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Felinine stability in the presence of selected urine compounds

S. M. Rutherfurd[1], **T. M. Kitson**[2], **A. D. Woolhouse**[3], **M. C. McGrath**[1], and **W. H. Hendriks**[4]

[1] Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand
[2] Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand
[3] Industrial Research Ltd., Lower Hutt, New Zealand
[4] Nutrition Group, Department of Animal Sciences, Wageningen University, Wageningen, The Netherlands

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Summary. The stability of felinine, an amino acid present in feline urine, was investigated. Synthetic felinine was unstable in the urine of a selection of mammals. Felinine was found to stable in feline urine in which urea had been degraded. Synthetic felinine was found to react specifically with urea and did not react with urea analogues such as biuret or thiourea or other nucleophilic compounds such as ammonia which is more nucleophilic or acetamide and water which are less nucleophilic than urea. The reaction of urea and felinine was independent of pH over the range of 3–10. Urea did not react with *N*-acetyl-felinine suggesting a felinine N-terminal interaction with urea. Mass spectral analysis of the reaction products showed the presence of carbamylated felinine and fragmentation ions derived from carbamyl-felinine. The physiological relevance of felinine carbamylation is yet to be determined.

Keywords: Felinine – Urea – Cats – Urine – Carbamylation – Smell

Introduction

Felinine (2-amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoic acid) ($HOOCCH(NH_2)CH_2SC(CH_3)_2CH_2CH_2OH$) is a sulphur amino acid found in large quantities in the urine of entire male domestic cats. Excretion rates per day by castrated male and female domestic cats is approximately a quarter of that of entire male cats. While the function of felinine is uncertain, it may be involved in the production of pheromone compounds used for the scent marking of territories in order to repel male cats and potentially attract female cats (Hendriks et al., 1995b). Felinine itself has been reported to be odourless (Brand, 1979; Hendriks et al., 1995a) but when stored at room temperature and at 4 °C, synthetic felinine develops the characteristic catty odour associated with feline urine (Hendriks et al., 1995b). Consequently, it is likely that breakdown or modification of felinine is required to elicit this characteristic and pungent odour. A number of felinine degradation products have been postulated with 3-mercapto-3-methyl-1-butanol being the prime candidate responsible for the catty odour (Joulain and Laurent, 1989; Mattina et al., 1991; Hendriks et al., 1995b). Other workers, however, have not been unable to detect the presence of 3-mercapto-3-methyl-1-butanol in entire male cat urine samples (Rutherfurd et al., 2004) indicating that other volatiles originating from felinine or felinine degradation products may be involved in the generation of the characteristic catty odour of feline urine urine or that the potency of 3-mercapto-3-methyl-1-butanol is such that the levels present in cat urine were below the level of detection.

Upon examination of the molecular structure of felinine, it is difficult to envisage the pathway by which felinine breaks down or is modified to produce odorous volatile compounds. Felinine is structurally similar to methionine and cysteine but does not appear to undergo the oxidation of the sulphur atom that these other sulphur containing amino acids are susceptible to (Rutherfurd et al., 2004). It is possible that felinine stability may be pH-dependent as synthetic felinine is either degraded or modified in the present of acids (Westall, 1953; Hendriks et al., 1995b). Recently, Rutherfurd et al. (2004) reported that felinine, naturally present in male cat urine degrades or disappears from solution much faster compared to synthetic felinine dissolved in water.

Urine, particularly of cats consuming a natural high protein diet, contains relatively high concentrations of urea (1.4 M) and ammonia (0.1 M) (Cottam et al., 2002) compared to urine of other species. Although it is difficult to

postulate a mechanism by which these compounds are involved in felinine modification in feline urine, Rutherfurd et al. (2004) showed that felinine, naturally present in feline urine, disappears at a faster rate compared to synthetic felinine dissolved in water when stored at higher temperatures. To date, there have been no reported studies investigating the interaction between felinine and specific urine compounds. The aim of this study was to understand the mechanism with which felinine degradation/modification occurs in feline urine and whether this mechanism is specific or general in nature.

Materials and methods

Study material

Norleucine, urease, ammonia, acetamide, thiourea, urea, biuret, cysteine, methionine, serine threonine, sodium citrate and phenol were obtained from either Sigma-Aldrich (St Louis, MO, USA) or BDH (VWR International Ltd., Poole, England). *t*-Butylcysteine was obtained from Frinton Laboratories, (Vineland, NJ, USA). Felinine was synthesised according to the procedure described by Hendriks et al. (1995a) while *N*-acetylfelinine was prepared as described by Hendriks et al. (2004).

