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Theoretical Studies of the Radiation Products of Hydroxyproline

Fuqiang Ban, James W. Gauld, and Russell J. Boyd*

Department of Chemistry, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J3

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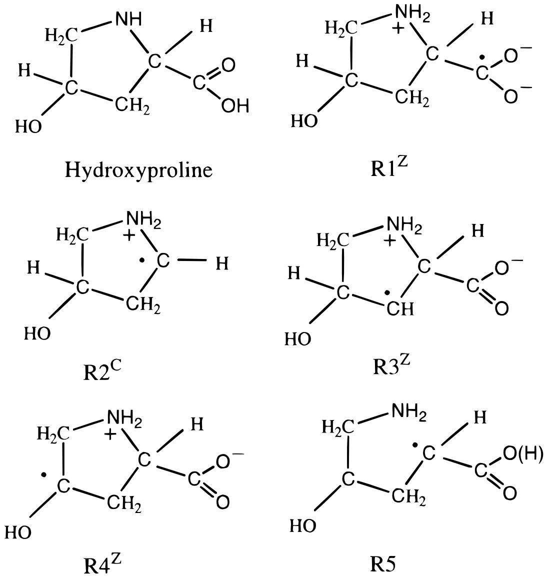
The radiation products of hydroxyproline have been investigated using density functional theory. In the resulting radicals, the choice of the apical ring atom is found to be dependent on the nature and strength of the intramolecular hydrogen bonding. The observed hyperfine couplings previously assigned to two zwitterionic conformers of the hydroxyproline primary radical anion are found to be better described by its nonzwitterionic isomers and corresponding neutral protonated isomers. Similarly, the observed hyperfine couplings for radicals formed by cleavage of the C_α−N bond (deamination) are in closest agreement with those calculated for their neutral forms. Theoretical proton hyperfine couplings support the experimental assignment of the radical cation formed by decarboxylation and the radicals resulting from hydrogen abstraction from the C_2 and C_3 positions. The proton hyperfine couplings are sensitive to the conformations of the radicals, which, in turn, are highly dependent upon the extent of intramolecular hydrogen bonding.

1. Introduction

Since the 1950's,[1] electron spin resonance (ESR) techniques have been used to study the radiation chemistry of amino acids. Upon irradiation, an amino acid may form either its primary radical cation or anion by ejection or capture of an electron. These primary radicals may then undergo further reactions, generally by decarboxylation or deamination of the primary radical cation or anion, respectively, to give various secondary radicals. Characterization and identification of the resulting radicals by ESR is often difficult, however, as found in the case of proline and related systems.[2−6]

Of all of the essential amino acids, proline is unique in having a five-membered ring with a secondary amino group. In the 1980's, a number of studies were performed on X-ray irradiated L-proline monohydrate,[5] DL-proline•HCl,[3] hydroxyproline•HCl[6] and hydroxyproline[2,4] single crystals. Two conformations were proposed for the hydroxyproline primary radical anion ($R1^A$). A number of other hydroxyproline-derived radicals were also proposed, including a secondary radical cation ($R2^C$) resulting from decarboxylation, three conformations of secondary radicals resulting from deamination, and radicals resulting from H-atom abstraction at the C_2 and C_3 positions. In addition, several possible zwitterionic radicals resulting from irradiation of hydroxyproline[2,4] were also proposed. Because of complex and overlapping ESR spectra, however, the exact identification and characterization of many radicals and reaction intermediates were not possible. The experimentally proposed hydroxyproline-derived radicals are shown in Figure 1.

Theoretical chemistry offers a complementary approach to experiment that can be used to gain greater insight into the radicals produced upon irradiation of amino acids and their reactions. Indeed, numerous studies have investigated the reliability of various computational schemes for the calculation of the hyperfine couplings of bioradicals.[7] In particular, density functional theory methods have been shown to give reliable structures and hyperfine coupling constants for a range of amino acids,[8−14] quinones,[15,16] and nucleic acid bases.[17] For the zwitterionic isomers, however, a slightly modified approach has been found to be necessary. This is due to the fact that the zwitterionic isomers of amino acids and their radicals often do not correspond to energy minima in the gas phase, as exemplified by glycine[18] and its radical.[19,8] Thus, to obtain such structures, the electrostatic effects of the crystalline environment must be taken into account. One approach is to treat electrostatic interactions of neighboring molecules as a uniform dielectric continuum. For example, in the Onsager model,[20] the interaction of the target molecule with the surrounding molecules is simulated by the interaction of the dipole of the target with an electric field produced by the dielectric. Previous calculations using the Onsager and related models for the zwitterions of glycine[8,9] and alanine[10] radicals have been found to yield



Hydroxyproline $R1^Z$ $R2^C$ $R3^Z$ $R4^Z$ R5

Figure 1. Schematic illustration of the structures of hydroxyproline and related radicals generated upon X-irradiation.

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* Author to whom correspondence should be addressed. E-mail: boyd@is.dal.ca.

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hyperfine coupling constants in good agreement with experiment.

In the present study, density functional theory methods have been employed to investigate possible radicals formed upon irradiation of hydroxyproline.

2. Computational Methods

All geometry optimizations were performed with the B3LYP hybrid density functional in conjunction with the 6-31+G(d,p) basis set using the Gaussian 98 suite of programs.[21] The B3LYP functional is a combination of Becke's three-parameter hybrid exchange functional,[22] as implemented in Gaussian 98,[23] and the Lee−Yang−Parr correlation functional.[24] Zwitterionic structures of hydroxyproline radicals were obtained using the standard Onsager model[25] with a dielectric constant for water of 78.39. All stationary points were confirmed to be local minima by harmonic vibrational frequency calculations. Relative energies of all conformers of R1^{A}, R1^{P}, R2^{C}, R3^{N}, R5^{A}, and R5^{N} were obtained by performing single-point calculations at the B3LYP level in conjunction with the 6-311G(2df,p) basis set using the above-optimized geometries and inclusion of the appropriate scaled[26] zero-point vibrational energy, ie., B3LYP/6-311G(2df,p)//B3LYP/6-31+G(d,p) + ZPVE.

Isotropic and anisotropic hyperfine coupling constant (HFCC) calculations were performed using the deMon program.[27] The PWP86 functional, a combination of Perdew and Wang's exchange functional (PW)[28] and Perdew's nonlocal correlation functional (P86),[29] in conjunction with the 6-311G(2d,p) basis set was employed. The (5,4;5,4) family of auxiliary basis sets was used to fit the charge density and the exchange correlation potential. This functional and basis set combination has been shown to give accurate hyperfine coupling constants in previous studies of amino acid[9,10,14] and model π radicals.[7]

Assessments of methods for the accurate calculation of HFCCs have been previously reported.[30] Accurate isotropic HFCCs require a good description of the electron correlation and a suitable basis set, whereas satisfactory anisotropic HFCCs can be obtained at fairly low levels of theory provided that the structure is qualitatively correct. However, good agreement of both theoretical isotropic and anisotropic components with experimental hyperfine coupling constants is necessary to confidently identify a new radical species.

