512. *Macrozamin. Part II.* The Aliphatic Azoxy Structure of the Aglycone Part.*

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Chemical behaviour of macrozamin is described which shows that the aglycone, an entity apparently incapable of independent existence, contains linked nitrogen atoms; this feature has not previously been met in a natural product. Combined with the results of ultra-violet and infra-red absorption measurements this leads to the formulation of macrozamin as a primeverosyloxyazoxymethane (III*a* or III*b*), which has been substantiated by similar absorption measurements on some synthetic aliphatic azoxy-compounds.

A compound prepared by Kijner now shown to be azoxy-*p*-menthane and azoxy*cyclo*hexane, now prepared similarly, are the only compounds so far known in which the carbon atoms adjacent to the azoxy-group carry hydrogen atoms, as in the proposed structure (III).

In Part I * the carbohydrate component of macrozamin, the toxic constituent of *Macrozamia* spp., was identified as primeverose, and it was shown that the rest of the molecule is uniquely attached to $C_{(1)}$ of the glucose unit through a β-glycosidic link. The present paper describes the structural elucidation of the aglycone part of the molecule, in which the toxicity must reside.

Analysis of the aglycone itself could not be carried out, as it appears incapable of independent existence, but analytical and molecular-weight determinations on macrozamin and its hexa-acetate indicate that the former has the composition $Pv{\cdot}C_2H_5O_2N_2$, where Pv is the primeverosyl residue, $C_5H_9O_4{\cdot}C_6H_{10}O_5{\cdot}$; this has been confirmed by a study of two stoicheiometric reactions of macrozamin, in which the fates of all the component atoms were determined. It is clear that the aglycone part contains two hydrogen atoms less than a corresponding saturated acyclic structure, and must therefore contain either a double bond or a ring system.

The speed of hydrolysis of macrozamin by dilute acids was that expected for an *O*- or *N*-glycopyranoside; acid concentrations less than 0·1N. or temperatures less than 70° gave an inconveniently slow reaction; with N-hydrochloric acid at 100° hydrolysis was complete within 2 hours. The liberation of the aglycone by hydrolysis, methanolysis, or acetolysis caused it to decompose; after the acidic hydrolysis one of its carbon atoms appeared as formaldehyde (1 mol. of the dimedone derivative was obtained per mol. of macrozamin hydrolysed). The second aglycone carbon atom appeared as methanol, which was shown by conversion into methyl iodide, determined iodometrically. 1 Molar volume of nitrogen gas was also liberated during the hydrolysis. The quantitative formation of xylose and glucose under these conditions having been demonstrated in Part I, the equation for the hydrolysis is

$$C_5H_9O_4{\cdot}C_6H_{10}O_5{\cdot}C_2H_5O_2N_2 + H_2O = C_5H_{10}O_5 + C_6H_{12}O_6 + CH_3{\cdot}OH + CH_2O + N_2 \quad . \quad (1)$$

The quantitative formation of molecular nitrogen in this reaction suggests strongly that macrozamin contains linked nitrogen atoms, a feature not previously met in a natural product.

* Part I, *J.*, 1949, 2716.

The presence of this feature is also indicated by the formation of molecular nitrogen (*ca.* 0·5 mol.) when macrozamin was decomposed by hot dilute sodium hydroxide solution, but this reaction was more complex than the acidic hydrolysis, and other identified products were cyanide ion (*ca.* 0·5 mol.) (cf. Cooper, *Proc. Roy. Soc. New South Wales*, 1940, **74**, 450), formic acid (*ca.* 0·5 mol.), and traces of methylamine and ammonia. Even weak alkalis sufficed to decompose macrozamin; the primeverose was liberated intact by the action of cold N-aqueous ammonia; this behaviour is noteworthy because relatively few alkali-labile glycosides are known.

The most direct evidence of the presence of linked nitrogen atoms in macrozamin was obtained by treating its hexa-acetate, dissolved in chloroform–ether, with dry hydrogen chloride, whereupon hydrazine dihydrochloride was precipitated in a yield of *ca.* 75%. Formaldehyde (*ca.* 0·8 mol.) was also produced in this reaction, but the sugar fragment could not be obtained pure; probably it was first set free as the hexa-acetate with an unprotected glycosidic centre and then decomposed by the vigorous conditions. The results of this reaction made it likely that the linked nitrogen atoms of macrozamin occupy an oxidation level higher than that of hydrazine, and underwent reduction to this level by an oxidation–reduction similar to that in which bis-ω-nitrosophenylmethane is converted into benzoylhydrazine by dry hydrogen chloride (Behrens, *Annalen*, 1902, **323**, 272).

