(52) (a) T. W. Bentley and P. v. R. Schleyer, *J. Am. Chem. Soc.*, **98**, 7658 (1976); (b) F. L. Schadt, T. W. Bentley, and P. v. R. Schleyer, *ibid.*, **98**, 7667 (1976).

(53) Recently E. N. Peters, *J. Am. Chem. Soc.*, **98**, 5627 (1976), suggested a correlation of rates of secondary tosylates with tertiary benzylic derivatives and applied it in the elucidation of anchimeric assistance.

# Stereochemical Consequences of Hydrogen Exchange as a Result of Tritium Atom Reactions on Solid Aliphatic Amino Acids[1]

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**Abstract:** The products and stereochemistry resulting from radicals generated by the interaction of tritium atoms with L-isoleucine and L-alloisoleucine in the solid phase were determined. Among the four possible tritiated stereoisomers for each amino acid the major product was the parent L-amino acid (approximately 70% in each case) with the major fraction of the labeling being in positions other than the $\alpha$ position. Approximately 30% of the labeling resulted in the diastereomeric product by reaction at either the $\alpha$ or $\beta$ position, with the major pathway being $\beta$-inversion. The yield of products from $\alpha$-carbon attack of L-isoleucine was minor (7.9%) and occurred with net retention. Labeling at the $\alpha$-carbon of alloisoleucine was <1%. Tritiated glycine was formed from both amino acids by cleavage of the alkyl side chain. This may result from the excitation decomposition of the intermediates formed from recombination of $\alpha$ (or $\beta$) amino acid radicals with tritium. Determination of the stereochemical and chemical consequences of radical formation at chiral centers provides a sensitive probe for studying the consequences of tritium (hydrogen or deuterium) atom reactions.

The nature (structure) and fate (i.e., chemistry and stereochemistry) of the highly reactive radicals formed by the reaction of hydrogen atoms with biologically important molecules is not well understood. Its delineation is necessary for understanding solid state atom replacement reactions and in the development of a model for in vivo radiation effects.[2-7]

Previous studies on the interaction of hydrogen atoms generated by microwave discharge or $\gamma$ irradiation with amino acids,[8-11] polyamino acids,[12-15] and proteins[13,16,17] have used electron spin resonance (ESR) as a qualitative measure mainly of the chemically stable radicals produced in the solid phase by such bombardment. However, the fate of the intermediate radicals, i.e., those important in terms of product formation and stereochemistry, cannot be determined under these conditions. Likewise, in solution Neta et al.[18-21] have measured (by ESR) the kinetics of such reactions by following the disappearance of hydrogen atoms, and Volkert[22] by competition of H-atom donor (amino acids) and scavenger species (allyl alcohol) for radiolytic hydrogen atoms. However, in no case, either in solution or solid phase, has a detailed product analysis been performed or the stereochemistry investigated.

In this study the reaction of hydrogen atoms, by the use of tritium atom reactions with L-isoleucine and L-alloisoleucine, is described. The diastereomeric relationship of these two amino acids gives one a sensitive probe, both as to the position of tritium (hydrogen or deuterium) atom attack as well as the stereochemical consequences of intermediate radicals. The dynamic reaction conditions employed make it probable that the observed products result predominantly from the highly reactive radicals formed, and not from the chemically stable species previously investigated by ESR.[8-17]

The formation of glycine by side chain cleavage of the parent amino acid was also investigated.

## Results

**A. Stereochemical Result.** The major product among the four possible stereoisomers in the tritium labeling of L-isoleucine and L-alloisoleucine using the microwave discharge activation of tritium gas was the production of the labeled parent amino acid with little tritium on the $\alpha$ position. The labeled diastereomeric amino acids were formed predominantly by inversion of the C(3) carbon at the $\beta$ (tertiary) position and not by inversion at the $\alpha$-amino carbon (Tables I and II). The labeling of L-isoleucine produced 26.7% alloisoleucine, 87.2% of which resulted from inversion at the $\beta$ carbon. Similarly the labeling of L-alloisoleucine resulted in the formation of 29.4% of its diastereomer (isoleucine), 99% coming from inversion at the $\beta$ carbon.

The amount of each diastereomer was determined directly by isolation of the pure material using Dowex 50W-X8 chromatography. The enantiomeric composition of each diastereomer was determined by GLC of the $N$-(L-2-chloropropionyl)amino acid methyl ester (Table II).

