167. *The Constitution of Yeast Ribonucleic Acid. Part VI. The Nature of the Carbohydrate Radicals.*

By J. Masson Gulland and Geoffrey R. Barker.

d-Ribose has come to be regarded as the carbohydrate of yeast nucleic acid and the related nucleotides. Examination of the evidence on which this conclusion is based shows it to be unsatisfactory and the question has been re-examined. *d*-Ribose, and *l*-lyxose in small amount, have been identified, in the form of benziminazole derivatives, in the products of hydrolysis of yeast nucleic acid, and *d*-ribose has been similarly identified as the carbohydrate of guanylic, adenylic and cytidylic acids prepared from yeast nucleic acid, which is therefore designated correctly as the ribonucleic acid of yeast. It is believed that this is the first occasion on which *l*-lyxose has been identified as occurring in a product of natural origin.

It seems now to be usually assumed that the carbohydrate constituent of the pentose nucleotides and poly-nucleotides is $d(-)$-ribose. Examination of the evidence for this conclusion, however, revealed inconsistencies which made a reinvestigation of the question desirable.

Kossel first reported (*Arch. Anat. Physiol.*, 1891, 181) the presence of carbohydrate in yeast nucleic acid and believed (*ibid.*, 1893, 157) it to consist of a hexose and a pentose. Hammarsten (*Z. physiol. Chem.*, 1894, **19**, 19) and Salkowski (*ibid.*, 1899, **27**, 507) isolated from hydrolysed ox pancreas nucleoprotein a compound thought to be a pentosazone. Neuberg (*Ber.*, 1902, **35**, 1467) obtained from the same source an osazone which he believed to be derived from $d(+)$-xylose.* Attention then turned to the inosic acid of Liebig's extract of meat, of which the sugar was considered to be $d(+)$-xylose by Neuberg and Brahn (*Biochem. Z.*, 1907, **5**, 438), *dl*-arabinose by Bauer (Hofmeister's *Beitrage*, 1907, **10**, 345), and $d(-)$-lyxose by Haiser and Wenzel (*Monatsh.*, 1909, **30**, 147, 377). Levene and Jacobs (*Ber.*, 1909, **42**, 1198) then succeeded in obtaining the crystalline sugar from inosic acid by a two-stage hydrolysis involving the isolation of pure inosine (*Ber.*, 1909, **42**, 335), and later (*ibid.*, p. 3247) concluded that the sugar was $d(-)$-ribose after showing that it gave a *p*-bromophenylosazone which was the optical antipode of *l*-arabinose *p*-bromophenylosazone and on oxidation yielded an optically inactive trihydroxyglutaric acid, thus excluding the possibility of the sugar being *d*-arabinose.

Levene and Jacobs (*Ber.*, 1909, **42**, 2102, 2469, 2474, 2703) also concluded that the sugar was $d(-)$-ribose in the guanylic acids of liver and pancreas, in yeast nucleic acid and in guanosine and adenosine derived from that acid. The evidence, however, was scanty, being either the specific rotation only of the crystalline sugar or the comparison of the melting point and rotation of the osazone with those of an impure sample of the osazone prepared from the sugar of inosic acid. It will be evident that the identification of the sugar in these nucleotides depends largely on the reliability of the designation of the sugar of inosic acid and not on a comparison with an authentic sample of $d(-)$-ribose; at that time such a sample was not available. Moreover, subsequent investigations detract from the value of the determination of specific rotation alone as a reliable method of identification of $d(-)$-ribose, since Phelps, Isbell, and Pigman (*J. Amer. Chem. Soc.*, 1934, **56**, 747) showed that this sugar exhibits a remarkable and rapid mutarotation, in which there is a minimum value, and the initial and the final specific rotation are not greatly different at rather higher than $-23°$. Levene's published specific rotations for $d(-)$-ribose, varying from $-19·2°$ to $-19·5°$, lie a little to either side of the mutarotation minimum at $-18·8°$, and the question of mutarotation does not seem to have been considered by him. Further uncertainty as to the validity of Levene's conclusions arises from the wide discrepancies between the melting points of the phenylhydrazone and the *p*-bromophenylhydrazone obtained from the sugar of inosic acid and the accepted values for these derivatives of ribose quoted by Levene and Jacobs (*Ber.*, 1909, **42**, 2102) :

