yellow oil which upon evaporative distillation afforded 160 mg. (80%) of colorless oil. Its infrared spectrum showed a strong ketone absorption band at 1720 cm.$^{-1}$ An oxime was prepared and recrystallized three times from ethanol to give small prisms, m.p. 234–236° dec.

Anal. Calcd. for $C_{15}H_{25}ON_3$: C, 68.40; H, 9.57; N, 15.96. Found: C, 68.67; H, 9.49; N, 15.89.

NOTE ADDED IN PROOF: Since this work was submitted two papers dealing with the structure of angustifoline have appeared. L. Marion, M. Wiewiorowski, and M. D. Bratek [*Tetrahedron, Letters*, No. 19, 1 (1960)] and F. Bohlmann and E. Winterfeldt [*Ber.*, **93**, 1956 (1960)] independently reinvestigated the chemistry of angustifoline. In distinction to the earlier work [M. Wiewiorowski, F. Galinovsky, and M. D. Bratek, *Monatsh.*, **88**, 663 (1957)], angustifoline was found to contain a secondary amino group, and our earlier observation[2] that jamaicensine and angustifoline were probably identical has been confirmed. Both groups arrived at the same structural proposal by converting angustifoline to epihydroxylupanine by formaldehyde addition. It is interesting to note that the chemical addition of formaldehyde to jamaicensine leads to jamaidine, and that both occur in *O. jamaicensis* seeds, while the 13-hydroxy epimer, hydroxylupanine, occurs in *Lupinus* species.

Modified Oppenauer oxidation of 13-hydroxysparteine. The oxidation of 0.2 g. of 13-hydroxysparteine (prepared by reduction of 13-hydroxylupanine) was run in the same manner as that of desoxyjamaidine. It yielded 0.18 g. (90%) of thick colorless oil. The infrared spectrum was identical to that of the preceding ketone. An oxime was also prepared, m.p. 234–236° dec.[8] It gave no depression in a mixed melting point with the oxime prepared from the oxidation product of desoxyjamaidine.

Epimerization of 13-hydroxysparteine. The epimerization was carried out essentially by Bohlmann's[4] method. Colorless needles, m.p. 178–179°, were obtained. A mixed melting point with desoxyjamaidine showed no depression. The infrared spectrum of this 13-epihydroxysparteine was identical in all respects to that of desoxyjamaidine. A picrate was also prepared and recrystallized from ethanol, m.p. and mixed m.p. with desoxyjamaidine dipicrate 122° dec.

Conversion of jamaicensine to jamaidine. A solution of 140 mg. of jamaicensine hydrochloride, 100 mg. of aqueous formaldehyde solution (36%), and 0.5 ml. of water was allowed to stand stoppered at room temperature. The *p*H of the solution was approximately 6.5. The course of the reaction was followed by paper chromatography. From time to time a drop of the reaction mixture was chromatographed on Whatman #1 paper in a solvent system of *sec*-butyl alcohol, hydrochloric acid, and water (100:20:36). The results are given in Table I in the text.

After 3 days the solution was made strongly basic with 50% sodium hydroxide and exhaustively extracted with chloroform. The extract dried and evaporated *in vacuo* yielded 121 mg. of pale yellow oil. Upon addition of ether crystalline material separated. It was recrystallized once from acetone to yield 69 mg. (51%) of colorless needles, m.p. 194–195°. The melting point was not depressed on admixture with jamaidine. The infrared spectrum was identical to that of jamaidine. The noncrystalline residue (45 mg.) examined by paper chromatography appeared to be a mixture of four products: two compounds of R_f 0.58 (jamaidine) and 0.75 in approximately equal amounts, and traces of material of R_f 0.82 (jamaicensine) and 0.90. This residue was chromatographed on alumina in an attempt to isolate the compound of R_f 0.75. No separation was accomplished. The compound was either retained on the column or it decomposed and only a small amount of impure jamaidine was recovered.

(8) Galinovsky[3] reports a melting point of 244–245° for this oxime.