Base exchange of each member of the diastereomeric pair (isoleucine, alloisoleucine) which resulted from the labeling of L-isoleucine resulted in a 7.9% loss of the total activity in the sum of the two diastereomers. Most of the loss (5.4%) was from the parent L-isoleucine, indicating that labeling at the $\alpha$-amino carbon favors retention of configuration by more than a factor of 2. However, base exchange of alloisoleucine and isoleucine generated from L-alloisoleucine resulted in little or no loss of tritium from either diastereomer. In both cases labeling at the C(2) $\alpha$-amino carbon was shown to be a minor process.

The amount of exchangeable tritium ($\alpha$-amino carbon tritium) was determined as the percent of decrease in specific activity after base exchange with 4 N $Ba(OH)_2$ at 110 °C (Table III).

These data can also be used as a check on the GLC determination of the amount of D-amino acid formed in the carrier-free diastereomer. The decrease in specific activity in each enantiomeric pair should be equal to the percent D-amino acid formed. Although there is some variation (Table IV), the agreement is good.

**B. Glycine Formation.** The fragmentation of the alkyl side

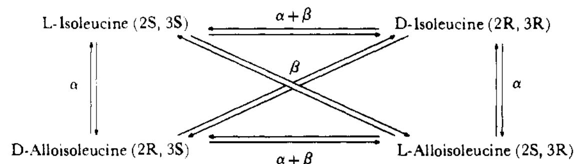


**Figure 1.** Diastereomeric relationship and interconversion of isoleucine and alloisoleucine by epimerization at the $\alpha$ or $\beta$ carbon. Absolute configurations are given in parentheses.

chains from the $\alpha$-amino carbons of L-isoleucine and L-alloisoleucine was observed by formation of carrier-free, tritium-labeled glycine. The glycine was identified both by Dowex 50W-X8 chromatography as well as by GLC with added [$^{14}$C]glycine, as the *N*-(2-chloropropionyl)glycine methyl ester derivative.

The amount of glycine (as well as the carrier-free diastereomers produced) was determined by reverse isotope dilution. A known amount of unlabeled glycine carrier was added to the crude labeled product prior to Dowex chromatography.

The purity of the glycine was further tested after isolation by Dowex chromatography by adding [$^{14}$C]glycine and comparing the T/$^{14}$C ratio before and after GLC analysis of its *N*-(2-chloropropionyl)glycine methyl ester. In each case there was a drop in the T/$^{14}$C ratio after GLC analysis, indicating some impurity remained in the glycine after Dowex chromatography. The amount of glycine formed listed in Tables V and VI was determined from the T/$^{14}$C ratio after the above GLC analysis.

Production of glycine and the amounts of each identified product are listed in Tables V and VI.

It should also be noted that small amounts of [$^3$H]-D-isoleucine (from L-isoleucine) and [$^3$H]-D-alloisoleucine (from L-alloisoleucine) were detected by GLC analysis of the parent amino acids after labeling (Tables V and VI). This could result from double labeling events at both chiral centers in the starting L-amino acid (Figure 1). Statistically, however, this is a highly unlikely process under our labeling conditions, since at most one molecule in $10^4$ is labeled either as parent amino acid, diastereomer, or glycine. This may result from radiation induced inversion, inversion of highly excited intermediates, or incomplete resolution of the peaks on GLC (see Experimental Section and ref 35).

## Discussion

The reactions of thermal hydrogen atoms with amino acids,[8-11] polyamino acids,[12-15] and proteins[13,16,17] in the solid phase has been extensively studied by electron spin resonance (ESR). The major processes proposed in the generation of amino acid and peptide radicals are: (1) hydrogen abstraction at $\alpha$-amino carbon; (2) hydrogen abstraction at a $\beta$ or $\gamma$ tertiary carbon; (3) carbon-carbon cleavage of the alkyl side chain; (4) functional group cleavage, i.e., amino ($-NH_2$) or carboxyl ($-CO_2H$).

It should be noted, however, that these long-lived stable radicals observed in the ESR spectra may not be responsible for the tritium-labeled products in a dynamic reaction system. Radicals that are initially formed may be short lived compared to the ESR time scale. These radicals in an isolated ESR system may be rapidly converted to stable radicals, whereas under the dynamic conditions of the labeling process may lead instead to the observed labeled products.

