THE CHEMISTRY OF ANTI-PERNICIOUS ANÆMIA FACTORS

PART IV.—BENZIMINAZOLE GLYCOSIDES

(1) THE PREPARATION AND PROPERTIES OF SOME *o*-NITROANILINE GLYCOSIDES

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IN experiments on the degradation of Vitamin B_{12}, Ellis, Petrow and Snook[1] extracted the coloured cobalt-containing complex formed on acidolysis and examined the colourless cobalt-free phase spectroscopically. Selective absorption in the ultraviolet was observed in the region of 2850 Å, the material responsible being designated " the 285-component." Subsequent work, by Beavan *et al.*, outlined in Part III[2] revealed the complex character of " the 285-component " which was resolved by paper chromatography into three related compounds designated *components* α, β and γ. Spectroscopic and chemical studies indicated the identity of *component* γ with 5 : 6-dimethylbenziminazole and of *components* α and β with 1-substituted 5 : 6-dimethylbenziminazoles. It was therefore inferred that *components* α, β and γ represented different stages of degradation of a common precursor. The isolation of 5 : 6-dimethylbenziminazole from acid hydrolysates of vitamin B_{12} was announced simultaneously[3] by Brink and Folkers[4].

The formation by acid hydrolysis of vitamin B_{12} of two 1-substituted 5 : 6-dimethylbenziminazoles as well as 5 : 6-dimethylbenziminazole itself, led to the conclusion that the 1-substituent was capable of stepwise degradation by acid. This fact, together with the structural similarity between vitamin B_{12} and riboflavine implicit in the existence of an N-substituted 4 : 5-dimethyl-*o*-phenylenediamine residue in both compounds, led to the further conclusion that the grouping may be glycosidic in character. The synthesis of benziminazole glycosides was accordingly undertaken with the object of examining their behaviour on acidolysis. In addition, such compounds were required for phosphorylation studies following the discovery that, on hydrolysis, the α-component passed smoothly into the β-component and phosphate.

The synthesis of sugar derivatives of benziminazole has not hitherto been reported in the literature. The synthesis of nucleosides, a group which bears a formal analogy to the benziminazole glycosides, however, has formed the subject of detailed study by a number of workers. Thus Fischer and Helferich[5] prepared the D-glucosides of adenine, guanine and hypoxanthine from the reaction product of acetobromglucose and trichloropurine silver, a method subsequently exploited by Levene and Sobotka[6] and by Davoll, Lythgoe and Todd[7]. A major contribution to the synthesis of nucleosides, however, is contained in a series of publications by Todd, Lythgoe and their collaborators[8]. In addition to extending the silver salt route, Todd *et al.* elaborated a new synthesis of nucleosides in which a 5-amino-4-glycosidamino pyrimidine is treated with dithio-

formic acid[9] when a 4-glycosidamino-5-thioformamidopyrimidine is obtained, which is converted into the purine by treatment with basic reagents[9,10,11].

Our own synthetic experiments followed, in outline, the pattern established for the nucleosides, methods analogous to the silver salt route and Todd's glycosidaminopyrimidine route being successfully developed.

o-Phenylenediamine glycosides (e.g.E) were required for the latter study and their preparation forms the subject of the present communication.

The glycosidation of *o*-nitroanilines by boiling with hexose and pentose sugars in ethyl alcoholic solution in the presence of ammonium chloride was described by Kuhn and Strobële[12] in 1937. As the reaction involves the anomeric centres of the sugar components it is not surprising that two D-arabinosides and two L-arabinosides of 5-nitro-*o*-4-xylidine were obtained, whilst the other glycosides described melted over a range of temperatures. Kuhn and Strobële (*loc. cit.*) claimed, however, that the isomerism disappeared on acetylation, the same 5-nitro-*o*-4-xylidine-triacetyl-L-arabinoside being obtained, for example, from the two modifications of the unacetylated glycoside.

We have now extended these observations to include the preparation of glycosides from D-glucose, D-mannose, D-galactose, L-arabinose, D-xylose, D-ribose and L-rhamnose as the sugar component and *o*-nitroaniline, *m*-nitro-*p*-toluidine, 3- and 5-nitro-*o*-4-xylidines as the aglycone. As nearly all these compounds proved heterogeneous in the crude state, it appears that a mixture of isomers is invariably formed in this reaction. The relative proportions obtained, however, vary over a wide range with the nature of the reactants and the experimental conditions employed. Furthermore one isomer is occasionally formed to the almost complete exclusion of the other.

When L-arabinose was employed as the sugar component the isomeric L-arabinosides were formed in approximately equal amounts and were conveniently separated by crystallisation from alcohol. Following the nomenclature devised by Kenner, Lythgoe and Todd[13] for the analogous aminopyrimidine glycosides, we differentiate these by the suffixes I and II. The distinction is an arbitrary one and in our usage is based solely upon rotational data, the L-arabinoside with the highest positive rotation being designated the series I isomer. Structural similarity within the series is assumed. The rotations obtained are recorded in Table I, column (i). Serious attempts to fractionate the isomeric glycosides were limited to those derived from L-arabinose. In all other instances the reaction products were directly acetylated with acetic anhydride in pyridine solution. In our hands, however, and in contrast to the observations recorded by Kuhn and Strobële (*loc. cit.*), these acetylated products were usually found to consist of two isomeric acetates. Thus, for example, acetylation of 5-nitro-*o*-4-xylidine-L-arabinosides (mixed isomers) gave the series I triacetate, m.pt. 213° to 214°C., $[\alpha]_D$ +142° (in chloroform), already described by Kuhn and Strobële, together with smaller quantities of 5-nitro-*o*-4-xylidine-triacetyl-L-arabinoside II, m.pt. 143° to 144°C., $[\alpha]_D$ +24° (in chloroform). Some of these results are collected in Table I, column (ii).