3. Results and Discussion

Structure and Hyperfine Couplings of the Hydroxyproline Radical Anion (R1^{A}) and its Protonated Neutral Radical (R1^{P}). Experimentally, upon irradiation of hydroxyproline, no carboxylate hydrogen interactions were detected.[2,4] Thus, the observed H$_\beta$ hyperfine tensors (see Table 1) were assigned to two different conformations of the zwitterionic radical R1^{Z}.[2,4] The optimized structures of possible conformers of R1^{Z} using the Onsager model with a radius of 4.35 Å are shown schematically in Figure 2, while the corresponding calculated isotropic and anisotropic HFCCs are given in Table 1.

Two possible conformers, R1^{Z}-I and R1^{Z}-II, of the zwitterionic radical anion R1^{Z} were obtained. The main difference between R1^{Z}-I and R1^{Z}-II is the orientation of $O_3{-}H_3$. However, both conformers possess a half-chair conformation with the $-C_3$-HOH− moiety forming the apex (see Figure 2). The two structures both contain a reasonably strong intramolecular $O_1 \cdots H_2{-}N$ hydrogen bond ($r_{O\cdots H}$ < 1.811 Å) between the positively charged ring $-NH_2^{+}-$ group and the almost-planar (see Table 2) anionic carboxylate group. As can be seen from Table 1, the calculated H$_\beta$ HFCCs of R1^{Z} differ considerably from the experimentally observed values. Hence, it would appear unlikely that either of the experimentally observed conformers is R1^{Z}.

TABLE 1: Calculated, PWP86/6-311G(2d,p), and Experimental H$_\beta$ HFCCs (MHz) of R1^{Z}, R1^{A}, and R1^{P} and Relative Energies (kJ mol^{-1}) of the Conformers of R1^{A} and R1^{P}

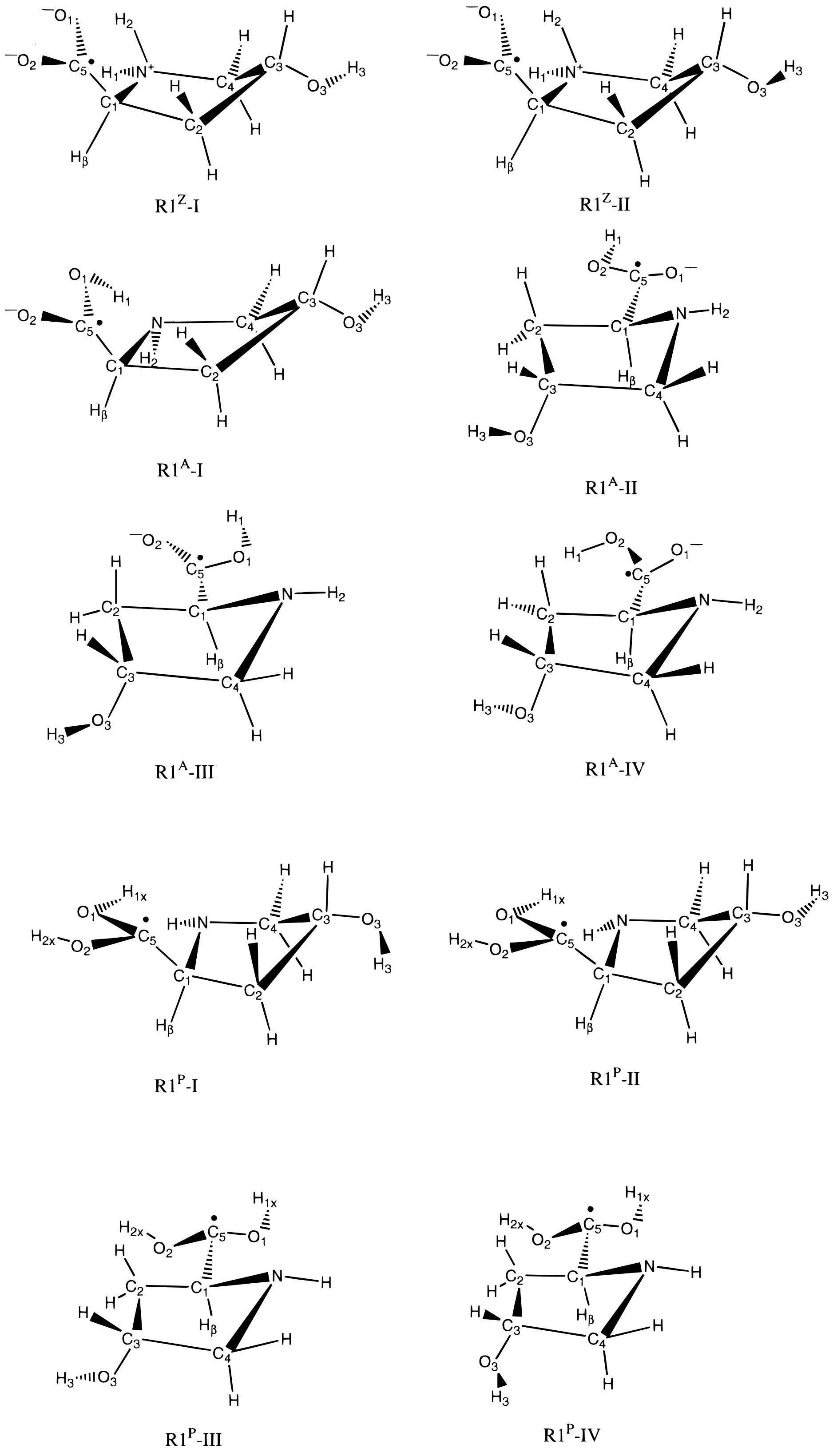
system	A_{iso}	T_{xx}	T_{yy}	T_{zz}	ΔE^{c}
R1^{Z}-I	2.0	−5.0	−0.9	5.8	
R1^{Z}-II	4.9	−4.8	−0.5	5.3	
R1^{A}-I	32.3	−4.4	−3.5	7.9	0.0
R1^{A}-II	17.9	−4.3	−3.7	8.0	10.3
R1^{A}-III	18.5	−4.5	−3.8	8.3	12.5
R1^{A}-IV	6.2	−3.7	−2.4	6.0	35.9
R1^{P}-I	59.9	−5.1	−3.8	8.9	0.0
R1^{P}-II	58.8	−5.1	−3.8	8.9	1.4
R1^{P}-III	55.9	−5.3	−3.5	8.8	22.0
R1^{P}-IV	56.2	−5.3	−3.6	8.9	22.4
exptl I^{a}	23.9	−6.2	−5.2	11.4	
exptl II^{b}	61.0	−4.7	−2.1	6.9	

^{a} Reference 2. ^{b} Reference 4. ^{c} B3LYP/6-311G(2df,p)//B3LYP/6-31+G(d,p) + ZPVE.

