THE CHEMISTRY OF ANTI-PERNICIOUS ANÆMIA FACTORS

PART IV. BENZIMINAZOLE GLYCOSIDES

(3) THE PREPARATION OF SOME BENZIMINAZOLE PENTOSIDES

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THE present paper describes the extension of the new synthetic methods elaborated in Part IV (2) to the preparation of some benziminazole pentosides[1]. The work herein contained completes the studies initiated in Part IV (1) of this series by providing potential routes to the ribose derivatives of benziminazole[2]. The synthesis of the latter compounds formed the *raison d'etre* for this series of investigations. Their preparation bears on the chemistry of vitamin B_{12}, however, and will form the subject of a subsequent communication.

The preparation of benziminazole pentosides by the " orthoformate route " presented certain points of difference from the observations recorded in Part IV (2). Thus the intermediate ethyl *iso*formanilides proved, in all cases, to be compounds of low crystallising power which could only be isolated as gums. Their subsequent conversion into benziminazole pentosides was effected by heating with 0·1N hydrochloric acid, or, more conveniently, by treatment with alcoholic picric acid. In the latter case the picrate separated only after the solution had been boiled for some minutes. This result leads to the conclusion that the benziminazole was not originally present in the mixture but was formed from the *iso*formanilide by the cyclising action of the picric acid. Picric acid, it may be added, failed to effect ring closure with such compounds as 2-ethoxymethylene-*o*-phenylenediamine-tetraacetyl-D-glucoside (see Part IV (2)) for which hot dilute hydrochloric acid is required.

o-Phenylenediamine-triacetyl-D-xyloside was converted by ethyl orthoformate into benziminazole-1β-triacetyl-D-xylopyranoside, the constitution of which followed from its alternative preparation employing benziminazole silver and α-acetobromxylose. Hydrolysis furnished benziminazole-1β-D-xylopyranoside, the pyranose character of the lactol ring being established by periodate titration. 4-Methyl-*o*-phenylenediamine triacetyl-D-xyloside behaved in a similar way, but the resulting 5-methylbenziminazole-1β-triacetyl-D-xylopyranoside could not, unfortunately, be prepared by the silver salt route. 5 : 6-Dimethyl-benziminazole-triacetyl-D-xyloside, in contrast, could not be prepared by either synthetic method, a failure no doubt due to an inability to form a picrate (cf. the corresponding glucoside), thereby rendering isolation extremely difficult.

Attempts to prepare the benziminazole-L-arabinosides by the orthoformate route proved only partly successful. Reaction of *o*-phenylenediamine-triacetyl-L-arabinoside and its 4-methyl derivative with ethyl orthoformate, with or without subsequent treatment with 0·1N hydrochloric acid, invariably gave intractable gums from which a crystalline product could not be isolated. When 4 : 5-dimethyl-*o*-phenylenediamine-

triacetyl-L-arabinoside was employed, however, reaction occurred to give 5 : 6-dimethyl-l-α-triacetyl-L-arabopyranoside, although in somewhat poor yield. The constitution assigned to this compound followed from its facile preparation from 5 : 6-dimethylbenziminazole silver and β-acetobrom-L-arabinose. The silver salt method likewise gave excellent yields of benziminazole-l-α-triacetyl-D-arabopyranoside (from β-acetobrom-D-arabinose), benziminazole-l-α-triacetyl-L-arabopyranoside (from β-acetobrom-L-arabinose) and 5-(or 6)-methylbenziminazole-l-α-L-triacetyl-arabopyranoside. The latter result is particularly interesting as previous attempts to employ 5-(6)-methylbenziminazole silver had invariably given mixtures of isomers which had proved incapable of separation. Hydrolysis with 6N hydrochloric acid furnished the corresponding benziminazole arabopyranosides.

5 : 6-Dimethylbenziminazole-l-triacetyl-L-rhamnoside was prepared in excellent yield by the orthoformate route and was smoothly de-acetylated to give 5 : 6-dimethylbenziminazole-l-L-rhamnopyranoside, the structure of which was confirmed by periodate titration.

The foregoing pentosides resembled the benziminazole glucosides in their remarkable stability to acids being recovered apparently unchanged after twelve hours' heating with 6N hydrochloric acid at 100°C. The bearing of these results on the structure of the "β-component" from vitamin B_{12} will be discussed in a subsequent communication.

