the stability of the *cis* isomer relative to the situation in the unsubstituted α-phenylcinnamic acids.

Experimental[9]

α-Phenylcinnamic Acids.—The preparation of the acids was carried out according to Fieser's directions.[1a] The only modifications were those made necessary by the relatively low solubility of many of the *trans* acids in ether. The pH adjustment required for precipitation of the *cis* isomer was checked with a Leeds and Northrup pH meter. The immiscible solvent used in the extraction, the recrystallization solvents, and the pH adjustment made to precipitate the *trans* acids are given in Table I.

The details are illustrated by the preparation of α-*p*-nitrophenyl-*cis*- and *trans*-*p*-nitrocinnamic acid from *p*-nitrophenylacetic acid and *p*-nitrobenzaldehyde (the low solubility of this *trans* acid and its salts requires the most extensive changes in conditions). A solution of 6.11 g. (0.040 mole) of *p*-nitrobenzaldehyde and 6.66 g. (0.037 mole) of *p*-nitrophenylacetic acid in 4 ml. of pyridine and 4 ml. of acetic anhydride was heated under reflux in an oil bath maintained at 150° for 30 min. A precipitate often forms during this heating period. The reaction mixture was acidified with 8 ml. of concentrated hydrochloric acid, the solid broken up and the whole mass transferred to a separatory funnel and dissolved in 1200 ml. of methylene chloride. This solution was washed with several 200-ml. portions of water and then extracted with three 200-ml. portions of 0.2% sodium hydroxide. The combined extracts were then acidified to a pH of 4.0 with about 10 ml. of acetic acid. The *trans* acid was filtered from the solution containing the salt of the *cis* acid and washed with water. The yield was 8.95 g., m.p. 255–265°. Recrystallization from 200 ml. of acetic acid gave 7.5 g., m.p. 265–270°. The *cis* isomer was precipitated by the addition of 10 ml. of concentrated hydrochloric acid to the above solution to give 1.2 g., m.p. 204–207°.

Alternately the reaction mixture, after acidification with 8 ml. of concentrated hydrochloric acid, can be stirred with 100 ml. of water, the solid collected, and washed with water. This solid can be extracted by stirring with successive portions of 0.2% sodium hydroxide and the *trans* and *cis* acids precipitated with acetic and hydrochloric acid, respectively, as described above. The reaction proceeds at lower temperature (100° for 2 hr.) or in the presence of larger amounts of either acetic anhydride or pyridine or both. The yield is not affected by these changes.

The separation of the *trans* and *cis* isomers sometimes is not satisfactory. This is easily detected by either the melting point or the infrared spectrum. In these cases the incompletely separated fraction can be redissolved in alkali and reprecipitated as described above. These difficulties may stem from the fact that the optimum pH for precipitation of the *trans* isomer is a function of the ionic strength, temperature, and concentrations of acids as well as the nature of the acids being separated.

Conversion of the α-Phenyl-*trans*-cinnamic Acids to the Equilibrium Mixtures of *cis* and *trans* Isomers.—Equilibration was accomplished by the method of Zimmerman[6] by heating 2.2 mmoles of the purified *trans* acid under reflux in a mixture of 5 ml. of triethylamine and 5 ml. of acetic anhydride for 22 hr., except in the case of the dinitro acids where triethylamine was replaced with pyridine and the reaction time decreased to 4 hr.

The composition of the acidic fractions of the equilibrium mixtures was determined by analysis of the infrared spectra of the acids and reaction product mixtures in potassium bromide.[10] Synthetic mixtures of the experimentally determined composition gave spectra that were identical with those obtained from the reaction mixtures. The fact that a ratio of concentration rather than absolute concentrations were determined decreases many errors encountered in infrared analyses in potassium bromide.

