[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF MICHIGAN]

The Isomerization of Alkyl Thiocyanates to Isothiocyanates

BY PETER A. S. SMITH AND DAVID W. EMERSON[1]

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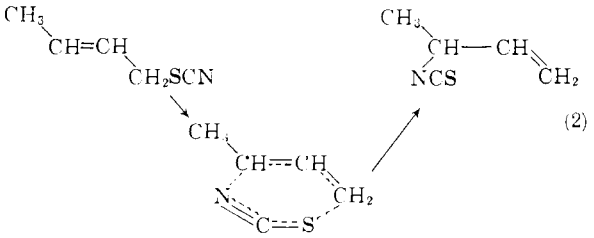
The rates of rearrangement of several thiocyanates have been found to be first order. Allyl and 2-methylallyl thiocyanates rearrange at the same rate in solvents of various polarity, and are believed to pass through a cyclic intermediate in which the thiocyanate group is not detached from the alkyl group. Cinnamyl thiocyanate, on the other hand, rearranges much more difficultly, and the rate is enhanced by an increase in the polarity of the solvent. Saturated thiocyanates could not be rearranged quantitatively, but their qualitative rates increased in the order of increasing stability of the carbonium ion derived from the alkyl group, and were enhanced by increased polarity of the environment. Cinnamyl and saturated thiocyanates are believed to rearrange by ionization and recombination.

The isomerization of alkyl thiocyanates to isothiocyanates (eq. 1) has been known since 1875, when Gerlich[2] and Billeter[3] independently observed the isomerization of allyl thiocyanate during distillation.

$$RSCN \longrightarrow RNCS \qquad (1)$$

The isomerization of methyl[4] and benzyl[5] thiocyanates was observed later, but much higher temperatures were required, and in more recent years the thermal isomerization of various other thiocyanates, both saturated and unsaturated, has been reported.[6] Catalysis of the isomerization by metal salts, such as cadmium iodide[7] and zinc chloride,[8] and by strong acids[8] also has been observed.

The mechanism of the isomerization was first discussed by Billeter,[9] who suggested a cyclic, intramolecular process for allylic thiocyanates. Evidence in support of this view was provided by Mumm and Richter's observation that crotyl thiocyanate isomerizes not to crotyl but to α-methylallyl isothiocyanate[10] (eq. 2), and that very



(2)

probably γ-ethylallyl thiocyanate isomerizes to α-ethylallyl isothiocyanate and *vice versa*. Such a path cannot account for the isomerization in saturated systems, of course, nor for the fact that cinnamyl thiocyanate isomerizes without allylic shift. The work we now describe was undertaken to provide additional information pertinent to the mechanism with the aim of reconciling the foregoing observations.

(1) From the doctoral dissertation of D. W. Emerson; Allied Chemical and Dye Corporation Fellow, 1955–1956.

(2) G. Gerlich, *Ann.*, **178**, 80 (1875).

(3) O. Billeter, *Ber.*, **8**, 462 (1875).

(4) A. W. Hofmann, *ibid.*, **13**, 1349 (1880).

(5) H. Hennicke, *Ann.*, **344**, 24 (1906).

(6) *E.g.*, (a) F. D. Jones, U. S. Patent 2,394,915 (*C. A.*, **40**, 2261 (1946)); (b) R. Bergmann, *J. Chem. Soc.*, 1361 (1935), and ref. 5.

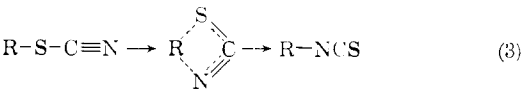
(7) A. Smits and H. Vixseboxe, *Verslag K. Akad. Wetenschappen*, 46 (1913); *C. A.*, **8**, 649 (1914); J. Gillis, *Rec. trav. chim.*, **39**, 330 (1920).

(8) E. Schmidt, W. Striewsky, M. Seefelder and F. Hitzler, *Ann.*, **568**, 192 (1950).

(9) O. Billeter, *Helv. Chim. Acta*, **8**, 337 (1925).

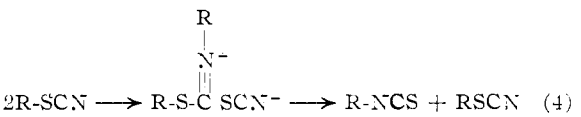
(10) O. Mumm and H. Richter, *Ber.*, **73**, 843 (1940).

In addition to the intramolecular path for rearrangement involving an allylic shift, there are other paths with sufficient *a priori* probability to warrant consideration. One such path is an analog of the Chapman rearrangement of imino esters, which has been shown to be an intramolecular process.[11] It would involve a four-membered ring transition state, and would preclude an allylic shift (eq. 3). Another conceivable path involves bimolecular displacement, which may be either



(3)

S$_N$2 or S$_N$2′, by the nitrogen of one molecule on the α-carbon of another, followed by a second displacement by the thiocyanate ion first produced (eq. 4). Yet another possibility is ioniza-



(4)

$$R\text{-}SCN \rightleftarrows R^+ + SCN^- \longrightarrow R\text{-}NCS \qquad (5)$$

tion and recombination (eq. 5). Finally, the analogous homolytic cleavage into alkyl and thiocyanogen radicals followed by recombination would accomplish the change, as might an α-elimination to a carbene and thiocyanic acid.

We originally planned to measure the rates of isomerization of thiocyanates of representative structural types in a variety of media and over temperature ranges sufficient to allow the estimation of thermodynamic characteristics. This plan was in part achieved for allyl and β-methallyl (2-methylallyl) thiocyanates but, for reasons that will become apparent, could not be extended quantitatively to saturated systems. The course of the reactions was followed by measuring the appearance of alkyl isothiocyanate titrimetrically.[12]

Allyl and β-Methallyl (2-Methylallyl) Thiocyanates.—The rearrangements were carried out in toluene, nitrobenzene and dimethylformamide as solvents. Only toluene gave essentially quantitative conversion. In the others, side-reactions reduced the conversion to the range of 86–92% and correspondingly affected the reliability of the kinetic data; the first-order constants calculated from the measurements showed only small varia-

(11) A. W. Chapman, *J. Chem. Soc.*, **127**, 1992 (1925); 1743 (1927); K. B. Wiberg and B. I. Rowland, THIS JOURNAL, **77**, 2205 (1955).