Following these unexpected observations we turned our attention to the direct acetylation of the pure *o*-nitroaniline glycosides listed in column (i) of Table I, and obtained, in each instance, only the pure

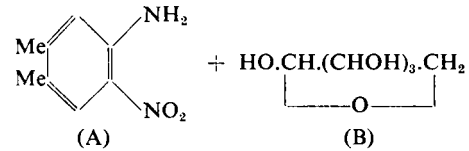
TABLE I

Aglycone		(i) L-arabinosides		(ii) triacetyl-L-arabinosides	
		M.pt.	$[\alpha]_D$ in pyridine c = 1	M.pt.	$[\alpha]_D$ in chloroform c = 1
o-Nitroaniline	Series I	159° to 161°C.	+55·5°	154°C.	+159·1°
	Series II	173° to 175°C.	+21·1°	90° to 91°C.	−23°
m-Nitro-p-toluidine ...	I	133° to 135°C.	+83·6°	194°C.	+146·5°
	II	141° to 142°C.	−10°	77° to 79°C.	−22·6°
5-Nitro-*o*-4-xylidine ...	I	187·5°C.	+71°	213° to 214°C.	+142°
	II	108° to 109°C.	−7·8°*	143° to 144°C.	+ 24°

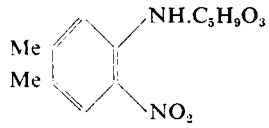
* Kuhn and Strobële (*loc. cit.*) give $[\alpha]_D$ +26°.

triacetate of the same series. The view expressed by Kuhn and Strobële that isomerisation of *o*-nitroaniline glycosides takes place on acetylation is thus shown to be erroneous and to lack experimental foundation. Their sample of 5-nitro-*o*-4-xylidine-L-arabinoside II which yielded the series I triacetate on acetylation must have been admixed with the Series I isomer and it is significant that the rotation of our sample of 5-nitro-*o*-4-xylidine-L-arabinoside II is considerably lower than the value recorded by the German authors.

We have, *inter alia*, made the interesting observation that the triacetyl-L-arabinosides II are converted into the series I compounds by simply

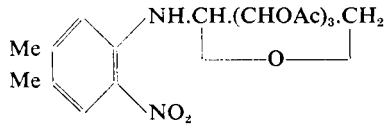


(A) (B)



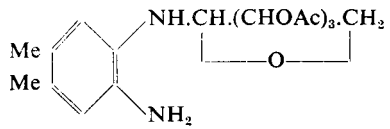
(C)

5-Nitro-*o*-4-xylidine-L-arabinosides I and II.



(D)

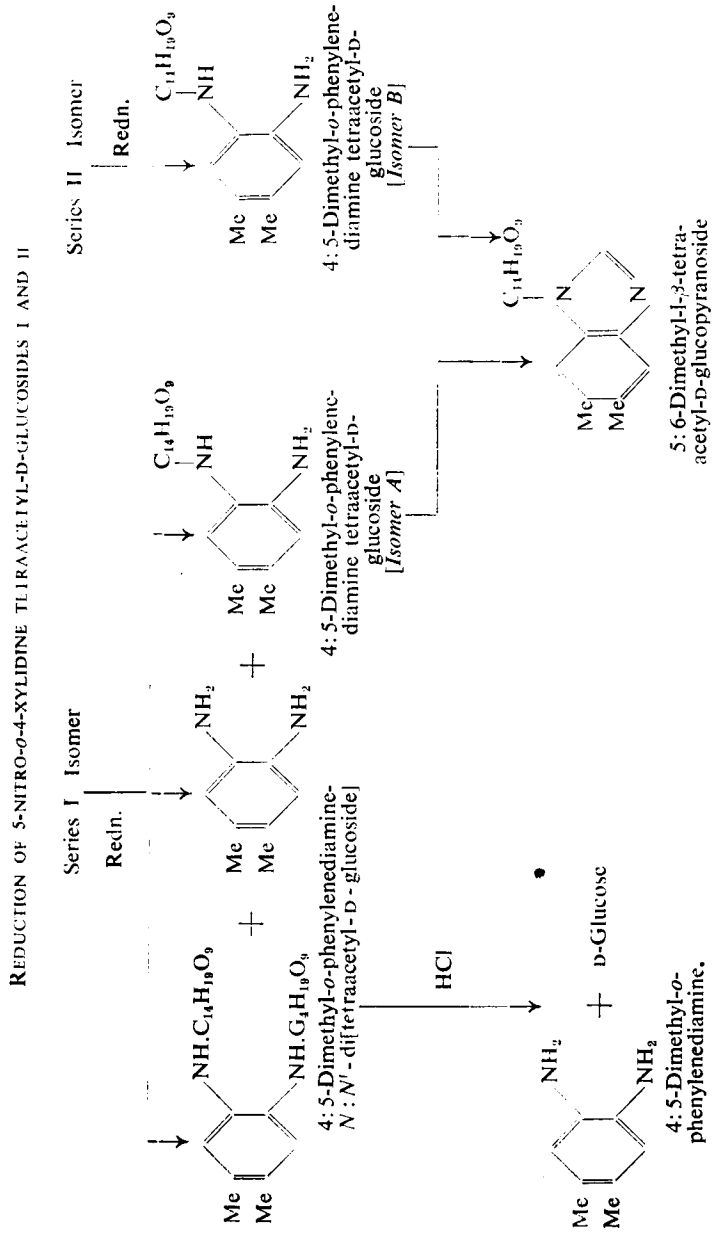
5-Nitro-*o*-4-xylidine triacetyl-L-arabinosides I and II.



