12. *The Action of Periodic Acid on Glucosamine Derivatives.*

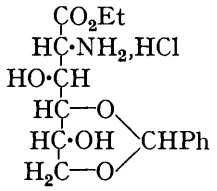
By Albert Neuberger.

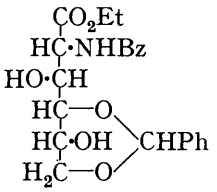
The action of periodic acid on derivatives of glucosamine and glucosamic acid has been investigated. In the latter case the oxygen uptake exceeds the theoretical value, unless the hydroxyl adjacent to the amino-group is blocked. For *N*-acylglucosaminides the oxygen uptake is normal, if metaperiodate is used. Free periodic acid, however, gives too high results and leads to secondary decomposition.

The original purpose of investigating the action of periodic acid on derivatives of glucosamine was to convert this amino-sugar into an amino-acid occurring in proteins for which the *l*-configuration has been well established and thus to demonstrate the configurational relationship between amino-acids and amino-sugars. This aim has not been attained, but certain observations have been made which show that periodic acid may be a useful tool for structural studies involving these compounds.

Karrer and Mayer (*Helv. Chim. Acta*, 1937, **20**, 407), starting from a compound first prepared by Levene and La Forge (*J. Biol. Chem.*, 1915, **21**, 348) and considered by the latter authors to be ethyl 5 : 6-benzylidene glucosamate hydrochloride, attempted to oxidise its *N*-carbethoxy-derivative between carbon atoms 3 and 4, using lead tetra-acetate.

Fission, however, took place between carbon atoms 2 and 3, leading to a glyoxylic acid–ammonia derivative. The formula of a 4 : 6-benzylidene compound was therefore assigned to the substance of Levene and La Forge (I).


(I.)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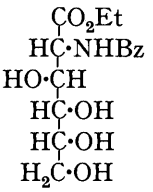
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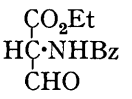
(IV.)

It was thought that the desired substituted amino-aldehyde might be obtained by removing the benzylidene residue and treating the resulting compound, which has four neighbouring hydroxyl groups, with either lead tetra-acetate or periodic acid. (I) was therefore benzoylated to give *ethyl* N-*benzoyl* 4 : 6-*benzylidene glucosamate* (II), from which on catalytic hydrogenation *ethyl* N-*benzoyl glucosamate* (III) was obtained. It was expected that on treatment with lead tetra-acetate (III) would use up three atoms of oxygen to produce (IV), two mols. of formic acid and one mol. of formaldehyde. It was found, however, that more than 4·5 mols. of oxygen were used up and that complete destruction of the molecule had taken place. Treatment of (III) with periodic acid or sodium metaperiodate also leads to an excessive uptake of oxygen and moreover to a liberation of iodine and ammonia. It is probable that the α-benzamido-aldehyde (IV) first formed is too unstable and/or is further oxidised and polymerised. Glucosamic acid itself behaves in a similar manner towards periodic acid.

If the hydroxyl group in position 3 is blocked as in 3-methylglucosamic acid (following paper), the oxygen uptake is normal: two equivalents of periodic acid are taken up and one mol. of formaldehyde and one mol. of a volatile reducing acid, presumably formic acid, are formed. An attempt to isolate the β-amino-aldehyde, the presumed reaction product, in the form of its hydrochloride was unsuccessful, nor was a catalytic reduction with platinum and ferric chloride (Carothers and Adams, *J. Amer. Chem. Soc.*, 1923, **45**, 1071) to the corresponding 2-amino-4-hydroxy-3-methoxybutyric acid possible.

The behaviour of *N*-acylglucosaminides towards metaperiodate is more normal. *N*-Acetyl-α-methylglucosaminide (Moggridge and Neuberger, J., 1938, 745), which has one pair of hydroxyl groups attached to adjacent carbon atoms, takes up one atom of oxygen with sodium metaperiodate; the reaction is slow, which is probably due to the fact that the two hydroxyl groups are in the *trans*-position to each other. If free periodic acid, however, is used, the oxygen uptake reaches two equivalents in 22 hours and iodine is liberated. Identical results were obtained with N-*benzoyl-α-methylglucosaminide* which was prepared from *N*-benzoyl tetra-acetyl glucosamine (Bergmann and Zervas, *Ber.*, 1931, **64**, 975) by treatment with 2·1% methyl-alcoholic hydrogen chloride. The dialdehyde presumably formed on treating the *N*-acylglucosaminides with sodium metaperiodate could not be isolated, nor could any dicarboxylic acids or their calcium and barium salts be obtained after oxidising the crude reaction products with bromine at p_H 5 or perbenzoic acid.

