640. *Some Glycosylbenziminazoles.*

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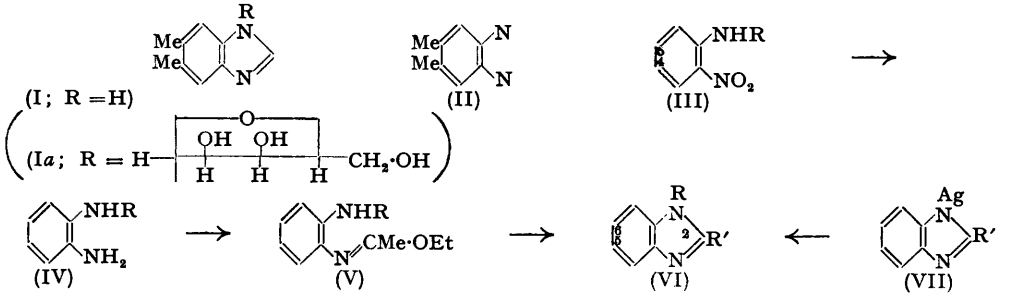
With the object of preparing metabolite inhibitors of vitamin B_{12} for study as potential chemotherapeutic agents, the synthesis of (i) 5-chloro- and 5 : 6-dichloro-1-glycosylbenziminazole and (ii) 2-methyl- and 2 : 5-dimethyl-1-glycosylbenziminazole (the "*N*-glycosides") has been undertaken.

Compounds of type (ii) were prepared by a novel route in which a *N*-glycosyl-*o*-phenylenediamine is treated with ethyl orthoacetate, and the resulting *iso*acetanilide is converted into the glycosylbenziminazole by treatment with dilute hydrochloric acid.

The isolation of 5 : 6-dimethylbenziminazole (I) (Brink and Folkers, *J. Amer. Chem. Soc.*, 1949, **71**, 2951; Holiday and Petrow, *J. Pharm. Pharmacol.*, 1949, **1**, 734; Beaven, Holiday, Johnson, Ellis, Mamalis, Petrow, and Sturgeon, *ibid.*, p. 957; Brink and Folkers, *J. Amer. Chem. Soc.*, 1950, **72**, 4442) and 5 : 6-dimethyl-1-α-D-ribofuranosylbenziminazole (5 : 6-dimethylbenziminazole-1-α-D-ribofuranoside) (I*a*) (Brink, Holly, Shunk, Peel, Cahill, and Folkers, *ibid.*, p. 1866; see also Cooley, Ellis, Mamalis, Petrow, and Sturgeon, *J. Pharm. Pharmacol.*, 1950, **2**, 579) from vitamin B_{12} makes it possible to draw a structural analogy between riboflavin and the "benziminazole glycoside" portion of the B_{12} molecule, and to postulate the existence of a common biogenetic precursor of the two vitamins containing the grouping (II). By a further extension of the concept it is reasonable to conclude that those structural alterations which effect conversion of riboflavin into a metabolite inhibitor of riboflavin would likewise be effective in the benziminazole glycoside series.

The preparation of a riboflavin antagonist had been effected fortuitously by Kuhn, Weygand, and Möller (*Ber.*, 1943, **76**, 1044), who, having noted the crystallographic similarity between methyl- and chloro-substituted benzene derivatives, prepared 6 : 7-dichloro-9-1′-D-ribityl*iso*-



alloxazine for biological study. This compound, in striking contrast with the parent vitamin, proved to be a potent metabolite inhibitor. Its 6 : 7-dibromo-analogue, however, exhibited only one-quarter to one-eighth of the activity of the corresponding dichloro-compound (Weygand, Löwenfeld, and Möller, *ibid.*, 1951, **84**, 101), an observation which emphasises the importance of atomic volume in relation to biological effect. Replacement of methyl by chlorine in riboflavin thus leads to complete reversal in the biological functions. Properly constituted analogues of (I*a*), such as 5-chloro- and 5 : 6-dichloro-1-glycosylbenziminazole may, on this basis, be expected to function as B_{12}-antagonists, the preparation of which formed the object of the present study.

