Reaction of Alkoxyuridines with Ammonia (1)

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2-Alkoxy- and 4-alkoxyuridines were converted into isocytidine and cytidine by reaction with ammonia at room temperature. Reaction with hydrogen sulfide produced 2-thio- and 4-thiouridine but the reaction was incomplete. The reaction may be helpful in the identification of alkylated pyrimidine nucleosides in DNA.

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Alkylation of DNA by carcinogens can result in the formation of covalent adducts or alkylated derivatives with all bases (3,4). The base adducts that carry the alkyl moiety on an oxygen have attracted particular interest because of their ability to miscode *in vitro* (5-7) and their suspected importance in carcinogenesis (8). The reaction described here offers a means to derivatize *O*-alkylated uridines and thymidines to known cytidines and isocytidines and thus derives the structure in cases where the *O*-alkylated nucleoside is not known.

The exchange of an alkoxy- against an amino group has been described for alkoxyuracils (9) but more vigorous conditions were required.

Both 2-*O*-methyluridine and 4-*O*-methyluridine are smoothly converted into isocytidine and cytidine respectively by reaction with liquid ammonia in a sealed tube at 20° with half-times of about 20 hours (Figure 1). Both reactions are essentially completed after one week (data points not shown).

Hydrogen sulfide converts both 2-*O*-methyluridine and 4-*O*-ethyluridine into 2-thiouridine and 4-thiouridine. The reaction is about 50% complete after 18 hours at 110° but no kinetics was studied because of the objectionable smell. The products were identical to the known reference compounds in their retention volumes on cation exchange and reverse phase hplc and uv spectra. There was no evidence of de-pyrimidation and the respective cytidine was the only product in the reaction with ammonia according to cation exchange chromatography. The reaction with hydrogen sulfide, even though incomplete, was free of any free pyrimidine by-products according to reverse phase chromatography and boronate chromatography. Such free pyrimidine by-products are reported to occur and even predominate in the hydrolysis of alkoxyuridines in aqueous buffers (10).

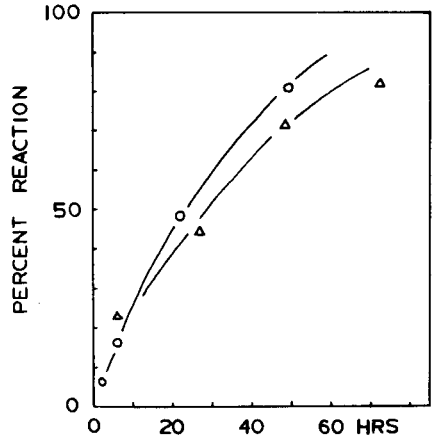

Figure 1. Reaction of 2-*O*-methyluridine (Δ) and 4-*O*-methyluridine (o) with ammonia expressed as percent conversion to isocytidine and cytidine, respectively.

EXPERIMENTAL

Reaction with Ammonia.

Solutions containing 0.25 μmoles of 4-*O*-methyluridine or 2-*O*-methyluridine were dried in a thick-walled vial. About 0.5 ml. of ammonia was condensed into each vial and the vial sealed, leaving a 1 ml. air space. Sealed samples were reacted at 20° and then opened. The entire sample was dissolved in 0.4 ml. of buffer for spectroscopic analysis.

Reaction with Hydrogen Sulfide.

Solutions containing 1.0 μmole of 2-*O*-methyluridine or 4-*O*-ethyluridine were dried in a thick-walled glass tube, one drop of acetic acid was added and about 1 ml. of liquid hydrogen sulfide was condensed into the tube under dry ice-acetone cooling. The tubes were sealed and heated at 110° for 18 hours. They were then cooled again, opened and the hydrogen sulfide allowed to escape. Pyridine, 0.3 ml., and acetic anhydride, 0.1 ml., were added and allowed to react for 18 hours at room temperature and then evaporated under vacuum. The nucleoside triacetates were then analyzed by reverse-phase Hplc-chromatography.

Cation Exchange Hplc-chromatography.

The system of Uziel and Cohn (11) was used to analyze the products. At a flow rate of 0.38 ml./minute, the elution volumes for 2-*O*-methyluridine, 4-*O*-methyluridine, cytidine and isocytidine were 5.7 ml., 9.6 ml., 20.4 ml. and 9.0 ml., respectively.

Reverse-phase Hplc-chromatography.

A Whatman Partisil PXS 10/25 ODS-2 was eluted with a linear gradient of 40 ml. of 10% aqueous ethanol to 40 ml. of 50% aqueous ethanol. Both reservoirs also contained 0.15% of acetic acid. At a flow rate of 1.5 ml./minute, the elution volumes for the triacetates of uridine, 2-*O*-methyluridine, 4-*O*-ethyluridine, 2-thiouridine and 4-thiouridine were 32 ml., 42 ml., 50 ml., 52 ml. and 40 ml. respectively.

Polyacrylamide Boronate Chromatography.

Bio-Rad Affi-Gel 601 boronate resin was loaded into 0.1*M* ammonium acetate, *p*H 10.5, and the ribonucleosides were eluted in 1*M* acetic acid.

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With this regimen the resin had a capacity of 0.8 mmoles of guanosine per 5 g. of dry resin. One gram of this resin was used to separate nucleosides from non-nucleosides.

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