THE CHEMISTRY OF ANTI-PERNICIOUS ANÆMIA FACTORS

PART V.

THE INTER RELATIONSHIP AND STRUCTURE OF THE α-, β-, AND γ-COMPONENTS

BY G. COOLEY, B. ELLIS, P. MAMALIS, V. PETROW AND B. STURGEON

*From the Research Laboratories, The British Drug Houses, Ltd., London, N.*1

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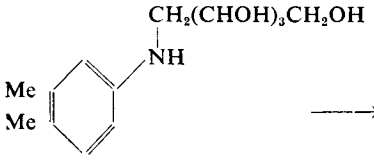
PREVIOUS work by Beaven, Holiday, Johnson, Ellis, Mamalis, Petrow and Sturgeon[1] had shown that: (i) hydrolysis of vitamin B_{12} leads to the formation of three closely related substances designated *components* α, β and γ, (ii) *component* γ is spectroscopically and chromatographically indistinguishable from 5:6-dimethylbenziminazole (VII)[2], (iii) *components* α and β are spectroscopically identical with certain 1-substituted 5:6-dimethylbenziminazoles (*vide infra*), (iv) the effect produced by the 1-substituent in the α- and β-*components* on the absorption spectrum of the parent 5:6-dimethylbenziminazole is similar to that exerted by a methyl grouping.

Consideration of these facts led to the following conclusions: 1. The substituent attached to N^1 in the α- and β-*components* is capable of stepwise degradation by acid. 2. The spectroscopic similarity of the α- and β-*components* with 1:5:6-trimethylbenziminazole excludes the identity of the 1-substituent present in these two compounds with any polar grouping capable of exerting an auxochromic effect. 3. The α- and β-*components* bear a structural similarity to the *N*-D-ribityl-4:5-dimethyl-*o*-phenylenediamine portion of the riboflavine molecule.

In so far as the facts allowed, Beaven *et al.*[1] interpreted the evidence as indicating that the α- and β-*components* might well prove to be sugar derivatives of 5:6-dimethylbenziminazole or their acid transformation products. Experiments on the synthesis of benziminazole glycosides were accordingly initiated in these laboratories.

From this point our studies on vitamin B_{12} developed simultaneously in two main directions. In the first place, procedures for the synthesis of benziminazole glycosides were elaborated[3]. Secondly, chromatographic studies were initiated with the object of determining the sequence in which phosphate[4], the "ninhydrin-reacting" fragment[5] (1-aminopropan-2-ol[6,7]) and the α-, β-, and γ-components were released from vitamin B_{12} during hydrolysis.

The quantity of vitamin B_{12} at our disposal at this stage was, unfortunately, extremely limited owing to heavy commercial demands upon available supplies. It was thus not possible to attempt the direct isolation and identification of the α- and β-*components* by the methods of classical organic chemistry. Nevertheless, we hoped to obtain the desired evidence regarding their structure by chromatographic comparison with synthetic glycosides of known structure.



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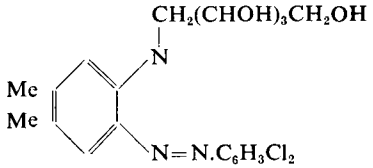

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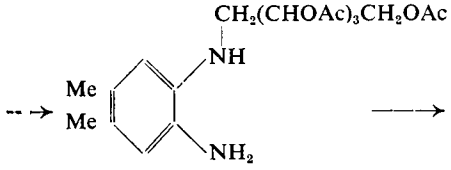

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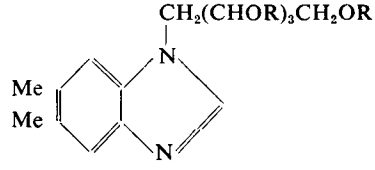

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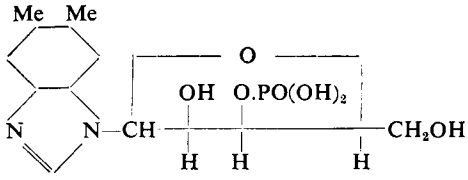
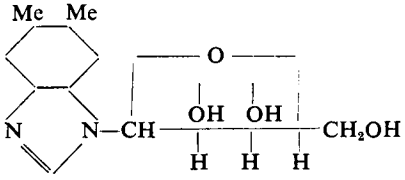


(V)

α—*Component*



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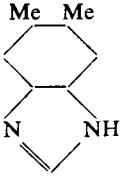
β—*Component*