(E)

4: 5-Dimethyl-*o*-phenylenediamine triacetyl-L-arabinoside

heating for a few seconds with 2N hydrochloric acid containing a little ethyl alcohol. The change is irreversible and prolonged heating results in fission of the molecule with regeneration of the components. This behaviour was found to be characteristic of all the Series II acetylated glycosides examined and indicates that the difference between the Series



I and Series II isomers involves only the anomeric centres of the sugars. It also excludes the possibility that the difference might be due to an Amadori rearrangement[14].

Catalytic reduction of *m*-nitro-*p*-toluidine-tetraacetyl-D-glucosides I and II gave the same 4-methyl-*o*-phenylenediamine-tetraacetyl-D-glucoside. It is thus evident that in this instance the isomerism existing between the two series of acetylated *o*-nitroaniline glycosides disappear upon reduction of the nitro-grouping. Similar results have been recorded in the pyrimidine series by Kenner, Lythgoe and Todd[13], who observed that reductive fission of the isomeric 6-amino-4-triacetyl-D-xylosidamino-5-(2′ : 5′-dichlorobenzeneazo)-pyrimidines I and II led to the same 5 : 6-diamino-4-triacetyl-D-xylosidaminopyrimidine.

Experiments on the reduction of 5-nitro-*o*-4-xylidine tetraacetyl-D-glycosides I and II gave somewhat different results. The Series II isomer, $[\alpha]_D+111°$ (in chloroform) passed into a crystalline 4 : 5-dimethyl-*o*-phenylenediamine tetraacetyl-D-glucoside [*isomer B**], m.pt. 128° to 129°C. $[\alpha]_D-31\cdot6°$ (in chloroform) in excellent yield. The Series I isomers $[\alpha]_D-62°$ (in chloroform), in contrast, gave rise to a product, $C_{36}H_{48}O_{18}N_2$, m.pt. 180° to 181°, $[\alpha]_D-19\cdot4°$ (in chloroform) together with smaller quantities of a 4 : 5-dimethyl-*o*-phenylenediamine tetraacetyl-D-glucoside (*isomer A*). The latter resembled *isomer B* in giving the same 5 : 6-dimethylbenziminazole-1-β-tetraacetyl-D-glucopyranoside (see Part IV (2)), but differed from it in the facility with which this transformation could be effected.

The constitution of a 4 : 5-dimethyl-*o*-phenylene-diamine-*N* : *N*′-di[tetraacetyl-D-glucoside], has been assigned to the main reduction product, m.pt. 180° to 181°C. of the Series I isomer. This formulation is based on its conversion by acid hydrolysis, into D-glucose, identified as the osazone, and 4 : 5-dimethyl-*o*-phenylenediamine identified as 5 : 6-dimethylbenziminazole. In addition, its ultraviolet absorption spectrum shows the typical characteristics of a substituted 4 : 5-dimethyl-*o*-phenylenediamine (see Fig. 1). Its formation presumably involves the dismutation of a tetraacetyl glucose residue between two *o*-phenylenediamine glycoside groupings, with parallel formation of the parent base, identified as present in the products of reduction by conversion into 5 : 6-dimethyl-benziminazole and isolation as the picrate (see page. 494).

The other *o*-phenylenediamine glycosides prepared during this phase of the work proved to be compounds of low crystallising power. In all the cases examined, however, the same benziminazole glycoside was obtained from both Series I and Series II nitroglycosides (see Parts IV (2) and IV (3)), so that merging of the two series must have occurred either during the reduction of the nitroaniline glycosides, or during the conversion of the resulting *o*-phenylenediamine glycosides into the corresponding benziminazole glycosides. As the latter proved to be glyco-

* The terminology Series I and II has not been employed in this instance as the $[\alpha]_D$ of *isomer B* would appear to indicate its adherence to Series I. Direct comparison with *isomer A* to settle this point was not possible, however, as attempts to isolate the latter compound in a state of purity proved unsuccessful. Its existence was inferred, however, by its facile conversion into the corresponding benziminazole glycoside (Part IV (2)).

pyranosides (see Parts IV (2) and IV (3)), the pyranose formulation must be adopted for both the *o*-nitroaniline- and *o*-phenylenediamine acetyl glycosides unless migration of an acetyl grouping be assumed, an eventuality which is, in our opinion, very slight. The nature of the isomerism in these two classes of compounds must consequently be of the α β type at the glycosidic centre, a view which accords well with the conversion of the Series II *o*-nitroaniline acetylglycosides into their Series I anomers

