[CONTRIBUTION FROM THE LABORATORY OF PHYSICAL CHEMISTRY OF THE UNIVERSITY OF UPSALA]

THE MOLECULAR WEIGHTS OF AMANDIN AND OF EXCELSIN

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In a recent communication from this Laboratory[1] it was shown that the edestin molecule has a weight of 212,000 in a P_H region from the isoelectric point P_H 5.5 to about P_H 10. At a P_H of 11.3 definite dissociation takes place. Molecules of one-half and one-third of the normal are formed at this alkalinity. On the acid side of the isoelectric point the edestin molecule was found to be quite unstable.

Chiefly thanks to the fundamental work done by Osborne[2] a considerable number of vegetable proteins are known. Especially those which like edestin belong to the globulin group seem to be very stable and are easy to prepare in a pure state. It was thought to be of interest to study some more representatives of this important class of proteins by means of the ultracentrifuge analysis. Amandin and excelsin were first worked with because of their widely differing elementary composition. Amandin has the lowest (0.44%) and excelsin the highest (1.08%) sulfur content of the oil-seed proteins so far analyzed.

Preparation of Material

Amandin was prepared in the following way. Almonds were soaked in cold water for some hours and freed from skins. The material was then ground in a mill, and 1000 g. of the paste suspended in 5000 cc. of 10% sodium chloride solution. Toluene was added as a preservative and the mixture was digested with stirring at room temperature for twenty-four hours. After settling the solution was siphoned off and the residue treated again in the same way with 2000 cc. of 10% sodium chloride solution and after that with 1000 cc. of the same solution. By means of centrifuging the collected liquid extracts were freed from oil and fat which had been partly dissolved in the toluene. To the protein solution was then added an equal volume of saturated ammonium sulfate and some more toluene. After standing for two days at 0° the precipitate was filtered off and washed with half saturated ammonium sulfate solution. The amandin was then dissolved in a phosphate buffer 0.095 M in KH_2PO_4 and 0.005 M in Na_2HPO_4 and further containing 1% of sodium chloride in order to increase the solubility of the amandin. The solution was dialyzed against the same buffer; the final volume was 50 cc. and the concentration 2.89%.

Excelsin was isolated in the following manner. Brazil nuts were freed from the shells and ground in a mill (without removing the skins from the kernels), and 1000 g. of the paste was suspended in 5000 cc. of a 3% ammonium sulfate solution. Toluene was added as a preservative and the mixture shaken at room temperature for twenty-four hours. It was then filtered and to the solution was added ammonium sulfate to 60% saturation. The precipitate formed was dissolved in 6% ammonium sulfate, the solution was filtered and reprecipitated with ammonium sulfate to the same degree of saturation. The excelsin precipitate was washed with ammonium sulfate solution of

[1] Svedberg and Stamm, THIS JOURNAL, **51**, 2170 (1929).

[2] Osborne, "Vegetable Proteins," Longmans, Green and Co., London, 1909.

the same strength. One portion of the excelsin precipitate was dissolved in phosphate buffer of P_H 5.5 (0.182 M in KH_2PO_4 and 0.018 M in Na_2HPO_4) and containing 1% ammonium sulfate and dialyzed at 0° against the same buffer. No precipitation occurred; volume of solution, 35 cc. and concentration, 3.79% (Material I). A second portion of the excelsin was dialyzed against pure water. The excelsin was precipitated in crystalline form and the crystals were dissolved as in the case of Material I: volume of solution, 50 cc.; concentration, 2.26% (Material II). The isoelectric points of these proteins are difficult to determine accurately, because of the high salt content necessary to keep them in solution. A value of P_H 5.5–5.7 was assumed as the most probable one.[3]

Specific Volume.—The partial specific volume was determined pycnometrically at 19.8° as described in previous communications.[4] For amandin the value 0.746 was obtained. The protein concentration was 1.4%, the solvent was 0.095 M in KH_2PO_4, 0.005 M in Na_2HPO_4 and contained 1% NaCl, giving a P_H of 5.7. For excelsin the value found was 0.743. The protein concentration was 1% and the solvent was 0.182 M in KH_2PO_4, 0.018 M in Na_2HPO_4 and contained 1% of ammonium sulfate, giving a P_H of 5.5.

