LXVIII. ON THE ISOLATION FROM TISSUES OF CERTAIN PENTOSE DERIVATIVES.

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IN a letter to *Nature* [Winter and Smith, 1923, 1] reference was made to a carbohydrate present in rabbits' tissues which appeared to be of an unusual type. At that time it was believed [Dudley and Marrian, 1923] that an animal at the point of insulin convulsions had little or no glycogen present in the tissues, and it was suggested that there might be a conversion of glycogen or glucose into this unidentified sugar under the action of the insulin. The chief point that was noted about this substance was its apparent instability during the process of isolation from the tissues. Extracts which had been kept for a short time in a semi-dry state showed a markedly diminished sugar content as evidenced by the lessened intensity of the Molisch reaction, and in some cases this test became negative. It was also found that boiling for a short time with very weak acid would appreciably restore the property of giving a positive α -naphthol reaction. The more recent experiments to be described have confirmed the truth of these observations. Though the fresh extracts were without action on Fehling's solution, before and after hydrolysis with mineral acid, it had been found that the incubation of such an extract with fresh muscle tissue led to an increase in the amount of reducing sugar, suitable control experiments being performed. This indicated that there was in the extracts a carbohydrate which was potentially a reducing sugar, yet combined in such a way that the reducing power was masked; and that hydrolysis with acid was not capable, or had been carried out under such conditions that it was not capable, of liberating the reducing sugar. It is only as a result of later work with the methylated substance that it has been possible to demonstrate definitely the reducing nature of the sugar. At that time there was great difficulty in attempting to isolate such a sugar, since there was only the α -naphthol reaction that could be used as a guide during the many operations. It was suggested by Berkeley [1923] that the unknown substance was of a pentose nature. Reasons were given [Winter and Smith, 1923, 2] for thinking that this was incorrect, since the extracts used were not sufficiently fresh to give more than minimal amounts of furfural. Later, when it had been recognised that the estimations must be carried out within a short time after extraction, it was found

that considerable quantities of furfural were formed on distillation with 12 % HCl, and since then this has been used as the method for checking the different fractions during the process of isolation. The work has been carried on continuously during the past three years, and although it is still incomplete, it must be interrupted for a time. The following is an account of the results which have so far been obtained.

EXPERIMENTAL.

In working with the liver and muscle of rabbits, alcoholic extraction was used. When it was found necessary to work on a large scale, the quantity of fat removed by the alcohol made the subsequent operations very difficult. The use of acetone is a great improvement in this respect. Normal goats were used as the source of fresh liver and muscle. Determinations of the pentose content (unpublished experiments with W. Smith) of the tissues of normal and insulinised animals did not show sufficient increase in the latter to warrant the use of large amounts of insulin, and in many cases the results were inconclusive. It was soon recognised that if the sugar was as unstable as the preliminary experiments had indicated, the greatest chance of success in isolation of the substance lay in stabilising it at an early stage after extraction. A means of alkylating the sugar was therefore looked for. To carry it out effectively it was desirable to precipitate the pentose sugar, as adsorption compound or otherwise, in order to obtain it relatively free from other substances. Though the precipitant adopted, silver, only carries down a part of the desired sugar, to methylate sugar contained in such a large syrupy mass as results from concentration of the acetone extract of material from only one goat was obviously out of the question; and the alternative of attempting to remove other constituents of the syrup, leaving the half gram of pentose, was equally impossible, even supposing that the sugar would not have been destroyed in the large number of necessary operations, and the preliminary experiments all tended to suggest that such destruction would take place. It has been found that, though precipitation with silver is effective in removing a considerable amount of pentose, the sugar cannot again be precipitated with this reagent. There is no difference in this respect whether the silver is removed as sulphide or chloride. After removal of hydrogen sulphide or chloride from the filtrate, followed by concentration of the solution *in vacuo*, the re-addition of silver sulphate causes the formation of a precipitate, which does not contain more than a trace of furaloid substance. This fact suggests that the unstable pentose is held as adsorption compound on the original silver precipitate, which probably consists largely of purine material. At this stage the only evidence of the presence of pentose was the formation of furfural on distillation with acid. Glycuronic acid reacts in this manner, and it was essential to be sure that the latter substance was not responsible for the facts observed. The naphthoresorcinol test was usually negative, even when a large amount of pentose was indicated by the quantitative estimation. On some occasions a

slight positive reaction was obtained, but there was no constancy in the results from this test.

