The finding that complexes **5** and **6** give high diastereofacial selectivity in their aldol reactions with chiral aldehydes should encourage the continued investigation of the origins of the selectivity and the synthetic applications of the reactions of aminocarbene complexes.

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Supplementary Material Available: Spectral data (^1H NMR, ^{13}C NMR, and IR) for all new compounds (6 pages). Ordering information is given on any current masthead page.

Ring Opening of Cyclic Pentapeptides by Electron Impact Mass Spectrometry: Correlation with Peptide Bond Nonplanarity

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We have designed and synthesized a series of cyclic pentapeptides as conformational models of reverse turns.[1] These molecules are highly constrained by formation of the cyclic backbone. Consistent with this, we have observed at least one strongly nonplanar peptide bond in crystal structures of these cyclic peptides.[2] As part of our characterization, we have obtained electron impact (EI) mass spectra of these synthetic cyclic peptides. The results of these studies suggest that ring opening of cyclic pentapeptide radical molecular ions, $M^{\bullet+}$s, produced from electron impact occurs preferentially in bonds adjacent to a nonplanar peptide bond in the parent molecule. If general, this mechanism could offer useful information about conformational properties of cyclic peptides.

Early EI studies showed that principal breakdown of linear peptides was by cleavage at peptide bonds.[3] Substantial evidence of rearrangements of fragments and secondary fragmentations[4] contributed to decreasing application of EI methods for sequence determination as the softer methods, principally fast atom bombardment, became available.[5] EI studies of cyclic peptides focused on the first loss process, which requires the cleavage of at least two bonds, the first a ring opening and the second analogous to fragmentation in a linear peptide. Multiple mechanisms have been proposed:[6] (1) Cleavage of a C^{α}—CO bond of a residue in $M^{\bullet+}$, with the loss of a neutral amine fragment carrying the side chain (NH=CHR), (2) cleavage at the peptide bond preceding an aromatic residue with migration of a β-hydrogen atom to the oxygen of the preceding residue, or (3) cleavage of a C^{α}—CO bond of a residue with the loss of neutral NHCO.

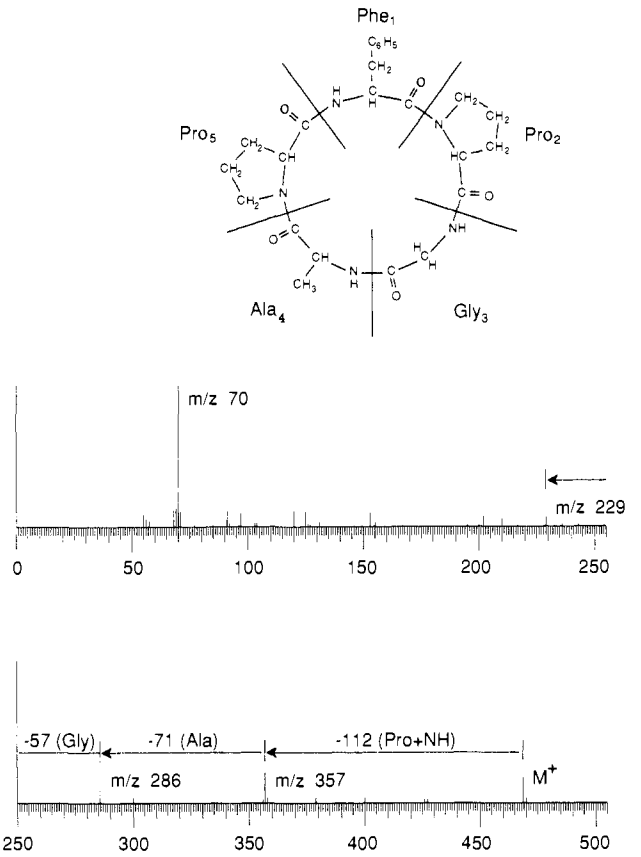

Figure 1. EI mass spectrum of cyclo(D-Phe_1-Pro_2-Gly_3-D-Ala_4-Pro_5). Data were collected on a DuPont 21-492B double focusing mass spectrometer equipped with a Hewlett-Packard 21MX computer. The samples were prepared by applying the peptide to the Teflon covered direct exposure probe tip: 70 eV.

Figure 1 shows a typical mass spectrum for a cyclic pentapeptide, cyclo(D-Phe_1-Pro_2-Gly_3-D-Ala_4-Pro_5), I. The peak at m/z 70, $C_4H_8N^+$, is a characteristic ion observed in the EI mass spectra of many proline-containing peptides.[6c,7] The most abundant high mass fragment ions (m/z 357, 286, 229) suggest the sequential losses of proline + NH (112), alanine (71), and glycine (57). Peaks corresponding to the molecular ion minus 43 and minus 69 are also observed, suggesting that the 112 loss may occur as two fragments, a peptide bond unit (CONH, 43), and a proline ring amine (C_4H_7N, 69). From the known sequence of the peptide and the assumption that these ions arise primarily from a single ring opening followed by sequential loss of residues, we infer that the proline lost is Pro_5 (not Pro_2) and that ring opening occurred

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Table I. Major Fragments of Four Cyclic Pentapeptides[a]