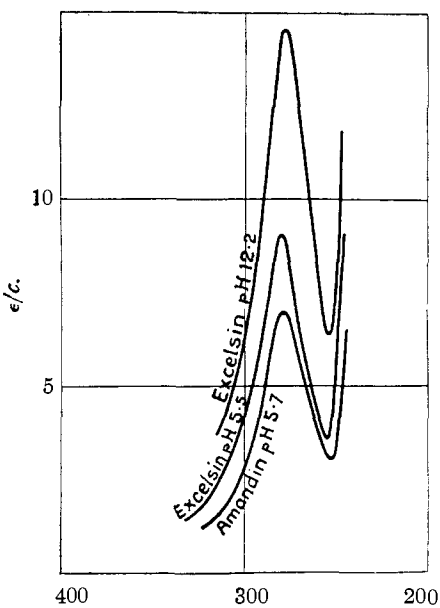

Fig. 1.

The specific volumes for these two proteins are identical within the limits of error and also identical with the specific volumes of egg albumin, Bence–Jones protein, hemoglobin, serum albumin, serum globulin, phycocyan, phycoerythrin and edestin.

Light Absorption.—The light absorption of amandin and excelsin solutions were studied by means of the Judd–Lewis spectrophotometer. The solutions were brought to the desired concentration and P_H directly before measuring the light absorption. Amandin was measured in 0.2 and 0.1% solutions and with a thickness of layer of 2.0 cm. The solvent was 0.095 M in KH_2PO_4 and 0.005 M in Na_2HPO_4 with 1% of NaCl, giving a P_H of 5.5. The absorption maximum was found to be 280$\mu\mu$ and the minimum 253$\mu\mu$. At high P_H values where the centrifugal analysis showed that splitting up of the molecule occurs, the light absorption was found to increase.

Excelsin was measured at two different P_H values, at 5.5 and at 12.2. In the first case two excelsin concentrations 0.10 and 0.05% made up from Material II were used in 2.0-cm. layer. The solvent was 0.182 M in KH_2PO_4 and 0.018 M in Na_2HPO_4 with 1% of ammonium sulfate. The absorption maximum was 279$\mu\mu$ and the minimum 253$\mu\mu$. In the second case the solution was 0.06% with regard to excelsin. The solvent was 0.021 M in KH_2PO_4, 0.013 M in Na_2HPO_4, 0.030 M in NaOH and 0.12% in ammonium sulfate. The maximum was 279$\mu\mu$ and the minimum 253$\mu\mu$. The position

[3] Csonka, Murphy and Jones, THIS JOURNAL, **48**, 763 (1926).

[4] Svedberg and Chirnoaga, *ibid.*, **50**, 1401 (1928).

of the absorption band therefore is not shifted by the change in P_H, but on the other hand the values of the extinction constant were much higher at the higher P_H. The centrifugal analysis showed that the excelsin molecule, which at the isoelectric point is built up of six units of weight 35,000, is at a P_H of 12.2 completely split up into the unit 35,000. This together with a slight formation of non-centrifugal substance accounts for the increase in light absorption at the high P_H. In Fig. 1 the specific extinction coefficient, $\epsilon/c = 1/cd \times \log I_0/I$ (where c is the concentration, d the thickness of the solution, I_0 the intensity of the light beam after passing through the solvent and I the intensity after passing through the same thickness of solution), is plotted against wave length. As shown by the diagram the excelsin has a stronger light absorption than amandin. This is probably caused by the higher tryptophane content of excelsin.[5]

Determination of Molecular Weight

Sedimentation Equilibrium Method.—As shown in previous communications[6,7] the molecular weight is given by the relation

$$M = \frac{2RT \ln (c_2/c_1)}{(1 - V\rho)\omega^2(x_2^2 - x_1^2)}$$

where R is the gas constant, T the absolute temperature, V the partial specific volume of the solute, ρ the density of solvent, ω the angular velocity and c_2 and c_1 are the concentrations at the distances x_2 and x_1 from the center of rotation.