In those cases where the infrared bands were not sufficiently resolved (III, IV, and VI) the mixtures were analyzed by carefully separating the isomers by the method described under the preparative experiments. When the equilibration reactions were carried out for preparative purposes the ratios for the *cis* and *trans* isomers were always within 3% of the analyses reported in Table III. The acidic fraction gave no evidence of decomposition. Any stilbene formed by decarboxylation would have been removed in the extraction process. The per cent recovery of the acids is given in Table III.

Infrared and Ultraviolet Spectra.—The ultraviolet spectra were measured in 95% ethanol with a Carey, Model 11, spectrophotometer. The infrared spectra in potassium bromide were recorded on a Perkin–Elmer, Model 21, spectrophotometer.

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(9) Melting points are uncorrected. Microanalyses are by the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley, Calif.

(10) Many of the substituted α-phenyl-*trans*-cinnamic acids were not sufficiently soluble in carbon disulfide which was the solvent that Zimmerman (ref. 6) used for his infrared analyses. Our results, therefore, probably are not so accurate as his.

The Preparation of Radioactive D-Galactose from Radioactive D-Glucose[1]

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D-Galactose-U-C^{14},4-T was prepared from D-glucose-U-C^{14} by conversion to 2,3:5,6-di-*O*-isopropylidene D-glucose-U-C^{14} dimethyl acetal. This substance was oxidized to di-*O*-isopropylidene-4-keto D-glucose-U-C^{14} dimethyl acetal which was subsequently reduced with lithium aluminum tritide, yielding the corresponding galactose and glucose derivatives from which the free sugars were obtained after hydrolysis.

The mechanism whereby biological systems bring about an epimerization of the hydroxyl group on carbon 4 of D-galactose to form D-glucose has been the subject of numerous investigations[3–9] and a number of possible mechanisms have been postulated and investigated. Largely from experiments using isotopes, it has been

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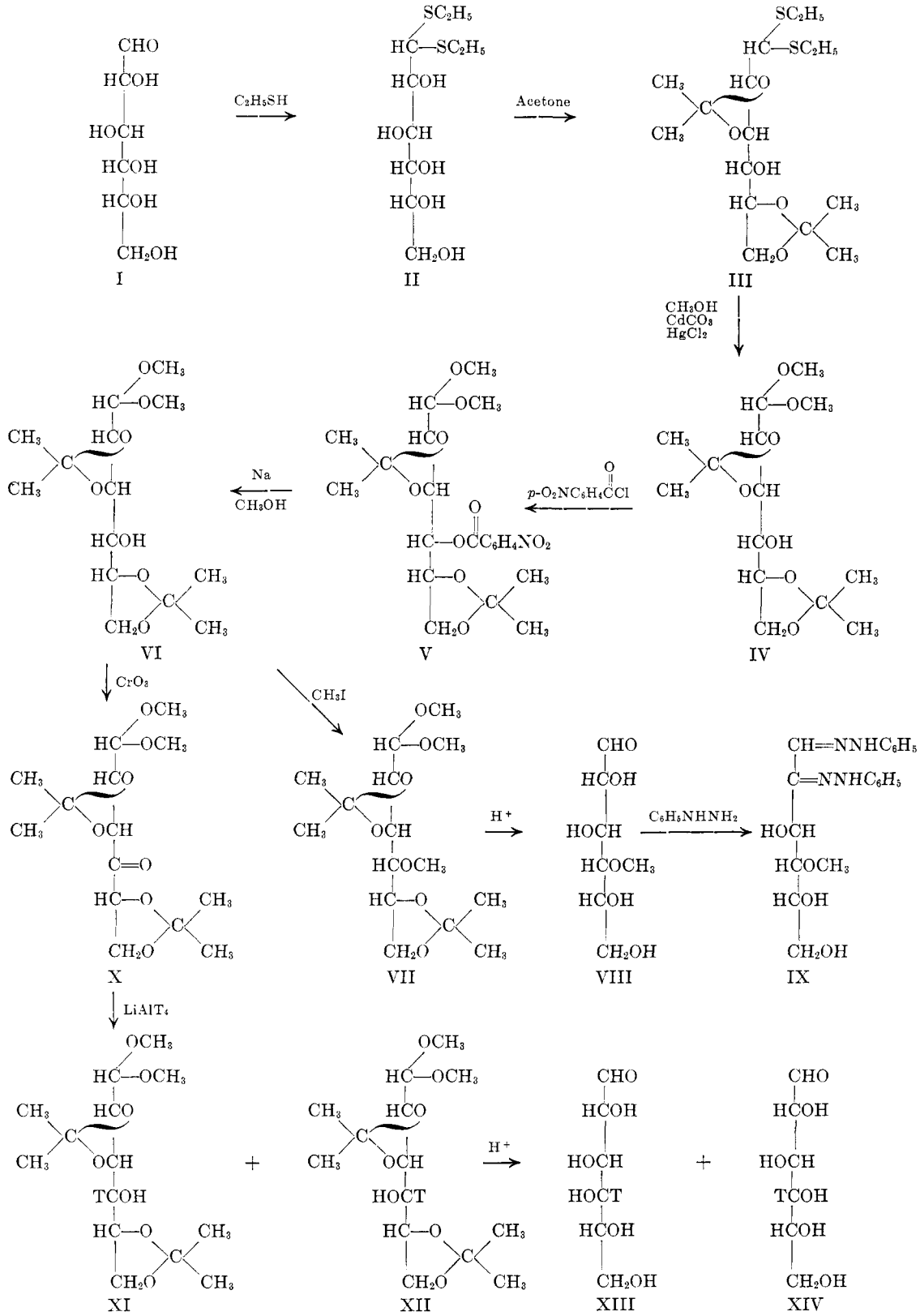

