574. *Macrozamin. Part I. The Identity of the Carbohydrate Component.*

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The toxic compound macrozamin, first isolated by Cooper (*Proc. Roy. Soc. New South Wales*, 1940, **74**, 450) from *Macrozamia spiralis*, has been obtained from the Western Australian plant *M. Riedlei*. Macrozamin is shown to have the molecular formula $C_{13}H_{24}O_{11}N_2$. It is a glycosidic compound, and the carbohydrate component has been identified as primeverose. Acetylation of macrozamin gives a *hexa-acetyl* derivative in which all six acetyl groups are attached to the primeverosyl residue.

Plants of the genus *Macrozamia*, species of which are distributed widely in Australia, have long been known to be toxic to humans and animals, and at times have caused severe losses of sheep and cattle. The chronic condition of cattle, known to farmers as "wobbles" or "staggers," and characterised by a permanent, partial paralysis and weakness of the hindquarters, is usually attributed to ingestion of the leaves or underground stems. Death may supervene through inability of the animal to obtain food, but sufficient recovery usually occurs after hand-feeding for the animal to forage for itself. The acute condition induced by ingestion of the seeds is characterised by diarrhœa, anæmia, and jaundice, and usually terminates rapidly and fatally (see Hurst, "Poison Plants of New South Wales," Sydney, 1942, for a full account).

Toxic material capable of producing the acute symptoms in sheep was first isolated in a state of purity from the seeds of *Macrozamia spiralis* by Cooper (*Proc. Roy. Soc. New South Wales*, 1940, **74**, 450), and named macrozamin. She described it as a colourless, crystalline, water-soluble compound, $[\alpha]_D^{21\cdot5}$ $-74\cdot8°$ (in water); analytical results suggested the formula $C_6H_{11}O_5N$ or $C_6H_{13}O_5N$; the molecular weight was not determined. The presence of an unidentified carbohydrate component was demonstrated following hydrolysis of macrozamin with dilute hydrochloric acid, and, although with this reagent no hydrocyanic acid was produced, it was detected after macrozamin had been treated with sodium hydroxide solution and then acidified. It may be recalled that two other natural products, *viz.*, hiptagin (Gorter, *Bull. Jard. bot. Buitenzorg*, 1920, [iii], **2**, 187) and karakin (Easterfield and Aston, *Proc. Chem. Soc.*, 1903, **19**, 191; Carrie, *J. Soc. Chem. Ind.*, 1934, **53**, 288T; Carter, *ibid.*, 1943, **62**, 238T) behave similarly in this respect; such compounds are perhaps best described as pseudo-cyanogenetic glycosides.

Preliminary experiments made by one of us (N. V. R.) at the University of Western Australia showed that the seeds of the local species, *M. Riedlei*, contained toxic water-soluble material, and by the use of an isolation procedure similar to that of Cooper (*loc. cit.*) a crystalline product was obtained which had the same m. p. and reactions as macrozamin, but a somewhat lower optical rotation. This discrepancy was later traced to the presence in the material from *M. Riedlei* of small quantities of a contaminant, the nature of which will be described in a separate note; its removal left the major part of the material in a pure condition, and with constants showing that it was undoubtedly macrozamin. The investigation of macrozamin was continued collaboratively in the Cambridge Laboratory, and our initial experiments are reported in this communication.

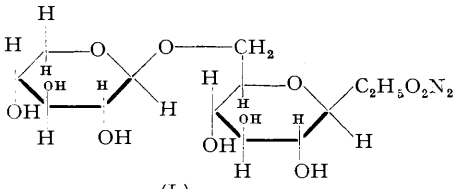
Elementary analysis and molecular-weight determinations showed that macrozamin has the molecular formula $C_{13}H_{24}O_{11}N_2$. Acetylation with acetic anhydride and pyridine gave a *hexa-acetyl* derivative, the nature of which follows from its formula, $C_{25}H_{36}O_{17}N_2$, from its reconversion into macrozamin by methanolic sodium methoxide, and from quantitative hydrolysis.

The carbohydrate component of the molecule was the first to receive investigation. After macrozamin had been hydrolysed with dilute hydrochloric acid, and the reagent removed, a gum remained from which D-glucose was isolated by crystallisation as the monohydrate. Indications that another carbohydrate fragment, probably a pentose, was present had been obtained at an earlier stage, since furfuraldehyde was formed by the action of hot, concentrated hydrochloric acid on macrozamin. The substance responsible was identified as D-xylose by the isolation of its crystalline dibenzylidene dimethyl acetal (Breddy and Jones, *J.*, 1945, 738) from the gummy hydrolysis product mentioned above. The optical rotation of the hydrolysate from a known quantity of macrozamin indicated that the two monosaccharide units were formed quantitatively and in equimolecular amount, and that they were the only optically active products of the reaction.