(12) F. Wild, "Estimation of Organic Compounds," Cambridge University Press, 1953 pp. 201–202.

tion up to conversions of about 70%, after which pronounced drifts to lower values generally occurred.

The results for allyl and β-methallyl thiocyanates in toluene are summarized in Table I. The rearrangements obeyed first-order kinetics over a temperature range from 57.8 to 86.4°. The energies and entropies of activation calculated from these data also are given.

TABLE I

ISOMERIZATION OF ALLYL AND 2-METHYLALLYL THIOCYANATES IN TOLUENE

	Allyl		2-Methylallyl	
T, °C.	C_0, mole/l.	k_1, hr.$^{-1}$	C_0, mole/l.	k_1, hr.$^{-1}$
86.4	0.832	1.37 ± 0.02	0.713	1.66 ± 0.07
	.418	1.36 ± .02	.356	1.66 ± .05
	.209	1.39 ± .03	.178	1.67 ± .02
80.9	.413	0.84 ± .02	.705	1.01 ± .01
	.209	.81 ± .03	.352	1.00 ± .01
68.2	.833	.225 ± .008	.715	0.279 ± .008
	.423	.224 ± .005	.355	.281 ± .003
	.206	.221 ± .006	.178	.277 ± .003
57.8	.828	.077 ± .001	.705	.089 ± .001
	.423	.077 ± .001	.354	.088 ± .002
	.208	.082 ± .005	.179	.091 ± .004
E_a, kcal./mole		23.8 ± .2		24.2 ± .2
S^*, e.u.		−9.4 ± 1		−8.7 ± 1

The results of the reactions carried out in nitrobenzene and dimethylformamide are given in Table II. Although the measurements lack precision, useful information can be drawn from them also. First-order kinetics were obeyed, and the rate constants were not significantly different from those in toluene. The solubility of inorganic salts in dimethylformamide allowed the effect of a further alteration in reaction medium to be studied; added salts did not appear to influence either the conversion or the rate, even when the salt was itself a thiocyanate.

TABLE II

EFFECT OF THE MEDIUM ON THE RATE OF ISOMERIZATION OF ALLYL THIOCYANATE AT 68.2°

Medium	Initial concn., mole/l.	k, hr.$^{-1}$
Toluene	Various	0.223
Nitrobenzene	0.826	.233[a] ± 0.008
Dimethylformamide	.408	.199[b] ± .004
0.40 M KSCN in dimethylformamide	.404	.193[c] ± .003
0.398 M KI in dimethylformamide	.404	.190[d] ± .007

[a] To 84.4% of completion. [b] To 66.5% of completion. [c] To 62.9% of completion. [d] To 64.4% of completion.

These observations are most consistent with the intramolecular process (eq. 2). All the paths but eq. 4 are consistent with the observed first-order kinetics, but the relative insensitivity of the rate to changes in the reaction medium is not in accord with the formation of ions in the rate-determining step. Another departure from implications of an ionization path is that the product from crotyl thiocyanate is largely if not entirely α-methylallyl isothiocyanate.[10] First-order displacements on crotyl chloride or α-methylallyl chloride, such as by ethoxide ion, give predominantly crotyl derivatives.[13] In support of eq. 2, the observed entropies of activation suggest a high degree of order in the transition state, such as occurs in the internal, cyclic processes. In this connection it is significant to note that the entropies of activation are of the same sign and order of magnitude as those reported for the first-order, gas-phase decompositions of several *gem*-diacetates and for acetic anhydride, and for the Claisen rearrangement of allyl vinyl ether, all of which are thought to proceed through pseudo-six-membered-ring transition states.[14] They do not, however, allow a differentiation between four-membered- and six-membered-ring transition states.

The analogy of the Chapman rearrangement is of course inconsistent with the allylic shift observed with crotyl thiocyanate (although it is not thereby ruled out for other examples). Furthermore, such a path would be expected to impose a considerably higher activation energy than we have observed, owing to the marked distortion of the linear thiocyanate system demanded for formation of the pseudo-four-membered-ring transition state.

While homolytic cleavage could conform to the observed first-order kinetics if it were followed by a simple recombination, a chain process (eq. 7) would be more likely. Furthermore, the presence of free radicals would be expected to lead, in the case of unsaturated alkyl groups, to side-products

$$\mathrm{R{-}SCN} \longrightarrow \mathrm{R\cdot} + \mathrm{\cdot SCN} \qquad (7)$$
$$\mathrm{R\cdot} + \mathrm{RSCN} \longrightarrow \mathrm{RNCS} + \mathrm{R\cdot}$$

resulting from olefinic polymerization and from addition of thiocyanogen to the ethylenic double bonds. We have found no evidence for such products when the isomerizations were carried out in non-polar solvents. The absence of high-boiling side-products was demonstrated by the complete evaporation on a steam-bath of a reaction mixture obtained from the isomerization of a sample of 2-methylallyl thiocyanate.

Cinnamyl Thiocyanate.—The isomerization of cinnamyl thiocyanate shows significant differences from that of allyl. Its outstanding difference is that allylic shift does not occur, and the product is cinnamyl isothiocyanate,[6b] instead of α-phenylallyl isothiocyanate as would have been expected from analogy with allyl thiocyanate. Our efforts to obtain kinetic measurements for this example disclosed another difference; the isomerization is much slower than that of allyl thiocyanate, the rate constant at 153° being of the same order of magnitude as that of 2-methylallyl thiocyanate at 68°, and the conversion is poor (maximum 78%) owing to the formation of much tar. This characteristic prevented us from determining accurate kinetics for the reaction, but it was nevertheless possible to show that the rate is roughly first-order and is sensitive to the nature of the solvent, being nearly ten times faster in diphenylmethane or nitrobenzene than in decalin. The isomeriza-

(13) A. A. Catchpole and E. D. Hughes, *Trans. Faraday Soc.*, **37**, 629 (1941); *J. Chem. Soc.*, 4 (1948).