The nitrogen atoms of macrozamin have no basic properties; its aqueous solutions are neutral and its hexa-acetate forms neither salts with acids nor a quaternary compound with methyl iodide. This affords evidence of the presence of linked nitrogen atoms which, unlike the chemical evidence presented, is not invalidated by the possibility of molecular rearrangement. To lose its basic character a nitrogen atom must be (*a*) triply linked, as in nitriles, (*b*) conjugated with a group like C=O, (*c*) doubly linked to a carbon and singly to an electronegative atom, like oxygen, as in oximes, or (*d*) have at least two valencies attached to oxygen or nitrogen. Spectroscopic results described below show that the first three conditions do not apply to macrozamin, so the fourth must be fulfilled in respect of both nitrogen atoms. This can only be done if they are linked together, so that each assists in weakening the basic character of the other.

Unlike most compounds containing linked nitrogen atoms, macrozamin did not give a hydrazine derivative when reduced. In neutral solution, hydrogen and palladium caused no reduction, and hydrogen and platinum only a partial reduction, after which much macrozamin was recovered unchanged. For success an acid seemed necessary, and the course of the reduction depended then both on the acid strength and the reagent used. In cold 5N-hydrochloric acid, stannous chloride caused the reduction

$$\mathrm{Pv{\cdot}C_2H_5O_2N_2 + 3SnCl_2 + 6HCl = PvOH + 3SnCl_4 + CH_3{\cdot}NH_2 + NH_3 + CH_2O} \quad . \quad . \quad (2)$$

This is the second of the two stoicheiometric reactions mentioned above; its quantitative nature was confirmed with respect to stannous chloride by iodometric titration, to total volatile base by distillation and titration with acid, to methylamine by a modification of Cromwell's method (*Biochem. J.*, 1949, **45**, 84), and to formaldehyde by weighing of the dimedone derivative. The formation of reducing sugar was shown qualitatively. When macrozamin was reduced with platinum and hydrogen in 0·2N-hydrochloric acid, 4 mols. of hydrogen were absorbed, the sugar was liberated as primeverose, and 2 mols. of a volatile base consisting essentially of methylamine were formed; this corresponds to a reaction

$$\mathrm{Pv{\cdot}C_2H_5O_2N_2 + 4H_2 = PvOH + 2CH_3{\cdot}NH_2 + H_2O} \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

Similar reduction in the presence of N-acid followed a mixed course in which reactions of equations (2) and (3) appeared to proceed simultaneously, and a similar mixed reaction was obtained with zinc dust and acetic acid. Sodium amalgam could not be used because of the alkali-lability of macrozamin.

It is surprising that no hydrazine derivatives were formed in these experiments; in only a few compounds containing linked nitrogen atoms is the N–N link split in a similar way, *e.g.* in *ON*-dimethyl-*N*-nitrosohydroxylamine (I) (Boese, Jones, and Major, *J. Amer. Chem. Soc.*, 1931, **53**, 3530). The reduction of macrozamin expressed by equation (2) shows that there is a link between one nitrogen and one carbon atom of the aglycone. There is probably also a link between the second nitrogen and the second carbon atom, as shown by the formation of 2 mols. of methylamine in equation (3), but some caution is necessary about this conclusion, as it is possible, though not probable, that the second molecule of methylamine might arise from reductive condensation of ammonia and formaldehyde produced as in equation (2).

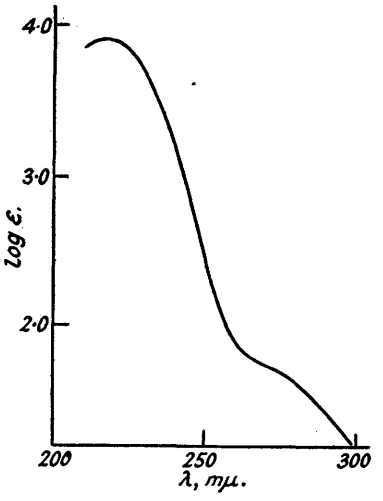
The reactions described are the only useful ones found for macrozamin. The latter was unaffected by nitrous acid and did not respond to tests for a peroxide link. Its hexa-acetate was not changed by treatment with bromine in chloroform, ethereal diazomethane, chromic acid in acetic acid, or perbenzoic acid in chloroform. Although at this stage of the work a possible structure could be suggested (cf. Lythgoe and Riggs, *Experientia*, 1949, **5**, 471), the only conclusions rigidly established were that (*a*) the aglycone contains the system C–N–N and (*b*) the primeverose is linked to it through oxygen or nitrogen. It was clear that wider conclusions could be drawn if it could be settled whether the aglycone contained a double bond. This point was decided spectroscopically.

