The HPLC assay procedure is rapid, precise, and specific for the analysis of cyclobenzaprine hydrochloride in tablets and represents a more convenient assay method than that of the USP.

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Derivatization of Chiral Amines with (*S*,*S*)-*N*-Trifluoroacetylproline Anhydride for GC Estimation of Enantiomeric Composition

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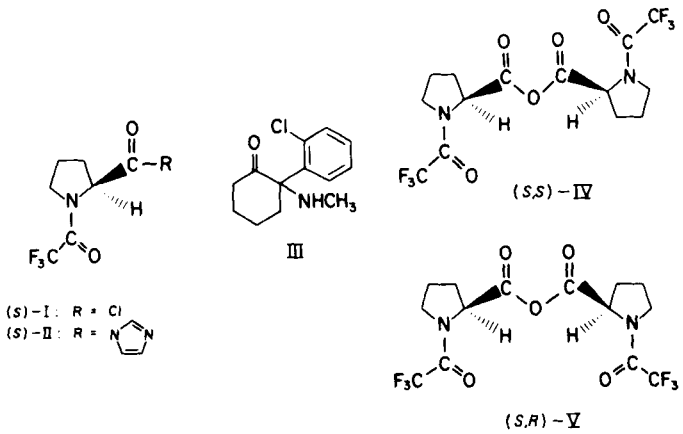
Abstract □ The reaction characteristics of (*S*,*S*)-*N*-trifluoroacetylproline anhydride were examined in an attempt to develop a quantitative GC assay of the enantiomers of the sterically hindered, chiral amine ketamine. With the aid of the individual enantiomers of ketamine and the corresponding synthetic *N*-trifluoroacetylprolyl amides, it was found that the derivatization reaction proceeds stereoselectively, in poor yield, and with some degree of racemization of the acylating reagent. The results indicate that care must be exercised when prolyl derivatizing reagents are chosen for assaying chiral amines.

Keyphrases □ (*S*,*S*)-*N*-Trifluoroacetylproline anhydride—derivatization of ketamine, GC estimation of enantiomeric composition □ GC analysis—detection of enantiomers of chiral amines with (*S*,*S*)-*N*-trifluoroacetylproline anhydride □ Enantiomers—derivatization of chiral amines with (*S*,*S*)-*N*-trifluoroacetylproline anhydride for GC estimation of enantiomeric composition

It is important to examine the extent to which chiral xenobiotics may undergo enantioselective metabolic transformations in an effort to characterize the effects which such processes may have on the pharmacological and toxicological properties of these substances (1, 2). In the case of chiral amines, quantitative estimations of enantiomeric composition have been achieved through GC analysis of the diastereomeric amides formed by derivatization with (*S*)-*N*-trifluoroacetylprolyl chloride [(*S*)-I] (3). Although commercially available as a solution in chloroform, this reagent is difficult to obtain in pure form and is susceptible to racemization (4). The corresponding imidazolide, compound (*S*)-II, is a relatively stable, crystalline solid (5). However, it was observed that this derivative reacts sluggishly with sterically hindered amines such as ketamine (III) (6). In an attempt to obtain a derivatizing reagent that can be prepared in crystalline form and that might react more readily with ketamine, the synthesis of (*S*,*S*)-*N*-trifluoroacetylproline anhydride [(*S*,*S*)-IV] was attempted. An attractive feature of (*S*,*S*)-IV is that inversion about one of the two chiral centers present in this molecule leads to the *meso*-diastereomeric species (*S*,*R*)-V which, in theory, should be separable from (*S*,*S*)-IV.