Using L-leucine as a model, a proposed reaction scheme for the reaction of L-isoleucine with tritium atoms is shown in Scheme I.[8-12,14]

**Stereochemical Result.** One can see in Figure 1 that abstraction of hydrogen at the $\alpha$ carbon of L-isoleucine (eq 1) and subsequent recombination with tritium can produce $\alpha$-labeled parent L-isoleucine by retention and the diastereomer D-alloisoleucine by inversion of configuration. Likewise, reaction at the $\beta$ carbon (eq 2) with retention will give the $\beta$-labeled L-isoleucine or by inversion will produce L-alloisoleucine. An analogous reaction pathway is available for L-alloisoleucine.

The results (Tables II and III) show clearly a very large preference for $\beta$ attack over $\alpha$ attack in the formation of the diastereomeric amino acids. We have also observed similar specificity in the labeling of the tripeptide L-isoleucyl-L-isoleucyl-D-valine, where 75% of the alloisoleucine produced resulted from $\beta$ attack.[23]

This preference can be explained, at least in part, by thermodynamic considerations. Both bond dissociation energies[24,25] as well as the energy of activation ($E_a$) of hydrogen atom abstraction by H atoms in the gas phase decrease as one goes from primary, secondary, to tertiary carbon,[26] with a resulting increase in the rate constants[27] (Table VII).

If no other factors were involved, it is clear that abstraction of hydrogen from a tertiary carbon is energetically favored over

**Table I.** Diastereomer Formation by Tritium Labeling of Isoleucine and Alloisoleucine[a]

| Amino acid labeled | % labeled parent | % labeled diastereomer |
|---|---|---|
| L-Isoleucine | 73.3 | 26.7 |
| L-Alloisoleucine | 70.6 | 29.4 |

[a] Determined by Dowex 50W-X8 chromatography as ($\mu$Ci of tritium in peak of interest/$\Sigma$ tritium in the two diastereomeric peaks) $\times$ 100.

**Table II.** Percent L, D Formed for Each Enantiomeric Pair[a]

| Amino acid labeled | Parent | | Diastereomer | |
|---|---|---|---|---|
| | % L | % D | % L($\beta$) | % D($\alpha$) |
| L-Isoleucine | 98.5 | 1.5 | 87.2 | 12.8 |
| L-Alloisoleucine | 95.8 | 4.2 | 99.0 | 1.0 |

[a] Determined by GLC.

**Scheme I.** Reaction of L-Isoleucine [(2*S*,3*S*)-2-amino-3-methylpentanoic acid] with Tritium Atoms

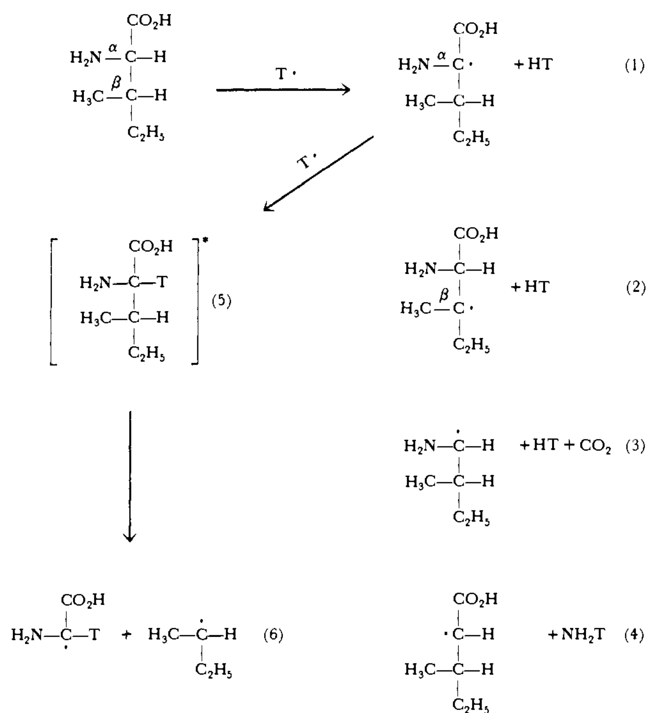

**Table III.** Distribution of Base Exchangeable Tritium between Labeled Diastereomeric Pair

| Amino acid exposed | Total activity in diastereomeric pair, $\mu$Ci | Base exchangeable tritium, $\mu$Ci | | % total activity base exchangeable[a] |
|---|---|---|---|---|
| | | Isoleucine | Alloisoleucine | |
| L-Isoleucine | 19.58 | 1.06 | 0.49 | 7.9 |
| L-Alloisoleucine | 75.02 | 0.40 | 0.00 | 0.4 |

