2. 4,8,9,10-Tetrachloro-2,6-dithia-adamantan V: 5,2 g III (0,025 *M*) werden in 35 ml Benzol gelöst und mit 5,2 g Schwefeldichlorid (0,05 *M*) versetzt. Danach wird 17 h zum Siedem erhitzt, im Vakuum eingeengt und der Rückstand in Äther suspendiert. Man saugt die abgeschiedenen Kristalle ab und reinigt das Rohprodukt durch Umkristallisation aus Benzol oder durch Sublimation. Smp. 225–226°, lässt sich bei ~ 150 bis 160°/10 Torr sublimieren. Bei erhöhter Temp. tritt campferartiger Geruch auf. Ausbeute: 1,5–1,7 g (20–22% bez. auf III). UV-Spektrum (MeOH): $\lambda_{max} = 235$ nm ($\log \varepsilon = 2{,}81$). IR-Spektrum (KBr): Banden bei 1324, 801 und 683 cm^{-1}. $C_8H_8Cl_4S_2$ (310,09) Ber. C, 30,99, H, 2,60, Cl, 45,73, S, 20,68%. Gef. C, 31,21, H, 2,59, Cl, 45,71, S, 20,41%.

Summary. Transannular addition of one mole sulphur dichloride to cyclooctatetraene yielded 2,6-dichloro-9-thiabicyclo-[3.3.1]-3,7-nonadiene. Transannular addition of a second mole sulphur dichloride led to the tetrachlorinated 2,6-dithia-adamantane.

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Studies on the Structure of Collagen V[1]. The Site of Binding of Trivalent Iron on Collagen

Much work has been devoted to determining the site where different metal tanning agents are attached to the collagen molecule (for review see CHAMBARD[2]). As far as we know, all reports up to the present have shown only that different metals are bound to a particular type of functional group in the collagen molecule.

In previous studies evidence has been accumulated showing that the carboxyl groups are involved in the binding of iron in collagen (CHAMBARD[2]). Nobody has tried to isolate the actual complex of Fe^{III} with a small peptide, i.e. isolate the active sites binding Fe^{III} to collagen. This was the aim of the work reported in this paper.

Experimental. Rat-tail tendon collagen was extracted 10 times with 10% sodium chloride to remove the non-collagenous proteins, and the tanning was performed by 0.1 *M* solution of ferric sulphate in 0.1 *M* citrate buffer (pH 5, μ 0.1) for a period of 1 h. The tendons were washed free from the excess of Fe^{III} in running water overnight. The tanned collagen was suspended in 0.05 *M* calcium chloride and treated with pronase for 24 h at 20 °C (collagen-pronase ratio 100:1). The resulting mixture was dialysed against water at 4 °C.

No Fe^{III} was detected in the dialysate and therefore the non-dialysable part was separated from pronase by precipitation with 15% KCl + 0.02 *M* K_2HPO_4 and denatured for 1 h in 0.05 *M* calcium chloride at 80 °C. It was then cooled and digested with pronase again for 24 h at 25 °C (collagen-pronase ratio 50:1).

The reaction mixture was dialysed a second time against water at 4 °C; this time a considerable amount of bound iron went into the dialysate.

The dialysate was concentrated and separated on a Dowex 50 X-2 column using pyridine-formate and pyridine-acetate buffers (starting buffer pH 3, μ 0.075; final buffer pH 7, μ 2). Five peptides containing Fe^{III} have been separated. The homogeneity of these peptides was proved by paper chromatography in butanol-pyridine-acetic acid-water (30:20:6:24).

The total and/or partial sequences of these peptides were determined according to GRAY and HARTLEY[3] and are summarized in the Table.

Peptide No. 4 appeared to be a mixture of peptides 3 and 5 as far as *N*-terminals and total composition are concerned; it contained however only 1 atom of iron, therefore this is presumably the peptide with the iron cross-link.

From the structure of peptides listed above, one can conclude that the binding site of trivalent iron in acidic media is aspartic acid in the sequence Ala-Asp-Gly.

A second interesting result of our work is the presence of 1 mole of cysteine in peptide No. 1, which suggests the presence of 1 cysteine molecule/tropocollagen molecule. This is in agreement with the results of MCBRIDE and HARRINGTON[4], who detected the presence of cystine in invertebrate collagen. The presence of cysteine in collagen from species other than rat-tail tendon collagen is under active investigation.

Peptide No.	Sequence	Moles Fe^{III}/mole of peptide
1	Cys-Ala-Asp-Gly	1
2	Gly-Ala-Asp-Gly	1
3	Ala-Asp-(4 Gly, 2 Glu, 2 Lys, 2 Ala)	1
5	Pro-(Ala, Asp, 4 Gly, Glu, 2 Lys, Arg)	1

Zusammenfassung. Die Reaktion zwischen Kollagen und Fe^{III} wurde studiert. Es zeigte sich, dass das Eisen an die Sequenzen des Typs Ala-Asp-Gly gebunden wird. Weiter wurde gefunden, dass 1 mol Ratten-Tropokollagen 1 mol Cystein als Sequenz Cys-Ala-Asp-Gly enthält.

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