where X = OCH_3), the present results at 20–70 eV[15,18] can be interpreted most simply in terms of the initial formation of benzyl ions II from unrearranged molecular ions I, followed by rearrangement of II to tropylium ions III (except where X = OCH_3) prior to further fragmentation. The anomalous behavior of methoxy substituents has been noted before,[6,7] and our metastable data (Table I) are in agreement with the proposal[7] that $CH_3OC_6H_4CH_2^+$ ions have different (probably benzylic) structures (II) when derived from isomeric precursors I (X = *m*- and *p*-OCH_3).

Acknowledgment. Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research. Purchase of the Atlas CH4B mass spectrometer was made possible through Grant No. GB-4939 from the National Science Foundation.

(15) At lower electron energies, relative ion abundances are affected by the proximity of the appearance potential of the product ion.[16,17]

(16) F. W. McLafferty and M. M. Bursey, *J. Org. Chem.*, **33**, 124 (1968).

(17) See ref 3b.

(18) Mass spectra were secured using an Atlas CH4B mass spectrometer equipped with a molecular beam inlet system,[19] operating under the following conditions: probe at room temperature, source at 210°, filament current 3–8 μA. All reported ion abundance measurements were made at least in duplicate.

(19) C. Brunnée, 14th Annual Conference on Mass Spectrometry, ASTM Committee E-14, Dallas, Texas, 1966, p 410.

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A Reagent for Peptide Synthesis. Copoly(ethylene-N-hydroxymaleimide)

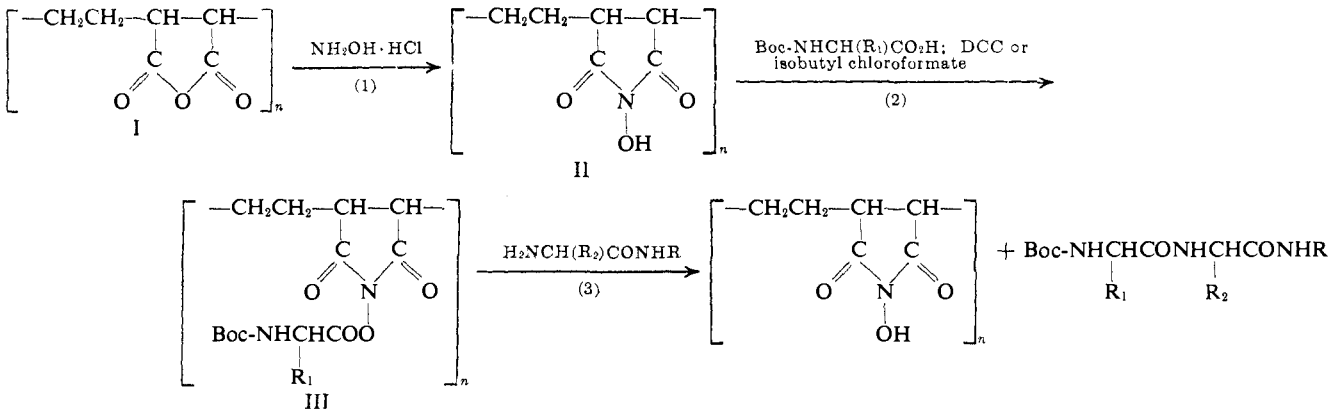
Sir:

The adaptability of reactions involving aminolysis of *t*-butyloxycarbonyl- (Boc-) amino acid N-hydroxysuccinimide (NHS) esters[1] to rapid work-up procedures in peptide synthesis has been recently demonstrated.[2] The use of polymers either as support systems[3] or as reagents[4] in stepwise syntheses of several peptides has been described. We report here a way to use NHS esters as polymeric reagents by (1) the synthesis of copoly(ethylene-N-hydroxymaleimide) (II) and its Boc-amino acid ester derivatives (III) and (2) the application of this polymeric form of NHS-active esters as a reagent in peptide synthesis.