For the nonzwitterionic isomer (R1^{A}) of R1^{Z}, four possible conformers were found: R1^{A}-I through R1^{A}-IV (see Figure 2). The main difference between the four conformers is the orientation of O_1 or $O_2{-}H_1$ in the carboxyl group. All are found to have a half-chair conformation with the −NH− moiety forming the apex, except for R1^{A}-I for which the $-C_3HOH-$ moiety forms the apex. Because R1^{A}-I possesses a strong intramolecular $O{-}H_1 \cdots N$ hydrogen bond ($r_{N\cdots H_1} = 1.818$ Å) while the other conformers all possess a weak $O \cdots H_2{-}N$ hydrogen bond ($r_{O\cdots H_2} > 2.692$ Å), it can be concluded that the apexes of the R1^{A} half-chair structures are dependent on the strength of the hydrogen bond. Interestingly, for R1^{A}-II and R1^{A}-III, the bases of the half-chairs are almost perfectly planar, whereas their carboxylic groups are more pyramidal than those of R1^{A}-I and R1^{A}-IV (see Table 2).

The calculated isotropic and anisotropic H$_\beta$ HFCCs for all R1^{A} conformers are given in Table 1. The coupling constants for R1^{A}-II and R1^{A}-III are very similar, reflecting the close similarities in structure, and are in close agreement with one of the experimentally observed values (exptl I, see Table 1). The isotropic coupling constants for R1^{A}-I (32.3 MHz) and R1^{A}-IV (6.2 MHz), however, differ considerably from either of the experimentally observed values of 23.9 and 61.0 MHz. Furthermore, they also differ significantly from the calculated coupling constants of R1^{A}-II and R1^{A}-III. These results suggest that the experimentally observed conformer denoted as exptl I in this present study is most probably R1^{A}-II or R1^{A}-III, or even possibly a mixture of the two (they are calculated to lie close in energy, see Table 1).

The large difference between the two experimentally observed H$_\beta$ HFCCs suggests that there may, in fact, be a net change in the charge between the two conformers exptl I and exptl II, i.e., exptl II may, in fact, be a protonated form of exptl I. Thus, possible protonated conformers of R1^{A} were investigated, the resulting radicals being neutral radicals. Four possible conformers were obtained, and they can be divided into two groups with respect to the five-membered ring skeletal structures: R1^{P}-I and R1^{P}-II (group I) and R1^{P}-III and R1^{P}-IV (group II) (see Figure 2). The main difference between the two conformers in each group is the $O_3{-}H_3$ orientation. Group I possesses relatively stronger intramolecular $O{-}H \cdots N$ hydrogen bonds ($r_{N\cdots H} \approx$ 2.044 Å), with the $-C_3HOH-$ moiety forming the apex of the



$R1^Z$-I

$R1^Z$-II

$R1^A$-I

$R1^A$-II

$R1^A$-III

$R1^A$-IV

$R1^P$-I

$R1^P$-II

$R1^P$-III

$R1^P$-IV

Figure 2. Optimized structures of $R1^Z$, $R1^A$, and $R1^P$.

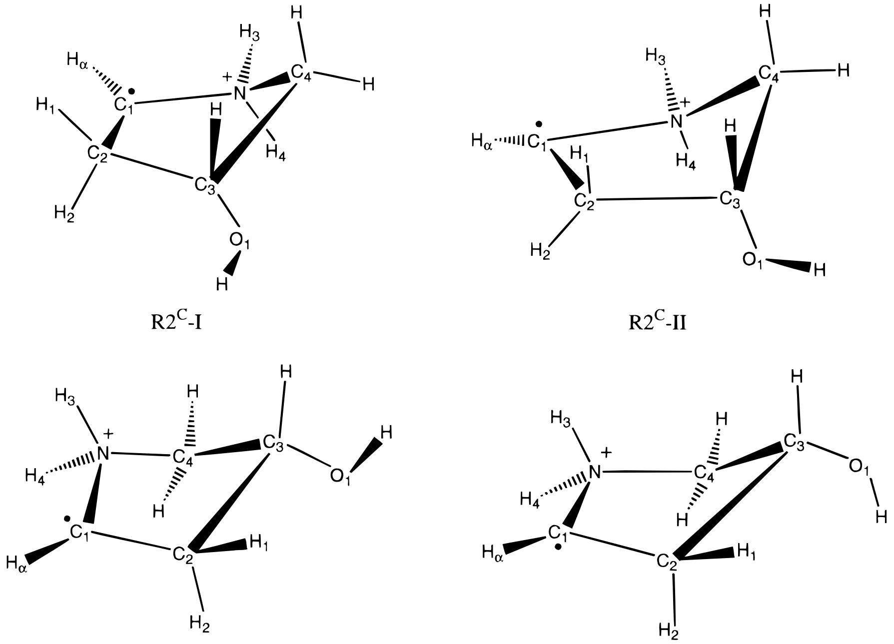
five-membered ring. In contrast, group II possesses weaker intramolecular O···H−N hydrogen bonds ($r_{N\cdots H} \approx 2.655$ Å), and the −NH− moiety forms the apex. In both groups, however, the carbon of the protonated carboxylic group has become more pyramidal, as shown in Table 2.

The calculated H_β HFCCs of $R1^P$-I through $R1^P$-IV are given in Table 1. Groups I and II give similar H_β HFCCs. Importantly, however, these values differ considerably from those calculated for $R1^A$. The calculated coupling constants of $R1^P$ are in good agreement with those previously experimentally assigned to a conformer of the zwitterion $R1^Z$ and denoted as exptl II in Table 1. This suggests that the experimentally observed couplings of exptl II in fact arise from a protonated derivative of the radical anion of hydroxyproline. The calculated relative energies (see

TABLE 2: Optimized Value of the Angle $\angle O_1C_5O_2C_1$[a] in $R1^Z$, $R1^A$, and $R1^P$

radical	$R1^Z$-I	$R1^Z$-II	$R1^A$-I	$R1^A$-II	$R1^A$-III	$R1^A$-IV	$R1^P$-I	$R1^P$-II	$R1^P$-III	$R1^P$-IV
$\angle O_1C_5O_2C_1$	178.9	−179.8	175.8	160.5	159.5	167.4	133.7	133.8	130.4	130.3

[a] Angles in degrees.



