reaction, and the temperature then allowed to rise to 0°. At about −30° a large amount of red selenium was liberated. The reaction mixture was poured onto ice, the ice allowed to melt and the mixture was filtered. The yellow filtrate was extracted with ether and the solid material boiled repeatedly with ether until the solvent remained colourless. The combined ether extracts were dried over sodium sulphate and the ether removed by distillation. The residual ether was removed in a vacuum desiccator. A yellow, crystalline product was obtained. Yield 22.0 g (97.5 %). M.p. 80−85°.

The red selenium was boiled with alcohol and water. The grey selenium thus obtained was filtered off and dried. Yield 7.6 g (46.5 % of the introduced amount in the form of potassium selenocyanate, or 93 % of the calculated amount according to Scheme 1).

The product was dissolved in methylene chloride, boiled with *norite*, and, after filtering, the solution was concentrated to 100 ml and diluted with 100 ml of light petroleum (b.p. 30−65°). Upon standing in a refrigerator the solution gave 15.7 g (70 %) of pale yellow crystals in three crops, m.p. 91−96°. After two more recrystallizations the product melted at 98.5−100°.

(Found: Mol. wt., ebullioscopically in benzene 239; C 48.92; H 2.77; N 12.52; Se 35.56. Calc. for $C_9H_6N_2Se$: Mol. wt. 221.1, C 48.89; H 2.74; N 12.67; Se 35.71.).

3,3'-Diindolyl diselenide (II) A solution of 10.8 g (0.049 mole) of 3-selenocyanoindole in 100 ml of methanol was prepared. To this solution were added *ca* 50 ml of a 5 % methanolic solution of potassium hydroxide. The reaction mixture immediately became yellow-brown. After standing at room temperature for a few minutes, the solution was diluted with *ca* 200 ml of water. A bright yellow precipitate was obtained, that was filtered off and dried in a desiccator. The yield was 9.5 g (100 %), m.p. 167−172°. After two recrystallization from methanol-water, the product was obtained as bright yellow needles. M.p. 178−179.5°.

(Found: Mol. wt., ebullioscopically in benzene 384; C 49.57; H 3.18; N 7.10; Se 40.29. Calc. for $C_{16}H_{12}N_2Se_2$: Mol. wt. 390.2, C 49.25; H 3.10; N 7.18; Se 40.47.)

The selenium analyses were carried out according to Fredga [8].

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Specific Activities of Free, Neutral-Salt Soluble and Insoluble Hydroxyproline after Administration of ^{14}C-Proline to Chick Embryos

KARI I. KIVIRIKKO

Department of Medical Chemistry, University of Helsinki, Finland

Since the investigations of Stetten [1], it has been known that the hydroxyproline of collagen is not derived from free hydroxyproline but from proline that is hydroxylated during the synthesis of collagen. It has been shown, however, that in carrageenan granuloma [2] and polyvinyl sponge implants [3] considerable amounts of free hydroxyproline are present even during early stages of the development of connective tissue. Tissues of chick embryos likewise have a relatively high content of free hydroxyproline [4-6]. Mitoma *et al.*[7] found that free hydroxyproline could be incorporated in small amounts into collagen in chick embryos, but the later work of Prockop *et al.*[8] indicated that free hydroxyproline cannot be a significant source for collagen hydroxyproline even in rapidly developing chick embryos. Therefore they suggested that the free hydroxyproline of

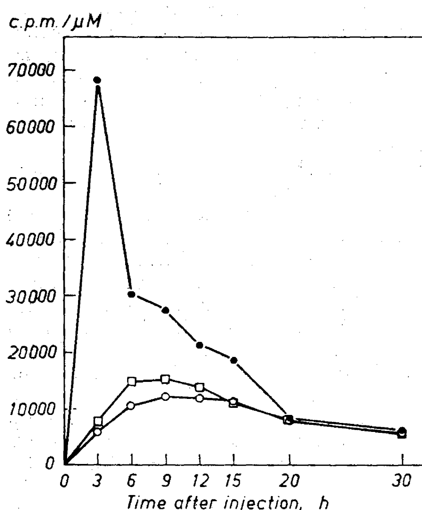

Fig. 1. Specific activities of free □—□, 1 M NaCl soluble collagen ●—● and insoluble ○—○ hydroxyproline after the administration of 2 μC of ^{14}C-proline onto the chorioallantoic membrane of 11-day-old chick embryos.

chick embryos is formed by degradation of one of the more highly labelled protein fractions. In a previous work [9], it was found that the changes in the content of free hydroxyproline during the development of chick embryos are similar to the changes in the content of protein-bound hydroxyproline in the fraction soluble in 1 M NaCl. Therefore it seemed worth while to follow the specific activities of free, 1 M NaCl soluble collagen and insoluble hydroxyproline after the administration of ^{14}C-proline into chick embryos.

After 11 days' incubation, 2 μC of uniformly labelled ^{14}C-proline (10.8 μC/μmole) was injected onto the chorioallantoic membrane of embryonated eggs. After 3 to 30 h the embryos were taken and homogenized in cold 1 M NaCl (2 ml/g embryo). The homogenates were stored at + 2°C for 24 h with occasional shaking, and the soluble fraction then separated from the insoluble fraction by centrifugation at 60 000 × *g*. The insoluble fraction was washed several times with cold 1 M NaCl, cold water, ethanol and ether and hydrolyzed with 6 N HCl. The proteins of the supernatant fraction were precipitated by adding 4 vol. of cold ethanol, and after several washings with 80 % ethanol, absolute ethanol and ether, the fraction was hydrolyzed. The hydroxyproline present in this 1 M NaCl soluble, ethanol insoluble fraction is called in the following 1 M NaCl soluble collagen hydroxyproline. The ethanol soluble fraction was evaporated to dryness and taken up in water, and after filtering, used for the determination of the specific activity of free hydroxyproline. The specific activities of hydroxyproline in the different fractions were determined by the method of Prockop *et al.*[10] Two series of experiments were made, the first containing 3 embryos at 3, 6, 9, and 12 h and one at 15 h after proline injection and the second containing 2 embryos at 6 h and 3 at 20 and 30 h. Each embryo was analysed separately.

