XXVIII. NOTE ON TYROSINE.

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Reference has already been made to a preliminary observation that tyrosine is not methylated by diazomethane in ethereal suspension [Geake and Nierenstein, 1914, 1, p. 292]. The present communication confirms this observation, which is of interest both for the chemistry of methylo-caseinogen [Geake and Nierenstein, 1914, 1], since this substance gives Millon's reaction, and for that of tyrosine. The fact that the carboxyl group of tyrosine is not methylated is quite in accordance with our experiences with other amino acids [Geake and Nierenstein, 1914, 2] which have led us to accept the "betaine" formula [Meyer and Jacobson, 1913]

for amino acids. It has further also been found that an hydroxyl-group in para-position to a nitrogen atom is not methylated by diazomethane, as for example in the cases of γ -pyridone, chelidamic and cuminaminic acids [Pechmann, 1895, Meyer, 1906, 1 and 2]. These substances yield unchanged γ -pyridone, the dimethyl ester of chelidamic acid and the methyl ester of methoxy-cuminaminic acid.

Meyer expresses the influence of the nitrogen atom on the hydroxyl group by dotted lines, but emphasises at the same time as follows: "das Wesen der Erscheinungen wird aber natürlich durch derlei Zeichensprache nicht im mindestens enthüllt und man kann nur den in jüngster Zeit wiederholt

gezogenen Schluss rekapitulieren, dass die derzeit herrschenden Anschauungen über Valenz nicht ausreichen, die Gesamtheit der Beobachtungen zu erklären." In this connection reference ought also to be made to the classical investigations of Kaufmann [1911] on valency with which our observations are in full accord.

The following formula therefore expresses the behaviour of tyrosine towards diazomethane:

In view of the fact that the hydroxyl group in the meta-position of cumininamic acid undergoes methylation when treated with diazomethane an experiment was also made with 3:4-dihydroxyphenylalanine [Funk, 1911] to which the formula:

is assigned. The reaction was accompanied by a strong evolution of nitrogen, which indicated that apparently methylation had taken place, especially as nitrogen is not evolved when tyrosine is treated with diazomethane. The material kindly sent by Dr Funk was, however, so small in quantity that we were not able to isolate any definite product. We intend to repeat the experiment on a larger scale later on, since dihydroxyphenylalanine is now more accessible through its isolation from *Vicia faba* by Guggenheim [1913].

Since the carboxyl groups of the amino acids and the peptides become accessible to methylation on acylation, when the "betaine"-ring is opened [Geake and Nierenstein, 1914, 2], the action of diazomethane on glycy-tyrosine was also investigated especially with the view of studying the behaviour of the tyrosine hydroxyls in caseinogen towards diazomethane. The product thus obtained was the glycy-tyrosine methyl ester, as we were

able to recover on hydrolysis glycyl-tyrosine and on oxydation *p*-hydroxybenzoic acid. It is apparent that the nitrogen atoms of the glycyl-tyrosine

continue to have an influence on the hydroxyl-group, which leads us, at least for the time-being, to the conclusion *that the tyrosine hydroxyls in methylo-caseinogen are not methylated by diazomethane.*

EXPERIMENTAL.

Action of diazomethane on l-tyrosine. One g. tyrosine from hair (m.p. 303–306°) was treated with 0.5 g. (2 mol.) diazomethane; no visible reaction took place and 0.85 g. unchanged tyrosine (m.p. 303°) was recovered. In another experiment 0.2 g. tyrosine from caseinogen and 0.1 g. (2 mol.) diazomethane were used and 0.2 g. unchanged tyrosine (m.p. 306°) recovered.

Action of diazomethane on dl-3 : 4-dihydroxyphenylalanine. 0.2 g. dihydroxyphenylalanine was treated with 0.25 g. (4 mol.) diazomethane. The substance dissolved with evolution of gas, leaving a residue (0.04 g.) which after recrystallisation from alcohol and water formed small needles which melted at 225°. It is possible that this was unchanged dihydroxyphenylalanine for which the following melting points are given: 238° (Funk) and 280° (Guggenheim). The ethereal solution gave an oil which we have not investigated.

Action of diazomethane on glycyl-l-tyrosine. One g. glycyl-l-tyrosine prepared according to Abderhalden and Oppler [1907] was treated with 1 g. diazomethane; on evaporation of the ether a solid was left, which crystallised from methyl alcohol in small needles and melted at 123–124°. The *glycyl-l-tyrosine methyl ester* thus obtained dissolves easily in alcohol, acetone and to some extent also in water. If the ester is melted, cooled and then remelted the melting point rises to about 295–300°, glycyl-l-tyrosine anhydride being formed during the melting process as in the case of leucyl-l-tyrosine [Fischer and Schrauth, 1907].

Analysis: 0.1488 g.: 13.2 cc. N₂ (moist) 19°, 772 mm.

0.3038 g.: 27.3 cc. N₂ (moist) 16°, 770 mm.

0.1206 g.: 0.2546 g. CO₂, 0.0722 g. H₂O.

0.1222 g.: 0.2558 g. CO₂, 0.0672 g. H₂O.

		Cal. for C ₁₂ H ₁₆ O ₄ N ₂
C ..	57.60, 57.09 %	C .. 57.14 %
H ..	(8.43), 7.06 %	H .. 6.35 %
N ..	10.32, 10.58 %	N .. 10.94 %

Hydrolysis of glycyl-L-tyrosine methyl ester. One g. of the ester was dissolved in 10 cc. alcohol and warmed on the water bath with 2.5 cc. N/10 alcoholic potash. It was then diluted with 10 cc. water and acidified with 2.5 cc. N/10 hydrochloric acid and the solid filtered off. The product consisted of glycyl-L-tyrosine and glycyl-L-tyrosine anhydride, which were separated by shaking with dilute hydrochloric acid. The anhydride was crystallised from water and melted at 293–297°. The glycyl-L-tyrosine recovered from the hydrochloride crystallised from dilute alcohol in needles, melting at 183°, the melting point not being depressed by glycyl-L-tyrosine; mixed melting point found, 182–183°.

Oxidation of glycyl-L-tyrosine methyl ester. Two g. of the ester were dissolved in 20 cc. of 30 per cent. potassium hydroxide and boiled for 3–4 hours with an excess of potassium permanganate. When acidified with hydrochloric acid a small precipitate was obtained which after several recrystallisations from water formed small prismatic needles, melting at 213–215° (m.p. of *p*-hydroxybenzoic acid, 213–215°). A mixed melting with this acid gave 213–215°. For further identification 0.3 g. of the oxidation product was also converted with dimethyl sulphate and alcoholic potash into anisic acid (m.p. found, 186°; m.p. of anisic acid, 184°).

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