α- and β-glycuronidase, but not between glucuronidase and galacturonidase activity in either the α- or β-series.

7. Heat treatment and the inhibitory effects of galacturonic acid suggested that there was more than one α-glycuronidase present in limpet preparations.

8. Mammalian β-glucuronidase preparations were found to possess β-galacturonidase activity; the rate of hydrolysis, the affinity for the substrate, and the pH optimum were all lower with the latter.

9. Mammalian β-galacturonidase activity was inhibited by boiled saccharate, boiled mucate, glucuronate and galacturonate solutions, in that order; the affinity constants agreed with those for β-glucuronidase, and it was concluded that the enzymes were identical.

10. Phenyl α- and β-*N*-acetylglucosaminuronide were not hydrolysed by α- or β-glucuronidase or by *N*-acetylglucosaminidase.

The authors are indebted to Dr W. P. K. Findlay, of the Forest Products Research Laboratory, for supplying cultures of wood-rotting fungi.

REFERENCES

Bernfeld, P., Bernfeld, H. C., Nisselbaum, J. S. & Fishman, W. H. (1954). *J. Amer. chem. Soc.* **76**, 4872.
Beyler, A. L. & Szego, C. M. (1951). *Amer. J. Physiol.* **167**, 768.
Bishop, C. T. (1953). *Canad. J. Chem.* **31**, 134.
Brooks, J. & Reid, W. W. (1955). *Chem. & Ind.* p. 325.
Conchie, J. (1954). *Biochem. J.* **58**, 552.
Conchie, J., Findlay, J. & Levvy, G. A. (1956). *Nature, Lond.*, **178**, 1469.
Conchie, J. & Levvy, G. A. (1957). *Biochem. J.* **65**, 389.
Cornforth, J. W., Daines, M. E. & Gottschalk, A. (1957). *Proc. chem. Soc.*, p. 25.
Dodgson, K. S., Lewis, J. I. M. & Spencer, B. (1953). *Biochem. J.* **55**, 253.
Dodgson, K. S. & Spencer, B. (1957). *Biochem. J.* **65**, 668.
Haldane, J. B. S. (1957). *Nature, Lond.*, **179**, 832.
Karunairatnam, M. C. & Levvy, G. A. (1949). *Biochem. J.* **44**, 599.
Kerr, L. M. H., Graham, A. F. & Levvy, G. A. (1948). *Biochem. J.* **42**, 191.
Klenk, E., Faillard, H., Weygand, F. & Schöne, H. H. (1956). *Hoppe-Seyl. Z.* **304**, 35.
Levvy, G. A. (1952). *Biochem. J.* **52**, 464.
Levvy, G. A., Hay, A. J. & Marsh, C. A. (1957). *Biochem. J.* **65**, 203.
Levvy, G. A. & Marsh, C. A. (1952). *Biochem. J.* **52**, 690.
Lineweaver, H. & Burk, D. (1934). *J. Amer. chem. Soc.* **56**, 658.
Marsh, C. A. (1952). *J. chem. Soc.* p. 1578.
Marsh, C. A. & Levvy, G. A. (1957). *Biochem. J.* **66**, 4P.
Marsh, C. A. & Levvy, G. A. (1958). *Biochem. J.* **68**, 617.
Masamune, H. (1938). *J. Biochem., Tokio*, **19**, 353.
Park, J. T. (1951). *J. biol. Chem.* **194**, 885.
Roudier, A. (1953). *C.R. Acad. Sci., Paris*, **237**, 662.
Sammons, H. G. & Williams, R. T. (1946). *Biochem. J.* **40**, 223.
Smith, E. E. B. & Mills, G. T. (1953). *Biochem. J.* **54**, 164.
Storey, I. D. E. & Dutton, G. J. (1955). *Biochem. J.* **59**, 279.
Strominger, J. L., Maxwell, E. S., Axelrod, J. & Kalckar, H. M. (1957). *J. biol. Chem.* **224**, 79.
Talalay, P., Fishman, W. H. & Huggins, C. (1946). *J. biol. Chem.* **166**, 757.
Utusi, M., Huzi, K., Matumoto, S. & Nagaoka, T. (1949). *Tohoku J. exp. Med.* **50**, 175.
Webster, M. E., Clark, W. R. & Freeman, M. E. (1954). *Arch. Biochem. Biophys.* **50**, 223.

