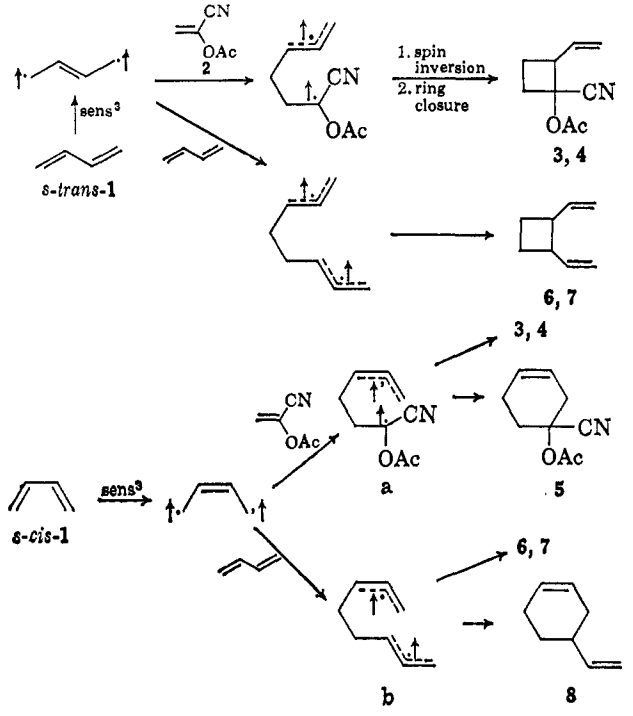
Table I. Composition of Products from Photosensitized Cross-Addition of Butadiene and α-Acetoxyacrylonitrile

Expt	Sensitizer[a]	E_T[b]	Distribution of cross-adducts and dimers, %[c] 3 + 4[d]	5	6	7	8
1	Acetophenone	73.6	34	0.7	56	7	2
2	Benzophenone	68.5	33	0.9	54	10	3
3	Triphenylene[e]	66.6	30	0.9	69	...[f]	...[g]
4	Anthraquinone[e]	62.4	60	3	31	6	...[g]
5	Flavone	62.0	32	1.6	52	12	3
6	β-Naphthyl phenyl ketone	59.6	41	7	47	4	2
7	Biacetyl	54.9	38	12	28	7	14
8	Benzil	53.7	34	12	30	5	18
9	Camphorquinone	50	28	12	23	7	29
10	Pyrene[e]	48.7	38	17	34	11	...[g]
11	Anthracene[e]	42.5	41	6	45	8	...[g]
12	9,10-Dibromoanthracene[e]	40.2	45	1	44	10	...[g]

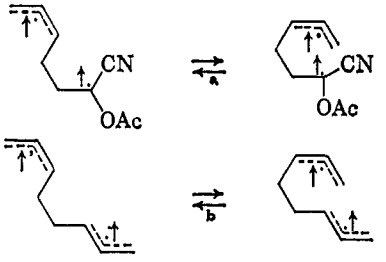
[a] Sensitizer (1.0 mmole) in 5.0 mmoles of **1** and 5.0 mmoles of **2**. [b] Lowest triplet energy level in kilocalories per mole: W. G. Herkstroeter, A. A. Lamola, and G. S. Hammond, *J. Am. Chem. Soc.*, **86**, 4537 (1964). See also ref 3c. [c] Determined by gas chromatography with each component corrected for thermal conductivity variation in detector. [d] The *cis* and *trans* isomers were not separated. The nmr spectrum of the product isolated from the benzophenone-sensitized reaction showed two acetyl methyl groups at −2.05 and −2.07 ppm from internal TMS of approximately equal intensity. [e] Saturated solution of sensitizer in equimolar mixture of **1** and **2**. [f] Not determined due to low over-all conversion. [g] Not determined due to interference by a large excess of **2** and the presence of a small amount of **8** in the starting diene **1**.

