amide nitrogen, for it is a well-known fact that proteins and peptide linkings very readily yield ammonia when boiled with 20% hydrochloric acid.

In the work here reported the free ammonia nitrogen varied from 6.33 to 12.04% of the water-soluble nitrogen not precipitated by colloidal iron. Hart and Bentley found by their method that the free ammonia nitrogen of immature and mature plants rarely exceeded 5% of the water-soluble nitrogen, and in some instances was wholly absent. Their low results for ammonia were probably due to the direct extraction of the food materials with *hot* water. We have confirmed the results of Hart and Bentley that an extract of alfalfa hay prepared with boiling water contains no free ammonia, or at least only a slight trace. On the other hand, as shown in the Table III, a cold water extract of alfalfa hay contained 6.33% of its total soluble nitrogen not precipitated by colloidal iron, in the form of free ammonia. Further, an extract of alfalfa hay prepared by hot water slightly acidified (0.185% hydrochloric acid), contained 4.44% of its total soluble nitrogen, not precipitated by colloidal iron, as free ammonia. A water extract of alfalfa hay is distinctly alkaline to litmus paper.

Further studies to determine the amount and the nature of the nonprotein nitrogenous constituents of feedingstuffs are now under way in this laboratory.

URBANA, ILL.

THE ISOMERIC PENTACETATES OF GLUCOSAMINE AND OF CHONDROSAMINE.[1]

BY C. S. HUDSON AND J. K. DALE.

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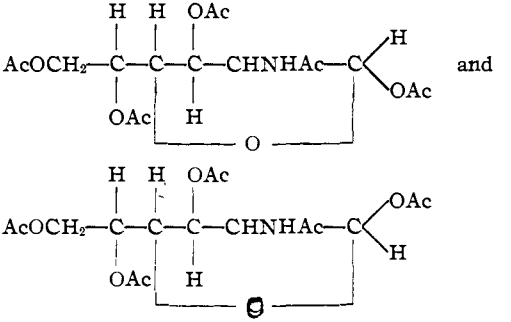
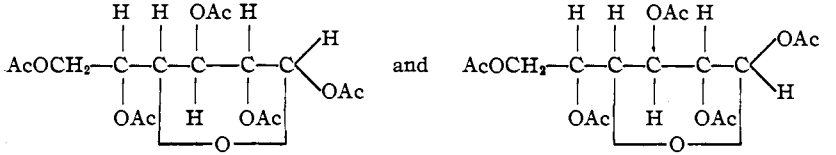
Two isomeric pentacetyl derivatives of glucosamine have been described by Lobry de Bruyn and Van Ekenstein.[2] If these compounds have the isomeric structures,



[1] Contribution from the Carbohydrate Laboratory, Bureau of Chemistry, United States Department of Agriculture.

[2] *Rev. trav. chim.*, 18, 83 (1899).

the molecular rotations of the substances may be expressed by the quantity (A + B) for one form and (—A + B) for the other, in accordance with considerations advanced by one of us,[1] and more recently developed in connection with the acetyl derivatives of the sugars.[2] The quantity A represents the rotation which is due to the end asymmetric carbon atom and B denotes the rotation due to the remainder of the molecule. In a similar manner the molecular rotations of the alpha and beta forms of glucose pentacetate,



may be written (A + B′) and (—A + B′) where B′ is of different value from B on account of the dissimilarity of the glucose and glucosamine chains. The difference between these values for the alpha and beta glucosamine pentacetates is 2A and the corresponding difference for the glucose pentacetates is also 2A. The deduction is drawn that the difference between the molecular rotations of the glucosamine pentacetates is equal to that of the glucose pentacetates. Lobry de Bruyn and Van Ekenstein found the specific rotation in chloroform solution of the more soluble glucosamine pentacetate to be +86.5° and the less soluble form to be optically inactive, thus a molecular[3] rotation difference of +33,600. The similar difference for the glucose pentacetates we have found to be +38,100.[4] The disagreement amounts to 4,500 or about 11.5° in specific rotation. This considerable divergence suggested to us that the specific rotation of one or both of the glucosamine pentacetates might be in error due to an incomplete separation of the isomers. Accordingly, the preparation and purification of these compounds was undertaken for a redetermination of their specific rotations. We have found for the more soluble glucosamine pentacetate, which we will call alpha because it is more dextrorotatory than the other isomer, $[\alpha]_D^{20} = +93.2°$, instead of +86.5°, and for the less soluble pentacetate, the beta form, $[\alpha]_D^{20} = +1.2°$, instead of optical inactivity. These values correspond to a molecular rotation difference of +35,800 which is 2,300 less than the difference for the glucose pentacetates, a disagreement of about 6° in specific rotation. Although this difference is more than the uncertainty of the mea-

