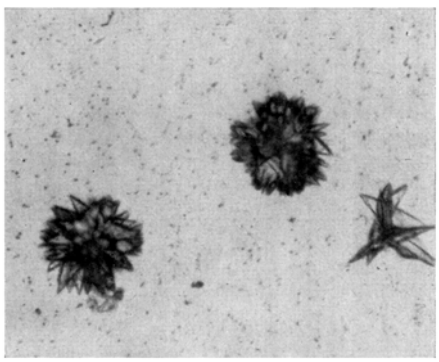
In order to have some indication of the existence of a biological mechanism of transulfuration between a sulfur-containing aminoacid and ethanolamine, it was found necessary to prepare the hypothetical intermediate: aminoethylcysteine (I) and its homologue aminoethylhomocysteine. We have started with the

$$NH_2CH_2{\cdot}CH_2{-}S{-}CH_2{\cdot}CHNH_2{\cdot}COOH \qquad (I)$$

synthesis of the cysteine derivative in order to have information on such types of synthesis and because this substance is more easily obtainable.



S-aminoethylcysteine monohydrochloride recrystallized with acetone from an hydroalcoholic solution (about 150 ×).

Experimental. 5 g of L-cysteine hydrochloride were dissolved in 10 ml of bidistilled water through which a flow of nitrogen was run for a period of 15 min. Keeping an atmosphere of nitrogen, 6 g of KOH dissolved in 10 ml oxygen-free water were added. Then by heating on a water bath at 60–70°, 7 g of β-bromoethylamine hydrobromide[1] were added in a period of 10 min. The solution was then left at room temperature under nitrogen for 3 h, neutralized with concentrated HBr and 80 ml alcohol added. After 4–5 h at 0°, a precipitate of KBr was removed and the filtrate was concentrated, on a boiling water bath at reduced pressure, to about 20 ml. 60 ml of alcohol were added and the suspension dissolved by warming and adding a small amount of water. The solution was left overnight at 0° and filtered from a new precipitate of KBr. The supernatant which contained the aminoethylcysteine as a mixed hydrobromide and hydrochloride was passed through a column, 2·5 × 40 cm, of Dowex 50, in the acid form, ground to 80–100 mesh. The column, washed with 500 ml of water, was eluted with NH_3 1 N and the effluent, from the appearance of the ammonia, was collected for a total amount of 200 ml. The collected solution was brought to dryness on a boiling water bath at reduced pressure. The oily residue was dissolved with 20 ml water and neutralized up to a slight acidity with concentrated HCl. Then 80 ml alcohol were added and the crude hydrochloride was precipitated by slowly adding, with vigorous shaking, 100 ml of acetone. In this way an oily precipitate is first obtained which, by shaking and rubbing, solidifies into a semi-crystalline mass. After few days at 0°, the supernatant was removed, the solid mass was mechanically detached from the walls of the container, broken in the presence of acetone, filtered and dried. The crude aminoethylcysteine hydrochloride weighed 4·8 g (75% of the theoretical value); a negative nitroprusside test was obtained before and after treatment with NaCN.

[1] F. CORTESE, *Organic Syntheses*, Coll. Vol. II, p. 91.

The recrystallization was complicated by the tendency of the compound to precipitate from aqueous solutions in a semi-oily form carrying water with it. We have obtained good crystallization by the following procedure: 1 g of the above precipitate was suspended in 30 ml 95% alcohol; when boiling, water was added in small portions until a complete solution was obtained. The solution was filtered and acetone was added, while shaking, until a slight permanent turbidity was reached; the solution was clarified by boiling and left at room temperature for a few hours. Acetone was then added in drops until a slight turbidity was obtained again: after rubbing the walls and after a permanence at 0° for 24 h, the compound begins to crystallize in the form of needleshaped crystals assembled in rosettes (Figure). The treatment with acetone up to a slight turbidity, followed by standing at 0° for several hours, was repeated 4–5 times. Finally the liquid was poured out, the crystals washed with alcohol, collected and dried in a dessiccator; yield 0·5 g. From the mother liquor, further 0·15 g of pure crystalline material were obtained following the same treatment for several days.

