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THE ROTATORY DISPERSION OF AMINO ACIDS

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ROTATORY dispersion studies on amino acids can reveal little concerning their structures since these are now so well established. In fact absolute configurations are now known for fairly complicated amino acids such as isoleucine, hydroxyproline, etc. It is only in relation to larger problems such as the polypeptides that such an investigation becomes of interest. I would like to review the line of thought which led to the group of experiments I am about to describe.

We are all aware of the very prominent work being done in the field of sequence determinations. It seems a great stroke of luck that, in many cases, optical rotation measurements ignore precisely this aspect of protein structure and respond instead to backbone configuration. This is only a working hypothesis but one gains confidence in its utility from the uniform rotatory properties of several synthetic polypeptides and the correlations established by Doty, Yang and Blout with the extensive measurements on proteins. A number of polypeptides known to be in helical form follow the same kind of dispersion curve, which is in accordance with the suggestion of Cohen that there is a distinct type of rotation attributable to the backbone helix *per se*.[1] Polypeptides and proteins known to be random follow another kind of curve. The properties of intermediate configuration can be deduced and are observed in many proteins.[2]

On the other hand optical rotation is a spectral property and one encounters many snags in this kind of interpretation. For example polybenzyl aspartate, polyhistidine, polytryptophane, etc. do not follow the rotatory dispersion that is believed established for right-handed helices. Bence-Jones proteins, pepsin and γ-globulin have a different kind of rotatory dispersion than the proteins first mentioned.† Finally with polypeptides and proteins containing large fractions of chromophoric or configurationally unusual side chains, specific residue effects can be expected.

Data on polypeptides and co-polypeptides are accumulating at a fast rate but it will certainly be some time before all polypeptides of interest will be investigated. We decided on the less reliable but very much faster approach of studying a large number of the amino acids themselves in order to detect as rapidly as possible the contribution of their side chains to the dispersion. The inference is not to be drawn that there will be a one to one correspondence between anomalous contributions in free amino acids with those in high molecular weight compounds, but a side chain chromophore which is highly active in an amino acid ought to be regarded as suspect in polypeptides until appropriate evidence proves otherwise. In the course of these studies a number of observations on the intrinsic rotatory capabilities of groups resulted.

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* Chemistry Department, University of Minnesota, Minneapolis, Minn.

† See paper of B. Jirgensons in this volume, p. 166.

[1] C. Cohen, *Nature, Lond.* 175, 1929 (1955).

[2] J. T. Yang and P. Doty, *J. Amer. Chem. Soc.* 79, 761 (1957).

[3] C. Djerassi, *Optical Rotatory Dispersion*. McGraw-Hill, New York (1960).

Experimental conditions

The bulk of our observations were performed in acid solution. Actual conditions varied to suit the requirement that the carboxylate group was completely titrated to COOH so that the rotation was on a plateau with respect to pH and was little affected by small changes in pH or concentration. In this region the bulk of the rotation appears rather clearly to be associated with the COOH chromophore at 2050 Å* *except* when other chromophores of higher wave length are present. Simpson at this meeting has stated that this transition is the same as the 2900 Å transition of the CO group of ketones so much discussed in the papers of Djerassi and Klyne.

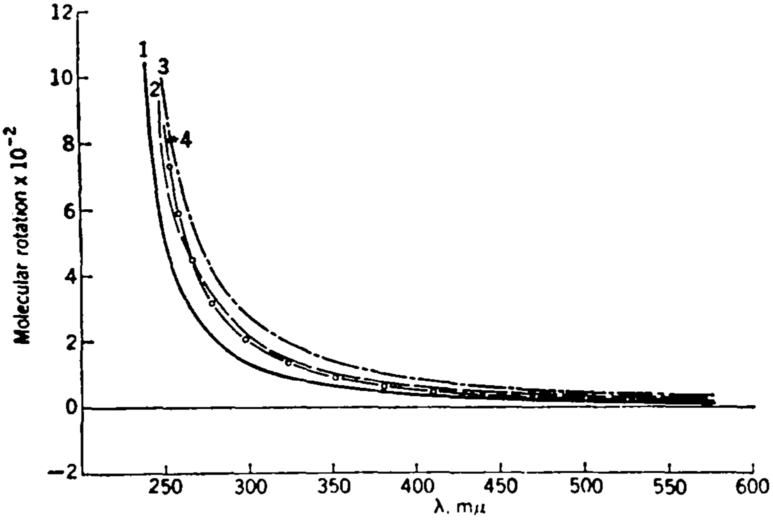

FIG. 1. RD curves of alkyl-substituted alanines: (1) alanine; (2) α-aminobutyric acid; (3) valine, and norvaline; (4) leucine.

