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Journal of Chromatography B, 811 (2004) 85–91
JOURNAL OF CHROMATOGRAPHY B
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Protopine alkaloids in horse urine

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Received 20 November 2003; accepted 26 March 2004
Available online 11 September 2004

Abstract

Protopine was extracted from *Fumaria officinalis* and purified by column chromatography. Urine samples were collected from horses and a human volunteer that had been administered either *F. officinalis* or protopine free base. Plant and urine samples were acetylated and analysed by GCMS after solid-phase extraction (SPE). The urinary metabolites of protopine were identified as 4,6,7,13-tetrahydro-9,10-dihydroxy-5-methyl-benzo[e]-l,3-benzodioxolo [4,5-1][2] benzazecin-12(5H)-one, 4,6,7,13-tetrahydro-10-hydroxy-9-methoxy-5-methyl-benzo[e]-1,3-benzodioxolo[4,5-1][2] benzazecin-12(5H)-one and 4,6,7,13-tetrahydro-9-hydroxy-10-methoxy–5-methyl-benzo[e]-1,3-benzodioxolo[4,5-l][2] benzazecin-12(5H)-one, chelianthifoline, isochelianthifoline and 2-O-desmethylchelianthifoline. The metabolic formation of the tetrahydroprotoberberines by closure of the bridge across N5 and C13 is rate limited and protopine-like metabolites accumulate only when the route is overloaded. Metabolism was qualitatively similar in the horse and human.

Keywords: Protopine; Alkaloids; Opiates; Chemotaxonomy

1. Introduction

Narcotic analgesics, including morphine, exhibit significant stimulatory activity in the horse when administered at low dose [1] and thus have a high potential for abuse in equine performance sports. In many cases, the detection of morphine in the urine of horses has been attributed to feed contaminated with *Papaver somniferum*. Several authors [2,3] have proposed that the detection of botanical markers in urine that tested positive to morphine can provide evidence of the source of the morphine. The method has significant forensic appeal as it depends only on analysis of the urine specimen and not on any later inspection of feed samples.

Duffield et al. [2,4] proposed that the urinary metabolites of protopine (I) and cryptopine (II) could be used as botanical markers for a cultivar of *P. somniferum* ssp. *setigerum* that was found as a contaminant of animal feed (Table 1). Unfortunately, neither alkaloid is unique to the *Papaver* species. The more abundant of the two, protopine, is also found in a number of other species including *Fumaria*, *Corydalis and Nandina* [5,6] that have been used as traditional medicines and might be used in racing stables. *Fumaria officinalis*, in which protopine is abundant, is also a common plant of both pasture and creek margins and is reputedly sought out by grazing animals [7]. It is one of the most likely sources of dietary protopine to which horses may be exposed.

Whether considered as phytometabolites derived from pasture species, herbal preparations or as markers in determining the source of urinary opiates, the metabolism and excretion of protopine and related alkaloids is of interest to the equine forensic chemist. This paper examines the dose dependant metabolism of protopine in the horse by comparison with that in humans. The elucidation of protopine metabolism in the horse also permits the re-evaluation of the botanical marker technique described by Duffield et al. [2,4] in their investigation of *P. somniferum* ssp. *setigerum* contaminated animal feed.

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doi:10.1016/j.jchromb.2004.03.077

Table 1
A 10 peak index of significant EI fragment ions and their abundances for alkaloids and their metabolites