Our second approach to the problem of B_{12}-inhibitors derived from the observation of Emerson, Brink, Holly, Koniuszy, Heyl, and Folkers (*J. Amer. Chem. Soc.*, 1950, **72**, 3084) that 5-methyl- and 5 : 6-dimethyl-benziminazole showed significant vitamin B_{12}-like growth-activity in the rat test, whilst 2 : 5-dimethylbenziminazole showed growth-depressant or -inhibitor properties. The synthesis of some 2-methyl- and 2 : 5-dimethyl-1-glycosyl-benziminazoles was, therefore, included in the present investigation.

The preparation of 5-chloro-1-β-D-glucopyranosylbenziminazole (cf. VI; R = glucosyl, R′ = H) was effected essentially by methods previously developed in this laboratory (Mamalis, Petrow, and Sturgeon, *J. Pharm. Pharmacol.*, 1950, **2**, 491, 503, 512). Condensation of 4-chloro-2-nitroaniline with D-glucose in ethanol in the presence of ammonium chloride (Kuhn and Strobële, *Ber.*, 1937, **70**, 773) furnished a mixture of *N*-D-glucosyl derivatives (cf. III; R = glucosyl) from which only one isomer was obtained in a state of purity. Acetylation of the crude mixture, however, followed by fractionation, yielded 4-chloro-1-(tetra-acetyl D-glucosyl)-2-nitroaniline I and II. Reduction of either isomer, followed by treatment with ethyl orthoformate, furnished 5-chloro-1-(β-tetra-acetyl *D*-glucosyl)benziminazole, from which the corresponding 5-chloro-1-β-D-glucopyranosylbenziminazole (cf. VI; R′ = H, R = β-D-glucopyranosyl) was obtained by ethanolysis in the presence of a catalytic amount of sodium ethoxide. Attempts to confirm the β-configuration of the glycosidic centre in the last compound by its alternative synthesis from the silver derivative of 5-chlorobenziminazole (cf. VII; R′ = H) and α-acetobromoglucose proved uniformly unsuccessful. The constitution assigned to the glycosidic centre follows, however, from the detailed studies previously referred to (Mamalis *et al.*, *loc. cit.*).

Attempts to employ D-xylose and L-arabinose as the sugar component in the foregoing synthesis proved disappointing. The corresponding *o*-nitroaniline "pentosides" I and II were readily obtained in each instance. Reduction of the triacetyl xylosides, followed by reaction with ethyl orthoformate, gave what was presumably 5-chloro-1-(triacetyl D-xylosyl)-benziminazole, isolated as the picrate, but the yield was too low to permit satisfactory identification of the product. Complete failure attended attempts to convert the triacetyl L-arabinosyl derivatives into the corresponding glycosylbenziminazole. 4-Iodo-2-nitroaniline condensed with D-glucose, but acetylation of the crude product, followed by fractionation, gave only a single 4-iodo-2-nitro-1-(tetra-acetyl D-glycosyl)aniline, from which a benziminazole could not be obtained.

The foregoing synthesis could not be extended to the preparation of 5 : 6-dichloro-1-glycosyl benziminazoles as the intermediate 4 : 5-dichloro-1-glycosyl-*o*-nitroanilines could not be prepared by Kuhn and Ströbele's method (*loc. cit.*). Condensation of the silver derivative

of 5 : 6-dichloro-2-methylbenziminazole (cf. VII; R′ = Me) with α-acetobromogluoose likewise proved unsuccessful, the unchanged dichloro-compound being recovered in high yield. Attention was, therefore, directed to the preparation of the corresponding 1-deoxyglycosides, which were readily obtained from the appropriate 4 : 5-dichloro-*N*-glycityl-2-nitroanilines (cf. III). The preparation of the latter compounds was achieved by the method of Kuhn *et al.* (*loc. cit.*). Reaction of 1 : 2-dichloro-4 : 5-dinitrobenzene with D-ribosamine, L-arabosamine, and D-glucosamine furnished the 4 : 5-dichloro-*N*-glycityl-2-nitroanilines (cf. III) (Weygand *et al.*, *loc. cit.*). Catalytic reduction of these compounds, followed by treatment with formic acid in 4N-hydrochloric acid (Philips, *J.*, 1929, 1143), gave the 1-D-ribityl, 1-L-arabityl, and 1-D-sorbityl derivatives of 5 : 6-dichlorobenziminazole (cf. VI; R′ = H). The last compound has recently been described by Holly, Peel, Cahill, and Folkers (*J. Amer. Chem. Soc.*, 1951, **73**, 332) in connection with studies on carcinolytic compounds. By employing acetic acid in place of formic acid in the above condensations, the 1-D-ribityl, 1-L-arabityl, and 1-D-sorbityl derivatives of 5 : 6-dichloro-2-methylbenziminazole (cf. VI; R′ = Me) were readily obtained.

