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.

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

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

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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-

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duced chondroitin[1] samples, which had been desulfated and had traces of carboxyl functions, were only 29% *N*-deacetylated, although obtained in high yield. This indicated that the absence of the carboxylate function of the uronic acid moiety reduced the efficiency of the hydrazine action. That the carboxyl group could be brought into sufficient proximity to the hydrogen of the acetamido function to form a hydrogen bond, was shown with molecular models. Increase in nitrogen content was noted only in carboxylate- and sulfate-containing modifications. No marked change in the infrared absorption spectrum was noted from the reaction, which increased the specific rotation of the polymer.

TABLE I

HYDRAZINOLYSIS[a] OF CHONDROITIN SULFATE A AND ITS MODIFICATIONS

	Non-dialyzable product, %		
	N-Deacetylation[b]	Yield	Desulfation
Chondroitin sulfate A, barium salt[c]	59–68	42–44	39–53[c]
Chondroitin sulfate A, sodium salt[c]	59	48	30[c]
Chondroitin sulfate modifications			
Sodium chondroitin sulfate A, 92% terminal carbonyl-reduced	60–64	66–67	28–33
Chondroitin, sodium salt	62–73	80–82	(100)[d]
Chondroitin, 96% carboxyl-reduced	30	82	(100)[d]
Chondroitin, 90% carboxyl-reduced	28	68	(100)[d]

[a] Details in Experimental section. [b] Acetyl analysis after Chaney and Wolfrom.[21] [c] Partially (18%) desulfated material. [d] Desulfated[6] starting material.

Sulfation of chondroitin sulfate A and its *N*-deactylated modifications gave products of slightly higher anticoagulant activity than chondroitin sulfate A but only 15% that of heparin. Prior *N*-deacetylation of the polysaccharide with hydrazine did not improve the activity of the sulfated preparation. Sulfated 62% *N*-deacetylated chondroitin sulfates with 3 and 2.5 sulfate groups per anhydrodisaccharide unit were obtained in 64 and 88% yields by the sulfur trioxide-N,N-dimethylformamide and by the chlorosulfonic acid–pyridine method, respectively. The anticoagulant activity was shown not to be a simple function of the degree of sulfation. The activity of sulfated chondroitin sulfate obtained was somewhat higher than previously reported.[7] Sulfation resulted in higher specific rotation of products.

Aside from the negative ninhydrin test, *N*-sulfation in the sulfated partially *N*-deacetylated chondroitin sulfate was established by its weak infrared NH (acetamido) absorption at 1,560 cm.$^{-1}$, absent in heparin,[8] but distinct in sulfated (non-deacetylated) chondroitin sulfate (Fig. 1). This absorption band was absent also in 2-deoxy-2-sulfoamino-D-glucose (sodium salt)[9] and in 2-hydroxyethylsulfamic acid hydrogen sulfate, disodium salt, trihydrate (I), herein synthesized, but was present in sulfamic acid

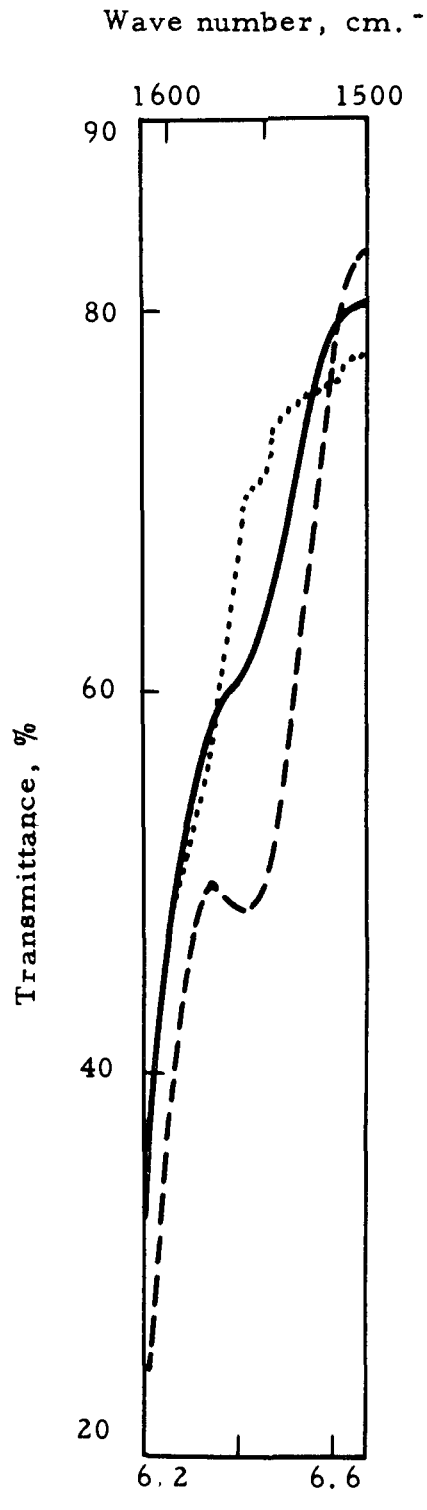
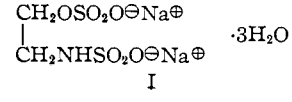


