The Chemistry and Biochemistry of Phenolic Glycosides

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1 Introduction

The existence of phenolic glycosides in plants has been known for more than a century. In 1845, Piria[1] isolated salicin from *Populus* and *Spiraea* species and showed that it was a compound of glucose and saligenin (*o*-hydroxybenzyl alcohol). Several hundreds of naturally occurring glycosides have since been identified, and their distribution is widespread.[2] Bourquelot and Fichtenholz[3] found glycosides in 205 of the 281 species of phanerogams that they examined. Bourquelot, Hérissey, Bridel, Aubry, and Ludwig were the first workers to carry out systematic biochemical studies *in vitro* on the structure of plant glycosides, using the glycoside-cleaving enzymes of sweet almond emulsin. With these and other enzymes, such as the α-galactosidase (α-D-galactoside galactohydrolase) and the α-glucosidase (α-D-glucoside glucohydrolase) of yeast they were able to synthesise the monoglycosides of a variety of mono- and di-hydric alcohols.[4]

2 The Occurrence and Distribution of Phenolic Glycosides in the Plant Kingdom

Phenolic glycosides occur to a greater or lesser extent in most parts of plants,[1,2d,5] and the majority of naturally occurring glycosides are phenolic derivatives. Conversely, most phenols in plants occur in combination with sugars.[2d,6] In an extensive review of phenolic glycosides in plants, Harborne[2d] states that these substances are contained in the following parts of the plant: flowers, seeds, pollens, roots, tubers, and woody tissues. Phenolic glycosides and free phenols

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[1] E. F. Armstrong and K. F. Armstrong in 'The Glycosides', Longmans, London, 1931, p. 12.

[2] (*a*) W. Pfeffer in 'Planzenphysiologie', 2nd edn., Wilhelm Engelmann, Leipzig, 1897, vol. 1, p. 492; (*b*) A. Goris, *Rev. gén. Sci. pure appl.*, 1921, **32**, 337; (*c*) M. Bridel, *ibid.*, 1926, **37**, 134; (*d*) J. Harborne in 'Biochemistry of Phenolic Compounds', ed. J. Harborne, Academic Press, London, 1964, p. 129.

[3] E. Bourquelot and A. Fichtenholz, *J. Pharm. et Chim.*, 1901, [6] **14**, 481; 1906, [6] **34**, 165; 1907, [6] **35**, 16, 378; 1911, [7] **3**, 5; 1912, [7] **5**, 49, 296; 1913, [7] **8**, 158; 1915, [7] **11**, 219; quoted by Beguin in *Pharm. Acta Helv.*, 1926, **1**, 90.

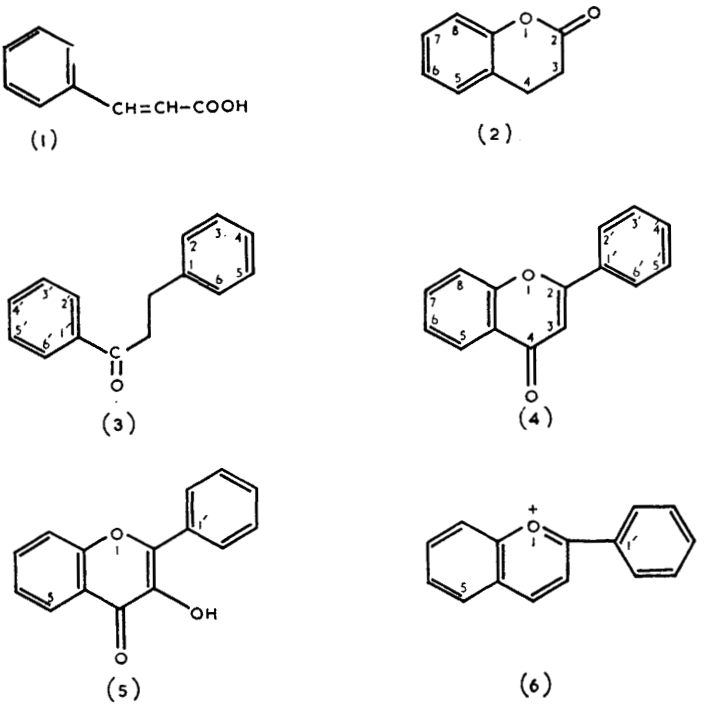
[4] (*a*) E. Bouquelot, H. Hérissey, and M. Bridel, *Compt. rend.*, 1911, **153**, 168, 330, 491, 1493, (*b*) E. Bourquelot and A. Aubry, *ibid.*, 1914, **158**, 70; 1915, **160**, 214; (*c*) E. Bourquelot and M. Bridel, *ibid.*, 1914, **158**, 206, 307, 898, 1219; 1919, **168**, 253; (*d*) H. Hérissey and A. Aubry, *ibid.*, 1914, **158**, 204; (*e*) E. Bourquelot and A. Ludwig, *ibid.*, 1914, **158**, 1037, 1377; (*f*) E. Bourquelot, A. Aubry, and M. Bridel, *ibid.*, 1915, **160**, 674.

[5] (*a*) W. Karrer in 'Konstitution und Vorkommen der organischen Pflanzenstoffe', Birkhauser Verlag, Basle, 1958; (*b*) R. Paris in 'Chemical Plant Taxonomy', ed. T. Swain, Academic Press, London, 1963, p. 337.

[6] (*a*) E. C. Bate-Smith, *Symp. Biochem. Soc.*, 1949, **3**, 62; (*b*) C. H. Ice and S. H. Wender, *J. Amer. Chem. Soc.*, 1953, **75**, 50; (*c*) C. G. Nordström and T. Swain, *J. Chem. Soc.*, 1953, 2764; (*d*) P. Baruah and T. Swain, *Biochem. J.*, 1957, **66**, 321; (*e*) P. Baruah and T. Swain, *J. Sci. Food Agric.*, 1959, **10**, 125; (*f*) S. A. Brown, *Phytochemistry*, 1963, **2**, 137.

applied to *Vicia faba* leaves have been shown to be translated to all parts of the plant, including the roots.[7]

The pattern of glycosides in plants varies from tissue to tissue. In flower petals, anthocyanins are the main scarlet, red, mauve, and blue pigments, and yellow colouring may be due to the glycosides of aurones, chalcones, or hydroxyflavones (Scheme 1), although much yellow is due to carotenoids. Flavone glycosides are



Scheme 1 *Some ring systems occurring in natural phenolic compounds:* (1) cinnamic acid, (2) coumarin, (3) chalcone, (4) flavone, (5) hydroxyflavone, (6) anthocyanin (flavylium)

present in many white or ivory-coloured flowers. Phenolic glycosides in fruit are simpler in structure than the flower constituents. In leaves, anthocyanin pigmentation is comparatively rare, but flavonol glycosides are widespread. Some seeds contain free phenols but there is a tendency for these organs to produce complex glycosides. Wheat bran, for example, contains nyacitin, which yields nicotinic acid, *o*-aminophenol, D-glucose, D-xylose, L-arabinose, ferulic acid, and sinapic acid on hydrolysis.[8] Woody tissues contain mainly free phenols, although a few flavanone glycosides are also present.

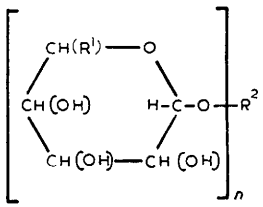
Williams,[9] in his study of the phenolics of fruit trees, showed that the glucoside phloridzin [3,5-dihydroxy-2-(*p*-hydroxyhydrocinnamoyl) phenyl β-D-gluco-

[7] N. J. Macleod and J. B. Pridham, *Phytochemistry*, 1966, **5**, 777.
[8] E. Kodicek and P. W. Wilson, *Biochem. J.*, 1960, **76**, 27P.
[9] A. H. Williams in 'Phenolics in Plants in Health and Disease', ed. J. B. Pridham, Pergamon Press, Oxford, 1960, p. 3.

pyranoside], the main phenolic of apple trees, is present in the leaf and bark, and to some extent in the seeds. The concentration is highest in the root-bark and negligible in the fruit flesh. All parts of the tree contain glycosides of quercetin (3,3′,4′,5,7-pentahydroxyflavone) and kaempferol (3,4′,5,7-tetrahydroxyflavone), in varying relative amounts (Scheme 1). Quercetin α-L-arabinoside predominates in the bark[10] and the β-D-galactoside in the leaf and fruit.[11] A phloretin arabinoglucoside occurs in the bark only. Pear trees differ considerably from apple trees in their glycoside components. Phloridzin is entirely replaced in pear by arbutin (*p*-hydroxyphenyl β-D-glucoside) which, like phloridzin, is present in leaf, bark, and seeds, but not in fruit flesh. The arbutin content of root-bark is low, however. Other differences include the structures of the hydroxyflavone glycoside components. In pear, kaempferol glycosides predominate, and neither kaempferol nor quercetin occurs as the α-L-rhamnoside. Hybrids of apple and pear contain superimposed glycoside patterns.[12] The universal occurrence of arbutin in *Pyrus* species and the widespread existence of phloridzin in *Malus* is taxonomically significant.

3 Properties of Phenolic Glycosides

Phenolic glycosides are optically active compounds which are readily hydrolysed by dilute mineral acids, dilute alkali, or a suitable enzyme. Methods for the isolation and structural determination of these compounds have been reviewed elsewhere.[13] The naturally occurring phenolic glycosides have the general structure shown in Scheme 2. The glycosidic linkage is almost invariably β with a D-sugar and α with an L-sugar.



Scheme 2 *The structure of natural phenolic glycosides:* R^1 = CH_2OH, H, Me, or CO_2H, R^2 = aryl, n = 1—3

The glycone moiety of the phenolic glycoside can be D-glucose, D-galactose, D-fructose, L-rhamnose, D-xylose, L-arabinose or D-glucuronic acid.[14] D-Glucose is the most common in the natural glycosides, whereas D-fructose and D-glucuronic acid are rare. Disaccharide glycosides of phenols are quite common, *e.g.* rutin, 3′,4′,5,7-tetrahydroxy-3-[α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyloxy]flavone and primeverin, 5-methoxy-2-methoxycarbonylphenyl β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside. Ten disaccharides combined as phenolic

[10] A. E. Bradfield and A. E. Flood, *Rep. E. Malling Res. Sta.*, 1949, 103.
[11] W. H. Siegelman, *J. Biol. Chem.*, 1955, **213**, 647.
[12] A. H. Williams, *Nature*, 1955, **175**, 213.
[13] J. B. Pridham, *Adv. Carbohydrate Chem.*, 1965, **20**, 371.
[14] R. J. McIlroy in 'The Plant Glycosides', Arnold, London, 1951, p. 5.

glycosides are known. Of these, sophorose (2-*O*-β-D-glucopyranosyl-β-D-glucopyranose), rutinose (6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranose), and sambubiose (2-*O*-β-D-xylopyranosyl-β-D-glucopyranose) are common. Six trisaccharides have been found in phenolic glycosides. All are uncommon. Two rare branched trisaccharides are also known.[2d,15] Many of the oligosaccharides mentioned here occur only in combination with phenols. The glycosidic linkages with these sugars are almost exclusively (1 → 2) and (1 → 6), and D-glucose is nearly always the terminal reducing unit. A kaempferol glucoside containing six glucose units has been reported in pea plants grown in the dark.[16]