The polymeric NHS (II) was prepared by condensing copoly(ethylenemaleic anhydride) (I) (Monsanto DX 840, 1 equiv) with hydroxylamine hydrochloride (4 equiv) in dimethylformamide (DMF) and distilling the solvent until the boiling point reached *ca.* 153° (Chart I, reaction 1). The exothermic reaction led to II in nearly quantitative yields as a buff-colored powder which was characterized by elemental analysis (*Anal.* Calcd for $C_6H_7O_3N$: C, 51.1; H, 5.0; N, 9.9. Found: C, 51.5; H, 5.7; N, 9.6) and by the characteristic infrared bands of the N-hydroxysuccinimide moiety (1780 (m), 1715 cm^{-1} (s); film cast from DMF). Polymer II was soluble in DMF and dimethyl sulfoxide and insoluble in H_2O, MeOH, EtOH, 2-PrOH, dimethoxyethane (DME), and acetonitrile.

Polymeric NHS esters of Boc-α-amino derivatives[5] of alanine, phenylalanine, O-benzylserine, threonine, β-benzylaspartic acid, methionine, *im*-benzylhistidine, ε-carbobenzyloxylysine, γ-benzylglutamic acid, nitroarginine, and leucine were synthesized in yields of *ca.* 70% either by the mixed anhydride[6] or dicyclohexylcarbodiimide (DCC)[1] methods (Chart I, reaction 2) using DMF as solvent. Use of equimolar quantities of all reactants usually resulted in 90–100% Boc-amino acid substitution of all NHS sites in polymer II. The extent of substitution on the polymer was estimated by amino acid analysis,[7] the characteristic infrared bands of the Boc-amino acid NHS ester moiety (1820 (w), 1740 cm^{-1} (s); films cast from DMF), and by subsequent test peptide coupling reactions. Amino acid substitution was varied (2–100%) by appropriate adjustment of the Boc-amino acid:polymer II molar ratio.

Chart I. Preparation and Reactions of Copoly(ethylene-N-hydroxymaleimide) in Peptide Synthesis



Use of polymeric NHS esters (III) as intermediates in syntheses of peptides listed in Table I was made in

(1) G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Am. Chem. Soc.*, **86**, 1839 (1964).

(2) D. A. Laufer and E. R. Blout, *ibid.*, **89**, 1246 (1967).

(3) R. B. Merrifield, *ibid.*, **85**, 2149 (1963); *Biochemistry*, **3**, 1385 (1964); *J. Org. Chem.*, **29**, 3100 (1964); G. R. Marshall and R. B. Merrifield, *Biochemistry*, **4**, 2394 (1965); R. B. Merrifield, *Recent Progr. Hormone Res.*, **23**, 451 (1967), and references cited therein; M. M. Shemyakin, Yu. A. Ovchinikov, A. A. Kiryushkin, and I. V. Kozhevnikova, *Tetrahedron Letters*, 2323 (1965).

(4) M. Fridkin, A. Patchornik, and E. Katchalski, *J. Am. Chem. Soc.*, **87**, 4646 (1965); **88**, 3164 (1966); T. Wieland and C. Birr, *Angew. Chem. Intern. Ed. Engl.*, **5**, 310 (1966); *Chimia* (Aarau), **21**, 581 (1967).

(5) Boc-L-amino acids were purchased from Cyclo Chemical Corp. The alanine and methionine derivatives were once crystallized before use.

(6) G. W. Anderson, F. M. Callahan, and J. E. Zimmerman, *J. Am. Chem. Soc.*, **89**, 178 (1967).

(7) Amino acid analyses of acid-hydrolyzed peptides were carried out with the Beckman-Spinco amino acid analyzer.

Table I. Peptides Synthesized with Copoly(ethylene-N-hydroxymaleimide)

