Carbon-13 Nuclear Magnetic Resonance Studies of Heterocycles Bearing Carbon–Sulfur and Carbon–Selenium Bonds: 1,3,4-Thiadiazole, 1,3,4-Selenadiazole, and Tetrazole Derivatives[1]

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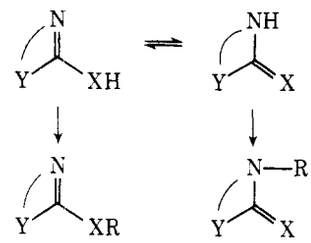
A number of 1,3,4-thiadiazole and selenadiazole thiols and selenols (**2, 3, 5, 6, 7,** and **9**) have been synthesized. ^{13}C NMR is shown to offer a reliable method for distinguishing thiones and selones from the corresponding thiol and selenol derivatives. All the thiols and selenols studied are shown to exist as their thione and selone tautomers, respectively. Substitution of selenium for exocyclic sulfur (at C-2) leads to a shielding effect at the carbon of attachment and (for selones) a deshielding effect at C-5 (see Table I), suggestive of transmission of inductive effects across the ring chalcogen. Substitution of selenium for ring sulfur results in deshielding at both C-2 and C-5; comparison with models shows this effect to be essentially independent of the number of ring nitrogens. The possible origin of this deshielding effect is discussed. These correlations have been extended to the tetrazole series, where they have been used to establish the structure of the tetrazolium salt (**27**) (obtained by alkylation of **24a**) and its mesoionic solvolysis products (**28** and **29**). Some ^{13}C–^{77}Se coupling data are presented.

^{13}C NMR is a powerful tool for structure determination in organic chemistry. Among other advantages, it makes possible the direct observation of functional groups such as carbonyl and thiocarbonyl. ^{13}C chemical shifts have been determined for a number of thiocarbonyl functions,[2] and a quantitative relationship between thiocarbonyl and carbonyl chemical shifts has been put forward.[2b] Our interest has lain in the application of ^{13}C NMR to functional groups containing carbon–selenium bonds, and their comparison with the corresponding carbon–sulfur functions. A further objective of our work has been to establish methods of distinguishing between substitution on nitrogen and on sulfur or selenium in derivatives of heterocyclic thiols and selenols (see eq 1). Since



(1)

X, Y = S, Se

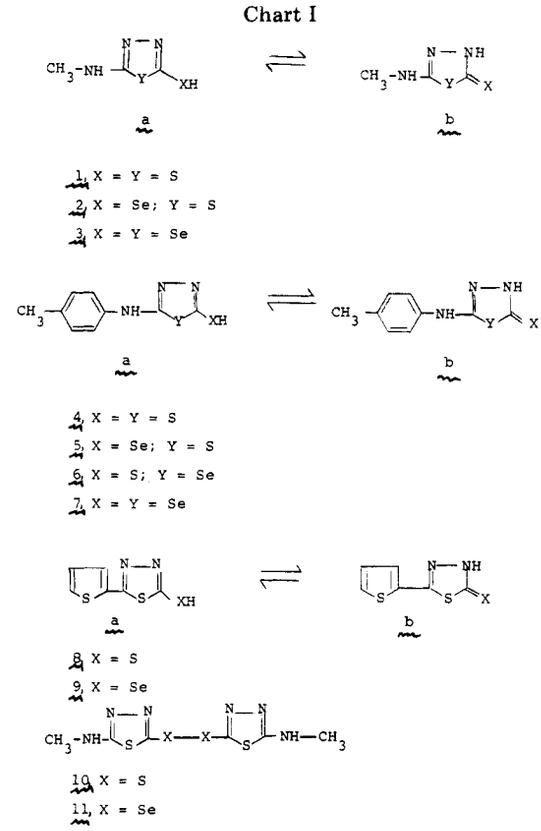
structural assignments for such materials are often difficult using existing methods, the development of ^{13}C NMR correlations in this field has considerable value.

Results and Discussion

1,3,4-Thiadiazole and 1,3,4-Selenadiazole Derivatives. The compounds examined in this phase of our study are shown in Chart I. Literature methods were used for the preparation of **1**[3] and **4**,[4] while **8** was obtained by treatment of potassium 3-(2-thenoyl)dithiocarbazate[5] with concentrated sulfuric acid at 10 °C. Treatment of 4-(*p*-tolyl)-3-selenosemicarbazide[6] with thiophosgene afforded **6.** Heterocyclic selenols **2, 3, 5, 7,** and **9** were prepared by the action of carbon diselenide[7] on the corresponding thio or seleno hydrazides. Oxidation of **1** with hydrogen peroxide gave the disulfide **10;** the corresponding diselenide **11** was obtained by air oxidation of a methanolic solution of **2.**

^{13}C NMR data for some 1,3,4-thiadiazole derivatives are presented in Table I. Substitution of selenium for sulfur leads, in general, to a decrease in line intensity for the carbon of attachment.[8] The data for **1** and **2** show that these materials exist predominantly as the thione and selone tautomers (b) (Chart I), respectively. This is shown by using disulfide **10** and

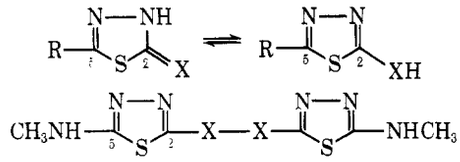
Chart I



diselenide **11** as model compounds; their ^{13}C NMR spectra should approximate those of the thiol (**1a**) and selenol (**2a**) tautomers. However, conversion of **1** into **10,** and of **2** into **11,** results in large changes in chemical shift, upfield for C-2 and downfield for C-5, such that the positions of the C-2 and C-5 resonances are reversed. This is consistent only with the conversion of the thione (**1b**) and selone (**2b**) tautomers into **10** and **11,** respectively. The assignments of the C-2 and C-5 signals are confirmed by the undecoupled spectra, in which the C-5 carbons appear as multiplets owing to long-range coupling with the methyl and NH protons, while the C-2 carbons still give rise to singlets.

The C-2 line shows a marked upfield shift on going from sulfur to selenium, both for the change thione → selone (**1, 2;** $\Delta\delta_{Se-S} = -12.1$ ppm) and for disulfide → diselenide (**10, 11;** $\Delta\delta_{Se-S} = -8.6$ ppm). The C-5 line shows a corresponding downfield shift on going from thione to selone ($\Delta\delta_{Se-S} = 3.6$

Table I. ^{13}C NMR Chemical Shifts of 1,3,4-Thiadiazole Derivatives[a]



Compd	δ[b] C-2	δ[b] C-5	δ[b] Other	Δδ Se−S[c] C-2	Δδ Se−S[c] C-5
1	180.6	161.6	CH_3: 29.9		
10	148.6	173.0	CH_3: 31.2	(−32.0)	(11.4)
2[d]	168.5	165.2	CH_3: 30.35	−12.1	3.6
11	140.0	173.1	CH_3: 31.2	−8.6	0.1
4	181.1	156.6	C-1′: 137.4 C-2′: 117.6 C-3′: 129.4 C-4′: 131.2 CH_3: 20.3	(−28.5)	(7.9)
5	170.5	160.2	C-1′: 137.2 C-2′: 117.6 C-3′: 129.4 C-4′: 131.4 CH_3: 20.3	−10.6	3.6
8	187.2	154.2			
9	177.8	158.2		−9.4	4.0

[a] In Me_2SO-d_6. [b] Parts per million downfield from internal tetramethylsilane. [c] Δδ values for the changes thione → disulfide and selone → diselenide are shown in parentheses. [d] Spectrum run at 18 °C.