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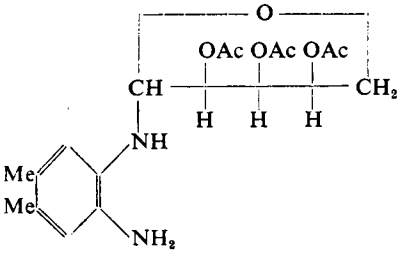
γ—*Component*



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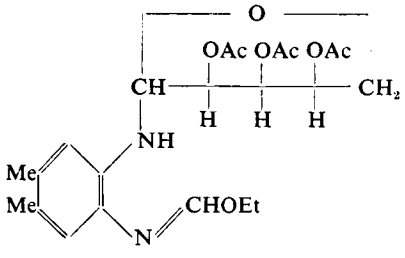

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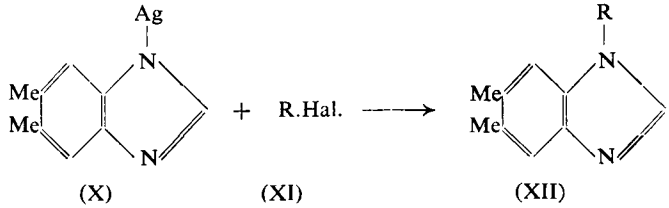
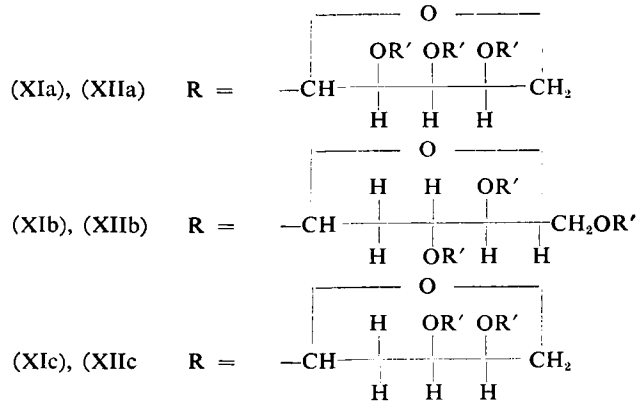


(X) (XI) (XII)



Treatment of vitamin B_{12} with 6N hydrochloric acid for very short periods at room temperature led to the rapid liberation of the α-*component*. Thus the latter fragment could be detected on chromatograms prepared from an aliquot of hydrolysate withdrawn and dispensed on to the paper only 5 minutes after solution of the vitamin in mineral acid. Spectrophotometric estimation of the α-*component* eluted from a chromatogram of a 5-hour hydrolysate indicated that not less than 0·7 mol. had been split off from the B_{12} molecule by this treatment. These results were obtained in collaboration with Dr. E. R. Holiday (M.R.C. Spectrographic Unit, The London Hospital, E.1), whose help we gratefully acknowledge.

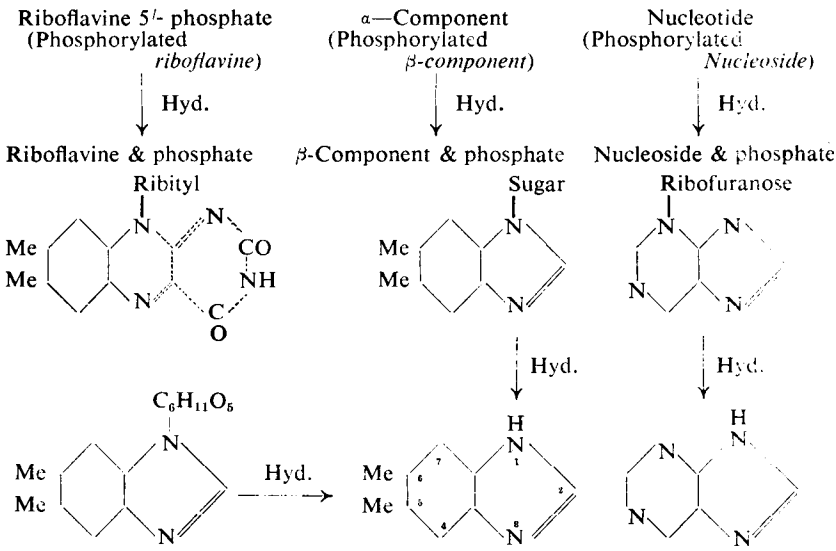
Further contact with 6N hydrochloric acid led, after not less than 18 hours, to the gradual release of 1-aminopropan-2-ol, which formed the second moiety liberated from vitamin B_{12} under these experimental conditions. Neither phosphate nor the β- or γ-*components* made their appearance on chromatograms at this stage.

