94. ISOLATION OF ADENINE-DESOXYRIBOSIDE FROM THYMUSNUCLEIC ACID

By THOMAS G. BRADY

From the Department of Biochemistry, University College, Dublin

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In the course of deamination experiments it was found necessary to prepare a sample of adenine-desoxyriboside. This substance has already been isolated by Klein [1934; 1938]. Using his method, which, however, it was found necessary to modify considerably, the substance obtained had a very much higher sintering point and a distinctly higher melting point than described by Klein. The crystalline nucleoside isolated in this Laboratory sintered at approximately 160° and melted sharply at 187–188°. ¹ The data given for adenine-desoxyriboside by Klein show that his product sintered between 120 and 125° and melted sharply at 181°. ¹ Klein [1934] showed that the M.P. varied with the rate of heating; with slow heating the substance melted some degrees below 181°. The same effect was observed with the higher-melting compound described in this paper, but at no time was the M.P. below 184°.

In other properties, e.g. solubility, crystalline form and rotation, the substance is very similar to Klein's. The analytical data for C, H and N, are in good agreement with the theoretical values, and the substance gives a quantitative yield of NH₃ when deaminated with tissue extracts. It therefore seems reasonably certain that the higher-melting substance is in fact an adenine-desoxyriboside. The possibility that it is an isomeride differing from Klein's in the attachment of the sugar molecule to the aglucone cannot, however, be overlooked.

Gulland & Story [1938], using a sample of Klein's adenine-desoxyriboside, proved by absorption spectrum analysis that the sugar molecule is attached to position 9 of the purine skeleton. The point of attachment of the higher-melting substance may conceivably be at position 7. This, of course, would be of considerable interest, as it would be the first example of a nucleoside with the sugar attachment at position 7. Unfortunately no final decision can be reached on this question, as the facilities at present available in this laboratory do not allow of an absorption spectrum measurement being carried out. The alternative, considerably strengthened by observations made on thymine-desoxyriboside, that the higher-melting substance is merely a purer form of adenine-desoxyriboside seems, however, more probable. It was found, for example, that an impure sample of thymine-desoxyriboside after as many as four recrystallizations from distilled water melted 10° below the correct M.P. of this substance. One recrystallization of this same impure sample from 50% water/alcohol gave a substance which melted correctly at 185°. It appears from this, that unless a suitable solvent is used, minute amounts of impurities may adhere to these nucleosides and cause a considerable depression of M.P. In the case of adenine-

¹ All melting points recorded in this paper are uncorrected. The possibility that the divergence in the melting points may be due to differences in the thermometers used, is ruled out by the fact that the thymine-desoxyriboside isolated in this laboratory melts at exactly the same temperature as that recorded by Klein, i.e. at 185°, almost midway between the divergent melting points.

desoxyriboside it was found advantageous to use a water/acetone mixture instead of absolute alcohol. By this means, adenine-desoxyriboside invariably crystallized out on standing overnight in the refrigerator, and the long and tedious procedure adopted by Klein of allowing the nucleoside to crystallize from absolute alcohol (1–2 weeks) was found to be unnecessary. At the same time it appears that a purer product has been obtained.

The various modifications of Klein's method are fully described in the experimental section and need not be further discussed here. There is, however, one point not mentioned by Klein in any of his communications on the desoxynucleosides and nucleotides, which appears to be of considerable importance, namely, the effect of the depolymerizing enzyme 'nucleogelase'. The work of Feulgen [1935] and Schmidt & Levene [1938] on nucleogelase has proved that this enzyme breaks down polymerized *a*-thymusnucleic acid to depolymerized *b*-thymusnucleic acid. One would expect Klein's thymusnucleic acid prepared by the method of Feulgen [1914] to contain much polymerized *a*-thymusnucleic acid, and this form is not attacked by nuclease [Schmidt & Levene, 1938]. Yet Klein obtained 96% dephosphorylation in the preparation of the nucleosides. Feulgen suggests that the thymonucleinase of Klein is associated with the nucleogelase, noting that the *pH* optima of these ferments are close together, but his figure of 6.5 for the thymonucleinase is incorrect, the true value being *pH* 9.2 [Klein, 1932]. Klein and his co-workers for preparative work [Klein, 1932; 1933; 1934; 1938; Bielschowsky & Klein, 1932] selected a slightly lower *pH* (8.8–9.0) than the optimum but at this point nucleogelase, according to Feulgen himself, is inactive.

