Paramagnetic Resonance Study of Liquids during Photolysis. XI. Citric Acid and Sodium Citrate in Aqueous Solution[1,2]

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Abstract: Paramagnetic resonance spectra of radicals present during photolysis of aqueous solutions of citric acid and sodium citrate were studied. In citric acid solution the radicals $(HO_2CCH_2)_2\dot{C}OH$, $HO_2CCH_2C(OH)(CO_2H)\dot{C}HCO_2H$, and $HO_2CCH_2C(OH)(CO_2H)\dot{C}H_2$ (I, II, and III) were identified. I and III result from the loss respectively of the central and an end carboxyl group of excited citric acid. II results from loss of a methylene H atom, probably to a reactive radical. II was prepared at much higher steady-state concentration by adding H_2O_2 to the solution. In sodium citrate solutions only the triply ionized form of II was found, and it too was prepared at much higher concentration by adding H_2O_2. Some of the radicals were also studied in D_2O solutions in order to properly assign hyperfine couplings.

We have been studying electron paramagnetic resonance (epr) spectra of short-lived radicals present during the photolysis of liquids.[2] Radicals have been formed from many molecules by loss of hydrogen to photolytically excited acetone or to $\cdot OH$ produced by photolysis of H_2O_2. In continuing this work we investigated a number of hydroxy acids and found that radicals could be seen during photolysis without the addition of radical initiators such as H_2O_2 or acetone. We here report epr spectra of radicals found during uv irradiation of aqueous solutions of citric acid and sodium citrate near room temperature. By adding H_2O_2 two of the radicals were prepared at much higher concentration, and this made possible a much more detailed study of their spectra.

Experimental Section

The experimental arrangement and methods for making measurements and calculating g values and hyperfine couplings have been described.[3] The estimated error limits are ±0.00004 for g and ±0.03 G for the couplings unless indicated differently. The microwave spectrometer operated at about 9.5 GHz and used 100-kHz field modulation. Photolysis took place in a flat silica cell in the microwave cavity as the solution flowed through it at a flow rate in the range 0.1–1 ml/min. The inside cross section of the cell was 0.4 mm thick (optical path length) × 9 mm wide. The solutions were freed of molecular oxygen by purging with helium or argon. Temperatures are given for the solutions shortly after leaving the cavity. The citric acid and sodium citrate were reagent grade. The H_2O_2 was 98% and supplied by FMC Corp.

Results and Discussion

Citric Acid. Figure 1 shows the spectrum for a 3.6 *M* citric acid solution. Three radicals are labeled by the stick spectra in the figure. Radical I has hyperfine splittings from four equivalent protons and one other proton which give a 1:4:6:4:1 pattern of doublets. It is identified as $(HO_2CCH_2)_2\dot{C}OH$ formed by the loss of the central carboxyl group of excited citric acid. The four equivalent protons are the methylene protons and the unique proton is from the hydroxyl group. The relative intensities of the lines are anomalous in that the high-field lines are stronger than the corresponding low-field lines. This kind of anomaly has been seen frequently[4] in photolytically produced short-lived radicals. Consistent with the assignment and as expected for a hydroxyl proton, its splitting vanished upon adding strong mineral acid. This is caused by rapid acid-catalyzed exchange.[5] Radical II shows a large splitting from a single proton in Figure 1. This radical was prepared at much higher concentrations (see later) in solutions containing H_2O_2, and very small splittings from three additional protons were then resolved. This radical is $HO_2CH_2C(OH)(CO_2H)\dot{C}HCOOH$, made by abstraction of H from citric acid, probably by a reactive radical. The large splitting in Figure 1 is from the $\cdot CH$ proton. Radical III has a large 1:2:1 splitting from two equivalent protons and further line structure probably arising from a single proton. This radical is identified as $HO_2CCH_2C(OH)(CO_2H)\dot{C}H_2$ made by loss of an end carboxyl group from excited citric acid. The large 1:2:1 splitting arises from equivalent $\cdot CH_2$ protons. It is not known whether the unresolved splitting is due to one of the two protons in the position γ to the carbon with the unpaired electron or to the hydroxyl proton. Parameters of the radicals measured for this solution are given in Table I.

