14. *Amino-sugars and Related Compounds. Part II.* Observations on the Acidic Hydrolysis of Derivatives of 2-Amino-2-deoxy-D-glucose (D-Glucosamine).†*

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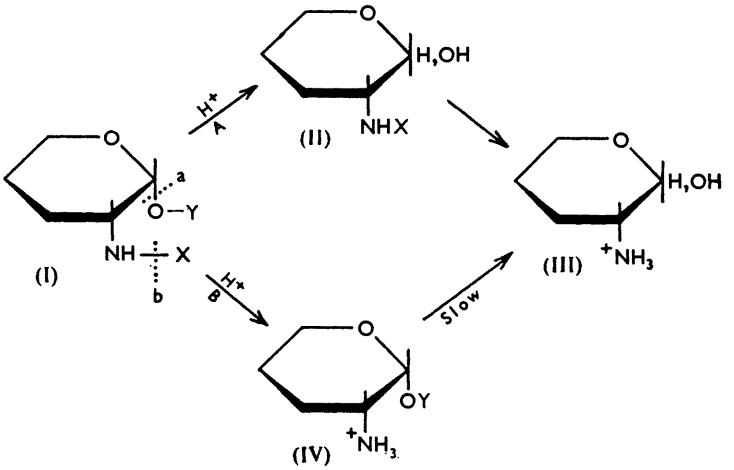
Acidic hydrolysis of a series of *O*-alkylglycosidic derivatives of 2-acylamino-2-deoxy-D-glucose (*N*-acyl-D-glucosamine) has been studied. In each case glycosidic hydrolysis was incomplete owing to the existence of two reaction pathways. The possible implications of these results in the hydrolysis of mucopolysaccharides are noted. The behaviour of the mono-*O*-methyl derivatives of 2-amino-2-deoxy-D-glucose in the Elson–Morgan test is described.

The acidic hydrolysis of glycosidic derivatives (I) of 2-amino-2-deoxy-D-glucose (D-glucosamine) may follow simultaneously the two pathways shown in the annexed scheme. Cleavage of the glycosidic substituent Y [*i.e.*, at *a* in (I)] may occur first to give the intermediate (II) which is further hydrolysed to yield the free amino-sugar (III) (pathway A). Alternatively, initial hydrolysis of the *N*-substituent X [*i.e.*, at *b* in (I)] will



Schematic representation of the acidic hydrolysis of 2-amino-2-deoxy-α-D-glucoside derivatives. A similar scheme operates for the β-anomers.

yield the 2-amino-2-deoxy-D-glucoside derivative (IV) (pathway B). The derivative (IV) is strongly resistant to further acidic hydrolysis since the positive charge acquired by the amino-group in the reaction medium electrostatically shields the neighbouring glycosidic substituent from attack by hydrions.[1,2] Although the alternative pathways A and B of hydrolysis were recognised as occurring [2] in the case of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (V) the potential implications appear not to have been appreciated.

The extent to which the pathways A and B are favoured depends on the nature of X and Y and on the configuration at the glycosidic centre. Thus, when X = SO_3H and Y = alkyl, the main pathway of hydrolysis is B. This situation occurs in heparin and it results in unsymmetrical fragmentation of the mucopolysaccharide on acidic hydrolysis [3]

* Part I, *J.*, 1956, 4531.

† A preliminary report of some of these results has been given in *Chem. and Ind.*, 1956, 175.

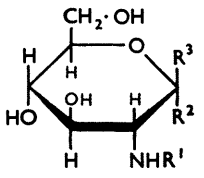
[1] See Foster and Overend, *Chem. and Ind.*, 1955, 566, for details of, and references to, the mechanism of acidic hydrolysis of glycosides.

[2] Moggridge and Neuberger, *J.*, 1938, 745.

[3] Wolfrom, Montgomery, Karabinos, and Rathgeb, *J. Amer. Chem. Soc.*, 1950, **72**, 5796; see also Foster and Huggard, *Adv. Carbohydrate Chem.*, 1955, **10**, 335.

with the ultimate formation of a resistant disaccharide in which the 2-amino-2-deoxy-D-glucosidic linkage is preserved. When X = acetyl and Y = alkyl (*e.g.*, in hyaluronic acid and the chondroitin hydrogen sulphates [4]) the main hydrolytic pathway is A. The examples considered herein fall into the latter category.