Feline urine was obtained from adult cats housed at Massey University's Centre for Feline Nutrition (Palmerston North, New Zealand). Canine urine was collected from a privately owned, entire male Labrador-Golden Retriever cross (35 kg, 2 years of age). Rat and hamster urine were obtained from the Small Animal Unit at Massey University (Palmerston North, New Zealand) while the human urine was donated by one of the authors (SR).

Felinine analysis

Synthetic felinine concentrations were determined in duplicate using a Waters ion-exchange HPLC system, utilizing post-column ninhydrin derivatization and detection using absorbance at 570 nm. Felinine concentrations present in urine samples were analysed in duplicate using a Waters ion-exchange HPLC system, utilising post-column o-phthalaldehyde (OPA) derivatisation and fluorescence detection since proline, which does not react with OPA, may be present in the urine and coelutes with felinine. Norleucine was added as an internal standard to the synthetic felinine reaction mixtures after incubation but not to the incubated urines.

Felinine stability in urines of different mammalian species

Urine from entire male, castrated male and female cats was used along with the urine from a male dog, rat, hamster and human. Each urine sample (0.9 ml) was pipetted (in duplicate) into a separate 1.5 ml Eppendorf tube and 0.1 ml of synthetic felinine (9 mg/ml) was added to each urine sample with the exception of the urine collected from the entire male cat to which 0.1 ml of deionised water was added. The diluted urine sample from the entire male cat had a felinine concentration of 4.6 mg/ml. A solution of synthetic felinine (0.9 mg/ml) was also prepared as a negative control. This set of tubes was incubated at 70 °C in a water bath for 24 h while at the same time an identical set of tubes was prepared and stored at −20 °C for 24 h. After incubation, both unincubated and incubated samples were diluted 1/10 with 67 mM sodium citrate (pH 2.0) containing 0.1% (w/v) phenol, transferred to HPLC vials and analysed for felinine concentration. Felinine recovery was calculated as described below.

The urea, ammonia and creatinine concentrations of the urine samples were determined in duplicate on a Cobas Fara II autoanalyser using the kinetic UV assay kit for urea/urea nitrogen (Roche Diagnostics), ammonia assay kit (Sigma Chemicals) and creatinine assay kit (Roche Diagnostics) respectively. These assays are based on the methods of Talke and Schubert (1965), Cheuk and Finne (1984) and Masson et al. (1981) respectively.

Stability of felinine in sterilized urine

1 ml of entire male cat urine was filtered through a sterile 0.2 µm syringe filter into two autoclaved 1.5 ml eppendorf tubes. The tubes were incubated at 70 °C for 24 h. After incubation, both unincubated and incubated samples were diluted 1/10 with 67 mM sodium citrate (pH 2.0) containing 0.1% (w/v) phenol, transferred to HPLC vials and analysed for felinine concentration. Felinine recovery was calculated as described below.

Stability of felinine in urea reduced urine

A "urea reduced" urine was prepared from entire male cat urine as described below. Seven hundred units of urease was added to 5 ml of urine at room temperature and gently stirred on a magnetic stirrer with constant monitoring of the pH. As the pH increased above 8, due to the generation of ammonia from urea, it was readjusted to approximately 7 with 10 µl aliquots of 6.0 M HCl. The reaction was allowed to proceed for 40 hr with additional urease (700 units) being added when the pH showed no increase. In all, urease was added five times with the final addition not resulting in an increase in pH. The urea concentration in the 5 times urease-treated urine was determined before 1 ml of this "urea-reduced" solution was incubated at 70 °C in a waterbath for 24 h (incubated sample) with another 1 ml being stored at −20 °C (unincubated sample) over the same time period. After incubation, both unincubated and incubated urea-reduced urine samples were diluted 1/10 with 67 mM sodium citrate (pH 2.0) containing 0.1% (w/v) phenol, transferred to HPLC vials and analysed for felinine concentration. Felinine recovery was calculated as described below.

