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Note

Resolution of (+)- and (−)-α-difluoromethylornithine by capillary gas chromatography

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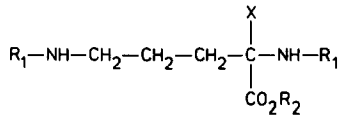
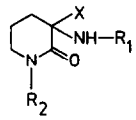
α-Difluoromethylornithine (DFMO, MDL 71.782, I, Fig. 1) is a selective, enzyme-activated, irreversible inhibitor of ornithine decarboxylase (ODC). Inhibition of this enzyme, which catalyses the initial, rate-limiting step in the biosynthesis of putrescine and the polyamines spermine and spermidine, leads to *in vivo* depletion of



Compound	R_1	R_2	X	Ion m/z
I	−H	−H	$-CHF_2$	-
II	−H	−H	−H	-
III	$-COCF_2CF_3$	$-C_3H_7$	−H	216
IV	$-COCF_2CF_3$	$-C_3H_7$	$-CHF_2$	266



Compound	R_1	R_2	X	Ion m/z
V	−H	−H	$-CHF_2$	-
VI	$-COCF_2CF_3$	−H	$-CHF_2$	270
VII	$-COCF_2CF_3$	$-COCF_2CF_3$	$-CHF_2$	266
VIII	$-COCF_2CF_3$	−H	−H	260
IX	$-COCF_2CF_3$	$-COCF_2CF_3$	−H	388

Fig. 1. Structural formulae of DFMO and derivatives. Numbers refer to compounds as cited in text.

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those compounds. The chemotherapeutic implications of the inhibition of polyamine biosynthesis have recently been reviewed[1]. While DFMO is currently available as a racemic mixture, only the (−)-isomer shows significant ODC-inhibiting activity. Hence, in any clinical studies with DFMO, it will be the plasma and tissue levels of the pharmacologically more active enantiomer which are of particular interest. The significance of such stereochemical considerations in pharmacokinetics and clinical pharmacology has recently been emphatically pointed out by Ariens[2]. A previously published assay for DFMO using automated amino acid analysis did not distinguish between the two enantiomers[3].

The enantiomers of ornithine (ORN, II) along with those of many other amino acids have previously been resolved as their di-pentafluoropropionamide propyl esters (*e.g.* di-PFP-ORN-isopropyl ester, III) by capillary gas chromatography on a commercially available chiral stationary phase[4]. The enantiomers of the analogous derivative of DFMO (di-PFP-DFMO-isopropyl ester, IV) failed to resolve on this phase under a wide range of operating conditions. A new approach, involving the lactamisation of DFMO to form 3-amino-3-(difluoromethyl)-2-piperidone (DFMO-lactam, MDL 71.880, V) was adopted.

This compound was further derivatised to the corresponding mono- or di-pentafluoropropionamide, the enantiomers of which were subsequently resolved by capillary gas chromatography. This method may provide a useful basis for establishing a stereospecific assay for DFMO in biological samples.

MATERIALS AND METHODS

(±)-DFMO (I), (+)-DFMO (I), and (±)-DFMO-lactam (V) were gifts from Merrell-Dow Pharmaceuticals. (±)-Ornithine, (+)-ornithine and (−)-ornithine were purchased from Sigma (St. Louis, MO, U.S.A.). Hexamethyldisilazane (HMDS) and pentafluoropropionic anhydride (PFPA) were purchased from Pierce (Rockford, IL, U.S.A.) and were used without further purification. Analytical reagent (AR) grade dichloromethane and acetonitrile were redistilled and dried over molecular sieve (BDH type 4A). AR grade benzene was redistilled and dried over sodium wire.

Preparation of derivatives

(±)-di-PFP-ORN-isopropyl ester (III) was prepared as described previously by Frank *et al.*[4].

(±)-di-PFP-DFMO-isopropyl ester (IV) was also prepared by this method.

(±)-DFMO-lactam (V) was prepared from (±)-DFMO by a modification of the method described by Pellegata *et al.*[5] for the corresponding lactamisation of ornithine. (±)-DFMO hydrochloride (2.36 mg, 10 μmol) was refluxed for 24 h in a mixture of acetonitrile (0.5 ml) and HMDS (0.2 ml). The reaction mixture was evaporated to dryness under a stream of dry nitrogen.

(±)-PFP-DFMO-lactam (VI) was prepared by dissolving the residue from the previous reaction in dichloromethane (200 μl) and adding PFPA (20 μl). The mixture was then allowed to stand in a sealed vessel at room temperature for 20 min, before evaporation to dryness under a stream of dry nitrogen. The residue was then redissolved in benzene (20 μl).