$R2^C$-I $R2^C$-II

$R2^C$-III $R2^C$-IV

Figure 3. Optimized structures of $R2^C$.

TABLE 3: Calculated, PWP86/6-311G(2d,p), and Experimental HFCCs (MHz) of H_α, H_1, H_2, H_3, and H_4 in $R2^C$-I through $R2^C$-IV and Relative Energies (kJ mol^{-1})

tensor	HFCC	$R2^C$-I	$R2^C$-II	$R2^C$-III	$R2^C$-IV	exptl[a]
H_α	A_{iso}	−62.2	−61.2	−66.0	−66.7	−46.3
	T_{xx}	−39.0	−38.8	−39.2	−39.3	−35.0
	T_{yy}	−1.6	−1.6	−1.3	−1.4	0.2
	T_{zz}	40.6	40.4	40.5	40.7	34.8
H_1	A_{iso}	137.2	138.8	163.9	153.9	133.3
	T_{xx}	−6.0	−6.0	−6.1	−5.9	−6.3
	T_{yy}	−4.0	−4.1	−2.9	−3.4	−3.9
	T_{zz}	10.0	10.1	9.0	9.3	9.7
H_2	A_{iso}	78.7	84.1	77.4	77.4	45.9
	T_{xx}	−5.3	−5.5	−5.3	−5.4	−5.1
	T_{yy}	−2.9	−2.7	−3.8	−3.4	−2.6
	T_{zz}	8.2	8.3	9.1	8.7	7.7
H_3	A_{iso}	42.5	41.8	61.7	59.0	58.9
	T_{xx}	−5.8	−5.7	−5.8	−5.8	−5.4
	T_{yy}	−5.1	−5.1	−4.5	−4.7	−5.0
	T_{zz}	10.8	10.8	10.3	10.6	10.3
H_4	A_{iso}	94.1	94.3	82.9	85.9	72.6
	T_{xx}	−6.3	−6.2	−6.6	−6.5	−7.7
	T_{yy}	−4.3	−4.3	−4.8	−4.5	−4.5
	T_{zz}	10.5	10.5	11.3	11.0	12.3
ΔE[b]		0.0	1.5	14.5	25.4	

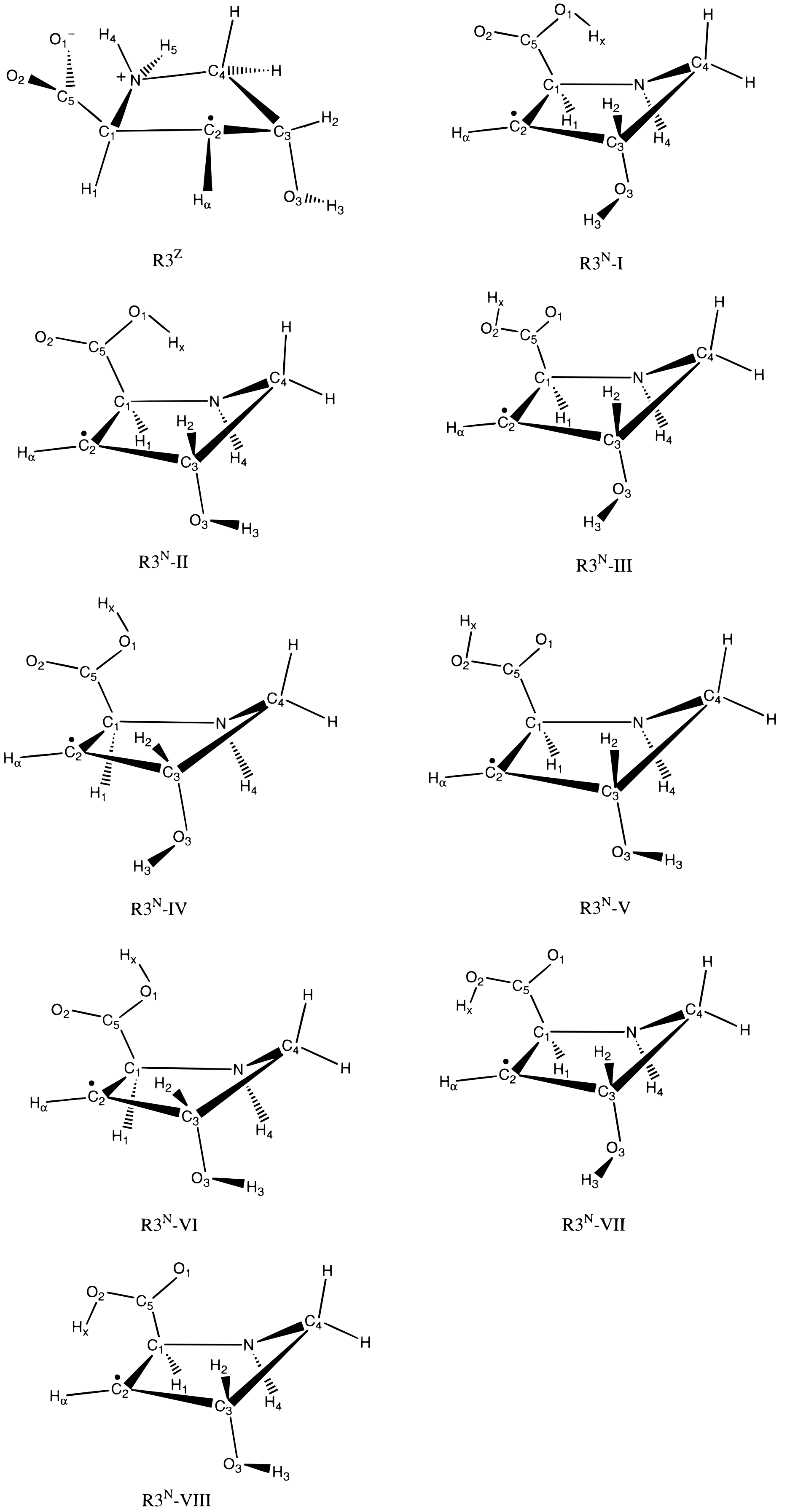
[a] Reference 2. [b] B3LYP/6-311G(2df,p)//B3LYP/6-31+G(d,p) + ZPVE.

Table 1) suggest that exptl II is most likely a conformer, or a mixture of both conformers, of group I.

Secondary Radical Cation Formed upon Decarboxylation: $R2^C$. Four conformations of $R2^C$ were found and are illustrated schematically in Figure 3. As seen in Table 3, the two lowest-energy conformers are $R2^C$-I and $R2^C$-II. In both radicals, the $-C_4(H_2)-$ moiety forms the apex of the five-membered ring because of the formation of the intramolecular $N-H_4\cdots O_1$ hydrogen bond. In $R2^C$-III and $R2^C$-IV, the $-C_3(HO_1H)-$ moiety is in the apical position of the five-membered ring, and both radicals lack intramolecular hydrogen bonds.

