Asymmetric Photolysis of (*RS*)-Leucine with Circularly Polarized Ultraviolet Light[1]

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Abstract: (*RS*)-Leucine in 0.1 M HCl solution has been subjected to photolysis with 212.8-nm right (R-) and left circularly polarized light (LCPL) obtained from a laser source. RCPL preferentially photolyzed the (*R*)-leucine component and LCPL the (*S*)-leucine component of the *RS* substrate. The enantiomeric excesses produced were 1.98% for the 59% conversion with RCPL and 2.50% for the 75% conversion with LCPL. These "equal and opposite" effects represent the second highest enantiomeric enrichments yet reported for an asymmetric photolysis and the first ever reported for a prebiotically important substrate—an amino acid. Implications regarding the origin of optical activity are briefly discussed.

The question of the primeval origin of optically active molecules in nature has fascinated scientists for well over a century, since certain of such chiral molecules are the basis of life as we know it. Hypotheses proposed since Pasteur's time for the origin of optical activity have recently been reviewed.[3-6] Since many of the historical experiments bearing on such hypotheses have been somewhat ambiguous, we have recently undertaken to reinvestigate some of the abiotic hypotheses previously proposed using modern instrumental techniques.

Before the turn of the century van't Hoff[7] suggested that optically active substances in nature might be formed by the action of right or left circularly polarized light (RCPL or LCPL), which was thought to be present in natural sunlight as a result of both reflections at the earth's surface[8] and the earth's magnetic field.[9,10] These ideas led to a number of unsuccessful experimental attempts to induce optical activity during degradative or synthetic reactions in the presence of CPL.[11] The first successful asymmetric photochemical reaction was that of Kuhn and Braun[12] in 1929, who irradiated ethyl (*RS*)-α-bromopropionate with 280-nm R- or LCPL and achieved maximum rotations of ca. 0.05° after roughly 50% photolysis. In later, more definitive experiments involving the photodestruction of *N,N*-dimethyl-(*RS*)-α-azidopropionamide to the extent of 40%, Kuhn and Knopf[13] reported rotations as high as 0.78 and −1.04° for the unphotolyzed residues, depending upon whether RCPL or LCPL (280–320 nm), respectively, was employed. The rotation of −1.04° observed[13] corresponded to an enantiomeric purity of ca. 0.5% for the residual unphotolyzed amide.[14] Several recent reviews[11,15,16] summarize the successful asymmetric photodegradative and photosynthetic reactions with CPL which have been conducted since Kuhn's experiments.

The most detailed theoretical analysis of asymmetric photochemical reactions, particularly the photodestruction of racemates, has been given recently by Kagan and coworkers.[14,16] These investigators emphasize (as do earlier workers[10,11]) the importance of the anisotropy factor g ($g = \Delta\epsilon/\epsilon$, where $\Delta\epsilon = \epsilon_{LCPL} - \epsilon_{RCPL}$ and $\epsilon = \frac{1}{2}(\epsilon_{LCPL} + \epsilon_{RCPL})$ = molecular absorption coefficient) in determining the enantiomeric purity of the products of such asymmetric photochemical processes. They deduce quantitatively, for example, the enantiomeric purity achievable during photolysis of a racemate as a function of the magnitude of g and the extent of reaction, and show that optical purity approaching 100% can be obtained with a sufficiently large g value and extent of photodestruction. Finally, Kagan et al.[14] have tested their theoretical conclusions by studying the photolysis of racemic camphor with 290–370-nm CPL, finding that, as predicted by their theory, an enantiomeric purity of 20% (the highest ever recorded for a CPL photolysis) was obtainable after ca. 99% photodestruction of the substrate. Our interest in the origin of optical activity[4] has now prompted us to undertake the asymmetric photolysis of a racemic substrate more pertinent from a prebiotic viewpoint, namely, an amino acid—a type of component generally thought to have been produced naturally from the reducing atmosphere of the primitive earth.[17] In doing so we hoped to provide a plausible and realistic experimental model for the abiotic origin of optical acitivity by the CPL mechanism.

