72. *Derivatives of Chondrosamine.*

By Maurice Stacey.

A convenient method is described for preparing chondrosamine hydrochloride. By application of processes developed for the glucosamine series, a number of acetylated and methylated derivatives of chondrosamine have been synthesised.

Attention has recently been directed to the widespread occurrence of biologically important polysaccharides containing amino-sugars (Stacey, *J. Soc. Chem. Ind.*, 1942, **61**, 110). Two naturally occurring aminohexoses have so far been recognised, *viz.*, glucosamine and chondrosamine.

The formulation of glucosamine as 2-aminoglucose has been established by Haworth, Lake, and Peat (J., 1939, 271) and independently by Cox and Jeffrey (*Nature*, 1939, **143**, 984), but the configuration of the amino-group in chondrosamine has not yet been established. This sugar has been less accessible than glucosamine; it occurs in combination with acetic acid, sulphuric acid, and glucuronic acid residues in the chondroitin sulphate of cartilaginous tissues. When this investigation was begun, the only known derivatives of chondrosamine were the penta-acetates and certain Schiff's bases, so it was decided to prepare characteristic derivatives of value in the elucidation of polysaccharide structure, but in the meantime work along identical lines has been published by Levene (*J. Biol. Chem.*, 1941, **137**, 29). Adopting methods worked out by Cutler, Haworth, and Peat (J., 1937, 1979), he prepared fully methylated *N*-acetylchondrosamine and oxidised this with mercuric oxide to the corresponding chondrosaminic acid, which was then degraded by means of chloramine-T to 2 : 3 : 5-trimethyl lyxose. On this evidence, a pyranose structure was ascribed to *N*-acetyl trimethyl methyl-chondrosaminide. Although Levene failed to obtain crystalline derivatives in the final oxidation sequence, there is no reason to doubt that his conclusions are correct, since the properties of the α- and the β-form of the fully methylated *N*-acetyl chondrosamine described below are those of pyranosides and bear a close analogy to the corresponding glucosamine derivatives which have been shown to have the pyranoside structure. Levene failed to separate and characterise the α- and the β-form of his fully methylated *N*-acetyl chondrosamine although it is evident that he had prepared a mixture of the two forms.

The source of the chondrosamine used in the present study was chondroitin sulphate prepared from the cartilage of bovine nasal septa. A convenient method for preparing the hydrochloride of the amino-sugar in good yield was devised. In collaboration with Dr. G. A. Jeffrey, in this department, it was discovered that the *X*-ray powder photograph of chondrosamine hydrochloride was quite distinct from that of glucosamine hydrochloride, and we have applied this property as a means of identifying and distinguishing small amounts of the two amino-sugars in biological material. When the hydrochloride is obtainable in crystalline form the method has distinct advantages over the identification methods described by Chargaff and Bovarnick (*J. Biol. Chem.*, 1937, **118**, 421) and by Jolles and Morgan (*Biochem. J.*, 1940, **34**, 1183).

Levene (" Hexosamine and Mucoproteins," 1925) has presented evidence that chondrosamine is either 2-amino-galactose or 2-amino-talose. We have confirmed that treatment of its hydrochloride with phenylhydrazine affords galactosazone, but the present investigation has provided no evidence as to the orientation of the amino-group on the second carbon atom in chondrosamine.

When the acetylation of chondrosamine was carried out by acetic anhydride and pyridine, the crystalline product obtained was the α-*penta-acetate* (yield 60%), m. p. 178°, $[\alpha]_D$ +102° in chloroform, the residue being an

amorphous mixture of the α- and the β-form of the penta-acetate. When, however, zinc chloride was used as catalyst in the acetylation, the sole crystalline product was the β-*penta-acetate* (yield 30%), m. p. 235°, $[\alpha]_D$ +7° in chloroform.

N-Acetyl chondrosamine was conveniently prepared by the action of silver acetate in dry methyl alcohol on chondrosamine hydrochloride. It crystallised as a *monohydrate* (m. p. 120—122°), and from the direction of its mutarotation in water ($[\alpha]_D$ +115° ⟶ +80°) it was evidently the α-form. Treatment of penta-acetyl chondrosamine with methyl-alcoholic hydrogen chloride (cf. Moggridge and Neuberger, J., 1938, 745) gave in quantitative yield N-*acetyl α-methylchondrosaminide*, m. p. 217—218°, $[\alpha]_D$ +170° in chloroform. From this compound, *N*-acetyl trimethyl α-methylchondrosaminide was obtained in good yield on methylation with silver oxide and methyl iodide. An alternative route to the same substance was found in the methylation, in alkaline solution, of α-penta-acetyl chondrosamine, Cutler, Haworth, and Peat's method (*loc. cit.*) for glucosamine being used. The *N*-acetyl group in the chondrosamine series exhibits a stability in the presence of alkali comparable with that of the *N*-acetyl group in corresponding glucosamine derivatives under the same conditions.

