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GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC INVESTIGATION OF DEXTROMORAMIDE (PALFIUM) METABOLISM IN THE HORSE

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SUMMARY

Dextromoramide (Palfium) was given by intravenous injection to a Thoroughbred horse at a dosage of 20 mg and urine was collected 2, 4, 6 and 8 h after drug administration. Enzymatic hydrolysis of the urine followed by solvent extraction gave a residue which was back-extracted into 0.1 *M* sulphuric acid. After basification to pH 9 and solvent extraction, the resulting residue was submitted to gas chromatographic–mass spectrometric analysis. Both electron-impact and ammonia chemical-ionization mass spectra were recorded and, based on the observed fragmentation patterns, the principal metabolites in horse urine were shown to be 2,2-diphenyl-3-methyl-4-morpholinobutyramide (compound **2**) and the product of hydroxylation of one phenyl ring in dextromoramide (compound **3**), respectively. The electron-impact mass spectra of compounds **2** and **3**, and of their derivatisation products from on-column methylation in the gas chromatograph, are reported.

INTRODUCTION

Dextromoramide (Palfium, compound **1**, see Fig. 1) is a potent analgesic, first described in 1956[1], which is commonly believed to enhance the racing performance of Thoroughbred and Standardbred horses. Methods used for the analysis of dextromoramide include paper and thin-layer chromatography[2–5] and ultraviolet spectrophotometry[6] but these methods lack the sensitivity and specificity required for this drug's detection in human or equine urine. Thermal degradation of dextromoramide was reported[7] to be a problem with a method based upon the gas chromatographic (GC) quantitation of **1**. Alternatively, dextromoramide was quantitated using a procedure incorporating its initial oxidation with permanganate to benzophenone which was then detected by GC or high-performance liquid chromatography[7].

Several pharmacokinetic parameters have been determined for dextromoramide in humans[7]. A report[7] has described the human metabolism of **1** as occurring by formation of the morpholine-N-oxide, *para*-hydroxylation of one of the phenyl rings and hydrolysis of the pyrrolidinylamide linkage in **1** to a substituted butyric acid. However, nothing has been published on the metabolic fate of dextromoramide in the horse.

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We were unable to detect dextromoramide in equine urine 2 h after an intravenous 20-mg dose. In the present study the urinary metabolites of dextromoramide have been identified as having structures **2** (2,2-diphenyl-3-methyl-4-morpholinobutyramide) and **3** (the product of hydroxylation of one phenyl ring in dextromoramide), respectively (see Fig. 1). These assignments were based upon their mass spectral fragmentation patterns and structure **2** was confirmed from direct comparison with a reference compound of known structure. It follows that the GC–mass spectrometric (MS) identification of **2** in an equine urine swab is direct evidence for the prior administration of dextromoramide. The method described can identify **2** in equine urine after a single intravenous dose of 20 mg of dextromoramide for at least 8 h after administration of this dose.

EXPERIMENTAL

Chemicals and solvents

All solvents were obtained from Mallinckrodt Australia (Clayton, Australia) and were of nanograde quality. MethElute was purchased from Pierce (Rockford, IL, U.S.A.).

Drug administration

Dextromoramide (Palfium) was a product of Janssen Pharmaceutica (Beerse, Belgium) and was purchased from F. H. Faulding (Thebarton, Australia). Four 5-mg tablets were crushed and extracted with dichloromethane to isolate dextromoramide which was suspended in water and administered intravenously to a healthy Thoroughbred horse. Under GC–MS conditions no indication was obtained for the presence of metabolites **2** and **3** in extracts of dextromoramide isolated from this procedure. Urine samples were collected 2, 4, 6 and 8 h after drug administration.

GC–MS instrumentation

The mass spectrometer used in this study was a Kratos MS-80RFAQ instrument interfaced to the same manufacturer's Model DS-90 data system. Electron-impact (EI) mass spectra were recorded at 70 eV with a trap current of 100 μA. Chemical-ionization (CI) mass spectra were obtained using alternating EI and CI scans and ammonia as the CI reagent gas. Scan speeds of 1 s decade^{-1} were used and the ion source temperature was maintained at 200°C. Typically the electron multiplier was operated at 4.5 kV.