possible to reach a tentative conclusion that the process involves an oxidation of the hydroxyl group at carbon 4 to a keto group, followed by a reduction to yield the isomer having the opposite steric configuration. Direct evidence for this mechanism has not been obtained, but the isotope studies apparently have ruled out all other mechanisms thus far proposed. In an attempt to elucidate the mechanism further, we wished to prepare galactose labeled with C^{14} and with tritium only on carbon 4, with the expectation that if oxidation to a keto group occurred, tritium would be lost and the reduced compound would have either no tritium or a considerably lowered tritium to carbon-14 ratio. Since the doubly-labeled galactose could be prepared by oxidation of C^{14}-labeled glucose at carbon 4 followed by reduction with tritium, it was necessary to obtain a suitable derivative with all of the hydroxyl groups blocked except that on carbon 4. A derivative of this

nature would also be of value for the preparation of 1→4 linked disaccharides.

Of those intermediates that may have been used for this investigation,[10-15] 2,3:5,6-di-*O*-isopropylidene D-glucose dimethyl acetal was prepared and purified through the crystalline *p*-nitrobenzoate. On a macro scale the preparation of this substance proceeds readily in good yield, and is therefore satisfactory for use in the preparation of disaccharides. This report describes the preparation of this compound from carbon-14 labeled D-glucose and its conversion to D-galactose labeled with both carbon-14 and tritium.

The initial reaction carried out was the preparation of D-glucose diethyl dithioacetal (II) from D-glucose (I) labeled in all carbons with carbon-14,[16] using the method of Hough and Taylor.[17] By treatment with acetone, II was converted to a mixture of isopropylidene derivatives[11,12,18] in which 2,3:5,6-di-*O*-isopropylidene D-glucose diethyl dithioacetal (III) predominates. The corresponding dimethyl acetals (IV) were prepared by the method of Wolfrom, *et al.*[19] From the mixture of compounds, obtainable only as oils, crystalline 2,3:-5,6-di-*O*-isopropylidene-4-*O*-*p*-nitrobenzoyl D-glucose dimethyl acetal (V) was prepared. Hydrolysis of V with sodium methoxide yielded 2,3:5,6-di-*O*-isopropylidene glucose dimethyl acetal (VI) as an oil. Its structure was demonstrated in two ways. The 4-*O*-methyl derivative (VII) of VI was prepared by the method of Freudenberg and Hixon.[20] On acid hydrolysis, 4-*O*-methyl D-glucose (VIII) was obtained as a sirup. Paper chromatography of the sirup revealed only two substances, one corresponding to glucose and the other, more rapidly moving than glucose, having an R_g = 2.34, similar to other monomethyl glucose derivatives.[21]