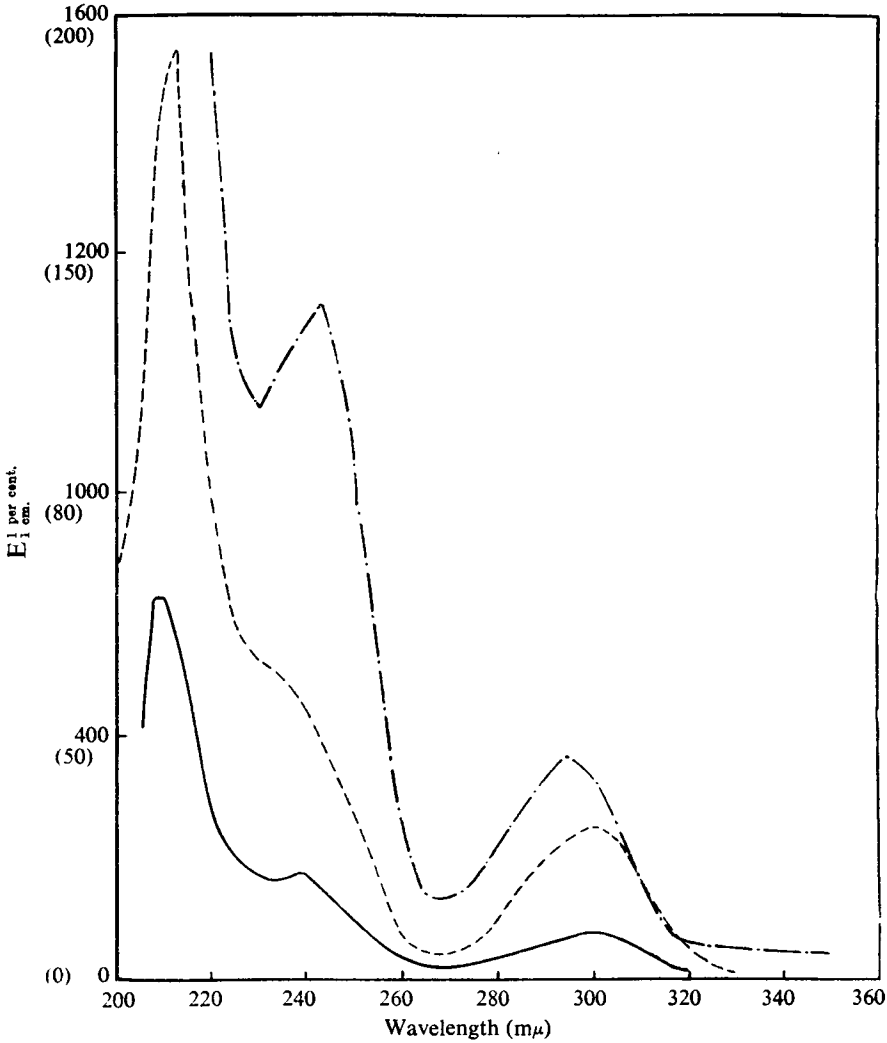


FIG. 1. Absorption curves.

———— 4 : 5-Dimethyl-*o*-phenylenediamine-tetraacetyl-D-glucoside.
—·—·— 4 : 5-Dimethyl-*o*-phenylenediamine-*N* : *N*′-di-(tetraacetyl-D-glucoside).
– – – – 4 : 5-Dimethyl-*o*-phenylenediamine.

In order to represent the absorption curve for 4 : 5-dimethyl-*o*-phenylenediamine-*N* : *N*′-di (tetraacetyl-D-glucoside) on this figure it has been necessary to plot the $E_{1\text{ cm.}}^{1\text{ per cent.}}$ values for this compound on the scale 0-200.

on warming with dilute acid (*vide supra*). Unfortunately it cannot be argued that the parent *o*-nitroaniline glycosides likewise have the pyranose-structure, as furanose → pyranose changes are known to occur on acetylation[15]. Kuhn and Strobële (*loc. cit.*) originally assigned furano-structure to their *o*-nitroaniline glycosides, but the experimental foundation for this postulate, the formation of trityl-derivatives, is now known to be unreliable. Howard, Kenner, Lythgoe and Todd[15] have, in fact, demonstrated that *o*-nitroaniline-L-arabinoside has the pyranose structure. The complete elucidation of the structures of the *o*-nitroaniline glycosides described in this communication, with this one exception, must, therefore, await further investigation.

EXPERIMENTAL

Melting points are corrected. Microanalyses are by Drs. Weiler and Strauss, Oxford. The specific rotations of the glycosides and of the acetylated glucosides were measured (c=1) in pyridine and chloroform solutions, respectively.

Preparation of the o-*Nitroaniline glycosides.* The following general method was employed (cf. Kuhn and Strobële, *loc. cit.*). The sugar (0·1 mol.), the appropriate *o*-nitroaniline (0·2 to 0·3 mol.), ammonium chloride (2 g.) and carefully dried alcohol (200 to 300 ml.) were heated under reflux on the steam bath with exclusion of moisture until the sugar had dissolved. Heating was then continued for a further 30 minutes. A portion of the solvent was removed by distillation, and the residual liquor poured onto a column of alumina. Excess *o*-nitroaniline present was removed by washing with benzene, whereafter the glycoside was eluted with alcohol (50 per cent.) or with water. The eluate was concentrated under reduced pressure to small volume, or until the glycoside began to separate. After standing at 0° to 5°C. for 24 hours the product was collected, washed with ice-water, and dried *in vacuo*.