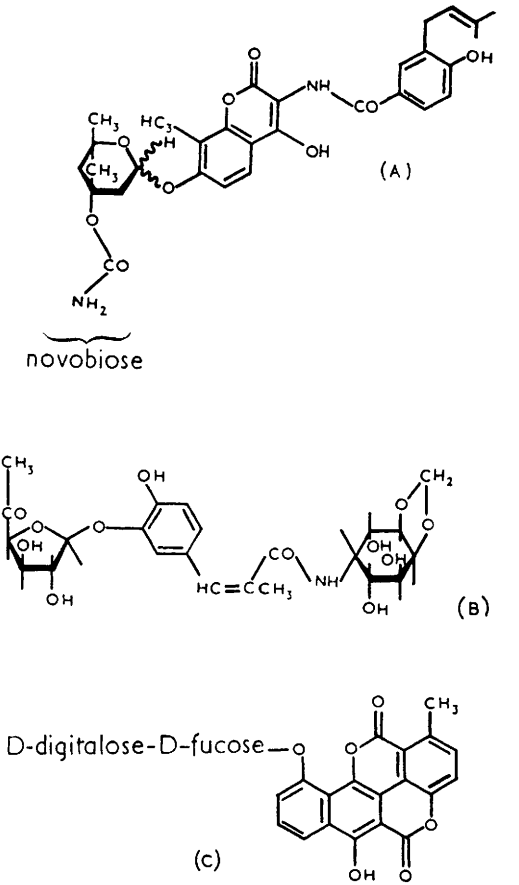
Other naturally occurring glycosides possessing two or more monosaccharide units have more than one glycosylated hydroxy-group in the aglycone[5b,17] *e.g.* cyanidin 3,5-diglucoside (3,5-di-β-D-glucopyranosyloxy-3′,4′,7-trihydroxyflavylium chloride). In some antibiotics of fungal origin, more complex sugars occur, *e.g.* the nitrogenous methylated deoxy-sugar, novobiose,[18] in novobiocin, and a disaccharide of D-fucose and D-digitalose (3-*O*-methyl-D-fucose) in chartreusin,[19] and 6-deoxy-*arabino*-5-hexosulose in homomycin[20] (Scheme 3). All these glycosides have antibiotic activity.

In the great majority of phenolic monoglycosides, the D-monosaccharide residue is in the pyranoid form and has the β-D-configuration at C-1. The only two α-D-glycosides known to occur naturally are 3-α-D-arabinosyloxy-3′,4′,5,7-tetrahydroxyflavylium chloride and 3-α-D-arabinosyloxy-3′,4′,5,7-tetrahydroxyflavone (avicularin), which both occur in several species.[2d] Pridham[13] has reviewed phenolic glycosides from the standpoint of the carbohydrate part.

The aglycones[5a] of natural phenolic glycosides may be simple phenols, *e.g.* saligenin (*o*-hydroxybenzyl alcohol) and catechol, or they may contain condensed ring systems (Scheme 1).

Free phenols are rare in plants, many being phytotoxic.[21] Where they do occur in higher plants, it is usually in storage tissues, *e.g.* heartwood.[2d] Dormant tea seeds contain phenols mainly in the bound state, whereas seed peels contain large amounts of free phenols. The latter appear to become bound during germination.[22] All groups of phenols occurring in higher plants form glycosides, but lower plants, particularly fungi, contain free anthraquinones, hydroxycinnamic acids, and hydroxybenzoic acids.[2d] The metabolism of dihydroxyphenols in micro-organisms has been reviewed by Dagley.[23]

[15] J. B. Harborne and E. Hall, *Biochem. J.*, 1963, **88**, 41P.
[16] F. E. Mumford, D. H. Smith, and P. G. Heytler, *Biochem. J.*, 1964, **91**, 517.
[17] Ref. 9, p. 9.
[18] Ref. 2*d*, p. 324.
[19] E. Simonitsch, W. Eisenhuth, O. A., Stamm, and H. Schmid, *Helv. Chim. Acta*, 1960, **43**, 58.
[20] M. Welsch in 'Comparative Biochemistry', ed. M. Florkin and H. Mason, Academic Press, London, 1964, vol. 7 suppl., p. 265.
[21] (*a*) Ref. 9, p. 25; (*b*) C. M. Stewart, *Nature*, 1960, **186**, 374; (*c*) G. B. Maksimov and G. I. Radkevitch, Regulatory Rosta i Rost. Rast., *Akad. Nauk S.S.S.R., Sibirsk. Otdel., Vost.-Sibirsk. Biol. Inst.*, 1964, 158.
[22] T. A. Shubert, *Biokhim. Chain, Proizv., Akad. Nauk S.S.S.R.*, 1966, 26 (Chem. Abs., 1967. **66**, 463).
[23] S. Dagley, *Soil Biochem.*, 1967, 287.



Scheme 3 *Some complex natural phenolic glycosides:* (A) Novobiocin, an aminocoumarin glycoside from *Streptomyces nivens* (see J. B. Harbourne in 'Biochemistry of Phenolic Compounds', Academic Press, London, 1964, p. 324); (B) Homomycin, a cinnamamide derivative (see M. Welsch in 'Comparative Biochemistry', Academic Press, London, 1964, vol 7 suppl.); (C) Chartreusin, from *Streptomyecs* spp (E. Simonitsch, *Helv. Chim. Acta*, 1960, **43**, 58)

Some free phenols stimulate the growth of fungi, *e.g. Fusicoccum amygdali*[24] with protocatechuic acid. The yeast *Debaromyces subglobosus* will even grow on phenol as its sole source of carbon.[25] At low concentrations, phenols stimulate the growth of the bacterium *Escherichia coli*, but at higher concentrations, the reverse effect becomes apparent, until a lethal dose is reached.[26]

Many free phenols, however, are toxic to fungi[24,27] and bacteria.[27c,28] There is

[24] M. W. Borys and N. F. Childers, *Acta Microbiol. Polon.*, 1964, **13**, 211.
[25] D. A. J. Vase and J. S. Hough, *J. Gen. Microbiol.*, 1966, **42**, 13, (1966).
[26] H. Commager and J. Judis, *J. Pharm. Sci.*, 1965, **54**, 1436.
[27] (*a*) R. I. Davies, C. B. Coulson, and D. A. Lewis, *J. Soil Sci.*, 1964, **15**, 299; (*b*) H. Oku, *Phytopathol. Z.*, 1962, **44**, 39; (*c*) Y. Uesegi and T. Suzuki, *Bull. Nat. Inst. Agric. Sci.* (*Japan*), 1964, **17**, 181.
[28] A. L. Schaal and G. Johnson, *Phytopathol.*, 1955, **45**, 626.

some suggestion that the un-ionised form of the phenol is the fungitoxic one, the ionised form being unable to penetrate the cell membrane.[29] In *E.coli*, phenolic disinfectants administered at bacteriostatic concentrations combine with the cell wall, damaging the membrane and affecting permeability, which in turn prevents the biosynthesis of β-galactosidase at that site and also inhibits the formation of protein, RNA, and DNA.[30] Higher concentrations of phenolic disinfectants cause serious membrane damage and loss of cell contents.

In comparison with the state of knowledge of higher plants, little is known of phenolic glycosides in fungi and lower plants, but they appear to be rare.[5b] Among the few known examples are complex structures (see Scheme 3).

C-Glycosides of natural products have recently attracted some attention. They appear to be widespread in plants.[31] They are glycosyl derivatives in which C-1 of the sugar is attached directly to the aromatic ring. Examples of thioglycosides (*S*-glycosides) have also been found in plants.[32] They have been extensively studied in *Brassica* plants, and reviewed.[33]

4 The Functions of Phenolic Glycosides in Plants

A number of functions have been ascribed to phenolic glycosides in plants, but their role is still far from clear. An early worker, Errera,[34] claimed that they protect plants from the voracity of animals! In 1897, Pfeffer[2a] made the first systematic investigation and concluded that phenolic glycosides acted as secondary food reserves by virtue of their carbohydrate part. He noted that their production was a periodic phenomenon and refuted the suggestion that these substances were merely waste products.

By the 1920's, workers in the field were strongly divided in their opinion of the physiological rôle of glycosides. Pfeffer's hypothesis was supported by the work of Bourquelot[35] on oleuropin in olives and by that of Vintelesco[35] on syringin in *Syringa* and *Ligustrum* species, which showed that glycosides accumulated in winter and disappeared during vegetative growth. Weevers,[36] however, detected seasonal variations in the salicin content of *Salix* and in the amounts of the other glycosides in *Pyrus*, *Populus*, and *Vaccinium* species. Wasicky,[37] however, attributed such variations to osmoregulation. Hérissey extensively reviewed the evidence at this period and decided in favour of Pfeffer's hypothesis.[38] Goris[2b]

[29] B. E. Winsley and V. Walters, *J. Pharm. Pharmacol.*, 1965, Suppl. 17, 22.
[30] J. E. Pullmann and B. L. Reynolds, *Austral. J. Pharm.*, 1965, **46**, S80.
[31] (*a*) H. W. Evans, A. McGookin, L. Jurd, A. Robertson, and W. R. N. Williamson, *J. Chem. Soc.*, 1957, 3510; (*b*) L. Hörhammer and H. Wagner in 'Recent Developments in the Chemistry of Natural Phenolic Compounds', ed. W. D. Ollis, Pergamon Press, Oxford, 1961, p. 185; (*c*) L. Haynes, *Adv. Carbohydrate Chem.*, 1963, **18**, 227; 1965, **20**, 371; (*d*) H. Wagner, *Comp. Phytochem.*, 1966, 309; (*e*) V. K. Bhatia, S. R. Gupta, and T. R. Seshadri, *Current Sci.*, 1965, **34**, 634; *Phytochemistry*, 1966, **5**, 177.
[32] (*a*) E. H. Fischer and E. A. Stein in 'The Enzymes', ed. P. D. Boyer, H. Lardy, and K. Myrbäck, Academic Press, New York, 1960, 2nd edn., vol. 4, p. 311; (*b*) D. Meakin, *Experientia*, 1967, **23**, 174; (*c*) G. A. Howard and R. D. Gaines, *Phytochemistry*, 1968, **7**, 585.
[33] E. Josefsson, *Phytochemistry*, 1967, **6**, 1617.
[34] L. Errera, *Bull. Soc. roy. Bot. Belg.*, 1886, [2] **25**, 86.
[35] E. Bourquelot and J. Vintelesco, *J. Pharm. et Chim.*, 1911, [7] **3**, 1; 1906, [6] **24**, 145.
[36] T. Weevers, *Jahrb. Wiss. Bot.*, 1904, **39**, 229.
[37] R. Wasicky, *Biochem. Z.*, 1921, **113**, 1.
[38] H. Hérissey, *Bull. Soc. chim. France*, 1923, [4] **33**, 349.

was the author of an equally comprehensive review supporting the theory that phenolic glycosides were the waste products of metabolism. He claimed that glycosides were present to a greater extent in the external parts of the plant and supposed this to be evidence of their end-product nature. This view was supported by Straub,[39] who observed that glycosides in *Digitalis* seeds underwent no change after formation.

In 1926, Bridel[2c] attempted to resolve the dilemma by showing that the two theories were compatible. He demonstrated that perennials, such as *Menyanthes*, showed no ability to utilise glycosides as food reserves, whereas annuals, such as *Rhinanthus*, used up some of their glycoside reserve before the onset of photosynthesis. Guignard[40] found that *Phaseolus lunatus* used about a third of its cyanohydrin glucoside when grown in the dark for a month. Bridel considered that the two theories differed only in the relative importance attached to the different reactions. On the one hand, the important part of the molecule was the aglycone, and the sugar entered the nutrition cycle as a secondary phenomenon; on the other hand, the aglycone was of lesser importance, and the glycoside was cleaved in order to utilise the glycone. He concluded that the food reserve was of greater consequence.

The waste product theory has never been proved, and seems unlikely in view of the wide variety of naturally occurring glycosidic residues.