	I, cyclo(D-Phe-Pro-Gly-D-Ala-Pro)									
intensity	14	14	7	3	4	5	7	9	100	
m/z	469 (M^+)	357	286	229	202	153	125	120	70	
	II, cyclo(Gy-Pro-Gly-D-Ala-Pro)									
intensity	10	13	8	3	6	6	100			
m/z	379 (M^+)	267	196	139	125	112	70			
	II*, cyclo(Gly-Pro-Gly($^2H_2$)-D-Ala-Pro)									
intensity	7	8	5	3	3	5	100			
m/z	381 (M^+)	269	198	139	125	112	70			
	III, cyclo(Gly-Pro-D-Phe-D-Ala-Pro)									
intensity	14	8	32	8	23	5	12	14	100	
m/z	469 (M^+)	426	357	343	286	210	139	120	70	
	IV, cyclo(Ala-Pro-Gly-D-Phe-Pro)									
intensity	4	6	3	2	7	3	5	7	6	100
m/z	469 (M^+)	357	329	286	210	168	153	125	120	70

[a] Experimental conditions as given in the legend to Figure 1.

Table II. Peptide Bond Torsion Angles

peptide	ω1	ω2	ω3	ω4	ω5	ref
I	180	180	179	179	−158	2c
II	174	−179	177	178	−160	2a
III	171	−177	−173	−176	−158	2d
IV	14	166	−177	175	176	1c

following the Pro_5-D-Phe_1 bond, specifically at the D-Phe N–C^α bond.

However, comparisons with fragmentation patterns of related peptides demonstrate that the aromatic residue is not essential in directing the ring opening (Table I). The major high mass fragment ions in the EI mass spectrum of II, cyclo(Gly_1-Pro_2-Gly_3-D-Ala_4-Pro_5), can be accounted for by sequential losses of 112, 71, and 57 or, proline + NH, alanine, and glycine, respectively. As above, peaks at the molecular ion minus 43 and 69 suggest that the 112 loss may occur in two steps. An isotopically enriched form of II, cyclo(Gly_1-Pro_2-Gly_3($^2H_2$)-D-Ala_4-Pro_5), II*, was used to corroborate the assignments of the observed fragment ions. The major high mass ions at m/z 269, 198, and 139 (Table I) are consistent with sequential losses of proline + NH (112), alanine (77), and glycine-d_2 (59). These observations suggest that the initial ring opening occurred at the Gly_1 N–C^α bond, following the Pro_5-Gly_1 peptide bond, and that the decomposition mechanism may not require an aromatic side chain. Moreover, inspection of the dihedral angles from crystal structures of peptides I and II reveals that initial cleavage occurs adjacent to the peptide bond that is the *most nonplanar* (Table II).

Fragmentation of a third cyclic pentapeptide, cyclo(Gly_1-Pro_2-D-Phe_3-D-Ala_4-Pro_5), III (Table I), parallels that observed in I and II. The major high mass ions at 357, 286, and 139 (Table I) are accounted for by sequential losses of proline + NH (112), alanine (71), and phenylalanine (147). These observations suggest that the initial ring opening occurred at the Gly_1 N–C^α bond, following the most nonplanar peptide bond in the crystal (Table II).

The analysis of the EI fragmentation pattern of a fourth cyclic pentapeptide, cyclo(Ala_1-Pro_2-Gly_3-D-Phe_4-Pro_5), IV (Table I), is less straightforward, possibly reflecting more than one ring opening site. Nonetheless, the greatest number of the observed ions can be explained by a ring opening adjacent to the Pro_2-Gly_3 peptide bond, one of two strongly nonplanar bonds in the crystal (Table II).

It thus appears that a *conformational feature* common to these four peptides influences ring opening: Substantial nonplanarity of a peptide bond correlates with ring cleavage at the following N–C^α bond. We suggest that the neutral and ionized cyclic peptide molecules retain a conformation in the gas phase that is similar to the crystal structure. The bond angle and length distortions associated with a nonplanar peptide bond appear to cause increased susceptibility to cleavage of proximal bonds in the vibrationally excited ions. Perturbation of the structure is plausible in the cases studied here, as the extents of nonplanarity are large. Theoretical estimates of the energy cost from a peptide bond distortion of 20° are from 5 to 6 kcal/mol.[8] The conformational influences from cyclization appear to be lost once the peptide ring is open, since the fragmentation appears to follow decomposition mechanisms for linear peptides.

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Measurement of NH–CαH Coupling Constants in Staphylococcal Nuclease by Two-Dimensional NMR and Comparison with X-ray Crystallographic Results

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Structure determination of proteins by NMR relies on measurement of cross relaxation rates (NOE effect)[1] which yields information about interproton distances and on measurements of J couplings that are related to dihedral angles through the well-known Karplus equations.[2] As first demonstrated by Marion and Wuthrich,[3] the J couplings in small proteins can be measured from the antiphase splittings within a cross peak in phase-sensitive COSY spectra provided that the spectra are recorded with very high resolution in the F_2 dimension. However, if the coupling is of the same order or smaller than the ^1H line width, measurement of the antiphase splitting can result in a serious overestimate of the actual J coupling.[4,5] This problem is particularly severe for

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