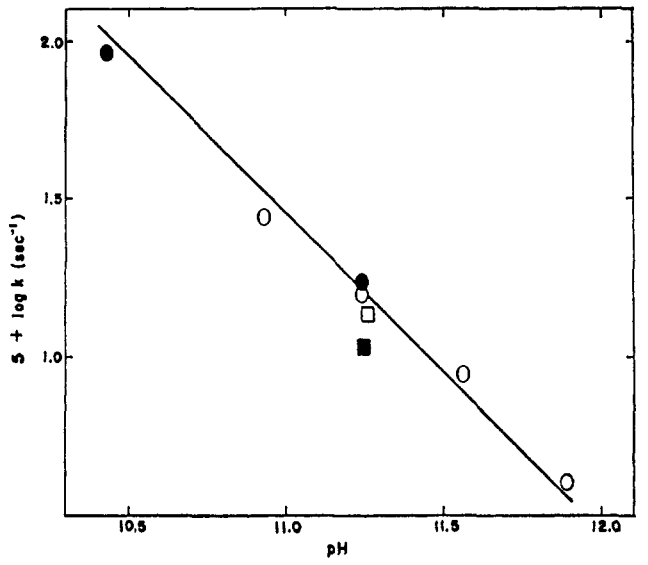

Figure 1. Plot of log k *vs.* pH. Straight line was drawn so that d log k/dpH = −1. ○, phosphate buffer, ionic strength 0.18–0.20; $C_6H_5N{=}NCOO^-$, 2.2–2.4 × 10^{-4} *M*; ●, carbonate buffer, ionic strength 0.18–0.20; $C_6H_5N{=}NCOO^-$, 2.2–2.4 × 10^{-4} *M*; □, phosphate buffer, ionic strength 0.45; $C_6H_5N{=}NCOO^-$, 2.4 × 10^{-4} *M*; ■, phosphate buffer, ionic strength 4.91; $C_6H_5N{=}NCOO^-$, 2.6 × 10^{-4} and 2.1 × 10^{-2} *M*.

(at 25° with a Cary Model 14 spectrophotometer) is begun within 30–70 sec. after mixing.

The half-life of **2** at the pH of the reaction solution (7.34) can be estimated from the figure as about 0.5 sec. Three new maxima are observed: 2140 (ϵ ~10,000), 2700 (ϵ ~7000), and 4000 Å. (ϵ ~160). The latter two correspond quite well to the expected $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions for a molecule like **1**.[4] The new absorptions decrease with a half-life of approximately 80,000 sec. The same phenomena are observed with more difficulty at pH 9.10 since the intermediate disappears at a much greater rate, $t_{1/2}$ *ca.* 1000 sec. Base-catalyzed decomposition of phenyldiimide is thus implied (eq. 2), as suggested by Cram and Bradshaw[5] for alkyldiimides, and in accord with the formation of 2-bromophenyl anion through the action of ethoxide ion on ethyl 2-bromophenylazoformate in ethanol.[6] In fact, **1** is completely destroyed within 100 sec. on raising the pH from 7.34 to 13.7.

$$C_6H_5N{=}NH + OH^- \longrightarrow HOH + [C_6H_5N{=}N^-] \longrightarrow \text{products} \quad (2)$$

The chief products of the decomposition of **1** are benzene, azobenzene, and hydrazobenzene. Oxygen reacts rapidly with **1**. In the presence of oxygen, **1** is not observed to form from **2**.

Many questions of interest may now be studied directly with solutions of phenyldiimide. The preparation and reactions of other aryldiimides and possibly alkyldiimides are being actively pursued. Phenyldiimide has been implicated as the compound responsible for the loss of glutathione in red blood cells treated with phenylhydrazine, acetylphenylhydrazine, and methyl phenylazoformate, a finding which throws light on the mechanism of drug-induced hemolysis and which might be useful in the design of antimalarial drugs.[7]

(4) Benzeneazomethane, $C_6H_5N{=}NCH_3$, has been reported to have λ_{max} 2605 Å. (ϵ 7800, ethanol); λ_{max} 4035 Å. (ϵ 87, hexane) [A. Burawoy, *J. Chem. Soc.*, 1177 (1939)].

(5) D. J. Cram and J. S. Bradshaw, *J. Am. Chem. Soc.*, **85**, 1108 (1963).

(6) R. W. Hoffman, *Chem. Ber.*, **97**, 2763 (1964).

(7) N. S. Kosower, G. A. Vanderhoff, E. M. Kosower, and P. C. Huang, *Biochem. Biophys. Res. Commun.*, 20, 469 (1965).

