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OPTICALLY ACTIVE AROMATIC CHROMOPHORES—V

CIRCULAR DICHROISM STUDIES OF β-ARYL-CARBOXYLIC ACIDS[1, 2]

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Abstract Circular dichroism (CD) data in the spectral region from 300 to ca. 200-nm are reported for a series of β-aryl-α-amino acids and for (−)-β-(5-imidazolyl)lactic acid. The aromatic amino acids examined are (−)-phenylalanine, ( )-tyrosine, (−)-3-hydroxytyrosine, ( )-tryptophan, ( )-5-hydroxytryptophan, and (−)-histidine, all of the L-configuration.

In the case of the amino acids the longest wavelength Cotton effect is positive and can be correlated with the absolute configuration. Compounds containing the benzene and indole chromophores exhibit positive Cotton effects in the 260-290 and 215-225 nm regions. The other members of the series have positive Cotton effects between 205 and 220 nm. Substitution of a OH group in the 3-position of tyrosine and the 5-position of tryptophan was found to exert a relatively small effect on the CD spectrum.

The results for (−)-histidine and (−)-β-(5-imidazolyl)lactic acid indicate the possibility of two overlapping optically active transitions in the region above 200-nm.

THE techniques of ORD and CD are potentially powerful tools for the study of optically active molecules in solution. However, since these methods do not yield absolute results the main problem is relating the Cotton effect curve to the molecular geometry of the compound under investigation. It is desirable initially to obtain optical activity data for molecules whose geometry[4] has been established by independent methods.

Much work along these lines, using ORD measurements, has been carried out.[5] The recent availability of precision spectropolarimeters capable of measurements to below 200 nm has stimulated interest in other chromophores which absorb in spectral regions previously inaccessible.[6] However, many Cotton effects are difficult to observe by means of ORD because they are obscured either by large background rotations caused by optically active transitions located at shorter wavelength or by other optically active absorption bands in close proximity.

[1] Part IV in preparation; data available upon request. Part III: L. Verbit, *J. Am. Chem. Soc.* **88**, 5340 (1966).

[2] Presented in part at the *152nd National Meeting of the American Chemical Society*, New York, N.Y., September 1966; Abstract C167

[3] Abstracted in part from the M.A. Thesis of P. J. Heffron, State University of New York at Binghamton, 1967.

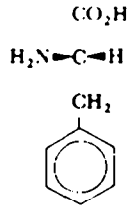
[4] By molecular geometry is meant a description of a chemical species in terms of bond angles, bond lengths, and relative positions of the atoms, sufficient to distinguish the species from its isomer. Each instantaneous specification thus corresponds to a *conformation*. *Absolute configuration* in this sense is then a broad term which includes all the conformations of a given optical isomer

[5] C. Djerassi, *Optical Rotatory Dispersion: Applications to Organic Chemistry* McGraw-Hill, New York (1960)

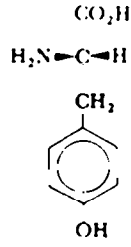
[6] P. Crabbé, *Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry* Holden-Day, San Francisco (1965)

In many cases CD, which measures the difference in absorption of left and right circularly polarized light, is the method of choice.[7] Cotton effects in the CD spectrum extend over a relatively narrow region around their maxima and are thus more easily resolvable than in the corresponding ORD measurement.

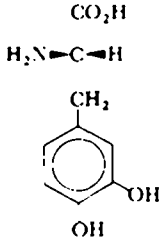
Currently, there is much interest in studying the geometry of proteins in solution as a means of understanding the biological functions of these molecules. ORD and CD are playing important roles in this work.[8] However, because of the complex nature of proteins we feel that an understanding of the optical properties of the constituent building blocks, the amino acids, is a prerequisite for the interpretation of protein optical activity data. In connection with our work on optically active aromatic chromophores[9] we report the results of a CD study of several β-aryl-α-substituted carboxylic acids, 1–7, in the UV region to about 200-nm.