APPENDIX

The Synthesis of Aryl Glycosiduronic Acids

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(*Received* 16 *September* 1957)

In connexion with the study of the relationships between different glycuronidases in the limpet (*Patella vulgata*) and in mammalian tissues (Marsh & Levvy, 1958), it was necessary to synthesize chromogenic α- and β-D-glucopyranosiduronic acids (glucuronides) and galactopyranosiduronic acids (galacturonides). For purposes of comparison, it was considered desirable in quantitative experiments to keep the aglycone constant, and all four derivatives of phenol were therefore prepared. Since, however, phenyl glycosides are unsuitable for the screening of crude-tissue preparations, owing to the presence of material that interferes in the determination of free phenol, certain nitrophenyl glycuronides were also prepared.

Catalytic oxidation by gaseous oxygen in the presence of platinum catalyst as a method whereby aliphatic and alicyclic hexosides in neutral aqueous solution are converted into the corresponding hexosiduronic acids has been already described (Marsh, 1952); attempts to oxidize aryl glycosides by the same procedure were unsuccessful. Subsequently, however, the syntheses of the 2-naphthyl (Tsou & Seligman, 1952) and the phenyl (Tsou & Seligman, 1953) β-D-glucopyranosiduronic acids by the same general method were reported: the latter

synthesis was confirmed by Bollenback (1956, personal communication), but not by Heyns & Kelch (1953). The preparation of phenyl α-D-glucopyranosiduronic acid in low yield (18%) by catalytic oxidation has also been described (Bollenback, Long, Benjamin & Lindquist, 1955). It appeared that aryl glycosides required more catalyst and a slightly higher temperature and pH than the aliphatic and alicyclic compounds, and we have prepared a number of new aryl hexosiduronic acids in this way, including 4-methylumbelliferone β-D-glucopyranosiduronic acid, described elsewhere (Marsh & Levvy, 1956). Freshly reduced platinum catalyst was essential, and unlike other authors (see above) we had no success with commercial platinum black. These more extreme conditions of oxidation were found to be unsuitable for the synthesis of borneol and (−)-menthyl α-D-glucopyranosiduronic acids; preparation of the former compound by the original procedure (Marsh, 1952) is described below.

Catalytic oxidation of phenyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranosides resulted, as anticipated, in the formation of the corresponding 2-acetamido-2-deoxy-glucopyranosiduronic acids (*N*-acetylglucosaminuronides). Heyns & Paulsen (1955) applied the oxidation to methyl and benzyl 2-carbobenzoxamido-2-deoxy-α-D-glucopyranoside, but had no success with an *N*-acetylated glucosaminide. Progressive decomposition of our *N*-acetylglucosaminuronides was followed during acid hydrolysis (Fig. 1), and the results were similar to those obtained for the hydrolysis of methyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranosides (Moggridge & Neuberger, 1938; Foster, Horton & Stacey, 1957). With the α-anomer, there was very slow and incomplete hydrolysis of the glycoside bond, due no doubt to rapid deacetylation to yield the resistant 2-amino-2-deoxy-α-glucopyranosiduronic acid (cf. Heyns & Paulsen, 1955). Release of phenol from the β-anomer rapidly approached the theoretical value, and in the initial stages was accompanied by liberation of 2-amino-2-deoxy-glucuronic acid in considerable amounts, the latter, however, being unstable in the acid solution (Heyns & Paulsen, 1955). Deacetylation must therefore follow hydrolysis of the β-glycoside bond or yield a relatively labile glucosaminuronide.