Since 6-(β-D-xylosido)-D-glucose (primeverose) is widely distributed in nature as the glycosidic component of numerous compounds, *e.g.*, ruberythric acid (Jones and Robertson,

J., 1933, 1167; Richter, *J.*, 1936, 1701) and monotropitin (Robertson and Waters, *J.*, 1931, 1881), it was thought this disaccharide might be present in macrozamin. That this is, in fact, the case was shown as follows. When macrozamin was treated with hot 50% acetic acid in the presence of a zinc–copper couple, and the product acetylated with acetic anhydride and pyridine, a nitrogen-free substance was obtained which had the composition and optical rotation expected for *α-hepta-acetyl primeverose*. This form of the hepta-acetate had not been described previously but, when it was deacetylated and the product reacetylated in the presence of sodium acetate, it gave the known β-hepta-acetate, identical with an authentic sample kindly supplied by Professor Alexander Robertson.

The reaction between macrozamin and sodium metaperiodate solution resulted in the consumption of 4 mols. of the oxidant, with the liberation of 2 mols. of formic acid, but no formaldehyde. Taken into consideration with the formation of the hexa-acetyl derivative, this establishes the following points: (*a*) in macrozamin all six secondary alcoholic hydroxyl groups of the primeverose residue are unsubstituted; (*b*) the glycosidic centre of the glucose unit is the only position available for the attachment of the remainder of the molecule; and (*c*) the aglycone component contains no readily acetylatable functions. The optical rotation of macrozamin shows that it is almost certainly a β-primeveroside, although the actual numerical value is perhaps somewhat greater than might be expected from its molecular weight; β-ethyl-primeveroside has $[\alpha]_D$ −58° (Rabaté, *Bull. Soc. Chim. biol.*, 1938, **20**, 449).



(I.)

The results obtained in the above work show that macrozamin is represented by the partial structure (I).

Experimental.

Isolation of Macrozamin from M. Riedlei.—Minced kernels of *M. Riedlei* (2·6 kg.; dry weight, 1·4 kg.) were steeped in cold water (1 l.) for 2 hours, the liquid filtered through coarse cloth, and the extraction repeated (6 times in all) until the extract, when boiled with sodium hydroxide solution and then acidified, no longer gave appreciable amounts of hydrocyanic acid. The combined extracts were set aside until most of the suspended starch had settled, and the supernatant liquid was decanted, treated with alcohol (1% by volume) and filtered through coarse paper. The filtrate was brought rapidly to the b. p. so as to coagulate the protein present in it, filtered, and concentrated to a thin syrup (*ca.* 2·6 l.) under diminished pressure at 50° in an atmosphere of coal gas, with octanol to suppress frothing. The syrup was treated with alcohol (4 l.), whereby most of the remaining starch and protein was precipitated. The liquid was filtered and the filtrate, after being concentrated as above to small volume (*ca.* 370 c.c.) (syrup *A*), was treated with alcohol, with constant swirling, until the precipitated gum adhered to the walls of the flask (*ca.* 1800 c.c. of alcohol were required); after the supernatant liquid had become clear, it was decanted into a clean flask, the walls of which were scratched to induce crystallisation, and set aside at room temperature for several days. The residual gum from the alcohol precipitation was dissolved in its own volume of water and treated with alcohol (*ca.* 5 volumes) exactly as described for syrup *A* above. The supernatant liquid furnished a further crop of crystals, and the residual gum was subjected to further similar treatments until no more crystals could be obtained from it; five such treatments were usually necessary. The combined crystal crops (12·5 g.; m. p. 192°) were dissolved in warm water (25 c.c.), alcohol (60 c.c.) was added, and the solution filtered and set aside. The colourless crystalline material so obtained had m. p. 199° (decomp.) and $[\alpha]_D^{15}$ −56° (*c*, 0·4 in water), unaltered by further recrystallisation. It was extracted with methanol in a Soxhlet apparatus for 8 hours, and the methanol-insoluble material recrystallised as before, whereby pure macrozamin was obtained as plates, m. p. 199—200° (decomp.), $[\alpha]_D^{16}$ −70° (*c*, 0·4 in water). It suffered no loss in weight when dried at 100°/0·1 mm. (Found: C, 40·8, 41·1; H, 6·2, 6·7; N, 7·1, 7·4. Calc. for $C_{13}H_{24}O_{11}N_2$: C, 40·6; H, 6·3; N, 7·3%). The molecular weight was kindly determined by Dr. R. H. Stokes by an application of the isopiestic technique of vapour-pressure measurement described by Robinson and Sinclair (*J. Amer. Chem. Soc.*, 1934, **56**, 1830); a series of different concentrations was investigated, and the plot of apparent molecular weight against concentration gave a straight line, which was extrapolated to zero concentration (Found: *M*, 385. Calc. for $C_{13}H_{24}O_{11}N_2$: *M*, 384).

