol–water as solvent, gave four spots which produced positive tests with the orcinol-TCA (trichloroacetic acid) spray test.[4,5]

The ordinary procedure for the isolation of IV from natural sources involves its acid conversion to the anhydride, I, which may be crystallized, a property not shared by the sugar itself.[6,7] The formation of compounds other than the free sugar provides a complicating factor and source of loss in such an isolation procedure. In addition, an inconvenience is introduced in that the acid-catalyzed reconversion to the free sugar takes place only to the extent of about 20% and requires an additional step to separate the sugar from residual I. These manipulations have been circumvented in recent work by the use of thick-paper chromatography for the isolation of radioactive IV extracted from plants.[8]

In view of these considerations and of the increasing importance of IV (and consequently of I) in biochemical studies, it was considered highly desirable to conduct a study of the acid treatment of I. This paper represents such a study. Two unknown compounds have been tentatively identified as 2,7-anhydro-β-D-*altro*-heptulofuranose (II) and 5-(1,2-dihydroxyethyl)-2-furfuraldehyde (VI). The proposed conversions which are completely analogous to similar reactions known to occur in the pentose and hexose sugars are given in the following scheme. Compounds I, II and VI are formed under the influence of acid and heat. In addition, the implication of these reactions in the application and limitations of the orcinol reaction for the quantitative determination of I are presented.

Experimental and Results

Crystalline I, often referred to as sedoheptulosan, was prepared for all experiments by the general procedure of La Forge and Hudson.[6,7] Absorption spectra were obtained with a model DU Beckman spectrophotometer. Colorimetric determinations were made with an Evelyn colorimeter equipped with filters giving the wave lengths indicated in the procedure. Because of the ease of removal, Dowex-50 was used instead of acid in several of the experiments.

Ion-exchange Chromatography of the Borate Complexes of Products Formed During the Acid Treatment of I.—A sample of I (420 mg.) in 20 ml. of distilled water containing

(1) Work performed under Contract No. W-7405-Eng-26 for the Atomic Energy Commission.

(2) L. P. Zill, J. X. Khym and G. M. Cheniae, THIS JOURNAL, **75**, 1339 (1953).

(3) G. R. Noggle, *Arch. Biochem. Biophys.*, **43**, 238 (1953).

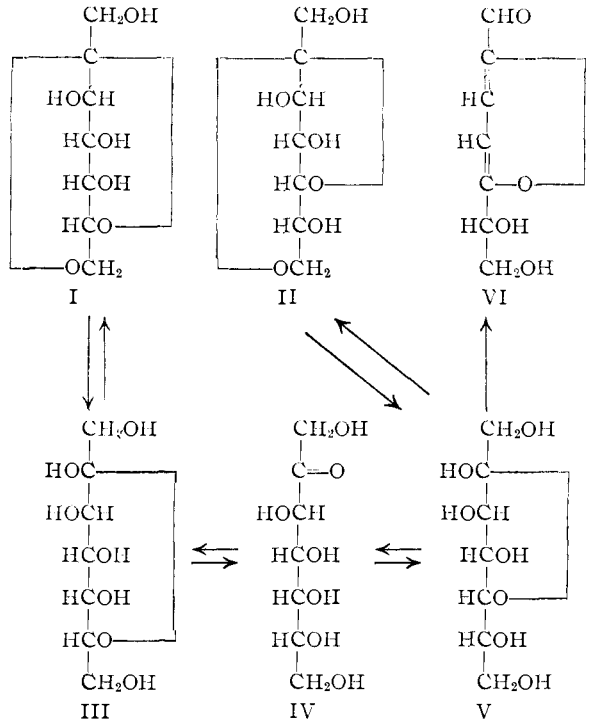
(4) R. Klevstrand and A. Nordal, *Acta Chem. Scand.*, **4**, 1320 (1950).

(5) A Bevenue and K. T. Williams, *Arch. Biochem. Biophys.*, **34**, 225 (1951).