	Sugar from inosic acid.	Ribose.	Xylose.	Arabinose.
Phenylhydrazone	124—127°	154—155°	—	153°
p-Bromophenylhydrazone	172—173	160	128°	165

* Throughout this paper pentoses are designated in accordance with modern nomenclature.

The recognition of the sugar in the pyrimidine nucleotides and nucleosides as $d(-)$-ribose was carried out (i) by simultaneous hydrolysis of the nucleoside and oxidation of the sugar to the aldonic acid (Levene and La Forge, *Ber.*, 1912, **45**, 608), which was identified as *d*-ribonic acid by the melting point of the phenylhydrazide, and (ii) by comparison of the osazone of the sugar, obtained by acid hydrolysis of the nucleoside after catalytic hydrogenation, with the osazone obtained in the earlier work (1909).

In 1909, Rewald (*Ber.*, 1909, **42**, 3134), in knowledge of Levene's results, reported the isolation from pancreas nucleoprotein of a sugar which yielded an osazone identical with that of $d(+)$-xylose.

The descriptions of various preparations of $d(-)$-ribose since the 1909—1912 period (Levene and Tipson, *J. Biol. Chem.*, 1931, **92**, 109; Levene and Stiller, *ibid.*, 1933, **102**, 187; 1934, **106**, 421; Steiger, *Helv. Chim. Acta*, 1936, **19**, 189; Bredereck, *Ber.*, 1938, **71**, 408) mention no physical properties, *e.g.*, crystalline form, melting points, optical rotations, by which the sugar then described could now be identified, or which could be used for comparison with the earlier results. Further the identification of the sugar in the nucleosides crotonoside (Cherbuliez and Bernhard, *Helv. Chim. Acta*, 1932, **15**, 978) and uric acid riboside (Davis, Newton, and Benedict, *J. Biol. Chem.*, 1922, **54**, 595) consisted of a comparison of the melting point and rotation of the sugar from these sources with those of a sample of ribose supplied by Levene. It is clear, therefore, that Levene and his collaborators alone were responsible for the identification of the sugar of the nucleotides as $d(-)$-ribose, whereas other investigators either preferred other alternatives or provided insufficient evidence to enable their agreement with Levene to be assessed. It is always possible, however, that pentoses other than $d(-)$-ribose may have been present, depending on the source of the nucleotides.

This was the position until our work was practically completed. Recently, however, Gurin and Hood (*J. Biol. Chem.*, 1941, **139**, 775) have shown that the carbohydrate of streptococcal nucleic acid is solely or mainly ribose, and v. Euler, Karrer, and Usteri (*Helv. Chim. Acta*, 1942, **25**, 323) and Schlenk (*J. Biol. Chem.*, 1942, **146**, 619) have concluded that the carbohydrate of cozymase is $d(-)$-ribose, in each case using methods different from those now described.

It was evident that independent investigation of the nature of the sugar in pentose nucleic acids and related compounds was required, and it was desirable that the method used should be capable of detecting more than sugar; the theoretical reasons for the latter proviso need not be discussed at present. It was decided to open this series of investigations with a study of yeast nucleic acid and its nucleotides.

