Experimental

A typical experiment is described as follows: Ten grams of lithium aluminum hydride was weighed directly into a lead foil in a fume hood. The lead foil was folded in such a manner that it could be manipulated quickly into a closed package. The hydride was then pulverized using a hard rubber mallet. The finely powdered material was added directly to 300 ml. of anhydrous ether in a 500-ml., round-bottomed flask fitted with a condenser having a nitrogen gas inlet, stirrer and drying tube. The slurry was stirred for 1–2 hours and then the sludge was allowed to settle.

One mole of thoroughly dried ester in approximately one and a half times its volume of anhydrous ether was cooled to Dry Ice temperature in a one-liter, round-bottomed flask fitted with a stirrer, condenser and addition funnel having a pressure equalizing arm. The addition funnel was connected to a nitrogen cylinder. The hydride slurry was then decanted into the addition funnel, fresh ether was added to the sludge, and this solution was then added dropwise in entirety to the reaction mixture. The total time for hydride addition and reaction was limited to 3 hours. At this point, 25 ml. of 95% ethyl alcohol was added *via* the addition funnel and the reaction mixture was permitted to come to room temperature. The reaction mixture was then poured into a two-liter beaker containing crushed ice and enough concentrated sulfuric acid, approx. 75 ml., to give a clear ether layer. The aqueous layer was separated and extracted twice with ether. The combined ether portions were distilled to remove the ether and alcohol present. An equal volume of concentrated sulfuric acid was slowly added to the residue with vigorous stirring and the aldehyde was collected in a cooled receiver. Redistillation results in a pure product. The small quantity of fluorine-containing alcohol formed in these reactions is removed during the course of the initial distillation prior to dehydration of the aldehydrol. In one experiment, the amount of lithium aluminum hydride was doubled resulting in a slight increase in yield of aldehyde and also formation of a larger quantity of the corresponding alcohol.

The experimental results and physical properties are summarized in Table I.

TABLE I

Product	B.p., °C.	Yield, %	2,4-Dinitrophenylhydrazone m.p., °C.
CF_3CHO[a]	−18	71	149
C_2F_5CHO[a]	1.5	75	129[d]
C_3F_7CHO[a]	28	76	106.5
$C_7F_{15}CHO$[b]	122	70	94.5[c]

[a] Physical properties in good agreement with those reported by D. R. Husted and A. H. Ahlbrecht, THIS JOURNAL, 74, 5422 (1952). [b] Not reported previously, $n^{20}D$ 1.2913. *Anal.* Calcd. for $C_8HF_{15}O$: C, 24.12; H, 0.25; F, 71.61. Found: C, 24.24; H, 0.42; F, 71.02. [c] Melting point of aldehydrol. *Anal.* Calcd. for $C_8H_3F_{15}O$: C, 23.08; H, 0.72. Found: C, 23.08; H, 0.74. [d] E. T. McBee, O. R. Pierce and J. F. Higgins, THIS JOURNAL, 74, 1387 (1952); reported value 128.5°.

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DEPARTMENT OF CHEMISTRY
PURDUE UNIVERSITY
LAFAYETTE, INDIANA

N-Acetylation of the Hexosamines

BY SAUL ROSEMAN[1] AND JULIO LUDOWIEG

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Due to the divergence in physical constants reported by various workers for N-acetylglucosamine (2-acetamido-2-deoxy-D-glucose) and for N-acetylchondrosamine (2-acetamido-2-deoxy-D-galactose), a new method for the preparation of these substances was investigated.

In contrast to the usual procedure (treatment of the hexosamine hydrochloride with silver acetate and acetic anhydride in dry methanol), it was found that N-acetylation could be performed by treating a cold, neutral, *aqueous* solution of the sugar with acetic anhydride. Adequate control of *p*H could be attained by the use of sodium bicarbonate, sodium hydroxide, barium hydroxide, etc., but the most convenient preparative method was by the use of a basic ion-exchange resin such as Dowex-1, carbonate form.[2]

In the course of this work, it became necessary to prepare galactosamine hydrochloride (2-amino-2-deoxy-D-galactose hydrochloride) in quantity. The difficulty of this preparation has been noted.[3] A procedure based upon the use of a cationic exchange resin was developed which yielded pure chrondrosamine hydrochloride from the commercially available, inexpensive, crude chondroitin sulfate.

The acetylation proceeds quantitatively under certain conditions, and application of this technique at a microgram level can be used for determination of the individual hexosamines in a mixture. Details of the analytical technique will be described elsewhere.