(14) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," John Wiley and Sons, Inc., New York, N. Y., 1953, Table 4, pp. 104–105.

tion is catalyzed by zinc chloride, which brought about slow reaction even at room temperature, with an ultimate conversion of 82%.

The foregoing observations suggest that cinnamyl thiocyanate isomerizes by a different path from allyl or 2-methylallyl thiocyanates. The dependence on solvent and catalysis by zinc chloride suggest that in this case ionization (presumably largely only to ion pairs) and recombination are involved. It is understandable that intramolecular rearrangement with allylic shift should not occur in this case, for such a path would be opposed by the gain in potential energy in converting the conjugated cinnamyl group to the non-conjugated α-phenylallyl group. The ionization path is not hindered by this consideration, for recombination of the ions can occur so as to produce the conjugated cinnamyl system, as has indeed been observed in the solvolysis of other cinnamyl and α-phenylallyl derivatives.[15] Such a path would also be favored by the considerably greater tendency for cinnamyl compounds to undergo SN1 solvolysis compared to allyl compounds.[16] Since we do not know whether the kinetic order for cinnamyl thiocyanate is first order, the possibility of some contribution from bimolecular displacement (eq. 4) must be considered, particularly inasmuch as such a process is also consistent with the observed influence of solvents. This path should be seriously interfered with by the introduction of a more strongly nucleophilic nitrogen atom than the rather weak one in the thiocyanate group. When the isomerization was carried out in the presence of tri-*n*-butylamine in nitrobenzene solution under conditions where isomerization would be essentially completed, the ratio of thiocyanate ion produced (presumably quaternary ammonium thiocyanate) to the amount of isomerization occurring in the absence of amine was 1:6. This ratio is very small relative to the large difference in nucleophilicities, and it thus seems unlikely that the displacement of thiocyanate ion by the nitrogen of cinnamyl thiocyanate as part of eq. 4 could be contributing significantly to the isomerization.[17] The ratio is larger than that observed for allyl thiocyanate, 1:14 under conditions similar except for temperature, but this is to be expected in view of the greater reactivity of cinnamyl halides in both SN1 and SN2 processes.[16]

Saturated and Benzylic Thiocyanates.—We also investigated *n*-butyl, *sec*-butyl, *t*-butyl, benzyl, α-phenylethyl and α-naphthylmethyl thiocyanates. With none of these compounds could a clean enough reaction be obtained to allow accurate kinetic measurements, although α-phenylethyl thiocyanate approached this requirement under catalysis by zinc chloride. Conversions were generally low, and usually there was much tar formed. On the other hand, qualitative comparisons of ease of isomerization can be made from the observations.

n-Butyl thiocyanate could not be made to isomerize at all. *sec*-Butyl thiocyanate was isomerized by boiling with anhydrous zinc chloride, either neat or in nitrobenzene solution, slowly and with low conversion (29% maximum observed). *t*-Butyl thiocyanate isomerizes more readily than this, as has been reported by Schmidt, *et al.*[8] It is evident that this is the order of stability of the carbonium ions corresponding to the respective alkyl groups.

Benzyl thiocyanate isomerized when heated with zinc chloride in nitrobenzene with something of the same ease as *sec*-butyl thiocyanate, as would be expected if carbonium ion formation is important. Both α-naphthylmethyl and α-phenylethyl cations in comparable reactions show greater stability or ease of formation than benzyl. Our findings are consistent with this, for we found that α-naphthylmethyl thiocyanate isomerized more readily, giving a 62% conversion in 20 hours at 50°, and that α-phenylethyl thiocyanate is almost completely isomerized in four days at room temperature or four hours at 100°, under the influence of zinc chloride.

The intermediate formation of carbonium ions during the isomerizations suggests the possibility of diverting the reaction along another path by exposure to a reagent capable of competing with thiocyanate ion for carbonium ions. However, in the absence of comparative rate data, we cannot tell whether the products that come from such diversion experiments actually result from the interception of an intermediate essential to the isomerization, or instead arise from the starting material by an independent path. Nevertheless, such diversion experiments have interest, for the formation of interception products is a consequence to be expected of the ionization path, and their complete absence would be evidence against such a mechanism.

The formation of quaternary ammonium thiocyanate, part of which may have arisen by an SN1 process, when the rearrangement of cinnamyl thiocyanate was carried out in the presence of tri-*n*-butylamine has already been mentioned. In a similar experiment with *n*-butyl thiocyanate, which does not rearrange, no detectable amount of thiocyanate ion was produced, but since the ratio of the SN2 reactivity of cinnamyl to butyl chlorides, as manifested toward ethoxide ion, is over 200,[16] this observation is of limited significance.

When a mixture of *t*-butyl thiocyanate, zinc chloride and phenol was heated under conditions known to effect the isomerization of the thiocyanate, *p*-*t*-butylphenol was indeed formed, among other products. However, both thiocyanates[18] and isothiocyanates[19] are known to react with phenols in the presence of zinc chloride to produce, respectively, imino thioester and thioamide derivatives of hydroxylbenzoic acids. That one or both of these side reactions also occurred in our experiment is shown by the fact that hydrolysis of the

(15) A. A. Catchpole and E. D. Hughes, *J. Chem. Soc.*, 1 (1948).

(16) C. A. Vernon, *ibid.*, 423, 4462 (1954).

(17) It is recognized that a large part of the observed thiocyanate ion may have arisen from the combination of cinnamyl cation, formed by unimolecular ionization, wth tri-*n*-butylamine. Also, at the temperature used (144°), there may have been regeneration of cinnamyl thiocyanate by bimolecular displacement between the quaternary ammonium ion and thiocyanate ion.

(18) R. J. Kaufman and R. Adams, THIS JOURNAL, **45**, 1744 (1923).

(19) H. Rivier and S. Kunz, *Helv. Chim. Acta*, **15**, 376 (1932).

reaction mixture yielded some *p*-hydroxybenzoic acid.