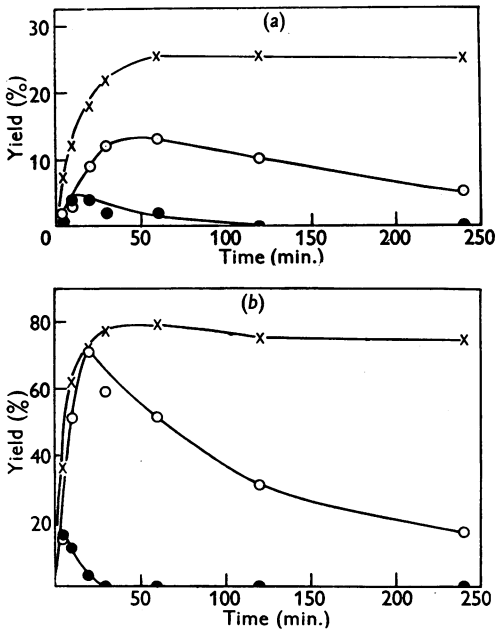

Fig. 1. Liberation of phenol (×), 2-acetamido-2-deoxy-D-glucuronic acid (●) and 2-amino-2-deoxy-D-glucuronic acid (○) during hydrolysis of (*a*) phenyl 2-acetamido-2-deoxy-α-D-glucopyranosiduronic acid and (*b*) phenyl 2-acetamido-2-deoxy-β-D-glucopyranosiduronic acid by 2N-HCl at 100°.

EXPERIMENTAL

Determinations of carbon, hydrogen and nitrogen are by Dr J. W. Minnis, Edinburgh University, and by Weiler and Strauss, Oxford. The latter also determined acetyl. M.p. values are corrected.

General oxidation procedure. The procedure for preparing the Pt catalyst and doing the oxidation has been described (Marsh, 1952). Fresh catalyst was added hourly, and the weight given below refers to the total. Evaporations were carried out below 50° at reduced pressure.

Phenyl α-D-glucopyranosiduronic acid. The following procedure gave a better yield than the one previously described (Bollenback *et al.* 1955). A solution of phenyl α-D-glucopyranoside monohydrate (2·16 g.) in water (50 ml.) at pH 8–10 with catalyst (1 g.) was oxidized at 90°, with frequent addition of 0·5N-$NaHCO_3$ soln. (total 15·5 ml.) to maintain the original pH, and the reaction was complete in 1½ hr. The filtered solution was evaporated to 10 ml. and hot ethanol (100 ml.) was added. The immediate precipitate was removed, and the sodium salt of phenyl α-D-glucopyranosiduronic acid (1·82 g.) separated on cooling. This was dissolved in water (18 ml.) and the pH brought to 2·2 with H_2SO_4. After continuous extraction with ethyl acetate for 2½ hr., the aqueous layer gave only a feeble Tollens reaction for uronic acid. Concentration of the ethyl acetate solution gave phenyl α-D-glucopyranosiduronic acid hemihydrate (1·35 g., 62%), m.p. 148–149°. After recrystallization from moist ethyl acetate, the m.p. was 149–150°; $[\alpha]_D^{22}$ +150° in water (*c*, 2); there was no depression of the mixed m.p. with an authentic specimen, m.p. 148–149°. According to Bollenback *et al.* (1955) the m.p. of the hemihydrate is 147–149°, $[\alpha]_D^{25}$ +153·6° in water (*c*, 1).

Phenyl β-D-glucopyranosiduronic acid. The following is a slight modification of the procedure of Tsou & Seligman (1953), who used commercial Pt black as the catalyst. Phenyl β-D-glucopyranoside dihydrate (2 g.) in water (25 ml.) together with catalyst (0·8 g.) was oxidized at 90°, with pH controlled to 8–10 by 0·5N-$NaHCO_3$ soln. (12·6 ml.).