The following compounds were prepared in this way :—

m-*Nitro*-p-*toluidine*-D-*glucoside* (yield 50 per cent.), golden-yellow needles from water, m.pt. 120° to 128°C. Found : C, 49·7 ; H, 6·3 ; N, 9·0. $C_{13}H_{18}O_7N_2$ requires C, 49·7 ; H, 5·8 ; N, 8·9 per cent.

o-*Nitroaniline*-D-*mannoside* (yield 60 per cent.), yellow needles from alcohol, m.pt. 214° to 215° (decomp.). Found : C, 48·4 ; H, 5·3. $C_{12}H_{16}O_7N_2$ requires C, 48·0 ; H, 5·4 per cent.

m-*Nitro*-p-*toluidine*-D-*mannoside* (yield 55 per cent.), crystallising in fine yellow needles from alcohol, m.pt. 205° to 206°C. (decomp.). Found : C, 48·6 ; H, 6·2. $C_{13}H_{18}O_7N_2$, $\frac{1}{2}H_2O$ requires C, 48·3 ; H, 5·9 per cent.

3-*Nitro*-o-4-*xylidine*-D-*mannoside* (yield 85 per cent.) formed silky orange needles from alcohol, m.pt. 215° to 216°C. $[\alpha]_D^{20°C.}$ −28·5°. Found : C, 51·2 ; H, 6·0 ; N, 8·4. $C_{14}H_{20}O_7N_2$ requires C, 51·2 ; H, 6·1 ; N, 8·5 per cent..

5-*Nitro*-o-4-*xylidine*-D-*mannoside* crystallised in orange needles from alcohol which turned yellow at 110° to 210°C. and melted at 213°C. (decomp.), $[\alpha]_D^{25°C.}$ −35·0°. Found : C, 51·0 ; H, 6·4 ; N, 8·3. $C_{14}H_{20}O_7N_2$ requires C, 51·2 ; H, 6·1 ; N, 8·5 per cent.

m-*Nitro*-p-*toluidine*-D-*galactoside* (yield 33 per cent.), crystallised from alcohol in yellow needles, m.p. 204°C. (decomp.). Found : C, 49·2 ; H, 5·8 ; N, 8·5. $C_{13}H_{18}O_7N_2$ requires C, 49·7 ; H, 5·8 ; N, 8·9 per cent.

o-*Nitroaniline*-L-*arabinosides*. The aqueous eluate from the column (*vide supra*) was allowed to stand in a refrigerator and the crystalline deposit collected after 24 hours. The latter comprised almost pure o-*nitroaniline*-L-*arabinoside* II, which, when recrystallised from alcohol, formed hard, orange-yellow needles, m.p. 173° to 175°C. $[\alpha]_D^{22°C.}$ + 21·1°. Found : C, 48·8 ; H, 5·2 ; N, 10·6. $C_{11}H_{14}O_6N_2$ requires C, 48·9 ; H, 5·2 ; N, 10·3 per cent. The mother liquors were taken to dryness *in vacuo*, and the residue carefully fractionated from alcohol. After separation of a further crop of the Series II isomer, the Series I arabinoside was crystallised by addition of light petroleum. o-*Nitroaniline*-L-*arabinoside* I formed orange needles from a mixture of alcohol and light petroleum, m.p. 159° to 161°C., $[\alpha]_D^{23°C.}$ +55·5°. Found : C, 49·3 ; H, 5·1 ; N, 10·0 per cent.

m-*Nitro*-p-*toluidine*-L-*arabinosides*. The crude mixture of arabinosides was fractionated in the manner described above. The series II L-arabinoside crystallised as the *hydrate* in orange needles from alcohol, m.p. 141° to 142°C. $[\alpha]_D^{20°C.}$ −10°. Found : C, 47·4 ; H, 6·0 ; N, 9·0. $C_{12}H_{16}O_6N_2$, H_2O requires C, 47·7 ; H, 6·0 ; N, 9·3 per cent. The Series II *isomer* formed rosettes of orange needles from alcohol, m.p. 133° to 135°C. $[\alpha]_D^{25°C.}$ +83·6°. Found : C, 50·9·; H, 5·7 : N, 9·7. $C_{12}H_{16}O_6N_2$ requires C, 50·7 ; H, 5·6 ; N, 9·9 per cent.

5-*Nitro*-o-4-*xylidine*-L-*arabinosides*. The mixture of isomers was fractionated from alcohol by the method described by Kuhn and Strobële (*loc. cit.*). The Series I compound had m.p. 187·5°C. $[\alpha]_D^{24°C.}$ +71·0° and the Series II, m.p. 108° to 109°C. $[\alpha]_D^{22°C.}$ −7·8°.

o-*Nitroaniline*-D-*xyloside*, yellow prisms from a mixture of alcohol and light petroleum, m.p. 172° to 176°C. Found : C, 48·7 ; H, 5·2 ; N, 10·3. $C_{11}H_{14}O_6N_2$ requires C, 48·9 ; H, 5·2 ; N, 10·3 per cent.

m-*Nitro*-p-*toluidine*-D-*xyloside* crystallised from aqueous alcohol m.pt., *ca.* 170°C. Found : C, 50·3 ; H, 5·9 ; N, 9·7. $C_{12}H_{16}O_6N_2$ requires C, 50·7 ; H, 5·6 ; N, 9·9 per cent.

5-*Nitro*-o-4-*xylidine*-D-*xyloside*, orange prisms from a mixture of methyl alcohol and ethyl acetate, m.pt. 212° to 213°C. (decomp.). Found : N, 9·5. $C_{13}H_{18}O_6N_2$ requires N, 9·4 per cent.

o-*Nitroaniline*-L-*rhamnoside* (yield 50 per cent.) formed silky yellow needles from alcohol, m.pt. 225°C. (decomp.). Found : C, 50·9 ; H, 5·3 ; N, 9 7. $C_{12}H_{16}O_6N_2$ requires C, 50·7 ; H, 5·7 : N, 9·9 per cent.

m-*Nitro*-p-*toluidine*-L-*rhamnoside* crystallised from alcohol in yellow needles, m.pt. 215°C. (decomp.). Found : C, 52·7 ; H, 6·1 ; N, 9·4. $C_{13}H_{18}O_6N_2$ requires C, 52·4 ; H, 6·1 ; N, 9·4 per cent.