Stability of felinine in the presence of urea, urea analogues and other nucleophiles

Solutions (1.4 M) of the nucleophiles ammonia, urea and acetamide were each prepared. This concentration was used as it was similar to the urea concentration found in entire male cat urine (Cottam et al., 2002). Solutions (0.36 M) of urea and its analogues, thiourea and biuret were also prepared. A concentration of 0.36 M was used for this experiment as biuret was much less soluble compared to urea and therefore did not permit the preparation of a 1.4 M solution as used before. Deionised water (0.9 ml, control) and each of the above solutions were pipetted, in duplicate, into separate 1.5 ml Eppendorf tubes where after 0.1 ml of a 9 mg/ml solution of felinine in water was added to each tube. These tubes were incubated at 70 °C in a water bath for 24 h (incubated samples) while a set of identical solutions were again stored at −20 °C (unincubated samples). After incubation, 950 nmol of norleucine was added to 0.1 ml of both unincubated and incubated samples. The tubes were then made up to 1 ml with 67 mM sodium citrate (pH 2.0) containing 0.1% (w/v) phenol, transferred to HPLC vials and analysed for felinine and norleucine content. Felinine recovery was calculated as described below.

Stability of felinine in the presence of urea

Urea solutions of concentrations ranging from 0 to 1.4 M were prepared. Solutions (0.9 ml) were aliquoted, in duplicate, into separate 1.5 ml Eppendorf tubes containing 0.1 ml of (9 mg/ml) felinine in water. These tubes

were incubated at 70 °C in a water bath for 24 h (incubated samples) while identical solutions were also prepared and stored at −20 °C (unincubated samples). After incubation, 950 nmol of norleucine was added to 0.1 ml of both unincubated and incubated samples. HPLC analysis was carried out as above.

Effect of reaction time on the stability of felinine in the presence of urea

Urea (0.9 ml of 1.4 M) and 0.1 ml of (9 mg/ml) felinine in water were incubated together in duplicate 1.5 ml Eppendorf tubes at either 20 °C (incubated samples) or −20 °C (unincubated samples) for 1–4, 7, 10, 14, 21, 28 and 38 days. Samples (0.1 ml) taken at the different time points from the tube incubated at 20 °C were stored at −20 °C until analysed. After day 38, the unincubated and incubated tubes were processed by adding 950 nmol of norleucine to each tube. HPLC analysis was carried out as above.

The effect of pH on the stability of felinine in the presence of urea

Phosphate buffers (20 mM) with a pH of 3.0, 4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 8.0, 9.0 and 10.0 containing 1.4 M urea were prepared. Each phosphate buffer solution (0.9 ml) and 0.1 ml of (9 mg/ml) felinine in water were added to duplicate 1.5 ml Eppendorf tubes. These tubes were incubated at 70 °C in a water bath for 24 h while identical solutions were stored at −20 °C. After incubation, 950 nmol of norleucine was added to 0.1 ml of both unincubated and incubated samples. HPLC analysis was carried out as above.

The effect of urea on felinine analogues and other selected amino acids

The effect of urea on the stability of selected amino acids including *t*-butyl-cysteine, cysteine, methionine, serine and threonine was investigated. Solutions of these amino acids were made up in water to the same molar concentration as the 9 mg/ml felinine solution and 0.1 ml of each amino acid solution was incubated in a 1.5 ml Eppendorf tube with 0.9 ml of 1.4 M urea at 70 °C for 24 h. An identical set of tubes were incubated at −20 °C. After incubation, 950 nmol of norleucine was added to 0.1 ml of both unincubated and incubated samples. HPLC analysis was carried out as above.

A solution of 14 mg/ml *N*-acetyl felinine was prepared using the method of Hendriks et al. (2004). Two and a half micro litres of *N*-acetyl felinine solution was added to two separate eppendorf tubes containing 39 µl of 1.4 M urea. One tube was incubated at 70 °C for 24 h while the second was kept at −20 °C for 24 h. After incubation, *N*-acetyl felinine concentration in the unincubated and incubated solutions was diluted to 100 µl with water and quantified by reversed-phase HPLC using an adaptation of the method by Hendriks et al. (2004) using a Phenomenex Luna Phenyl-hexyl 10, 5 µ column (4.6 × 150 mm) and a Waters HPLC system (Waters Corporation, Milford, MA). A linear 40 min gradient was run, from 100% solvent A (0.1% trifluoroacetic acid (TFA)) to 65% solvent A, and 35% solvent B (0.1% TFA and 90% acetonitrile), which commenced 10 min after sample injection. *N*-acetyl-felinine was detected by absorbance at 214 nm and peaks integrated using Millennium[32], Chromatography Manager® software (Waters Corporation, Milford, MA).

The acetyl-felinine standard was synthesised as described by Hendriks et al. (2004).

N-acetyl-felinine recovery was calculated as described below.

Mass spectrometry of felinine-urea reaction products

Urea (0.9 ml of 1.4 M) was incubated with 0.1 ml of felinine (9 mg/ml) in a 1.5 ml Eppendorf tube at 70 °C for 24 h. Following this, the sample was subjected to mass spectral analysis using electrospray ionisation in positive ion mode on a Waters Micromass-ZMD single quadrupole mass spectometer.

