partitioned on Celite diatomaceous earth using solvent system V[14]. A major fraction of 224 mg. of 11β,21-diacetate and a fraction, 421 mg. of diacetate–monoacetate mixture were obtained. Recrystallization of the pure diacetate fraction from acetone–petroleum ether gave crystals, m.p. 215–216, $[\alpha]^{22}D$ +134°, λ_{max} 236 mμ (ε 15,800); λ_{max}^{KBr} 2.90, 3.40, 5.73, 5.77(shoulder), 6.01, 6.15(shoulder), 7.29, 8.10, 9.07 μ, etc.; papergram mobility in system V[14] R_f 0.71.

Anal. Calcd. for $C_{25}H_{33}O_7F$: C, 64.64; H, 7.16; F, 4.09; acetyl, 18.53. Found: C, 64.10; H, 7.39; F, 4.04; acetyl, 19.23.

11β,21-Diacetoxy-17α-hydroxy-4-pregnene-3,20-dione (VIIb).—One gram of hydrocortisone 21-acetate was dissolved in 10 ml. of pyridine and 5.0 ml. of acetic anhydride, warmed at 80–85° for 24 hr., and worked up in the usual manner. The residue obtained was crystallized from ethyl acetate–petroleum ether, yielding 0.96 g. of the 11β,21-diacetate, m.p. 185.0–187.5° (Kofler), $[\alpha]^{22}D$ +168°, λ_{max} 238 mμ (ε 15,880).[17] Infrared spectra and papergram mobilities were consistent with the assigned 11β,21-diacetate structure.

11β,21-Diacetoxy-9α-fluoro-16α,17α-isopropyldienedioxy-1,4-pregnadiene-3,20-dione (X).—Acetylation of 0.6 g. of triamcinolone 16α,17α-acetonide[3,9] with 10 ml. of pyridine and 2.5 ml. of acetic anhydride by heating at 90° for 15 hr. afforded 0.7 g. of crystalline 11β,21-diacetate when worked up in the usual manner. Recrystallization from acetone–petroleum ether gave crystals, m.p. 230–232°, $[\alpha]^{22}D$ +138°, λ_{max} 236 mμ (ε 15,300); λ_{max}^{KBr} 3.40, 5.70, 5.75(shoulder), 5.98, 6.10, 6.19, 7.26, 8.13, 8.57, 9.25, 9.53, 10.98, 11.20, 11.68 μ, etc.; papergram mobiliy in system V[14] R_f 0.92.

(17) Hydrocortisone 11β,21-diacetate has been characterized[4a,4b] as follows: (a) m.p. 188–189, $[\alpha]^{25}D$ +167.1° (chloroform), λ_{max} 238 mμ (ε 17,200); (b) m. p. 191.0–191.8°, $[\alpha]D$ +167.1° (chloroform), $\lambda_{max}^{95\%EtOH}$ 240 mμ (17,200).

Anal. Calcd. for $C_{28}H_{35}O_8F$: C, 64.85; H, 6.80; F, 3.66; acetyl, 16.21. Found: C, 64.43; H, 7.33; F, 3.97; acetyl, 15.58.

Acetylation of triamcinolone 11β-acetate 16α,17α-acetonide with acetic anhydride and pyridine afforded the same 11β,21-diacetate 16α,17α-acetonide, as evidenced by infrared spectra, papergram mobility and melting point criteria.

11β-Acetoxy-9α-fluoro-21-hydroxy-16α,17α-isopropylidenedioxy-1,4-pregnadiene-3,20-dione (IX). (A). From Triamcinolone 11β-Acetate.—A solution of 1.5 g. of triamcinolone 11β-acetate in 500 ml. of acetone containing 2.25 ml. of concentrated hydrochloric acid was allowed to stand at room temperature for 24 hr., at which time the solution was neutralized with sodium bicarbonate solution, diluted with water, and concentrated in vacuum to a volume of 200 ml. The crystals formed were filtered, washed with water, and dried. The crystals, 1.4 g., were recrystallized from 50% aqueous methanol, m.p. 215–217°, $[\alpha]^{22}D$ +126.1°, λ_{max} 234 mμ (ε 15,490); λ_{max}^{KBr} 2.90, 3.40, 5.72, 5.80, 5.99, 6.10, 6.19, 6.91, 7.27, 8.13, 9.25, 9.55, 11.20, 11.67 μ, etc. papergram mobility in system V[14] R_f 0.69.