Fig. 1.—Infrared absorption spectra of the sodium salts of sulfated chondroitin sulfate (---------), sulfated partially *N*-deacetylated chondroitin sulfate (———) and commercial heparin (·····) (potassium bromide pellet).



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$H_3\overset{\oplus}{N}-SO_2-O^{\ominus}$, signifying the limitation of this property to mono-*N*-substituted sulfamates, $RNH-SO_2^{\ominus}Na^{\oplus}$. Crystalline I was obtained by the sulfation of 2-aminoethanol with sulfur trioxide–pyridine and conversion of the product to the disodium salt. The strong sulfate absorption at 1,240 cm.$^{-1}$ and the appearance of new bands at 990, 808 and 780 cm.$^{-1}$, characteristic of equatorial sulfate groups,[10] were noted on sulfation of chondroitin sulfate modifications since all the hydroxyl groups of the polysaccharide, except the sulfated C4 of the 2-acetamido-2-deoxy-D-galactose moiety, are equatorial.

Crude keratosulfate[11,12] has sulfate absorption bands at 1210–1250 cm.$^{-1}$ and at 998, 820 and 775 cm.$^{-1}$ consistent for an equatorial sulfate group.[10] Although the structure of this mucopolysaccharide is still unknown,[11,12] of its component sugars, 2-acetamido-2-deoxy-D-glucose and D-galactose, only the C4 hydroxyl of the latter is axial, making assignment of the sulfate group attachment from infrared data complicated. Infrared examination of model compounds showed that the 1,000 cm.$^{-1}$ absorption band was absent in 2-deoxy-2-sulfoamino-D-glucose (sodium salt),[9] but was present in I, denoting that this band was characteristic only of the ester sulfate function. The keratosulfate preparation also gave the characteristic acetamido bands[8] at 1,648 and 1,565 cm.$^{-1}$, and no carboxylate maximum at 1,612 cm.$^{-1}$. The crude keratosulfate was only partially desulfated[6] with methanolic hydrogen chloride. This is of interest since although chondroitin sulfates A and C were completely desulfated with the reagent,[6] chondroitin sulfate B (β-heparin) and heparin have been only partially desulfated.[13,14] However, Meyer and co-workers[15] reported complete desulfation of chondroitin sulfate B by a modified technique.

The preparation of the sulfated, partially *N*-deacetylated chondroitin sulfates of high activity (40% that of heparin), previously reported with Summers,[5] were unduplicatable. Further attempts to obtain a reasonably intact and *N*-deacetylated chondroitin sulfate A with strong alkali were unsuccessful.[16] Although hydrazine *N*-deacetylation was unsuitable for the preparation of sulfated *N*-deacetylated chondroitin sulfate of high activity, it should find more use, especially in deamination studies,[4,17] which could yield fragments of potential value for sequence determination of the component monosaccharides.

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Experimental

Barium Chondroitin Sulfate A.—Commercial sodium chondroitin sulfate[18] (100 g.) was purified by treatment with Magnesol–Celite.[19] It was converted to the barium salt by ethanol fractionation from dilute barium chloride solution[20]; yield 68 g. (53%) of white powder. An amount of 0.5 g. was dialyzed against distilled water for 3 days, concentrated, lyophilized and used for analysis; $[\alpha]^{20}D$ −20° (*c* 2.51, water), reported[20] −20°.