Compound	Number	Derivative	MWt	Fragment ions and percentage abundances									
Protopine	I	None	353	148	163	190	267	89	134	149	252	353	91
				100	*25*	*10*	*9*	*9*	*9*	*7*	*6*	*5*	*5*
Cryptopine	II	None	367	148	367	366	149	368	176	91	324	220	89
				100	*51*	*40*	*15*	*11*	*10*	*10*	*5*	*4*	*3*
Stylopine	III	None	323	148	323	322	174	149	91	324	89	179	116
				100	*66*	*42*	*17*	*16*	*10*	*10*	*8*	*4*	*3*
Protopine metabolite	IV[a]	None	355	148	165	190	149	136	191	91	253	325	355
				100	*21*	*10*	*9*	*8*	*8*	*7*	*6*	*4*	*4*
Protopine metabolite	IV[a]	Acetyl	397	148	165	190	149	312	91	269	253	354	397
				100	*15*	*13*	*10*	*9*	*7*	*7*	*6*	*5*	*4*
Protopine metabolite	VII	Acetyl	425	148	190	298	269	149	193	340	91	381	425
				100	*17*	*15*	*9*	*9*	*8*	*4*	*4*	*3*	*3*
Chelanthifoline (from *Nandina domestica*)	VIII[b]	None	325	148	325	324	149	326	176	91	89	165	310
				100	58	*51*	9	8	8	7	6	*4*	3
Chelanthifoline (from *Nandina domestica*)	VIII[b]	Acetyl	367	148	367	366	368	149	176	91	89	324	352
				100	*75*	*48*	*24*	*16*	*14*	*7*	*6*	*5*	*3*
O-desmethylchelanthifoline	IX	Acetyl	395	148	395	149	394	396	91	352	310	282	162
				100	*37*	*16*	*12*	*10*	*7*	*5*	*5*	*4*	*4*
Chelanthifoline metabolite (from *Nandina domestica*)	XI	Acetyl	411	150	410	352	411	176	368	326	135	218	220
				100	*98*	*83*	*72*	*27*	*22*	*14*	*13*	*10*	*10*

[a] Identical mass spectrum observed for isomeric compound VI.
[b] Identical mass spectrum observed for isomeric compound X.

2. Experimental

2.1. Reagents and chemicals

Solvents (EMD Chemicals, Gibbstown, USA) and reagents (BDH, Poole, England) for extraction and column chromatography and acetylation were of analytical grade. Deionised water was produced with an Elix 3 system (Millipore, Molsheim, France).

2.2. Collection of F. officinalis and isolation of protopine

Fresh whole specimens of *Fumaria officinalis* were harvested during December and January of 1997–2001 on an improved heavy black-clay soil in Victoria, Australia. The plant was air dried in dehumidified air at 20 °C for 3–4 weeks out of direct sunlight and then stored at −20 °C until required for use.

The dried plant (200 g) was coarsely chopped and soaked overnight in methanol (1 L). The methanol was decanted, filtered and concentrated in vacuo at 40 °C to a volume of approximately 75 mL. The crude methanolic concentrate was treated with aqueous acetic acid solution (5%, v/v; 500 mL) and extracted sequentially with petroleum ether (500 mL) and diethyl ether (500 mL). The aqueous solution was adjusted to pH 12 with the addition of 5.0 M sodium hydroxide solution and extracted with dichloromethane–methanol (3:1, 500 mL). The organic extract was back-extracted into water (200 mL) adjusted to pH 3 with the addition of 1.0 M aqueous hydrochloric acid and the aqueous extract was separated and decolourised with charcoal. The charcoal was removed by gravity filtration and the filtrate adjusted to pH 12 with the addition of 2.0 M sodium hydroxide solution. Non-phenolic alkaloids were extracted with dichloromethane (2 mL × 100 mL). The organic extracts were combined, dried over anhydrous sodium sulphate and evaporated to dryness in vacuo at 40 °C.

Crude protopine was separated from the non-phenolic alkaloid fraction in chloroform–methanol (10 mL) with crystallisation occurring over 1 week at −20 °C. A subsequent crop of crystals was recovered by removing the crystals and concentrating the chloroform–methanol solution. The remaining liquid was chromatographed on a 360 mm × 18 mm alumina column (90 g, 90 active neutral aluminium oxide, 0.063–0.200 mm, Merck, Darmstadt, Germany) with hexane–diethyl ether (1:1, 100 mL), diethyl ether (100 mL), diethyl ether–chloroform (9:1, 100 mL), diethyl ether–chloroform (1:1, 100 mL) and chloroform (100 mL). Fraction volumes of 30 mL were collected and those that contained protopine as the major component (>90% of the detectable alkaloid pool based on GCMS response for the crude alkaloid extract) were combined, evaporated to dryness and crystallised as described above. The crude protopine was recrystallised from ethyl acetate to yield a pale cream powder (340 mg, mp 205–7 °C lit [8]. 208 °C; IR (KBr) 2700–2980 (CH_2), 1650 (C=O), 1520, 1500 (aromatic), 1460 (CH_2), 925 (dioxolo), ^{1}H NMR ($CHC1_3$) 1.95 ppm (**CH**$_3$–N, s, 3H), 2.3–2.7 (**CH**$_2$, broad peak, 2H), 2.6–3.1 (**CH**$_2$, broad peak,