In earlier studies, Moggridge and Neuberger [2] employed reducing power to follow the hydrolytic release of 2-amino-2-deoxy-D-glucose from glycosidic derivatives. We have used the colorimetric method of Belcher, Nutten, and Sambrook.[5] Results obtained on acidic hydrolysis of a series of 2-amino-2-deoxy-D-glucose derivatives are recorded in Table 1. Both 2-acetamido-2-deoxy-D-glucose (V) and 2-benzyloxycarbonylamino-2-



(V): R^1 = Ac; R^2R^3 = H, OH
(VI): R^1 = Ph·CH_2·O·CO; R^2R^3 = H, OH
(VII): R^1 = Ac; R^2 = OMe; R^3 = H
(VIII): R^1 = Ac; R^2 = H; R^3 = OMe
(IX): R^1 = Ph·CH_2·O·CO; R^2 = OMe; R^3 = H
(X): R^1 = Ph·CH_2·O·CO; R^2 = H; R^3 = OMe
(XI): R^1 = Ph·CH_2·O·CO; R^2 = OEt; R^3 = H
(XII): R^1 = R^3 = H; R^2 = OMe
(XIII): R^1 = R^3 = H; R^2 = OMe

deoxy-D-glucose (VI) underwent complete and rapid hydrolysis. However, a much slower and incomplete release of 2-amino-2-deoxy-D-glucose occurred on acidic hydrolysis of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (VII). Similar results were obtained when the *N*-acetyl group in (VII) was replaced by an *N*-benzyloxycarbonyl group (IX) and the glycosidic methyl group by an ethyl residue (XI), indicating that the pattern of hydrolysis is probably a general one. In those cases, *e.g.*, with (VII) and (IX), where the

TABLE 1.

Derivative	X	Y	Time of half hydrolysis (min.)	2-Amino-2-deoxy-D-glucose release (%)
2-Acetamido-2-deoxy-D-glucose (V)	Ac	H	4—6	100
2-Benzyloxycarbonylamino-2-deoxy-D-glucose (VI)	Ph·CH_2·O·CO	H	4—6	100
Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (VII)	Ac	OMe	36	78
Methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (VIII)	Ac	OMe	4—8	82
Methyl 2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside (IX)	Ph·CH_2·O·CO	OMe	42	63
Methyl 2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (X)	Ph·CH_2·O·CO	OMe	21	86
Ethyl 2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside (XI)	Ph·CH_2·O·CO	OEt	27	70
Methyl 2-amino-2-deoxy-α-D-glucopyranoside hydrochloride (XII)	H,HCl	OMe	$8{\cdot}5 \times 10^3$ [a]	2
Methyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride (XIII)	H,HCl	OMe	$2{\cdot}8 \times 10^3$ [a]	6
Disaccharide A [b]	—	—	15	69

[a] By extrapolation. [b] Obtained by acid reversion [7] of 2-acetamido-2-deoxy-D-glucose and thought to be 1–6α-linked; m. p. 213°, $[\alpha]_D^{15}$ +106° (equil.) (*c* 3·0 in H_2O) (Found: N, 6·6. $C_{16}H_{28}O_{11}N_2$ requires N, 6·6%).

final hydrolysate was examined by chromatography it was found that the initial glycoside had been completely destroyed and that components corresponding to 2-amino-2-deoxy-D-glucose and methyl 2-amino-2-deoxy-α-D-glucopyranoside (III and IV respectively) were present. The time of half hydrolysis (Table 1) of the latter substance indicates that it would disappear very slowly during hydrolysis. Table 2 records a comparison of data reported here and calculated from the results of Moggridge and Neuberger [2] and emphasises their similarity.

[4] Stacey, *Adv. Carbohydrate Chem.*, 1946, **2**, 161.
[5] Belcher, Nutten, and Sambrook, *Analyst*, 1954, **79**, 201.