RESULTS AND DISCUSSION

The synthesis of (*S*,*S*)-IV from (*S*)-proline and trifluoroacetic anhydride was reported originally by Weygand (7). Attempts to repeat this synthesis initially led to the isolation of a species (mp 114–115°) which proved to be an isomer of the Weygand compound; longer reaction times however provided the Weygand compound (mp 138–140°). The electron impact mass spectra and NMR spectra of these products were essentially identical, which suggested that the two compounds were diastereomerically related. Since the high-melting isomer did not rotate plane polarized light, whereas the low-melting isomer was strongly levorotatory, the low-melting isomer was tentatively assigned the asymmetric structure (*S*,*S*)-IV and the Weygand compound the *meso*-structure, (*S*,*R*)-V.

Consistent with these assignments, reaction of (*S*,*S*)-IV with (*S*)-

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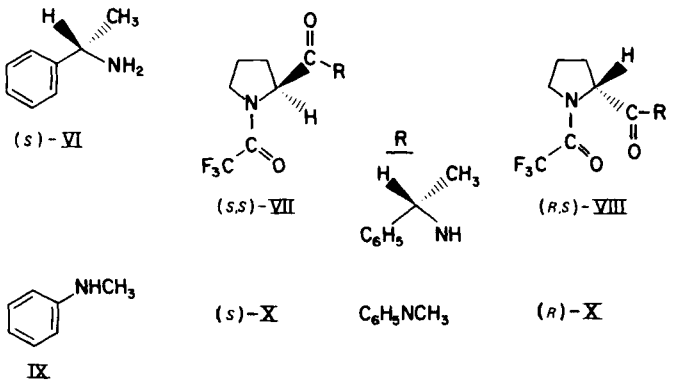
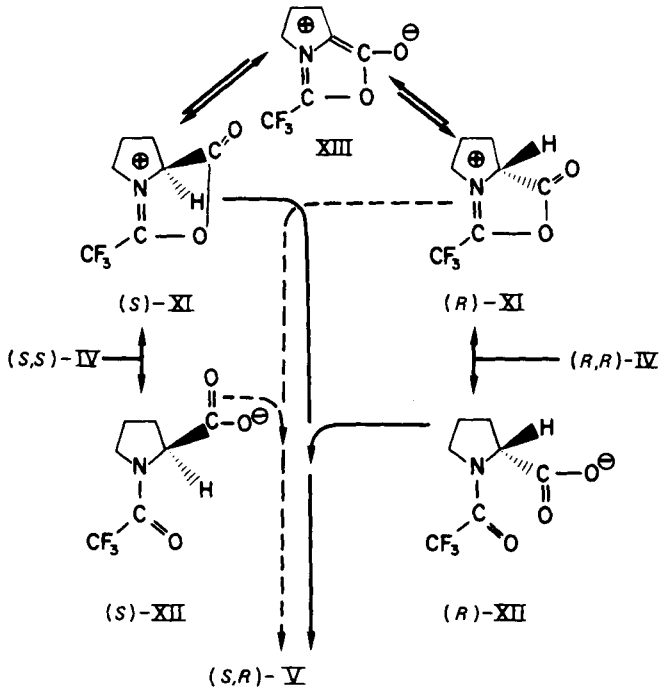
α-methylbenzylamine [(S)-VI] yielded a product that displayed a single GC peak and that presumably corresponds to structure (S,S)-VII. Reaction of (S)-VI with (S,R)-V, on the other hand, yielded a product displaying two equally intense GC peaks corresponding to (S,S)-VII and (R,S)-VIII[1]. Additionally, reaction of (S,S)-IV with N-methylaniline (IX) yielded a levorotatory anilide [(S)-X], while the corresponding reaction with compound (S,R)-V yielded a racemic mixture of (S)-X and (R)-X.



