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Gas chromatographic–electron-impact chemical ionization mass spectrometric identification of cinmetacin and its metabolites in human urine

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Abstract

Cinmetacin and its three metabolites were identified by gas chromatography–electron-impact positive-ion chemical ionization–negative chemical ionization mass spectrometry. After a single oral dose of 30 mg of cinmetacin to a healthy man, urine was collected and hydrolysed. The three metabolites were identified as O-desmethylated, N-descinnamoyl and C–C double bond reduced cinmetacins. The identification of cinmetacin metabolites in human urine was established by comparison of their GC retention times and electron impact and methane chemical ionization mass spectra with those of the synthesized authentic standards.

1. Introduction

Cinmetacin [5-methoxy-2-methyl-1-(1-oxo-3-phenyl-2-propenyl)-1*H*-indole-3-acetic acid] is an indole derivative possessing a marked direct inhibiting activity on acute inflammatory processes, accompanied by an analgesic effect comparable in strength to that of amidopyrine [1]. Its pharmacokinetic behaviour has been studied [2,3], but its biotransformations have not been described. In view of the paucity of information concerning the metabolism of cinmetacin, we studied the metabolic fate of this drug after its administration to humans. Detection of this drug using high-performance liquid chromatography (HPLC) [3] and polarography [4] has been described. However, no method has been reported for the simultaneous detection of cinmetacin and its metabolites.

The identification of cinmetacin (CIN) and its three urinary metabolites (Fig. 1) using electron impact (EI) and chemical ionization (CI) mass spectrometry after a single oral dose of 30 mg of cinmetacin to a healthy may is described in this paper. Pentafluoropropionyl (PFP) and methyl derivatives of the compounds were used in the subsequent structural characterization of cinmetacin and its metabolites. Synthetic procedures for two urinary metabolites, desmethylated

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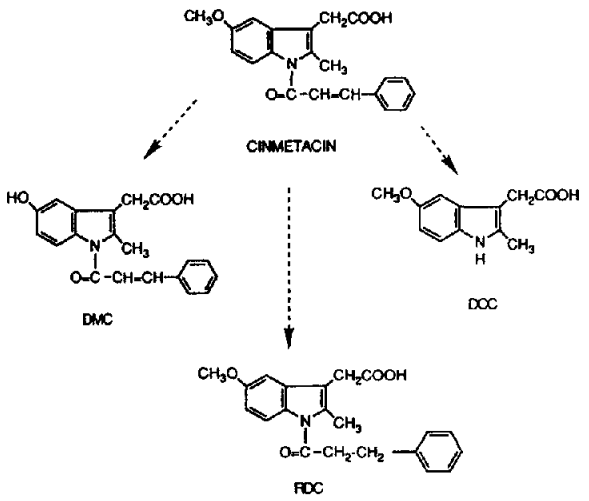

Fig. 1. Proposed metabolic pathway of cinmetacin in humans.

cinmetacin (DMC) and reduced cinmetacin (RDC), are presented.

2. Experimental

2.1. Instrumental

A Hewlett-Packard Model 5988A mass spectrometer coupled to an HP 5890A gas chromatograph controlled by an HP 59970C MS ChemStation was used. The mass spectrometer was operated with a filament current of 300 μA and an electron energy of 70 eV in the EI mode and 230 eV in the CI mode. Methane reagent gas was introduced into the ion source through a transfer line after passing through a trap of activated charcoal and molecular sieve. The ion source was held at 200°C and at 106 Pa in the CI mode. A cross-linked methylsilicone capillary column (12 m long × 0.2 mm I.D. with a film thickness of 0.33 μm) was installed in the gas chromatograph and inserted directly into the ion source of the mass spectrometer. Helium was used as the carrier gas at a flow-rate of 0.9 ml/min. The oven temperature was held at 100°C for 0.5 min, increased to 300°C at 20°C/min and finally held at 300°C for 3 min. The splitting ratio was 1:10. The injector and transfer line temperatures were at 290°C.

2.2. Reagents and chemicals

All chemicals and reagents were of analytical-reagent grade. Cinmetacin was obtained from Sam Jin Pharmaceutical (Seoul, South Korea), β-Glucuronidase from *Escherichia coli* from Boehringer (Mannheim, Germany), Amberlite XAD-2 resin (100–200 mesh) from Serva (Westbury, NJ, USA), pentafluoropropionic anhydride (PFPA), 2,2,3,3,3-pentafluoro-1-propanol, sodium tetrahydroborate and 5-methoxy-2-methyl-3-indoleacetic acid (DCC) from Aldrich (Milwaukee, WI, USA) and methyl iodide from Sigma (St. Louis, MO, USA).

2.3. Hydrolysis and extraction

A 3-mm diameter glass ball was introduced into a 28-cm pasteur capillary pipette from Costar (Cambridge, MA, USA) and XAD-2 slurry, washed with acetone, methanol and distilled water, was filled into the pasteur pipette until a bed height of 25 mm was achieved.