The ultra-violet extinction curve of macrozamin (Fig. 1) is a single band characterised by a maximum at 217 mμ. and an inflexion at *ca.* 275 mμ.; it was clear from this curve that macrozamin is covalently unsaturated. No chromophoric group showing the same kind of absorption has so far been described, but certain features of similarity are present in the ultra-violet absorption spectra of primary and secondary nitramines (R. N. Jones and Thorn, *Canadian J. Res.*, 1949, **27**, 828), and of certain *C*-nitro-compounds and their *aci*-forms (Kortüm, *Z. physikal. Chem.*, 1939, **43**, *B*, 271).

Fig. 2 reproduces part of the infra-red absorption spectra of macrozamin and its hexa-acetate. That of macrozamin shows no bands between 1600 and 1800 cm.$^{-1}$, showing that double bonds involving carbon are absent from the molecule; the band at *ca.* 1760 cm.$^{-1}$ in the spectrum of the hexa-acetate is due to the ester carbonyl groups. Characteristic of both spectra is the strong band near 1530 cm.$^{-1}$, which is not shown by hepta-acetyl primeverose, and was apparently due to an unsaturated centre in the aglycone of a kind not previously studied by infra-red spectral methods. Relatively few groups are known which cause absorption in this region; among them are the C–NO_2 group (1540—1590 cm.$^{-1}$) and the N–NO_2 group of certain nitramines (1500—1590 cm.$^{-1}$) (R. N. Jones, personal communication).

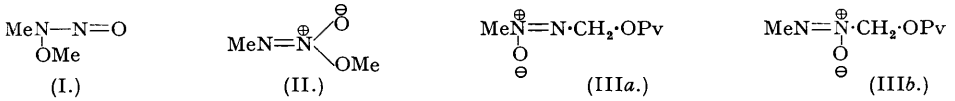
FIG. 1.

Light absorption of macrozamin in water.



The acidic hydrolysis of macrozamin (equation 1) could now be analysed to determine the oxidation level of the linked nitrogen atoms. If no oxidation–reduction were involved this level would be that of molecular nitrogen; but hydrolyses of organic compounds in which nitrogen is liberated frequently proceed by oxidation–reduction, in which the nitrogen system may either become oxidised during the hydrolysis, as with formamylazo*iso*butyronitrile (Thiele and Stange, *Annalen*, 1894, **283**, 34), or reduced, as with *ON*-dimethyl-*iso*nitramine (II) (Franchimont and Umbgrove, *Rec. Trav. chim.*, 1896, **15**, 213). In the present case it was therefore necessary to consider the adjacent lower (di-imide) and higher (nitrous oxide) oxidation levels as well as that of molecular nitrogen. The first of these possibilities would imply that macrozamin is an azo-compound, and can be discarded because these compounds show two ultra-violet absorption bands at about 220 and 340 mμ. (Kortüm and Finckh, *Z. physikal. Chem.*, 1940, **45**, *B*, 32; Kortüm, *ibid.*, 1941, **50**, *B*, 361). The possibility that macrozamin is a representative of the higher (nitrous oxide) level must also be discarded, although attractive at first sight, because similar representatives, such as (I) and (II), are isomeric with the hypothetical aglycone of macrozamin and when hydrolysed give the same products as the latter; all such representatives, if they contain a double bond, as established for macrozamin, provide no position for the glycosidic attachment of the primeverosyl group to oxygen or nitrogen. The level of the nitrogen system of macrozamin is therefore that of molecular nitrogen, a level which is represented by diazo-compounds, azo-ethers, nitrosamines, and azoxy-compounds. A diazo-structure is excluded by the stability towards cold dilute acids, and an azo-ether structure by the relatively high thermostability of macrozamin [the known azo-ethers decompose explosively at room temperature (Bamberger, *Ber.*, 1895, **28**, 225)]. A nitrosamine structure is excluded because nitrosamines show two absorption bands in the ultra-violet near 235 and 340 mμ. (Goldberg and Kirchenstein, *Helv. Chim. Acta*, 1943, **26**, 288; R. N. Jones and Thorn, *loc. cit.*). Macrozamin must therefore be an aliphatic azoxy-compound, namely, a primeverosyl-

oxyazoxymethane (III*a* or III*b*); the evidence is inadequate to decide between the two alternatives.



(I.) (II.) (III*a*.) (III*b*.)

Pv = $C_5H_9O_4 \cdot C_6H_{10}O_5 \cdot$

Very few aliphatic azoxy-compounds have been obtained until now; little is known of their chemical, and nothing of their spectroscopic, properties. The *s*-dinitrotetramethylazoxymethane described by Scholl and Born (*Ber.*, 1895, **28**, 1366) and Wieland's azoxyformic acid bisamidoxime (*Ber.*, 1905, **38**, 1452), are probably genuine, but the " bisazoxymethane "

FIG. 2.

