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Note

Identification of a major metabolite of the new hypolipidaemic agent, isopropyl 2-[4'(*p*-chlorobenzoyl)phenoxy]-2-methylpropionate (procetofene) in humans by gas chromatography–mass spectrometry

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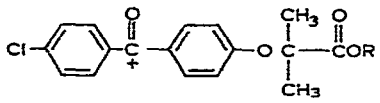
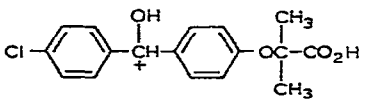
Isopropyl 2-[4'(*p*-chlorobenzoyl)phenoxy]-2-methylpropionate (procetofene, from Laboratoires Fournier, Dijon, France; LF 178; I, R = $CHMe_2$) is a new hypolipidaemic agent which although chemically related to clofibrate, is apparently more effective in lowering plasma concentrations of cholesterol and triglycerides.



(I)



(II)

+, Position of ^{14}C.

As part of the safety evaluation and development of this drug for clinical use, the metabolic fate of the ^{14}C-labelled drug has been studied in rats, dogs and man[1]. A chromatographic examination of the radioactivity in human plasma samples after oral administration suggested that major metabolites were the corresponding carboxylic acid (LF 153; I, where R = H) and/or the benzhydrol (II). Components corresponding to both these compounds were also detected in urine, where they occurred partly as conjugates. Mass spectrometry has now been used to provide unambiguous evidence for the formation of these metabolites.

EXPERIMENTAL

Details of the studies in human subjects have been described elsewhere[1]. A mean of 38.9% of the administered dose was excreted in the urine during 0–12 h. A sample (50 ml) of this urine from a human volunteer, who had received an oral dose of [^{14}C]-procetofene (50 μCi, 300 mg) was applied to a column (20 × 2 cm) of Amberlite XAD-2 resin. The urine was washed through with water (100 ml) and the radioactivity finally eluted with methanol (100 ml). The methanol eluate containing more than 90% of the applied radioactivity was evaporated to dryness at 40°. The residue was reconstituted in acetate buffer (2 ml, pH 5) and incubated with about

1000 units of β-glucuronidase–sulphatase (Type H1; Sigma, London, Great Britain) at 37° for 18 h. The solution was evaporated to dryness and the residue triturated with methanol. The methanol solution was applied to 0.25 mm kieselgel F_{254} thin-layer chromatography plates which were developed in a solvent system of acetone–water–5% ammonia (95:5:0.5, v/v). The major radioactive band (R_F 0.1–0.3) was located by autoradiography using Kodak Kodirex X-ray film, the corresponding area of silica gel removed, and the metabolites eluted with chloroform–ethanol (9:1, v/v). This extract would contain the metabolites LF 153 and the benzhydrol. Before examination by gas chromatography–mass spectrometry (GC–MS), the metabolites were methylated by treatment with diazomethane in methanol.

A pooled sample of plasma was extracted with acetone (10 volumes) and the extract evaporated to dryness under reduced pressure at 40°. More than 90% of the total radioactivity in the plasma sample was recovered by this process. The residue was dissolved in methanol and treated with diazomethane.

Spectroscopy

GC–MS was performed using a Pye 104 gas chromatograph interfaced to a VG Micromass 16F mass spectrometer through a single stage jet separator. The GC separation was carried out using a 200 × 0.4 cm I.D. glass column containing 1% Carbowax 20M on Gas-Chrom Q (80–100 mesh). Helium was used as carrier gas with a flow-rate of 40 ml/min. The temperatures of the GC column, separator and ion source were 220°, 200° and 220°, respectively. The mass spectrometer was operated in the electron impact mode with an electron beam energy of 70 eV and a trap emission of 100 μA. A programmable ion voltage supply was used for multiple ion monitoring of up to eight ions in the mass range 0–300% of the lowest mass.

RESULTS AND DISCUSSION

The mass spectrum of procetofene shows a molecular ion at *m/e* 360 with the corresponding isotope peak at *m/e* 362, due to ^{37}Cl, with these two peaks being in the ratio 3:1. The spectrum of the methyl ester of the carboxylic acid, LF 153 (I, R = H) (Fig. 1a) shows a molecular ion at *m/e* 332 and fragment ions at *m/e* 273 and 232, all with associated isotope peaks (Scheme I). The methyl ester of the benzhydrol (II) shows molecular ion peaks at *m/e* 334 and 336. Preliminary chromatography showed that the methyl esters of LF 153 and the benzhydrol had retention times of about 15 and 8 min, respectively, under the GC conditions employed.

