73. *The Synthesis of* 1-*Glycosylbenziminazoles.*

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Methods for the synthesis of 1-D-galactosyl- and 1-(2-deoxy-D-galactosyl)-benziminazole have been compared. Best results are obtained from the direct condensation in dry dioxan of the *O*-acetylglycosyl halide and benziminazole and deacetylation of the product by the Zemplén procedure. A series of compounds of this class has been synthesised and examined for bacteriostatic action against *E. coli* 15. The stability of some of the compounds in 6N-hydrochloric acid at 100° and 150° has been investigated. It is less if a deoxy-group is present in the sugar moiety.

In connexion with an investigation of certain anti-metabolites, a series of glycosylbenziminazoles was required. Two main methods are available for the synthesis of 1-glycosylbenziminazoles. Either a mono-*N*-glycosyl derivative of *o*-phenylenediamine can be converted into a benziminazole derivative,[1-5] or a suitable benziminazole derivative can be condensed with an *O*-acetylglycosyl halide.[4-9] These methods have been examined for the preparation of 1-β-D-galactopyranosylbenziminazole in reasonable yield.

[1] Brink, Holly, Shunk, Peel, Cahill, and Folkers, *J. Amer. Chem. Soc.*, 1950, **72**, 1866.
[2] Holly, Shunk, Peel, Cahill, Lavigne, and Folkers, *ibid.*, 1952, **74**, 4521.
[3] Buchanan, Johnson, Mills, and Todd, *J.*, 1950, 2845.
[4] Cooley, Ellis, Mamalis, Petrow, and Sturgeon, *J. Pharm. Pharmacol.*, 1950, **2**, 579.
[5] Mamalis, Petrow, and Sturgeon, *ibid.*, 1950, **2**, 491, 503, 512.
[6] Weygand, Wacker, and Wirth, *Z. Naturforsch.*, 1951, **6**b, 25.
[7] Davoll and Brown, *J. Amer. Chem. Soc.*, 1951, **73**, 5781.
[8] Johnson, Miller, Mills, and Todd, *J.*, 1953, 3061.
[9] Heyl, Chase, Shunk, Moore, Emerson, and Folkers, *J. Amer. Chem. Soc.*, 1954, **76**, 1355.

2 : 3 : 4 : 6-Tetra-*O*-acetyl-α-D-galactosyl bromide, condensed with benziminazolylsilver, gave a dark product from which crystalline 1-β-D-galactosylbenziminazole was isolated (overall yield 17·2%) after chromatography to remove unchanged benziminazole and deacetylation. The compound was smoothly oxidised by 2 mol. of sodium periodate with the liberation of 1 mol. of formic acid, which showed it to be a pyranoside. The β-configuration was assigned on the assumption that a Walden inversion occurred during the condensation. In all experiments with this procedure considerable decomposition occurred. Although lowering the temperature prevented this, it also slowed the reaction and so improved yields were not obtained. An excess of vigorously stirred finely divided benziminazolylsilver should be used since it becomes coated with silver bromide as the reaction proceeds: this slows the reaction. Deacetylation of the crude product by the Zemplén–Pacsu method [10] was satisfactory and the objections to the method mentioned by Mamalis *et al.*[5] were not encountered. These workers and also Johnson *et al.*[8] used boiling mineral acid to deacetylate similar compounds: the stability of the *N*-glycosyl linkage apparently precludes hydrolysis but it has now been shown (see below) that in some cases isomerisation can occur and for this reason the Zemplén deacetylation method is preferable.

Condensing tetra-*O*-acetyl-α-D-galactosyl bromide with chloromercuribenziminazole in boiling xylene (cf. Davoll and Brown [7]) followed by deacetylation gave a slightly better yield (23%) of the galactosylbenziminazole. [The chloromercuribenziminazole was made by the addition of a solution of benziminazole and sodium hydroxide to one of mercuric chloride instead of *vice versa*, to prevent the formation of dibenziminazolylmercury, which apparently was a contaminant in Davoll and Brown's product.[7]] The best method of synthesis was to heat tetra-*O*-acetyl-α-D-galactosyl bromide with an excess of benziminazole in dry dioxan. After deacetylation 1-β-D-galactosylbenziminazole was obtained in 37% overall yield. Undesired side-reactions undoubtedly occur, probably base-catalysed reactions leading to products of the *O*-acetylglycoseen and the *O*-acetyl-1 : 6-anhydroglycose type. This method has been employed by Johnson *et al.*[8] to give acetylated glycosylbenziminazoles but was developed independently in the present work which was completed in 1953 [11] before the publication by Johnson *et al.*[8] An attempt to condense the galactosyl halide with benziminazole at room temperature using the improved Koenigs–Knorr technique [12] was unsuccessful, as was an attempt to prepare 1-β-D-galactosylbenziminazole by cyclisation of the appropriate *N*-glycosyl-*o*-phenylenediamine with ethyl orthoformate. 1-β-D-Galactosylbenziminazole gave a glassy tetra-acetate and solid 6-*O*-triphenylmethyl and 3 : 4(?)-*O*-*iso*propylidene derivatives. As the latter compound was prepared by treating the glycosylamine with acetone and acid it is not certain that the β-configuration is retained (see p. 411).

