XXII. AMINO-ACIDS, ACYL-AMINO-ACIDS, DIPEPTIDES, ACYL-DIPEPTIDES, AND DERIVATIVES OF THESE COMPOUNDS

I. ABSORPTION SPECTRA¹

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IN connexion with a study of the action of various radiations on amino-acids and their derivatives, it became necessary for us to determine the absorption spectra of all the compounds of that category which were available for investigation. The spectral region investigated was from 2100 to 6000 Å.

The ultraviolet absorption spectra of glycine [Kober, 1915; Castille & Ruppel, 1926; Shibata & Asahina, 1927; Ley & Arends, 1928; 1932], alanine [Kober, 1915; Shibata & Asahina, 1927; Ley & Arends, 1928; 1932; Barnés, 1930], α -aminoisobutyric acid [Ley & Arends, 1928; 1932], valine [Kober, 1915], leucine [Kober, 1915; Shibata & Asahina, 1927], β -phenylalanine [Kober, 1915; Shibata & Asahina, 1927; Abderhalden & Rossner, 1928, 1; Feraud *et al.* 1935] and glycyl-leucine [Shibata & Asahina, 1927; Abderhalden & Haas, 1926; Abderhalden & Rossner, 1928, 2] may be found in the literature. The determinations have been repeated for purposes of comparison with the other compounds studied. Throughout this report, statements concerning the relative absorption by two or more compounds apply only to the wave-lengths shown on the Figures.

EXPERIMENTAL METHODS

Preparation of compounds. The derivatives of amino-acids used in this and another investigation (see Part II) are compounds already known. All of them, with the exception of the three following, had been prepared in the ways described in the chemical literature [Steiger, 1934, 1; Levene & Steiger, 1931].

Benzoyl- α -aminoisobutyric acid [Mohr & Geis, 1908; 1910; Gabriel, 1913; Heller & Lauth, 1919]. A solution of α -aminoisobutyric acid in *N* sodium hydroxide was treated in the cold with benzoyl chloride, *N* sodium hydroxide being used to remove the hydrogen chloride formed in the reaction.

Glycyl-dl-leucine [Fischer, 1905]. Chloroacetyl-dl-leucine was aminated, at room temperature, with 10 times its weight of ammonium hydroxide of sp. gr. 0.90.

Acetyl-dl- α -phenylalanyl- α -aminoisobutyric acid. Cold water was added to the pure azlactone [Steiger, 1934, 2] of acetyl-dl- α -phenylalanyl- α -aminoisobutyric acid. The opening of the azlactone ring takes place at room temperature. By this procedure a much purer product is obtained than the one resulting on mere recrystallization of the crude acetyl-dl- α -phenylalanyl- α -aminoisobutyric acid as formed by the action of the azlactone of acetyl-dl- α -phenylalanine on α -aminoisobutyric acid in glacial acetic acid.

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Technique. The solutions were prepared as follows: the dry powders (at least 99.0 % pure) were weighed in 10 ml. volumetric flasks and dissolved in the appropriate solvents and the solutions were diluted to volume at room temperature.

A medium size Bausch and Lomb quartz spectrograph was used throughout these experiments. Its entire range from 2100 to 8000 Å. can be taken in one exposure on a 10-inch plate (Eastman Kodak Company No. 33 plates were used). Hilger echelon cells [Twyman, 1933; McDonald, 1936] with the liquid thicknesses ranging from 0.1 to 1.0 cm. were used. The curves show the absorption of light by a 1 cm. layer of the solutions under examination, i.e. $\epsilon = \frac{1}{d} \log \frac{I_0}{I}$, d being the layer thickness in centimetres. A water-cooled hydrogen arc served as a source of light [McDonald, 1936]. This light is nearly continuous, showing only a few lines near the visible part of the spectrum. The measurements were made at room temperature.

The positions of the absorption bands are correctly represented on the Figures; however, the extinction coefficients are not the absolute ones. In order to indicate the magnitude of the error in our values, we have determined the absorption spectrum of azobenzene, the absorption of which has been determined by Brode [1926]; the two curves are given in Fig. 12.

EXPERIMENTAL RESULTS

In Fig. 1 are to be seen the absorption spectra of some of the amino-acids which entered into the composition of the more complex compounds studied in this work. The formulae of these amino-acids are given below, the arrows indicating an increase in the absorption of ultraviolet light.