The food reserve role of glycosides cannot be stated with certainty. It is possible that they are used up under starvation conditions, and Hutchinson, Roy, and Towers[41] showed that phlorin synthesis decreased and the phloroglucinol content increased in *Pelargonium* and *Gerbera* species under starvation. Rozier[42] found that rutin (quercetin 3-rhamnoglucoside), the major hydroxyflavone of potato plants, was present in a much greater concentration in the leaves of plants grown from young seed tubers than in those grown from old tubers. This difference only become apparent under favourable growth conditions. The glycosides may be important sources of the less common sugars such as L-rhamnose and L-arabinose, at all growth stages.

Reviews by Armstrong[43] and Frey-Wyssling[44] suggested that no single function can be assigned to the glycosides. They perform a variety of functions resulting from the diversity of their glycones and aglycones. Frey-Wyssling believed that glycoside formation was a means of solubilising the phenols in the cell sap. However, some glycosides, *e.g.* hesperidin, are insoluble in aqueous systems and others are less soluble than the corresponding aglycones, for example salicin.

The only well established function of phenol glycosylation is the detoxification of phytotoxic aglycones. Several workers have shown that foreign phenols are rapidly converted into mono-β-D-glucosides when fed to leaves and seedlings of

[39] W. Straub, *Biochem. Z.*, 1917, **82**, 48.
[40] L. Guignard, *Compt. rend.*, 1908, **147**, 1023.
[41] A. Hutchinson, C. Roy, and G. H. N. Towers, *Nature*, 1958, **181**, 841.
[42] C. Rozier. *Compt. rend.*, 1966, **263**, *D*, 1841.
[43] Ref. 1, ch. 8.
[44] A. Frey-Wyssling, *Naturwiss.*, 1942, **33**, 500.

higher plants.[45] Kosuge and Conn[46] showed that *o*-coumaric acid fed to sweet clover shoots was rapidly and almost entirely converted into the phenolic β-D-glucoside. In contrast, Harborne and Corner[47] found that other cinnamic acid derivatives gave rise mainly to D-glucose esters, when fed to a variety of plants. Only caffeic acid gave some of the phenolic D-glucose (3-β-D-glucosyloxycaffeic acid). Harborne suggested that esterification rather than phenol glucosylation occurred *in vivo* with this group of compounds in order to neutralise the acid groups and enhance sap-solubility. There may be a connection between these facts and the special rôle of phenol glucosylation in the biosynthesis of coumarins and related compounds from cinnamic acid derivatives.[48] This is one of the few cases so far known where glucosides, rather than free aglycones, are essential intermediates in biosynthesis. El Basyouni and Neish[49] have shown that cinnamic acid glucose esters are the metabolically active intermediates in pathways which yield, *inter alia*, flavonoids and lignins in wheat and barley plants. The rôle of glycosides in lignin formation has already been reviewed in detail.[50]

It is interesting to note that 3-β-D-glucosyloxycaffeic acid has been found in wild, tuberous *Solanum* species, but is absent from the non-tuberous, cultivated species. The latter, however, contains considerable amounts of a glucose ester, 1-*p*-hydroxycinnamoyl-β-D-glucose, which is absent from the wild species. Pridham[51] showed that the ability to glucosylate foreign phenols is widespread in the plant kingdom. It is prominent among gymnosperms and angiosperms with the exception of a few water plants. The various ferns and the single liverwort tested all formed glucosides. Many of the mosses that were investigated gave traces of glucoside, but this ability was entirely absent from the algae tested. However, Frederick[52] has reported pairs of glucosyltransferases in three different groups of algae. The conversion of phenols into their glucosides in the plant kingdom is analogous to the formation of phenolic glucuronides as a mechansim for phenol detoxification in animals.

A further function of phenol glycosylation in plants may be the protection of the phenol from enzymatic oxidation. In studies on potato phenolics, by Baruah and Swain[6e] and on tea phenolics, by Roberts and Wood,[53] the actions of the accompanying polyphenol oxidase (*o*-diphenol:oxygen oxidoreductase, EC 1 . 10 . 3 . 1) on the glycosides phloridzin, aesculin, chaerophyllin, quercitrin,

[45] (*a*) A. Hutchinson, C. Roy, and G. H. N. Towers, *Nature*, 1958, **181**, 841; (*b*) J. B. Pridham, *ibid.*, **182**, 1958, 795; (*c*) G. Ciamician and C. Ravenna, *Atti. Accad. naz. Lincei, Rend. Classe Sci. fis. mat. nat.*, 1916, [5] **25**, 3; (*d*) T. Miwa, S. Nakamura, and A. Shibata, *Kôso Kagaku Shinpojiuma*, 1957, **12**, 48 (*Chem. Abs.*, 1958, **52**, 1314); (*e*) C. W. Nystrom, N. E. Tolbert, and S. F. Wender, *Plant Physiol.*, 1959, **34**, 442.

[46] T. Kosuge and E. E. Conn., *J. Biol. Chem.*, 1959, **234**, 2135.

[47] J. B. Harborne and J. J. Corner, *Biochem. J.*, 1961, **81**, 242.

[48] (*a*) S. A. Brown, 2nd Meeting, Federation of European Biochemical Societies, 1965, Abstracts p. 253; (*b*) D. J. Austin and M. B. Myers, *Phytochemistry*, 1965, **4**, 255.

[49] S. J. El Basyouni and A. C. Neish, *Phytochemistry*, 1966, **5**, 686.

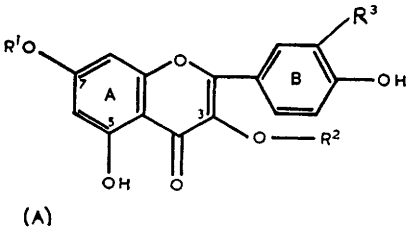
[50] (*a*) K. Freudenberg, *Fortschr. Chem. org. Naturstoffe*, 1962, **20**, 41; (*b*) F. A. Isherwood in 'Biosynthetic Pathways in Higher Plants', ed. J. B. Pridham and T. Swain, Academic Press, London, 1965, p. 133.

[51] J. B. Pridham, *Phytochemistry*, 1964, **3**, 493.

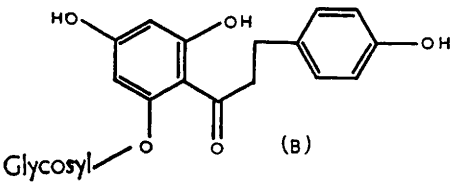
[52] J. F. Frederick, *Phytochemistry*, 1967, **6**, 1041.

[53] E. A. H. Roberts and D. J. Wood, *Nature*, 1951, **167**, 608.

rutin, and myricitrin, and on their free aglycones were compared. The latter were all substrates for the enzyme, whereas all glycosides except phloridzin and chaerophyllin were total inhibitors of enzyme action (Scheme 4). All the non-substrates for the enzyme were glycoslylated at C-3 and Roberts[54] pointed out that such compounds are non-planar, owing to steric effects between the C-3 glycosyl group and the aryl group on C-2. Chaerophyllin, which is glycosylated at C-7, and phloridzin, which is glycosylated at C-5, are not, however, sterically hindered in this way. Hence the shape of the glycoside molecule determines its inhibitor action on polyphenol oxidase. Similar results were obtained by Siegelman,[11] who examined quercetin glycosides with apple-skin polyphenol oxidase. Phenolic glycosides have been found in tumorous tissue from interspecific *Nicotiana* hybrids.[55] Compared with the parent species, the levels of scopoletin (7-hydroxy-6-methoxy-coumarin), and particularly of its 7-glucoside, scopolin, were considerably higher. On the other hand, Asen and Emsweller[56] showed that a tumorous *Lilium* interspecific hybrid had poor ability to glucosylate ferulic acid, whereas in an intraspecific hybrid, with normal growth, 94% of the ferulic acid occurred as the glucose ester. Plant hybrids have patterns of phenolic glycosides which represent superimposition of those in the parent plants. A study of the phenolic compounds in a supposed hybrid has been used to determine whether or not hybridisation has, in fact, occurred.



(A)



(B)

Scheme 4 (A) *Hydroxyflavone glycosides:* 3-glycoside: R^1 = H, R^2 = glycosyl, R^3 = H or OH; 7-glycoside: R^1 = glycosyl, R^2 = H, R^3 = H or OH. (B) *Phloridzin*

[54] E. A. H. Roberts, *Nature*, 1960, **185**, 536.
[55] T. C. Tso, L. G. Burk, L. J. Dieterman, and S. H. Wender, *Nature*, 1964, **204**, 779.
[56] S. Asen and S. L. Emsweller, *Phytochemistry*, 1962, **1**, 169.

Deysson and Truhaut[57] showed that phenolic substances interfere with mitosis and that glycosylation eliminates this effect to a large extent.

The interactions of plants and fungi may well be partially controlled by the presence of phenolic compounds in the plants or in the fungi.[58] Sherwood[59] found that the fungus *Rhizoctonia solani* produces *o*-nitrophenyl β-D-glucoside, which is phytotoxic, whereas fungitoxic oxidised phenols have been found in plants, *e.g.* in potato wounds.[60] Strains of rice resistant to fungal leaf spot disease (*Helminthosporium oryzae*) were found to brown rapidly and intensely owing to oxidised phenol formation in areas attacked by the fungus, whereas susceptible plants showed only weak ability to form oxidised phenols. Synthesis of phlorin was found to be inversely proportional to the disease due to rust on wheat. Comparison of the glucoside formation rates in healthy and diseased plants has been used to evaluate the resistance to a given disease.[27b]

It has been suggested that the flavonol glycosides almost universally present in plant leaves may act as growth regulators.[2d,61] In general, glycosides of the kaempferol type with a single hydroxy-group at the 4′-position of the B-ring (see Scheme 4A; R^3 = H) were inhibitors of indoleacetic oxidase (*N*-acetylindoxyl : oxygen oxidoreductase, EC 1 . 7 . 3 . 2), whereas glycosides of quercetin, with two hydroxy-groups in the B-ring, at the 3′- and 4′- positions, (*e.g.* Scheme 4A; R^3 = OH), acted as cofactors for the enzyme.

The range of sugar components in phenolic glycosides is striking and, although its significance is not yet known, future work may reveal relationships between the repeating units of polysaccharides and the glycones present in the plant.[2d]

The function of oligosaccharides is not known, but Pridham[62] has tentatively suggested that they might be used as a starting point for biosynthesis of complex heteroglycans, where they might be pre-formed as phenol oligoglycosides and then transferred *in toto* to the heteroglycan by transglycosylation. In this connection, disaccharidases with transferase activity, which hydrolyse primeverosides and rutinosides to the aglycone and disaccharide have been found in *Rhamnus* species.[63]

5 The Biosynthesis of Phenolic Glycosides

The biosynthesis of the aglycone and glycone components of phenolic glysosides has been partially understood for some time. The carbohydrates[64] arise from chlorophyllic assimilation of carbon dioxide in sunlight, and the phenols are derived from shikimic acid, from condensations of acetyl coenzyme A units or

[57] G. Deysson and R. Truhaut, *Compt. rend., Soc. Biol.*, 1957, **151**, 1719.
[58] P. Molot, *Compt. rend.*, 1967, **264**, *D*, 1724.
[59] R. T. Sherwood, *U.S.P.*, 3, 179, 653/1965 (*Chem. Abs.*, 1965, **63**, 1170).
[60] L. V. Melitskii, O. L. Ozeretskovskaya, G. I. Chelenko, and G. A. Strokova, *Doklady Akad. Nauk. S.S.S.R.*, 1965, **160**, 964.
[61] (*a*) V. I. Kefeli, R. K. Turetskaya, and L. Sarapuu, *Fiziol. Rast.*, 1964, **11**, 853; (*b*) H. W. Hilton, *Adv. Carbohydrate Chem.*, 1966, **21**, 377.
[62] Ref. 9, p. 9.
[63] (*a*) C. R. Charaux, *Compt. rend.*, 1924, **178**, 1312; (*b*) M. Bridel, *ibid.*, 1924, **179**, 780.
[64] L. Hough and J. K. N. Jones, *Adv. Carbohydrate Chem.*, 1965, **11**, 185.

from phenylpropanoid precursors.[65] Until recently, the mechanism for the formation *in vivo* of phenolic glycosides from these components was unknown. It was generally supposed that the sugar portion was attached at a late stage in the process, after the formation of the phenol.