No attempt has been made to keep the extracts of liver and muscle separate, since the sole object of the experiments was the isolation of as much material as possible. It has been found impossible to work separately with the liver of a goat, since the large amount of reducing sugar which is formed in the short time before removal of the organ causes rapid reduction of the silver solution. When the liver is minced and mixed with the muscle, the combined extract can be dealt with without difficulty, and it has therefore been possible to infer that the liver brings to the silver precipitate approximately the same amount of pentose as does the whole of the muscle. Two goats are usually used. They are pithed, bled and skinned; the livers and as much muscle as possible are removed, minced, and placed in cold commercial acetone in the proportion 1.25 l. per kg. of fresh tissue (about 14 kg.). The mixture is allowed to stand with occasional shaking for two days at room temperature. The liquid is then strained off and filtered, and as much acetone as possible removed from the tissue by means of a press. The acetone is then distilled off, and the watery extract concentrated under reduced pressure at 40° until the volume is about 500 cc., the concentration being completed about nine hours after the commencement of filtering. The thin syrup is thoroughly cooled, and treated with saturated baryta (cold) until no further precipitation occurs; this point is readily observed with experience owing to the flocking of the precipitate which takes place. The fluid is then centrifuged, and the clear solution poured off. The precipitate is stirred up very thoroughly with cold water. After centrifuging, the washings are added to the main bulk, and the whole neutralised with dilute sulphuric acid. Using a large machine of 1200 cc. capacity, the liquid need not be alkaline for more than 30 minutes; it is unlikely that in this time the alkalinity will have done more than bring about equilibrium between stereoisomers, certainly no appreciable resinification of sugars occurs. It has not been found possible to avoid the use of baryta. The barium sulphate is not removed from the neutralised fluid, but excess of silver sulphate is added. Approximately 6 l. of the saturated solution are required for about 14 kg. tissue, and when the precipitate flocks and begins to settle appreciably within two or three minutes after the last addition, the right point is reached. The mixture is then placed in the dark for an hour, and as much as possible of the clear fluid is siphoned off. The remainder, usually about 3 l., is centrifuged. It is important to protect the material from the action of light at this stage. The solid in each cup is stirred up with 95 % alcohol, and again spun down. Then follow two washings with absolute alcohol, and two with dry ether. It was originally decided to methylate the sugar contained in the silver precipitate with methyl iodide and silver oxide, as in the methods developed by Purdie and Irvine; the last ether washing was therefore poured off, and the solid transferred to a flask. 100 cc. dry methyl alcohol, and 50 g. methyl iodide were added, and the mixture was heated at 50–60° for two hours.

After the addition of 10 g. silver oxide, and 10 g. methyl iodide, heating was maintained for a further four hours. The silver was filtered off and washed with methyl alcohol; the filtrate was evaporated to dryness, and heated at 70–80° *in vacuo* overnight. Next day about 20 cc. cold methyl alcohol were necessary to dissolve the syrup; 20 g. methyl iodide were added, followed by 10 g. silver oxide, in small amounts. For the third methylation 20 g. methyl iodide were used, and the syrup was partly soluble in this medium, only 2 cc. methyl alcohol being necessary to take the whole into solution. 16 g. silver oxide were added slowly, followed later by 20 g. methyl iodide, and 8 g. silver oxide. Two further alkylations were performed using methyl iodide alone. The syrup finally obtained was distilled at 13 mm. pressure. The first fraction began to come over at 110°, and the temperature continued to rise to 180°, with a bath-temperature that was gradually increased to 240°. Three fractions were collected. Considerable charring took place during distillation. The distillates were soluble in water and ether, and gave a Molisch reaction. After hydrolysis with acid a reducing substance was present in the solution. It is remarkable that the distillates all contained organic phosphorus, the amount increasing with the boiling-point. This result was confirmed in all cases in which the methylation was carried out in the above manner. Though the nature of the phosphorus-containing substance in the distillates has not been established, it is very probable that it is derived from a pentose-phosphoric acid compound, such as inosinic acid which is very likely to be present in the silver precipitate. The phosphorus is only slowly liberated on hydrolysis with acid.

0.3 g. of the fraction boiling between 115° and 130° was heated on the boiling water-bath with 4 cc. of *N* sulphuric acid.