2H), 3.5–3.7 (**CH**$_2$–N, broad peak, 2H), 3.7–3.9 (**CH**$_2$–N, broad peak, 2H), 5.91 (O–**CH**$_2$–O, s, 2H), 5.93 (O–**CH**$_2$–O, s, 2H), 6.62 (Ar–**H**, s, 1H), 6.612 and 6.651 (Ar–**H**, d, J = 7.9 Hz, 1H), 6.659 and 6.669 (Ar–**H**, d, J = 7.9 Hz 1H), 7.239 (Ar–**H**, s, 1H); ^{13}C NMR ($CDC1_3$) 31.2 (Ar–**C**H$_2$–CH$_2$), 41.6 (**C**H$_3$–N), 45.8 (**C**H$_2$–CO), 51.2 (N–**C**H$_2$–) 57.5 (Ar–**C**H$_2$–N), 106.9, 108.0, 110.3, 125.1 (Ar–H), 117.1, 128.4, 132.0, 135.3, 146.0, 146.05, 146.1, 148.05 (Ar, no H); EI GCMS 148 (100%), 163 (25), 190 (10), 267 (9), 89 (9), 134 (9), 353 (5); PICI GCMS (acetonitrile) M + 1354 Da). NMR spectra were recorded on a Gemini A200 (Varian, Palo Alto, CA, USA) and mass spectra were obtained using a Saturn 2000 GCMS (Varian, Walnut Creek, CA, USA).

2.3. Administrations and sample collection

Air-dried *F. officinalis* was blended with 7–10 L of dry feed and fed to a Thoroughbred gelding to give a dose of 50 mg/kg body weight (equivalent to a dose of approximately 0.1 mg protopine/kg body weight). Partial volumes of naturally voided urine were collected over the 24 h following administration. Urine samples were frozen within 2 h of collection and stored at −20 °C until required for analysis.

For comparison of metabolism between species, *F. officinalis* was administered with the informed consent of one of the authors. To improve the palatability of the herb, it was prepared as a tea containing 20 mg herb/mL boiling water. The tea was brewed for 2 h, sweetened with sugar and administered orally at a rate of 0.71 mL/kg body weight (equivalent to a dose of approximately 0.04 mg protopine free base/kg body weight). Urine samples were collected at 0, 0.5, 1.5, 3, 4, 5, 7.5, 9 and 16 h after administration. The tea was prepared as recommended in authoritative herbal literature [9]. In the investigation of the dose dependent metabolism of protopine, the alkaloid free base was self-administered by the same volunteer at an oral dose rate of 3.5 mg/kg body weight. Urine samples were collected at 0,1, 2, 3, 5, 7, 9,13 and 22 h following administration. In both experiments, samples were frozen at −20 °C immediately after collection and stored until required for analysis.

2.4. Extraction of urine and plant specimens

For the analysis of urine, aliquots of each specimen (3 mL) were diluted with 0.1 M potassium phosphate buffer (pH 6.0, 4.5 mL) and the pH adjusted to 6.2–6.3. Each sample was then enzyme hydrolysed with β-glucuronidase (2500 IU, *Escherichia coli*, Sigma, St Louis, USA) for 2 h at 50 °C and extracted on a solid-phase extraction column (120 mg, 3 mL, Bond-Elut Certify^{TM}, Varian, Harbor City, USA) as described previously [10]. The basic fraction was evaporated to dryness under a stream of nitrogen at a flow rate of 1 mL/min/port and 20 °C. Residues were dissolved in 200 μL of pyridine and 100 μL of acetic anhydride and heated at 60 °C in a culture tube with a Teflon^{TM} lined screw cap for 20 min. In each case the sample was then cooled and evaporated to dryness under a stream of nitrogen. The residues were reconstituted in 100 μL of ethyl acetate (nanograde, EMD Chemicals) and analysed by electron impact (EI) GCMS.

Prior to extraction, plant material was prepared by soaking overnight in sufficient volume of 0.05 M sulphuric acid solution to completely immerse the sample. The supernatant fraction was decanted and filtered, if necessary, to remove suspended material. A portion of the liquor, representing approximately 50 mg of dry plant, was adjusted to pH 6.0 prior to dilution and extracted as described previously for hydrolysed urine samples.

