SPECTROSCOPICAL STUDY OF AMINO-ACID ANHYDRIDES. II. · LIGHT ABSORPTIONS OF SOME AMINO-ACIDS, THEIR ESTERS, PEPTIDES AND ANHYDRIDES.[1]

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Received November 24 1927. Published December 28, 1927.

In the preceding paper[2] we have described the results of spectrochemical studies on the constitution of some diketopiperazines, which are regarded, from the experimental and theoretical points of view of the recent protein chemistry, as the important fundamental molecules of protein substances. The conclusion, at which we have experimentally arrived, was that glycine anhydride, alanine anhydride and sarcosine anhydride are in the keto-form only, although they are often considered to take possibly the enolic form. Quite recently, however, E. Abderhalden and E. Schwab[3] have synthesised these compounds which have surely the enolic form, in using the other mode of preparation than those generally known.[4] This latter fact, that is to say, the new samples of diketopiperazines obtained by Abderhalden are not identical in their constitution with those hitherto known, seems to us to be in good accordance with our conclusion just mentioned.

In the present work, we have further carried out spectrochemical studies on various amino-acids, their esters, dipeptides and anhydrides. Among these compounds, tyrosine and phenylalanine containing chromophore groups in their molecules absorb selectively in ultraviolet: the absorption maximum of tyrosine exists at 3580 of frequency (Fig. 3), while those of phenylalanine are observed at 3780 and 3900 of frequencies (Fig. 1). These absorptions remain unchanged even when substitutions occur in the molecules of those amino-acids, so far as the substituents themselves contain no chromophores. Only phenylalanine anhydride shows an exceptional results: it has an end absorption only[5] and the characteristic absorption bands of phenylalanine are thus vanished on anhydride formation (Fig. 1).

(1) Read before the annual meeting of the Chemical Society of Japan, April 5, 1927.

(2) This journal, **1** (1926), 71.

(3) *Z. physiol. Chem.*, **149** (1925), 100 & 298; **152** (1926) 80. Abderhalden, u. Gebelein, ibid., **152** (1926), 125.

(4) Compare E. Fischer, "Untersuchungen über Aminosäuren, Polypeptide und Proteine." Maillard, *Comp. rend.*, **153** (1911), 1078. Balbiano, *Ber.* **33** (1910), 2323; **34** (1911), 1501. K. Shibata, *Acta Phytochim.*, **2** (1925), 39.

(5) It is well known that diphenyl and triphenyl carbinol give no selective absorptions, although they contain two or more benzene nuclei in their molecules [Baly and Tuck, *J. Chem. Soc.*, **93** (1908), 1913. Baker, ibid., **91** (1907), 1495].

As for the absorption of tyrosine, we may expect that it will show an analogy with that of phenol, for the former contains phenol nucleus in its molecule. In fact, it was proved to be the case by Ward[1] and Blyth[2] and further it was observed by Stenström and Reinhard[3] that, when tyrosine and phenol were respectively dissolved in media with varying H-ion concentrations, the both substances display analogous changes in their extinction coefficients. Our present spectrochemical investigation confirmed too the results obtained by these authors.

Except tyrosine and phenylalanine, we have found that so far as our study concerns, there are neither amino-acids nor their derivatives which absorb selectively in any region of spectrum.

Experimentals.

Amino-acids.—*Glycocoll.* After careful recrystallisation from water, was studied in 1 mol solution in using the Adam-Hilger's quarz spectrograph; its spectrogram showed no selective absorption.

Alanine. Kahlbaum's synthetical pure material was recrystallised from water; its 0.1 mol solution gave no selective absorption.

Leucine. Crude *l*-leucine obtained by hydrolysis of gluten was recrystallised several times from water and dilute alcohol; 0.01 mol solution of this substance gave no selective absorption.

Phenylalanine. *l*-Phenylalanine obtained also by hydrolysis of gluten was purified as in the previous case; so purified sample showed the rotatory power $[\alpha]_D = -34°$, and absorption maxima at 3780 and 3900 of frequencies in 0.01 mol solution (Fig. 1). Synthetical racemic phenylalanine gave the identical absorption with the active body.[4] The analogous phenomenon has previously been observed by one of the present authors (Y. Shibata) in the spectrochemical investigation of metallic complex salts; i.e., he found that the optically active cobalt-ammines absorb identically with their racemic modifications.

