A New Synthesis of Isoxazoles from 1,4 Dianions of Oximes Having an α Hydrogen. Mass Spectrometry[1]

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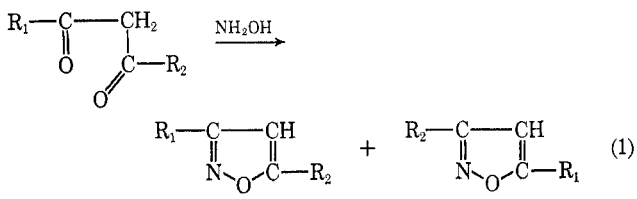
Received September 19, 1969

The 1,4 dianions of deoxybenzoin oxime, acetophenone oxime, and *para*-substituted acetophenone oximes were prepared and condensed with aromatic esters followed by acid cyclization to give unsymmetrical 3,4,5-tri- and 3,5-disubstituted isoxazoles. The synthesis of other isoxazole types, using various oxime 1,4 dianions, by this convenient and unequivocal method is presented. Mass spectra of unsymmetrical isoxazoles are also discussed.

Probably the most general method for the synthesis of isoxazoles involves the condensation–cyclization of hydroxylamine with a β diketone; the reaction of unsymmetrical β diketones or their enol ethers with hydroxylamine gives unsymmetrically substituted isoxazoles; however, the reaction can and does give both possible isomers, although some selectivity has been achieved by controlling the pH of the reaction[3-6] (eq 1).

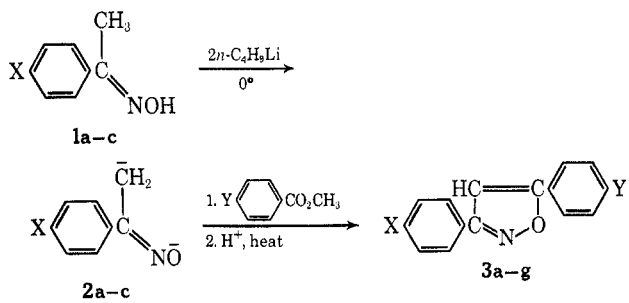


(1)

The present paper describes an unequivocal method for the synthesis of substituted isoxazoles from the oxime of a ketone having an α hydrogen and from an aromatic ester. The oxime was converted to its 1,4-dilithio salt with 2 molar equiv of *n*-butyllithium[7] and 0.5 molar equiv of ester was added.[8] The presumed intermediate keto oximes were not isolated, but were cyclized directly under acidic conditions to give substituted isoxazoles in good yields.

An unequivocal synthesis of unsymmetrical 3,5-diarylisoxazoles was undertaken by aroylation of several acetophenone and *para*-substituted acetophenone oxime dianions with methyl benzoate or methyl *para*-substituted benzoates followed by acid cyclization to the isoxazole (Scheme I). Dianion **2** was conveniently formed at 0° instead of −80°[7] by the reaction of 2 mol of *n*-butyllithium/mol of oxime. Aroylation was accomplished in the manner of the Claisen aroylation[8] with 0.5 mol of ester/mol of **2**, and upon acid cyclization isoxazoles **3a–g** were obtained in 50–60% yield

SCHEME I



based upon the ester. The structures of isoxazoles **3a–g** were supported by analysis and/or absorption spectra. The melting points of known compounds **3a–e** were in agreement with those previously reported.[9]

The infrared spectrum of **3a** was essentially identical with the spectrum reported for this compound.[10] The infrared spectrum of each of the other six isoxazoles **3b–g** was consistent with the assigned structure. For the three pairs of isomeric 3,5-diarylisoxazoles **3b** and **3d**, **3c** and **3e**, and **3f** and **3g**, the infrared spectrum of one isomer was similar to but not identical with that of the other isomer. No consistent pattern was discernible in the three pairs of spectra to permit identification of one isomer of such a pair of unsymmetrically disubstituted isoxazoles.

The nmr of the seven isoxazoles **3a–g** contained aromatic absorptions, plus a singlet due to the methoxy group in **3c,e–g**. Each isoxazole had an absorption signal in the region δ 6.8–7.2[11] which was assigned to the proton at the 4 position of the isoxazole ring. When this resonance signal was not complicated by other aromatic absorptions, it appeared as a singlet which integrated for one proton.