2.5. Gas chromatography mass spectrometry (GCMS) analysis

EI-GCMS was performed on a Hewlett Packard 6890 GC-5973 MSD with a 7683 injector (Palo Alto, USA). The GC was equipped with a BPX5 column (12 m × 0.2 mm, 0.25 μm film thickness, SGE, Melbourne, Australia) and used helium as the carrier gas with a constant flow of 1.0 mL/min. The oven temperature was held at 75 °C for 2 min then increased at 30 °C/min to 300 °C with a final holding time of 9.0 min. Injections of 2 μL were pulsed splitless with a nominal head pressure of 60 kPa pulsed to 170 kPa for 1 min after injection. A scan range of 50–650 Da at 2.48 scan/s was used.

2.6. Data analysis

Alkaloids or their urinary metabolites were identified by comparison of their EI mass spectra with spectral data reported in the literature [2,5,11] or the Wiley mass spectral library (HPG1035A, purchased from Agilent Technologies, Forest Hill, Australia). For compounds for which no data was available assignments were supported by extraction chemistry, response to acetylating reagents, retention time, EI and PICI mass spectral data (including common fragmentation pathways established for structurally analogous compounds) and alkaloid extracts obtained from plants in which the target compounds had previously been characterised. The species *Corydalis cava* and *Nandina domestica* were sources of tetrahydroprotoberberines chelanthifoline, O-desmethylchelanthifoline and 5,8,13,13a-tetrahydro-2,3-dihydroxy-6H-dibenzo[4,5-a:5,6-g]quinolizine. The significant EI mass spectral fragments for the named alkaloids and their metabolites are summarised in Table 1.

3. Results and discussion

F. officinalis, which contains several important alkaloids including protopine and stylopine, showed no significant variation in alkaloid composition when propagated over several growing seasons. A typical chromatogram showing the distribution of alkaloids for the species is shown in Fig. 1.

Following administration of *F. officinalis* or protopine base to both a horse and a human volunteer, urine samples

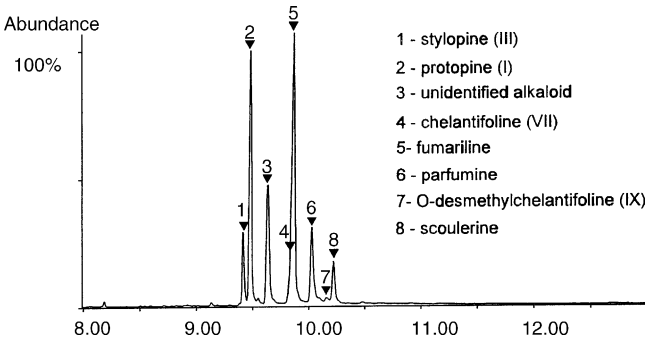


Fig. 1. The basic alkaloids of *Fumaria officinalis* in an acetylated basic extract.

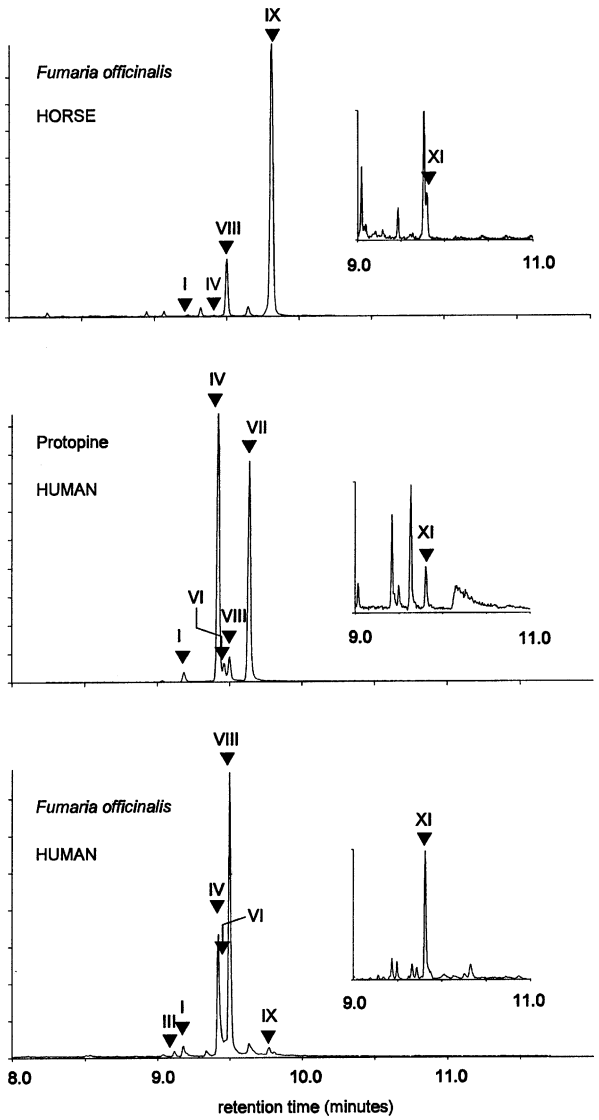


Fig. 2. Extracted ion chromatograms for *m*/*z* 148 and *m*/*z* 150 (inset) for urine samples collected from a horse administered *Fumaria officinalis* (top), a human administered protopine (middle) and a human administered *F. officinalis* (bottom).

