CXXVIII. A NEW HYDROLYSIS PRODUCT FROM ELASTIN.

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It is generally believed that Millon's reaction obtained with proteins is due to the presence of tyrosine. However, such a substance as gelatin also gives this reaction, although no tyrosine is formed upon hydrolysis. Similar conditions prevail in the case of elastin, which in its chemical composition shows some relation to gelatin; no tyrosine could be isolated from the crystallised crude leucine originally obtained from elastin in a joint investigation by W. Biehler and myself¹. Upon examination of another sample of elastin only such a small amount as 0.15 % of crude tyrosine could be found, which hardly explains the strong Millon reaction obtained with the original material.

It occurred to the author therefore that, in addition to tyrosine, other substances, thus far unknown as decomposition products, also give Millon's reaction. This suspicion was fully verified in the case of elastin. First, it was shown that the basic portion, precipitated from the hydrolysis products by phosphotungstic acid, gave the reaction, even when the precipitate was treated in a manner which precluded any retention of the filtrate. The substance yielding the reaction possesses therefore pronounced basic properties. It was isolated in the following manner.

Air-dry elastin was boiled for 5 hours with about five times the quantity of concentrated hydrochloric acid, and the liquid evaporated upon the water-bath to a syrupy consistence. This was diluted with sufficient water to bring the weight of the liquid to about eight times the weight of elastin used. 50 % phosphotungstic acid was then added and the precipitate after standing for several hours carefully washed or triturated with 5 % sulphuric acid.

This precipitate, as stated in greater detail in the above-mentioned communication¹, was treated with lead hydroxide and after careful washing decomposed with lukewarm baryta solution in the well-known manner. Overheating or great excess of baryta was carefully avoided by adding the baryta solution very slowly in small portions and removing it by suction, when the reaction had remained alkaline for several hours. The residue was treated twice in the same manner. The decomposition took about 2 days and the solution of the basic substances contained only traces of excess of baryta as well as some lead. The lead, after addition of animal charcoal, was removed

¹ R. Engeland and W. Biehler, "New Decomposition Products of Elastin," to be published shortly.

with hydrogen sulphide, and the filtrate was evaporated *in vacuo* to a thin syrup, then boiled with copper hydroxide and filtered. The filtrate was again greatly concentrated, mixed with methyl alcohol until a strong turbidity occurred and set aside at 0° for several days. A considerable precipitate was then noted, partly crystalline, partly consisting of brown flakes. These flakes, on account of their low specific gravity, could mostly be washed off with ice-cold water. The deep blue residue, very slightly soluble in water, was suspended in a little water, and freed from copper by passing hydrogen sulphide through the heated liquid. Upon concentration of the slightly coloured filtrate, long white needles were obtained which were recrystallised from dilute methyl alcohol.

2.782 mg. subst. :	5.997 mg. CO ₂ and 1.901 mg. H ₂ O		
2.470 mg. „ :	5.328 mg. CO ₂ and 1.536 mg. H ₂ O		
3.426 mg. „ :	0.277 cc. N at 708 mm. 11°		
Calculated for C ₁₆ H ₂₄ O ₅ N ₂ :	C=59.22 %, H=7.46 %, N=8.64 %		
Found	C=58.83 %, H=7.65 %, N=9.06 %		
	58.85 %, 6.96 %		

The values calculated for formulae with 15 C-atoms would also be very close to those found:

Calculated for C ₁₅ H ₂₂ O ₅ N ₂ :	C=58.03 %, H=7.15 %, N=8.41 %		
C ₁₅ H ₂₀ O ₅ N ₂	C=58.41 %, H=6.54 %, N=9.09 %		

However the values found for the copper salt strongly suggest the formula with 16 carbon-atoms. The copper salt forms small, dark blue needles, which are very sparingly soluble in water. The crystals purified by crystallisation from hot water gave the following analysis:

(1) 4.524 mg. subst. :	0.950 mg. CuO			
(2) 3.388 mg. „ :	0.691 mg. CuO			
4.350 mg. „ :	7.875 mg. CO ₂ and 2.240 mg. H ₂ O*			
3.960 mg. „ :	0.275 cc. at 723 mm. and 18°*			
Found:	Cu=16.78 %, C=49.39 %, H=5.76 %, N=7.75 %			
	16.30 %			
Calculated for C ₁₆ H ₂₂ O ₅ N ₂ Cu:	Cu=16.48 %, C=49.74 %, H=5.75 %, N=7.38 %			

* These two analyses were kindly carried out by Dr F. Reindl.

The free substance tastes slightly bitter, and is rather readily soluble in water as well as in acetic (glacial) acid. The aqueous solution upon evaporation on the water-bath is coloured slightly brownish and gives off a flower-like, very characteristic odour. Upon treatment with oxidising agents such as NaClO₃ and HCl this odour may be obtained from very small quantities. Upon boiling with concentrated nitric acid a slight yellow coloration is observed, which is intensified when ammonia is added in excess. Millon's reagent produces even in the cold a pink colour, which however is not so intense as with tyrosine. With diazobenzenesulphonic acid in alkaline (Na₂CO₃) solution an orange coloration is observed. An infusion of the conidiophores of two different forms of *Russula* rapidly colours the solution, in presence of air, red with a blue tinge. After several hours the colour changes to bluish brown. The intensity of this colour is seemingly more pronounced than that obtained in the same way with tyrosine. By phosphotungstic acid the substance is precipitated even from very dilute solution. Upon heating

in the melting point apparatus the substance begins to be discoloured at about 200° and sinters at about 220°.

The substance was isolated by the same method from two different hydrolyses. I consider the substance isolated, therefore, as a constant hydrolysis product of elastin and call it *hyphasmine* (from τὸ ὑφασμα = the tissue).

The yield amounted to almost 1 %, when very carefully purified elastin was used, and was very much less when another less purified product was examined. Inasmuch as the solution of the substance is decomposed upon heating, it is certain that a considerable portion, originally present, is lost through the method of preparation described above.

With the same method I have tested another material, namely, the membrane of hen's eggs, which also upon hydrolysis yields no tyrosine. However, the substance could not be isolated, nor could I isolate the two new hydrolysis products which were obtained in the investigation jointly carried out with W. Biehler and referred to above.

Concerning the constitution of hyphasmine, one could be tempted to think that we have here a dipeptide, and that tyrosine takes part in its formation. The second component would thus be a hitherto unknown amino-acid—and the compound be characterised by a peculiarly strong resistance to hydrolysis. The composition of the copper salt, however, seems to speak against such an assumption.