Two different types of ultracentrifuges for medium speed previously described[8,9] were used.

In Table I the result of a typical run with amandin is given and in Table II are collected the data of the five equilibrium runs made on amandin and excelsin.

TABLE I

AMANDIN, SEDIMENTATION EQUILIBRIUM RUN

Concn., 0.18%; phosphate buffer, P_H 5.7 (0.095 M in KH_2PO_4, 0.015 M in Na_2HPO_4, 1% in NaCl); $V = 0.746$; $\rho = 1.017$; $T = 293.2°$; length of col. of soln., 0.51 cm.; thickness of col., 0.80 cm.; dist. of outer end of soln. from axis of rotation, 5.95 cm.; speed, 5350 r.p.m. ($\omega = 178.3\pi$); light absorption standard, $M/300$ in K_2CrO_4; source of light, mercury lamp; light filters, chlorine and bromine; aperture of objective, F:25; plates, Imperial Process; time of exposure, 60, 120 and 180 seconds; exposures made after 38, 53 and 68 hours of centrifuging.

Distances, cm.		Mean concn., %		Number of exposures	Mol. wt.
x_2	x_1	c_2	c_1		
5.79	5.74	0.238	0.195	9	222,200
5.74	5.69	.195	.162	9	208,500
5.69	5.64	.162	.137	9	190,200
5.64	5.59	.137	.115	8	200,400
5.59	5.54	.115	.096	6	205,200
				Mean	205,300

[5] Jones, Gersdorff and Moeller, *J. Biol. Chem.*, **62**, 183 (1924).

[6] Svedberg and Fåhraeus, THIS JOURNAL, **48**, 430 (1926).

[7] Svedberg and Nichols, *ibid.*, **48**, 3081 (1926).

[8] Svedberg and Heyroth, *ibid.*, **51**, 550 (1929).

[9] Svedberg and Sjögren, *ibid.*, **51**, 3594 (1929).

TABLE II

AMANDIN AND EXCELSIN, SUMMARY OF SEDIMENTATION EQUILIBRIUM MEASUREMENTS

Excelsin Material II was used

Solvent			PH of soln.	Concn. of protein at start, %	Mean speed, r.p.m.	Mol. wt.
KH_2PO_4, *M*	Na_2HPO_4, *M*	1% in				
0.095	0.005	NaCl	5.7	Amandin, 0.18	5,350	205,300
.095	.005	NaCl	5.7	Amandin, 0.72	4,600	210,400
.182	.018	$(NH_4)_2SO_4$	5.5	Excelsin, 0.25	4,600	213,000
.182	.018	$(NH_4)_2SO_4$	5.5	Excelsin, 0.30	4,600	212,000
.1	.1	$(NH_4)_2SO_4$	6.5	Excelsin, 0.20	4,800	210,500

As mean values for the molecular weights we find for amandin 208,000 ± 5000 and for excelsin 212,000 ± 5000.

Sedimentation Velocity Method.—For the sedimentation velocity runs the high-speed oil-turbine ultracentrifuge was used.[10] The cells were of the type described in the previous paper.[9] In most of the runs the wave length region was 290–240 $\mu\mu$ isolated from the mercury arc by means of chlorine and bromine filters. For the study of extremely low protein concentrations the wave length region 231–214$\mu\mu$ produced by means of a cadmium spark-lamp as described in the previous paper was used.[9]

In the case of amandin and excelsin the same kind of irregularities with regard to diffusion constant were observed as in the case of the Bence-Jones protein.[9] The measurement of the sedimentation constant on the other hand presented no difficulties. In the following, therefore, only a report of the latter determination will be given.