Time (mins.)	0	10	20	65
<i>a</i>	+ 1.72°	+ 1.12°	+ 1.15°	+ 1.15°

The acid was removed as barium sulphate, and the filtrate was concentrated to dryness. The syrup was extracted with ether, and distilled after removal of the solvent. The distillate reduced Fehling's solution vigorously, and contained organic phosphorus in considerable amount. As it was unlikely that the sugar sought was in combination with phosphorus, for the pentose of inosinic acid is stable and not likely to show the same phenomenon of a diminished capacity to form furfural when kept for any length of time, a method of removing the phosphorus compound was desirable. This was found to be very difficult to effect by fractional distillation, and, as none of the distillates showed signs of crystallising, the only solution lay in preventing the phosphorus compound from being methylated at the same time as the other pentose. Preliminary extraction of the silver precipitate with methyl alcohol failed to remove the phosphorus-containing substance, but the use of methyl sulphate, as in Haworth's method of alkylating sugars, proved successful in keeping back the phosphoric acid from the outset. The silver precipitate was suspended in dry ether in a dark bottle and decomposed by passing dry HCl gas through.

it. This stage was always reached on the same day as the acetone extract was filtered off. Twelve hours later the ether was removed, and the solid shaken with a further quantity of ether (about 100 cc.). After standing for a further 12 hours the ether was replaced with 75 cc. dry methyl alcohol, and the precipitate allowed to stand in contact with the alcohol for two days at room temperature with frequent shaking. The alcohol was then removed and the precipitate shaken with a further 75 cc. of alcohol. Meanwhile the first extract was being shaken with silver carbonate. The second alcoholic solution was separated after 24 hours, added to the first, and the last traces of acid removed. After this treatment a water extract of the silver residues showed only traces of pentose remaining. The pentose content of the alcohol extract was estimated as a routine procedure by removing a known small amount, distilling with hydrochloric acid, and weighing the precipitate of phloroglucide. The solvent alcohol was first removed on the water-bath. In order to methylate the sugar contained in the alcoholic extract with methyl sulphate it was necessary to remove almost the whole of the alcohol, and, whilst it is possible that some destruction of the furaloid substance might occur during concentration, the test samples were treated in a similar manner and would indicate the amount of pentose which was available at the time of methylation. Up to 0.8 g. pentose has been shown in the extract from two goats; sometimes it has been much less than this. For a considerable time it was customary to keep the methyl alcohol solution, freed from acid, of each pair of animals at 0°, until four such extracts were available, and then to combine them and concentrate for methylation. It was assumed that the pentose would be stable under these conditions. The four combined extracts were then concentrated under reduced pressure, or directly on the water-bath (no apparent difference in the yield being observed in this respect), down to 3–4 cc. The syrup was then transferred to an apparatus fitted with mechanical stirrer and dropping funnels, and treated with methyl sulphate and sodium hydroxide. A very vigorous reaction ensued, and the temperature was not allowed to rise above 50° until half the necessary quantity of the reagents had been added, in successive small amounts. For this amount of extract 30 cc. methyl sulphate and 48 cc. sodium hydroxide solution (40 g. in 85 cc. water) were used. The whole of the reagents having been added, the bath-temperature was raised to 70° and maintained at this level for an hour. After boiling for half an hour, the contents were cooled, and extracted twice with chloroform. The chloroform extracts were dried by standing over anhydrous magnesium sulphate for several days, filtered into a flask and the solvent removed. Methylation was then continued with methyl iodide and silver oxide, the whole of the syrup being soluble in the iodide. After heating for six hours, the silver residues were extracted with ether; the solution was dried, the solvent removed, and the syrup distilled under 13 mm. pressure. 0.6 g. was collected boiling between 125° and 131° and, on changing the receiver, no further distillate was collected when the temperature of the bath was raised. A considerable amount of non-volatile

residue remained in the flask. The distillate was neutral and entirely free from phosphorus, while an intense Molisch reaction was obtained. After heating a sample with *N* sulphuric acid at 100° for an hour, the solution reduced Fehling's solution. The next four pairs which were dealt with in a similar manner gave a very poor yield of the methylated derivative, only 0.2 g. being obtained. The initial pentose content of the two batches as deduced from the test sample taken from each alcohol extract was very similar, and it became evident that there was some secondary change proceeding which must account for the wide variation in the yield.

Examination of the alcoholic solution before methylation.