Accordingly we isolated the α-*component* on the micro-scale and submitted it to further degradation with 6N hydrochloric acid at 100°C. Slow hydrolysis occurred to give, after 6 hours, unchanged α-*component*, some β-*component*, and phosphate, an observation which led us to conclude that the α-*component* was a phosphorylated derivative of the β-*component*. Finally, hydrolysis of the β-*component* with 6N hydrochloric acid at 150°C. in a sealed tube gave the γ-*component* 5:6-dimethylbenziminazole. The fate of the erstwhile 1-substituent which had been eliminated during the last stage could not be determined.

This sequence of changes brought to mind the behaviour of the nucleotides on acidolysis. These compounds are known to be converted, with

liberation of phosphate, into nucleosides, which then undergo further hydrolysis into the heterocyclic base and the sugar (see below). On this basis the α-*component* would correspond to the nucleotide, and the β-*component* to the nucleoside.

Pursuing this line of reasoning further, we were led to compare the behaviour, on acidolysis, of the β-*component* with that of 5:6-dimethylbenziminazole-1-β-D-glucopyranoside, the synthesis of which had already been accomplished at this stage. A remarkable degree of similarity was observed. Thus the two compounds were recovered unchanged after 12 hours' heating with 6N hydrochloric acid at 100°C. whilst at 150°C. profound degradation occurred to give the γ-*component*.



This result furnished us with the first experimental evidence, although admittedly of an indirect character, that the β-*component* could rationally be formulated as a benziminazole glycoside. It left unanswered, however, the nature of the sugar residue involved in glycosidic union with the benziminazole residue.

The structural analogy between riboflavine and the β-*component* (see above) appeared to indicate that the latter compound might be a ribityl-benziminazole. The resemblance to a nucleoside, on the other hand, pointed to a ribosido-benziminazole formulation. Unfortunately we had no direct means of determining the accuracy of these speculations and were thus forced to adopt a purely empirical approach and to attempt the synthesis of the ribosido- and ribityl-derivatives of 5:6-dimethylbenziminazole.

1-D-Ribityl-5:6-dimethylbenziminazole (IV; R=H), prepared as described in the Synthetical Section proved completely stable to 6N hydrochloric acid at 150°C., an observation which eliminated it from further consideration. 5:6-Dimethylbenziminazole-1-β-D-ribopyranoside (XIIa;

R′=H), in contrast, closely resembled the β-*component* both in its stability to 6N hydrochloric acid at 100°C. and in its degradation to 5:6-dimethylbenziminazole at 150°C. In addition, the R_F values of the two compounds on paper chromatography proved to be identical in three different solvent systems.

The results are collected in the Experimental Section together with parallel data obtained with some related benziminazole glycosides synthesised during the course of this work.

Cooley, Ellis and Petrow[8] have previously shown that chromatographic methods, *per se*, cannot be used to identify an unknown compound in the absence of supporting chemical data. We therefore attempted to obtain evidence of this character by periodate oxidation of (XIIa; R′=H) and of the β-*component*, followed by chromatographic examination of the products. The results proved disappointing. Both compounds underwent facile oxidation, as was indeed expected, but the chromatograms obtained from the oxidation products were not satisfactory.

Nevertheless, in spite of these results, we felt convinced that, on biogenetic grounds alone, the β-*component* could not be formulated as (XIIa; R′=H) but, if our speculations were correct, should be assigned the constitution of a 5:6-dimethylbenziminazole-1-ribofuranoside. This conviction was based upon the universal occurrence of D-ribose in the furanose form in such naturally occurring substances as the nucleosides. We therefore turned our attention to the reaction between 5:6-dimethylbenziminazole silver and acetobromribofuranose, hoping thereby to obtain the desired product. Before this work could be completed, Brink, Holly, Shunk, Peel, Cahill and Folkers[9] reported the isolation of 5:6-dimethylbenziminazole-1-α-D-ribofuranoside (VI) from acid hydrolysates of vitamin B_{12} and its synthesis by an unambiguous route. This compound (VI) is undoubtedly identical with the β-*component* of Beaven *et al.* (Part III)[1], the complete structure of which may now be considered as finally elucidated.