A number of the *o*-nitroaniline-glycosides were not purified as such, but were directly acetylated as described below :—

Acetylation of the o-*Nitroaniline-glycosides and Fractionation of the resulting mixtures of Acetates*. Acetic anhydride (1·0 to 1·2 mols.) was

added to a cooled solution of the glycoside (0·1 mol.) in pyridine (200 to 250 ml.). After standing at room temperature for 24 hours, the excess of acetic anhydride was destroyed by addition of alcohol, and the solvents removed under reduced pressure. The residue was re-evaporated once or twice with alcohol, and then recrystallised once from the minimum of alcohol (with charcoal if necessary) to give the mixture of acetates. The latter was fractionated by crystallisation from ethyl acetate. The first crop, which comprised the series I isomer, was collected and washed with a little ethyl acetate. The Series II acetate was recovered from the filtrate either by evaporation to dryness, or by addition of light petroleum. Both acetates were then crystallised to constant melting-point and rotation from the appropriate solvent. The total yield of the acetates generally exceeded 85 per cent. of the theoretical yield.

o-*Nitroaniline-tetraacetyl*-D-*glucoside* crystallised from a mixture of alcohol and ethyl acetate in yellow prisms, m.pt. 184°C. (cf. Kuhn and Strobële *loc. cit.*). Evaporation of the filtrates gave only small amounts of β-pentacetyl-D-glucose, m.pt. 129° to 130°C.

m-*Nitro*-p-*toluidine-tetraacetyl*-D-*glucoside* I crystallised from alcohol in yellow needles or prisms, m.pt. 180°C. $[\alpha]_D^{23°C.}$ −51·7°. Found: C, 52·9; H, 5·3; N, 5·9. $C_{21}H_{26}O_{11}N_2$ requires C, 52·3; H, 5·4; N, 5·8 per cent. The Series II *isomer* separated from alcohol in yellow needles, m.pt. 129°C. $[\alpha]_D^{23°C.}$ +107·7°. Found: C, 52·5; H, 5·3; N, 5·7 per cent.

3-*Nitro*-o-4-*xylidine-tetraacetyl*-D-*glucoside* I formed prismatic yellow needles from alcohol, m.pt. 157°C. $[\alpha]_D^{24°C.}$ −172·5°. Found: C, 53·4; H, 5·8; N, 5·7. $C_{22}H_{28}O_{11}N_2$ requires C, 53·2; H, 5·7; N, 5·6 per cent.

5-*Nitro*-o-4-*xylidine-tetraacetyl*-D-*glucoside* was described by Kuhn and Strobële (*loc. cit.*) as a compound of indefinite melting point. This mixture of isomers was readily separated by crystallisation from ethyl acetate. The Series I isomer formed yellow needles from ethyl acetate, m.pt. 168° to 169°C. $[\alpha]_D^{24°C.}$ −62°. Found: C, 53·6; H, 5·5; N, 5·8. $C_{22}H_{28}O_{11}N_2$ requires C, 53·2; H, 5·7; N, 5·6 per cent. The Series II compound separated from alcohol in prismatic yellow needles, m.pt. 150° to 151°C. $[\alpha]_D^{22°C.}$ +111°. Found: C, 53·2; H, 5·6; N, 5·8 per cent.

No attempts were made to fractionate the following galactosides and mannosides :—

o-*Nitroaniline-tetraacetyl*-D-*galactoside* separated in fine yellow needles from alcohol, m.pt. 178°C. $[\alpha]_D^{25°C.}$ −34·6°. Found: C, 51·3; H, 5·4; N, 5·9. $C_{20}H_{24}O_{11}N_2$ requires C, 51·3; H, 5·2; N, 6·0 per cent.

m-*Nitro*-p-*toluidine-tetraacetyl*-D-*galactoside* formed yellow platelets from alcohol, m.pt. 190°C. $[\alpha]_D^{22°C.}$ −21·6°. Found: C, 52·6; H, 5·3; N, 5·8. $C_{21}H_{26}O_{11}N_2$ requires C, 52·3; H, 5·4; N, 5·8 per cent.

3-*Nitro*-o-4-*xylidine-tetraacetyl*-D-*galactoside* crystallised in prismatic orange-yellow needles from ethyl acetate, m.pt. 196° to 197°C. $[\alpha]_D^{23°C.}$ −149·2°. Found: C, 53·3; H, 5·5; N, 5·3. $C_{22}H_{28}O_{11}N_2$ requires C, 53·2; H, 5·7; N, 5·6 per cent.