As evidence was accumulating as a result of the alkylation experiments that the pentose substance when methylated was capable of reducing an alkaline copper solution after hydrolysis, it seemed desirable to test the solution in methyl alcohol for the presence of a potentially reducing sugar, and to ascertain whether the process of methylation was after all necessary for the isolation of the pentose. It was possible that a reducing sugar might have been liberated during the decomposition of the silver precipitate with HCl in ether, which would condense with the solvent during the extraction with methyl alcohol under the influence of the HCl which was present before removal of the acid with silver carbonate. An extract was used which showed a pentose content of 0.51 g. The alcohol was removed under reduced pressure, and the residue dried in a desiccator for one day. The syrupy mass was then extracted with cold methyl alcohol (30 cc.). A white solid was left which on examination was found to be hypoxanthine. No pentose residue was found insoluble in cold methyl alcohol. The solution was cleared with charcoal in the cold, filtered, and evaporated again in the desiccator. More solid separated and was removed by extraction as before with alcohol, the syrup going into solution very readily. A test sample showed a pentose content of only 0.170 g., and since the residues had been quantitatively examined each time for pentose with negative results, it must be concluded that decomposition of the desired substance had taken place or some alteration which prevented the formation of furfural on distillation with acid. It was clear, therefore, that if the pentose underwent such a profound change during the mild conditions of the above experiments, it was improbable that the sugar could be isolated without recourse to the stabilisation conferred by methylation. The solution was again evaporated; three weeks later the pentose content had not appreciably altered. It is likely that the residual pentose was ribose, and in combination with phosphoric acid and hypoxanthine. There was organic phosphorus present, and hypoxanthine had also been separated from the mixture. Since silver precipitates purines, it was to be expected that such substances would be present. The syrup was taken up in *N* sulphuric acid, and heated at 100°. There was a steady fall in the polarimeter readings, and a very slight reduction of Fehling's solution was observed.

Time (mins.)	0	10	20	30	90
α	- 0.40°	- 0.31°	- 0.19°	- 0.17°	- 0.15°

The silver precipitate from another goat was dried with alcohol and ether and decomposed in the usual manner. During the second extraction with ether, the HCl was removed with silver carbonate. The precipitate was shaken for one hour with a small amount of methyl alcohol, and then filtered. To a part of the clear solution was added some HCl in dry methyl alcohol so that the concentration of acid was 1 %. The optical activity was observed at once, and at varying intervals during the course of 24 hours, but was unaltered. No apparent change was taking place in the carbohydrate under the action of the acid and alcohol, at room temperature. The methyl alcohol extracts are laevo-rotatory, and the methylated derivative finally obtained is dextro-rotatory, the positive value due to the sugar being evidently masked by a large amount of impurity; yet any change in the sugar due to condensation with the solvent should be observed in a change in the optical activity of the mixture. A silver precipitate was dried with alcohol and ether, and then extracted for 5 minutes with methyl alcohol containing 2 % HCl. The solution was centrifuged off and examined, but no change in the rotation was observed on standing. The result was therefore the same whether the sugar was subjected to the action of hydrochloric acid in ether or in methyl alcohol. No evidence was obtained of condensation of the sugar with the alcohol, and if it does proceed it must be completed very rapidly. It was impossible to examine the solution immediately, since a short time must be allowed for some extraction of the precipitate, and the solution has then to be cleared by filtering or by the centrifuge.

The instability of the pentose in the alcoholic extract led to a reconsideration of the advisability of leaving the extract from each pair of animals in cold storage until three or four such solutions were available. It was not possible to deal with more than two goats in one week, the extraction with methyl alcohol not being complete until the evening of the 7th day after the animals were killed. By the end of 3 or 4 weeks the first sample might have a considerably diminished pentose content, even though the mixture was maintained at 0°. This was found to be the case, though the difference was not large as determined by the furfural estimation. When the distillation of the final methylated derivative came to be carried out, however, the difference was remarkable, both qualitatively and quantitatively. While only traces had before been obtained of distillate boiling under 124–125°, now the main fraction was over when this temperature was reached; also, provided that a reasonable amount of pentose had been indicated in the small test samples, a proportional yield of the final product was obtained. In the previous series of experiments there was no certainty that this would be so. Each methyl alcohol extract is now methylated with Haworth's reagents as soon as possible. Directly the acid has been removed, the solution is filtered and concentrated *in vacuo* to a small volume, and treated with 20 cc. methyl sulphate and 32 cc. sodium hydroxide. The united chloroform extracts are kept in the cold store