If, however, the hydroxyl group in position 3 is blocked as in 3-methyl *N*-acetyl α-methylglucosaminide (following paper), no oxygen uptake with either periodic acid or sodium metaperiodate occurs. It may be assumed that similarly no reaction will occur if position 4 is substituted, since it has been found that a hydroxyl group adjacent to a carbon atom to which an acylamido-group is attached reacts with periodic acid only very slowly, if at all (Nicolet and Shinn, *J. Amer. Chem. Soc.*, 1939, **61**, 1615).

The usefulness of sodium metaperiodate as a tool for elucidating the structure of a partially substituted glucosaminide is shown in the following paper; the results here reported may also help to explain the experiments on the oxidation of heparin by periodate (Charles and Todd, *Biochem. J.*, 1940, **34**, 112).

Experimental.

Ethyl N-*Benzoyl* 4 : 6-*Benzylidene Glucosamate.*—To ethyl 4 : 6-benzylidene glucosamate hydrochloride (3·6 g.), dissolved in water (40 ml.) containing sodium bicarbonate (1·95 g.), benzoyl chloride (1·35 ml.) was added in small portions during ½ hour; the mixture was shaken intermittently and kept at 5°. The crystalline precipitate which separated at once was filtered off and recrystallised first from chloroform and light petroleum (b. p. 60—80°) and then from alcohol; m. p. 173—174°, $[\alpha]_D$ −80° in chloroform (c = 1·1) (Found: C, 63·4; H, 5·9; N, 3·4. $C_{22}H_{25}O_7N$ requires C, 63·6; H, 6·0; N, 3·4%).

Ethyl N-*Benzoyl Glucosamate.*—Ethyl *N*-benzoyl 4 : 6-benzylidene glucosamate (2·7 g.), dissolved in alcohol (50 ml.), was treated with palladium (0·7 g.) and hydrogen for 6 hours; 4 atoms of hydrogen per mol. had then been taken up. The catalyst was filtered off, and the solution evaporated to low bulk. On addition of ether the *substance* crystallised in needles (yield, 70%), m. p. 144—145°, $[\alpha]_D$ + 11·8° in water (c = 0·8) (Found: C, 55·0; H, 6·3; N, 4·3. $C_{15}H_{21}O_7N$ requires C, 55·1; H, 6·4; N, 4·3%).

N-*Benzoyl* α-*Methylglucosaminide.*—*N*-Benzoyl tetra-acetyl glucosamine (23 g.) was refluxed with 2·1% methyl-alcoholic hydrogen chloride for 2 hours. The excess of acid was removed by lead carbonate, and the *substance* crystallised by evaporation of the solution to low bulk and recrystallised from alcohol (yield, 75%); m. p. 225—226°, $[\alpha]_D$ + 114° in water (Found: C, 56·3; H, 6·3; N, 4·7. $C_{14}H_{19}O_6N$ requires C, 56·6; H, 6·4; N, 4·7%).

Oxidation with Periodic Acid.—Periodic acid was determined according to Fleury and Lange (*J. Pharm. Chim.*, 1933, **17**, 107). Sodium metaperiodate was prepared by adding 1 equiv. of sodium hydroxide to the standard periodic acid solution, the final p_H being about 6.

Oxidation of Ethyl N-*Benzoyl Glucosamate by Lead Tetra-acetate.*—327 Mg. of the ester (1 millimol.) were dissolved in 10 ml. of acetic acid which had been distilled over lead tetra-acetate, and 60 ml. of 0·15M-lead tetra-acetate in acetic acid (4·5 millimols.) were added. After 20 mins. at room temperature no tetra-acetate could be detected in the solution. In an experiment on a larger scale where the theoretical amount of the oxidising agent was added slowly, an attempt was made to isolate the products; chloroform extracts, however, gave an uncrystallisable gum having only very weak reducing properties and from which no crystalline derivatives could be obtained. Benzoic acid, hippuric acid and *N*-benzoyl *dl*-serine are stable to lead tetra-acetate under the conditions used.