[a] Determined as ($\mu$Ci lost from parent + $\mu$Ci lost from diastereomer during base exchange/$\mu$Ci parent + $\mu$Ci diastereomer prior to base exchange) $\times$ 100.

**Table IV.** Percent Tritium Lost During Base Exchange Compared to Percent D-Amino Acid Present by GLC Determination

| Amino acid exposed | % D by base exchange[a] | | % D by GLC | |
|---|---|---|---|---|
| | Isoleucine | Alloisoleucine | Isoleucine | Alloisoleucine |
| L-Isoleucine | | 9.4 | | 12.8 |
| L-Alloisoleucine | 1.3 | | 1.0 | |

[a] Determined by the decrease in specific activity after base exchange.

**Table V.** Amount of Each Identified Product Formed From the Labeling of L-Isoleucine (Ile)

| Compd | $\mu$Ci produced | % each isomer[b] | $\mu$Ci formed/mg Ile labeled |
|---|---|---|---|
| L-Isoleucine | 14.13 | 72.2 | 3.62[a] |
| D-Isoleucine | 0.22 | 1.1 | 0.06 |
| L-Alloisoleucine | 4.56 | 23.3 | 1.17 |
| D-Alloisoleucine | 0.67 | 3.4 | 0.17 |
| Glycine | 4.41 | | 1.13 |

[a] Also represents specific activity of parent L-isoleucine. [b] ($\mu$Ci in isomer/$\Sigma$ $\mu$Ci in each of the four possible stereoisomers) $\times$ 100.

**Table VI.** Amount of Each Identified Product Formed from the Labeling of L-Alloisoleucine (Alloile)

| Compd | $\mu$Ci produced | % each isomer[b] | $\mu$Ci formed/mg Alloile labeled |
|---|---|---|---|
| L-Alloisoleucine | 50.71 | 67.6 | 14.01[a] |
| D-Alloisoleucine | 2.25 | 3.0 | 0.62 |
| L-Isoleucine | 21.83 | 29.1 | 6.03 |
| D-Isoleucine | 0.23 | 0.3 | 0.06 |
| Glycine | 1.69 | | 0.47 |

[a] Also represents the specific activity of parent L-alloisoleucine. [b] ($\mu$Ci in isomer/$\Sigma$ $\mu$Ci in the four possible stereoisomers) $\times$ 100.

$\alpha$ abstraction or abstraction from the rest of alkyl side chain. This specificity for $\beta$ attack is also consistent with the rates of H-atom reactions with aliphatic amino acids in solution.[18,22] Amino acids with a tertiary carbon reacted most rapidly, with the rate falling off as one goes to amino acids with secondary or only primary carbon atoms in the alkyl side chain (val, ile, leu > norval > ala).

There are, however, other factors to be considered which cannot be quantitated, the main factor being the effect of the crystal structure, since these reactions take place on a solid crystalline surface of the amino acid. These factors may influence both the availability of various hydrogens in the amino acid to attack by H atoms (or tritium atoms) as well as the fate of the radical once formed; i.e., its disposition toward recombination (radical stability) as well as the ability of the recombined excited amino acid to redistribute the exothermicity, leading to a stable labeled product. The effect of charge and the influence of the functional groups may also play an unknown role, since this is certainly the case in solution.[22]

One result which may be a manifestation of crystal structure differences is the difference in specific activity between isoleucine and alloisoleucine, in which alloisoleucine is favored by nearly a factor of 4 (Tables V and VI). In this case other effects are probably unimportant in the actual amount of tritium incorporation due to the structural similarity of the diastereomers. This is borne out since the relative labeling patterns and diastereomer formation are very similar, and are significantly different only in absolute yields.

Although labeling at the $\alpha$ carbon is small in both cases (<8%) the stereochemical result is clear. The labeling of L-isoleucine favored retention by a factor of 2 at the $\alpha$ carbon (68/32), whereas little or no reaction at the $\alpha$ carbon occurred in the labeling of L-alloisoleucine (Table III).