5-*Nitro*-o-4-*xylidine-tetraacetyl*-D-*galactoside* formed yellow octahedra

from alcohol, m.p. 180°C. $[\alpha]_D^{23°C.}$ −23·4°. Found : C, 53·3 ; H, 5·6 ; N, 5·3. $C_{22}H_{28}O_{11}N_2$ requires C, 53·2 ; H, 5·7 ; N, 5·6 per cent.

o-*Nitroaniline-tetraacetyl*-D-*mannoside* formed lemon yellow prismatic needles, m.p. 127° to 128°C. $[\alpha]_D^{21°C.}$ −103·0°. Found : C, 51·7 ; H, 5·2 ; N, 6·1. $C_{20}H_{24}O_{11}N_2$ requires C, 51·3 ; H, 5·2 ; N, 6·0 per cent.

m-*Nitro*-p-*toluidine-tetraacetyl*-D-*mannoside* separated from a mixture of ethyl acetate and light petroleum in golden-yellow needles, m.p. 144° to 145°C. $[\alpha]_D^{21°C.}$ −97·8°. Found : C, 52·8 ; H, 5·2 ; N, 5·7. $C_{21}H_{26}O_{11}N_2$ requires C, 52·3 ; H, 5·4 ; N, 5·8 per cent.

3-*Nitro*-o-4-*xylidine-tetraacetyl*-D-*mannoside* crystallised in silky bright yellow needles from ethyl acetate, m.p. 154° to 155°C. $[\alpha]_D^{21°C.}$ −258°. Found : C, 53·0 ; H, 5·5 ; N, 5·9. $C_{22}H_{28}O_{11}N_2$ requires C, 52·2 ; H, 5·7 ; N, 5·6 per cent.

o-*Nitroaniline-triacetyl*-L-*arabinoside* I crystallised from a mixture of ethyl acetate and light petroleum in yellow needles, m.p. 154°C. $[\alpha]_D^{22°C.}$ +159° (Kuhn and Strobële *loc. cit.* give m.p. 151°C. $[\alpha]_D$ + 133·8°). The Series II isomer separated in pale yellow needles from light petroleum (b.p. 60° to 80°C.), containing a trace of ethyl acetate, m.p. 90° to 91°C. $[\alpha]_D^{21°C.}$ −23°. Found : C, 51·5 ; H, 5·3 ; N, 7·5. $C_{17}H_{20}O_9N_2$ requires C, 51·5 ; H, 5·1 ; N, 7·1 per cent.

m-*Nitro*-p-*toluidine-triacetyl*-L-*arabinoside* I, hard yellow needles from ethyl acetate, m.p. 194°C. $[\alpha]_D^{22°C.}$ +146·5°. Found : C, 53·0 ; H, 5·4 ; N, 6·5. $C_{18}H_{22}O_9N_2$ requires C, 52·7 ; H, 5·4 ; N, 6·8 per cent. and the Series II *isomer*, orange cubes from a mixture of ethyl acetate and light petroleum, m.p. 77° to 79°C. $[\alpha]_D^{21°C.}$ −22·6°. Found : C, 52·5 ; H, 5·5 ; N, 6·8 per cent.

3-*Nitro*-o-4-*xylidine-triacetyl*-L-*arabinoside* crystallised in yellow prisms from ethyl acetate, m.p. 167°C. $[\alpha]_D^{23°C.}$ −140·8°. Found : C, 53·6 ; H, 5·9 ; N, 6·3. $C_{19}H_{24}O_9N_2$ requires C, 53·8 ; H, 5·7 ; N, 6·6 per cent.

5-Nitro-*o*-4-xylidine-triacetyl-L-arabinoside I, m.p. 213° to 214°C. $[\alpha]_D$ +142° has been described by Kuhn and Strobële *loc. cit.* The Series II glycoside separated from a mixture of ethyl acetate and light petroleum in prismatic needles, m.p. 143° to 144°C. $[\alpha]_D^{22°C.}$ +24°. Found : C, 53·9 ; H, 5·7 ; N, 7·2. $C_{19}H_{24}O_9N_2$ requires C, 53·8 ; H, 5·7 ; N, 6·6 per cent.

o-*Nitroaniline-triacetyl*-D-*xyloside* I has been described by Kuhn and Strobële (*loc. cit.*). A second isomer appeared to be present in the mother liquors but could not be isolated in a crystalline state.

m-*Nitro*-p-*toluidine-triacetyl*-D-*xyloside* I formed fluffy yellow needles from alcohol, m.p. 183°C. $[\alpha]_D^{22°C.}$ −87·2°. Found : C. 52·8 ; H. 5·4 ; N, 6·8. $C_{18}H_{22}O_9N_2$ requires C, 52·7 ; H, 5·4 ; N, 6·8 per cent. The Series II *isomer*, yellow needles, m.p. 130°C. to 132°C. $[\alpha]_D^{26°C.}$ +7·9°. Found : C, 52·5 ; H, 5·4 ; N, 7·0 per cent.

5-*Nitro*-o-4-*xylidine-triacetyl*-D-*xyloside* separated from aqueous alcohol in yellow needles, m.p. 168° to 169°C. Found : C, 53·5 ; H, 5·7 ; N, 6·6. $C_{19}H_{24}O_9N_2$ requires C, 53·8 ; H, 5·7 ; N, 6·6 per cent. The mother liquors were not investigated.

o-*Nitroaniline-triacetyl*-L-*rhamnoside* crystallised in yellow needles from alcohol, m.p. 185°C. $[\alpha]_D^{23°C.}$ +117·6°. Found : C, 52·7 ; H, 5·4 ; N, 6·4. $C_{18}H_{22}O_9N_2$ requires C, 52·7 ; H, 5·4 ; N, 6·8 per cent.

m-*Nitro*-p-*toluidine-triacetyl*-L-*rhamnoside* formed yellow flakes from alcohol, m.p. 161° to 162°C. $[\alpha]_D^{23°C.}$ +104·9°. Found : C, 54·5 ; H, 5·5 ; N, 6·7. $C_{19}H_{24}O_9N_2$ requires C, 53·8 ; H, 5·7 ; N, 6·6 per cent.