While the evidence as a whole supports the unimolecular ionization path for the isomerization of all but simple allylic thiocyanates, we should not accept this path without first considering whether the behavior of carbonium ions required in it is consistent with the characteristics of carbonium ions formed in other reactions. In particular, the mechanism requires that carbonium ions combine with thiocyanate ion to give sensible amounts of isothiocyanates. That this requirement is met has been shown recently by Taft and Cannell,[20] who found that isobutyl, *t*-butyl and benzhydryl cations, generated in various ways, combined with thiocyanate ion in such a way that 11 to 35% of the product had the isothiocyanate structure. Triphenylmethyl halides significantly gave rise exclusively to isothiocyanate; rearrangement of any thiocyanate formed would of course be expected to be rapid with a group that can exist as a carbonium ion as readily as can triphenylmethyl. Another example of this behavior exists in the reaction of acid chlorides and metal thiocyanates. The products, heretofore assumed to be thiocyanates ("rhodanides"), are apparently isothiocyanates; we found that the infrared spectra of the compounds obtained from benzoyl and 3,4,5-trimethoxybenzoyl chlorides and lead thiocyanate show wide, strong absorption bands in the 1940–2000 cm.$^{-1}$ region, characteristic of the isothiocyanate structure, and none in the 2130–2160 cm.$^{-1}$ region, where thiocyanates absorb.[21]

Methyl thiocyanate, which has been reported[7] to rearrange, albeit slowly, at its boiling point (131°), especially when assisted by dissolved salts, may be a special case. The intermediate formation of methyl cations, methyl radicals or carbene (CH_2) is energetically sufficiently unlikely that it does not appear probable that rearrangement proceeds through any of them. We nevertheless attempted to detect the possible formation of carbene by carrying out the rearrangement at temperatures up to 200° in cyclohexene solution, which should give rise to thiocyanic acid to the extent that carbene is intercepted. The spectrum of the resulting solution showed the absence not only of thiocyanic acid, but also of methyl isothiocyanate. It thus appears that the rearrangement that occurs readily in the neat compound is quite prevented by the relatively non-polar solvent. The bimolecular path (eq. 4), which would require a polar environment, would be especially favored by the lack of steric hindrance about the carbon atom. The fact that *n*-butyl thiocyanate appears to rearrange much more difficultly than methyl thiocyanate is consistent with the concept that saturated primary thiocyanates rearrange, if at all, by a bimolecular displacement.

The catalytic influence of zinc chloride (and other heavy metal halides) on thiocyanate isomerizations can be understood in terms of either the bimolecular or the unimolecular ionization paths, inasmuch as it would, by complex formation or similar association, promote the separation of thiocyanate ion. In those isomerizations carried out at 100° or below, the catalytic activity remained essentially unchanged throughout a run, but with reactions at higher temperatures the catalytic activity fell markedly, evidently owing to destruction of zinc chloride by secondary reactions as shown by the formation of precipitates of zinc sulfide. In such cases, the catalytic activity could be temporarily restored by addition of fresh zinc chloride.

The thiocyanates used in this work were prepared by the general method of reaction of an alkyl halide with a metal or ammonium thiocyanate, but carried out under conditions modified so as to minimize isomerization during preparation. In addition, *p*-nitrocinnamyl thiocyanate was prepared, but no kinetic measurements were made with it owing to the unsatisfactory results from cinnamyl thiocyanate.

Experimental

Preparation of Thiocyanates.—Allyl and 2-methylallyl thiocyanates were prepared from allyl bromide or 2-methylallyl chloride, sodium thiosulfate and potassium cyanide by the method of Footner and Smiles.[22] Allyl thiocyanate, obtained in 66% yield, boiled at 35° (2.7 mm.) and showed strong, sharp infrared absorption at 2160 cm.$^{-1}$. 2-Methylallyl thiocyanate, obtained in 67% yield, boiled at 35–36° (1.6 mm.).

t-Butyl thiocyanate was prepared by the method of Schmidt[8] and co-workers in 48% yield, b.p. 40° (10 mm.). *sec*-Butyl thiocyanate was prepared in 17% yield by the reaction of 68 g. (0.5 mole) of *sec*-butyl bromide with 38 g. (0.5 mole) of ammonium thiocyanate in acetone for one week at room temperature; b.p. 75° (32 mm.) (reported[23] 48.5–49.5° (7 mm.)).

Benzyl thiocyanate was prepared in 47% yield by the reaction of 63 g. (0.5 mole) of benzyl chloride with 38 g. (0.5 mole) of ammonium thiocyanate in 150 ml. of acetone for five hours at 0°; b.p. 97.8° (1 mm.), (reported[24] 256°). 1-Phenylethyl thiocyanate (α-methylbenzyl thiocyanate) was prepared in 58% yield by the reaction of 22 g. (0.29 mole) of ammonium thiocyanate in acetone with 32.5 g. (0.24 mole) of α-phenylethyl chloride for five days at room temperature; b.p. 81° (0.2 mm.) (reported[25] 157–159° (36 mm.)). Cinnamyl thiocyanate was prepared by the method of Bergmann[6b] in 46% yield, m.p. 69.5–70.5°. 1-Naphthylmethyl thiocyanate was prepared by the method of Jones[6a] from 1-naphthylmethyl chloride.[26] The material used, recrystallized from benzene–petroleum ether mixture, had m.p. 88–89° (reported[7] 91–91.5°).

3,4,5-Trimethoxybenzoyl Isothiocyanate.—To 50 ml. of a benzene solution containing 6.14 g. (0.027 mole) of 3,4,5-trimethoxybenzoyl chloride was added 6.46 g. (0.02 mole) of lead thiocyanate. The mixture was refluxed for 3.5 hours. A portion of Norit was added and the mixture refluxed for 5 minutes, then filtered. The filtrate gave a negative Beilstein test for halogen. A 20-ml. portion of the filtrate was concentrated in a stream of dry air until a solid formed. This was redissolved by heating, petroleum ether was added, and the product crystallized on cooling in good yield. A portion pressed dry on a clay plate melted sharply at 98.8°. The material was recrystallized from carbon tetrachloride–petroleum ether mixture, and showed m.p. 94–98.8°. Qualitative analysis showed the presence of N and S. The infrared spectrum showed strong bands at 1940 to 1990, 1690 and 1580 cm.$^{-1}$.

Anal. Calcd. for $C_{11}H_{11}NO_4S$: C, 52.16; H, 4.38. Found: C, 52.34; H, 4.32.