Anal. Calcd. for $C_{14}H_{19.18}Ba_{0.50}NO_{11}(SO_3Ba_{0.50}\cdot 5H_2O)_{0.82}$: C, 26.19; H, 4.28; N, 2.18; S, 4.10; $COCH_3$, 6.70; uronic acid, 32.1; ash (as sulfate), 33.10. Found: C, 25.02; H, 4.91; N, 2.26; S, 4.25; $COCH_3$,[21] 6.43; uronic acid,[22] 27 (cor., 34); ash (as sulfate), 32.10.

Paper chromatographic analysis on Whatman No. 1 filter paper using 1-butanol, pyridine and water (3:2:1.5 by vol.) developer and aniline hydrogen phthalate[23] indicator showed only glucuronic acid and 2-amino-2-deoxy-D-galactose (D-galactosamine).[24] Infrared spectral analysis showed sulfate absorption bands at 920, 848 and 720 cm.$^{-1}$, characteristic for chondroitin sulfate A.[10]

***N*-Deacetylated Chondroitin Sulfate A, Sodium Salt.**—An amount of 2.28 g. of barium chondroitin sulfate A was heated (caution) with 11 ml. of anhydrous hydrazine in a sealed tube for 19 hr. at 100°.[4] The turbid reaction mixture was concentrated to dryness under reduced pressure to remove the excess hydrazine, dissolved in 30 ml. of water and dialyzed against distilled water for 3 days. The dialyzate was rid of inorganic residue by filtration through asbestos, the filtrate and washings were passed through a column (100 × 13 mm., diam.) of Dowex 50[25] (H^+ form). Effluent and washings were carefully neutralized with dilute sodium hydroxide, concentrated under reduced pressure and lyophilized; light-cream powder, $[\alpha]^{20}D$ −14° (*c* 1.19, water); see Table I.

Anal. Calcd. for $C_{12}H_{18.01}N_{1.72}Na_{0.64}O_{9.64}(COCH_3)_{0.38}(SO_3Na)_{0.69}$: N, 5.44; S, 5.00; $COCH_3$, 3.70; uronic acid, 44.2. Found: N, 5.46; S, 5.07; $COCH_3$,[21] 3.72; uronic acid,[22] 40 (cor., 50); ninhydrin test (+). The infrared spectrum was similar to that of the starting material.

Similar results were obtained on hydrazine treatment, as described above, of sodium chondroitin sulfate A (see Table I).

***N*-Deacetylated Carbonyl-reduced Chondroitin Sulfate A, Sodium Salt.**—Magnesol–Celite-purified[19] sodium chondroitin sulfate A was reduced with sodium borohydride[16,26] at room temperature for 72 hr. A 92% reduction was determined[27] for the product, $[\alpha]^{23}D$ −20° (*c* 1.06, water). Hydrazine treatment, as above, yielded a cream-colored powder, $[\alpha]^{23}D$ −11° (*c* 1.03, water); see Table I.

Anal. Calcd. for $C_{12}H_{16.93}NNaO_{10}(COCH_3)_{0.40}(SO_3Na)_{0.67}$: S, 4.81; $COCH_3$, 3.86. Found: S, 4.83; $COCH_3$,[21] 3.90; ninhydrin test (+).

***N*-Deacetylated Chondroitin, Sodium Salt.**—Sodium chondroitin sulfate A was converted to the calcium salt by ethanol fractionation from calcium acetate buffer.[11] The product was treated with methanolic hydrogen chloride (0.06 *N*) to form chondroitin methyl ester,[6] presumably with terminal-group glycosidation. This ester was exactly saponified by careful portion-wise additions of dilute sodium hydroxide; yield 81% of a white powder, $[\alpha]^{23}D$ −23° (*c* 1.09, water). Sodium chondroitin was subjected to hydrazine *N*-deacetylation as described above; white powder, $[\alpha]^{24}D$ −7.5° (*c* 0.66, water); see Table I.

Anal. Calcd. for $C_{12}H_{18.06}N_{1.22}Na_{0.89}O_{10.89}(COCH_3)_{0.27}$: N, 4.62; $COCH_3$, 3.14. Found: N, 4.60; $COCH_3$,[21] 3.14; ninhydrin test (+).

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***N*-Deacetylated Carboxyl (and Carbonyl)-reduced Chondroitin.**—Carboxyl-reduced (96%) chondroitin,[1] $[\alpha]^{19}D$ +11° (*c* 0.46, dimethyl sulfoxide), was treated with hydrazine as described above and the water-insoluble product was dialyzed for 3 days against distilled water, carefully neutralized with dilute sodium hydroxide and lyophilized; white powder, $[\alpha]^{24}D$ +13° (*c* 0.47, dimethyl sulfoxide); see Table I.