It is well known [1] that β-glycopyranosides are generally less resistant towards acidic hydrolysis than are the α-anomers and it is seen that methyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranoside conform to this rule (Table 1). However, it is surprising that the release of 2-amino-2-deoxy-D-glucose from the β-anomer is little greater (4%) than from

TABLE 2. *Hydrolysis* [a] *of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside.*[b]

Concn. of glycoside (mg./ml.)	Acid strength (N)	Temp.	Time of half hydrolysis	Release (%) of 2-amino-2-deoxy-D-glucose
1·825	1·0	61·25°	19 hr.[c]	—
1·825	1·0	80	1·5 hr.[c]	77—80 [c]
0·5	1·16	100	36 min.	78
4·0	1·16	100	—	74

[a] Hydrolyses performed in aqueous hydrochloric acid. [b] The configuration of the derivative studied by Moggridge and Neuberger [3] was not stated but it is undoubtedly α. [c] Calc. from the data of Moggridge and Neuberger.[3]

the α-anomer on acidic hydrolysis. In the corresponding *N*-benzyloxycarbonyl derivatives (IX) and (X) the β-anomer is hydrolysed more rapidly than the α-anomer and appreciably more (23%) 2-amino-2-deoxy-D-glucose is released. No case has yet been encountered of the complete glycosidic hydrolysis of compounds of the type (VII)—(XI) under acidic conditions.

It is not unreasonable to infer that the reaction pattern of the scheme will operate during the acidic hydrolysis of mucopolysaccharides and mucoproteins (or oligosaccharides derived therefrom), many of which contain *N*-acetylated β-glycosidically linked amino-sugars.[4] The possibility of incomplete release of amino-sugars in such hydrolyses appears to have been largely neglected. The results in Table 1 suggest that care must be exercised in interpreting, from an analytical standpoint, the colorimetric determinations of amino-sugars in mucopolysaccharide or tissue hydrolysates. The same considerations apply to the separation, by resin columns, of amino-sugars from such hydrolysates.[6] A further, relevant example is provided by the acidic hydrolysis of the disaccharide A, obtained by acid reversion [7] of 2-acetamido-2-deoxy-D-glucose, which gave a 69% release of 2-amino-2-deoxy-D-glucose on acidic hydrolysis.

Alternative methods for the complete hydrolytic release of amino-sugars from mucopolysaccharides are being studied.

A number of the derivatives in Table 1 have been prepared by modifications of known methods or by the use of methods not previously applied in the carbohydrate field (see Experimental).

Examination of the behaviour of the mono-*O*-methyl derivatives of 2-amino-2-deoxy-D-glucose in the Elson–Morgan colorimetric test [8] as modified by Belcher, Nutten, and Sambrook [5] showed (Table 3) that the 4- and 6-*O*-methyl derivatives gave a colour identical with (maximal absorption at 511 mμ) but less intense than that given by the parent amino-sugar. The 3-*O*-methyl derivative, however, gave a colour which was visibly different from that given by 2-amino-2-deoxy-D-glucose and in fact had maximal absorption at 503 mμ. Anderson and Percival [9] report that 2-amino-2-deoxy-D-glucose 6-phosphate gives a colour with maximal absorption at 518 mμ. These results contrast with those obtained [10] by application of the Morgan–Elson test [11] to derivatives of 2-acetamido-2-deoxy-D-glucose, where substitution at position 3 or 6 gives respectively an increased and identical intensity of the colour in comparison with that of the parent amino-sugar,

[6] Gardell, *Acta Chem. Scand.*, 1953, **7**, 201.
[7] Foster and Horton, unpublished results; cf. Ricketts, *J.*, 1954, 4031.
[8] Elson and Morgan, *Biochem. J.*, 1933, **27**, 1824.
[9] Anderson and Percival, *J.*, 1956, 814.
[10] Jeanloz and Trémège, *Fed. Proc.*, 1956, **15**, 282; Kuhn, Gauhe, and Baer, *Chem. Ber.*, 1954, **87**, 1138.
[11] Morgan and Elson, *Biochem. J.*, 1934, **28**, 988; Aminoff, Morgan, and Watkins, *ibid.*, 1952, **51**, 379.