(6) F. B. La Forge and C. S. Hudson, *J. Biol. Chem.*, **30**, 61 (1917).

(7) J. W. Pratt, N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **74**, 2200 (1952).

(8) N. E. Tolbert and L. P. Zill, *Plant Physiol.*, in press.



5 ml. (moist packed volume) of Dowex-50 was heated in a boiling water-bath for one hour. The Dowex-50 was removed by filtration and the filtrate volume reduced *in vacuo* to 2.5 ml. This was made 0.005 *M* in borate ions and placed on a column of Dowex-1 in the borate form. Elution and analysis of the sample were carried out as previously described.[2] The results presented in Fig. 1 demonstrate the presence of three components which are separable by this technique. The fractions of each peak were pooled, and the borate removed.[2]

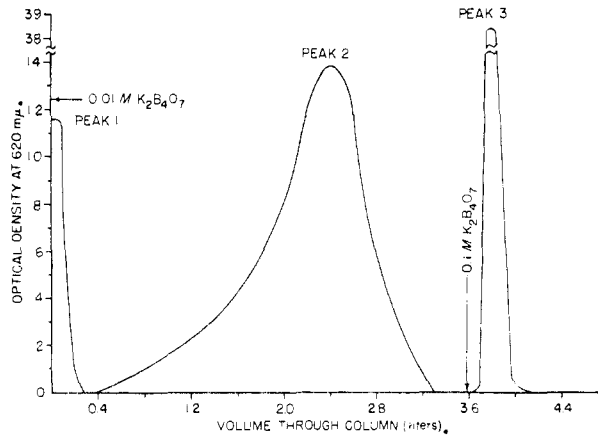


Fig. 1.—Separation of the products formed from acid treatment of sedoheptulosan (I). Exchanger, 0.85 sq. cm. × 11 cm., strong-base anion-exchange resin, *ca.* 300 mesh, borate form; eluting agent, potassium tetraborate as shown at 1 ml./min. Peak 1 contains 2,7-anhydro-β-D-*altro*-heptulofuranose; peak 2 contains 2,7-anhydro-β-D-*altro*-heptulopyranose; peak 3 is a mixture of D-*altro*-heptulose and 5-(1,2-dihydroxyethyl)-2-furfuraldehyde.

Paper Chromatography Separation of Compounds.—Samples of each peak from the ion-exchange chromatography were then subjected to paper chromatography in which a phenol–water solvent was used. The components were detected on the chromatogram by spraying with orcinol-TCA and heating for at least 20 minutes. Peak 1, which will be shown to be compound II, gave a single spot with an R_f value (0.66); this is very close to that of one of the unknowns reported by Noggle. Peak 2 was 2,7-anhydro-β-D-*altro*-heptulopyranose (I), indicated by a single spot with an R_f value identical with that given by an authentic sample. Peak 3 gave two spots—one was characteristic of D-*altro*-heptulose (IV) with an R_f of 0.44 and the other, having an R_f of 0.93 and corresponding to the other unknown reported by Noggle, has been identified as compound VI. Therefore, peak 3 which was initially thought to contain only one substance[2] is shown to contain two components. Attempts to resolve this mixture through their borate complexes on anion exchangers were unsuccessful.

The ion-exchange procedure has been routinely used for the preparation of compound II which is represented by peak 1 of Fig. 1. Yields of slightly over 2% were obtained in all cases. Borate could be removed from the pooled fractions of peak 1 by passage through a column of Dowex-1 (chloride form) followed by passage through Amberlite Monobed exchanger to remove the KCl. This procedure is applicable only to the first peak, since the borate concentration is low and borate complexing of the compound is negligible if not entirely absent.