(8) (a) Alfred P. Sloan Fellow, 1960–1964, (b) National Institutes of Health Predoctoral Fellow, 1964–1966; (c) support from the National Institutes of Health, the National Science Foundation, and the Army Research Office (Durham) is gratefully acknowledged.

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A Synthesis of Cyclic Peptides Utilizing High Molecular Weight Carriers

Sir:

Cyclic peptides are usually prepared from linear peptides by intramolecular cyclization. The carboxylic end of the peptide is as a rule activated by the formation of active esters, anhydrides, azides, or chlorides, and the free terminal amino end allowed to react with the active terminal carbonyl group at high dilution.[1-4] Because of intermolecular condensation occurring even under these conditions, linear oligopeptides are formed in addition to the desired cyclic peptide. The techniques available so far thus lead to reaction mixtures from which cyclic peptides are usually isolated only in relatively low yields. In the following we report on the development of a new method for the synthesis of cyclic peptides in which high molecular weight peptide active esters of type II (see Figure 1), in which the peptide is bound to a high molecular weight polyalcohol carrier, are used as intermediates. When insoluble esters of this type are employed condensation between the activated peptide moieties is suppressed, and internal aminolysis leads to the formation of the desired cyclic peptide (IV) which is released from the insoluble polyhydroxy carrier (III). Intermolecular condensation might be expected to be markedly reduced even when soluble high molecular weight peptide esters of type II are used.

Two high molecular weight poly(nitrophenol) derivatives have been used in the preparation of the peptide active esters: cross-linked poly-4-hydroxy-3-nitrostyrene (IIIa) and a branched copolymer of DL-lysine and 3-nitro-L-tyrosine (IIIb) in which free amino groups have been blocked by acetylation. The former has been prepared according to the literature[5] and is insoluble in the usual organic solvents. The latter has been prepared by total acetylation, with acetic anhydride, of a branched copolymer of DL-lysine and L-tyrosine (molar residue ratio 3:1),[6] removal of the O-acetyl groups in alkali, and nitration in concentrated nitric acid at 0°. IIIb is insoluble in dioxane, ether, and acetone, but is soluble in dimethylformamide (DMF),

(1) R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, 40, 624 (1957).
(2) T. Wieland and K. W. Ohly, *Ann. Chem.*, **605**, 179 (1957).
(3) K. Vogler, R. O. Studer, W. Lergier, and P. Lanz, *Helv. Chim. Acta*, **43**, 1751 (1960).
(4) H. Gerlach, J. A. Owtschinnikow, and V. Prelog, *ibid.*, **47**, 2294 (1964).
(5) D. I. Packham, *J. Chem. Soc.*, 2617 (1964).
(6) M. Sela, S. Fuchs, and R. Arnon, *Biochem. J.*, **85**, 223 (1962).

ethanol, and water at pH values above 10. N-Benzyloxycarbonyl derivatives of peptides were coupled with both poly(nitrophenol) compounds in DMF using N,N′-dicyclohexylcarbodiimide as the coupling reagent. Highest yields of the N-benzyloxycarbonyl active esters (I) were obtained when the reaction mixture contained at least a threefold excess of the N-benzyloxycarbonyl peptide in comparison with the nitrophenol content of the polymer. Excess of unreacted peptide was removed from Ia by washing and from Ib by dialysis. The protecting groups of I were removed with anhydrous hydrogen bromide in glacial acetic acid,[7] and the high molecular weight peptide ester hydrobromides were isolated by filtration (esters derived from IIIa) or by precipitation with ether (esters derived from IIIb). The free amino groups of the peptide esters (II) were liberated from the corresponding hydrobromides by neutralization with triethylamine in DMF, and the cyclization reaction was allowed to take place at room temperature in the same solvent. The DMF-insoluble free peptide esters studied (IIa), derived from IIIa, yielded under the experimental conditions used chromatographically pure cyclopeptides in 60–80% yield. The latter could readily be isolated from the DMF solution. The DMF-soluble peptide esters studied (IIb), derived from IIIb, yielded the corresponding cyclic peptides, also in 60–80% yield. The cyclic peptides in this case, however, which were only identified chromatographically, were contaminated with some peptide oligomers.