The above observations, together with those recorded in Part IV (2) provide a basis for discussing the mechanism of the "orthoformate route." The first stage in the reaction between an *o*-phenylenediamine glycoside and ethyl orthoformate is assumed to be the formation of a 2-ethoxy-methylene-*o*-phenylenediamine glycoside (I). Reaction of the latter compound with hydrogen ions, supplied by picric acid or, where necessary, by hydrochloric acid, leads to formation of the benziminazole glycoside (II) with concomitant elimination of ethylalcohol. The *iso*-formanilide derived from 3 : 4-dimethyl-*o*-phenylenediamine-tetraacetyl-D-glucoside forms an exception, however, in passing spontaneously into the benziminazole glycoside on heating at 100° in excess orthoformate employed as a solvent.

Consideration of these facts leads to the reaction scheme which is presented in simplified form on page 514.

The facility with which the change (I) → (II) may be effected is thus directly related to the basicity of the glycosidic nitrogen atom[3] which, in turn, is influenced by the following factors :

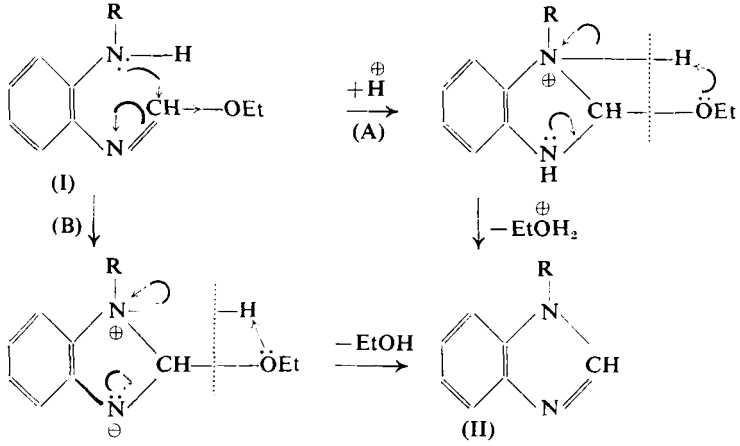
(i) The presence of substituents in the aryl nucleus of (I)

(ii) The presence of hydrogen ions which provide the driving force for reaction-scheme (A)

(iii) The possibility of chelation between an acetyl grouping of the sugar residue and glycosidic hydrogen which, as already pointed out by Brownlie, Sutherland and Todd[4], would have the effect of inducing a negative charge on the glycosidic nitrogen atom thereby increasing its basicity

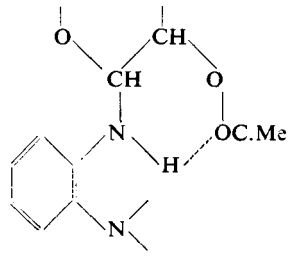
(iv) The nature of the sugar residue

(v) The α β-character of the glycosidic linkage.



(R = Hexose or Pentose Residue)

In so far as the present evidence allows, however, it seems clear that the inductive effect exerted by the sugar residue on the free electron pair of the glycosidic nitrogen represents the determining factor in the change



(I) → (II). The inductive effect, of course, will vary with the sugar residue, but its qualitative evaluation, unfortunately, is impossible at this juncture.

EXPERIMENTAL

Melting points are corrected. Microanalyses are by Drs. Weiler and Strauss, Oxford.

*Benziminazole-1β-triacetyl-*D*-xylopyranoside.* (i) *o*-Nitroaniline-triacetyl-D-xylopyranoside (1·4 g.) was reduced in warm ethyl acetate solution by shaking with hydrogen in the presence of 10 per cent. palladised charcoal. When hydrogen uptake was complete, the catalyst was filtered and ethyl orthoformate (5 ml.) added to the filtrate, which was then slowly evaporated on a steam bath for 3 hours. The remaining solvents were removed under reduced pressure and the residue heated with 0·1N hydrochloric acid (8 ml.) at 100°C. for 10 minutes. After basification with potassium bicarbonate, the product was extracted with chloroform and converted into the picrate (yield 800 mg.). *Benziminazole-1β-triacetyl-*D*-xylopyranoside picrate* separated from alcohol in flat yellow needles, m.pt. 192° to 193°C. Found : C, 47·8 ; H, 4·0 ; N, 11·4.

$C_{18}H_{20}O_7N_2$, $C_6H_3O_7N_3$ requires C, 47·6 ; H, 3·8 ; N, 11·6 per cent. Alternatively, the gum remaining after evaporation of the ethyl orthoformate was treated with alcoholic picric acid under reflux, when the picrate separated in slightly increased yield.