The preparation of 1-glycosyl-2-methylbenziminazoles has not hitherto been recorded in the literature, but has now been effected by extension of the methods developed by Mamalis *et al.* (*loc. cit.*). Thus reaction of *N*-(tetra-acetyl D-glucopyranosyl)-*o*-phenylenediamine (cf. IV), which was obtained crystalline for the first time, with ethyl orthoacetate, furnished the acetimidate (V; R = tetra-acetyl D-glucopyranosyl) in excellent yield. Dilute hydrochloric acid then effected ring closure to give 2-methyl-1-(β-tetra-acetyl D-glucopyranosyl)-benziminazole (cf. VI; R′ = Me). The corresponding 2 : 5-dimethyl-1-β-(tetra-acetyl D-glucopyranosyl) compound, converted by dilute hydrochloric acid into the hydrochloride of the 1-(β-D-glucopyranosyl) compound, was prepared in the same way, as well as 2-methyl- and 2 : 5-dimethyl-1-(β-triacetyl D-xylopyranosyl)benziminazole.

Experimental.

M. p.s are uncorrected.

4-Chloro-N-D-*glucosyl*-*2-nitroaniline*.—4-Chloro-2-nitroaniline (71 g.), glucose (30 g.), and ammonium chloride (5 g.) were heated under reflux in absolute ethanol (400 ml.) for 6 hours. After removal of part of the ethanol by distillation, the solution was poured through a column of alumina (B.D.H.; 1¾ × 15″), and excess of 4-chloro-2-nitroaniline was washed out with benzene. Elution with ethanol, followed by concentration of the eluate, gave *4-chloro*-N-D-*glucosyl*-*2-nitroaniline* as yellow needles (from acetone–ethanol), m. p. 185°, $[\alpha]_D^{24}$ +7·96° (*c*, 2·194 in pyridine) (Found: C, 43·5; H, 4·6; N, 8·9. $C_{12}H_{15}O_8N_2Cl$ requires C, 43·0; H, 4·4; N, 8·3%).

4-Chloro-*2-nitro*-1-(*tetra-acetyl* D-*glucosyl*)*aniline* I *and* II.—The crude mixture of glucosyl derivatives (12 g.), obtained as described above, was dissolved in pyridine (90 ml.) and treated with acetic anhydride (100 ml.) at room temperature. After 24 hours excess of anhydride was destroyed by addition of ethanol, and the solution concentrated under reduced pressure to a small bulk. Crystallisation of the syrupy residue from ethyl acetate–light petroleum (b. p. 40—60°) gave *4-chloro*-*2-nitro*-1-(*tetra-acetyl* D-*glucosyl*)*aniline* I, yellow needles, m. p. 150°, $[\alpha]_D^{24}$ −63·4° (*c*, 1·35 in chloroform) (Found: C, 48·3; H, 4·5; N, 5·7. $C_{20}H_{23}O_{11}N_2Cl$ requires C, 47·7; H, 4·6; N, 5·6). Concentration of the mother-liquors gave the *isomer* II, yellow needles (from ethanol), m. p. 126°, $[\alpha]_D^{24}$ +115·8° (*c*, 2·07 in chloroform) (Found: C, 47·4; H, 4·5; N, 6·4%).

4-Chloro-N-D-*xylosyl*-*2-nitroaniline* crystallised from aqueous ethanol in yellow needles, m. p. 180°, $[\alpha]_D^{23}$ −59·6° (*c*, 1·016 in pyridine) (Found: C, 42·9; H, 4·4; N, 9·7. $C_{11}H_{13}O_6N_2Cl$ requires C, 43·3; H, 4·2; N, 9·1%).

4-Chloro-*2-nitro*-N-(*triacetyl* D-*xylosyl*)*aniline* I crystallised from ethanol in yellow needles, m. p. 162°, $[\alpha]_D^{21}$ −90·1° (*c*, 1·118 in chloroform) (Found: C, 47·6; H, 4·4; N, 6·8. $C_{17}H_{19}O_9N_2Cl$ requires C, 47·3; H, 4·4; N, 6·5%). The *isomer* II separated from ethanol in yellow needles, m. p. 117°, $[\alpha]_D^{22}$ +96·7° (*c*, 0·716 in chloroform) (Found: C, 47·0; H, 4·3; N, 6·3%).