Data analysis

The recovery of the amino acids felinine, *N*-acetyl-felinine, *t*-butyl-cysteine, cysteine, methionine, serine and threonine was calculated as:

$$\text{Amino acid recovery (\%)} = \frac{\text{[amino acid] (mg/ml) in the incubated samples}}{\text{[amino acid] (mg/ml) in the unincubated samples}}$$

Results

The mean standard error between duplicate determinations calculated for all felinine recoveries where felinine could be detected over all experiments was less than 5.0%. The concentration of felinine in the entire male, castrated male and female cat urines were 4.6, 1.2 and 1.8 mg/ml.

Felinine stability in urines of different mammalian species

The recovery of felinine in entire male cat urine and the spiked urine of other selected mammals after incubation at 70 °C for 24 h is shown in Table 1. There was little loss of synthetic felinine when incubated in water alone (8%) compared to its unincubated counterpart. In contrast, when felinine was present in the urines, the losses of felinine ranged from 86% in the entire male cat urine to 98% in the hamster urine. The exception was the rat urine for which the loss of felinine was only 25%.

The urea, ammonia and creatinine concentrations of the mammalian urines were also determined and are shown in Table 1. The urea concentration in the urines ranged from 195 mM in the rat to 1640 mM in the entire male cat urine, while the ammonia concentrations ranged from

Table 1. The urea, ammonia and creatinine concentration in a selection of mammalian urines and the recovery of felinine in spiked mammalian urine after incubation at 70 °C for 24 h

Urine	Felinine recovery (%)	Concentration (mM)		
		Urea	Ammonia	Creatinine
Synthetic felinine[a]	92			
Entire male cat	14	1640	182	36
Castrated male cat[b]	1	902	31	28
Female cat[b]	9	940	65	30
Hamster[b]	2	ND[c]	ND	ND
Dog[b]	5	705	46	11
Human[b]	11	535	41	13
Rat[b]	75	195	64	10

[a] Synthetic felinine (0.9 mg/ml) in water
[b] These urines were spiked with felinine to give a concentration of 0.9 mg/ml
[c] Not determined

31 mM in the castrated male cat urine to 182 mM in the entire male cat urine. Creatinine concentration was found to be approximately three times higher in feline urine (28–36 mM) compared to dog, human and rat urine (range 10–13 mM).

Stability of felinine in sterilized urine

When felinine was incubated at 70 °C for 24 h in urine sterilised by filtration, no felinine could be recovered.

Stability of felinine in urea-reduced urine

The urea concentration of the entire male cat urine before urease treatment was 1640 mM and was decreased as a result of the urease treatment to 135 mM. The felinine concentration in the unincubated, urea-reduced urine as a result of dilution was 1.6 mg/ml. This decreased to 1.4 mg/ml after incubation at 70 °C for 24 h. Overall, the recovery of felinine after incubation was 88%.

Stability of felinine in the presence of urea, urea analogues and other nucleophiles

The recovery of felinine in the presence of a range of nucleophiles (ammonia, urea, acetamide and water) after incubation at 70 °C for 24 h is shown in Table 2. The recovery of felinine was greater than 90% when incubated in 1.3 M ammonia, 1.3 M acetamide and water in comparison with their unincubated counterparts. In contrast, no felinine was recovered when incubated in 1.3 M urea.

Table 2. The recovery of felinine in the presence of selected urea analogues and a range of nucleophiles after incubation at 70 °C for 24 h

Compounds[a]	Felinine recovery (%)
Nucleophiles	
Water	92
Acetamide	98
Urea	0
Ammonia	91
Urea and urea analogues	
Urea	0
Thiourea	95
Biuret	75

[a] The final concentration of the nucleophiles and urea analogues in the incubation mix was 1.3 and 0.3 M, respective

When incubated in the presence of the urea analogues, felinine recovery, in comparison with the unincubated solutions, was high, 75% for biuret and 95% with thiourea. In contrast, when incubated in the presence of urea, even though the urea concentration was one quarter that used in the previous experiment, no felinine could be recovered.

Stability of felinine in the presence of urea

The recovery of felinine after incubation with varying concentrations of urea is shown in Fig. 1. The recovery of felinine decreased in a curvilinear manner from 100% when felinine was incubated with no urea to 0% when felinine was incubated with 100 mM urea. The urea concentration at which half the felinine was lost was approximately 30 mM.