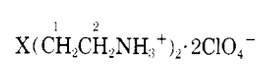
Table II. ^{13}C NMR Chemical Shifts for Simple Chalcogenides

Compd	C-1	C-2	C-3	C-4
12[a]	28.9	39.6		
13[a]	20.5	40.7		
$\Delta\delta_{Se-S}$ ($\delta_{13} - \delta_{12}$)	−8.4	+1.1		
14[b]	136.0	127.2	129.3	127.4
15[b]	130.1	130.8	129.3	127.7
$\Delta\delta_{Se-S}$ ($\delta_{15} - \delta_{14}$)	−5.9	+3.6	0	+0.3

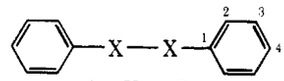
[a] Solvent: D_2O (Me_4Si external reference). [b] Solvent: Me_2SO-d_6 (Me_4Si internal reference).

ppm), but in the disulfide–diselenide pair this line remains essentially unchanged. Other thione–selone pairs (**4, 5** and **8, 9**) show similar trends.

Comparison with some simple chalcogenides (**12, 13, 14,** and **15**; see Table II) suggests that these shifts are due mainly to



12, X = S
13, X = Se

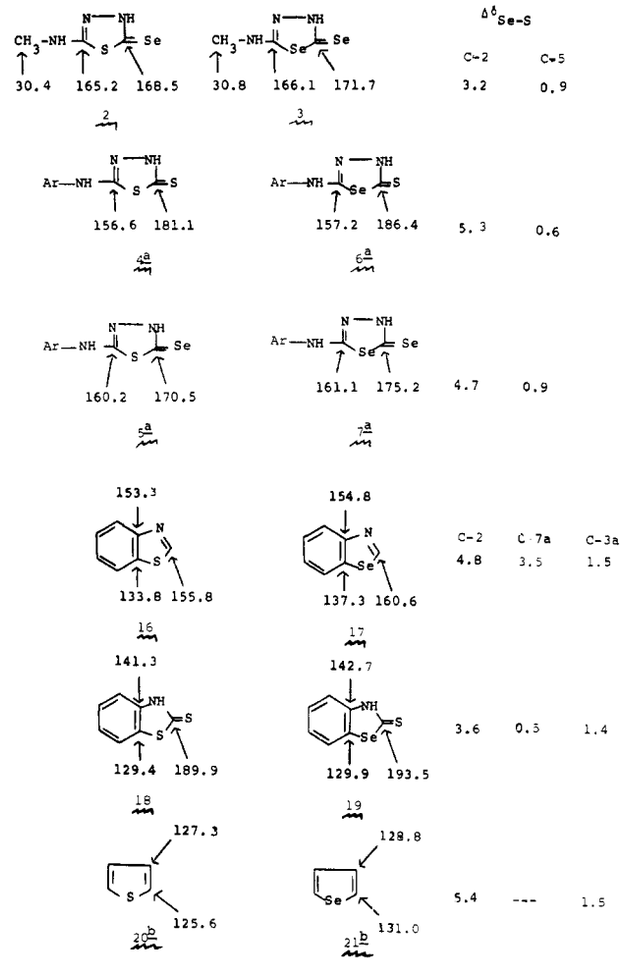


14, X = S
15, X = Se

inductive effects, resulting primarily from the lower electronegativity of selenium as compared to sulfur. What is remarkable, however, is the strong deshielding effect of selenium on C-5 in the thiadiazole-2-selones. It is of the same order as the deshielding of the ortho carbon in phenyl diselenide, and implies transmission of a strong inductive effect via the ring sulfur. Similar effects have been observed in comparative studies of ^{13}C NMR spectra of 2-substituted furans, thiophenes, selenophenes, and tellurophenes.[9]

In contrast, substitution of ring sulfur by selenium results in a marked deshielding effect (3–5 ppm) at C-2 and a much smaller deshielding effect at C-5. Similar effects are observed (see Scheme I) when one compares benzoselenazoles and selenophene with the sulfur analogues. The data on thiophene

Scheme I



[a] Ar = *p*-tolyl. [b] Values taken from ref 9.

and selenophene are taken from the work of Gronowitz and his co-workers;[9] their findings are consistent with earlier studies.[10,11] Introduction of a thione function at C-2 of benzoselenazole decreases the deshielding effect of selenium at C-7a, in conformity with the trends already noted for 1,3,4-selenadiazoles. This deshielding effect may be accounted for by a decrease in total charge density on the α carbons, resulting from a greater tendency for the d orbitals of the heteroatom to accept electrons from the ring for selenium than for sulfur. The latter effect apparently outweighs the opposite trend anticipated on the basis of differences in electronegativity between the two heteroatoms. Recent correlations of the ^{13}C NMR shifts of thiophene and selenophene with CNDO/2 calculations of total charge densities are consistent with this view.[9] The smaller deshielding effect observed for C-3a in the benzazole pairs **16** and **17**, and **18** and **19**, is insensitive to substitution at C-2 and is similar to that observed[9] for C-3 in thiophene (**20**) and selenophene (**21**).

The effect of selenium on the ^{13}C chemical shifts of azoles may thus be summarized as follows. Substitution of exocyclic sulfur by selenium results in an upfield shift at the carbon of attachment, whereas substitution of ring sulfur by selenium results in a downfield shift at the adjacent carbon, and these effects are essentially independent of the number of ring nitrogens.

Tetrazole Derivatives. Table III shows the ^{13}C chemical shifts of some tetrazole derivatives. 1-Phenyl-1,2,3,4-tetrazole-5-thiol (**22**) exists predominantly as the thione tautomer (**22b**), in agreement with the findings of Lieber et al.[12] The chemical shift of the tetrazole carbon (C-5; see Chart II) comes close to those observed for 1-phenyl-4-(1′-piperidinometh-

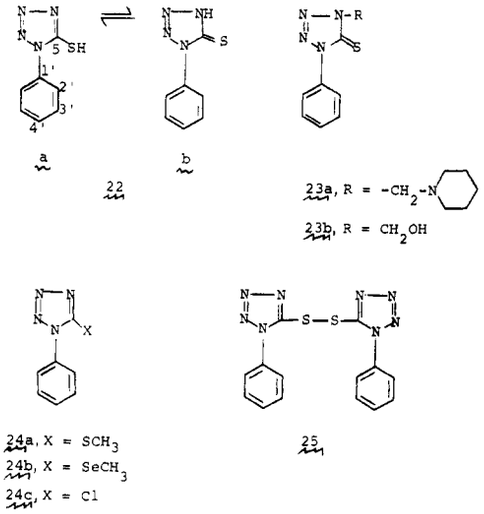
Table III. ^{13}C NMR Chemical Shifts of Tetrazole Derivatives[a]

