The Direct Formation of a 3′,5′-Cyclic Mononucleotide from an Adenine Nucleoside

By M. HUBERT-HABART and L. GOODMAN*

(*Stanford Research Institute, Menlo Park, California* 94025)

THE 3′,5′-cyclic monophosphate of adenosine is an intermediate in the action of many peptide hormones.[1] A number of adenine nucleosides, such as 9-(β-D-xylofuranosyl)adenine (I) and 9-(β-D-arabinofuranosyl)adenine (III) possess anti-tumour activity[2,3] and we have been interested in the biological consequences of converting some of these compounds into their cyclic phosphates. The direct phosphorylation method of Yoshikawa, Kato, and Takenishi[4] proved to be especially useful for these syntheses.

When xylofuranosyladenine (I) was treated with phosphoryl chloride in trimethyl phosphate[4] for 2 hr., followed by hydrolysis, conversion into the barium salt, and finally treatment with Dowex 50 (H), a 38% yield of the crystalline 3′,5′-cyclic phosphate (II) was obtained.† Both paper-chromatographic and electrophoretic data‡ established that (II) was the cyclic compound and not the simple 5′-phosphate. The n.m.r. spectrum of (II) (2·5% NaOD) showed H-1′ as a sharp singlet quite similar to the appearance of

---

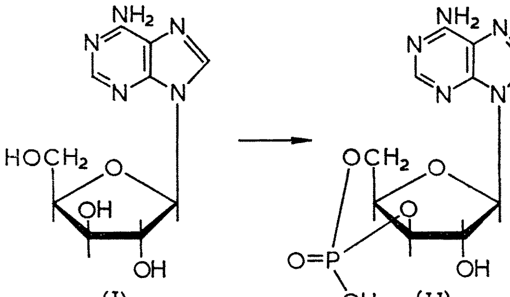
† Satisfactory analytical and spectral data were obtained for (II), (IV), and (V). We attribute the rather low yields of isolated nucleotides [(II) and (IV)] to the difficulty of separating their barium salts from the inorganic barium salts during the work-up procedures.

‡ Kindly provided by Dr. M. R. Atkinson, Flinders University of South Australia, on leave at Stanford University, who also provided useful information on the phosphorylation technique.

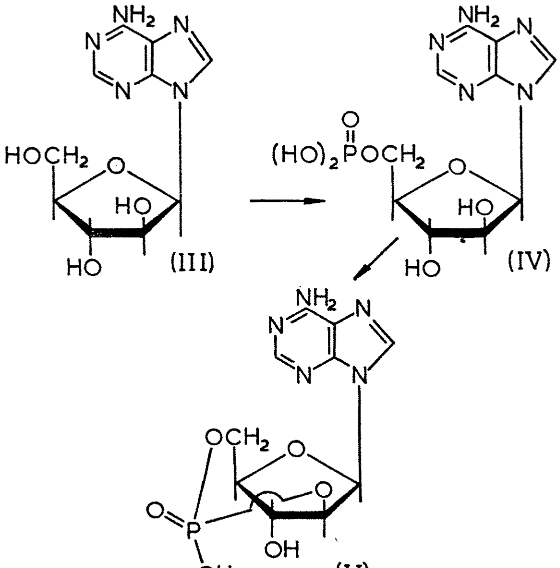
that resonance in 3′,5′-AMP[5] and in contrast with the situation in adenosine, 5′-adenylic acid and xylofuranosyladenine where H-1′ appears as a well-resolved doublet.

When 9-(β-D-arabinofuranosyl)adenine was treated similarly with phosphoryl chloride and trimethyl phosphate[4] a 40% yield of "spongoadenylic acid" (IV) was isolated as a crystalline solid. This was conveniently cyclized in high yield with dicyclohexylcarbodi-imide using the procedure of Smith, *et al.*[6] The n.m.r. spectrum of (V) as compared with that of (IV) clearly showed that cyclization gave the 2′,5′-cyclic nucleotide (V) in agreement with Wechter's results on the cyclization of N^4-benzoyl-*ara*-cytidine 5′-phosphate.[7] The pattern of the sugar proton resonances in (V) was identical with those reported for *ara*-cytidine 2′,5′-cyclic phosphate.[7] Cohen[8] has described the preparation of (IV) in 33% yield by the Tener[9] procedure and described a by-product cyclic phosphate which accompanies (IV). This latter compound appears to be the cyclic phosphate (V) by comparison of the described properties[10] with our compound. In our direct phosphorylation procedure[4] on (III), we carefully looked for the presence of (V) in the product and found only traces of it. This suggests that the larger quantities of cyclic phosphate described in Cohen's[8] work may have formed during the base treatment to convert the 5′-β-cyanoethylphosphate into (IV).

Biological studies with (II) and (V) are in progress.



(I) (II)



(III) (IV) (V)

Financial support was partly provided by a Contract with Chemotherapy, National Cancer Institute, National Institutes of Health, Public Health Service. M. H-H., from I.N.S.E.R.M., Paris, France, was the holder of an Eleanor Roosevelt International Cancer Fellowship awarded by the International Union Against Cancer.

(*Received, May 5th,* 1969; *Com.* 618.)

---

[1] E. W. Sutherland, I. Oeye, and R. W. Butcher, *Recent Progr. Hormone Res.*, 1965, **21**, 623.
[2] G. A. LePage and I. G. Junga, *Cancer Res.*, 1963, **23**, 739.
[3] D. B. Ellis and G. A. LePage, *Canad. J. Biochem.*, 1965, **43**, 617.
[4] M. Yoshikawa, T. Kato, and T. Takenishi, *Tetrahedron Letters*, 1967, 5065.
[5] E. D. Jardetzky, *J. Amer. Chem. Soc.*, 1962, **84**, 62.
[6] M. Smith, G. I. Drummond, and H. G. Khorana, *J. Amer. Chem. Soc.*, 1961, **83**, 698.
[7] W. J. Wechter, *J. Org. Chem.*, 1969, **34**, 244.
[8] J. J. Furth and S. S. Cohen, *Cancer Res.*, 1967, **27**, 1528.
[9] G. M. Tener, *J. Amer. Chem. Soc.*, 1961, **83**, 159.
[10] S. S. Cohen, *Progr. Nucleic Acid Res. Mol. Biol.*, 1966, **5**, 33.