(1) *Biochem. J.*, **17** (1923), 891.
(2) *J. Chem. Soc.*, **75** (1899), 1162.
(3) *J. Phys. Chem.*, **29** (1915), 1477.
(4) Racemic phenylalanine procured from Kahlbaum, when it was examined without any purification, gave thoroughly different absorption from the active substance (Fig. 2). On exhaustive extraction with ether we have obtained from this Kahlbaum's sample certain quantities of white non-nitrogenous substance with the melting point 132–133°. On removing this impurity, the racemic body showed quite the same absorption with the active compound (Fig. 6).

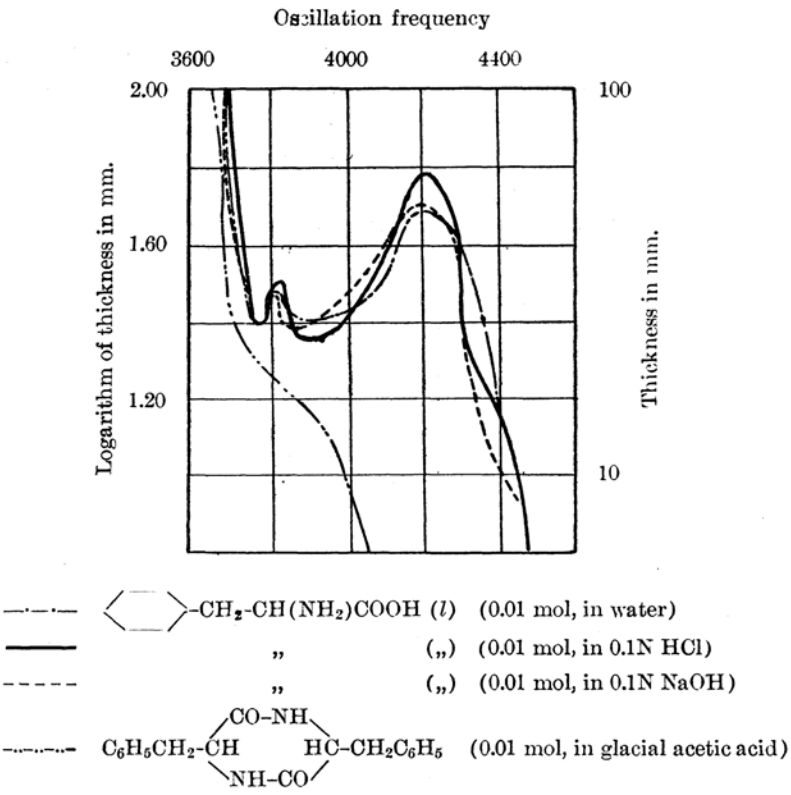


Fig. 1.

Tyrosine. *l*-Tyrosine was repeatedly recrystallised from water and examined; its absorption is mentioned in Fig. 3. It is known that, tyrosine shows, like phenol, different absorptions according to the varying P_H-value of the medium, especially in the alkaline side.[1] We have carried out, therefore, comparative studies between these two substances, both in 0.001 mol solution in 0.1 N sodium hydroxide and pure water. On changing the proportion between alkaline solution and water, i.e. on varying the P_H-value of the medium, shifts of absorption bands, quite parallel in both substances, were observed (Fig. 3, 4 and 5). It is obvious, therefore, that, the absorption of tyrosine is certainly due to the phenol nucleus in its molecule.

(1) Stenström and Reinhard, loc. cit.