Anal. Calcd. for $C_{26}H_{33}O_7F$: C, 65.53; H, 6.98; F, 3.98; acetyl, 9.03. Found: C, 65.25; H, 7.13; F, 3.80; acetyl, 12.00.

(B) **From Triamcinolone 16α,17α-Acetonide 11β,21-Diacetate.**—A solution of 170 mg. of triamcinolone 16α,17α-acetonide 11β,21-diacetate in 16 ml. of methanol was diluted with 6.1 ml. of water and 2.0 ml. of concentrated hydrochloric acid, and the mixture was refluxed for 3 hours. After dilution with 15 ml. of water, the solution was concentrated in vacuum to about 15 ml. The crystals formed, 0.12 g., were recrystallized from aqueous methanol. Identity of the acetonide 11β-acetates formed by methods A and B was established by melting point, infrared spectral and paper chromatographic evidences.

PEARL RIVER, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY, CALIF.]

Reaction of D-Erythrose and 2,4-O-Ethylidene-D-erythrose with Methanolic Hydrogen Chloride[1]

BY CLINTON E. BALLOU

RECEIVED OCTOBER 6, 1959

The reaction of 2,4-O-ethylidene-D-erythrose with methanol containing hydrogen chloride gives, as the main product, methyl 2,3-O-ethylidene-β-D-erythroside. The migration of the ethylidene group and the formation of two fused five-membered rings apparently accounts for the stability of this product. Free D-erythrose reacts under the same conditions to give mostly methyl β-D-erythroside, contaminated with a little of the α-anomer. Pure methyl β-D-erythroside has a specific rotation of −149°. 2,4-O-Ethylidene-D-erythrose hydrolyzes completely in aqueous acid only if the acetaldehyde is allowed to escape. There is a strong tendency for the formation of 2,3-O-ethylidene-D-erythrose by the migration of the ethylidene group, but the same product is formed from free D-erythrose in aqueous acid containing 5% acetaldehyde. The reduction of the 2,3-O-ethylidene-D-erythrose gives 2,3-O-ethylidene-erythritol.

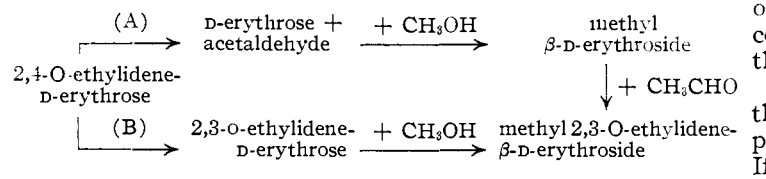
Several past investigations, particularly by Hockett and Maynard,[2] have been concerned with the reaction of the tetrose, erythrose, with methanol in the presence of an acid catalyst to give acetal derivatives. The only well defined product obtained has been a methyl D-erythroside; although the possible formation of acyclic acetals, as well as acetals of dimers of a dioxane type structure, has been the basis of conjecture.

During an attempt to prepare methyl D-erythroside by treating 2,4-O-ethylidene-D-erythrose with methanol containing 1% hydrogen chloride, we were surprised to find the rotation of the solution become strongly levorotatory. On the assumption that the ethylidene group was coming off by alcoholysis and the resulting free D-erythrose was being converted to methyl D-erythroside, the rotation indicated that the product must be primarily a β-anomer. This would be at variance with the observations of Hockett and Maynard that a solution of D-erythrose in methanolic hydrogen chloride became only slightly levorotatory. When the products from the above reaction of 2,4-O-ethylidene-D-erythrose were isolated, the major component was found to be methyl 2,3-O-ethylidene-D-erythroside, indicating that the ethylidene group had migrated, but had remained, for the most part, attached to the sugar molecule. A lesser amount of a methyl D-erythroside also was obtained. The rotations of both of these products were strongly levorotatory and suggestive of β-anomeric forms.

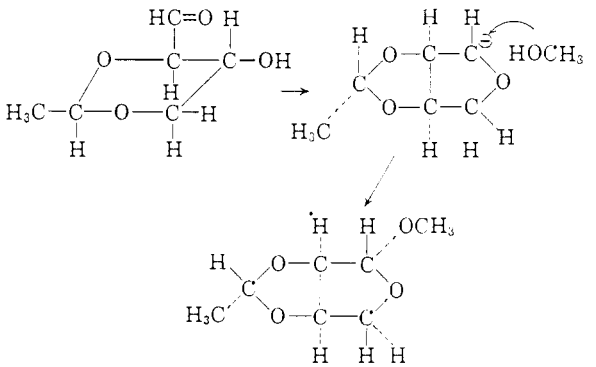
(1) Presented in part at the Meeting of the American Chemical Society in Chicago, Ill., September, 1958.