Results and Discussion

Our plan was to photolyze a racemic amino acid to a known extent using ultraviolet R- and LCPL of an appropriate wavelength, then to examine the enantiomeric composition of the residual undestroyed amino acid for its enantiomeric ratio by gas chromatography (GC) to detect any enantiomeric excess engendered during photolysis. The advantages of GC over other methods for the determination of enantiomeric excesses have recently been discussed.[18] (*RS*)-Leucine was selected as a suitable substrate, since it has one of the larger g factors among the proteinaceous amino acids,[19] and since the gas chromatographic determination of the enantiomeric composition of (*R*)- and (*S*)-leucine mixtures has been extensively studied.[20]

To establish the most suitable conditions for the asymmetric photolysis of (*RS*)-leucine, we first measured the circular dichroism (CD) of (*R*)- and (*S*)-leucine, as well as λ_{max} and ϵ_{max} for these in 1 N HCl. Our measurements of λ_{max} and ϵ_{max}, respectively, averaged 207 nm and 67.7, while our CD measurements showed an average molar ellipticity maximum (Figure 1) of ca. 5460 at λ_{CD} 211 nm, permitting the calculation[21] of $\Delta\epsilon = 1.65$ and $g = \Delta\epsilon/\epsilon = 0.0244$. This value, fitted to the table of g values, percent degradation, and enantiomeric excess developed by Kagan and coworkers,[14] suggests that an enantiomeric excess of ca. 2% should develop if (*RS*)-leucine is photolyzed to the extent of 80% with R- or LCPL of 211-nm wavelength.

For our photolyses we used R- and LCPL of 212.8 nm (closely approximating the desired 211 nm), which was generated using the 1064-nm coherent infrared radiation from a Nd/YAG laser and a recently demonstrated nonlinear optical process[22] for efficient production of its 212.8-nm fifth harmonic. The 212.8-nm beam generated in this way is linearly polarized, and it was converted to circular polarization by passing it through a LiF Fresnel rhomb. The R- or LCPL was finally directed into dilute solutions of (*RS*)-leucine in 0.1 N HCl contained in a quartz-window reaction vessel, and irradiations were conducted for time periods calculated (by preliminary experiments) to achieve 60–80% total photolysis. Finally, aliquots of each irradiated solution were removed and the residual leucine component in each aliquot was converted

Table I. Photolysis of (*RS*)-Leucine with 212.8-nm CPL

Light polarization	Energy input, mW-h	Sample decomposition, %	Sample composition[a]			Enantiomeric excess	
			%*R*	%*S*	(±)[b]	Gross[f]	Per unit % dec[g]
RCPL	44.0	59	49.01	50.99[c]	0.22	1.98 ± 0.31	0.0336
LCPL	50.4	75	51.25	48.75[d]	0.25	2.50 ± 0.35	0.0333
Unpolarized	40.0	54	50.15	49.85[e]	0.23	0.30 ± 0.33	

[a] Compared to (*RS*)-leucine standard corrected to *R*/*S* = 50/50. [b] (±) represents standard deviation. [c] Eight sample analyses; eight (*RS*)-leucine standard analyses. [d] Twelve sample analyses; 12 (*RS*)-leucine standard analyses. [e] Six sample analyses; six (*RS*)-leucine standard analyses. [f] Absolute value of %*S* enantiomer − %*R* enantiomer. [g] Gross enantiomeric excess divided by percent sample decomposition.

to its *N*-trifluoroacetyl (N-TFA) isopropyl ester derivative and examined by GC to determine both the extent of photolysis[18] and the enantiomeric enrichment produced. The results are shown in Table I.

Table I shows that the (*R*)-leucine component of (*RS*)-leucine, which preferentially absorbs RCPL (Figure 1[21]), was degraded more extensively than was the (*S*)-leucine component. Conversely, LCPL photolyzed the (*S*)-leucine component preferentially. Thus the gross enantiomeric excess induced by RCPL (1.98%) was reversed when LCPL was employed (2.50%), and the almost quantitative "equal and opposite" nature of the effect is seen in the last column of Table I, where the enantiomeric enrichment per unit of sample decomposition is indicated. The enantiomeric excesses shown in Table I are somewhat higher than those predicted using the table developed by Kagan and co-workers[14] (ca. 1.1 and 1.7%, respectively, for 59 and 75% decomposition and $g = 0.0244$), suggesting that the present photodecomposition may not strictly obey the simple first-order kinetics upon which Kagan's equation is derived.