**Table VII.** Bond Dissociation Energy $E_a$ and Rate Constants for Hydrogen Abstraction from Primary, Secondary, and Tertiary Carbons in Simple Alkanes

| Bond | Bond dissociation energy,[24,25] kcal/mol | $E_a$ (H·),[26] kcal/mol | H abstraction rate constants,[27] $cm^3\ mol^{-1}\ s^{-1}$ |
|---|---|---|---|
| $C_2H_5$-$CH_3$(p) | 100 | 9-12 | $0.25 \times 10^9$ |
| $(CH_3)_2$-$CH_2$(s) | 94.5 | 7-8 | $1.10 \times 10^9$ |
| $CH_3)_3$-CH(t) | 89 | 5-6 | $7.75 \times 10^9$ |

This result is in contrast with equilibration of isoleucine and alloisoleucine in solution by both acid[28] and base.[29] In acid, the equilibrium constant for isoleucine, alloisoleucine epimerization was 1.29, giving an observed equilibrium mixture of 44% isoleucine and 56% alloisoleucine. In these studies we have found the equilibrium mixture by base treatment resulted in 43.4% isoleucine and 56.7% alloisoleucine, again in favor of alloisoleucine formation.

**Glycine Formation.** Formation of glycyl radicals by reaction of H atoms with polyamino acids by alkyl side chain cleavage has been proposed in several systems.[12,14,15] However, formation of glycyl radicals from free amino acids other than glycine itself has not yet been reported. The formation of glycine may result from the excitation decomposition of the intermediate formed by the recombination of the $\alpha$ (or $\beta$) radical with tritium (Scheme I, reaction 5).

Other modes of decomposition are also available for the above excited intermediate, such as decarboxylation (eq 3) or deamination (eq 4). Products from these modes of fragmentation (1-amino-2-methylbutane, 3-methylpentonoic acid) may be present in the reaction mixture. Further investigation would be needed to elucidate these possibilities.

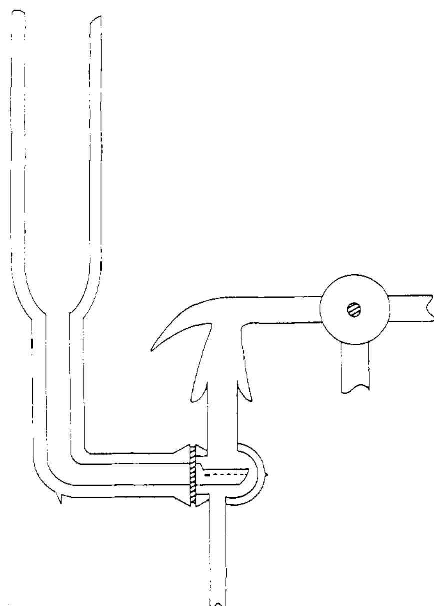

Figure 2. Modified tritium labeling system.


## Summary

By the use of tritium atoms to investigate H-atom reactions the products and stereochemical course of the labeling reaction of tritium atoms with two amino acids have been determined. From this information insight has also been gained into the nature of the product forming radicals as well as possible reactions of hydrogen atoms in vivo. The results indicate with both L-isoleucine and L-alloisoleucine that $\beta$-hydrogen abstraction greatly predominates over abstraction of hydrogen at the $\alpha$ carbon. Recombination of the resulting radicals with tritium may lead to labeled parent, to the diastereomer, or to fragmentation products such as glycine. Although the product distribution for each amino acid was qualitatively similar, quantitative differences were observed which may be attributable to structural effects.

The stereochemical implications of these results as applied to both tritium labeling as well as the reactions of H atoms in vivo are promising. Since the extent of labeling at the chiral $\alpha$ carbon is small, problems arising from conformational changes due to the formation of peptide diastereomers by $\alpha$-carbon inversion may be minimal. Reaction (and/or labeling) at tertiary carbons on the other hand would be very desirable, since these positions are stable to exchange in biological systems and, except in rare cases where the amino acid has two chiral centers (e.g., isoleucine, threonine and hydroxyproline), the stereochemistry of these positions are unimportant.