Structure	δ, ppm C-5	C-1′	C-2′	C-3′	C-4′	Other
22	163.8	134.0	124.3	129.2	129.5	
	165.0	135.4	124.1	129.6	129.6[b]	
23a	164.7	135.0	123.8	129.1	129.4	NCH_2N: 70.4; C-2″: 51.6; C-3″: 25.9; C-4″: 23.6[c]
	165.4	136.0	124.3	129.5	129.6	NCH_2N: 70.6; C-2″: 52.0; C-3″: 26.4; C-4″: 24.2[b]
23b	163.2	134.2	124.3	129.5	129.7	CH_2: 70.4
24a	155.1	133.1	124.3	130.0	130.5	CH_3: 15.2
24b	148.0	133.7	124.4	130.0	130.6	CH_3: 8.6
25	152.3	132.8	124.9	129.7	130.8	
24c	146.1	132.3	125.3	129.9	131.1	

[a] In Me_2SO-d_6. [b] In dioxane-d_8. [c] In chloroform-d.

yl)-Δ^2-1,2,3,4-tetrazoline-5-thione (**23a**) and the 4-(hydroxymethyl) analogue **23b**, the structures of which have been established by the work of Postovskii and Nirenburg.[13] The

Chart II



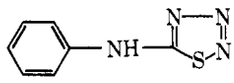
thio ether **24a**, prepared by methylation of **22** with methyl iodide,[14] shows a signal at 155.1 ppm for C-5. The corresponding seleno ether **24b**, obtained somewhat surprisingly by the action of methanolic bis(methoxymagnesium) diselenide[15] on 5-chloro-1-phenyl-1,2,3,4-tetrazole (**24c**), shows its C-5 resonance at 148.0 ppm, giving $\Delta\delta_{Se-S} = -7.1$ ppm, similar to the value ($\Delta\delta_{Se-S} = -8.7$ ppm) observed for the 1,3,4-thiadiazole disulfide/diselenide pair (**10, 11**) considered earlier. The methyl resonances show an upfield shift of the same order ($\Delta\delta_{Se-S} = -6.6$ ppm). The tetrazolyl disulfide **25**, as expected, behaves like the thio ether **24a**, while the chlorotetrazole **24c** shows aromatic ^{13}C shifts like those of **24b**. The tetrazole series thus shows trends similar to those observed for the 1,3,4-thiadiazole and 1,3,4-selenadiazole series. Furthermore, recent work by L'abbé and his co-workers[16] includes ^{13}C NMR spectral data for 1-benzyl- and 1-phenyl-Δ^2-1,2,3,4-tetrazoline-5-thione and their N- and S-substituted derivatives, and their values for C-5 chemical shifts are very similar to ours.

The following example illustrates the diagnostic value of the ^{13}C chemical shift correlations just described. Alkylation of **24a** with triethyloxonium fluoroborate gives a stable, crystalline tetrazolium salt, which on treatment with sodium hydrogen sulfide yields a yellowish white solid A, $C_9H_{10}N_4S$, presumably formed by nucleophilic attack by SH^- with loss of methanethiolate anion. Treatment with sodium hydrogen selenide gives the yellow selenium analogue B, $C_9H_{10}N_4Se$. The C-5 chemical shifts of these materials are 173.5 and 165.8 ppm, respectively. These values are inconsistent with a 1,4-disubstituted thione (selone) structure, for which our correlations would predict C-5 chemical shifts in the vicinity of 164 (thione) and 155 (selone) ppm. UV spectral data (Table IV) support this conclusion, as well as indicating that these compounds are structural analogues, differing only in the chalcogen. The UV spectrum of 5-anilino-1,2,3,4-thiatriazole (**26**)

Table IV. UV Spectral Data[a]

Compd	λ_{max}, nm	ε
A. $C_9H_{10}N_4S$	228	12 400
	255	9 600
	340	2 600
B. $C_9H_{10}N_4Se$	234	11 600
	270	7 800
	362	1 840
23a	285	4 800
26	240	8 600
	284	8 400
	302	11 400

[a] Solvent, 95% ethanol; concn, 5×10^{-5} M.



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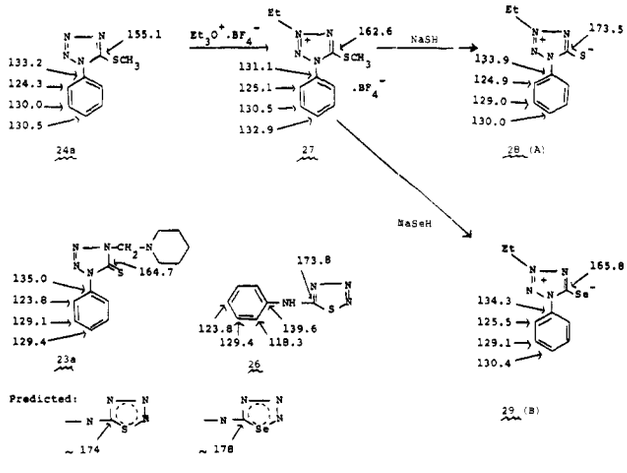
is quite different, suggesting that we are not dealing with a 1,2,3,4-selenatriazole/thiatriazole pair either.

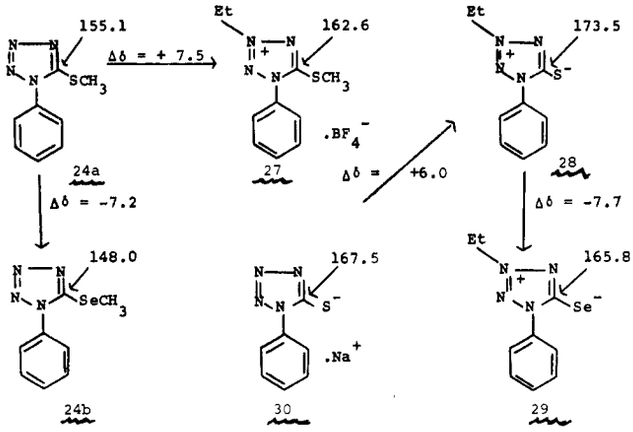
We propose structure **27** for the tetrazolium salt and structures **28** and **29** for compounds A and B, respectively (Scheme II), on the basis of the ^{13}C NMR and UV evidence. First, the ^{13}C benzenoid shifts in these materials are similar to those of other 1-phenyltetrazoles, but very different from those of 5-anilino-1,2,3,4-thiatriazole (**26**), despite the close-

Scheme II



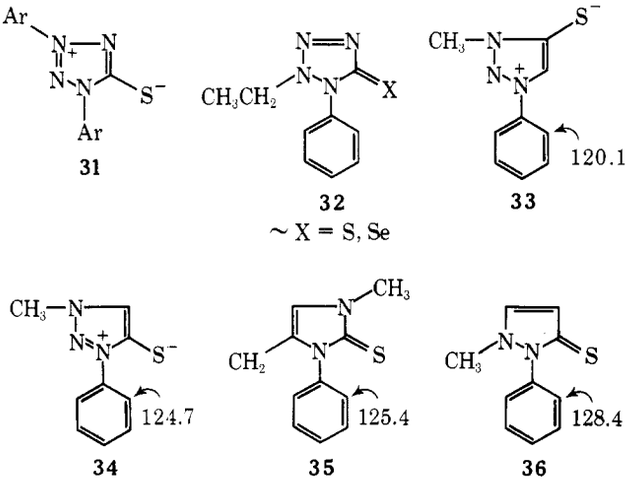
ness of the thiatriazole C-5 signal (173.8 ppm) to the observed shift (173.5 ppm) in A. Second, if the latter were a thiatriazole derivative, one would expect the selenium analogue B to show the deshielding effect of ring selenium. The C-5 signal should appear around 178 ppm. Instead, changing from sulfur to selenium gives rise to a strong shielding effect. Third, this shielding effect is almost exactly what one would expect for *exocyclic* selenium in the tetrazole series (Scheme III). In