N-L-*Arabinosyl*-*4-chloro*-*2-nitroaniline* formed yellow needles (from aqueous ethanol), m. p. 110° (Found: C, 42·6; H, 4·4; N, 9·6%).

4-Chloro-*2-nitro*-N-(*triacetyl* L-*arabinosyl*)*aniline* I separated from aqueous ethanol in yellow needles, m. p. 194°, $[\alpha]_D^{21}$ +150·9° (*c*, 1·26 in chloroform) (Found: C, 47·8; H, 3·9; N, 6·5%). The *enantiomer* II formed yellow needles [from ethyl acetate–light petroleum (b. p. 60—80°)], m. p. 117°, $[\alpha]_D^{21}$ +10·5° (*c*, 0·894 in chloroform) (Found: N, 6·1%).

N-D-*Glucosyl*-*4-iodo*-*2-nitroaniline* crystallised from ethanol in yellow plates, m. p. 136° (decomp.), $[\alpha]_D^{23}$ +8·6° (*c*, 1·19 in pyridine) (Found: C, 33·0; H, 3·7; N, 6·2. $C_{12}H_{15}O_7N_2I$ requires C, 33·8; H, 3·5; N, 6·6%). The *tetra-acetyl* derivative formed yellow plates (from ethanol), m. p. 135°, $[\alpha]_D^{20}$ −69·8° (*c*, 0·866 in pyridine) (Found: C, 39·9; H, 4·1; N, 4·9. $C_{20}H_{23}O_{11}N_2I$ requires C, 40·0; H, 3·8; N, 4·7%).

5-Chloro-1-(*β-tetra-acetyl* D-*glucosyl*)*benziminazole*.—4-Chloro-2-nitro-1-(tetra-acetyl D-glucosyl)aniline I or II (7 g.) was reduced catalytically in ethanolic solution (50 ml.) in the presence of 5% palladised charcoal (3 g.), to the corresponding diamine which was heated with ethyl orthoformate (30 ml.) for 3 hours. Excess of ortho-ester was removed by distillation under reduced pressure, and ring-closure effected by heating with N/40-aqueous-alcoholic hydrochloric acid for 1 hour. The product yielded

5-chloro-1-(β-tetra-acetyl D-*glucopyranosyl)benziminazole picrate*, yellow needles (from ethanol), m. p. 173° (Found: C, 45·7; H, 3·6; N, 9·4. $C_{21}H_{23}O_9N_2Cl,C_6H_3O_7N_3$ requires C, 45·2; H, 3·6; N, 9·8%), which was decomposed by percolation of its chloroform solution through a column of alumina. The *5-chloro-1-(β-tetra-acetyl* D-*glucosyl)benziminazole* so obtained formed needles [from ethyl acetate–light petroleum (b. p. 60—80°)], m. p. 156°, $[\alpha]_D^{22}$ −39·0° (*c*, 0·95 in chloroform) (Found: C, 52·3; H, 4·7; N, 6·0. $C_{21}H_{23}O_9N_2Cl$ requires C, 52·2; H, 4·7; N, 5·8%). Deacetylation with ethanol containing a trace of sodium ethoxide furnished *5-chloro-1-β-*D-*glucopyranosylbenziminazole*, rosettes of needles (from aqueous ethanol), m. p. 247°, $[\alpha]_D^{19}$ −24·6° (*c*, 1·644 in pyridine) (Found: C, 50·0; H, 4·7; N, 8·7. $C_{13}H_{15}O_5N_2Cl$ requires C, 49·6; H, 4·7; N, 8·8%).

5 : 6-Dichloro-2-methylbenziminazole.—4 : 5-Dichloro-2-nitroaniline (7 g.) in ethanol (70 ml.) was catalytically hydrogenated in the presence of 5% palladised charcoal (3 g.) to the corresponding diamine. After filtration and removal of the ethanol by distillation, acetic acid (5 ml.) and 4N-hydrochloric acid (40 ml.) were added and the mixture was heated under reflux for 40 minutes. *5 : 6-Dichloro-2-methylbenziminazole*, isolated in the usual way, formed pale yellow needles (from aqueous ethanol), m. p. 249° (Found: C, 47·8; H, 3·0; N, 13·7. $C_8H_6N_2Cl_2$ requires C, 47·7; H, 3·0; N, 13·9%). The *picrate* crystallised from ethanol in yellow needles, m. p. 252° (decomp.) (Found: N, 16·6. $C_8H_2N_2Cl_2,C_6H_3O_7N_3$ requires N, 16·3%).