For $R2^C$-I through $R2^C$-IV, the calculated and experimentally observed HFCCs of H_α, H_1, H_2, H_3, and H_4 are listed in Table 3. On the basis of their calculated coupling constants, the four conformers can be divided into two groups. One group consists of $R2^C$-I and $R2^C$-II, while the other consists of $R2^C$-III and $R2^C$-IV. The coupling constants of the two groups differ significantly, in particular for H_1, H_3, and, to a lesser extent, H_4 (see Table 3). The calculated coupling constants of H_α and H_2 appear to be less sensitive to the conformation. The calculated HFCCs of H_1 in $R2^C$-I and $R2^C$-II are in closest agreement with experiment, whereas $R2^C$-III and $R2^C$-IV give HFCCs for H_3 and, to a lesser degree, H_4 in closer agreement with experiment. For all four conformers, the calculated anisotropic HFCCs of each of the five hydrogens listed in Table 3 are in close agreement with the corresponding experimental values. These results suggest that the experimentally observed radical is indeed the decarboxylated secondary radical cation. However, a more precise determination of the conformation is not possible. Explicit consideration of hydrogen bonding and other interactions between the radical cation and the crystalline environment is necessary for a more precise assignment. This is, however, beyond the scope of the methods employed in this study.

Radicals Formed upon Hydrogen Abstraction from the C_2 or C_3 Position. The experimental observation of a single hydrogen HFCC of −54.1 MHz was assigned to the zwitterion of the radical formed upon hydrogen abstraction from the C_2 position: $R3^Z$.[2] In the present study, the optimized structure of $R3^Z$ was obtained using the Onsager model, with an estimated



R3^Z R3^N-I

R3^N-II R3^N-III

R3^N-IV R3^N-V

R3^N-VI R3^N-VII

R3^N-VIII

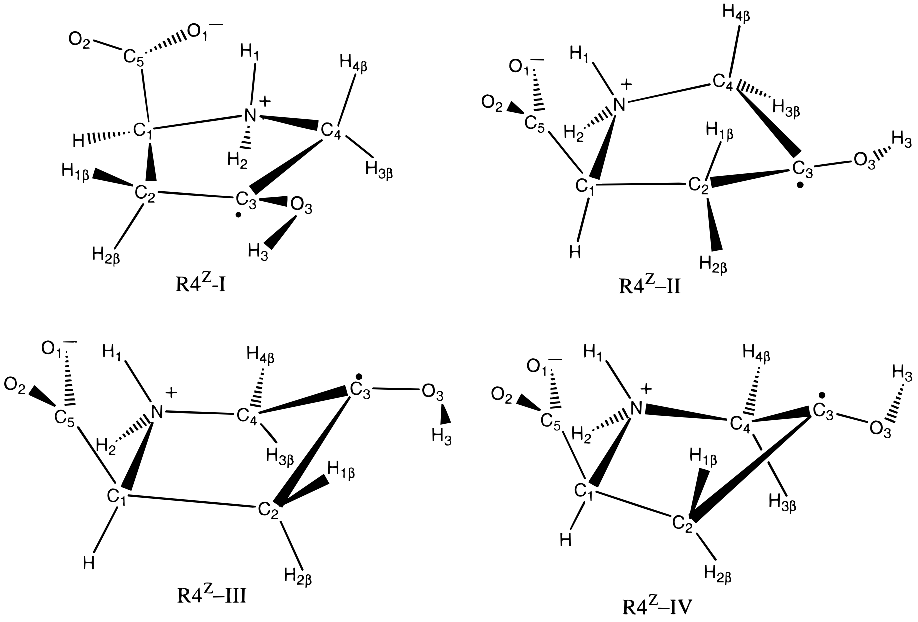
Figure 4. Optimized structures of R3^Z and R3^N.

radius of 4.19 Å, and is illustrated schematically in Figure 4. R3^Z contains a strong intramolecular $O_1 \cdots H_4{-}N$ hydrogen bond ($r_{O \cdots H}$ = 1.762 Å). The $-C_3HOH-$ moiety forms the out-of-plane apex of the ring, causing the radical center situated at the

TABLE 4: Calculated, PWP86/6-311G(2d,p), and Experimental HFCCs (MHz) of H_α, H_1, and H_2 in $R3^Z$ and $R3^N$ and Relative Energies (kJ mol^{-1}) of the Conformers of $R3^N$

	H_α				H_1				H_2				
tensor	A_{iso}	T_{xx}	T_{yy}	T_{zz}	A_{iso}	T_{xx}	T_{yy}	T_{zz}	A_{iso}	T_{xx}	T_{yy}	T_{zz}	ΔE^b
$R3^Z$	−48.7	−32.3	−1.1	33.5	106.9	−3.9	−3.1	6.9	13.8	−5.4	−3.2	8.6	
$R3^N$-I	−57.6	−35.9	−0.2	36.0	98.8	−4.7	−3.4	8.1	12.6	−5.5	−3.1	8.6	0.0
$R3^N$-II	−58.0	−36.3	−0.7	37.1	100.6	−4.8	−3.4	8.2	30.2	−5.5	−3.5	9.0	8.8
$R3^N$-III	−58.0	−36.3	−0.2	36.5	82.8	−4.6	−3.3	7.9	19.9	−5.5	−2.9	8.4	14.7
$R3^N$-IV	−58.5	−36.7	−0.6	37.3	81.1	−4.5	−3.5	8.1	18.6	−5.6	−2.9	8.6	15.9
$R3^N$-V	−58.3	−36.7	−0.6	37.4	82.2	−4.7	−3.3	8.0	40.4	−5.5	−3.3	8.9	19.2
$R3^N$-VI	−59.5	−37.2	−1.1	38.3	80.5	−4.7	−3.5	8.2	39.4	−5.6	−3.4	9.0	21.1
$R3^N$-VII	−57.3	−36.1	−0.3	36.4	80.6	−4.3	−3.6	7.9	18.4	−5.4	−2.9	8.3	38.7
$R3^N$-VIII	−57.9	−36.6	−0.6	37.2	80.4	−4.4	−3.5	8.0	39.4	−5.4	−3.3	8.7	42.2
exptl[a]	−54.1	−31.6	0.4	31.4					98				

[a] Reference 2. [b] B3LYP/6-311G(2df,p)//B3LYP/6-31+G(d,p) + ZPVE.



$R4^Z$-I

$R4^Z$–II

$R4^Z$–III

$R4^Z$–IV

Figure 5. Optimized structures of $R4^Z$.

adjacent $-C_2-$ position to become quite pyramidal by approximately 16°.