Our first experiments were made with 2 : 4-dinitrophenylhydrazine as reagent, but were abandoned because the compounds isolated were osazones instead of the hoped-for hydrazones. Yeast nucleic acid was hydrolysed with 2% sulphuric acid, and a solution of 2 : 4-dinitrophenylhydrazine hydrochloride was added to the solution obtained by removal of the pyrimidine nucleotides, purines and phosphoric acid. The product was fractionated into two portions, melting at 259° (decomp.) and 290—291° (decomp.), both of which had the composition of a pentose 2 : 4-dinitrophenylosazone. By refluxing a solution of 2 : 4-dinitrophenylhydrazine and $d(-)$-arabinose, *arabinose-2 : 4-dinitrophenylosazone* was obtained, melting point 259° (decomp.), which was not depressed by admixture with the lower-melting compound obtained from nucleic acid. This was, therefore, the 2 : 4-dinitrophenylosazone of $d(-)$-ribose or $d(-)$-arabinose. The fraction melting at 290—291° obtained from nucleic acid constituted only a small part of the total yield, and it was not possible to establish its constitution. In view of the existence of isomeric derivatives of 2 : 4-dinitrophenylhydrazine (Bredereck, *Ber.*, 1932, **65**, 1833; Bredereck and Fritsche, *Ber.*, 1937, **70**, 802), it could not be assumed that the compound was the 2 : 4-dinitrophenylosazone of xylose or lyxose, and attempts to obtain this derivative from $d(+)$-xylose were unsuccessful.

It was then decided to abandon experiments with dinitrophenylhydrazine and apply the procedure of Moore and Link (*J. Biol. Chem.*, 1940, **133**, 293) whereby the sugar is oxidised to the aldonic acid, which is condensed with *o*-phenylenediamine to give an aldobenziminazole (polyhydroxyalkylbenziminazole). At the time this work was carried out, the properties of *d*-xylo-, *l*-arabo- and *d*-lyxo-benziminazoles had been recorded by Moore and Link, but *d*- and *l*-ribobenziminazoles had not been prepared.

d-*Ribobenziminazole* was therefore made from synthetic *d*-ribonic acid, obtained from calcium *d*-gluconate by Steiger's method (*Helv. Chim. Acta*, 1936, **19**, 189). The properties observed differed widely from those published by Richtmeyer and Hudson (*J. Amer. Chem. Soc.*, 1942, **64**, 1612) subsequently to the completion of our work. In order, therefore, to confirm that our starting material was ribonic acid, the phenylhydrazide was prepared. This compound had the same melting point as that recorded for ribonic phenylhydrazide by Fischer and Piloty (*Ber.*, 1891, **24**, 4214), and depressed the melting point when mixed with the phenylhydrazide of *d*-lyxonic acid which we prepared from *d*-galactose (Kiliani, *Ber.*, 1885, **18**, 1552; Ruff and Ollendorf, *Ber.*, 1900, **33**, 1779; Wohl and List, *Ber.*, 1897, **30**, 3107); the melting points of lyxonic and ribonic phenylhydrazides are the same. Moreover, our *d*-lyxonic acid yielded a benziminazole which had the same melting point as that recorded for this derivative by Moore and Link (*loc. cit.*), and differed widely in properties from our *d*-ribobenziminazole. It was, therefore, concluded that the acid we used was really *d*-ribonic acid, and that our *d*-ribobenziminazole was correctly identified. We are unable to account for the discrepancy between our results and those of Richtmeyer and Hudson.

l-*Ribobenziminazole* was prepared by epimerisation of *l*-arabobenziminazole. This is an example of a method of epimerisation which is more convenient than the normal procedure using the acids, owing to the ease with which the resulting mixture of two benziminazoles may be separated. This method will form the subject of a later communication.

Yeast nucleic acid was hydrolysed with 2% sulphuric acid; this hydrolyses the purine nucleosides to purine, sugar and phosphoric acid, but has little effect on the pyrimidine nucleotides. The syrup remaining after the removal of the pyrimidine nucleotides, purines and phosphoric acid was oxidised and condensed with *o*-phenylenediamine. Two fractions were obtained from the condensation product; the less soluble fraction, the bulk of the product, was identical with synthetic *d*-ribobenziminazole. The more soluble fraction, which was obtained in very small yield, had the same melting point as *d*- and *l*-lyxobenziminazole and was shown to be identical with *l*-lyxobenziminazole; the melting point of a mixture with *d*-lyxobenziminazole was depressed but not that of a mixture with *l*-lyxobenziminazole. The *l*-lyxonic acid required was prepared from *l*-arabinose by oxidation to *l*-arabo-trihydroxyglutaric acid (Kiliani, *Ber.*, 1888, **21**, 3007), of which the monolactone was reduced, following the method of Fischer and Herz (*Ber.*, 1892, **25**, 1249), to a mixture of *l*-arabonic and *l*-lyxonic acids, which were separated as the benziminazoles.