Ciamician and Ravenna[45c,66] were the first workers to demonstrate the biogenesis of phenolic glycosides from phenols by feeding saligenin, catechol, and quinol to *Zea mays* and other plants. Pridham and Saltmarsh,[67] however, obtained mainly the alcoholic glucoside (*o*-hydroxybenzyl β-D-glucoside), when feeding saligenin to *Zea mays* and *Salix daphnoides*, and Bourquelot and Fichtenholz[3] found the same product when D-glucose and saligenin were incubated with almond emulsin. Recent work with *Vicia faba* suggests that both the phenolic and alcoholic glucosides are formed in significant amounts *in vivo* from saligenin, but that the latter predominates.[68] Miwa, Nakamura, and Shibata[45d] found that the formation of phenolic glucosides from several simple phenols and D-glucose infused into leaf discs of various plants requires oxygen, and occurs more rapidly in the presence of α-D-glucose 1-phosphate. Hutchinson *et al.*[45a] demonstrated that glucosides of simple phenols could be formed in the presence of D-glucose with infused *Malus* and *Gerbera* leaf discs, but that no glycosylation occurred with D-fructose, L-rhamnose, D-xylose, and L-arabinose, under these conditions. In contrast to the observations of Harborne and Corner,[47] mentioned previously, that many species convert cinnamic acid derivatives almost entirely into D-glucose esters, Runeckles and Woolrich[69] found that tobacco leaf discs, infused with cinnamic acids, formed appreciable amounts of both phenolic glucosides and glucose esters. The former predominated, in the ratios of 2:1 and of 4:1 for *p*-coumaric and ferulic acids, respectively. Pridham and Saltmarsh[67] showed that the major products of mono-, di-, and tri-hydric phenols fed to *Vicia faba* seeds were the mono-β-D-glucosides. Phenol itself proved to be highly toxic to the plant, but Nystrom, Tolbert, and Wender[70] obtained phenyl β-D-glucoside when traces of phenol were fed to barley leaves.

The rule of attachment of the sugar portion to the phenol after the formation of the latter appears to have a notable exception, that is in the biosynthesis of coumarins, mentioned previously.

The phenolic glycosides formed in the biosynthesis discussed above are all β-D-anomers and the D-glucose donor *in vivo* is believed to be the 'high energy' nucleotide sugar, uridine 5′-diphosphate glucose (UDPG), which occurs very

[65] (*a*) A. J. Birch, *Fortschr. Chem. org. Naturstoffe*, 1957, **14**, 204; (*b*) Ref. 31*b*, p. 1; (*c*) H. Friedrich, *Pharm. Weekblad.*, 1967, **102**, 371; (*d*) K. R. Hanson, M. Zucker, and E. Sondheimer, *Phenolic Compounds Metab. Regul.*, 1967, 68; (*e*) M. H. Zenk, *Phytochemistry*, 1966, **6**, 245.

[66] G. L. Ciamician and C. Ravenna, *Atti Accad. naz. Lincei, Rend. Classe Sci. fis. mat. nat.*, 1909, [5] **18** (1), 419; [5] **18** (2), 594.

[67] J. B. Pridham and M. J. Saltmarsh, *Biochem. J.*, 1960, **74**, 42P; 1963, **87**, 218.

[68] M. A. Young, personal communication.

[69] V. C. Runeckles and K. Woolrich, *Phytochemistry*, 1963, **2**, 1.

[70] C. W. Nystrom, N. E. Tolbert, and S. H. Wender, *Plant Physiol.*, 1959, **34**, 142.

widely in plant tissues[71] and was first isolated by Cardini and Leloir[72] in 1949. They found that UDPG was involved in the 'isomerisation' of α-D-galactose 1-phosphate to α-D-glucose 1-phosphate under the action of UDPG:α-D-galactose 1-phosphate uridylyltransferase (EC2.7.7.12) and that phenols incubated with UDPG and wheat germ extract gave the corresponding β-D-glucosides. Other potential glucose donors, *e.g.*, α-D-glucose 1-phosphate, sucrose, cellobiose, maltose, uridine diphosphate maltose, and deoxyadenosine diphosphate glucose (deoxy ADPG) did not function with this system. ADPG proved to be more efficient than UDPG, and the guanosine, and to a lesser extent the cytidine analogues, were weak glucose donors. Pridham and Saltmarsh[67] showed that UDPG was necessary for the synthesis of phenolic β-D-glucosides, using *Vicia faba* extracts. Uridine diphosphate derivatives of other sugars[71,73] are known in plant tissues and presumably act as glycosyl donors for the corresponding phenolic glycosides. The subject of glycoside biosynthesis from nucleotides has been reviewed by Kelleher.[74] All attempts to synthesise phenolic β-D-glucosides *in vitro* by use of enzymes from higher plants and 'low energy' glucose, *e.g.* α-D-glucose 1-phosphate, D-glucose, cellobiose, and maltose, have failed.[73,75] Glucosylation of phenols is characteristic of most higher plants, but as previously mentioned, it occurs slowly if at all, with Bryophytes and Thallophytes and lower plants. Since UDPG is ubiquitous in plant tissues, inability to glucosylate phenols appears to be due to the lack of the glucosyltransferase.[76]

The first synthesis *in vitro* of a phenolic glucoside from a 'low energy 'glucosyl donor was reported in 1961.[77] This was achieved with an *Aspergillus niger* mycelial extract, with maltose or isomaltose as donor and various simple phenols as acceptors. The corresponding α-D-glucosides were formed. Glucose donors which were not substrates in this reaction were α-D-glucose 1-phosphate, sucrose, methyl-α-D-glucoside, and cellobiose.[78]

Two other instances of the conversion of phenols into their glycosides in the presence of 'low energy' donors and a lower plant or bacterial enzyme have been reported. Nakamura and Miwa[79] transferred D-fructose from sucrose to quinol to give the β-D-fructoside, by use of yeast invertase (β-D-fructoside fructohydrolase, EC 3.2.1.26) and Pridham and Wallenfels[80] obtained phenolic β-D-fucosides using *o*-nitrophenyl β-D-fucopyranoside as the donor with *E.coli* β-galactosidase

[71] V. Ginsburgh, P. J. Stumpf, and W. J. Hassid, *J. Biol. Chem.*, 1956, **223**, 977.
[72] (*a*) R. Caputto, L. F. Leloir, R. E. Trucco, C. E. Cardini, and A. C. Paladini, *J. Biol. Chem.*, 1949, **179**, 497; (*b*) C. E. Cardini, A. C. Paladini, R. Caputto, and R. F. Leloir, *Nature*, 1950, **182**, 1446; (*c*) C. E. Cardini and L. F. Leloir, *Ciencia Invest.* (*Buenos Aires*), 1957, **13**, 514.
[73] C. E. Cardini and T. Yamaha, *Nature*, 1948, **182**, 1446.
[74] W. J. Kelleher, *J. Pharm. Sci.*, 1965, **54**, 1091.
[75] (*a*) J. B. Pridham, *Biochem. J.*, 1960, **76**, 13; (*b*) J. D. Anderson, L. Hough, and J. B. Pridham, *Biochem. J.*, 1960, **77**, 564.
[76] J. B. Pridham, *Phytochemistry*, 1964, **3**, 493.
[77] J. B. Pridham, *Chem. and Ind.*, 1961, 1172.
[78] S. M. Hopkinson and J. B. Pridham, *Biochem. J.*, 1967, **105**, 655.
[79] S. Nakamura and T. Miwa, *Nature*, 1964, **202**, 291.
[80] J. B. Pridham and K. Wallenfels, *Nature*, 1964, **202**, 488.

(β-D-galactoside galactohydrolase, EC 3.2.1.23). With lactose, β-D-galactosides were formed, possibly together with *O*-digalactosyl derivatives.

Miller[81] was the first to report the biosynthesis of a phenolic diglucoside in feeding experiments. He obtained *o*-chlorophenyl β-gentiobioside by the treatment of gladioli corms with *o*-chlorophenol. Pridham[82] observed the production of *p*-hydroxyphenyl β-gentiobioside with arbutin and *Populus grandidentata* extract. Almond emulsin and a *Vicia faba* enzyme preparation gave similar results with arbutin.[75b] Cardini and Yamaha[73,83] found two enzymes in wheat germ, one of which converted quinol into *p*-hydroxyphenyl β-gentiobioside, and the other of which converted the monoglucoside, arbutin, into the gentiobioside. Both required UDPG. In the presence of the *Vicia faba* enzyme of Pridham, quinol and D-glucose, or quinol and gentiobiose, produced neither arbutin nor the gentiobioside. The β-D-glucosidase and synthetic activities appeared to be due to a single enzyme, and it was suggested that arbutin molecules act as both donors and acceptors in the gentiobioside formation. UDPG was not involved in this reaction.

Barber[84] converted quercetin to quercetin 3-β-D-glucoside by use of UDPG or the thymidine analogue, and thence to the diglycoside, rutin, by use of thymidine diphosphate L-rhamnose in the presence of a *Phaseolus aureus* leaf preparation.

6 Enzymes Responsible for the Formation of Glycosides

These enzymes fall into the large group known as the transferases in the classification recommended by the International Union of Biochemistry.[85] In particular they belong to the sub-group of glycosyltransferases, many members of which have been extensively studied and reviewed.[86] This sub-group includes enzymes which use 'high energy' nucleotide sugars as donors, as well as those which use oligosaccharides or glycosides. Members of both types of glycosyltransferases are capable of forming phenolic glycosides, but we shall be concerned here mainly with 'simple transferases', which cause the production of phenolic glycosides in the presence of 'low energy' donors, with the retention of configuration at C-1 of the aglycone. The formation of phenolic glycosides in the presence of 'high energy' donors has been reviewed by Kelleher.[74]

Many enzymes of the 'simple transferase' type are capable of catalysing the reverse reaction, *i.e.* the hydrolysis of glycosides. Thus, they may also be classified as hydrolases, in the 'sub-sub-group' glycoside hydrolases,[85] the members of which are often known by the trivial name 'glycosidases'. They include those enzymes which hydrolyse low molecular weight glycosides (*i.e.* alkyl and aryl glycosides and oligosaccharides) and also those which are responsible for the

[81] L. P. Miller, *Contribut. Boyce Thompson Inst.*, 1941, **12**, 168.
[82] J. B. Pridham, *Analyt. Chem.*, 1957, **29**, 1167.
[83] T. Yamaha and C. E. Cardini, *Arch. Biochem. Biophys.*, 1960, **86**, 133.
[84] G. A. Barber, *Biochemistry*, 1962, **1**, 463.
[85] 'Enzyme Nomenclature', 1964 Report of the Commission on Enzymes, *IUB Symposium Series*, no. 20, Elsevier, London, 1965, pp. 98, 136.
[86] (*a*) Ref. 32*a*, pp. 311, 369, and 409; (*b*) M. A. Jermyn, *Rev. Pure Appl. Chem.*, 1961, **11**, 92; (*c*) R. A. Dedonder, *Ann. Rev. Biochem.*, 1961, **30**, 347; (*d*) J. B. Pridham in 'The Enzyme Chemistry of Phenolic Compounds', ed. J. B. Pridham, Pergamon, Oxford, 1963, p. 73.

build-up and breakdown of polysaccharides, in the absence of 'high energy' glycosyl donors.