In a manner similar to the procedure described above, certain 5-*para*-substituted 3,4,5-triarylisoxazoles were prepared. The dianion of deoxybenzoin oxime **4** was prepared and condensed with various methyl and methyl *para*-substituted benzoates to give the corresponding isoxazoles **5a–c** in 75–85% yield (Scheme II). The structures of **5a–c** were confirmed by absorption spectra and compounds **5b** and **5c** were also supported by analysis (Table I).

The general applicability of this method of isoxazole synthesis was partially investigated. Isoxazoles were successfully prepared by the condensation–cyclization

(1) Supported by the Public Health Service, Research Grant CA-04455 from the National Cancer Institute and the National Science Foundation.

(2) (a) National Aeronautics and Space Administration Trainee, 1967–1969. (b) Deceased.

(3) See A. Quilico, "The Chemistry of Heterocyclic Compounds," Vol. 17, R. L. Wiley, Ed., John Wiley & Sons, Inc., New York, N. Y., 1962, Chapter 1.

(4) See, for example, the discussion in K. M. Johnston and R. G. Schotter, *J. Chem. Soc., C*, 1774 (1968).

(5) (a) See R. A. Barnes, "Heterocyclic Compounds," Vol. 5, R. C. Elderfield, Ed., John Wiley & Sons, Inc., New York, N. Y., 1957, p 454; (b) N. K. Kochetkov and S. D. Sokolov, *Advan. Heterocycl. Chem.* **2**, 366 (1963).

(6) See U. Teurck and H. Behrenger, *Chem. Ber.*, **98**, 3020 (1965).

(7) F. E. Henoch, K. G. Hampton, and C. R. Hauser, *J. Amer. Chem. Soc.*, **91**, 676 (1969).

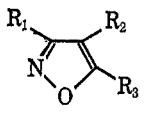
(8) The proportions correspond to the use of 2 molar equiv of base and ketone and one of ester in the analogous acylation of a ketone, which has been one of the two recommended procedures for such Claisen condensations with ester; see also C. R. Hauser, F. W. Swamer, and J. T. Adams, *Org. React.*, **8**, 113 (1954).

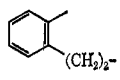
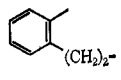
(9) C. Weygand, E. Bauer, and W. Heynemann, *Justus Leibigs Ann. Chem.*, **459**, 123 (1927).

(10) A. R. Katritsky and A. J. Boulton, *Spectrochim. Acta*, **17**, 238 (1961).

(11) K. Sirakawa, O. Aki, S. Tsushima, and K. Konishi, *Chem. Pharm. Bull.* (Tokyo), **14**, 89 (1966).

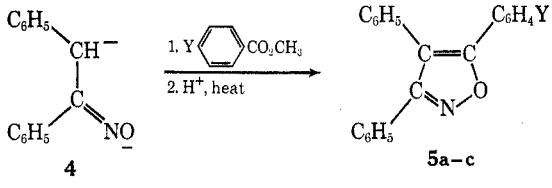
TABLE I



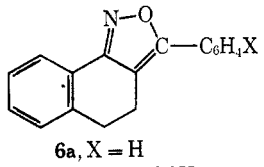
Compd	R_1	R_2	R_3	% yield	Mp, °C	Lit. mp, °C (ref)	Mol wt M^+ [a]	Nmr, δ [b] C_4 H isoxazole
3a	C_6H_5–	H	C_6H_5–	59	141–142.5 [c]	141 [f]		6.83
3b	C_6H_5–	H	*p*-C_6H_4Cl–	59	179.5–180.5 [d]	178–179 [g]	255	7.18
3c	C_6H_5–	H	*p*-$C_6H_4OCH_3$–	51	127–128 [c]	128 [i]	251	6.89
3d	*p*-C_6H_4Cl–	H	C_6H_5–	65	176.5–177.5 [d]	175 [h]	255	7.19
3e	*p*-$C_6H_4OCH_3$–	H	C_6H_5–	54	120.5–121.5 [c]	121 [i]	251	6.75
3f	*p*-C_6H_4Cl–	H	*p*-$C_6H_4OCH_3$–	66	179–181 [d,e]		285	7.22
3g	*p*-$C_6H_4OCH_3$–	H	*p*-C_6H_4–Cl–	54	185.5–186.5 [d,e]		285	7.04
5a	C_6H_5–	C_6H_5–	C_6H_5–	72	210–211 [c]	210 [j]	297	
5b	C_6H_5–	C_6H_5–	*p*-C_6H_4Cl–	80	174–176 [c,e]		331.0764 (C) 331.0760 (F)	
5c	C_6H_5–	C_6H_5–	*p*-$C_6H_4OCH_3$–	79	162–163 [c,e]		327.1259 (C) 327.1257 (F)	
6a			C_6H_5–	19	87–89 [c,e]			
6b			*p*-$C_6H_4OCH_3$	37	146–147 [c,e]		277.1103 (C) 277.1102 (F)	
7a	$-(CH_2)_3-$		C_6H_5–	74	106–107 [c,e]	108–109 [k]	185	
7b	$-(CH_2)_4-$		C_6H_5–	62	65–67 [c]	67 [k]	199	
8a	*p*-$C_6H_4OCH_3$–			47	139–141 [c,e]		252.0899 (C) 252.0899 (F)	6.85
8b	C_6H_5–	H	$-CH_2C_6H_5$	28	79–80 [c]	81 [l]	235.0997 (C) 235.0992 (F)	6.82

