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Synthesis of Felinine, 2-Amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoic Acid

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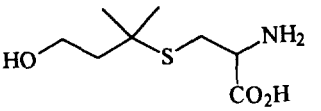
Several published synthesis procedures reported to lead to (±)-felinine, 2-amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoic acid, a sulfur-containing urinary amino acid of cats (*Felis domesticus*), were evaluated for their yield. Most of the procedures were found to produce an amino acid isomeric with felinine and the structural assignment of this isomer has been determined as 2-amino-7-hydroxy-7-methyl-4-thiaoctanoic acid. The yield for the only evaluated synthesis procedure shown to produce felinine was found to be low. A new high-yielding method for the synthesis of (±)-felinine is presented.

INTRODUCTION

During a recent study at our institute on the amino acid metabolism of adult domestic cats it became necessary to quantify the amount of an important sulfur-containing amino acid, felinine, known to be present in large amounts in cat urine (*1*, *2*). This amino acid was first isolated from cat urine by Westall (*2*) in 1953 and tentatively assigned structure **1**, 2-amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoic acid, on the basis of a Raney nickel-induced desulfurization of the felinine and subsequent isolation of alanine and isopentenol (Structure **1**).

Confirmation of the structural assignment, by synthesis, was achieved in 1957 by Trippett (*3*) and subsequent attempts by various research groups (*4–6*) to substantiate this assignment have all purported to lead to this compound. In these procedures characterization was accomplished, for the most part, using two-dimensional paper chromatography in addition to melting-point determinations on products obtained by solvent-induced precipitation from water. No spectroscopic



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STRUCTURE 1

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or high-resolution chromatographic validation of any of these products has hitherto been reported.

Recently, bobcat (*Lynx Rufus*) urine has been shown to have a repellent effect on the behavior of several herbivorous animals (*7–9*). This effect is believed to be caused by several volatile sulfur-containing compounds which could originate from the degradation of felinine (*10*), although direct evidence is lacking. Since felinine has been found in bobcat urine and the levels of this amino acid have been reported to be higher in the urine of entire male than entire female cats (*11*), felinine may have a role as a pheromone or precursor to a pheromone in Felidae.

To obtain a standard for high-performance liquid chromatography (HPLC), the synthesis procedures of Trippett (*3*), Schöberl *et al.* (*5*), and Schöberl *et al.* (*6*) and the two procedures published by Eggerer (*4*) which used isopentenol were followed. This contribution reports the results of the evaluation of the latter-mentioned synthesis procedures and gives the structural reassignment of the product obtained in three of these procedures. Nuclear magnetic resonance (NMR), mass spectrometry (MS), and HPLC data for natural and synthetic felinine are also presented to corroborate these findings and a new procedure for the synthesis of felinine is reported.

EXPERIMENTAL

Chemicals

L-Cysteine, L-cysteine hydrochloride, L-cysteine methyl ester hydrochloride, (±)-β-chloroalanine, 3-methylbut-2-en-1-ol, and dimethylformamide were purchased either from Aldrich Chemical Co. (St. Louis, MO) or Sigma Chemical Co. (St. Louis, MO). The 3-methylbutan-1,3-diol was synthesized by performing a Reformatsky reaction between ethylbromoacetate and acetone and subjecting the resulting β-hydroxy ester to reduction by lithium aluminum hydride. The diol was purified by distillation under vacuum. The 3-methyl-3-thiobenzylbutan-1-ol and 3-mercapto-3-methylbutan-1-ol were prepared by Michael addition of benzylmercaptan and hydrogen sulfide, respectively, to methyl-3,3-dimethylacrylate followed by reductions by lithium aluminum hydride of each of the adducts. The structures of these synthesized compounds were authenticated by NMR spectroscopy.

All other reagents were of AR grade and solvents were distilled prior to use.

Apparatus and Methods

^1H and ^{13}C NMR spectra were recorded as solutions in either D_2O or deuterochloroform ($CDCl_3$) on a Bruker AC300 instrument operating at 20°C and all chemical shift data obtained in D_2O solutions are cited with respect to the HOD signal corrected to δ 4.90.

Fast atom bombardment–mass spectrometry (FAB–MS) was performed on a VG70-250S double-focusing magnetic sector mass spectrometer (VG Analytical, Manchester) equipped with standard LSIMS ion source and associated ion gun.

Chemical ionization (CI) mass spectrometry data were obtained using a dedicated instrument identical to the FAB–MS instrument.

Amino acid analyses were performed on a Waters (Millipore, Milford, MA) ion-exchange HPLC system employing postcolumn derivatization with *O*-phthalaldehyde and detection by fluorescence spectrometry. A Waters ion-exchange amino acid analysis column was used with HPLC conditions as described by the manufacturer. Chromatograms were integrated using dedicated software (Waters Maxima 820) and the retention times of the synthesized amino acids were compared with those of a standard amino acid mixture.