The investigation of tritium atom reactions with biologically important molecules has been extended to peptides (synthetic as well as natural). Several important observations have already been made:[23] (1) $\alpha$-carbon labeling is again a minor process (<10%); (2) diastereomeric amino acids were formed predominantly by reaction at the $\beta$ carbon (75% $\beta$, 25% $\alpha$); (3) the specific activities of labeled peptides (and their component amino acids after hydrolysis) were in *all* cases investigated 100–1000-fold greater than those obtained by the labeling of the free amino acids. It is thought that this observation may reflect a basic physicochemical difference between the structure of amino acids and peptides. Quantitation and studies designed to explain this startling result are in progress.[23]


## Experimental Section

**Materials.** L-Isoleucine (allo-free) and L-alloisoleucine as purchased from Fox Chemical Co. were optically pure by GLC, Dowex chromatography, and optical rotation. Amino acids were chromatographed on Dowex 50W-X8 (200–400 mesh) using pyridine–acetate buffers. Pyridine was distilled from KOH–ninhydrin mixture before use. Triethylamine, used in the formation of GLC derivatives, was distilled from KOH, $\alpha$-naphthylisocyanate mixture before use. Tritium was purchased from Oak Ridge National Laboratory.

**Labeling Apparatus.** All labeling reactions were carried out in a reaction system previously described,[30] with the following modification: light traps were installed at a right angle bend 5 cm above the reaction area to eliminate short wavelength UV light from reaching the sample (Figure 2).

**Labeling of Isoleucine.** L-Isoleucine (3.90 mg, 0.0297 mmol) was placed onto a 8 × 10 mm glass reaction tray. After being under vacuum for 1 h it was exposed to 1 Ci of pure tritium gas at 4 mmHg pressure for 5 min with liquid nitrogen cooling. The tritium discharge was sustained by a microwave power of 20 W. The tritium gas cycling pump was pulsing at 175 cpm.

After the exposure the tritium gas was removed and the isoleucine remained under vacuum for an additional hour to remove "volatile" tritium.

Upon removal of the isoleucine from the reaction system, carrier L-alloisoleucine (1.68 mg, 0.0128 mmol), D-alloisoleucine (1.71 mg, 0.0130 mmol), and glycine (2.54 mg, 0.0338 mmol) were added prior to purification.

Purification was carried out on three successive ion exchange columns using Dowex 50W-X8 (200–400 mesh) with a pyridine–acetate buffer (0.1 M, pH 3.15).

**Labeling of L-Alloisoleucine.** L-Alloisoleucine (3.62 mg, 0.0276 mmol) was labeled in the same manner as L-isoleucine. After the reaction L-isoleucine (1.81 mg, 0.0138 mmol) and glycine (2.11 mg, 0.0281 mmol) were added as carriers prior to purification. Purification was the same as for L-isoleucine.

**Base Exchange of Isoleucine and Alloisoleucine.** The amino acid sample (1–2 $\mu$Ci) was equilibrated in 2 mL of 4 N $Ba(OH)_2$ at 110 °C.[31] The recovered amino acid was acidified (pH 2) with dilute HCl and chromatographed as above. The amino acid peak was then pooled and the specific activity of the $\alpha$ carbon exchanged amino acid was determined. This method was also used to determine the equilibrium mixture of isoleucine–alloisoleucine under base exchange conditions.

**Preparation of Amino Acid Methyl Esters (I).** A solution of the amino acid containing 500 $\mu$g was lyopholized in a small reaction vessel and esterfied in methanol (2 mL) saturated with anhydrous HCl at 0 °C.

**Preparation of *N*-(L-2-Chloropropionyl)amino Acid Methyl Esters (II).** The above amino acid methyl ester HCl (I) was dissolved in 1 mL of $CHCl_3$ and placed in a small screw cap vial. The sample was cooled in an ice bath at 0 °C and L-2-chloropropionyl chloride[32,33] (25 $\mu$L) and triethylamine (50 $\mu$L) were added and stirred at room temperature. After 5 min methanol (25 $\mu$L) was then added with continued vigorous stirring. The sample was then washed with water and dried over sodium sulfate prior to GLC analysis.

**GLC Analysis of *N*-(L-2-Chloropropionyl) Methyl Ester Derivatives of Isoleucine, Alloisoleucine, and Glycine.**[33] Analyses were carried out on a 1/8 in. × 12 ft 10% Carbowax 20 M Chromosorb-W (HMDS) column at a flow rate of 37 mL/min and a column temperature of 170 °C. Under these conditions a single analysis could be done in <25 min with nearly a baseline separation of the diastereomers.