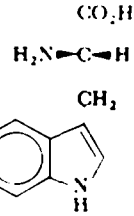
(−)-1



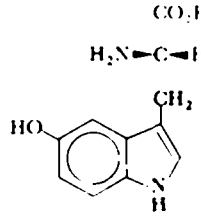
(−)-2



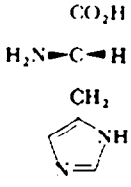
(−)-3



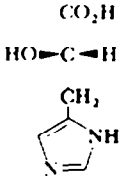
(−)-4



(−)-5



(−)-6



(−)-7

[7] L. Velluz, M. Legrand, and M. Grosjean, *Optical Circular Dichroism*. Academic Press, New York (1965).

[8] W. F. Harrington, R. Josephs, and D. M. Segal in *Ann. Rev. Biochem.* **35**, 599 (1966); D. D. Ulmer and B. L. Vallee in *Adv. Enzymology* **27**, 37 (1965); J. A. Schellman and C. Schellman in *The Proteins* **2**, 1 (1964); G. D. Fasman in *Methods in Enzymology* **6**, 928 (1963).

[9] [a] L. Verbit, *J. Am. Chem. Soc.* **88**, 5340 (1966); [b] L. Verbit, S. Mitsui, and Y. Senda, *Tetrahedron* **22**, 753 (1966); [c] L. Verbit, *J. Am. Chem. Soc.* **87**, 1617 (1965).

The compounds investigated were (−)-phenylalanine[10] (−)-1, (−)-tyrosine (−)-2, (−)-3-hydroxytyrosine (−)-3, (−)-tryptophan (−)-4, (−)-5-hydroxytryptophan (−)-5, (−)-histidine (−)-6, and (−)-β-(5-imidazolyl) lactic acid (−)-7.[11,12] All optical activity data are reported in terms of compounds of the L-configuration (see projection formulas). These molecules possess the advantage of being relatively simple, i.e. they contain only a single asymmetric center. In addition, their absolute configuration is firmly established.[14]

RESULTS

Because of the acid-base properties of amino acids, at least three species in rapid

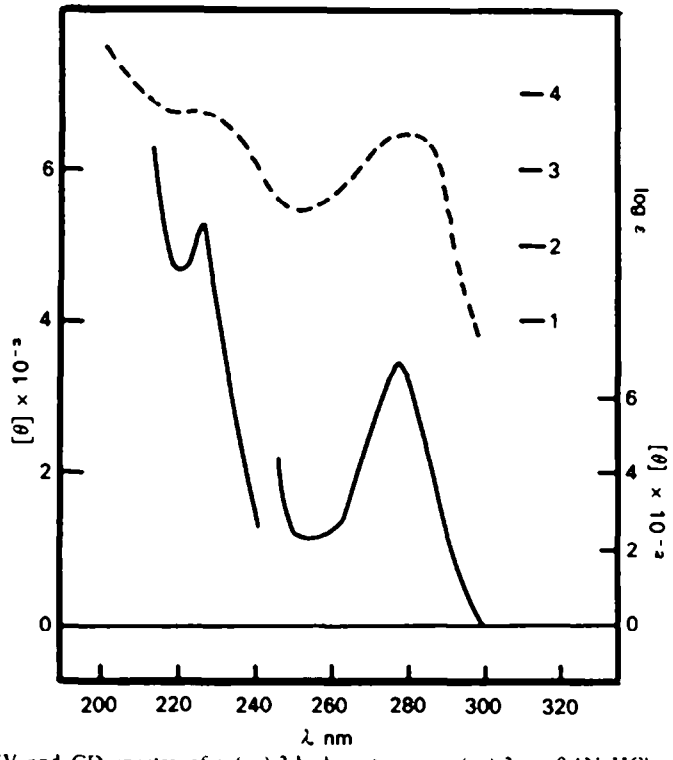


FIG. 1. UV and CD spectra of L-(−)-3-hydroxytyrosine, (−)-3, in 0·1N HCl solution. See footnote 16 for explanation of the ordinate scales.