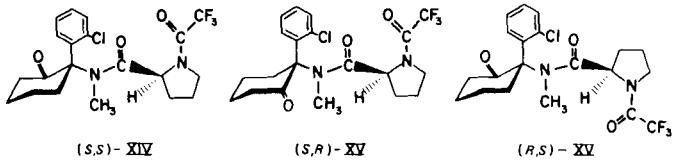
The experimental evidence described previously is sufficient to assign unambiguously the structure of the low-melting isomer. Parallel experimental results have led to the same conclusions (8). The data supporting the structure assignment of (S,R)-V, however, are also consistent with a racemic mixture of (S,S)-IV and (R,R)-IV. As has been reported with related systems, the ability to distinguish between such species is often not a trivial task (9). Therefore, the preparation of the racemate of IV was attempted by mixing equal amounts of (R,R)-IV [prepared from (R)-proline and trifluoroacetic anhydride] and (S,S)-IV. The product obtained after crystallization proved to be identical in every way to the high-melting isomer. The IR spectra of the presumed racemate and the high-melting isomer obtained from the Weygand procedure also were identical but were different from that of (S,S)-IV. Based on these results and the facile interchange which is reported to occur with mixtures of acetic anhydride and trifluoroacetic anhydride (10), it is possible that upon recrystallization, the racemic mixture of IV undergoes disproportionation to yield the thermodynamically more stable *meso*-isomer (S,R)-V.

The pathway leading to the formation of the *meso*-compound is likely to involve the oxazolinium intermediate (XI) (Scheme I). Recombination of the (R)-N-trifluoroacetylprolyl anion [(R)-XII] with (S)-XI leads to (S,R)-V. Compound (S,R)-V may also be formed from (S)-XII and (R)-XI. It seems reasonable to speculate that the formation of compound (S,R)-V from (S)-proline and trifluoroacetic anhydride also proceeds through XI, which may undergo inversion to (R)-XI through reversible tautomerism to the symmetric mesoionic species (XIII). A similar process has been proposed for the racemization of (S)-N-p-nitrobenzoylproline in the presence of acetic anhydride and a trace of acid (11).



Scheme I

The utility of (S,S)-IV for the quantitative estimation of the enantiomeric composition of ketamine was examined next. GC analysis of the reaction product obtained between (S,S)-IV and racemic ketamine in the presence of triethylamine gave a pair of sharp peaks with baseline separation and mass spectra consistent with the expected amide structures (S,S)-XIV and (S,R)-XV[2]. With the aid of the pure ketamine enantiomers, the diastereomer with the shorter retention time was shown to be the (S)-N-trifluoroacetylprolyl amide of (S)-ketamine. Under a variety of reaction conditions, however, product formation appeared to favor the diastereomer having the longer retention time, *i.e.*, (S,R)-XV. For reasons to be discussed, reaction of racemic ketamine with (S,S)-IV also may yield significant amounts of the enantiomeric species (R,S)-XV.



The two N-trifluoroacetylprolyl diastereomeric amides of (R)- and (S)-ketamine were obtained in analytically pure form from the reaction of (S)-N-trifluoroacetylprolyl chloride and individual ketamine enantiomers. Although the reaction with (R)-ketamine proceeded smoothly, attempts to prepare the corresponding amide of (S)-ketamine were accompanied by extensive inversion of the derivatizing reagent. The main product obtained in this reaction was the diastereomeric (R)-N-trifluoroacetylprolyl amide of (S)-ketamine, *i.e.*, (R,S)-XV. The N-trifluoroacetylproline recovered from the reaction mixture proved to be racemic, which indicates that racemization of the prolyl reagent during the reaction with (S)-ketamine is extensive.

GC analysis of the two diastereomers showed that the detector responses of these compounds were essentially identical. Consequently, the different peak heights observed in the analysis of racemic ketamine must be due to the stereoselective formation of the (S)-N-trifluoroacetylprolyl amide of (R)-ketamine and/or the (R)-N-trifluoroacetylprolyl amide of (S)-ketamine.

An additional frustration encountered in the attempts to develop a ketamine assay with (S,S)-IV was the low yields realized in these reactions. Based on peak heights found with the synthetic N-trifluoroacetylprolyl amides, the maximum combined yield of the two diastereomeric amides obtained upon reaction of racemic ketamine with (S,S)-IV was only 17% (Table I). The use of a large excess of the anhydride or introduction of additional anhydride during the course of the reaction did not influence the overall yield. Although somewhat better yields were obtained with (S)-I, the derivatization of ketamine with (S)-I was more stereoselective (Table I).