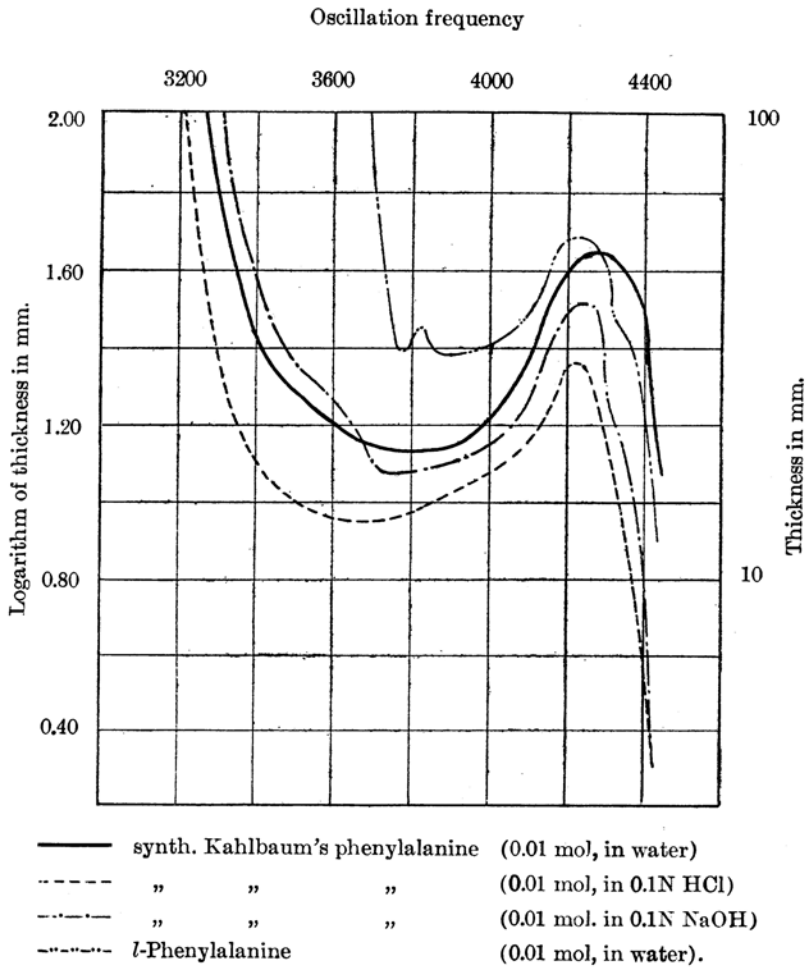
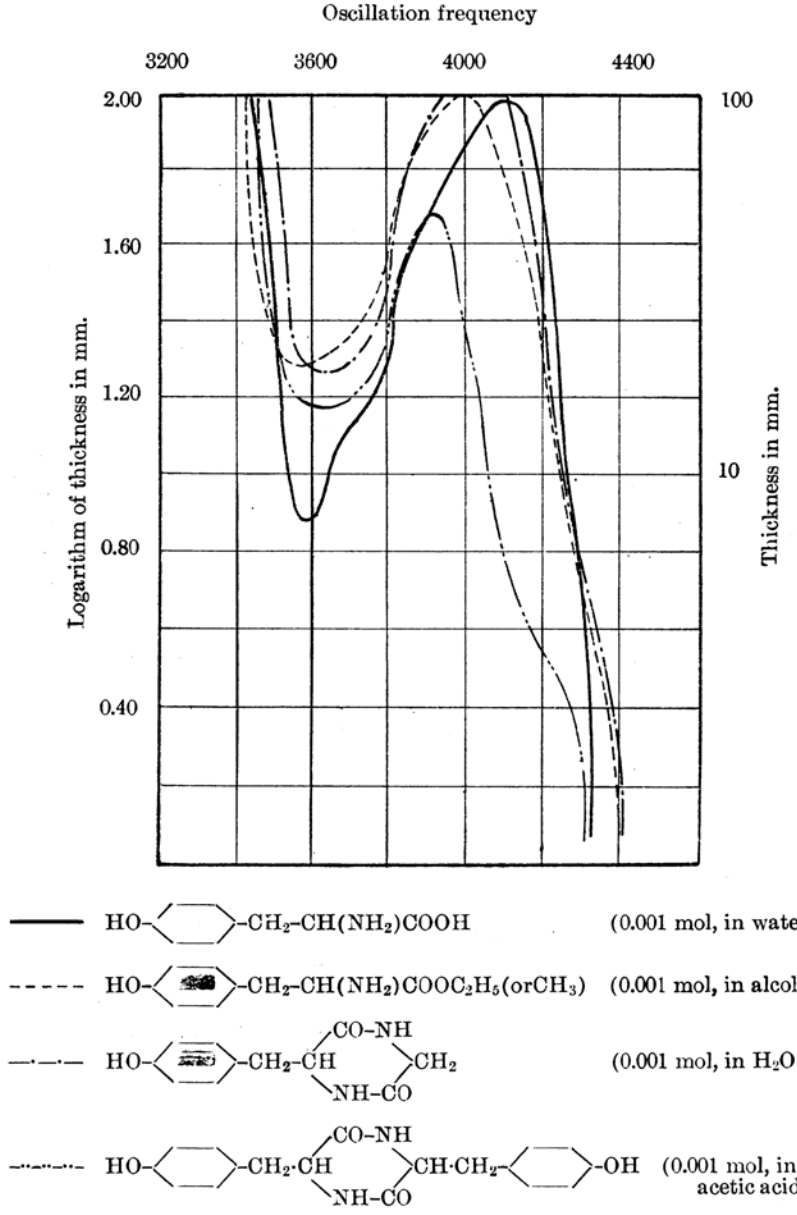