All published synthesis procedures were carried out precisely as described in the original publications.

Isolation of "Natural Felinine"

Urine, from male cats housed in metabolic cages, was collected, pooled, and immediately frozen prior to processing. The urine (200 ml) was filtered, deproteinized, treated with activated charcoal (2), and acidified to pH 1.5 with hydrochloric acid. The liquor was applied to a cation exchange column (Dowex-W50 in the acid form) and eluted with a sodium citrate buffer (67 mM, pH 2.1). Fractions were collected and analyzed by HPLC for the presence of the peak believed to be felinine. Those fractions containing what was believed to be relatively pure felinine were pooled and concentrated to dryness under reduced pressure. The resulting solid (0.5 g) was redissolved in a small volume of water and desalted by adsorption on a Dowex-W50 (H^+ form) column and eluted with an aqueous ammonia solution (0.2 M). The eluate was reconcentrated and the desalting procedure repeated. In this manner, an homogeneous white lyophilisate (20 mg) was obtained. FAB–MS: (−) *m/z* 205, 206; (+) *m/z* 207, 208 ($C_8H_{17}NO_3S$ requires *m/z* 207 amu). ^1H NMR (D_2O): δ 1.46 (s, 3H), 1.49 (s, 3H), 2.01 (2× overlapping t, J = ca. 7.4 Hz, 2H), 3.10 (m, 2H (H_β)), 3.92 (2× overlapping t, J = ca. 7.4 Hz, 2H), 4.32 (t, J = 5.4 Hz, 1 H (H_α)). ^{13}C NMR: 164.1 (C_Q), 61.56/61.48 (CH_2), 58.9 (CH), 47.08/46.77 (C_Q), 45.9 (CH_2), 34.0 (CH_2), 31.06/31.02 (CH_3).

Synthesis of "Schöberl Felinine," 2-Amino-7-hydroxy-7-methyl-4-thiaoctanoic Acid ***(7a)***

The use of either 3-methylbutan-1,3-diol or 3-methylbut-2-en-1-ol (isopentenol) in the procedures of Schöberl *et al.* (*5, 6*) gave rise to the same crude product that was shown by NMR spectroscopy and HPLC analysis to mainly comprise a single amino acid. However, attempts to purify the product either by crystallization or ion-exchange chromatography were unsuccessful. Spectral data recorded on the lyophilized crude product were clearly resolved so as to permit a structure assignment. FAB–MS: (−) *m/z* 206; (+) *m/z* 208 ($C_8H_{17}NO_3S$ requires *m/z* 207 amu). ^1H NMR (D_2O): δ 1.34 (s, 6H), 1.90 (m, 2H), 2.74 (t, J = 8.2 Hz, 2H), 3.20 (qd, J_{AB} = 14.6 Hz, $J_{A\alpha}$ = 7.3 Hz, $J_{B\alpha}$ = 4.4 Hz, 2H (H_β)), 4.01 (dd, $J_{A\alpha}$ = 7.3 Hz, $J_{B\alpha}$ = 4.4 Hz, 1H (H_α)). ^{13}C NMR: 175.4 (C_Q), 74.0 (C_Q), 55.0 (CH), 45.2 (CH_2), 34.0 (CH_2), 30.5 (CH_3), 30.5 (CH_3), 29.6 (CH_2).

The Eggerer (4) synthesis procedures followed were essentially the same reactions as that of Schöberl *et al.* (*6*) except that the reaction of L-cysteine hydrochloride with 3-methylbut-2-en-1-ol (isopentenol) was carried out in the presence of pyridine.