Our observation that conversion of the α-*component* into the β-*component* is accompanied by release of phosphate (*vide supra*) has recently been confirmed by Buchanan, Johnson, Mills and Todd[10], who have successfully isolated the former fragment of the B_{12} molecule as its barium salt. Like ourselves, they find the α-*component* stable to periodic acid, and accordingly assign it the constitution of a 2′- or 3′-phosphoryl-5:6-dimethylbenziminazole-1-α-D-ribofuranoside (V). We accept these conclusions, but are unable to adopt their view that the D-1-aminopropan-2-ol present in vitamin B_{12} is likewise attached to the phosphoryl grouping. Our own results (*vide supra*) show that release of the α-*component* is not necessarily accompanied by concomitant liberation of 1-aminopropan-2-ol, for which an alternative location must obviously be found. On general grounds of symmetry, too, the existence of two molecules[11] of 1-aminopropan-2-ol in the B_{12} molecule would appear to render the attachment of only one of them to the phosphoryl grouping somewhat unlikely.

SYNTHETICAL SECTION

Ribityl-and-2'-Deoxyribosido-benziminazoles

The preparation of 1-D-ribityl-5:6-dimethylbenziminazole (IV; R=H) was effected in the following way.

N-D-Ribityl-*o*-4-xylidine (I) was condensed with 2:4-dichlorophenyldiazonium chloride when 5(2':4'-dichlorophenylazo)-N-D-ribityl-*o*-4-xylidine (II) was readily obtained in excellent yield. Acetylation of this product, followed by reduction with zinc dust and acetic acid in ethyl acetate solution, furnished tetraacetyl-D-ribityl-4:5-dimethyl-*o*-phenylene diamine (III). The latter compound was not isolated but was condensed *in situ* with ethyl orthoformate to give 1-tetraacetyl-D-ribityl-5:6-dimethylbenziminazole (IV; R=Ac), isolated as the picrate. Hydrolysis in the usual way furnished 1-D-ribityl-5:6-dimethylbenziminazole (IV; R=H).

In addition to the foregoing compound, the preparation of the related 2'-deoxyriboside was undertaken, as 2-deoxy-D-ribose is known to occur in certain nucleosides. Before embarking on its synthesis, however, we explored the preparation of the corresponding deoxy-D-glucoside.

Reaction of 1-bromo-3:4:6-triacetyl-2-deoxy-D-glucose (XIb, R'=Ac) with 5:6-dimethylbenziminazole silver (X) gave a non-crystalline mixture of αβ-isomers which could not be resolved by fractionation procedures. Deacetylation, followed by treatment with picric acid, however, readily gave a crystalline 5:6-dimethylbenziminazole-1-(2'-deoxy-D-glucopyranoside) picrate from which, by acetylation and regeneration of the base, 5:6-dimethylbenziminazole-1-(3':4':6'-triacetyl-2'-deoxy-D-glucopyranoside) (XIIb; R'=Ac) was obtained in crystalline form. Hydrolysis of the latter now furnished the pure deoxy-D-glucoside (XIIb; R'=H). The second isomer, which was no doubt present in the initial reaction mixture, was not isolated.

In contrast to these results, reaction between 5:6-dimethylbenziminazole silver (X) and 1-chloro-3:4-diacetyl-2-deoxy-D-ribose (XIc; R'=Ac) in xylene solution at 100°C. readily gave a homogeneous 5:6-dimethylbenziminazole-1-(3':4'-diacetyl-2'-deoxy-D-ribopyranoside) (XIIc; R'=Ac). Hydrolysis furnished 5:6-dimethylbenziminazole-1-(2'-deoxy-D-ribopyranoside) (XIIc; R'=H) characterised by conversion to the picrate.

Similar results were obtained by reaction between benziminazole silver and (XIc; R'=Ac). The product readily gave crystalline benziminazole-1-(3':4'-diacetyl-2'-deoxy-D-ribopyranoside) picrate, decomposed to the glassy base (XIIc; R'=Ac). Hydrolysis of the latter compound, followed by purification *via* the picrate, gave benziminazole-1-(2'-deoxy-D-ribopyranoside) (XIIc; R'=H), isolated as the hydrochloride.

These observations contrast somewhat with those recorded by Davoll and Lythgoe[12], who obtained two isomeric deoxyribosides and only one deoxyglucoside by condensing (XIb; R'=Ac) and (XIc; R'=Ac) with theophylline silver.

5:6-Dimethylbenziminazole-1-β-D-ribopyranoside

The ribopyranoside (XIIa; R'=H) required for comparison with the

β-*component* was readily prepared by an extension of the synthetic methods elaborated in Part IV[3].