A final washing with 2 ml of distilled water was carried out before applying the urine. A 10-ml volume of urine sample was loaded and the column was washed with an equal volume of water. It was then eluted with 4 ml of methanol. The methanolic solution was evaporated to dryness using a vacuum rotary evaporator. A 1-ml volume of 0.2 *M* potassium phosphate buffer (pH 7.0) was added to the residue, followed by 25 μl of the enzyme β-glucuronidase (activity 10 U/ml, from *E. coli*).

Hydrolysis was performed at 50°C for 1 h. After cooling, 5 ml of diethyl ether were added and shaken at 130 cycles/min on a horizontal shaker for 15 min. After discarding the aqueous phase, the organic phase was evaporated using the vacuum rotary evaporator.

2.4. Derivatization

PFPA derivatization

The procedure used by Wilk and Orlowski [5] was modified for the derivatization of carboxylic

acids. The dried residue was treated with 100 μl of a mixture of 2,2,3,3,3-pentafluoro-1-propanol in pentafluoropropionic anhydride (PFPA) (1:4, v/v) and heated at 40°C for 10 min. After cooling and evaporation just to dryness with nitrogen, the reaction was completed by further heating with an additional 100 μl of PFPA at 40°C for 10 min. After evaporation just to dryness with nitrogen gas, the residue was dissolved in 200 μl of ethyl acetate for injection into the GC–MS system.

Methyl derivatization

The dried residue was dissolved in 180 μl of acetone, 20 μl of methyl iodide and 40 mg of potassium carbonate were added and the tube was heated for 20 min at 45°C.

2.5. Synthesis of desmethylated cinmetacin

Desmethylated cinmetacin (DMC)

For the synthesis of desmethylated cinmetacin the procedure from Strachan et al. [6] for the preparation of desmethylated indomethacin was modified slightly. A 2-g amount of cinmetacin was added to 10 g of molten pyridine hydrochloride under nitrogen at 170°C. The mixture was stirred at 170°C for 20 min, cooled slightly and poured into 40 g of ice. The resulting solid product was filtered, dried and washed successively with 10 ml of methylene chloride and 30 ml of diethyl ether. The mass spectrum obtained by LC–MS of synthesized DMC is shown in Fig. 2.

Cinmetacin (m.p. 171°C): ^{1}H NMR (in C^2HCl_3), 7.80 (1H, d, $J = 15.6$ Hz), 7.58 (2H, m), 7.56 (1H, d, $J = 2.4$ Hz), 7.38 (3H, m), 6.93 (1H, d, $J = 2.4$ Hz), 6.78 (1H, dd, $J = 2.4$, 9.0 Hz), 3.80 (3H, s), 3.65 (2H, s), 2.55 (3H, s).

DMC: ^{1}H NMR (in $C^2H_3O^2H$), 7.76 (1H, d, $J = 15.6$ Hz), 7.69 (3H, m), 7.60 (d, 1H, $J = 2.4$ Hz), 7.40 (2H, m), 7.35 (1H, d, $J = 15.6$ Hz), 6.92 (1H, d, $J = 2.4$ Hz), 6.70 (dd, 1H, $J = 2.4$, 9.0 Hz), 3.55 (1H, s), 2.58 (2H, s). In DMC, the methyl peak of the methoxy group was not shown by NMR spectrometry.

Reduced cinmetacin (RDC)

A 0.16-g amount of palladium–charcoal in THF was added to 0.25 g of cinmetacin and the mixture was shaken for 2 h at 2 bar under hydrogen. The residue was filtered and dried. The mass spectrum obtained by LC–MS of synthesized RDC is shown in Fig. 3.

RDC (m.p. 161°C): ^{1}H NMR (in C^2HCl_3), 7.76 (1H, d, $J = 9.0$ Hz), 7.33 (5H, m), 6.93 (1H, d, $J = 2.4$ Hz), 6.84 (1H, dd, $J = 2.4$, 9.0

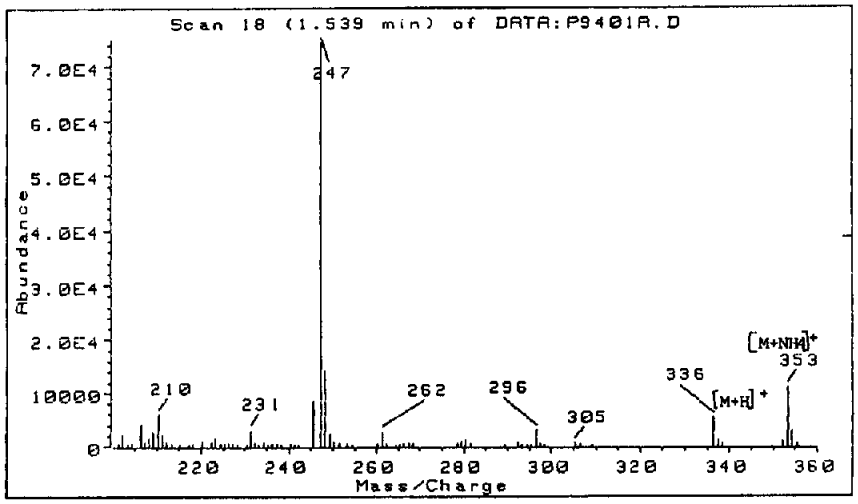

Fig. 2. Mass spectrum obtained by LC–MS of synthesized DMC.