Percolation of a chloroform solution of the picrate through a column of alumina afforded the *triacetyl*-D-*xylopyranoside*, which separated from a mixture of chloroform and light petroleum in colourless cubes, m.p. 163°C. $[\alpha]_D^{25°C.}$ −44·8° (in chloroform, c=1). Found : C, 58·0 ; H, 5·2 ; N, 7·6. $C_{18}H_{20}O_7N_2$ requires C, 57·5 ; H, 5·3 ; N, 7·5 per cent.

(ii) A suspension of benziminazole silver (2·4 g.) in xylene (120 ml.) was dried azeotropically by distillation of one half of the solvent. α-Acetobromxylose (3·7 g.) was added and the mixture heated under reflux for 4 hours, whereafter the silver bromide was filtered, washed with xylene, and the filtrate and washings taken to dryness under reduced pressure. The crude xyloside was purified by passing an ethyl acetate solution through alumina, and was then isolated as the picrate, which separated from alcohol in yellow needles, m.p. 193°C. Found : C, 46·8; H, 3·8 per cent. This compound showed no depression in m.p. when mixed with a sample prepared by method (i).

Benziminazole-1β-D-*xylopyranoside.* The foregoing triacetate (700 mg.) and 6N hydrochloric acid (20 ml.) were heated at 100°C. for 2 hours. The solution was evaported to dryness, and the residue recrystallised from aqueous acetone. *Benziminazole*-1β-D-*xylopyranoside hydrochloride monohydrate* formed soft, colourless needles, m.p. 148° to 150°C., $[\alpha]_D^{25°C.}$ −25·4° (in water, c=1). Found : C, 47·4 ; H, 5·5 ; N, 9·4 ; Cl, 12·3. $C_{12}H_{14}O_4N_2$, HCl, H_2O requires C, 47·3 ; H, 5·6 ; N, 9·2 ; Cl, 11·7 per cent.

An aqueous solution of this compound was percolated through a column of Amberlite IR-4B, and the *xylopyranoside* recovered by evaporation to dryness. It crystallised from a mixture of benzene and alcohol in platelets, m.p. 237° to 238°, $[\alpha]_D^{21°C.}$ −89·1 (c=1, in pyridine). Found : C, 57·5 ; H, 5·6 ; N, 11·2. $C_{12}H_{14}O_4N_2$ requires C, 57·6 ; H, 5·6 ; N, 11·2 per cent. The pyranoside structure of this compound was confirmed by oxidation with periodic acid, when 2·05 moles were consumed.

5-*Methylbenziminazole*-1β-*triacetyl*-D-*xylopyranoside.* 4-Methyl-*o*-phenylenediamine-triacetyl-D-xylopyranoside (prepared by reduction of 2 g. of the corresponding nitro-compound I or II), ethyl acetate (50 ml.), and ethyl orthoformate (8 ml.), were heated on the steam bath for 3 hours. The solvents were removed *in vacuo* and the residue treated with alcoholic picric acid. The *picrate* (1·1 g.) crystallised from alcohol in fine yellow needles, m.p. 201°C. Found : C, 48·5 ; H, 3·5 ; N, 11·1. $C_{19}H_{22}O_7N_2$, $C_6H_3O_7N_3$ requires C, 48·5 ; H, 4·0 ; N, 11·3 per cent. Decomposition of this picrate on alumina afforded 5-*methylbenziminazole*-1α-*triacetyl*-D-*xylopyranoside*, colourless needles, m.p. 183°C. $[\alpha]_D^{23°C.}$ −67·0° (c=1, in chloroform). Found : C, 58·5 ; H, 5·6 ; N, 6·9. $C_{19}H_{22}O_7N_2$ requires C, 58·5 ; H, 5·6 ; N, 7·2 per cent.

5-*Methylbenziminazole*-1β-D-*xylopyranoside.* Deacetylation of the

foregoing compound (500 mg.) with 6N hydrochloric acid (12 ml.) at 100°C. followed by evaporation to dryness and passage of an aqueous solution of the resulting hydrochloride through an ion exchange resin, gave *5-methylbenziminazole-1β-D-xylopyranoside*, fine felted needles from alcohol, m.pt. 215° to 216°C. $[\alpha]_D^{20°C.}$ −50·7 (c=1, in water). Found: N, 10·4, $C_{13}H_{16}O_4N_2$ requires N, 10·4 per cent. Accurate analytical figures for carbon and hydrogen could not be obtained.

The lactol ring structure of this compound was proved to be of the pyranoside type by oxidation with periodic acid, when 2·09 moles were consumed.