Results.

In order to make as brief as possible the task of presenting a large quantity of data, most of which is happily repetitious, the amino acids will be divided into groups. Readers who are unfamiliar with the structures of the amino acids will find them in a glossary at the end of this paper.

Group 1

Amino acids with no chromophores beyond the β carbon. We define a chromophore as a group with an absorption band above 1500 Å. Fig. 1 shows the type of data which was obtained. The curves are uniform and the substances involved are shown in the collective Table 1.

Group 2

Amino acids with chromophores beyond the β carbon atom. A typical set of data is shown in Fig. 2. Other examples differing little in dispersion will be found in Table 1. It would appear that the side-chain groups are sufficiently far from the COOH group in this case that they do not differentiate these substances from Group 1. Unfortunately no chromophores of the very near U.V. were studied. These would have provided

* This point is discussed in Ref. 3, Chapter 15.

TABLE 1. GROUPING OF AMINO ACIDS ACCORDING TO STRUCTURAL FEATURES IMPORTANT TO ROTATORY PROPERTIES

Group 1: aliphatically substituted amino acids	Group 2: amino acids lacking chromophore on β-carbon atom	Group 3: amino acids with chromophore on β-carbon atom	Group 4: amino acids with two asymmetric centers	Group 5	Group 6
Alanine Aminobutyric acid Norvaline Valine Norleucine Leucine	Methionine Arginine Ornithine Lysine Glutamic acid	Serine Cysteine Aspartic acid Asparagine *Tyrosine *Phenylalanine *Tryptophan †Histidine Cysteic acid *S-Ethylcysteine *S-Methylcysteine	Isoleucine *Threonine	*Proline *Hydroxy-proline	*Cystine and derivatives

* The dispersion curves for these substances are qualitatively as well as quantitatively distinct from alanine which shows a plain positive curve.

† Histidine shows anomalous behavior in alkaline, but not in acid solution.

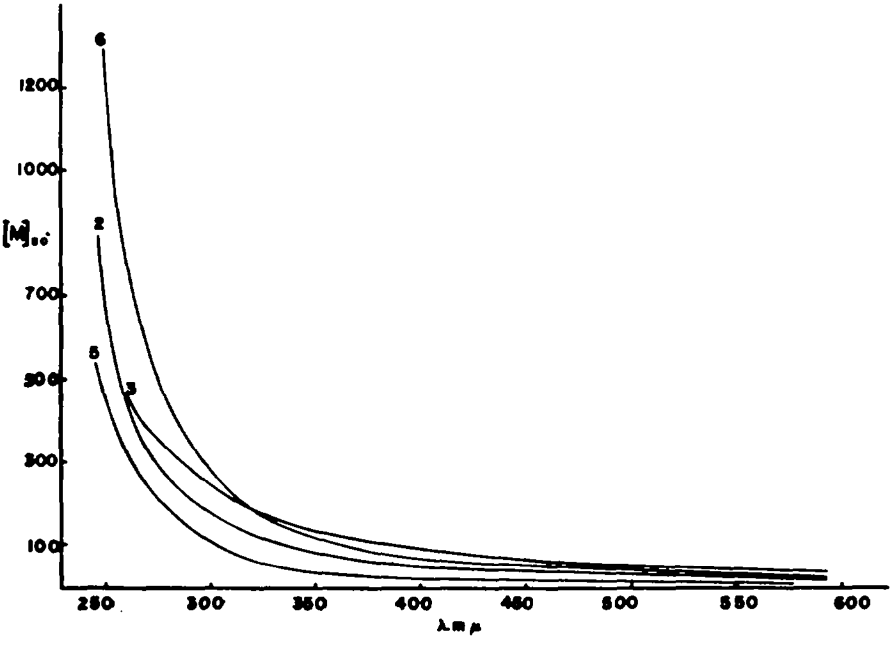

FIG. 2. RD curves of amino acids with chromophores beyond the β carbon atom; (1) alanine (reference); (2) arginine; (3) methionine; (4) lysine; (5) glutamic acid.

an additional stringent test of the effect of distance along the chain, though no common biological materials are of this kind.