(±)-di-PFP-DFMO-lactam (VII) was prepared in the same manner except that the acylation reaction was carried out at 80°C for 1 h.

(±)-PFP-ORN-lactam (VIII) and (±)-di-PFP-ORN-lactam (IX) were prepared from (±)-ornithine by the methods described above for the analagous derivatives of DFMO.

Corresponding derivatives of individual enantiomers were prepared by the same methods using the appropriate enantiomer of the starting material.

Gas chromatography–mass spectrometry

Results were acquired using a Pye 204 gas chromatograph equipped with an SGE Unijector capillary injection system and directly coupled, via a heated, glass-lined stainless-steel tube (0.5 mm I.D.) to a VG 70/70F mass spectrometer with 2035 datasystem. Chromatography was carried out on Chirasil-Val columns (Applied Science (Deerfield, IL, U.S.A.), 25 or 50 m long and 0.3 mm I.D. with a film thickness of 0.23 μm. Helium was used as the carrier gas at a flow-rate of 2 ml/min measured at atmospheric pressure and with the column at ambient temperature. Injections were made in the split mode with a split-ratio of 10:1. The oven temperature was programmed from 140 to 200°C at 4°C/min with the injection port and interface temperatures held at 250 and 220°C respectively.

Full mass spectra of reference compounds were recorded initially by direct insertion probe in the electron-impact ionisation mode at 70 eV and 4 kV accelerating voltage. Representative ions for selected ion monitoring were chosen from the resulting spectra.

RESULTS AND DISCUSSION

Under chromatographic conditions which gave baseline resolution of the enantiomers of (±)-di-PFP-ORN-isopropyl ester, no resolution of the corresponding derivative of (±)-DFMO [(±)-di-PFP-DFMO-isopropyl ester] was achieved (Fig. 2). Similarly, the enantiomers of the analogous *n*-propyl, *tert.*-butyl and methyl ester derivatives of DFMO could not be resolved. The failure of the present system to resolve the DFMO derivatives must then be attributed to steric and/or electronic influences of the difluoromethyl substituent on the formation of the diastereomeric association complex responsible for the resolution of the analogous derivative of ornithine, as discussed by Frank *et al.*[6]. It was noted, however, that in some full-scan chromatograms of these DFMO derivatives, two small, partially resolved peaks of equal size were eluted after the derivatives of immediate interest. The mass spectra of these two peaks were identical and corresponded with that of PFP-DFMO-lactam derived from an authentic sample of DFMO-lactam. A second pair of peaks of equal size, baseline-resolved and eluting earlier than any of the other derivatives also occurred in some samples. Full-scan chromatograms showed that these peaks also had identical spectra and corresponded to di-PFP-DFMO-lactam.

Attempts to achieve lactamisation of DFMO via its methyl ester (as described for preparative scale work by Bey *et al.*[7]) proved unsuccessful at the analytical level. Subsequently, a method described by Pellegata *et al.*[5] to achieve lactamisation of ornithine was successfully adapted to the analytical scale.

By controlling the conditions of the subsequent halocylation step it was possible to limit the product of this reaction exclusively to PFP-DFMO-lactam. Although di-PFP-DFMO-lactam, formed under more vigorous conditions, gave some-