Cytidylic, guanylic and adenylic acids derived from yeast nucleic acid all yielded only *d*-ribobenziminazole after simultaneous hydrolysis and oxidation by a mixture of bromine and hydrobromic acid.

The results described above show that *d*(−)-ribose is to be regarded as the chief carbohydrate constituent of yeast nucleic acid, which is therefore correctly designated as the ribonucleic acid of yeast. Levene's conclusions are therefore confirmed. *l*-Lyxose was present to a small extent as a subsidiary sugar in the commercial sample examined. As far as is known, this is the first record of the detection of *l*-lyxose in naturally occurring material.

The examination of the sugar of nucleotides and polynucleotides from other sources is being undertaken.

EXPERIMENTAL.

Isolation of d-*Ribo- and* l-*Lyxo-benziminazoles from Yeast Nucleic Acid.*—Yeast nucleic acid (B.D.H.) (100 g.) was refluxed with 2% sulphuric acid (1 l.) for 2 hours, and after removal of the guanine sulphate from the resulting solution, cytidylic and uridylic acids were removed by Bredereck and Richter's method (*Ber.*, 1938, **71**, 718). The resulting sugar solution, free from other organic nucleic acid constituents, was concentrated under reduced pressure to about 50 c.c., made alkaline with excess of hot saturated baryta, filtered to remove barium phosphate and brucine, and finally freed from brucine by repeated extraction with chloroform. Barium ions were exactly removed with dilute sulphuric acid, and the barium sulphate was centrifuged and washed with water until free from pentose-containing material. The combined centrifugate and washings contained 2 g. of pentose (8·6% of the purine nucleotide pentose in the nucleic acid used) when analysed by Hinton and Macara's method (*Analyst*, 1924, **49**, 2). The solution was evaporated under reduced pressure to a thick syrup, which was oxidised by Moore and Link's method (*loc. cit.*). The resultant potassium aldonate (A, see below) was collected and condensed with *o*-phenylenediamine by Moore and Link's method, and the product crystallised from water. *d*-Ribobenziminazole separated in rosettes of fine needles, m. p. 239° (decomp.), not depressed by the benziminazole from synthetic *d*-ribonic acid.

Ammoniacal cupric acetate was added to the filtrate from the ribobenziminazole until no further precipitate formed. The solid was collected, washed with water, suspended in water (5 c.c.), and decomposed with hydrogen sulphide. The solution was filtered from copper sulphide, and evaporated under reduced pressure at room temperature over phosphoric oxide. A small quantity of *l*-lyxobenziminazole separated, and after crystallisation from water formed needles, m. p. 189·5°, not depressed by synthetic *l*-lyxobenziminazole, m. p. 189°, but depressed to 178° by *d*-lyxobenziminazole, m. p. 189°.

When barium iodide in dry methyl alcohol was added to the filtrate from the potassium aldonate (see A above), a small precipitate was obtained, but this yielded no benziminazole on condensation with *o*-phenylenediamine either at 130—140° or at 180° (cf. Moore and Link, *loc. cit.*). This excludes the presence of aldonic acids other than ribonic and lyxonic.