Group 3

β-substituted chromophores. These are of two kinds: those which give rise to anomalous dispersion, and those which produce plain curves similar to groups 1 and 2. The latter are shown in Fig. 3. Individualism is beginning to appear but above 3500 Å

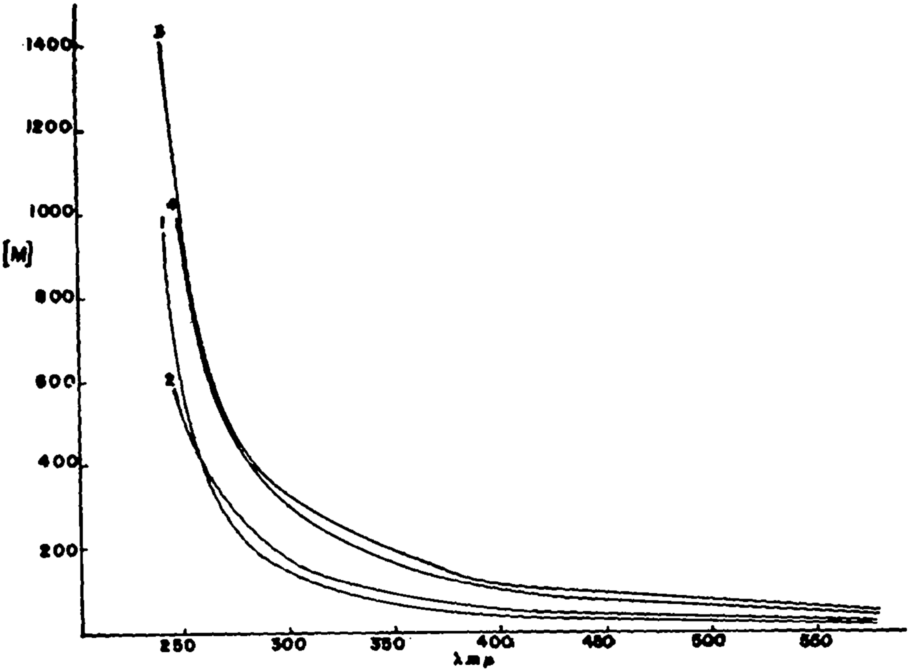

Fig. 3. RD curves of amino acids with β chromophores in the deep ultraviolet. (1) serine; (2) cysteine; (3) aspartic acid; (4) cysteic acid.

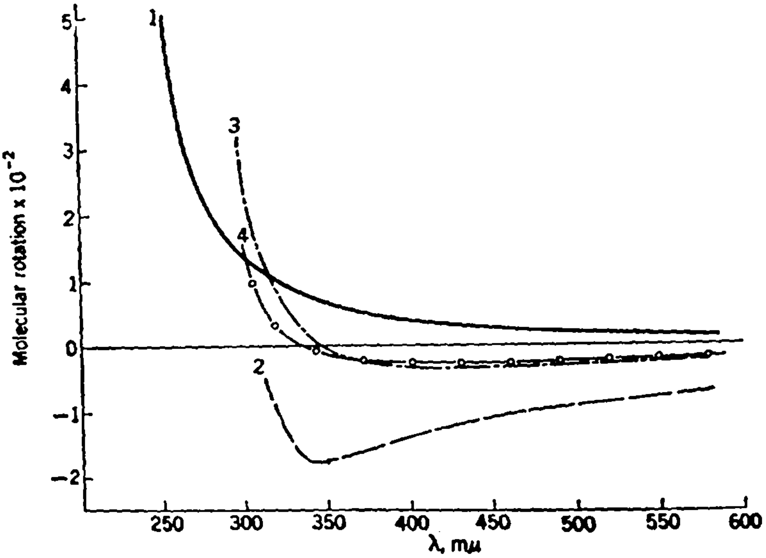

Fig. 4. RD curves of amino acids with β chromophores in the near ultraviolet. (1) alanine (reference); (2) tryptophane; (3) tyrosine; (4) phenylalanine.

where most polypeptide and protein dispersion runs are performed, the curves are definitely banded together. On the other hand phenylalanine, tyrosine and tryptophane show distinctly anomalous behavior (Fig. 4), very likely arising from their absorption bands above 2500 Å. Cotton effect curves could not be obtained because of absorption but two term dispersion curves calculated with the formulas of Lowry[4] led in each case to a λ_0 corresponding to the appropriate absorption band. For tyrosine similar results had been obtained over a more limited wave length region. Ethyl and methyl cysteine also gave anomalous curves at 20°. Both are not reproduced, but Fig. 5 displays an unusual feature of the temperature dependence of the dispersion of S-ethyl cystine: the curve is anomalous at 20° with a broad minimum at about 4750 Å, and simple at 50°. It seems reasonably plain from the closeness of the curves that this does not represent a structural change but a gentle raising of the curve across the zero axis. Results of this kind point out a warning against attributing special significance to anomalous dispersion.