Anal. Calcd. for $C_{11}H_{17.30}NO_8(CH_2OH)_{0.96}(COCH_3)_{0.70}(CO_2Na)_{0.04}$: N, 3.95; $COCH_3$, 8.49. Found: N, 3.52; $COCH_3$,[21] 8.51.

Sulfated N-Deacetylated Chondroitin Sulfate, Sodium Salt. (a) Sulfur Trioxide-N,N-Dimethylformamide Method.[28]—Prior activation of the 62% *N*-deacetylated sodium chondroitin sulfate A (6.00 g.) involved precipitation from aqueous solution with 3 vol. of ethanol,[29] collecting in a fritted-glass filter (medium pore) and, without exposure to air, washing successively with 80% ethanol, 95% ethanol, diethyl ether and N,N-dimethylformamide. The activated suspension in N,N-dimethylformamide was transferred to a 1-liter two-necked, round-bottomed flask fitted with a dropping funnel, drying tube and containing a Teflon-covered magnetic stirrer. Sulfur trioxide solution[28] in N,N-dimethylformamide (3.36 *N*, 200 ml.) was added in portions, with stirring, to the polysaccharide over a period of 2 hr. at room temperature. All the suspension dissolved within 2 hr., forming a clear brownish-red solution. After stirring for 10 hr. more, the reaction mixture was neutralized with solid sodium bicarbonate and the inorganic residue was removed by filtration. The filtrate and N,N-dimethylformamide washings were diluted with 2 vol. of ethanol[29] and the tan precipitate was collected and dissolved in water. To this was added the water washings from the inorganic residue above. This solution was made slightly alkaline with sodium hydroxide, dialyzed against distilled water for 4 days, filtered, concentrated and lyophilized; yield 5.95 g. (64%) of tan powder, $[\alpha]^{25}D$ −1° (*c* 1.0, water). Aside from the characteristic sulfate bands, the infrared spectrum showed a weak band at 1,560 cm.$^{-1}$, characteristic of the acetamido function.

Anal. Calcd. for $C_{12}H_{15.63}N_{1.70}Na_{0.64}O_{9.64}(COCH_3)_{0.38}(SO_3Na)_{3.08}$: N, 3.52; S, 14.40; $COCH_3$, 2.38. Found: N, 3.28; S, 13.58; $COCH_3$,[21] 2.46; ninhydrin test (−); anticoagulant activity,[28,30] 8.2 International Units per mg.

(b) **Chlorosulfonic Acid–Pyridine Method.**[5]—All pyridine employed was of high purity and was dried by distillation over barium oxide. Prior activation of 62% *N*-deacetylated chondroitin sulfate A (0.30 g.) involved precipitation[29] from aqueous solution by the addition of 3 vol. of methanol, decantation of the supernatant and washing 5 times with pyridine before storing over phosphorus pentoxide. Pyridine (5–10 ml.) was cooled (salt–ice–water-bath) in a 100-ml. 3-necked, round-bottomed flask fitted with a mercury-sealed mechanical stirrer, dropping funnel and reflux condenser with a drying tube outlet. To this was added, with stirring, chlorosulfonic acid (0.6–1.2 ml.), forming the white solid complex. The activated polysaccharide suspension in pyridine was added quickly and the mixture was heated over a boiling water-bath for 1 hr. with stirring. The sulfated product formed a viscous mass at the bottom of the flask. On cooling, the clear supernatant was carefully decanted and the tan residue was dissolved and neutralized with dilute sodium hydroxide. The solution was then dialyzed against distilled water for 3 days, filtered, concentrated and lyophilized; yield 0.34 g. (88%) of light tan powder, $[\alpha]^{25}D$ +10° (*c* 0.40, water).

Anal. Calcd. for $C_{12}H_{15.08}N_{1.72}Na_{0.64}O_{9.64}(COCH_3)_{0.38}(SO_3Na)_{2.54}$: S, 13.0. Found: S, 13.01; ninhydrin test (−); anticoagulant activity,[28,30] 9.4 I.U. per mg.