Several other solvent systems for paper-chromatographic separation were investigated in an attempt to obtain better resolution of the various compounds and to aid in establishing their identity through their R_f values. The results are given in Table I. Phenol–water and butanol–propionic acid–water were prepared according to Benson, *et al.*,[9] acc-

TABLE I

R_f VALUES FOR THE VARIOUS COMPOUNDS AS OBTAINED BY PAPER CHROMATOGRAPHY (WHATMAN NO. 1)

Compound	Solvent: Phenol–water	Butanol–propionic acid–water	Butanol saturated with 2 *N* NH_4OH	Butanol–ethanol–water	Acetone–urea–water
I	0.69	0.30	0.12	0.13	0.73
II	.66	.28	.20	.22	.76
IV	.44	.22	.12	.13	.73
VI	.93	.73	.66	.65	.92

tone–urea–water according to Bentley and Whitehead,[10] butanol–ethanol–water according to Bevenue and Williams,[5] and butanol saturated with 2 *N* ammonium hydroxide according to Hird and Trikojus.[11] All of the four components formed by the acid treatment of compound I give a positive blue-green color after spraying the chromatogram with orcinol–TCA and heating to 105°. Compound VI gives the color immediately upon heating while D-*altro*-heptulose requires 5–10 minutes heating. Although sedoheptulosan can be detected after about 20 minutes heating, much better color development has been obtained by removing the chromatogram from the oven after about 10 minutes, respraying, and reheating. Sedoheptulosan appears first, followed by compound II. Bevenue and Williams[5] have reported that I does not give a positive test. This discrepancy, which has also been pointed out by Noggle,[3] can probably be attributed to differences in time of heating.

Isolation of Compounds II and VI by Thick-Paper Chromatography.—Sedoheptulosan (508 mg.) was dissolved in 50 ml. of 0.5 *N* H_2SO_4 and boiled under reflux for 17 hours. Sulfate was then removed by barium precipitation and final traces of ions removed by ion exchange. The volume was reduced *in vacuo* to *ca.* 4 ml. This solution was applied in the form of bands on four sheets of Whatman 3 MM filter paper equipped with wicks.[12] The chromatograms were developed with butanol–ethanol–water which, as is apparent from Table I, gives a suitable resolution of the desired compounds. The band of compound VI was easily located under ultraviolet light. Compound II and the equilibrium mixture of D-*altro*-heptulose–sedoheptulosan were located by spraying a narrow strip from the edge of the chromatogram with orcinol-TCA. These bands were then cut out,

(9) A. A. Benson, J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Haas and W. Stepka, THIS JOURNAL, **72**, 1710 (1950).

(10) H. R. Bentley and J. K. Whitehead, *Biochem. J.*, **46**, 341 (1950).

(11) F. J. P. Hird and V. M. Trikojus, *Aust. J. Sci.*, **10**, 185 (1948).

(12) J. H. Mueller, *Science*, **112**, 405 (1950).

and the compounds eluted with water. About 12 mg. of compound II and 21 mg. of VI were obtained, the remainder being present in the single band of I and IV.

Ultraviolet Absorption Spectra of Eluted Compounds.—The ultraviolet absorption spectra of the compounds prepared by thick-paper chromatography are given in Fig. 2.

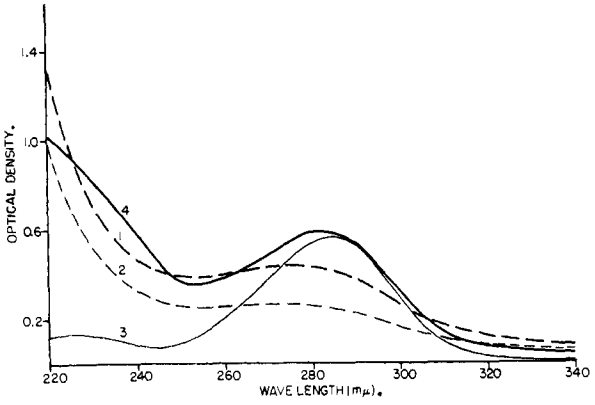


Fig. 2.—Ultraviolet absorption spectra: 1, a mixture of compounds I and IV; 2, compound II; 3, compound VI; 4, a mixture of compound IV and VI from peak 3 of Fig. 1.