were collected and examined for evidence of protopine or its metabolites.

Chromatograms showing the excretion pattern for protopine and stylopine (III) related metabolites are shown for each case in Fig. 2.

In both the horse and human, protopine and stylopine were almost completely metabolised with little of the unchanged parent excreted in the urine. The metabolite pool was qualitatively similar in both species, with the same metabolites detected in each case, although significant differences in the relative importance of the metabolites were readily observed.

3.1. Protopine like metabolites

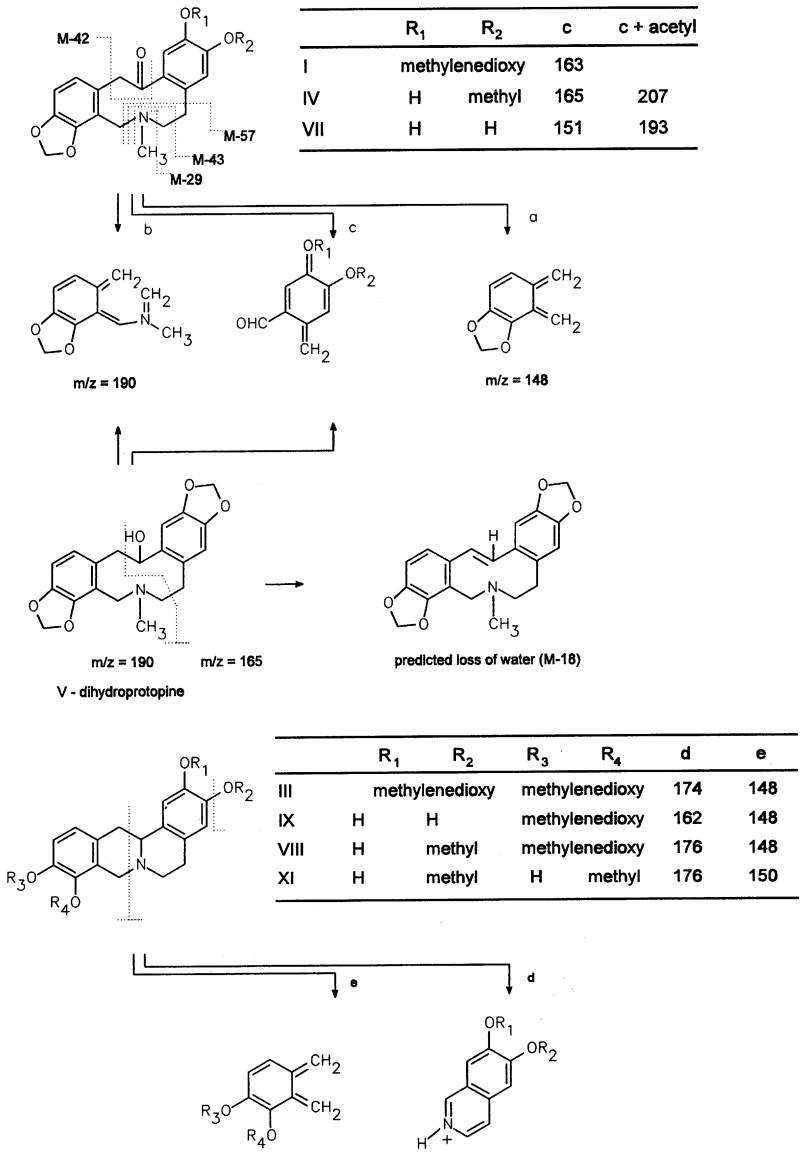
A reduced metabolite of protopine (IV) that gave a molecular ion at *m*/*z* 397 following acetylation was detected at very low levels relative to other metabolites in the horse. In the absence of any other candidates with the same molecular weight, it is likely that this compound was the one assigned as the ketone reduction metabolite, dihydroprotopine (4,6,7,13,14-pentahydro-5-methyl-bis[1, 3]-benzodioxolo[4,5-c:5′,6′-g]azecin-13(5H)-ol, V), by previous investigators [2,4]. A minor isomeric compound (VI), with an abundance of approximately 5% of IV and with an identical mass spectrum, was also detected.