[1] Hudson, THIS JOURNAL, 31, 66 (1909).

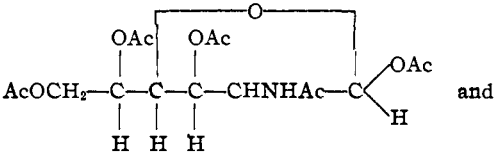
[2] *Ibid.*, 37, 1264, 1270, 1276, 1280, 1589, 1591, 2748 (1915).

[3] The molecular weights used are 389 for glucosamine pentacetate and 390 for glucose pentacetate.

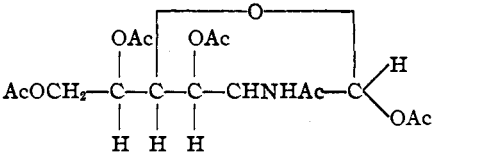
[4] THIS JOURNAL, 37, 1264 (1915).

surements, on the other hand, the agreement with the theory is close enough to justify the nomenclature we have adopted for the two substances.[1]

The Isomeric Pentacetates of Chondrosamine.—Chondrosamine hydrochloride, prepared by Levene and LaForge,[2] is isomeric with glucosamine hydrochloride. The existence of two pentacetates of glucosamine suggested the existence of two similar derivatives of chondrosamine. Both of these compounds have been prepared as described in detail farther on and their specific rotations in chloroform carefully measured. Since chondrosamine hydrochloride has been shown to be the hydrochloric acid salt of either *l*-allose- or *l*-altrose-amine,[3] the structures of its pentacetyl derivatives will be, for one form,



and



for the other. The end asymmetrical carbon atom in these compounds has the same configuration as in glucosamine pentacetate and glucose pentacetate, but the remainder of the molecule is different, hence the molecular rotations of the alpha and beta forms may be written $(-A + B'')$ and $(A + B'')$.[4] The difference is $-2A$, identical with the quantity that was found for the glucosamine pentacetates and the glucose pentacetates, but opposite in sign, hence the difference of the molecular rotations of the chondrosamine pentacetates should be equal and opposite to that of the glucosamine pentacetates and of the glucose pentacetates. In Table I is given the specific rotation of each of these compounds with the corresponding molecular rotation and the differences in molecular rotation.

[1] Lobry de Bruyn and Van Ekenstein named these compounds in the opposite sense, the less soluble form, alpha, and the more soluble form, beta, their reason seeming to be that the less soluble isomer was obtained first.

[2] *J. Biol. Chem.*, 18, 126 (1914).

[3] Levene and LaForge, *J. Biol. Chem.*, **20**, 433 (1915).

[4] Since chondrosamine is genetically related to *l*-glucose, the lower rotating pentacetyl derivative is to be named the alpha form and the higher rotating isomer the beta. See THIS JOURNAL, 31, 66 (1909).

TABLE I.