Sulfated *N*-Deacetylated Carbonyl-reduced Chondroitin Sulfate A, Sodium Salt.—Sulfation of 60% *N*-deacetylated 92% terminal carbonyl-reduced sodium chondroitin sulfate A (0.35 g.) by the chlorosulfonic acid–pyridine[5] method, described above, gave a light tan powder; yield 0.39 g. (75%), $[\alpha]^{25}D$ +5° (*c* 1.04, water).

Anal. Calcd. for $C_{12}H_{14.81}NNaO_{10}(COCH_3)_{0.40}(SO_3Na)_{2.79}$: S, 13.50. Found: S,13.50; ninhydrin test (−); anticoagulant activity,[28,30] 16 I. U. per mg.

Sulfated Chondroitin Sulfate, Sodium Salt.—Chondroitin sulfuric acid A (0.50 g.), derived from the barium salt by decationizing through Dowex 50[25] (H^+ form), upon treatment with chlorosulfonic acid–pyridine, as described above, gave a light tan powder; yield 0.63 g. (83%), $[\alpha]^{24}D$ −11° (*c* 1.02, water). Infrared spectral analysis showed, besides the observed sulfate absorptions at 1,220–1,270 cm.$^{-1}$ and in the region 700 to 1,000 cm.$^{-1}$, the distinct band at 1,560 cm.$^{-1}$ characteristic of the acetamido group, but absent in heparin (Fig. 1).

Anal. Calcd. for $C_{14}H_{16.99}NNaO_{11}(SO_3Na)_{2.01}$: S, 10.63. Found: S, 10.63; anticoagulant activity,[28,30] 16 I. U. per mg., reported[7] inactive.

Sulfated *N*-Deacetylated Chondroitin, Sodium Salt.—Chlorosulfonic acid–pyridine sulfation of 73% *N*-deacetylated sodium chondroitin (0.22 g.) provided a tan powder; yield 0.24 g. (81%), $[\alpha]^{25}D$ +8° (*c* 0.40, water).

Anal. Calcd. for $C_{12}H_{17.21}N_{1.22}Na_{0.89}O_{10.89}(COCH_3)_{0.27}(SO_3Na)_{0.85}$: S, 5.97. Found: S, 5.95; anticoagulant activity,[28,30] <3.3 I. U. per mg.

Sulfated *N*-Deacetylated Carboxyl-reduced Chondroitin, Sodium Salt.—Chlorosulfonic acid–pyridine sulfation of water-insoluble 30% *N*-deacetylated 96% carboxyl-reduced chondroitin (0.35 g.) gave a white water-soluble powder; yield 0.60 g. (98%), $[\alpha]^{23}D$ +7° (*c* 1.0, water).

Anal. Calcd. for $C_{11}H_{14.71}NO_8(CH_2OH)_{0.96}(COCH_3)_{0.70}(CO_2Na)_{0.04}(SO_3Na)_{2.59}$: S, 13.42. Found: S, 13.42; ninhydrin test (−); anticoagulant assay,[28,30] 11 I. U. per mg.

Sodium Chondroitin Sulfate A.—Barium chondroitin sulfate A, prepared as described above, was converted to the sodium salt by cation exchange on Dowex 50[25] (H^+ form) and neutralization of the effluent. The sodium chondroitin sulfate, $[\alpha]^{24}D$ −19° (*c* 1.42, water), thus obtained had an anticoagulant activity[28,30] of less than 3.3 I. U. per mg. Summers[5] cites −25° and Wolfrom and McNeely[7] cite no activity for this preparation.

Crude Keratosulfate.—Commercial sodium chondroitin sulfate[18] (60 g.) was purified by treatment with Magnesol-Celite.[19] It was converted to the calcium salt by ethanol fractionation from a 4% solution in calcium acetate buffer[11]; yield 42.9 g. (74%) of white powder, $[\alpha]^{22}D$ −24.3° (*c* 2.18, water), reported[11] −28 to −32°. The 50% ethanol mother liquor from the above precipitation of calcium chondroitin sulfate A was concentrated under reduced pressure and dialyzed against distilled water for 3 days; yield 1.40 g. of tan powder. Paper chromatographic analysis showed the presence of galactose, 2-amino-2-deoxy-D-glucose (D-glucosamine),[24] glucuronic acid and 2-amino-2-deoxy-D-galactose,[24] indicating chondroitin sulfate contamination. This preparation was ethanol-refractionated in calcium acetate buffer,[11] and the supernatant was dialyzed for 5 days against distilled water, filtered through asbestos, concentrated under reduced pressure and lyophilized; yield 0.32 g. (0.5%) of light-cream powder, $[\alpha]^{22}D$ +0.4° (*c* 0.69, water), reported[11] −10 to +6°.

