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THE REDUCTION OF PYRIMIDINE NUCLEOSIDES WITH SODIUM AND ETHANOL IN LIQUID AMMONIA*†

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In connection with work on the structure of some pyrimidine nucleosides obtained from marine invertebrates (1), it became necessary to develop a method for the identification of the carbohydrate moiety of these nucleosides. The resistance of pyrimidine nucleosides to mild acid hydrolysis is well known, and the glycosidic link can only be hydrolyzed readily and without destruction of the carbohydrate residue after hydrogenation of the heterocyclic ring (2). High pressure catalytic hydrogenation of nucleosides is inconvenient on a small scale and does not always proceed readily (1). However several authors have shown that heterocyclic rings can be reduced with sodium or with sodium and ethanol in liquid ammonia (3–5), and preliminary experiments showed that treatment of uridine with sodium in liquid ammonia led to about 50% reduction (as judged by the absorption at 261 mμ). Addition of ethanol to the reaction mixture led to complete reduction.

Following the reduction, sodium ions were removed by passing the solution through a column of Dowex 50 resin. Free ribose could then be demonstrated by paper chromatography, by paper ionophoresis in a borate buffer, and by isolation as the phenylosazone. Similar reduction of a number of synthetic pentopyranoside nucleosides gave the respective pentose in each case. Parallel experiments with adenosine, which contains a normal glycosidic bond, indicate that this bond is probably split by the resin rather than by the ethanol functioning as an acid in liquid ammonia (6). The nature of the reduction product of the pyrimidine ring was not established, but Batt, *et al.* (7) have shown that catalytic hydrogenation of the pyrimidines gives the corresponding dihydro compounds which decompose in alkali to give the β-ureidoacids. Evaporation of the eluate from the Dowex 50 column gave a residue which was acid to litmus paper, and this may be due to the acid obtained by fission of the pyrimidine ring. The acidity was sufficient to decompose 2-deoxyribose and a little ammonia was routinely added before evaporation.

Reduction of thymidine and deoxycytidine by this method gave 2-deoxyribose, identified by paper chromatography and paper ionophoresis in a borate buffer. This is the first chemical demonstration of the presence of 2-deoxyribose as the carbohydrate moiety of the pyrimidine deoxyribosides, although it has been demonstrated enzymatically (8, 9). Attempted reduction of nucleotides and the nucleic acids failed presumably because of their insolubility in liquid ammonia.

The application of this work to the nucleosides from marine invertebrates will

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be published elsewhere. A preliminary account of this work has already been published (10).

EXPERIMENTAL

Materials. The author is indebted to J. J. Fox of the Sloan-Kettering Institute, New York, for the gifts of the pentopyranosyl nucleosides and to W. G. Overend of the University of Birmingham, England for the 2-deoxy-L-ribose. The ribonucleosides and ribonucleotides were obtained from Schwarz Labs., Mount Vernon, N. Y., and the deoxyribose derivatives and the nucleic acids from the California Foundation for Biochemical Research, Los Angeles, California.

General method. The nucleoside (10–20 mg.) was dissolved in dry liquid ammonia (50 cc.) and dry ethanol (1 cc.); sodium (30–50 mg.) was added with stirring. After the mixture had been stirred for three hours in a methanol-Dry Ice bath, water (20 cc.) was added cautiously. The excess ammonia was removed at 25° and 20 mm. to give a solution showing no selective absorption in the ultraviolet. This solution then was passed down a column of Dowex 50 (10 cm. x 0.75 cm^2, hydrogen form). After addition of 0.88 *N* ammonium hydroxide (1 cc.) to the eluate, it was evaporated at 40–50° and 20 mm. and the residue was chromatographed on paper. The sugars were detected by the method of Partridge (11).

Treatment of the four naturally occurring pentoses in this way, followed by paper chromatography (descending system) with *n*-butanol saturated with water (12) as solvent, showed that no detectable isomerization had occurred.

Pyrimidine ribosides. Reduction of cytidine (8 mg.) and of uridine (7 mg.) in the above manner followed by paper chromatography with *n*-butanol saturated with water and *n*-butanol:water:ethanol (5:4:1) (upper layer) (13) as solvents revealed one spot identical with that given by ribose. This identification was confirmed by paper ionophoresis in borate buffer (14).

