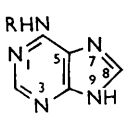
1144. *The Oxidation of Nucleic Acid Derivatives by Manganese Dioxide.*

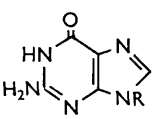
By A. S. Jones, R. T. Walker, and A. R. Williamson.

Manganese dioxide in aqueous suspension at 100° oxidised certain purines, but not pyrimidines, to urea derivatives and in some cases to guanidine. Purine, but not pyrimidine, nucleosides were degraded to the corresponding bases and their oxidation products, and thymidine-5′ phosphate was degraded to thymine. An oligodeoxyribonucleotide fraction gave small amounts of free pyrimidines and oxidation products of the purines. Deoxyribonucleic acid was also oxidised, but much less readily than the oligodeoxyribonucleotides.

A preliminary communication [1] reported that treatment of thymidine-5′ phosphate with manganese dioxide at 100° gave thymine, whereas thymidine was unaffected. It appeared that the reaction might involve oxidation of the 3′-hydroxyl group, and, therefore, might be specific for the terminal residue of an oligodeoxyribonucleotide. To ascertain this, a study has been made of the oxidation of nucleic acid derivatives with manganese dioxide.

When adenine (I; R = H), 6-methylaminopurine (I; R = Me), guanine (II; R = H), and 9-ethylguanine (II; R = Et) were heated in water at 100° with ten times their weight of manganese dioxide, there was considerable destruction of the purine ring system, as shown by a decrease in optical absorption at 250 or 260 mμ. Chromatography of the

(I) 

 (II)

products and the use of specific spray reagents showed that adenine gave urea and biuret. The latter must have arisen from N-1, N-3, and the 6-amino-group, while the urea could have been produced from N-7 and N-9 and/or from the biuret. 6-Methylaminopurine gave urea, methylurea, and small amounts of adenine, biuret, and methylbiuret. It was probable that the methylbiuret was breaking down to methylurea.

Guanine was oxidised more rapidly than adenine and gave guanidine and urea. The former must have arisen from N-1, N-3, and the 2-amino-group and the latter from N-7 and N-9. 9-Ethylguanine was oxidised less readily than guanine and gave guanidine, urea, and ethylurea.

The pyrimidines, uracil, cytosine, and thymine were not affected by this treatment with manganese dioxide.

[1] Jones and Williamson, *Chem. and Ind.*, 1960, 1624.

The nucleosides, adenosine and guanosine, were also oxidised, but more slowly than the corresponding purines. Hydrolysis may have preceded the oxidation. The pyrimidine nucleosides, uridine, cytidine, and thymidine, were, however, not attacked by manganese dioxide under these conditions. In view of this resistance it was surprising that thymidine-5′ phosphate was readily degraded to thymine. Deoxycytidine-5′ phosphate and deoxyadenosine-5′ phosphate also gave mainly the corresponding base and a trace of the nucleoside, but in view of the ready hydrolysis of the glycosidic linkage in these cases [2] this can possibly be attributed to an enhancement of hydrolysis.

When this reaction was applied to oligodeoxyribonucleotides, the pH was maintained at 8—9 to avoid hydrolysis of the purine glycosidic linkages. In these conditions, from an oligodeoxyribonucleotide fraction (average chain length, 7·6 nucleotides; terminal 3′-hydroxyl group and terminal 5′-phosphate group), obtained from a pancreatic deoxyribonuclease digest of deoxyribonucleic acid, 4—6% of the pyrimidines and oxidation products of the purines (urea and guanidine) were liberated. These had not been detected in our previous work.[1] When highly polymerised deoxyribonucleic acid was similarly treated, liberation of pyrimidines was barely detectable. However, when ten times the usual amount of manganese dioxide was used, there was appreciable degradation of the deoxyribonucleic acid with the liberation of free pyrimidines, thus showing that non-terminal nucleotide units were affected. The oligodeoxyribonucleotide fraction was more strongly absorbed by the manganese dioxide than was deoxyribonucleic acid, and this may have been the cause of the easier oxidation of the former. The method cannot be used, therefore, for stepwise degradation of an oligodeoxyribonucleotide.