but substitution at position 4 inhibits colour development. It is difficult to reconcile these results with the structures allocated to the chromophores in the Elson–Morgan and in the Morgan–Elson test.[12]

Experimental

Colorimetric Determination of 2-Amino-2-deoxy-D-glucose (D-Glucosamine) and Related Compounds.—2-Amino-2-deoxy-D-glucose in solution was determined by the colorimetric procedure described by Belcher, Nutten, and Sambrook;[5] a "Spekker" photoelectric absorptiometer, model H760 with 1 cm. cells and Ilford No. 604 filters, was used. Standard graphs were prepared simultaneously with each determination.

The following derivatives were found not to interfere with the determination of 2-amino-2-deoxy-D-glucose when present in the relative molar concentrations shown in parentheses: 2-amino-2-deoxy-D-glucitol hydrochloride (5), acetic acid (1), 2-acetamido-2-deoxy-D-glucitol (1), and methanol (10). Solutions of 2-amino-2-deoxy-D-glucose hydrochloride and each of these substances separately in 1·16N-hydrochloric acid when heated at 95° for 4 hr. gave the theoretical content of amino-sugar. The following substances gave no colour with the Elson–Morgan test:[8] methyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranosides, methyl 2-acetamido-2-deoxy-4-*O*-methyl-α-D-glucopyranoside, methyl 2-amino-2-deoxy-α- and -β-D-glucopyranoside hydrochloride, 2-acetamido-2-deoxy-D-glucose and 2-benzyloxycarbonylamino-2-deoxy-D-glucose.

The results obtained by application of the colorimetric procedure[5,8] to 2-amino-2-deoxy-D-glucose and its mono-*O*-methyl derivatives are shown in Table 3.

TABLE 3. *Colorimetric determination of 2-amino-2-deoxy-D-glucose and its mono-O-methyl derivatives by the method of Belcher, Nutten, and Sambrook.*[5]

Derivative	Relative optical density at 515 mμ
2-Amino-2-deoxy-D-glucose hydrochloride [a]	1·00
2-Amino-2-deoxy-3-*O*-methyl-D-glucose hydrochloride	0·66 [b]
2-Amino-2-deoxy-4-*O*-methyl-D-glucose hydrochloride	0·58 [c]
2-Amino-2-deoxy-6-*O*-methyl-D-glucose hydrochloride [a]	0·80

[a] Maximal absorption at 511 mμ. [b] Maximal absorption at 503 mμ where it had higher optical density than that of 2-amino-2-deoxy-D-glucose. [c] Determined after acidic hydrolysis of methyl 2-acetamido-2-deoxy-4-*O*-methyl-α-D-glucopyranoside, release of amino-sugar being assumed identical with that from methyl 2-acetamido-2-deoxy-α-D-glucopyranoside under the same conditions.

Methyl 2-Benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside.—A solution of 2-benzyloxycarbonylamino-2-deoxy-D-glucose (5·0 g.; dried *in vacuo* at 55° over P_2O_5) in methanolic hydrogen chloride (100 ml.; 2·5% w/v) was boiled under reflux for 4 hr. Recrystallisation of the residue, isolated in the usual way, from ethyl acetate–methanol (10 : 1) gave the product (3·3 g., 69%). After a further recrystallisation it had m. p. 158—159°, $[\alpha]_D^{21}$ +88° (*c* 0·4 in pyridine). Neuberger[13] reports m. p. 159—160° for the same product prepared by a different procedure.

From the mother-liquors an unidentified product (1·5 g.) was isolated which after recrystallisation from ethyl acetate–methanol had m. p. 118—121°, resolidified, melting again at 154°, $[\alpha]_D$ +20° (*c* 0·4 in pyridine) (Found: C, 51·55; H, 6·45; N, 3·9%). It consumed 1 mol. of periodate and gave no formic acid or formaldehyde.