Compound VI has an absorption spectrum which is almost identical with that of 5-(hydroxymethyl)-2-furfuraldehyde[13,14] but the absorption spectra for the other compounds are apparently non-specific. The ultraviolet absorption spectrum of peak 3 from the ion-exchange chromatograph of Fig. 1 is given in curve 4 of Fig. 2 and shows an absorption maximum at 285 mμ. This curve thus represents a composite spectrum of the non-specific absorption of D-*altro*-heptulose and the specific absorption of compound VI and confirms the paper chromatography identification of the two components of peak 3.

Periodate Oxidation of Compound II (Peak 1).—On the preliminary assumption that the molecular weight of compound II was the same as that of sedoheptulosan, 29 μmoles (5.60 mg.) was subjected to periodate oxidation according to established procedure.[15] After 24 hours 7.35 μmoles of periodate had been consumed.

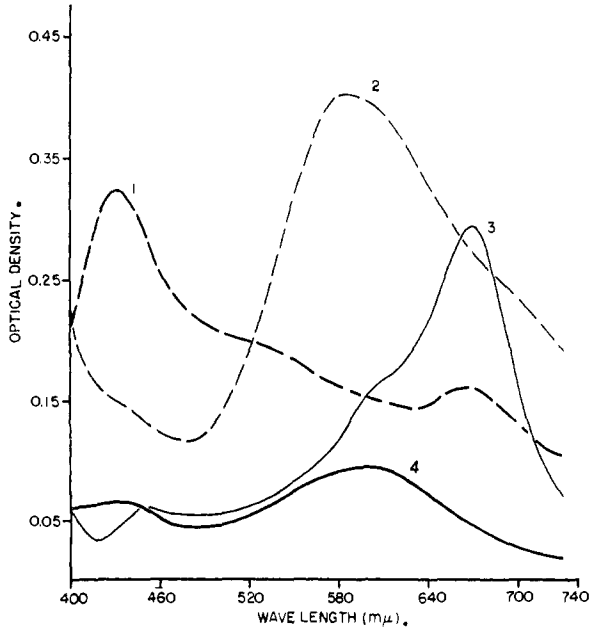


Fig. 3.—Absorption spectra of compounds found in orcinol reaction with fructose (curve 1), sedoheptulosan (curve 2), ribose (curve 3), and compound VI (curve 4). All samples were heated 10 minutes.

Action of Acid on Compounds II and VI.—Samples of these two compounds were subjected to the action of Dowex-50 for one hour in a boiling water-bath and then chromatographed with phenol–water and butanol–ethanol–water. Compound VI was recovered unchanged, whereas II had partially reverted back to a mixture of D-*altro*-heptulose, sedoheptulosan and compound VI.

Orcinol Reaction of Sedoheptulosan and Compound VI.—The absorption spectra of the reaction products of orcinol with sedoheptulosan and compound VI are given in Fig. 3. For comparative purposes the spectra obtained from the reaction of orcinol with D-ribose and D-fructose are included. The orcinol test was carried out according to Brown.[16] The effect of time of heating on the absorption spectra is given in Fig. 4. Curve 1, Fig. 5, shows the use of the absorption peak at 580 mμ for the quantitative determination of sedoheptulosan. A straight-line relation for samples up to 50 μg. may be obtained by preheating the sample in a boiling water-bath with 0.5 ml. of concentrated HCl before carrying out the orcinol reaction (curve 2).