The alloisoleucine diastereomers (LD, LL) had retention times 19.1 and 21.1 min, respectively,[34] with a resolution of 1.97.[35] Isoleucine diastereomers (LD, LL) had retention times of 20.1 and 22.1 min[34] with a resolution of 1.16.[35] The glycine derivative had a retention time of 23 min under similar conditions.

The samples were collected in a 1/4 × 2 in. glass tube packed with glass wool. The glass wool was then removed, placed in a liquid scintillation vial, and counted.

**Determination of Percent D and L in the Labeled Amino Acids.** Prior to this calculation, the optical purity of the resolving reagent, L-2-chloropropionyl chloride was determined by GLC of its derivative formed with optically pure L-valine, L-isoleucine, L-alloisoleucine, and L-alanine. Peak area analysis of the two diastereomeric peaks (LD, LL) were carried out on a Dupont 310 curve resolver. The L-2-chloropropionyl chloride was determined to contain 3.75% D and 96.25% L. The following equation was used to determine % D, L composition of the labeled amino acids and corrects for the percent (3.75% D) impurity in the resolving reagent.

$$A = 50(A \text{ apparent} - k)/(50 - k)$$

where $A$ is the percent isomer to be determined, $A$ apparent = (cpm in peak $A$/cpm $A$ + cpm $B$) × 100, and $k$ is percent optical impurity in the resolving reagent (3.75).[34]

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### References and Notes

(1) (a) Brookhaven National Laboratory; (b) Columbia University.
(2) (a) D. V. Bent and E. Hayon, *J. Am. Chem. Soc.*, **97**, 2599 (1975); (b) *ibid.*, **97**, 2606 (1975).
(3) R. Livingston, D. G. Doherty, and H. Zeldes, *J. Am. Chem. Soc.*, **97**, 3198 (1975).
(4) H. Taniguchi et al., *J. Phys. Chem.*, **72**, 1926 (1968).
(5) N. N. Lichtin and A. Schafferman, *Radiat. Res.*, **69**, 432 (1974).
(6) N. N. Lichtin, J. Ogdan, and G. Stein, *Radiat. Res.*, **55**, 69 (1973).
(7) H. Jung and K. Kurzinger, *Radiat. Res.*, **36**, 369 (1968).
(8) V. Nothig-Laslo and J. N. Herak, *Croat. Chem. Acta*, **43**, 39 (1971).
(9) W. Snipes and J. Schmidt, *Radiat. Res.*, **29**, 194 (1966).
(10) H. Jensen and T. Henriksen, *Acta Chem. Scand.*, **22**, 2263 (1968).
(11) H. Shields and W. Gordy, *J. Phys. Chem.*, **62**, 789 (1958).
(12) F. G. Liming, Jr., *Radiat. Res.*, **39**, 252 (1969).
(13) F. G. Liming and W. Gordy, *Proc. Natl. Acad. Sci. U.S.A.*, **60**, 794 (1968).
(14) R. C. Drew and W. Gordy, *Radiat. Res.*, **18**, 552 (1963).
(15) G. McCormick and W. Gordy, *J. Phys. Chem.*, **62**, 783 (1958).
(16) P. Riesz and F. H. White, *Adv. Chem. Ser.*, **No. 81**, 496–520 (1968).
(17) R. Holroyd, J. Glass, and P. Riesz, *Radiat. Res.*, **44**, 59 (1970).
(18) P. Neta, *Chem. Rev.*, **72**, 533 (1972); P. Neta and R. H. Schuler, *Radiat. Res.*, **47**, 612 (1971).
(19) P. Neta, R. W. Fessenden, and R. H. Schuler, *J. Phys. Chem.*, **75**, 1654 (1971).
(20) P. Neta, M. Simic, and E. Hayon, *J. Phys. Chem.*, **74**, 1214 (1970).
(21) R. A. Witter and P. Neta, *J. Org. Chem.*, **38**, 484 (1973).
(22) W. A. Volkert and R. R. Kuntz, *J. Phys. Chem.*, **72**, 3394 (1968).
(23) A. P. Wolf, S. Lieberman, W. C. Hembree, and R. L. E. Ehrenkaufer, manuscript in preparation.
(24) J. A. Kerr, *Chem. Rev.*, **66**, 465 (1966).
(25) A. Lieberles, "Introduction to Theoretical Organic Chemistry", Macmillan, New York, N.Y., 1968, p 220.
(26) A. F. Trotman-Dickenson and G. S. Milne, "Tables of Bimolecular Gas Reactions", *Natl. Stand. Ref. Data Ser., Natl. Bur. Stand.*, **No. 9**, 5–7, 41–49 (1967).
(27) T. J. Hardwick, *J. Phys. Chem.*, **66**, 117 (1962).
(28) S. Nakapapksin, E. Gil-Av, and J. Oro, *Anal. Biochem.*, **33**, 374 (1970).
(29) W. C. Hembree, R. L. E. Ehrenkaufer, and A. P. Wolf, unpublished results.
(30) W. C. Hembree, R. E. Ehrenkaufer, S. Lieberman, and A. P. Wolf, *J. Biol. Chem.*, **248**, 5532 (1973).
(31) S. Blackburn, "Amino Acid Determination. Methods and Techniques", Marcel Dekker, New York, N.Y., 1968, pp 23–24.
(32) (a) M. Renard, *Bull. Soc. Chim. Biol.*, **28**, 497 (1946); (b) S.-C. J. Fu, S. M. Birnbaum, and J. P. Greenstein, *J. Am. Chem. Soc.*, **76**, 6058 (1954); (c) H. C. Brown, *ibid.*, **60**, 1325 (1938); (d) W. Fickett, H. K. Gardner, and H. J. Lucas, *ibid.*, **73**, 5063 (1951).
(33) (a) B. Halpern and J. W. Westley, *Chem. Commun.*, 256 (1965); (b) B. Halpern, J. W. Westley, and B. Weinstein, *Nature (London)*, **210**, 837 (1966).
(34) R. Pettijohn, Dissertation, University of Nebraska, 1973, pp 131–158 (University Microfilms No. 74-13 011).
(35) $R = 2d/(W_1 + W_2)$ where $d$ is the peak-to-peak separation of the diastereomers and $W_1$ and $W_2$ are extrapolated to baseline, peak widths. $R$ = 1 corresponds to approximately 98% resolution; $R$ = 1.5 indicates a baseline separation with a resolution of 99.7%.[36] These values represent optimized resolutions. The actual resolution may vary slightly from one injection to the next.
(36) H. M. McNair and E. J. Bonelli, "Basic Gas Chromatography", 5th ed, Varian Aerograph, Walnut Creek, Calif., 1968.