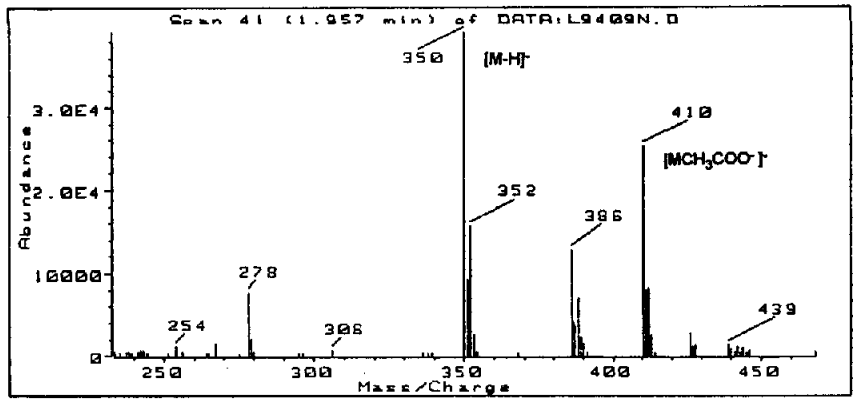

Fig. 3. Mass spectrum obtained by LC–MS of synthesized RDC.

Hz), 3.85 (3H, s), 3.67 (2H, s), 3.31 (2H, m), 3.19 (2H, m), 2.57 (3H, s).

3. Results and discussion

By comparing the total ion chromatogram (TIC) of the pre-dose sample with that of the post-dose sample, three PFP-derivatized metabolites were detected at retention times of 6.01, 10.12 and 10.22 min and cinmetacin was detected at 10.89 min (Fig. 4).

The metabolites were identified by comparison of retention times and the EI and CI mass spectra of their PFP derivatives with those of standards. The mass spectra of CIN using the EI and positive-ion CI (PCI) conditions are shown in Fig. 5. The EI mass spectrum shows the base peak at m/z 131 and other ions at m/z 77, 103, 174, 351 and 481 $[M]^+$. The methane PCI mass spectrum shows the pseudo-molecular ion of m/z 482 $[M+H]^+$, and characteristic ions of m/z 510 $[M+C_2H_5]^+$, 522 $[M+C_3H_5]^+$ and 462 $[MH-HF]^+$. The EI mass spectrum of the methyl derivative is shown in Fig. 6.

3.1. Identification of descinnamoylcinmetacin (DCC)

One metabolite was detected at a retention time of 6.01 min. EI and PCI mass spectra for the PFP derivative are shown in Fig. 7. The metabolite was assumed to be DCC from the interpretation of these spectra. The presence of this compound in human urine after administration of indomethacin has been reported [7,8]. An authentic sample of DCC was obtained from Aldrich and both the retention time and mass spectra of the authentic standard were identical with those of the detected DCC. Under EI-MS conditions, the molecular ion of this metabolite was observed at m/z 497 and $[M-COOCH_2C_2F_5]^+$ was observed at m/z 320. The PCI mass spectrum showed a pseudo-molecular ion of m/z 498 $[M+H]^+$, and other ions of m/z 526 $[M+C_2H_5]^+$, 538 $[M+C_3H_5]^+$ and 478 $[MH-HF]^+$. Hence the metabolite was identified as an N-descinnamoylated product from cinmetacin. The EI mass spectrum of its methyl derivative is shown in Fig. 8.

3.2. Identification of desmethylated cinmetacin (DMC)

Another metabolite was detected at 10.12 min. The mass spectra of the PFP derivative of this metabolite are shown in Fig. 9. The metabolite was assumed to be 5-hydroxy-2-methyl-1-(1-oxo-3-phenyl-2-propenyl)-1*H*-indole-3-acetic acid (DMC) from its mass spectra. DMC was derivatized with PFPA. The molecular ion of this metabolite produced by EI-MS was observed at m/z 613. It could not be detected by PCI-MS because the amount of this metabolite was too small.