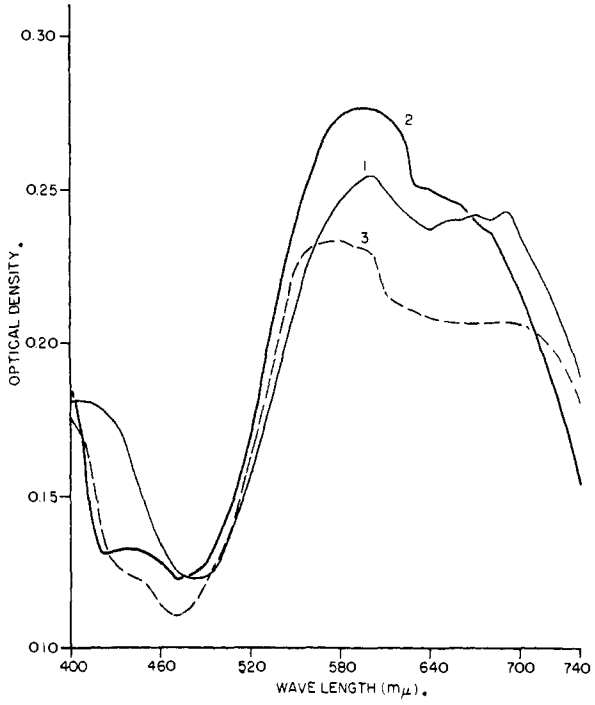


Fig. 4.—Absorption spectra of compounds formed in orcinol reaction of sedoheptulosan for various times of heating: 22 min., curve 1; 35 min., curve 2; 50 min., curve 3.

Effect of Prolonged Treatment of Sedoheptulosan with Acid.—Two samples of sedoheptulosan (112 mg. each) were exposed to the action of acid for 23 hours under reflux conditions. One sample was dissolved in 50 ml. of 0.5 *N* H_2SO_4 and the other in 50 ml. of water containing 12 ml. (wet packed volume) of Dowex-50 in the hydrogen form. At various time intervals samples were withdrawn and analyzed for reducing power by the method of Somogyi.[17] The samples containing H_2SO_4 were neutralized with NaOH before application of this procedure. The results presented in Fig. 6 indicate that the maximum amount of D-*altro*-heptulose is formed within 40 minutes with either treatment. After this time there is demonstrated a continual, gradual decrease in the amount of D-*altro*-heptulose. The greater

(13) M. L. Wolfrom, R. D. Schuetz and L. F. Cavalieri, THIS JOURNAL, **70**, 514 (1948).

(14) B. Singh, G. R. Dean and S. M. Cantor, *ibid.*, **70**, 517 (1948).

(15) E. L. Jackson in "Organic Reactions," (Editor, Roger Adams), Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 361.

(16) A. H. Brown, *Arch. Biochem.*, **11**, 269 (1946).

(17) M. Somogyi, *J. Biol. Chem.*, **160**, 61 (1945).

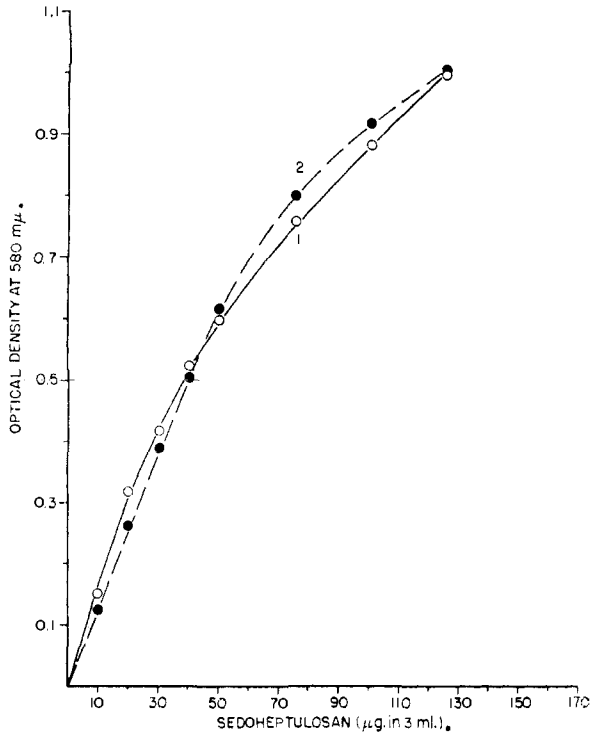


Fig. 5.—Variation of optical density with sedoheptulosan concentration for the orcinol reaction.

loss of reducing power obtained with the resin can probably be attributed to an increase in the effective acid concentration since none of the resin was removed with the samples. Samples chromatographed for each of the time intervals demonstrated a continual increase in the amount of compound VI whereas the amount of compound II appeared to remain almost constant.