To synthesise 1-(2-deoxy-D-hexosyl)benziminazoles, acetohalogeno-2-deoxyhexoses are required as intermediates. The method of preparation adopted was to add hydrogen halide to an acetylated glycal in benzene containing benzoyl peroxide. In this way a crude tri-*O*-acetyl-2-deoxy-D-galactosyl bromide was synthesised from tri-*O*-acetyl-D-galactal. Treatment with aniline and subsequent deacetylation yielded 2-deoxy-*N*-phenyl-D-galactosylamine. Condensing the crude bromide with benziminazolylsilver in xylene gave after deacetylation a lævorotatory form (*A*) of 1-(2-deoxy-D-galactosyl)-benziminazole (14%), also obtained (in 5% yield) by using chloromercuribenziminazole instead of benziminazolylsilver. Treatment of the acetyldeoxygalactosyl bromide with an excess of benziminazole in dioxan at 100° yielded a small amount of the above lævorotatory compound together with more (30%) of a dextrorotatory isomer (*B*). The formation of two isomers, which are probably α- and β-forms, is not surprising in view of the syrupy nature of the *O*-acetylglycosyl halide employed, but it is not clear why isomer (*B*) should be the

[10] Zemplén and Pacsu, *Ber.*, 1929, **62**, 1613.
[11] Cleaver, Final Report to D.S.I.R., 1953.
[12] Reynolds and Evans, *J. Amer. Chem. Soc.*, 1938, **60**, 2559.

main product of condensation with benziminazole whereas isomer (*A*) is the main product from salts of the base. Isomer (*B*) was converted into (*A*) by boiling dilute mineral acid and as far as the authors are aware this is the first time that interconversion of α- and β-forms of sugars attached to a tertiary nitrogen atom has been observed. Several derivatives of these compounds were prepared. A syrupy triacetate of isomer (*B*) yielded a crystalline picrate which could be used as a means of isolating and purifying the condensation product of tri-*O*-acetyl-2-deoxy-D-galactosyl bromide and benziminazolylsilver. Two crystalline mono*iso*propylidene derivatives of 1-(2-deoxy-D-galactosyl)benziminazole (*A*) were isolated, both of which gave correct analyses and were hydrolysed rapidly by dilute hydrochloric acid at 100° to the initial material (*A*). These derivatives are probably also α- and β-forms although the possibility that the *iso*propylidene residue is linked in different positions (*e.g.*, 3 : 4 and 4 : 6) cannot be excluded. That 1-(2-deoxy-D-galactosyl)-benziminazole (*B*) is isomerised by 0·01N-hydrochloric acid would account for the formation of (*A*) on hydrolysis of both *iso*propylidene derivatives.

1-(2 : 6-Dideoxy-D-galactosyl)benziminazole was synthesised as follows: D-galactose was converted into D-fucose from which 3 : 4-di-*O*-acetyl-D-fucal (3 : 4-di-*O*-acetyl-6-deoxy-D-galactal) was prepared. When treated with hydrogen chloride in dry benzene this afforded 3 : 4-di-*O*-acetyl-2 : 6-dideoxy-D-galactosyl chloride which was condensed in the crude state with benziminazolylsilver to give 1-(di-*O*-acetyl-2 : 6-dideoxy-D-galactosyl)-benziminazole, which was then deacetylated. 1-(Di-*O*-acetyl-2-deoxy-L-ribosyl)benziminazole picrate [4] and 1-(2-deoxy-L-ribosyl)benziminazole were prepared likewise. The latter compound slowly gave a blue colour with the Dische reagent,[13] indicating that slow hydrolysis occurred under the hot strongly acid conditions of this colour test. Cooley *et al.*[4] prepared a 1-(di-*O*-acetyl-2-deoxy-D-ribosyl)benziminazole which differs in melting point but has the same sign for its optical rotation as our product. It is probable that the two compounds have different glycosidic configurations (cf. results of Davoll and Lythgoe [14] who obtained two isomeric 2-deoxyribosides by condensing di-*O*-acetyl-2-deoxy-D-ribosyl chloride with theophyllinylsilver).

1-(2-Deoxy-D-glucosyl)benziminazole was also prepared.

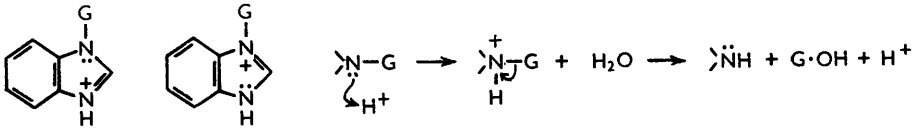
In addition to various glycosyl derivatives of benziminazole, the corresponding D-galactosyl and 2-deoxy-D-galactosyl derivatives of 5 : 6-dimethylbenziminazole were prepared.

The stability of some of the 1-glycosylbenziminazoles in 6N-hydrochloric acid was examined chromatographically. 1-β-D-Galactosylbenziminazole (*a*), 1-(2-deoxy-D-galactosyl)benziminazole (*b*), and 1-(2 : 6-dideoxy-D-galactosyl)benziminazole (*c*) were separately heated in the acid at 100° and 150°. The results of the chromatographic analysis of the products are shown in the Experimental section. Although the compounds are glycosylamines they are very resistant. Introduction of the 2-deoxy-group increases the lability and this is accentuated in the 2 : 6-dideoxy-derivative. This is a further example of the greater lability of 2-deoxyhexosides than of hexosides. Cooley *et al.*[4] showed a similar relation for 5 : 6-dimethylbenziminazole pentose and deoxypentose derivatives. It is of interest that the isomerisation mentioned previously was encountered for the glycosides of a 2-deoxy-sugar. The acid-stability of 1-glycosylbenziminazoles may be due to the existence in acid solution of compounds of this class as substituted benziminazolium ions, the positive charge being distributed throughout the ring system.

