tetrachloride, and 100% chloroform, and weighed 500 mg., 675 mg. and 129 mg., respectively.

When fraction 1 was crystallized from absolute ethanol, it gave 193 mg. of a white crystalline compound, m.p. 135–148.5°; recrystallization gave 105 mg., m.p. 146.5–154.5°.

When fraction 2 was crystallized from absolute ethanol, there sesulted 390 mg. of white crystals, m.p. 150–153°. An analytical rample was taken from this product, and the recrystallized product melted at 157–158°.

Anal. Calcd. for $C_{18}H_{15}ClO_2$: C, 72.36; H, 5.06. Found: C, 72.31; H, 4.89.

When fraction 3 was recrystallized from absolute ethanol, it yielded 22 mg. of white crystals, m.p. 145–155°. Mixture melting points with fractions 1 and 2 were not depressed. Mixture melting points and solution infrared spectra of this compound showed it to be identical with the chloroacetate obtained from the acetolysis of II or III.[8]

***syn*-8-Chlorodibenzobicyclo[3.2.1]octadien-*exo*-2-ol. (XIII).**—A slurry of 40 mg. of crushed lithium aluminum hydride in approximately 20 ml. of ether, which had been dried over sodium ribbon, was stirred while an ethereal solution of 134 mg. (0.448 mmole) of the combined fractions 1 and 2 above (compound XII) was slowly added. The reaction mixture was stirred at room temperature for 2.5 hr., and the excess lithium aluminum hydried was destroyed by the addition of water. The solution was decanted, the residual aluminum hydroxide was washed several times with ether, and the decantates were combined. The solvent was evaporated in an air stream and the residue was dride under vacuum over phosphorus pentoxide, leaving 112 mg. (97%) of white crystals, m.p. 111–122°. An infrared spectrum taken in carbon disulfide solution showed a strong hydroxyl peak at 2.80 μ and no acetate carbonyl absorption.

The product was recrystallized from absolute ethanol, yielding 35 mg. of white crystals, m.p. 130–134.5°. A second crop of crystals was taken, yield 13 mg., m.p. 127.5–136.5°. The crude yield was 42%. Crop 1 was recrystallized twice from absolute ethanol to give pure XIII, m.p. 137.5–138.5°.

Anal. Calcd. for $C_{16}H_{13}ClO$: C, 74.78; H, 5.10. Found: C, 74.91; H, 5.03.

***syn*-8-Chlorodibenzobicyclo[3.2.1]octadien-2-one (XIV).**—A mixture of 312 mg. (1.21 mmoles) of crude XIII (m.p. 120–135°), 40 ml. of benzene, 2 g. of potassium permanganate (12.7 mmoles), 20 ml. of *t*-butyl alcohol and 6 ml. of water was heated at 60° for 75 hr. with constant stirring. The excess potassium permanganate was destroyed by the addition of aqueous sodium bisulfite. The solution was dried under vacuum, 40 ml. of water was added, and the manganese dioxide precipitate was filtered and washed with water. The filter cake was extracted with hot chloroform, and the extract dried over sodium sulfate. The organic solvent was removed under vacuum, leaving 190 mg. of a white crystalline material, m.p. 106–112°. The product was dried over phosphorus pentoxide under vacuum, and the infrared spectrum showed no hydroxyl absorption and a strong carbonyl absorption at 5.88 μ.

Of the 190 mg. obtained, 72 mg. of the product was chromatographed on an alumina column. By dissolving in hot petroleum ether (b.p. 60–70°) and eluting with chloroform, a fraction weighing 66 mg. (60%), m.p. 114.7–116°, was obtained. Recrystallization from absolute ethanol gave *syn*-8-chlorodibenzobicyclo[3.2.1]octadien-2-one (XIV), m.p. 115.5–116.5°.

Anal. Calcd. for $C_{16}H_{11}ClO$: C, 75.44; H, 4.35. Found: C, 75.47; H, 4.38.

The Reduction of *syn*-8-Chlorodibenzobicyclo[3.2.1]octadien-2-one (XIV).—A mixture of 110 mg. (0.432 mmole) of XIV, 5 g. of amalgamated zinc, 6 ml. of concentrated hydrochloric acid, 5 ml. of acetic acid, and 5 ml. of toluene was heated at reflux for 22 hr. During this time, three 5-ml. portions of concentrated hydrochloric acid were added. The reaction mixture was extracted three times with 30-ml. portions of benzene. The benzene extracts were washed with sodium carbonate solution, followed by several washings with water, and dried over anhydrous sodium sulfate. The solvent was removed under vacuum, leaving a thick, yellow oil. The product was chromatographed on an alumina column (25 g. of Merck Co., acid-washed alumina packed in petroleum ether, b.p. 60–70°) and was eluted with carbon tetrachloride. The crude yield was 73 mg., m.p. 110–143°. Recrystallization from absolute ethanol yielded a product weighing 32 mg., m.p. 142–144.5° (36%).