The sirup was subjected to chromatographic separation on paper. A radioautogram was prepared and the area corresponding to the methyl derivative was eluted from the paper with water. From this aqueous solution of VIII, 4-*O*-methyl D-glucosazone (IX) was prepared.[22] Since only 4-*O*-methyl glucose and glucose were detected, VI must have had a free hydroxyl only at carbon 4. A second demonstration of this was deduced as a consequence of those reactions leading to galactose.

Compound VI was oxidized to 2,3:5,6-di-*O*-isopropylidene-4-keto D-glucose dimethyl acetal (X)[23] and the reaction mixture was chromatographed on silica gel.[24] From the column both X and unoxidized VI were recovered. Those fractions with an absorption band in the 1705–1725-cm.$^{-1}$ region due to ketone groups and no absorption band in the 3590–3650-cm.$^{-1}$ region due to hydroxyl groups[25] were pooled and reduced[26] with $LiAlT_4$,[27] yielding the diisopropylidene dimethyl acetal derivatives of D-glucose (XI) and D-galactose (XII) containing tritium on carbon 4, as well as carbon-14 in the carbons of the carbohydrate. The mechanism of the reduction is such that tritium does not appear in the hydroxyl group on carbon 4.[18] Paper chromatography following acid hydrolysis showed the presence of galactose (XIII) and glucose (XIV) only. It therefore follows that the reduction must have been carried out on a compound having a keto group at carbon 4 only. If any hydroxyl group other than that at carbon 4 had been free during the oxidation, the oxidation product would have been subsequently reduced and carbohydrates other than glucose and galactose would have been produced. Glucose and galactose were separated by paper chromatography and eluted from the paper with water. Studies of the biological conversion of galactose to glucose using the doubly labeled galactose will be the subject of a separate report.

Experimental[29]

D-Glucose-U-C^{14} Diethyl Dithioacetal (II) and 2,3:5,6-Di-*O*-isopropylidene D-Glucose-U-C^{14} Diethyl Dithioacetal (III).—From D-glucose-U-C^{14} (1 g., 5.55 mmoles), 0.96 g. (60%) of D-glucose-U-C^{14} diethyl dithioacetal, m.p. 129–130°, was obtained by treatment with ethanethiol.[17] On treatment with acetone this material was converted to a mixture of isopropylidene derivatives, in which III predominates.[11,12] An oil weighing 1.1 g. was obtained.

2,3:5,6-Di-*O*-isopropylidene D-Glucose-U-C^{14} Dimethyl Acetal (IV).—The oil (1.1 g.) containing III and other isopropylidene derivatives was converted to a mixture of the corresponding dimethyl acetals by the method of Wolfrom, *et al.*,[19] modified by the use of absolute methanol in place of ethanol and employing a temperature of 65–70°.

2,3:5,6-Di-*O*-isopropylidene-4-*p*-nitrobenzoyl D-Glucose-U-C^{14} Dimethyl Acetal (V).—The oil, IV, (0.8 g.) was dissolved in 5 ml. of dry pyridine and 750 mg. (4 mmoles) of *p*-nitrobenzoyl chloride was added. The reaction mixture was allowed to stand at room temperature for 13 hr. and then poured into a mixture of water and crushed ice. A precipitate formed. The aqueous mixture containing the precipitate was extracted three times with 50-ml. portions of petroleum ether (b.p. 60–70°). The petroleum ether layer was washed twice with 10-ml. portions of 5% sodium bicarbonate and three times with 10-ml. portions of water. The solution was then concentrated to a sirup which spontaneously formed a crystalline mass. After recrystallization from methanol, 650 mg. of V was obtained, m.p. 106–107°, $[\alpha]^{20}D$ +0.79° (*c*, 20.0; chloroform).