Synthesis of Methyl 2-Amino-7-hydroxy-7-methyl-4-thiaoctanoate ***(7b)***

The use of L-cysteine methyl ester in place of the free amino acid in each of the procedures described by Schöberl *et al.* (*5, 6*) gave rise to identical (NMR) crude products. These were isolated by extraction of the aqueous acid phase with dichloromethane (2 × 20 ml), followed by neutralization with saturated aqueous sodium bicarbonate solution and extraction again with dichloromethane (2 × 20 ml). The organic phase was dried and concentrated to a mobile liquid and subjected to flash chromatography over silica (50% ethylacetate/hexane) to give a colorless mobile liquid (ca. 40% yield, based upon cysteine methyl ester). CI(+): MH^+ *m/z* 222.1160 (Calcd for $C_9H_{20}NO_3S$ *m/z* 222.1164); $[MH\text{-}H_2O]^+$ *m/z* 204.1058 (Calcd for $C_9H_{18}NO_2S$ *m/z* 204.1058). ^1H NMR ($CDCl_3$): δ 1.62 (s, 6H), 2.02 (m, 2H), 2.73 (m, 2H), 2.88 (qd, J_{AB} = 13.0 Hz, $J_{A\alpha}$ = 7.1 Hz, $J_{B\alpha}$ = 4.9 Hz, 2H (H_β)), 3.68 (dd, $J_{A\alpha}$ = 7.1 Hz, $J_{B\alpha}$ = 4.9 Hz, 1H (H_α)), 3.77 (s, 3H). ^{13}C NMR: 174.4 (C_Q), 69.7 (C_Q), 54.2 (CH), 52.1 (CH_3), 45.9 (CH_2), 37.3 (CH_2), 32.4 (CH_3), 32.3 (CH_3), 28.0 (CH_2).

Synthesis of "Trippett Felinine,"
2-Amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoic Acid ***(1)***

Using the method of Trippett (*3*) of reductive S-debenzylation of 3-methyl-3-thiobenzylbutan-1-ol in the presence of (±)-β-chloroalanine, a crude product comprising two species was isolated. NMR spectra revealed the presence of the mono (or di-)-sodium salt of 3-mercapto-3-methylbutan-1-ol as the major (≥80%) component and a compound displaying ^1H and ^{13}C signals characteristic of felinine **1**. The crude material was dissolved in a small volume of water, acidified to pH 4, and extracted with dichloromethane (4 × 15 ml) to remove the free mercaptoalcohol and the aqueous phase filtered through celite and freeze–dried, to give an off-white lyophilisate. This product was not subjected to further purification. HPLC analysis confirmed the presence of a compound coeluting with natural felinine. FAB–MS: (−) *m/z* 205; (+) *m/z* 207 ($C_8H_{17}NO_3S$ requires *m/z* 207 amu). ^1H NMR (D_2O): δ 1.56 (s, 6H), 2.06 (t, *J* = 7.4 Hz, 2H), 3.30 (qd, J_{AB} = 16.3 Hz, $J_{A\alpha}$ = 7.3 Hz, $J_{B\alpha}$ = 4.4 Hz, 2H (H_β)), 3.95 (t, *J* = 7.4 Hz, 2H), 4.16 (dd, $J_{A\alpha}$ = 7.3 Hz, $J_{B\alpha}$ = 4.4 Hz, 1H (H_α)). ^{13}C NMR: 175.3 (C_Q), 61.3 (CH_2), 56.9 (CH), 47.7 (C_Q), 45.8 (CH_2), 30.9 (CH_2), 30.9 (CH_3), 30.8 (CH_3).

Synthesis of (±)-Felinine, 2-Amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoic Acid ***(1)****, an Adapted Procedure*

To a stirred solution of 3-mercapto-3-methylbutan-1-ol (2.0 g, 16.7 mmol) and (±)-β-chloroalanine (1.9 g, 15.2 mmol) in dry dimethylformamide (40 ml), sodium hydride (0.67 g, 60% dispersion, 16.7 mmol) was added in a single portion. The

solution was stirred at ambient temperature for 0.5 h, after which time a second equivalent was added. A third equivalent of sodium hydride was added after an additional 1 h and the solution stirred for 4 h before being quenched with dilute hydrochloric acid (pH 2, 30 ml) and extracted with ether (3× 20 ml). The aqueous acid phase was filtered through celite and concentrated under reduced pressure to give a light yellow semisolid (2.5 g) that could be freed of dimethylformamide by pumping at 0.2 mm Hg. The homogeneity of the product was subsequently confirmed by HPLC analysis which showed a single amino acid coeluting with natural felinine. A sample of this product (0.2 g) was dissolved in dilute hydrochloric acid and applied to a Dowex-W50 column in the H^+ form and washed to neutrality with 3 vol water before being eluted with dilute ammonia solution (ca. 0.2 M) and prepared as a white lyophilisate (0.1 g). A sample of this material was crystallized (with ≤30% recoveries) by dissolving in a tetrahydrofuran/methanol mixture to give (±)-felinine as white rosettes, mp 185–187°C (dec). Elemental analysis: C, 44.27; H, 8.32; N, 6.35 ($C_8H_{17}NO_3S.0.5H_2O$ requires C, 44.44; H, 8.33; N, 6.54). FAB–MS: (−) *m/z* 206, (+) *m/z* 208 ($C_8H_{17}NO_3S$ requires *m/z* 207 amu). ^1H NMR (D_2O): δ 1.56 (s, 6H), 2.06 (t, J = 7.4 Hz, 2H), 3.30 (qd, J_{AB} = 16.3 Hz, $J_{A\alpha}$ = 7.3 Hz, $J_{B\alpha}$ = 4.4 Hz, 2H (H_β)), 3.95 (t, J = 7.4 Hz, 2H), 4.16 (dd, $J_{A\alpha}$ = 7.3 Hz, $J_{B\alpha}$ = 4.4 Hz, 1H (H_α)). ^{13}C NMR: 175.3 (C_Q), 61.3 (CH_2), 56.9 (CH), 47.7 (C_Q), 45.8 (CH_2), 30.9 (CH_2), 30.9 (CH_3), 30.8 (CH_3). No change in the chemical shifts of these signals was observed after treating the sample with dilute ammonia (to pH 10.5) and lyophilizing the resulting solution.