[10] The CD of (−)-phenylalanine is reported in Ref. 1 in connection with a study of the effect of separating the aromatic chromophore from the asymmetric center

[11] The signs of the Na D line rotations given are for compounds of the L-configuration in water. Some of the D line rotations change sign in aqueous solution at pH 1, the chief solvent in the present work. In order to minimize confusion the sign of the D line rotations for the L-compounds of the present series in 0·1N HCl solution follow: (−)-1, (−)-2, (−)-3, (+)-4, (+)-5, (+)-6, and (−)-7

[12] While this work was in progress a CD study of a series of α-amino acids appeared.[13] For most compounds the authors presented data at only a few selected wavelengths, thereby limiting the value of their work. We have included in our studies three compounds measured by the French workers, (−)-2, (−)-4, and (−)-6, in order to use the data, run under identical conditions, for comparison with the related compounds (−)-3, (−)-5, and (−)-7. CD data for all compounds are given in the Experimental section

[13] M. Legrand and R. Viennet, *Bull. Soc. Chim. Fr.* 679 (1965).

[14] J. P. Greenstein and M. Winitz, *Chemistry of the Amino Acids*, Vol. 1; Chap. 2. Wiley, New York (1961)

equilibrium may exist in solution.

$$\overset{+}{N}H_3RCHCO_2H \cdots \overset{+}{N}H_3RCHCO_2^- \rightleftharpoons NH_2RCHCO_2^-$$

The ORD spectra of amino acids are known to be dependent on the concentrations of the species present at equilibrium.[15] Therefore, all compounds were measured in 0·1N HCl solution in which the amino acid exists predominantly in the completely protonated form. In addition, CD measurements were made on some compounds at pH 6 and also in ethanol solution but in no case was the sign of a Cotton effect found to be pH or solvent dependent.

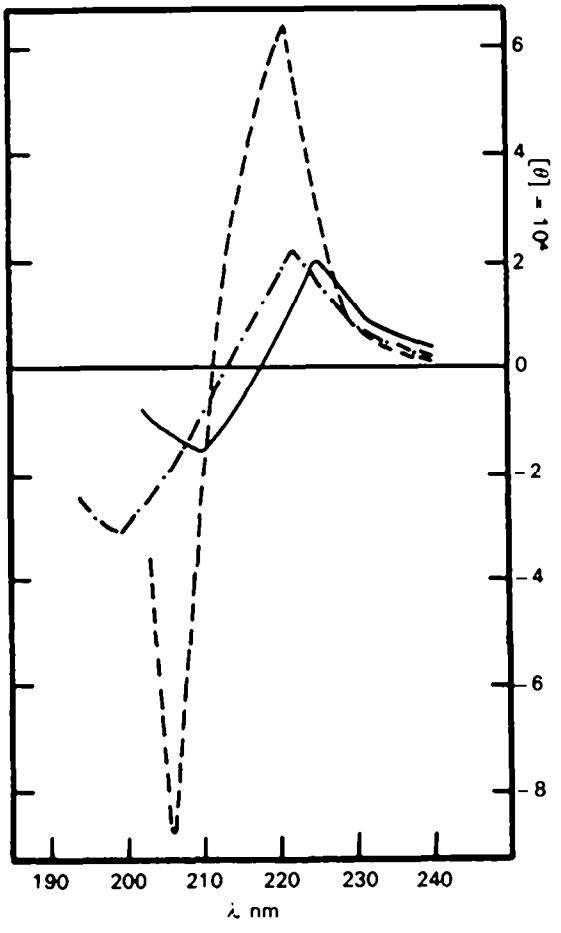


FIG. 2. CD spectra of L-(−)-tryptophan, ( )-4, in 0·1N HCl; - - - water, pH 5·85; and absolute ethanol.

[15] For example, see J. A. Schellman in Ref 5

The UV and CD spectra of compounds (−)-3, (−)-5, and (−)-7 in 0·1N HCl solution are shown in Figs 1, 3, and 4.[16]

As in the case of other optically active aromatic compounds,[1] the CD measurements of this work were characterized by low signal-to-noise levels due to the small anisotropy ratios, $\Delta\varepsilon/\varepsilon$, of the aromatic acids. In part this is due to the fact that the aromatic

