Biochemical Pharmacology, Vol. 18, pp. 1549–1551. Pergamon Press. 1969. Printed in Great Britain

THE METABOLISM OF TREMORINE— THE IDENTIFICATION OF 1,4-DI (2-OXOPYRROLIDINO)-2-BUTYNE (SYMMETRIC DIOXOTREMORINE)

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(*Received* 21 *October* 1968; *accepted* 10 *December* 1968)

Abstract—A new metabolite of tremorine, symmetric dioxotremorine [1,4-di(2-oxopyrrolidino)-2-butyne] has been identified in rats by the use of combined gas chromatography–mass spectrometry.

STUDIES of the metabolism of tremorine (TMN), 1,4-dipyrrolidino-2-butyne, have shown that the central effects produced by the compound e.g. tremor and hypothermia, are mediated by the metabolite oxotremorine[1] (OTMN), 1-pyrrolidino-4(2-oxopyrrolidino)-2-butyne. This paper reports the identification of a new metabolite of TMN in rats, symmetric dioxotremorine (DOT), which does not cause hypothermia or any gross central cholinergic effects.

METHODS

Preparation of urine samples

Male Sprague–Dawley rats, weighing 300–400 g were injected repeatedly i.p. with tremorine dihydrochloride (10 mg/kg). Urine from several rats was collected free of faeces and pooled. The urine was alkalinized with NaOH and put on Dowex 1-resin in the fluoride form. The resin was washed with 100 ml of water. The non-adsorbed material from the resin was freeze-dried and dissolved in 20 ml of 0·1 N NaOH and extracted three times with 20 ml of chloroform. The combined extracts were evaporated to dryness and the residue taken up in 0·2 ml of methanol.

Gas–liquid chromatography (GLC)

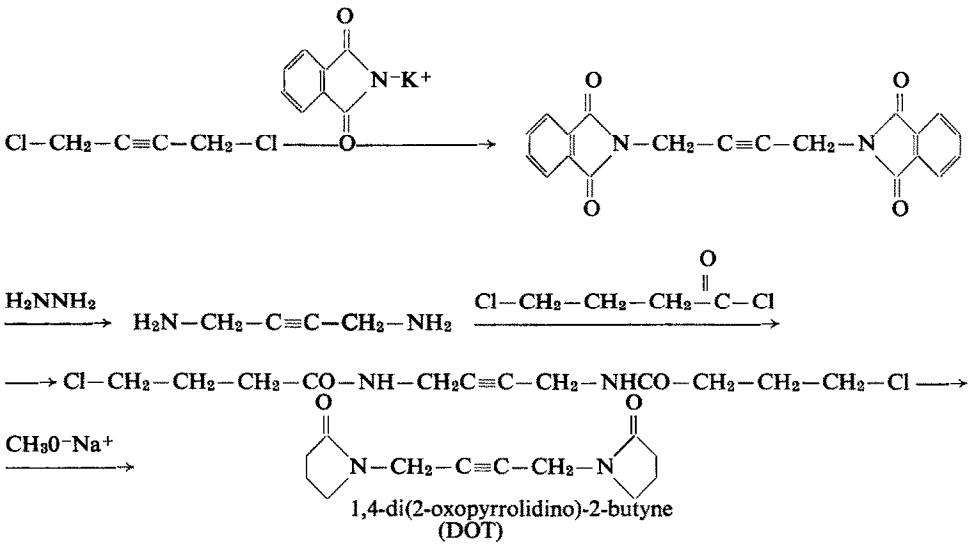
GLC was undertaken as described previously.[2] The column used was 5 per cent OV-17 on silanized gaschrom P (100–120 Mesh) and operated at 250°.

Gas–liquid chromatography–mass spectrometry (GLC–MS)

For procedure see Hammer *et al.*[2] Spectra of authentic DOT and of the studied component of the urinary extract were obtained under identical conditions.

Synthesis of DOT

DOT was synthesized accordingly:



The purity and identity of the final product was verified by GLC and GLC–MS (Fig. 1), respectively.