Oscillation frequency
3200
3600
4000
4400
2.00
1.60
1.20
0.80
0.40
Logarithm of thickness in mm.
100
10
Thickness in mm.
synth. Kahlbaum's phenylalanine (0.01 mol, in water)
" " " (0.01 mol, in 0.1N HCl)
" " " (0.01 mol. in 0.1N NaOH)
l-Phenylalanine (0.01 mol, in water).

Fig. 2


Oscillation frequency
3200
3600
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4400
2.00
1.60
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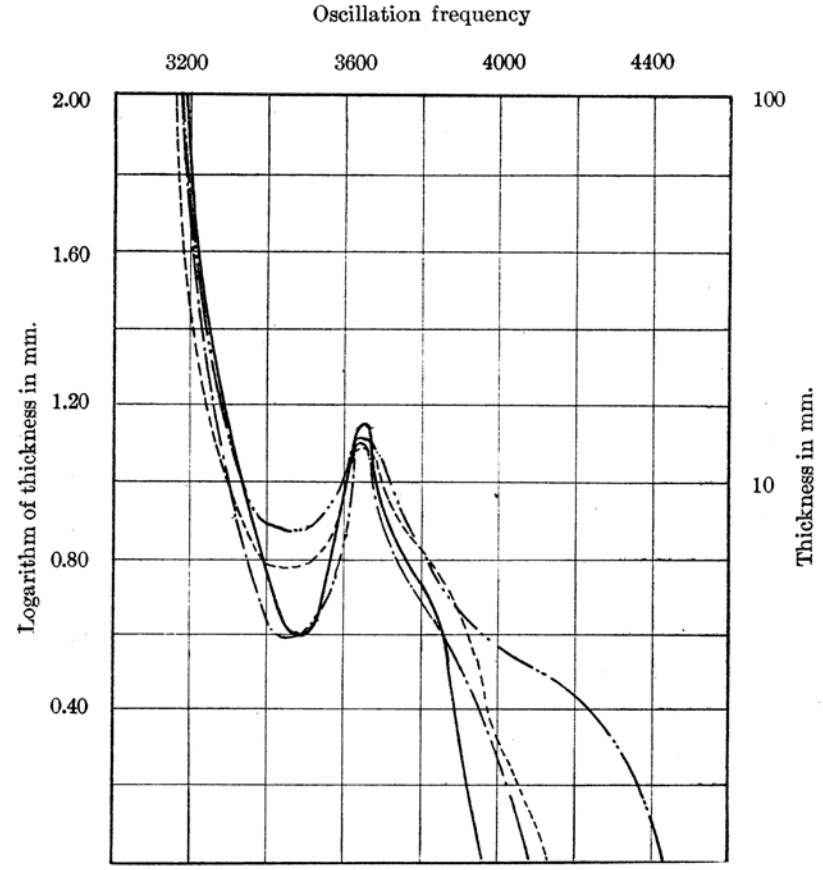
0.80
0.40
Logarithm of thickness in mm.
100
10
Thickness in mm.
HO-C6H4-CH2-CH(NH2)COOH (0.001 mol, in water)
HO-C6H4-CH2-CH(NH2)COOC2H5(orCH3) (0.001 mol, in alcohol)
HO-C6H4-CH2-CH(CO-NH, NH-CO)CH2 (0.001 mol, in H2O)
HO-C6H4-CH2·CH(CO-NH, NH-CO)CH·CH2-C6H4-OH (0.001 mol, in glacial acetic acid)

Fig. 3.



——— HO-C$_6$H$_4$-CH$_2$-CH(NH$_2$)COOH (0.001 mol, in 0.1N NaOH)

– – – – – HO-C$_6$H$_4$-CH$_2$-CH(NH$_2$)-COOC$_2$H$_5$(or CH$_3$) („)

—·—·— HO-C$_6$H$_4$-CH$_2$-CH(NH-CO / CO-NH)CH$_2$ („)

—·—·—·— HO-C$_6$H$_4$-CH$_2$-CH(CO-NH / NH-CO)CH-CH$_2$-C$_6$H$_4$-OH („)

Fig. 4.