Structures (*X*) and (*Y*) would be expected to be the main contributors although the resonance energy will not be so great as in the unsubstituted benziminazolium ion where the two main contributors to the system are identical. The contribution made by (*Y*) effectively places a fractional positive charge on $N_{(1)}$. This will tend to prevent protonation at this site, which is a primary step in the acid hydrolysis of these compounds and which can be depicted as shown. Introduction of a 2-deoxy-group will lower the $-I$

[13] Dische, *Mikrochemie*, 1930, **8**, 4.
[14] Davoll and Lythgoe, *J.*, 1949, 2526.

effect of the glycosyl residue, assisting in the splitting of the >C–N< linkage. In addition certain stereochemical factors will be operative which will assist the hydrolysis.[15] It is interesting to contrast the acid-stability of 1-glycosylbenziminazoles with the acid-lability of 9-glycosylpurines. In the latter type of compound the positive charge can be



partly accommodated on the nitrogen atoms of the six-membered ring and so protonation of the glycosylated nitrogen atom is not effectively prevented as in the glycosylbenziminazoles. We should note Kenner's ideas [16] on this problem and work is in progress to obtain further evidence.

A series of benzimidazole derivatives were tested for bacteriostatic action on *E. coli* 15 (see p. 417).

Experimental

Benziminazolylsilver.—Phillips's procedure [17] was modified as follows: To a stirred solution of benziminazole (11·8 g.) in water (500 c.c.) at 95° an aqueous solution of silver nitrate (17 g. in 80 c.c.) and aqueous ammonia (20 c.c.; *d* 0·880) were added slowly. After 0·5 hr. the white precipitate was collected, washed successively with boiling water, ethanol, and ether, and dried (21·8 g.). Before being used the product was finely pulverised and heated for 12 hr. at 115°.

5 : 6-Dimethylbenziminazolylsilver was prepared in almost identical fashion.

Chloromercuribenziminazole.—Davoll and Brown's method [7] was adapted. A solution of benziminazole (11·8 g.) and sodium hydroxide (4·0 g.) in hot 10% ethanol (1 l.) was added slowly to a stirred solution of mercuric chloride (27·2 g.) in ethanol (300 c.c.). The precipitate which separated on storage of the mixture was washed with water and dried (32 g.).

1-β-D-*Galactosylbenziminazole.*—(*a*) A mixture of benziminazolylsilver (21·9 g.) and xylene (500 c.c.) was distilled until 50 c.c. of distillate had been collected, then cooled. Powdered tetra-*O*-acetyl-α-D-galactosyl bromide (40 g.) was added, the distillation was repeated, and then the mixture was heated under reflux (moisture excluded) for 2 hr. and filtered. (Longer refluxing had no effect on the yield.) The residue was washed with hot xylene. On storage at 0° the combined filtrate and washings deposited crystalline benziminazole (2 g.), m. p. 173°. The xylene liquors were evaporated under diminished pressure to a black gum which was transferred in chloroform (50 c.c.) to an alumina column (40 × 3 cm.). The residue from evaporation of chloroform washings (400 c.c.) of the column was dissolved in dry methanol (200 c.c.) containing a trace of sodium methoxide. After 5 days solid material (5 g.) had crystallised. Further material was obtained from the mother-liquors by treatment with carbon dioxide and evaporation. Recrystallisation from water afforded 1-β-D-*galactosylbenziminazole* (4·7 g.), m. p. 257° (decomp.), $[\alpha]_D^{15}$ −31·5° (*c* 1·18 in C_5H_5N) (Found: C, 55·45; H, 5·8; N, 9·8. $C_{13}H_{16}O_5N_2$ requires C, 55·7; H, 5·75; N, 10·0%). The compound is soluble in hot water, hot ethanol, and pyridine. It does not give a Molisch test. The *picrate* crystallised from water as yellow needles, m. p. 169—170°, $[\alpha]_D^{19}$ −15·3° (*c* 1·05 in C_5H_5N) (Found: C, 44·4; H, 3·8; N, 13·75. $C_{13}H_{16}O_5N_2,C_6H_3O_7N_3$ requires C, 44·8; H, 3·8; N, 13·75%). The *hydrochloride monohydrate* had m. p. 197° (Found: C, 46·9; H, 5·6; N, 8·2; Cl, 10·9. $C_{13}H_{16}O_5N_2,HCl,H_2O$ requires C, 46·6; H, 5·7; N, 8·4; Cl, 10·6%). 1-β-D-Galactosylbenziminazole (0·0993 g.) consumed 2·04 mol. of sodium periodate in 240 min. and liberated 0·94 mol. of formic acid.

(*b*) A suspension of finely divided chloromercuribenziminazole (8·6 g.) in xylene (700 c.c.) was distilled (300 c.c. of distillate) and, after cooling, tetra-*O*-acetyl-α-D-galactosyl bromide (10 g.) was added and boiling was continued for 2 hr. (moisture excluded). The xylene was decanted and the brown residue was extracted with hot chloroform. The filtered extracts

[15] Foster and Overend, *Chem. and Ind.*, 1955, 566.
[16] Kenner, in "The Chemistry and Biology of Purines," London, J. & A. Churchill, Ltd., 1957, p. 312.
[17] Phillips, *J.*, 1931, 1143.

were evaporated under diminished pressure to 150 c.c. Successive washing with water (2 × 100 c.c.), 10% sodium iodide solution (3 × 100 c.c.), and water (3 × 100 c.c.), drying (Na_2SO_4), and evaporation afforded a material which was purified by passage through alumina and deacetylated as above. 1-β-D-Galactosylbenziminazole (1·56 g.) was obtained as prisms, m. p. 257° (decomp.), $[\alpha]_D^{19}$ −31·1° (*c* 1·09 in C_5H_5N).

