ON THE REACTION OF ETHYLENE OXIDE WITH cAMP[1]

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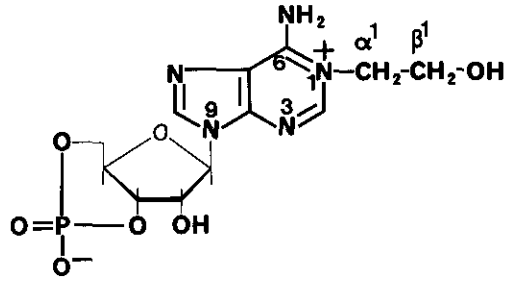
Abstract - Contrary to recent suggestions, proton coupled carbon-13 spectra show that the title reaction occurs on N^1 of the base rather than on the phosphate diester group.

Several synthetic methods have been proposed to convert nucleoside 3',5'-cyclic monophosphates into corresponding (electrically neutral) phosphotriesters.[2-5] These reactions were generally reported to produce a pair of diastereomeric triesters[3-5] in low yield[3] and a number of by-products corresponding to simultaneous substitution at the endo- or exocyclic heteroatoms (or groups) of the base.[5] In contrast to these observations, it has been recently reported[6] that the treatment of cAMP triethylammonium salt with ethylene oxide in aqueous solution for several hours gives one of the diastereomeric P-(2-hydroxyethyl) triesters as the major product stereospecifically and selectively in acceptable yields (approx. 30%). This finding appears to be quite unusual because ethylene oxide has been widely used as the alkylating agent of the bases of nucleosides and nucleotides,[7,8] whereas earlier attempts to convert phosphodiesters into phosphotriesters by use of ethylene oxide proved unsuccessful.[7,8] Supporting the triester structure, Zielinski *et al.*[6] reported their pertinent ^1H and ^{13}C NMR results. Remarkably however, both hydroxyethyl methylene carbon atoms were shown to exhibit unusually low (< 0.8 Hz) coupling constants with phosphorus which, according to these authors reflects a particular side chain conformation stabilized by an intramolecular H bond. Although the geometric dependence of vicinal $^3J_{COOP}$ coupling interaction in alkoxyl-substituted organophosphorus compounds has been the subject of extensive scrutiny,[9,10] no precedents are known in the literature where both geminal ($^2J_{COP}$) and vicinal ($^3J_{COOP}$) couplings of contiguous methylene carbon atoms assume similarly low values. In addition, the resonances of the hydroxyethyl methylene carbons were reported to occur at 59 and

54 ppm which values are approx. 10 ppm too low to account for the presence of oxygen substituents at both ends of the alkoxyl group. These difficulties in the interpretation, however, can be eliminated by assumption that the reaction with ethylene oxide occurs on the base rather than on the phosphodiester link.[11] In order to test this hypothesis, we have repeated the reaction of cAMP with ethylene oxide following the procedure given in Ref. 6.[12] The major product proved to be identical in every respect with the substance in the paper cited. Its broad band proton decoupled carbon-13 spectrum[13] revealed that, within the limits of resolution (approx. 0.2 Hz), the hydroxyethylene carbons, in fact, show no discernible couplings with phosphorus. The site of hydroxyethyl substitution, on the other hand, could be readily identified by recording the proton coupled ^{13}C spectrum (in D_2O) of the same product. Unlike with unreacted cAMP,[14] the components of the C^2-H^2 and C^6-H^2 doublets now appeared as triplets with splittings of 5.4 and $\sim$ 4.5 Hz, respectively, which were assigned to three-bond C^2-N^1-C-H and C^6-N^1-C-H coupling interaction. This finding unambiguously shows the hydroxyethyl group to be located at N^1 (1). Alkaline treatment of the product (NH_4HCO_3 in D_2O, pD = 10, 50°, 24 h) afforded N^6-(2-hydroxyethyl)-cAMP[15] via



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Dimroth rearrangement[16] which provided an independent corroboration for the above conclusion. Performing the reaction of cAMP with ethylene oxide at ambient temperature, we have isolated 1,3-di-(2-hydroxyethyl)-cAMP[17] as the second major product in addition to 1. The interaction of ethylene oxide with AMP,[1] on the other hand, afforded 1-(2-hydroxyethyl)-AMP-P-O-(2-hydroxyethyl) ester.[8,18] Our attempts to isolate phosphotriesters from the reaction products proved unsuccessful also with cyclic monophosphates of pyrimidine nucleosides, despite the known higher resistance of pyrimidine bases to alkylation with epoxides.[7] On a

novel synthetic route permitting to obtain the diastereomeric phosphotriesters in high (> 50%) yields, we will report in a later communication.

REFERENCES AND NOTES

1. Abbreviations used in this paper: cAMP = 9-β-D-ribofuranosyladenine 3',5'-cyclic phosphate; AMP = 9-β-D-ribofuranosyladenine 5'-phosphate.
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11. After completion of this work, we learned about the paper by J.B. Hobbs, in 'Organophosphorus Chemistry', eds. by D.W. Hutchinson and S. Trippett (Specialist Periodical Reports), The Chemical Society, London, 1979, Vol. 10, p. 169, suggesting a similar explanation for the observations made in Ref. 6.
12. A major alteration in the procedure was the substantial increase in the reaction time (7 to 10 days) required to attain the yield published. Satisfactory purification of the products could be achieved on a DEAE Sephadex A-25(HCO_3^-) column.
13. Spectra were recorded with a Varian XL-100/15 disk-augmented FT NMR system (25.16 MHz). Proton coupled ^{13}C spectra were obtained by the gated decoupling method, using 32K data words. Dioxane served for internal reference

(67.71 ppm).

14. Relevant proton coupled ^{13}C data for cAMP (D_2O) : C2 (d, J(C2,H2) 219.2), C6 (d, J(C6,H2) 10.8).

15. ^{13}C NMR (D_2O) : δ 43.70 (α-CH_2), 60.98 (β-CH_2), 68.05 (C5' d, J(P,C) 7.3), 72.47 (C4' d, J(P,C) 3.7), 73.06 (C2' d, J(P,C) 7.7), 77.98 (C3' d, J(P,C) 4.6), 92.28 (C1'), 119.46 (C5), 139.74 (C8), 147.94 (C4), 153.43 (C2 d, J(C2,H2) 219.2), 155.12 (C6, d, t, J(C6,H2) 10.9, J(C6,αH) 4.5) ppm.

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17. ^{13}C NMR (D_2O) : δ 47.88 (α^3-CH_2), 54.45 (α^1-CH_2), 59.40 (β^1-CH_2) 61.30 (β^3-CH_2), 68.23 (C5' d, J(P,C) 7.0), 73.30 (C4' d, J(P,C) 4.6), 73.56 (C2' d, J(P,C) 7.8), 78.08 (C3' d, J(P,C) 4.6), 93.43 (C1'), 120.65 (C5), 141.37 (C8), 147.35 (C4), 149.46 (C6), 149.95 (C2) ppm.

18. ^{13}C NMR (D_2O) : δ 53.69 (α^1-CH_2), 58.94 (β^1-CH_2), 62.15 (β-CH_2 d, J(P,C) 7.5), 65.59 (C5' d, J(P,C) 5.4), 67.85 (α-CH_2 d, J(P,C) 5.7), 71.20 (C2'), 75.36 (C3'), 85.12 (C4' d, J(P,C) 8.3), 89.26 (C1'), 119.8 (C5), 144.5 (C8), 147.8 (C6), 150.0 (C2) ppm.

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