The calculated and experimentally observed HFCCs of H_α, H_1, and H_2 in $R3^Z$ are given in Table 4. For H_α, the calculated and experimentally observed coupling constants are in close agreement. The calculated isotropic hyperfine couplings of the beta hydrogens H_1 and H_2 are 106.9 and 13.8 MHz, respectively. Experimentally, only one beta hydrogen coupling of approximately 98 MHz was observed and was suggested to arise from H_2, although we note that it is, in fact, close to the HFCC calculated for H_1. It is feasible that the second, considerably smaller, beta hydrogen coupling was too small to be observed. These results appear to support the assignment of the zwitterionic radical $R3^Z$ as the radical observed; however, a reversal of the assignment of the beta hydrogen HFFCs is suggested.

In addition, the neutral nonzwitterionic isomer of $R3^Z$, hereafter denoted as $R3^N$, was also investigated. Eight conformers of $R3^N$ were obtained and are shown schematically in Figure 4. The major differences between each of the conformers are in the orientations of the carboxylic group and the hydroxyl group at the C_3 position.

The calculated HFCCs of the eight conformers of $R3^N$ are shown in Table 4. The value of H_α is similar for all eight conformers. It can be clearly seen that the calculated H_1 and H_2 HFCCs of $R3^N$-III through $R3^N$-VIII differ significantly from the experimentally observed values. Although the H_1 HFCC of $R3^N$-II is close to the experimentally observed value of 98.8 MHz, the H_2 HFCC of 30.2 MHz is too large to suggest that it may not have been experimentally observed. For $R3^N$-I, however, the H_1 HFCC is in close agreement with the beta coupling observed experimentally, while the H_2 HFCC is quite small. Indeed, the calculated isotropic HFCCs of $R3^N$-I are in closer agreement with the experimentally observed values than those of $R3^Z$. Thus, it is not possible to exclude $R3^N$-I as the observed radical, although we note that the H_α anisotropic components of $R3^Z$ are in closer agreement with the experimental values than those of $R3^N$-I.

Experimentally,[2] a radical was observed that possessed four beta hydrogen couplings. These were proposed as arising from a radical in which hydrogen abstraction from the C_4 position had occurred. In the present study, four possible conformers of the zwitterionic isomer of such a radical were found ($R4^Z$-I through $R4^Z$-IV) and are shown schematically in Figure 5. The structures were obtained using the Onsager model with an estimated radius of 4.30 Å. All four conformers contain a strong intramolecular $O_1 \cdots H_1-N$ hydrogen bond ($r_{O\cdots H}$ < 1.686 Å) and a radical center that is quite pyramidal by approximately 18°. However, in $R4^Z$-I, the $-C_4-$ atom forms the apex of the five-membered ring, whereas in all other conformers, the $-C_3-$ radical center forms the apex. In $R4^Z$-II, the $-C_3-$ radical center can be considered to be trans to the carboxyl group, whereas it can be considered to be cis in $R4^Z$-III and $R4^Z$-IV.

TABLE 5: Calculated, PWP86/6-311G(2d,p), and Experimental HFCCs (MHz) of $R4^Z$

tensor	HFCC	$R4^Z$-I	$R4^Z$-II	$R4^Z$-III	$R4^Z$-IV	exptl[a]
$H_{1\beta}$	A_{iso}	89.0	98.1	49.8	54.3	89.8
	T_{xx}	−5.0	−4.2	−5.2	−5.0	−4.1
	T_{yy}	−2.1	−2.6	−4.1	−4.2	−2.5
	T_{zz}	7.0	6.8	9.2	9.3	6.5
$H_{2\beta}$	A_{iso}	62.4	49.9	94.3	92.8	85.6
	T_{xx}	−5.6	−4.9	−4.2	−4.2	−5.1
	T_{yy}	−4.4	−4.2	−3.1	−2.9	−4.6
	T_{zz}	10.1	9.2	7.4	7.1	9.9
$H_{3\beta}$	A_{iso}	53.2	51.7	84.1	89.4	62.5
	T_{xx}	−5.1	−5.5	−4.2	−4.4	−4.4
	T_{yy}	−4.0	−4.0	−3.5	−3.4	−3.2
	T_{zz}	9.1	9.5	7.7	7.8	11.3
$H_{4\beta}$	A_{iso}	95.3	87.7	53.3	47.0	101.4
	T_{xx}	−4.2	−4.6	−5.1	−5.4	−4.6
	T_{yy}	−2.9	−3.0	−4.0	−3.8	−1.6
	T_{zz}	7.2	7.6	9.1	9.2	

[a] Reference 2.

The calculated HFCCs of the four beta hydrogens of the four conformers of $R4^Z$ are listed in Table 5. The HFCCs for the four conformers differ significantly, with ranges of 48.3, 44.4, 37.7, and 48.3 MHz for $H_{1\beta}$, $H_{2\beta}$, $H_{3\beta}$, and $H_{4\beta}$, respectively. This is due to the quite different orientations of the $-C_3^{\bullet}OH-$ moiety. The calculated HFCCs of $R4^Z$-I are in reasonable agreement with experiment, although the HFCC of $H_{2\beta}$ is still considerably lower than that observed experimentally. This difference is most likely due to the effects of the crystalline environment on the structure of the radical. A more detailed and explicit consideration of the effects of the environment, however, is beyond the scope of the methods employed in this present study.

Secondary Radicals Formed upon Cleavage of $C_\alpha-N$ (Deamination): R5. Hyperfine couplings of two radicals have been observed experimentally and have been assigned to different conformations of the radical formed upon deamination, R5. One conformation was observed at 77 K,[4] denoted as exptl I, whereas the second conformer was observed at 125 K,[4] denoted as exptl II. For temperatures intermediate between 77 and 125 K, a mixture of conformations was observed.[2] It was proposed that the conformational difference between exptl I and exptl II may simply be due to protonation of the carboxyl group $-COO^-$. However, no couplings of a carboxylic proton for the two conformations were observed. Thus, it was unclear whether R5 was in its anionic ($R5^A$) or neutral protonated ($R5^N$) form.

Two conformers, $R5^A$-I and $R5^A$-II, of the anionic form of R5 were obtained and are shown schematically in Figure 6. Both contain a strong intramolecular $O_1\cdots H_3-N$ hydrogen bond ($r_{O\cdots H}$ = 2.151 and 2.240 Å, respectively). The corresponding calculated HFCCs are listed in Table 6. The considerable differences between the calculated and experimentally observed HFCCs, particularly for H_1, clearly indicate that the observed radicals are not different conformers of the anionic form of R5.