Substance.	$[\alpha]_D^{20.1}$	Molecular rotation.	Difference.
α-Glucosamine pentacetate.....	+ 93.5°	+36,400 = +A + B	35,930 = 2A
β-Glucosamine pentacetate.....	+ 1.2°	+ 470 = —A + B	
α-Chondrosamine pentacetate.....	+ 10.5°	+ 4,100 = —A + B″	—35,400 = —2A
β-Chondrosamine pentacetate....	+101.3°	+39,500 = +A + B″	
α-Glucose pentacetate.....	+101.6°	+39,600 = +A + B′	38,100 = 2A
β-Glucose pentacetate.....	+ 3.8°	+ 1,500 = —A + B′	

The values of 2A for the acetylated amino sugars agree closely with each other, the disagreement corresponding to only about 1.5° in specific rotation. With the glucose pentacetates the agreement is not so good, the divergence amounting to about 6° in specific rotation in one case and 7.5° in the other. These facts seem to indicate that the nature of the groups on the chain have in this case a definite, though small, influence upon the rotation of the end asymmetrical carbon atom.

Experimental.

Preparation of Glucosamine Hydrochloride and the α- and β-Glucosamine Pentacetates.—Glucosamine hydrochloride was prepared both from lobster shells and crab shells. Both materials gave a good yield of the amino sugar, but crab shells had the advantage in being cleaner and easier to work with. The lobster shells required a preliminary treatment with caustic soda whereas the crab shells were immediately digested with 5% HCl. This treatment was continued with one or two renewals of the acid until the evolution of CO_2 had ceased. The softened shells were washed and boiled with concentrated HCl, more acid being added from time to time until the shells were completely decomposed and the whole mass had become a thick, black mush. This was diluted with three times its volume of water, warmed with the addition of some active decolorizing carbon such as "eponite" or "norit" and filtered. The filtrate, which was clear and colorless, on evaporation under diminished pressure, crystallized in the flask. The crystals were filtered off and washed with 75% alcohol. The product thus prepared was nearly pure hydrochloride of glucosamine.

The acetylation of this compound was carried out with sodium acetate and acetic anhydride according to the directions of Lobry de Bruyn and Van Ekenstein.[2] The less soluble isomer, the β-pentacetate, was recrystallized from alcohol until its specific rotation became constant. The pure substance melted at 118–189° corr. With chloroform as solvent 1.0828 g. of this compound per 25 cc. of the solution gave a reading to the right +0.1° in a two decimeter tube with sodium light, hence

[1] All specific rotation measurements were made in chloroform (*chloroformum purificatum*, U. S. P.) solution.

[2] *Loc. cit.*

$[\alpha]_{20}^{D} = +1.2°$. After another recrystallization a duplicate measurement in which 1.1092 g. of the compound were used gave $[\alpha]_{20}^{D} = +1.3°$.

An acetyl determination made by boiling 0.3034 g. of the acetate with 20% H_2SO_4, distilling off the acetic acid into a flask and titrating with 0.1 *N* NaOH gave 77.13% acetic acid, which agrees with the theoretical value 77.12% for pentacetyl glucosamine.

The more soluble isomer, the alpha pentacetate, was recrystallized from water, then from alcohol and then from ether until its specific rotation became constant. The pure compound melted at 139–140° corr. With chloroform as solvent, 1.2762 g. gave a reading to the right +9.55° in a two decimeter tube with sodium light, hence $[\alpha]_{20}^{D} = +93.5°$. A duplicate measurement using 1.0561 g. of the substance gave $[\alpha]_{23}^{D} = +93.3°$.

An acetyl determination gave 77.25% acetic acid.

Preparation of α-Chondrosamine Pentacetate.—The first supply of chondrosamine hydrochloride was obtained through the courtesy of Dr. P. A. Levene, of the Rockefeller Institute for Medical Research. A later supply was prepared by Mr. E. P. Clark, of this laboratory, according to the directions of Levene and LaForge.[1]

The acetylation of this amino sugar was easily accomplished. To 80 cc. of acetic anhydride in which had been dissolved twelve grams of zinc chloride were added 10 g. of chondrosamine hydrochloride, which went rapidly into solution with the development of considerable heat. The reaction mixture was poured into about 200 cc. of water and the acid nearly neutralized with sodium bicarbonate. From this solution crystals of a compound separated out which proved to be a pentacetyl derivative of chondrosamine. It was recrystallized from alcohol until its specific rotation became constant. The yield of the pure substance was about equal to the weight of the hydrochloride used.