The mean values for the specific activities of the hydroxyproline in the different fractions after the administration of ^{14}C-proline are presented in Fig. 1. The figure summarizes the results of both series. The specific activity of the 1 M NaCl soluble collagen hydroxyproline showed a maximum after as little as 3 h, and during the observation period there occurred a continuous decrease. At 20 and 30 h the value did not differ significantly from that of the other fractions. The specific activity of the free hydroxyproline was considerably lower than that of the 1 M NaCl soluble collagen hydroxyproline during the first 12 h, but in all embryos the value during this time was higher than that of insoluble hydroxyproline. It is of interest that incorporation of radioactivity into the insoluble hydroxyproline is also rapid in chick embryos. The decrease in the specific activities towards the end of experimental period is to a large extent due to synthesis of new unlabelled hydroxyproline. Since the rate of increase in the content of hydroxyproline in the different fractions is similar at this stage of development, the increase cannot account for the different rates of decrease in the specific activities of the different fractions.

The results of the present investigation suggest that free hydroxyproline is derived at least partly from the soluble collagen fraction, since its specific activity was higher than that of the insoluble hydroxyproline during the first 12 h. This is in agreement with the findings that part of the urinary free and peptide hydroxyproline is derived from soluble collagens[11,12]. The recent work of Hurych and Chvapil [13] indicates the presence of hydroxyproline of high specific activity in the ultrafiltrable fraction during the incubation of skin slices

of chick embryos with ^{14}C-proline. Evidently most of the bound hydroxyproline of their ultrafiltrable fraction is in the 1 M NaCl soluble collagen fraction of the present study, for the content of bound hydroxyproline was 60 – 30 % of their ultrafiltrable hydroxyproline but it is only about 10 % of the ethanol soluble hydroxyproline in the present investigation.

The details of this and related work will be published later elsewhere. This work was supported by a grant from the *Reumaliitto*.

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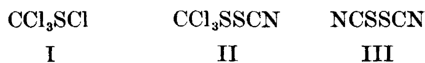
Trichlormethansulfenylrhodanid

ALEXANDER SENNING

Chemisches Institut der Universität Aarhus, Aarhus, Dänemark

Trichlormethansulfenylchlorid (I) ist das weitaus stabilste aller organischen Sulfenylchloride und zeichnet sich durch ein im Vergleich zu allen anderen Vertretern der Verbindungsklasse stark herabgesetztes Reaktionsvermögen aus.



CCl_3SCl	CCl_3SSCN	NCSSCN
I	II	III

Eine Kernsubstituierung aromatischer Verbindungen lässt sich mit Trichlormethansulfenylchlorid nur in Parastellung zu einer N,N-Dialkylaminogruppe erzielen, während Sulfenylchloride im allgemeinen auch mit anderen reaktiven Aromaten reagieren.

Da das bisher unbekannte Trichlormethansulfenylrhodanid (II) strukturell mit dem hochreaktiven Dirhodan (III) nahe verwandt ist, erschien es wünschenswert, diese Verbindung darzustellen und zu untersuchen, ob sich nicht mit ihrer Hilfe Trichlormethylmerkaptogruppen besonders leicht in aromatische Verbindungen einführen lassen. Trichlormethansulfenylrhodanid wurde in siedendem Benzol aus Trichlormethansulfenylchlorid und Kaliumrhodanid dargestellt. Die Verbindung war, wie das Sulfenylchlorid, gelb gefärbt, hatte einen ähnlichen unangenehmen Geruch und zeigte keine gesteigerte Reaktionsfähigkeit gegenüber N,N-Dimethylanilin oder Phenol. Wie das Sulfenylchlorid oxydiert Trichlormethansulfenylrhodanid Kaliumjodid unter Jodausscheidung und reagiert mit Natriumazid in Azetonitril unter heftiger Gasentwicklung. Das Infrarotspektrum enthält eine charakteristische Absorption bei 2170 cm^{-1}; es handelt sich also nicht um das isomere Trichlormethansulfenylisothiocyanat, CCl_3SNCS. Lieber und Mitarbeiter [1] fanden die charakteristische Absorption organischer Rhodanide bei 2140 cm^{-1}, die organischer Isothiocyanate bei 2060 – 2105 cm^{-1}.

Versuchsbeschreibung. Käufliches Kalium rhodanid wurde über Nacht im Trockenschrank bei 130° getrocknet. 19,4 g (0,2 Mol) des so getrockneten Kaliumrhodanids wurden mit 200 ml Benzol gerührt und langsam 37,2 g (0,2 Mol) Trichlormethansulfenylchlorid zugetropft. Danach wurde noch 3 1/2 Stunden am Rückfluss gekocht, die erkaltete Benzollösung filtriert und im Vakuum destilliert. Zwischen 94° und 95° (10 mm) gingen 33,5 g (80 % der Theorie) Produkt über. Die analysenreine Substanz hatte Kp. 94°/10 mm und n_D^{22} 1,5820. (Gef. Cl 50,90; N 6,89; S 30,54. Ber. für $C_2Cl_3NS_2$: Cl 51,01; N 6,72; S 30,75).

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