A pathway to account for these results would involve cleavage of the anhydride to the (S)-oxazolinium species [(S)-XI] and (S)-N-trifluoroacetylproline anion [(S)-XII]. Conversion of (S)-XI to XIII would deplete the acylating reagent and also generate 1 mole of acid. Although a 5:1 *M* ratio of triethylamine to ketamine was used, the large excess of (S,S)-IV could result in adequate acid production to protonate (and hence inactivate) the ketamine. The yellow color which developed during the course of the reaction is consistent with the report that XIII is yellow (12).

Analytical scale reactions with the individual isomers of ketamine again demonstrated the poor reactivity of (S)-ketamine with both (S,S)-IV and (S)-I (Table I). Extensive racemization occurred during these reactions. Since the chiral center of ketamine is tetrasubstituted, and therefore unlikely to racemize, the formation of the undesired diastereomer is attributed to inversion of the prolyl moiety. The reactions of (S,S)-IV and (S)-I with (R)-III proceeded in reasonable yields and were accompanied by only a limited amount of inversion (Table I). If it is assumed that the transition state energy for the formation of the prolyl

[1] For all such designations, the first symbol refers to the chirality of the N-trifluoroacetylprolyl moiety and the second symbol to the chirality of the amine.

[2] The absolute stereochemistry of ketamine-free base has been found to be R(+) and S(−). These data will be published in the near future.

Table I—GC Analysis of Ketamine following Derivatization with *N*-Trifluoroacetylpropyl Reagents[a]

Reactant	Reagent	Temperature (Time, hr)	Peak Areas[b] (*S,S*)-/(*R,S*)-XIV	Peak Areas[b] (*S,R*)-/(*R,S*)-XV	Yield, %[c]
(*R,S*)-III	(*S,S*)-IV	75° (2)	3	6	2
(*R,S*)-III	(*S,S*)-IV	75° (2)	3	8	2
(*R,S*)-III	(*S,S*)-IV	100° (1)	14	68	16
(*R,S*)-III	(*S,S*)-IV	100° (2)	21	63	17
(*R,S*)-III	(*S,S*)-IV	100° (4)	20	58	16
(*S*)-III	(*S,S*)-IV	75° (2)	2	0	1
(*S*)-III	(*S,S*)-IV	100° (2)	49	14	25
(*R*)-III	(*S,S*)-IV	75° (2)	0	6	3
(*R*)-III	(*S,S*)-IV	100° (2)	12	86	39
(*R,S*)-III	(*S*)-I	75° (2)	33	206	48
(*R,S*)-III	(*S*)-I	75° (4)	32	196	46
(*R,S*)-III	(*S*)-I	100° (1)	49	211	52
(*R,S*)-III	(*S*)-I	100° (2)	29	134	33
(*S*)-III	(*S*)-I	75° (2)	27	19	18
(*R*)-III	(*S*)-I	75° (2)	18	208	90

[a] The assays were performed according to the procedure described in the Experimental section. [b] Since (*S,S*)-IV and (*S*)-I are susceptible to inversion, the peak areas reported for reactions involving racemic ketamine represent the sum of the responses of the enantiomeric prolyl amides. [c] Yields were calculated on the basis of detector responses obtained with pure (*S,S*)-XIV and (*R,S*)-XV. Note that the individual enantiomers were run at one-half the concentration of racemic ketamine.

amide with (*S*)-ketamine is greater than with (*R*)-ketamine, the competing reaction pathway (Scheme I) leading to isomerization of (*S,S*)-IV and (*S*)-I would proceed to a greater extent in the reaction with (*S*)-ketamine than with (*R*)-ketamine. Once inversion has occurred, the energetically more favored reaction of (*S*)-ketamine with the (*R*)-prolyl reagent could occur preferentially.