Scheme I



large number of conformations ranging from cyclic to extended (Scheme II), at the moment of spin inversion

Scheme II



the diradicals may be at least partially in the extended conformation. If the ring closure of a is more rapid than b, then a larger proportion of the cyclobutanes **3** and **4** would be expected. By virtue of its selectivity, b has a greater chance to reach a conformation which will lead to the more stable product, namely the cyclohexene **8**.[8]

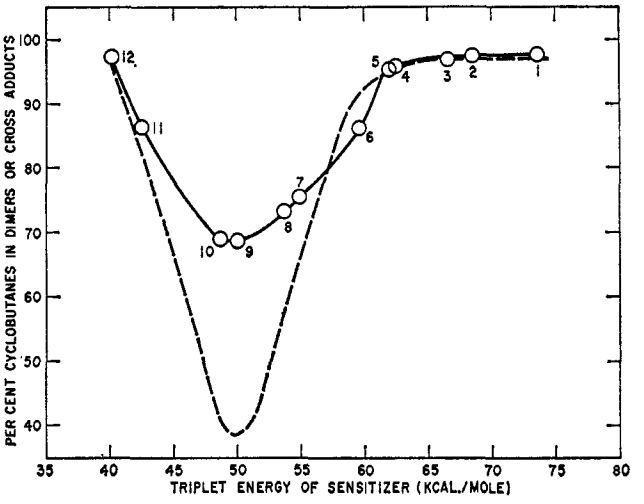


Figure 1. Butadiene–α-acetoxyacrylonitrile cross-adduct composition *vs.* triplet energy of sensitizer (solid line); butadiene dimer composition (dashed line, data of Hammond, *et al.*[3c]).

Acknowledgment. The author wishes to thank Dr. J. C. Little, Dr. T. Alfrey, Jr., and Dr. V. R. Sandel for many helpful discussions during this work, and Mr. K. C. Whitman for experimental assistance.

(8) "Steric Effects in Organic Chemistry," M. S. Newman, Ed., John Wiley and Sons, Inc., New York, N. Y., 1956; (a) W. G. Dauben and K. S. Pitzer, p 38; (b) F. H. Westheimer, p 533.

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A New Peptide Coupling Reagent

Sir:

In recent years the potential utility of activated esters as coupling agents in peptide synthesis has been extended, first by the development of the isoxazolium salts and their derivatives[1] which generate enolic or

(1) (a) R. B. Woodward, R. A. Olofson, and H. Mayer, *J. Am. Chem.*

Table I

	$\lambda_{max}^{CH_2Cl_2}$, μ	$\lambda_{max}^{H_2O}$ phenol, mμ (ϵ)	λ_{max} anion, mμ (ϵ)	pK_a (H_2O)
3-Hydroxy-2-methoxy-N-ethylbenzamide	1.460 (0.63), H-bonded NH 1.502 (0.33), H-bonded OH	282 (1610)	306 (2000)	9.0
3-Methoxy-2-hydroxy-N-ethylbenzamide	1.484 (0.59), NH stretch	306 (2600) 246 (6280)	331 (4850)	8.3
3-Acetoxy-2-hydroxy-N-ethylbenzamide (IIa)	1.485 (0.62), NH stretch	299 (3800) 238 (8120)	326 (6530)	6.7

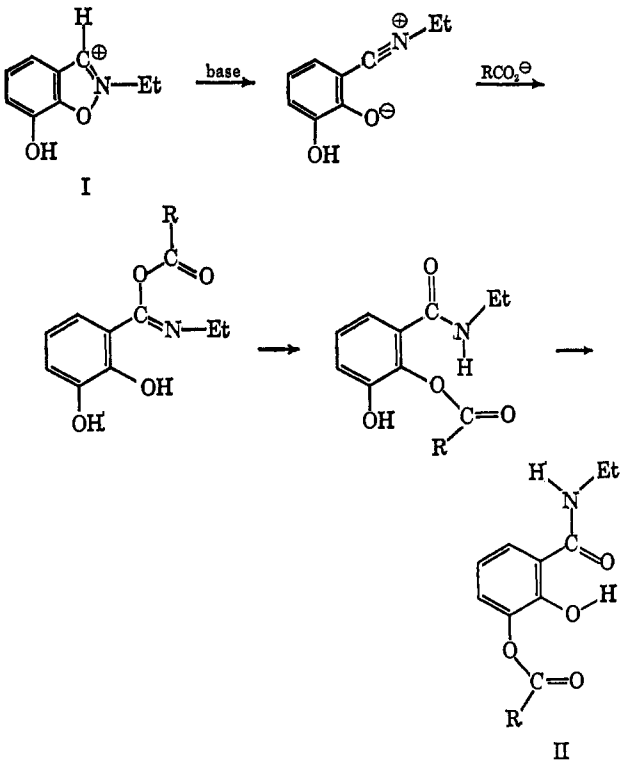
phenolic esters of peptide acids under convenient and mild conditions, and second by the discovery of peptide activated esters which discriminate effectively between aminolysis and racemization, even in polar solvents and under strongly basic conditions.[2] We wish to report the preparation of 2-ethyl-7-hydroxybenzisoxazolium cation (I), a substance which combines both features in one system.