*5 : 6-Dichloro-1-*D-*sorbitylbenziminazole.*—4 : 5-Dichloro-2-nitro-*N*-D-sorbitylaniline (1 g.) in ethanol (30 ml.) was catalytically hydrogenated in the presence of 5% palladised charcoal (1 g.) to the corresponding diamine. After filtration and removal of the alcohol by distillation, the residue was treated with 4N-hydrochloric acid (8 ml.) and formic acid (0·5 ml.). The mixture was heated on the steam-bath for 20 minutes. It was then filtered, cooled, and basified with aqueous ammonia. The product was collected and crystallised from aqueous ethanol, yielding *5 : 6-dichloro-1-*D-*sorbitylbenziminazole*, needles, m. p. 206° with a transition at 147° (Found: C, 43·6; H, 5·0; N, 7·8. Calc. for $C_{13}H_{16}O_5N_2Cl_2$: C, 44·4; H, 4·6; N, 7·9. Holly *et al.* (*loc. cit.*) give m. p. 198—200° with a transition at 147°.

*5 : 6-Dichloro-1-*L-*arabitylbenziminazole* formed needles (from aqueous ethanol), m. p. 191° (Found: C, 45·5; H, 5·0; N, 9·2. $C_{12}H_{14}O_4N_2Cl_2$ requires C, 44·9; H, 4·4; N, 8·7%), and *5 : 6-dichloro-1-*D-*ribitylbenziminazole* crystallised from aqueous ethanol in needles, m. p. 187° (transition at 143°) (Found: C, 45·3, 45·1; H, 5·3, 5·2; N, 8·5%).

*5 : 6-Dichloro-2-methyl-1-*D-*sorbitylbenziminazole* formed needles (from aqueous ethanol), m. p. 225° (Found: C, 46·9; H, 5·0; N, 7·5. $C_{14}H_{18}O_5N_2Cl_2$ requires C, 46·0; H, 4·9; N, 7·6%). 1-L-*Arabityl-5 : 6-dichloro-2-methylbenziminazole* separated from aqueous ethanol in needles, m. p. 247° (Found: C, 46·7; H, 5·4; N, 8·4. $C_{13}H_{16}O_4N_2Cl_2$ requires C, 46·5; H, 4·7; N, 8·4%), and *5 : 6-dichloro-2-methyl-1-*D-*ribitylbenziminazole* formed needles (from aqueous ethanol), m. p. 207° (Found: C, 46·7; H, 4·8; N, 8·3%).

*2-Methyl-1-(β-tetra-acetyl-*D-*glucopyranosyl)benziminazole.*—*o*-Nitro-*N*-(tetra-acetyl D-glucopyranosyl)aniline (15 g.) in ethanol (100 ml.) was catalytically hydrogenated in the presence of 5% palladised charcoal (5 g.) to give N-(*tetra-acetyl* D-*glucopyranosyl*)-*o*-*phenylenediamine*, needles (from ethanol), m. p. 182°, $[\alpha]_D^9$ −89·5° (*c*, 0·668 in chloroform) (Found: C, 54·2; H, 6·0; N, 6·8. $C_{20}H_{26}O_9N_2$ requires C, 54·7; H, 5·9; N, 6·4%).

This diamine was heated with ethyl orthoacetate (30 ml.) on the steam-bath for 2 hours, after which excess of ortho-ester was removed by distillation under reduced pressure. The residue, on crystallisation from alcohol–light petroleum (b. p. 40—60°), gave *o*-(1-*ethoxyethylideneamino*)-N-(*tetra-acetyl* D-*glucopyranosyl*)*aniline*, needles, m. p. 189°, $[\alpha]_D^{22}$ −91·7° (*c*, 2·864 in chloroform) (Found: C, 57·0; H, 6·3; N, 5·8. $C_{24}H_{32}O_{10}N_2$ requires C, 56·6; H, 6·1; N, 5·5%).