The results obtained in this study clearly point to a number of difficulties with prolyl derivatizing reagents to which researchers should be alerted. Whenever possible, quantitative estimations based on GC analysis of reaction mixtures should be based on detector responses measured with the pure synthetic diastereomeric amides. Secondly, the possibility of stereoselective reactions with chiral amines and prolyl derivatizing reagents should be carefully evaluated. Finally, the stability of the derivatizing reagent with respect to racemization should be examined with the aid of the individual enantiomers of the amine. In the present study it has been found that the reaction of the anhydride reagent (*S,S*)-IV with ketamine proceeds stereoselectively, in poor yield, and is accompanied by inversion. Unfortunately, the corresponding reaction between the commercially available (*S*)-*N*-trifluoroacetylprolyl chloride suffers from similar limitations.

EXPERIMENTAL[3]

(*S,S*)-*N*-Trifluoroacetylproline Anhydride [(*S,S*)-IV]—Trifluoroacetic anhydride (84 g, 0.40 mole) was added dropwise with stirring under nitrogen and external cooling to an ice cold suspension of (*S*)-proline (23 g, 0.20 mole). After 3 hr, the volatile components were removed under vacuum and the residual yellow mass was recrystallized 3 times from ether to yield 22.9 g (0.06 mole, 57%) of white crystals, mp 114–115° [lit. (8) mp 114–115°]; NMR (deuterochloroform): 1.8–2.5 (m, 8H, CH_2), 3.79 (m, 4H, NCH_2), and 4.62 (t, J = 6.2 Hz, 2H, CH) ppm; IR (oil mull): 1822, 1760, 1690, 1460, 1380, 1360, 1350, 960, 940, 930, 910, 878, 840, 815, 795, and 768 cm^{-1}; electron impact mass spectrum (relative intensity): 404 (M^+, 0.4), 376 (0.5), 211 (1.4), 194 (44), 166 (100), 98 (9.8), 96 (35), and 69 (82); $[\alpha]_D^{22} = -98.6°$ (C, 0.7 mg/ml; benzene).

Anal.—Calc. for $C_{14}H_{14}N_2O_5F_6$: C, 41.60; H, 3.49; N, 6.93. Found: C, 41.53; H, 3.51; N, 6.83.

[3] Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. IR spectra were obtained using a Beckman Aculab 2 spectrophotometer. NMR spectra were recorded on a Varian FT-80 instrument. Chemical shifts are reported in parts per million (ppm) relative to trimethylsilane. Chemical ionization mass spectra were taken on an Associated Electronics Inc. Model MS-902 double-focus mass spectrometer equipped with a direct inlet system and modified for chemical ionization. The electron impact mass spectra were recorded on a Hitachi model M-52 instrument. Specific rotation measurements were performed on a Perkin-Elmer 141 electronic polarimeter. GC analyses of the derivatized amines were recorded on a Hewlett-Packard 5700A machine equipped with a nickel electron capture detector. The column was a 3 m × 6-mm i.d. glass column packed with 3% SP 2250 coated on 100/120 mesh Supelcoport. Argon–methane carrier gas flow rate was 30 ml/min and the column temperature was 250°. Peak area integration was done automatically by a Hewlett-Packard 3380A integrating recorder. Microanalyses were performed by the Microanalytical Laboratory, University of California at Berkeley.

The corresponding reaction with (*R*)-proline provided the enantiomeric anhydride (*R,R*)-IV, in 36% yield: mp 114–115°; $[\alpha]_D^{22} = +106.5°$ (C, 0.7 mg/ml, benzene).