Group 4

Amino acids with more than one asymmetric center. Unusual features might be expected here and they are observed. The threonine dispersion curve is displaced downward from the norm to become anomalous; the isoleucine curve is displaced upwards to be unusually dextrotatory.

Group 5

Proline and hydroxyproline. Here again the curves deviate from the norm (Fig. 7). The amino acids are levorotatory, the trough of a Cotton effect curve is observed, and the rotations are very large, in accordance with the rule of Kauzmann and Eyring[5] for ring structures. Pyroglutamic acid also shows these features (unpublished results).

Group 6

Cystine. Cystine has long been known as one of the many disulfide compounds which show exceptionally large rotations at the sodium D line. Fig. 8, which compares the dispersion of cystine and alanine demonstrates not only the great magnitude of the rotation at all wave lengths, but shows the trough of a Cotton wave of very large amplitude at 2650 Å. This is undoubtedly associated with the absorption band of cystine at 2500 Å*, and demonstrates that the bulk of the optical rotation is associated with the disulfide group itself. This probably arises from the fact that the disulfide link is intrinsically asymmetric as is shown in Fig. 9. The 90° dihedral angle gives rise to a screw sense in all disulfides. With no asymmetric center, right and left senses occur with equal probability. But since both R groups in cystine are asymmetric, it is more than conceivable that one or the other configuration will be preferred, thus producing an intrinsic rotation associated with the disulfide link. It is interesting that the Cotton effect is positive in homocystine (unpublished observations).

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* Some disulfides have absorption maxima and Cotton curves at higher wave lengths. See references to Schotte and Sjoberg.

[4] T. M. Lowry, *Optical Rotatory Power*. Longmans, Green, London (1935).

[5] W. Kauzmann and H. Eyring, *J. Chem. Phys.* 9, 41 (1941).