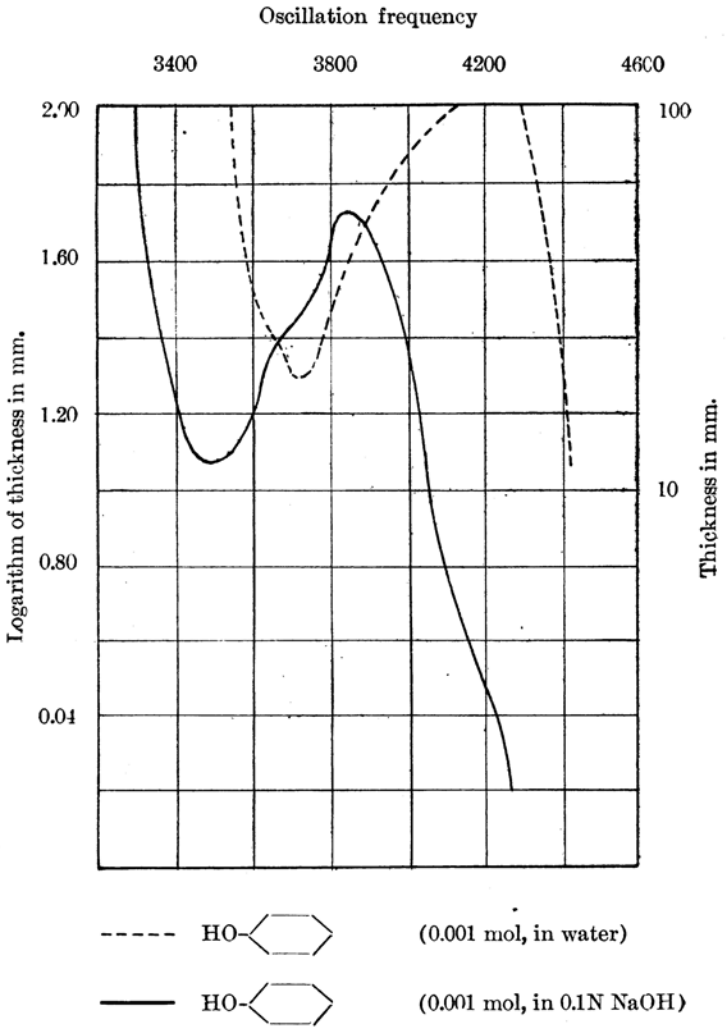

Oscillation frequency
3400
3800
4200
4600
2.00
1.60
1.20
0.80
0.04
Logarithm of thickness in mm.
100
10
Thickness in mm.
HO-
(0.001 mol, in water)
HO-
(0.001 mol, in 0.1N NaOH)

Fig. 5.

Esters of Amino-acids.—*Glycocoll ester.* Its hydrochloride was first prepared according to the method described by V. Auger[1] and it was then transformed into ester.[2] The solution of ester in alcohol which had been freshly redistilled over sodium gave no selective absorption.

Tyrosine esters, Ethyl and methyl esters of tyrosine were prepared according to mainly the methods given by E. Fischer[3] and his collaborators, only slight modifications being applied to it.

Ethyl-ester (m.p. 108°) and methyl-ester[4] (m.p. 133.5–134.5°) thus prepared and carefully purified, were dissolved in ethyl alcohol, both in 0.001 mol solution. Their absorption spectrograms coincide with each other (Fig. 3 and 4) and the shifts of these bands in alkaline solutions with the same P_H-value are also the same in both cases.