over anhydrous magnesium sulphate until a number are available. Though not fully methylated, the sugar must be sufficiently stabilised by this time, and there is no advantage in carrying out the alkylation with Purdie's reagents on individual extracts. Three such chloroform extracts were concentrated under reduced pressure, and on the addition of 25 g. methyl iodide the whole of the syrup went into solution. 10 g. silver oxide were added gradually, and the mixture was heated at 55–60° for 7 hours. The silver residues were extracted three times with ether. The ether extract was dried, and some weeks later combined with that obtained in a similar manner from three other pairs of goats. The solvent was removed, and the residue distilled at the water-pump. 1.5 g. of a colourless liquid was collected, boiling between 118° and 122° (fraction 1). The remainder (0.7 g.) distilled at 125–135° (fraction 2). There was a dark viscous syrup left in the flask, which was extracted with ether. Several of these extracts have since been combined, and the contained syrup distilled under a high vacuum. A crystalline fraction was obtained, but these distillates have not yet been examined. The fractions referred to as 1 and 2 were neutral, soluble in water and ether, and gave an intense Molisch reaction. Nitrogen and phosphorus were absent. Kept for a year they have shown no sign of crystallising. Fraction 1 showed $[\alpha]_{\text{Hg green}} = +10.85^\circ$ in water, $c = 1.94$. OMe = 53.51 %. Hydrolysis was not effected by boiling with $N/100$ acid. $N/10$ sulphuric acid slowly liberated reducing sugar. The following observations were made on a very dilute solution (58 mg. in 4 cc. $N/10$ acid).

$\alpha =$	+ 0.14°	0.16°	0.16°	0.16°	0.14°	0.25°	0.24°	0.24°
Time (hours)	0	$\frac{1}{2}$	1	3	6	12	20	28

With normal acid, hydrolysis was rapid, as will be shown later. Fraction 2 showed $[\alpha]_{\text{Hg green}} = +19.00^\circ$ in water, $c = 1.53$. OMe = 52.52 %. This fraction was more readily hydrolysed with $N/10$ acid, and the change in the polarimeter readings is in the opposite direction to that shown by fraction 1. The solution contained 45 mg. in 4 cc. acid.

$\alpha =$	+ 0.16°	0.10°	0.08°	0.07°	0.08°	0.09°
Time (hours)	0	$\frac{1}{2}$	1	$1\frac{1}{2}$	2	$2\frac{1}{2}$

At the end of this time the solution reduced Fehling's solution markedly. To undertake the methylation of the small quantities of material that were now being dealt with, it was necessary to devise special apparatus. This was constructed by Messrs Flaig and Sons, 57, Hatton Garden, E.C. It consists of a short boiling tube, $3\frac{1}{2} \times \frac{3}{4}$ ins., ground at the neck to receive a condenser or a bent tube. The boiling tube is slightly expanded at the top, so that when the condenser or distilling tube is fitted into the ground joint, mercury can be poured on to the joint to act as a seal. The condenser is of the double surface type, and measures only $5\frac{1}{2} \times \frac{7}{8}$ ins. Fraction 1 was transferred to the boiling tube and methylated with 13.5 g. methyl iodide, and 9 g. silver oxide for 7 hours at 50–55°. The silver residues were extracted with ether. The solution was dried and filtered. After removal of the ether, which is accompanied by

unavoidable loss on account of the volatility of the methylated substance, the syrup was distilled at 13 mm. pressure. The distillate was remethylated, using the same quantities of the reagents as before, and again distilled. The distillate was collected in three fractions. The main (middle) fraction was analysed¹.

3.505 mg. gave 15.552 mg. AgI: 56.00 % OMe.
 3.599 mg. gave 15.145 mg. AgI: 55.72 % OMe.
 0.0780 g. gave 0.1481 g. CO₂, 0.0615 g. H₂O: C, 51.78; H, 8.76.
 0.0877 g. gave 0.1669 g. CO₂, 0.0700 g. H₂O: C, 51.85; H, 8.86.
 C₆H₅O(OMe)₄ requires C, 52.42; H, 8.74.

A cryoscopic determination of the molecular weight in water gave a value of 211. The specific rotation in water for mercury green light was + 17.6° (*c* = 1.81).