When β-penta-acetyl chondrosamine was methylated with methyl sulphate and sodium hydroxide, the crystalline product (45% yield) was *N*-acetyl trimethyl β-methylchondrosaminide. Hence, it was expected that methylation of a mixture of α- and β-penta-acetyl chondrosamine would yield the α- and the β-form of *N*-acetyl trimethyl methylchondrosaminide. This was found to be the case, and the two isomers were separated by fractional crystallisation from ethyl acetate in which the β-form was the less soluble.

The relationship between the two isomers was established when it was found that by boiling the β-form with 1% methyl-alcoholic hydrogen chloride it was quantitatively transformed into the α-form. This conversion exactly parallels that obtained by Cutler, Haworth, and Peat (*loc. cit.*) with the corresponding glucosamine derivative.

On hydrolysis with N-hydrochloric acid, *N*-acetyl trimethyl α-methylchondrosaminide gave trimethyl chondrosamine hydrochloride, m. p. 178°, $[\alpha]_D$ +114° in water, having properties essentially the same as those described by Levene (*loc. cit.*).

Attempts to make acetobromochondrosamine gave products in which the bromine was unexpectedly labile and which readily yielded *tetra-acetyl chondrosamine monohydrate*.

Experimental.

Chondrosamine Hydrochloride.—The barium salt of chondroitin sulphate (11 g.) was dissolved in cold concentrated hydrochloric acid (250 c.c.), and the solution boiled under reflux (12 hours) until it no longer gave a Molisch test for carbohydrate or a naphtharesorcin test for uronic acid. The dark solution was boiled with charcoal (2 g.) for a further 15 minutes, filtered, and evaporated to a syrup under diminished pressure. Crystalline barium chloride which separated was stirred with methyl alcohol and filtered off. The methyl-alcoholic solution was evaporated to a syrup from which a further small quantity of barium chloride was separated by stirring it with methyl alcohol containing 10% of acetone. On concentration of the solution the residue set to a solid mass. It was kept for 24 hours and then stirred with methyl alcohol–acetone and filtered off (yield 4·3 g.). A further quantity was isolated from the concentrated mother-liquors. The product, recrystallised from aqueous methyl alcohol, had $[\alpha]_D^{20°}$ +105° ⟶ +96°, equilibrium value in water after 24 hours (Found : N, 6·4; Cl, 14·5. Calc. for $C_6H_{14}O_5NCl$: N, 6·4; Cl, 14·8%). The crystallographic and *X*-ray analysis will be described elsewhere. When heated at 80° in aqueous solution with an excess of phenylhydrazine acetate, the compound afforded galactosazone in 40% yield.

α-Penta-acetyl Chondrosamine.—Chondrosamine hydrochloride (4 g.) was suspended in dry pyridine (100 c.c.) and shaken with acetic anhydride (100 c.c.) for 60 hours. The pale yellow solution was decanted from a small amount of undissolved material (100 mg.) and evaporated to a syrup in a vacuum. The syrup was dissolved in chloroform (300 c.c.) and the solution washed 3 times with distilled water. The chloroform solution was dried over calcium chloride and concentrated to a syrup, which rapidly crystallised. Recrystallised from alcohol–light petroleum, the crystals (2·5 g.) had m. p. 178°; $[\alpha]_D^{20°}$ +102° in chloroform (c, 1·6) (Found : C, 49·5; H, 6·1; N, 3·9; OAc, 55·1. $C_{16}H_{23}O_{10}N$ requires C, 49·3; H, 6·0; N, 3·6; OAc, 55·3%). The aqueous washings gave on evaporation a syrupy mixture of the α- and the β-form of the penta-acetate, which failed to crystallise.

