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Separation of 5′-Nucleotides by Carbon Chromatography. Isolation of Each Nucleotide from the Enzymic Digestion Products of RNA and DNA

In the chemistry of nucleotides, activated carbon has been used only in simple procedures such as removal of salts. The authors have found that nucleotides can be separated from each other by activated carbon chromatography because their adsorptivity differs according to the kind of the purines and pyrimidines they contain. By the use of this method, each nucleotide was successfully isolated from the enzymic digestion products of RNA and DNA,*1 and the results are set herein.

Activated carbon chromatography of nucleic acid components was reported for the first time in 1960.[1] According to this report, as much as 5 g. of activated carbon is required for about 2~3 μmoles of nucleic acids and, though elution is effected with diluted alcohol containing ammonia (gradient elution method), the eluate amounts to a large volume because the acid components are eluted inefficiently. On the other hand, there is a report[2] on the separative purification of CMP and 2′,3′-cyclic CMP by activated carbon. In the present experiments nucleotides were adsorbed on activated carbon at acid pH and the carbon, after washing with water, was eluted with 1.5~2% aqueous ammonia. This method will be usable industrially because it requires a small amount of activated carbon, the eluate is comparatively small, and each nucleotide is separated efficiently.

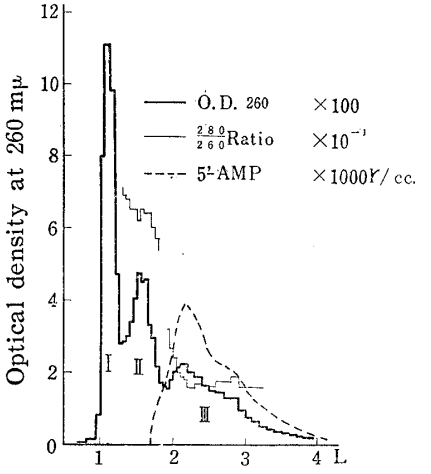

Fig. 1.

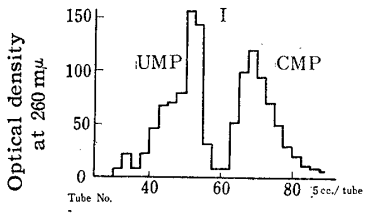

Fig. 2.

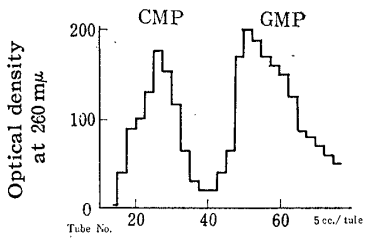

Fig. 3.

*1 Abbreviations used : RNA, ribonucleic acid; 5′-AMP, adenosine 5′-monophosphate; 5′-GMP, guanosine 5′-monophosphate; 5′-CMP, cytidine 5′-monophosphate; 5′-UMP, uridine 5′-monophosphate; DNA, deoxyribonucleic acid; 5′-dAMP, deoxyadenosine 5′-monophosphate; 5′-dGMP, deoxyguanosine 5′-monophosphate; 5′-dCMP, deoxycytidine 5′-monophosphate; TMP, thymidine 5′-monophosphate.

1) R. L. Stambaugh, D. W. Wilson : J. Chromatog., **3**, 221 (1960).
2) E. M. Crook, A. P. Mathias, B. W. Rabin : Biochem. J., **74**, 230 (1960).

At first, attempt was made to separate 5′-ribonucleotide from a solution of enzymic digestion products of RNA (containing 5′-AMP, 5′-GMP, 5′-CMP, and 5′-UMP). The solution was passed through a column of activated carbon weighing 8～9 times the total nucleotides at pH 1.5～2, the column was washed with water, and eluted with 1.5～2% aqueous ammonia. The washing contained no UV-absorbing substance. The eluate was divided into three fractions as shown in Fig. 1. The third fraction seems to contain 5′-AMP from its UV-absorbing ratio and enzymic assay, the second fraction is assumed to contain 5′-GMP, from its UV-absorbing ratio, and consequently the first fraction ought to contain 5′-UMP and 5′-CMP. To identify the components of each fraction, each was subjected to ion exchange chromatography on Amberlite CG-400 (chloride form) and, as chief components, 5′-UMP and 5′-CMP were detected in Fraction 1, 5′-GMP in Fraction 2, and 5′-AMP in Fraction 3 as expected, though they were more or less contaminated with each other. For separating 5′-UMP and 5′-CMP in Fraction 1, they were further chromatographed on activated carbon weighing 80～90 times their amount, when 5′-UMP was eluted first, and then 5′-CMP (Fig. 2).