The g value for I, 2.00314, is typical of values for radicals derived from alcohols by loss of a hydrogen atom from the α carbon. A good radical for comparison is $(CH_3)_2\dot{C}OH$ whose carbon skeleton near the unpaired electron is similar and which has the value[5b] 2.00313 in aqueous solutions. The four β protons of I are equivalent. The β coupling constant of 14.52 G is small compared to that expected for unhindered rotation about the bond C_α–C_β. The β proton coupling may be estimated from the relation[6]

$$a_{H\beta} = (B_{H\beta} \cos^2 \theta)\rho \qquad (1)$$

where $a_{H\beta}$ is the coupling for the β proton, ρ is the electron spin density for the unpaired electron in the $2p_z$ orbital of the α carbon, and θ is the angle of rotation about the C_α–C_β bond which would bring the C_β–H

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[1] Research sponsored by the U. S. Atomic Energy Commission under contract with Union Carbide Corp.

[2] The preceding paper of this series is R. Livingston, J. K. Dohrmann, and H. Zeldes, *J. Chem. Phys.*, 53, 2448 (1970).

[3] (a) R. Livingston and H. Zeldes, *ibid.*, 44, 1245 (1966); (b) *ibid.*, 47, 4173 (1967).

[4] (a) R. Livingston and H. Zeldes, *ibid.*, **53**, 1406 (1970); (b) H. Zeldes and R. Livingston, *J. Phys. Chem.*, 74, 3336 (1970).

[5] (a) H. Fischer, *Mol. Phys.*, **9**, 149 (1965); (b) H. Zeldes and R. Livingston, *J. Chem. Phys.*, 45, 1946 (1966).

[6] (a) C. Heller and H. M. McConnell, *ibid.*, 32, 1535 (1960); (b) E. W. Stone and A. H. Maki, *ibid.*, 37, 1326 (1962).

Table I. Parameters of Radicals[a]

Solution (temp, °C)	Radical	g	Proton couplings
3.6 M citric acid (31)	I, $(HO_2CCH_2)_2\dot{C}OH$	2.00314	$a_\beta{}^4 = 14.52$, $a_{OH}{}^1 = 0.63$
	III, $HO_2CCH_2C(OH)(CO_2H)\dot{C}H_2$	2.00266 ±0.00015	$a_\alpha{}^2 = 22.3 \pm 0.2$
	II, $HO_2CCH_2C(OH)(CO_2H)\dot{C}HCO_2H$	2.00324	$a_\alpha{}^1 = 20.90$
1.2 M citric acid, 0.4 M H_2O_2 in D_2O (34)	II, $DO_2CCH_2C(OD)(CO_2D)\dot{C}HCO_2D$	2.00319	$a_\alpha{}^1 = 20.92$, $a_\gamma{}^2 = 0.30$
1.2 M citric acid, 0.4 M H_2O_2 (35)	II, $HO_2CCH_2C(OH)(CO_2H)\dot{C}HCO_2H$	2.00320	$a_\alpha{}^1 = 20.88$, $a_\gamma{}^2 = 0.30$,[b] $a_{OH}{}^1 = 0.32 \pm 0.06$
1.7 M sodium citrate (30)	II^{3-}, $^-O_2CCH_2C(OH)(CO_2{}^-)\dot{C}HCO_2{}^-$	2.00325	$a_\alpha{}^1 = 20.32$, $a_{\gamma 1}{}^1 = 0.55$, $a_{\gamma 2}{}^1 < 0.15$, $a_{OH}{}^1 = 0.27$
1.6 M sodium citrate, 0.6 M H_2O_2 (38)	II^{3-}, $^-O_2CCH_2C(OH)(CO_2{}^-)\dot{C}HCO_2{}^-$	2.00325	$a_\alpha{}^1 = 20.30$, $a_{\gamma 1}{}^1 = 0.55$, $a_{\gamma 2}{}^1 = 0.11$, $a_{OH}{}^1 = 0.27$
1.7 M sodium citrate, 0.7 M H_2O_2 in D_2O (38)	II^{3-}, $^-O_2CCH_2C(OD)(CO_2{}^-)\dot{C}HCO_2{}^-$		$a_\alpha{}^1 \approx 20$, $a_{\gamma 1}{}^1 = 0.53$

[a] Where $a_i{}^n$ is the coupling (G) for n protons of kind i. [b] The value of $a_\gamma{}^2$ was taken from the D_2O solution.