For the neutral protonated form of R5, eight possible conformers ($R5^N$-I through $R5^N$-VIII) were obtained and are shown schematically in Figure 6. The conformers can be divided into two groups depending on whether they contain an intramolecular hydrogen bond between the carboxylic group and the $-NH_2$ moiety. The conformers $R5^N$-I, $R5^N$-II, $R5^N$-III, $R5^N$-V, and $R5^N$-VI, hereafter referred to as group I, contain a slightly shortened hydrogen bond between O_2 of the carboxylic group and H_4 of the $-NH_2$ moiety ($2.316 < r_{O\cdots H} < 2.431$ Å). The conformers $R5^N$-IV, $R5^N$-VII, and $R5^N$-VIII, hereafter referred to as group II, contain no such hydrogen bond ($r_{O\cdots H} > 3.661$ Å). Consequently, the structures of group II conformers are more open than those of group I.

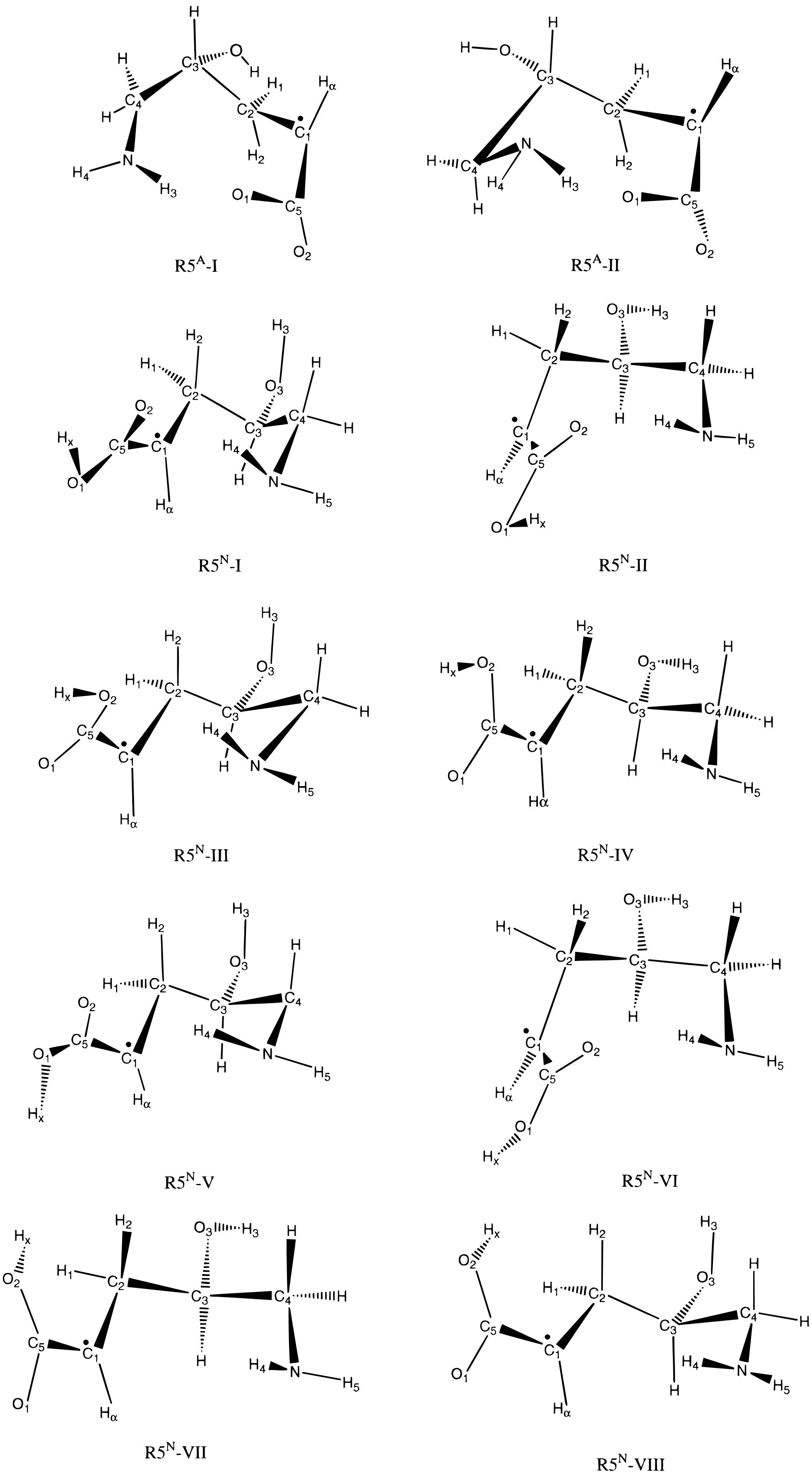
As can be seen in Table 6, these differences in the structures are also reflected in the calculated HFCCs. For the conformers in group I, the calculated H_α, H_1, and H_2 HFCCs are all grouped in the approximate ranges of −46 to −49, 70−80, and 2−4 MHz, respectively. These coupling constants are in close agreement with those experimentally observed at 77 K, i.e., exptl I. The small calculated coupling constants of H_2 support the experimental postulation that the second beta-hydrogen coupling may have been too small to have been observed. The calculated HFCCs of the group I conformers are very similar to each other. Thus, we are unable to determine if exptl I is due to one conformer or a mixture of the group I conformers. For conformers in group II, the H_α, H_1, and H_2 HFCCs are all grouped in the approximate ranges of −50 to −53, 130−140 and 21−34 MHz, respectively. These coupling constants are in good agreement with the conformer observed at 125 K, i.e., exptl II, with those of $R5^N$-VIII in closest agreement. These results suggest that the major difference between the two experimentally observed conformers is the presence of intramolecular hydrogen bonding at lower temperatures.

Conclusions

The structures and HFCCs of possible experimentally observed hydroxyproline-derived radicals have been investigated using density functional theory. The calculated HFCCs have been compared with experimental values.

For the radical anion of hydroxyproline ($R1^A$), the zwitterionic isomer ($R1^Z$) was obtained using the Onsager model. The calculated isotropic H_β HFCC of $R1^Z$ differs significantly from the two experimentally observed values that were previously assigned to different conformations of $R1^Z$. Thus, it is unlikely that either of the conformations observed is the zwitterion $R1^Z$. Four possible conformations of the nonzwitterionic form of the radical were also found. Of these, the isotropic and anisotropic H_β HFCCs of two possible conformations, $R1^A$-II and $R1^A$-III, are found to be in good agreement with one of the experimentally observed radicals. The second H_β HFCC observed experimentally is found to agree closely with the H_β HFCCs calculated for four possible conformations of the protonated derivative of the radical anion, $R1^P$, in which the carboxylic group has been protonated. On the basis of these results, the two radicals observed, and previously assigned as different conformations of the zwitterion of the primary radical anion, are concluded to be a nonzwitterionic conformer of the primary radical anion and a derivative in which the carboxylic group has been protonated.