Mixture melting point showed no depression with the hydrogenolysis product of compound II, and the infrared spectrum was identical with that of product VIII.

Isomerization of Dichloride II to III in Liquid Sulfur Dioxide.—In a sealed Pyrex tube was placed 1 ml. of *o*-cresol, 398 mg. of II, and approximately 50 ml. of liquid sulfur dioxide. The mixture was allowed to stand for 4 hr. at 0° and then at room temperature for 10 hr. The sulfur dioxide was allowed to evaporate, and the solution was chromatographed on alumina by dissolving in petroleum ether (b.p. 60–70°) and eluting with carbon tetrachloride. Two fractions were obtained. The first was 220 mg. (55%) of a white crystalline product, m.p. 95–99°. After recrystallization from absolute ethanol, a melting point of 100–100.5° was obtained. The product had an infrared spectrum identical with that of III, and a mixture melting point with that compound showed no depression. A mixture melting point with II was depressed. The second fraction was an oil, 23 mg. (6%), which was not investigated.

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The Synthesis of 2-Acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-glucose[1]

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The synthesis of 2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-glucose, a product of degradation of a tetrasaccharide isolated from human milk and of various glycoproteins, is described, starting from D-galactose and D-glucosamine.

The structure of 2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-D-glucose (VI) has been assigned to a disaccharide of D-galactose and D-glucosamine, which has been isolated from the products resulting from the degradation of oligosaccharides obtained from human milk.[3,4] This compound has also been found, together with 2-acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-D-glucose, in the controlled acid-hydrolyzate of blood group A substance,[5–7] in which it forms an essential

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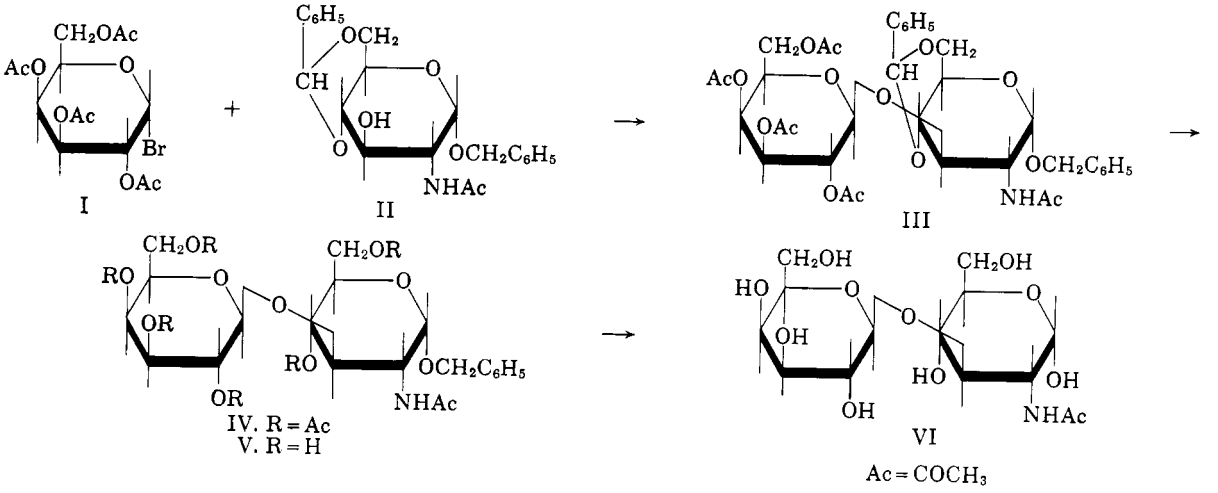

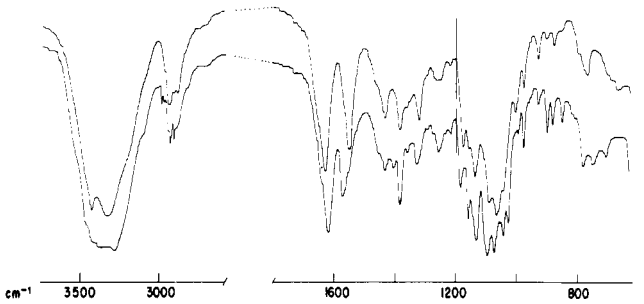

Fig. 1.—Infrared spectra of 2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-glucose measured immediately after preparation of compound (lower curve) and after compound had been stored six months in desiccator (upper curve). Both samples dried at 80°, over phosphorus pentoxide, under high vacuum, overnight; concentration 0.8 mg. in 200 mg. of potassium bromide.

part of the antigen. The disaccharide VI has been synthesized by an extract of bull testes[8] and by an enzymic extract of *L. bifidus var. pennsylvanicus*,[9] which also synthesizes the 4-isomer and 2-acetamido-2-deoxy-6-*O*-(β-D-galactopyranosyl)-D-glucose.