bond in eclipse with the z axis. The value of $B_{H\beta}\langle\cos^2\theta\rangle$ for a freely rotating methyl group is $B_{H\beta}/2$, and we use the value of 29.3 G determined by Fessenden and Schuler,[7] which gives the value 58.6 G for $B_{H\beta}$. To evaluate ρ we use Fischer's[8] relation

$$\rho = \prod_{i=1}^{3}[1 - \Delta(X_i)] \qquad (2)$$

where $\Delta(X_i)$ is an empirical parameter depending upon the substituent X_i on the α carbon. It is assumed that each substituent other than H decreases the spin density in proportion to the factor $[1 - \Delta(X_i)]$. Equation 2 is an extension of a similar relation used by Fessenden and Schuler[7] for a series of simple alkyl radicals. For $\Delta(OH)$ we use the value[9] 0.160, and for $\Delta(CH_2COOH)$ the value[8] 0.080. These give the value 0.711 for ρ. If there were unhindered rotation about the C_α–C_β bond, then $a_{H\beta}$ would have a value close to 58.6 × 0.5 × 0.711, or 20.8 G, rather than the observed value of 14.52 G. We interpret the value for the coupling of the β protons and their equivalence with a model given by Fischer.[8] The time dependence of θ for each proton is approximated by assuming that the radical is most of the time in one of the two conformations, a and b of Figure 2. The substituents X_1 and X_2 are on the α carbon, and the substituents on the β carbon are X_3 and the two β protons labeled $H_{\beta 1}$ and $H_{\beta 2}$. The substituents X_1, X_2, and X_3 for I are OH, HO_2CCH_2, and CO_2H, respectively. Conformations a and b are mirror related, and they may be interconverted by proper rotations about single bonds. This is possible only because no asymmetric atoms are present in the molecule. Since the conformations are energetically equivalent, they must be equally populated. Interconversion, however, interchanges the roles of $H_{\beta 1}$ and $H_{\beta 2}$; their couplings interchange. The amplitude of oscillation about the axis C_α–C_β is assumed to be small, but it is assumed that the frequency with which rotations occur which convert one conformation to the other is fast compared to the difference in coupling constants that the two β protons would have in a static conformation. Under these conditions the protons appear equivalent, and each proton exhibits a coupling given by the average of couplings for the two static conformations

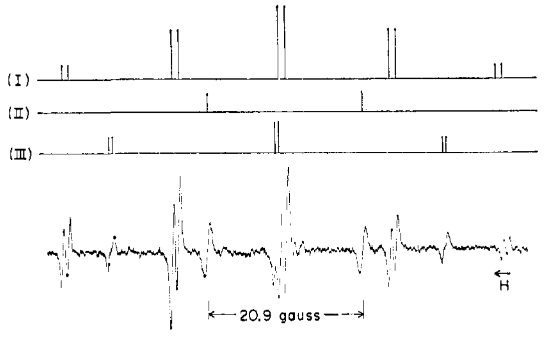

Figure 1. Spectrum for 3.6 M citric acid at 39°. Stick spectra I, II, and III, respectively, locate lines of $(HO_2CCH_2)_2\dot{C}OH$, $HO_2CCH_2C(OH)(CO_2H)\dot{C}HCO_2H$, and $HO_2CCH_2C(OH)(CO_2H)\dot{C}H_2$.

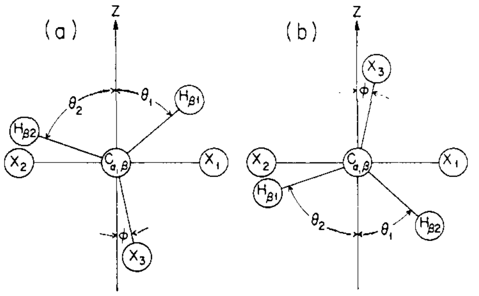

Figure 2. Equivalent rotational conformations of $X_3CH_2\dot{C}X_1X_2$. The direction from C_α to C_β is perpendicular to the page. The z axis of the $2p_z$ orbital is in the plane of the page.

$$\begin{aligned} a_{H\beta} &= {}^1/_2 B_{H\beta}(\cos^2\theta_1 + \cos^2\theta_2)\rho \\ &= {}^1/_4 B_{H\beta}(2 - \cos 2\phi)\rho \end{aligned} \qquad (3)$$

With this relation we compute the value 26° for ϕ. The values for θ_1 and θ_2 are then given by 60° ± ϕ or 34 and 86°. Each pair of β protons of I has the same value for ϕ, since all four protons are equivalent, but there is ambiguity in the sense of rotation of ϕ.