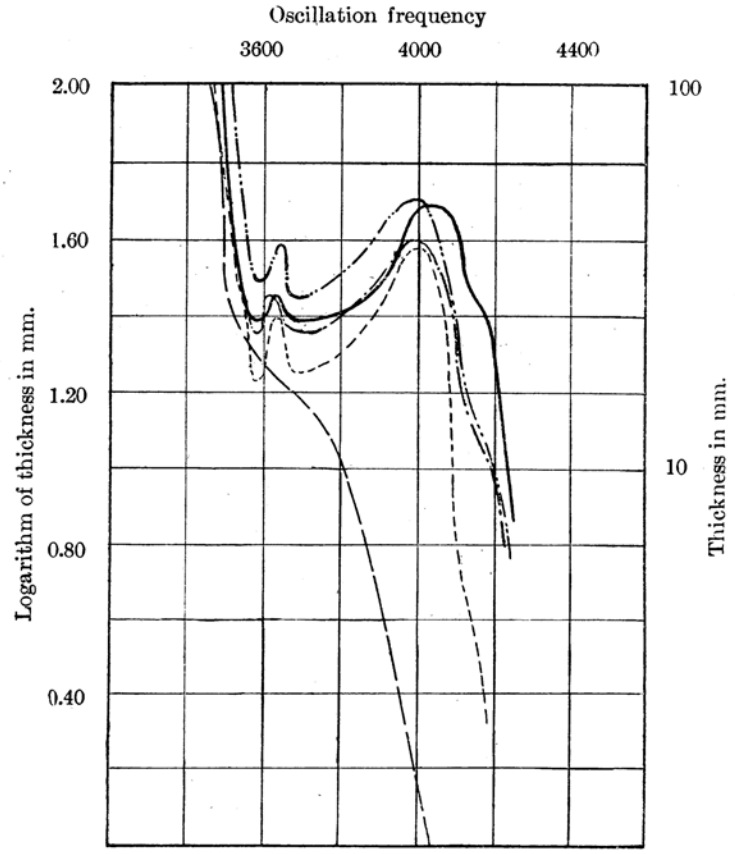
Dipeptides.—*Glycylleucine.* This substance was prepared according to the method given by E. Fischer[5]; the crude sample thus obtained was once dissolved in a small bulk of water and rendered to crystallise out again by adding some quantities of 90% ethyl alcohol to the solution. 0.01 Mol aqueous solution of glycyl-*l*-leucine so purified gave no selective absorption.

Glycyl-l-phenylalanine. This substance was also prepared after Fischer's method[6] and purified by repeating the alternative dissolution in water and precipitation by adding alcohol. 0.01 Mol solution of this peptide gave the absorption identical with that of *l*-phenylalanine (Fig. 6).

Glycyl-d,l-phenylalanine. This peptide was prepared in the same manner as the case of active body, in using Kahlbaum's racemic phenylalanine as one component. The spectrogram of the solution of this substance was also identical with both the active compound and phenylalanine itself (Fig. 6).

Anhydrides.—*Glycyl-l-tyrosine anhydride.* This substance was prepared according to the method described by Fischer and Schrauth,[7] and spectroscopically studied in neutral and alkaline solutions; the former solution being made by dissolving 0.0110 gr. of the anhydride in 50 c.c. of pure water, while in the latter the same quantity of the substance being contained in 50 c.c. of 0.1 N sodium hydroxide solution. The absorptions of these two solutions were found to be identical respectively with the corresponding solutions of tyrosine itself (Fig. 3 and 4).

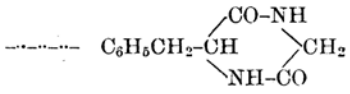
(1) *Bull. soc. chim.*, [3], **21** (1899), 5.
(2) E. Fischer, *Ber.*, **34** (1901), 436.
(3) *Ber.*, **34** (1901), 433.
(4) E. Fischer u. Schrauth, *Ann.*, **354** (1907) 21.
(5) E. Fischer u. J. Steingroever, *Ann.*, **340** (1905) 157.
(6) Fischer u. Schoeller, *Ann.*, **357** (1907), 1.
(7) Fischer u. Schrauth, *Ann.*, **354** (1907), 21.



——— $C_6H_5CH_2CH(NH_2)COOH(d,l)$ (0.01 mol, in water)

– – – – – NH_2CH_2–CO–(*l*)–NH–CH(–$CH_2C_6H_5$)–COOH (0.01 mol, in water)

—·—·— NH_2–CH_2–CO(*d,l*)–NH–CH(–$CH_2C_6H_5$)–COOH („)

—·—·—·—  („)

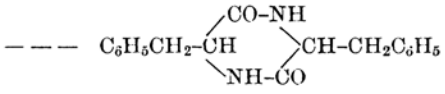
———  (0·01 mol, in glacial acetic acid)

Fig. 6.

Tyrosine anhydride. This anhydride was prepared in two ways: we have namely followed faithfully the preparatory method given by Fischer and Schrauth,[1] and, on the other hand, tyrosine was heated in 8 times of

(1) Fischer u. Schrauth, *Ann.*, **354** (1907), 21.

its weight of glycerol at 170°–180° for 25 hours. The glycerol solution so treated was added with alcohol in order to let separate out the desired anhydride, which was washed with hydrochloric acid to remove unchanged tyrosine and then was recrystallised from 25% ammonia solution.