Methyl 2-Benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside.—A solution of 2-benzyloxycarbonylamino-2-deoxy-D-glucose (3 g.) in methanolic hydrogen chloride (180 ml.; 0·7% w/v) was stored at 25° (α_D +0·46° ⟶ +0·11° in 88 hr.). Thereafter the product (1·2 g., 41%) was isolated as for the α-anomer. After two recrystallisations it had m. p. 168—170°, $[\alpha]_D$ −35° (*c* 0·4 in pyridine). Neuberger and Rivers[14] give m. p. 166—168°, $[\alpha]_D$ −38° in pyridine. The method described by these authors could not be reproduced.

From the mother-liquors an unidentified product (0·5 g.) was obtained having m. p. 152—156°, $[\alpha]_D$ +15° (*c* 0·4 in pyridine).

[12] See Foster and Stacey, *Adv. Carbohydrate Chem.*, 1952, **7**, 247.
[13] Neuberger in "Biochemistry of the Amino Sugars" by Kent and Whitehouse, Butterworths, 1955, p. 229, footnote.
[14] Neuberger and Rivers, *J.*, 1939, 122.

Methyl 2-Amino-2-deoxy-α-D-glucopyranoside Hydrochloride.—A solution of 2-acetamido-1 : 3 : 4 : 6-tetra-*O*-acetyl-2-deoxy-αβ-D-glucose, prepared [15] from 2-amino-2-deoxy-D-glucose hydrochloride (10 g.), in methanolic hydrogen chloride (200 ml.; 7% w/v) was boiled under reflux for 1 day. Thereafter the solution was neutralised and concentrated, whereupon 2-amino-2-deoxy-D-glucose hydrochloride (1 g.) crystallised. Paper-chromatographic examination of the mother-liquors, with use of the organic phase of butanol–ethanol–water–ammonia (40:10:49:1), and development with ninhydrin revealed 2-amino-2-deoxy-D-glucose (R_F 0·16) and methyl 2-amino-2-deoxy-α-D-glucopyranoside (R_F 0·30). The mixture was separated on a column (50 × 5·5 cm.) of cellulose powder by elution with the organic phase of butanol–ethanol–water (4 : 1 : 5), to yield methyl 2-amino-2-deoxy-α-D-glucopyranoside (2·1 g.) as a colourless glass, $[\alpha]_D^{21}$ +130° (*c* 1·0 in H_2O). (Neuberger [13] gives $[\alpha]_D$ +145° in H_2O for the crystalline product prepared by a different method.) The compound was chromatographically homogeneous and gave a good yield of methyl 2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside on reaction with benzyloxycarbonyl chloride.

Methyl 2-Amino-2-deoxy-β-D-glucopyranoside Hydrochloride.—(*a*) A solution of methyl 2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (1·03 g.) in liquid ammonia (50 ml.) was treated with sodium until a permanent blue colour was obtained.[16] After 15 min. an excess of ammonium chloride was added, the solvent removed by evaporation, and the residue was extracted with methanol (150 ml.). Evaporation of the extract gave a residue which was extracted with dimethylformamide. Evaporation of this extract gave a residue (0·7 g.) which after recrystallisation from methanol–ether gave the product (0·446 g., 62%), m. p. 189—190°, $[\alpha]_D^{20}$ −24° (*c* 0·1 in H_2O).

(*b*) A solution of methyl 3 : 4 : 6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside hydrobromide, (0·2 g.), prepared according to the method of Irvine, McNicoll, and Hynd [17] in 0·069N-hydrochloric acid (12 ml.), was heated on a boiling-water bath until a constant optical rotation was obtained (55 min.). The residue obtained after neutralisation and evaporation of the solution was dissolved in cold concentrated hydrochloric acid (2 ml.), and the product (0·10 g., 88%) precipitated by the addition of acetone. It had m. p. 184—190°, $[\alpha]_D$ −20° (*c* 0·1 in H_2O). Irvine and Hynd [18] give m. p. 190°, $[\alpha]_D$ −24·2° in H_2O. The product was contaminated with 2-amino-2-deoxy-D-glucose (2·5% as determined colorimetrically [5]) which was not removed by further recrystallisation of the glycoside from ethanol.