Scheme III



addition, comparison with the 1-phenyltetrazole-5-thiolate anion (**30**) shows that the C-5 (tetrazole) resonance is shifted downfield on going from the latter to the mesoionic *N*-ethyl derivative **28** by an amount comparable with the deshielding effect observed on going from 5-methylthio-1-phenyl-1,2,3,4-tetrazole (**24a**) to the 3-ethyl-5-methylthio-1-phenyl-1,2,3,4-tetrazolium cation (**27**). This effect must be due largely to lowering of the electron density of the tetrazole ring. The mesoionic structures **28** and **29** are thus aryl–alkyl analogues of the known 1,3-diaryl-1,2,3,4-tetrazolium 5-thiolates **31**,[17] whose structures have recently been confirmed by x-ray crystallographic studies.[18]

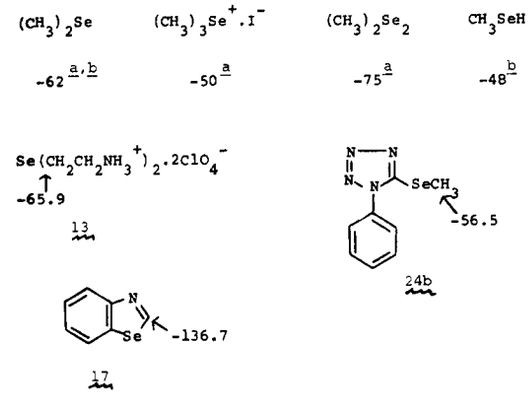
Structures substituted in the 2 position, such as **32**, may be



excluded on the basis of the ^{13}C chemical shift of the ortho benzenoid carbons in **27**, **28**, and **29**. Begtrup's studies[19] on phenyl-substituted azoles show wide variations in the chemical shifts of the ortho benzenoid carbon, which are ascribed to the effect of steric hindrance on the extent of interannular conjugation, the direction of shift being downfield with increasing steric hindrance. Pertinent examples from Begtrup's work[19] are **33**, **34**, **35**, and **36**. **27**, **28**, and **29** show signals for the ortho benzenoid carbon near 125 ppm, like the model compounds **34** and **35**, and so must have only one substituent adjacent to the phenyl group, whereas **32** should show an ortho carbon signal near 128 ppm, as does **36**. Further support for this conclusion comes from recent work[20] by Lippmann and his co-workers on the acylation of tetrazole-5-thiols, which suggests that the 1-phenyl group exerts a considerable steric effect at the 2 position. This effect may be expected to operate in the alkylation of 5-methylthio-1-phenyltetrazole, since quaternization reactions are frequently sensitive to steric factors. It is possible, too, that alkylation at the 3 position is facilitated by electron release from the 1-nitrogen. Finally, the mass spectra of **28** and **29** both show a prominent peak at *m/e* 105, consistent with the ion $C_6H_5N{\equiv}N^+$. The latter would be an expected fragmentation product of **28** and **29**, but not of **32**.

^{13}C–^{77}Se Coupling. ^{13}C–^{77}Se coupling data reported in the literature[21,22] encouraged us to look for such coupling in the present work. The natural abundance (7.58%) of ^{77}Se permits observation of ^{77}Se satellites in ^{13}C NMR spectra, provided the primary is sufficiently intense. Our results, together with representative literature data, are summarized in Scheme IV.

Scheme IV. ^{13}C–^{77}Se Coupling Constants for C–Se Bonds, Hz



[a] Values taken from ref 21. [b] Values taken from ref 22.

The signs of the coupling constants are assumed to be negative, in consonance with the earlier findings.[21,22] Bis(2-aminoethyl) selenide perchlorate (**13**) and the tetrazole derivative **24b** both give values for sp^3-hybridized carbons similar to those reported[21,22] in the literature, whereas a value of −136.7 Hz is observed for C-2 of benzoselenazole (**17**; sp^2), suggesting that direct ^{13}C–^{77}Se coupling constants for sp^2 carbons should be roughly twice as large as those for sp^3 carbons. We had hoped to investigate the question of whether ^{13}C–^{77}Se coupling might be sensitive to the state of hybridization of selenium as well as of carbon, but the low intensity of signals due to nonprotonated carbons attached to selenium[8] have so far prevented us from doing so.

Experimental Section

^{13}C NMR spectra were recorded on a Varian CFT-20 spectrometer at ambient temperature and for 0.25–1.0 M solutions in Me_2SO-d_6 containing tetramethylsilane as internal reference, unless otherwise stated. For decoupled spectra flip angles were normally 18–27°, and sensitivity enhancement and apodization parameters were −0.4 and 0.187, respectively. Coupled spectra were observed by the gated decoupling technique, using a 2-s pulse delay. Spectra of organoselenium compounds were run in the dark. IR spectra were determined on Perkin-Elmer 421 or 621 spectrophotometers, and UV spectra on a Cary 14 spectrophotometer. ^1H NMR spectra were recorded on a Varian T-60 spectrometer. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Carbon diselenide was obtained from Strem Chemicals, Inc., Danvers, Mass., and used as received; all experiments with this reagent were performed in a good hood and in subdued light, with exclusion of oxygen and moisture. Residues were destroyed using ethanolic potassium hydroxide or ethanol–piperidine mixtures. Benzene was dried over Linde Molecular Sieve, pore size 4 Å.

Reactions with Carbon Diselenide: 1,3,4-3*H*-Thiadiazoline-2-selone (2). A solution of 4-methyl-3-thiosemicarbazide (1.05 g, 10.0 mmol) in refluxing benzene (250 mL) was dried azeotropically (Dean–Stark trap), after which the trap was replaced by an Allihn condenser connected to an absorption trap containing ethanolic potassium hydroxide. The apparatus was then purged with dry nitrogen, after which carbon diselenide (0.80 mL, 2.14 g, 12.6 mmol) in dry benzene (50 mL) was added dropwise to the stirred, refluxing mixture during 0.5 h. Stirring and reflux were continued for a further 2 h, by which time evolution of hydrogen selenide had virtually ceased. The mixture, still under nitrogen, was allowed to cool, and the precipitated product collected, washed with benzene, and immediately dried in vacuo over phosphoric oxide, potassium hydroxide, and paraffin wax shavings. The selone (2) (1.80 g; 93%) was a fluffy pink solid: mp 123.5–124 °C dec; UV λ_{max} (95% ethanol) 338 nm (ϵ 12 800); ^{1}H NMR δ_{Me_4Si} (Me_2SO-d_6) 2.86 (d, J = 5 Hz, 3), 7.69 (q, J = 5 Hz, 1), 13.99 (br s, 1).

Anal. Calcd for $C_3H_5N_3SSe$: C, 18.56; H, 2.60; N, 21.65; S, 16.52; Se, 40.68. Found: C, 18.45; H, 2.50; N, 21.69; S, 16.74; Se, 40.54.