Following administration of protopine free base to a human volunteer, the two isomeric metabolites IV and VI were readily detected (see Fig. 2). An additional metabolite (VII) with a molecular weight of 425 was also observed and although abundant, this compound showed no evidence of any minor isomer. The three compounds were also readily detected following administration of *F. officinalis* tea to the same volunteer.

The mass spectral data alone does not demonstrate unequivocally that the metabolites IV and VI are isomers of dihydroprotopine (V). In the three acetylated metabolites and in protopine the EI-fragment ion *m*/*z* 148 is considered to be the [1,3]benzodioxolo-dimethide fragment (fission process “a”) and thus its presence in the spectrum indicated that the dioxolo-ring proximal to the *N*-methylated hetero-atom remained intact (Fig. 3). The fragment ion *m*/*z* 163 in protopine from fission process “b” was shifted to *m*/*z* 165 (and 207 with the addition of an acetyl group) in the metabolites IV and VI and to *m*/*z* 193 after acetylation of metabolite VII. The three metabolites were also similar to protopine in that they showed the presence of the fragment ion *m*/*z* 190 attributable to fission process “c” at approximately the same relative intensity.

The similarity of the fragmentation patterns for IV, VI and VII suggest that the compounds are structurally similar but the molecular weight of 425 for VII is difficult to rationalise in terms of a dihydroprotopine analogue.

The rationalisation of the data is more reasonable for the putative structures of the catechol analogue of protopine formed by ring opening of the *N*-distal dioxolo-ring and the isomeric *O*-methylated secondary metabolites of the



	R_1	R_2	c	c + acetyl
I	methylenedioxy		163	
IV	H	methyl	165	207
VII	H	H	151	193

	R_1	R_2	R_3	R_4	d	e
III	methylenedioxy		methylenedioxy		174	148
IX	H	H	methylenedioxy		162	148
VIII	H	methyl	methylenedioxy		176	148
XI	H	methyl	H	methyl	176	150

Fig. 3. Rationalisation of the EI mass spectral fragments for protopine like alkaloids (top) and stylopine like alkaloids (bottom).

catechol. Thus, the metabolites may be assigned as 4,6,7,13-tetrahydro-9,10-dihydroxy-5-methyl-benzo[e]-1,3-benzodioxolo[4,5-l][2] benzazecin-12(5H)-one (VII), 4,6,7,13-tetrahydro-10-hydroxy- 9-methoxy-5-methyl-benzo[e]-l,3-benzodioxolo[4,5-l][2] benzazecin-12(5H)-one (IV) and 4, 6,7,13-tetrahydro- 9-hydroxy-10-methoxy–5-methyl-benzo[e]-l,3-benzodioxolo[4,5-l][2] benzazecin-12(5H)-one (VI). These metabolites are consistent with the structures reported by Paul and Maurer [11] following the administration of protopine containing *Eschscholtzia californica* to rats. The EI mass spectral fragmentation is rationalised in Fig. 3. The dominance of IV over VI is undoubtedly a result of the specificity of 3-catechol-O-methyltransferase (3-COMT, EC 2.1.1.6) towards the two possible orientations of the catechol (VII) with the β-phenethyl orientation directing methylation toward the 9-methoxy isomer. The catechol metabolite (VII) was not found in the horse urine samples.

Although the reasons for this remain unknown, it is possible to surmise that the difference reflects species differences in the 3-COMT activities for first-pass metabolism or the further rate limited metabolism of these compounds to secondary or tertiary metabolites.

3.2. Tetrahydroprotoberberine metabolites

Following *F. officinalis* administration to the horse, the two most abundant metabolites detected, VIII and IX, gave protonated molecular ions in PICI GCMS at *m/z* 368 and 396. A very minor compound (X) that was isomeric with VIII was also detected.

In the human, the three compounds (VIII–X) were detected following the administration of both *F. officinalis* tea and protopine free base, although their relative abundance differed for each dose form. Their formation following

administration of protopine base demonstrated, at least in the human, that they are metabolites of protopine.