RESULTS AND DISCUSSION

Felinine isolated from tom cat urine has been reported to coelute with citrulline between glutamic acid and glycine on a cation exchange column (*12, 13*). In the present study when adult tom cat urine was analyzed by HPLC, a large peak was observed (Fig. 1, peak 6) to elute between glutamic acid and glycine with clear resolution on both sides. Variation of the chromatographic conditions and a citrulline standard showed that citrulline was not present in the cat urine. The species believed to be felinine was subsequently isolated by semipreparative HPLC, but remained contaminated with small amounts of impurities (Fig. 1). Nonetheless, the 1D and 2D NMR (^1H–^1H and ^1H–^{13}C COSY) spectral data for this species, as isolated, were consistent with expectations of the structure of felinine (*2*). The two contiguous methylene groups appear as multiplets at δ 2.01 and δ 3.92 and are disconnected to the methine and methylene groups of the cysteinyl moiety which appear as multiplets at δ 4.32 and δ 3.10 (Fig. 2). The multiplicities of these resonances are not, however, those expected of a first-order analysis, a phenomenon that we believe is attributable to diastereotopic effects due to the chiral α-carbon of the amino acid unit. FAB–MS data clearly confirmed that the isolated compound had a molecular weight which corresponds to that for structure **1**. It was concluded, therefore, that the compound isolated from tom cat urine was the same compound studied by Westall (*2*) and named felinine.

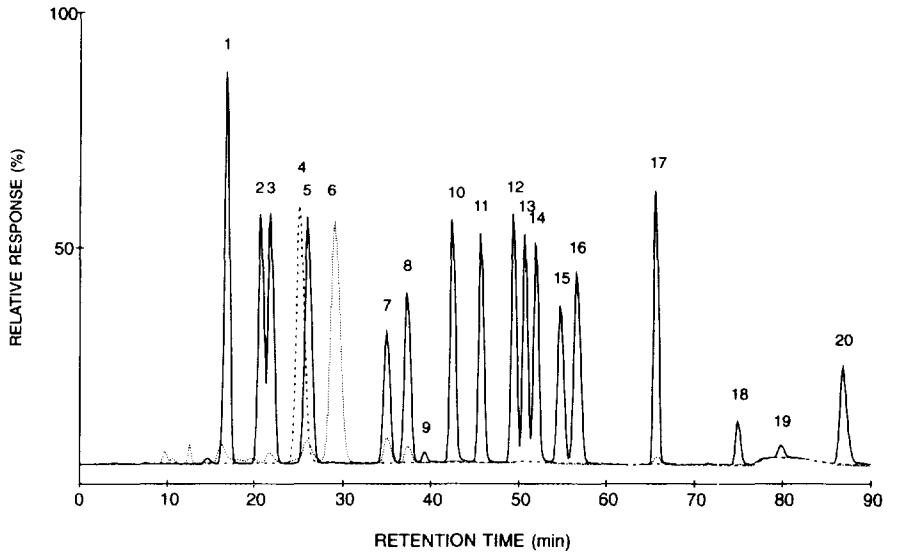
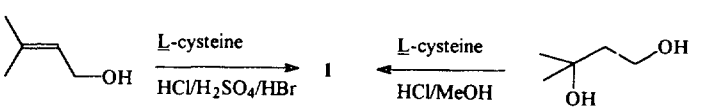


FIG. 1. Superimposed HPLC chromatograms of a standard amino acid mixture (—), the product mixture from the Schröberl *et al.* (*5*) procedure (---), and felinine isolated from tom cat urine (-----). Peak 1, Asp; 2, Thr; 3, Ser; 4, 2-amino-7-hydroxy-7-methyl-4-thiaoctanoic acid; 5, Glu; 6, Felinine; 7, Gly; 8, Ala; 9, Cys; 10, Val; 11, Met; 12, Iso; 13, Leu; 14, Norleucine; 15, Tyr; 16, Phe; 17, His; 18, Lys; 19, NH_3; 20, Arg.