(*c*) A solution of tetra-*O*-acetyl-α-D-galactosyl bromide (10 g.) and benziminazole (6·32 g.) in dry dioxan (25 c.c.) was heated at 100° for 0·5 hr. (moisture was excluded) and dry benzene (50 c.c.) was added to the cooled solution. A deposit of benziminazole mixed with its hydrobromide slowly separated and was collected and washed with benzene. From the washings and filtrate a brown syrup was isolated. In chloroform solution (100 c.c.) this was washed with 2N-ammonia (3 × 50 c.c.) and water (3 × 100 c.c.) and by the procedure described above 1-β-D-galactosylbenziminazole (2·5 g.), m. p. 257° (decomp.), $[\alpha]_D^{26}$ −31·5° (*c* 1·21 in C_5H_5N), was isolated.

An attempt was made to condense dry powdered benziminazole and tetra-*O*-acetyl-α-D-galactosyl bromide by an improved Koenigs–Knorr procedure. Benziminazole (2·87 g.), dry silver carbonate (6·7 g.), freshly ignited calcium sulphate (25 g.), and dry chloroform (50 c.c.) were stirred together for 1 hr. in the dark. After addition of iodine (1·25 g.) a solution of the acetylgalactosyl bromide (10 g.) in dry chloroform (40 cc.c.) was added dropwise during 1 hr. Stirring was continued for 24 hr. and the mixture was filtered through "Celite." Only unchanged benziminazole and tetra-*O*-acetyl-α-D-galactosyl bromide were isolated. The bromide was recovered quantitatively.

Acetylation (anhydride in pyridine) of the glycoside yielded 1-(2 : 3 : 4 : 6-*tetra*-O-*acetyl*-β-D-*galactosyl*)*benziminazole* as a colourless glass, $[\alpha]_D^{20}$ −17·6° (*c* 1·71 in $CHCl_3$) (Found: N, 6·3. $C_{21}H_{24}O_9N_2$ requires N, 6·25%).

1-(O-iso*Propylidene*-β-D-*galactosyl*)*benziminazole*.—To a swirled suspension of dry finely powdered 1-(β-D-galactosylbenziminazole) (2·0 g.) in dry acetone (120 c.c.) at 0° concentrated sulphuric acid (4·6 c.c.) was added dropwise. After 2 hr. at room temperature the cooled solution was added slowly to a stirred ice-cold solution of sodium carbonate (20 g.) in water (20 c.c.). After removal of the acetone at 50° the white solid remaining in suspension was filtered, washed with water, and dried. Recrystallisation from aqueous ethanol afforded the *mono*-O-iso*propylidene derivative* (1·1 g.) as prisms, m. p. 240—241° (decomp.), $[\alpha]_D^{22}$ +43·8° (*c* 0·914 in C_5H_5N) (Found: C, 60·2; H, 6·3; N, 9·0. $C_{16}H_{20}O_5N_2$ requires C, 60·0; H, 6·3; N, 8·75%).

1-(6-O-*Trityl*-β-D-*galactosyl*)*benziminazole*.—1-β-D-Galactosylbenziminazole (1 g.) was kept with triphenylmethyl chloride (0·973 g.) in pyridine (8 c.c.) at 100° for 6 hr. The product was isolated in standard fashion and the 6-O-*trityl derivative* (0·9 g.) was obtained as a granular white solid, m. p. 234° (decomp.), $[\alpha]_D^{22}$ −15·8° (*c* 1·01 in C_5H_5N) (Found: N, 5·1. $C_{32}H_{30}O_5N_2$ requires N, 5·4%).

Crude Tri-O-*acetyl*-2-*deoxy*-D-*galactosyl Bromide*.—Tri-*O*-acetyl-D-galactal (20 g.) and benzoyl peroxide (250 mg.) were dissolved in dry benzene (150 c.c.), and the solution was saturated at room temperature with dry hydrogen bromide. The solution was evaporated under reduced pressure at 30° and dry benzene was distilled in a vacuum over the residue. The product was a straw-coloured syrup which on storage decomposed with evolution of hydrogen bromide. The bromide (from 10·5 g. of tri-*O*-acetyl-D-galactal) was dissolved in dry ether (100 c.c.), and silver carbonate (10 g.) was added. To the stirred mixture aniline (4 c.c.) was added and after 12 hr. the product was isolated and deacetylated (Zemplén method). 2-Deoxy-*N*-phenyl-D-galactosylamine (2·1 g., 23% based on tri-*O*-acetyl-D-galactal) was obtained, having m. p. 130°, $[\alpha]_D^{15}$ −52·2° (equilibrium) (*c* 1·11 in MeOH). Butler *et al.*[18] give m. p. 134—135° and $[\alpha]_D^{18}$ −53° (*c* 1·3 in MeOH).