Hexa-acetyl Macrozamin.—A solution of macrozamin (1 g.) in pyridine (10 c.c.) was cooled, treated with acetic anhydride (10 c.c.), and set aside for 4 days. It was then evaporated under reduced pressure, and the residue evaporated several times with alcohol under reduced pressure in order to remove traces of pyridine and acetic acid, dissolved in hot alcohol (20 c.c.), and set aside till crystallisation was complete. Recrystallisation from ethyl acetate–acetone–light petroleum (b. p. 80—100°) gave *hexa-acetyl macrozamin* as needles, m. p. 144—145°, $[\alpha]_D^{16}$ −49° (*c*, 0·85 in chloroform) (Found: C, 47·3, 47·3; H, 6·1, 5·6; N, 4·5, 4·2. $C_{25}H_{36}O_{17}N_2$ requires C, 47·2; H, 5·7; N, 4·4%). The same material

was obtained from vigorous treatment of macrozamin with pyridine and acetic anhydride at 100°. Quantitative hydrolysis of this material gave an apparent acetyl value of 45·2%, but macrozamin itself gave titratable acid under the same conditions, equivalent to an acetyl value of 4·9%. Taking this into account gives the following result. Found: $CH_3{\cdot}CO$, 40·3. $C_{13}H_{18}O_{11}N_2(CO{\cdot}CH_3)_6$ requires $CH_3{\cdot}CO$, 40·6%.

Deacetylation of Hexa-acetyl Macrozamin.—An ice-cold solution of the hexa-acetate (500 mg.) in dry chloroform (5 c.c.) and dry methanol (10 c.c.) was treated with 5 drops of 0·7N-methanolic sodium methoxide and kept at 0° overnight. After acidification with glacial acetic acid (0·5 c.c.) the solution was evaporated under reduced pressure, the residue dissolved in water (1 c.c.), and alcohol (4 c.c.) added. The crystals (230 mg.) which separated after some time had m. p. 198·5° (decomp.), undepressed on admixture with macrozamin.

Isolation of D-*Glucose from Macrozamin.*—Macrozamin (1 g.) and N-hydrochloric acid (40 c.c.) were heated together at 100° for 2 hours, and the solution treated with an excess of lead carbonate with stirring, cooled, and filtered. The filtrate was evaporated under reduced pressure to a syrup, which was extracted with 3 portions of hot alcohol (10 c.c. each), the extracts cooled, filtered, and set aside for crystallisation. The brown crystals were collected and recrystallised from alcohol, giving colourless material (100 mg.), $[\alpha]_D^{16}$ 45° (*c*, 0·96 in water containing a trace of ammonia); D-glucose monohydrate has $[\alpha]_D$ 48° under these conditions. Acetylation of the above material with acetic anhydride and sodium acetate in the usual way gave β-penta-acetyl D-glucose, m. p. 130° alone or on admixture with authentic material, $[\alpha]_D^{16}$ 5·7° (*c*, 0·9 in chloroform).

Identification of D-*Xylose.*—(*a*) Macrozamin (1 g.) was heated under reflux for 1 hour with hydrochloric acid (12%; 15 c.c.), and the solution diluted with water (15 c.c.) and distilled. The distillate (20 c.c.) was treated with a few drops of glacial acetic acid, and then with phenylhydrazine (0·5 g.), and the precipitate recrystallised from dilute alcohol, giving furfuraldehyde phenylhydrazone, m. p. 94—96° alone or on admixture with authentic material.