Anal. Calcd. for $C_{14}H_{22.28}NO_{10}(SO_3Ca_{0.5})_{0.77}$: S, 5.59. Found: S, 5.59.

Paper chromatographic analysis showed galactose and 2-amino-2-deoxy-D-glucose[24] with traces of 2-amino-2-deoxy-D-galactose.[24] Infrared spectral examination revealed strong acetamido bands[8] at 1,648 and 1,565 cm.$^{-1}$, and the broad sulfate band at 1,210–1,250 cm.$^{-1}$. In the region 790 to 1,000 cm.$^{-1}$, sulfate absorption peaks at 998, 820 and 775 cm.$^{-1}$ were noted. No carboxylate band at 1,612 cm.$^{-1}$ was present.

An amount of 0.05 g. of crude keratosulfate was treated with 20 ml. of methanolic hydrogen chloride (0.06 *N*) after Kantor and Schubert[6]; yield 0.02 g. of tan powder. Infrared spectral analysis still showed the characteristic sulfate bands in the region 700 to 1,000 cm.$^{-1}$ and a weak uronate ester absorption at 1,749 cm.$^{-1}$, showing chondroitin sulfate contamination of the preparation.

Anal. Calcd. for $C_{14}H_{22.56}NO_{10}(SO_3H)_{0.44}$: S, 3.23. Found: S, 3.24.

2-Hydroxyethylsulfamic Acid Hydrogen Sulfate, Disodium Salt, Trihydrate (I).[31]—An amount of 7.5 ml. of sulfur tri-

(28) M. L. Wolfrom and T. M. Shen Han, THIS JOURNAL, **81**, 1764 (1959); T. M. Shen Han, Ph.D. dissertation, The Ohio State University, 1954.

(29) Accelerated by adding a few ml. of a saturated aqueous sodium chloride solution.

(30) O. F. Swoap and M. H. Kuizenga, *J. Am. Pharm. Assoc.*, **38**, 563 (1949).

(31) Experimental work by Mr. C. G. Summers.

oxide[32] was added slowly (1 hr.), with stirring, to 30 ml. of distilled pyridine in a three-necked flask fitted with a dropping funnel, an air-tight mechanical stirrer and a water condenser with a drying tube outlet. The solid complex which formed was diluted with 10 ml. of pyridine and cooled in an ice-bath. An amount of 5.0 ml. of freshly distilled 2-aminoethanol was then added dropwise, with stirring, over a period of 4 hr. The reaction mixture was heated to 60° for 30 min. and kept at room temperature overnight. The pyridine supernatant was decanted carefully and the viscous residue was carefully neutralized, under vigorous stirring and cooling, with *N* methanolic sodium methoxide. The voluminous white precipitate which formed was collected by filtration, washed well with diethyl ether and stored over phosphorus pentoxide under reduced pressure; yield 19.1 g. (72%) of a white powder, very soluble in water, slightly soluble in abs. methanol but insoluble in diethyl ether. This product exhibited a negative ninhydrin test. An amount of 1.0 g. of the above powder was dissolved in 20 ml. of distilled water and 9 vol. of methanol was added. The cloudy solution was centrifuged and diethyl ether was added with stirring to incipient turbidity. Crystallization was initiated by storing at 0° for a week. Recrystallization was effected in the same manner; yield 0.9 g. of white crystals, m.p. 220–221° (water of hydration evolved at *ca.* 80°); X-ray powder diffraction data: 11.8[33]s,[34] 9.12vs(1), 6.36m, 5.44s, 4.50s, 4.34w, 4.12w, 3.87vs(1), 3.70w, 3.55s, 3.21m, 3.10w, 3.03m, 2.94vw, 2.77w, 2.68w, 2.53s. Its infrared spectrum showed bands at 1,560 cm.$^{-1}$ and at 1,050 and 993 cm.$^{-1}$.

Anal. Calcd. for $C_2H_5NNa_2O_7S_2 \cdot 3H_2O$: C, 7.52; H, 3.47; N, 4.39; S, 20.09; Na, 14.41; H_2O, 16.93. Found: C, 7.57; H, 3.26; N, 4.13; S, 19.78; Na, 14.65; H_2O, 16.78.