The reaction was complete in 3 hr. After filtration the solution was concentrated to 10 ml., treated with charcoal, filtered and evaporated to give a gum. Evaporation with methanol, followed by addition of ethanol (50 ml.) yielded the insoluble crude sodium salt of phenyl β-D-glucopyranosiduronic acid. This was dissolved in water (5 ml.), the pH adjusted to 2·4 with H_2SO_4, and extracted repeatedly with ethyl acetate (total 100 ml.). Evaporation of the dried ethyl acetate solution yielded a gummy solid which was recrystallized from moist ethyl acetate, yielding phenyl β-D-glucopyranosiduronic acid dihydrate (0·55 g., 27%), m.p. 156–159°. After a further recrystallization the m.p. was 159–160°, alone and mixed with an authentic biosynthetic specimen, m.p. 160–161°. Tsou & Seligman (1953) give m.p. 160–161° and yield 32%.

p-*Nitrophenyl* α-D-*glucopyranosiduronic acid.* Oxidation of *p*-nitrophenyl α-D-glucopyranoside (1·5 g.) in water (25 ml.) and with catalyst (0·6 g.), with pH adjustment to 8–10 with 0·5 N-$NaHCO_3$ soln. (9·6 ml.), was complete in 2 hr. at 90°. Acidification of the filtrate with 3 N-HCl caused immediate crystallization of p-*nitrophenyl* α-D-*glucopyranosiduronic acid monohydrate*; after recrystallization from 50% (v/v) aqueous ethanol, m.p. 211–212° (decomp.), (0·80 g., 48%), $[\alpha]_D^{24}$ + 161° in ethanol (*c*, 1) (Found: loss at 100°, 5·6. $C_{12}H_{13}O_9N,H_2O$ requires H_2O, 5·4. Found, for anhydrous compound: C, 45·5; H, 4·0; N, 4·7. $C_{12}H_{13}O_9N$ requires C, 45·7; H, 4·2; N, 4·45%). The compound was slightly soluble in water, moderately soluble in ethanol, and very soluble in alkali with decomposition and liberation of *p*-nitrophenol, especially in the presence of protein. It slowly reduced Fehling's solution, and did so much more rapidly after hydrolysis with 2 N-HCl (15 min. at 100°). A strong Tollens reaction for uronic acid was given.

Phenyl α-D-*galactopyranosiduronic acid.* Phenyl α-D-galactopyranoside monohydrate (1 g.) dissolved in water (25 ml.) with catalyst (0·4 g.) was completely oxidized in 1½ hr. at 90°, the pH being maintained at 8–10 with 0·5 N-$NaHCO_3$ soln. (7 ml.). The filtered solution was treated with charcoal, filtered and evaporated to dryness. Ethanol (50 ml.) was added to the white solid residue, which was filtered off and redissolved in water (8 ml.); the pH was adjusted to 2–3 with HCl and the solution extracted with ethyl acetate (total 100 ml.). After being dried over anhydrous Na_2SO_4, the ethyl acetate solution was evaporated to yield *phenyl* α-D-*galactopyranosiduronic acid.* This was precipitated from methanolic solution with ether (10 vol.) and finally recrystallized from moist ethyl acetate; m.p. 192–193° (decomp.), (0·28 g., 28%), $[\alpha]_D^{22}$ + 156° in water (*c*, 1) (no loss of weight on drying at 100°. Found: C, 53·3; H, 5·2. $C_{12}H_{14}O_7$ requires C, 53·3; H, 5·2%). The white crystalline product was soluble in water and ethanol, and sparingly soluble in ethyl acetate. It gave a strong Tollens reaction for uronic acid, and had reducing power only after hydrolysis with 2 N-HCl (15 min. at 100°).

Phenyl β-D-*galactopyranosiduronic acid.* The oxidation of phenyl β-D-galactopyranoside (1 g.) dissolved in water (20 ml.) with catalyst (0·3 g.) and pH adjustment to 8–10 with 0·5 N-$NaHCO_3$ soln. (7·6 ml.) was complete in 1½ hr. at 90°. The filtered solution was concentrated and *phenyl* β-D-*galactopyranosiduronic acid monohydrate* (0·55 g., 47%) precipitated after acidification with HCl. After two recrystallizations from water the product had m.p. 173° (decomp.), $[\alpha]_D^{23}$ − 73° in methanol (*c*, 1) (Found: loss at 100°, 6·4. $C_{12}H_{14}O_7,H_2O$ requires H_2O, 6·3. Found for anhydrous product: C, 52·9; H, 5·6. $C_{12}H_{14}O_7$ requires C, 53·3; H, 5·2%). This compound was only slightly soluble in cold water, but quite soluble in ethanol. It gave a strong Tollens reaction for uronic acid, and had reducing power only after hydrolysis with 2 N-HCl (15 min. at 100°).