With the purpose of preparing a synthetic standard for quantitation experiments, we elected to utilize the simple procedure described by Schöberl *et al.* (*5*) for the synthesis of felinine. In contrast to the results reported by these authors, the procedure in which cysteine is added as an electrophile to 3-methylbut-2-en-1-ol **2** (isopentenol) (Scheme **1**), gave a multicomponent mixture that contained no amino acid that coeluted (HPLC) with natural felinine. Employing the second method described by Schöberl *et al.* (*6*) that utilizes 3-methylbutan-1,3-diol **3** instead of isopentenol **2** (Scheme **1**), a similar outcome was observed. HPLC analysis again showed no compound coeluting with natural felinine.

The crude products from each of these reactions, when prepared as lyophilisates and subjected to HPLC analysis, did reveal the presence of a single amino acid species (≥85% product) but this did not coelute with natural felinine. This amino acid was found to be clearly resolved and to be more mobile than natural



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SCHEME 1

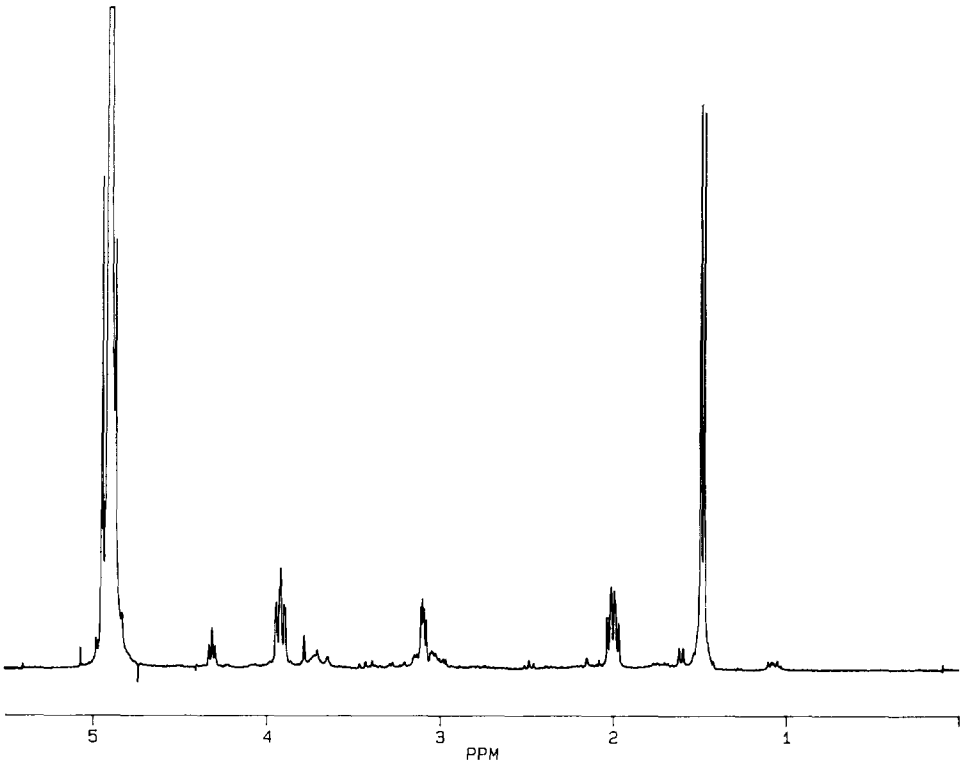
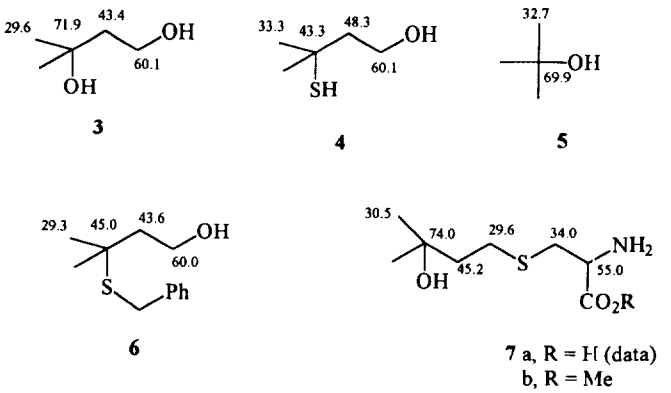


FIG. 2. ^1H NMR spectrum of felinine isolated from tom cat urine.

felinine (Fig. 1, peak 4). ^1H and ^{13}C NMR data recorded for this compound were consistent with a species *isomeric* (FAB–MS (−) *m/z* 206, (+) *m/z* 208) with the expected structure in that the correct number of carbon types was observed in addition to the anticipated ^{13}C–^1H and ^1H–^1H connectivities. Chemical shift data, however, were not consistent with that expected for felinine. Particularly obvious was the absence of a signal attributable to a methylene bearing a hydroxyl function. In addition, the appearance of a single quaternary carbon resonance at 74.0 ppm is consistent with oxygen substitution rather than sulfur substitution at this center (*14*). Based upon chemical shift data recorded here for the model compounds **3–6**, the structure we have assigned to this isomeric species is 2-amino-7-hydroxy-7-methyl-4-thiaoctanoic acid **7a** (Structures **3–7b**).