The behavior of amandin was studied within the PH region 4.3–12.2. Excelsin was investigated from PH 5.5 to PH 11.9. The solutions were

TABLE III

AMANDIN, SUMMARY OF SEDIMENTATION VELOCITY MEASUREMENTS

Speed, about 25,000 r.p.m. in the five first runs and about 42,000 r.p.m. in the last two runs

Solvent				PH of soln.	Concn. of protein, %	$s_{20}° \times 10^{13}$
HAc, *M*	NaAc, *M*		NaCl, %			
0.016	0.004		2.5	4.27	0.10	11.4[11]
KH_2PO_4, *M*	Na_2HPO_4, *M*	NaOH, *M*				
0.095	0.005	..	1	5.7	.18	11.3
.095	.005	..	1	5.7	.50	11.2
.095	.005	..	1	5.7	1.45	12.1
.050	.050	..	1	7.05	0.29	11.3
.006	.032	0.002	0.07	9.40	.20	11.2
.003	.013	.031	.03	12.16	.10	[a]

[a] Decomposition, 59% of molecules of 208,000 and 41% of 34,500.

[10] Svedberg and Nichols, THIS JOURNAL, **49**, 2920 (1927); Svedberg, "Colloid Chemistry," 2d ed., The Chemical Catalog Co., Inc., New York, **1928**, p. 153.

[11] The directly measured value, 11.1×10^{-13}, was corrected for the increased density and viscosity of the solvent due to the high salt content in the same way as described by Svedberg and Stamm, Ref. 1.

brought to the desired concentration and P_H immediately before starting a run. In Table III are summarized the determinations on amandin, in Table IV the determinations on excelsin.

TABLE IV

EXCELSIN, SUMMARY OF SEDIMENTATION VELOCITY MEASUREMENTS

Material I was used for Runs 2–5, Material II for Runs 1 and 6–10

No.	Solvent KH_2PO_4, M	Solvent Na_2HPO_4, M	Solvent $(NH_4)_2SO_4$, %	P_H of soln.	Concn. of protein, %	Speed, r.p.m.	$s_{20}° \times 10^{13}$ Obs.	$s_{20}° \times 10^{13}$ Corr.
1	0.182	0.018	1	5.5	0.03[a]	25,600	10.3	11.4
2	.182	.018	1	5.5	.12	24,800	10.6	11.8
3	.182	.018	1	5.5	.20	24,300	10.7	11.8
4	.182	.018	1	5.5	.76	23,400	11.1	12.4
5	.182	.018	1	5.5	1.03	44,900	10.5	11.7
6	.182	.018	1	5.5	1.13	23,500	10.9	12.1
7	.1	.1	1	6.47	0.32	24,500	10.9	12.2
8	.013	.187	0.13	7.46	.13	23,400	..	11.3
9[b]	.022	.030	.12	9.81	.11	40,900	..	11.3
10[c]	.021	.013	.12	11.88	.11	....	..	3.54

[a] Cadmium light was used. [b] 0.005 M in sodium hydroxide. [c] 0.040 M in sodium hydroxide.

As shown by Table III amandin is stable within the P_H range 4.3–9.4. The mean value of the sedimentation constant is 11.4×10^{-13}. A non-centrifugible substance was not present in the solutions studied. In Fig. 2 a diagram of the microphotometer curves from the run at P_H 12.16 is given. The diagram shows that at this P_H the solution contains two different centrifugible substances (together with a small amount of a non-centrifugible substance). In Fig. 3 the last curve corresponding to the state of the solution after two hours of centrifuging is given after being reduced to represent relative concentrations (Curve D) instead of merely galvanometer deflections as in Fig. 2. In the same diagram are given (in dotted lines) the curves for the undecomposed amandin (A), for a protein of molecular weight one-sixth of the normal amandin (B) and for a mixture of 59% normal amandin and 41% of amandin of weight one-sixth of the normal substance (C). The closeness of the two curves C and D makes it very probable that the normal amandin at a P_H of 12.16 is to a large extent split up into molecules of one-sixth of