0.6 g. of the methylated derivative obtained in a similar manner from eight animals was hydrolysed with 20 cc. of *N* sulphuric acid at 100°.

Time (hours)	0	$\frac{1}{2}$	$1\frac{1}{2}$	$2\frac{1}{2}$
α =	+ 0.49°	0.53°	0.70°	0.70°
[α]Hg green =	+ 16.33°	17.66°	23.33°	23.33°

The acid was removed with barium carbonate, and the solution filtered and concentrated under reduced pressure. The residue was extracted with absolute ethyl alcohol, and the solvent removed by distillation. A viscid acid syrup was left and decomposition occurred on attempting to distil it at 13 mm. pressure. Only a drop of distillate was obtained which was dextro-rotatory, gave the Molisch reaction, and reduced Fehling's solution. This failure to separate the reducing sugar from the products of hydrolysis was not unexpected in view of the results obtained by Carruthers and Hirst [1922] with trimethyl-methyl-xyloside. Apparently it is necessary to start with the pure α - or β -compound; when a mixture is present a large proportion of breakdown products is formed during the heating with acid. Though in the above experiment the acid used was as weak as possible, and the hydrolysis was stopped as soon as the rotation had ceased to alter, it is possible that the failure to isolate the reducing sugar was due to the presence of two stereo-isomers in the original liquid.

The nature of the parent sugar and of the methylated derivative is as yet uncertain. That the latter is a stable type is clear from the strength of acid necessary to bring about hydrolysis. It is hydrolysed at a similar rate to trimethyl- β -methyl-arabinoside. The following figures were obtained with a solution of the latter compound in *N* sulphuric acid.

Time (hours)	0	$\frac{1}{2}$	$1\frac{1}{2}$	$2\frac{1}{2}$
α =	+ 0.24°	0.31°	0.35°	0.34°
[α]Hg green =	+ 52.9°	68.5°	77.8°	74.9°

The hydrolysis is complete in the same time in both cases. Though the rather low results obtained in the methoxyl determinations suggest that the substance

¹ Methoxyl determinations were made by the Pregl micro-Zeisl method; for valuable advice on this and other analyses I am indebted to Mr H. I. Coombs. The values for C and H were determined by Mr F. C. Hall, of the Dyson Perrins Laboratory, Oxford.

is not completely methylated, this is not sufficient to account for the large difference between the specific rotations of the unknown sugar and trimethyl-methyl-arabinoside. Xylose can be excluded at once. Methylated derivatives of lyxose and ribose have apparently not been prepared. Even if future work results in the identification of the methylated sugar from the silver precipitate with an alkylated riboside or lyxoside, there remains the problem of the labile nature of the sugar before methylation.

Isolation of a derivative of d-ribose.

The ether extracts of the silver precipitates were examined for pentose at an early stage of the work, and it was found that a small amount of furfural was obtained on distilling the residue after evaporation of the ether. When such a syrup was kept for some months there was found to be no disappearance of pentose as occurred in the methyl alcohol extract. It seemed possible, therefore, that the ether had extracted a small amount of another pentose derivative. The substance was finally isolated from each extract as follows. The acid having been removed from the ether with silver carbonate, the solution was dried with potassium carbonate, followed by sodium sulphate. The filtered solution was distilled slowly through a fractionating column, until the volume of ether was reduced to about 10 cc. 50 cc. of light petroleum were added, and distillation was continued. It is important that the petroleum should have previously been distilled. Only that fraction is used which will come through the column on the boiling water-bath. When the flask contains only 5–10 cc. of fluid, it is removed from the bath, and the liquid at once poured out while still hot. On cooling, fat begins to separate. In the flask remains a very small amount of a semi-solid yellow substance from which, however, nearly the whole of the fat has been removed by the treatment with petroleum. The residue in the flask is taken up in a few cc. of cold absolute ethyl alcohol, in which it is very soluble, and filtered. The solution is evaporated in a desiccator, and the resulting syrup crystallises readily. The crystals are dissolved in water, and the solution is cleared with charcoal to remove the last traces of fatty matter. After evaporating to dryness in a desiccator, the substance is recrystallised from absolute alcohol. The slightly hygroscopic compound was dried *in vacuo* over phosphorus pentoxide until the weight was constant. The pentose content was estimated in the usual manner.