Peptide	Crystal solvent[a]	Mp, °C[b]	Yield,[c] %	Amino acid analysis	Elemental analysis, % Calculated C	H	N	Found C	H	N
Boc-Ala-Ala-OBzl	B	73–74	86		61.7	7.5	8.0	61.7	7.5	7.9
Boc-Met-Ala-OMe	B	86–88	84	$Met_{0.98}Ala_{1.02}$	50.3	7.8	8.4	50.2	7.6	8.3
Z | Boc-Lys-Phe-OMe	AB	105–106	85	$Lys_{1.02}Phe_{0.98}$	64.3	7.3	7.8	64.1	7.2	7.7
Boc-Thr-Ala-OBzl	AB	98–101	55	$Thr_{1.07}Ala_{0.93}$	60.0	7.4	7.4	60.2	7.4	7.4
OBzl | Boc-Asp-Ala-OBzl	AB	66–68	75	$Asp_{0.98}Ala_{1.02}$	64.4	6.7	5.8	64.2	6.6	5.6
Bzl | Boc-Gln-His-OBzl	AB	119–121	42		61.9	6.8	12.1[d]	62.3	6.9	12.1
Bzl | Boc-Ser-(Ala)$_2$-OBzl	C	168–169	100	$Ser_{0.96}Ala_{2.04}$	61.8	7.3	9.0[d]	61.9	7.3	9.2
Z | Boc-Ala-Lys-Phe-OMe	AB	111–114	83	$Ala_{1.06}Lys_{0.98}Phe_{0.96}$						
Boc-(Ala)$_3$-OBzl	AB	140–141	76		59.8	7.4	10.0	59.8	7.4	9.8
Bzl | Boc-Thr-Ser-(Ala)$_2$-OBzl	AB	168–171	88	$Thr_{1.02}Ser_{0.94}Ala_{2.04}$						
Boc-Thr-(Ala)$_3$-OBzl	DE	210–211	77	$Thr_{0.98}Ala_{3.02}$	57.4	7.3	10.7	57.6	7.3	...
Z | Boc-(Ala)$_2$-Lys-Phe-OMe	AB	162–165	91	$Ala_{2.01}Lys_{1.01}Phe_{0.94}$	61.5	7.2	10.2	61.2	7.3	...
Bzl Bzl | | Boc-Ser-Thr-Ser-(Ala)$_2$-OBzl	C	187–189	94	$Ser_{1.96}Thr_{0.98}Ala_{2.06}$	61.2	6.9	8.5[d]	61.2	6.9	8.5
Z | Boc-(Ala)$_3$-Lys-Phe-OMe	AB	198–203	43	$Ala_{3.06}Lys_{0.99}Phe_{0.95}$	60.5	7.2	11.1	60.3	7.3	10.9
Bzl Bzl | | Boc-(Ser)$_2$-Thr-Ser-(Ala)$_2$-OBzl	AB	202–204	79	$Ser_{2.98}Thr_{0.96}Ala_{2.06}$	62.4	6.8	8.4[d]	61.9	6.6	8.2
OBzl Bzl Bzl | | | Boc-Asp-(Ser)$_2$-Thr-Ser-(Ala)$_2$-OBzl	AB	218–219	84	$Asp_{1.03}Ser_{2.99}Thr_{0.96}Ala_{2.01}$						
OBzl Bzl Bzl | | | Boc-Met-Asp-(Ser)$_2$-Thr-Ser-(Ala)$_2$-OBzl[e]		219–224	57	$Met_{0.46}Asp_{0.94}Ser_{2.98}$ $Thr_{0.99}Ala_{2.00}$	61.9	6.5	8.5	61.8	6.6	8.2

[a] A = EtOAc, B = hexane, AB = EtOAc–hexane, C = dimethoxyethane, DE = DMF–ether. [b] Determined on a Kofler block, uncorrected. [c] Refers to one cycle in a stepwise synthesis, *e.g.*, the removal of the Boc-amino protecting group from (n − 1) peptide and coupling of the product with polymeric NHS ester III to yield Boc-amino-n-peptide which was once crystallized from the indicated solvent. [d] Calculated for the monohydrate. [e] Purified by column chromatography on carboxymethyl cellulose using DMF as eluent.

the following way. Polymer III (1–2 equiv) was suspended in an anhydrous DME or ethyl acetate solution containing the aminopeptide salt (1 equiv) and triethylamine (1 equiv) and stirred for 1–5 hr (Chart I, reaction 3). The extent of reaction was followed by tlc on silica gel H. The insoluble by-products were removed by filtration or centrifugation. Flash evaporation *in vacuo* of the supernatant led to chromatographically pure peptides in near quantitative yields. All products were obtained in crystalline form and characterized as shown in Table I. Several peptides crystallized upon flash evaporation of the supernatant; the remainder were crystallized from the solvents indicated in Table I.

Removal of the Boc-protecting groups from α-amino-protected peptide intermediates was carried out with trifluoroacetic acid[8] or anhydrous HCl in either DME[2] or ethyl acetate.[9] Removal of excess acid and trituration with appropriate solvents led to chromatographically pure products in nearly quantitative yields.