[a] Molecular weight determined by low- and high-resolution mass spectrometry. C = calculated; F = found. [b] Trifluoroacetic acid solvent. [c] Recrystallized from ethanol. [d] Recrystallized from benzene. [e] Satisfactory analyses (±0.30%) for C, H, N, and Cl (where applicable) were obtained for all new compounds reported (Editor). [f] C. Goldschmidt, *Chem. Ber.*, **28**, 2540 (1895). [g] P. Grunanger, *Gazz. Chim. Ital.*, **89**, 1771 (1959). [h] G. Bianchetti, D. Pocar, and P. D. Croce, *ibid.*, **93**, 1714 (1963). [i] Reference 9. [j] D. E. Worrall, *J. Amer. Chem. Soc.*, **57**, 2299 (1935). [k] G. Bianchetti and P. Grunanger, *Chim. Ind.* (Milan), **46**, 425 (1964). [l] G. S. d'Alcontres and G. L. Vecchio, *Gazz. Chim. Ital.*, **90**, 1239 (1960).

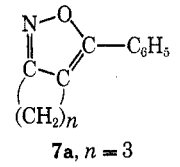
SCHEME II



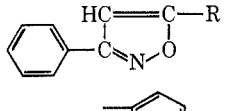
of methyl or a methyl *para*-substituted benzoate with the oxime dianions of α-tetralone, cyclopentanone, and cyclohexanone. The yields of the isoxazoles obtained ranged from 19 to 74% (**6a**,**b** and **7a**,**b**). Isoxazoles **6a**



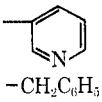
6a, X = H
b, X = *p*-OCH_3



7a, $n = 3$
b, $n = 4$

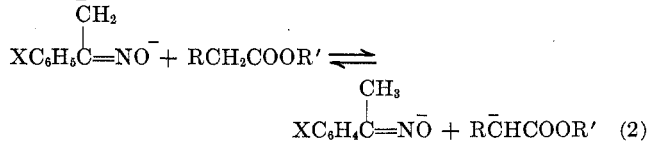


8a, R =



b, R = $-CH_2C_6H_5$

and **b** were characterized by analysis and absorption spectra. The yields reported (19 and 37%) are probably not the maximum obtainable, because of diminished solubility of this dianion compared with the solubility of the dianions previously mentioned. Isoxazoles **7a** and **7b** are known and confirmation of structure was possible from comparison of melting points with those reported and their absorption spectra. The ester component was also varied, and isoxazoles were prepared by condensation–cyclization with ethyl nicotinate and ethyl phenylacetate to give **8a** and **8b** in yields of 47 and 28%, respectively. Interestingly, precursor pyridyl β diketones for **8a** have not been reported; thus the present procedure represents a convenient and direct synthetic route to this new type of 5-substituted isoxazole. The low yield of **8b** may be attributed in part to an acid–base reaction between the oxime dianion and the active hydrogen α to the carbonyl as shown in eq 2. This equilibrium may destroy some of the oxime



$$XC_6H_5\bar{C}{=}NO^- + RCH_2COOR' \rightleftharpoons XC_6H_4\bar{C}{=}N\bar{O} + R\bar{C}HCOOR' \quad (2)$$

dianion before the dianion could condense with the ester; also, formation of the ester monoanion should deactivate the ester toward attack by the oxime dianion.