The (*RS*)-leucine employed was actually an "artificial" racemate containing equal quantities of ordinary (*R*)-leucine and carboxyl-labeled (*S*)-leucine-^{13}C (cf. Experimental Section). The purpose of using an artificial racemate isotopically enriched in one enantiomer was to allow subsequent evaluation of the enantiomeric composition of the unphotolyzed leucine residue by mass spectrometric measurement of the $^{13}CO_2/^{12}CO_2$ ratio obtained on decarboxylation[23] of the residue, should our proposed gas chromatographic technique prove insufficiently sensitive.[24] Despite the internally consistent "equal and opposite" results of the two CPL experiments in Table I, however, we felt it additionally desirable to convince ourselves that the observed data were not related to any isotope effect associated with the $^{13}C/^{12}C$ isotopic composition of our artificial racemate. Accordingly, a portion of our stock solution was photolyzed with *unpolarized* ultraviolet light to the extent of 54%, then was examined gas chromatographically as before for any enantiomeric inequality in the residue. The last experiment in Table I, constituting a "control", indicates that an isotope effect could not be appreciably influencing the enantiomeric selectivity observed in the first two experiments, since the latter enantiomeric selectivity is within experimental error of zero. Furthermore, although a normal isotope effect ($k^{12}/k^{13} > 1$) might have explained the preferential destruction of (*R*)-leucine-^{12}C with RCPL in the first experiment of Table I, a *reverse* isotope effect ($k^{13}/k^{12} > 1$) would have to be operative to explain the preferential degradation of (*S*)-leucine-^{13}C with LCPL in the second experiment.

Since the γ-irradiation of aqueous solutions of mandelic acid has been observed to engender not only radiolysis but also racemization,[25] the question of the possible photoracemization of leucine by ultraviolet light was also of interest to us, since competing photoracemization could impair the efficiency of our photolyses (but not, presumably, their overall asymmetric bias due to the CPL). Accordingly an (*S*)-leucine solution was irradiated with 253.7-nm light for a 7-h period, after which the solute was examined by GC both for gross decomposition and racemization. The sample proved to be ca. 96% decomposed, but no racemization could be detected, suggesting that photoracemization was at best a negligible side reaction in the above photolyses.

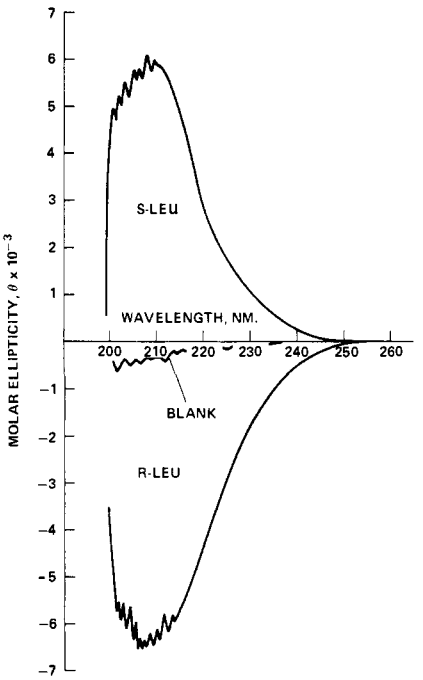

Figure 1. Circular dichroism curves for (*R*)- and (*S*)-leucine.