β-Penta-acetyl Chondrosamine.—Chondrosamine hydrochloride (6 g.) was suspended in acetic anhydride (75 c.c.) and shaken with powdered anhydrous zinc chloride (2 g.) for 24 hours. The solution was poured into ice-water (4 vols.), cautiously neutralised with sodium carbonate, made faintly alkaline with sodium hydroxide, and then extracted six times with chloroform (75 c.c. portions). The combined extracts were dried over anhydrous magnesium sulphate and then evaporated in a vacuum. When crystallisation commenced, the solution was diluted with ethyl alcohol and kept overnight at 0°. Crystallised from chloroform–ethyl alcohol, the crystals (3·2 g.) had m. p. 235°; $[\alpha]_D^{20°}$ +7° (*c*, 0·6) in chloroform (Found : C, 48·9; H, 6·0; N, 3·5; OAc, 54·8%). A further crop of crystals (0·4 g.) was separated from the mother-liquors, the main constituent of which was a syrupy mixture of the α- and the β-form of penta-acetyl chondrosamine. It was converted (see p. 274) into tetra-acetyl chondrosamine.

N-*Acetyl Chondrosamine.*—Chondrosamine hydrochloride (2 g.) and silver acetate (1 g.) were suspended in dry methyl alcohol containing a few drops of acetic anhydride, and then shaken vigorously for 3 hours. The solution was heated for a few minutes at 50°, diluted with a little ether, filtered, and concentrated to a syrup which crystallised on being kept. Recrystallised from ethyl acetate–methyl alcohol, it had m. p. 120—122°; $[\alpha]_D^{21°}$ +115° ⟶ 80° after 50 hours (equilibrium value in water) (Found : C, 39·8; H, 7·2; N, 5·9. $C_8H_{15}O_6N,H_2O$ requires C, 40·1; H, 7·1; N, 5·9%). A Van Slyke estimation showed the absence of a primary amino-group.

N-*Acetyl α-Methylchondrosaminide.*—α-Penta-acetyl chondrosamine (1·5 g.) was dissolved in 2% methyl-alcoholic hydrogen chloride (150 c.c.) and boiled for 90 minutes ($[\alpha]_D^{21°}$ +107° ⟶ +83°). The solution was neutralised with silver carbonate, filtered, and evaporated in a vacuum, leaving a crystalline mass. This was dissolved in a small amount of alcohol, and ether added to precipitate a little colloidal silver salt which was filtered off. After removal of the solvent, the product (0·9 g.) was recrystallised several times from alcohol–light petroleum. It had m. p. 217—218°, $[\alpha]_D^{21°}$ +170° in chloroform, and did not reduce Fehling's solution (Found : C, 45·6; H, 7·2; N, 6·3; OMe, 13·7. $C_9H_{17}O_6N$ requires C, 45·9; H, 7·3; N, 6·0; OMe, 13·2%). The product tended to separate in a gelatinous form. It was also prepared in quantitative yield from an amorphous mixture of α- and β-penta-acetates.

N-*Acetyl Trimethyl α-Methylchondrosaminide* (This substance and its β-isomer were first isolated in 1938 from methylated chondrosine in these laboratories in collaboration with Dr. T. White).—*From* N-*acetyl α-methylchondrosaminide.* The glucoside (0·5 g.) was dissolved in methyl iodide (20 c.c.) containing dry methyl alcohol (1 c.c.), and the solution boiled with silver oxide (2 g.) for 4 hours. Chloroform (50 c.c.) was added, and the filtered solution concentrated to a semi-solid mass, which was again methylated by the same reagents. The product obtained on evaporation of the solvent was crystallised from ethyl acetate–light petroleum (yield 0·6 g.); m. p. 185°, $[\alpha]_D^{20°}$ +121° in chloroform (*c*, 1·4) (Found: C, 51·8; H, 8·3; N, 5·0; OMe, 43·8. Calc. for $C_{12}H_{23}O_6N$: C, 52·0; H, 8·3; N, 5·1; OMe, 44·8%). The substance readily sublimed at 187°, forming long, feathery needles. A Van Slyke estimation showed the absence of a primary amino-group.

From α-penta-acetyl chondrosamine. The crystalline α-penta-acetate (p. 273) was methylated by sodium hydroxide and methyl sulphate in the presence of carbon tetrachloride by essentially the same method as that described by Cutler, Haworth, and Peat (*loc. cit.*). The product had m. p., and mixed m. p. with the methylated derivative described above, 185°; $[\alpha]_D^{20°}$ +120° in chloroform (*c*, 1·0) (Found: C, 51·9; H, 8·3; N, 5·1; OMe, 44·3%). It was soluble in chloroform, ether, alcohol, and acetone, and insoluble in light petroleum.