These two samples,[1] however, gave the same spectrogram and further the shifts of their absorption bands in alkaline media were identical between them (Fig. 3 and 4), all these absorptions not differing from those of tyrosine itself and its derivatives.

Glycyl-l-leucine anhydride. 1 Gr. of glycyl-*l*-leucine was heated in 8 gr. glycerol at 170°–180° for 9 hours[2]; crude anhydride thus obtained was carefully recrystallised from water (found, N=16.8, calc., 16.5%) and examined in 0.01 mol aqueous solution. This solution gave no selective absorption.

Leucine anhydride. 9 Gr. leucine was well shaken with 20 Gr. glycerol and the mixture was heated for 20 hours at 160°–170°. After cooling, a little water was added to the liquid and yellowish crystals were drained, and well washed with hot water in order to remove the adhering amino-acid (the yield of the substance was 6.6 gr., i.e., 85% of theory). In starting from racemic leucine and in following the same mode of preparation just described, leucine anhydride was prepared. (Found, N=12.5, calc., 12.4%) 0.005 Mol alcoholic solutions of both samples gave the same spectrogram without any selective absorption.

Glycyl-l-phenylalanine anhydride. This substance was prepared according to the method described by Fischer and Schoeller.[3] 0.01 Mol aqueous solution of this anhydride gave the identical absorption either with phenylalanine or with glycylphnylanine.

Phenylalanine anhydride. 3 Gr. *l*-phenylalanine suspended in 10 gr. glycerol was heated in an oil bath at 180°–200° for 3 hours, until the suspended matters were entirely disappeared and a clear solution with slightly yellowish colour was made. From this solution, after about half an hour, anhydride began to separate out as yellowish crystalline masses, which were drained, well washed with hot water and recrystallised from boiling alcohol in addition of animal charcoal. So purified colourless crystals of phenylalanine anhydride (found, N=9.58, calc., 9.53%) were dissolved in glacial acetic acid (0.01 mol). The spectrogram of this solution, as was remarked in the introductory part, gave no selective absorption. Only a few substances are known, which show no selective absorption, in spite of that they contain more than one benzene nucleus in their molecules. This anomality in light absorption of the substances of this category, such as diphenyl

(1) Glacial acetic acid was used as solvent.

(2) Compare Abderhalden und Schwab, *Z. physiol. Chem.*, **148** (1925), 254.

(3) Fischer und Schoeller, loc. cit.

and some of triphenyl compounds probably is due to the relative position of phenyl groups in molecule.

Summary.

(1) Glycocoll, alanine, leucine, phenylalanine, tyrosine, glycocoll ester, tyrosine esters, glycylleucine, glycyl-*l*-phenylalanine, glycyl-*d*,*l*-phenyl alanine, glycyl-*l*-tyrosine anhydride, tyrosine anhydride, glycyl-*l*-leucine anhydride, leucine anhydride, glycyl-*l*-phenylalanine anhydride and phenylalanine anhydride were spectrochemically studied in aqueous, alcoholic or glacial acetic acid solutions.

(2) Among these compounds only those which contain phenyl and oxy-phenyl groups in their molecules show selective light absorptions; i.e., tyrosine, phenylalanine and their derivatives, except phenylalanine, anhydride absorb selectively.

(3) Although two phenyl groups exist in its molecule, phenylalanine anhydrid show only end absorption, perhaps due to the mutual effect of these two chromophores symmetrically situated to each other.

(4) Derivatives of either tyrosine or phenylalanine absorb identically with their mother substances.

(5) Other amino-acids, their esters, pepetides and anhydrides show no selective absorptions, as in the cases of glycocoll anhydride, alanine anhydride and sarcosine anhydride which were described in our previous paper.

(6) As for the constitution of amino-acid anhydrides, the same conclusion as was previously mentioned, may be applied again to the substances now studied, that is to say, all these anhydrides are in keto-form.

The experiments are going on with other amino-acid compounds and anhydrides. The authors' best thanks are due to Prof. Keita Shibata who was not unwilling to make valuable remarks and also to Dr. Yoshitaro Takayama who kindly supplied them with amino-acids. The cost of the present investigation was defrayed from the grant of the Imperial Academy of Science, for which they wish to record their gratefulness.

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