d-*Ribobenziminazole.*—(i) *From guanylic acid.* A mixture of guanylic acid (4 g.), prepared from B.D.H. yeast nucleic acid by Jones and Perkins' method (*J. Biol. Chem.*, 1924—5, **62**, 557), and N-hydrobromic acid (100 c.c.) was refluxed for 2 hours, during which period bromine (60 g.) was added in four batches, and then shaken at room temperature for 8 hours. After the excess of bromine had been removed by distillation, the hydrogen bromide was largely removed by the addition of lead carbonate to the hot solution, thorough cooling, and filtration from lead bromide. Excess of lead was removed by precipitation with hydrogen sulphide, and the remainder of the bromide ions by grinding the filtrate from the lead sulphide with freshly prepared silver oxide, after it had been freed from hydrogen sulphide. The solution was filtered and, after removal of silver ions by precipitation with hydrogen sulphide, the filtrate from silver sulphide was evaporated under reduced pressure to a thick syrup, in which the content of aldonic acid was determined by titrating an aliquot portion (about 20 mg.) with N/100-sodium hydroxide, with phenolphthalein as indicator. The main portion of the syrup (0·57 g. of aldonic acid) was heated at 130—140° for 2 hours with *o*-phenylenediamine (0·48 g.), glacial phosphoric acid (0·37 c.c.), and diethylene glycol (1·36 c.c.) (compare Lomar, Dimler, Moore, and Link, *J. Biol. Chem.*, 1942, **143**, 552). The resulting syrup was dissolved in water (2 c.c.), and the solution decolorised with charcoal and made just alkaline to litmus by addition of aqueous ammonia (*d* 0·880). Needles began to form immediately, and after being cooled thoroughly, were collected, washed successively with water, alcohol and ether, and dried. *d*-Ribobenziminazole had m. p. 239—240°, not depressed by a sample prepared from *d*-ribonic acid. Second and third crops of ribobenziminazole were obtained by concentration of the mother-liquors, but no other product could be isolated.

(ii) *From yeast adenylic acid.* A mixture of adenylic acid (2 g.), prepared from B.D.H. yeast nucleic acid by Jones and Perkins' method (*loc. cit.*), N-hydrobromic acid (50 c.c.), and bromine (10 c.c.) was shaken at room temperature for 48 hours, a further quantity of bromine (5 c.c.) being added after 24 hours. The mixture was refluxed for 2 hours to complete the reaction. The resulting solution was freed from bromine and hydrogen bromide as described above and concentrated to a thick syrup under reduced pressure. After titration of an aliquot part with N/100-sodium hydroxide, the syrup (0·12 g. of aldonic acid) was heated for 2 hours at 130—140° with *o*-phenylenediamine (0·1 g.), concentrated hydrochloric acid (0·08 c.c.), glacial phosphoric acid (0·08 c.c.), and diethylene glycol (0·3 c.c.). The product, when worked up as already described, yielded 0·05 g. of *d*-ribobenziminazole, m. p. 239°, identical with a sample prepared from *d*-ribonic acid. No other compound could be isolated from the reaction.

(iii) *From cytidylic acid.* A mixture of cytidylic acid (2·7 g.), prepared from B.D.H. yeast nucleic acid by Bredereck's method (*Ber.* 1938, **71**, 718), N-hydrobromic acid (100 c.c.), and bromine (14 c.c.), was refluxed for 8·5 hours and shaken at room temperature for 48 hours. After the solution had been freed from bromine and hydrobromic acid and its aldonic acid content determined as before, condensation was carried out at 130—140° for 2 hours with *o*-phenylenediamine

(0·14 g.), concentrated hydrochloric acid (0·11 c.c.), glacial phosphoric acid (0·11 c.c.), and diethylene glycol (0·4 c.c.). The product was worked up as already described, and *d*-ribobenziminazole, m. p. 239—240°, was obtained identical with that obtained from *d*-ribonic acid.

A careful examination of the mother-liquors for other benziminazoles or for aldonic acids not undergoing condensation at 130—140° (*e.g.*, xylonic acid) showed that these compounds were absent.