Chemical shift data and the corresponding multiplicities were discernable and showed the signals associated with the cysteinyl moiety with H_α at δ 4.01 (dd $J_{A\alpha}$ = 7.3 and $J_{B\alpha}$ = 4.4 Hz) and H_β at δ 3.20 (qd J_{AB} = 14.6 Hz, $J_{A\alpha}$ = 7.3, and $J_{B\alpha}$ = 4.4 Hz) and those of the contiguous methylene units of the "isopentenol" moiety as triplets (J = 8.2 Hz) at δ 2.74 and δ 1.90. Moreover, in accordance with expectation, discrete ^1H–^1H and ^1H–^{13}C correlations were observed for each "unconnected" moiety. Mechanistically, compound **7a** appears to result from a



STRUCTURES 3–7

nucleophilic displacement of the protonated primary hydroxyl function. Capture of any incipient tertiary electrophilic center at C3 (derived from **2** or **3**), which would give rise to felinine appears to be the less-favored pathway.

As the purification of **7a** was consistently unsuccessful we undertook the same reaction but with L-cysteine methyl ester instead of L-cysteine and isolated, by conventional flash chromatography, a mobile homogeneous liquid in ca. 40% yield. This compound **7b** was found, from examination of the ^{13}C and ^{1}H NMR (Fig. 3) spectra, to be the methyl ester of **7a**. Connectivity plots and accurate mass measurements were consistent with expectations. In addition, when both the Schöberl *et al.* (*5, 6*) procedures were evaluated by two-dimensional paper chromatography according to the procedure of Datta *et al.* (*15*) using phenol and collidine/lutidine as solvents, the isomer **7a** was found to have the same R_f values as natural and "Trippett's" felinine. The color of this amino acid after reaction with ninhydrin, however, was a distinct purple compared to that of natural and Trippett's felinine which gave a yellow-purple colour. Since the procedure from (*5*), and therefore also the procedure from (*6*) which was compared with that from (*5*), relied mainly on paper chromatography for the identification of felinine (*16*), the identical R_f values might be the reason why these procedures were believed to yield felinine.

Reaction of cysteine at the primary center in **3** (to give **7a**) is not without a precedent. A by-product having the stoichiometry of a 2 : 1 adduct **8** has already been isolated by Schöberl *et al.* (*5*) from the acid-mediated condensation of cysteine with the diol **3** (Structure **8**). Moreover, this compound, for which no analytical or spectroscopic data were presented, is purported to be formed by prolonging the same reaction at 80°C from 1 h to 4 days and also by reacting the (his) putative felinine with 6 M HCl at 80°C for 4 h.

Under the basic conditions (stoichiometric pyridine) described in two of Eggerer's (*4*) three routes, which utilize isopentenol and which might perhaps be expected to disfavor reaction at the primary hydroxyl center, we observed the formation of an insoluble precipitate (precisely as described, and believed to be