By condensing 4:5-dimethyl-*o*-phenylenediaminetriacetyl-D-ribopyranoside (VIII) with ethyl orthoformate, the *iso*formanilide (IX) was obtained in excellent yield. Treatment with dilute hydrochloric acid furnished 5:6-dimethylbenziminazole-1-β-triacetyl-D-ribopyranoside (XIIa; R′=Ac). The β-configuration assigned to the anomeric centre in this compound followed from its alternative synthesis from 5:6-dimethylbenziminazole silver (X) and α-acetobromribopyranose (XIa; R′=Ac). Hydrolysis with hydrochloric acid gave the desired 5:6-dimethylbenziminazole-1-β-D-ribopyranoside (XIIa; R′=H) which was isolated as the hydrochloride hemihydrate. The pyranoside character of the lactol ring in the latter compound was confirmed by periodate titration.

EXPERIMENTAL

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

Section A

*The preparation of 1-*D*-Ribityl-5:6-dimethylbenziminazole. 5-(2′:4′-Dichlorophenylazo)-N-*D*-ribityl-o-4-xylidine.*—2:4-Dichloroaniline (1·65 g.) was diazotised by treating an ice-cold solution in water (75 ml.) and concentrated hydrochloric acid (5 ml.) with sodium nitrite (0·75 g.) in water. A paste of *N*-D-ribityl-*o*-4-xylidine (2·5 g.) in a little water was added with stirring, followed at once by sufficient sodium bicarbonate to make the mixture just alkaline. The *azo*-compound (2·0 g.) was collected after 24 hours, and was recrystallised from aqueous alcohol, forming red needles, m.pt. 158° to 160°C. (decomp.) Found: C, 52·8; H, 5·2; N, 9·7. $C_{19}H_{23}O_4N_3Cl_2$ requires C, 53·3; H, 5·4; N, 9·8 per cent. The compound gave a syrupy tetraacetate which could not be crystallised.

*1-Tetraacetyl-*D*-ribityl-5:6-dimethylbenziminazole.*—The acetyl-derivative of the foregoing compound (1·6 g.) was dissolved in ethyl acetate (240 ml.) and zinc dust (24 g.) added. The suspension was boiled and mechanically stirred whilst acetic acid (12 g.) in ethyl acetate (110 ml.) was slowly added over 1 hour. The colourless filtrate and washings were evaporated to dryness and the 2:4-dichloroaniline removed by washing with light petroleum. The residue was heated at 100°C. for 3 hours with ethyl orthoformate (5 ml.) and then concentrated to a syrup. 1-Tetraacetyl-D-ribityl-5:6-dimethylbenziminazole was isolated as the *picrate*, which crystallised from alcohol in yellow needles. M.pt. 153° to 154°C. Found: C, 49·7; H, 4·8; N, 10·8. $C_{22}H_{28}O_8N_2$, $C_6H_3O_7N_3$ requires C, 49·6; H, 4·6; N, 10·9 per cent.

The foregoing picrate (60 mg.) was decomposed by passing a chloroform solution through a column of alumina, and the tetraacetate hydrolysed by treatment with methyl alcoholic sodium methoxide. 1-D-Ribityl-5:6-dimethylbenziminazole was obtained as a syrup which was used without further purification for stability and chromatographic studies.

*5:6-Dimethylbenziminazole-1-(3′:4′:6′-triacetyl-2′-deoxy-*D*-glucopyranoside).* 5:6-Dimethylbenziminazole silver (9·6 g.), 1-bromo-3:4:6-

triacetyl-2-deoxy-D-glucose [prepared from 7·7 g. of triacetyl-D-glucal by the method of Davoll and Lythgoe (loc. cit.)] and xylene (80 ml.) were heated at 100°C. for 30 minutes with frequent shaking. The product, isolated in the usual way, was dissolved in alcohol (100 ml.) and treated with picric acid (5 g.). An insoluble, gummy picrate separated, which was washed several times by decantation and then decomposed by percolation through alumina in chloroform solution. The resulting syrupy triacetate (3 g.) was allowed to stand for 3 days with methyl alcohol containing a trace of sodium methoxide, whereafter the deacetylated deoxy-glucoside was isolated as the picrate (1·5 g.). 5:6-*Dimethylbenziminazole*-1-2′-*deoxy*-D-*glucopyranoside picrate* crystallised from alcohol in glistening yellow needles. M.pt. 189° (decomp.), [α] −4·9° (c=1, in pyridine). Found: C, 47·7; H, 4·7; N, 13·5. $C_{15}H_{20}O_4N_2$, $C_6H_3O_7N_3$ requires C, 48·4; H, 4·5; N, 13·4 per cent. The foregoing picrate was acetylated with acetic anhydride/pyridine and after removal of the solvents, the crude triacetate picrate was decomposed on alumina in chloroform solution. 5:6-*Dimethylbenziminazole*-1-(3′:4′:6′-*triacetyl*-2′-*deoxy*-D-*glucopyranoside*) crystallised from chloroform and light petroleum in sparkling needles, m.pt. 125° to 127°C. $[\alpha]_D^{25°C.}$ −39·4° (c=1, in chloroform). Found: C, 60·8; H, 6·6; N, 6·0. $C_{21}H_{26}O_7N_2$ requires C, 60·3; H, 6·3; N, 6·1 per cent.