This compound (7 g.) was treated with a mixture of ethanol (80 ml.), 0·1N-hydrochloric acid (40 ml.), and water (40 ml.) under reflux for 1 hour on the steam-bath. After removal of part of the ethanol the product crystallised on cooling. Crystallisation from ethanol–light petroleum gave *2-methyl-1-(β-tetra-acetyl* D-*glucopyranosyl)benziminazole*, needles, m. p. 202°, $[\alpha]_D^{22}$ −30·2° (*c*, 0·109 in chloroform) (Found: C, 55·8; H, 5·4; N, 6·6. $C_{22}H_{26}O_9N_2$ requires C, 57·1; H, 5·6; N, 6·1%).

2 : 5-Dimethyl-1-(β-tetra-acetyl D-*glucopyranosyl)benziminazole.*—4-Methyl-3-nitro-*N*-(tetra-acetyl D-glucopyranosyl)aniline (14 g.) in ethanol (100 ml.) was catalytically reduced in the presence of 5% palladised charcoal to the corresponding diamine, which was not isolated but was treated with ethyl orthoacetate (30 ml.). The mixture was heated on the steam-bath for 3 hours. Removal of excess of ortho-ester by vacuum-distillation gave the *ethoxyethylidene* compound, needles [from ethanol–light petroleum (b. p. 40—60°)], m. p. 138°, $[\alpha]_D^{22}$ −85·7° (*c*, 3·018 in chloroform) (Found: C, 57·5; H, 6·5; N, 5·6. $C_{25}H_{34}O_{10}N_2$ requires C, 57·4; H, 6·5; N, 5·4%).

The foregoing product (7 g.) was heated under reflux with ethanol (80 ml.), 0·1N-hydrochloric acid (40 ml.), and water (40 ml.) for 1 hour, giving *2 : 5-dimethyl-1-(β-tetra-acetyl* D-*glucopyranosyl)-benziminazole*, needles (from ethanol), m. p. 220°, $[\alpha]_D^{22}$ −43·4° (*c*, 1·938 in chloroform) (Found: C, 58·2; H, 6·0; N, 6·3. $C_{23}H_{28}O_9N_2$ requires C, 57·9; H, 5·9; N, 5·9%).

Hydrolysis with 6N-hydrochloric acid for 1 hour under reflux then gave *2 : 5-dimethyl-1-(β-*D-*glucopyranosyl)benziminazole hydrochloride*, needles (from aqueous acetone), m. p. 236° (decomp.), $[\alpha]_D^{22}$ +20° (*c*, 1·262 in water) (Found: C, 52·2; H, 6·1; N, 8·1. $C_{15}H_{21}O_5N_2Cl$ requires C, 52·0; H, 6·6; N, 7·9%).

2-Methyl-1-(β-triacetyl D-*xylopyranosyl)benziminazole.*—Reduction of *o*-nitro-*N*-(triacetyl D-xylopyranosyl)aniline gave the corresponding *diamine*, white plates (from ethanol), m. p. 179°, $[\alpha]_D^{23}$ −50° (*c*, 2·954 in chloroform) (Found: C, 54·6; H, 5·6; N, 8·3. $C_{17}H_{22}O_7N_2$ requires C, 55·7; H, 6·0; N, 7·6%). The *ethoxyethylidene* compound crystallised from ethanol in needles, m. p. 139°, $[\alpha]_D^{19}$ −62·1° (*c*, 2·872 in chloroform) (Found: C, 57·7; H, 6·4; N, 6·5. $C_{12}H_{28}O_9N_2$ requires C, 57·8; H, 6·4; N, 6·4%). Cyclisation then gave *2-methyl-1-(β-triacetyl* D-*xylopyranosyl)benziminzole*, needles (from

aqueous ethanol), m. p. 228° $[\alpha]_D^{22}$ −76·3° (*c*, 1·16 in chloroform) (Found : C, 59·2; H, 5·5; N, 7·2. $C_{19}H_{22}O_7N_2$ requires C, 58·4; H, 5·6; N, 7·2%).

2 : 5-*Dimethyl*-1-(*β-triacetyl* D-*xylopyranosyl*)*benziminazole* formed needles (from ethanol), m. p. 258°, $[\alpha]_D^{22}$ −93·9° (*c*, 1·026 in chloroform) (Found : C, 59·4; H, 6·0; N, 6·9. $C_{20}H_{24}O_7N_2$ requires C, 59·4; H, 5·9; N, 6·9%).

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