The synthesis of some representative cyclic peptides by our new method is described: *Cyclo(Gly-Gly)*. Z-Gly-Gly was coupled with IIIa in DMF to yield the corresponding ester Ia containing 0.8 mmole of peptide/g. The hydrobromide formed after removal of the benzyloxycarbonyl group (1 hr. in 30% HBr in anhydrous acetic acid) was filtered, washed with ether, suspended in DMF, and neutralized with triethylamine. After 12 hr. at room temperature the polymer was filtered off and the filtrate brought to dryness *in vacuo*. Crystalline cyclo(Gly-Gly) was obtained on trituration with ether; yield 75% of the theoretical; m.p. 310° (fast heating). The identity of the product obtained as diketopiperazine was ascertained by the appearance of one spot on thin layer partition chromatography (t.l.p.c.) with R_f values identical with those of an authentic sample when developed with *t*-butyl hypochlorite–starch–KI[8] using 1-butanol–acetic acid–water (BAW, 4:1:1, v./v.) (R_f 0.40) or 1-butanol–pyridine–acetic acid–water (BPAW) (15:10:3:12, v./v.) (R_f 0.56) as solvents. As expected, cyclo(Gly-Gly) gives a negative ninhydrin test.

On coupling Z-Gly-Gly with IIIb in DMF a quantitative yield of the corresponding ester Ib was obtained. Excess Z-Gly-Gly was removed by dialysis against water. Removal of the protecting groups and neutralization with triethylamine was carried out as above. The amount of cyclo(Gly-Gly) formed in DMF after 12 hr. at room temperature corresponded to 75% of the theoretical as assayed by quantitative t.l.p.c.

By a procedure analogous to the above *cyclo*(L-*Ala-Gly*) [R_f 0.43 (BAW), 0.64 (BPAW); m.p. 240° dec. (lit. 240°[9]; 241°[10]); $[\alpha]^{23}D$ −2.5° (*c* 1.2, water)], *cyclo*(L-*Ala*-L-*Ala*) [R_f 0.53 (BAW)], and *cyclo*(L-*Phe-Gly*) [R_f 0.77 (BAW); m.p. 260–265° dec. (lit. 260–265°[11]); $[\alpha]^{23}D$ −12.4° (*c* 3.68, dichloroacetic acid)] were prepared from the corresponding high molecular weight esters IIa and IIb.

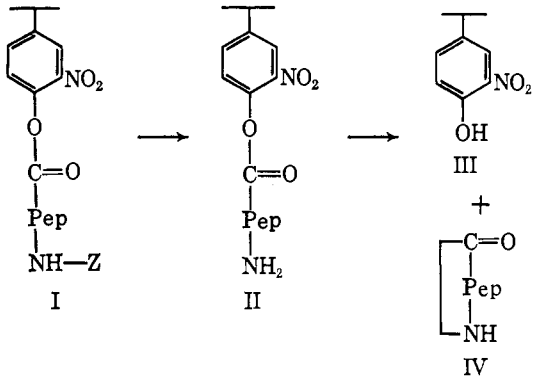

Figure 1. Scheme describing the method employed for the synthesis of cyclic peptides. IIIa stands for poly-4-hydroxy-3-nitrostyrene; Ia and IIa are the corresponding N-benzyloxycarbonyl (Z) peptide and free amino peptide ester derivatives of IIIa. IIIb stands for a branched copolymer of DL-lysine and 3-nitro-L-tyrosine in which free amino groups have been blocked by acetylation; Ib and IIb are the corresponding N-benzyloxycarbonyl (Z) peptide and free amino peptide ester derivatives of IIIb.

Cyclo(tetra-L-*Ala)* [R_f 0.60 (BAW), 0.68 (BPAW); m.p. 250°; $[\alpha]^{22}_{484}$ −68°, $[\alpha]^{22}_{366}$ −157° (*c* 0.5, DMF)] was prepared from three different tetra-Ala active esters: the active esters derived from IIIa and IIIb, as well as from tetra-L-Ala-*p*-nitrophenyl ester. Cyclization of the high molecular weight tetra-L-Ala esters IIa and IIb was carried out in DMF solution at room temperature (12 hr.). Cyclization of tetra-L-Ala *p*-nitrophenyl ester was carried out in dilute pyridine solution at 90–100° (12 hr.). A chromatographically pure cyclic peptide in 50–65% yield was obtained from the high molecular weight active esters. Considerably lower yields (38–42%) were obtained from the low molecular weight tetra-L-Ala ester. Furthermore, the pyridine reaction mixture was found to contain linear tetra-Ala oligomers in addition to the cyclic peptide. Cyclo(tetra-L-Ala) gave on mild alkaline hydrolysis (*N*/15 LiOH, 1 hr., 100°)[12] tetra-, tri-, di-, and monoalanine, which could be separated by high voltage paper electrophoresis at pH 1.5.