Infra-red absorption spectra of (A) macrozamin and (B) hexa-acetyl macrozamin.

(Samples were examined as pastes with Nujol, to which the bands near 6·85 and 7·25μ. are due.)

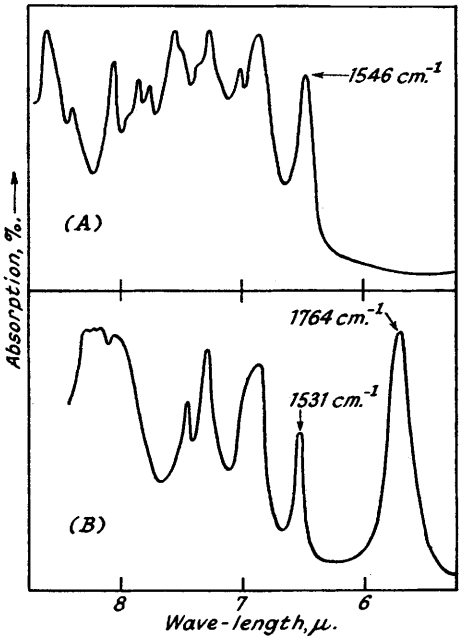
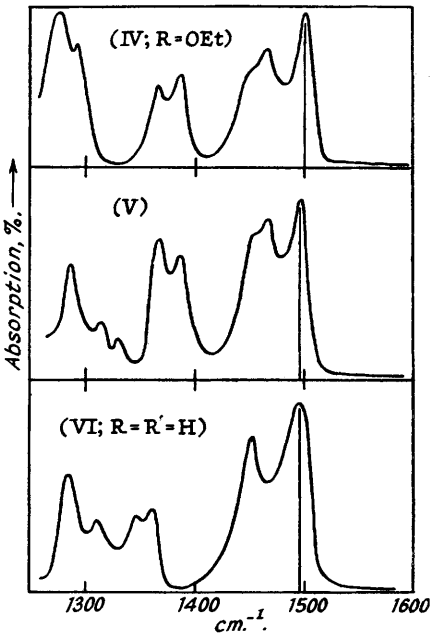
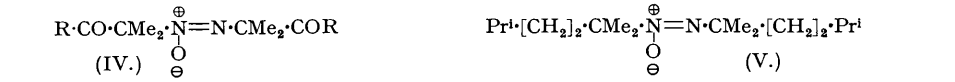


FIG. 3.

Infra-red absorption of ethyl 2-azoxyisobutyrate (IV; R = OEt), *2-azoxy-2 : 5-dimethylhexane* (V) *and azoxycyclohexane* (VI; R = R′ = H) *(liquid films).*



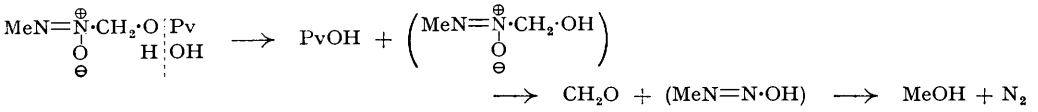
described by Hantzsch and Lehmann (*Ber.*, 1900, **33**, 3669) was shown by Curtius and his colleagues (*Ber.*, 1908, **41**, 3161) to be 1 : 2 : 4 : 5-tetrazine; and the nature of Bamberger's " bisazoxybenzyl " (*Ber.*, 1897, **30**, 2281) has never been clarified. The only well-authenticated aliphatic azoxy-compounds are those described by Aston and his collaborators; (IV; R = Me) and (IV; R = OEt) were obtained (*J. Amer. Chem. Soc.*, 1932, **54**, 1530; 1934, **56**, 1387) by graduated reduction of the corresponding nitroso-dimers; (IV; R = OEt) was also prepared by oxidation of ethyl 2-azo*iso*butyrate with hydrogen peroxide in acetic acid. By condensing the appropriate nitroso- and hydroxylamino-octanes, Aston and Ailman (*ibid.*, 1938, **60**, 1930) prepared the only known azoxy-paraffin, 2-azoxy-2 : 5-dimethylhexane (V).



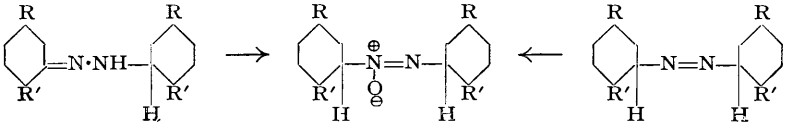
(IV.) (V.)