Both 2-acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-D-glucose[10,11] and the 6-isomer[12] have been chemically synthesized. In view of the major importance of the 3-isomer VI in the study of the chemical structure of glycoproteins, its chemical synthesis was investigated, and is reported in the present paper.

Condensation of tetra-*O*-acetyl-α-D-galactopyranosyl bromide (I)[13] with benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (II)[14] in a mixture of nitromethane and benzene in the presence of mercuric cyanide gave the crystalline disaccharide III in 53% yield after purification. The optical rotation, $[\alpha]_D$ +40°, was the expected one for the methyl α-D-glucoside of a β-linked disaccharide, and no α-linked disaccharide was observed.

Condensation of glycosyl halides with alcohols in the presence of mercuric cyanide was introduced by Zemplén and Gerecs,[15] who showed that, in benzene solution, only the β-anomer was formed. Helferich and associates carried out a series of investigations, using nitromethane as solvent, establishing that the α-anomer was produced only when the acetyl halide was condensed with phenols under heating,[16,17] whereas alcohols or acetylated hexoses, at room temperature, gave only the β-anomer.[17,18] This course of the reaction has been recently confirmed in the condensation of tetra-*O*-acetyl-α-D-glucopyranosyl bromide and of (methyl tri-*O*-acetyl-α-D-glucopyranosyluronate) bromide with methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside, in which only the β-linked disaccharides could be isolated.[19] Matsuda, however, using the same conditions, has reported the formation of α-linked disaccharides in preponderant yield when tetra-*O*-acetyl-α-D-glucopyranosyl bromide was condensed with 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose,[20] or with 2,3,-4,6-tetra-*O*-acetyl-α-D-glucopyranose.[21]

Hydrolysis of the benzylidene group of III, followed by acetylation, gave IV, which was saponified to V. Both reactions proceeded with excellent yields, and gave crystalline compounds. Catalytic hydrogenolysis of the benzyl group gave 2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-glucose (VI) in 77% yield. This product was identical to the "lacto-biose I" derived from human milk,[3,4] on the basis of melting point, optical rotation, infrared spectra, and paper chromatography. The infrared spectra observed presented some differences from those already reported.[9] Similar differences were found to be present in the infrared spectra of a sample kept for six months in a desiccator, despite the fact that melting point, mutarotation, and speed of migration in paper chromatography were unchanged (see spectra).

Experimental

Melting points were taken on a hot stage, equipped with a microscope, and correspond to "corrected melting point." Rotations were determined in semimicro- or micro- (for amounts smaller than 3 mg.) tubes with lengths of 100 or 200 mm., using a Rudolph photoelectric polarimeter attachment, Model 200: the chloroform used was A. R. grade and contained approxi-

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mately 0.75% of ethanol. Infrared spectra were determined on a Perkin-Elmer spectrophotometer, Model 237. Chromatograms were made with the flowing method using silica gel; "Silica Gel Davison," from the Davison Co., Baltimore 3, Md. (grade 950; 60–200 mesh) was used without pretreatment. When deactivation by contact with moist air occurred, reactivation was obtained by heating to 170–200° (manufacturer's instructions). The sequence of eluents was hexane, benzene or dry chloroform, ether, ethyl acetate, acetone, and methanol individually or in binary mixtures. The proportion of weight of substance to be absorbed to weight of adsorbent was 1 to 50–100. The proportion of weight of substance in g. to volume of fraction of eluant in ml. was 1 to 100. The ratio of diameter to length of the column was 1 to 20. Evaporations were carried out *in vacuo*, with an outside bath temperature kept below 45°. Amounts of volatile solvent smaller than 20 ml. were evaporated under a stream of dry nitrogen. The microanalyses were done by Dr. M. Manser, Zürich, Switzerland, and Dr. S. M. Nagy, Cambridge, Mass.

Benzyl 2-Acetamido-4,6-benzylidene-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoside (III).—A solution of 0.80 g. of benzyl 2-acetamido-4,6,*O*-benzylidene-2-deoxy-α-D-glucopyranoside[14] (II), 0.82 g. of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (I),[13] and 0.56 g. of mercuric cyanide in a mixture of 50 ml. of nitromethane and 30 ml. of benzene was stirred at 40° for 24 hr. with exclusion of moisture. An additional quantity of II (0.40 g.) and mercuric cyanide (0.28 g.) was added and stirring continued for an additional 24 hr. at 40°. The solution was allowed to cool to room temperature, diluted with excess benzene, and washed several times with cold sodium bicarbonate solution and water, dried, and concentrated *in vacuo*. The residue (2.0 g.), dissolved in a mixture of benzene and ether (1:1), was chromatographed on silicic acid. A crystalline fraction was obtained by elution with a mixture of ether and ethyl acetate (9:1). On recrystallization from a mixture of acetone and ether, it gave 0.80 g. of needles (53%), m.p. 175–177°, $[\alpha]^{20}D$ +40° (in chloroform, *c* 1.43).