N-*Acetyl Trimethyl β-Methylchondrosaminide.*—β-Penta-acetyl chondrosamine (3 g.) was methylated with potassium hydroxide and methyl sulphate at 45—50° in the presence of acetone and carbon tetrachloride. The solution was stirred for 5 hours, neutralised, and extracted with chloroform, removal of which left a crystalline residue (0·55 g.), m. p. 212—215°. The aqueous mother-liquor was evaporated to a solid, which was extracted with ethyl alcohol. This was also evaporated, and the syrupy product extracted with chloroform. On evaporation of the solvent there remained a syrup which was boiled for 10 minutes in methyl alcohol containing acetic anhydride, and the solvents were then distilled off. The residue was methylated with silver oxide and methyl iodide and there was obtained a product (1·25 g.), m. p. 220°, which was combined with the first crop (0·55 g.) and recrystallised (twice) from ethyl acetate–light petroleum; m. p. 232°, $[\alpha]_D^{20°}$ +7° in chloroform (*c*, 0·95) (Found: C, 51·8; H, 8·3; N, 5·3; OMe, 44·1%). A Van Slyke estimation showed the absence of a primary amino-group. The product can be purified by sublimation at 235°.

N-*Acetyl Trimethyl αβ-Chondrosaminide.*—When an amorphous mixture of α- and β-penta-acetyl chondrosamine (2 g.) was methylated with potassium hydroxide and methyl sulphate by the method described above, there was obtained a crystalline mass (1·2 g.), m. p. 160—170°, which when fractionally crystallised from dry ethyl acetate gave *N*-acetyl trimethyl α-methylchondrosaminide (1·5 g.), m. p. 185°, $[\alpha]_D^{21°}$ +121°, and its β-isomer (0·35 g.), m. p. 233°, $[\alpha]_D^{20°}$ +14° in chloroform (*c*, 1·1). These two compounds could be partly separated by fractional sublimation in a high vacuum.

Conversion of N-*Acetyl Trimethyl β-Chondrosaminide into its α-Isomer.*—The β-chondrosaminide (0·2 g.) was dissolved in 25 c.c. of 1% methyl-alcoholic hydrogen chloride, and the solution boiled for 8 hours; $[\alpha]_D^{21°}$ +7° → $[\alpha]_D^{21°}$ +110°. The hydrochloric acid was neutralised by addition of lead carbonate, and the filtered solution evaporated to dryness. The solid residue (0·2 g.) was recrystallised from ethyl acetate–light petroleum, giving white needles, m. p. and mixed m. p. with the α-form previously described, 185°; $[\alpha]_D^{20°}$ +120° in chloroform (*c*, 1·0).

Triacetyl N-*Acetyl Chondrosamine.*—Amorphous penta-acetyl chondrosamine (3 g.) was dissolved in glacial acetic acid (30 c.c.) saturated with hydrogen bromide. After being kept for 4 hours, the liquid was dissolved in chloroform, and the solution washed with water until free from acid. The dried chloroform solution was concentrated to a syrup which slowly crystallised. Recrystallised from ethyl alcohol–light petroleum, the crystals had m. p. 152° and consisted essentially of acetobromochondrosamine (Found: Br, 17·1. Calc. for $C_{14}H_{20}O_8NBr$: Br, 19·5%). The bromine in this compound was relatively labile, since on recrystallising the above material several times from 90% aqueous ethyl alcohol a bromine-free product, m. p. 183°, $[\alpha]_D^{20°}$ +60° in chloroform (*c*, 1·0), was obtained (Found: C, 46·4; H, 6·2; N, 3·6. *Triacetyl* N-*acetyl chondrosamine monohydrate*, $C_{14}H_{21}O_9N,H_2O$ requires C, 46·0; H, 6·3; N, 3·8%).

Hydrolysis of N-*Acetyl Trimethyl α-Methylchondrosaminide.*—This compound (1·0 g.) was dissolved in N-hydrochloric acid (60 c.c.) and heated on the boiling water-bath until hydrolysis was complete ($[\alpha]_D^{21°}$ +139° → 112° in 4 hours). The solvent was distilled off under diminished pressure, and the residue partly crystallised (0·5 g.); recrystallised from alcohol–ether, it had m. p. 178°, $[\alpha]_D$ +114° equilibrium value in water (*c*, 1·0) (Found: Cl, 13·5; OMe, 36·0. Calc. for trimethyl chondrosamine hydrochloride, $C_9H_{20}O_5NCl$: Cl, 13·8; OMe, 36·1%). Attempts to prepare the free amine gave unstable liquids.

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