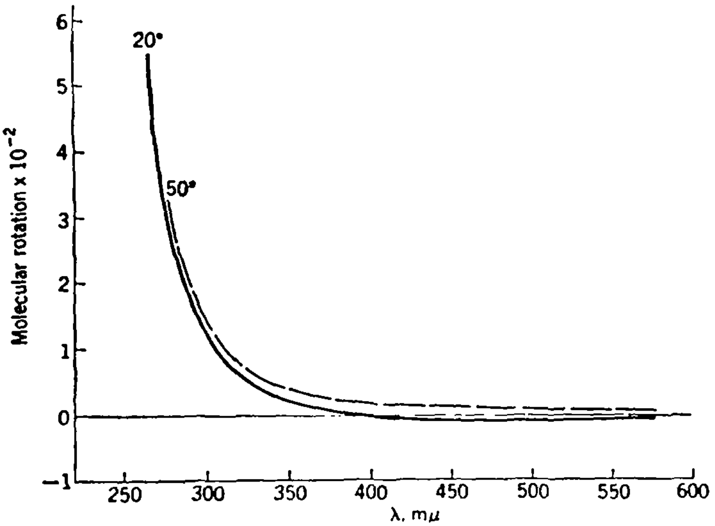

FIG. 5. Effect of temperature on the rotatory dispersion of S-ethylcysteine,

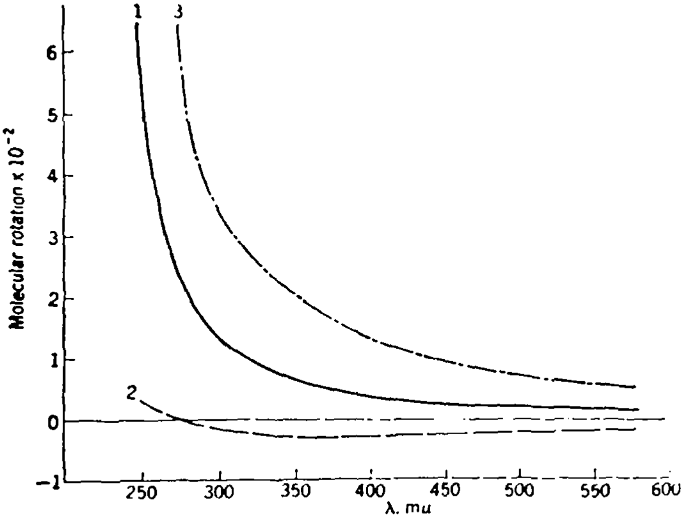

FIG. 6. RD curves of amino acids with two centers of asymmetry: (1) alanine (reference); (2) threonine; (3) isoleucine.

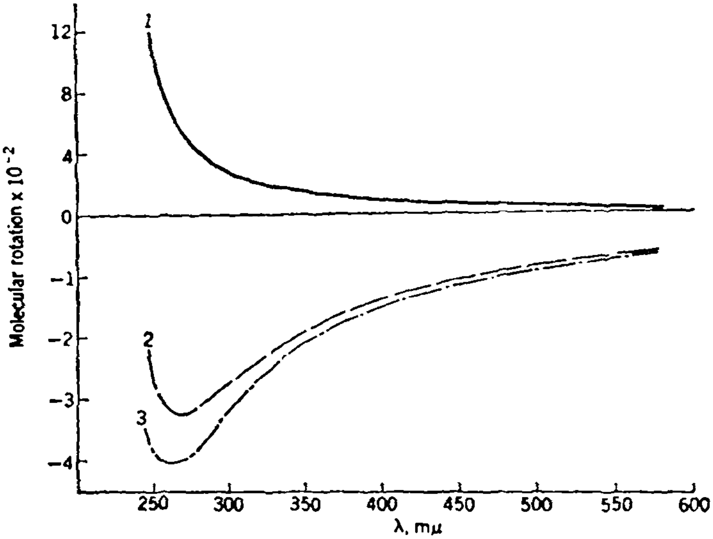

FIG. 7. RD curves of (1) norvaline (reference); (2) proline; (3) hydroxyproline.

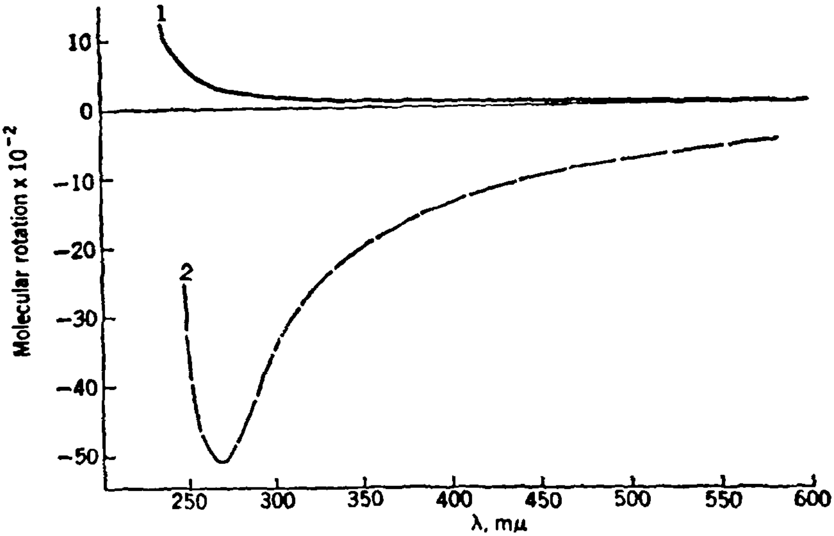

FIG. 8. RD curves of (1) alanine; (2) cystine.

Discussion

First, it must be emphasized that a comparison between the side chain contribution of a chromophore in an amino acid and in a polypeptide is not a rigorous procedure. In an amino acid the chromophore "sees" a very different set of vicinal groups than in a polypeptide, especially if the polypeptide is in the helical conformation. Consequently these studies should be regarded more as a scouting expedition than the firm establishment of fact. Yet they have their value because the only way in which the contribution of a chromophore to the dispersion can be established or eliminated in high molecular weight compounds is by the measurement of dispersion within its absorption band and this is difficult work. To my knowledge only two investigations of this kind have been carried out, one on proteins,[6] and the other on polypeptides reported in Blout's paper at this meeting*.

