to the National Institutes of Health (GM 08321) for partial support of this research.

(15) Alfred P. Sloan Research Fellow, 1971–1973.
(16) Research Career Development Awardee of the National Institutes of Health.

L. J. Altman,[15] Lucienne Ash, R. C. Kowerski
Department of Chemistry, Stanford University
Stanford, California 94305

W. W. Epstein, B. R. Larsen
Department of Chemistry, University of Utah
Salt Lake City, Utah 84112

H. C. Rilling,*[16] F. Muscio, D. E. Gregonis
Department of Biochemistry, University of Utah
Salt Lake City, Utah 84112

Received January 24, 1972

Phenyl Esters for C-Terminal Protection in Peptide Synthesis

Sir:

The potential of phenyl esters as intermediates in peptide synthesis has been pointed out,[1] but the preliminary experiments have not been followed up hitherto. We were prompted to resuscitate the method by the need to synthesize peptides with the awkward sequence[2] -Asp-Gly- in the course of our lysozyme program. We now describe practical conditions for synthesis of oligopeptide phenyl esters, and for *removal of the phenyl group under very mild conditions without any racemization.*

A synthesis of the protected sequence 11–16 of calcitonin M exemplifies the construction of intermediates and the disposition of phenyl esters to crystallize. Treatment of Z-Phe-OPh[3] with HBr–HOAc yielded H_2^+-Phe-OPhBr$^-$, mp 232–233°,[4] which was coupled (mixed anhydride with isobutyl carbonate) with Z-Asp(OBu^t)-OH. The dipeptide derivative (68%) was an oil, which yielded, however, crystalline H_2^+-Asp(OBu^t)-Phe-OPhCl$^-$, mp 153–156° (78%), on hydrogenation (10% palladium/carbon) in dimethylformamide containing 1 equiv of HCl in dioxane.[5] The tripeptide derivative, Z-Gln-Asp(OBu^t)-Phe-OPh, mp 192–194°, was obtained (78%) by coupling (mixed anhydride with pivalic acid) with Z-Gln-OH, and then the tetrapeptide derivative, mp 137–139° (83%), by hydrogenolysis and coupling (mixed anhydride with isobutyl carbonate) with Z-Thr(Bu^t)-OH. Hydrogenolysis yielded the tetrapeptide phenyl ester, mp 119° (87%), which was coupled (dicyclohexylcarbodiimide–1-hydroxybenzotriazole)[6] with Z-Thr(Bu^t)-Tyr-OH. The resultant Z-Thr(Bu^t)-Tyr-Thr(Bu^t)-Gln-Asp(OBu^t)-Phe-OPh, mp 211–214° (58%), was hydrolyzed in 15 min at 20°, pH 10.5 (autotitrator), in aqueous dimethylformamide containing 0.80 equiv of hydrogen peroxide yielding the carboxylic acid, mp 193° dec (63%). In general, hydrolysis of C-terminal phenyl esters of peptide derivatives was accomplished efficiently in times between 7 and 20 min at pH 10.5, 20°, in mixtures of water (about 40%) and an organic solvent, which could be acetone, dioxane, or dimethylformamide.

The extraordinary susceptibility of phenyl esters to nucleophilic attack by peroxides is well documented by Jencks and Gilchrist.[7] Presumably the peptide peracids are the initial product, but conversion to the carboxylic acids is rapid, and only the latter have been isolated. Decomposition of peracids is a general phenomenon,[8] which is especially marked in alkaline solution in the case of the chloroacetyl compound,[9] which must have a pK_a similar to that of an α-amido acid; presumably the electron withdrawing of the α substituent promotes decomposition of the peracid. We have investigated racemization during peroxide-catalyzed hydrolysis by the general method of Manning and Moore,[10] which would easily detect 0.1% of the L,D dipeptide;[11] no trace was found in hydrolyses of the L,L dipeptide esters, Z-Ala-Phe-OPh and Z-Leu-Ala-OPh. In the absence of peroxide the rate of hydrolysis was an order of magnitude less and much racemization was observed. In contrast to the results with phenyl esters, normal saponification of the corresponding methyl esters (1 equiv of 0.25 *N* NaOH diluted with 3 vol of acetone, 1 hr at 20°) gave, respectively, 2.8 and 0.8% racemization. To our knowledge this danger of racemization in an *unactivated* peptide methyl ester has not been reported previously.