Attempts to prepare isoxazoles *via* aroylation of possible dianions of the oximes of *m*-nitroacetophenone,

phenacyl chloride, and α-dimethylaminoacetophenone have been nonreproducible or unsuccessful. Isoxazoles, if formed, were in trace amounts and were contaminated with intractable tarry material.

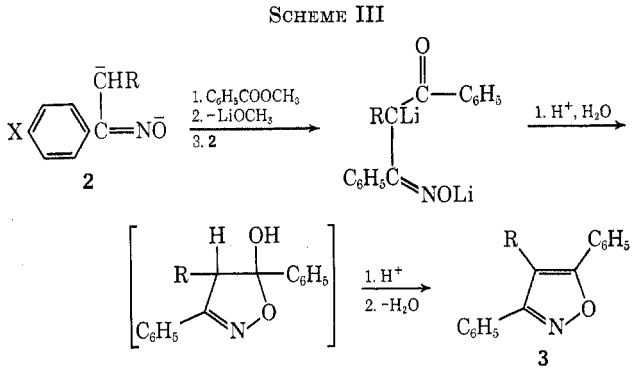
Table I summarizes the isoxazoles which were prepared during this investigation and gives significant data for each compound.

Discussion

The synthesis described in the present work has several advantages over previous methods: an unequivocal route to unsymmetrically substituted isoxazoles, readily available starting materials, compatibility with a variety of substituent groups, and a short, simple experimental procedure.

The formation of the oxime dianion by the action of *n*-butyllithium probably takes place by a stepwise process where protons of significantly different acidities are involved. In the case of a simple oxime of type **1**, the first equivalent of base removes the more acidic hydroxyl proton of the oxime, and the second equivalent of base removes a proton α to the oxime function. Evidence for the existence of such dianions has been obtained by alkylation of **2** with benzyl chloride and *n*-butyl bromide.[7] In both cases, the alkylation occurred at the more nucleophilic carbanionic site rather than at the oxygen site. The aroylation of the dilithio salt **2** (R = H) with methyl benzoate is similar to a Claisen aroylation and should involve a similar mechanism (see Scheme III).[8]

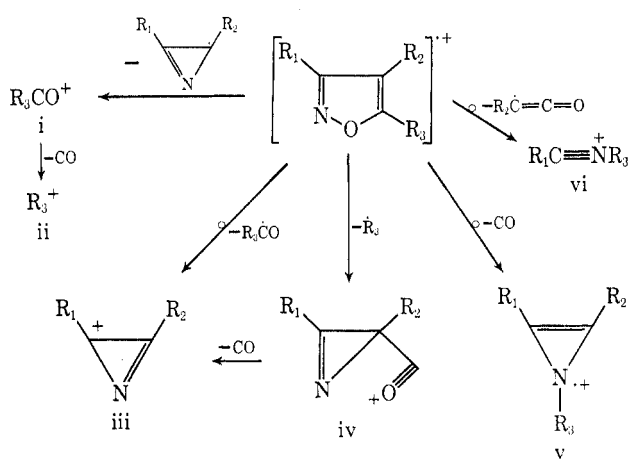
SCHEME III



In an attempt to find a readily available and simple method of identifying the isomers of a pair of unsymmetrically substituted 3,5-diarylisoxazoles, the mass spectra of many compounds prepared in this work were examined. In all cases an ion of relative intensity ≥50% arising from a fragmentation involving the 5 substituent was observed. Thus, mass spectrometry permits the identification of the isomer obtained and provides the needed check on the unequivocal nature of various isoxazole syntheses.

Our findings have been confirmed by the recent work of Bowie, Kallury, and coworkers[12,13] and Nakata and coworkers.[14,15] These investigators have correctly suggested on the basis of their work with alkylphenyl- and 3,5-diphenylisoxazoles[13,14] that the substituent at the 5 position of the isoxazole ring will be indicated by the presence of fragments arising from the loss of the substituent as a radical (M − R_3· peak),[12,15] or from cleavage of the heterocyclic ring to give an aroyl cation (R_3—C≡O+)[12-15] (Scheme IV). The latter fragment, when present, will lose CO and give the aryl cation, R_3+. The loss of a 5 substituent as a radical occurs in significant amounts only when a stable radical is formed (*e.g.*, the benzyl radical in **8b**). The M − R_3· ion then loses CO to give iii. The presence of metastable peaks for the latter fragmentation confirms the contribution of this pathway to the intensity of ion iii.