1-(2-*Deoxy*-D-*galactosyl*)*benziminazole*.—(*a*) The crude bromo-derivative (from 31·6 g. of tri-*O*-acetyl-D-galactal) in dry xylene (500 c.c.), and dry benziminazolylsilver (26·1 g.) were kept at 100—120° for 1 hr. Chloroform (100 c.c.) was added and after filtration the product was isolated and deacetylated as described for the corresponding galactosyl derivative. After recrystallisation from water 1-(2-*deoxy*-D-*galactosyl*)*benziminazole* (A) (4·3 g., 14%) was obtained as needles, m. p. 214°, $[\alpha]_D^{25}$ −18·9° (*c* 1·06 in C_5H_5N) (Found: C, 58·8; H, 6·4; N, 10·6. $C_{13}H_{16}O_4N_2$ requires C, 59·1; H, 6·1; N, 10·6%) {*picrate*, yellow needles (from water), m. p. 160°, $[\alpha]_D^{19}$ −9·07° (*c* 1·98 in C_5H_5N) (Found: C, 46·5; H, 3·8; N, 14·3. $C_{13}H_{16}O_4N_2,C_6H_3O_7N_3$

[18] Butler, Laland, Overend, and Stacey, *J.*, 1950, 1433.

requires C, 46·25; H, 3·9; N, 14·2%); *hydrochloride*, m. p. 152° (decomp.), $[\alpha]_D^{20}$ +5·17° (*c* 1·55 in H_2O) (Found: C, 51·8; H, 5·7; N, 9·6; Cl, 11·5. $C_{13}H_{16}O_4N_2$,HCl requires C, 51·9; H, 5·7; N, 9·4; Cl, 11·8%)}.

(*b*) The 1-bromo-derivative (from 11·7 g. of tri-*O*-acetyl-D-galactal) was added in a small volume of dry xylene to a suspension of chloromercuribenziminazole (15·2 g.) in xylene (400 c.c.), and the mixture was kept at 110—120° for 2 hr. Working up was as for the galactosyl analogue and 1-(2-deoxy-D-galactosyl)benziminazole (*A*) (0·57 g.), m. p. 214°, $[\alpha]_D^{20}$ −17·9° (*c* 1·12 in C_5H_5N), was obtained.

(*c*) Tri-*O*-acetyl-D-galactosyl bromide (from 10·2 g. of tri-*O*-acetyl-D-galactal) and dry powdered benziminazole (9·7 g.) were swirled for 1 hr. in dry dioxan at 100°. Dry benzene (100 c.c.) was added and after 12 hr. the precipitate (11·1 g.) was collected and washed with benzene. The combined filtrate and washings were evaporated to a syrup. In chloroform solution this was washed with 2N-ammonia (2 × 50 c.c.) and water (3 × 100 c.c.), dried (Na_2SO_4), evaporated to small bulk, and placed on an alumina column (24 × 3 cm.). The column was washed with chloroform (250 c.c.) and from the eluate a syrup was obtained which was deacetylated in dry methanol (200 c.c.) by the Zemplén procedure. Needles of 1-(2-*deoxy*-D-*galactosyl*)*benziminazole* (B) (2·92 g.), m. p. 199—200°, $[\alpha]_D^{23}$ +21·6° (*c* 1·11 in C_5H_5N) (Found: C, 59·4; H, 5·9; N, 10·7%), were obtained. On storage of the aqueous mother-liquors a small quantity of 1-(2-deoxy-D-galactosyl)benziminazole (*A*) (0·24 g.), m. p. 214°, $[\alpha]_D^{22}$ −16·0° (*c* 1·0 in C_5H_5N), was deposited. A mixture of the isomers melted at 199—205°.

Isomerisation of 1-(2-*Deoxy*-D-*galactosyl*)*benziminazole* (B).—The glycosylamine (100 mg.) in 0·1N-hydochloric acid (4·4 c.c.) and water (5·6 c.c.) was boiled under reflux. The reaction was followed polarimetrically and was complete in 15 min. The solution was neutralised with sodium hydroxide and concentrated. On cooling, 1-(2-deoxy-D-galactosyl)benziminazole (*A*), m. p. 212—213°, separated. The yield was low and the mother-liquors were brown. As neither benziminazole isomer gave a brown solution when boiled with dilute alkali some hydrolysis was indicated during the acid treatment. 2-Deoxy-D-galactose was shown to give no colour in boiling dilute hydrochloric acid but did yield a brown solution when boiled with dilute alkali.

1-(*Tri*-O-*acetyl*-2-*deoxy*-D-*galactosyl*)*benziminazole Picrate*.—1-(2-Deoxy-D-galactosyl)benziminazole (*A*) (250 mg.) was acetylated with fused sodium acetate (250 mg.) and acetic anhydride (10 c.c.) at 100° for 1 hr. The syrupy product was isolated by standard procedures and was treated with a slight excess of saturated ethanolic picric acid: 1-(*tri*-O-*acetyl*-2-*deoxy*-D-*galactosyl*)*benziminazole picrate* (250 mg.) was obtained as yellow needles, m. p. 158°, $[\alpha]_D^{14}$ ±0° (*c* 1·2 in $CHCl_3$) (Found: C, 48·6; H, 4·1; N, 11·3. $C_{19}H_{22}O_7N_2$,$C_6H_3O_7N_3$ requires C, 48·4; H, 4·1; N, 11·3%).

1-(2-*Deoxy*-3 : 4(?)-O-iso*propylidene*-D-*galactosyl*)*benziminazole*.—Concentrated sulphuric acid (4·8 c.c.) was added dropwise to a suspension of finely powdered 1-(2-deoxy-D-galactosyl)-benziminazole (*A*) (2·0 g., dried for 3 hr. at 61°/20 mm. over P_2O_5) in dry acetone (120 c.c.) at 0°. After 1 hr. at room temperature the solution was cooled at 0° and added to a stirred solution of anhydrous sodium carbonate (20 g.) in ice-water (80 c.c.). The solution was evaporated at 50° to remove acetone, and the resulting suspension was extracted with chloroform (3 × 100 c.c.). The extracts were washed with water, dried (Na_2SO_4), and evaporated to a solid which was recrystallised from aqueous ethanol. 1-(2-*Deoxy*-3 : 4(?)-O-iso*propylidene*-D-*galactosyl*)*benziminazole* (0·63 g.) was obtained as needles, m. p. 218—219° (decomp.), $[\alpha]_D^{23}$ −11·5° (*c* 3·12 in C_5H_5N) (Found: C, 62·8; H, 6·55; N, 9·3. $C_{16}H_{20}O_4N_2$ requires C, 63·2; H, 6·6; N, 9·2%). When the mother-liquors were allowed to evaporate slowly more needles separated accompanied by some large colourless prisms. The latter were separated and crystallised from aqueous-ethanol. Colourless prisms of the *isomer* (0·16 g.), m. p. 204—205°, $[\alpha]_D^{17}$ −64·7° (*c* 3·09 in C_5H_5N) (Found: C, 62·9; H, 6·6; N, 9·5%), were obtained.