For the preparation of hexa- and higher peptides, the use of cross-linked, surface-substituted polymers was investigated. Polymer II was cross-linked[10] by exposure to 20 Mrads of high-energy electrons at 2.5 Mrads per pass using a High-Voltage Engineering ICT 500 electron processing system.[11] Preparation of Boc-amino acid esters of cross-linked polymer III was carried out by the mixed anhydride procedure[6] using 30% DMF in DME as solvent. Extent of substitution was *ca.* 2%. Polymer III was used in coupling reactions in the manner indicated (Chart I, reaction 3).

(8) H. Kappeler and R. Schwyzer, *Helv. Chim. Acta*, **43**, 1453 (1960); R. Schwyzer and H. Kappeler, *ibid.*, **44**, 1991 (1961).

(9) R. Schwyzer and P. Sieber, *ibid.*, **43**, 1910 (1960); R. Schwyzer, H. Kappeler, B. Iselin, W. Rittel, and H. Zuber, *ibid.*, **42**, 1702 (1959).

(10) Exposure reduced (1) the solubility of the polymer in DMF and (2) its maximum extent of substitution from 100 to *ca.* 2.5%. The infrared spectra of irradiated samples were unchanged.

(11) We thank David R. Francis and Robert C. Bradley of High Voltage Engineering Corp., Burlington, Mass., for carrying out the radiation cross-linking of polymer II, and Dr. Gian P. Lorenzi for helpful suggestions.

The optical purity of two peptides (with side-chain blocking groups removed), H-(Ala)$_2$-Lys-Phe-OMe (IV) and H-Ser-Thr-Ser-(Ala)$_2$-OH (V), was investigated by enzymic hydrolysis. Peptide IV was incubated with aminopeptidase M,[12] and peptide V was treated with leucine aminopeptidase.[13] Amino acid analysis and paper and thin layer chromatograms of the hydrolysates showed that both peptides were completely hydrolyzed, thus indicating that no detectable racemization occurred during synthesis.

The results reported above indicate that the use of polymeric NHS esters combines high reactivity and steric homogeneity in peptide synthesis with the facile work-up of polymer reagent and support systems. Application of this method to the synthesis of higher peptides is being investigated.[14]

Acknowledgments. We thank Dr. Elizabeth R. Simons for valuable assistance in enzymic hydrolyses and Miss Mary Jane Becherer for amino acid analyses. This work was supported in part by the U. S. Public Health Service, Grants AM 07300 and AM 10794, and by the Office of the Surgeon General, Department of the Army.

(12) G. Pfleiderer and P. G. Celliers, *Biochem. Z.*, **339**, 186 (1963); K. Hoffmann, F. M. Finn, M. Limetti, J. Montibeller, and G. Zannetti, *J. Am. Chem. Soc.*, **88**, 3633 (1966).

(13) K. Hofmann and H. Yajima, *ibid.*, **83**, 2289 (1961); K. Hofmann, H. Yajima, T.-Y. Liu, N. Yanaihara, C. Yanaihara, and J. L. Humes, *ibid.*, **84**, 4481 (1962).

(14) Subsequent to the completion of the manuscript we noted a brief reference to the use of the polymer reported here: A. Patchornik, M. Fridkin, and E. Katchalski, "Proceedings of the Eighth European Peptide Symposium," John Wiley and Sons, Inc., New York, N. Y., 1967, p 92.

(15) National Institutes of Health Postdoctoral Fellow 1965–1966.

(16) National Science Foundation Postdoctoral Fellow 1965–1967.

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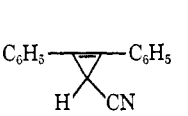
Antiaromatic Effects in Cyanocyclopropenyl Anions

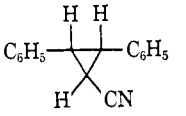
Sir:

We have reported[1-3] evidence for conjugative destabilization in some cyclopropenyl anions. The most convincing data involve a comparison[3] of deuterium exchange rates for cyclopropyl and cyclopropenyl ketones, sulfones, and esters; in spite of the greater formal conjugation in the related anions, exchange in the cyclopropenes is considerably slower. This striking effect may result from antiaromaticity in the cyclopropenyl anions, or various steric differences which might make rehybridization to a trigonal carbon more difficult in the cyclopropene case. Previous arguments[3] indicated that the steric explanation is less likely. We wish now to report further evidence which supports antiaromaticity as the major factor in these rate differences.