GC–MS analysis of the major radioactive component(s) in urine gave an integrated ion current chromatogram showing a component with a retention time identical to the methyl ester of the acid, LF 153. A scanned mass spectrum of this component was identical to that of the authentic standard (Fig. 1b). In order to detect the presence of other minor components, particularly the benzhydrol, the more sensitive multiple ion detection facility was used. Three ions were monitored, namely *m/e* 336, 334, and 332; the ions *m/e* 334 and 332, representing the molecular ion of methylated LF 153, and *m/e* 334 and 336, representing the molecular ion of the methylated benzhydrol. Again there was no indication of the presence of a component corresponding to the methylated benzhydrol, while the ions *m/e* 334 and 332 occurred at a retention time corresponding to LF 153 methyl ester.

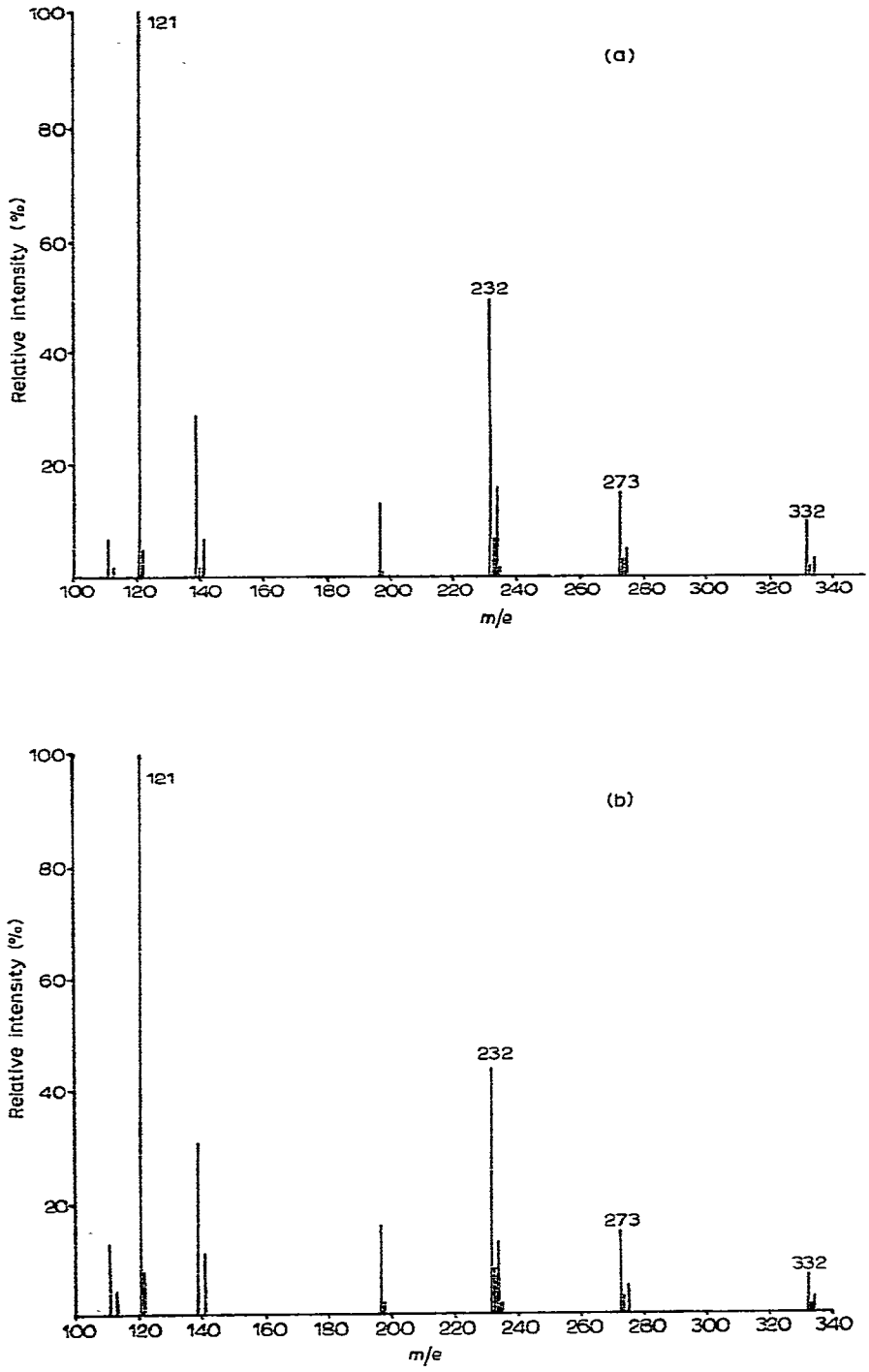
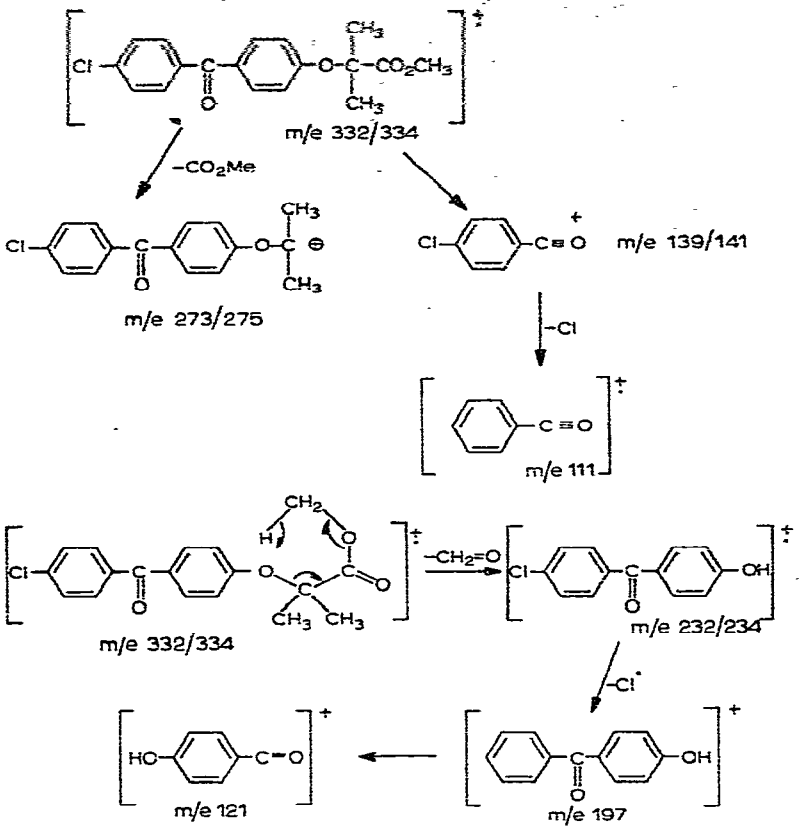