SCHEME IV[a]



[a] Looped arrows designate skeletal rearrangements during fragmentation to the indicated ion or ion radical.

In addition to these two primary fragmentation pathways, rearrangement–fragmentation involving the migration of the 5 substituent has been found to occur.[12,13] In the mass spectra of triarylisoxazoles **5a–c**, fragmentation involving the loss of phenyl ketene radical recently noted by Kallury and Bowie[13] has been confirmed. That the aromatic ring lost in the ketene radical is not the 5 substituent was demonstrated by retention of *para*-substituted phenyl groups of 5-*para*-substituted triarylisoxazoles in the ion vi.

Loss of stable neutral fragments was observed in systems where the 3 and 4 positions of the isoxazole ring were bridged by an aliphatic side chain (Scheme V).

In addition to the fragmentations involving cleavage of the isoxazole ring, fragmentations involving cleavage of aryl substituents were observed. These were consistent with previously reported cleavages, *e.g.*, loss of CH_3· in those compounds containing a methoxy group. Several doubly charged ions were present in the spectra of the compounds studied, and in all cases an ion corresponding to M^{2+} was observed. No attempt was made to describe the fragmentation pathways for such doubly charged species.

Table II summarizes the major fragmentations which were observed in the compounds studies.

Experimental Section

All analyses were performed by M-H-W Laboratories, Garden City, Mich. Infrared spectra were obtained on Perkin-Elmer Model 137 and 237 spectrometers. The nmr spectra were ob-

(12) J. H. Bowie, R. K. M. R. Kallury, and R. G. Cooks, *Aust. J. Chem.*, **22**, 563 (1969).

(13) B. K. Simons, R. K. M. R. Kallury, and J. H. Bowie, *Org. Mass Spectrom.*, **2**, 739 (1969).

(14) H. Nakata, H. Sakurai, H. Yoshizumi, and A. Tatematsu, *ibid.*, **1**, 199 (1968).

(15) H. Nakata, *et al.*, *ibid.*, **2**, 195 (1969).

TABLE II
MASS SPECTRAL DATA OF CERTAIN ISOXAZOLES[a]

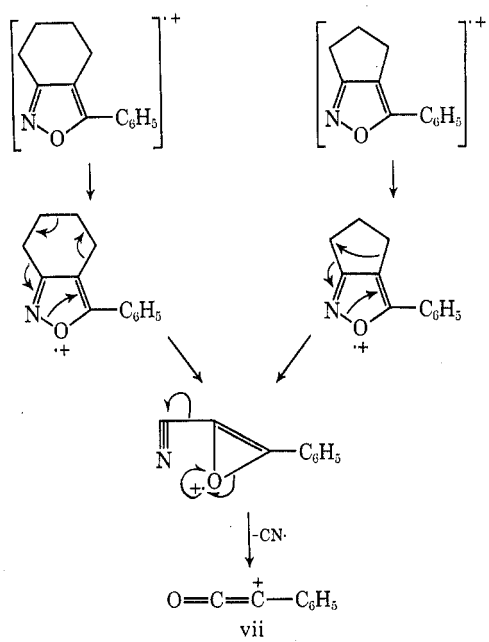
Compd no.	M^+	M^{2+}	i	ii	iii	iv	v	vi	vii
3b	255 (91)	127.5 (6.9)	139 (100)*	111 (19.5)*	116 (3)	144 (16)	227 (3.6)*		
3c	251 (63.2)	125.5 (6)	135 (100)*	107 (5.5)*	116 (1.5)	144 (2)	223 (0.3)*		
3d	255 (63)	127.5 (6.3)	105 (100)	77 (27)*	150 (2.5)	178 (5.5)	227 (1.5)*		
3e	251 (100)	125.5 (9.9)	105 (51)	77 (25)*	146 (4)	174 (8.5)	223 (0.6)*		
3f	285 (85)	142.5 (5.5)	135 (100)*	107 (4.5)*	150 (1.7)	178 (1.1)			
3g	285 (100)	142.5 (7)	139 (58)	111 (15)*	146 (31)	174 (9.2)			
5a	297 (100)	148.5 (3.9)	105 (92.2)	77 (33.3)*	192 (2.2)	220 (1.9)	269 (2.5)*	180 (33.3)*	
5b	331 (97)	166.5 (3.1)	139 (100)	111 (30.3)*	192 (2.7)	220 (1.6)	303 (3.2)*	214 (54.4)*	
5c	327 (95)	163.5 (5.3)	135 (100)	107 (5.7)*	192 (2.7)		229 (2.4)*	210 (52.5)*	
6b	277 (100)	138.5 (1.2)	135 (50.0)	107 (7.1)*	142 (1.5)		249 (5.1)*		
7a	185 (30.6)	92.5 (0.8)	105 (100)*[b]	77 (83.3)*	80 (3.1)	108 (2.2)	157 (4.4)		117 (8.3)
7b	199 (100)	99.5 (21.0)	105 (63.2)*[c]	77 (26.3)*	94 (21.1)	122 (1.8)	171 (1.1)*		117 (2.9)
8a	252 (100)	126 (10)	106 (33.4)	78 (26.2)*	146 (11.3)*[d]	174 (24.2)			
8b	235 (608)	117.5 (2.5)		91 (8.4)[e]	116 (8)*[d]	144 (100)*			