Evidence similar to that we have already reported comes from a comparison of the rate of exchange[4] of diphenylcyclopropenyl cyanide[5] (I) with that of the corresponding cyclopropane derivative[6] (II). Some of the steric effects cited[3] for the corresponding benzoyl derivatives should be smaller with a cyano group, but PPP–SCF calculations predict that the antiaromaticity effect should be larger. This results essentially from less effective charge removal by the cyano group than by a benzoyl group. Experimentally, the exchange of I was carried out at 60.3° in 40% dimethoxyethane and 60% *t*-butyl alcohol-O-*d* with 0.06 *M* potassium *t*-butoxide, with aliquots analyzed by mass spectroscopy. Good reproducible pseudo-first-order data were obtained, $k_I = 1.86 \times 10^{-6}$ sec^{-1}. The much faster exchange of II was examined in the same medium at three lower temperatures and extrapolated to 60.3°: $k_{II}(-0.8°) = 1.82 \times 10^{-4}$ sec^{-1}, $k_{II}(6.9°) = 3.84 \times 10^{4}$ sec^{-1}, $k_{II}(16.4°) = 8.75 \times 10^{-4}$ sec^{-1}. The ratio at 60.3° is $k_{II}/k_I = 10{,}000$. As predicted by the MO calculations, this is larger than the corresponding number (6000) for ketone activation.

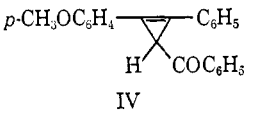


I

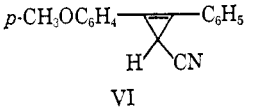


II

Evidence of a different sort comes from studies on racemization *vs.* exchange in optically active derivatives. 1-*p*-Anisyl-2-phenylcyclopropene-3-carboxylic acid[6] (III), mp 179–180°, was resolved with cinchonine, α_D(III) −20.3°. With phenyllithium this afforded optically active 1-*p*-anisyl-2-phenyl-3-benzoylcyclopropene[6] (IV), mp 100–102°, a_D −35.8°; the ORD shows a Cotton curve with α_{trough} −235°. Exchange with potassium ethoxide (0.2 *M*) in 1:1 dimethoxyethane and ethanol-O-*d* at 100.7° was followed by mass spectroscopy and ORD: $k_{exchange}/k_{racemization}$ was reproducibly 1.0 ± 0.1. For an optically active benzoylcyclopropane Walborsky has found $k_e/k_r = 1.6$ in methanol. We have also converted optically active III to the carboxamide V, mp 238–240°, and thence to optically active 1-*p*-anisyl-2-phenyl-3-cyanocyclopropene (VI), mp 95–96°. This was treated at 60.2° with 0.42 *M* potassium *t*-butoxide in *t*-butyl alcohol-O-*d* and aliquots were examined by mass spectroscopy and ORD. The ratio $k_{exchange}/k_{racemization}$ was 4.0 ± 0.2. By contrast, Walborsky has found $k_e/k_r = 77$ for optically active 2,2-diphenylcyclopropenyl cyanide in *t*-butyl alcohol with potassium *t*-butoxide.



IV
$k_e/k_r = 1.0 \pm 0.1$



VI
4.0 ± 0.2

Exchange in the cyclopropene system is thus accompanied by more racemization than in the cyclopropane series. The stereochemical retention for

(1) R. Breslow and M. Battiste, *Chem. Ind.* (London), 1143 (1958).

(2) R. Breslow and P. Dowd, *J. Am. Chem. Soc.*, **85**, 2729 (1963).

(3) R. Breslow, J. Brown, and J. Gajewski, *ibid.*, **89**, 4383 (1967).

(4) W. van Wijnen, M. van Wijnen, H. Steinberg, and Th. J. de Boer, *Tetrahedron*, **23**, 3763 (1967), suggest that the rate-determining step in this reaction comes after proton removal, but we feel their observed k_H/k_D of 1.9 in methanol is consistent with rate-determining ionization, as is expected in this solvent.

(5) R. Breslow, J. Lockhart, and H. W. Chang, *J. Am. Chem. Soc.*, **83**, 2375 (1961).

(6) New compounds had reasonable nmr, infrared, and ultraviolet spectra, and were further characterized by analysis or mass spectrum.