Asymmetric photolyses of amino acids have not been reported in the past, possibly because sufficient CPL flux at short enough wavelengths has not been available with conventional ultraviolet sources and monochromators to permit excitation of the carboxyl chromophore. Our present laser source providing pure 212.8-nm CPL has been able to circumvent this difficulty, and the 2.50% enantiomeric enrichment reported in Table I is the second highest ever reported during the asymmetric photolysis of a racemate, as well as the first ever to be recorded for a prebiotically implicated substrate. Accordingly, we believe that our present observations on the stereoselective photolysis of racemic leucine reinforces the hypothesis[26] that circularly polarized light might have been involved in the prebiotic genesis of optical activity on earth and perhaps on other planets. These results, as well as our earlier observations wherein optical activity was engendered abiotically in racemic amino acids with the aid of quartz[27-30] or longitudinally polarized electrons,[31] suggest moreover that one should apply the utmost caution in interpreting any future observation of optical activity per se in extraterrestrial matter as a definitive indicator of extraterrestrial life.

Experimental Section

Circular Dichroism and Anisotropy Factor for Leucine. The circular dichroism curves for (*R*)- (Sigma Chemical Co.) and (*S*)-leucine (Calbiochem, A grade) were measured[32] in 0.01 and 0.02 M solutions made up in 1 N HCl using both a Jasco ORD/UV-5 optical rotatory dispersion recorder modified for CD measurements and a Jasco J40 automatic recording spectropolarimeter, which plotted the molar ellipticity, θ, as shown in Figure 1. The average of eight separately measured values was $\theta_{max} = 5460 \pm 75$ at λ_{CD} 211 nm, allowing the calculation[21] of $\Delta\epsilon = 1.65 \pm 0.02$. The latter is in good agreement with a literature value[33] of 1.68, but in less satisfactory agreement with another value[19] of 1.85. Measurement of the ultraviolet absorption spectrum of the above (*R*)- and (*S*)-leucine samples each at four different concentrations in 1 N HCl using a Cary 14 ultraviolet spectrophotometer gave an average of eight measured values for ϵ of 67.7 ± 2.4 at λ_{max} 207 nm (literature value[19] 95 at 206 nm). From our values we calculate $g = 0.0244$ for leucine, in fair agreement with a literature value[19] of 0.0195.

Right and Left Circularly Polarized Light Source (212.8-nm). The ca. 211-nm CPL required for our photolyses was obtained using the following optical system.[22] Coherent infrared radiation at 1064 nm was generated by a flash-pumped, Q-switched Nd/YAG laser system comprising an oscillator and amplifier. The infrared pulses were produced at a 10-Hz repetition frequency, with a pulse duration of 10 ns and an average power near 1 W. This laser beam was passed through a crystal of KH_2PO_4 which converted a fraction of the 1064-nm radiation to 532 nm by second harmonic generation.[34] These two wavelengths were separated by a dichroic mirror into two beams. The 532-nm beam was then passed through a crystal of $NH_4H_2PO_4$ which generated the 266-nm fourth harmonic of the original 1064-nm radiation. A fused silica prism system was used to separate the 266- and 532-nm beams and also to combine the 1064- and 266-nm beams so that they were made to propagate collinearly. The fifth harmonic at 212.8 nm was generated by means of a recently demonstrated parametric mixing or "upconversion" [22,34] technique in which the 1064 + 266 nm beams pass through a 2.2-cm 45° Z-cut crystal of $NH_4H_2PO_4$ maintained near −45 °C in a thermostatically controlled vacuum cryostat.[35] Final separation of the 1064-, 266-, and 212.8-nm radiation was accomplished using a fused silica prism. The 212.8-nm component was linearly polarized with an average power near 2 mW.[22] Right or left circular polarization was obtained by passage of this 212.8-nm beam through an appropriately oriented (±45° to the plane of the incident 212.8-nm polarization) LiF Fresnel rhomb (Karl Lambrecht Corp., Chicago, Ill.) mounted in a calibrated, rotatable bezel. A portion of the 212.8-nm power was monitored by a silicon detector device and recorded during the photolysis of each sample, so that the total exposure could be computed.