(20) R. W. Taft, Jr., and L. G. Cannell, National Meeting of the American Chemical Society, Dallas, Tex., April, 1956; Abstracts of Papers, Organic Division, p. 40-N.

(21) L. S. Luskin, G. E. Gantert and W. E. Craig, THIS JOURNAL, **78**, 4965 (1956).

(22) H. B. Footner and S. Smiles, *J. Chem. Soc.*, **127**, 2887 (1925).

(23) M. V. Likhosherstov and V. I. Butrimov, *Acta Univ. Voronegiensis*, **8**, No. 4, 86 (1935); *C. A.*, **32**, 6624 (1938).

(24) L. Henry, *Ber.*, **2**, 636 (1869).

(25) H. L. Wheeler and T. B. Johnson, *Am. Chem. J.*, **26**, 185 (1901).

(26) Kindly prepared by Dr. G. E. Hein.

p-**Nitrocinnamyl Thiocyanate.**—Approximately 0.5 g. of *p*-nitrocinnamyl alcohol[27] was dissolved in ether, pyridine was added, and then thionyl chloride. The mixture was shaken frequently for a period of 0.5 hr. and then poured into water and the layers separated. The ether layer was warmed with a solution of ammonium thiocyanate in acetone for a few minutes, whereupon a precipitate formed. Water was added and the ether was evaporated in a stream of air, leaving a mixture of oil and crystals. The oil slowly solidified, and then was recrystallized from a mixture of benzene, ligroin and absolute alcohol, giving *p*-nitrocinnamyl thiocyanate in moderate yield. An analytical sample, m.p. 69–70°, was obtained by a second recrystallization after decolorization with charcoal in methanol solution. Its infrared spectrum showed sharp absorption at 2160 cm.$^{-1}$.

Anal. Calcd. for $C_{10}H_8N_2O_2S$: C, 54.50; H, 3.65. Found: C, 54.52; H, 3.62.

Estimation of Allyl Isothiocyanate and β-Methylallyl Isothiocyanate.—The general procedure for the estimation of isothiocyanates described by Wild[12] makes use of the reaction of an isothiocyanate with a known excess of a primary amine; titration with standard acid gives the amount of unconsumed amine and thus by difference the amount of isothiocyanate. The usual directions specify heating, and are thus unsatisfactory for mixtures of isothiocyanate and an easily isomerized thiocyanate. We found by experiments with pure allyl isothiocyanate and *n*-butylamine in pure anhydrous dioxane that reaction is essentially complete in 13 to 15 minutes; we therefore omitted heating in our analyses, and obtained satisfactory results. A small correction factor determined from a plot of observed allyl isothiocyanate concentration *vs.* the mole ratio of *n*-butylamine to allyl isothiocyanate had to be applied. This correction was in the range of 2–4% depending on the amine/isothiocyanate mole ratio. No such correction was necessary for 2-methylallyl isothiocyanate.

Kinetic Experiments.—The reactions were carried out in 23 mm. o.d. × 250 mm. Pyrex tubes in a thermostat capable of controlling the temperature to within ±0.2°. The solvents used were sodium-dried toluene, reagent grade nitrobenzene and redistilled reagent grade dimethylformamide (DMF). The standard *n*-butylamine solution was prepared from Eastman Kodak White Label *n*-butylamine which was dried over potassium hydroxide pellets and distilled, and technical grade dioxane which was purified by the method described by Fieser[28] and stored in a glass-stoppered bottle over sodium ribbon. Freshly prepared solutions containing known weights of thiocyanate were brought up to temperature and analyzed periodically during the run. The rate constants were calculated for each experimental increment from the integrated first-order rate equation, and the enthalpy and entropy of activation were calculated in customary manner.[29] Detailed experimental results are given in Tables III and IV only for some representative experiments; the net results of all the experiments have been summarized in Table I.

Isomerization of Allyl Thiocyanate in Nitrobenzene in the Presence of Tri-*n*-butylamine.—Fifty ml. of a solution was prepared which contained 2.04 g. (0.412 *M*) of allyl thiocyanate, 3.89 g. (0.42 *M*) of tri-*n*-butylamine and nitrobenzene as a solvent. The solution was kept at 68.2° for 30 hours. On mixing the reagents a red color developed which had become more intense at the end of the experiment. A 5.0-ml. portion of the solution was shaken with 25.0 ml. of distilled water; 20.0 ml. of the aqueous extract required 0.98 ml. of 0.1028 *N* silver nitrate by Volhard titration for thiocyanate. The amount of thiocyanate ion thus amounted to 6.1% of the original allyl thiocyanate present. Analysis of the original solution for allyl isothiocyanate showed 91.8% conversion.

Isomerization of Allyl Thiocyanate in Toluene in the Presence of Tri-*n*-butylamine.—Fifty ml. of a solution was prepared containing 2.04 g. (0.412 *M*) of allyl thiocyanate, 3.89 g. (0.42 *M*) of tri-*n*-butylamine and sufficient toluene to make up the volume. The solution was kept at 68.2° for 30 hours. No appreciable color change was noted except for a slight yellowing of the solution. An aqueous extract of a portion of the solution gave no color with ferric nitrate, indicating the absence of thiocyanate ion. Another portion of the original solution was assayed for allyl isothiocyanate and the yield was found to be 98.5% of the allyl thiocyanate originally put in.

TABLE III

ISOMERIZATION OF ALLYL THIOCYANATE AT 68.2° IN VARIOUS MEDIA

Time, hr.	In toluene (0.833 *M*) R-NCS, %	k_1, hr.$^{-1}$	In DMF (0.404 *M*) with 0.4 *M* KSCN R-NCS, %	k_1, hr.$^{-1}$	In DMF (0.404 *M*) with 0.4 *M* KI R-NCS, %	k_1, hr.$^{-1}$
0	1.4	...	4.5	...	11.0	...
1	21.0	0.223	21.1	0.191	25.6	0.182
2	38.2	.234	35.3	.195	39.7	.195
3			47.1	.197	51.7	.203
4	61.8	.236	55.5	.191	57.5	.185
5	[a]	[a]	62.9	.189	64.4	.183
10	88.7	.217				
27			84.2	.066	82.5	.060

[a] Additional values: at t = 6.1 hr., 75.8% RCNS, k_1 = 0.223 hr.$^{-1}$; at t = 8 hr., 83.6% R-NCS, k_1 = 0.217 hr.$^{-1}$.