5-*Nitro*-o-4-*xylidine-triacetyl*-L-*rhamnoside* I, yellow prisms from ethyl acetate, m.p. 169°C. $[\alpha]_D^{22°C.}$ +100·8°. Found : C, 55·0 ; H, 6·1 ; N, 6·4. $C_{20}H_{26}O_9N_2$ requires C, 54·7 ; H, 6·0 ; N, 6·4 per cent. A Series II compound was isolated in very low yield and crystallised in yellow needles from light petroleum, m.p. 106° to 107°C.

Isomerisation of the o-*Nitroaniline-acetyl glycosides* II. The following examples illustrate the methods employed :—

(i) *m*-Nitro-*p*-toluidine-triacetyl-L-arabinoside II (50 mg.) and 2N hydrochloric acid (3 ml.) were boiled together. The molten solid rapidly resolidified and, after cooling, was collected and recrystallised from a mixture of ethyl acetate and light petroleum to give the Series I isomer, m.p. and mixed m.p. 194°C. Yield 40 mg.

(ii) 5-Nitro-*o*-4-xylidine-triacetyl-L-arabinoside II (60 mg.), and 2N hydrochloric acid (4 ml.) were heated to boiling and alcohol added dropwise until solution was complete. After rapid cooling, the product which separated was collected, recrystallised, and identified as the Series I isomer by m.p. and mixed m.p. determinations.

o-*Phenylenediamine-acetylglycosides* : The following general method was employed. The nitroglycoside, Series I or II (5g.) dissolved in ethyl acetate (100 ml.) was shaken with hydrogen in the presence of a 10 per cent. palladised charcoal catalyst at 40° to 50°C. When uptake ceased, the catalyst was separated and the solvent removed *in vacuo*. The crude amines were generally used directly for benziminazole synthesis (see Part IV (2)). The following compounds were examined in detail and obtained in crystalline form :—

4-*Methyl*-o-*phenylenediamine-tetraacetyl*-D-*glucoside* crystallised from a mixture of benzene and light petroleum in felted needles, m.p. 130° to 131°C. $[\alpha]_D^{23°C.}$ −47·0°. Found : 55·4 ; H, 5·8 ; N, 6·4. $C_{21}H_{28}O_9N_2$ requires C, 55·7 ; H, 6·2 ; N, 6·2 per cent.

The same amine was obtained from both the Series I and Series II nitro-compounds.

3 : 4-*Dimethyl*-o-*phenylenediamine-tetraacetyl*-D-*glucoside* crystallised from alcohol in white prismatic needles, m.p. 128° to 129°C. $[\alpha]_D^{22°C.}$ −36·4°. Found : C, 56·7 ; H, 6·5 ; N, 6·1. $C_{22}H_{30}O_9N_2$ requires C, 56·6 ; H, 6·5 ; N, 6·0 per cent.

4 : 5-*Dimethyl*-o-*phenylenediamine-tetraacetyl*-D-*glucoside* (*isomer* B) was prepared by reduction of the nitro-compound (Series II) and crystallised from a mixture of ethyl acetate and light petroleum in colourless needles, m.p. 128° to 129°C. $[\alpha]_D^{20°C.}$ −31·6°. Found : C, 56·8 ; H, 6·8 ; N, 5·9. $C_{22}H_{30}O_9N_2$ requires C, 56·6 ; H, 6·5 ; N, 6·0 per cent.

Reduction of 5-*nitro*-o-4-*xylidine-tetraacetyl*-D-*glucoside* I. The nitroglycoside I (4 g.) was reduced in ethyl acetate and the product isolated in the usual way. 4 : 5-*Dimethyl*-o-*phenylenediamine-N* : *N'-ditetraacetyl*-D-*glucoside* separated from a mixture of ethyl acetate and light petroleum in silky white needles, m.pt. 181°C. $[\alpha]_D^{22°C.} -19·4°$. Found : C, 54·4, 54·4 ; H, 6·1, 6·2 ; N, 3·7, 3·4. $C_{36}H_{48}O_{18}N_2$ requires C, 54·3 : H, 6·1 ; N, 3·5 per cent. This compound (200 mg.) was heated with 4N hydrochloric (5 ml.) at 100°C. for 15 minutes, whereafter the solution was divided into two portions. One yielded glucosazone m.pt. 200°C. (decomp.), on treatment with phenylhydrazine and sodium acetate. The other portion yielded 5 : 6-dimethylbenziminazole, isolated as the picrate after heating with formic acid.

Summary and Conclusions

1. Glucosidation of *o*-nitroaniline gives, in general, a mixture of two isomers designated *o*-nitroaniline glycosides I and II.

2. In contrast to results reported by Kuhn and Strobële[12] acetylation of these isomers gives the corresponding *o*-nitroaniline acetylglycosides I and II.

3. Conversion of the Series II isomer into the Series I isomer has been effected in certain cases by very short contact with hot dilute hydrochloric acid.

4. Reduction of the *o*-nitroaniline acetylglycosides I and II is accompanied, in certain instances, by a merging of the two series and the formation of only one *o*-phenylenediamine acetylglycoside.

5. Similar results have been obtained and are here recorded, employing *m*-nitro-*p*-toluidine, 3-nitro-*o*-4-xylidine and 5-nitro-*o*-4-xylidine.

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