Infrared Absorption Spectral Data.—Infrared absorption spectral data of the samples (potassium bromide pellets) were obtained with the Baird Associates infrared recording spectrophotometer (model B). Results are noted in Fig. 1 and under compound descriptions. A sample of sulfamic acid showed the 1,560 cm.$^{-1}$ band, whereas 2-deoxy-2-sulfoamino-D-glucose (sodium salt),[9] previously prepared in this Laboratory, had no bands at 1,560 and 1,000 cm.$^{-1}$.

Acknowledgments.—A commercial sample of heparin and a standardized heparin sample (110 I. U. per mg.) were kindly supplied by The Upjohn Co., Kalamazoo, Mich. The counsel of Dr. T. M. Shen Han on the anticoagulant assays is gratefully acknowledged.

(32) "Sulfan B," a product of The General Chemical Division, Allied Chemical and Dye Corp., New York, N. Y.

(33) Interplanar spacing, Å., CuKα radiation.

(34) Relative intensity, estimated visually; s, strong; m, medium; w, weak; v, very.

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[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS, URBANA, ILLINOIS]

Reactions of 2*H*,3*H*-Thieno[3,2-b]pyrrol-3-one. I.[1]

BY WAYNE CARPENTER[2] AND H. R. SNYDER

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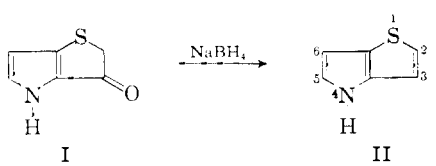
Carbethoxylation of 2*H*,3*H*-thieno[3,2-*b*]pyrrol-3-one(I) by reaction with ethyl carbonate catalyzed by sodium ethoxide occurs not only by attack on the α-methylene group of I but also by attack on the nitrogen atom, yielding a mixture of mono- and dicarbethoxy derivatives. Both the products (V and VI) exist as enolic dimers, and a similar "mixed dimer" forms from one of each of the monomeric units corresponding to V and VI. The reactions of the compounds with diazomethane, methyl iodide and potassium carbonate, and lithium aluminum hydride are studied. Structures of the various substances are proposed on the basis of chemical and spectral data.

The recent synthesis of thieno[3,2-*b*]pyrrole[3,4] from 2*H*,3*H*-thieno[3,2-*b*]pyrrol-3-one(I) suggests that derivatives of the ketone (I) may be useful intermediates in the synthesis of substituted thieno[3,2-*b*]pyrroles. Perhaps the simplest reaction by means of which I could be converted to a substituted thienopyrrole is acylation, which would be expected to yield 2-acyl-3-hydroxy derivatives. In the present work the carbethoxylation of the ketone I is examined.



I II

The reaction of I with ethyl carbonate was effected in the same manner as the carbethoxylation of ketones and esters,[5] that is, in the presence of excess ethyl carbonate with sodium ethoxide as catalyst. A mixture of the sodium salts III and IV precipitated from the reaction mixture. These salts could be separated by virtue of their different solubilities in tetrahydrofuran, and the N-carbethoxyl group of IV could be removed by the action of boiling water or ethanol, effecting the conversion of IV to III. Acetic acid converted the salts III and IV to the enols V and VI, each of which was found to be dimeric by molecular weight determination in boiling methyl ethyl ketone. A third dimeric enol (IX) was obtained when the mixture of III and IV was acidified or when equimolar amounts of V and VI were mixed in cyclohexane solution.

That the ring structure was not altered in the carbethoxylation was shown by the hydrolysis and decarboxylation of V with the regeneration of I in high yield. The fact that V and VI give colors with ferric chloride, whereas I does not, shows that a carbethoxyl group is located at position 2 in V and VI. In the n.m.r. spectra of compounds V, VI and IX, the peaks due to the hydroxyl proton are observed in the same region as the corre-

(1) Abstracted from a portion of the Thesis submitted by Wayne Carpenter to the Graduate College of the University of Illinois in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1959.

(2) National Science Foundation Fellow, 1956–1959.

(3) H. R. Snyder, L. A. Carpino, J. F. Mills and J. F. Zack, THIS JOURNAL, **79**, 2556 (1957).

(4) D. S. Matteson and H. R. Snyder, *J. Org. Chem.*, **22**, 1500 (1957).

(5) V. H. Wallingford, A. H. Homeyer and D. M. Jones, THIS JOURNAL, **63**, 2252 (1941).