Isolation of ribose as the phenylosazone. Uridine (187 mg.) in dry ammonia (100 cc.) was stirred while dry ethanol (5 cc.) and sodium (0.1 g.) were added. The reaction mixture then was worked up as above to give a syrup (90 mg.) $[\alpha]_D$ −10°, which was shown by paper chromatography to contain ribose as the only carbohydrate constituent. Treatment with phenylhydrazine in the usual way gave a phenylosazone; the latter, when recrystallized from aqueous methanol, gave material, the melting point of which, 156–158°, was not depressed by admixture with the phenylosazone from ribose. The infrared spectrum was identical with that of the phenylosazone from ribose (in dioxane), but different from that of the phenylosazone from xylose (in the 9.75 μ region).

Synthetic pentopyranosylpyrimidine nucleosides. Reduction of D-arabinopyranosylcytosine (29 mg.) (15) and D-arabinopyranosyluracil (15 mg.) (16), followed by paper chromatography in *n*-butanol-water and *n*-butanol-water-ethanol systems, revealed a single spot identical with that given by arabinose. Similar treatment of D-xylopyranosylthymine (17 mg.) (15) gave a spot identical with that given by xylose.

Adenosine. (*a*). Treatment of adenosine (20 mg.) with sodium and ethanol in liquid ammonia led to complete reduction, and working up as above, followed by paper chromatography and paper ionophoresis in a borate buffer, showed the presence of ribose.

(*b*). Adenosine (30 mg.) was stirred in dry ammonia (50 cc.) and absolute ethanol (5 cc.) for three hours. Evaporation and paper chromatography failed to reveal any adenine or ribose.

(*c*). Adenosine (20 mg.) was dissolved in water (20 cc.), methanol (5 cc.) and sodium hydroxide (50 mg.) were added, and the solution was passed down a column of Dowex-50. Evaporation of the eluate and paper chromatography showed ribose to be present.

Deoxyribose pyrimidine nucleosides. Reduction of thymidine (28 mg.) and deoxycytidine (27 mg.), followed by chromatography in the butanol-water and butanol-water-ethanol systems and by paper ionophoresis in a borate buffer, revealed a single spot identical with that given by 2-deoxy-L-ribose.

Nucleotides. Treatment of uridylic acid (25 mg.) and cytidylic acid (23 mg.) with sodium and ethanol in liquid ammonia gave 80% and 90% recovery, respectively, of unchanged nucleotide (estimated spectroscopically). The nucleotides appeared to be insoluble in the liquid ammonia and also in mixtures of liquid ammonia and dimethylformamide. Deoxycytidylic acid (20 mg., as the diammonium salt) also appeared to be insoluble and was recovered in 70% yield (estimated spectroscopically). Working up in the usual way afforded a solution which gave a negative Dische test.

Nucleic acids. Both ribonucleic acid and deoxyribonucleic acid appeared to be insoluble in liquid ammonia and were recovered in 88% and 87% yield (estimated spectroscopically) and after treatment with sodium and ethanol.

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SUMMARY

1. A simple method for the identification of the carbohydrate moiety of small quantities of pyrimidine nucleosides has been described.

2. The carbohydrate moiety of the pyrimidine desoxyribosides has been shown by chemical means to be 2-deoxyribose.

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BIBLIOGRAPHY

(1) BERGMANN AND FEENEY, *J. Org. Chem.*, **16**, 981 (1951).
(2) LEVENE AND LAFORGE, *Ber.*, **45**, 608 (1912).
(3) KNOWLES AND WATT, *J. Am. Chem. Soc.*, **65**, 410 (1943).
(4) CAPPEL AND FERNELIUS, *J. Org. Chem.*, **5**, 40 (1940).
(5) BIRCH AND MCALLAN, *Nature*, **165**, 899 (1950).
(6) BIRCH, *Quart. Reviews (London)*, **4**, 69 (1950).
(7) BATT, MARTIN, PLOESER, AND MURRAY, *J. Am. Chem. Soc.*, **76**, 3663 (1954).
(8) FRIEDKIN AND ROBERTS, *J. Biol. Chem.*, **207**, 257 (1954).
(9) MACNUTT, *Biochem. J.*, **50**, 384 (1952).
(10) BURKE, *Chemistry & Industry*, **45**, 1393 (1954).
(11) PARTRIDGE, *Nature*, **164**, 443 (1949).
(12) ZAMENHOF, LEIDY, FITZGERALD, ALEXANDER, AND CHARGAFF, *J. Biol. Chem.*, **203**, 695 (1953).
(13) HIRST AND JONES, *Discussions Faraday Soc.*, No. **7**, 268 (1949).
(14) FOSTER, *Chemistry & Industry*, **71**, 828 (1952).
(15) FOX AND GOODMAN, *J. Am. Chem. Soc.*, **73**, 3256 (1951).
(16) VISSER, DITTMER, AND GOODMAN, *J. Biol. Chem.*, **171**, 377 (1947).