Apart from their stability to oxidising agents and lack of basic properties, Aston's compounds seem to have little in common chemically with macrozamin. Thus (V) was stable to alkali, and

was decomposed by stannous chloride and hydrochloric acid, but not by the latter alone, giving nitrogen, octenes, etc.; (IV; R = Me) was reduced by stannous chloride and hydrochloric acid giving hydrazine. This difference in behaviour is not, however, surprising, for the proposed structure (III) has two features not possessed by (IV) or (V), both of which must influence decisively the behaviour of the molecule. The first of these features is the primeverosyloxy-group of (III), which probably determines the course of the acidic hydrolysis; if, as the speed of the latter suggests, the initial attack is at the glycosidic link, then the elimination of formaldehyde and nitrogen must follow, as shown, for example, by the scheme below, in which possible intermediates are formulated :



A second feature of structure (III) is that the carbon atoms adjacent to the azoxy-group carry hydrogen atoms; until now no azoxy-compound of this kind was known. Since a similar feature in azo-compounds is known to lead to instability (isomerisation to hydrazones) it was thought desirable to show that azoxy-compounds of this kind are capable of existence; this was done as follows. Kijner (*J. Russ. Phys. Chem. Soc.*, 1899, **31**, 894) obtained, by the action of concentrated nitric acid on menthone menthylhydrazone, a very stable compound of the composition $(menthyl)_2ON_2$ to which he tentatively assigned a structure which, if translated into modern terms, would represent 3-azoxy-*p*-menthane (VI; R = Me, R′ = Pr^i). It is shown below that this, and the analogous azoxy*cyclo*hexane (VI; R = R′ = H), now obtained by oxidising azo*cyclo*hexane with nitric acid, are true secondary azoxy-compounds; their stability is probably due to resonance in the azoxy-group.



(VI.)

The accuracy of the proposed structure (III) could now be tested; if it is correct, the synthetic azoxy-compounds (IV; R = OEt), (V), (VI; R = R′ = H), and (VI; R = Me, R′ = Pr^i) should show absorption bands in the infra-red and ultra-violet closely similar to the very characteristic bands found for macrozamin. The relevant parts of the infra-red absorption spectra of the first three of these compounds are shown in Fig. 3. Dr. Sheppard, to whom we are indebted for his advice, reports on them as follows.

" These spectra all showed a strong band in the region 1495—1505 cm.$^{-1}$ which undoubtedly arises from a vibration of the azoxy-group; no other bands were found in the region 1500—1800 cm.$^{-1}$, usually characteristic of double bonds, except in the spectrum of (IV; R = OEt), where the strong band near 1745 cm.$^{-1}$ is attributable to the ester carbonyl groups. It is probable that the band near 1500 cm.$^{-1}$ corresponds to the asymmetric stretching mode of the azoxy-group, and it is of interest that the corresponding mode of vibration of the electronically similar C–NO_2 group has a frequency of 1555—1750 cm.$^{-1}$ in the liquid state (Smith, Pan, and Nielson, *J. Chem. Phys.*, 1950, **18**, 706).

" The characteristic azoxy-frequency near 1500 cm.$^{-1}$ correlates only approximately with the absorption band of macrozamin near 1530 cm.$^{-1}$, but it must be borne in mind that the compounds so far investigated contain heavy secondary or tertiary hydrocarbon residues attached to the azoxy-group, and since the corresponding frequency of the nitro-group shifts from 1570 to 1558 cm.$^{-1}$ on passing from nitromethane to 2-nitropropane it is likely that the azoxy-group would have a considerably higher frequency if it bore a methyl group as postulated in structure (III) for macrozamin. The extent of this frequency shift could not be assessed without an investigation of azoxy-compounds bearing groups of this kind, but pending this it can be concluded that the absorption band near 1530 cm.$^{-1}$ shown by macrozamin is consistent with the presence of an azoxy-group."

The ultra-violet absorption maxima of the synthetic azoxy-compounds are listed in the Table,

and the extinction curves of the tertiary compound (V) and the secondary compound (VI; R = R′ = H) are shown in Fig. 4.

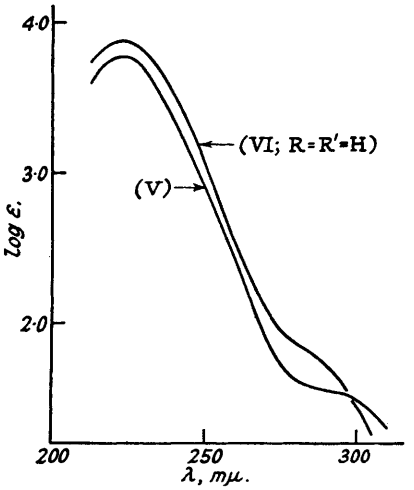
Light Absorption of Aliphatic Azoxy-compounds in Alcohol.