The first *iso*propylidene derivative A (50 mg.) was heated under reflux in water (2·8 c.c.) containing 0·1N-hydrochloric acid (2·2 c.c.). The reaction was complete in 5 min., as indicated polarimetrically. After neutralisation 1-(2-deoxy-D-galactosyl)benziminazole (*A*) (16 mg.), m. p. 214°, was obtained. Similar procedure with the second isomer (75 mg.) also gave compound (*A*).

1-(2-*Deoxy*-D-*glucosyl*)*benziminazole*.—Crude tri-*O*-acetyl-2-deoxy-D-glucosyl bromide (from 20 g. of tri-*O*-acetyl-D-glucal) was added to a suspension of benziminazolylsilver (16·6 g.) in xylene (500 c.c.). After 30 minutes' heating at 120° the mixture was worked up and

deacetylated according to the procedure developed, and isolated as the picrate. 1-(2-*Deoxy*-D-*glucosyl*)*benziminazole picrate* (5·54 g.) was obtained as orange-yellow needles, m. p. 153—154° (decomp.), $[\alpha]_D^{15}$ −17·3° (*c* 2·08 in C_5H_5N) (Found: C, 45·7; H, 3·9; N, 14·3. $C_{13}H_{16}O_4N_2,C_6H_3O_7N_3$ requires C, 46·25; H, 3·9; N, 14·2%). This picrate (5·2 g.), water (200 c.c.), nitrobenzene (100 c.c.), and concentrated hydrochloric acid (5 c.c.) were shaken together vigorously until the solid had dissolved. The aqueous layer was separated and extracted with nitrobenzene (100 c.c. and 3 × 50 c.c.) and then chloroform (3 × 25 c.c.). After concentration (to 40 c.c.) under reduced pressure the solution was made alkaline (5 c.c. of concentrated ammonia) and rapidly filtered. Solid separated which was collected and recrystallised from ethanol. 1-(2-*Deoxy*-D-*glucosyl*)*benziminazole* was obtained as large prisms, m. p. 196°, $[\alpha]_D^{22}$ −35·2° (*c* 1·19 in C_5H_5N) (Found: C, 59·2; H, 6·1; N, 10·8. $C_{13}H_{16}O_4N_2$ requires C, 59·1; H, 6·1; N, 10·6%).

1-(2 : 6-*Dideoxy*-D-*galactosyl*)*benziminazole*.—Di-*O*-acetyl-D-fucal (m. p. 50—52°, $[\alpha]_D^{25}$ −7·1° in acetone) (2·25 g.) was dissolved in dry benzene (50 c.c.) at 0° and the solution was saturated with dry hydrogen chloride. Evaporation at 40° and re-evaporation with benzene (2 × 50 c.c.) yielded crude di-*O*-acetyl-2 : 6-dideoxy-D-galactosyl chloride as a pale yellow syrup which was added in the minimum of dry xylene to a suspension of benziminazolylsilver (5 g.) in dry xylene (75 c.c.), and the stirred mixture was heated at 110° for 1 hr. After evaporation of the filtrate the residue was washed down an alumina column with chloroform. The washings were evaporated to a pale brown syrup (2·2 g.) which was treated in ethanol (20 c.c.) with a slight excess of saturated ethanolic picric acid. 1-(*Di*-O-*acetyl*-2 : 6-*dideoxy*-D-*galactosyl*)*benziminazole picrate* (1·4 g.) was obtained as pale yellow needles, m. p. 168°, $[\alpha]_D^{20}$ +107·8° (*c* 2·54 in C_5H_5N) (Found: C, 48·9; H, 4·0; N, 12·45. $C_{17}H_{20}O_5N_2,C_6H_3O_7N_3$ requires C, 49·2; H, 4·1; N, 12·5%). This compound (1·35 g.) was freed from picric acid by dissolution in the minimum amount of chloroform and by passage through alumina (50 g.; 8 × 3 cm.). The column was washed with more chloroform (200 c.c.). Evaporation yielded a syrup which crystallised on trituration with ethanol. Recrystallisation from ethanol–light petroleum (b. p. 60—80°) afforded 1-(*di*-O-*acetyl*-2 : 6-*dideoxy*-D-*galactosyl*)*benziminazole* (0·45 g.) as prisms, m. p. 206—207°, $[\alpha]_D^{20}$ +201·7° (*c* 2·34 in $CHCl_3$) (Found: C, 61·6; H, 6·0; N, 8·7. $C_{17}H_{20}O_5N_2$ requires C, 61·4; H, 6·1; N, 8·4%). The material (0·40 g.) was deacetylated in dry methanol (50 c.c.) containing sodium methoxide. On recrystallisation from water 1-(2 : 6-*dideoxy*-D-*galactosyl*)*benziminazole hemihydrate* was obtained as needles, m. p. 185—186°, $[\alpha]_D^{18}$ −52·4° (*c* 2·1 in C_5H_5N) (Found: C, 61·05; H, 6·8; N, 10·9. $C_{13}H_{16}O_3N_2,0{\cdot}5H_2O$ requires C, 60·7; H, 6·7; N, 10·9%). The mother-liquors were adjusted to pH 2 by addition of dilute hydrochloric acid and an excess of saturated aqueous picric acid was added. Recrystallisation from water of the yellow precipitate yielded 1-(2 : 6-*dideoxy*-D-*galactosyl*)*benziminazole picrate* as yellow needles, m. p. 161—162° (decomp.) (Found: C, 48·1; H, 3·8; N, 14·9. $C_{13}H_{16}O_3N_2,C_6H_3O_7N_3$ requires C, 47·8; H, 4·0; N, 14·7%).