(2) R. C. Hockett and C. W. Maynard, Jr., THIS JOURNAL, **61**, 2111 (1939); G. F. Felton and W. Freudenberg, *ibid.*, **57**, 1637 (1935).

The formation of the methyl 2,3-O-ethylidene-D-erythroside in acidic methanol is obviously related to its two fused 5-membered rings, a fairly stable system.[3] It is expected that the migration of the ethylidene group, as contrasted with its solvolysis, would be promoted by the formation of such a ring system. That the product is primarily a β-glycoside is also to be expected. The presumed intermediate carbonium ion of 2,3-O-ethylidene-D-erythrose would be most readily attacked in the *exo* position on carbon 1, an attack which would yield the β-anomer exclusively.



This interpretation is also consistent with the previous report that 2,3-O-isopropylidene-D-erythrose yields, with methanolic hydrogen chloride, methyl 2,3-O-isopropylidene-β-D-erythroside.[4]



Again the acetone group was not removed by the acidic methanol, and the product had a large negative rotation ($[\alpha]^{25}D$ −137°) expected in a β-glycoside. In this case, the acetone group did not have to migrate; and the reaction, as indicated by the rotation, was complete in 2 hours. Since the comparable reaction starting with 2,4-O-ethylidene-D-erythrose takes about 24 hours, it appears as if the rate-controlling step is the migration of the ethylidene group. Felton and Freudenberg,[2] as well as Overend, Stacey and Wiggins,[5] have shown that the treatment of D-erythrose with a mixture of acetone and methanol containing hydrogen chloride gives a methyl 2,3-O-isopropylidene-D-erythroside. However, the product in this reaction had a specific rotation of −55 to −58° (in chloroform), which suggests that the strong directive influence of the two fused 5-membered rings did not have a chance to play its full part under these conditions.

When it was found that methyl D-erythroside could not be made in good yield directly from 2,4-O-ethylidene-D-erythrose, it became necessary to carry out the hydrolysis of the ethylidene group first. Rather than use the strenuous conditions employed by Rappoport and Hassid,[6] a 10% solution of ethylidene-erythrose in 0.1 *N* sulfuric acid was heated at 60° for two days in a closed container. At this time the solution had reached a constant rotation and the periodate uptake had reached a maximum (about 1 mole per mole of starting compound). When the product was isolated and converted to the methyl glycoside, a major component was again the methyl 2,3-O-ethylidene-D-erythroside. Thus, the ethylidene group was only partly hydrolyzed under the above conditions. This partial hydrolysis represented the extent of an equilibrium reaction; since, if the hydrolysis were carried out in an open vessel so that the acetaldehyde could escape, it went completely with the formation of free D-erythrose.

The methyl 2,3-O-ethylidene-β-D-erythroside obtained above could have been produced by two routes as shown If route B were involved, the first step, that is, the migration of the ethylidene group, would take place in aqueous acid as well as in methanolic hydrogen chloride. Therefore, the product from an acid hydrolysis in 0.1 *N* sulfuric acid, carried out as above, was treated with sodium borohydride to reduce the aldehyde group, and the ethylidene-erythritol was isolated. This product was optically inactive and proved to be 2,3-O-ethylidene-erythritol. Thus, migration had occurred.

Further attempts to make methyl D-erythroside were undertaken, this time starting with free D-erythrose. Again the rotation became strongly levorotatory when hydrogen chloride was added to a methanol solution of the sugar. The products from this reaction were an oil (b.p. 70–90° at 0.1 mm.) in 30% yield, and a black gum that remained in the still pot. The distillate analyzed for a methyl erythroside and the rotation ($[\alpha]^{25}D$ −114°) indicated that it was primarily the β-anomer. On fractional distillation of this material, a pure component, b.p. 87–90° at 0.1 mm., was obtained that showed a specific rotation of −149° (*c* 1, chloroform). When this substance was oxidized with sodium periodate, it consumed exactly one mole per mole almost instantaneously, and the resulting dialdehyde had a rotation that corresponded to the periodate oxidation product from methyl β-D-arabinopyranoside. Thus, it is methyl β-D-erythrofuranoside.