Fig. 1. Mass spectra of (a) the authentic methyl ester of LF 153 and (b) the methyl ester of LF 153 isolated from human urine.



Scheme 1. Postulated fragmentation pattern of the methyl ester of LF 153.

Similarly, mass fragmentograms were recorded during GC–MS analysis of the plasma extract (Fig. 2). No unchanged drug could be detected by monitoring the ions *m/e* 360 and 362 corresponding to the molecular ion of procetofene. A single component was detected, containing the peaks *m/e* 332 and 334, with a retention time corresponding to LF 153 methyl ester. There was no evidence for the presence of the benzhydrol methyl ester.

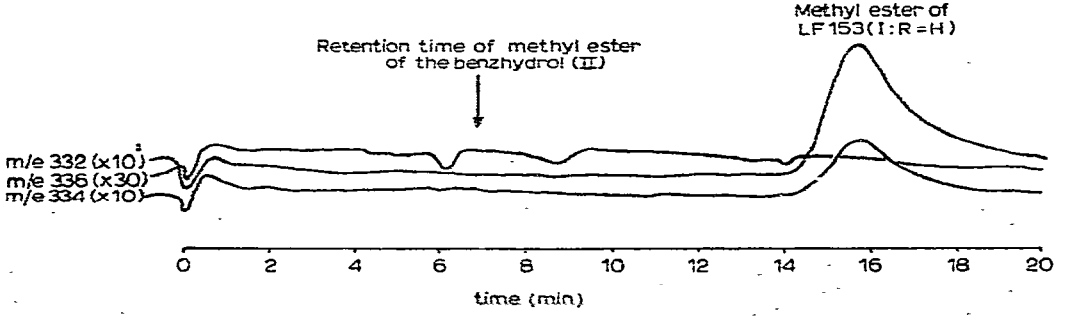


Fig. 2. Mass fragmentogram of plasma extract monitoring *m/e* values of 332, 334, and 336.

These results confirmed unambiguously, that a major metabolite of procetofene in the urine was the corresponding carboxylic acid, LF 153, which was probably excreted as a mixture of the free compound and the ester glucuronide conjugate. LF 153 was also the only component detected in a plasma extract containing more than 90% of the drug-related material in plasma. Preliminary studies on the chromatographic examination of human urine metabolites had suggested the presence of LF 153 and the benzhydrol, representing approximately 70 and 20% of the total urine radioactivity, respectively. However, as only LF 153 was detected unequivocally in either urine or plasma, these results demonstrate the caution required in drawing conclusions concerning the identity of metabolites from a chromatographic comparison with authentic reference compounds alone.

ACKNOWLEDGEMENT

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REFERENCE

1 R. B. Brodie, L. F. Chasseaud, L. F. Elsom, E. R. Franklin and T. Taylor, *Arzneim.-Forsch.*, 11 (1971) in press.