By means of procedures described previously,[1b] 2,3-dihydroxybenzaldehyde[3] can be converted in 75% yield to the fluoroborate salt of I, mp 136–137°.[4] Addition of this salt as a powder to a vigorously stirred, chilled aqueous solution of sodium salts of carboxylic acids, overlayered with ethyl acetate and maintained at pH 4.5 with a pyridine buffer, results in the formation of 3-acyloxy-2-hydroxy-N-ethylbenzamides, II.[5] Reactions are complete within 5 min under these conditions ($t_{1/2}$ < 1 min, 25°, H_2O, pH 5) and the products can be isolated from the organic phase after acid and bicarbonate extractions. By analogy with the behavior of the 2-ethylbenzisoxazolium cation (III),[6] the mechanism shown in Scheme I is assigned to this conversion. Under conditions which with III result in predominant azlactone formation,[1b,c] I combines with ZGly-L-PheOH to yield IIc with no detectable formation (<0.5%) of the corresponding azlactone; I thus provides a method of converting peptide acids to activated esters without concomitant racemization.

That structure II corresponds to the products of these reactions follows from a study of the acetic acid product. This substance, infrared 1645 and 1760 cm^{-1}, whose spectra are compared in Table I with models, is also formed in 73% yield by aqueous acetylation of 2,3-dihydroxy-N-ethylbenzamide; its reaction with ethereal diazomethane–fluoroboric acid followed by hydrolysis yields 3-hydroxy-2-methoxy-N-ethylbenzamide.

Although otherwise similar to simple phenolic esters of peptide acids, peptide esters of structure II are remarkably resistant to racemization by tertiary amines. After 12 hr in dry DMF 0.4 *M* in triethylamine IIc is racemized to the extent of less than 1 %.[7] Under identical conditions O(-ZGly-L-Phe)-N-ethylsalicylamide is racemized over 4000 times faster.

Scheme I



The remarkable base stability of these esters suggested their use in coupling reactions with salts of amino acids. When IIb and the tetramethylammonium salt of L-phenylalanine were combined in dry DMF, a quantitative precipitation of L-phenylalanine occurred and the soluble tetramethylammonium salt of IIb was formed. Addition of excess L-phenylalanine salt resulted in a rapid reaction ($t_{1/2}$ ~ 1.5 min at 0.2 *M* amine), and isolation of the product after 15 min by addition of aqueous bicarbonate, extraction with ethyl acetate, and acidification yielded, after crystallization, 91% carbobenzoxyglycyl-L-phenylalanine, mp 129–130°, $[\alpha]^{23}D$ +39.9° (*c* 2.0, EtOH). Similarly, the tripeptide acid carbobenzoxyglycyl-L-phenylalanylglycine was obtained in 89% yield, mp 162–163°, $[\alpha]^{22}D$ −14.8° (*c* 1.3, EtOH), by condensation of IIc with glycine tetramethylammonium salt in DMSO. As a test of optical purity,[6] the tripeptide acid was dissolved in aqueous base and converted with triethyloxonium ion to its ethyl ester in 60% yield; less than 0.5% of racemic ester was observed.

The favorable properties of these activated species appear to result from an interaction of several inde-

Soc., **83**, 1007 (1961); (b) D. S. Kemp and R. B. Woodward, *Tetrahedron*, **21**, 3019 (1965); (c) D. S. Kemp, Ph.D. Thesis, Harvard University, 1964.