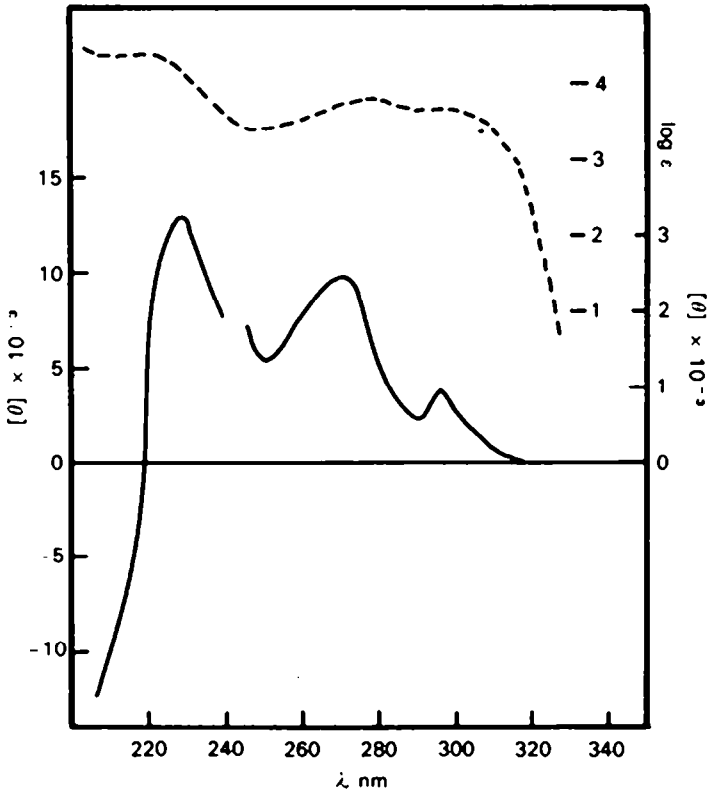


FIG. 3. UV and CD spectra of L-(−)-5-hydroxytryptophan, (−)-5, in 0·1N HCl solution.

group is one carbon removed from the asymmetric center and the steep dependence of rotational strength on distance is well known.[17] However, appropriate precautions in the measurements were taken to minimize instrumental and experimental error.[18]

[16] Because the long wavelength Cotton effects are so much weaker than those below ca. 240-nm, the CD curves (*which are continuous*) were broken into two parts: the right hand ordinate refers to the longer wavelength Cotton effect and the left hand scale refers to the bands below ca. 240-nm. The log ε scale corresponds to the isotropic absorption spectrum.

[17] For examples in a series of aldehydes and ketones, see C. Djerassi and L. E. Geller, *J. Am. Chem. Soc.* **81**, 2789 (1959).

[18] See the discussion of this important point in Ref 1.

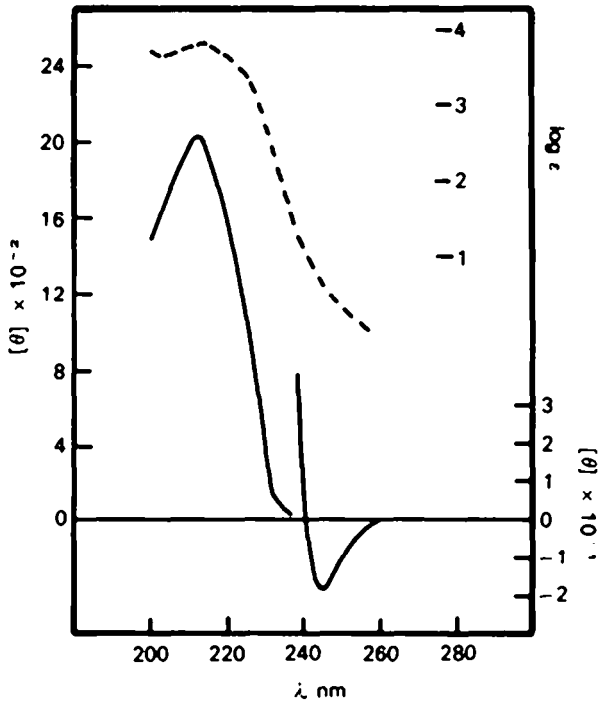


FIG. 4. UV and CD spectra of L-(−)-β-(5-imidazolyl)lactic acid, (−)-7, in 0·1N HCl solution.

DISCUSSION

The CD spectrum of (−)-phenylalanine at pH 1 exhibits two positive Cotton effects in the region from 300 to 200-nm;[1,13] a weak band in the 260-nm region associated with the symmetry-forbidden $\pi \rightarrow \pi^*$ transition[19] and a more intense Cotton effect at 217-nm.