The absorption of light by a dipeptide, e.g. glycyl-*dl*-leucine, is greater than that of either or both of its constituent amino-acids (curves 1, 4 and 13; Fig. 2). When a hydrogen atom of the amino-group of either an amino-acid (α -aminoisobutyric acid) or a dipeptide (glycyl-*dl*-leucine) is replaced by an acetyl group, the absorption curve of the resulting derivative, compared with that of the original compound, lies on the side of the longer wave-lengths (curves 6 and 7; 13 and 19; Fig. 2).

When the hydrogen atom of the imino-group of an acetylamino-acid (acetyl- α -aminoisobutyric acid) is replaced by a methyl group, the absorption of ultraviolet light by the resulting compound (acetyl-*N*-methyl- α -aminoisobutyric acid) is greater than that by the original substance (curves 7 and 10; Fig. 3). The absorption of ultraviolet light by a benzoylamino-acid (benzoyl- α -aminoisobutyric acid) is much greater than the absorption by the corresponding acetylamino-acid (acetyl- α -aminoisobutyric acid) (curves 7 and 8; Fig. 3). The spectrum (Fig. 4) of a benzenesulphonylamino-acid (benzenesulphonyl- α -

Fig. 1. 1, glycine; 2, *dl*-alanine; 3, *dl*-valine; 4, *dl*-leucine; 5, *dl*- β -phenylalanine; 6, α -aminoisobutyric acid. 0.05 *M* in water.

Fig. 2. 1, glycine; 4, *dl*-leucine; 6, α -aminoisobutyric acid; 7, acetyl- α -aminoisobutyric acid; 13, glycyl-*dl*-leucine; 19, acetylglycyl-*dl*-leucine. 0.05 *M* in water.

Fig. 3. 7, acetyl- α -aminoisobutyric acid; 8, benzoyl- α -aminoisobutyric acid; 10, acetyl-*N*-methyl- α -aminoisobutyric acid. 0.01 *M* in water.

Fig. 4. 8, benzoyl- α -aminoisobutyric acid; 9, benzenesulphonyl- α -aminoisobutyric acid. 0.01 *M* in 95% EtOH.

Fig. 5. *a*, acetic acid and α -aminoisobutyric acid; *b*, acetyl- α -aminoisobutyric acid and α -aminoisobutyric acid; 7, acetyl- α -aminoisobutyric acid; 22, acetyl- α -aminoisobutyryl- α -aminoisobutyric acid. 0.01 *M* in water.

Fig. 6. 14, acetyl-*dl*-alanyl-glycine; 15, acetylglycyl-*dl*-alanine; 16, acetyl-*dl*-valylglycine; 17, acetylglycyl-*dl*-valine; 18, acetyl-*dl*-leucylglycine; 19, acetylglycyl-*dl*-leucine. 0.05 *M* in water.

Fig. 7. 15, acetyl-glycyl-*dl*-alanine; 20, acetyl-glycyl- α -aminoisobutyric acid; 21, acetyl-*dl*-alanyl- α -aminoisobutyric acid; 22, acetyl- α -aminoisobutyryl- α -aminoisobutyric acid; 0.05 *M* in water. 23, acetyl-*dl*- α -phenylalanylglycine; 24, acetyl-*dl*- α -phenylalanyl-*dl*-alanine; 25, acetyl-*dl*- α -phenylalanyl- α -aminoisobutyric acid. 0.01 *M* in 95% EtOH.

Fig. 8. 21, acetyl-*dl*-alanyl- α -aminoisobutyric acid; 25, acetyl-*dl*- α -phenylalanyl- α -aminoisobutyric acid; 26, acetyl-*dl*- β -phenylalanyl- α -aminoisobutyric acid. 0.01 *M* in 95% EtOH.

Fig. 9. 19, acetyl-glycyl-*dl*-leucine; 23, acetyl-*dl*- α -phenylalanylglycine; 24, acetyl-*dl*- α -phenylalanyl-*dl*-alanine; 26, acetyl-*dl*- β -phenylalanyl- α -aminoisobutyric acid; 27, acetyl-glycyl-*dl*-leucyl-anilide; 28, acetyl-*dl*- α -phenylalanylglycylanilide; 29, acetyl-*dl*- α -phenylalanyl-*dl* (or *ld*)-alanyl-anilide; 31, acetyl-*dl*- β -phenylalanyl- α -aminoisobutyryl-anilide. 0.01 *M* in 95% EtOH.

Fig. 10. 9, benzenesulphonyl- α -aminoisobutyric acid; 12, benzenesulphonyl- α -aminoisobutyrylamide; 25, acetyl-*dl*- α -phenylalanyl- α -aminoisobutyric acid; 30, acetyl-*dl*- α -phenylalanyl- α -aminoisobutyrylamide. 0.01 *M* in 95% EtOH.