(*b*) Macrozamin (479 mg.; 1·246 millimols.) and N-hydrochloric acid (15 c.c.) were heated together at 100° for 2 hours, and the cooled solution was diluted accurately to 25 c.c.; it then had α_D^{16} 0·59° (*l*, 1 dm.). A similar solution containing D-glucose and D-xylose in equimolar amounts (1·246 millimols. each) should have α_D^{16} 0·61° (*l*, 1 dm.). The hydrolysate was neutralised with lead carbonate and then filtered, and the filtrate evaporated to a syrup under reduced pressure. Evaporation of the alcohol-soluble part of this syrup with alcohol in order to remove traces of moisture gave a gum, which was treated with the xylose reagent (10 c.c.) described by Breddy and Jones (*loc. cit.*). The white solid so obtained separated from solution in chloroform as needles (200 mg.), m. p. 208° alone or in admixture with authentic dibenzylidene D-xylose dimethyl acetal; $[\alpha]_D^{16}$ −9° (*c*, 1·0 in chloroform). Breddy and Jones record for this compound, m. p. 211°, $[\alpha]_D^{20}$ −9° (*c*, 1·2 in chloroform).

Isolation of α-Hepta-acetyl Primeverose from Macrozamin.—To a solution of macrozamin (300 mg.) and copper sulphate (a trace) in 50% acetic acid (10 c.c.), maintained at 100°, zinc dust (1 g.) was added gradually during 15 minutes, and heating continued for 2 hours longer. The filtered solution was evaporated under reduced pressure and the residue heated at 100° for 2 hours with pyridine (10 c.c.) and acetic anhydride (10 c.c.). The cooled solution was poured into water (200 c.c.), the mixture extracted with chloroform, and the chloroform extract, after being washed and dried as usual, was evaporated. Crystallisation of the residue from ethyl acetate–light petroleum (b. p. 80—100°) gave *α-hepta-acetyl primeverose* (270 mg.), m. p. 202—204°, $[\alpha]_D^{16}$ 33° (*c*, 0·6 in chloroform) (Found: C, 49·4; H, 5·7. $C_{25}H_{34}O_{17}$ requires C, 49·5; H, 5·6%). The rotation of this and the isomeric form of the hepta-acetate are in reasonably close conformity with the isorotation laws. For the two hepta-acetyl primeveroses, $M_\alpha - M_\beta = 606(33° + 23·5°) = 34{,}300°$. For the two penta-acetyl glucoses, $M_\alpha - M_\beta = 390(101·6° — 3·8°) = 38{,}200°$.

Isolation of β-Hepta-acetyl Primeverose.—A solution of the α-hepta-acetate (100 mg.), obtained as described above, in chloroform (5 c.c.) and methanol (10 c.c.) was treated with a few drops of N-methanolic sodium methoxide and set aside at room temperature for 1 hour. It was acidified with glacial acetic acid (0·5 c.c.), and evaporated to dryness under reduced pressure. The residue was heated at 100° for 2 hours with acetic anhydride (5 c.c.) containing freshly fused sodium acetate (0·5 g.), and the mixture poured into ice–water (100 c.c.). The product, isolated by extraction with chloroform in the usual manner, separated from alcohol as crystals, m. p. 212°, $[\alpha]_D^{16}$ −22° (*c*, 0·5 in chloroform). A mixture with an equal amount of authentic β-hepta-acetyl primeverose (m. p. 216°) had m. p. 214°.

Periodate Oxidation of Macrozamin.—Macrozamin (257 mg.) was dissolved in 0·217M-sodium metaperiodate solution (20 c.c.), and the solution diluted to 100 c.c. and set aside at room temperature for 30 hours, after which no further reaction took place. Periodate consumed, 4·07 mols.; formic acid liberated, 1·99 mols. per mol. of macrozamin. A portion of the solution was distilled, and formaldehyde sought in the distillate by the quantitative method of Reeves (*J. Amer. Chem. Soc.*, 1941, **63**, 1476), but none was present. In another experiment an attempt to isolate the periodate fission product was made; the reaction solution was evaporated under reduced pressure after precipitation of iodate and periodate by barium hydroxide, and the fission product extracted from the residue with alcohol. Evaporation of the extract gave a colourless glass which did not crystallise. When heated with sodium hydroxide solution and then acidified it yielded hydrogen cyanide, indicating that the action of the periodate was confined to the primeverose part of the molecule, the aglycone remaining unaffected

We thank Professor A. R. Todd, F.R.S., for his interest in this work, Professor Alexander Robertson for the gift of a sample of β-hepta-acetyl primeverose, and Dr. R. H. Stokes for carrying out the molecular-weight determination. One of us (N. V. R.) acknowledges gratefully the facilities afforded for the preliminary work by the University of Western Australia, and the award of a Senior Studentship by the Australian Council of Scientific and Industrial Research.

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