The following compounds were prepared similarly.

5-Methylamino-1,3,4-3*H*-selenadiazoline-2-selone (3) (93% from 4-methyl-3-selenosemicarbazide[23]): pale orange solid; mp 143–144 °C dec; UV λ_{max} (95% ethanol) 267 (ϵ 2400), 343 nm (13 600); ^{1}H NMR δ_{Me_4Si} (Me_2SO-d_6) 2.81 (d, J = 4 Hz, 3), 7.57 (br q, 1), 13.75 (br s, 1).

Anal. Calcd for $C_3H_5N_3Se_2$: C, 14.95; H, 2.09; N, 17.43; Se, 65.52. Found: C, 14.67; H, 2.30; N, 16.88; Se, 66.15.

5-(*p*-Toluidino)-1,3,4-3*H*-thiadiazoline-2-selone (5) [95% from 4-(*p*-tolyl)-3-thiosemicarbazide[4]]: pale pink felted needles; mp 156–157 °C dec; UV λ_{max} (95% ethanol) 239 (ϵ 13 600), 296 (5500), 343 nm (16 800); ^{1}H NMR δ_{Me_4Si} (Me_2SO-d_6) 2.28 (s, 3), 7.17 (m, 2), 7.32 (m, 2), 10.18 (s, 1), 14.25 (br s, 1).

Anal. Calcd for $C_9H_9N_3SSe$: C, 40.01; H, 3.36; N, 15.55; S, 11.86; Se, 29.22. Found: C, 40.08; H, 3.34; N, 15.65; S, 11.79; Se, 29.05.

5-(*p*-Toluidino)-1,3,4-3*H*-selenadiazoline-2-selone (7) [95% from 4-(*p*-tolyl)-3-selenosemicarbazide[6]]: pale yellowish fluffy needles; mp 168.5–170 °C dec; UV λ_{max} (95% ethanol) 236 (ϵ 16 400), 286 (sh) (6600), 354 nm (19 200); ^{1}H NMR δ_{Me_4Si} (Me_2SO-d_6) 2.25 (s, 3), 7.19 (m, 2), 7.33 (m, 2), 10.10 (br s, 1), 13.67 (br s, 1).

Anal. Calcd for $C_9H_9N_3Se_2$: C, 34.09; H, 2.86; N, 13.25; Se, 49.80. Found: C, 34.19; H, 2.93; N, 13.12; Se, 49.98.

5-(2-Thienyl)-1,3,4-3*H*-thiadiazoline-2-selone (9) (72% from thiophene-2-thiocarbohydrazide[24]): greenish-yellow plates; mp 137–139 °C dec; UV λ_{max} (95% ethanol) 240 (ϵ 7000), 287 (7500), 372 nm (14 000); ^{1}H NMR δ_{Me_4Si} (Me_2SO-d_6) 7.25 (d × d, J = 3.6, 5.0 Hz, 1), 7.71 (d × d, J = 3.6, 1.2 Hz, 1), 7.91 (d × d, J = 5.0, 1.2 Hz, 1).

Anal. Calcd for $C_6H_4N_2S_2Se$: C, 29.15; H, 1.63; N, 11.33; S, 25.94; Se, 31.94. Found: C, 28.92; H, 1.59; N, 11.30; S, 25.75; Se, 31.76.

5-(*p*-Toluidino)-1,3,4-3*H*-selenadiazoline-2-thione (6). 4-(*p*-Tolyl)-3-selenosemicarbazide[6] (0.9 g, 3.9 mmol) was dissolved in dimethylformamide (25 mL) by warming on the steam bath. The solution was cooled to 25 °C and a solution of thiophosgene (0.3 mL, 4.6 mmol) in ether (25 mL) added dropwise with stirring. The resulting mixture was stirred for a further 2.5 h at room temperature and then poured into water (200 mL). The oil which appeared solidified slowly on standing. The crude product was stirred with 1 N hydrochloric acid for 15 min, then collected and digested with 1 N sodium hydroxide. Reacidification of the alkaline digest with concentrated hydrochloric acid furnished the thione as a pale yellow flocculent solid, mp 199–201 °C dec (0.5 g, 47%). Recrystallization from ethanol gave yellow-brown prisms: mp 198–200 °C dec; IR ν_{max} 3110, 2900 (NH), 1600, 1560, 810 cm^{-1}; ^{1}H NMR δ_{Me_4Si} (Me_2SO-d_6) 2.25 (s, 3), 7.13 (m, 2), 7.28 (m, 2), 9.88 (s, 1), 13.30 (br s, 1).

Anal. Calcd for $C_9H_9N_3SSe$: C, 40.01; H, 3.36; N, 15.55; S, 11.86; Se, 29.22. Found: C, 40.16; H, 3.40; N, 15.54; S, 11.71; Se, 29.16.

5-(2-Thienyl)-1,3,4-3*H*-thiadiazoline-2-thione (8). Thiophene-2-carboxylic acid hydrazide (25 g, 0.18 mol) was added to a solution of potassium hydroxide (11.8 g, 0.18 mol) in anhydrous ethanol (200 mL). To the resulting yellow solution was added carbon disulfide (25 mL, 0.42 mol) and the mixture was stirred at room temperature. After 5 min, a thick yellow precipitate formed. The mixture was stirred for a further 10 min, after which the product was collected, washed with ethanol and then with ether, and dried in vacuo, giving potassium 3-(2-thenoyl)dithiocarbazate, mp 273–276 °C dec (42 g, 93%) (lit.[5] mp 284–285 °C). Without further purification, the foregoing dithiocarbazate salt (41.5 g, 0.16 mol) was added slowly, with stirring, to concentrated sulfuric acid (200 mL), the temperature being maintained at 10–15 °C. After addition was complete, the mixture was stirred for a further 1 h, during which time the temperature was allowed to rise to 25 °C. The resulting slightly turbid solution was poured into ice–water (1500 mL) and the yellow precipitate that formed was collected, washed with water, and dissolved in 1% aqueous potassium hydroxide (1500 mL). After filtration to remove traces of insoluble material, the yellow solution was reacidified with concentrated hydrochloric acid, and the resulting yellow precipitate was collected and washed with water. The product was partially dried and then recrystallized from an ethanol–water mixture (260 mL, 2:1). The thione was obtained as pale yellow needles: mp 199–200 °C (10.1 g, 32%; lit.[5] mp 193–194 °C); IR ν_{max} 3067, 2860 (NH), 1550, 1500, 1416, 1288, 736, 729 cm^{-1}; UV λ_{max} (95% ethanol) 232 (ϵ 6700), 265 (8100), 350 nm (ϵ 16 600); ^{1}H NMR δ_{Me_4Si} (Me_2SO-d_6) 7.31 (d × d, J = 4, 5 Hz, 1), 7.51 (d × d, J = 4, 1.5 Hz, 1), 7.80 (d × d, J = 5, 1.5 Hz, 1), 14.51 (br s, 1).

Anal. Calcd for $C_6H_4N_2S_3$: C, 35.98; H, 2.01; N, 13.99; S, 48.02. Found: C, 35.80; H, 1.92; N, 13.83; S, 47.82.