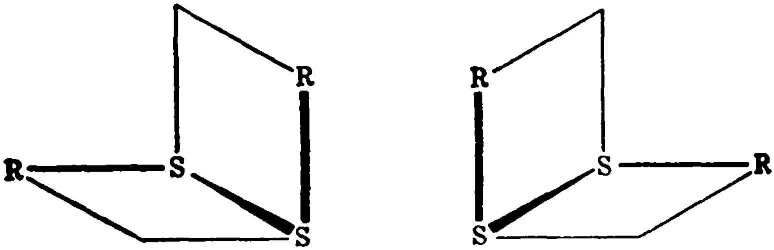

FIG. 9. The asymmetric disulfide group.

The overall results are shown in Table 1 which lists most of the amino acids which have been investigated. Substances marked with asterisks are those whose dispersion curves are qualitatively as well as quantitatively distinct from alanine which shows a plain positive curve. (Glycine, of course, also falls into the category of an amino acid with unusual rotatory properties.)

It appears to us that it is rather a dangerous procedure to correlate rotatory dispersion directly with backbone conformation with any polypeptide or protein containing relatively large quantities of these amino acids.

Roughly 25 per cent of the natural amino acids are of the "anomalous" variety. Yet an inspection of the amino acid composition of most proteins reveals that they usually contain at least 90 per cent of the alanine-like amino acids. Thus the above measurements are in accordance with a conformational interpretation of rotatory dispersion. One investigation of dispersion on serum albumin and ribonuclease between 2500 and 3000 Å[6] revealed that the contribution of the aromatic side chains is negligibly small compared to the bulk rotation of the backbone, presumably because of their small mole ratios in these proteins. In contrast Würtz and Haurowitz[7] have been recently investigating the effect of disulfides on total rotation in protein and have concluded that the contribution is significant.

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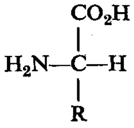
* *Note added in proof:* Recently Simmons, Cohen, Szent-Gyorgyi, Wetlaufer and Blout have extended their measurements below 2500 Å and have observed Cotton effects originating from the peptide bond with a number of proteins and helical polypeptides. *J. Amer. Chem. Soc.* In press.[7]

[6] J. and C. Schellman, *Arch. Biochem. Biophys.* **65**, 58 (1956).

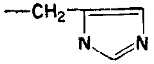
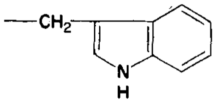
[7] H. Würtz and F. Haurowitz, *Abstract of Paper Presented at the American Chemical Society Meeting*, Cleveland (1960).

One final instance of where conformational rules might break down is in the presence of a prosthetic group which absorbs in the visible; e.g. heme groups and colored metal complexes. We have seen in Eichorn's paper that their bands are optically active and their proximity to the region of measurement could well cause them to dominate, completely obscuring the conformational aspects of dispersion.

Glossary of structures of amino acids



$$\begin{array}{c} CO_2H \\ | \\ H_2N—C—H \\ | \\ R \end{array}$$

R	Amino acid
$—CH_3$	Alanine
$—CH_2CH_3$	Aminobutyric acid
$—(CH_2)_2CH_3$	Norvaline
$—(CH_2)_3CH_3$	Norleucine
$—CH(CH_3)_2$	Valine
$—CH_2CH(CH_3)_2$	Leucine
$—CH(CH_3)CH_2CH_3$	Isoleucine
$—CH_2OH$	Serine
$—CH_2SH$	Cysteine
$—CH_2CO_2H$	Aspartic acid
$—CH_2CONH_2$	Asparagine
$—CH(OH)CH_3$	Threonine
	Histidine
$—CH_2C_6H_5$	Phenylalanine
$—CH_2C_6H_4OH$	Tyrosine
	Tryptophan
$—CH_2SO_3H$	Cysteic acid
$—CH_2SCH_3$	S-Methylcysteine
$—CH_2SC_2H_5$	S-Ethylcysteine
$—CH_2CH_2CO_2H$	Glutamic acid
$—CH_2CH_2SCH_3$	Methionine
$—(CH_2)_3NHC(=NH)NH_2$	Arginine
$—(CH_2)_3NH_2$	Ornithine
$—(CH_2)_4NH_2$	Lysine
$—CH(CO_2H)CH_2CO_2H$	Amino tricarballylic acid