Compound.	$\lambda_{max.}$ (mμ.).	log ε.	Compound.	$\lambda_{max.}$ (mμ.).	log ε.
Ethyl 2-azoxy*iso*butyrate ...	223	3·74	3-Azoxy-*p*-menthane	225	3·88
2-Azoxy-2 : 5-dimethylhexane	223	3·75	Azoxy*cyclo*hexane	223·5	3·86

The curves of Fig. 4 resemble that of macrozamin closely; the maxima are near 223 mμ. and the inflexions in the region 280—285 mμ., and are thus only 5—10 mμ., removed from those of macrozamin, which is well within the range of variation found in compounds with the same

FIG. 4.

*Light absorption of azoxy*cyclo*hexane* (VI; R = R′ = H) *and 2-azoxy-2 : 5-dimethylhexane* (V) *in alcohol.*



unsaturated group but different substituents. The ultra-violet results provide strong evidence that Aston's tertiary compounds, the azoxy*cyclo*hexane derivatives, and macrozamin all possess the same chromophore.

For a conclusive proof of structure (III), a decision between the alternatives (III*a*) and (III*b*), and a proper interpretation of the behaviour of macrozamin towards alkalis and other reagents, the synthesis and examination, both chemical and spectroscopic, of a range of aliphatic azoxy-compounds more closely related to these structures than those so far prepared are necessary, and this work is in progress.

EXPERIMENTAL.

Hydrolysis of Macrozamin with Dilute Acid.—Determination of formaldehyde. Macrozamin (36·1 mg.) and N-hydrochloric acid (2 c.c.) were heated under reflux at 100° for 2 hours. The condenser was rinsed with N-sodium acetate solution (5 c.c.) and water (6 c.c.), and the solution and washings were treated with a solution of dimedone (160 mg.) in alcohol (2 c.c.), kept at 100° for 10 minutes, and then refrigerated. The derivative which separated was dried to constant weight (26·9 mg.) in a vacuum-desiccator over sulphuric acid. After recrystallisation from dilute alcohol it had m. p. 187—189°; Reeves (*J. Amer. Chem. Soc.*, 1941, **63**, 1476) gives 189—190° for the dimedone derivative of formaldehyde (Found: 0·98 mol. of formaldehyde per mol. of macrozamin).

Determination of methanol. A Kipp carbon dioxide generator was connected through a washing flask to a small hydrolysis flask from which a delivery tube led to a Zeisel apparatus of the type described by Belcher and Godbert (" Semi-micro Quantitative Organic Analysis," Longmans, Green & Co., 1945, p. 119) so modified that the gas inlet tube reached almost to the bottom of the flask containing the hydriodic acid. In the hydrolysis flask were placed macrozamin (30 mg.), acetic acid (2 c.c.), and N-hydrochloric acid (2 c.c.). The washing and the hydrolysis flask were kept at 100° during the determination, which was allowed to continue for 3 hours; the methyl iodide formed was determined iodometrically according to Belcher and Godbert's method (Found: 0·51 mol. of methanol per mol. of macrozamin). Under similar conditions methyl oxalate gave 2 × 0·66 mol. of methanol per mol. of ester.

Detection and determination of nitrogen. The macrozamin (479 mg.) was placed in a 25 c.c.-flask, which was filled almost completely with N-hydrochloric acid; the flask was then closed with a stopper having a short glass tube which was fillled with air-free distilled water. The flask was submerged in a beaker of air-free water and a filled, inverted burette brought over the exit tube. The beaker was heated in a boiling-water bath till gas evolution had ceased (2 hours), the apparatus allowed to cool, and the water in the burette levelled. Corrected to N.T.P., the gas volume was 28·4 c.c., corresponding to 1·02 mols. of nitrogen per mol. of macrozamin. Standard observations on samples of gas prepared in this way, showed that it was nitrogen, uncontaminated with oxygen.

Behaviour of Macrozamin with Alkalis.—Methanolic sodium methoxide and hexa-acetyl macrozamin. A solution of the hexa-acetate (1·28 g.) in chloroform (5 c.c.) was treated with 2N-methanolic sodium methoxide (15 c.c.) and kept at room temperature for 1 hour. The white crystalline precipitate was collected, washed with a little cold methanol, dried, and heated at 100° for 1 hour with acetic acid (2 c.c.), acetic anhydride (5 c.c.), and fused sodium acetate (0·5 g.). The cooled mixture was poured into water, and, when the excess of acetic anhydride had decomposed, the solid was collected and recrystallised from alcohol, giving β-hepta-acetyl primeverose, m. p. 212—214° undepressed on admixture with authentic material.

The alkaline filtrate from which the sodium derivative of primeverose had separated contained sodium cyanide and formaldehyde; determination of the latter as its dimedone derivative showed the presence of 0·45 mol. per mol. of macrozamin.