The dephosphorylation experiments carried out in this Laboratory gave consistently low values for hydrolysed phosphorus (50% of total organic P) and in consequence the yield of adenine-desoxyriboside was only 50% of that obtained by Klein. Depolymerization experiments, using Feulgen's method, but not using Merck's pancreatin, were attempted, but with little success. It is probable that the pancreatin employed contained very little nucleogelase. The quantity of nucleophosphatase was increased to threefold that used by Klein, but even then only 60% of the total organic P was hydrolysed.

Another factor influencing the dephosphorylation very considerably was the addition of Ag-ion as a deaminase inhibitor. This reduced the hydrolysis of PO_4 -ion by about one-third. The average amount hydrolysed was approximately 20% of the total organic P.

EXPERIMENTAL

Enzyme solution. The preparation and purification of the enzyme nucleophosphatase from calves' intestines were carried out according to the direction of Klein [1938]. It was found an advantage, however, to modify slightly the initial step in the purification by using 2 volumes instead of 1 volume of acetate buffer, and addition of the glycerol extract to the acetate solution. Although this procedure increased the volume of solution to be centrifuged, it counteracted any tendency for the precipitate to come out in a colloidal form, which by the original method sometimes occurred, and was found very difficult to rectify.

Hydrolysis of thymusnucleic acid. The enzymic hydrolysis was carried out on 10 g. of thymusnucleic acid. The procedure adopted was almost identical with that of Klein [1938], except that the concentrations of enzyme and Ag used were trebled. The hydrolysed P was estimated colorimetrically [Bomskov, 1932].

Treatment of the hydrolysed thymusnucleic acid. Klein's technique was used for the precipitation of the $\text{PO}_4^{=}$ and for concentrating the solution. The centri-

fuging of the cooled gel was attempted but was found to be unsatisfactory, and absolute alcohol was added until the precipitate which formed was quite powdery. To ensure complete extraction of adenine-desoxyriboside the powder was shaken three times with alcohol. The combined extracts were concentrated *in vacuo* at 40° and removal of inorganic ions (NH_4 , Mg, SO_4) and neutralization, were carried out as outlined by Klein [1938]. Amorphous substances, probably of protein nature, were removed by repeated precipitation with absolute alcohol. The filtrate was concentrated to about 5 ml. Further concentration was effected by passing a filtered stream of air through a suction tube, containing the filtrate and washings in a small test tube, heated to 40° in a water bath. When all the alcohol had evaporated, acetone was added giving a gummy residue. On addition of a few drops of water, this dissolved, and on standing overnight in the refrigerator, adenine-desoxyriboside crystallized out in typical block-shaped crystals.

Most of the acetone was removed by evaporation in an air stream as described above. On standing in the refrigerator for a few hours the residue had set to a solid mass. The crystals were filtered off on a small filter, using a glass button. The crystalline mass, which was sometimes admixed with gummy material, was dissolved in water heated to 60°. A small quantity of a water-insoluble, noncrystalline material was filtered off. After a few hours in the refrigerator adenine-desoxyriboside crystallized out. Yield 120 mg., sinters 162–165° M.P. 187–188° (uncorr.).

It was recrystallized three times for analysis without alteration of M.P. $[\alpha]_D^{20} - 26.9$ (in water). Found: C, 48.00; H, 5.42; N, 28.30; H_2O , 6.35. Calc. for $\text{C}_{10}\text{H}_{13}\text{O}_5\text{N}_5$, H_2O : C, 47.78; H, 5.22; N, 27.88; H_2O , 6.69%. (Analysis by Drs Weiler & Strauss, Oxford.)

Quantitative estimation of amino-N by the Conway technique [Conway, 1935; Conway & Cook, 1939]. 0.5 ml. of a solution containing 0.5 mg. adenine-desoxyriboside was added to 3.5 ml. phosphate buffer pH 6.1. To this was added 1 ml. of the enzyme solution, made by shaking 50 mg. of acetone-dried intestinal mucosa with 10 ml. water. Found amino-N, 5.08; 5.35. Calc. 5.2%.

Isolation of thymine-desoxyriboside. After removal of all the adenine-desoxyriboside the water-acetone mother liquors were concentrated to a small volume. On standing for some time in the refrigerator thymine-desoxyriboside crystallized out in long needles. After one recrystallization from 50% alcohol it melted at 185°. Yield 140 mg.

SUMMARY

A modified method, which considerably shortens the isolation of adenine-desoxyriboside from thymusnucleic acid has been described.

The product obtained differs from that already described in the literature [Klein, 1934; 1938] in having a considerably higher M.P.

Adenine-desoxyriboside isolated in this Laboratory sinters at 162–165° and melts sharply at 187–188°.

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