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Methyl 2-Deoxy-2-sulfoamino-β-D-glucopyranoside Trisulfate and the Preparation of Tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosyl Bromide[1]

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A knowledge of the fundamental chemistry of derivatives of 2-amino-2-deoxy-D-glucose (D-glucosamine) containing both sulfoamino and ester acid sulfate groups is important for the proper understanding of the chemical nature of heparin and related polysaccharides. Meyer and Schwarz[3] reported the preparation of the amorphous 2-deoxy-2-sulfoamino-D-glucose (ammonium salt) and its instability toward acid hydrolysis. Wolfrom and McNeely[4] investigated the inactivation of heparin by mild acidity and reported a loss of only 8% of the total sulfur content. This low value of sulfate release is now believed to be due to the peptization sequestration of the barium sulfate by the intact heparin molecule. Jorpes and associates[5] reported that on subjecting alkali-treated heparin to hydrolysis in very dilute hydrochloric acid, exactly equivalent amounts of sulfate and amino nitrogen were released.

We report herein the details of our preparation of an amorphous barium salt of methyl 2-deoxy-2-sulfoamino - β - D - glucopyranoside trisulfate dihydrate by the sulfation of methyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride with chlorosulfonic acid and pyridine. The glycoside used was made by the reaction of tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosyl bromide hydrobromide with methanol and subsequent deacetylation. The glycosyl halide was first prepared by

(1) Preliminary communication: *J. Am. Chem. Soc.*, **75**, 1519 (1953).

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(3) K. H. Meyer and D. E. Schwarz, *Helv. Chim. Acta*, **33**, 1651 (1950).

(4) M. L. Wolfrom and W. H. McNeely, *J. Am. Chem. Soc.*, **67**, 748 (1945).

(5) J. E. Jorpes, H. Boström, and V. Mutt, *J. Biol. Chem.*, **183**, 607 (1950).

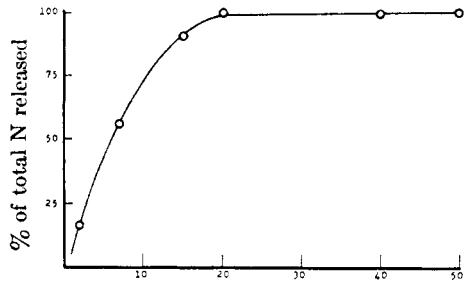

Fig. 1. Rate of free amino group release from methyl 2-deoxy-2-sulfoamino-β-D-glucopyranoside trisulfate, barium salt dihydrate

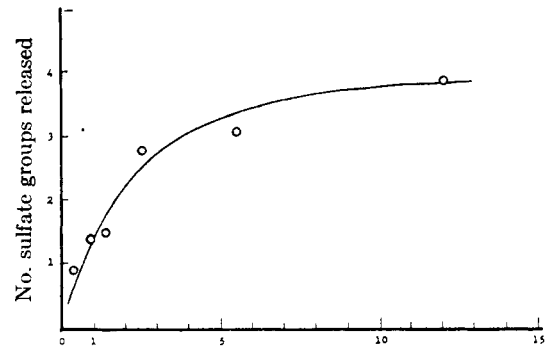

Fig. 2. Rate of sulfate release from methyl 2-deoxy-2-sulfoamino-β-D-glucopyranoside trisulfate, barium salt dihydrate

Irvine and co-workers[6] by the direct action of acetyl bromide upon 2-amino-2-deoxy-D-glucose in an adaptation of the original procedure of Colley[7] for the synthesis of the analogous compound by reaction of D-glucose with acetyl chloride. Considerable difficulty was encountered in obtaining this compound[6] and we report herein our detailed modifications which allowed its preparation in pure form in 85% yield.