Cyclo(L-*Ala-Gly*-L-*Ala*-L-*Ala*) (R_f 0.54 (BAW), 0.64 (BPAW)] was prepared from the high molecular weight active ester of L-Ala-Gly-L-Ala-L-Ala with IIIa. In its chromatographic behavior it was found to be identical with a sample of the cyclic peptide obtained from Gly-L-Ala-L-Ala-L-Ala *p*-nitrophenyl ester [R_f 0.54 (BAW), 0.64 (BPAW)].

The above examples illustrate the use of high molecular weight polyalcohol carriers of the types IIa and IIb in the synthesis of cyclic peptides. It should be mentioned, however, that although the yields of the desired cyclic peptides on intramolecular aminolysis of

(7) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, 17, 1564 (1952).
(8) R. H. Mazur, B. W. Ellis, and P. S. Cammarata, *J. Biol. Chem.*, 237, 1619 (1962).
(9) J. L. Bailey, *J. Chem. Soc.*, 3461 (1950).
(10) T. I. Orlova and N. I. Gavilov, *Zh. Obshch. Khim.*, 27, 3314 (1957).
(11) H. T. Huang and C. Niemann, *J. Am. Chem. Soc.*, 72, 921 (1950).
(12) R. Schwyzer, B. Iselin, W. Rittel, and P. Sieber, *Helv. Chim. Acta*, 39, 872 (1956).

II (II → III + IV) are good, the amount of the N-benzyloxycarbonyl peptide which can be bound to the insoluble carrier IIIa is rather low. Finally, it is pertinent to note that we were able to utilize the high molecular weight active esters of N-masked peptides and amino acids described here in the synthesis of linear peptides by their interaction with the suitable amino acid or peptide esters.

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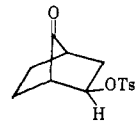
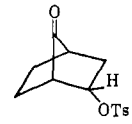
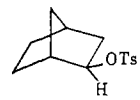
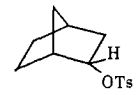
The Solvolysis of 7-Ketonorbornyl Tosylates

Sir:

Much of the attention accorded the solvolysis of various derivatives of bicyclo[2.2.1]heptane[1] has been directed toward establishing the existence[2] or nonexistence[3] of a nonclassical carbonium ion intermediate in the solvolysis of 2-substituted bicyclo[2.2.1]heptanes. Solvolytic results obtained from bicyclo[2.2.1]heptyl tosylates in which the bicyclic system was substituted with groups capable of stabilizing a positive charge (*i.e.*, methyl or phenyl) have been used as evidence both for and against the existence of nonclassical carbonium ions. Since stabilizing the positive charge yields data which are inconclusive, we felt that destabilizing the norbornyl cation might lead to more definitive results.

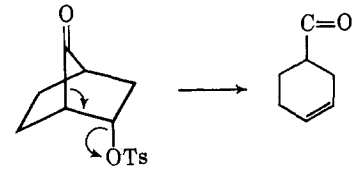
We report here on the effect of the carbonyl function on the acetolysis of 2-*exo*-hydroxybicyclo[2.2.1]heptan-7-one tosylate (**1**) and 2-*endo*-hydroxybicyclo[2.2.1]-heptan-7-one tosylate (**2**).[4,5] The solvolyses of **1** and **2** were carried out in anhydrous acetic acid buffered with sodium acetate at 75, 90, and 100° to yield the specific rate constants listed in Table I.[6] To our knowledge this is the first case of an *endo*-tosylate solvolyzing faster than the *exo* epimer. We envisage three possible rationalizations of this unique *exo/endo* rate ratio: (1) the *exo* and *endo* isomers are solvolyzing by different mechanisms,[7] (2) an unprecedented dipole–dipole interaction is occurring which either accelerates the *endo* solvolysis or inhibits the *exo* solvolysis by a factor $>10^3$,[8] (3) the *exo*-tosylate, **1**, is solvolyzing without anchimeric assistance while all other known *exo*-norbornyl tosylates solvolyze with anchimeric assistance. This latter rationalization appears to be the most attractive.[7,8]

Table I. Acetolysis Rates of Various Norbornyl Tosylates

Compound	Ref.	Temp., °C.	Rate, sec.$^{-1}$	ΔH^*, kcal./mole	ΔS^* e.u.
 **1**		100.00	(1.84 ± 0.01) × 10^{-4} [a]	27.1	−3.5
		90.00	(5.96 ± 0.06) × 10^{-5}		
		75.71	(1.33 ± 0.01) × 10^{-5}		
		(25.0)	1.44 ± 10^{-8}		
 **2**		100.00	(4.66 ± 0.00) × 10^{-4}		
		90.00	(1.79 ± 0.03) × 10^{-4}	24.7	−8.1
		75.74	4.28 × 10^{-5}		
		(25.0)	8.66 × 10^{-8}		
	6	25	2.33 × 10^{-6}	21.6	−7.2
	6	(25)	8.28 × 10^{-8}	25.8	−4.4

[a] A similar rate for solvolysis of the *exo*-tosylate has been obtained by K. Mislow and W. Meyer. For details see W. E. Meyer, Ph. D. thesis, New York University, 1964.