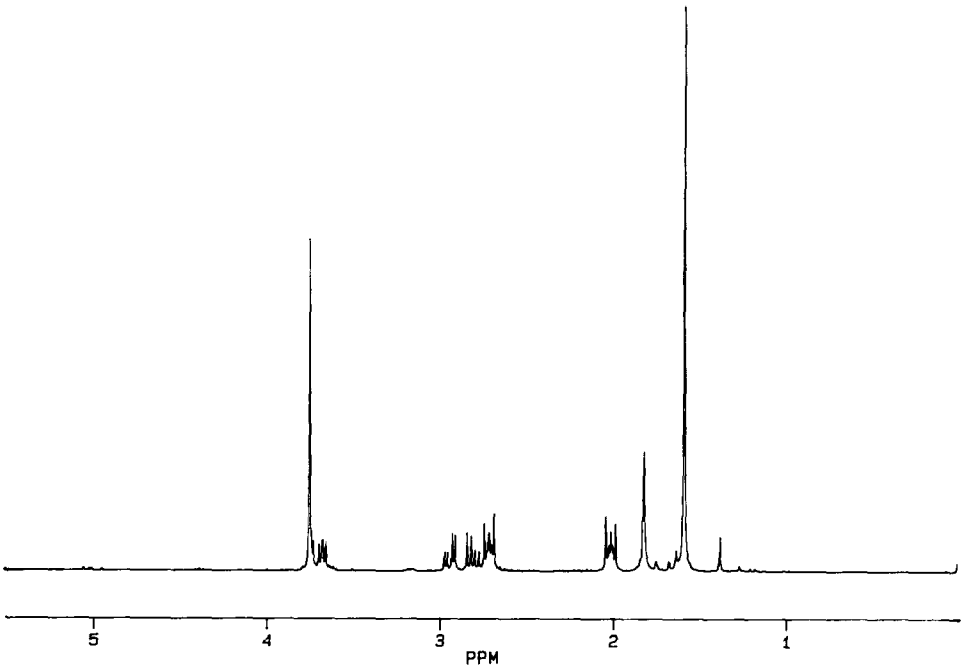
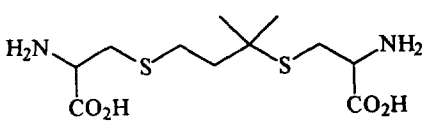


FIG. 3. ^{1}H NMR spectrum of the compound obtained from the use of L-cysteine methyl ester instead of L-cysteine in the Schöberl *et al.* (*5*) procedure.

cystine) with only small recoveries of product mixtures, which contained no felinine as analyzed by HPLC, but only a small amount of the isomer **7a**. The main procedure published by Eggerer (*4*) that utilizes a phosphate-protected derivative of isopentenol in a reaction with L-cysteine hydrochloride has not been evaluated in the present work. We have no reason to doubt that the route is other than as described.

The Trippett's (*3*) synthesis of felinine, involving a reductive S-debenzylation of 3-methyl-3-thiobenzylbutan-1-ol **6**, followed by nucleophilic addition to (±)-β-chloroalanine **9** (Scheme **2**), was successful. In addition to the supposed mono- or disodium salt of 3-mercapto-3-methylbutan-1-ol **10**, a small amount (≤10%) of a product component shown to coelute (HPLC) with natural felinine was observed.

When reacted with 1 M sulfuric acid and repeatedly (3×) extracted with ether,



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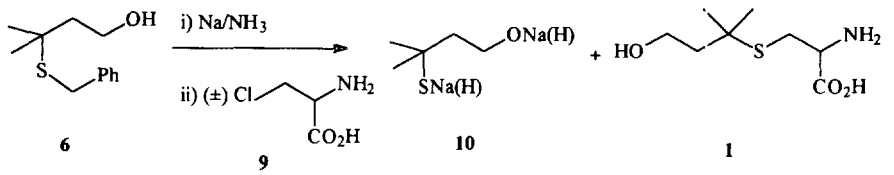
STRUCTURE 8



SCHEME 2

the original lyophilized product could easily be freed from the mercaptobutanol "salt" **10** to give a product (judged to be ≥80% pure) showing resonances and ^{13}C–^{1}H and ^{1}H–^{1}H connectivities characteristic of those expected of felinine. In particular, the ^{1}H–^{1}H COSY plot revealed again two discrete sets of vicinal connectivities with the chemical shifts of the resonances consistent with those already described. ^{1}H and ^{13}C signals characteristic of the hydroxyl-bearing methylene carbon were observed at δ 3.95 (t, J = 7.3 Hz) and 61.3 ppm, respectively. The ^{13}C signal due to the sulfur-substituted quaternary carbon was observed at 47.7 ppm. Chemical shifts and multiplicities of the contiguous cysteinyl methylene (δ 3.30 qd, J_{AB} = 16.3, $J_{A\alpha}$ = 7.3, and $J_{B\alpha}$ = 4.4 Hz) and methine (δ 4.16, dd, J_{AB} = 16.3, $J_{A\alpha}$ = 7.3, and $J_{B\alpha}$ = 4.4 Hz) protons were clearly resolved. The stoichiometry of this compound was confirmed by FAB–MS which revealed an identical spectrum to that recorded for the natural felinine with an obvious and prominent molecular ion at m/z 207 (40%).

By modifying this reductive S-debenzylation route (*4*) we have achieved a quantitative synthesis of felinine. Thus, treatment of an equimolar mixture of 3-mercapto-3-methylbutan-1-ol **4** and (±)-β-chloroalanine **9** in anhydrous dimethylformamide with stepwise additions of three molar equivalents of sodium hydride gives felinine **1** in yields of ca. 65%. Purification could be achieved, although with considerable loss, using ion-exchange chromatography to give (±)-felinine as a white lyophilisate. A sample of this product was crystallized by judicious dissolution in a tetrahydrofuran/methanol mixture to give the amino acid as white rosettes, mp 185–187°C. This purified synthetic compound coeluted with natural felinine as analyzed by HPLC and the FAB–MS(+) spectrum showed an obvious molecular ion at m/z 208 (MH^+). The ^{1}H NMR spectrum of the lyophilized product is shown in Fig. 4.