5:6-*Dimethylbenziminazole*-1-2′-*deoxy*-D-*glucopyranoside* slowly crystallised from a solution of the triacetate (300 mg.) in methyl alcohol (20 ml.) containing sodium (5 mg.) and was recrystallised from methyl alcohol/light petroleum forming colourless prismatic needles, m.pt. 250°C. Found: C, 62·0; H, 7·0; N, 8·4. $C_{15}H_{20}O_4N_2$ requires C, 61·6; H, 6·9; N, 8·3 per cent. 5:6-*Dimethylbenziminazole*-1-(3′:4′-*diacetyl*-2′-*deoxy*-D-*ribopyranoside*). Diacetyl-D-arabinal (2 g.) was converted into 1-chloro-3:4-diacetyl-2-deoxy-D-ribose by the method of Davoll and Lythgoe[12]. The crude compound was reacted with 5:6-dimethylbenziminazole-silver (3 g.) in xylene (60 ml.) at 100°C. for 4 hours. Isolation in the usual way gave 5:6-*dimethylbenziminazole*-1-(3′:4′-*diacetyl*-2′-*deoxy*-D-*ribopyranoside*) *picrate* (1 g.), yellow needles from alcohol and chloroform, m.pt. 203°C. Found: C, 49·9; H, 4·3; N, 11·8. $C_{18}H_{22}O_5N_2$, $C_6H_3O_7N_3$ requires C, 50·1; H, 4·4; N, 12·2 per cent. The *diacetyl*-2′-*deoxy*-D-*riboside* formed tablets from ethyl acetate/light petroleum, m.pt. 118°C. $[\alpha]_D$ +2·6° (c=1, in chloroform). Found: C, 62·7; H, 6·2; N, 8·2. $C_{18}H_{22}O_5N_2$ requires C, 62·4; H, 6·4; N, 8·1 per cent.

5:6-*Dimethylbenziminazole*-1-2′-*deoxy*-D-*ribopyranoside.* A solution of the foregoing diacetate (500 mg.) in dry methyl alcohol (20 ml.) containing a trace of sodium was allowed to stand at room temperature for 3 days. Carbon dioxide was bubbled in and the solution then evaporated to dryness. Crystallisation of the residue from alcohol-light petroleum gave 5:6-dimethylbenziminazole-1-2′-deoxy-D-ribopyranoside as the *hydrate* in silky needles, m.pt. 160°C.$[\alpha]_D^{25°C.}$ +30·9° (c=0·5, in pyridine). Found: C, 60·1; H, 7·2; N, 9·8. $C_{14}H_{18}O_3N_2$. H_2O requires C, 60·0; H, 7·2; N, 10·0 per cent. The *picrate* formed yellow needles from

alcohol, m.pt. 203°C. Found: C, 48·7; H, 4·4. $C_{14}H_{18}O_3N_2,C_6H_3O_7N_3$ requires C, 48·9; H, 4·3 per cent.