The analyses made on the crystalline compound gave the following results:

$C_5H_{12}O_2N_2S{\cdot}HCl$	calculated	S 15·97%	N 13·96%
200·69	found	S 15·66%	N 13·40%

The nitrogen was shown to be entirely present as amino nitrogen by the VAN SLYKE procedure: 98% of the theorical value. M.P. 192°–192·5°; at the microstage with the KOFLER apparatus. $[\alpha]_D^{25} = +7{\cdot}2°$: 1% in aqueous solution.

The ninhydrin test and the iodoplatinate test for sulfur-containing amino acids[1] were strongly positive. By paper chromatography only one spot was shown to be present with the following R_f: 0·80 in phenol; 0·19 in collidine-lutidine.

Acknowledgments: The present work was supported by a grant of the Consiglio Nazionale delle Ricerche. We are grateful to Prof. MAROTTA, Director of the Istituto Superiore di Sanità, for the permission given to us to have the microanalyses made in his Institute by Dr. MARZADRO.

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Institute of Biological Chemistry, University of Rome, June 24, 1954.

Riassunto

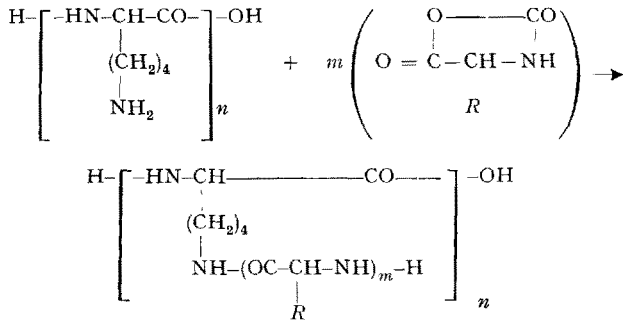
L'aminoetilcisteina, composto intermedio ipotetico di transulfurazione tra cisteina e etanolamina, è stata preparata cristallina sotto forma di monocloridrato, trattando la cisteina con bromoetilamina in ambiente alcalino.

[1] H. M. WINEGARD, G. TOENNIS, and R. J. BLOCK, Science *108*, 506 (1948).

Branched Polyamino Acids

Most of the known poly-α-amino acids were prepared from N-carboxy-α-amino acid anhydrides, using water or amines as polymerization initiators. The linear polypeptides thus obtained are usually of an average degree of polymerization of 10 to 100. Branched polyamino acids of a considerably higher molecular weight have been prepared by us, using polylysine[1] as a polyvalent amine initiator, according to the following scheme:

[1] E. KATCHALSKI, I. GROSSFELD, and M. FRANKEL, J. Amer. Chem. Soc. *70*, 2094 (1948).



Since N-carboxy-α-amino acid anhydrides react with amines considerably faster than with water, it was possible to prepare various branched polyamino acids in a water-dioxane mixture, under conditions similar to those used by FRAENKEL-CONRAT[1] for the preparation of peptide derivatives of proteins.

As an example of the synthesis of the branched polyamino acids, the preparation of poly-(ε,N-poly-DL-alanyl)-L-lysine will be described: An ice-cooled solution of N-carboxy-DL-alanine anhydride (m.p.45°) (18 g) in anhydrous dioxane (250 ml) was added to an ice-cooled solution of poly-L-lysine hydrobromide (*n* average 20[2]) (500 mg) in M/150 phosphate buffer pH 7 (200 ml). The reaction mixture was left for 24 h in the refrigerator, and then dialysed against water for 5 to 6 days, until the biuret and ninhydrin reactions of the dialysate were negative. The solution left in the cellophane bag was concentrated in vacuo and the residue was triturated with acetone and dried (5 g). The nondiffusible material thus obtained dissolves readily in water and glacial acetic acid, and is insoluble in dimethylformamide, pyridine and ethanol. It may be salted out from its aqueous solution by the addition of sodium chloride or ammonium sulphate.