Oxidation of Ethyl N-*Benzoyl Glucosamate by Periodic Acid.*—4·5 Ml. of 0·5M-periodic acid were added to an aqueous solution of ethyl *N*-benzoyl glucosamate (0·330 g.); the solution turned brown and ammonia was liberated instantly. A titration indicated that all the periodic acid had been taken up. Oxidation with sodium metaperiodate in acetic acid buffer led to similar results. Attempts to isolate the products were unsuccessful. A colour reaction with ferric chloride, described by Erlenmeyer and Stoop (*Annalen*, 1907, **337**, 236) for the compound, was negative.

Oxidation of 3-*Methyl Glucosamic Acid by Periodic Acid.*—2 Ml. of 0·445M-periodic acid were added to 58 mg. of 3-methyl glucosamic acid in water (10 ml.). After 1 hour 1·24 ml. were used up (calc. for three hydroxyl groups, 1·26 ml.). At the same time 1 equiv. of acid was formed. If methyl-red is used as indicator, periodic and iodic acid behave as monobasic acids; an increase in acidity, therefore, must be due to acid formed during the oxidation. 30·2 Mg. of 3-methyl glucosamic acid were treated with 0·9 ml. of 0·445M-periodic acid and left for 2 hours. The solution now required 5·65 ml. of 0·1N-sodium hydroxide, while the same amount of periodic acid uses up 4·20 ml., the difference being 1·45 ml. (calc., 1·44 ml.). The acid was identified as formic acid as follows: 0·5 g. of 3-methyl glucosamic acid was oxidised with the theoretical amount of periodic acid, the iodic acid formed was removed by barium hydroxide, and the neutral solution was evaporated to low bulk. In this distillate the presence of formaldehyde was proved by the formation of its dimedon derivative. The residue was acidified, water added, and the solution again distilled; the second distillate contained an acidic substance reducing silver nitrate. No ammonia or iodine was liberated during the oxidation.

Glucosamic acid, on the other hand, reacts with periodic acid instantly with the appearance of iodine and ammonia in the solution.

Oxidation of N-*Acetyl* α-*Methylglucosaminide by Periodic Acid.*—To a solution of *N*-acetyl α-methylglucosaminide (0·235 g.; 1 millimol.) were added 5 ml. of 0·395M-periodic acid (1·98 millimols.), and the solution made up to 25 ml. The rotation after 24 hours had dropped to zero, the initial $[\alpha]_D$ being + 105°. The oxygen uptake was as follows: 2·5 hrs., 1·13 atoms; 9 hrs., 1·8 atoms; 22 hrs., 1·98 atoms. After 2 hrs. a brown colour appeared in the solution

which increased in intensity as the reaction proceeded. If 1 mol. of periodic acid is added per mol. of substance, 65% of the available oxygen is taken up in 2·5 hrs. Similar results were obtained with *N*-benzoyl α-methylglucosaminide.

If the reaction is carried out at neutral p_H, only one atom of oxygen is taken up and the curves obtained by plotting the changes in rotation and the oxygen uptake against time are identical. This indicates that probably only one primary reaction occurs at neutral p_H. *N*-Acetyl α-methylglucosaminide (0·235 g.) was dissolved in water (25 ml.) containing 5 ml. of 0·422M-periodic acid (2·11 millimols.) and 2·25 ml. of N-sodium hydroxide. Samples were removed at intervals and titrated. The initial oxygen uptake was more rapid than in acid solution, but the oxidation became very slow after one atom of oxygen had been taken up. The figures were as follows: after 1 hour, 0·83 atom; after 3 hrs., 0·99 atom; after 7 hrs., 1·02 atoms; after 17 hrs., 1·06 atoms. The changes in $[\alpha]_D$ were as follows: 1 hour, +48°; 3 hrs., + 33·3°; 7 hrs., + 28·5°; 17 hrs., + 27·9°. The solution remained colourless during the period. Similar results were obtained with the *N*-benzoyl compound.

Attempted Oxidation of N-*Acetyl* 3-*Methyl* α-*Methylglucosaminide by Periodic Acid.*—The α-methylglucosaminide (50 mg.) was dissolved in water (10 ml.) containing 1 ml. of 0·445M-periodic acid. The rotation remained constant during 24 hours, $[\alpha]_D$ being + 114°; no oxygen uptake occurred. Equally negative results were obtained with sodium metaperiodate.

The author wishes to thank Sir F. G. Hopkins for his encouragement. Acknowledgment is also made to the Beit Memorial Trustees for a Fellowship.

Biochemical Laboratory, Cambridge. [*Received, November 11th,* 1940.]