(iv) *From synthetic* d-*ribonic acid.* Barium *d*-ribonate (1·6 g.), prepared from calcium gluconate by Steiger's method (*Helv. Chim. Acta*, 1936, **19**, 189), was dissolved in water (5 c.c.) and freed from barium ions exactly with sulphuric acid. The solution, to which hydrochloric acid (0·8 c.c.) had been added, was evaporated under reduced pressure to a syrup, which was heated with *o*-phenylenediamine (0·7 g.) and glacial phosphoric acid (0·5 c.c.) for 2 hours at 130—140°. The product was dissolved in water (5 c.c.), and the solution decolorised with charcoal and diluted to 15 c.c. with water. After the solution had been cooled in running water, a small quantity of non-nitrogenous material was removed; the filtrate, on standing, yielded d-*ribobenziminazole* as rosettes of fine needles, m. p. 239—240°, after being washed successively with water, alcohol, and ether (Found : N, 11·6. $C_{11}H_{14}O_4N_2$ requires N, 11·8%). The m. p. was not altered by further recrystallisation from water. In 5% aqueous citric acid ($c = 1·19$), $[a]_D^{20°}$ was $-50·4°$. Richtmeyer and Hudson (*loc. cit.*) record that their product formed prismatic needles, m. p. about 190° (decomp.), with $[a]_D^{20°}$ $+21·6$ ($c = 2·0$).

The picrate crystallised from water in long yellow needles, m. p. 179—180°.

It is convenient to state here that in our preparations of benziminazoles from pentose aldonic acids, we have observed that the more soluble members of the group are liable to be contaminated with ammonium phosphate (from the reaction mixture), which melts at approximately 187°.

d-*Ribonic Phenylhydrazide.*—(i) *From synthetic* d-*ribonic acid.* Barium ions were removed exactly with sulphuric acid from a solution of synthetic barium *d*-ribonate (0·3 g.) in water (5 c.c.). The filtrate was evaporated under reduced pressure to a syrup, which was heated with phenylhydrazine (0·1 c.c.) for 2 hours at 100°. The phenylhydrazide, crystallised three times from industrial methylated spirit, formed long fine needles, m. p. 163° (Found : N, 11·1. Calc. for $C_{11}H_{16}O_5N_2$: N, 10·9%). Fischer and Piloty (*loc. cit.*) give m. p. 162—164°.

(ii) *From guanosine.* A mixture of guanosine (56 mg.), 2% hydrobromic acid (10 c.c.), and bromine (0·5 c.c.) was refluxed for 3 hours, and the solution freed from bromine and hydrobromic acid as previously described; after removal of the bromine, guanine hydrobromide separated and was removed. The syrup, obtained by evaporating the final solution under reduced pressure, was condensed with phenylhydrazine (0·1 c.c.) as described above; the phenylhydrazide had m. p. 163° (Found : N, 10·9%), not depressed by the phenylhydrazide from synthetic *d*-ribonic acid.

d-*Lyxonic Phenylhydrazide and* d-*Lyxobenziminazole.*—*d*-Lyxonic acid was prepared by oxidation of *d*-galactose with bromine (Kiliani, *Ber.*, 1885, **18**, 1552), degradation of the calcium *d*-galactonate thus obtained with hydrogen peroxide in presence of ferric acetate (Ruff and Ollendorf, *Ber.*, 1900, **33**, 1798), and oxidation of the resulting syrupy *d*-lyxose by Moore and Link's method (*loc. cit.*).

Condensation with phenylhydrazine as already described (Fischer and Bromberg, *Ber.*, 1896, **29**, 581) yielded the phenylhydrazide in fine needles, m. p. 162—163° (Found : N, 10·9. Calc. for $C_{11}H_{16}O_5N_2$: N, 10·9%). A mixture of this phenylhydrazide with that of *d*-ribonic acid melted at 148—152°.