Experimental[4]

D-Galactosamine Hydrochloride.—Chondroitin powder, Wilson Laboratories,[5] (100 g.) was shaken mechanically for 12 hours with 1 l. of water, and the dark solution was passed through a column containing 600 ml. of Amberlite IR-120, barium form. The effluent and washings were concentrated to 900 ml. and the solution was refluxed for 12 hours after the addition of 450 ml. of concentrated hydrochloric acid. The mixture was treated with 15 g. of Norit A, cooled, shaken mechanically with 1 l. of Dowex-1, carbonate form[6] and then passed through a column containing 500 ml. of Dowex-1, carbonate form. The effluent was acidified with acetic acid and concentrated to dryness *in vacuo*. Fractionation of the residue was accomplished by dissolving it in 26 ml. of water, transferring the solution to the top of a 350-ml. column of Dowex-50, hydrogen form (200–400 mesh)[6] and eluting with 0.05 *N* hydrochloric acid solution at a flow rate of about 5 ml. per minute. The fractions (500 ml. each) were tested with Benedict solution and positive tests were obtained in fractions 1 and 8–11. Fraction 1 was brown in color and the reducing substances were presumably neutral decomposition products. Fractions 8–11 were combined and concentrated to dryness yielding a pale yellow or colorless sirup. Occasionally, when the chondroitin powder was of unusually poor quality, the sirup was quite dark and it was refractionated by means of the ion-exchange resin. Crys-

(1) The Rackham Arthritis Research Unit, University of Michigan, Ann Arbor, Michigan.

(2) The hydroxyl form of the resin cannot be used for reasons previously described: S. Roseman, R. H. Abeles and A. Dorfman, *Arch. Biochem. Biophys.*, **36**, 232 (1952).

(3) A. B. Foster and M. Stacey in "Advances in Carbohydrate Chemistry," Vol. 7, Academic Press, Inc., New York, N. Y., 1952, p. 280; S. Gardell, *Acta Chem. Scand.*, **5**, 195 (1951).

(4) All m.p.'s are corrected.

(5) Supplied through the courtesy of Dr. David Klein; a mixture of crude chondroitin sulfate salts.

(6) Dowex-1 and Dowex-50 were supplied by the National Aluminate Corp., Chicago, 38, Ill. Amberlite IR-120 was supplied by the Rohm and Haas Co., Resinous Products Division, Washington Square, Philadelphia, Pa. Dowex-1, or Nalcite SBR, is a strong basic resin of the quaternary ammonium type with 3 methyl groups per N atom. It is prepared from polymerized styrene crosslinked with divinylbenzene. Dowex-50, or Nalcite HCR, is a strong cationic exchange resin—a monofunctional, sulfonated, copolymer of styrene and divinylbenzene. Amberlite-120 is similar in properties to Dowex-50.

tallization was accomplished by dissolving the sirup in a small volume of water and adding 3 volumes of alcohol followed by acetone until a faint turbidity was observed. Long needles (about 10 g.) were deposited after standing overnight in the refrigerator. The crude material was recrystallized for analysis from water–alcohol–acetone mixture, $[\alpha]^{28}D$ +96.4° (2% in water).

Anal.[7] Calcd. for $C_8H_{14}O_5NCl$: C, 33.42; H, 6.54; N, 6.50; Cl, 16.44. Found: C, 33.58; H, 6.45; N, 6.59; Cl, 16.40.

It is of interest that the hexosamines are eluted from the resin[8] before the amino acids,[9] suggesting that the mechanism of action in either or both cases is not simple ion exchange. The separation of glucosamine from amino acids by this procedure was tested by adding inactive glucosamine hydrochloride to radioactive amino acids. The isolated glucosamine hydrochloride possessed less than 0.5% of amino acid contamination. The details of these experiments will be presented elsewhere.

N-Acetyl-D-glucosamine.—A solution containing 43.2 g. of glucosamine hydrochloride (Nutritional Biochemicals Corp.) in 1 l. of water and 100 ml. of methanol was stirred for 90 minutes at 0–5° with 1200 ml. of Dowex-1, carbonate form and 26 ml. of acetic anhydride. The mixture was filtered and the filtrate and washings were passed through a column containing 200 ml. of Amberlite IR-120, acid form.[8] The colorless effluent and washings were heated to boiling and the solution was concentrated to dryness *in vacuo* with the temperature of the water-bath below 55°. The N-acetyl-D-glucosamine crystallized during this procedure as white needles (40 g.), m.p. 193–195° (dec., preliminary browning). Recrystallization could be effected as described by White.[10] Better results were obtained by dissolving the crude material (37 g.) in water (100 ml.) and ethanol (75 ml.), heating the solution on the steam-bath and gradually adding, with vigorous stirring, 1 l. of boiling dimethoxyethane (Arapahoe Chemicals Co.). The major portion of the purified compound crystallized out of the boiling solution as long, colorless needles. After slow cooling, 32 g. of product was obtained, m.p. 210° (dec., turns tan at about 203°), $[\alpha]^{28}D$ +41.0° (*c* 1.0, water, final). Additional material (about 2 g.) was obtained from the mother liquors.