Benziminazole-1-2′-deoxy-D-ribopyranoside. 1-Chloro-3:4-diacetyl-2-deoxy-D-ribose, from D-arabinal (4·9 g.) was condensed with benziminazole silver (7·4 g.) in xylene solution at 100°C. The filtrate and washings were taken to dryness and treated with alcoholic picric acid (5 g.). The resulting picrate was extracted with chloroform and the soluble fraction (2·8 g.) recrystallised from alcohol to give *benziminazole-1-(3′:4′-diacetyl-2′-deoxy-D-ribopyranoside picrate* in yellow needes, m.pt. 167° to 168°C. $[\alpha]_D^{26°C.}$ −8·6° (c=1, in pyridine). Found: C, 48·3; H, 3·9; 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 per cent. Decomposition of this compound by passing a chloroform solution through alumina gave only a resinous triacetate which was therefore directly deacetylated with sodium methoxide in methanol. The deoxy-D-riboside so obtained was isolated as the *picrate*, yellow needles from alcohol, m.pt. 170°C.$[\alpha]_D^{22°C.}$ −14·8° (c=1, in pyridine). Found: C, 46·5; H, 3·7; N, 14·7. $C_{12}H_{14}O_5N_2$, $C_6H_3O_7N_3$ requires C, 46·6; H, 3·7; N, 15·1 per cent., which was decomposed by shaking with dilute hydrochloric acid and nitrobenzene. Evaporation of the aqueous layer gave *benziminazole-1-2′-deoxy-D-ribopyranoside hydrochloride hydrate,* long needles from alcohol, m.pt. 150°C.$[\alpha]_D^{24°C.}$ −34·5° (c=0·5, in water). Found: C, 50·0; H, 6·0; N, 9·7. $C_{12}H_{14}O_3N_2HCl,H_{20}$ requires C, 49·9; H, 5·9; N, 9·7 per cent.

5:6-*Dimethylbenziminazole-1-β-triacetyl-D-ribopyranoside.* (i) 5-Nitro-*o*-4-xylidine-triacetyl-D-riboside (3 g. Kuhn and Strobële[13]) in ethyl acetate (50 ml.) was shaken with hydrogen in the presence of a palladised charcoal catalyst until hydrogen uptake was complete. After removal of the catalyst, ethyl orthoformate (10 ml.) was added and the solution heated on the steam bath in an open flask for 3 hours. The resin remaining after removal of the solvent was heated with 0·1N hydrochloric acid (15 ml.) for 10 minutes, whereafter the solution was basified with potassium carbonate and the product extracted with chloroform and converted to the picrate. 5:6-*Dimethylbenziminazole-1-β-triacetyl-D-ribopyranoside picrate* (500 mg.) formed yellow needles from alcohol, m.pt. 186° to 187°C. Found: C, 48·9; H, 4·7; 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 *base* crystallised in colourless plates from chloroform and light petroleum, m.pt. 155°C.$[\alpha]_D^{24°C.}$ −40·4° (c=1, in chloroform). Found: C, 59·6; H, 6·2; N, 7·0. $C_{20}H_{24}O_7N_2$ requires C, 59·4; H, 6·0; N, 6·9 per cent. (ii) 5:6-Dimethylbenziminazole silver (1·3 g.) and acetobrom-D-ribose (1·65 g.) were condensed together in xylene (40 ml.) at 140°C. in the usual way. The product was purified by passing a chloroform solution through a short column of alumina and then converted into the *picrate* (600 mg.), which crystallised from alcohol in yellow needles identical in m.pt. and mixed m.pt. with the compound prepared by method (i).

5:6-*Dimethylbenziminazole-1-β-D-ribopyranoside hydrochloride hemihydrate* was prepared by hydroylsis of the foregoing triacetate (500 mg.) with 2N hydrochloric acid (50 ml.) at 100°C. followed by evaporation to

dryness. It separated from alcohol/ether in small needles, m.pt. 229° to 230°C. (decomp.). Found: C, 51·5; H, 6·3. $C_{14}H_{18}O_4N_2,HCl,\frac{1}{2}H_2O$ requires C, 51·9; H, 6·2 per cent. The *riboside* crystallised from alcohol-light petroleum in flat needles, m.pt. 250° to 251°C. (decomp.). Found: S, 60·2; H, 6·2; N, 10·0. $C_{14}H_{18}O_4N_2$ requires C, 60·4; H, 6·5; N, 10·1 per cent. This compound consumed 2·05 moles. of periodic acid.

Section B. Whatman No. 1 filter paper was employed for the chromatograms. The benziminazole derivatives examined on chromatograms were detected by inspection of the papers under the ultra-violet light transmitted by a low-pressure mercury resonance lamp fitted with a Chance OX7 filter, when they appeared as blue-violet fluorescent spots. The latter filter was found to be just as suitable as the Corning 9863 filter previously employed for this purpose[1]. The α- and β-*components* present in hydrolysates of vitamin B_{12} and required for degradation studies, were obtained from chromatogram segments which were eluted by the method of Dent[14].

The stabilities to hydrochloric acid of the following compounds were investigated.

(a) The β-*component.*
(b) 5:6-Dimethylbenziminazole-1-β-D-ribopyranoside (XIIa; R′=H).
(c) 1-D-Ribityl-5:6-dimethylbenziminazole (IV; R=H).
(d) 5:6-Dimethylbenziminazole-1-(2′-deoxy-D-ribopyranoside) (XIIc; R=H).