The parameters for III (Table I) are consistent with the structure of the radical. The g value of 2.00266 ± 0.00015 is close to the value[7] 2.00260 for the alkyl radical $CH_3\dot{C}H_2$. Substitution of either OH or CO_2H in the α position of an alkyl radical increases the g value appre-

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[7] R. E. Fessenden and R. H. Schuler, *J. Chem. Phys.*, **39**, 2147 (1963).

[8] H. Fischer, *Z. Naturforsch. A*, **19**, 866 (1964).

[9] H. Fischer, *ibid.*, **20**, 428 (1965).

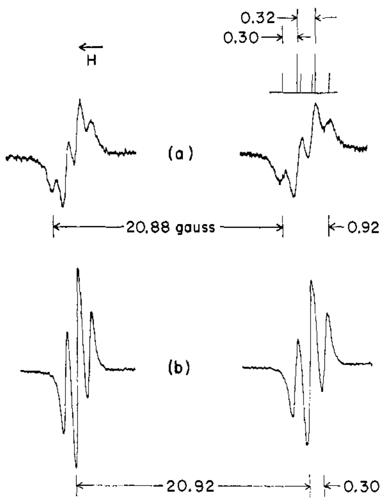

Figure 3. Spectra for solutions which contain both citric acid and H_2O_2: (a) $HO_2CCH_2C(OH)(CO_2H)\dot{C}HCO_2H$ in 1.2 *M* citric acid and 0.4 *M* H_2O_2 at 35°, (b) $DO_2CCH_2C(OD)(CO_2D)\dot{C}HCO_2D$ in 1.2 *M* citric acid and 0.4 *M* H_2O_2 in D_2O at 34°.

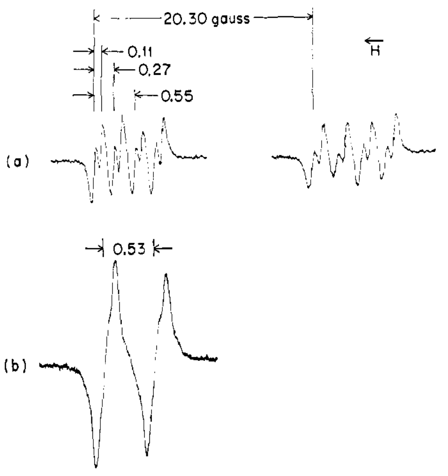

Figure 4. Spectra for solutions which contain both sodium citrate and H_2O_2: (a) $^-O_2CCH_2C(OH)(CO_2^-)\dot{C}HCO_2^-$ in 1.6 *M* sodium citrate and 0.6 *M* H_2O_2 at 38°; (b) high-field lines of $^-O_2CCH_2C(OD)(CO_2^-)\dot{C}HCO_2^-$ in 1.7 *M* sodium citrate, 0.7 *M* H_2O_2 in D_2O at 38°.

ciably. Values for $CH_3\dot{C}HOH$ and $CH_3\dot{C}HCO_2H$ are 2.00315[5b] and 2.00327,[10] respectively. These *g* values indicate that the two equivalent protons of III are in the α rather than the β position. The proton coupling of (22.3 ± 0.2) G for the two α protons is reasonable when compared with the values 22.38,[7] 22.00,[3a] and 22.4[11] for the related radicals $\cdot CH_2CH_3$, $\cdot CH_2CH_2OH$, and $\cdot CH_2CH_2CO_2H$, respectively.

