Bromination of Adenine Nucleoside and Nucleotide

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THE halogenation of nucleic acid as well as its components (bases, nucleosides, and nucleotides) have been studied extensively by many investigators.[1] Especially, on the bromination reaction, several workers have reported[2] the introduction of bromine into uracil, cytosine, and guanine moieties of nucleotides. Despite the use of a variety of halogenating agents and reaction conditions, the direct bromination of adenine nucloetides has not yet been reported. Although recently Holmes and Robins[3] developed a unique method for bromination of adenosine, the use of organic solvent for the reaction limits its use in the adenine nucleotides,[4] mainly because of their insolubility in the organic solvent.

We report a versatile method for the bromination of adenine nucleosides and nucleotides.

Adenosine 5′-monophosphate (A5′P) disodium salt* was dissolved in 0·1N-sodium hydroxide solution (containing 1 equiv. of sodium hydroxide). Bromine water (0·3 mmole/ml.) was added to this solution until one molar equivalent of bromine was contained in the mixture. The reaction was carried out with occasional shaking at 20° for 3·5 hr. and further at room temperature (15—20°) for 7 hr. Examination of the reaction mixture by paper

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* The salt with other cations may be used as such in this reaction.

chromatography showed the conversion of A5′P into the bromo-derivative [R_f (A)† 0·08, R_f (B) 0·31]. Ultraviolet absorption properties of the upper spot were λ_{max} (H^+) 262 mμ, λ_{max} (H_2O) 263 mμ.‡ Purification of this product on Dowex 1 × 8 (formate form) column eluted with 0·1N-formic acid gave 8-bromoadenosine 5′-monophosphate (disodium salt) in 81% yield. The structure of this nucleotide was confirmed by the comparison with an authentic sample synthesised from 2′,3′-*O*-isopropylidene-8-bromoadenosine[5] by phosphorylation, followed by the acidic removal of the protecting group.[6]

Adenosine was brominated in an analogous procedure. Although in this case the reaction proceeded slowly, at the end of 96 hr. adenosine was converted completely into 8-bromoadenosine [R_f (A) 0·61, R_f (B) 0·62, R_f (D) 0·41; λ_{max} (H^+) 264·5 mμ, λ_{max} (H_2O) 266·5 mμ, λ_{max} (OH^-) 265 mμ]. 8-Bromoadenosine isolated by paper chromatography (solvent C) showed identical properties with an authentic sample[3] (yield 78%). Bromination of 2′-deoxyadenosine was achieved by an analogous procedure, and 8-bromo-2′-deoxyadenosine was obtained in 66% yield.

When the bromination was applied to the protected adenosine, the following procedure was used. 2′,3′-*O*-Isopropylideneadenosine (1 mmole) was dissolved in 1 : 1 (vol./vol.) mixture (30 ml.) of dioxan and 10% disodium hydrogen phosphate solution, followed by the addition of 1·5 equiv. of bromine. The mixture was shaken for 5 hr. at room temperature and kept overnight at room temperature. Extraction of the reaction mixture with chloroform and the usual work-up gave 8-bromo-2′,3′-isopropylideneadenosine (80·5%) [m.p. 224—225° (from ethanol) λ_{max} (H^+) 264 mμ, λ_{max} (H_2O) 266 mμ, λ_{max} (OH^-) 265 mμ]. Paper chromatography: R_f (C) 0·77. The sample was identical with an authentic specimen.[5]

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† Solvent A, propan-2-ol–conc. ammonia–water, 7 : 1 : 2; solvent B, n-butanol–acetic acid–water, 5 : 2 : 3; solvent C, n-butanol–water, 86 : 14; solvent D, water adjusted to pH 10 with ammonia.

‡ Although these values were not completely consistent with those reported for 8-bromoadenosine (ref. 3), the introduction of the 5′-phosphoryl residue may cause a slight shift in ultraviolet absorption maxima.

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