GC separations were achieved using a Carlo Erba gas chromatograph operating under the following conditions. A cross-linked fused-silica 5% phenylmethylsilicone column (Hewlett-Packard, North Ryde, Australia) (20 m × 0.31 mm I.D.; 0.17 μm film thickness) was used in all the studies reported and it terminated in the MS ion source. Helium served as the GC carrier gas at a flow-rate of 1 ml min^{-1}. Urine extracts were injected at an initial oven temperature of 100°C which was programmed 2 min after sample injection at 20°C min^{-1} to 200°C, then at 30°C to a final temperature of 280°C. The GC injector was maintained at 250°C and the interface line between the GC and MS ion source at 250°C.

Urine extraction

Urine (9 ml), collected 2 h after dextromoramide administration, was hydrolysed at 37°C overnight by β-glucuronidase/arylsulphatase (Boehringer Mannheim, North Ryde, Australia). The hydrolysed solution was extracted with dichloromethane (1 × 10 ml) and the organic phase washed with 0.1 *M* sulphuric acid (1 × 1 ml). The aqueous phase was adjusted to pH 9 (sodium carbonate) and the organic bases were extracted into dichloromethane (2 × 10 ml). After drying over anhydrous sodium sulphate the residue was dissolved in ethyl acetate (20 μl) prior to GC–MS analysis.

Reference standard of structure 2

Compound **2** was a gift from Janssen Research Foundation (Beerse, Belgium) and it had an identical GC retention time and EI and ammonia CI mass spectra with the metabolite of dextromoramide occurring in equine urine. Under CI conditions using a resolving power of 10 000 (10% valley) the $[MH]^+$ ion of **2** was measured to be 339.2059 (calculated for $C_{21}H_{27}N_2O_2$: 339.2066).

RESULTS AND DISCUSSION

The EI mass spectrum of **1** has been published[8]. The base peak of this spectrum occurs at *m/z* 100 with prominent ions at *m/z* 128 and 265 with a low-abundance (3%) molecular ion visible at *m/z* 392. This fragmentation can formally be accommodated by the bond fissions depicted in Fig. 1. Thus, metabolic transformations of dextromoramide should be readily identified by MS from displacement of the *m/z* values of these diagnostic ions.

In contrast to the EI mass spectrum[8] of **1** where high mass ions are of low abundance, the ammonia CI mass spectrum of this compound has the protonted molecular ion $[MH]^+$ at *m/z* 393 as the base peak.

Equine urine, collected 2 h after drug administration, was enzymatically hydrolysed (β-glucuronidase) and the products were analysed by GC–EI-MS. The total ion

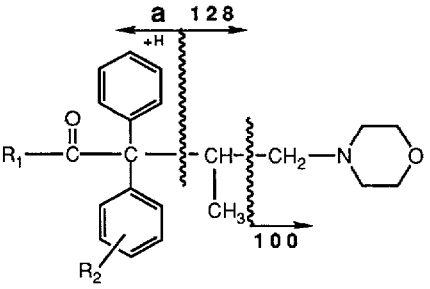

Fig. 1. Rationalisation of the principal bond fissions in the EI mass spectrum of dextromoramide (**1**), its urinary metabolites (**2** and **3**) and their products after derivatisation with MethElute. Structures **4** and **5** derived from metabolite **2** and structure **6** from metabolite **3**. **1**: R_1 = N-pyrrolidinyl; R_2 = H; a = *m/z* 265; **2**: R_1 = NH_2; R_2 = H; a = *m/z* 211; **3**: R_1 = N-pyrrolidinyl; R_2 = OH; a = *m/z* 281; **4**: R_1 = $NHCH_3$; R_2 = H; a = *m/z* 225; **5**: R_1 = $N(CH_3)_2$; R_2 = H; a = *m/z* 239; **6**: R_1 = N-pyrrolidinyl; R_2 = OCH_3; a = *m/z* 295.