Benziminazole-1α-triacetyl-L-arabopyranoside. Benziminazole silver (2·0 g.) was added to boiling xylene (70 ml.) and after distillation of 30 ml. of the solvent to remove traces of moisture, β-acetobrom-arabinose (3 g.) was added. The mixture was heated in an oil bath at reflux temperature for 4 hours, filtered from silver bromide, and the filtrate and washings taken to dryness *in vacuo*. The residue was dissolved in alcohol under reflux and picric acid (2 g.) added, when *benziminazole-1-α-triacetyl-L-arabopyranoside picrate* (2·2 g.) was obtained, yellow needles from alcohol, m.p. 182° to 185°C. Found: C, 47·2; H, 3·5; N, 11·1. $C_{18}H_{20}O_7N_2$, $C_6H_3O_7N_3$ requires C, 47·6; H, 3·8; N, 11·6 per cent. Filtration of a chloroform solution of this compound through a bed of alumina gave the *triacetyl-L-arabopyranoside*, which crystallised from a mixture of benzene and light petroleum in prismatic needles, m.pt. 165°C. $[\alpha]_D^{25°C.}$ −3·7° (c=1, in chloroform). Found: C, 57·4; H, 5·2; N, 6·9. $C_{18}H_{20}O_7N_2$ requires C, 57·4; H, 5·4; N, 7·4 per cent.

Benziminazole-1α-L-arabopyranoside was prepared by deacetylation of the foregoing triacetate and removal of the hydrochloric acid in the usual way. It separated from alcohol in prismatic needles, m.pt. 225°C. $[\alpha]_D^{20°C.}$ +22·6 (c=1 in water). Found: C, 57·9; H, 5·7; N, 11·1. $C_{12}H_{14}O_4N_2$ requires C, 57·6; H, 5·6; N, 11·2 per cent. This pyranoside consumed 1·93 moles of periodic acid.

Benziminazole-1α-D-arabopyranoside was prepared by an identical sequence of reactions starting from β-acetobrom-D-arabinose. It crystallised in colourless needles from a mixture of alcohol and light petroleum, m.pt. 226°C. $[\alpha]_D^{15°C.}$ −24·9° (Found: C, 57·4; H, 5·8; N, 11·3 per cent.), and consumed 2·05 moles of periodic acid. The *triacetate* separated in colourless needles from a mixture of ethyl acetate and light petroleum, m.pt. 165°C. $[\alpha]_D^{24°C.}$ +7·1° (Found: C, 57·7; H, 5·6; N, 7·5 per cent.), and was characterised as the *picrate*, yellow needles from a mixture of alcohol and β-ethoxyethanol, m.pt. 181° to 183°C. Found: C, 47·2; H, 3·7; N, 12·3 per cent.

5 (or 6)-*Methylbenziminazole-1α-L-arabopyranoside.* 5 (or 6)-*Methylbenziminazole-1α-triacetyl-L-arabopyranoside*, prismatic needles from ethyl acetate/light petroleum, m.pt. 181° to 183°, $[\alpha]_D^{24°C.}$ −15·7° (c=1, in chloroform) (Found: C, 58·2; H, 5·8 $C_{19}H_{22}O_7N_2$ requires C, 58·5; H, 5·7 per cent.) was prepared in 20 per cent. yield by reacting 5-methylbenziminazole silver (1·7 g.) with β-acetbromo-L-arabinose (2·7 g.) in xylene solution, and was characterised as the *picrate*, which formed yellow needles from β-ethoxyethanol, m.pt. 217° to 218°C.

(decomp.). Found: C, 48·9; H, 4·1; N, 11·0. $C_{19}H_{22}O_7N_2$, $C_6H_3O_7N_3$ requires C, 48·5; H, 4·1; N, 11·3 per cent. Hydrolysis of the triacetate with 6N hydrochloric acid etc. gave 5 (or 6)-*methyl-benziminazole*-1α-L-*arabopyranoside*, white needles from a mixture of alcohol and light petroleum, m.p. 229°C $[\alpha]_D^{22°C.}$ +14·8°. Found: C, 59·7; H, 6·3. $C_{13}H_{16}O_4N_2$ requires C, 59·1; H, 6·1 per cent. This compound consumed 2·06 moles of periodic acid.