TABLE IV

ISOMERIZATION OF 2-METHYLALLYL THIOCYANATE (0.356 *M* IN TOLUENE) AT 86.4°

Time, hr.	Vol. acid[a]	RNCS, %	k_1, hr.$^{-1}$
0	42.75	13.4	..
0.25	39.80	42.5	1.71
.50	37.75	62.8	1.70
.75	36.42	76.2	1.72
1.00	35.70	83.3	1.65
1.25	35.11	89.0	1.66
1.50	34.85	91.7	1.57
1.75	34.50	95.1	1.65

[a] 0.1059 *N* HCl taken to titrate the excess butylamine reagent after combination with 3-ml. samples of the reaction mixture; the blank required 44.10 ml.

Treatment of *n*-butyl Thiocyanate with Tri-*n*-butylamine in Nitrobenzene.—To a 50.0-ml. volumetric flask was added 1.85 g. (0.321 *M*) of *n*-butyl thiocyanate,[30] 3.89 g. (0.42 *M*) tri-*n*-butylamine and nitrobenzene to the mark. After 30 hours at 68.2°, no thiocyanate ion could be detected with ferric nitrate. No *n*-butyl isothiocyanate was formed in the original solution.

Reaction of *t*-Butyl Thiocyanate with Phenol.—To a 125-ml. erlenmeyer flask was added 18.8 g. (0.2 mole) of phenol, 23 g. (0.2 mole) of *t*-butyl thiocyanate and 26.5 g. (0.2 mole) of anhydrous zinc chloride and the flask was fitted with a drying tube. The reaction mixture began to warm up and turn yellow with the evolution of gas bubbles. After 3 days, the flask was heated for 2 hours on a steam-bath, then cooled whereupon the contents solidified to a glassy orange mass. The reaction mixture was dissolved in acetone and placed in a 1-l. 3-neck flask and a steam distillation was carried out. A mass of solid white material, m.p. 56–83°, separated from the distillate. Sublimation did not change the melting point. Sodium fusion did not reveal the presence of elements other than carbon, hydrogen and oxygen. The substance formed an insoluble precipitate with 15% aqueous sodium hydroxide, and the filtrate, when acidified, yielded a white solid which when recrystallized from petroleum ether gave 3.45 g. (11.5%) of *p*-*t*-butylphenol, m.p. 98.8–99.8° (reported[31] 99°). A portion was treated with concentrated nitric acid in acetic acid; on dilution with water, 2,6-dinitro-4-*t*-butylphenol, m.p. 92.8–93.8°, was obtained (reported[31] 93°).

Another portion was treated with chloroacetic acid and sodium hydroxide and yielded *p*-*t*-butylphenoxyacetic acid, m.p. 86.7–88.7° (reported[32] 86.5°).

(27) H. Meerwein, B. von Bock, Br. Kirschnick, W. Lenz and A. Migge, *J. prakt. Chem.*, **147**, 211 (1936).

(28) Louis F. Fieser, "Experiments in Organic Chemistry," 2nd Ed., D. C. Heath and Co., Boston, Mass., p. 369.

(29) A. A. Frost and R. G. Pearson, ref. 14, pp. 96–98.

(30) R. J. Kaufmann and R. Adams, THIS JOURNAL, **45**, 1744 (1923).

(31) A. Studer, *Ber.*, **14**, 1474 (1881).

(32) W. P. Bradley and F. Kniffen, *Am. Chem. J.*, **19**, 70 (1897).

The aqueous layer was decanted from the residue in the steam distillation flask and the remaining gummy orange material was taken up in ether. The aqueous layer was extracted once with ether, the layers were combined, and the solution was concentrated to a thick, red oil. On addition of benzene, a yellow solid was precipitated which was recrystallized from ethyl acetate and petroleum ether to give a buff solid which melted at 196–204° with decomposition and the evolution of a gas with a strong sulfurous odor. A portion of the buff solid was heated on a steam-bath with 10% aqueous sodium hydroxide for two days, and on cooling and acidification a considerable quantity of *p*-hydroxybenzoic acid, m.p. 210.7–212.7°, was obtained (reported[33] 213°). A portion treated with *p*-bromophenacyl bromide yielded a solid of m.p. 192.5–194.6° (reported[34] for the *p*-bromophenacyl ester of *p*-hydroxybenzoic acid, 192°).

Isomerization of *sec*-Butyl Thiocyanate with Zinc Chloride.—To 31.0 g. of *sec*-butyl thiocyanate was added 1.0 g. of anhydrous zinc chloride, which promptly dissolved. After standing at room temperature for 10 days, no isomerization had occurred. The solution was placed in a flask and heated to boiling, whereupon it darkened rapidly. The solution was refluxed for 2 hours, diphenylmethane was added as a "chaser" and the mixture was distilled, yielding a fraction boiling at 62–63° (21 mm.). Analysis showed this fraction to be 48% isothiocyanate. A portion of this material was treated with ammonium hydroxide and alcohol at 5° for three days. Concentration of the solution yielded a white solid which, after recrystallization from benzene and petroleum ether followed by recrystallization from benzene and alcohol, gave N-*sec*-butylthiourea, m.p. 130–133°, in moderate yield (reported[10] 133°).

Isomerization of *sec*-Butyl and Benzyl Thiocyanates with Zinc Chloride in Nitrobenzene.—Solutions were prepared of 0.17 g. of anhydrous zinc chloride, 2.95 g. of *sec*-butyl or 4.65 g. of benzyl thiocyanate, and sufficient nitrobenzene to make 25.0 ml. The solutions were placed in tubes and heated to 153° with the vapors of boiling bromobenzene, and portions were removed periodically, cooled, and 2.0-ml. samples were analyzed for isothiocyanate. The results are summarized in Table V. At the end of the experiment, gray solids had deposited in both solutions. That from benzyl thiocyanate was examined, and found to be insoluble in water, but to dissolve in hydrochloric acid with the evolution of an odor like hydrogen sulfide.