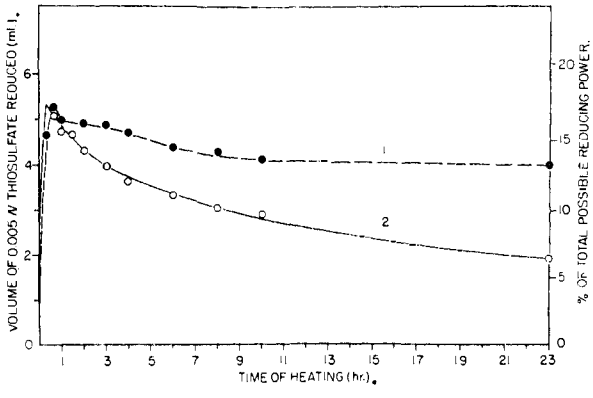


Fig. 6.—Effect of time of heating on the formation of D-*altro*-heptulose from sedoheptulosan in the presence of H_2SO_4 (curve 1) and strong-acid ion-exchange resin (curve 2).

Chromotropic Acid Test for Formaldehyde.—It has been shown that the hexoses and common disaccharides containing hexoses produce a violet color when heated with a sulfuric acid solution of chromotropic acid.[18] The reaction is caused by the conversion of hexoses to 5-(hydroxymethyl)-2-furfuraldehyde with subsequent splitting of the methylol group to form formaldehyde which then reacts with the chromotropic acid. Pentoses, which form furfural, do not react. Positive tests were not obtained with the acid concentration employed by Klein and Weissman but were obtained with several hexoses and disaccharides by using the reagent for the West and Rapoport modification of the chromotropic acid test.[19] A negative test was obtained with compounds I, II, IV and VI, which indicated the absence of formaldehyde formation.

(18) B. Klein and M. Weissman, *Anal. Chem.*, **25**, 771 (1953).

Discussion

On the basis of previous studies of the behavior of borate complexes of sugars and related polyhydroxy compounds on strong-base anion-exchange resins[2,20] it is possible to draw certain inferences from the behavior of the borate complexes of two previously unidentified compounds formed by the acid treatment of sedoheptulosan. It was shown in Fig. 1 that compound II appeared as a breakthrough fraction having no affinity for the resin. It has been adequately demonstrated[2,20] that such behavior of a polyhydroxy compound is caused by the absence of vicinal *cis*-hydroxyl groups which are requisite for the formation of a highly ionized borate complex. It is therefore concluded that compound II lacks such a grouping as part of its structure. On the other hand, compound VI which shows a strong affinity for the resin, must certainly possess the requisite *cis*-hydroxyl grouping. Other than this, no information as to the structural configuration with which the grouping is associated can be deduced from this type of data. The reversion of compound II to D-*altro*-heptulose, sedoheptulosan and compound VI under acid conditions strongly suggests a second anhydride of D-*alto*-heptulose. Most pertinent to such a possibility is the report by Stewart and Richtmyer[21] that two crystalline anhydrides, 1,6-anhydro-D-*glycero*-β-D-*gulo*-heptopyranose and 1,7-anhydro-D-*glycero*-β-D-*gulo*-heptopyranose may be obtained by the acid treatment of D-*glycero*-D-*gulo*-heptose. The finding that compound II was resistant to periodate oxidation (the small amount consumed is probably owing to partial reversion to sedoheptulose) is explained, not on the basis of absence of vicinal hydroxyl groups (in this case, necessarily *trans*), but on the basis that such groups, although present, are resistant to periodate oxidation. Both D-glucose and D-galactose form two crystalline anhydrides.[22,23] The 1,6-anhydro-β-D-glucofuranose and the 1,6-anhydro-β-D-galactofuranose do not react with periodate even though they contain a pair of vicinal —CHOH groups. The most likely second anhydride of D-*altro*-heptulose would then be the furanose form, 2,7-anhydro-β-D-*altro*-heptulofuranose. Additional support is furnished by the observation that compound II is even more difficult to hydrolyze than sedoheptulosan, as indicated by the relative rates of color development on chromatograms sprayed with the orcinol-TCA reagent and also with the relative amount of reversion under acid conditions to compounds I, IV and VI. The 2,7-anhydrofuranose ring system does not undergo the rapid acid hydrolysis which is usually associated with the normal furanoside structure but presents a relatively high stability toward acid