The discrepancy between the observations of Hockett and Maynard[2] those reported here with respect to the rotation change that occurs when a methanol solution of D-erythrose is treated with hydrogen chloride is unanswered, although it could well be due to the quality of the D-erythrose used in each investigation. Hockett and Maynard[2] apparently obtained a mixture of anomers containing about equal parts of each form. Some of the fractions of methyl D-erythroside obtained by redistilling the crude product with $[\alpha]^{25}D$ −114° had very low rotations and one fraction showed $[\alpha]^{25}D$ +47°. From the rotations of the dialdehyde produced on periodate oxidation of these fractions, it is possible to calculate that pure methyl α-D-erythroside will be found to have a specific rotation very near to +150° in chloroform.

(3) J. A. Mills, *Advances in Carbohydrate Chem.*, **10**, 1 (1956).
(4) C. E. Ballou, THIS JOURNAL, **79**, 165 (1957).
(5) W. G. Overend, M. Stacey and L. F. Wiggins, *J. Chem. Soc.*, 1358 (1949).
(6) D. A. Rappoport and W. Z. Hassid, THIS JOURNAL, **73**, 5524 (1951).

Experimental

2,4-O-Ethylidene-D-erythrose was prepared from 4,6-O-ethylidene-D-glucose[6,7] as described by Rappoport and Hassid,[6] except that the first extraction was made with absolute ethanol, and on concentration of this extract to dryness the residue was extracted with ethyl acetate. The ethyl acetate extract gave, on concentration to dryness, a sirup that corresponded to a 95–100% yield of ethylidene-erythrose.

Action of Methanolic Hydrogen Chloride on 2,4-O-Ethylidene-D-erythrose.—A solution of 17 g. of ethylidene-D-erythrose in 150 ml. of dry methanol had a rotation, α, of $-1.190°$ (0.5 dcm. tube), which changed slowly to a constant value of $-1.343°$. The specific rotation was calculated to be $-25°$ (c 1, methanol). Dry hydrogen chloride gas (1.0 g.) was bubbled into the solution, and the mixture was kept at 40°. The rotation, α, was measured from time to time and found to change as follows: 5 minutes $-1.340°$, 15 minutes $-2.000°$, 50 minutes $-2.474°$, 100 minutes $-2.716°$, 5 hours $-3.540°$, 24 hours $-5.440°$. The solution was neutralized by stirring it with 11 g. of silver carbonate for about 30 minutes, and was then filtered by suction through Filter-cel. The filtrate was concentrated to a sirup that weighed 15 g. and had a specific rotation of $-117°$ (c 2, chloroform).

This sirup was distilled at water-pump pressure, about 20 mm. There was a slight forerun, with the major fraction (I, 6.8 g.) distilling at 77–80°. A second fraction (II, 2.4 g.) distilled at 140–160°. Fraction I had a specific rotation of $-147°$ (c 1, chloroform), while II showed a specific rotation of $-87.5°$ (c 1, chloroform). Compound I did not react with sodium periodate, while II consumed 1 mole per 130 g. of compound. These data and the elemental analyses indicate that II is a methyl erythroside, while I is a methyl ethylidene-erythroside.

Anal. Calcd. for $C_7H_{12}O_4$ (160) (I): C, 52.4; H, 7.5; OCH_3, 19.4. Found: C, 52.0; H, 7.6; OCH_3, 19.4. Calcd. for $C_8H_{10}O_4$ (134) (II): C, 44.7; H, 7.5; OCH_3, 23.2. Found: C, 44.5; H, 7.9; OCH_3, 23.7.

Hydrolysis of 2,4-O-Ethylidene-D-erythrose.—A solution of 20 g. of ethylidene-erythrose in 100 ml. of 0.1 N sulfuric acid had a specific rotation of $-32°$. The mixture was kept at 60° in a closed container. Aliquots were removed from time to time and analyzed for uptake of sodium periodate. The reaction with periodate became constant in about 5 hours and corresponded to 1 mole of periodate per mole of ethylidene erythrose, indicating that only about one-third of the starting material was hydrolyzed at equilibrium. The acid was removed by treating the solution with a weak base resin, and the neutral solution was concentrated to a sirup, 17 g., which was dissolved in 170 ml. of dry methanol to give a solution with a specific rotation of $-28.3°$. About 2 g. of dry hydrogen chloride was bubbled into the solution, which was then kept at 40°. After 18 hours the rotation was constant, $[\alpha]^{25}D$ $-95.5°$.