5:6-*Dimethylbenziminazole*-1α-*triacetyl*-L-*arabopyranoside*. (i) 5-Nitro-*o*-4-xylidine-triacetyl-L-arabinoside (series I or II, 3 g.) was reduced in the usual way and the solution, freed from catalyst, slowly evaporated with ethyl orthoformate (6 ml.) for 4 hours. The residue, after evaporation to dryness, was heated at 100°C. with 0·05N hydrochloric acid (15 ml.). After neutralisation, the product was isolated with chloroform and converted into the picrate (1·0 g.). 5:6-*Dimethyl-benziminazole*-1α-*triacetyl*-L-*arabopyranoside picrate* crystallised from a mixture of β-ethoxyethanol and alcohol in prismatic yellow needles, m.p. 234° to 236°C. (decomp.). Found: C, 49·0; H, 4·2; N, 10·9. $C_{20}H_{24}O_7N_2$, $C_6H_3O_7N_3$ requires C, 49·3; H, 4·3; N, 11·0 per cent.). The free *base* formed colourless needles from a mixture of benzene and light petroleum, m.p. 141° to 143°C. $[\alpha]_D^{25°C.}$ −31·9° (in chloroform). Found: C, 59·3; H, 6·0; N, 6·3. $C_{20}H_{24}O_7N_2$ requires C, 59·4; H, 6·0; N, 6·9 per cent.

(ii) 5:6-Dimethylbenziminazole silver (2·4 g.), β-acetobrom-L-arabinose (3·3 g.) and xylene (35 ml.) were heated under reflux in the usual way. The product was isolated as the picrate (2·2 g.), obtained in yellow needles from β-ethoxyethanol, m.p. 234° to 236°C. (decomp.). Found: C, 49·3; H, 4·2 per cent. $C_{20}H_{24}O_7N_2$, $C_6H_3O_7N_3$ requires C, 49·3; H, 4·3 per cent., not depressed in admixture with the compound prepared by the "ethyl orthoformate route." Regeneration of the base gave 5:6-dimethylbenziminazole-1α-triacetyl-L-arabopyranoside, m.p. 142° to 143°C.

5:6-*Dimethylbenziminazole*-1α-L-*arabopyranoside* crystallised from alcohol/light petroleum in rosettes of white needles, m.p. 280° to 281°C. (decomp.) $[\alpha]_D^{23°C.}$ −70·5° (c=1, in pyridine). Found: C, 60·0; H, 6·8; N, 10·3. $C_{14}H_{18}O_4N_2$ requires C, 60·4; H, 6·5; N, 10·1 per cent. It consumed 2·15 moles of periodic acid. The picrate formed rosettes of yellow needles from alcohol, m.p. 216° to 217°C. (decomp.).

5:6-*Dimethylbenziminazole*-1-*triacetyl*-L-*rhamnopyranoside*. 4:5-Dimethyl:*o*-phenylenediamine-triacetyl-L-rhamnoside (prepared by reduction of 5 g. of nitro-compound) in ethyl acetate (70 ml.) was heated on the steam bath with ethyl orthoformate (7 ml.) for 5 hours. Following treatment with 0·05N hydrochloric acid, the product was isolated as the *picrate* (3·3 g.), which crystallised from aqueous alcohol in slender yellow needles, m.p. 184°C. Found: C, 50·3; H, 4·8; N, 11·0. $C_{21}H_{26}O_7N_2$, $C_6H_3O_7N_3$ requires C, 50·1; H, 4·5; N, 10·8 per cent. 5:6-*Dimethyl-benziminazole*-1-*triacetyl*-L-*rhamnopyranoside* formed small white needles from light petroleum, m.p. 92° to 95°. Found: C, 59·8; H, 6·3. $C_{21}H_{26}O_7N_2$ requires C, 60·3; H, 6·3 per cent.

5:6-*Dimethylbenziminazole*-1-L-*rhamnopyranoside* crystallised in wisps

from alcohol/light petroleum, m.pt. 252°C. Found : C, 61·5 : H, 6·9 ; N, 9·3. $C_{15}H_{20}O_4N_2$ requires C, 61·6 ; H, 6·9 ; N, 9·6 per cent. The pyranoside structure followed from periodate oxidation, when 2·2 moles of the oxidant were consumed.

Summary and Conclusions

1. New methods elaborated in Part IV (2) for the synthesis of benziminazole glucosides have been extended by the preparation of some benziminazole pentosides.

2. Results obtained employing the "orthoformate route" show that ethoxymethylene-*o*-phenylenediamine acetylpentosides undergo more facile ring closure than the corresponding glucosides, being readily converted into the benziminazole pentosides by the action of alcoholic picric acid.

3. The pyranoside structures assigned to the foregoing compounds have been confirmed by periodate titrations.

4. Benziminazole pentosides show unexpected stability to acids and are recovered unchanged after heating for 12 hours with 6N hydrochloric acid at 100°C.

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