9.6 mg. gave 6.6 mg. phloroglucide. 8.0 mg. gave 5.2 mg. phloroglucide. Using for comparison a pure sample of β -methyl-arabinoside, 10.2 mg. gave 6.9 mg. phloroglucide.

The substance contains only carbon, hydrogen, and oxygen; it does not reduce Fehling's solution until after hydrolysis with acid. Heated in a capillary tube, it begins to soften at 58°, and melts at 65–66° (uncorr.). The specific rotation was determined in absolute alcohol. 0.1634 g. in 4.926 g. alcohol gave $\alpha = -3.61^\circ$ ($c = 3.31$, $l = 1$). $[\alpha]_{\text{Hg green}} = -108.8^\circ$. The solubility in water and alcohol and the liberation of reducing sugar on hydrolysis suggest that

the substance is an alkyl-pentoside. Owing to the hygroscopic nature of the compound accurate determination of methoxyl was not possible; a value of 12.27 % OMe was obtained, an approximation which gives support to the suggested nature of the substance. The first step in the identification of the pentose was the preparation of the phenylosazone. A small amount of the pentoside was hydrolysed with 2 % sulphuric acid for $1\frac{1}{2}$ hours on the boiling water-bath. Phenylhydrazine hydrochloride and sodium acetate were added, and the mixture heated at 100° for 20 minutes. The crystals deposited on cooling were recrystallised from dilute pyridine. The specific rotation for mercury green was -70.2° , Neuberg's pyridine-alcohol mixture being used. The crystalline form of the osazone was identical with that prepared from a sample of arabinose, and the sugar is therefore *d*-arabinose or *d*-ribose. A sample was then hydrolysed, and the optical changes recorded. 0.1408 g. in 10.27 g. 2 % sulphuric acid was heated under reflux on the boiling water-bath.

Time (mins.)	0	45	65	85	135
$\alpha =$	-1.19°	0.56°	0.40°	0.27°	0.29°
$[\alpha]_{\text{Hg green}} =$	-86.8°	40.9°	29.4°	19.7°	21.2°

The final specific rotation is very nearly that of *d*-ribose ($[\alpha]_D - 19.0$), and the identity of the sugar is established. It is uncertain whether the crystalline substance is a methyl- or ethyl-riboside, for there was only a small quantity available for investigation. The yield of the pure substance is only about 2.5 mg. per kg. fresh tissue. If the riboside does not pre-exist as such in the tissues, but is formed from ribose set free from inosinic or guanylic acids during the extraction with ether, it seems likely that it will be the ethyl compound due to reaction with traces of ethyl alcohol, persisting from the operation of drying the silver precipitate, under the catalytic influence of the hydrochloric acid. On the other hand, it is to be expected that a mixture of the α - and β -forms would be produced under these conditions, and the complete crystallisation which takes place when the solution in absolute alcohol is evaporated suggests that only one isomer is present, from the optical activity probably the α -riboside. The curve of polarimeter readings on hydrolysis is regular and indicates the absence of the β -compound, assuming the difference usually found in the rates of hydrolysis of α - and β -sugar derivatives. There is no evidence then for the existence of both isomers in the crystalline material obtained, and it might be that the more soluble form was left behind in the light petroleum used for precipitating the substance. Examination of this solution, however, showed no trace of pentose. Using arabinose, no formation of an ethyl-arabinoside was observed when a suspension of the sugar was treated in a similar manner to the silver precipitate. 200 mg. arabinose were shaken with 200 cc. dry ether, containing 2 cc. absolute alcohol, and 2 % HCl, for 24 hours. The sugar was apparently unchanged, and no weighable residue was obtained on evaporating the ether after removal of acid. It seems unlikely, therefore, that the riboside which has been isolated is artificially produced from nuclear material during the processes used for its extraction, and

it is probable that it is present in the fresh tissues. The pentose which was isolated in the form of its methylated derivative is present in relatively large amount as compared with the alkyl-riboside, approximately 0.005 g. as compared with 0.0002 g. per 100 g. fresh tissue. Actually, however, the quantity is very small, and this fact in conjunction with the marked lability of the compound renders it probable that the sugar is an intermediate in carbohydrate metabolism.

SUMMARY.

Results are given of an investigation of two pentose derivatives present in animal tissues. One contains *d*-ribose, and is probably an alkyl-riboside. The other is unstable, and only the methylated derivative has been isolated. Its nature has not been determined.

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