(−)-Tyrosine. (−)-2, which differs from (−)-1 only in having a hydroxyl group in the *para* position exhibits a CD spectrum quite different from that of (−)-1. The ^1L_b band[20] at 274-nm in the protonated species is considerably more intense and lacks the fine structure found in (−)-1. The intensity of the transition has been explained in terms of the availability of non-bonding orbitals of the ring OH for mixing with the electric dipole transition moment of the aromatic ring.[19] A positive Cotton effect is found at 225-nm. This band corresponds to a peak in the isotropic absorption spectrum assigned to a $\pi \rightarrow \pi^*$ transition of the aromatic ring.[21]

[19] A. Moscowitz, A. Rosenberg, and A. E. Hansen, *J. Am. Chem. Soc.* **87**, 1813 (1965).
[20] J. R. Platt, *J. Chem. Phys.* **17**, 484 (1949).
[21] Y-H. Pao, R. Longworth, and R. L. Kornegay, *Biopolymers* **3**, 519 (1965); H. Grinspan, J. Birnbaum, and J. Feitelson, *Biochim. Biophys Acta* **126**, 13 (1966).

The 225-nm Cotton effect of (−)-tyrosine is overlapped by a shorter wavelength band which is still ascending at 198-nm (see data in Experimental). A negative Cotton effect must exist in the spectral region below 198-nm in order to account for the negative ORD curve in the visible region.

The effect on the CD spectrum of activating the aromatic nucleus of (−)-2 by an additional OH group was examined. The UV and CD data for (−)-3-hydroxytyrosine (−)-3, Fig. 1, are similar to (−)-2. The addition of the second ring OH introduces only a small perturbation to the CD spectrum in the accessible region. However, the chemical effect of ring activation was dramatically evident upon attempting to carry out CD measurements on (−)-3 at pH 13. Even under an inert atmosphere and using deoxygenated solvent, a solution of (−)-3 turned a deep red color.

A CD examination of (−)-tryptophan, (−)-4, was of interest since this amino acid contains the indole chromophore and exhibits very intense absorption in the 260–290-nm region. The CD spectra of (−)-4 in three different solvents; 0·1N HCl, aqueous solution at pH 5·85, and absolute ethanol (see data in Experimental), all exhibit a positive broad band in the 260–290-nm region. As with previous aromatic amino acids in the series, a positive Cotton effect is found in the 220-nm region. However, in (−)-4 which contains the indole system, a negative Cotton effect with a maximum[22] at 209-nm (pH 1) is found overlapping the 225-nm band. The CD results for (−)-4 in the above three solvents in the region from 240 to 190-nm are compared in Fig. 2. It is seen that the peaks are shifted towards shorter wavelength in aqueous solution relative to 0·1N HCl solution. Similar behavior is found in the CD spectra of aliphatic amino acids[13] and alkyl carboxylic acids.[23] This hypsochromic shift in the CD spectrum of (−)-4 is presumably due to the presence in aqueous solution of the negatively charged carboxylate anion. The enhanced magnitude of the Cotton effects in ethanol compared to water is characteristic of a change to a less polar solvent.[9a]

The effect of substitution of a ring OH group was studied in (−)-5-hydroxytryptophan, (−)-5, Fig. 3. This compound is of interest since it is an intermediate in the conversion of tryptophan to the physiologically important base 5-hydroxytryptamine.

As found for (−)-3-hydroxytyrosine, no large changes in the CD spectrum accompany the introduction of an −OH group into the 5-position of tryptophan. However, the 225-nm peak of (−)-4 is shifted 2-nm to the red while the negative maximum in the CD spectrum of (−)-4 (see data in Experimental) has shifted by at least 4-nm to shorter wavelength, Fig. 3.