Bis(5-methylamino-1,3,4-thiadiazol-2-yl) Disulfide (10). Aqueous hydrogen peroxide (30%; 1.7 mL, 15 mmol) was added dropwise to a solution of 5-methylamino-1,3,4-thiadiazole-2-thiol (1.47 g, 10 mmol) in methanol (50 mL) during 5 min. The resulting bright yellow solution was stirred until precipitation of the product was complete (2 h). Collection furnished the disulfide as a yellow solid, mp 205–206 °C dec (1.35 g, 92%); IR ν_{max} (KBr) 3320 (sh), 3220 (NH); UV λ_{max} (2-methoxyethanol) 282 (sh) (ϵ 8200), 324 nm (10 300); ^{1}H NMR δ_{Me_4Si} (Me_2SO-d_6) 2.96 (d, J = 5 Hz, 3), 8.19 (br q, J = 5 Hz, 1).

Anal. Calcd for $C_6H_8N_6S_4$: C, 24.65; H, 2.76; N, 28.74; S, 43.86. Found: C, 24.66; H, 2.76; N, 28.88; S, 43.96.

Bis(5-methylamino-1,3,4-thiadiazol-2-yl) Diselenide (11). A solution of the selone (**2**; 100.4 mg) in methanol (5 mL) was freed from traces of elemental selenium (Celite), and the clear filtrate was stored in an open vessel in the dark. After 3 days the product was collected, washed with methanol, benzene (to remove any colloidal selenium), and finally again with methanol, and air dried. The diselenide formed orange needles or prisms: mp 200–202 °C dec (78.5 mg, 79%); IR ν_{max} 3400 (sh), 3200 (NH); UV ν_{max} (2-methoxyethanol) 264 (ϵ 9300), 280 (9600), 325 nm (7600); ^{1}H NMR δ_{Me_4Si} (Me_2SO-d_6) 2.93 (d, J = 4.5 Hz, 3), 7.99 (br q, J = 4.5 Hz, 1).

Anal. Calcd for $C_6H_8N_6S_2Se_2$: C, 18.66; H, 2.09; N, 21.76; S, 16.60; Se, 40.89. Found: C, 18.72; H, 2.09; N, 21.73; S, 16.70; Se, 40.61.

Bis(2-aminoethyl) Sulfide Perchlorate (12). Perchloric acid (70%; 2.87 g, 0.02 mol) was added dropwise to a stirred solution of bis(2-aminoethyl) sulfide (1.20 g, 0.01 mol) in anhydrous ethanol (25 mL), which was maintained at 0 °C. The mixture was stirred for a further 15 min after addition was complete, and the solid was then collected and washed sparingly with anhydrous ethanol, followed by ether. The product (2.74 g) had mp 187.5–188.5 °C. Recrystallization by dissolution in methanol, followed by gradual addition of absolute ethanol to the boiling solution until all the methanol had been displaced, gave the pure perchlorate salt as cream-colored needles, mp 189.5–190.5 °C (2.45 g).

Anal. Calcd for $C_4H_{14}Cl_2N_2O_8S$: C, 14.96; H, 4.39; Cl, 22.08; N, 8.72; O, 39.86; S, 9.98. Found: C, 15.07; H, 4.39; Cl, 21.86; N, 8.72; O, 39.98; S, 10.05.

Bis(2-aminoethyl) Selenide Perchlorate (13). 2,2′-Bis(2-aminoethyl) selenide was prepared from aziridine and hydrogen selenide by the method of Kroll and Bolton.[25] A solution of the diamine (26.35 g) in absolute ethanol (400 mL) was saturated with hydrogen chloride and the crystalline precipitate (31.17 g; mp 147–148.5 °C) was recrystallized twice by dissolution in methanol, followed by addition of ethanol to the boiling solution until all methanol had been displaced, giving needles, mp 149.5–150 °C (21.75 g). A further recrystallization gave an analytical sample of the hydrochloride: mp 150–151 °C; ^{1}H NMR δ_{Me_4Si} (Me_2SO-d_6) 2.94, 3.04 (aa′ bb′ multiplet, 8), 8.40 (br s, 6).

Anal. Calcd for $C_4H_{14}Cl_2N_2Se$: C, 20.02; H, 5.88; Cl, 29.54; N, 11.67; Se, 32.90. Found: C, 20.09; H, 5.83; Cl, 29.74; N, 11.69; Se, 32.87.

A solution of the foregoing hydrochloride (2.4 g) in water (5 mL) was treated with aqueous sodium hydroxide (4 mL; 50%) at 0 °C and the liberated diamine was isolated by extraction of the mixture with ether, followed by evaporation of the dried ethereal extracts. The oil so obtained was taken up in absolute ethanol and treated with perchloric acid (2.87 g; 70%) at 0 °C, and the resulting precipitate was collected and recrystallized from methanol–ethanol by the method described above. The perchlorate formed needles, mp 162–163 °C (2.32 g).

Anal. Calcd for $C_4H_{14}Cl_2N_2O_8Se$: C, 13.05; H, 3.83; Cl, 19.27; N, 7.61; Se, 21.45. Found: C, 13.28; H, 4.06; Cl, 18.81; N, 7.65; Se, 21.70.

5-Methylthio-1-phenyl-1,2,3,4-tetrazole (24a). 1-Phenyl-1,2,3,4-tetrazole-5-thiol (35.6 g, 0.20 mol) was added to a solution of potassium hydroxide (14.02 g, 0.25 mol) in methanol (178 mL), and

the resulting solution was stirred while methyl iodide (126.5 g, 0.89 mol) was added dropwise. The mixture was stirred under reflux for 4.5 h, then cooled and poured into water (300 mL). Extraction of the mixture with ether (2 × 300 mL), followed by evaporation of the dried (sodium sulfate) ether phase, gave a residue which was recrystallized (Norit) from ethanol. The methylthio derivative was obtained as plates: mp 78.5–80 °C (27.0 g; 70%; lit.[14] mp 84 °C); UV λ_{max} (95% ethanol) 228 (ϵ 7700), 242 (sh) nm (7000); ^{1}H NMR δ_{Me_4Si} (Me_2SO-d_6) 2.84 (s, 3), 7.80 (s, 5).

Anal. Calcd for $C_8H_8N_4S$: C, 49.98; H, 4.19; N, 29.14; S, 16.68. Found: C, 49.96; H, 4.26; N, 29.20; S, 16.73.

5-Methylseleno-1-phenyl-1,2,3,4-tetrazole (24b). This preparation was carried out in subdued light under an atmosphere of nitrogen. A methanolic solution of bis(methoxymagnesium) diselenide was prepared, according to the procedure of Günther,[15] from magnesium (0.75 g, 31 mg-atom), red selenium (1.97 g, 25 mg-atom), and methanol (40 mL), in the presence of a trace of iodine. The reagent was allowed to cool somewhat and a warm solution of 5-chloro-1-phenyl-1,2,3,4-tetrazole (4.52 g, 25 mmol) in methanol (100 mL) was added dropwise with stirring during 15 min. Stirring was continued for 1 h at room temperature (color change from red-brown to gray) and then for 2 h under reflux. The mixture was then filtered (Celite), and the filtrate was evaporated under reduced pressure (bath temperature <40 °C). The semisolid residue (8.23 g) was triturated with ether and the insoluble material removed. Evaporation of the ether filtrate under reduced pressure and trituration of the residue with hexane gave a grayish solid, mp 60–70 °C (2.22 g), which on dissolution in ethyl acetate, followed by gradual addition of hexane to the cloud point, gave 5-methylseleno-1-phenyl-1,2,3,4-tetrazole as pale orange masses: mp 88–89 °C (0.82 g, 14%); IR ν_{max} (CsI) 1596, 1571, 1499, 1410, 1371, 1269, 1231, 1012, 768, 692, 540, 398 cm^{-1}; UV λ_{max} (95% ethanol) 230 nm (ϵ 6800); ^{1}H NMR δ_{Me_4Si} ($CDCl_3$) 2.77 (s, 3), 7.58 (s, 5).