1-(2-*Deoxy*-L-*ribosyl*)*benziminazole*.—A solution of di-*O*-acetyl-L-arabinal (5 g.) in dry benzene (35 c.c.) was saturated at room temperature with dry hydrogen chloride. After evaporation at 30° dry benzene (80 c.c.) was distilled over the residue. Crude di-*O*-acetyl-2-deoxy-L-ribosyl chloride in the minimum of dry xylene was added to a suspension of benziminazolylsilver (8·4 g.) in dry xylene. The mixture was heated at 110° for 1 hr. and worked up by the usual procedure to give a brown gum (6·05 g.), a portion (250 mg.) of which afforded a *picrate* as yellow needles (222 mg.) (from ethanol), m. p. 176°, $[\alpha]_D^{17}$ −13·9° (*c* 3·6 in C_5H_5N) (Found: C, 48·3; H, 3·7; N, 12·7. $C_{16}H_{18}O_5N_2,C_6H_3O_7N_3$ requires C, 48·3; H, 3·9; N, 12·8%). Cooley *et al.*[4] give m. p. 167—168°, $[\alpha]_D^{26}$ −8·6° (C_5H_5N), for a compound from 2-deoxy-D-ribose. The main bulk of the gum was deacetylated in dry methanol (150 c.c.) containing a trace of sodium methoxide. To the glassy product excess of picric acid was added and the oily deposit was crystallised and collected (3·25 g.). Recrystallisation from water yielded a yellow solid which was a mixture of the required product and benziminazole picrate. The solid was dissolved in aqueous methanol (100 c.c.) and shaken with Amberlite resin IRA-400(OH) (30 g.) until the yellow colour was discharged. Evaporation of the filtrate gave a colourless gum which crystallised after 3 months. Recrystallisation from water yielded 1-(2-*deoxy*-L-*ribosyl*)*benziminazole* (0·107 g.), m. p. 183°, $[\alpha]_D^{17}$ −33·9° (*c* 1·89 in C_5H_5N) (Found: C, 61·2; H, 6·4; N, 12·35. $C_{12}H_{14}O_3N_2$ requires C, 61·5; H, 6·0; N, 12·0%). This compound gave a Dische diphenylamine test, but much more slowly than 2-deoxy-L-ribose.

1-D-*Galactosyl*-5 : 6-*dimethylbenziminazole*.—A mixture of tetra-*O*-acetyl-α-D-galactosyl

bromide (5·34 g.), 5 : 6-dimethylbenziminazole (4·17 g.), and dry dioxan (15 c.c.) was heated at 100° for 1 hr. Dry benzene (30 c.c.) was added to the cooled mixture and after 2 hr. 5 : 6-dimethylbenziminazole hydrobromide (2·7 g.) was collected and washed with benzene. Removal of solvent from the filtrate and washings gave a brown syrup from which 5 : 6-*dimethyl*-1-(*tetra*-O-*acetyl*-D-*galactosyl*)*benziminazole picrate* was obtained as yellow needles, m. p. 182—184°, $[\alpha]_D^{23}$ +55·8° (*c* 1·18 in $CHCl_3$) (Found: C, 49·2; H, 4·7; N, 10·0. $C_{23}H_{28}O_9N_2,C_6H_3O_7N_3$ requires C, 49·4; H, 4·4; N, 9·9%). This acetate was freed from picric acid by passage in chloroform through alumina (16 × 3 cm.). Evaporation yielded a pale yellow glass (3·6 g.) which was deacetylated (Zemplén). 1-D-*Galactosyl*-5 : 6-*dimethylbenziminazole* (0·84 g.) was obtained, having m. p. 269° (decomp.) (from ethanol), $[\alpha]_D^{17}$ −29·4° (*c* 0·54 in C_5H_5N) (Found: C, 58·6; H, 6·5; N, 9·0. $C_{15}H_{20}O_5N_2$ requires C, 58·45; H, 6·5; N, 9·1%) [*picrate*, orange needles (from ethanol), m. p. 211° (decomp.) (Found: C, 46·55; H, 4·5. $C_{15}H_{20}O_5N_2,C_6H_3O_7N_3$ requires C, 46·9; H, 4·3%)].