TABLE V

ISOMERIZATION OF *sec*-BUTYL AND BENZYL THIOCYANATES IN NITROBENZENE WITH ZINC CHLORIDE

Time, hr.	*sec*-Bu-NCS, %	Time, hr.	Benzyl-NCS, %
0	3.4	3	20.4
1	14.6	5	27.0
2	23.0	15.5[a]	14.5
4.5	29.2	19	27.3
7	25.6		

[a] After this measurement, 0.8 g. more zinc chloride was added.

It can be seen that in both cases the isothiocyanate reaches a maximum concentration, after which it is destroyed faster than it is formed. This phenomenon is apparently associated with removal of zinc chloride, for when fresh zinc chloride was added to the benzyl thiocyanate reaction after the maximum had been passed, the isothiocyanate concentration rose again. This deterioration of the catalytic effectiveness of zinc chloride was generally noticeable in those experiments carried out above 100°, but did not occur appreciably in experiments at lower temperatures (*cf.* the isomerization of 1-phenylethyl thiocyanate at 100°).

Isomerization of Benzyl Thiocyanate in Nitrobenzene with Aluminum Chloride; Detection of Thiocyanate Ion in the Solution.—A solution was prepared containing 3.51 g. of benzyl thiocyanate, 0.66 g. of aluminum chloride and nitrobenzene to make 25.0 ml. The solution was heated to 153° by the vapors of boiling bromobenzene for 1.5 hours. The solution was cooled, diluted with ether and extracted with water. A small portion of the aqueous layer gave a blood-red color with ferric chloride. The bulk of the aqueous solution was heated with nitric acid, evaporated nearly to dryness, diluted with water, heated to boiling, and an excess of dilute barium chloride solution was added. The solution was heated for 2 hours on a steam-bath, distilled water was added and the solution was buffered to *p*H 4 with sodium acetate. After standing overnight the barium sulfate was filtered off and ignited for 3 hours. The yield of barium sulfate was 0.71 g., which corresponds to a sulfur/aluminum ratio of 0.6 and a sulfur/benzyl thiocyanate ratio of 0.13. The ether was evaporated from the organic layer and the residue was assayed for benzyl isothiocyanate; conversion was 48%.

A similar reaction mixture was diluted with ether, extracted with water, and aniline was added. The solution was decolorized with Norit, filtered and the ether evaporated. On dilution of the oil with petroleum ether and benzene, a greenish solid was obtained. The color was removed by washing with ethyl acetate and the white solid was purified by two recrystallizations from absolute alcohol. The purified material, obtained in moderately good yield, melted at 151.2–151.7° and showed no depression of melting point when admixed with an equal weight of authentic N-benzyl-N′-phenylthiourea.

Isomerization of 1-Phenylethyl Thiocyanate with Zinc Chloride.—To 4.0 g. (0.0245 mole) of 1-phenylethyl thiocyanate containing 9.7% isothiocyanate was added 0.1 g. (0.00074 mole) of anhydrous zinc chloride. The flask was stoppered and the zinc chloride gradually dissolved. After 4 days, a sample was removed and found to be 99% isothiocyanate.

A portion of the reaction mixture was removed and treated with 33% aqueous dimethylamine and sufficient alcohol to make a homogeneous solution. The solution was filtered to remove zinc hydroxide and allowed to stand for 12 hours. On cooling, a solid separated in good yield, and was twice recrystallized from carbon tetrachloride–petroleum ether to give a white solid, m.p. 106.8–108.9°. The reported melting point of N-1-phenylethyl-N′,N′-dimethylthiourea is 108–109°.[9]

Another portion of the solution was treated with aniline and the resulting oil was crystallized by triturating with absolute alcohol and petroleum ether. One recrystallization from this solvent pair gave a moderately good yield of N-phenyl-N′-1-phenylethylthiourea, m.p. 102.3–103.8° (reported[9] 106°).

Isomerization of 1-Phenylethyl Thiocyanate with Zinc Chloride in Diethyl Carbitol.—Diethyl Carbitol (diethylene glycol diethyl ether, Union Carbide technical grade) was purified by distillation, refluxing with metallic sodium, and a second distillation. A solution containing 2.19 g. of 1-phenylethyl thiocyanate (0.538 *M*), 0.1003 g. of anhydrous zinc chloride (0.029 *M*) and sufficient diethyl Carbitol to make 25.0 ml. was placed in a tube and heated to 100° by steam. Portions were removed periodically, cooled, and 2.0-ml. samples were analyzed with *n*-butylamine reagent. During the course of the reaction a small quantity of colloidal yellow solid appeared. The results are summarized in Table VI.

TABLE VI

ISOMERIZATION OF α-PHENYLETHYL THIOCYANATE WITH ZINC CHLORIDE IN DIETHYL CARBITOL

Time, hr.	RNCS, %	k_1, hr.$^{-1}$
0	12.0	0.29
1.5	43.1	.35
2.5	63.8	.35
3.5	74.0	.36
4.0	78.9	
>12	96	

Isomerization of Cinnamyl Thiocyanate in Decalin, Diphenylmethane and Nitrobenzene.—Cinnamyl thiocyanate is insoluble in decalin (decahydronaphthalene) at room temperature, so the solutions were prepared at steam-bath temperatures. A solution of 4.313 g. in 18.932 g. of decalin (total volume 25.0 ml. at room temperature) was heated to 153° with the vapors of boiling bromobenzene. Samples were removed periodically and analyzed for isothiocyanate

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(34) H. Lund and T. Langvad, THIS JOURNAL, **54**, 4107 (1932).

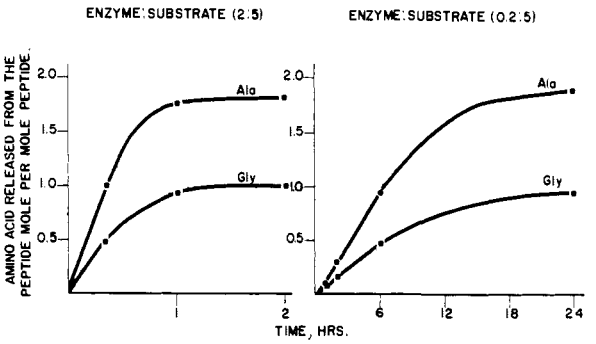

Fig. 3.—Rate of release of amino acids from carbobenzoxy-L-alanyl-I-alanyl-L-alanyl-glycine by CPase (2 mg. of the enzyme to 5 mg. of the substrate).