NCI-MS was therefore used. The methane

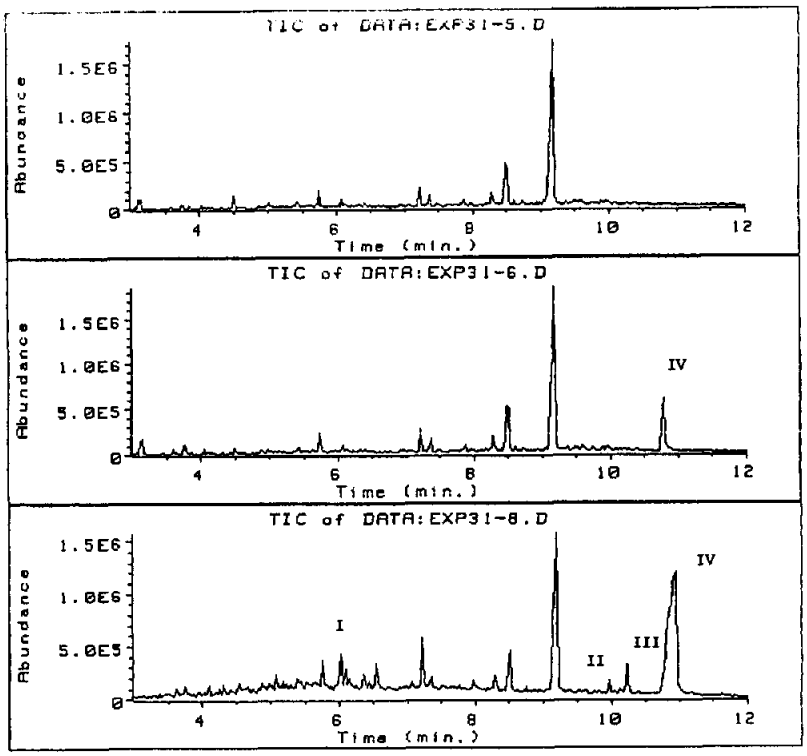

Fig. 4. Total ion chromatograms of a PFP-derivatized extract of a blank urine (top), a blank urine to which cinmetacin was added (middle) and urine from a human subject administered cinmetacin (bottom). Peaks: I = DCC; II = DMC; III = RDC; IV = cinmetacin.

NCI mass spectrum of this metabolite showed the molecular ion $[M]^-$ at m/z 613. The retention time and the characteristic ions of the metabolite were identical with those of the synthesized DMC. Because the methyl derivative of DMC is identical with methylated cinmetacin, it did not appear in the TIC of the methylated urine samples (Fig. 10).

3.3. Identification of reduced cinmetacin (RDC)

Another metabolite was found at a retention time of 10.22 min. The metabolite was assumed to be cinmetacin with carbon–carbon double bond reduced in the cinnamoyl group. As shown in the EI mass spectrum (Fig. 11) the molecular ion at m/z 483 of the metabolite is 2 u greater than that of cinmetacin (m/z 481). The base peak at m/z 131 present in the spectrum of CIN is hardly visible in this spectrum. Also, the ion of m/z 105 was 2 u greater than the ion of m/z 103 present in the spectrum of CIN. This indicates that the double bond of $-CH{=}CHC_6H_5$ was reduced to a single bond, $-CH_2{-}CH_2C_6H_5$. Another diagnostic ion is the ion of m/z 91, which corresponds to the rupture of the benzylic bond giving the resonance-stabilized benzylic ion [9]. This ion was absent in the mass spectrum of CIN.

The methane PCI mass spectrum of this metabolite showed $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$ and $[MH-HF]^+$ ions at m/z 484, 512, 524 and 464, respectively. The EI mass spectrum of its methylated derivative is shown in Fig. 12.

4. Conclusion

Unchanged cinmetacin and its three metabolites were detected in human urine. By compar-