(*S,R*)-*N*-Trifluoroacetylproline Anhydride [(*S,R*)-V]—The reaction of (*S*)-proline (5.75 g, 0.05 mole) and trifluoroacetic anhydride (42.0 g, 0.20 mole) in 20 ml of methylene chloride at room temperature for 48 hr yielded 2.5 g (0.006 mole, 25%) of the *meso*-anhydride, (*S,R*)-V, which was recrystallized from ether: mp 139–140° [lit. (7) mp 138–140°]; NMR and electron impact mass spectrum were indistinguishable from (*S,S*)-IV; $[\alpha]_D^{22} = 0.0°$ (C, 0.66 mg/ml; benzene); IR (oil mull): 1822, 1760, 1690, 1465, 1380, 1368, 1355, 940, 930, 914, 874, 812, and 760 cm^{-1}.

Anal.—Calc. for $C_{14}H_{14}N_2O_5F_6$: C, 41.60; H, 3.49; N, 6.93. Found: C, 41.77; H, 3.62; N, 6.96.

(*S*)-*N*-(*N*-Trifluoroacetylprolyl)-*N*-methylaniline [(*S*)-X]—A mixture of (*S,S*)-IV (1.8 g, 4.5 mmoles) and *N*-methylaniline (5 g, 46.7 mmoles) was heated for 2 hr under nitrogen with stirring. The cooled reaction mixture was washed twice with 10 ml of hexane and the residual oil was subjected to a short path distillation under reduced pressure. The distillate was crystallized from chloroform to yield 0.6 g (2.0 mmoles, 22.4%) of a colorless solid: mp 136–138°; NMR (deuterochloroform): 1.5–2.3 (m, 4H, CH_2), 3.28 (s, 3H, CH_3), 3.76 (m, 2H, NCH_2), 4.43 (t, J = 6.3 Hz, 1H, CH), and 7.40 (m, 5H, phenylH) ppm; $[\alpha]_D^{22} = -102.9°$ (C, 0.7 mg/ml; benzene).

Anal.—Calc. for $C_{14}H_{15}N_2O_2F_3$: C, 56.00; H, 5.04; N, 9.33. Found: C, 55.65; H, 5.07; N, 9.28.

The same reaction carried out with (*S,R*)-V provided (*S,R*)-X in 17% overall yield: mp 102–104°; $[\alpha]_D^{22} = 0.0°$ (C, 0.7 mg/ml, benzene).

Anal.—Calc. for $C_{14}H_{15}N_2O_2F_3$: C, 56.00; H, 5.04; N, 9.33. Found: C, 56.05; H, 5.04; N, 9.31.

(*S,S*)-2-[*N*-(*N*-Trifluoroacetylprolyl)-*N*-methyl]amino-2-*o*-chlorophenylcyclohexanone [(*S*)-*N*-Trifluoroacetylprolyl Amide of (*S*)-Ketamine, (*S,S*)-XIV]—A solution of (*S*)-*N*-trifluoroacetylprolyl chloride (536 mg, 2.33 mmoles in 23 ml of methylene chloride) and (*S*)-III (13) (500 mg, 2.11 mmoles in 10 ml of toluene) was heated at 75° with stirring under nitrogen for 1.5 hr. After cooling, the reaction mixture was stirred with 30 ml of 0.2% NaOH, separated, and washed with 10 ml of 0.5% HCl. After drying with sodium sulfate, hexane was added and the resulting solution was cooled to 5° to produce 300 mg (0.7 mmole, 33.2%) of a solid: $[\alpha]_D^{22} = +41.6°$ (C, 0.77 mg/ml; benzene); mp 197–200°. The NMR, electron impact mass spectrum, and GC retention time of the product were identical to the corresponding values for (*S,R*)-XV and therefore, its structure is assigned as (*R,S*)-XV. Treatment of the mother liquor with an additional 5 ml of hexane caused the precipitation of a second solid (45 mg, 0.2 mmole) which proved to be racemic *N*-trifluoroacetylproline: mp 53–55°; $[\alpha]_D^{22} = 0.0°$ (C, 0.7 mg/ml; benzene); chemical ionization mass spectrum, 212 (MH^+).