A check with Z-Asp(OBu^t)-Gly-OPh (*viz.* successive peroxide-catalyzed hydrolysis, hydrogenolysis, and treatment with trifluoroacetic acid) confirmed the absence of an $\alpha \rightarrow \beta$ aspartyl shift.[12] An obvious risk in peroxide catalysis is destruction of indole and sulfur side chains. Indeed BOC-Met-OPh yielded 78% of the sulfoxide and 9% of sulfone under the standard conditions, but oxidation was not detected when 30 mol equiv of dimethyl sulfide was added. Studies with BOC-Ala-Cys(Acm)-Gly-OPh and Z-Trp-Gly-OPh showed that destruction of the side chains was completely obviated by inclusion of dimethyl sulfide, which did not diminish the rates of hydrolysis.[13]

The heptapeptide derivative, Z-Asp(OBu^t)-Ile-Thr(Bu^t)-Ala-Ser(Bu^t)-Val-Gly-OPh, mp 260–262° dec, was synthesized stepwise from the C-terminus as in the first example above, except that 2,4,5-trichlorophenyl esters were used in adding the residues of Val, Ala, Ile, and Asp(OBu^t). All the intermediate *N*-carbobenzoxy phenyl esters were crystalline and their hydrolysis was tested in each case. The autotitrator showed reaction to be complete in 15 min (dipeptide), 7 min (tri-

(1) G. W. Kenner, *Angew. Chem.*, **71**, 741 (1959).
(2) M. A. Ondetti, A. Deer, J. T. Sheehan, J. Pluscec, and O. Kócy, *Biochemistry*, 7, 4069 (1968).
(3) Abbreviations according to IUPAC-IUB tentative rules, *Biochemistry*, **5**, 2485 (1966), and "Peptides 1969," E. Scoffone, Ed., North-Holland Publishing Co., Amsterdam, 1971, p xvii.
(4) All compounds gave satisfactory CHN (±0.4) and amino acid analyses (±5%). Cited yields are of analytically pure products.
(5) If methanol was included in the solvents for hydrogenolyses, some transesterification was detected.
(6) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
(7) P. Jencks and M. Gilchrist, *J. Amer. Chem. Soc.*, **90**, 2622 (1968).
(8) J. d'Ans and W. Frey, *Z. Anorg. Allg. Chem.*, **84**, 145 (1913); D. Swern in "Organic Peroxides," Vol. 1, Wiley-Interscience, New York, N. Y., 1970, Chapter 6.
(9) E. Koubek, M. L. Haggett, C. J. Battaglia, K. M. Ibne-Rasa, H. Y. Pyun, and J. O. Edwards, *J. Amer. Chem. Soc.*, **85**, 2263 (1963).
(10) J. Manning and S. Moore, *J. Biol. Chem.*, **243**, 5591 (1968).
(11) JEOLCO JLC-5AH analyzer.
(12) Retention times of α and β dipeptides at 58 ml/hr, pH 3.25 buffer, were 88 and 42 min (column at 57°).
(13) In other examples, which do not have C-terminal Gly, it has later been found necessary to add intermittently fresh portions of hydrogen peroxide, usually no more than 3 equiv, in order to maintain the rate of hydrolysis. The products were, however, still of good quality.

peptide, tetrapeptide), 8 min (pentapeptide), and 25 min (hexapeptide).

The phenyl ester method is an integral part of our program in the synthesis of lysozyme analogs, and therefore we expect to report results of its more extensive application in due course. It should be noted that acylation, coupled with deacylation by peroxide anion, provides a *potentially general method for protection of phenolic hydroxyl groups.*

Acknowledgment. We are grateful to Drs. P. M. Hardy and J. R. McDermott for preliminary studies in this area and to Dr. R. C. Sheppard for helpful discussions.