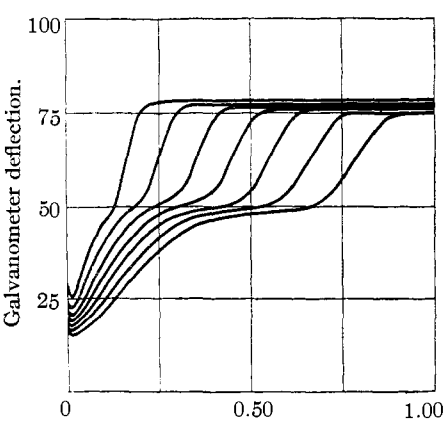

Fig. 2.

the normal weight. Because of the difficulty of taking into account the difference in light absorption between the normal and the decomposed amandin, the calculation of the relative amounts of the two components in the mixture should be regarded only as a first approximation.

Table IV shows that excelsin is stable within the P_H range 5.5–9.81, with a mean corrected sedimentation constant of 11.8×10^{-13}. A small amount of a non-centrifugible substance was present. At a P_H of 11.88 the protein was completely broken up into a substance possessing a sedimentation constant of 3.54×10^{-13}. Now this is, within the limit of error, the same value as that found for egg albumin (3.32×10^{-13}) and for Bence-Jones protein (3.55×10^{-13}), which have been shown to have a molecular weight of one-sixth of that of normal excelsin. It is therefore extremely probable that in the solution of P_H 11.88 the excelsin is broken up into molecules of one-sixth the weight of normal excelsin.

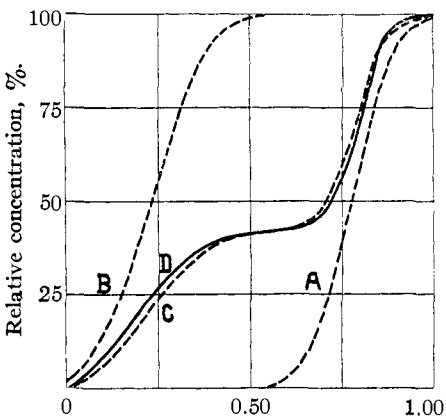

Fig. 3.

A test of the reversibility of the disaggregation of the excelsin molecule was performed. A solution identical with the one used for Run 10 (of P_H 11.88) which had been shown to possess a sedimentation constant of 3.54×10^{-13}, indicating a molecular weight of one-sixth of normal excelsin, was dialyzed for seven days against a phosphate buffer of P_H 6.5 (0.1 M in KH_2PO_4, 0.1 M in Na_2HPO_4 and 1% in ammonium sulfate). As shown by Table IV, excelsin has its normal molecular weight at the P_H of 6.5. During the dialysis a small amount of insoluble substance appeared and was filtered off. The determination of the sedimentation constant gave 11.7×10^{-13} (corrected value). A small amount of non-centrifugible substance was present and also traces of a centrifugible substance of lower sedimentation constant. The light absorption had very nearly assumed the value corresponding to undecomposed excelsin (compare Fig. 1). The disaggregation of the excelsin molecule into molecules one-sixth of normal excelsin is therefore practically reversible.

Discussion of Results

The sedimentation equilibrium measurements have shown that amandin has a molecular weight of 208,000 ± 5000 and excelsin a weight of 212,000 ± 5000 near their isoelectric points. These values are, within the limits of error, identical with those previously found for edestin,[1] R-phycoeryth-

rin,[12,13] R-phycocyan and C-phycocyan[13] near their isoelectric points. At higher P_H values the molecules of all these proteins have been found to break up into simple sub-multiples of the isoelectric molecule. With regard to P_H stability R-phycoerythrin resembles amandin inasmuch as at a P_H of 11.0 its molecules are to an amount of about 25% disaggregated into molecules of the weight one-sixth of that of the normal molecule. The sedimentation velocity measurements have shown that at a P_H of 12.2 the amandin molecule is to an extent of about 41% disaggregated into molecules of weight one-sixth of the normal.