Anal.—Calc. for $C_7H_8NO_3F_3$: C, 39.81; H, 3.79; N, 6.64. Found: C, 39.80; H, 3.88; N, 6.60.

The residue obtained from the mother liquor filtrate was chromatographed on 10 g of silica. Elution with benzene–ethanol (95:5) provided a solid, which after crystallization from toluene–hexane yielded 7 mg (0.016 mmole, 0.8%) of the desired amide (*S,S*)-XIV: mp 184–186°; chemical ionization mass spectrum (*m/z*, relative intensity): 433 and 431 (MH^+, 25, 100), 397 (10), 227 and 225 (50, 100), 211 and 209 (60, 100); GC retention time, 16.8 min.

Anal.—Calc. for $C_{20}H_{22}N_2O_3F_3Cl$: C, 55.75; H, 5.15; N, 6.50. Found: C, 55.56; H, 5.30; N, 6.46.

(*R*)-2-[*N*-(*N*-Trifluoroacetyl-(*S*)-prolyl)-*N*-methyl]amino-2-*o*-chlorophenylcyclohexanone [(*S*)-*N*-Trifluoroacetylprolyl Amide of (*R*)-Ketamine, (*S,R*)-XV]—(*S*)-*N*-Trifluoroacetylprolyl chloride and (*R*)-ketamine were allowed to react under the same conditions as described previously. After treatment with 0.2% NaOH, extraction with 0.5% HCl, and drying with sodium sulfate, addition of hexane and cooling led to the crystallization of 490 mg (1.14 mmoles, 54%) of (*S,R*)-XV: mp 200–202°; NMR (deuterochloroform): 1.5–2.6 (m, 11H, CH_2), 3.24 (s, 3H, NCH_3), 3.3–3.5 (q, 1H, CH), 3.7–3.9 (m, 2H, CH_2), 5.09 (t, 1H, CH), and 7.1–7.5 (m, 4H, phenylH) ppm; $[\alpha]_D^{22} = -41.2°$ (C, 0.68 mg/ml; benzene); chemical ionization mass spectrum was identical to (*S,S*)-XIV; GC retention time, 19.2 min.

Anal.—Calc. for $C_{20}H_{22}N_2O_3F_3Cl$: C, 55.75; H, 5.15; N, 6.50. Found: C, 55.70; H, 5.24; N, 6.32.

Amine Derivatization—Derivatizations of racemic ketamine and the individual enantiomers of ketamine for GC analysis were performed under similar conditions except that the concentration of racemic ketamine was twice that of the individual enantiomers. Reactions were carried out in 50 μl of toluene–methylene chloride–triethylamine (95:5:0.02) containing (*R,S*)-ketamine (1.68 m*M*) or the ketamine enantio-

mers (0.84 mM), and in each case a 30 M excess of (S,S)-IV or (S)-I. The reactions were performed in screw cap sealed, polytef-lined reaction vials which were heated to 75–100° for 1–4 hr in a block heater. At the end of the reaction, the mixtures were washed with 0.2% NaOH (50 μl) followed by 0.5% HCl (50 μl) and then dried over sodium sulfate. The solutions were carefully transferred with the aid of a Pasteur pipet to a second reaction vial and the solvent removed under a stream of dry nitrogen. The residues were dissolved in 100 μl of toluene, and 1 μl of the resulting solution was analyzed by GC. Retention times for (S,S)-XIV and (S,R)/(R,S)-XV were 16.8 and 19.2 min, respectively. Derivatization of (S)-α-methylbenzylamine with (S,S)-IV and (R,S)-V proceeded in a similar fashion. GC retention times for (S,S)-VII and (S,R)-VIII were 15.5 and 13.3 min, respectively, using the same conditions as for the ketamine analyses.