Other fragments[f]

3b: 258 (5.5) [M + 3]; 257 (30) [M + 2]; 256 (19.5) [M + 1]; 254 (14) [M − 1]; 141 (33) [i + 2]; 113 (7.5) [ii + 2]; 89 (5.5); 81 (9.5); 77 (7.7); 75 (5.9); 73 (5.2); 69 (18.3); 57 (7.3); 55 (7.0); 51 (6); 43 (7.4); 41 (7.3). **3c**: 252 (19) [M + 1]; 136 (11) [i + 1]; 92 (7.2) [ii − CH_3]; 77 (22.1). **3d**: 257 (22) [M + 2]; 256 (11) [M + 1]; 106 (7.6); 69 (5.5); 51 (5) [77 − C_2H_2]. **3e**: 252 (22) [M + 1]; 135 (48)*.[g] **3f**: 288 (5.2) [M + 3]; 287 (29.1) [M + 2]; 286 (15) [M + 1]; 136 (9.1) [i + 1]; 92 (6.2) [ii − CH_3]; 81 (5); 77 (10); 69 (11). **3g**: 288 (6) [M + 3]; 287 (33) [M + 2]; 286 (19) [M + 1]; 270 (6.8) [M − CH_3]; 149 (16); 141 (17) [i + 2]; 138 (7.5); 137 (10); 97 (5.7); 95 (6.2); 83 (7.5); 82 (6); 81 (19); 73 (9.5); 71 (9.5); 70 (6.5); 69 (48); 68 (6.5); 67 (5); 60 (8.5); 57 (17); 56 (5.5); 55 (14); 43 (16.5); 41 (17.6). **5a**: 298 (24.2) [M + 1]; 166 (6.3); 165 (14.5); 106 (7.1); 89 (9.4); 51 (5.5) [ii − C_2H_2]. **5b**: 334 (8.4) [M + 3]; 333 (34.9) [M + 2]; 332 (25.4) [M + 1]; 216 (17) [vi + 2]; 165 (19.1); 148 (5.5); 141 (31.7) [i + 2]; 140 (7.9); 113 (21.4) [ii + 2]; 112 (7.9); 89 (13.9); 51 (5.2). **5c**: 328 (24) [M + 1]; 211 (7.9) [vi + 1]; 196 (5.8); 181 (5.3); 136 (9.5) [i + 1]; 92 (7.4); 89 (7.4); 78 (13.1); 77 (15.3). **6a**: 278 (13.7) [M + 1]; 262 (7.1) [M − CH_3]*; 248 (23) [M − HCO]; 234 (11.1) [M − (CH_3 + CO)]; 233 (5.1) [M − (HCO + CH_3)]; 218 (9.1); 133 (6.0); 92 (9.4) [ii − CH_3]; 89 (5.8); 77 (14.6). **7a**: 186 (4.9) [M + 1]; 156 (14.7) [M − HCO]; 130 (9.3); 129 (12.8); 115 (9.4); 106 (8) [i + 1]; 103 (13.3); 78 (8.6) [ii + 1]; 53 (5.6); 52 (6.1); 51 (30.6) [ii − C_2H_2]; 50 (7.5); 42 (8.3). **7b**: 200 (14.7) [M + 1]; 143 (7.1) [M − C_4H_8]; 106 (5.5) [i + 1]; 104 (5.5); 91 (21); 51 (6.3) [ii − CH_2]$_2$. **8a**: 253 (17.5) [M + 1]; 237 (21) [M − CH_3]*; 221 (5), [M − CH_3O]*; 51 (6.3) [ii − HCN). **8b**: 236 (18.4) [M + 1]; 145 (12.5) [iv + 1]; 89 (4.1) [iii − HCN]*; 77 (16); 76 (10)