Excelsin seems to be somewhat less stable than amandin. Its P_H stability region is smaller and it shows a tendency to form a non-centrifugible decomposition product, which is not the case with amandin.

The values of the sedimentation constants 11.41×10^{-13} for normal amandin and 11.78×10^{-13} for normal excelsin are within the limits of error identical with the values found for the proteins just mentioned in the vicinity of their isoelectric points. A calculation of the molar frictional constants f at 20° by means of the formula, $f = [M(1 - V\rho)]/s$, gives for amandin 4.63×10^{16} and for excelsin 4.63×10^{16}, while the values of f_s to be expected for spherical molecules of the same weight and specific volume, calculated by means of the formula $f_s = 6\pi\eta N(3MV/4\pi N)^{1/3}$, are for amandin 4.50×10^{16} and for excelsin 4.52×10^{16}. The ratio f/f_s, which for a spherical molecule should be unity, is for amandin 1.03 and for excelsin 1.02. The radius of the molecule, derived from the formula $r = (3MV/4\pi N)^{1/3}$, is for amandin 3.94$\mu\mu$ and for excelsin 3.96$\mu\mu$.

It seems to be of interest to be able to compare the values for the frictional constant and the radii of edestin, R-phycoerythrin, R-phycocyan, and C-phycocyan with the values for amandin and excelsin. Those other values have therefore been calculated. In order to give a more complete review of the data of the six proteins with molecular weights near 208,000. Table V has been arranged so as to contain molecular weight, M, sedimentation constant, s, molar frictional constant, f, molar frictional constant for a spherical molecule, f_s, dissymmetry ratio f/f_s and radius r.

TABLE V

COLLECTED VALUES

Protein	Molecular weight	$s \times 10^{13}$	$f \times 10^{-16}$	$f_s \times 10^{-16}$	f/f_s	r, $\mu\mu$
Amandin	208,000 = 6.04 × 34,500	11.4	4.63	4.50	1.03	3.94
Excelsin	212,000 = 6.15 × 34,500	11.8	4.63	4.52	1.02	3.96
Edestin	208,000 = 6.04 × 34,500	12.8	4.16	4.50	0.93	3.94
R-Phycoerythrin	209,000 = 6.06 × 34,500	11.5	4.61	4.51	1.02	3.95
R-Phycocyan	206,000 = 5.97 × 34,500	11.1	4.51	4.51	1.00	3.95
C-Phycocyan	208,000 = 6.04 × 34,500	11.2	4.74	4.50	1.05	3.94

[12] Svedberg and Lewis, THIS JOURNAL, **50**, 525 (1928).

[13] Svedberg and Katsurai, *ibid.*, **51**, 3573 (1929).

Within the limit of error all the corresponding data of Table V are equal for the six proteins in question.[14] Thus it is obvious that the mass and the shape of the molecules of these proteins are almost identical. The six proteins in question have different chemical composition, different isoelectric points and different light absorption. Their P_H stability ranges are also different but the molecular weights of the products of alkaline disaggregation are simple sub-multiples of the normal molecule. So far only the sub-multiples $^1/_2$, $^1/_3$, $^1/_6$ have been observed. It is very remarkable that these sub-multiples are identical with the normal molecular weights of other proteins. Thus all the 12 native and stable proteins so far investigated in this Laboratory as well as their disaggregation products—with the possible exception of the hemocyanins, which have molecular weights of the order of millions—can all as a first approximation be derived from the unit 34,500 by multiplying with the integers *two*, *three* and *six*.

The expenses connected with these experiments have been defrayed by grants from the foundation "Therese och Johan Anderssons Minne" and from the Nobel Fund of Chemistry.

Summary

1. The molecular weights and P_H stability regions of amandin and excelsin have been studied by means of the ultracentrifugal methods.

2. Amandin is stable from P_H 4.3 to about 10 with a molecular weight of 208,000 ± 5000. At a P_H of 12.2 it is partly disaggregated, forming molecules with a weight one-sixth of the normal ones. The sedimentation constant is 11.41×10^{-13} and the molar frictional constant 4.63×10^{16} for the normal molecules. The molecules of normal amandin are spherical with a radius of 3.94 $\mu\mu$.