(14) Fellow of the Canadian Medical Research Council.

G. W. Kenner,* John H. Seely[14]
The Robert Robinson Laboratories, University of Liverpool
Liverpool, England
Received October 22, 1971

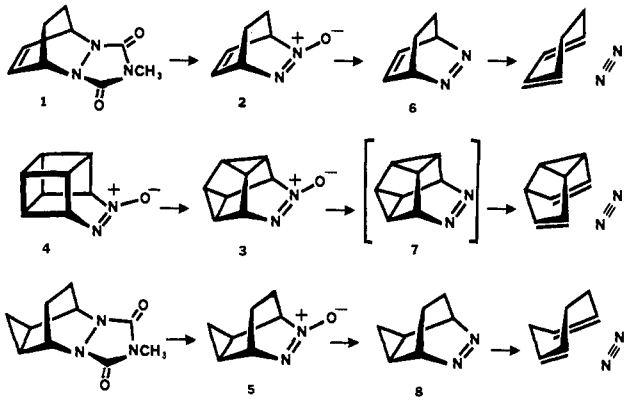
cis-Azoxyalkanes. III. A Dichotomy in the Thermal Stability of Azo- and Azoxyalkanes

Sir:

cis-Azoxyalkanes belong to a compound class of recent vintage. Although a variety of saturated examples exist,[1] only a few highly condensed derivatives[1a,2] and no unsaturated cases are known. We now report the synthesis of two new examples and elaborate on the remarkable stability of azoxyalkanes relative to their deoxy relatives, the azoalkanes.

Diels–Alder addition of *N*-methyl-1,2,4-triazolinedione and cyclohexadiene proceeds smoothly to give adduct **1**.[1a] Careful oxidative hydrolysis (ethylene glycol–water (1:1), excess 30% aqueous H_2O_2, 120°) generates 2,3-diazabicyclo[2.2.2]octadiene *N*-oxide (**2**) in a single experimental step (57% yield; mp 48.5–49.5°; ir ν (KBr) 1230 (s, NNO), 1485 cm^{-1} (s, NNO); nmr ($CDCl_3$) τ 3.46 (2 H, unsym t), 4.65 (2 H, envelope), 7.75–8.65 (4 H, envelope)). Alternatively, heterocycle **3**, 9,10-diazapentacyclo[3.3.2.$0^{2,4}$.$0^{3,7}$.$0^{6,8}$]decane *N*-oxide (60% yield; mp 133–134°; ir ν ($CHCl_3$) 1502 (s, NNO), 1265 cm^{-1} (s, NNO); nmr ($CDCl_3$) τ 4.4–5.0 (2 H, envelope), 7.3–8.1 (6 H, envelope)), arises by silver ion promoted rearrangement of diazabasketene *N*-oxide (**4**)[2] ($AgBF_4$, refluxing $CHCl_3$, inert atmosphere, 5 days). This conversion may be viewed as an allowed [$_\sigma 2_s$ + $_\sigma 2_s$] sigmatropic reorganization.[3] The structure of compound **3** follows from its spectroscopic properties and facile conversion to semibullvalene as discussed below.

The thermal stability of *N*-oxides **2**, **3**, and **5** stands in stark contrast to that of the corresponding azo series **6**, **7**, and **8**. Compound **6**, generated at −78°, decomposes to cyclohexadiene rapidly upon formation ($t_{1/2}^{-78°} \leq 30$ sec).[4] The strained heterocycle **7** has been implicated as an unobserved intermediate in the formation of semibullvalene (<0°).[3a] Homolog **8** cycloreverts to 1,4-cycloheptadiene and nitrogen at room temperature ($k_1^{-3.5°} = 1.7 \times 10^{-4}$ sec^{-1}).[5] Azoxyalkanes **2**, **3**, and **5**, on the other hand, resist thermal change below 175° either neat or in solution. In fact the cis azo systems mentioned above are conveniently stored as their *N*-oxide derivatives. Treatment of each of the latter with excess hexachlorodisilane[6] in an nmr tube (25°) causes a mildly exothermic reaction which leads to the instantaneous disappearance of the azoxy spectrum. Only the corresponding hydrocarbon absorption (cyclohexadiene, semibullvalene,[7] and 1,4-cycloheptadiene, respectively) remains. Vacuum pyrolysis of compounds **2** (325°) and **5** (400°) over a quartz bed delivers 1,3-cyclohexadiene and 1,4-cycloheptadiene, respectively, as the exclusive products.