[a] Tabulated are *m/e* (relative intensity) for the molecular ion, doubly charged molecular ion, and ions corresponding to general structures i–vii in Schemes IV and V. Asterisks indicate fragmentations for which metastable peaks were observed. All observed metastable peaks agree to ±0.2 of the calculated value. [b] Metastable peaks at 77.2 and 51.4 indicate that two fragmentations 143 → 105 and 185 → 105 contribute to the intensity of this ion fragment. [c] Metastable peak at 77.1 indicates that fragmentation 143 → 105 contributes to the intensity of the 105 ion peak. [d] Metastable peaks indicate that these fragments arise in part that iv by loss of CO or ĊO. [e] This fragment shows no evidence of arising from fragmentation, Scheme IV. [f] Ions of relative intensity ≥5%. Data which are in brackets indicate postulated origin. Since high resolution was not employed except for select cases, ions of other elemental composition than those indicated may contribute to the reported peak. [g] High resolution indicates that this ion has the composition $C_8H_7O_2$ (mol wt calcd 135.0445, found 135.0449); metastable peak indicates that it arises from the molecular ion.

SCHEME V



tained using a Varian A-60 nmr spectrometer using trifluoroacetic acid as a solvent, and shifts are reported in parts per million downfield (δ) from an internal tetramethylsilane (TMS) standard. Mass spectra were taken at the Research Triangle Institute for Mass Spectrometry, Durham, N. C., on an MS-902 mass spectrometer.[16] Melting points were taken on samples in open capillary tubes in a Thomas–Hoover melting point apparatus and are uncorrected. Tetrahydrofuran (THF) was distilled from lithium aluminum hydride immediately before use. The *n*-butyllithium was obtained from Alfa Inorganics, Inc., Beverly, Mass., and was used as supplied.

General Procedure for Conversion of an Oxime to Its Dilithio Salt.—To a stirred solution of 0.025 mol of oxime in 100 ml of THF, which was cooled to 0° under a nitrogen atmosphere, was added during 5 min 22.5 ml (0.05 mol) of 2.25 *M* *n*-butyllithium. After 30 min, the solution was assumed to contain 0.025 mol of dilithio salt, which was condensed with an ester as described below.

General Procedure for Aroylation[17] of Dilithio Salt Followed by Acid Cyclization to Form Isoxazole.—A 0.0125-mol sample of the ester dissolved in 15 ml of THF was added during 5 min to a solution containing 0.025 mol of dilithio salt (prepared as described above). After stirring at 0° for 15 min, the mixture was neutralized with 100 ml of 3 *N* hydrochloric acid. The mixture was then heated at reflux temperature for 1 hr and cooled, and

(16) The authors thank Dr. David Rosenthal for the mass spectral determinations, which were done at the Research Triangle Mass Spectrometry Center supported by Special Facility Grant No. Fr-00330-01, National Institutes of Health.

(17) Condensations of the oxime dianion with ethyl phenyl acetate followed by acid cyclization were carried out using this procedure.

the layers were separated. The aqueous layer was neutralized with sodium bicarbonate and extracted with three 50-ml portions of ether. The combined ether extracts were concentrated at reduced pressure on the steam bath.[18] If the residue contained a mixture of solid and an oil, it was washed with 10 ml of cold (0°) methanol and filtered immediately. If a solid was not formed, 5–10 ml of methanol was added and crystallization occurred upon refrigeration of the mixture. Recrystallization was effected with ethanol or benzene (see Table I).

(18) Conveniently, crystallization was found to be hastened if the ether extracts were not dried before removal of excess solvents, especially when *p*-methoxyaryl isoxazoles were synthesized.

Registry No.—**3b**, 1148-87-4; **3c**, 3672-51-3; **3d**, 24097-17-4; **3e**, 3672-52-4; **3f**, 24097-19-6; **3g**, 24097-20-9; **5a**, 22020-72-0; **5b**, 24097-22-1; **5c**, 24097-23-2; **6a**, 24097-24-3; **6b**, 24097-25-4; **7a**, 24097-26-5; **7b**, 24097-27-6; **8a**, 24162-37-0; **8b**, 18753-56-5.