(19) C. D. West and S. Rapoport, *Proc. Soc. Exptl. Biol. Med.*, **70**, 141 (1919).

(20) J. X. Khym and L. P. Zill, THIS JOURNAL, **74**, 2090 (1952).

(21) L. C. Stewart and N. K. Richtmyer, Abstracts of Papers, 122nd Meeting, Amer. Chem. Soc., 1952, p. 1R.

(22) R. J. Dimler, H. A. Davis and G. E. Hilbert THIS JOURNAL, **68**, 1377 (1946).

(23) B. H. Alexander, R. J. Dimler and C. L. Mehltretter, *ibid.*, **73**, 4658 (1951).

hydrolysis, presumably as a result of the presence of the double lactol ring system.[24] The structural similarity of compound II with sedoheptulosan is further reflected in the similar R_f values obtained with several different solvent systems.

It has been demonstrated that the ketohexose, fructose, under the influence of HCl, is partially converted to two di-D-fructose dianhydrides.[25] The possibility of compound II being, in a similar manner, di-D-*altro*-heptulose dianhydride is excluded since several moles of periodate should be readily consumed. Conclusive proof of the structure of compound II must await the production of sufficient material for degradative studies, and the characterization of derivatives.

The formation of compound VI is amenable to explanation since the production of analogous compounds by the acid treatment of hexoses and pentoses is well known.[26] The absorption spectrum of compound VI is almost identical with that of 5-(hydroxymethyl)-2-furfuraldehyde. This spectrum is at variance with the absorption spectrum of furfural owing to substitution in the 5-position. Although the absorption spectrum indicates that the furan ring is still intact in compounds VI, the non-formation of formaldehyde on treatment with strong acid indicates that the absorption is not caused by 5-(hydroxymethyl)-2-furfuraldehyde. This is also shown by the comparative absorption spectra of glucose, ribose, sedoheptulosan and compound VI when reacting with orcinol. On the basis of the analogous reaction of acid on fructose[27] this compound would then be 5-(1,2-dihydroxyethyl)-2-furfuraldehyde. This compound contains a pair of *cis*-hydroxyl groups (or at least potentially of *cis* configuration) thereby explaining the affinity of the borate complex for the anion-exchange resin. The presence of the furan ring is shown by the obtained absorption spectrum.

The absorption spectrum of compound VI with orcinol exhibits the same maximum as does sedoheptulosan. It thus appears that the formation of compound VI is a requisite for the orcinol reaction of D-*altro*-heptulose and sedoheptulosan since compound VI is formed irreversibly from sedoheptulosan. The non-linearity of the orcinol reaction with larger amounts of sedoheptulosan is probably attributable to the cleavage of furan compounds in acid media. The formation of furfural from 5-(1,2-dihydroxyethyl)-2-furfuraldehyde is indicated by the increased absorption at 670 mμ with longer times of heating for the orcinol reaction. Pretreatment of sedoheptulosan with HCl before carrying out the orcinol reaction has been reported by Dische,[28] who has also applied the cysteine and diphenylamine reactions to the quantitative determination of sedoheptulosan. These latter reactions appear to be more applicable to the determination of larger amounts of sedoheptulosan. The non-linearity of the orcinol reaction with sedoheptulose (above *ca.* 12 μg./ml.) has been reported.[29]

Acknowledgment.—The authors wish to thank Dr. Nelson K. Richtmyer and Dr. James W. Pratt of the National Institutes of Health for suggesting the structure of compound II.