The general transglycosylation may be represented by the following two-step scheme:[86d,87]

$$G—OR^1 + EH \rightleftharpoons G—E + H—OR^1 \quad (1)$$

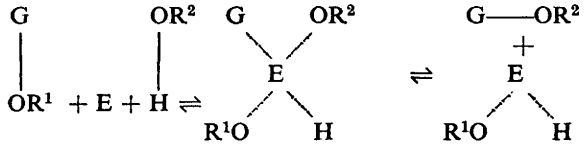
$$G—E + H—OR^2 \rightleftharpoons G—OR^2 + EH \quad (2)$$

E = glycosyltransferase, G = glycosyl group

Scheme 5

Step (1) is the formation of a complex or complexes between the glycosyl donor and the active site of the enzyme. The group R^1 may be alkyl, aryl, or glycosyl. Step (2) involves an attack on the complex by the acceptor molecule, R^2OH.[88] It may be a transfer reaction, if R^2 is aryl, glycosyl, or alkyl (primary or secondary alcoholic, but not usually tertiary), or a hydrolysis, if R^2 is H. Hence, hydrolysis is really only a special case of transfer and the two activities usually run parallel during enzyme purifications, indicating that a single enzyme is responsible for them both.[89] There are few transferases known which do not possess hydrolase activity, and most hydrolases are also transferases. One transferase without hydrolase activity is the D-enzyme of potato (α-1,4-glucan : α-1,4-glucan 4-glucosyltransferase, EC 2.4.1.25), which transfers polyglucosyl chains from starch and maltodextrins to D-glucose, with no trace of hydrolytic products.[90] It is possible, therefore, that some enzymes in this group operate by markedly different mechanisms from the one outlined in Scheme 5.

One possible mechanism for glycosidase action was suggested by Jermyn.[91] This involves the formation of a ternary complex between the substrate, enzyme, and acceptor, which then breaks down to give the products (Scheme 6)



Scheme 6

In this model, the displacement is a frontal one, which accounts for the retention of the anomeric configuration of the glycone. The scheme does not, however, explain the high specificity towards the glycone, as compared with the aglycone. The ternary complex hypothesis is supported by extensive kinetic

[87] (*a*) Ref. 32*a*, p. 369; (*b*) S. A. Barker and E. J. Bourne, *Quart. Rev.*, 1953, **7**, 56.
[88] Ref. 32*a*, p. 409.
[89] (*a*) J. E. Courtois and M. Leclerc, *J. Biol. Chem.*, 1956, **38**, 365; (*b*) T. Miwa and K. Takano, *Kôso Kagaku Shinpojiuma*, 1950, **4**, 76 (*Chem. Abs.*, 1952, **46**, 2097b).
[90] (*a*) S. Peat, W. J. Whelan, and W. R. Rees, *J. Chem. Soc.*, 1956, 44; (*b*) G. J. Walker and W. J. Whelan, *Biochem. J.*, 1957, **67**, 548.
[91] M. A. Jermyn, *Science*, 1957, **125**, 12.

studies with various acceptors and *Stachybotrys atra* β-glucosidase[92] by Jermyn; Stringer and Tsuchiya[93] and Thompson and Neely[94] showed that it is probably the mechanism for dextransucrase (α-1,6-glucan : D-fructose 2-glucosyltransferase, EC 2.4.1.5) which transfers α-D-glucosyl residues from sucrose to suitable carbohydrate acceptors. This latter enzyme is unusual, however, since it is not a hydrolase.

Koshland[95] proposed another possible mechanism for glycosidase action, which gives overall retention of the anomeric configuration by double displacement. This is illustrated in Scheme 7.

$$E: + G—OR^1 \rightleftharpoons E—G + :OR^1 \quad [1]$$

$$E—G + :OR^2 \rightleftharpoons E + G—OR^2 \quad [2]$$

Scheme 7

In step [1], a nucleophile of the enzyme active site attacks the substrate causing the splitting of the gycosidic bond and formation of a covalent bond between the glycosyl residue and the enzyme surface. Step [2] is the nucleophilic attack on this species by the group OR^2 from the acceptor. In both steps, the nucleophilic attack is on the side opposite to the outgoing group, giving overall retention of the anomeric configuration. An instance of this mechanism is known in the exchange of phosphate by sucrose phosphorylase (disaccharide glycosyltransferase, EC 2.4.1.7),[96] a non-specific enzyme which catalyses the general reaction:

$$G—OR^1 + H—OR^2 \rightleftharpoons G—OR^2 + H—OR^1$$

in which R^1OH and R^2OH may be various ketoses, L-arabinose, phosphate, or arsenate.

Lysozyme (mucopeptide *N*-acetylmuramylhydrolase, EC 3.2.1.17) earned fame as the first enzyme for which the entire primary, secondary, and tertiary structure was elucidated, together with many details about its mode of action at the active site. It has been extensively reviewed elsewhere.[97] Although it has transferase activity, its main action is the cleavage of the cell-wall material of bacteria, a complex mucopolysaccharide, where side groups in the substrate, which are absent in the substrates of 'simple glycosidases', may assist the catalytic action of the enzyme.[98]

The enzyme of this group which deserves special mention is the β-galactosidase of *Escherichia coli* (EC 3.2.1.23), because it was probably the first glycosyl-

[92] M. A. Jermyn, *Austral. J. Biol. Sci.*, 1958, **11**, 114; 1966, **19**, 903, 919, 927, 935, 1153.
[93] C. S. Stringer and H. M. Tsuchiya, *J. Amer. Chem. Soc.*, 1958, **80**, 6620.
[94] W. B. Neely and C. F. Thompson, *Nature*, 1959, **184**, B.A. 64.
[95] D. E. Koshland, in 'Mechanism of Enzyme Action', ed. W. D. McElroy and B. Glass, Hopkins, Baltimore, 1954, p. 608.
[96] M. Doudoroff, S. A. Barker, and W. J. Hassid, *J. Biol. Chem.*, 1947, **168**, 725, 733.
[97] (*a*) A. C. T. North, *Sci. J.*, 1966, **2**, 55; (*b*) P. Jollès, *Exposés Ann. Biochim. Med.*, 1966, 1; (*c*) Symposium (various authors), *Proc. Roy. Soc.*, 1967, *B.*, **167**, 350.
[98] (*a*) D. Piskiewicz and T. C. Bruice, *J. Amer. Chem. Soc.*, 1967, **89**, 6237; (*b*) 1968, **90**, 2156.

transferase to be completely purified and crystallised, and to be studied extensively by chemical and physical methods.

Samples of *E.coli* β-galactosidase were prepared from the strain ML 309 by Wallenfels, Zarnitz, Laule, Bender, Keser, and Arens.[99] Other samples were extracted by Hu, Wolfe, and Reithel[100] from the strain ML 308 and by Sund and Weber[101] from the strain ML 35. All appeared to have very similar chemical properties. Evidence from several sources[99c,101,102] suggests that the molecule exists as a tetramer, of moleculer weight $5{\cdot}2 \times 10^5$. Each sub-unit has a *C*-terminal lysine unit, and a probable hexamer,[88] of molecular weight $7{\cdot}5 \times 10^5$, had four *N*-terminal threonine residues and two *N*-terminal glutamic acid units. Anfinsen[103] suggested that the sub-units of the molecule were chemically different. Appel, Alpers, and Tompkins[104] formed by induction several higher polymers from single, tetrameric β-galactosidases from various *E.coli* strains and hybrids. More recent work suggests that the sub-units consist of at least three polypeptide chains, each of molecular weight about 40,000.[105]

Wallenfels and co-workers[88,106] studied the chemical properties of *E.coli* β-galactosidase. It has both hydrolase and transferase activities, which are enhanced by Na^+, K^+, Mn^+, and Mg^+ ions.[107] Sugars, alcohols and phenols,[80] and water all function as acceptors, and transfer products can be detected after only one minute of reaction time. The glycone specificity is comparatively high, being restricted to β-D-galactopyranosides with unsubstituted hydroxy-groups on C-2, C-3, C-4, and C-6. Replacement of the primary alcohol group by H or CH_3 (giving α-L-arabinosides or β-D-fucosides, respectively) resulted in a decreased rate of hydrolysis. In contrast, the aglycone specificity was wide, embracing glycosyl, alkyl, and aryl groups. The hydrolysis rate, however, depends on the aglycone. Inhibitor studies indicated the presence of L-cysteine and L-histidine in the active site. These observations led Wallenfels to postulate a similar mechanism to that outlined in Scheme 7. His more detailed scheme is illustrated in Scheme 8.[88] The galactosyl residue may be held to the enzyme surface by hydrogen bonds, between carbonyl groups of peptide bonds in the active site and hydroxy-groups on C-2, C-3, C-4, and C-6 of the sugar portion of the substrate.

Gorin, Spencer, and Phaff[108] combined a mechanism of this type with Koshland's[95] double displacement theory to explain the action of *Sporoboromyces singularis* β-galactosidase (lactose:lactose 4-galactosyltransferase), which

[99] (*a*) K. Wallenfels, M. L. Zarnitz, G. Laule, H. Bender, and M. Keser, *Biochem. Z.*, 1959, **331**, 459; (*b*) K. Wallenfels and M. L. Zarnitz, *Angew. Chem.*, 1957, **69**, 482; (*c*) K. Wallenfels and A. Arens, *Biochem. Z.*, 1960, **332**, 247.

[100] A. S. L. Hu, R. G. Wolfe, and R. J. Reithel, *Arch. Biochem. Biophys.*, 1959, **81**, 500.

[101] H. Sund and K. Weber, *Biochem. Z.*, 1963, **337**, 24.

[102] (*a*) U. Karlsson, S. Koorijian, I. Zabin, F. S. Sjöstrand, and A. Miller, *J. Ultrastruct. Res.*, 1964, **10**, 457; (*b*) S. Koorijian and I. Zabin, *Biochem. Biophys. Res. Comm.*, 1965, **18**, 381; (*c*) G. D. Zipster, *J. Biol. Chem.*, 1963, **7**, 113.

[103] G. R. Craven, E. Steers, C. B. Anfinsen, and J. L. Bethune, *J. Biol. Chem.*, 1965, **240**, 2478.

[104] S. H. Appel, D. H. Alpers, and G. M. Tompkins, *J. Mol. Biol.*, 1965, **11**, 12.

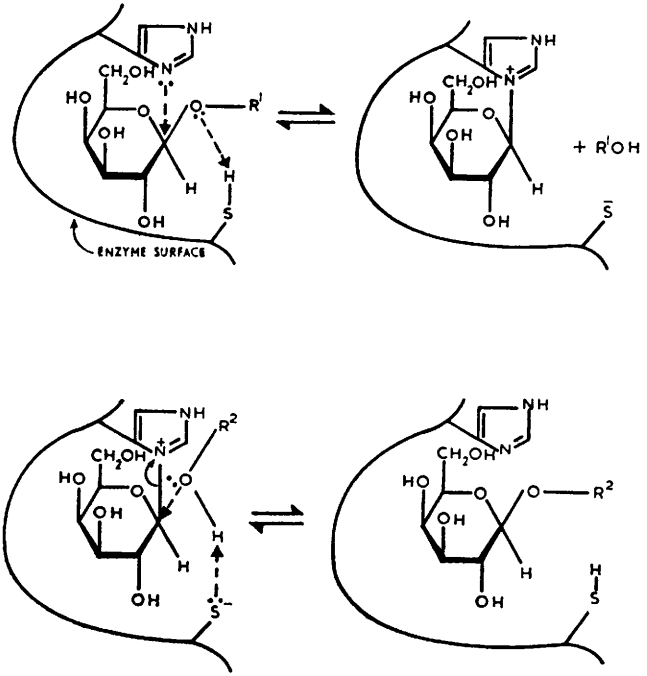
[105] C. Gölker, Dissertation, University of Freiburg, 1967.