When the ^{1}H NMR spectra are compared to that recorded for natural felinine (Fig. 2) several contrasting features warrant explanation. First, the appearance of two discrete quaternary methyl singlets and multiplicities due to the three methylene and methine units in the ^{1}H NMR spectrum of natural felinine which are dissimilar to those observed for the racemic synthetic product (Fig. 4). This is unusual, but not unexpected. The effect of chirality at the α-carbon of the amino acid is evidently transmitted through the entire molecule to the point where different components of the molecule experience different chiral environments (diastereotopism), at least in aqueous media. It is perhaps unusual that this phenomenon is not observed in the spectrum recorded for the felinine isomer **7a**, since this was

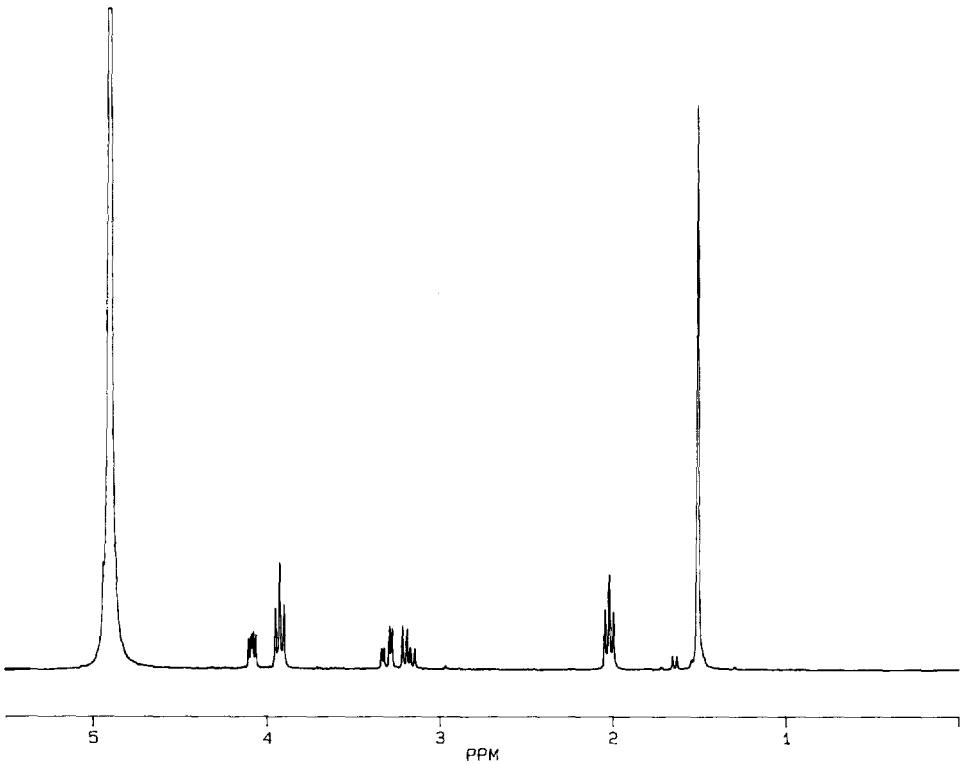


FIG. 4. ^{1}H NMR spectrum of the compound obtained from the new synthesis procedure of felinine.

synthesized from L-cysteine, but isolated as a neutral species. However, two discrete methyl carbon resonances were clearly discernable. Second, in all the ^{1}H NMR spectra recorded on various preparations of felinine (which have been shown to coelute by HPLC with the natural amino acid) we observed minor variations in chemical shifts, most notably with the α-cysteinyl proton. These effects are no doubt concentration dependent and they are seen to be pH dependent as samples of each of the synthetic materials, prepared either from **4** or **6** and lyophilized from acid or alkaline pH give rise to α-CH resonances with a range of ca. δ 0.5. The difference is seen to be more significant (δ 0.4) simply as a result of the use of sulfuric acid in place of hydrochloric acid in the quenching process.

It is notable that during the studies into the synthesis of (±)-felinine, synthetic and natural felinine when stored as a lyophilisate at −20°C or room temperature were observed to slowly decompose, with the development of a characteristic "catty" odor. The process by which this appears to take place and its relevance to the production of (urinary) volatile sulfur-containing compounds is currently being investigated.

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