o-*Nitrophenyl* β-D-*galactopyranosiduronic acid.* *o*-Nitrophenyl β-D-galactopyranoside (1 g.) dissolved in water (20 ml.) with catalyst (total 0·4 g.) was oxidized at 90° with pH adjustment to 8–10 with 0·5 N-K_2CO_3 soln. (6·2 ml.). After 1½ hr. no further pH change occurred; the filtered solution was concentrated, treated with charcoal and evaporated to dryness. The crude potassium salt (0·9 g.) was well washed on the filter with ethanol, then redissolved in water (8 ml.); $HClO_4$ (approx. 5%, v/v) was added at 0° until the pH was 2·4. An equal volume of methanol was added and the filtered solution was evaporated to dryness; the residue was extracted with warm methanol (10 ml.) and filtered from residual $KClO_4$. Evaporation of the methanolic solution yielded *o-nitrophenyl* β-D-*galactopyranosiduronic acid*, which crystallized from warm water as the dihydrate (0·3 g., 26%), m.p. 171–172° (decomp.), $[\alpha]_D^{23}$ − 96° in water (*c*, 1) (Found: loss at 100°, 10·5. $C_{12}H_{13}O_9N,2H_2O$ requires H_2O, 10·2. Found for anhydrous compound: C, 45·3; H, 4·7; N, 4·2. $C_{12}H_{13}O_9N$ requires C, 45·7; H, 4·2; N, 4·45%). The pale-yellow crystalline product was very soluble in water and ethanol; it gave a strong Tollens reaction for uronic acid and slowly reduced Fehling's solution, much more rapidly after hydrolysis with 2 N-HCl (10 min. at 100°).

Borneol α-D-*glucopyranoside.* The method was similar to that used to prepare menthyl α-D-glucopyranoside (Fischer & Bergmann, 1917). A mixture of borneol (optically inactive, 20 g.), tetra-*O*-acetyl-α-D-glucopyranosyl bromide (10 g.) and redistilled quinoline (5 g.) was heated on an oil bath at 105–110° for 2 hr. with frequent shaking. The melt was cooled, and after adding water (50 ml.) extracted with ether (total 200 ml.); the ether solution was then washed with 2 N-H_2SO_4, saturated $NaHCO_3$ soln. and finally with water. Evaporation of the ether yielded a yellow gum which was steam-distilled to remove much of the excess of borneol. The residue was re-extracted with ether to yield a gum (11·8 g.) containing a mixture of borneol tetra-*O*-acetyl α- and -β-D-glucopyranosides, which could not be further purified. This was dissolved in ethanol (25 ml.) and a solution of KOH (5 g.) in water (15 ml.) added. The mixture was heated for 1 hr. at 60° with stirring, made neutral with HCl and steam-distilled to remove more borneol. The residual solid was filtered off and recrystallized thrice from ethyl acetate, yielding *borneol* α-D-*glucopyranoside* (0·46 g.), m.p. 142–144°, $[\alpha]_D^{23}$ + 59° in methanol (*c*, 1·5) (Found: no loss of weight on drying at 100°. Found: C, 60·3; H, 9·2. $C_{16}H_{28}O_6$ requires C, 60·8; H, 8·9%). This was moderately soluble in hot water, very soluble in ethanol.