The pure compound did not melt, but commenced to turn brown at 220° and was completely decomposed at 235°. It was insoluble in cold water, alcohol and ether, and only slightly soluble in chloroform and acetone. In chloroform solution 0.2500 g. per 50 cc. of the solution gave a reading to the right +0.21° in a four decimeter tube with sodium light, hence $[\alpha]_{20}^{D} = +10.5°$. After another recrystallization a duplicate measurement gave $[\alpha]_{20}^{D} = +11.0°$.

An acetyl determination made by boiling 0.3050 g. of the compound with 20% H_2SO_4, distilling off the acetic acid and titrating with 0.1 *N* NaOH gave 77.22% acetic acid. A duplicate gave 76.91% acetic acid. The theoretical amount for chondrosamine pentacetate is 77.12%.

0.2481 g. subst. gave 0.4502 g. CO_2 and 0.1359 g. H_2O, corresponding to 49.48% C and 6.13% H; 1.000 g. of the substance gave 3.25% N by the Kjeldahl method.

Calc. for $C_{16}H_{23}O_{10}N$: 49.33% C, 5.96% H, and 3.61% N.

[1] *J. Biol. Chem.*, **18**, 126 (1914).

Transformation of α-Chondrosamine Pentacetate to the Isomeric Beta Form.—When the β-chondrosamine pentacetate was dissolved in acetic anhydride containing a small amount of $ZnCl_2$, the rotation was found to change upwards from an initial specific rotation of about 12° to a final value of about 65°. After the rotation had reached a constant value the solution was poured into water and the acid nearly neutralized with sodium bicarbonate. A quantity of the original pentacetate separated out at this point. On extracting the mother liquor with chloroform and evaporating the chloroform extract, crystals of a substance were obtained which proved to be the isomeric alpha chondrosamine pentacetate. The following has proved to be a convenient method for the preparation of this compound. Twelve grams of α-chondrosamine pentacetate were dissolved at the temperature of the steam bath in 100 cc. of acetic anhydride containing about two grams of $ZnCl_2$. The heating was continued until no further change in the rotation was noticed. The solution was poured into 300 cc. of water and the acid nearly neutralized with sodium bicarbonate. The solution was cooled in ice and the crystals of the original substance which here separated out were filtered off. The filtrate was extracted with chloroform and the chloroform extract evaporated to a small volume. On stirring in absolute ether, crystals of the new compound mixed with a small amount of the α-form separated out. The β-pentacetate was freed from the α-isomer by dissolving the mixture of crystals in a small amount of absolute alcohol and adding five times this volume of absolute ether. The α-pentacetate immediately crystallized out and was filtered off. The β-pentacetate was crystallized by evaporating the filtrate in a current of air with the addition from time to time of small quantities of absolute ether. The substance was recrystallized by the above method until its specific rotation became constant and then once more by adding absolute ether to its solution in chloroform. The melting point of the pure substance was 182–183° corr. This compound is very soluble in cold water, alcohol, ethyl acetate and chloroform, which is in marked contrast to the extreme insolubility of the α-isomer. In chloroform solution 0.8053 g. of the compound per 25 cc. of the solution gave a reading to the right, +6.58° in a two decimeter tube with sodium light, hence $[\alpha]_{17}^{D} = +102.1°$. A duplicate measurement in which 0.5343 g. of the substance was used gave $[\alpha]_{18}^{D} = +101.3°$.

Two acetyl determinations made by boiling 0.3075 g. and 0.4169 g. of the β-pentacetate with 50 cc. of 20% H_2SO_4, distilling off the acetic acid and titrating it against 0.1 *N* NaOH gave 77.03% and 77.30% acetic acid, which agrees with the theoretical, 77.12%.

0.3390 g. gave 0.6122 g. CO_2 and 0.1832 g. H_2O, corresponding to 49.25% C and 6.05% H; 1.000 g. gave 3.55% N by the Kjeldahl method.

Calc. for $C_{16}H_{23}O_{10}N$: 49.33% C; 5.96% H; 3.61% N.

WASHINGTON, D. C.