This solution was neutralized with silver carbonate and then worked up as described above. On distillation, it yielded 5.0 g. of I with b.p. 77–80° (20 mm.), and 4.2 g. of II with b.p. 90–98° (0.1 mm.) or 140–150° (20 mm.). Compound I showed $[\alpha]^{25}D$ $-142°$ (c 1, chloroform) and II showed $[\alpha]^{25}D$ $-96°$ (c 1, chloroform).

Preparation of D-Erythrose from 2,4-O-Ethylidene-D-erythrose.—The above experiments demonstrated that the acetaldehyde must be removed from the hydrolysis mixture if the reaction is to go to completion. When the hydrolysis was carried out in 0.1 N sulfuric acid at 60° in an open flask to allow the acetaldehyde to escape, the solution showed the expected uptake of 3 moles of periodate per mole of starting ethylidene-erythrose after 2 days.

A solution of 50 g. of ethylidene-erythrose in 500 ml. of 0.1 N sulfuric acid was kept in an open flask at 60° for 2 days. The acid was then removed by use of a weak base resin, and the neutral solution was concentrated to a light-brown colored sirup. This was taken up in absolute ethanol, and the colored impurity was precipitated by adding ethyl ether. Most of the erythrose remained in solution. After an hour, the solution was decanted from the insoluble gum and concentrated to a light-yellow sirup. Chromatography of this sirup on Whatman #1 paper in butanol–ethanol–water (45:5:49)[8] showed mainly erythrose with a small amount of glucose.

Action of Methanolic Hydrogen Chloride on D-Erythrose.—A solution containing 50 g. of D-erythrose, 400 ml. of dry methanol and 2.0 g. of hydrogen chloride was left at 40° for 2 days. The rotation changed to a final value of $[\alpha]^{25}D$ $-98°$. The reaction mixture was neutralized by stirring the solution with 13 g of silver carbonate. The filtrate from the removal of the silver salts was concentrated to a sirup which was distilled in a high vacuum. The major distillable fraction boiled at 70–90° (0.1 mm.), with a bath temperature of 120–145°. The material had $[\alpha]^{25}D$ $-114°$ (c 1, chloroform). The residue in the still-pot was a dark, thick sirup.

The volatile methyl erythroside fraction was redistilled at 0.1 mm. and separated into fraction IIA (1 g.) with b.p. 70–87° and $[\alpha]^{25}D$ $+47°$ (c 1, chloroform), fraction IIB (5.0 g.) with b.p. 81–89° and $[\alpha]^{25}D$ $-81°$ (c 1, chloroform), and fraction IIC (8 g.) with b.p. 89–92° and $[\alpha]^{25}D$ $-149°$ (c 1, chloroform). Each fraction analyzed for a methyl erythroside, and each consumed 1 mole of periodate per mole assuming that structure. It is apparent that the fractions represent methyl α- and β-D-erythroside in varying proportions. To determine the anomeric composition of each fraction, they were oxidized with periodate and the rotation of the resulting dialdehydes were compared with those produced by oxidation of methyl α- and β-D-arabinopyranoside. Fraction IIC, with the most negative rotation, gave a dialdehyde with specific rotation of $-114°$. Since the dialdehyde from pure methyl-β-D-arabinopyranoside has $[\alpha]D$ $-124°$,[9] it can be calculated that fraction IIC is 96% methyl β-D-erythrofuranoside, and 4% of the α-anomer. Fraction IIA gave a dialdehyde with $[\alpha]D$ $+35°$, while fraction IIB gave $-60°$.