Borneol α-D-*glucopyranosiduronic acid.* Borneol α-D-glucopyranoside (0·5 g.) dissolved in water (25 ml.) with catalyst (0·2 g.) was completely oxidized in 2½ hr. at 50–55°, 0·5 N-$NaHCO_3$ (2·3 ml.) being periodically added to keep the solution at neutrality. The filtered solution was concentrated to about 1 ml., filtered and made acid to Congo red with 3 N-HCl. A yellowish oil separated, which rapidly crystallized on standing at 0°. This was recrystallized twice from moist ethyl acetate (2 ml.) to give *borneol*

α-D-glucopyranosiduronic acid (0·15 g., 27%) as the hydrate, m.p. 149–153° with softening above 130°, $[\alpha]_D^{23}+46°$ in methanol (*c*, 1), corresponding to $[\alpha]_D^{23}+49°$ for the anhydrous compound (Found: loss on drying at 100°, 6·7. $C_{16}H_{26}O_7,H_2O$ requires H_2O, 5·2; $C_{16}H_{26}O_7,1\frac{1}{2}H_2O$ requires H_2O, 7·6. Found on anhydrous compound: C, 58·7; H, 7·8. $C_{16}H_{26}O_7$ requires C, 58·3; H, 7·9%). This compound gave a strong Tollens test for uronic acid, and possessed reducing properties only after hydrolysis with 2N-HCl (15 min. at 100°). Unlike the β-anomer (Quick, 1927), it did not form an insoluble zinc salt. It was not hydrolysed by β-glucuronidase.

Phenyl 2-acetamido-2-deoxy-α-D-glucopyranosiduronic acid. The oxidation of phenyl 2-acetamido-2-deoxy-α-glucopyranoside (0·4 g.) in solution in water (25 ml.) with catalyst (0·2 g.) was complete in 2 hr. at 90°; the reaction mixture was maintained at pH 8–10 with 0·5N-$NaHCO_3$ (2·6 ml.). The filtered solution was concentrated to about 10 ml., made definitely alkaline with dil. aq. NH_3 soln. and basic lead acetate added. The precipitated lead salt was centrifuged off, washed with aq. 0·01N-NH_3 soln., and decomposed with H_2S. Evaporation of the filtered solution yielded *phenyl 2-acetamido-2-deoxy-α-D-glucopyranosiduronic acid monohydrate*, which had m.p. 229–230° (decomp.) after two recrystallizations from water (0·13 g., 29%), $[\alpha]_D^{20}+174°$ in water (*c*, 2) (Found: no loss on drying at 100°; C, 51·3; H, 5·7; N, 4·6; CH_3CO, 12·4; equivalent weight by titration with NaOH under N_2, 314. $C_{14}H_{17}O_7N,H_2O$ requires C, 51·2; H, 5·8; N, 4·3; CH_3CO, 13·1%; equivalent weight, 329. Found: loss on drying at 120°, 5·25. $C_{14}H_{17}O_7N,H_2O$ requires H_2O, 5·5%). This compound possessed only slight reducing power after hydrolysis with 2N-HCl (20 min. at 100°) and did not give a normal Tollens test for uronic acid.

Phenyl 2-acetamido-2-deoxy-β-D-glucopyranosiduronic acid. A solution of phenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (Conchie & Levvy, 1957) (0·5 g.) in water (20 ml.) with catalyst (0·3 g.) was oxidized for 1 hr. at 90°, with maintenance at pH 8–10 by 0·5N-$NaHCO_3$ soln. (3·1 ml.). The filtered product was then worked up as for the α-anomer. After two recrystallizations from 95% (v/v) aq. ethanol pure *phenyl 2-acetamido-2-deoxy-β-D-glucopyranosiduronic acid monohydrate* was obtained, m.p. 183° (decomp.) (0·27 g., 49%), $[\alpha]_D^{20}-17°$ in water (*c*, 1·5) (Found: no loss on drying at 100°; C, 51·8; H, 5·8; N, 4·6; CH_3CO, 11·6; equivalent weight by titration 343. $C_{14}H_{17}O_7N,H_2O$ requires C, 51·2; H, 5·8; N, 4·3; CH_3CO, 13·1%; equivalent weight 329. Found: loss on drying at 120°, 5·9. $C_{14}H_{17}O_7N,H_2O$ requires H_2O, 5·5%). The product gave a negative Tollens test for uronic acid, and reduced Fehling's solution only after acid hydrolysis (10 min. at 100° with 2N-HCl).