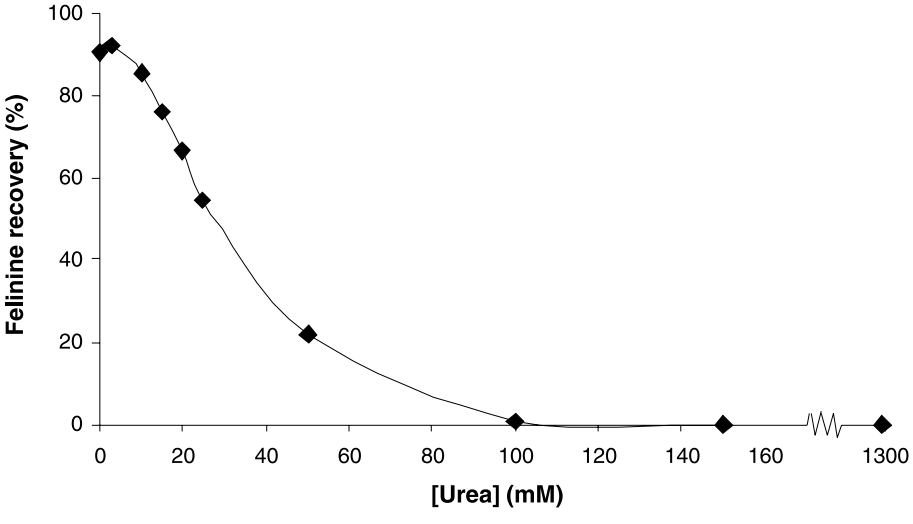

Fig. 1. Recovery of synthetic felinine in the presence of varying concentrations of urea. Felinine was incubated with 0 to 1.3 M varying concentrations of urea at 70 °C for 24 h

Effect of reaction time on the stability of felinine in the presence of urea

The recovery of felinine when incubated at 20 °C with 1.3 M urea over 0–38 days was determined and the results are shown in Fig. 2. The recovery of felinine decreased from 100% in the unincubated sample to approximately 12% after 38 days of incubation at 20 °C. Loss of felinine was most rapid between 1 and 4 days incubation. 50% of the felinine disappeared after approximately 10 days incubation.

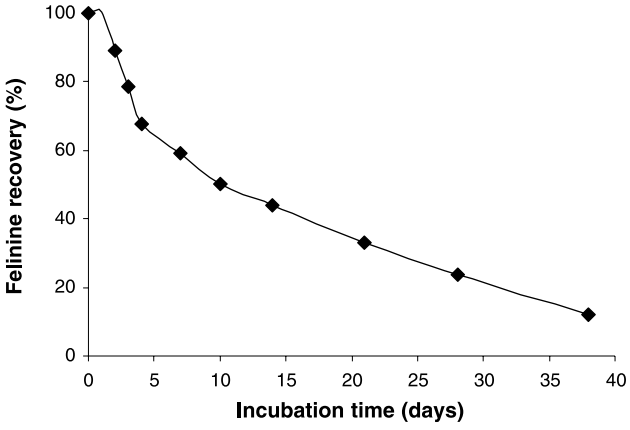

Fig. 2. Recovery of synthetic felinine (%) in the presence of urea over time. Felinine was incubated with 1.3 M urea at 20 °C for 0–38 days

The effect of pH on the stability of felinine in the presence of urea

Felinine was incubated with 1.3 M urea at 70 °C for 24 h over a pH range of 3–10 and the recovery of felinine was determined. For all pH's tested, no felinine was recovered.

The effect of urea on felinine analogues and other selected amino acids

N-acetyl-felinine, *t*-butyl-cysteine, serine, threonine, methionine and cysteine were incubated with 1.3 M urea at 70 °C for 24 h and the recovery of each amino acid was determined. The recovery of *N*-acetyl felinine was 107%. In contrast, the recovery of all the other amino acids was 0%.