(a) *Action of sodium hydroxide solution on macrozamin. The determination of nitrogen and formic acid.* Macrozamin (35 mg.) and N-sodium hydroxide solution (2 c.c.) were allowed to interact in an apparatus of the van Slyke type. The reaction was completed by heating the reaction tube by an external jacket through which boiling alcohol vapour was passed. When the apparatus had been cooled and the mercury levelled the gas volume was read and corrected to N.T.P.; 0·45 mol. (0·9 c.c.) was obtained per mol. of macrozamin used. The usual tests showed that the gas was nitrogen, and that it was uncontaminated by oxygen.

Alkaline reaction solutions similar to that which remained at the end of the above experiment were acidified and distilled in an apparatus of the kind used for the determination of acetyl groups (Belcher and Godbert, *loc. cit.*, p. 123); the distillates were titrated with alkali, methyl-red being used as indicator. The neutral solutions so obtained reduced ammoniacal silver nitrate solution, indicating that the volatile strong acid was formic acid, of which 0·45 mol. was obtained per mol. of macrozamin used.

(b) *Action of sodium hydroxide solution on macrozamin. The determination of volatile bases and cyanide ion.* Aliquots (5 c.c.) of a solution containing macrozamin (392 mg.) in water (25 c.c.) were mixed with sodium hydroxide solution (10 c.c.; 10%) and distilled with steam in an apparatus for the determination of acetyl groups. The distillate was collected in 0·0266N-hydrochloric acid and the excess of acid titrated with alkali; 0·18 equiv. of base was formed per mol. of macrozamin used. The alkaline solution left after the steam-distillation was acidified with dilute sulphuric acid and distilled into alcoholic silver nitrate. The precipitated silver cyanide was collected, dried, and weighed; 0·52 mol. were obtained per mol. of macrozamin used.

The volatile base obtained in the above experiment was converted into the chloroplatinate, analysis of which showed that approximately equal parts of methylamine and ammonia were present [Found: C, 3·2; H, 2·2. Calc. for $(NH_3Me)_2PtCl_6$: C, 5·1; H, 2·5. Calc. for $(NH_4)_2PtCl_6$: C, 0·0; H, 1·8%].

Action of Chloroformic Hydrogen Chloride on Hexa-acetyl Macrozamin.—A solution of the hexa-acetate (1·284 g.) in dry chloroform (20 c.c.) and dry ether (5 c.c.) was saturated with dry hydrogen chloride (30 minutes), and the solution kept at room temperature with the exclusion of moisture for 60 hours. The precipitated solid was collected, washed with ether and chloroform, and dried (160 mg.). Material prepared in this way was shown to be hydrazine dihydrochloride by the following tests: (*a*) it reduced Fehling's solution and ammoniacal silver solutions immediately in the cold with the evolution of nitrogen gas; (*b*) a sample (120 mg.) dissolved in water (0·6 c.c.) and treated with 30% sulphuric acid (0·3 c.c.) gave a sparingly soluble precipitate of hydrazine sulphate (132 mg.) which, after one recrystallisation from hot water, had m. p. 252° (decomp.) undepressed on admixture with authentic material (Found: N, 21·8. Calc. for N_2H_4,H_2SO_4: N, 21·5%); a portion of the sulphate (113·8 mg.) titrated with 0·025M-potassium iodate under Andrews's conditions required 34·8 c.c. (theoretical titre for hydrazine sulphate: 35·0 c.c.).

Titration of the crude reaction product with potassium iodate showed it was hydrazine dihydrochloride of 97% purity with respect to hydrazine. Similar titration of the chloroform filtrate revealed the presence of further small amounts of hydrazine, the total yield being 79·4%. The filtrate also contained formaldehyde, which was determined colorimetrically by the chromotropic acid method; 0·85 mol. was formed per mol. of macrozamin used; this figure includes the small amounts of formaldehyde found in the crude precipitate of hydrazine dihydrochloride.

The chloroform filtrate obtained from an experiment similar to that described above was washed with water till free from hydrochloric acid, dried, and evaporated under reduced pressure. The residue of partially acetylated sugar had strong reducing properties towards Fehling's solution, and was insoluble in sodium carbonate solution, so it was not an acetylated aldonic acid. It contained minor amounts of acetylated 1-halogeno-sugar, since some silver chloride was formed when it was boiled with alcoholic silver nitrate. When it was crystallised from alcohol, ill-defined material, m. p. 215—230° (decomp.), was obtained, which could not be purified by the use of other solvents or by chromatography. Acetylation with acetic anhydride and pyridine gave an equally ill-defined material, m. p. 185—195°, which could not be purified.

