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Note

Oxetorone metabolism *in vitro*

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Oxetorone, 6-(3-dimethylaminopropylidene)[2,3-*e*]benzofuro-12H-benzo[*b*]oxepine hydrogen fumarate, is an antagonist of 5-hydroxytryptamine, histamine and catecholamines[1]. In addition, it shows antiemetic, antianaphylactic, analgesic properties and it dilates the brain vessels, but is only a weak neuroleptic agent[2]. These properties have suggested the use of oxetorone in the treatment of migraine[3].

This paper describes the identification of metabolites such as oxetorone-N-oxide and desmethyloxetorone, formed *in vitro* after incubation of oxetorone with rat liver microsomes.

EXPERIMENTAL

Standards and reagents

Oxetorone and desmethyloxetorone were supplied by S. A. Labaz N.V. Brussels, Belgium) as a mixture of two isomers, E and Z.

The following reagents were used: glucose-6-phosphate, glucose-6-phosphate dehydrogenase and NADP (Boehringer, Mannheim, G.F.R.), and magnesium chloride and sodium hydroxide (Merck, Darmstadt, G.F.R.).

The solvents used were of analytical-reagent grade.

Incubation system

Microsomes were obtained from livers of rats by a procedure[4] involving centrifugation (11,000 *g*) of liver homogenate in a 0.05 *M* phosphate buffer (pH 7.4), containing potassium chloride (1.15%) and magnesium chloride (5 m*M*). The supernatant was ultracentrifuged at 104,000 *g* for 1 h in order to obtain the microsomal fraction.

The final concentrations of co-factors, added to the microsomal suspension in 0.05 *M* phosphate buffer (pH 7.4), were NADP 0.3 m*M*, glucose-6-phosphate 10 m*M* and glucose-6-phosphate dehydrogenase 0.3 U/ml; oxetorone was added as an ethanolic solution (100 μg) and the mixture was incubated at 37° for 1 h, with gentle shaking.

Extraction procedure

The incubation mixture (5 ml) was adjusted to pH 9 with 1 *N* sodium hydroxide

solution and extracted twice with 10 ml of ethyl acetate. The organic phase was evaporated to dryness in a water-bath at 60° under nitrogen.

The residue, dissolved in methanol, was used for thin-layer chromatography (TLC), gas–liquid chromatography (GLC) and mass spectrometry (MS) using a direct inlet system (DIS).

Thin-layer chromatography

Thin-layer chromatograms were prepared on glass plates (5 × 20 cm) coated with a 0.25-mm layer of silica gel F_{254} (Merck, Darmstadt, G.F.R.) and developed at room temperature, using benzene–acetone–ammonia (50:50:1) as solvent system. Oxetorone and its *in vitro* metabolites were detected under UV light at 254 and 365 nm.

Gas chromatography–mass spectrometry

An LKB 9000 mass spectrometer equipped with a gas chromatograph was used. The chromatographic column was a glass tube (2 m × 4 mm I.D.) packed with 3% OV-1 on Gas-Chrom Q (100–120 mesh) (Applied Science Labs., State College, Pa., U.S.A.). The injection port temperature was 290° and the oven temperature 270°; the carrier gas (helium) flow-rate was 30 ml/min. The mass spectrometer was operated under the following conditions: molecular separator temperature, 290°; ion source temperature, 290°; energy of ionization beam, 70 eV; ionization current, 200 μA. Samples were introduced either by a GLC procedure or by the DIS at a probe temperature between 20° and 150°.

Synthesis of oxetorone-N-oxide

A 100-mg amount of oxetorone fumarate (E and Z isomers) was dissolved in 50 ml of distilled water and made alkaline with 1 *N* sodium hydroxide solution. The suspended free base was extracted twice with 50 ml of diethyl ether; the organic phase was evaporated to dryness and the residue dissolved in 10 ml of methanol.

A 5-ml volume of 30% hydrogen peroxide was dissolved in an equal volume of methanol and added to the solution of the free base. The mixture was kept at room temperature for 12 h and checked by GLC and TLC. The product had an R_F value of 0.70 in the TLC system.

In the NMR ($CDCl_3$) spectrum, $-N(CH_3)_2$ appears as a singlet at 2.5 δ in the drug and as two singlets at 3.2 and 3.4 δ in the N-oxide.

TABLE I

R_F VALUES OF OXETORONE AND ITS *IN VITRO* METABOLITES

Silica gel F_{254} glass plates, 0.25 mm layer thickness. Solvent system: benzene–acetone–ammonia (50:50:1).