Anal.[7] Calcd. for $C_8H_{15}O_6N$: C, 43.43; H, 6.84; N, 6.33. Found: C, 43.43; H, 7.02; N, 6.37.

The silver acetate–methanol procedure was reported to yield products melting at 190[11] and 196°.[10] When obtained by acyl migration during methanolysis of 1,3,4,6-tetraacetyl-D-glucosamine, however, the compound was reported to melt at 205°.[12] This is confirmatory evidence that the silver acetate procedure yields impure preparations.

N-Acetyl-D-galactosamine.—Chondrosamine hydrochloride was treated as described for glucosamine hydrochloride. Concentration of the final solution to dryness gave a sirup which was readily crystallized by the addition of a little absolute ethanol; yield was from 85–95%, m.p. varying from 162 to 171°. Recrystallization of the product was unsatisfactory since losses were appreciable, although second and third crop material could be recovered from the mother liquors. The solvent system employed was the usual methanol–ethyl acetate mixtures. The purified product melted at 172–173° (preliminary softening), $[\alpha]^{27}D$ +86.1° (*c* 1.0, water, final). The m.p. varied about 3° (higher or lower) depending upon the rate of heating.

Anal.[7] Calcd. for $C_8H_{15}O_6N$: C, 43.43; H, 6.84; N, 6.33. Found: C, 43.68; H, 6.88; N, 6.13.

Previous reports on the physical constants of the compound as obtained by the silver acetate method were: m.p. 120–122°, $[\alpha]^{21}D$ +80° (equil.)[13]; m.p. 154°[14]; m.p. 159–160°, $[\alpha]^{5}D$ +94.5° (equil.)[15]; m.p. 162–164° (uncor.), $[\alpha]_{6461}$ +98° (equil.).[16] The suggestion[13] that the low melting compound was a hydrate is of interest since the compound isolated from aqueous solution by the present procedure is high melting and anhydrous.

It was reported[16] that the N-acetylhexosamines showed very similar R_f values in a wide variety of solvents. Investigations in this Laboratory confirmed this point, although small differences in R_f (about 5–8%) were noted when butanol–pyridine–water mixtures (6:4:3; upper phase) were used with Whatman #1 paper.

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(7) Analyses were performed by Micro-Tech Laboratories, Skokie, Ill.

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DEPARTMENTS OF PEDIATRICS AND BIOCHEMISTRY
UNIVERSITY OF CHICAGO
AND LARABIDA JACKSON PARK SANITARIUM
CHICAGO, ILLINOIS

An Improved Procedure for the Preparation of Alkyl Halide Derivatives of Saccharin

BY HAROLD L. RICE[1] AND GEORGE R. PETTIT[2]

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The preparation of alkyl derivatives of saccharin (I) by using the sodium salt of saccharin with an alkyl halide has been accomplished previously by methods which have resulted in poor yields,[3–6] and which generally required a long reaction time. The work of Sheehan and Bolhofer[7] suggested to us that the poor yields obtained by previous workers might have been due to an inadequate solvent. Sheehan described the preparation of alkyl phthalimides from the halide and sodium salt using a dimethylformamide solvent. Recently many alkyl 4-nitrophthalimides have been prepared by Billman and Cash[8] using the same solvent.

Our own work, these previous reports, and an unpublished observation by Stacy and Gortatowski[9] have led us to believe that dimethylformamide is especially effective as a solvent for weakening halogen carbon bonds. Its activating influence appears to extend beyond its powerful solvating ability into the realm of catalysis.

In our investigations dimethylformamide has been found to be an excellent solvent for the reaction of alkyl halides with sodium saccharin. Previous investigators have not reported exact yields for individual derivatives but they appeared

(1) E. I. du Pont, Electrochemicals Department, Niagara Falls, N. Y.

(2) This work was carried out by George R. Pettit as an undergraduate research project.

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(4) C. Fahlberg and A. List, *Ber.*, **20**, 1598 (1887).

(5) H. Eckenroth and G. Koerppen, *ibid.*, **30**, 1265 (1897).

(6) T. Sacks, T. von Wolff and A. Ludwig, *ibid.*, **37**, 3254 (1904).

(7) J. C. Sheehan and W. A. Bolhofer, THIS JOURNAL, **72**, 2786 (1950).

(8) J. H. Billman and R. V. Cash, *ibid.*, **75**, 2499 (1953).

(9) G. W. Stacy and M. Gortatowski observed that alkylation occurred swiftly and practically quantitatively between *p*-nitrobenzyl chloride and a sodium enolate in dimethylformamide.