Methyl β-D-Erythroside 2,3-Di-O-p-nitrobenzoate (III).—A mixture of 0.5 g. of methyl β-D-erythroside, $[\alpha]^{25}D$ $-149°$, and 2.5 g. of p-nitrobenzoyl chloride in 10 ml. of dry pyridine was left overnight at room temperature. A few drops of water was then added to destroy the excess chloride, and the mixture was taken up in 100 ml. of benzene. The organic layer was washed with water, 1 N acid, 1 N bicarbonate and water. It was then dried with anhydrous sodium sulfate and concentrated to a sirup that weighed 1.4 g. This was dissolved in 100 ml. of 95% ethanol and left at room temperature. After several weeks crystals formed in the solution. The crystallization was completed at 5°. The product was filtered off and recrystallized from the same solvent. The yield of air-dry material was 0.8 g., m.p. 60–75°, $[\alpha]^{25}D$ $-40°$ (c 1, chloroform). This corresponds to a molecular rotation of $-17{,}200°$. The methyl β-D-erythroside has a molecular rotation of $-20{,}500°$.

Anal. Calcd. for $C_{19}H_{16}O_{10}N_2$(432): C, 52.7; H, 3.7; N, 6.5. Found: C, 52.1; H, 4.0; N, 6.5.

1,4-Di-O-benzoyl-2,3-ethylidene-erythritol (V).—A solution of 25 g. of 2,4-ethylidene-erythrose in 125 ml. of 0.1 N sulfuric acid was kept at 60° in a closed vessel for 24 hours. The acid was then removed by stirring the solution with IR-45 resin, and the neutral solution was treated with 3 g. of sodium borohydride to reduce the aldehyde group. After 2 hours, the solution was brought to pH 8 with acetic acid and concentrated to dryness. The residue was acetylated with 75 ml. of pyridine and 75 ml. of acetic anhydride. After 18 hours, the excess reagent was destroyed by adding water, and the acetylated product was extracted out with benzene. The benzene layer was washed with water, 1 N hydrochloric acid, 1 M sodium bicarbonate and then with water again. The benzene layer was dried with sodium sulfate and was then concentrated to dryness. The dry sirup was deacetylated in dry methanol by the addition of barium methoxide. After 2 hours, the reaction mixture was concentrated to dryness. On addition of 50 ml. of acetone, some crystals formed. These were filtered off, and found to consist of about 3 g. of erythritol, m.p. 116–119° after one recrystallization from water–ethanol. The melting point of a mixture with authentic erythritol was not depressed.

The acetone filtrate was concentrated to a sirup that weighed 15 g. It was distilled at a pressure of 0.2 mm., and the major component came over with a b.p. 103–104°. The yield was 10 g. This substance was optically inactive and analyzed for an ethylidene-erythritol (IV).

(7) R. Barker and D. L. MacDonald, unpublished procedure.

(8) S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).

(9) E. L. Jackson and C. S. Hudson, THIS JOURNAL, **59**, 994 (1937).

Anal. Calcd. for $C_6H_{12}O_4$ (148): C, 48.6; H, 8.1. Found: C, 48.5; H, 8.1.

A sample, 1.5 g., of the above ethylidene-erythritol was benzoylated in pyridine with 3.5 ml. of benzoyl chloride. The product crystallized from 95% ethanol to give 2.4 g. of the dibenzoate with m.p. 110–111° (V).

Anal. Calcd. for $C_{20}H_{20}O_6$(356): C, 67.4; H, 5.6. Found: C, 66.9; H, 5.4.

1,4-Di-O-benzoyl-erythritol (VI).—The above dibenzoyl-ethylidene-erythritol, 1.1 g., was heated in 15 ml. of 80% acetic acid on a steam-bath for 1 hour. The solution was then concentrated to a sirup that crystallized. This was dissolved in the minimum amount of hot benzene and allowed to crystallize. The product was recrystallized from methanol, giving about 0.5 g., m.p. 152–154°. The recorded[10] m.p. of 1,4-di-O-benzoyl-erythritol is 148°. When we prepared a sample of the dibenzoate according to Ohle and Melkonian, we found a m.p. of 153–155°. The m.p. of a mixture of the above two products was not depressed.

Anal. Calcd. for $C_{18}H_{18}O_6$(330): C, 65.5; H, 5.5. Found: C, 65.3; H, 5.7.