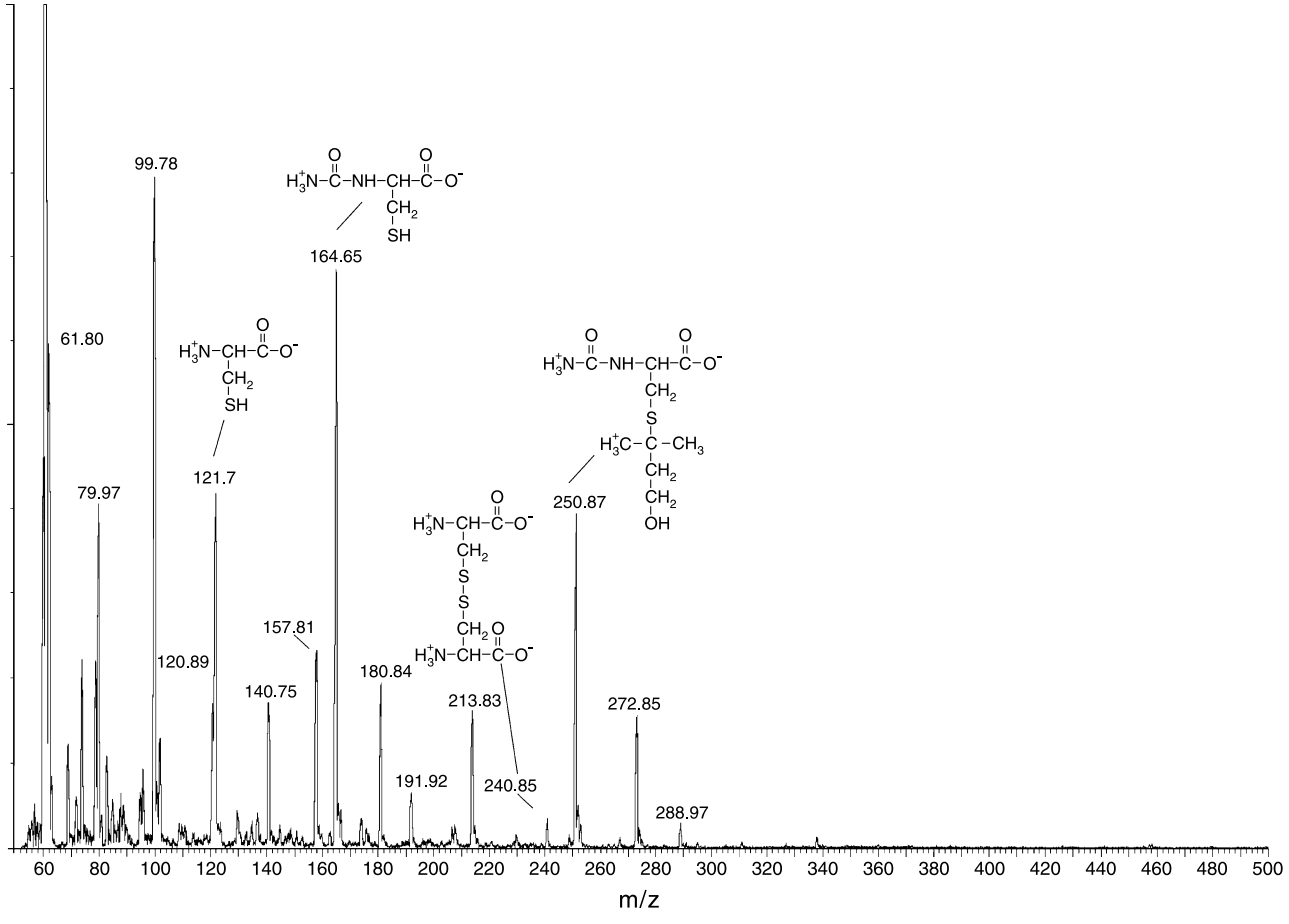

Fig. 3. Mass spectrometry spectrum of the reaction mixture of urea and synthetic felinine

Mass spectrometry of felinine-urea reaction products

In the reaction mixture of synthetic felinine incubated with 1.3 M urea at 70 °C for 24 h, several monoisotopic masses were recorded, with the major peaks being at 60.00, 79.97, 99.78, 121.70, 164.65 and 250.87 (m/z) (Fig. 3). The higher mass of 250.87 Da was consistent with the structure of protonated carbamyl-felinine ($H_3{}^+NCONHCH(CH_2SC(CH_3)_2CH_2CH_2OH)COOH$), while the masses 240.85 and 121.7 Da are consistent with the structure of cystine and cysteine respectively. The mass of 164.65 Da corresponds with the molecular weight of protonated carbamyl-cysteine. The molecular mass of approximately 60 was consistent with unreacted urea present in the reaction mixture. The masses of 99.78 and 79.97 Da could not be assigned to a structure. No mass corresponding to alanine was observed.