Condensation of *d*-lyxonic acid with *o*-phenylenediamine by the standard procedure yielded *d*-lyxobenziminazole, m. p. 189°, as described by Moore and Link.

l-*Lyxobenziminazole.*—*l*-Arabinose (2·5 g.) was oxidised to *l*-arabo-trihydroxyglutaric acid with nitric acid (Ruff, *Ber.*, 1899, **32**, 558), and the syrupy acid thus obtained was converted into the lactone by being heated at 100° under reduced pressure for 1·5 hours. The lactone was reduced by sodium amalgam in faintly acid (sulphuric) solution (Fischer and Bromberg, *Ber.*, 1896, **29**, 584) until the reducing power of the solution, measured by Folin and Wu's method (*J. Biol. Chem.*, 1920, **41**, 367), fell to a minimum value after its initial rise; this indicated that the aldehyde group formed by reduction of the monolactone had undergone further reduction to a primary alcoholic group. After removal of the mercury, sodium sulphate and sulphuric acid, the solution was evaporated to a syrup, which was condensed with *o*-phenylenediamine in the usual way. The aqueous solution of the product was evaporated in a vacuum desiccator and three crops of needles were collected. The first two consisted of pure *l*-lyxobenziminazole, which formed fine needles, m. p. 189°, the third needed one crystallisation from water to reach this purity owing to contamination with ammonium phosphate. This compound will be described in more detail in a later paper.

Epimerisation of l-*Arabobenziminazole.*—A solution of *l*-arabobenziminazole (0·2 g.), prepared from *l*-arabinose (Moore and Link, *loc. cit.*), in water (5 c.c.) containing 10% of pyridine was refluxed for 48 hours. After cooling, the pyridine was removed by distillation under reduced pressure and the solution was cooled, filtered from a slight impurity, and diluted to 50 c.c. Unchanged *l*-arabobenziminazole separated in needles, m. p. 234—235°. The filtrate was evaporated to 15 c.c. and, on cooling, deposited *l*-ribobenziminazole in rosettes of fine needles, m. p. 239°. A mixture with *d*-ribobenziminazole had m. p. 228°.

Isolation of 2 : 4-*Dinitrophenylosazones from Yeast Nucleic Acid.*—Yeast nucleic acid (B.D.H.) (100 g.) was hydrolysed as described above in the isolation of ribo- and lyxo-benziminazoles, and the sugar solution, after removal of the other fission products of the nucleic acid and of brucine as before, was mixed with a solution of 2 : 4-dinitrophenylhydrazine (0·3% in 2N-hydrochloric acid) (2 l.). A precipitate formed slowly and was collected at intervals of several days. After several weeks, the combined precipitates were dissolved in the minimum quantity of pyridine, and water was added until a precipitate formed, which was redissolved by warming. On cooling, a mixture of long fine needles and very small, hexagonal plates separated. The needles were obtained free from hexagonal plates by filtration through a coarse filter paper (Whatman No. 1) and the hexagonal plates were collected by filtration through a fine filter paper (Whatman No. 42). The needles were recrystallised three times from dry pyridine and formed brilliant red rhombs, m. p. 259° (decomp.). (Found : C, 40·0; H, 3·3; N, 22·4. $C_{17}H_{16}O_{11}N_8$ requires C, 40·1; H, 3·1; N, 22·05%). The hexagonal plates, after three crystallisations from dry pyridine, formed an orange microcrystalline powder, m. p. 290—291° (Found : C, 40·9; H, 3·1. $C_{17}H_{16}O_{11}N_8$ requires C, 40·1; H, 3·1%).

d(−)-*Arabinose*-2 : 4-*dinitrophenylosazone.*—A mixture of *d*(−)-arabinose (0·5 g.), dissolved in water (5 c.c.), and 2 : 4-dinitrophenylhydrazine (2 g.), dissolved in 50% ethyl alcohol–glacial acetic acid, was refluxed for 1·5 hours. After cooling, the solution was evaporated under reduced pressure to 50 c.c., diluted with water (100 c.c.), and cooled to 0°. The dark red *osazone* which separated was collected and after crystallisation three times from dry pyridine formed brilliant red rhombs, m. p. 259—260° (Found : N, 21·7. $C_{17}H_{16}O_{11}N_8$ requires N, 22·1%), not depressed by the 2 : 4-dinitrophenylosazone of the same m. p. obtained from yeast nucleic acid.