[106] K. Wallenfels, *Bull. Soc. Chim. biol.*, 1960, **42**, 1715.

[107] M. Cohn and J. Monod, *Biochim. Biophys. Acta*, 1951, **7**, 153.

[108] P. A. J. Gorin, J. F. T. Spencer, and H. J. Phaff, *Canad. J. Chem.*, 1964, **42**, 2307.

synthesises digalactosyl- and trigalactosyl-glucosides from lactose *in vivo*. If D-glucose was added to the system, there was galactosyl transfer to the 3- and 6-positions. The mechanism of Gorin *et al.* was essentially the same as that of Wallenfels, except that the positions of the cysteine and the histidine residues on the active sites were interchanged, relative to the position of the aglycone, in the intermediate complex. Thus, the nucleophilic attack on the anomeric carbon atom took place on the opposite side from the outgoing aglycone, and the galactose in the galactose–enzyme complex had the α-D-configuration. The latter under-



Scheme 8 *A possible mechanism of* E.coli *β-galactosidase action* (by courtesy of Prof. K. Wallenfels, from 'The Enzymes', ed. P. Boyer, H. Lardy, and K. Myrbäck, Academic Press, London, 2nd edn., 1964, vol. 4, p. 409)

went a second nucleophilic attack to revert to the β-D-configuration. This, Gorin considered, was more in accord with the energy requirements for the reaction at the anomeric carbon atom, *i.e.* the reaction was probably an S_N2 type, where the nucleophile attacks on the opposite side from the outgoing hydroxy-group, while the other substituents on C-1 lie approximately in a plane. This arrangement minimises the exchange energy between bonds which are breaking and those which are forming and gives rise to inversion.[109] Gorin *et al.* noted that, unlike many enzymes of this type, which glycosylate the C-6 primary alcohol group the

[109] C. K. Ingold in 'Structure and Mechanism in Organic Chemistry', Bell, London, 1953, p. 378.

most readily, the *S.singularis* enzyme *in vivo* preferentially glycosylated secondary hydroxy-groups of the acceptor, which had a vicinal hydroxy-group. This explained the comparative lack of hydrolysis in the system and suggested, as in Jermyn's hypothesis for *Stachybotrys atra* β-glucosidase mentioned previously, that the acceptor might also be bound to the enzyme surface by its hydroxy-group. Hopkinson[110] found some evidence of possible bonding of the acceptor to the glucosyltransferase of *Aspergillus niger* mentioned earlier, which catalyses the formation of α-D-glucosides, isomaltosides, and maltosides from maltose and a phenol. The same author[111] has evidence of acceptor–enzyme binding for certain acceptors with *E.coli* β-galactosidase. Phenolic carboxylic acids would appear to be bound, possibly by hydrogen bonding at their CO_2H group, to the CO_2H group of either glutamic or aspartic acid, while phenols, halogenophenols, and hydroxypryidines, containing no carboxylic groups, appear to be bound to the enzyme surface at a group of higher pK value. This could be NH_2 in lysine, C_6H_5O in tyrosine, or SH in cysteine, and binding may be by hydrogen bonding to an OH group in the acceptor. Hasnain[112] reports, from pH studies on its activity, that almond emulsin β-glucosidase requires histidine and a dicarboxylic acid (aspartic or glutamic acid) for its activity. He proposes a mechanism similar to that of Wallenfels[88] for *E.coli* β-galactosidase, or that of Gorin,[108] for *Sporoboromyces singularis* β-galactosidase, with a carboxy-group, or carboxylate ion, replacing the mercapto-group, or sulphide ion, respectively. Histidine and a dicarboxylic amino-acid have been implicated at the active site of several glycosidases, in particular dextransucrase[113] (EC 2.4.1.5) from *Leuconostoc mesenteroides* and bean and potato invertases[114] (β-fructofuranoside fructohydrolase, EC 3.2.1.26).

Glycosidases and glycosyltransferases often occur in pairs or even in multiple forms in living tissue. Jermyn[115] found evidence of more than one β-glucosidase in *Aspergillus oryzae* and a pair of β-glucosidases with transferase activity in *Stachybotrys atra*.[91,92] Legler[116] has found no less than six β-glucosidases in *Aspergillus wentii*. Some pairs of enzymes appear to have similar specificities, while differing in their physicochemical properties, *e.g.* the β-galactosidases of *Neurospora crassa*,[117] which differ in their pH optima and thermal stabilities, as do also the glucosyltransferases of *Aspergillus niger*.[78]

One commonly occurring pair of β-glycosidases is cellobiase (cellobiose glucohydrolase) and aryl β-glucosidase (aryl β-D-glucoside glucohydrolase), which have been reported as coexisting *inter alia* in barley,[118] *Trifolium repens*,[119]

[110] S. M. Hopkinson, Ph.D. Thesis, University of London, 1965.
[111] S. M. Hopkinson, in press.
[112] S. N. Hasnain, Dissertation, University of Bonn, 1968.
[113] W. B. Neely, *J. Amer. Chem. Soc.*, 1958, **80**, 2010.
[114] G. M. Frost, R. N. Greenshields, and F. J. W. Teale, *Biochem. J.*, 1968, **107**, 625.
[115] M. A. Jermyn, *Austral. J. Biol. Sci.*, 1952, *B*, **5**, 433.
[116] G. Legler, *Z. physiol. Chem.*, 1967, **348**, 1359.
[117] W. K. Bates and D. O. Woodward, *Science*, 1964, **146**, 777.
[118] F. Anderson, W. L. Cunningham, and D. J. Manners, *Biochem. J.*, 1964, **90**, 30.
[119] G. W. Butler, R. W. Bailey, and L. D. Kennedy, *Phytochemistry*, 1965, **5**, 369.

Neurospora crassa,[120] *Aspergillus wentii*[121] and *Saccharomyces cerevisiae*.[122]

A single glycosidase may have more than one activity. Much of the work of Helferich[123] and others[124] suggests that the β-glucosidase and β-galactosidase activity of sweet almond emulsin are due to a single enzyme, with no specificity for C-4 of the substrate. However, other reports[125] indicate that two distinct enzymes, a β-glucosidase and a β-galactosidase, exist in emulsin. Higham, Kent, and Pritchard[126] report finding two β-glucosidases in emulsin, both with β-xylosidase activity, *i.e.* these enzymes are lacking in C-6 specificity. However, Manners and Taylor[124c] consider that β-D-glucosidase, β-D-galactosidase, β-D-xylosidase, and α-L-arabinosidase activity in emulsin are all due to a single enzyme. Emulsin β-glucosidase will hydrolyse aryl β-glucofuranosides, as well as the pyranosides.[127] Robinson[128] has found that the β-glucosidase and β-galactosidase activities of *Medicago sativa* are distinct, but difficult to separate. His work on this pair of activities in 11 other sources showed them all to be due to separate enzymes.[129]

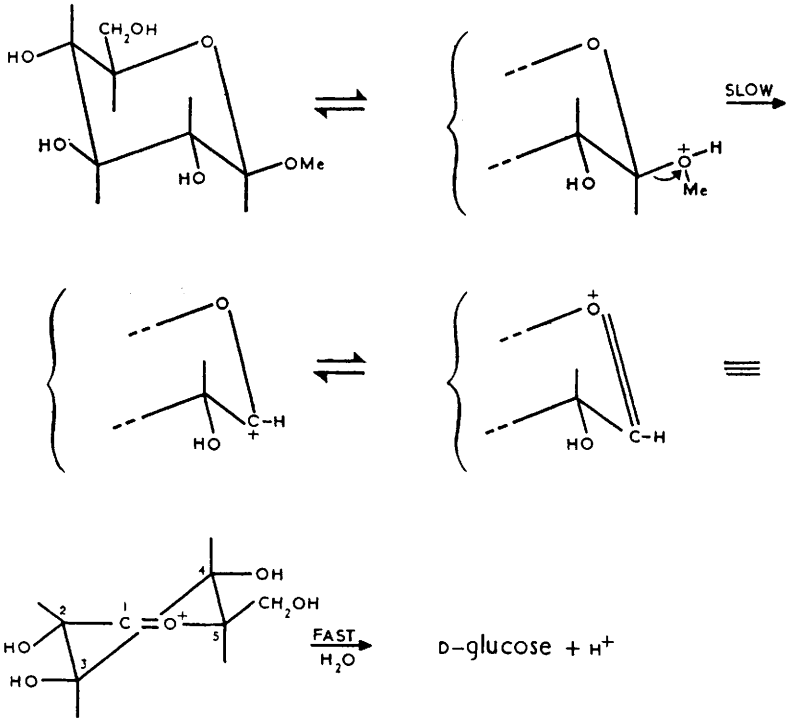
A similarity has often been noted between the hydrolyses of glycosides catalysed by acid and by enzymes,[130] which both occur by aldose–oxygen fission of the glycosidic bond. This does not accord with all the observed facts, however. The acid hydrolysis mechanism, [A] (see Scheme 9), is the one favoured by the majority of workers.[131] In this mechanism, which is of the *A*1 type, there is rapid protonation of the glycosidic oxygen atom, followed by the slower heterolysis of the aldose–oxygen bond as a rate-determining step, to give the C-1 carbonium ion. This is stabilised by resonance involving the ring oxygen atom, as shown, and probably exists in the half-chair conformation. The final step is the rapid reaction of the carbonium ion with water, to give the free sugar.

BeMiller[132] and Wagner and Nuhn[133] have reviewed the acid-catalysed hydrolysis of glycosides. The latter authors consider that the alternative mechanism of

[120] (*a*) P. R. Mahadevan and B. Eberhart, *Biochim. Biophys. Acta*, 1964, **91**, 214; (*b*) B. Eberhart, D. F. Cross, and L. R. Chase, *J. Bacteriol.*, 1964, **87**, 761.
[121] G. Legler, *Z. physiol. Chem.*, 1967, **348**, 1359.
[122] (*a*) J. G. Kaplan, *J. Gen. Physiol.*, 1965, **48**, 873; (*b*) A. N. Inamdar and J. G. Kaplan, *Canad. J. Biochem.*, 1966, **44**, 1099.
[123] B. Helferich and T. Kleinschmidt, *Naturwiss.*, 1966, **53**, 132.
[124] (*a*) M. V. Keleman and W. J. Whelan, *Arch. Biochem. Biophys.*, 1966, **117**, 423; (*b*) J. Conchie, A. L. Gelman, and G. A. Levvy, *Biochem. J.*, 1967, **103**, 609; (*c*) D. J. Manners and D. C. Taylor, *Carbohydrate Res.*, 1968, **7**, 497.
[125] S. Veibel, J. Wangel, and G. Østrup, *Biochim. Biophys. Acta*, 1947, **1**, 126.
[126] M. Highham, P. W. Kent, and P. Pritchard, *Biochem. J.*, 1966, **98**, 46P.
[127] K. Yoshida, T. Kamada, N. Harada, and K. Kato, *Chem. and Pharm. Bull. (Japan)*, 1966, **14**, 583.
[128] D. Robinson, *Phytochemistry*, 1966, **5**, 699.
[129] R. G. Price and D. Robinson, *Comp. Biochem. Physiol.*, 1966, **17**, 129.
[130] (*a*) G. Wagner and R. Metzner, *Naturwiss.*, 1965, **52**, 61; (*b*) C. A. Vernon, *Proc. Roy. Soc.*, 1967, *B*, **167**, 389.
[131] (*a*) C. A. Bunton, T. A. Lewis, D. R. Llewellyn, and C. A. Vernon, *J. Chem. Soc.*, 1955, 4419; (*b*) C. A. Vernon, C. A. Bunton, S. Patai, L. H. Selman, and C. A. Vernon, *ibid.*, 1961, 412; (*c*) B. E. C. Banks, Y. Meinwald, A. J. Rhind-Tutt, I. Sheft, and C. A. Vernon, *ibid.*, 1961, 3240; (*d*) W. G. Overend, C. W. Rees, and J. S. Sequeira, *ibid.*, 1962, 3429; (*e*) G. Bamford, B. Capon, and W. G. Overend, *ibid.*, 1962, 5138.
[132] J. N. BeMiller, *Adv. Carbohydrate Chem.*, 1967, **22**, 25.
[133] P. Nuhn and G. Wagner, *Pharmazie*, 1966, **21**, 261.

acid-catalysed glucoside hydrolysis, [B] (Scheme 10), cannot be excluded. This involves rapid protonation of the ring oxygen atom as a first step, followed by slow heterolysis of the bond between this atom and C-1, that is, a ring opening. This step is rate-determining and the final step of loss of the alcohol and re-formation of the ring is rapid.