3. Excelsin is stable from P_H 5.5 to about 10, possessing a molecular weight of 212,000 ± 5000. At a P_H of 11.9 excelsin is completely disaggregated into molecules of one-sixth of the molecular weight of normal excelsin. The sedimentation constant is 11.78×10^{-13} and the molar frictional constant 4.63×10^{16} for the normal molecules. The sedimentation constant is 3.54×10^{-13} for the disaggregated molecules. The aggregation and disaggregation processes are reversible. The normal excelsin molecules are spherical with a radius of 3.96 $\mu\mu$.

4. The molecular weight, sedimentation constant, molar frictional constant and molecular radius of amandin and excelsin have been compared with the corresponding constants of edestin, R-phycoerythrin, R-phycocyan and C-phyccocyan and have been found to be identical within the limits of error.

5. The disaggregation products of these six proteins have been found

[14] The sedimentation constant for edestin and the other data containing this constant are uncertain because of the high salt content of the solution.

to belong to one or more than one of the sub-multiples $^1/_2$, $^1/_3$ or $^1/_6$ of the normal molecule. The molecular weights of these sub-multiples correspond to normal molecular weights of other proteins.

UPSALA, SWEDEN

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

CYCLIC QUATERNARY AMMONIUM SALTS FROM HALOGENATED ALIPHATIC TERTIARY AMINES

BY E. R. LITTMANN AND C. S. MARVEL

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The formation of rings from open-chain compounds of various types has been the subject of many recent investigations. The work of Ruzicka[1] and his collaborators on the large carbon rings has led to this study of the nitrogen compounds in the hope of obtaining large rings containing a nitrogen atom in place of one of the carbon atoms. The largest simple ring containing a nitrogen atom heretofore described is the seven-membered ring, hexamethylene-imine, which has recently been characterized by Müller and Sauerwald.[2]

In a recent study of bromo tertiary amines[3] it was observed that 4-bromobutyldiethylamine on standing changed into a quaternary ammonium salt, which was thought to be a pyrrolidine derivative. The ease with which this reaction occurred suggested that other brominated tertiary amines might give the desired larger rings containing nitrogen.

Knorr and Roth[4] have studied bromo-ethyldimethylamine and chloropropyldimethylamine and have found that they reacted to give six- and eight-membered rings containing two nitrogen atoms, according to the general reaction

$$2\mathrm{Br(CH_2)}n\mathrm{NR_2} \longrightarrow \mathrm{R_2N}\begin{matrix}\diagup\mathrm{(CH_2)}_n\diagdown\\ \mathrm{Br}\qquad\quad\mathrm{Br}\\ \diagdown\mathrm{(CH_2)}_n\diagup\end{matrix}\mathrm{NR_2}$$

Von Braun and his students[5] have studied bromo-alkyl primary amines of the type $\mathrm{Br(CH_2)}_n\mathrm{NH_2}$ and have found that when $n = 4, 5$ or 6 some of the cyclic nitrogen compound was obtained, although the yields were low and by-products due to secondary reactions formed the greater part of the final product. When $n = 7$, they were unable to obtain a reaction product that could be identified as the cyclic amine. In addition to these

[1] The fourteenth paper on large carbocyclic rings by Ruzicka and his co-workers was published in *Helv. Chim. Acta*, **11**, 1174 (1928).

[2] Müller and Sauerwald, *Monatsh.*, **48**, 527, 731 (1927).

[3] Marvel, Zartman and Bluthardt, THIS JOURNAL, **49**, 2299 (1927).

[4] Knorr and Roth, *Ber.*, **39**, 1425 (1906).

[5] Von Braun and Steindorff, *ibid.*, **38**, 173, 3089 (1905); Von Braun and Müller, *ibid.*, **39**, 4116 (1906); Von Braun and Beschke, *ibid.*, **39**, 4121 (1906).