In considering the large *exo/endo* rate ratio which is generally observed in the solvolysis of norbornyl tosylates, the two published explanations[1,2,9–11] of this phenomenon require evaluation. According to both **1** and **2** yield products with the 7-ketonorbornane skeleton intact, this rationalization is ruled out. A discussion of the product analysis of **1** and **2** will be published in the near future.



(1) J. A. Berson, "Molecular Rearrangements," Vol. 1, P. de Mayo, Ed., Interscience Publishers, Inc., New York, N. Y., 1963, p. 111.

(2) For a leading reference see S. Winstein, *J. Am. Chem. Soc.*, **87**, 381 (1965).

(3) For a leading reference see H. C. Brown and M. H. Rei, *ibid.*, **86**, 5008 (1964).

(4) The *exo*-tosylate, **1**, was synthesized from 7,7-dimethoxybicyclo-[2.2.1]heptene *via* a four-step synthesis consisting of epoxidation, lithium aluminum hydride reduction, hydrolysis, and reaction with *p*-toluenesulfonyl chloride. The isomer **2** was prepared from 7,7-dimethoxybicyclo[2.2.1]heptan-*exo*-2-ol *via* oxidation, Meerwein–Ponndorf–Verley reduction, hydrolysis, and reaction with *p*-toluenesulfonyl chloride. The stereochemistry of the epimeric 7,7-dimethoxybicyclo-[2.2.1]heptan-2-ols was established by n.m.r., near-infrared hydrogen bonding studies, and chemical conversion to known compounds.

(5) Correct analytical data have been obtained for all new compounds named with the exception of **2**. This tosylate could not be obtained in greater than 90% purity. Thus the acetolysis rates on **2** are based on infinity titers of *ca.* 90%.

(6) P. von R. Schleyer, M. M. Donaldson, and W. E. Watts, *J. Am. Chem. Soc.*, **87**, 375 (1965). It must be stressed at this point that comparison of the absolute rates of solvolyses of the 7-keto compounds with other absolute rate constants is complicated by the inductive effect of the carbonyl and the change in geometry of the system which results from the incorporation of the carbonyl in the 7 position.

(7) (a) The possibility that the rate of solvolysis of **2** is accelerated by hemiacetal formation followed by an intramolecular S_N2 displacement requires consideration. Solvent interactions of this type could occur. However, if this type of internal displacement was responsible for an acceleration of the solvolysis of **2** by a factor of 10^2–10^3, changing solvent should produce a drastic change in the absolute rate of solvolysis of **2** and in the *exo/endo* rate ratio. In fact, ethanolysis of **1** and **2** gave rates which were very close to the acetolysis rates both in absolute rate values and in the *exo/endo* rate ratio of 2.1 (at 100°) *vs.* 0.4 (at 100°) for acetolysis. (b) A concerted bond cleavage process leading to an acyl carbonium ion is an alternate mechanism for the solvolysis of **2**. Since both **1** and **2** yield products with the 7-ketonorbornane skeleton intact, this rationalization is ruled out. A discussion of the product analysis of **1** and **2** will be published in the near future.

(8) H. Kwart and T. Takeshita, *J. Am. Chem. Soc.*, **86**, 1161 (1964), have shown that not only the presence of an inductive group, but also its orientation relative to the reaction site, can influence solvolysis rates. The orientation factors studied by these workers generally changed the rates by a factor of less than ten. In comparing our rates with those of the epimeric norbornyl tosylates we find a change in the *exo/endo* rate ratio of 1.7 × 10^3. We doubt that an orientation factor could be that large; however, we cannot unequivocally rule out this possibility. Experiments are in progress to determine the orientation effect of the carbonyl group on solvolyses rates in rigid systems.

(9) H. C. Brown, "The Transition State," Special Publication No. 16, The Chemical Society, London, 1962.

(10) H. C. Brown, F. J. Chloupek, and M. H. Rei, *J. Am. Chem. Soc.*, **86**, 1248 (1964).

(11) A. Streitwieser, Jr., "Solvolytic Displacement Reactions," McGraw-Hill Book Co., Inc., New York, N. Y., 1962.