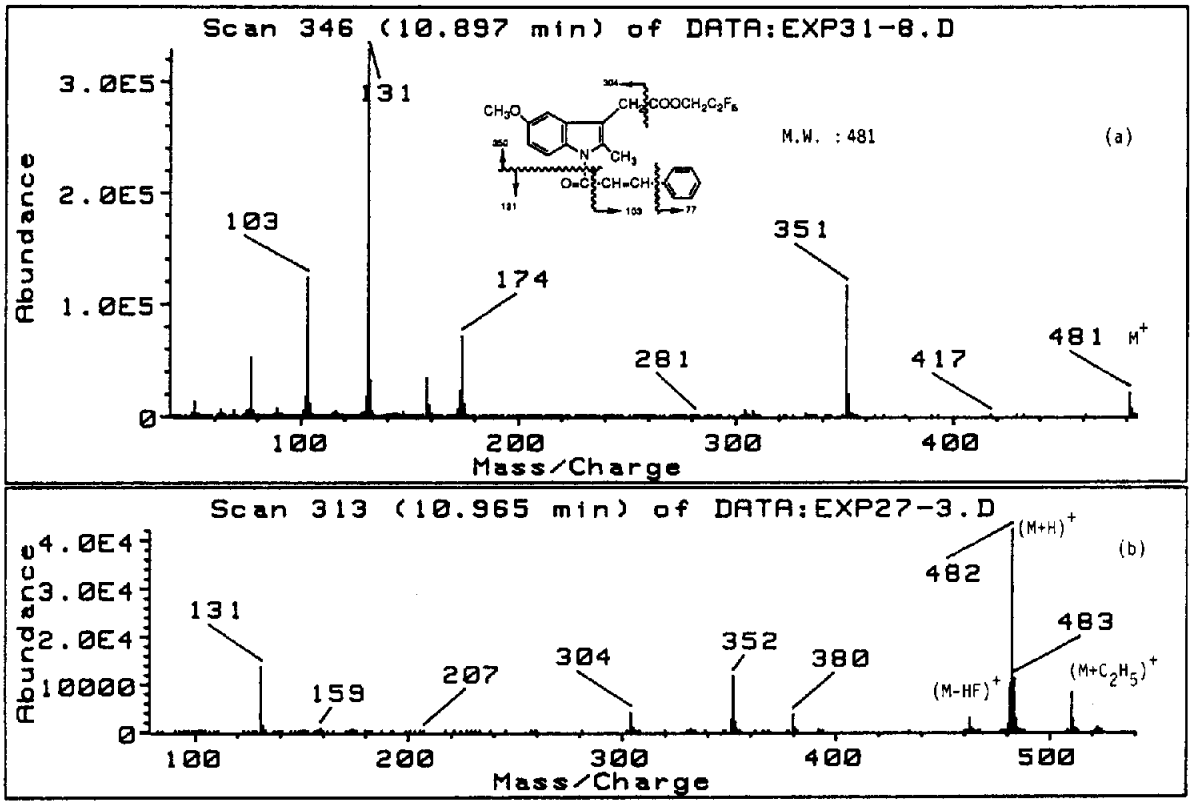

Fig. 5. (a) EI and (b) methane PCI mass spectra of PFP-derivatized cinmetacin.

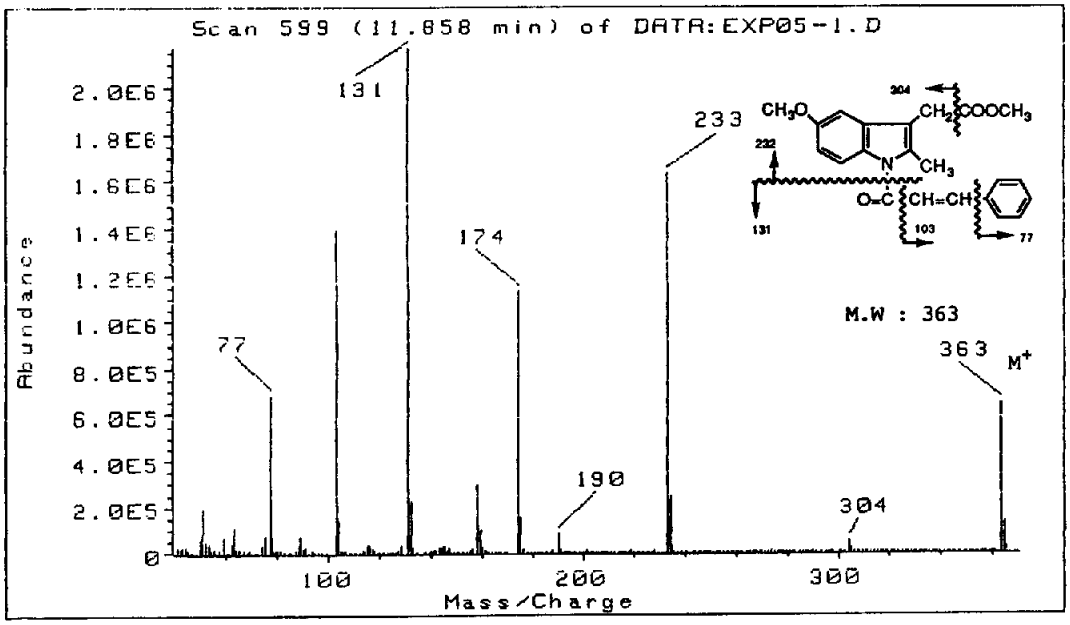

Fig. 6. EI mass spectrum of methylated cinmetacin.