Radical II, with *g* value of 2.00324 and α proton coupling of 20.90 G, may be compared favorably with the similar radicals $CH_3\dot{C}HCO_2H$ and $HO_2CCH_2\dot{C}HCO_2H$. For $CH_3\dot{C}HCO_2H$ the corresponding parameters[10] in aqueous solution are 2.00327 and 20.25 G. For $HO_2CCH_2\dot{C}HCO_2H$ the α proton coupling is 20.87 G.[12]

Radical II was made at a much higher steady-state concentration by adding H_2O_2 to the citric acid solutions. Photolysis of H_2O_2 produced the OH radical which quickly abstracted a methylene hydrogen of citric acid to form the radical. The higher concentration made it possible to analyze additional hyperfine structure, Figure 3a, which, however, was not completely resolved. A similar solution prepared with D_2O rather than H_2O and containing six D atoms for every exchangeable H atom was also examined under high-resolution conditions; Figure 3b. The latter spectrum shows resolved structure from two equivalent protons with hyperfine coupling of 0.30 G which can only be from the γ protons, as these are the only nonexchangeable protons in the radical other than the α proton. Using these γ proton splittings it was then possible to analyze the partially resolved structure of Figure 3a. Only one additional proton splitting is reasonable, and this is from the OH proton. To our knowledge, a splitting from a CO_2H proton has never been observed, and, moreover, rapid proton-transfer reactions in this solution would eliminate this kind of splitting. The stick spectrum in Figure 3a shows this analysis; the OH proton splitting is 0.32 G. The couplings and *g* values for II in these solutions are given in Table I. It is interesting that there is a detectable proton splitting from the OH group on the β carbon. This is similar to the finding[3a] of an 0.316-G proton coupling for the OH group on the β carbon of $\cdot CH(OH)CH_2OH$.

The above measurements were made for 3.6 and 1.2 *M* citric acid solutions. It is very likely that the carboxylic radicals studied in these solutions were largely undissociated because of the high concentration of citric acid and because these radical acids are not expected to be a good deal stronger than citric acid (p*K* = 3.08). For instance, pK_1 for the radical $HO_2CCH_2\dot{C}HCO_2H$ was determined[12] to be 3.35, whereas pK_1 for $HO_2CCH_2CH_2CO_2H$ is 3.75. The radical is the stronger acid by 0.4 p*K* unit. If we assume that the radicals made from citric acid are stronger acids by 0.4 p*K* unit, then in 3.6 and 1.2 *M* citric acid 4 and 7%, respectively, of the molecules are singly ionized. Since a very strong spectrum of II was present in solutions containing H_2O_2, hydrochloric acid was added to see if this affected the shape of the two partly resolved groups of lines. Unfortunately, the presence of 0.1 *N* HCl very nearly caused the spectrum to disappear. Radical II was also observed in solutions containing H_2O_2, where the citric acid concentration ranged from 0.6 to 2.4 *M*. There was not much change in the shape of the partly resolved lines. This gave added assurance that the spectrum arose mainly from neutral radicals.

Sodium Citrate. Photolysis of 1.7 *M* sodium citrate (no added H_2O_2) produced a weak spectrum from a single radical, II^{3-}, which showed splittings from one large proton coupling and three small inequivalent proton couplings. Measured parameters are in Table I. This radical is identified to be $^-O_2CCH_2C(OH)(CO_2^-)\dot{C}HCO_2^-$, the triply ionized derivative of II. It was probably produced from citrate ion by the loss of H to a reactive radical. As in the case of II a very much higher steady-state concentration of II^{3-} was obtained by the addition of H_2O_2. The spectrum is shown in Figure 4a, and measurements are in Table I. The α proton coupling of II is 20.88 G and that for II^{3-} is 20.30 G. These values are remarkably similar to those of the radical $HO_2C\dot{C}HCH_2CO_2H$, its once ionized, and its twice ionized derivatives, which have the values[12]

(10) R. Livingston and H. Zeldes, unpublished results.

(11) W. T. Dixon, R. O. C. Norman, and A. L. Buley, *J. Chem. Soc.*, 3625 (1964).

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20.87, 20.55, and 20.41 G, respectively. The close similarity may be fortuitous, as our measurements were made for two rather different solutions, and there may be some effect of the medium. The decrease in coupling with increasing ionization has been explained[12] by the increasing delocalization of the unpaired electron into the carboxylate group. Radical II^{3-} has been identified[13] in γ-irradiated single crystals of sodium citrate pentahydrate, and the isotropic α-hydrogen coupling which results from the reported principal values is 60.7 MHz. Our value in the same units is 56.9 MHz. The difference due to media seems rather large for an α hydrogen.