Photolyses with 212.8 nm Circularly Polarized Light. A 3.2×10^{-3} M stock solution of labeled (*RS*)-leucine hydrochloride was prepared by dissolving the appropriate quantity of a mixture of 90% carboxyl-labeled (*S*)-leucine-^{13}C (Merck, Sharp, and Dohme International) and an equal amount of unlabeled (*R*)-leucine in 0.1 N HCl. Aliquots (25-mL) of this stock solution were placed in a 10-cm path length cell equipped with Suprasil (SiO_2) windows, and the solutions were irradiated (while stirring and purging with a slow stream of nitrogen) with 212.8-nm CPL from the above laser source for periods of time which pilot experiments had shown would engender 60–80% overall photolysis. The first target solution was exposed to 44 mW-h of 212.8-nm RCPL and the second solution to 50.4 mW-h of LCPL. Each solution (1.00 mL) was removed and treated with 1.00 mL of a 3.2×10^{-3} M solution of (*S*)-leucine in 0.1 N HCl. Each mixture was rotary evaporated to dryness under aspirator vacuum at 70 °C, and the residues were converted to their N-TFA isopropyl ester derivatives for GC analysis as described below, with results permitting calculation by the enantiomeric marker technique[18] of the percent degradations shown in Table I. Samples (1–2 mL) of each irradiated target solution were similarly evaporated to dryness and the crude residues were also converted to N-TFA isopropyl ester derivatives for GC analyses of their enantiomeric composition.

The N-TFA isopropyl ester derivatives were prepared[20] by dissolving each residue in 4 mL of 2-propanol saturated with anhydrous HCl, then heating under reflux for 3 h, stripping the solvent under vacuum and "chasing" the ester hydrochloride residue by adding dichloromethane (DCM) and vacuum evaporating. The residue was next heated for 30 min with 2 mL of trifluoroacetic anhydride and 2 mL of DCM (reflux), then was stripped of solvents and "chased" with DCM as before. The final derivatives were dissolved in DCM for GC analyses.

Our GC analyses were conducted using 150-ft × 0.02-in. i.d. stainless steel capillary columns coated with the optically active phases of *N*-lauroyl-(*S*)-valyl-*tert*-butylamide[20,36] or *N*-docosanoyl-(*S*)-valyl-*tert*-butylamide.[37] These were installed in both a Perkin Elmer 900 and a Hewlett-Packard 5700A gas chromatograph, each coupled to a Hewlett-Packard 3380A digital electronic integrator-recorder. All irradiated samples were so analyzed in replicate, interspersed "back-to-back" with similar replicate control analyses of the same derivatives prepared similarly from the original nonirradiated (*RS*)-leucine stock solution. The analytical precision of these techniques for estimating enantiomeric ratios has been recently evaluated.[20] The control analyses, corrected to 50/50 *R*/*S*, were used in turn to give the corrected enantiomeric compositions of the irradiated samples shown in Table I..

Attempted Photoracemization of (*S*)-Leucine. Solutions of (*S*)-leucine (1 mg) in water (6 mL) were placed in two 1.4 × 10-cm quartz test tubes each located 2.5 cm from a Penray 11SC-1 ultraviolet lamp (whose bulb was 5-cm long and whose measured power output was ca. 2.5 mW/cm^2 at a distance of 2.5 cm). The solutions were stirred magnetically and irradiated during a 7-h period, then were evaporated to dryness. One residue was examined as above by the GC enantiomeric marker technique[18] and found to be 96% decomposed. The other residue was examined similarly by GC for its enantiomeric composition and was found to be completely nonracemized.

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An ESR Study of the Acid Dissociation of NH Protons.[1] 2. Cyclic Peptide Radicals and Related Radicals