Acid hydrolysis of phenyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranosiduronic acids. Measured portions (1 ml.) of a solution of the aryl glycoside (1·5 mg./ml.) in 2N-HCl were heated on the boiling-water bath in tubes fitted with water-cooled thimbles to prevent evaporation. After predetermined periods of time, the tubes were withdrawn, cooled, the pH was adjusted to 7 and the solutions were diluted to 10 ml. Portions (1 ml.) of each were then taken for determination of (*a*) phenol, (*b*) 2-acetamido-2-deoxyglucuronic acid or (*c*) 2-amino-2-deoxyglucuronic acid. All colorimetric measurements were made on the Spekker model 760 absorptiometer. Free phenol was estimated with Folin–Ciocalteu reagent (Kerr, Graham & Levvy, 1948) with Ilford no. 608 red filter (wavelength of peak transmission, 680 mμ); 2-acetamido-2-deoxyglucuronic acid was estimated by the method of Morgan & Elson (1934) with Ilford no. 605 yellow–green filter (wavelength of peak transmission, 550 mμ); it was necessary to assume that this compound, not available as a standard, gave the same equivalent colour intensity as 2-acetamido-2-deoxyglucose. 2-Amino-2-deoxyglucuronic acid was estimated by the Elson–Morgan reaction, as modified by Rondle & Morgan (1955), with Ilford no. 604 green filter (wavelength of peak transmission, 520 mμ); an authentic sample of this compound (glucosaminuronic acid dihydrate) was found to produce the same equivalent colour intensity as glucosamine (cf. Heyns & Paulsen, 1955). The unhydrolysed phenyl glycosides gave no colour in these reactions, nor did glucosaminuronic acid give the Morgan–Elson reaction; correction for the probable very slight colour given by 2-acetamido-2-deoxyglucuronic acid in the Elson–Morgan reaction could not be made.

Liberation of phenol from the α-anomer was slow and never exceeded 25% of theory, even after prolonged heating, whereas 80% of the total phenol was liberated from the β-anomer after about 30 min. In both cases, small amounts of 2-acetamido-2-deoxyglucuronic acid were detected, but only after short periods of hydrolysis. 2-Amino-2-deoxyglucuronic acid (about 10%) was present in hydrolysates of the α-anomer after heating for 1 hr., and approached phenol in concentration in the early stages of β-glycoside hydrolysis; after attaining a maximum the concentration fell markedly in both experiments (Fig. 1).

SUMMARY

1. The specific oxidation of the primary alcohol group in glycosides with gaseous oxygen in the presence of a platinum catalyst has been extended to the synthesis of aryl glycopyranosiduronic acids, and a number of derivatives of glucuronic acid and galacturonic acid have been thus prepared, including phenyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranosiduronic acids. Decomposition of the latter compounds with acid has been studied. The preparation of borneol α-D-glucopyranosiduronic acid is also described.

We are grateful to Professor K. Heyns for a gift of D-glucosaminuronic acid dihydrate, and Dr G. N. Bollenback for a specimen of phenyl α-D-glucopyranosiduronic acid hemihydrate.

REFERENCES

Bollenback, G. N., Long, J. W., Benjamin, D. G. & Lindquist, J. A. (1955). *J. Amer. chem. Soc.* **77**, 3310.
Conchie, J. & Levvy, G. A. (1957). *Biochem. J.* **65**, 389.
Fischer, E. & Bergmann, M. (1917). *Ber. dtsch. chem. Ges.* **50**, 711.
Foster, A. B., Horton, D. & Stacey, M. (1957). *J. chem. Soc.* p. 81.
Heyns, K. & Kelch, C. (1953). *Chem. Ber.* **86**, 601.
Heyns, K. & Paulsen, H. (1955). *Chem. Ber.* **88**, 188.