Anal. Calcd. for $C_{21}H_{29}O_{10}N$: C, 55.38; H, 6.37; N, 3.08. Found: C, 55.07; H, 6.53; N, 3.06.

Deacylation of V.—Non-isotopic V was added as carrier to the isotopic material prepared above. A portion of the mixture (910 mg., 2 mmoles) was suspended in 8 ml. of absolute methanol and 5 ml. of sodium methoxide solution (10 mg. sodium per ml. of methanol) was added. The reaction mixture was allowed to stand at room temperature for 3 hr., poured into 200 ml. of 0.3 *N* sodium hydroxide, warmed until a small amount of solid

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material dissolved and a slight cloudiness disappeared, and allowed to stand for 3 hr. at room temperature.

The solution was extracted five times with 50-ml. portions of chloroform. The chloroform extract was washed once with 50 ml. of 5% sodium bicarbonate and twice with 50-ml. portions of water, dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated to an oil weighing 630 mg.

2,3:5,6-Di-*O*-isopropylidene-4-*O*-methyl D-Glucose-U-C^{14} Dimethyl Acetal (VII).[20]—The oil (630 mg.) obtained by alkaline hydrolysis of V was dissolved in 5 ml. of anhydrous ethyl ether. Thin plates of freshly cut sodium were added and the reaction mixture was placed on a shaker at 25° overnight. The mixture was filtered through glass wool and concentrated under reduced pressure to a red-brown solid. On addition of 5 ml. of methyl iodide the solid dissolved and the solution was placed in a water bath at 35°. After 1 hr. the mixture had solidified, an additional 5 ml. of methyl iodide was added and the precipitate was broken up with a glass stirring rod. After 1 hr. more, anhydrous ether was added and the suspension was filtered by gravity. The precipitate was washed several times with small volumes of ether. The filtrate was concentrated to an oil in a 12-ml. centrifuge tube. Paper chromatography indicated the presence of two major substances, and the oil was presumed to be a mixture of VI and VII.

4-*O*-Methyl D-Glucose-U-C^{14} (VIII).—To the oily mixture of VI and VII, 2 ml. of 1 *N* hydrochloric acid was added and the tube was placed in a boiling water bath. After 30 min. the oil had dissolved. The tube was removed from the bath after 1 hr. A small aliquot gave a strongly positive Benedict's test for reducing sugars. The solution was transferred to a separatory funnel, diluted to about 10 ml., and extracted twice with 15-ml. portions of 5% di-*n*-octylmethylamine[30] in chloroform. The chloroform layer was washed once with water. The neutral aqueous solution was concentrated under reduced pressure at 35–40° to a small volume. A trial chromatogram on a small aliquot of the sample showed the presence of two radioactive substances, one of which corresponded to glucose. The second substance, which moved more rapidly than glucose, as do other monomethyl ethers of glucose,[21] was assumed to be 4-*O*-methyl glucose. Determination of the isotope content of the two spots with a Tracerlab chromatogram strip scanner showed 580 c.p.m. in the glucose spot and 940 c.p.m. in the methyl glucose spot, indicating a 62% yield of the methyl derivative. The remainder of the solution was streaked on a sheet of Whatman 3-mm. filter paper. The chromatogram was developed by descending technique with *t*-amyl alcohol–water (11:2) for 48 hr. A radioautogram was prepared, the more rapidly moving substance was eluted from the paper with water and the solution was concentrated to a sirup which exhibited only one radioactive reducing spot on paper chromatography in three different solvent systems.