(2) S. M. Beaumont, B. D. Hanford, J. H. Jones, and G. T. Young, *Chem. Commun.*, **4**, 54 (1965); H. D. Jakubke and A. Voigt, *Chem. Ber.*, **99**, 2419 (1966).

(3) K. W. Mertz and J. Fink, *Arch. Pharm.*, **289**, 353 (1956).

(4) Satisfactory elemental analyses were obtained for all new substances.

(5) *E.g.*, IIa, RCO = CH_3CO, 77% yield, mp 136–137°; IIb, RCO = ZGly, 85% yield, mp 121–122°; IIc, RCO = ZGly-L-Phe, 93% yield, mp 115–116°, $[\alpha]^{22}D$ −29.2° (*c* 2.0, CH_2Cl_2).

(6) D. S. Kemp, *Tetrahedron*, in press.

(7) Determined by its conversion by reaction with glycine ethyl ester to ZGly-L-Phe-GlyOEt in 80% yield; 0.5% DL isolated.[8]

(8) G. W. Anderson and F. M. Callahan, *J. Am. Chem. Soc.*, **80**, 2902 (1958).

pendently favorable factors, among which the high acidity of the esters II (IIb is ~20% dissociated in DMF 0.2 *M* in Et_3N), the inhibitory effect of acids on the racemization of peptide esters,[9] the reluctance of salts of II to assume a second negative charge, and their high aminolytic reactivity[10] all figure prominently.

Further investigations into the mechanism of these processes and their general applicability to peptide coupling reactions are in progress and will be reported subsequently.

Acknowledgment. The support of the U. S. Public Health Service through Grants GM 13453-01 and -02 is gratefully acknowledged.

(9) *Cf.* D. S. Kemp and S. W. Chien, *J. Am. Chem. Soc.*, **89**, 2745 (1967).

(10) For a discussion of propinquity catalysis, see T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. I, W. A. Benjamin, Inc., 1966, pp 150–169.

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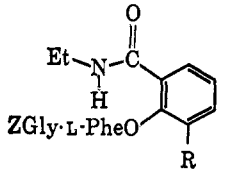
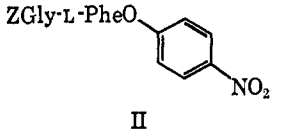
Specific Base Catalysis of Azlactone Formation

Sir:

Recent work[1,2] has established azlactones as essential intermediates in the racemization of many peptide activated species. While this and earlier work has made clear a dependence of azlactone formation on the presence of base, the precise nature of the dependence has not been explored. By analogy with Winstein's work on the cyclization of 2-benzamidoethyl tosylates,[3] one would anticipate for a primary or secondary amide a dual nucleophilicity, dependent on the relative concentrations of neutral amide and amide anion present in the reaction medium. We wish to present evidence which supports the presence of equilibrated amide anions as the reactive intermediates leading to azlactone formation from four peptide activated esters.



Ia, R=H
b, R=OMe
c, R=Me



II

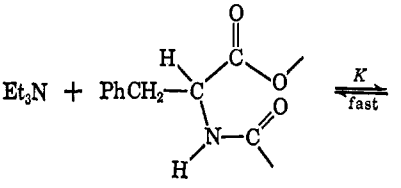
Table I presents first-order rate constants observed for the triethylamine-catalyzed racemization of O-(carbobenzoxyglycyl-L-phenylalanyl)-N-ethylsalicylamide (Ia)[4] in dimethylformamide containing tri-

Table I[a]

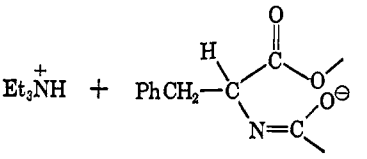
$[Et_3N]$	$[Et_3N^+H]$[b]	$[Et_3N]/[Et_3N^+H]$	First-order[c] rate constant, min^{-1}	Calcd rate[d]
0.2	No salt	...	12×10^{-2}	
0.2	0.000	...	20×10^{-2}	
0.2	0.01	20	3.0×10^{-2}	3.0×10^{-2}
0.2	0.015	13.3	2.4×10^{-2}	2.1×10^{-2}
0.2	0.02	10	1.7×10^{-2}	1.7×10^{-2}
0.2	0.05	4	0.86×10^{-2}	0.83×10^{-2}
0.2	0.2	1	0.38×10^{-2}	0.37×10^{-2}
0.4	0.04	10	1.9×10^{-2}	
0.1	0.01	10	1.7×10^{-2}	
0.05	0.005	10	1.5×10^{-2}	
0.02	0.002	10	1.1×10^{-2}	