Experimental

Paper chromatograms were developed by the descending method with the solvents: butan-1-ol–ethanol–water (4 : 1 : 5) (solvent I); propan-2-ol–water (70 : 30) (solvent II); propan-2-ol–ammonia (*d* 0·88)–water (35 : 3 : 15) (solvent III); propan-2-ol–10N-hydrochloric acid–water (68 : 20·5 : 11·5) (solvent IV); ethyl acetate–formic acid–water (60 : 5 : 35) (solvent V).

Derivatives of urea and biuret were detected with the Ehrlich reagent [3] with which they gave a characteristic yellow colour, and guanidine with the alkaline ninhydrin spray [4] with which it gave a green fluorescent spot in ultraviolet light. These compounds could also be detected as white spots on a brown background by use of the silver nitrate spray.[5]

Manganese dioxide was prepared by heating manganese carbonate at 200° for 3 days.[6]

Oxidation of Purines.—A solution of the purine in water (1 mg./ml.) was heated at 100° with manganese dioxide (10 mg./ml.). The ultraviolet absorption of the solution decreased as follows: adenine, 32% after 6 hr.; 6-methylaminopurine, 62% after 7 hr.; guanine, 100% after 3 hr.; 9-ethylguanine, 28% after 3 hr. The products were identified by paper chromatography in solvents I, II, and IV. Adenine gave urea and biuret; 6-methylaminopurine gave adenine, methylurea, urea, biuret (trace), and methylbiuret (trace); guanine gave guanidine and urea; 9-ethylguanine gave guanidine, urea, and ethylurea.

Treatment of Pyrimidines.—When thymine, cytosine, and uracil were similarly treated with manganese dioxide there was no change in their ultraviolet absorption spectra.

Oxidation of Nucleosides.—The conditions were as for the purines and pyrimidines. Control solutions of the purine nucleosides, however, showed some hydrolysis.

Adenosine. After 6 hr. the absorption at 260 mμ decreased by 24%. Adenine, urea, and biuret were detected chromatographically.

Guanosine. After 3 hr. the absorption at 250 mμ decreased by 64%. Guanidine, urea, and a trace of guanosine, but no guanine, were detected.

Pyrimidine nucleosides. When thymidine, uridine, and cytidine were similarly treated

[2] Greer and Zamenhof, *J. Mol. Biol.*, 1962, **4**, 123.
[3] Hubener, Bode, Mollat, and Wehner, *Z. physiol. Chem.*, 1952, **290**, 136.
[4] Jones and Thompson, *J. Chromatography*, 1963, **10**, 248.
[5] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.
[6] Harfenist, Bavley, and Lazier, *J. Org. Chem.*, 1954, **19**, 1608.

there was no decrease in ultraviolet absorption. The reaction with thymidine was examined in detail as follows:

A solution of thymidine (500 mg.) in water (100 ml.) was heated at 100° for 6 hr. with manganese dioxide (5 g.). The manganese dioxide was centrifuged off and a portion (0·03 ml.) of the supernatant liquid examined by ascending paper chromatography on Whatman No. 3 paper in aqueous ammonia (pH 10) with untreated thymidine solution (0·02 ml.) as marker. The reaction solution contained only one component and this had the same R_F as thymidine. Its spectrum was identical with that of thymidine and differed from that of thymine. The bulk of the reaction solution was freeze-dried, to give a white solid (439 mg., 88% recovery) which crystallised from water as needles, m. p. 183—185° (mixed m. p. with thymidine 182·5—183·5°).

Oxidation of Deoxyribonucleotides.—Thymidine-5′ phosphate. A solution of calcium thymidylate trihydrate (16·5 mg.) in water (5 ml.) was boiled under reflux for 4 hr. with manganese dioxide (200 mg.). The manganese dioxide was filtered off and the filtrate (which contained only 10% of the phosphorus of the starting material) evaporated to dryness. The residue (4 mg., 80%) crystallised from water as needles which had an infrared spectrum and chromatographic behaviour in solvents III and V identical with those of thymine (Found: C, 47·4; H, 4·85. Calc. for $C_5H_6N_2O_2$: C, 47·6; H, 4·8%). A control solution in which calcium thymidylate was boiled with water did not produce thymine.