4-*O*-Methyl D-Glucosazone (IX).—From 17 mg. of sirupy VIII, the osazone was prepared as described by Kohn, *et al.*[22] The product melted at 158–160°, $[\alpha]^{18}D$ −15.0° (*c*, 0.33; ethanol; equilibrium after 24 hr.) in agreement with the reported values for 4-*O*-methyl D-glucosazone, m.p. 158–160°, $[\alpha]^{15}D$ −15.46° (*c*, 0.58; alcohol; equilibrium after 21 hr.).[11,12,18]

2,3:5,6-Di-*O*-isopropylidene-4-keto D-Glucose-U-C^{14} Dimethyl Acetal (X).[23]—A three-necked flask containing 620 mg. (6.2 mmoles) of chromium trioxide dissolved in 6.2 ml. of dry pyridine and equipped with a stirrer and a reflux condenser having a calcium chloride tube, was immersed in an oil bath at 70°. While the contents were being vigorously stirred, a solution of 620 mg. of VI in 6.2 ml. of dry pyridine was added. After 45 min. the mixture was poured into 100 ml. of water and crushed ice and the aqueous mixture was extracted once with 150 ml. of a 1:1 benzene–ethyl ether mixture and three times with 50-ml. portions of the same solvent mixture. The extract was washed four times with 25-ml. portions of water, dried over sodium sulfate, and concentrated under reduced pressure at 30° to an oil weighing 575 mg.

The products of the oxidation were separated by chromatography on silica gel as follows.[24]

To 40 g. of silica gel (Davison, grade 923, 100–200 mesh) 8 ml. of water was added and the mixture was thoroughly stirred. Sufficient petroleum ether was added to produce a heavy suspension, and the mixture was allowed to stand for 2 hr. The slurry was poured into a column 1 cm. in diameter to a height of 42 cm. and allowed to settle for 2 hr. The oil (575 mg.) obtained from the oxidation was dissolved in a small volume of benzene–hexane (1:1) and applied to the top of the column. Elution of the column was carried out as shown in Table I. Each of the fractions collected were examined by infrared spectrophotometry to detect those fractions which contained compounds with a keto group, an hydroxyl group, or both. As noted in Table I, fractions 13–24 contained a compound or compounds having a keto group. These fractions were pooled as fraction A. The spectra on fractions 27–34 indicated the presence of compounds having free-hydroxyl groups, but no keto compounds could be detected. The fractions were pooled as fraction B.

TABLE I

SILICA GEL CHROMATOGRAPHY FOLLOWING CrO_3 OXIDATION OF 2,3:5,6-DI-*O*-ISOPROPYLIDENE D-GLUCOSE-U-C^{14} DIMETHYL ACETAL

Fraction number	Eluent	Fraction volume, ml.	Effluent[a] content
1	Benzene–hexane (1:1)	50	Nil
2	Benzene	50	Nil
3–7	Benzene–ether (49:1)	10	Nil
8–12	Benzene–ether (24:1)	10	Nil
13–17	Benzene–ether (24:1)	10	Keto group
18–24	Benzene–ether (19:1)	10	Keto group
25–26	Benzene–ether (9:1)	10	Nil
27–28	Benzene–ether (9:1)	10	Hydroxyl group
29–33	Benzene–ether (3:1)	10	Hydroxyl group
34	Benzene–ether (1:1)	100	Hydroxyl group

[a] Effluent fractions were screened by infrared spectrophotometry solely to detect those fractions containing substances having keto and/or hydroxyl groups.

Fraction A was concentrated under reduced pressure to an oil weighing 145 mg. In some runs traces of hydroxyl groups could be detected in those fractions containing compounds having a keto group. This was presumed to be due to traces of water and could be eliminated by rechromatogramming on silica gel exactly as described except that the silica gel was not pre-treated with water. Fractions eluted with benzene–ether (3:1) contained only compounds having keto groups, indicating that the anhydrous conditions employed altered the elution characteristics of the substance present.