1-(2-*Deoxy*-D-*galactosyl*)-5 : 6-*dimethylbenziminazole*.—This compound was prepared as outlined above from crude tri-*O*-acetyl-2-deoxy-D-galactosyl bromide (from 10·25 g. of tri-*O*-acetyl-D-galactal) in dry xylene (200 c.c.) and 5 : 6-dimethylbenziminazolylsilver (10 g.). 5 : 6-*Dimethyl*-1-(*tri*-O-*acetyl*-2-*deoxy*-D-*galactosyl*)*benziminazole picrate* (4·5 g.) was obtained as yellow needles (from ethanol), m. p. 181°, $[\alpha]_D^{22}$ −3·5° (*c* 1·15 in $CHCl_3$) (Found: C, 50·0; H, 4·4; N, 11·1. $C_{21}H_{26}O_7N_2,C_6H_3O_7N_3$ requires C, 50·1; H, 4·5; N, 10·8%). Removal of picric acid from this material (4·0 g.) was effected by passage in chloroform through alumina. Evaporation of the effluent gave a straw-coloured glass (2·1 g.) which was deacetylated (Zemplén) to 1-(2-*deoxy*-D-*galactosyl*)-5 : 6-*dimethylbenziminazole* (1·15 g., 10%), prisms (from aqueous ethanol), m. p. 225°, $[\alpha]_D^{23}$ −32·4° (*c* 1·05 in C_5H_5N) (Found: C, 61·9; H, 6·8; N, 9·7. $C_{15}H_{20}O_4N_2$ requires C, 61·7; H, 6·9; N, 9·6%) {*picrate* (from ethanol), orange needles, m. p. 193° (decomp.) (Found: C, 48·2; H, 4·3; N, 13·4. $C_{15}H_{20}O_4N_2,C_6H_3O_7N_3$ requires C, 48·4; H, 4·45; N, 13·4%)}.

Acid Hydrolysis of 1-*Glycosylbenziminazoles*.—The following compounds were examined: (*a*) 1-β-D-galactosylbenziminazole; (*b*) 1-(2-deoxy-D-galactosyl)benziminazole (*A*); (*c*) 1-(2 : 6-dideoxy-D-galactosyl)benziminazole hemihydrate. A solution of each substance (10 mg.) in 6N-hydrochloric acid (2 c.c.) was heated in a sealed tube for 12 hr. at (i) 100° and (ii) 150°. Some charring occurred in all the tubes except (*a*) (i). The contents of each tube were evaporated to dryness at 30° and in each case an aqueous solution of the gummy residue was examined chromatographically on Whatman No. 1 paper by the ascending technique [19] with an irrigating solvent comprising the organic layer of a butan-1-ol–acetic acid–water (4 : 1 : 5, v/v) mixture for compounds (*a*) and (*b*), and the organic layer of butan-1-ol–water for compound (*c*). The reference compounds were the original glycosylamines (200 μg. per spot) and benziminazole (100 μg. per spot). The hydrolysate spots were neutralised with ammonia before drying. The various substances were located on the developed chromatograms by photography in filtered ultraviolet light.[20] The benziminazole spots were identified by spraying the papers with tetramminocupric sulphate solution, the free base being revealed (after drying) as a red spot on a pale blue background. Ammoniacal solutions of cobaltous chloride or nickel sulphate could be used as spray reagents for the detection of benziminazoles. A violet spot on a brown, and a violet spot on a green, background respectively were obtained but the colour contrasts were inferior to that obtained with tetramminocupric sulphate.

A qualitative estimate of the extent of hydrolysis is shown in the Table.

	Compounds detected on chromatogram of hydrolysate	Hydrolysis	
100°	150°	100°	150°
(*a*) Glycosylamine only	Glycosylamine + benziminazole (faint spot)	Nil	Slight
(*b*) Glycosylamine + benziminazole	Benziminazole only	~50%	Complete
(*c*) Benziminazole only	Benziminazole only	Complete	Complete

In a further experiment, 1-β-D-galactosylbenziminazole (0·0086 g.) was heated in a sealed tube at 100° for 3 days with 6N-hydrochloric acid (5 c.c.). No charring was observed. The solution was neutralised with sodium hydroxide, diluted (to 60 c.c.), and treated with 0·25N-sodium

[19] Williams and Kirby, *Science*, 1948, **107**, 481.
[20] Markham and Smith, *Biochem. J.*, 1949, **45**, 294.

periodate. On the assumption that any D-galactose liberated by hydrolysis would be destroyed in the experimental conditions, the periodate oxidation indicated 97% recovery of the glycosylamine.

Biological Activity of Some Benziminazole Derivatives.—The following compounds were tested for growth inhibition of *E. coli* 15: (i) 1-β-D-galactosylbenziminazole; (ii) 1-(2-deoxy-D-galactosyl)benziminazole (*B*); (iii) 1-(2-deoxy-D-galactosyl)benziminazole (*A*); (iv) 1-(2 : 6-dideoxy-D-galactosyl)-, (v) 1-β-D-galactosyl-5 : 6-dimethyl-, (vi) 1-(2-deoxy-D-galactosyl)-5 : 6-dimethyl-, (vii) 4-trifluoromethyl-,[21] and (viii) 5-trifluoromethyl-benziminazole.[21] The basal nutrient medium was a solution of peptone 1%, " Lemco " 1%, and sodium chloride 0·5%, and a series of 10 tubes were prepared for each compound to be tested. The first tube (T_1) of each series contained the test compound at a concentration of 1 part in 2000 and the dilution was doubled for each successive tube. The growth attained by the organism at a suitable time after inoculation was estimated visually by comparison with controls. Results were as follows:

Benziminazole	Time of growth (hr.)	T_1	T_2	T_3	T_4—T_{10}
4-Trifluoromethyl-	16	—	−+−	±	+
5-Trifluoromethyl-	16	—	−+−	±	+
1-(2-Deoxy-D-galactosyl)-5 : 6-dimethyl-	72	—	−+−	±	+

(+ = Growth equal to control; − = no growth.)

All the other compounds allowed growth equal to that of the control. The first two compounds also showed some activity against *A. aerogenes* when tested in a similar manner.

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[21] Sykes and Tatlow, *J.*, 1952, 4078.