Compound	*Isomer*	R_F
Desmethyloxetorone	E	0.22
	Z	0.18
Oxetorone	E	0.47
	Z	0.41
Oxetorone-N-oxide (Cope degradation product)	—	0.70

RESULTS AND DISCUSSION

TLC of the microsomal extract gave five spots that were not present in the control sample; their R_F values are reported in Table I.

GLC analysis of the microsomal extract showed five peaks that were not present in the control (Fig. 1, A and B) at a column temperature of 270°.

The eluates of the spots at R_F 0.41 and 0.47, analyzed both by using the DIS into the ion source of the mass spectrometer and by GLC (peaks 2 and 3), gave virtually identical mass spectra, corresponding to isomers E and Z of unchanged oxetorone, as shown in Fig. 2.

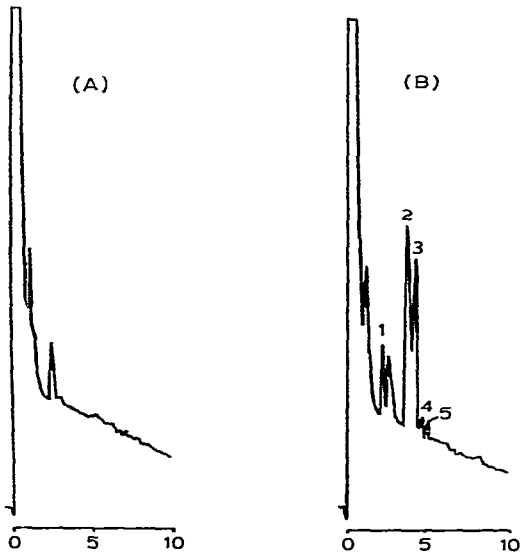

Fig. 1. Gas chromatograms of (A) control sample extract and (B) incubation mixture extract.

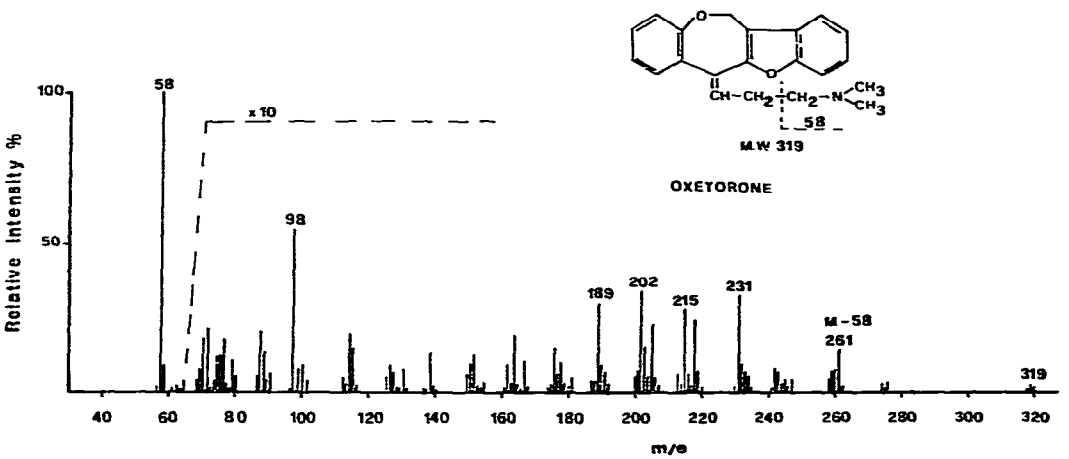

Fig. 2. Mass spectrum of oxetorone from microsomal extract, identical with that of the authentic compound. (The mass spectrum of isomer E is virtually identical with that of isomer Z.)

The base peak at *m/e* 58 corresponds to β-bond fission with respect to the nitrogen atom of the side-chain, giving rise to N,N-dimethylformimine, with retention of the positive charge. The mass spectrum includes the ion at *m/e* 261, due to the loss of 58 a.m.u. from the molecular ion at *m/e* 319.