Kerr, L. M. H., Graham, A. F. & Levvy, G. A. (1948). *Biochem. J.* **42**, 191.
Marsh, C. A. (1952). *J. chem. Soc.* p. 1578.
Marsh, C. A. & Levvy, G. A. (1956). *Nature, Lond.*, **178**, 589.
Marsh, C. A. & Levvy, G. A. (1958). *Biochem. J.* **68**, 610.
Moggridge, R. C. G. & Neuberger, A. (1938). *J. chem. Soc.* p. 745.
Morgan, W. T. J. & Elson, L. A. (1934). *Biochem. J.* **28**, 988.
Quick, A. J. (1927). *J. biol. Chem.* **74**, 331.
Rondle, C. J. M. & Morgan, W. T. J. (1955). *Biochem. J.* **61**, 586.
Tsou, K.-C. & Seligman, A. M. (1952). *J. Amer. chem. Soc.* **74**, 5605.
Tsou, K.-C. & Seligman, A. M. (1953). *J. Amer. chem. Soc.* **75**, 1042.

Some Aspects of the Destruction of Lysine under Conditions of Acid and Enzymic Hydrolysis of Protein Materials Containing Carbohydrates

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(*Received* 30 *July* 1957)

The injurious effects of dry heat on the nutritive value of many proteins has been repeatedly demonstrated (e.g. Morgan, 1931; Block, Jones & Gersdorff, 1934; Greaves, Morgan & Loveen, 1938). Greaves *et al.* (1938) reported that heating at 130° for 8 hr. produced only a slight decrease in the digestibility of dry casein but considerably reduced its nutritive value for rats. They also observed that growth of the rats could be restored by supplementing the heated casein with lysine. Block *et al.* (1934), using a chemical method for lysine assay, had previously observed that after acid hydrolysis as much lysine could be isolated from the heat-treated casein as from the untreated. They postulated that when a protein is heated in the dry state the free carboxyl groups of dicarboxylic amino acids might react with the ϵ-amino groups of lysine, forming a new peptide linkage which is resistant to enzymic digestion, but not to acid hydrolysis. Eldred & Rodney (1946), using the decarboxylase technique of Gale (1945) for the assay of lysine, confirmed the observations of Block *et al.* (1934). In contrast with protein-bound lysine, free lysine is remarkably stable to dry heat in the presence and absence of other free amino acids (Evans & Butts, 1948).

A second, different type of destruction of protein-bound lysine has been observed when proteins are heated with carbohydrates (Block *et al.* 1946; Clandinin, Cravens, Elvehjem & Halpin, 1947; Evans & Butts, 1948). Patton, Hill & Foreman (1948*a*, *b*), using a microbiological assay for the estimation of the amino acids, demonstrated that carbohydrates are concerned in the destruction of lysine. Patton & Hill (1948) postulated that the destruction in this case was due to compound-formation between reducing sugars and those amino acid residues possessing free functional nitrogen groups, and that the chemical bond formed was not split by acid hydrolysis. On heating proteins with carbohydrate, or on heating carbohydrates alone for long periods, a brown colour appears and after long heat treatment a chocolate-brown precipitate (humin) forms. Mohammad, Contrat & Olcott (1949), studying the progressive disappearance of the free amino groups in bovine serum albumin, concluded that these groups were involved in the browning reaction. Many workers (e.g. Stevens & McGinnis, 1947; Patton & Hill, 1948) have demonstrated that lysine can also react with carbohydrates.

The studies mentioned above were all conducted in the absence of acid. The behaviour of free and bound lysine in acid solution is also of interest since amino acid analysis of proteins usually involves acid hydrolysis. Tristram (1939) studied the effect of refluxing amino acids with 8 N-sulphuric acid in the presence and absence of carbohydrates, and concluded that arginine was significantly affected, whereas lysine was not. An interaction between amino acids and reducing sugars was claimed by Maillard (1912), and Roxas (1916) suggested that furan compounds were responsible for this Maillard reaction. Scallet & Gardener (1945) showed that aqueous solution of glucose became highly coloured when heated for several hours at 100° owing to the formation of 5-hydroxymethylfurfuraldehyde, followed by its polymerization to form humin substances. Wolfrom, Shuetz & Cavalieri (1948, 1949) and Wolfrom, Cavalieri & Cavalieri (1947) also suggested that 5-hydroxymethylfurfuraldehyde reacted with the free amino groups of amino acids. If pentoses were substituted for glucose, then the

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