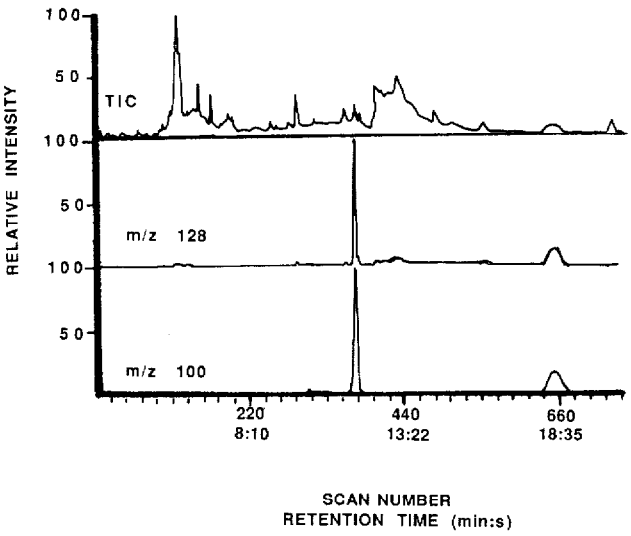

Fig. 2. Total ion chromatogram and mass chromatograms of *m/z* 100 and 128 of the basic fraction from horse urine collected 2 h after intravenous administration of dextromoramide (Palfium, 20 mg).

chromatogram (Fig. 2) was searched for the peak profiles of *m/z* 100 and 128, respectively. This identified two GC peaks with the first occurring at a retention time of 11 min 40 s. The EI mass spectrum of this component (Fig. 3) did not detect an obvious molecular ion and no ions were observed above *m/z* 211. However, using the alternating EI–CI scanning technique, the ammonia CI mass spectrum clearly identified a protonated molecular ion ($[MH]^+$) at *m/z* 339. The ions of mass 100 and 128 in the EI mass spectrum (Fig. 3) of this compound indicated that the morpholine ring system

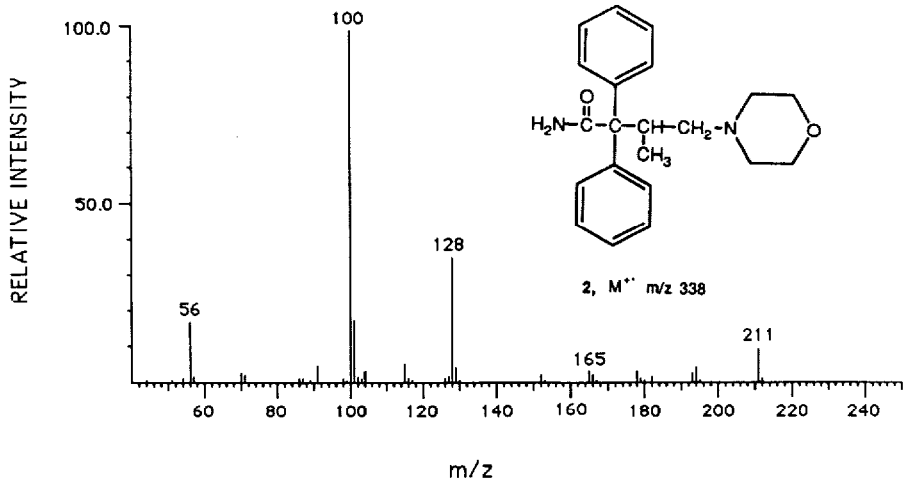

Fig. 3. EI mass spectrum (70 eV) of 2,2-diphenyl-3-methyl-4-morpholinobutyramide (**2**) corresponding to retention time 11 min 40 s in Fig. 2.

and the three carbon atoms attached to the nitrogen were present in this metabolite (see Fig. 1). The ion at *m/z* 211 in the mass spectrum of **2** (Fig. 3) corresponds in origin to that at *m/z* 265 ($[M - 127]^+$) in the mass spectrum of **1**[8] and formally corresponds to $[NH_2CO{-}CH[C_6H_5)_2]^+$ and must incorporate a hydrogen rearrangement from the N-alkylmorpholine system. A molecular weight of 338 for this metabolite indicated that 54 mass units had been eliminated from **1**. This is consistent with the assignment of structure **2** to this metabolite, and positive confirmation of this metabolite's structure was achieved by comparison with an authentic sample of **2** when an identical GC retention time and EI and CI mass spectra were obtained.