Four possible conformers of the secondary radical cation ($R2^C$) formed by decarboxylation of hydroxyproline were obtained. The calculated HFCCs of the four conformers support the experimental assignment; however, none completely accounts for the experimentally observed values. This is most likely due to interactions between the radical and the crystalline environment, i.e., intermolecular hydrogen-bond formation and crystal-packing effects, affecting the experimental values. The exact effect on the HFCCs is unclear. Thus, a precise determination of the conformation of the observed radical is not possible without the inclusion of specific interactions between the radical and the crystalline environment. This is not feasible for the methods employed in this present study.



R5^A-I R5^A-II

R5^N-I R5^N-II

R5^N-III R5^N-IV

R5^N-V R5^N-VI

R5^N-VII R5^N-VIII

Figure 6. Optimized structures of R5^A and R5^N.

Several possible conformers for radical zwitterions formed by hydrogen abstraction from the C_2 or C_3 position of the five-membered ring were obtained. The calculated HFCCs for the zwitterionic radical formed by hydrogen abstraction from the

TABLE 6: Calculated, PWP86/6-311G(2d,p), and Experimental HFCCs (MHz) of R5$^{\mathrm{A}}$ and R5$^{\mathrm{N}}$ and Relative Energies (kJ mol^{-1}) of the Conformers of R5$^{\mathrm{N}}$

	H$_\alpha$				H$_1$				H$_2$				
system	A_{iso}	T_{xx}	T_{yy}	T_{zz}	A_{iso}	T_{xx}	T_{yy}	T_{zz}	A_{iso}	T_{xx}	T_{yy}	T_{zz}	ΔE^b
R5$^{\mathrm{A}}$-I	−44.2	−32.5	−1.3	33.8	57.8	−4.2	−4.0	8.2	9.1	−4.4	−4.0	8.5	0.0
R5$^{\mathrm{A}}$-II	−40.9	−32.2	−1.3	33.5	60.1	−4.2	−4.0	8.2	8.8	−4.3	−3.8	8.1	7.4
R5$^{\mathrm{N}}$-I	−47.5	−29.8	−1.9	31.6	72.4	−4.4	−3.2	7.6	3.7	−3.7	−2.8	6.5	0.0
R5$^{\mathrm{N}}$-II	−49.0	−30.6	−1.9	32.6	79.1	−4.6	−3.2	7.8	3.3	−4.0	−2.8	6.8	2.1
R5$^{\mathrm{N}}$-III	−47.0	−28.9	−2.3	31.2	78.3	−4.4	−3.2	7.6	2.5	−3.4	−3.3	6.7	7.2
R5$^{\mathrm{N}}$-IV	−50.9	−30.5	−2.2	32.7	139.5	−5.4	−2.5	7.9	22.2	−3.9	−3.2	7.1	10.7
R5$^{\mathrm{N}}$-V	−46.3	−29.8	−1.5	31.3	71.6	−4.4	−3.1	7.5	3.5	−3.7	−2.7	6.4	19.6
R5$^{\mathrm{N}}$-VI	−47.5	−30.6	−1.6	32.2	80.2	−4.6	−3.1	7.7	2.9	−3.8	−2.8	6.7	22.2
R5$^{\mathrm{N}}$-VII	−52.8	−30.8	−1.9	32.7	136.8	−5.2	−2.5	7.7	34.1	−3.9	−3.3	7.3	34.8
R5$^{\mathrm{N}}$-VIII	−50.5	−30.4	−2.5	32.9	130.6	−5.1	−2.6	7.7	21.4	−3.7	−3.3	7.1	37.7
exptl I^a	−57.2	−32.4	1.9	30.6	78.0	−4.8	−3.4	8.3					
exptl II^a	−56.5	−32.4	1.3	31.1	123.1	−5.1	−2.2	7.4	21.4	−4.3	−3.2	7.6	

[a] Reference 4. [b] B3LYP/6-311G(2df,p)//B3LYP/6-31+G(d,p) + ZPVE

C$_2$ position, R3$^{\mathrm{Z}}$, are in good agreement with the experimental values. However, we also find that a neutral nonzwitterionic conformer, R4$^{\mathrm{N}}$-I, also gives HFCCs in close agreement with the experimental values. Thus, we are unable to determine whether the radical observed is the zwitterion. Four conformers, R4$^{\mathrm{Z}}$-I, R4$^{\mathrm{Z}}$-II, R4$^{\mathrm{Z}}$-III, and R4$^{\mathrm{Z}}$-IV, for the radical zwitterion formed by hydrogen abstraction from the C$_3$ position were obtained. The HFCCs of all conformers are found to be dependent on the orientation of the $-\mathrm{C_3^{\bullet}OH}-$ moiety. The calculated HFCCs of R4$^{\mathrm{Z}}$-I are in closest agreement with experiment. Thus, it is concluded that the radical observed is R4$^{\mathrm{Z}}$, as previously assigned, and that it possesses a conformation similar to that of R4$^{\mathrm{Z}}$-I.

Two possible conformations of the radical anion formed on deamination were obtained. The calculated HFCCs of both conformers are quite similar; however, they differ significantly from the experimentally observed values. Eight possible conformations of the neutral radical, R5$^{\mathrm{N}}$, formed on protonation of the carboxyl group of the radical anion, were obtained. The conformers can be divided into two groups: those that contain an intramolecular hydrogen bond between the carboxylic group and the amino group and those that do not. The calculated HFCCs of those conformers containing such an intramolecular hydrogen bond (R5$^{\mathrm{N}}$-I, R5$^{\mathrm{N}}$-II, R5$^{\mathrm{N}}$-III, R5$^{\mathrm{N}}$-V, and R5$^{\mathrm{N}}$-VI) are in reasonable agreement with each other and are in good agreement with the experimental values observed at 77 K. The calculated HFCCs of those conformers not containing such an intramolecular hydrogen bond (R5$^{\mathrm{N}}$-IV, R5$^{\mathrm{N}}$-VII, and R5$^{\mathrm{N}}$-VIII) are also similar to each other, but differ significantly from those of the conformers that do contain such a bond. However, they are in close agreement with the experimental values observed at 125 K, with those of conformer R5$^{\mathrm{N}}$-VIII in closest agreement. On the basis of these assignments, the change in HFCCs observed on going from 77 to 125 K is due to the breaking of the intramolecular carboxyl−amino hydrogen bond resulting in a more open structure.

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Supporting Information Available: Optimized B3LYP/6-31+G(d,p) geometries in Cartesian coordinates and total energies for all radicals in the present study (Table S1) are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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