# Thiosilanes, a Promising Class of Reagents for Selective Carbonyl Protection

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**Abstract:** The thermal and catalyzed carbonyl addition reactions of alkyl- and arylthiosilanes, $RSSiMe_3$, have been studied. Contrary to earlier literature reports, it has been found that thiosilanes react with aldehydes and ketones to form either thioketals or $O$-silylhemithioketals when various acid catalysts are employed. With $\alpha,\beta$-unsaturated ketones and aldehydes anion-initiated reactions result in exclusive 1,4-addition. The synthetic procedures reported in this paper constitute an exceptionally mild procedure for selective carbonyl protection.

In recent years organosilicon derivatives have played an ever increasing role in synthetic organic chemistry.[2] Much of the logic behind the development of organosilane reagents has relied upon the "proton–silicon correlation". For example, organosilanes undergo a number of thermal rearrangements[3] which phenomenologically have their direct counterparts in analogous proton systems.[4] A number of other processes such as olefin hydrosilylation,[5] carbonyl silicon pseudohalide[6] addition,[7] and silicon transfer to Lewis bases[8] are just a few of the other reactions of tetravalent silicon for which the proton analogy can be drawn. Organosulfur derivatives of silicon have been extensively studied over the years and a multitude of methods have been developed for their synthesis.[9,10] Based upon the relatively weak silicon–sulfur bond (ca. 70 kcal/mol)[11] these reagents should be good oxygenophiles. However, little definitive work has been reported on the applications of these reagents to useful synthetic transformations.[12]

The aims at the outset of this study were to develop organosilicon reagents that would selectively mask carbonyl groups under exceptionally mild conditions (eq 1-3). In part, our in-