Thus, it was found that a mixture of a pyrimidine nucleotide and a purine nucleotide can be efficiently separated into its components by chromatography on activated carbon. For example, Fig. 3 shows that a mixture of 5′-CMP and 5′-GMP can be separated by chromatography on activated carbon weighing 8 times their amount.

Next, a similar experiment was conducted on the enzymic digestion products of DNA (containing 5′-dAMP, 5′-dGMP, 5′-dCMP, and TMP), and Fractions 1 (5′-dCMP, TMP), 2 (5′-dGMP), and 3 (5′-dAMP) were obtained. Further chromatography of Fraction 1 on a larger amount of activated carbon exhibited two peaks (Fig. 4), but their separation was very difficult. Investigation by electrophoresis of the eluate from the above activated carbon showed that the former peak is due to 5′-dCMP and the latter to TMP. As described before, in the case of RNA series 5′-UMP was eluted first and 5′-CMP next. However, it is interesting to note that in the case of DNA series 5′-dCMP was eluted first and then TMP, which resembles 5′-UMP. This may be due to the methyl group in the pyrimidine ring. To examine the effect of the ribose and d-ribose in 5′-CMP and 5′-dCMP, a mixture of the authentic samples of 5′-CMP and 5′-dCMP was subjected to chromatography on activated carbon, but they were not separated at all. When Fraction 1 was submitted to chromatography on Amberlite IR-120 (acid form), TMP flowed out with the effluent and 5′-dCMP was eluted with 0.5*N* aqueous ammonia (Fig. 5) as in the case of 5′-UMP plus 5′-CMP.[3]

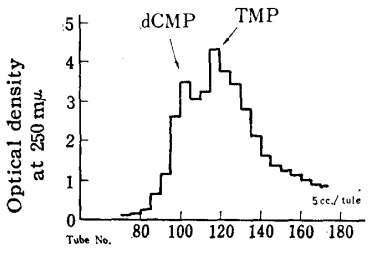

Fig. 4.

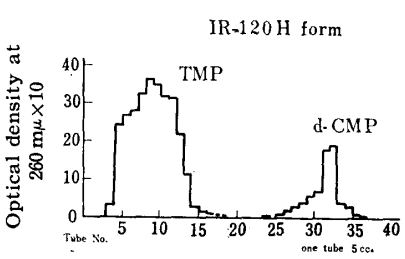

Fig. 5.

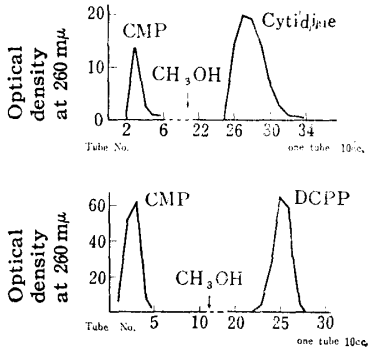

Fig. 6.

3) W.E. Cohn : Science, **109**, 377 (1949).

Finally, with a view to examining the behavior of nucleosides and dinucleotides in chromatography a mixture of 5′-CMP and cytidine, and a mixture of 5′-CMP and P,P_2-dicytidine pyrophosphate (DCPP) were chromatographed on activated carbon separately, whereupon cytidine and DCPP were clearly separated from 5′-CMP as shown in Fig. 6. Since these were hardly eluted with aqueous ammonia but readily with dilute methanol containing ammonia, it was found that the adsorptivity of nucleoside and dinucleotide on activated carbon is higher than that of mononucleotide.

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