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Abstract: Free radicals formed by the reaction of OH or O^- radicals with alicyclic compounds containing a peptide group (-CONH-) and structurally related compounds have been studied by the in situ radiolysis-steady state ESR method. Eight cyclic peptide radicals resulting from hydrogen abstraction from the C-H bond adjacent to the peptide group have been observed. They are divided into two groups on the basis of the skeletal structure of the radicals: >Ċ-NH-CO- and >Ċ-CO-NH-. In basic solutions significant changes occur in the ESR parameters of these radicals which can be interpreted in terms of the dissociation of the NH proton in a peptide group. The pK_a values for the NH proton dissociation have been determined to be in the range of 7.6 to 13.6 for eight cyclic peptide radicals from 5-methyl-2-pyrrolidinone, 2-pyrrolidinone, 2-pyrrolidone-5-carboxylic acid, hydantoin (for both first and second NH proton dissociations), 1-methylhydantoin, 2-thiohydantoin (second dissociation only), succinimide, and 2,5-piperazinedione (first dissociation only) and 10.9 for a related cyclic radical from 2-oxazolidone. These pK_a values are considerably lower than those for corresponding linear peptide radicals, partly because π-electron density on the nitrogen atom decreases in cyclic peptide radicals with a more delocalized π-electron system. Within each group of cyclic peptide radicals with >Ċ-NH-CO- or >Ċ-CO-NH- the same trend of changes in ESR parameters was observed upon the dissociation of peptide proton. In the first group with structure similar to linear peptide radicals the g value, and the γ- or δ-proton and nitrogen coupling constants, increase while α- and β-proton coupling constants decrease upon the dissociation. Apparently 0.07 to 0.09 of spin density flows from the α-carbon atom to the peptide carbon atom after NH proton dissociation in the three pyrrolidinone radicals. In the second group the g values and nitrogen coupling constants decrease upon dissociation and no remarkable changes in α-proton coupling constant are observed, suggesting that local rearrangement of π-electron distribution occurs in the dissociating peptide group upon the dissociation of peptide proton.

In a previous paper,[4] it was reported that nine linear peptide radicals (-CONHĊ<) formed by hydrogen abstraction with OH or O^- radicals were studied by the in situ radiolysis-steady state ESR method.[5] Significant changes in the ESR parameters occurred in strongly basic solutions and these changes could be interpreted in terms of the dissociation of a peptide proton. The pK_a values for the NH proton dissociation were determined to be 13.3 to 14.6 for six linear peptide radicals.

In this paper an ESR study of the acid dissociation of NH protons has been extended to include cyclic peptide and related radicals. In a spectrophotometric pulse radiolysis study[6] the pK_a value for the NH proton dissociation was determined to be 9.6 for two cyclic peptide radicals from glycine anhydride and alanine anhydride. It was demonstrated that the pK_a value (9.6) is less than that of the corresponding linear peptide radicals because of the effect of cyclization and resonance delocalization.[6] General decrease of pK_a values for the NH proton dissociation was found in linear peptide radicals from $-(CONHCHR)_n-$ with increasing n and the decrease might be correlated with the effect of cyclization.[4,7]

In the present paper a detailed *in situ* radiolysis ESR study of the acidity of several cyclic peptide and related radicals is described. These radicals are formed by hydrogen abstraction with OH or O^- radicals. It should be pointed out that the ESR method has many advantages for following an acid-base equilibrium, such as ability to identify and relate the acid and basic forms of a radical directly and independence of radical yields, side reactions, or impurities.[8]

Experimental Section

The experimental arrangement and procedures for the observation of cyclic peptide and related radicals were essentially the same as described previously.[4] All materials were obtained from commercial sources and used without further purification. To provide an acidity scale above the pH scale, Yagil's H_- or H_2- acidity function[9] was used for concentrated aqueous potassium hydroxide solutions. Since these acidity functions were determined from the ionization of indoles,[9] they are quite appropriate for the structurally related cyclic compounds studied in this work.

When the ESR spectrum for the pure basic form could not be observed, the limiting value a_B of hyperfine coupling constants for the basic form was determined by an extrapolation using eq 1

$$a = a_B + (1/K_a)(a_A - a)10^{-H_x} \quad (1)$$

where a is the observed hyperfine coupling constant at H_x (the value of the acidity function or pH of a solution), K_a is the acid dissociation constant, and a_A is the limiting value for the acid form. Using the limiting values a_A and a_B, pK_a values were determined from eq 2.

$$pK_a = H_x + \log [(a - a_B)/(a_A - a)] \quad (2)$$

The titration curves for the dissociation of NH protons were calculated and plotted with a Hewlett-Packard 9100A calculator and a 9125A plotter, according to eq 3.

$$a = [a_A + a_B \times 10^{(H_x - pK_a)}]/[1 + 10^{(H_x - pK_a)}] \quad (3)$$

The calculation of the peak-to-peak width in a first derivative ESR spectrum was carried out on a Hewlett-Packard 9830A calculator.