BIOCHEMISTRY

A Metabolite of 'Carbromal'

'CARBROMAL' (α-bromo diethylacetyl urea) is in widespread use as a sedative and hypnotic. None of its metabolites has, as yet, been fully characterized.

I recently isolated diethylacetyl urea from the intestine contents of a woman who was taking a proprietary drug containing 'Carbromal' 240 mgm. and 'Bromvaletone' 80 mgm. The method of isolation was that using acid ammonium sulphate, described by Nickolls[1]; 5 mgm. of the ureide crystallized readily from the neutral, ether-soluble fraction. Purification was by sublimation at 10^{-2} mm. at 100° C. It was at first thought that the product was α-ethyl crotonyl carbamate, which was noted as a product of the reaction of sodium hydroxide on 'Carbromal' by Newberry[2]. Both these compounds and their mixture melted within 5 deg. (200–205°); but because of their rapid sublimation these determinations were inadequate. Infra-red spectroscopy clearly differentiated the compound from α-ethyl crotonyl carbamate and from 'Sedormid' (allyl *iso*propyl, acetylurea). As a result of an examination of the spectra of several compounds in the series a specimen of diethylacetyl urea was prepared by hydrogenation of α-ethylcrotonylcarbamate (Dr. S. Trippett). After sublimation its spectrum in 'Nujol' was identical with that of the compound isolated from the intestines (Fig. 1). It seems probable that this is the compound observed by Turner[3].

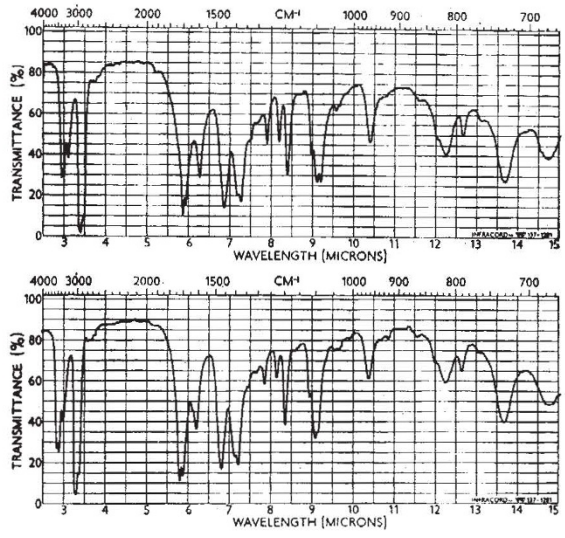

Fig. 1. The top curve shows the spectra of diethyl acetyl urea; the bottom curve is that of the crystals from the intestines

Thanks are accorded to D. Grime of Imperial Chemical Industries, Harrogate, for the infra-red spectroscopy.

A. S. CURRY

Home Office Forensic Science Laboratory, Harrogate.

[1] Nickolls, "The Scientific Detection of Crime", 365 (Butterworth, 1956).
[2] Newberry, *J. Chem. Soc.*, **127**, 295 (1925).
[3] Turner, *Med. J. Australia*, **i**, 729 (1959).

Synthesis of Nicotinic Acid Nucleotides

NICOTINIC acid nucleotide (5′-phospho-β-D-ribofuranosylpyridinium-3-carboxylate) and its adenylyl derivative, adenine-nicotinic acid dinucleotide, have recently been identified as intermediates in the biosynthesis of pyridine nucleotide coenzymes[1]. These compounds have been found in fungi[2,3], and the mononucleotide has been isolated in small amounts from yeast[4]. Both nucleotides have also been obtained in very low yields by enzymic modification of adenine-nicotinamide dinucleotide (coenzyme I)[2,3,5].

It has now been found that nicotinamide nucleotides, which are commercially available, may be conveniently converted into nicotinic acid nucleotides by treatment with nitrous anhydride. Preliminary work showed that nitrous acid or hydrogen chloride in amyl nitrite deaminates adenine nucleotides to hypoxanthine nucleotides, but does not liberate the amide nitrogen from nicotinamide nucleotides.

β-Nicotinamide nucleotide was suspended in dry acetic acid (10 ml. per millimole) and kept at −10° while an equal volume of nitrous anhydride[6] was condensed in the reaction vessel. The suspension was then kept at 10–15° for 1 hr. After removal of volatile material in a stream of dry air, the reaction products were applied to a column of 'Dowex-1-formate' and the nicotinic acid nucleotide was recovered by elution with formic acid[1]. The nucleotide was identified by electrophoresis, ion-exchange chromatography and paper chromatography, and by spectrophotometry in the presence and absence of cyanide[1,3,4].

When coenzyme I was treated with nitrous anhydride in acetic acid both amide and amine nitrogens were displaced, and hypoxanthine-nicotinic acid dinucleotide was obtained. This dinucleotide was quantitatively hydrolysed to nicotinic acid nucleotide and hypoxanthine nucleotide (inosine-5′-phosphate) by nucleotide pyrophosphatase from potato.

With adenosine triphosphate and magnesium ion at *p*H 7·6 in the presence of highly purified nicotinamide nucleotide-adenylyl transferase (coenzyme I pyrophosphorylase) from pig liver nuclei the nicotinic acid nucleotide was quantitatively converted into its adenylyl derivative. This derivative was likewise identified by the physical methods described above, and further by its conversion into coenzyme I in the presence of glutamine, adenosine triphosphate, magnesium ion and coenzyme I synthetase from pig liver cytoplasm. The coenzyme I was identified by reduction with ethanol and alcohol dehydrogenase from yeast.

Nicotinic acid nucleotide obtained in this way is a competitive inhibitor of enzymic adenylyl transfer from adenosine triphosphate to β-nicotinamide nucleotide, and is evidently identical with the natural coenzyme precursor.

M. R. ATKINSON
R. K. MORTON

Department of Agricultural Chemistry, Waite Agricultural Research Institute, University of Adelaide.

[1] Preiss, J., and Handler, P., *J. Biol. Chem.*, **233**, 488, 493 (1958).
[2] Serlupi-Crescenzi, G., and Ballio, A., *Nature*, **180**, 1203 (1957).
[3] Ballio, A., and Russi, S., *Arch. Biochem. Biophys.*, **85**, 567 (1959).
[4] Wheat, R. W., *Arch. Biochem. Biophys.*, **82**, 83 (1959).
[5] Lamborg, M., Stolzenbach, F. E., and Kaplan, N. O., *J. Biol. Chem.*, **231**, 685 (1958).
[6] Dox, A. W., "Organic Syntheses", edit. by Gilman, H., and Blatt, A. H., second ed., **1**, 266 (John Wiley and Sons, Inc., New York, 1958).