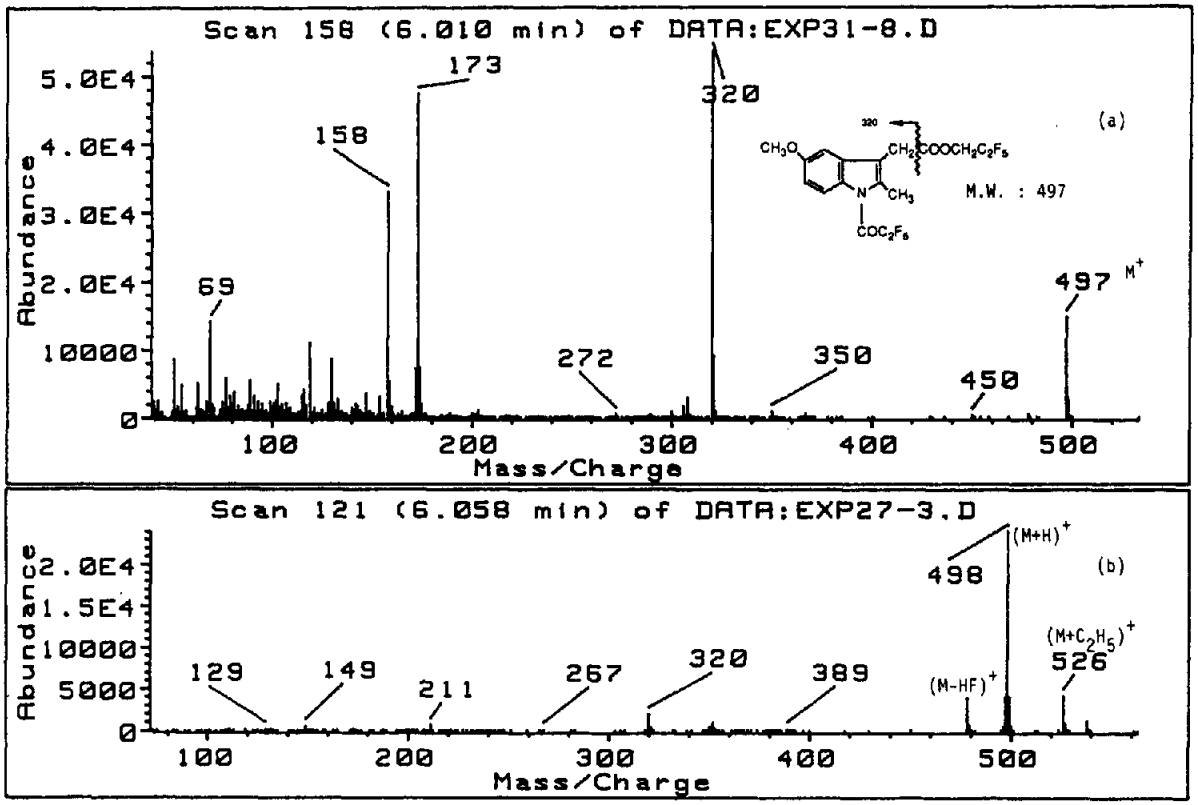

Fig. 7. (a) EI and (b) methane PCI mass spectra of PFP-derivatized DCC.

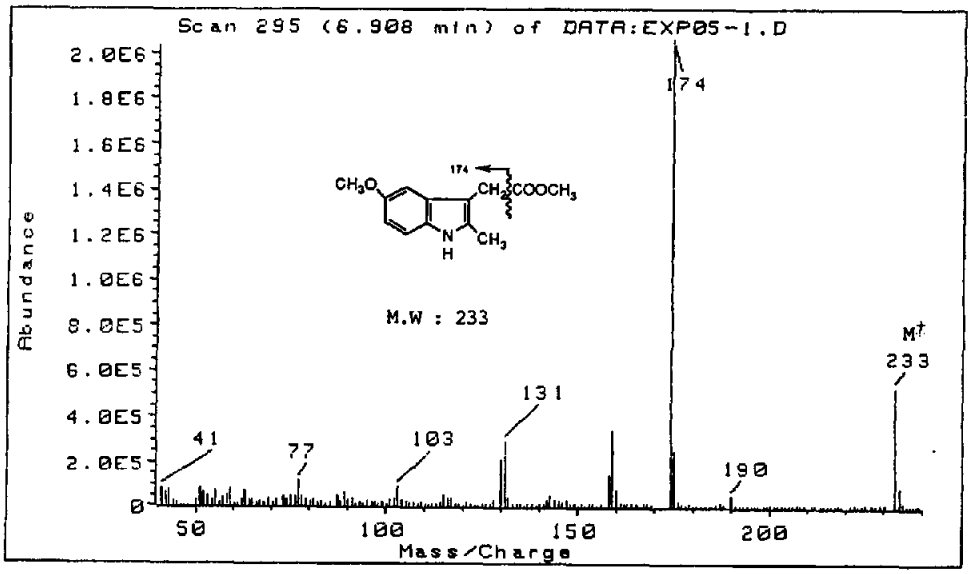

Fig. 8. EI mass spectrum of methylated DCC.