Fig. 3 shows the mass spectrum obtained from the eluate of the spot at R_F 0.18, identical with that obtained from the eluate of the spot at R_F 0.22, and corre-

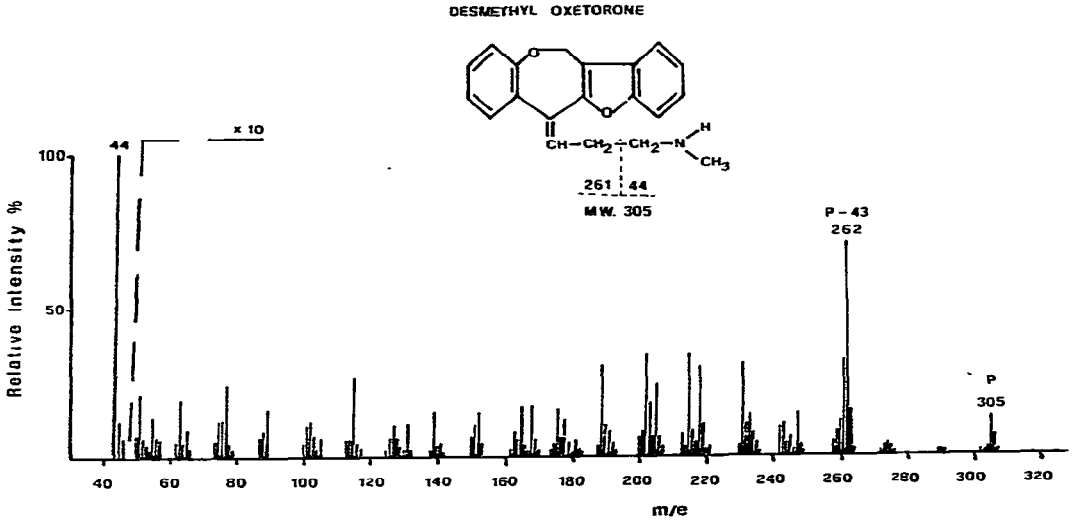

Fig. 3. Mass spectrum of desmethyloxetorone from microsomal extract, identical with that of the authentic compound. (The mass spectrum of isomer E is virtually identical with that of isomer Z.)

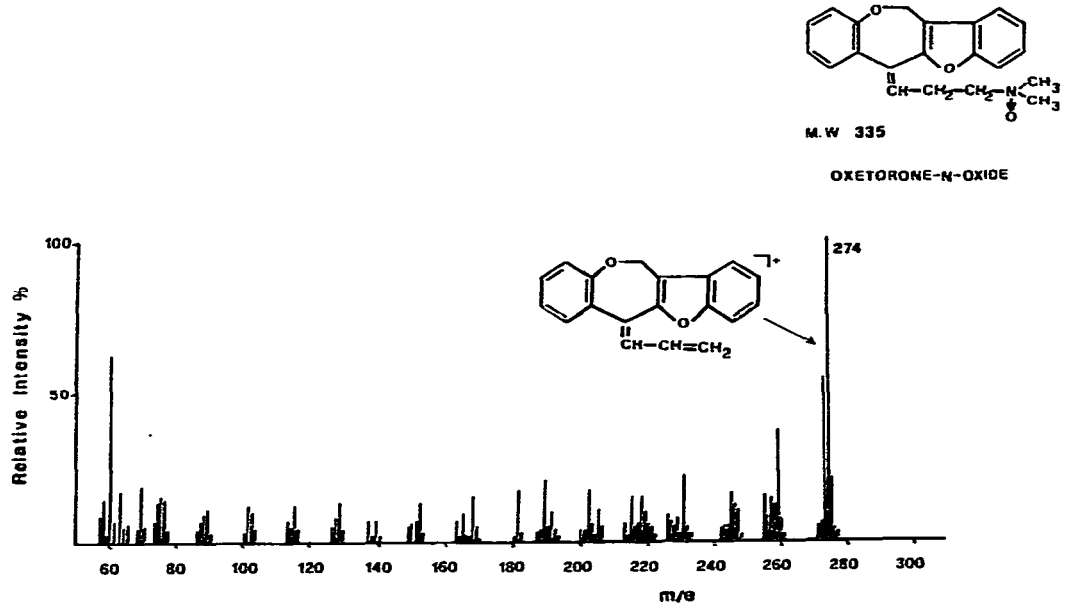

Fig. 4. Mass spectrum of oxetorone-N-oxide (Cope degradation product).

sponding to GLC peaks 4 and 5. The molecular ion at *m/e* 305, the base peak at *m/e* 44 and the ion at *m/e* 262 suggest that this compound has the structure of a desmethyloxetorone derivative, as a mixture of the two isomers E and Z. This was confirmed by comparison with an authentic sample of desmethyloxetorone, which had the same TLC R_F value, the same GLC retention time and the same mass spectrum as the metabolic product.