Action of Reducing Agents.—Attempted hydrogenation of macrozamin with palladium and hydrogen. A solution of macrozamin (2·334 g.) in water (50 c.c.) and alcohol (50 c.c.) containing palladous oxide (85 mg.) and a trace of palladium on charcoal was shaken in a hydrogen atmosphere. No hydrogen was absorbed either at 15° or at 50°, and when the catalyst was removed, and the solution concentrated and diluted with alcohol, the macrozamin was recovered unchanged.

Action of stannous chloride on macrozamin in cold 5N-*hydrochloric acid.* A 5·5 c.c. flask containing macrozamin (100 mg.) and A.R. stannous chloride dihydrate (572 mg.) was filled to a volume of 5·47 c.c. with 5N-hydrochloric acid, stoppered, and kept at room temperature for 7 days. A similar 5·39 c.c. flask containing stannous chloride dihydrate (560 mg.) and 5N-hydrochloric acid to a volume of 5·36 c.c. was kept for the same time as a control; titration of an aliquot iodometrically showed that no stannous chloride had been used by aerial oxidation. Iodometric titration of an aliquot from the experimental flask showed that 2·93 mols. of stannous chloride had been oxidised per mol. of macrozamin used.

An aliquot of the experimental solution (1 c.c.) was evaporated twice with water (4 c.c.) in a small Kjeldahl tube in order to remove formaldehyde, and then transferred to a Kjeldahl apparatus, made alkaline, and distilled into standard hydrochloric acid. The excess of acid was titrated with alkali, the results showing that 1·92 equivs. of volatile base were formed per mol. of macrozamin used.

The neutral solution so obtained was made up to 50 c.c. with water, and the methylamine present in an aliquot (1 c.c.) determined by Cromwell's colorimetric micro-method (*loc. cit.*). We wish to comment that Cromwell appears to carry out the oxidative deamination of methylamine at pH 5·5; at this pH we found that the reaction gives a yield of formaldehyde which is irregular and only about 50% of the theoretical, so that although a calibration curve was used, good results could not be obtained. When the oxidation was carried out at pH 7, the yield was 95% or better, and the use of a calibration curve permitted an accuracy of ±3%. It was found that in the reduction 1·03 mols. of methylamine were formed per mol. of macrozamin used.

Formaldehyde was determined in an aliquot (2 c.c.) of the reduction solution by distillation with water and treatment of the buffered distillate with dimedone. Formaldehyde produced: 0·95 mol. per mol. of macrozamin used.

Macrozamin, platinic oxide, and hydrogen in 0·2N-*hydrochloric acid.* When macrozamin (202 mg.) was hydrogenated at room temperature in 0·2N-hydrochloric acid (25 c.c.) containing platinic oxide, 48 c.c. of hydrogen were absorbed (4·06 mols. per mol.). The filtered solution was made alkaline and distilled into hydrochloric acid. Evaporation of the latter gave a crystalline residue (74 mg.; 2 mols. of $MeNH_2$,HCl per mol. requires 71 mg.). Only traces of this material were extractable with chloroform, but it was readily and completely soluble in alcohol, so that substantial amounts of ammonium chloride or dimethylamine hydrochloride were absent. The chloroplatinate was analysed [Found: C, 5·5; H, 2·2; Pt, 43·2. Calc. for $(MeNH_3)_2PtCl_6$: C, 5·1; H, 2·6; Pt, 41·3%].

Note on Hexa-acetyl Macrozamin.—A crystalline form different from that described in Part I (*loc. cit.*) was obtained from alcohol as needles, m. p. 150—151° [Found: *M* (Rast), 631. Calc. for $C_{25}H_{36}O_{17}N_2$: *M*, 636].

3-*Azoxy*-p-*menthane.*—Made by Kijner's method (*loc. cit.*) our sample had m. p. 82°, unchanged on further recrystallisation (Found: C, 74·2; H, 11·7; N, 9·2. Calc. for $C_{20}H_{38}ON_2$: C, 74·5; H, 11·7; N, 8·7%). Kijner reports m. p. 84—84·5°.

*Azoxy*cyclo*hexane.*—Azo*cyclo*hexane (1 g.) (Harkins and Lochte, *J. Amer. Chem. Soc.*, 1924, **46**, 450) was added in small amounts during 5 minutes to vigorously stirred nitric acid (5 c.c.; *d* 1·4), stirring was continued for 1 hour and the mixture then poured into cold water (100 c.c.). Extraction with ether and evaporation of the washed and dried extract gave *azoxy*cyclo*hexane* (0·7 g.), m. p. 22—23° (Found: C, 68·5; H, 11·1; N, 13·4. $C_{12}H_{22}ON_2$ requires C, 68·6; H, 10·5; N, 13·3%).

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