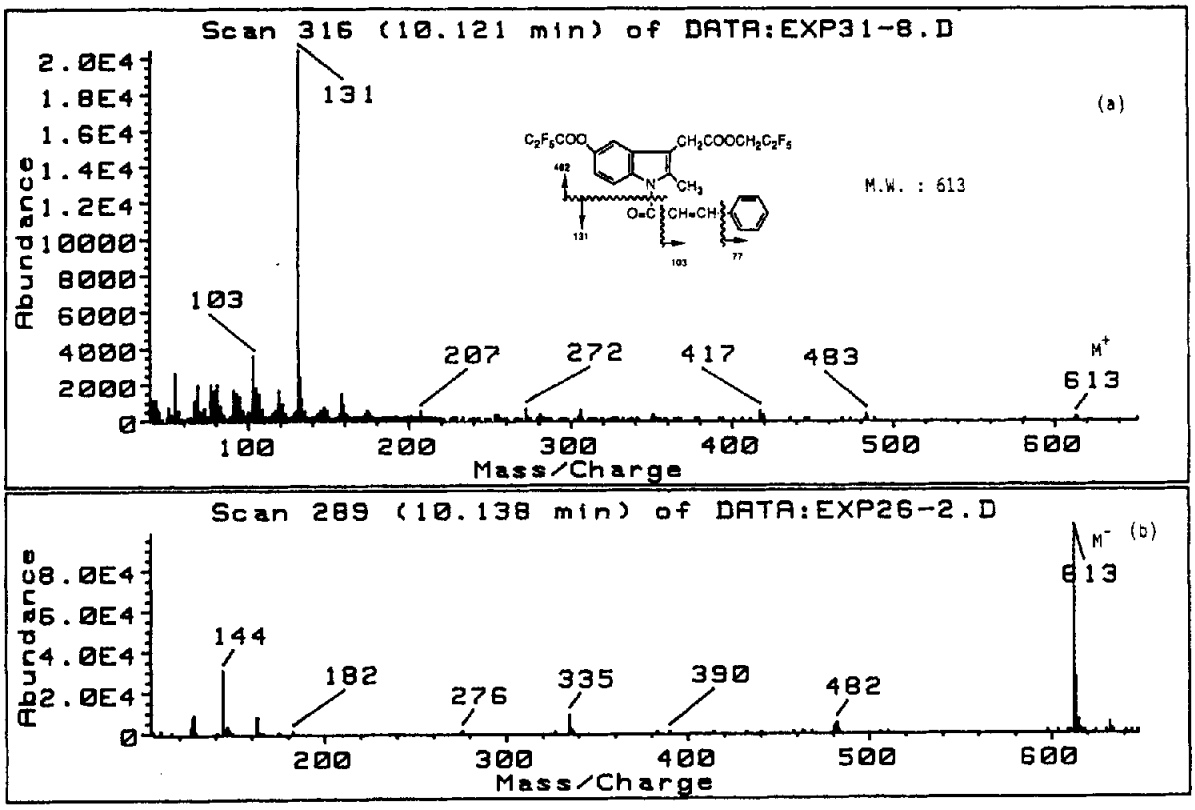

Fig. 9. (a) EI and (b) methane NCI mass spectra of PFP-derivatized DMC.

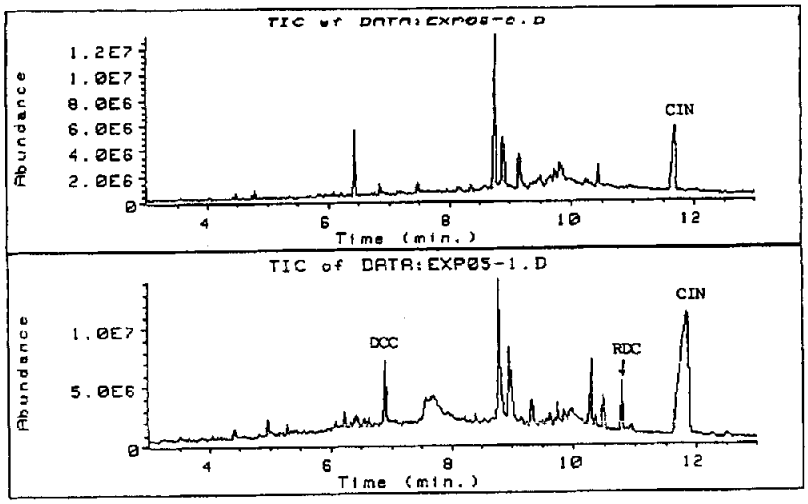

Fig. 10. Total ion chromatograms of a methyl-derivatized extract of a blank urine to which were added cinmetacin (top) and a urine sample from a human subject administered cinmetacin (bottom).