Discussion

Felinine, present in the urine of entire male cats, has been reported to be degraded or modified when stored or heated (Rutherfurd et al., 2004; Hendriks et al., 1995a). However, characterisation of this degradation product or the mechanism by which this occurs has to date not been reported in the literature. The initial experiment examined the degradation of felinine in urine from different mammalian species. The result that synthetic felinine was relatively stable when incubated in water at 70 °C for 24 h is consistent with the observation by Rutherfurd et al. (2004) who investigated the stability of felinine using a newly developed capillary (zone) electrophoresis method. In contrast, when synthetic felinine was added to castrated male and female cat urine, and the urine of a male dog, hamster and human and incubated under identical conditions, almost all of the felinine was degraded. Similarly, 86% of the felinine, naturally present in entire male cat urine, when incubated under these conditions was degraded. In rat urine, however, the added felinine was only partially degraded, with 75% of the felinine being recovered. It appeared that the factor(s) involved in the degradation of felinine previously reported are not unique to feline urine and also occur in the urine of other species.

Since urea, ammonia and creatinine are major and ubiquitous components of all mammalian urines, the concentrations of these compounds were also determined in the mammalian urines. As expected, given their carnivorous diet, the urea concentration in cat urines was the highest (Cottam et al., 2002), while the rat urine contained the lowest concentrations of urea. The urea concentration also appeared to reflect the disappearance of felinine after incubation at 70 °C for 24 h, with low felinine recoveries being observed in urine that contained high levels of urea (entire male, castrated male and female cat, dog, human and hamster) and higher in urine with low concentrations of urea (rat). No similar trend was observed for ammonia or creatinine. Based on this finding, it was hypothesised that urea, at least, may be involved in some manner in the degradation of felinine.

Since it appeared that urea may react with felinine in urine, a urea-reduced urine sample was prepared from entire male cat urine in order to investigate the influence of urea on felinine. Even after extensive urease treatment, urea could still be measured in the urine. However, it is possible that the high ammonia levels, a product of the urease action on urea, interfered with the urea assay giving an overestimate of the urea present in the urea-reduced urine. Nevertheless, the urea content had been reduced by a minimum of 90% of that in the original urine sample. In this urine the recovery of felinine after incubation at 70 °C for 24 h, compared to its unincubated counterpart, was high (88%). This was consistent with the relatively high felinine stability observed in the rat urine which also had a low urea content. The urea-reduced urine was not sterilized and as such most likely possessed the full complement of microorganisms normally found in cat urine. This suggests that microorganisms may not play a role in felinine degradation since the felinine recovery was high in this urea-reduced urine. This finding was confirmed in the sterilized urine, where no felinine could be recovered after incubation.

Felinine stability appeared to be influenced by the presence of urea and we conjectured that urea may possibly react with felinine nucleophically. Consequently, the stability of felinine in the presence of urea and other compounds of various nucleophilicity was examined. Ammonia, urea, acetamide and water, listed in decreasing nucleophilicity/basicity, were each incubated with synthetic felinine at 70 °C for 24 h. Over 90% of the synthetic felinine present was recovered after incubation with water, acetamide and ammonia but in the presence of urea no felinine was recovered. These results conclusively show that urea is involved in the degradation or modification of felinine. Furthermore, it would appear that this interaction is independent of nucleophilic potential since ammonia, which is more nucleophilic than urea, and acetamide and water, which are less nucleophilic than urea, did not appear to react with felinine.

When felinine was incubated with the urea analogues thiourea and biuret, it appeared to be relatively stable,

although some losses of felinine were observed when biuret (25%) was present. This was again in contrast to urea where a complete loss of felinine after incubation at 70 °C for 24 h was observed. This would suggest that the interaction between urea and felinine is highly specific, with felinine appearing to be relatively unreactive with compounds of similar nucleophilicity and structure to urea.

While urea appeared to be involved in the degradation of felinine, it was not known whether this involvement was in the form of a reactant or a catalyst. Consequently, a study was conducted where different amounts of urea were incubated with felinine and the recovery of felinine determined. The minimum urea concentration for which all the felinine disappeared was 100 mM. At this urea concentration, the molar ratio of urea to felinine was 20:1. The molar ratio of urea to felinine in the solution where half the felinine disappeared was approximately 7:1. This preliminary study would suggest that urea plays the role of a reactant rather than a catalyst.

The next experiment in this series of studies investigating the degradation of felinine involved an examination of the effect of pH on felinine degradation in the presence of urea. The pH of feline urine is highly variable and depends on the diet consumed, time of measurement, gender, etc. Urine pH of feral cats has been reported to vary from 5.54 to 7.39 with female feral cats having on average a significantly lower urine pH compared to male cats. Westall (1953) and Hendriks et al. (1995b) reported that synthetic felinine degraded in the presence of acids (2–6 M HCl). In this study felinine appeared to react with urea irrespective of pH, certainly at elevated temperatures.