(24) R. J. Dimler, "Advances in Carbohydrate Chemistry," Academic Press, Inc., New York, N. Y., Vol. 7, 1952, p. 37.

(25) M. L. Wolfrom and M. G. Blair, THIS JOURNAL, **70**, 2406 (1948).

(26) A. P. Dunlop and F. N. Peters, "The Furans," Reinhold Publ. Corp., New York, N. Y., 1953.

(27) W. N. Haworth and W. G. M. Jones, *J. Chem. Soc.*, 65 (1944).

(28) Z. Dische, *J. Biol. Chem.*, **204**, 983 (1953).

(29) A. Nordal and R. Klevstrand, *Anal. Chim. Acta*, **4**, 411 (1950).

OAK RIDGE, TENNESSEE

[CONTRIBUTION FROM THE PHARMACEUTICAL INSTITUTE, MEDICAL FACULTY, UNIVERSITY OF KYUSHU]

Cholesterol and Related Compounds. I. Structure of a New Non-conjugated Cholestadienol from 7-Bromocholesterol

BY KYOSUKE TSUDA, KO ARIMA[1] AND RYOICHI HAYATSU[1]

RECEIVED DECEMBER 28, 1953

Treatment of 7α- and 7β-bromocholesteryl acetate (Ia) (benzoate, Ib) with pyridine or other bases gives, as the chief reaction product, $\Delta^{5,8(9)}$-cholestadienyl acetate (IIa) (benzoate IIb). Nitric acid oxidation of IIa gives methylpyromellitic acid (VI) while hydrogenation of IIa in ethyl acetate in the presence of platinum oxide gives $\Delta^{8(9)}$-cholestenyl acetate (III). Reduction of III with platinum oxide in acetic acid yields cholestanyl acetate (V). Chromic acid oxidation of IIa gives 7-keto-$\Delta^{5,8(9)}$-cholestadien-3β-ol acetate (VIII) which on catalytic hydrogenation with palladium is converted into 7-keto-cholestan-3β-ol acetate (IX). Dienone–phenol rearrangement of VIII with acetic anhydride and sulfuric acid gives a steroidal phenol (Xb) whose methyl ether (Xc) forms methylnitrobenzenetetracarboxylic acid (XI) by oxidation with nitric acid and an anthracene series hydrocarbon, $C_{19}H_{18}$ (XIIa and b), by dehydration with selenium.

A. E. Bide, *et al.*,[2] obtained $\Delta^{4,6}$-cholestadienyl acetate by treating 7α-bromocholesteryl acetate[3] (Ia) with 2,6-lutidine, and $\Delta^{4,6}$-cholestadienyl acetate and 7-dehydrocholesteryl acetate by the treatment of Ia with diethylaniline. H. Schalteggar, *et al.*,[4] obtained 7-dehydrocholesteryl benzoate by the treatment of 7α-bromocholesteryl benzoate (Ib) with dimethylaniline. One of the authors, Arima,[5] treated Ia with ammonium thiocyanate and obtained a new, non-conjugated dienol of m.p. 147–148°. The present paper describes the detailed examinations of conditions for formation of

(1) Takamine Research Laboratory, Sankyo Co., Ltd., Tokyo, Japan.

(2) A. E. Bide, H. B. Henbest, E. R. H. Jones, R. W. Peevers and P. A. Wilkinson, *J. Chem. Soc.*, 1783 (1948).

(3) In the present paper the more dextrorotatory isomer is named "7β."

(4) H. Schalteggar and F. X. Mullner, *Helv. Chim. Acta*, **34**, 1096 (1951).

(5) K. Arima, *Pharm. Bull. (Japan)*, **1**, 224 (1953), *et seq.*