RESULTS AND DISCUSSION

The figure shows mass spectra of the gas-chromatographic peak of synthetic DOT and of the peak from the urine extract, which had the same retention time as DOT. The bond cleavages of the symmetrical DOT molecule occur mainly in the chain. The sites of these cleavages are both in α- and β-positions to the ring nitrogens. Both the heavy and the light fragments of the molecules, formed by the mentioned cleavages, are found in the mass spectrum. The α-cleavage gives rise to the fragments m/e = 136 and/or m/e = 84. However, this cleavage gives mainly rise to the fragment m/e 137, which has the highest intensity and is probably formed by an intramolecular rearrangement of a hydrogen.

Two other fragments which are characteristic in the mass spectrum are formed by the β-cleavage and have m/e values equal to 98 and 122. As the α-cleavage is dominant these smaller fragments have a lower intensity.

DOT was injected i.p. in rats and mice in doses of 5–10 mg/kg. In contrast to OTMN, DOT had no effect on the body temperature and no central or peripheral cholinergic effects were observed. OTMN produces tremor, salivation and in the mouse a marked hypothermia in doses of 0·2–0·5 mg/kg. It therefore appears that symmetric DOT is a biologically inactive metabolite of TMN. Interestingly, no evidence was obtained by GLC-MS for the formation of assymetric DOT[*N*-(4-pyrrolidino-2-butynyl)-succinimide] which produces hypothermia in mice in a dose of 5 mg/kg i.p. The present observation, previous data and experiments in progress ndicate that the hypothermia produced by TMN is caused by OTMN, formed

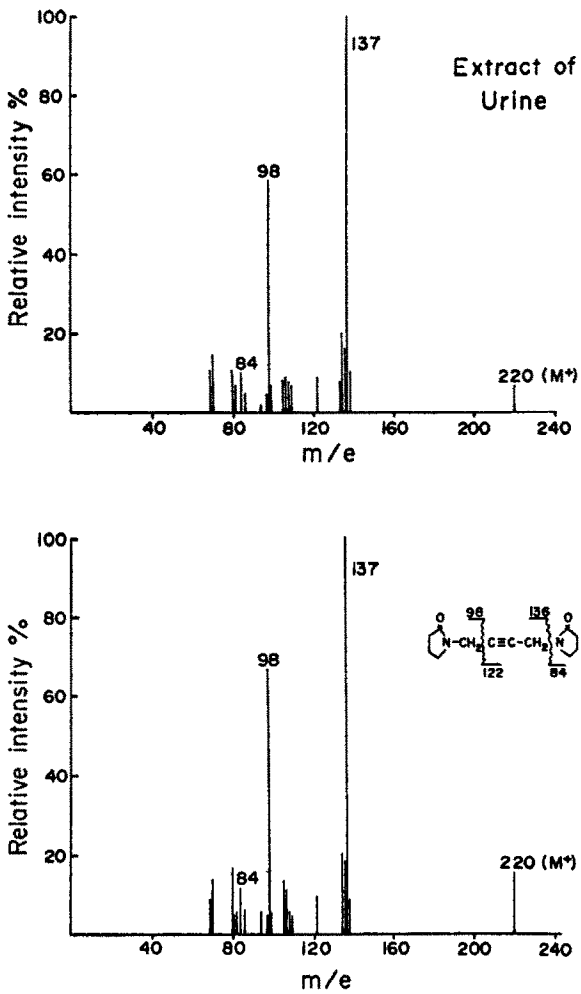


FIG. 1. Mass spectra obtained from gas chromatographic peaks with identical retention times. Upper panel: Rat urine extract. Lower panel: Synthetic 1,4-di(2-oxopyrrolidino)-2-butyne. Gas chromatographic conditions, see text.

either directly from TMN or by ring closure of the metabolite *N*-(4-pyrrolidino-2-butynyl)-γ-aminobutyric acid.[2] The formation of the latter metabolite is of interest in relation to the possible inhibitory transmittor action of GABA.

REFERENCES

1. A. K. CHO, W. L. HASLETT and D. J. JENDEN, *Biochem. Biophys. Res. Comm.* **5**, 276 (1961).
2. W. HAMMER, B. HOLMSTEDT, B. KARLÉN, F. SJÖQVIST and J. VESSMAN, *Biochem. Pharmac.* **17**, 1931 (1968).