Reaction of Free D-Erythrose with Aqueous Acetaldehyde.—To a solution of 20 g. of D-erythrose in 125 ml. of 0.1 *N* sulfuric acid was added 5.5 ml. of paraldehyde. The solution was kept in a closed container at 60° for 28 hours, at which time 20 g. of Amberlite IR-4B was added to neutralize the acid. The mixture was filtered and the filtrate was concentrated to dryness. This residue was taken up in 50 ml. of methanol and 5.6 g. of sodium borohydride was added in 35 ml. of water. After 3 hours, the excess borohydride was decomposed with glacial acetic acid and the solution was evaporated to dryness. The solid was taken up in 150 ml. of dry pyridine and acetylated with 150 ml. of acetic anhydride. After shaking the mixture for 24 hours, water was added to decompose the excess acetylating reagent, and the product was extracted out into chloroform (400 ml.). The chloroform layer was washed with 1 *N* hydrochloric acid, 1 *M* sodium bicarbonate and then with water. After drying over sodium sulfate, it was concentrated to a sirup *in vacuo*. The sirup weighed 25 g.

This diacetyl ethylidene-erythritol was deacetylated in 100 ml. of dry methanol using barium methoxide. After removal of the methanol, a light-brown sirup was obtained. It was distilled at 0.5 mm. pressure, giving a fraction of 7 g. boiling at 80–84°. One gram of this ethylidene-erythritol was benzoylated and yielded 1.8 g. of a dibenzoate with m.p. 112–113°, which was not depressed when mixed with 1,4-dibenzoyl-2,3-ethylidene-erythritol.

Acknowledgment.—This work was supported in part by the United States Public Health Service, Grant A884. The preparation by Mr. Stephen Freer of many of the intermediates is acknowledged with thanks.

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(10) H. Ohle and G. A. Melkonian, *Ber.*, **74B**, 291 (1941).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Chondroitin Sulfate Modifications. II.[1] Sulfated and *N*-Deacetylated Preparations

BY M. L. WOLFROM AND BIENVENIDO O. JULIANO[2]

RECEIVED SEPTEMBER 28, 1959

Hydrazine treatment of barium chondroitin sulfate A gave a partially desulfated and highly (59–68%) *N*-deacetylated polymer. The effect (on the hydrazine reaction) of desulfation, the nature of the inorganic cation and reduction of the carboxyl groups were also studied. Increase in nitrogen content was noted with the uronate- and sulfate-containing modifications. Sulfur trioxide–N,N-dimethylformamide and chlorosulfonic acid–pyridine sulfation of chondroitin sulfate A and its *N*-deacetylated modifications increased their anticoagulant activity to only 15% that of heparin. Prior *N*-deacetylation with hydrazine did not affect the activity of the sulfated products. The absence of the 1560 cm.$^{-1}$ absorption in the infrared of mucopolysaccharides was established as characteristic of the replacement of the acetamido function by the sulfoamino. Infrared bands at 998, 820 and 775 cm.$^{-1}$, consistent for an equatorial sulfate group, characterized crude keratosulfate, which was only partially desulfated with methanolic hydrogen chloride. The synthesis of 2-hydroxyethylsulfamic acid hydrogen sulfate, disodium salt, trihydrate (I) is reported.

Structural studies of chondroitin sulfates and *N*-acetylated mucopolysaccharides have been hampered by the lack of mild *N*-deacetylating agents. Acid reagents effect both desulfation and glycosidic cleavage, whereas aqueous alkaline reagents promote β-elimination.[3] Matsushima and Fujii[4] employed hydrazine to prepare 90% *N*-deacetylated chondroitin sulfate. The yield reported by these workers was low (< 20%) and the product was characterized by Van Slyke amino nitrogen assay.

We report herein the preparation of a cream-colored 60–70% *N*-deacetylated chondroitin sulfate A in 43% recovery by hydrazine action through a modification of the procedure of Matsushima and Fujii.[4] This was done in conjunction with the preparation of sulfated *N*-deacetylated chondroitin sulfates in this Laboratory.[5] Partial desulfation and increase in nitrogen content accompanied the reaction, which consisted of heating barium chondroitin sulfate A with excess anhydrous hydrazine in a sealed tube at 100° for 10 hr. The normal uronic acid assay indicated no considerable degradation of this moiety.

In attempts to improve both the efficiency and yield of the reaction, various chondroitin sulfate modifications were also subjected to hydrazine treatment (Table I). The nature of the inorganic cation had little effect on the reaction. Reduction of the terminal carbonyl with sodium borohydride increased the recovery by 50%, consistent with suppression of β-elimination in the alkaline medium. Desulfation with methanolic hydrogen chloride[6] had a negligible influence on the reaction efficiency, but increased the yield markedly, probably because of terminal group glycosidation concomitantly effected. However, the re-

(1) Part I, THIS JOURNAL, **82**, 1673 (1960).

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