Deoxyadenosine-5′ and deoxycytidine-5′ phosphate. When treated as above, these gave the corresponding nucleoside and free base. The amount of free base formed was about 50% greater than in control solutions which contained no manganese dioxide.

Oligodeoxyribonucleotide Fraction.—Preparation. Commercial herring-sperm deoxyribonucleic acid (12 g.) and magnesium sulphate heptahydrate (0·5 g.) in water (150 ml.) were treated with crude pancreatic deoxyribonuclease (65 mg.) at pH 7 for 42 hr. (the pH was maintained by the addition of dilute ammonia at intervals). The solution was then filtered and proteins were removed by Sevag's method.[7] The deproteinised solution was adjusted to pH 4 and lanthanum acetate solution (at pH 4) added until no further precipitation occurred. The precipitate was centrifuged off, washed with water, and dissolved in sodium hydrogen carbonate solution. After 18 hr. at 0°, the precipitate of lanthanum carbonate was centrifuged off and the supernatant liquid passed down a column of Zeo-Karb 225 (Na^+ form) and freeze-dried. The product (5 g.) was free from mononucleotides but contained 25% of inorganic material. It was analysed for purines and pyrimidines by Wyatt's method.[8] The results (moles of base/4 g.-atoms of P) were: guanine, 1·07; adenine, 1·04; cytosine, 0·641; uracil, 0·235; thymine, 0·994. The uracil was present in the commercial deoxyribonucleic acid and was presumably produced during manufacture since ribonucleic acid was absent. The average chain length of the fraction, determined by measurement of the amount of inorganic phosphate liberated by the action of prostatic phosphomonoesterase,[9] was 7·6.

Oxidation. A solution of the oligodeoxyribonucleotide fraction (1 mg./ml.) in 0·05M-ammonium hydrogen carbonate was boiled under reflux for 4·5 hr. with manganese dioxide (10 mg./ml.). The absorption of the solution at 260 mμ decreased by 8·5% whereas that of a control solution, which contained no manganese dioxide, remained constant. The reaction solution was chromatographed in solvent I. Uracil, thymine, and cytosine were identified and found to be present to the extent of 2·8, 5·8, and 4·0%, respectively, of the total base content of the original material. There were also considerable amounts of guanidine and urea.

Adsorption of Oligonucleotides on Manganese Dioxide.—The oligodeoxyribonucleotide fraction (20 mg.) was dissolved in 0·05M-ammonium hydrogen carbonate (20 ml.), and manganese dioxide was added with shaking as tabulated. After 1 min. the manganese dioxide was centrifuged off and the amount of ultraviolet-absorbing material remaining in the solution determined. The results were:

MnO_2 added (mg.)	0	100	200	400	600	800	2000
Oligonucleotides absorbed (%)	0	0	31	59	77	88	100

Oxidation of Deoxyribonucleic Acid.—A solution of highly polymerised calf-thymus deoxyribonucleic acid (1 mg./ml.) in 0·05M-ammonium hydrogen carbonate (30 ml.) was boiled under

[7] Sevag, *Biochem. Z.*, 1934, **273**, 419.
[8] Wyatt, *Biochem. J.*, 1951, **48**, 581, 584.
[9] Burton and Petersen, *Biochem. J.*, 1960, **75**, 17.

reflux for 4·5 hr. with manganese dioxide (100 mg./ml.). The manganese dioxide was centrifuged off and the supernatant liquid dialysed against distilled water. The solution outside the dialysis bag was freeze-dried and the residue chromatographed in solvent IV. It contained 2·5% of the cytosine and 3·0% of the thymine of the original nucleic acid. Urea was also detected. When only 10 mg./ml. of manganese dioxide was used, the diffusable material contained barely detectable amounts of thymine, cytosine and urea.

The authors thank Professor M. Stacey, F.R.S., for his interest, the Department of Scientific and Industrial Research for financial assistance and a scholarship (to A. R. W.), and the Salter's Institute of Industrial Chemistry for a research fellowship (to R. T. W.). They also thank Professor A. R. Katritsky for the sample of 9-ethylguanine.

CHEMISTRY DEPARTMENT, THE UNIVERSITY,
EDGBASTON, BIRMINGHAM 15.

[*Received, July 1st,* 1963.]