Anal. Calcd for $C_8H_8N_4Se$: C, 40.20; H, 3.37; N, 23.42; Se, 33.01. Found: C, 40.31; H, 3.33; N, 23.57; Se, 32.79.

3-Ethyl-5-methylthio-1-phenyl-1,2,3,4-tetrazolium Fluoroborate (27). A solution of triethyloxonium fluoroborate (14.2 g, 75 mmol) in dry dichloromethane (100 mL) was added dropwise to a stirred solution of 5-methylthio-1-phenyl-1,2,3,4-tetrazole (14.4 g, 75 mmol) in dichloromethane (100 mL), and stirring was continued for 8 days. The solid which had separated was collected, dissolved in dichloromethane, and reprecipitated with ether. The pure tetrazolium salt had: mp 156.5–157.5 °C (9.9 g); IR ν_{max} 1598, 1500, 1450, 1424, 1055 (vs, BF_4^-), 776, 695 cm^{-1}; UV λ_{max} (CH_2Cl_2) 271 (sh) nm (ϵ 4000); ^{1}H NMR δ_{Me_4Si} (Me_2SO-d_6) 1.69 (t, J = 7.5 Hz, 3), 2.88 (s, 3), 5.05 (q, J = 7.5 Hz, 2), 6.80 (s, 5).

Anal. Calcd for $C_{10}H_{13}BF_4N_4S$: C, 38.98; H, 4.25; B, 3.51; F, 24.65; N, 18.18; S, 10.41. Found: C, 39.05; H, 4.28; B, 3.52; F, 24.37; N, 18.36; S, 10.33.

Evaporation of the remaining dichloromethane filtrate gave further product, which, after dissolution in dichloromethane and reprecipitation with ether, gave material with mp 145–149 °C (14.4 g). Despite the lower melting point, this fraction was identical (^{1}H NMR) with the analytical sample. Prolonged drying in vacuo reduced the total weight of product from 24.3 to 23.1 g (100%).

3-Ethyl-1-phenyl-1,2,3,4-tetrazolium 5-thiolate (28). Sodium (313.6 mg, 13.64 mmol) was dissolved in methanol, and the resulting solution was cooled to 5 °C and saturated (stirring) with hydrogen sulfide during 2.5 h. A slurry of 3-ethyl-5-methylthio-1-phenyl-1,2,3,4-tetrazolium fluoroborate (1.85 g, 6.00 mmol) in methanol (75 mL) was then added slowly during 0.5 h, with continued passage of hydrogen sulfide. After all the tetrazolium salt had been added, passage of gas and stirring were continued for a further 0.5 h, during which time the mixture was allowed to reach room temperature. The hydrogen sulfide supply was then disconnected and the mixture stirred gently in a closed system overnight. The resulting yellow solution was freed from hydrogen sulfide by passage of a stream of nitrogen for 2.5 h, after which the mixture was taken to dryness under reduced pressure. Addition of water to the residue removed most of the color, leaving a crystalline product which was collected, mp 104–109 °C (1.13 g). Recrystallization from an ethyl acetate–hexane mixture (2:1 v/v) gave 3-ethyl-1-phenyl-1,2,3,4-tetrazolium 5-thiolate as faintly yellowish-white prisms: mp 114.5–115 °C (0.90 g, 73%); IR ν_{max} 1594, 1589, 1496, 1359, 1178, 774, 769, 733, 693, 689 cm^{-1}; ^{1}H NMR δ_{Me_4Si} (Me_2SO-d_6) 1.60 (t, J = 7 Hz, 3), 4.70 (q, J = 7 Hz, 2), 7.4–8.1 (m, 5).

Anal. Calcd for $C_9H_{10}N_4S$: C, 52.41; H, 4.89; N, 27.16; S, 15.54. Found: C, 52.35; H, 4.93; N, 27.20; S, 15.37.

3-Ethyl-1-phenyl-1,2,3,4-tetrazolium 5-Selenolate (29). This reaction was carried out in subdued light under an atmosphere of nitrogen. A deaerated mixture of sodium hydrogen carbonate (0.84 g, 10 mmol), water (67 mL), and ethanol (22 mL) was stirred at 0 °C while hydrogen selenide [generated by addition of 1.5 N sulfuric acid (13.2 mL, 20 mequiv) to aluminum selenide (0.97 g, 3.3 mmol)] was introduced. To the resulting solution of sodium hydrogen selenide was quickly added a solution of 3-ethyl-5-methylthio-1-phenyl-1,2,3,4-tetrazolium fluoroborate (1.54 g, 5 mmol) and sodium hydrogen carbonate (0.42 g, 5 mmol) in water (77 mL) and ethanol (35 mL). The resulting amber solution was allowed to reach room temperature during 2 h, after which stirring was continued for a further 22 h. The mixture was then freed from hydrogen selenide in a stream of nitrogen. All effluent gases were passed through sodium hydroxide and lead acetate traps. The reddish-tan solid which had separated was collected, washed with water, dried, and recrystallized from an ethyl acetate–hexane mixture to give 3-ethyl-1-phenyl-1,2,3,4-tetrazolium 5-selenolate as yellow needles: mp 128.5–129 °C (0.90 g, 71%); IR ν_{max} 1600, 1501, 1345, 1335, 1170, 780, 724, 700 cm^{-1}; ^{1}H NMR δ_{Me_4Si} (Me_2SO-d_6) 1.58 (t, J = 7.5 Hz, 3), 4.72 (q, J = 7.5 Hz, 2), 7.4–8.0 (m, 5).

Anal. Calcd for $C_9H_{10}N_4Se$: C, 42.70; H, 3.98; N, 22.13; Se, 31.19. Found: C, 42.91; H, 3.89; N, 22.24; Se, 30.94.

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Registry No.—**1b,** 27386-01-2; **2b,** 63528-58-5; **3b,** 63528-59-6; **4b,** 14731-25-0; **5b,** 63528-60-9; **6b,** 63528-61-0; **7b,** 63528-62-1; **8b,** 41526-33-4; **9b,** 63528-63-2; **10,** 32873-72-6; **11,** 63528-64-3; **12,** 63528-65-4; **13,** 63528-66-5; **13** HCl analogue, 63528-67-6; **14,** 882-33-7; **15,** 1666-13-3; **16,** 95-16-9; **17,** 273-91-6; **18,** 149-30-4; **19,** 10486-58-5; **22b,** 86-93-1; **23a,** 16618-41-0; **23b,** 32550-63-3; **24a,** 1455-92-1; **24b,** 62638-96-4; **24c,** 14210-25-4; **25,** 5117-07-7; **26,** 13078-30-3; **27,** 62638-94-2; **28,** 62681-14-5; **29,** 62638-95-3; carbon diselenide, 506-80-9; 4-methyl-3-thiosemicarbazide, 6610-29-3; 4-methyl-3-selenosemicarbazide, 5943-43-1; 4-(*p*-tolyl)-3-thiosemicarbazide, 13278-67-6; 4-(*p*-tolyl)-3-selenosemicarbazide, 14223-52-0; thiophene-2-thiocarbohydrazide, 63528-68-7; thiophene-2-carboxylic acid hydrazide, 2361-27-5; 3-(2-thenoyl)dithiocarbazate, 63528-69-8; bis(2-aminoethyl) sulfide, 871-76-1; bis(2-aminoethyl) selenide, 27974-50-1; bis(methoxymagnesium) diselenide, 14310-09-9; triethyloxonium fluoroborate, 368-39-8.