A paper chromatogram of an acid hydrolysate of the nondialysable material yielded, after spraying with ninhydrin, two spots identified as alanine and lysine. On the other hand, the dialysable material, after hydrolysis and chromatographic analysis, yielded alanine exclusively. As polylysine (*n* 20) passed the cellophane membrane under similar conditions of dialysis, it is evident that all the polylysine served as initiator in the formation of the branched poly-DL-alanine. The absence of a spot corresponding to ε-hydroxy-α-aminocaproic acid on a paper chromatogram of the acid hydrolysate of the branched polymer after treatment with nitrous acid, indicates that practically all the ε-amino groups of poly-L-lysine reacted with the N-carboxy DL-alanine anhydride to form ε-amide bonds.

A quantitative chromatographic analysis of the hydrolysate of the branched nondiffusible polymer proved that, on the average, 25 alanine residues are attached to each lysine residue. An independent evaluation of the average chain length (*m* 23) of the alanine side chains was obtained from the content of the terminal amino and carboxyl groups[2].

Since the diffusible material contained alanine residues, but no lysine, it is obvious that the peptides of this fraction were formed by water-initiated polymerization. A water-initiated linear poly-DL-alanine synthesized under conditions used for the preparation of the branched polymer—but in the absence of an amine initiator—passed the cellophane membrane to the extent of more than 90% during 6 days. It seems, therefore, that the nondiffusible fraction may contain only a minute amount of linear water-initiated polyalanine.

The electrophoretic mobility of poly-(ε,N-poly-DL-alanyl)-L-lysine (*n* 20, *m* 25) was compared with that of linear poly-DL-alanine (*n* 25), of a linear poly-L-lysine (*n* 20), as well as with that of a mixture of linear polyalanine (*n* 25) and polylysine (*n* 20) in a ratio of 25 alanine residues to one lysine residue. The measurements were carried out on a Whatman No. 1 filter paper in an acetate buffer of pH 3·6 and ionic strength 0·2, at a potential gradient of 10V/cm at room temperature. The polymers were revealed, after 2 h, with ninhydrin. The linear poly-DL-alanine did not move from the origin; the linear poly-L-lysine spread into an elongated spot of 8·6 cm length, towards the cathode; the mixture of the two linear polymers gave a round spot of polyalanine at the origin, and a thin elongated spot corresponding to polylysine. The branched poly-DL-alanine migrated as one circular spot for a distance of 2·8 cm from the origin towards the cathode. The specific viscosity of the branched polymer in water at 20°C was found to be η sp = 0·20. The viscosity of the linear polyalanine (*n* 25) was η sp = 0·030, that of polylysine hydrobromide (*n* 20) was η sp = 0·045, and that of a mixture of the linear polymers in a ratio of 25 alanine residues to one lysine residue was η sp = 0·033. All viscosity measurements were carried out on one per cent solutions. The diffusion coefficient of the linear poly-DL-alanine (*n* 25) in water at 18°C was found to be $D = 28 \cdot 10^{-7}$ cm²/s (*c*, 1 g in 100 ml). A diffusion coefficient of $D = 2 \cdot 10^{-7}$ cm²/s (*c*, 0·8 g in 100 ml glycine buffer of pH 9·0 and ionic strength 0·1) was found for poly-(ε,N-poly-DL-alanyl)-L-lysine (*n* 20, *m* 25) at 18°C. The above physicochemical data show that the properties of the nondiffusible material prepared differ greatly from those of a mixture of polyalanine and polylysine in the same monomer ratio. The relatively low diffusion coefficient and the high specific viscosity of the branched polymer indicate that its average molecular weight is considerably higher than that of the linear poly-amino acids.