These observations suggest that the rate of nitrogen extrusion in a reverse [4 + 2] cycloaddition is at the very least 10^6 times that for N_2O expulsion.[8] The mechanism by which the heterocycles decompose is not altogether resolved. However, cis–trans isomers of 1,2-diaza-3,6-dimethylcyclohexadiene-1,4 and the corresponding azo cycles in which the carbon–carbon double bond is replaced by a cyclopropane moiety cyclorevert with a very high degree of stereospecificity.[9] Furthermore, the reactions proceed rapidly below room temperature analogous to the fates of compounds **6**, **7**, and **8**. Nitrogen loss by way of a single, concerted step is strongly suggested. If the potential surfaces associated with *N*-oxides **2**, **3**, and **5** are similarly constituted, the disparate thermal behavior of unsaturated or pseudounsaturated azo- and azoxyalkanes may be rationalized by considering the form of the molecular orbitals for nitrogen and nitrous oxide.

For purposes of analyzing the heteroextrusion reaction, the frontier orbitals[10] (Figure 1) are of singular importance. SCF–MO–CNDO calculations[11] indicate

(1) (a) J. P. Snyder and V. T. Bandurco, *Tetrahedron Lett.*, 4643 (1969); (b) F. D. Greene and S. S. Hecht, *ibid.*, 575 (1969); W. R. Dolbier, Jr., and W. M. Williams, *J. Amer. Chem. Soc.*, **91**, 2818 (1969); W. Luttke and V. Schabacker, *Justus Liebigs Ann. Chem.*, **687**, 236 (1969); J. P. Freeman, *J. Org. Chem.*, **27**, 1309, 2881 (1962).

(2) J. P. Snyder, L. Lee, and D. G. Farnum, *J. Amer. Chem. Soc.*, **93**, 3816 (1971).

(3) (a) R. M. Moriarity, C. Yeh, and N. Ishibi, *ibid.*, **93**, 3085 (1971); L. A. Paquette, *ibid.*, **92**, 5765 (1970); R. Askani, *Tetrahedron Lett.*, 3349 (1970); (b) W. G. Dauben, C. H. Schallhorn, and D. L. Whalen, *J. Amer. Chem. Soc.*, **93**, 1446 (1971).

(4) N. Rieber, J. Alberts, J. A. Lipsky, and D. M. Lemal, *ibid.*, **91**, 5668 (1969).

(5) M. Martin and W. R. Roth, *Chem. Ber.*, **102**, 811 (1969); E. L. Allred and J. C. Hinshaw, *J. Chem. Soc. D*, 1021 (1969).

(6) K. Naumann, G. Zon, and K. Mislow, *J. Amer. Chem. Soc.*, **91**, 7012 (1969).

(7) H. E. Zimmerman, R. W. Binkley, R. S. Givens, G. L. Grunewald, and M. A. Sherwin, *ibid.*, **91**, 3316 (1969).

(8) Calculated on the basis of a 1%/week (25°) decomposition of azoxyalkanes **2**, **3**, and **5**. It should be noted that the latter appear to be stable indefinitely at ambient temperature.

(9) J. A. Berson and S. S. Olin, *J. Amer. Chem. Soc.*, **91**, 777 (1969).

(10) (a) K. Fukuii, *Accounts Chem. Res.*, **4**, 57 (1971); (b) R. G. Pearson, *ibid.*, **4**, 152 (1971).

(11) R. J. Boyd and M. A. Whitehead, *J. Chem. Soc. A*, 3579 (1971); *J. Chem. Soc., Dalton Trans.*, 73, 78, 80 (1972); core repulsion and in-