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Mode of Attack by Crude Papain on Racemic Z-Dipeptides That Contain a β-Alanine Residue during Anilide and Phenylhydrazide Syntheses

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A crude extract of papain exclusively attacked the carbonyl of an α-amino acid residue of Z-β-Ala-DL-Ala and Z-DL-Ala-β-Ala during catalyzed reactions with aniline or phenylhydrazine as the nucleophiles. The optical purities of the resultant, insoluble products, Z-β-Ala-L-Ala-NHPh, Z-β-Ala-L-Ala-NHNHPh, Z-L-Ala-NHPh, and Z-L-Ala-NHNHPh, were all substantially above 90%.

In papain-catalyzed reactions with nucleophiles, attack on a reactive Z dipeptide[1] can occur either at the carboxyl terminal[2] or at the carbonyl group[2,3] of the amide structure that joins the two amino acid residues. It was the purpose of the current study to examine the reactions of aniline and phenylhydrazine with the two racemic Z dipeptides that combine β-alanine with DL-alanine, when a crude papain extract was used as the catalyst. Although β-alanine is a well-known residue of the natural dipeptides, carnosine or anserine, it is not a component residue of proteins. Crude papain[4] contains a mixture of sulfhydryl proteases,[5,6] Enz–SH. It has frequently been utilized as a chiral, catalytic agent for resolutions of N-blocked DL-amino acids,[7,8] using a variety of nucleophiles.[8-11]

The reaction of Z-β-Ala-DL-Ala with RNH_2 in the presence of papain gave the Z-β-Ala-L-Ala-NHR derivatives with the peptide bond intact. Optical purities of the resultant anilide or phenylhydrazide were both approximately 99%.

Z-β-Ala-CO-NH-DL-Ala-COOH

$$\xrightarrow[\text{papain}]{RNH_2} \underset{\text{(insoluble)}}{\text{Z-β-Ala-CO-NH-L-Ala-CO-NHR}} + \underset{\text{(soluble)}}{\text{Z-β-Ala-CO-NH-D-Ala-COOH}}$$

On the other hand, with the β-Ala residue as the carboxyl terminal residue, the attack occurred at the peptide structure to give Z-L-Ala-NHR plus β-Ala. Respective optical purities of the anilide and phenylhydrazide were about 94 and 99%.

Z-DL-Ala-CO-NH-β-Ala-COOH

$$\xrightarrow[\text{papain}]{RNH_2} \underset{\text{(soluble)}}{\text{Z-D-Ala-CO-NH-β-Ala-COOH}} + \underset{\text{(insoluble)}}{\text{Z-L-Ala-CO-NHR}} + \underset{\text{(soluble)}}{NH_2\text{-β-Ala-COOH}}$$

Similar reactions were encountered when the L enantiomers, Z-β-Ala-L-Ala and Z-L-Ala-β-Ala, replaced the corresponding racemic modifications. Important details are summarized in Tables I and II. As might have been anticipated, on the basis of the absence of a β-Ala residue in proteins, crude papain did not catalyze such reactions with Z-β-Ala or Z-β-Ala-β-Ala. Furthermore, Z-β-Ala-D-Ala and Z-D-Ala-β-Ala were equally unproductive when appropriately tested.

An investigation of the pH dependence of yield was made for reactions of Z-β-Ala-Gly and Z-Gly-β-Ala with NH_2Ph and NH_2NHPh. Again, the nucleophilic attack was made exclusively on the carbonyl of the α-amino acid residue. pH optima are shown for the sole, insoluble products: Z-β-Ala-Gly-NHPh (pH 4.75); Z-β-Ala-Gly-NHNHPh (pH 4.25); Z-Gly-NHPh (pH 4.50); Z-Gly-NHNHPh (pH 4.25).

Thin-layer chromatography[2] on plastic plates coated with silica gel established that each successful catalysis yielded an insoluble product with a single structure. R_f values of reference compounds are recorded in Table III.

Experimental Section

Preparation of Active Crude Papain. The crude, active papain necessary for these experiments was prepared by a slight modification of the procedure outlined by Bennett and Niemann.[4]

Reactions of Z-β-Ala-L-Ala and Z-β-Ala-DL-Ala with Aniline and Phenylhydrazine. A mixture of 0.5000 g of Z-β-Ala-L-Ala, 0.26 mL of aniline or phenylhydrazine, 0.1000 g of papain, 0.1000 g of L-cysteine·HCl·H_2O, 25 mL of 0.50 buffer at pH 4.5, and 2 mL of hexamethylphosphoramide was filtered and then incubated at 40 °C. At appropriate time intervals, the solid reaction product was removed by suction filtration, washed with distilled water, dried in the incubator for several days, and weighed. When necessary, the solid was treated with carbon in methanol and filtered four times by suction filtration, with the terminal filtration through a fritted glass funnel. Sufficient methanol was used each time to remove all product from the funnel. Purified product was isolated either by rotary evaporation under reduced pressure or else evaporation in a Petri dish under the hood: % N Calcd for Z-β-Ala-L-Ala-NHPh 11.38, found 11.10; % N Calcd for Z-β-Ala-L-Ala-NHNHPh 14.68, found 14.32. Reactions of Z-β-Ala-DL-Ala were done on four times the scale of Z-β-Ala-L-Ala. Mixture melting points with corresponding products from Z-β-Ala-L-Ala showed no change.

The Behavior of Z-L-Ala-β-Ala and Z-DL-Ala-β-Ala toward Analine and Phenylhydrazine. For Z-L-Ala-β-Ala the solution contained 0.5000 g of Z-L-Ala-β-Ala, 0.52 mL of aniline or phenylhydrazine, 0.1000 g of papain, 0.1000 g of L-cysteine·HCl·H_2O, 25 mL of 0.50 M buffer at pH 4.5, and 2.0 mL of hexamethylphosphoramide. Following filtration, the solution was incubated at 40 °C. After appropriate time intervals, insoluble product was removed by suction filtration, dried for several days in the incubator, and then weighed. A mixture melting point for the anilide product with known Z-L-Ala-NHPh[2,7] exhibited no change. Similarly, the Z-L-Ala-NHNHPh from this study when mixed with known compound[12] showed no change in melting point. After purification: % N Calcd for Z-L-Ala-NHPh 9.39, found 9.62; % N Calcd for Z-L-Ala-NHNHPh 13.41, found 13.52. Reactions of Z-DL-Ala-β-Ala were performed in exactly twice the quantities used for Z-Ala-β-Ala. After purification: % N Calcd