The second equine urinary metabolite of dextromoramide eluted as a broad GC peak at a retention time of 18 min 20 s (Fig. 2). Its molecular ion was identified at *m/z* 408 from the ammonia CI mass spectrum ($[MH]^+$ at *m/z* 409) and the base peak under EI conditions (Fig. 4) occurred at *m/z* 100 with a prominent ion at *m/z* 128. A significant ion was located at *m/z* 281 [*m/z* 265 in 1 (Fig. 1) plus an oxygen atom] and this product can be assigned structure **3**.

On-column methylation of the metabolites **2** and **3** was investigated in order to improve the GC characteristics of the phenol **3**. Co-injection into the hot GC injection port of MethElute and hydrolysed horse urine extract resulted in partial (**4**) and complete methylation (**5**) of the amide nitrogen in **2**. Thus two GC peaks were observed (retention times of 13 min 8 s and 13 min 15 s, respectively) and their EI mass spectra were consistent with structures **4** and **5** and had the ubiquitous fragment ions at *m/z* 100 and 128 (Fig. 1). Ions corresponding to the ion at *m/z* 211 in **2** were now located at *m/z* 225 and 239, respectively, in **4** and **5**. The ammonia CI mass spectra of **4** and **5** had their respective $(MH]^+$ ions located at *m/z* 353 and 367 consistent with these structural assignments.

The phenolic metabolite, **3**, after methylation in the GC injector with MethElute, yielded **6**, which eluted from the GC as a sharp peak with a retention time of 15 min 30 s. This product had an EI mass spectrum consistent with the fragmentation depicted in Fig. 1. The *m/z* 265 fragment in the mass spectrum of **1** was now located at *m/z* 295 but the molecular ion (*m/z* 422) was not detected. However, using ammonia CI a $[MH]^+$ ion was readily identified at *m/z* 423.

Thus the two major metabolites of dextromoramide in the horse have been

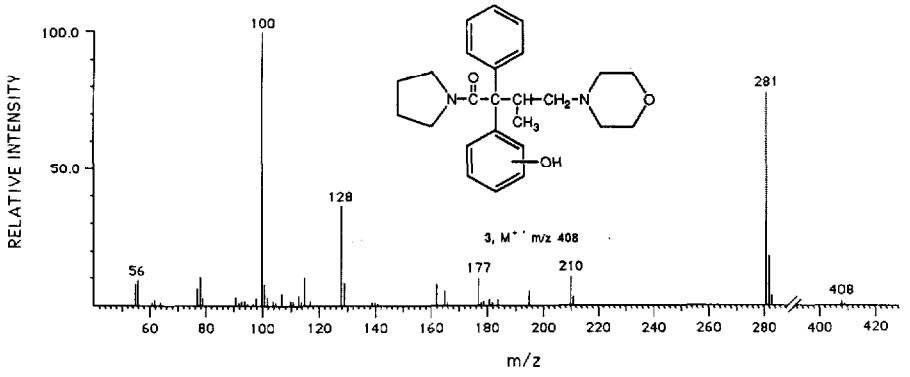

Fig. 4. EI mass spectrum (70 eV) of compound **3** corresponding to retention time 18 min 20 s in Fig. 2.

identified to have structures **2** and **3**, respectively. The ions of mass 100 and 128, using EI-MS, or of 339 and 409, using ammonia CI-MS, can be used under GC–MS conditions as a sensitive and specific method to detect these metabolites of dextromoramide in equine urine swabs. On-column methylation can also be used as additional evidence for the presence of **2** and **3** in equine urine extracts. The availability of an authenticated sample of **2** now provides the racing analyst with the necessary standard for the positive identification of dextromoramide (Palfium) in equine swabs.

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