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In Vivo and *In Vitro* Studies with Sulfamate Sweeteners

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Abstract □ The sweet compounds 2-methyl- and 3-methylcyclohexyl- and 2-cyclohexenylsulfamates were fed to Wistar albino rats. The urine (and feces in the case of 2-cyclohexenylsulfamate) was examined for possible amine, ketone, and alcohol metabolites. The total percent of metabolites formed was low and the hexenyl compound gave a particularly small quantity of metabolite. The results with these compounds are compared with those obtained from earlier *in vivo* studies with cyclamate and other sulfamates. In complementary *in vitro* studies, the four sweetest sulfamates, namely, cyclamate, cycloheptyl-, cyclooctyl-, and cyclopentylsulfamates were incubated with the cell-free extract of bacteria isolated from the feces of cyclamate fed rats. Some correlation was apparent between these *in vitro* experiments and previous *in vivo* studies. Preliminary mutagenicity testing (the Ames test) of some amines (corresponding to the sulfamates studied) has been carried out.

Keyphrases □ Sulfamate sweeteners—*in vivo* and *in vitro* studies of amine, ketone, and alcohol metabolites □ Metabolism—amine ketone, and alcohol metabolites of sulfamate sweeteners □ Cyclamates—*in vivo* and *in vitro* studies of amine, ketone, and alcohol metabolites

The controversial ban on the use of cyclohexylsulfamate (cyclamate) salts as nonnutritive sweeteners has provided a strong impetus for wide and varied toxicity studies of these compounds and their metabolites (1). Though at least some other sulfamates have a sweetness potency similar to the banned parent compound (2), and it has been suggested that certain of these sulfamates might be less readily metabolized than is cyclamate (3), nevertheless toxicological studies have been carried out on just a few such compounds (4). In the present work, *in vivo* animal feeding studies employed three other sweet sulfamates: 2- and 3-methylcyclohexyl- and 2-cyclohexenylsulfamates. In a complementary *in vitro* study, some of the sweetest known sulfamates have been incubated in cell-free extracts of the microorganisms responsible for the metabolism of cyclamate. Some work has been carried out on the mutagenicity of the amines to which these sulfamates give rise when metabolized.

EXPERIMENTAL

Reagents and Chemicals—The amines, ketones, and alcohols were commercially available and were used as obtained. The following compounds were synthesized by known literature methods: 2-methylcyclohexanol (5), cyclohexyl- (6), 2-methyl- (6), 3-methyl- (6), 2-cyclohexenyl- (7), *n*-octyl- (8), and phenyl- (9) sulfamates. Cyclopentyl-, cycloheptyl-, and cyclooctylsulfamates were previously prepared (4). All the synthesized sulfamates (as their sodium salts) gave a positive "sulfamate test" (2), satisfactory C, H, and N analysis, and the characteristic IR bands for sulfamates. 2-Cyclohexenylsulfamate gave an additional band in the 1640–1615 cm^{-1} region, which is characteristic of an ethylenic double bond.

***In Vivo* Experiments**—Female Wistar albino rats (200–257 g) were kept on solid food and water in rat metabolism cages[1]. The sodium salts of 2- and 3-methylcyclohexyl- and 2-cyclohexenylsulfamates were administered orally in water (~20 ml) at a level of 1.45 g/kg of body weight to groups of six rats (five in the case of 2-methylcyclohexylsulfamate). Prior to administration of the sulfamates, the rats were deprived of water for 24 hr. Prior to feeding, the urine of each rat was examined for metabolites by GLC. Similarly, the three sulfamates fed were screened for occluded amine, also by GLC. After administration of each sweet compound, the urine (and feces in the case of 2-cyclohexenylsulfamate) was collected for 3 days, bulked, and refrigerated for no more than 1 day when GLC analysis for the metabolites was carried out.

The urine and feces recovered from rats, fed 2-cyclohexenylsulfamate, was not analysed until the eighth day (instead of the customary fourth day), and accordingly, a study of the stabilities of 2-cyclohexenylamine and 2-cyclohexenone in urine and feces over a 7-day period was made.

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