Water-soluble poly-(ε,N-polysarcosyl)-L-lysine (*n* 20, *m* 7) was prepared from poly-L-lysine and N-carboxy-sarcosine anhydride[1], in a similar manner to the branched polyalanine. The dialysable fraction contained sarcosine residues only, while the ratio between sarcosine and lysine residues in the undialysable fraction, was in good agreement with that calculated from end-group analysis[2]. Polymerization of ε,N-carbobenzoxy-α,N-carboxy-L-lysine anhydride[3] in the presence of poly-L-lysine under conditions similar to those given above, yielded a branched polymer from which, after removal of the carbobenzoxy groups with anhydrous hydrogen bromide, a branched basic polyelectrolyte poly-(ε,N-poly-L-lysyl)-L-lysine (*n* 30, *m* 15) was obtained.

The chemical and physicochemical data given above for poly-(ε,N-poly-DL-alanyl)-L-lysine (*n* 20, *m* 25), as well as the fact that also the other water-soluble branched polyamino acids do not pass through the cellophane membrane on dialysis, seem to indicate that the average molecular weight of the branched polyamino acids prepared [38,000 in the case of the poly-(ε,N-poly-DL-alanyl)-L-lysine (*n* 20, *m* 25) described] lies in the range of that of proteins. Such water-soluble polymers may be of considerable interest in the study of multichain polymers,

[1] H. FRAENKEL-CONRAT, Bioch. bioph. Acta *10*, 180 (1953).
[2] M. SELA and A. BERGER, J. Amer. Chem. Soc. *75*, 6350 (1953).
[1] F. SIGMUND and F. WESSELY, Z. physiol. Chem. *157*, 91 (1926).
[2] M. SELA and A. BERGER, J. Amer. Chem. Soc. *75*, 6350 (1953).
[3] E. KATSCHALSKI, I. GRÜNFELD, and M. FRANKEL, J. Amer. Chem. Soc. *70*, 2094 (1948).

of branched polyelectrolytes, and of proteins. Some of these branched peptides might possibly be of use as blood volume expanders.

This investigation was supported by a research grant (PHS G-3677) from the U.S.A. National Institutes of Health, Public Health Service.

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Department of Biophysics, Weizmann Institute of Science, Rehovot, July 17, 1954.

Résumé

Des polyacides aminés ramifiés, avec des poids moléculaires approchant ceux des protéines, ont été préparés par la polymérisation des anhydrides des N-carboxy-acides aminés en présence du polylysine qui sert comme un initiateur polyvalent. Les polypeptides ramifiés suivants sont décrits: poly-(ε,N-poly-DL-alanyl)-L-lysine (*n* 20, *m* 25), poly-(ε,N-polysarcosyl)-L-lysine (*n* 20, *m* 7) et poly-(ε,N-poly-L-lysyl)-L-lysine (*n* 30, *m* 15). Quelques propriétés chimiques et physicochimiques des polyacides aminés ramifiés ont été comparées avec celles de polyacides aminés linéaires preparés par initiation avec un amine monovalent.

Rauwolfia Alkaloids, XVI[1]. Deserpidine, a New Alkaloid from *Rauwolfia canescens*[2]

During the course of an investigation of commercially available Indian *Rauwolfia canescens*, we have isolated a new alkaloid similar in its properties to reserpine. We propose the name deserpidine for this new and pharmacologically interesting alkaloid.

The description of its isolation will be given in a forthcoming experimental paper. Deserpidine crystallizes as colorless prismes or needles, m.p. 228–232°; $[\alpha]_D^{24.5} - 137° \pm 1°$ ($CHCl_3$); ε_{217} max. 64,000; ε_{272} max. 17,750; ε_{244} min. 6,300 (ethanol).

Analysis.
Calculated for $C_{32}H_{38}O_8N_2$: C, 66·42; H, 6·62; N, 4·84; OCH_3 (5), 26·81.

Found: C, 66·42; H, 6·76; N, 4·89; OCH_3, 26·90.