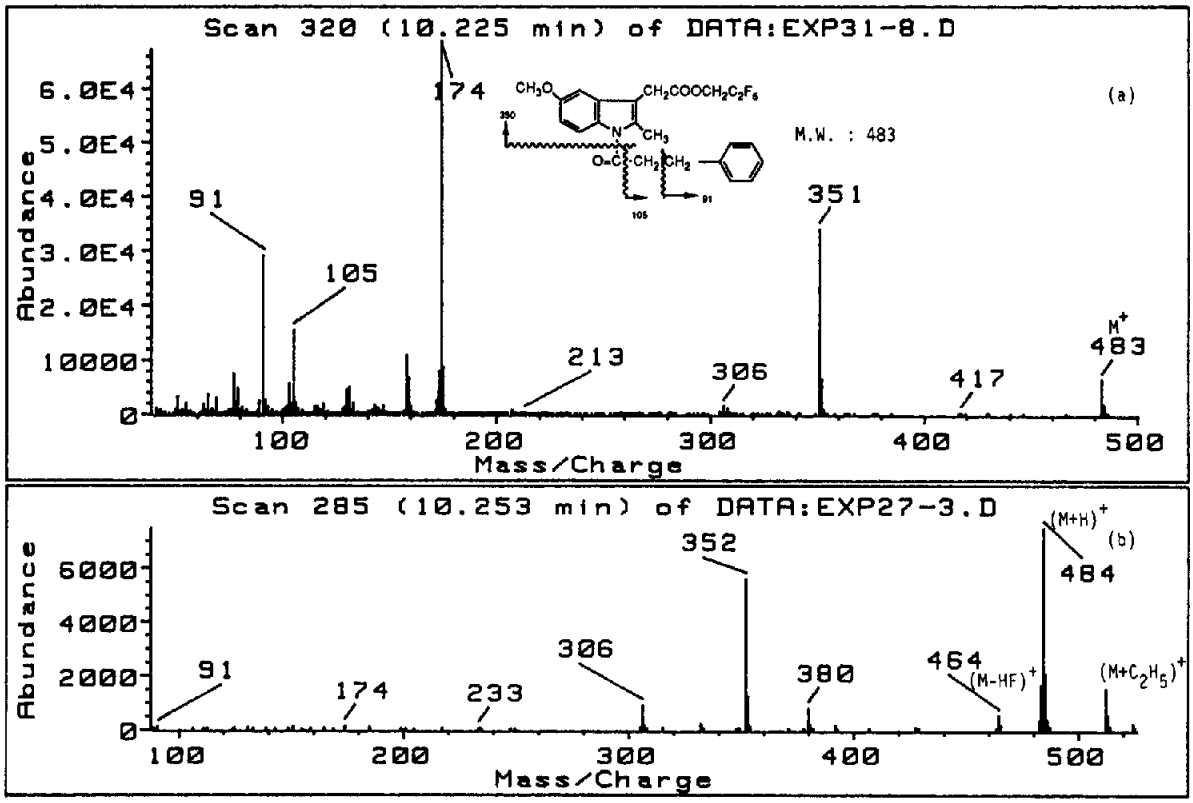

Fig. 11. (a) EI and (b) methane PCI mass spectra of PFP-derivatized RDC.

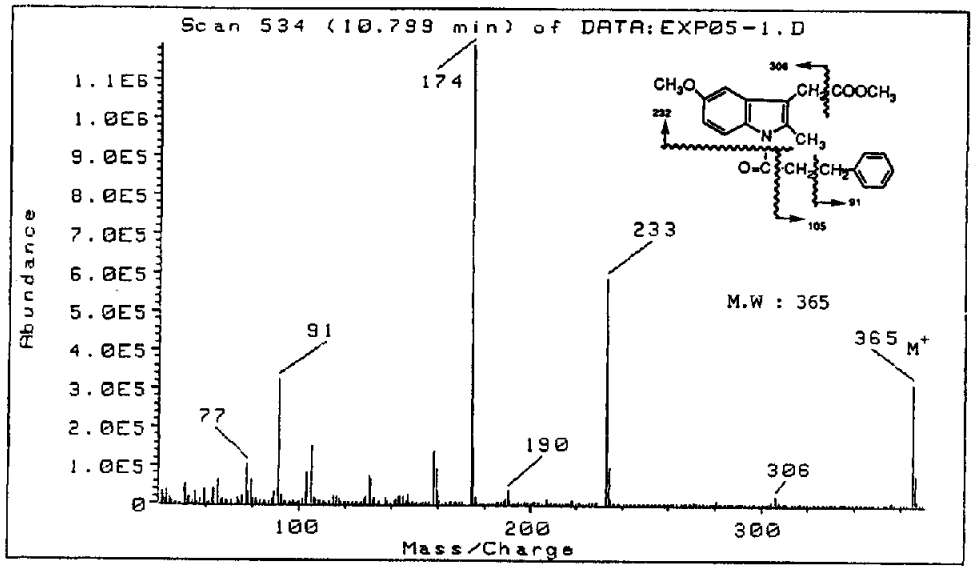

Fig. 12. EI mass spectrum of methylated RDC.

ing their EI and CI mass spectra and GC retention times with those of an authentic standard, the three metabolites were identified as O-desmethylated (DMC), N-descinnamoyl (DCC) and C–C double bond reduced cinmetacin (RDC).

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