Fraction B was concentrated under reduced pressure, yielding 410 mg. of an oil which was dissolved in 3.5 ml. of pyridine. To the solution, 580 mg. (3.1 mmoles) of *p*-nitrobenzoyl chloride was added, yielding 430 mg. of recrystallized 2,3:5,6-di-*O*-isopropylidene-4-*p*-nitrobenzoyl D-glucose-U-C^{14} dimethyl acetal, prepared as previously described, m.p. 107–108°, undepressed mixed with V. This material was set aside to be hydrolyzed to VI, which could then be subjected to chromic oxide oxidation.

Reduction of X with Lithium Aluminum Tritide.[25]—The oil (145 mg.) recovered from the silica gel column was dissolved in 15 ml. of anhydrous diethyl ether. To the solution, 10 mg. of $LiAlT_4$ (specific activity 2.5 mc. per mg.)[27] was added and the suspension was refluxed for 1 hr. Reduction was continued by addition of non-isotopic lithium aluminum hydride in two further portions, 10 mg. with refluxing for 1 hr. and 125 mg. with refluxing for 2 hr.

Excess lithium aluminum hydride was destroyed by addition of a small amount of water. Ice-cold 2 *N* sulfuric acid and diethyl ether were added and the mixture was shaken vigorously. The ether layer was separated, washed with 5% sodium carbonate and water, and dried over anhydrous sodium sulfate. The salt was removed by filtration and the filtrate was concentrated to an oil weighing 100 mg., consisting of a mixture of 2,3:5,6-di-*O*-isopropylidene D-glucose-U-C^{14},4-T dimethyl acetal (XI) and 2,3:5,6-di-*O*-isopropylidene D-galactose-U-C^{14},4-T dimethyl acetal (XII).

D-Galactose-U-C^{14},4-T (XIII) and D-Glucose-U-C^{14},4-T (XIV).—To the mixture of XI and XII (100 mg. of oil) 9 ml. of water and 0.3 ml. of concentrated hydrochloric acid were added and the mixture was placed in a boiling water bath for 1 hr. The solution was extracted with 15-ml. portions of a 5% solution of di-*n*-octylmethylamine in chloroform until the aqueous layer was neutral. The chloroform layer was washed with water and the aqueous solution was concentrated at reduced pressure to a sirup weighing 70 mg. Paper chromatography and radioautograms revealed the

(30) Obtained from K. and K. Laboratories, Jamaica, N. Y.

presence of only two radioactive reducing substances, which corresponded in mobility to glucose and galactose.

The two carbohydrates were separated by large scale paper chromatography. The areas containing each of the carbohydrates were located with radioautograms, and the sugars were eluted with water and concentrated to sirups under reduced pressure at 35°. The sirup containing glucose-U-C^{14},4-T was stored at −20°.

The sirup containing galactose-U-C^{14},4-T (45 mg.) was dissolved in methanol and a methanol solution containing 40 mg. of unlabeled galactose was added in order to reduce rapid decomposition attributed to a high tritium content. After refrigeration of the methanolic solution a crystalline precipitate formed which was recrystallied twice from methanol, yielding 38 mg. of galactose, m.p. 166–168°, $[\alpha]^{18}D$ +79.4° (*c*, 1, water, equilibrium), having a specific activity of 3.97×10^4 disintegrations per minute of C^{14} per mg. and 4.18×10^6 disintegrations per minute of H^3 per mg.[31] Further amounts of galactose can be obtained from the mother liquors.

(31) Assays for radioisotopes were carried out by New England Nuclear Assay Corporation, Boston, Mass.