The sulfation of methyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride with chlorosulfonic acid and pyridine at 60° yielded the dextrorotatory, amorphous, water-soluble, barium salt of methyl 2-deoxy-2-sulfoamino-β-D-glucopyranoside trisulfate dihydrate. The relative stability of the sulfoamino and ester acid sulfate linkages in this compound was demonstrated by mild acid hydrolysis. It was found that a solution of this substance in dilute hydrochloric acid at 95° readily released one equivalent of amino nitrogen in twenty minutes (Fig. 1) as determined by ninhydrin assay. The total sulfate group release is shown in Fig. 2 and indicates that the first group was lost very quickly. The molar concentration of amino nitrogen released was 0.00036*M*, an amount which would affect the acidity of the solution in a negligible manner. The ester acid sulfate was removed more slowly and only completely so after twelve hours. These acid-stabilities of the sulfoamino and the sulfate acid ester groups in this compound closely resemble those of heparin.[5]

EXPERIMENTAL

Tri-O-acetyl-2-amino-2-deoxy-α-D-glucopyranosyl bromide, hydrobromide. The procedure of Irvine, McNicoll, and Hynd[6] was modified. An amount of 10 g. (1.2 moles) of 2-amino-2-deoxy-α-D-glucose hydrochloride was placed in a dry three-necked flask fitted with a drying tube and sealed stirrer. An amount of 25 g. (5 moles) of acetyl bromide was added to the vigorously stirred mixture which was then heated cautiously to a bath temperature of 60–70° at which point the mixture turned red and hydrogen bromide was evolved. The bath was maintained at 60–70° until the mixture suddenly solidified (*ca.* 2 hr.). At this point the flask was removed from the oil bath and connected to a series of four 8-in. U-tubes containing, alternately, calcium chloride and soda lime, all connected to a water pump vacuum. After all the hydrogen bromide was absorbed (*ca.* 30 min.), the residue was extracted with warm ethanol-free chloroform (prepared by washing successively four times with concentrated sulfuric acid, water, aqueous sodium bicarbonate, and water, and drying over sodium sulfate for 30 min.). Then to the cooled and filtered extract was added dry ether (ethanol-free) to incipient crystallization; yield 16.9 g. (85%) m.p. 149–150° (recorded[6] m.p. 149–150°).

Methyl 2-deoxy-2-sulfoamino-β-D-glucopyranoside trisulfate, barium salt dihydrate. The above tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosyl bromide hydrobromide was converted to the methyl β-D-glycoside and this was deacetylated and the product isolated as the hydrochloride, following the procedure described by Irvine and co-workers.[6] An amount of 1 g. of methyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride was treated for 1 hr. at 60° with a solution of 1 ml. of chlorosulfonic acid in 25 ml. of anhydrous pyridine. The cooled reaction mixture was poured into 100 ml. of water and the solution was neutralized with saturated aqueous sodium carbonate solution. The resultant solution was evaporated to dryness under reduced pressure and the residue was dissolved in 100 ml. of water. To this was added aqueous barium acetate solution until no more precipitate formed. The barium sulfate precipitate was removed by filtration through a filter-aid (Filter Cel) and ethanol was added to the filtrate to effect precipitation of an amorphous solid, which was filtered and washed with hot absolute ethanol. Finally, the product was again precipitated from its aqueous solution by ethanol; yield 0.57 g. (17%), dec. 225°, $[\alpha]_D^{25}$ +4° (*c* 3.4, water).

Anal. Calcd. for $C_7H_{11}O_{17}S_4Ba_2 \cdot 2H_2O$: Ba, 33.48; S, 15.63. Found: Ba, 32.98; S, 15.64.

Acid hydrolysis of methyl 2-deoxy-2-sulfoamino-β-D-glucopyranoside trisulfate, barium salt dihydrate. This water-soluble salt (*c* 0.02; 3.6×10^{-4} *M*) was subjected to acid hydrolysis (0.04*N* hydrochloric acid at 95°) in the presence of 0.02*M* barium chloride and 0.58*M* sodium chloride. In our preliminary communication,[1] the acid-normality was given incorrectly. The rate of free amino group release (Fig. 1) was determined colorimetrically in the neutralized (0.01*N* sodium hydroxide) solution, with ninhydrin according to the procedure of Moore and Stein,[8] utilizing a Klett-Summerson photoelectric colorimeter. The rate of sulfate release was determined turbidimetrically with a Hellige turbidimeter and the data are plotted in Fig. 2.

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