Unlike the structure reported previously [2,4] for the compound with a molecular weight of 367, VIII and X showed fragmentation consistent with protoberberines rather than a protopine or dihydroprotopine-like compound. The fragmentation of compound VIII is rationalised in Fig. 3 as being consistent with chelianthifoline (5,8,13,13a-tetrahydro-2-hydroxy- 3- methoxy- 6H-benzo[a]1,3-benzodioxolo[5, 6-g]quinolizine), a dioxolo-ring fission metabolite of stylopine (III). Again, the minor isomer (X) is likely to be the alternate 3-COMT product 5,8,13,13a-tetrahydro-3-hydroxy-2-methoxy-6H-benzo[a] 1,3-benzodioxolo[5,6-g] quinolizine. Similarly, compound IX, with a molecular weight of 395, gave EI fragmentation consistent with the protoberberine 2-0-desmethylchelianthifoline(5,8,13,13a-tetrahydro-2,3-dihydroxy-6H-benzo[a]-[1, 3]benzodioxolo[5, 6-g]quinolizine).

Little or no evidence of urinary stylopine (III) was found in either species even though it might reasonably be considered as the parent to the tetrahydroberberine metabolites of protopine. This finding indicates that either stylopine is itself rapidly metabolised by fission of the benzodioxolo-ring or alternatively, formation of VIII, IX and X does not proceed via a stylopine intermediate. The metabolite IX accounted for approximately 90% of the basic protopine metabolites in the horse. In the human administered protopine, VIII–X were estimated on the basis of their mass spectral response as representing only 10, 3 and <0.2%, respectively, of the basic metabolite pool (Fig. 2). At the lower dose rate, when the alkaloids were administered as herbal tea, the distribution of metabolites was significantly different with VIII becoming the dominant basic product (Fig. 2).

In the human subject, there was an apparent dose dependence on the metabolic profile with the protopine like metabolites (IV, VI and VII) only becoming significant after the higher dose of protopine. It is likely that the metabolic formation of the tetrahydroprotoberberines by closure of the bridge across N5 and C13 is rate limited and when the pathway is overloaded an accumulation of dioxolo fission metabolites IV, VI and VII results. As very little unchanged protopine or stylopine were excreted, the fission of the dioxolo-ring and subsequent *O*-methylation of the catechol metabolites is unlikely to be rate limited.

Other protopine metabolites were sought on the basis of the distinctive fragment ion *m*/*z* 148 and analogous fragments with *m*/*z* 150,136 and 178 arising from the reductive cleavage of the benzodioxolo ring proximal to the nitrogen. The fragment ion at *m*/*z* 150 is attributed to a 4-hydroxy-3-methoxy-benzene-1,2-dimethide cation and those at *m*/*z*