[a] Temperature 25°, DMF solvent. [b] Unless otherwise specified, sufficient $Et_4N^+BF_4^-$ was added to bring the total salt concentration to 0.2 *M*. [c] Ester concentration 10–20 mg/ml; rates followed polarimetrically, first-order rate law followed for at least three half-lives. [d] For rates at 0.2 *M* Et_3N, a plot of the observed first-order rate constant *vs.* $[Et_3N]/[Et_3N^+H]$ was linear with a slope, k_a, of 1.4×10^{-3} min^{-1} and a zero intercept, k_b, of 2.3×10^{-3}. Calculated rate $= k_a[Et_3N]/Et_3N^+H] + k_b$.

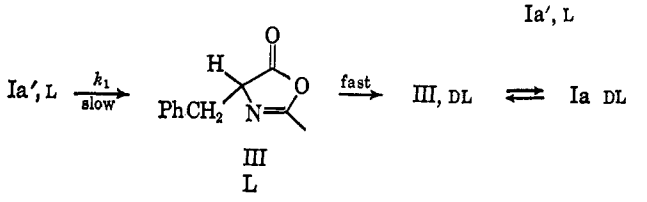
ethylammonium fluoroborate. The observed linear dependence of rate on the amine:amine salt ratio, together with the striking insensitivity of rate to the absolute amine concentration at constant amine:amine salt ratio, are most easily interpreted as requiring the intermediacy of a conjugate base of Ia. A scheme consistent with this result is shown below.[7]



Ia, L



Ia′, L



III
L

$$d[DL\text{-Ia}]/dt = k_1[L\text{-Ia}'] = k_1K[L\text{-Ia}][Et_3N]/Et_3\overset{+}{N}H] \quad (1)$$

Similar behavior is observed for the esters Ib, Ic, and II (Table II). It is of interest that the intercepts, k_b, which are the limiting rates expected at constant amine concentration as the amine salt concentration is raised, most include all general base catalyzed terms; their magnitudes therefore bound the rates of simple base-catalyzed enolization for these esters. Although these results must be generalized with caution since they stand in contrast to other well-known salt effects on peptide racemization,[2] it would appear that in DMF tertiary amine catalyzed racemization of peptide phe-

(1) M. Goodman and K. C. Steuben, *J. Org. Chem.*, 27, 3409 (1962); M. Goodman and L. Levine, *J. Am. Chem. Soc.*, **86**, 2918 (1964); M. Goodman and W. J. McGahren, *ibid.*, **87**, 3028 (1965).

(2) M. W. Williams and G. T. Young, *J. Chem. Soc.*, 3701 (1964); I. Antonovics and G. T. Young, *Chem. Commun.*, 398 (1965).

(3) F. L. Scott, R. E. Glick, and S. Winstein, *Experientia*, **13**, 183 (1957).

(4) Ia, mp 140–141°, $[\alpha]^{22}D$ −21.7° (*c* 2.2, CH_3CN); Ib, mp 149–150°, $[\alpha]^{25}D$ −25.2° (*c* 2.0, DMF); Ic, mp 125–126°, $[\alpha]^{25}D$ −47.8° (*c* 2.0, DMF); these esters were prepared in optically pure form by reaction of the sodium salt of ZGly-L-PheOH in an aqueous pyridine buffer with the appropriate 7-substituted N-ethylbenzisoxazolium salt.[5,6]

(5) Satisfactory elemental analyses were obtained for all new compounds.

(6) D. S. Kemp and R. B. Woodward, *Tetrahedron*, **21**, 3019 (1965).

(7) Although the formation of azlactones need not result in racemization, the reaction conditions of this study (polar solvent, excess of nonnucleophilic base) ensure that II racemizes faster than it reacts with phenolate anion, and therefore that k_1 is rate-determining.[1]