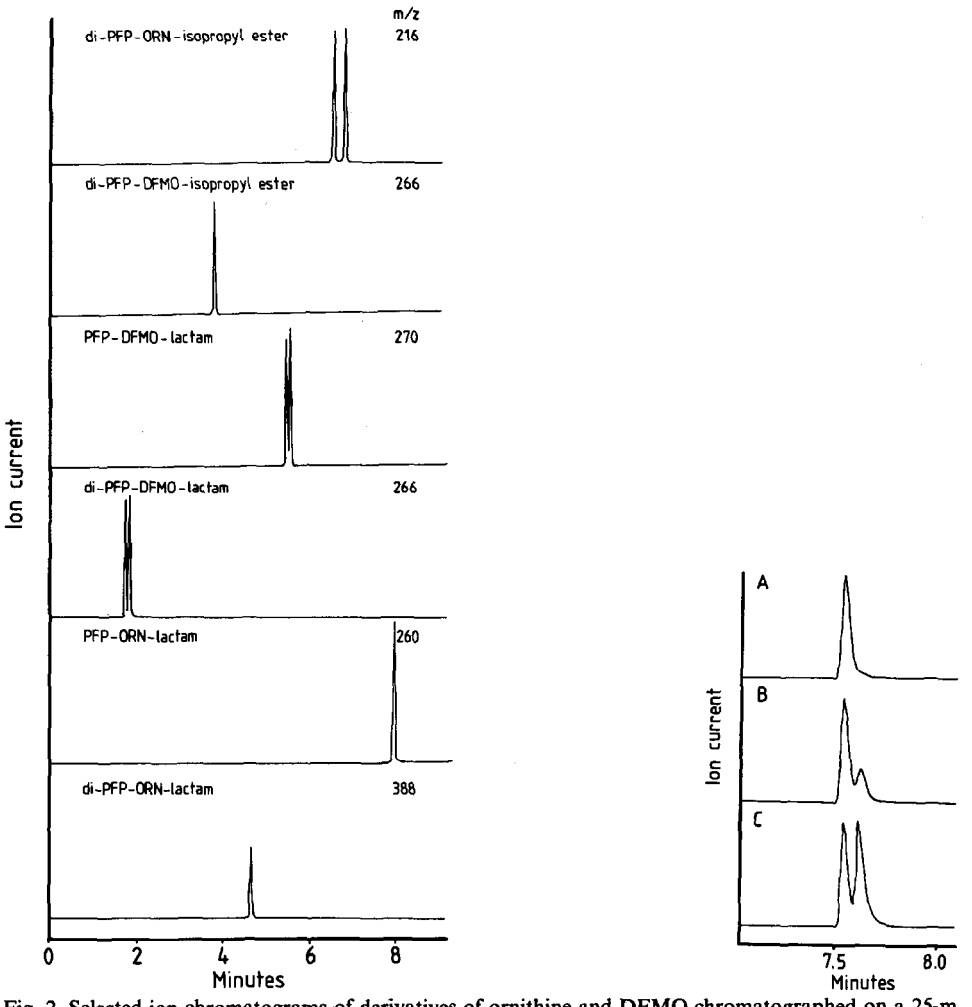

Fig. 2. Selected ion chromatograms of derivatives of ornithine and DFMO chromatographed on a 25-m Chirasil-valine column. Ion current in arbitrary units.

Fig. 3. Selected ion chromatograms (*m/z* 270) of PFP-DFMO-lactam. (A) Derived from (+)-DFMO; (B) derived from (+)-DFMO enriched by 25% with (±)-DFMO; (C) derived from (±)-DFMO. Ion current in arbitrary units.

what better resolution of the enantiomers and had an appreciably shorter retention time than PFP-DFMO-lactam (Fig. 2), it was found that the yield of the former derivative was capricious and hence would not be suitable as a basis for routine analytical work.

Initially, chromatography was carried out using a 25-m column. In an attempt to improve resolution of the more readily produced PFP-DFMO-lactam, subsequent studies were carried out using a 50-m column. Surprisingly, this yielded no measurable improvement in resolution at a flow-rate optimised according to the method of Davies[8], suggesting that factors other than those generally considered to influence

resolution using non-chiral stationary phases may have a predominant effect when using the present chiral system.

Paradoxically, both (±)-PFP-ORN-lactam and (±)-di-PFP-ORN-lactam were not resolved by the present system (Fig. 2) suggesting that the stereospecific interaction of the DFMO derivatives with the chiral stationary phase depends on the presence of both the lactam ring and the difluoromethyl substituent and that the basis of this interaction differs considerably from that responsible for the resolution of ornithine.

Previous studies have shown that the (−)-isomers of amino acids derivatised in the more conventional manner described by Frank *et al.*[4] consistently elute first when chromatographed on Chirasil-valine columns. However, the study of Pellegata *et al.*[5] noted that the lactamisation of ornithine proceeded with inversion of optical activity. In the absence of sufficient (+)-DFMO to determine the optical activity of PFP-DFMO-lactam derived from it (and hence whether inversion of optical activity had also occurred in the lactamisation of DFMO) it was necessary to demonstrate which of the two PFP-DFMO-lactam peaks was derived from (+)-DFMO. This was achieved by chromatographing a sample derived from (+)-DFMO and subsequently rechromatographing the same material enriched by 25% (on a molar basis) with material derived from (±)-DFMO. This clearly demonstrated that the first peak of the enantiomeric pair was due to material derived from (+)-DFMO (Fig. 3).

Resolution of the enantiomers of DFMO as the PFP-DFMO-lactam derivatives may thus provide a useful basis for the stereospecific analysis of DFMO in biological samples and further work to this end is being undertaken.

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