Treatment of deserpidine with sodium methylate[3] gave methyl, 3,4,5-trimethoxybenzoate identified by its m.p. mixed m.p. analysis, and I.R. as well as by its conversion to 3,4,5-trimethoxybenzoic acid. Also isolated from the sodium methylate treatment was the alkaloid acid ester, methyl deserpidate, which gave a crystalline nitrate; m.p. 271–276°.

Analysis. Calculated for $C_{22}H_{28}O_4N_2 \cdot HNO_3$: C, 59·05; H, 6·53; N, 9·39

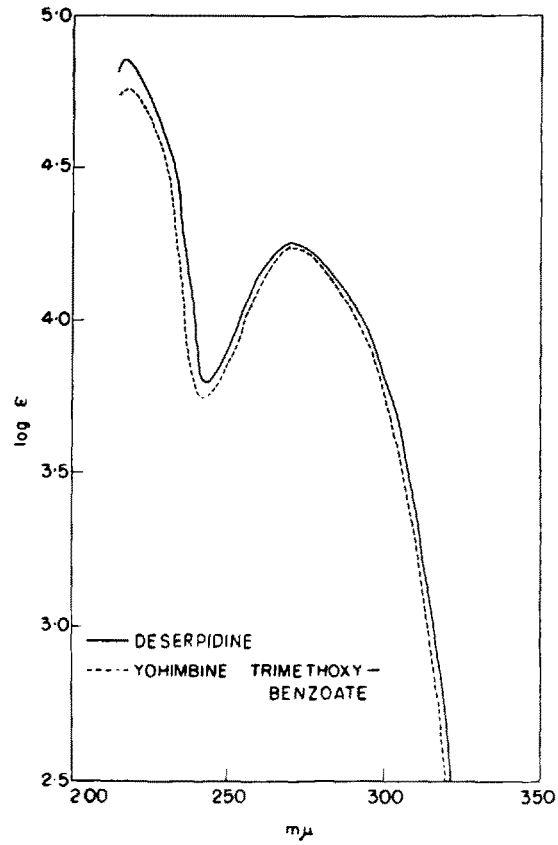
Found: C, 58·73; H, 6·74; N, 9·36.

The tosylate of methyl deserpidate was prepared; m.p. 226–228°.

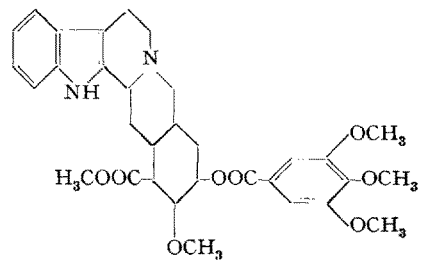
Analysis.
Calculated for $C_{29}H_{34}O_6N_2S$: C, 64·67; H, 6·36; N, 5·20.

Found: C, 64·51; H, 6·42; N, 4·97.

Other esters of methyl deserpidate are being prepared.



On the basis of analytical data, the isolation of 3,4,5-trimethoxybenzoic acid by hydrolysis, interpretation[1] of infrared and ultraviolet absorption spectra, and by analogy to reserpine, the following structure is proposed for deserpidine. Further work on this problem is in progress.



Preliminary pharmacological experiments[2] indicate that deserpidine exhibits both hypotensive and sedative activity comparable to that of reserpine.

[1] Communication XV, C. F. HUEBNER *et al.*, J.A.C.S. (in press).

[2] From lecture given at Columbia University, New York, January 12th, 1955.

[3] L. DORFMAN *et al.*, Helv. chim. acta *37*, 59 (1954).

[1] Absorption in the ultraviolet is similar to that of the 3,4,5-trimethoxybenzoate of yohimbine (see figure). This fact together with the appearance in the infrared absorption spectrum of bands at 730 and 760 K, characteristic of o-disubstituted benzene rings, and the disappearance of the band at 1625 K point to the absence of methoxyl in ring A.

[2] J. A. SCHNEIDER, A. J. PLUMMER, A. E. EARL, W. E. BARRETT, R. REINHART, and R. C. DIBBLE (*in press*).