Ethyl 2-Benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside.—A solution of 2-benzyloxycarbonylamino-2-deoxy-D-glucose (5 g.) in ethanolic hydrogen chloride (100 ml.; 0·7% w/v) was boiled under reflux for 18 hr. and then worked up in the usual way, to give the *product* (3·0 g., 54%), m. p. 133°, $[\alpha]_D$ +100·6° (*c* 0·4 in EtOH) (Found: N, 4·0. $C_{16}H_{23}O_7N$ requires N, 4·1%).

Hydrolysis of the Derivatives of 2-Amino-2-deoxy-D-glucose.—In addition to the compounds described above the following derivatives were prepared by the appropriate methods in the literature: 2-acetamido-2-deoxy-D-glucose,[19] m. p. 198°; 2-benzyloxycarbonylamino-2-deoxy-D-glucose,[20] m. p. 216—217°; methyl 2-acetamido-2-deoxy-α-D-glucopyranoside,[3] m. p. 189°; methyl 2-acetamido-2-deoxy-β-D-glucopyranoside,[14] m. p. 194—196°.

Standard conditions of hydrolysis were adopted as follows:

Aliquot parts (1 ml.) of a standard solution of the amino-sugar derivative (1 mg. per ml.) were each diluted with 2·32N-hydrochloric acid (1 ml.), and the solutions heated on a boiling-water bath. At suitable times a reaction solution was cooled, treated with N-sodium carbonate solution (2 ml.), and diluted to standard volume (10 ml.). A portion (1 ml.) was examined for 2-amino-2-deoxy-D-glucose content by the method of Belcher, Nutten, and Sambrook.[5] From graphs plotted for the release of 2-amino-2-deoxy-D-glucose with time, the times of half hydrolysis and the extents of hydrolysis shown in Table 1 were determined.

Chromatographic Examination of the Hydrolysates.—The hydrolysate obtained by the acid treatment of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (VII) was evaporated to dryness and examined chromatographically, the organic phase of butanol–ethanol–water–ammonia

[15] Lobry de Bruyn and Van Ekenstein, *Rec. Trav. chim.*, 1899, **18**, 83.
[16] Sifferd and du Vigneaud, *J. Biol. Chem.*, 1935, **108**, 753.
[17] Irvine, McNicoll, and Hynd, *J.*, 1911, **99**, 250.
[18] Irvine and Hynd, *J.*, 1912, **101**, 1128.
[19] Roseman and Ludwieg, *J. Amer. Chem. Soc.*, 1954, **76**, 301.
[20] Chargaff and Bovarnick, *J. Biol. Chem.*, 1937, **118**, 421.

(40 : 10 : 49 : 1) being used with ninhydrin as the detection reagent. Spots corresponding to 2-amino-2-deoxy-D-glucose (R_F 0·16) and methyl 2-amino-2-deoxy-α-D-glucopyranoside (R_F 0·30) were observed. There was no starting material left in the hydrolysate (detection of non-reducing *N*-substituted derivatives accomplished by the method of Trevelyan, Proctor, and Harrison [21]). Similar examination of the hydrolysate of disaccharide A, which was deacidified by the use of methyldi-*n*-octylamine,[22] showed the presence of 2-amino-2-deoxy-D-glucose and a spot (R_F 0·08) of similar mobility to chitobiose (detection by ninhydrin and the hexosamine reagent described by Foster and Ashton [23]). The hydrolysate from methyl 2-acetamido-2-deoxy-4-*O*-methyl-α-D-glucopyranoside contained components (R_F 0·39 and 0·62) with the behaviour expected for 2-amino-2-deoxy-4-*O*-methyl-D-glucose and methyl 2-amino-2-deoxy-4-*O*-methyl-α-D-glucopyranoside. The latter component did not react with hexosamine spray [22] but was detected with ninhydrin.

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[21] Trevelyan, Proctor, and Harrison, *Nature*, 1950, **166**, 444.
[22] Lester-Smith and Page, *J. Soc. Chem. Ind.*, 1948, **67**, 48.
[23] Foster and Ashton, *Nature*, 1953, **172**, 958.