Fig. 4.—Rate of release of amino acids from carbobenzoxy-L-alanyl-L-alanyl-L-alanyl-glycine by CPase (0.2 mg. of the enzyme to 5 mg. of the substrate).

the new C-terminal group of the digested HA, and alanine was only 0.05 mole per mole of protein. After 24 hr. of digestion, 0.2 mole of glycine was released from the original HA; and after 48 hr., 0.3 mole was released. The new C-terminal glycine of the digested protein was 0.7 and 0.6 mole, respectively. The sum of the released glycine and the new C-terminal glycine was always about one mole.

Synthesis of Carbobenzoxy-L-alanyl-L-alanyl-L-alanyl-glycine.—Carbobenzoxy-L-alanyl-L-alanyl-L-alanine hydrazide (Compound I) was synthesized by the procedure of Erlanger, Brand and Sachs.[13,14] It was then converted to its azide by the action of nitrous acid and allowed to react with glycine ethyl ester to give carbobenzoxy-L-alanyl-L-alanyl-L-alanyl-glycine ethyl ester (Compound II), which had a melting point of 224–225°. The amino acid composition of this peptide ester was determined, after hydrolysis with hydrochloric acid, by the DNP method. The molar ratio of glycine to alanine was estimated to be 1.07:3.00.

One hundred mg. of Compound II was dissolved in 2 ml. of dioxane and 2 ml. of water and then saponified with 0.27 ml. of 1 *N* sodium hydroxide for 1.5 hr. in a refrigerator. Upon addition of a slight excess of 1 *N* hydrochloric acid, the solution was returned to the refrigerator and allowed to stand overnight. The carbobenzoxy-L-alanyl-L-alanyl-L-alanyl-glycine (Compound III) crystallized by this treatment was filtered and washed with cold water. The crystals recrystallized from a mixture of dioxane–ether had a melting point of 241–242°.

Anal. Calcd. for $C_{19}H_{26}O_7N_4$: C, 54.02; H, 6.02; N, 13.26. Found: C, 53.98; H, 6.11; N, 13.30.

The C-terminal amino acid of the peptide (Compound III) was determined to be 0.9 mole of glycine per mole of peptide by the hydrazinolysis method.[12]

Digestion of Carbobenzoxy-L-alanyl-L-alanyl-L-alanyl-glycine (Compound III) with CPase.—To 5 ml. of a 0.1% solution of Compound III (adjusted to *p*H 7.5) was added 0.5 ml. of CPase solution containing 2 mg. of the enzyme. The digestion was carried out at room temperature (25°). The rate of release of the amino acids from the peptide is given in Fig. 3, which shows that the ratio of glycine to alanine released from the peptide was 1:1.9 at any time during the digestion.

Figure 4 shows the rate of release of the amino acids from the same peptide when the amount of enzyme used for the digestion was reduced to one tenth (5 mg. of the substrate and 0.2 mg. of the enzyme in 5.5 ml. of the reaction mixture).

Discussion

From a partial acid hydrolysate of DNP-HA Thompson obtained DNP-Asp, DNP-Asp.Ala, and a DNP-tripeptide for which the third amino acid in the sequence could not be determined.[5] He suggested, however, from the chromatographic behavior of the DNP-tripeptide, that the third amino acid might have some aromatic group. By the experiments described herein, the third amino acid was found to be mono-DNP(im)-His. This result is in agreement with Thompson's suggestion. It is of interest that only DNP-Asp.Ala.DNP(im)-His was found in the ethyl acetate extract of the enzymic digestion product of DNP-HA. As has been indicated in Fig. 1 and in Table I, neither DNP-Asp nor DNP-Asp.-Ala was found. This might be explained as follows: The enzyme cannot combine with the first or second peptide bond from the N-terminal group due to steric hindrance by such large radicals as DNP-groups, one of which was combined with the N-terminal amino group and another with the imidazole radical of the third amino acid. The third peptide bond was more easily attacked by the enzyme, so that only DNP-Asp.Ala.DNP(im)-His was obtained by such enzymic hydrolysis. Chymotrypsin, trypsin and papain were also tried; however, the DNP-protein could not be digested with such enzymes.

In experiments concerning the rate of release of amino acids from HA by CPase, results were similar to those of White, *et al.*, and seemed at first to support his suggestion that the C-terminal amino acid sequence of HA might be-(Gly,Val) Ala.Leu.[9]

Alanine was expected to be in the C-terminal position of the digested protein, which was separated after liberation of the C-terminal leucine from the original HA by CPase. Contrary to such expectation, however, glycine was found to be the C-terminal amino acid of the protein, as shown in Table II and Fig. 2. These results show that the the C-terminal sequence of HA might be-Gly.Leu.

In order to explain the release of alanine from HA by CPase as though it were the amino acid next to the C-terminal group of the protein, three possibilities were considered:

(1) That an endopeptidase, such as trypsin or chymotrypsin, might have been present as a contaminant in the CPase and caused the splitting of some peptide bond. This could expose a new C-terminal alanine to attack by CPase. To investigate the probability of such action, the N-terminal amino acid of the HA digested for 2 hr. was determined by the DNP method; one mole of aspartic acid was the only N-terminal amino acid found for one mole of protein. This possibility should, therefore, be given up.

(2) That HA might have two C-terminal groups, one being leucine and another being alanine amide. By the action of CPase, or some other enzyme (an amidase?) present as a contaminant in the CPase, the C-terminal amide could have been split off initially (along with leucine) and alanine would have been released next by the CPase as the new C-terminal group. In such a case, ammonia would have been liberated from the HA; however, no ammonia was detected in the progress of the enzymic action.

(3) That the greater specificity of CPase for peptide bonds containing alanine might account for the greater release of this amino acid if more units

(13) B. F. Erlanger and E. Brand, THIS JOURNAL, **73**, 3508 (1951).
(14) E. Brand, B. F. Erlanger and H. Sachs, *ibid.*, **74**, 1849 (1952).