Anal. Calcd. for $C_{36}H_{41}NO_{15}$: C, 59.42; H, 5.68; N, 1.92. Found: C, 58.95; H, 6.03; N, 1.97.

Benzyl 2-Acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoside (IV).—A solution of 0.80 g. of III in 5 ml. of 60% acetic acid was heated on a steam bath for 15 min. The clear solution obtained was evaporated and the residue, after being dried by repeated azeotropic distillation with toluene, was acetylated with 2 ml. of acetic anhydride and 2 ml. of pyridine at room temperature overnight. Evaporation of this solution and recrystallization of the residue from a mixture of acetone and ether afforded 0.65 g. of needles (82%), m.p. 173–175°. A further recrystallization from the same solvent mixture raised the m.p. to 175–176°, $[\alpha]^{25}D$ +45° (in chloroform, *c* 1.22).

Anal. Calcd. for $C_{33}H_{43}NO_{17}$: C, 54.61; H, 5.97. Found: C, 54.57; H, 6.14.

Benzyl 2-Acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-glucopyranoside (V).—Saponification of 0.30 g. of IV in 1 ml. of 2 *N* methanolic sodium methoxide solution gave, on cooling, 0.165 g. of needles (84%), m.p. 243–245°. The melting point remained unchanged on recrystallization from methanol, $[\alpha]^{22}D$ +101°(in 95% ethanol, *c* 1.03).

Anal. Calcd. for $C_{21}H_{31}NO_{11}$: C, 53.26; H, 6.59; N, 2.95. Found: C, 53.11; H, 6.76; N, 3.11.

2-Acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-glucose (VI).—A solution of 160 mg. of V in 5 ml. of 90% ethanol was hydrogenated catalytically with 10% palladium on charcoal, overnight, at room temperature, and atmospheric pressure. The residue, obtained after evaporation, was recrystallized from methanol. After filtration, it was dried for 48 hr. *in vacuo* over phosphorus pentoxide at 80°, giving 100 mg. (77%) of needles, melting at 193–194° dec., after sintering at 184°[22]; the product mutarotated from $[\alpha]^{23}D$ + 32° (0 min.) to +14.5° (after 24 hr.) (in water, *c*, 1.58).[23] The product migrated in descending chromatography, on paper Whatman no. 1, in the mixture of solvents *n*-butyl alcohol, ethanol, and water 10:1:2[24] with an $R_{glucose}$ 0.49.[22]

Anal. Calcd. for $C_{14}H_{25}NO_{15}$: C, 43.85; H, 6.57; N, 3.65. Found: C, 43.75; H, 6.52; N, 3.62.

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A Reimer–Tiemann Reaction with 6-Trichloromethylpurine[1]

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Phenol undergoes C-acylation at the *para* position with 6-trichloromethylpurine (I) under mild, basic conditions to yield purin-6-yl 4-hydroxyphenyl ketone (III). With *p*-cresol, acylation proceeds at the *ortho* position to yield purin-6-yl 2-hydroxy-5-methylphenyl ketone (VII). An uncharged, reactive intermediate is postulated to account for these and other acylation reactions of I.

In the course of an investigation of the chemical reactivity of 6-trichloromethylpurine (I),[3] it was found that reaction of I with sodium phenoxide in methanol did not lead to the expected 6-(triphenoxymethyl)-purine. Instead, an unstable product, II, was obtained, which, upon mild treatment with aqueous acid, gave rise to a *yellow* ketone, $C_{12}H_8O_2N_4$, III. The isolation of II proved to be difficult; III could be obtained in 79% yield, directly from the reaction mixture of I and sodium phenoxide, by treatment with dilute, aqueous hydrochloric acid.

Oxidative degradation of III by the use of hydrogen peroxide in acetic or trifluoroacetic acid solution gave rise to hypoxanthine (IV); in sodium hydroxide solution, it gave rise to purinoic acid, V.[4] A boiling solution of sodium hydroxide had no effect on III, which means, therefore, that it was not a phenyl ester of purinoic acid. Finally, a solution of ferric chloride gave a blue color reaction with III suggesting the

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(4) The term "purinoic" has been proposed by the authors in an earlier publication [S. Cohen, E. Thom, and A. Bendich, *Biochem.*, **2**, 176 (1963)] to replace the less convenient purine-6-carboxylic.