The reactivity of urea was tested with different felinine analogues and amino acids of similar structure in order to determine whether the reaction with urea is specific for felinine. The compounds tested included *N*-acetyl-felinine, *t*-butyl-cysteine, serine, threonine, methionine and cysteine. Urea reacted with all amino acids tested with the exception of *N*-acetyl-felinine, suggesting that urea reacts with the amino group present on the amino acids. Urea is known to form an equilibrium with isocyanic acid in solution. Cyanates can react with amino (Lapko et al., 2003; Van Driessche et al., 2002) and sulphydryl (Lippincott and Apostol, 1999) moieties. Mass spectrometry analysis of the products of the reaction between urea and felinine was conducted to determine if carbamyl-felinine was a product in these studies. A compound possessing the mass 250.87 was identified in the reaction mixture which is consistent with the presence of carbamyl-felinine. Furthermore, compounds with molecular masses consistent with the presence of cystine, cysteine and carbamyl-cysteine were also present in the urea-felinine reaction mixture. There were also 2 major unidentified peaks (79.97 m/z and 99.78 m/z) in the mass spectra. It would appear that felinine does react with urea via carbamylation with isocyanic acid. It also appears that felinine may be able to be degraded to produce carbamyl-cysteine and cysteine, although carbamylation may not necessarily be a prerequisite for degradation to cysteine. Interestingly, there was no mass corresponding to alanine or carbamylated alanine present in the reaction mixture. This indicates that carbamylation is unlikely to facilitate the release of the sulphur containing molecule from felinine that would generate a volatile organic compound. Hendriks et al. (1995b) hypothesised that 3-mercapto-3-methyl-1-butanol (MMB) would be one of the compounds potentially contributing to the typical tom cat smell of feline urine as MMB has been reported to have a typical "catty smell" in concentrations of 10–1000 ppb (Joulain and Laurent, 1989). Others (McGugan and Emmons, 1967; Badings, 1967; Aylward et al., 1967) have reported the presence of the smell of cat urine in foodstuffs, such as cheese and black currants and identified two (structurally similar), sulphur containing compounds (4-mercapto-4-methyl)-pentan-2-one and 4-methoxy-2-methylbutane-2-thiol) to MMB. The presence of MMB and a number of di- and trisulphide derivatives in bobcat urine (a felinine excreting species) has been hypothesised to occur through the action of *C-S*-lyase enzyme on felinine (Mattina et al., 1991). 3-Mercapto-3-methyl-butanol and its di- and trisulphide derivatives have also been identified in the urine of the domestic cat (Joulain and Laurent, 1989).

A study investigating the degradation of synthetic felinine in the presence of urea over time was conducted in order to ascertain the relative importance of carbamylation in felinine degradation. The incubation was carried out at 20 °C, which is a more relevant temperature compared to 70 °C to observe whether the timeframe for carbamylation was consistent with the potential for felinine to act as a precursor to urinary volatiles. Half of the felinine disappeared after 10 days incubation while after 38 days almost one quarter of the original felinine remained present. Whether the carbamylation of felinine is involved in the generation of sulphur containing volatiles in feline urine remains to be determined. However, the rate of felinine carbamylation is such that carbamylation of felinine would be a significant competing reaction to the mechanism by which sulphur containing volatiles are generated from felinine.

The present study shows that felinine reacts with urea in urine to produce carbamylated felinine via carbamylation with isocyanic acid, formed in equilibrium with urea. Carbamylation appears to be a quantitatively significant pathway in felinine degradation and if felinine is involved in pheromone production, carbamylated felinine might be an intermediate in the pathway to release the sulphur-containing felinine side chain. Yet, based on the molecular structure, carbamylated felinine would not be predicted to generate a sulphur-containing volatile as no alanine or carbamylated alanine was observed in any of the experiments conducted. It is possible that micro-organisms play a role in degradation of felinine or carbamylated felinine but that is also contrary to the findings of this study. If carbamylation is not a mechanism by which urinary volatile are generated from felinine, then it is competing directly for felinine molecules, which from a physiological point of view would appear to be evolutionary contradictionary. Whether the carbamylation of felinine is in some way involved in the production of pheromones is uncertain, but it is clear that this area of research requires further investigation.

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Authors' address: S. M. Rutherfurd, Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand, Fax: +64 6 350 5657, E-mail: S.M.Rutherfurd@massey.ac.nz