Fig. 11. 8, benzoyl- α -aminoisobutyric acid; 11, benzoyl- α -aminoisobutyrylmethylamide. 0.01 *M* in water.

Fig. 12. 0.0066% azobenzene in 95% EtOH. A, Brode's curve; B, our curve.

aminoisobutyric acid) is quite different from that of the corresponding benzoyl-amino-acid (benzoyl- α -aminoisobutyric acid).

It is to be noted (curves *a* and 7; Fig. 5) that the absorption of light by an acetylamino-acid (acetyl- α -aminoisobutyric acid) is greater than the absorption by a mixture of equimolecular amounts of its constituent parts (acetic acid and α -aminoisobutyric acid). In all likelihood, the absorption of light by an acetyl-dipeptide would also be greater than that by an equimolecular mixture of acetic acid and the corresponding dipeptide. More interesting is the fact (curves *b* and 22; Fig. 5) that an acetyl-dipeptide (acetyl- α -aminoisobutyryl- α -aminoisobutyric acid) exerts a greater absorption than the equimolecular mixture of the constituent parts consisting of acetylamino-acid (acetyl- α -aminoisobutyric acid) and amino-acid (α -aminoisobutyric acid).

The absorption curves of 6 acetyl-dipeptides are given in Fig. 6. Invariably, the acetyl-dipeptide in which the amino-acid of higher molecular weight (*dl*-alanine, *dl*-valine, *dl*-leucine) occupies the central position of the chain (formula I) shows less absorption than its position isomeride (formula II). The arrow indicates an increase in the absorption of ultraviolet light.

In Fig. 7 (curves 15, 20, 21, 22) is seen the effect of progressive introduction of methyl groups into acetyl-dipeptides. The absorption increases as the methylene hydrogen atoms of the peptide chain are replaced by methyl groups:

The same variation in absorption of light with the introduction of methyl groups is seen in Fig. 7 (curves 23, 24, 25) in another series of acetyl-dipeptides, the formulae of which are given below:

When a hydrogen atom of an acetyl-dipeptide containing only aliphatic radicals is replaced by a phenyl group, the absorption of ultraviolet light is increased and characteristic absorption bands are produced (Fig. 8). Introduction of the phenyl group into the *alpha* position of the alanyl unit of acetyl-*dl*-alanyl- α -aminoisobutyric acid produces 3 bands, while introduction of the phenyl group into the *beta* position of the same unit produces 5 bands. It is to be noted that the absorption of light by the α -phenyl derivative is greater than that by the corresponding β -phenyl derivative.

When the hydroxyl of the carboxyl group of an acetyl-dipeptide is replaced by an NHC_6H_5 residue, a marked increase in absorption results and there are two characteristic bands produced (at approximately 2800 and 2450 Å.), regardless of whether the acetyl-dipeptide is composed of aliphatic or aromatic amino-acids. This is shown in Fig. 9 (curves 19 and 27; 23 and 28; 24 and 29; 26 and 31). However, when the hydroxyl of the carboxyl group of an acetyl-dipeptide or of a benzenesulphonylamino-acid is replaced by an NH_2 residue (curves 25 and 30; 9 and 12; Fig. 10) there is only a slight change in absorption. Such slight change in absorption is also observed upon replacement of the hydroxyl of the carboxyl by an NHCH_3 residue (Fig. 11).

SUMMARY

The absorption spectra of some amino-acids and their derivatives have been determined.

The absorption of light by a dipeptide is greater than that of either or both of its constituent amino-acids.

When a hydrogen atom of the amino- or imino-group of either an amino-acid or of a dipeptide is replaced by an acetyl, benzoyl, or benzenesulphonyl group, the absorption curves of the resulting compounds when compared with those of the original compounds lie on the side of the longer wave-lengths.

In comparing the absorption of light by isomeric acetyl-dipeptides it was found that greater absorption occurs with the isomeride in which the amino-acid of higher molecular weight occupies the terminal position in the chain.

The absorption of light by acetyl-dipeptides increases as the methylenic hydrogen atoms of the peptide chain are replaced by methyl groups.

When a hydrogen atom of an acetyl-dipeptide is replaced by a phenyl group, the absorption of ultraviolet light is increased and characteristic absorption bands are produced.

When the hydroxyl of the carboxyl group of an acetyl-dipeptide is replaced by an NHC_6H_5 residue, a marked increase in absorption results. Replacement of the hydroxyl group by an NH_2 or NHCH_3 group causes only a slight change in absorption.

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