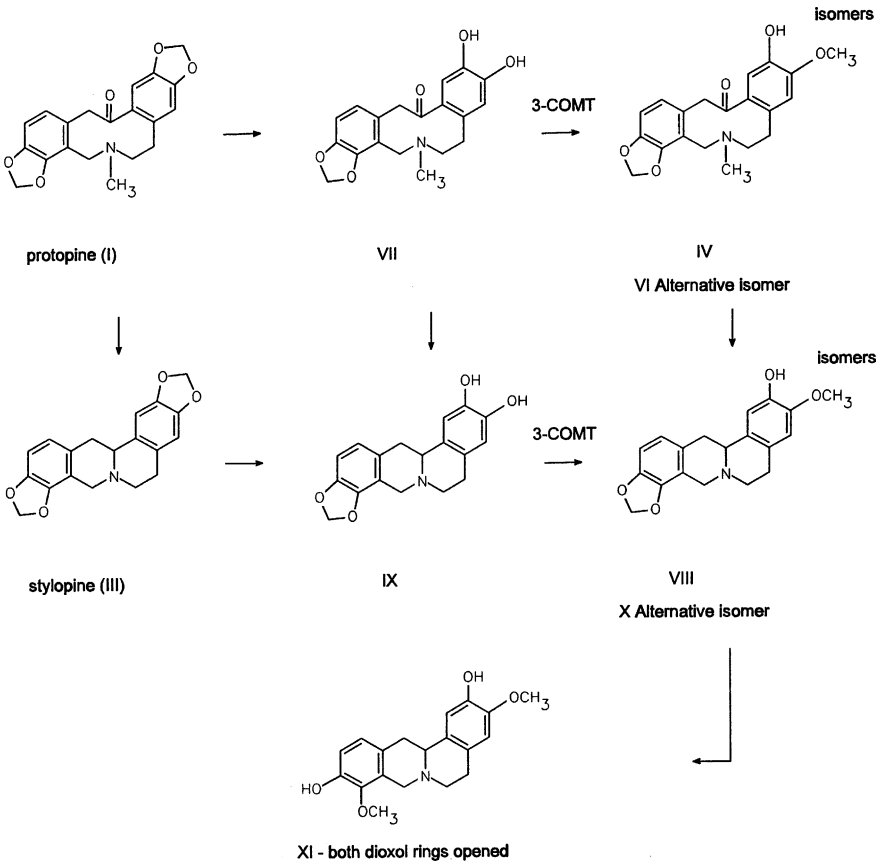


Fig. 4. The proposed urinary metabolites of protopine and stylopine in the horse and the human.

136 and 178 to a 3,4-dihydroxybenzene-1,2-dimethide and a 3 (or 4)-hydroxy-4 (or 3)-acetoxybenzene-dimethide cation, respectively.

Only one of the compounds was detected in the acetylated basic fraction from human urine. It gave an EI mass spectrum that was consistent with a tetrahydroprotoberberine and a protonated molecular ion of *m*/*z* 412 in PICI. On the basis of this data, the metabolite was assigned as 5,8,13,13a-tetrahydro-2,10-dihydroxy-3,9-dimethoxy-6H-dibenzo[4,5-a:5,6-g]quinolizine (XI).

The EI mass spectrum is rationalised as shown in Fig. 3 and the compound found to be identical with the same alkaloid isolated from *N. domestica* [5]. The compound was also detected in the horse but was quantitatively less important than was observed in humans.

With the identification of the metabolic pathways described above, a metabolic scheme is proposed for protopine and is shown in Fig. 4.

4. Conclusion

In the study described in this paper, *F. officinalis* was administered to a horse at a dose equivalent to approximately 0.1 mg protopine/kg body weight. As with the administration of protopine to the human at this dose rate, chelianthifoline (VIII) and O-desmethyl-chelianthifoline (X) were observed as the major urinary metabolites. In humans, a significant shift in the distribution of protopine metabolites to favour dioxolo-ring cleaved metabolites of protopine was observed when the dose was increased from 0.04 to 3.5 mg protopine/kg body weight.

The human metabolism provides a useful model on which to re-evaluate the equine metabolism of protopine when the dose of alkaloid is increased. Thus, in the absence of any evidence for the formation of dihydroprotopine by the horse, it appears likely that the metabolites identified by Duffield et al. [2] were the dioxolo-ring opened compounds IV, VI and VII. A similar dose dependence in the horse would be consistent with these compounds forming in equo as a result of a high dose of protopine having been administered with the result that the pathway to the tetrahydroprotoberberines metabolites was overwhelmed.

The demonstration of dose dependent metabolism does not consider the influence that concomitant administration of other the *Fumaria* alkaloids might have on the composition of the urinary metabolite pool. However, the other major alkaloids detected in *F. officinalis* (an uncharacterized isoquinoline alkaloid, fumariline and parfumine) are structurally unrelated to protopine and unlikely metabolic precursors to the tetrahydroprotoberberines. As the inhibited or rate limited conversion of protopine (and like alkaloids) to the tetrahydroprotoberberines is observed for high doses of protopine in the absence of these other alkaloids, it is also reasonable to conclude that they do not act as metabolic inhibitors.

The metabolic fate of parfumine and fumariline in the horse is the subject of continuing work. Preliminary and unpublished experiments suggest strongly that urinary metabolites of these alkaloids may be used as indicators for protopine metabolites that are derived from *Fumaria* ingestion. Neither parfumine or fumariline have been detected in the protopine containing opium *P. somniferum* ssp. *setigerum*.

The distribution of protopine in a common agricultural species and other plants used in herbal medicine does not discount the usefulness of protopine metabolites as a probe for feed contamination with *P. somniferum* ssp. *setigerum*. However, the technique should be used with caution and be considered in support of a feed contamination hypothesis only when alternative sources of protopine have been eliminated on the basis of a dietary audit of the index case in the investigation.

Acknowledgments

The authors wish to thank Drs. J.P. McCaffrey and R.C.E. Cust for collecting urine samples and Racing Victoria Limited, Harness Racing Victoria and Greyhound Racing Victoria for their financial support.

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