The three small proton couplings of II^{3-} must arise from the two γ protons and the OH proton. In order to make the proper assignments, a solution was made with D_2O in place of H_2O. The ratio of D atoms to exchangeable H atoms in this solution was 8.9. The high-field group of lines for this solution is shown in Figure 4b. Only the largest of the three small splittings can be seen, but the lines are broad enough that the smallest splitting (0.11 G) would not give resolved lines. Clearly the H atom giving the intermediate coupling of 0.27 G was replaced by D which caused pairs of lines with a splitting of 0.27 G to be replaced by triplets with a line to line spacing of 0.04 G. These triplets were not resolved, but they introduced enough width that the 0.11-G splitting was also not resolved. The OH proton is the exchangeable proton and has the coupling of 0.27 G.

The two γ protons have strikingly different couplings of 0.55 and 0.11 G. This is not surprising in II^{3-} where the energy barrier between conformations is expected to be large (larger than in II). It is interesting to compare with findings[7] for $C_2H_5\dot{C}H_2$ in liquid propane. At temperatures below −145° the γ protons become nonequivalent showing that the γ coupling is strongly dependent upon orientation of the methyl group.

It is probable that most of the molecules of II^{3-} are not protonated in the measured solutions, otherwise pK_3 for II would have to be more than 3 units larger than pK_3 for citric acid. The epr spectrum of II^{3-} was also observed in solutions containing the same amount of H_2O_2 but progressively less sodium citrate. The concentration of sodium citrate was reduced by a factor of 2 for successive solutions starting with a 1.6 *M* solution. For each solution a high-resolution scan was made for one of the two widely spaced groups of eight lines. After the first dilution, the lines were much better resolved. With further dilution the lines which were separated by the smallest splitting became more poorly resolved. After the fourth dilution, there was no indication of the smallest splitting. It was not possible to tell whether the smallest coupling (0.11 G) was changing in the different solutions or whether there were changes in width or both. However, it was clear that very little change if any occurred in the two larger splittings. After a fifth dilution, the absorption abruptly became weaker by more than a factor of 100. It was also found that the addition of NaOH to a 1.7 *M* sodium citrate solution containing H_2O_2 made it unstable. With 0.05 *M* NaOH present, the spectrum very nearly disappeared, and gas formed in the solution.

[13] D. B. Russell, *J. Chem. Phys.*, **43**, 1969 (1965).

Relaxation Spectra of L-Phenylalanine– and L-Dopa (3,4-Dihydroxyphenylalanine)–Copper(II) Complexes[1]

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Abstract: Temperature-jump experiments of the Cu(II)-phenylalanine and Cu(II)-dopa (amino acid end) systems have been interpreted according to the reaction $CuL_{n-1} + L \rightleftharpoons CuL_n$ (k_n, forward; k_{-n}, reverse), where L is the anionic form of the ligand and charges have been neglected. The results, at 25° and $\mu = 0.1$ *M*, are: phenylalanine, $k_1 = 1.2 \times 10^9\ M^{-1}\ sec^{-1}$, $k_{-1} = 22\ sec^{-1}$, $k_2 = 3 \times 10^8\ M^{-1}\ sec^{-1}$, $k_{-2} = 30\ sec^{-1}$; dopa, $k_1 = 1.1 \times 10^8\ M^{-1}\ sec^{-1}$, $k_{-1} = 8.3\ sec^{-1}$, $k_2 = 4.2 \times 10^7\ M^{-1}\ sec^{-1}$, $k_{-2} = 22\ sec^{-1}$. The dissociative, water-loss mechanism appears to be operative, although the hydroxyl groups of the dopa may have a misorienting effect on this ligand's reactivity.

Transition metal ion α-amino acid complexes are generally formed *via* a mechanism in which the rate-determining step is the loss of a water molecule from the metal ion's inner coordination sphere.[2,3] Ring closure does not appear to be rate determining for certain ligands forming five-membered chelate rings,[4] of which an α-amino acid is an example.

L-β-Phenylalanine forms five-membered chelates with metal ions through the carboxylate and α-amine groups. L-Dopa (3,4-dihydroxyphenylalanine) coordinates to

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[1] The authors gratefully acknowledge partial support from Public Health Service Research Grant No. GM-08893-08 from the National Institute of General Medical Sciences, Public Health Service; from the National Science Foundation for College Teacher Research Participation Grant No. GY3796 (A. K.); and from the Petroleum Research Fund, administered by the American Chemical Society, for Grant No. 2982B (R. F. P.).

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