Solutions of each of the above substances in 6N hydrochloric acid were heated in sealed tubes for 12 hours at (i) 100°C. and (ii) 150°C. and the products submitted to paper chromatography employing *n*-butyl alcohol-acetic acid[15] as the irrigation solvent. The results are recorded in Table I.

TABLE I

Compound	R_F value of compound	R_F values of products obtained after 12 hours at		R_F value of 5:6-dimethyl-benziminazole
		(i) 100°C.	(ii) 150°C.	
(a)	0·76	0·76	0·84	0·84
(b)	0·76	0·76	0·84	0·84
(c)	0·76	0·76	0·76	0·84
(d)	0·79	0·84 (0·79)	0·84	0·84

Compounds (a) and (b) behaved identically in every respect. Thus both were characterised by identical R_F values, were stable under conditions (i), and were completely degraded into the parent benziminazole under conditions (ii). Compound (c) proved to be completely stable even under the latter drastic conditions of acidolysis. In contrast to these results, compound (d) under conditions (i) gave rise on the chromatogram to two spots of differing intensity, the R_F value of the less intense spot being shown in brackets in the above Table. The degradation of this substance into the parent benziminazole was therefore largely complete under conditions (i).

Compounds (a) and (b) were also chromatographically indistinguishable when *n*-butyl-alcohol-acetic acid-water (4:1:1) or aqueous saturated secondary butyl alcohol were employed as the irrigation solvents. With these systems, R_F values of 0·72 and 0.90 respectively were obtained.

The chromatographic behaviour and stabilities to 6N hydrochloric acid of a number of synthetic benziminazole glycosides[3] were determined in a like manner. The compounds examined were: —

1. Benziminazole-1-β-D-glucopyranoside.
2. 4:5-Dimethylbenziminazole-1-β-D-glucopyranoside hydrochloride.
3. 5:6-Dimethylbenziminazole-1-β-D-glucopyranoside.
4. Benziminazole-1-α-L-arabopyranoside.
5. Benziminazole-1-β-D-xylopyranoside.
6. 5-Methylbenziminazole-1-β-D-xylopyranoside.
7. 5:6-Dimethylbenziminazole-1-L-rhamnopyranoside.

The R_F values obtained employing *n*-butyl-alcohol-acetic acid[15] as the irrigation solvent are shown in Table II.

TABLE II

Compound No.	R_F value of compound	R_F values of products obtained after 12 hours at		R_F value of parent benziminazole unsubstituted in 1-position
		(i) 100°C.	(ii) 150°C.	
1	0·46	0·46	0·76 (0·47)	0·75
2	0·61	0·61	0·84 (0·61)	0·84
3	0·59	0·60	0·83 (0·61)	0·84
4	0·50	0·51	0·76	0·75
5	0·58	0·58	0·75	0·75
6	0·62	0·61	0·75	0·74
7	0·71	0·71	0·83 (0·72)	0·84

Glycosides nos. 1, 2, 3 and 7 each gave rise to two benziminazole spots of differing intensity under conditions (ii). In each of these cases the R_F value of the less intense of the two spots is given in brackets.

The close correspondence between the R_F values of the compounds themselves with those obtained under (i) indicates that all the glycosides examined were unaffected by 6N hydrochloric acid for 12 hours at 100°C. On the other hand, comparison of the figures under (ii) with those given in the last column of Table II indicates that under the more drastic conditions of acidolysis employed, four of the glycosides were mostly, and the other three completely, degraded into the parent benziminazoles unsubstituted in the 1-position.

Summary and Conclusions

1. The order in which phosphate, the "ninhydrin-reacting" fragment (1-aminopropan-2-ol), and the *α-, β- and γ-components* are released from vitamin B_{12} on acid hydrolysis has been determined.

2. The presence of phosphorus in the *α-component* is reported.

3. The bearing of the results on the inter-relationship and structure of *α-, β-* and *γ-components* is discussed.

4. 1-D-Ribityl-5:6-dimethylbenziminazole and 5:6-dimethylbenziminazole-1-β-D-ribopyranoside have been synthesised and each compared with the *β-component* in respect to both stability towards hydrochloric acid and behaviour on paper chromatography.

5. 5:6-Dimethylbenziminazole-1-β-D-ribopyranoside and the *β-component* behaved identically on acidolysis and also on paper chromatograms irrigated with three different solvent systems.

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