Compound 7 was of interest because of its structural similarity to histidine. This compound differs from histidine only in the replacement of a $-\overset{+}{N}H_3$ by an —OH group. We have shown that these groups do not make important changes to the observed optical activity; for example, the CD spectra of L-leucine[13] and of L-isopropyl lactic acid[1] are almost identical. The CD spectrum of (−)-7, Fig. 4, is similar to that of (−)-histidine with a positive Cotton effect at 212-nm. However, an important difference is the finding of a weak negative band at 245-nm which overlaps the shorter wavelength Cotton effect. That this very weak Cotton effect ($[\theta]_{245}$ -18) is real is substantiated by its observation in the CD spectrum of (−)-7 in 95% ethanol

[22] Ref. 6, p. 20

[23] P. Swender, unpublished observations in this laboratory.

5% 0·1N HCl solution, $[\theta]_{247}$ -27. Both this band and the shorter wavelength positive Cotton effect were shifted bathochromically by 2-nm in this solvent, indicating the main components of both bands to be of similar origin. The absence of this long wavelength Cotton effect in the observed CD spectrum of (−)-histidine may simply be due to the extreme weakness of the band although we tried several combinations of concentration and path length in order to observe it.

The possibility that the negative Cotton effect at 245-nm might be due to the availability of non-bonding electrons of the hydroxyl group for mixing with an appropriate transition of the aromatic ring was ruled out when no analogous band was found in the CD spectrum of (−)-histidine at pH 13 where the unprotonated amino group would exist.

An alternative explanation is that the weak negative band is due to a Cotton effect centered at much shorter wavelength. The 245-nm band is then the negative part of the resultant due to overlapping positive and negative Cotton effects.[24] The absence of such a band in the CD spectrum of (−)-histidine can be attributed to another factor; namely, that (−)-histidine does possess a band to longer wavelength, but that it is *positive* and unresolved from a positive band at shorter wavelength. The unresolved resultant could then be the apparent Cotton effect at 216-nm.[25] Alternatively, when the CD spectrum of (−)-7 was resolved into isolated Gaussian bands using analog simulation[26] it was found that a small displacement (3-nm) of the negative, long wavelength curve to shorter wavelength was sufficient to cause the disappearance of this band from the composite CD curve. It is difficult at this time to distinguish between the above two alternatives.

EXPERIMENTAL

CD measurements were carried out using a JASCO Model ORD UV CD-5 instrument. The entire optical system and cell compartment were under a constant N_2 purge obtained from the boil-off of liquid N_2. The salient features of this instrument and some precautions in the measurement of highly absorbing, circularly dichroic compounds have been discussed.[1]

Isotropic absorption spectra were run on the JASCO and on a Cary Model 14 spectrophotometer. The Bouger Lambert-Beer law was found to obtain for each compound within the limits pointed out by Woldbye and Bagger.[27]

The JASCO CD instrument records the difference in absorbance of a sample for left and right circularly polarized radiation as a function of wavelength. This difference is converted to the corresponding difference in molar absorptivity, $(\varepsilon_l - \varepsilon_r)$ and thence to molecular ellipticity, $[\theta]$.[28]

$$[\theta] = 2{\cdot}303\left(\frac{4500}{\pi}\right)(\varepsilon_l - \varepsilon_r)\ \frac{\text{deg cm}^2}{\text{decimole}}$$

The compounds were the highest purity available commercially and were found to be homogeneous using paper chromatography in two solvent systems of different net polarity. In addition, both enantiomers of 1[10] and 4 were measured and mirror image CD spectra were obtained with the limits of the somewhat lower optical purity of the D-isomers. Therefore, data are reported for the L-enantiomer only.

[24] K. M. Wellman, P. H. A. Laur, W. S. Briggs, A. Moscowitz, and C. Djerassi, *J. Am. Chem. Soc.* **87**, 66 (1965).

[25] We are indebted to a referee for suggesting this explanation.

[26] The Du Pont 310 Curve Resolver at the Bell Telephone Research Laboratories was used through the kindness of Dr. Frank A. Bovey.

[27] F. Woldbye and S. Bagger, *Acta Chem. Scand.* **20**, 1145 (1966).

[28] A. Moscowitz in Ref. 5.

L-(−)-*Tyrosine*, (−)-2, CD in 0·1N HCl, 4·31 × 10^{-4}M.

$[\theta]_{280}$ +900, $(\theta)_{274}$ +1200 (max), $[\theta]_{240}$ +810, $[\theta]_{232}$ +4830, $[\theta]_{223}$ +8240 (max), $[\theta]_{217}$ +2300, $[\theta]_{205}$ +4600, $[\theta]_{198}$ +7000.