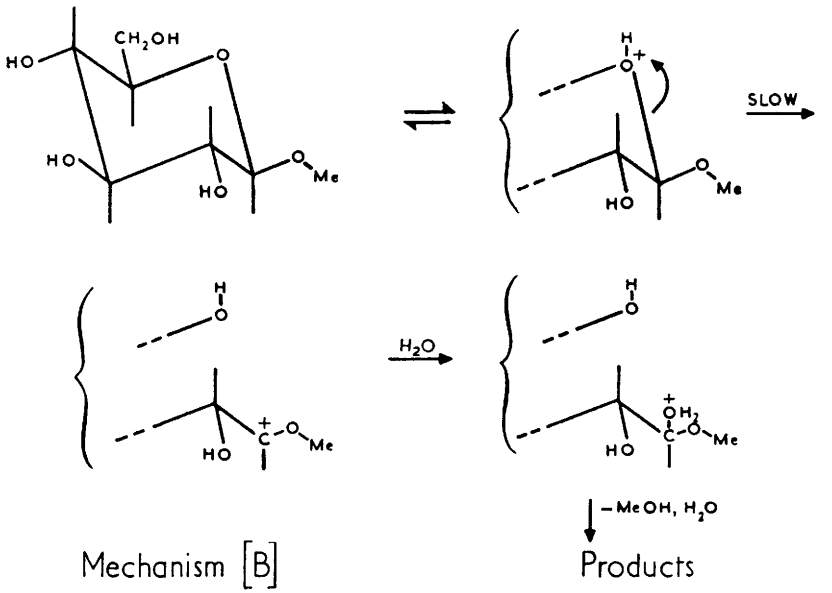
Some doubt has been cast upon mechanism [A] by the recent report by Timell and Saunders[134] that the electron affinity of the aglycone has no effect on the rate of hydrolysis of β-D-glucopyranosides. This was refuted by Feather and Harris,[135] who compared data from a number of different sources on the relative rates of the hydrolysis of glycosides by acid, in an attempt to show that the aglycone has a considerable effect on the reaction rate, a feature of mechanism [A]



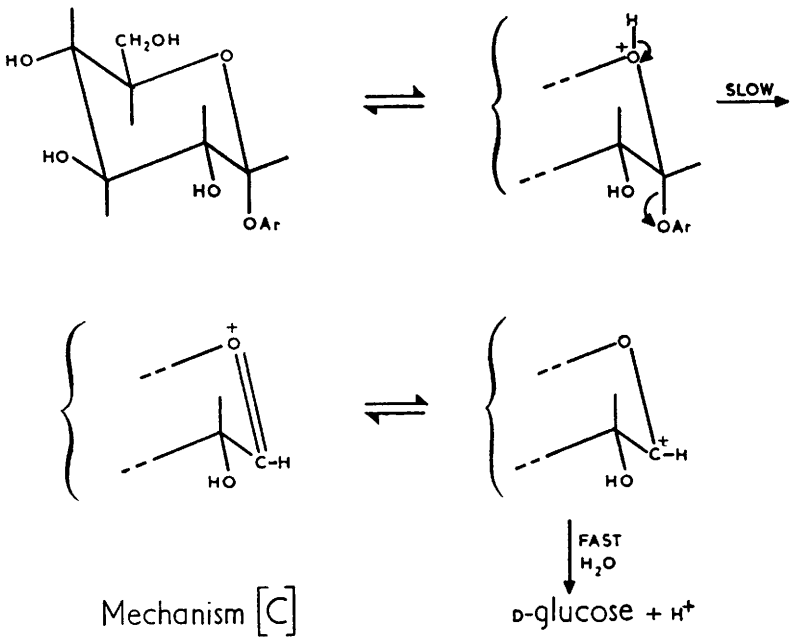
Scheme 9 *A proposed mechanism for acid-catalysed hydrolysis of glycosides* (ref. 131)

Other work by Timell and De[136] also seems to support this mechanism. They showed that glucopyranosides monomethylated at C-2, C-3, or C-4 hydroxy-groups are more stable than the corresponding unmethylated compounds in acid.

[134] M. D. Saunders and T. E. Timell, *Carbohydrate Res.*, 1967, **5**, 453.
[135] M. S. Feather and J. F. Harris, *J. Org. Chem.*, 1965, **30**, 153.
[136] K. K. De and T. E. Timell, *Carbohydrate Res.*, 1967, **4**, 72.



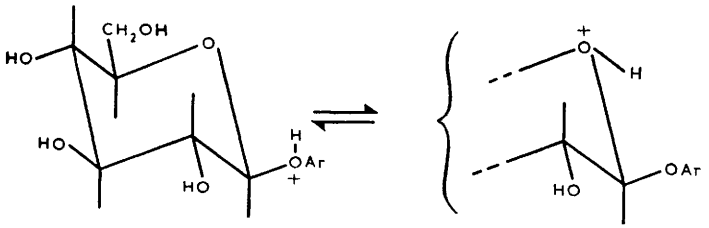
Scheme 10 *An alternative mechanism for acid-catalysed hydrolysis of glycosides* (ref. 131)



Scheme 11 *A possible mechanism for the acid-catalysed hydrolysis of aryl glycosides* (ref. 137)

Since rotation about the C(2)—C(3) and C(4)—C(5) bonds is necessary for the formation of the half-chair conformation of the carbonium ion in mechanism [A], the introduction of methoxy-groups at C-2, C-3, or C-4 would hinder rotation and, in turn, make the methylated glycosides more stable to acid.[135]

Hall, Hollingshead, and Rydon[137] consider that another mechanism, [C] (Scheme 11), is more likely for the case of aryl α-D-glycosides, based on their observation that, whereas in the β-D-series there was increased activity due to electron-repelling groups and decreased activity due to electron-attracting groups in the aglycone (*o*- and *p*-positions), similar polar substituents in the α-D-series had no effect. In this mechanism, protonation is axial (owing to the shielding of the equatorial position by the aryloxy-group) and decomposition of the conjugate acid by *trans*-elimination of two axial groups is controlled by steric, rather than electronic, effects. With aryl glycosides, the ring oxygen atom is more basic than the glycosidic oxygen atom (owing to the electron-withdrawing effect of the aryl group) and the apparent anomaly of protonation of the glycosidic oxygen in the aryl β-D-series was explained by invoking mesomerisation of the conjugate acid in this case, caused by proximity of the two oxygen atoms (Scheme 12). Timell,[138] however, believed that the apparent lack of effect of polar



Scheme 12 *Mesomerisation of the conjugate acid intermediate in acid-catalysed hydrolysis of aryl β-D-glycosides*

groups in the α-D-case was due to the coincidental cancelling of the opposite effects which such groups have on the protonation of the glycosidic oxygen, on the one hand, and on the heterolysis of the conjugate acid, on the other. In the β-D-series, these effects only partially cancel, but their difference is small.

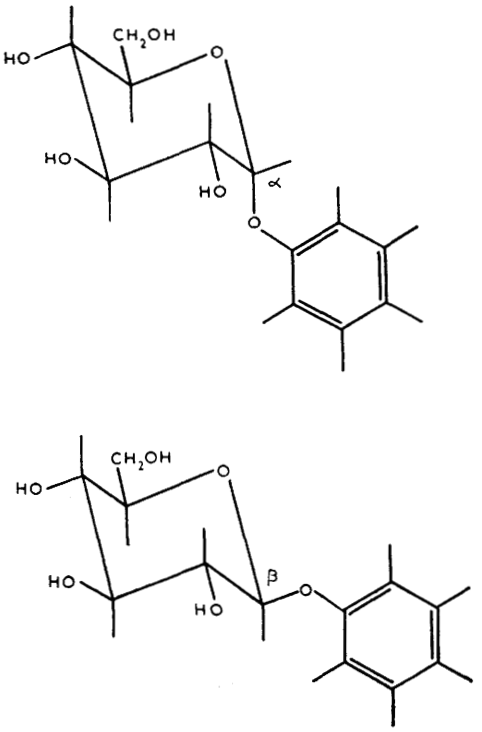
The early data of Moelwyn-Hughes[139] and of Heidt and Purves,[140] on activation energies of glycosides during acid hydrolysis, were augmented by Overend, Rees, and Sequeira.[131d] Values for aryl glycosides were generally lower than those for their alkyl analogues, those for aryl α-D-glycosides being lowest of all. The reason for this difference in the stability of the aryl α-D- and β-D-glycosides is a conformational one (Scheme 13).

In the C1 conformation, the α-D-anomer has an (Ar_{1a}) group, which is subjected to more repulsion than the (Ar_{1e}) group of the β-D-anomer. It is questionable

[137] A. N. Hall, S. Hollingshead, and H. N. Rydon, *J. Chem. Soc.*, 1961, 4290.
[138] T. E. Timell, *Canad. J. Chem.*, 1964, **42**, 1456.
[139] E. A. Moelwyn-Hughes, *Trans. Faraday Soc.*, 1958, **24**, 309, 321; 1959, **25**, 81, 503.
[140] L. J. Heidt and C. B. Purves, *J. Amer. Chem. Soc.*, 1944, **66**, 1385.

whether the aryl α-D-glucopyranosides exist in the C1 conformation, since it is conceivable that the combined (H_{3a})–(Ar_{1a}) and (H_{5a})–(Ar_{1a}) repulsions might be greater than (OH_a) interactions of some other conformation having an (Ar_{1e}) group, *e.g.* the 1C conformation. However, n.m.r. measurements[141] have shown that in maltose, which is an α-D-glucoside with a large aglycone, both pyranose rings have the C1 conformation. This, therefore, is probably the stable conformation of aryl α-D-glucopyranosides.

Overend *et al.*[131d] found that acid hydrolysis rate differences between anomeric pairs arose from a difference in entropy changes (ΔS^*) rather than in energy changes (ΔE^*). ΔE^* values were almost identical for most pairs. This showed that the α-D-anomers were the more highly orientated in the ground state, undergoing greater entropy changes upon activation. Reactivity was therefore determined by the increase in entropy due to interaction of the aglycone with (H_{3a})



Scheme 13 *The conformational difference between the α- and β-anomers of an aryl* D-*glucopyranoside*

and (H_{5a}), rather than by the increase in potential energy of the molecule, due to repulsion between these substituents and the aglycone (Scheme 13).