The mass spectrum obtained from the eluate of the spot with R_F 0.70, corresponding to GLC peak 1, is shown in Fig. 4. The even molecular ion at *m/e* 274 and the absence of the peak at *m/e* 58, which is the base peak in the mass spectrum of oxetorone, is in agreement with a modification of the side-chain containing the nitrogen atom. Because of the loss of 45 a.m.u. from the molecular ion of the drug, it was assumed that this compound might be the degradation product of the oxetorone-N-oxide, which undergoes Cope thermal degradation, losing dimethylhydroxylamine (Fig. 5)[5–8]. To confirm this result, a sample of oxetorone-N-oxide was synthesized and subjected to TLC, GLC and DIS–MS. In all instances its behaviour was similar to that of the metabolite. Moreover, the NMR spectrum described confirmed the structure of the compound as the N-oxide.

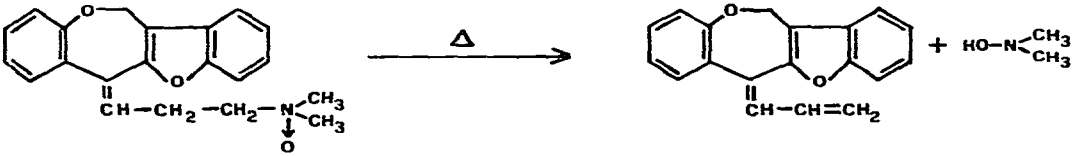

Fig. 5. Cope thermal degradation of oxetorone-N-oxide.

The Cope reaction provides further confirmation of the degradation that occurs in compounds with a terminal amino group in the side-chain, such as doxepin[9] and cyclobenzaprine[5].

In order to ascertain that the formation of this N-oxide and of desmethyloxetorone was not due to a chemical reaction occurring within the incubation medium, but to enzymatic reactions, the drug was incubated under the various experimental conditions described in Table II; the N-oxide and the desmethyl derivative are formed only in the presence of the complete microsomal system.

TABLE II

EXPERIMENTAL CONDITIONS FOR *IN VITRO* METABOLITE FORMATION

Experimental condition	*Oxetorone N-oxide formation*	*Desmethyloxetorone formation*
Microsomes + cofactors	+	+
Boiled microsomes* + cofactors	—	—
Microsomes + cofactors–NADP	—	—
Cofactors only	—	—

* Boiled microsomes were obtained by heating microsomes, re-suspended in phosphate buffer (0.2 *M*), at 100° for 10 min.

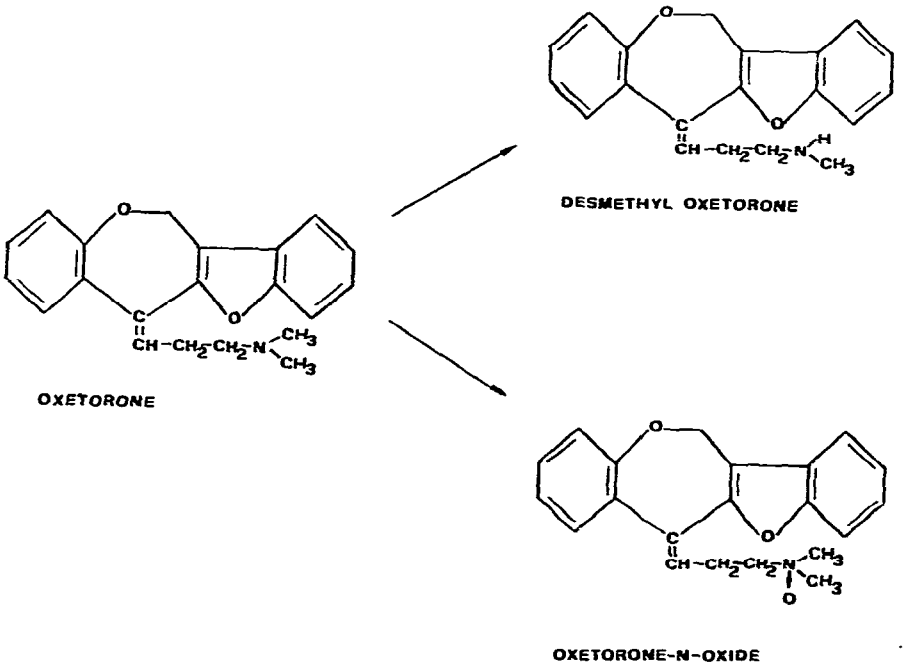

Fig. 6. *In vitro* formation of desmethyloxetorone and oxetorone-N-oxide.

The metabolism of oxetorone (Fig. 6), a tetracyclic compound, therefore shows considerable analogy with that of several tricyclic antidepressant drugs.

Studies on oxetorone metabolism *in vivo* are in progress in different animal species, such as the rat, dog and man. Preliminary results show the formation of oxetorone-N-oxide, as *in vitro*, while the desmethyl derivative was not found.

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