1,6-Anhydro-2,3-di-*O*-methyl-β-L-idopyranose and 1,6-Anhydro-2,3,4-tri-*O*-methyl-β-L-idopyranose[1]

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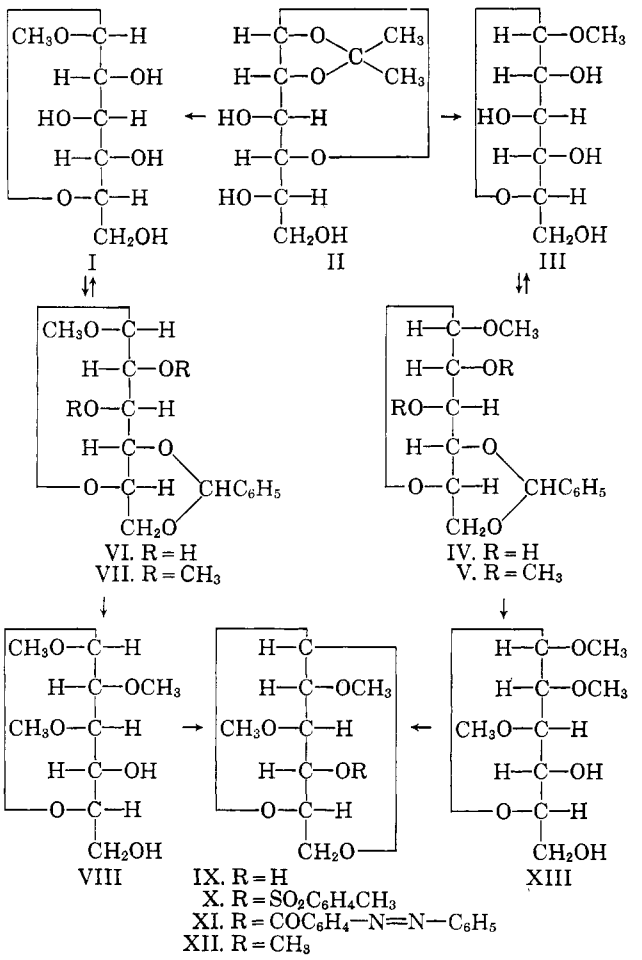
The syntheses of 1,6-anhydro-2,3-di-*O*-methyl-β-L-idopyranose, a reference substance used in the elucidation of the structure of dermatan sulfate, and of 1,6-anhydro-2,3,4-tri-*O*-methyl-β-L-idopyranose are described.

Recently, it has been shown that the uronic acid component of dermatan sulfate (also named β-heparin or chondroitin sulfate B), a polysaccharide isolated from various connective tissues, is L-iduronic acid.[4] Investigation of the structure of this polysaccharide by the methylation procedure resulted in the isolation of a dimethyl ether of 1,6-anhydro-β-L-idopyranose, which was found to be identical to the 2,3-di-*O*-methyl ether described in the present publication.[5]

In order to obtain the methyl ether derivatives of L-idose to be used as reference substances, the synthesis of methyl α-L-idopyranoside (I) and methyl β-L-idopyranoside (III) was undertaken, starting from 1,2-*O*-isopropylidene-L-idofuranose (II).[6] Reaction with methanolic hydrochloric acid gave a sirupy mix ture, and a partial separation of the two anomers could be achieved by partition chromatography on cellulose powder. A more convenient separation was obtained by condensing the sirupy mixture of glycosides with benzaldehyde in the presence of zinc chloride. The resulting mixture was chromatographed on silicic acid and gave two crystalline products: one (VI) melting at 148–149° and having an optical rotation $[\alpha]^{22}D$ −47° in chloroform; the other (IV) melting at 159–161° and having an optical rotation $[\alpha]^{22}D$ +87° in the same solvent.

The corresponding compounds in the D-series have been prepared and their structures firmly established: methyl 4,6-*O*-benzylidene-α-D-idopyranoside melts a 148–149° and has $[\alpha]^{14}D$ +49°, whereas the β-anomer melts at 163–164° with $[\alpha]^{20}D$ −88°, both rotations



measured in chloroform.[7] Consequently, on this basis, the α-L-configuration was allocated to the anomer VI, and the β-L-configuration to the anomer IV. Further substantiation of these allocated structures was obtained by study of the periodate oxidation of both methyl α-L-idopyranoside (I) and β-L-idopyranoside (III), obtained from VI and IV by reductive debenzyli-

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