Racemization of Amino Acid Derivatives. Rate of Racemization and Peptide Bond Formation of Cysteine Active Esters[1]

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Received December 1, 1969

It is demonstrated that N-carbobenzoxy-S-benzyl-L-cysteine active esters do not racemize *via* a "β-elimination–readdition" mechanism in the presence of triethylamine. Racemization and coupling rate constants of several N-carbobenzoxy-S-benzyl-L-cysteine esters are reported. The rate data indicate that (a) the required coupling time in some cases is considerably less than usually used in preparative work and (b) an N-protected amino acid containing a fast-coupling active ester can be joined with a slowly reacting active ester to yield optically pure carboxyl-activated intermediates which are useful for the preparation of high molecular weight sequential polypeptides. Comparison and evaluation of the coupling and racemization rate data allows selection of the "best suited" active ester for peptide bond formation under the conditions employed.

The most important problem in peptide synthesis is to avoid racemization. Racemization through an oxazolone[2] intermediate has been studied in detail and is well understood. However, some amino acid derivatives, where oxazolone formation is believed to be absent, have been found to racemize in the presence of base.[3] It has been suggested that racemization of these derivatives proceeds through α-hydrogen abstraction.[3] The unusual facility with which cysteine[4] and serine[5] derivatives racemize has been attributed to a "β-elimination–readdition" mechanism.[6] Even N-carbobenzoxy- and N-*t*-butoxycarbonyl-S-benzyl-L-cysteine active esters racemize in the presence of triethylamine.[3a,c]

In this paper we report studies on the mechanism of racemization and the comparison of the rates of racemization and peptide bond formation of N-carbobenzoxy-S-benzyl-L-cysteine active esters as a model for peptide synthesis. These studies led to important conclusions concerning the choice of an active ester for the synthesis of oligopeptides as well as high molecular weight sequential polypeptides.

Evaluation of the β-Elimination–Readdition Mechanism.[6]—The racemization of N-carbobenzoxy-S-benzyl-L-cysteine pentachlorophenyl ester with excess triethylamine was studied in the presence of benzyl mercaptan-^{35}S. The partially racemized active ester was isolated without any incorporation of radioactive sulfur.[7] On the other hand, racemization of N-carbobenzoxy-S-benzyl-L-cysteine *p*-nitrophenyl ester under identical conditions resulted in partially racemized N-carbobenzoxy-S-benzylcysteine thiobenzyl ester[8] (77% yield) which contained one equivalent of radioactive sulfur. The position of the sulfur-35 was established by hydrazinolysis of the thiobenzyl ester. The corresponding hydrazide showed complete absence of the incorporated sulfur-35.

These experiments clearly confirm that β-elimination–readdition is not the mechanism for the racemization of N-carbobenzoxy-S-benzyl-L-cysteine active esters under these basic conditions. This result leads one to conclude that racemization of cysteine derivatives proceeds through abstraction of the α hydrogen.

Racemization of N-Carbobenzoxy-S-benzyl-L-cysteine Active Esters.—The racemization of the active esters listed in Table I was carried out in tetrahydrofuran solution in the presence of triethylamine under strictly anhydrous conditions. When anhydrous solvents were used but manipulations were not carried out in a drybox, the racemization of some of the active esters was accompanied by hydrolysis which usually

(1) Part III of a series on racemization studies of amino acid derivatives. For parts I and II see ref 6.

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(7) The readdition of benzyl mercaptan to N-carbobenzoxydehydroalanine pentachlorophenyl ester yielded racemic N-carbobenzoxy-S-benzylcysteine pentachlorophenyl ester. On the other hand, N-carbobenzoxydehydroalanine *p*-nitrophenyl ester on reaction with 1 equiv of benzyl mercaptan under similar conditions yielded a complex mixture, two components of which are N-carbobenzoxy-S-benzyl-DL-cysteine *p*-nitrophenyl ester and N-carbobenzoxy-S-benzyl-DL-cysteine thiobenzyl ester. When the above reaction was run with 2 equiv of benzyl mercaptan, N-carbobenzoxy-S-benzyl-DL-cysteine thiobenzyl ester was isolated in high yield.

(8) This unexpected difference in the behavior between the *p*-nitrophenyl ester and the pentachlorophenyl ester in ester exchange reaction with benzyl mercaptan led to the investigation of the reaction of several other active esters with benzyl mercaptan. The data in Table IV suggest that steric effects may play a role in this ester exchange reaction.