L-(−)-3-*Hydroxytyrosine*, (−)-3, Fig. 1, CD in 0·1N HCl, 5·05 × 10^{-3}M.

$[\theta]_{290}$ +200, $[\theta]_{278}$ +693 (max), $[\theta]_{240}$ +1350, $[\theta]_{226}$ +5190 (max), $[\theta]_{214}$ +6340.

L-(−)-*Tryptophan*, (−)-4, CD in 0·1N HCl, 4·79 × 10^{-4}M.

$[\theta]_{286}$ +1610 (max), $[\theta]_{276}$ +2000 (max), $[\theta]_{269}$ +2590 (max), $[\theta]_{240}$ +3160, $[\theta]_{230}$ +11,000, $[\theta]_{225}$ +20,100 (max), $[\theta]_{218}$ 0, $[\theta]_{209}$ −15,400 (max), $[\theta]_{201}$ −7,300.

CD in water, pH 5·85, 4·85 × 10^{-3}M.

$[\theta]_{285}$ +1070 (max), $[\theta]_{279}$ +1160 (max), $[\theta]_{274}$ +1640 (max), $[\theta]_{240}$ +2140, $[\theta]_{222}$ +21,700 (max), $[\theta]_{213}$ 0, $[\theta]_{199}$ −30,000 (max), $[\theta]_{194}$ −24,300.

CD in absolute EtOH, 0·931 × 10^{-4}M.

$[\theta]_{289}$ +2100 (max), $[\theta]_{283}$ +2800 (max), $[\theta]_{275}$ +3200 (max), $[\theta]_{240}$ +1060, $[\theta]_{230}$ +8100, $[\theta]_{221}$ +65,000 (max), $[\theta]_{211}$ 0, $[\theta]_{206}$ −83,800 (max), $[\theta]_{203}$ −36,000.

L-(−)-5-*Hydroxytryptophan*, (−)-5, Fig. 3. CD in 0·1N HCl, 5·44 × 10^{-4}M.

$[\theta]_{296}$ +970 (max), $[\theta]_{271}$ +2460 (max), $[\theta]_{240}$ +4170, $[\theta]_{230}$ +12,600, $[\theta]_{227}$ +12,900 (max), $[\theta]_{220}$ 0, $[\theta]_{214}$ −7440, $[\theta]_{205}$ −12,400.

CD in water, 5·44 × 10^{-4}M.

$[\theta]_{276}$ +1460 (max), $[\theta]_{240}$ +2700, $[\theta]_{230}$ +10,800, $[\theta]_{222}$ +16,100 (max), $[\theta]_{212}$ 0, $[\theta]_{206}$ −16,100 (max), $[\theta]_{202}$ −9400.

L-(−)-*Histidine*, (−)-6, CD in 0·1N HCl, 0·271 × 10^{-3}M.

$[\theta]_{230}$ +1710, $[\theta]_{220}$ +5480, $[\theta]_{216}$ +7660 (max), $[\theta]_{205}$ +3040.

L-(−)-β-(5-*Imidazolyl*)*lactic acid*, (−)-7, Fig. 4, CD in 0·1N HCl, 2·56 × 10^{-2}M.

$[\theta]_{245}$ −18 (max), $[\theta]_{240}$ 0, $[\theta]_{230}$ +130, $[\theta]_{220}$ +1560, $[\theta]_{212}$ +2000 (max), $[\theta]_{200}$ +1500.

CD in 95% EtOH 5% 0·1N HCl, 1·24 × 10^{-3}M.

$[\theta]_{247}$ −27 (max), $[\theta]_{242}$ 0, $[\theta]_{230}$ +850, $[\theta]_{220}$ +1930, $[\theta]_{214}$ +2100 (max), $[\theta]_{210}$ +510.

Acknowledgement—This investigation was supported in part by U.S. Public Health Service Grant GM 14068 from the National Institute of General Medical Sciences. We are grateful to Professor B. E. Norcross of this Department for a helpful discussion and to Professor A. Moscowitz, University of Minnesota, and Dr. Frank A. Bovey, Bell Telephone Research Laboratories, for their interest in this work.