Similar results were found by Timell[138] for the anomeric pair maltose and

[141] V. S. R. Rao and J. F. Foster, *J. Phys. Chem.*, 1963, **67**, 951.

cellobiose. This supports the claim that aryl α-D-glucosides are the conformational analogues of maltose.

It is interesting to note that, in contrast to the case of acid hydrolysis, in irradiation hydrolysis the presence of an aryl group, either as an aglycone, or in other positions on the sugar, gives considerably enhanced stability. This is thought to be due to preferential absorption of energy by the aryl group. The aryl group also confers stability on neighbouring links. In phenyl hepta-*O*-acetyl-β-D-maltoside, the disaccharide link, while being less stable to irradiation than the glycosidic bond, is twenty times more resistant to γ-irradiation than in maltose.[142] Substituents in the aryl group, whether electropositive or electronegative, have no effect upon the radiation stability of aryl glycosides.

Rydon and his co-workers[137,148] produced the first data which give a clue to the probable nature of enzymatic hydrolysis and synthesis of glycosides, if their data are reinterpreted.[110] These workers compared the actions of acid and alkali upon a comprehensive group of aryl α- and β-D-glucosides with the enzymatic hydrolysis, using brewers' yeast α-glucosidase and almond emulsin β-glucosidase, respectively. They noted a resemblance between alkaline and enzymatic hydrolysis of the two series of glucosides, both being facilitated by electron-attracting substituents in the aglycone. Inspection of their data, however, shows that nearly all the electron-repelling substituents tested also increased the enzymatic hydrolysis rate, which is a feature of acid hydrolysis and a fact which these authors overlooked. Another important feature emerges from their values for the breakdown rate constants for the enzyme–substrate complexes. In all groups of compounds tested (*i.e.* Cl, CH_3O, CN, and NO_2), with one minor exception, the rates for the three positional isomers were *o*- > *m*- > *p*- (although data for the substituted α-D-glucosides are restricted mainly to *meta*- and *para*-examples). Such an order of rates can only be attributed to steric factors, which must therefore be relatively more important in enzymatic hydrolysis. It is interesting to note that Wallenfels[144] found with *E.coli* β-galactosidase that the order of reactivity of a series of thiogalactosides is 2,4,6-trinitrophenyl *S*-β-D-thiogalactosides ⩾ *o*-nitrophenyl *S*-β-D-thiogalactosides > phenyl *S*-β-D-thiogalactosides. This might be due to electronic effects, but could also be due to a steric selectivity of the enzyme for *ortho*-substituted aryl aglycones. In this connection, Wagner and Pflegel[145] found in the hydrolysis of 2-*S*- and 4-*S*-β-D-glycosyl-mercaptophenyl *O*-β-D-glucosides that the rate of hydrolysis by almond emulsin β-glucosidase is much lower for the 4-mercaptophenyl derivatives (*para*-form) than for the 2-mercaptophenyl isomers (*ortho*-isomers).

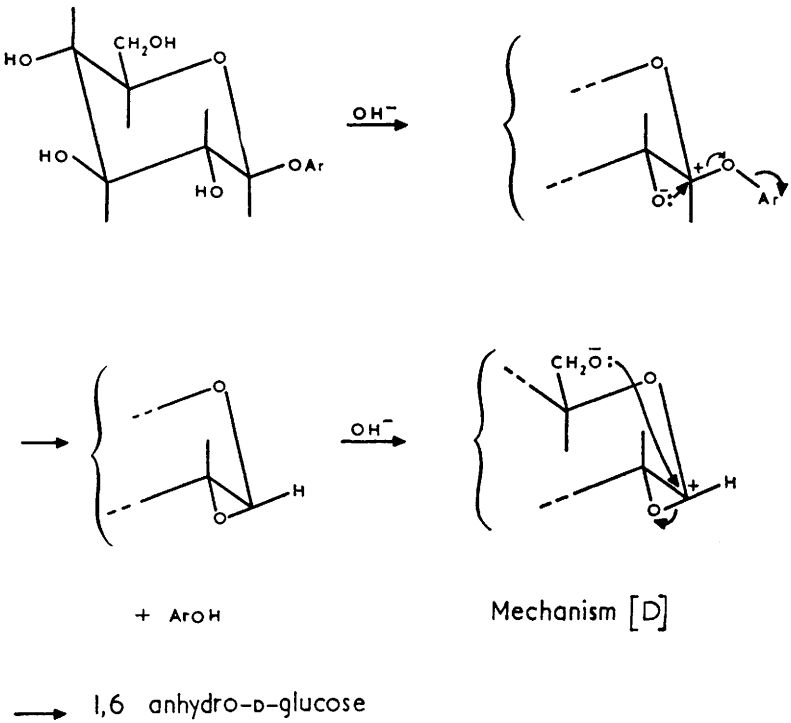
Alkaline hydrolysis of phenyl β-D-glucosides probably occurs by the mechanism [D] (Scheme 14), in which the glycosidic bond splits by aglycone-oxygen fission.[146] The nucleophilic attack on the anomeric carbon atom is enhanced by

[142] G. O. Phillips, F. A. Blouin, and J. C. Arthur, *Nature*, 1964, **202**, 1328.
[143] (*a*) A. N. Hall, S. Hollingshead, and H. N. Rydon, *Biochem. J.*, 1962, **84**, 390; (*b*) R. L. Nath and H. N. Rydon, *ibid.*, 1954, **57**, 1.
[144] K. Wallenfels, *Angew. Chem. Internat. Edn.*, 1964, **3**, 1.
[145] G. Wagner and P. Pflegel, *Pharmazie*, 1965, **20**, 140.
[146] C. E. Ballou, *Adv. Carbohydrate Chem.*, 1954, **9**, 59.

electron-attracting substituents in the aglycone, which make the anomeric carbon atom more acidic.

Phenyl α-D-glucosides react only very slowly with alkali. If mechanism [D] is correct, this slowness would be due to the difficulty of forming an epoxide ring where there is a *cis*-, rather than a *trans*-, arrangement of the C-2 hydroxy-group and the aglycone. Another alternative intramolecular mechanism, [E],[146] has been suggested for the α-D-anomers (Scheme 15). Overend, Ferrier, and Ryan[147] showed that aryl 2-deoxy-α-D-glucopyranosides are also cleaved by alkali, which appears to preclude mechanism [D] for aryl α-D-anomers, since 2-deoxy-sugars cannot form 1,2-epoxides. They also found that 1,6-anhydrides were only produced in relatively strong alkali. At lower concentrations, solvolysis predominates to give 2-deoxy-D-glucose (together with its 3,6-anhydride, which arose from it by the action of alkali).

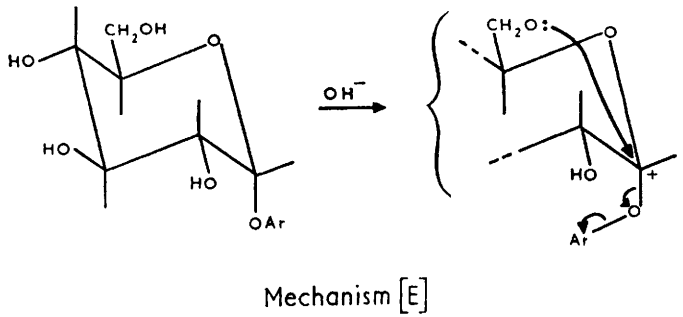
In contrast to these results, however, Gasman and Johnson[148] found that *p*-nitrophenyl 2-*O*-methyl-β-D-galactopyranoside and *p*-nitrophenyl 2-*O*-methyl-



Scheme 14 *Proposed mechanism for the alkali-catalysed hydrolysis of aryl β-D-glucosides* (ref. 146)

[147] R. J. Ferrier, W. G. Overend, and A. E. Ryan, *J. Amer. Chem. Soc.*, 1965, **87**, 3484.
[148] R. C Gasman and D. C. Johnson, *J. Org. Chem.*, 1966, **31**, 1830.

α-D-mannoside were hydrolysed by alkali considerably more slowly than the corresponding unmethylated compounds. These authors give evidence that, in general, alkaline hydrolysis proceeds by mechanism [D], but in the case of the



Mechanism [E]

Scheme 15 *An alternative intramolecular mechanism for alkali-catalysed hydrolysis of aryl* α-D-*glucosides* (ref. 146)

2-*O*-methyl compounds, the neighbouring group participation of the C-2 oxyanion is not possible, and the strong electron-attracting properties of the *p*-nitrophenyl group favour an S_N2 mechanism for hydrolysis of these compounds.

Unlike Rydon *et al.*, Wagner and Nuhn[149] and Wagner and Metzner[130a] found an apparent similarity between acid and enzymatic hydrolysis in their studies of acid, alkaline, and almond emulsin hydrolysis of an isologous series of *o*- and *p*-phenyl β-D-glucosides and nitrophenyl β-D-glucosides. The order of reactivity for the acid and enzymatic hydrolyses of the *para*-isologues was $O \gg Se > S$. This was also the order of hydrolysis with acid in the *ortho*-series, while that for the enzyme in this series was $O \gg S \gg Se$, an order which was attributed to steric repulsion between the nitro-group and the selenium atom. The latter order, however, is that which would be expected from the decreasing electronegativity of oxygen, sulphur, and selenium. The order $O \gg Se > S$ is not readily explicable. The order for alkali in all cases was quite different, *i.e.*, $Se > O > S$.

Complete analogies cannot therefore be drawn between enzymatic and acid-catalysed hydrolysis of glycosides, on the one hand, or between enzymatic and alkali-catalysed hydrolysis, on the other.[130b] The duality which exists in the reassessment of Nath and Rydon's results strongly suggests that glycosidase and glycosyltransferase action is of a general acid–base catalysis type,[150] as proposed by Pigman,[151] Wallenfels,[144] and Eigen [152] *inter alia*. Inspection of the postulated mechanism of Wallenfels (Scheme 8), or the similar one of Gorin, shows this acid–base nature. There is both protonation of the glycosidic oxygen atom by

[149] G. Wagner and P. Nuhn, *Arch. Pharm.*, 1965, **298**, 92.
[150] C. G. Swain, *J. Amer. Chem. Soc.*, 1950, **72**, 4578.
[151] W. Pigman, in 'The Carbohydrates', ed. W. Pigman, Academic Press, New York, 1957, p. 556.
[152] M. Eigen, *Angew. Chem. Internat. Edn.*, 1964, **3**, 1.

L-cysteine and nucleophilic attack on C-1 of the glycone by the basic nitrogen atom of L-histidine. Glycosides resemble esters in their ability to be cleaved by both acids and bases, and hydrolase action in general, including that of esterases, has been attributed to acid–base catalysis.[153]

Rydon *et al.* suggested that, in addition to the hydrogen bonding of the donor molecule to the active site of the enzyme, the acceptor was also bonded, but by weaker forces. This they attributed to van der Waals attraction.[137] Recently, binding of aryl glycosides to a plant protein, which depends upon the hydrophobic effect of *meta*-substituents in the aryl ring, has been found by Poretz and Goldstein.[154] An investigation of this concept in the case of aryl glycosides as enzyme substrates might be fruitful.

The work of Gorin[108] and of Hopkinson[111] suggests that a group (*e.g.* OH) in the acceptor, as well as those in the donor, may form hydrogen bonds with the enzyme. In the cases studied, one group in the acceptor appeared sufficient, whereas the donor was more rigidly bound to the enzyme by up to four hydrogen-bonded hydroxy-groups in the glycone. This would explain both the weaker bonding of the acceptor as compared with the donor and the relatively low specificity of the enzyme towards the acceptor.

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[153] M. L. Bender, *Chem. Rev.*, 1964, **60**, 53.
[154] R. D. Poretz and I. J. Goldstein, *Arch. Biochem. Biophys.*, 1968, **125**, 1034.