TABLE I

PHYSICAL CONSTANTS AND ANALYSES OF THE N-PHENYLPIPERAZINO-N'-ALKYL ACETATES

Ester	M. p., °C. corr.	B. p., °C. corr.	Press., mm.	Bath, °C.	d^{20}_{20}	n^{25}	Analyses, % N: Free base Calcd.	Free base Found	Dihydrochloride[c] Calcd.	Dihydrochloride[c] Found
Methyl	Oil	175–177	4–5	245	1.121	1.548			9.12	9.09
Ethyl	Oil	194–195	7–8	235	1.112	1.543	8.09[a]	8.09[a]	8.72	8.56
n-Propyl	Oil	185–186	3–4	250	1.089	1.536			8.36	8.43
n-Butyl	54–54.5	218–220[b]	8–9	270	...	...	10.14	10.02		

[a] Sulfate. [b] Slight decomposition. [c] Oven-dried.

liquid members of the piperazine series. The solubilities of the compounds in the N-phenylpiperazine series are greater and include a wider range of solvents than those in the piperazine series.

Experimental

N - Phenylpiperazino - N' - alkyl Acetates.—(Table I.) These arise from the action of N-phenylpiperazine[2] on the appropriate chloroacetic ester in ethanol solution in the presence of sodium carbonate. The average yield is 65–75%.

N-Phenylpiperazino-N'-ethanamide.—(a) From N-phenylpiperazino-N'-ethyl acetate and concentrated ammonium hydroxide in 85% yield, m. p. 169–170°. Calcd. for $C_{12}H_{17}ON_3$: N, 19.17. Found: N, 19.22. (b) From N-phenylpiperazine, chloroethanamide and sodium carbonate in quantitative yield, m. p. 169–170°. Found: N, 19.12. Toluene is the best solvent for recrystallization.

N-Phenylpiperazino-N'-ethanenitrile.—(a) From N-phenylpiperazine, methanal and hydrogen cyanide in 40% yield, m. p. 65–65.5°. Calcd. for $C_{12}H_{15}N_3$: N, 20.89. Found: N, 20.70. (b) From N-phenylpiperazine, chloroethanenitrile and sodium carbonate in hexane in 70% yield, m. p. 65–65.5°. Found: N, 20.71. Purification is effected by recrystallization from hexane.

Reduction of N-Phenylpiperazino-N'-ethyl Acetate.—This is accomplished by means of sodium and n-butyl alcohol. After removal of the latter by steam distillation the strongly alkaline solution is extracted with hot benzene which is cooled and dried over sodium carbonate. Evaporation of the benzene yields N-phenylpiperazino-N'-β-ethanol (yield, 35%), purified by repeated crystallization from hexane, m. p. 82.5–83°. A mixed melting point with an authentic specimen[3] shows no depression. Although the products of these two independent syntheses prove to be identical, Prelog and Blazek[4] report a third synthesis of the alcohol with a melting point of 91°. Upon decolorization and filtration of the hot concentrated alkaline reduction solution, N-phenylpiperazino-N'-sodium acetate is obtained. Calcd. for $C_{12}H_{15}O_2N_2Na$: N, 11.57. Found: N, 11.39. A sample of the latter prepared by refluxing N-phenylpiperazine, sodium chloroacetate and sodium carbonate in aqueous solution and purified by crystallization from water shows similar properties and 11.48% N. The yield is quantitative.

(2) Pollard and MacDowell, THIS JOURNAL, 56, 2199 (1934).

Summary

1. The preparation and properties of compounds arising from the reaction between N-phenylpiperazine and derivatives of monochloroacetic acid have been described.

2. A comparison has been made between these compounds and those of the piperazine series.

(3) Adelson, MacDowell and Pollard, unpublished work.

(4) Prelog and Blazek, *Collection Czechoslov. Chem. Communications*, 6, 549 (1934).

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Physical Chemistry of Lipoids. II. The Protective Power of Cephalin[1]

BY MONA SPIEGEL-ADOLF

The protective power of colloids can be investigated in different ways. It can be tested, for instance, on colloidal gold in the manner used by Schulz and Zsigmondy[2] for the classification of protein fractions or protein derivatives.[3] Furthermore, the protective power of a colloid may be used with reference to other colloids. For the biologist especially the interaction between colloids of the human body is of interest.

Although lipoids are known to behave in aqueous solutions like lyophilic colloids, no figures for their protective power could be found. The results to be reported here were obtained with the intention of supplying this deficiency. Investigations of the interactions between lecithin and proteins have been reported previously;[4]

(1) Read before the Meeting of the American Chemical Society at New York, April 23, 1935.

(2) Fr. N. Schulz and R. Zsigmondy, *Hofmeister's Beitr.*, 3, 137 (1902).

(3) E. Zunz, *Arch. int. d. Physiol.*, 1, 427 (1904); M. Spiegel-Adolf, *Biochem. J.*, 28, 1201 (1934).

(4) M. Spiegel-Adolf, *ibid.*, 26, 2183 (1932).

experiments were now extended to cephalin. This phospholipid seemed particularly promising for the study of the protective power, since like proteins, it is an ampholyte. According to Pauli and Valkó[5] the characteristic features of the proteins in their behavior toward colloids depend on their ampholytic nature. Besides, according to Singer[6] in human brain tissues cephalin prevails quantitatively over lecithin. However, in order to have material for comparison, further experiments were made with sols of human lecithin and egg lecithin.

Material.—The cephalin was obtained from human brains, cleaned from blood and meninges, dried at 37° and subsequently pulverized. This powder was extracted with ether and then treated with absolute alcohol, a precipitation resulting. The precipitate was redissolved in ether and reprecipitated with absolute alcohol. This procedure was repeated three times. The preparation thus obtained contained 2.64% of nitrogen and 5.6% of phosphorus. It was entirely soluble in water, ether and chloroform, insoluble in absolute alcohol and acetone. The conductance of a 1% aqueous solution is $K_{25}° = 3.10.10^{-4}$ mhos, the cH = $1.38.10^{-6}$. After further purification by means of dialysis the conductance dropped to $9.3.10^{-5}$ mhos and the cH rose to $4.71.10^{-6}$.

Cephalin and Colloidal Gold.—The colloidal gold used in these tests was prepared by the formaldehyde method. However, the results were essentially the same when dialyzed samples of colloidal gold or preparations of Bredig gold were used. Dialyzed cephalin showed the same behavior as undialyzed samples. Amounts of a 1% cephalin sol greater than 0.1 cc. protected 1 cc. of the red gold sol when 0.2 cc. of normal potassium chloride was added; 0.05 cc. of the cephalin sol failed to protect completely, since the red gold changed to red-violet in color. Smaller quantities showed still less protective action.

Using similar quantities of gold sol and normal potassium chloride, human lecithin showed protective action in quantities of 0.3 cc. of a 1% lecithin sol and above, some protective action in quantities of 0.2 cc.; little or no protection active in smaller amounts. These experiments demonstrated that cephalin did not precipitate the colloidal gold sol, that when it was present in appreciable concentration it protected the colloidal gold against precipitation by potassium chloride, and that the protective action of cephalin toward colloidal gold is greater than is the protective action of human lecithin from the same brain tissue. The protective power of human lecithin is greater than the protective power of egg lecithin (Merck). Both lecithin sols have been made by the method of Keeser.[7] In its behavior toward colloidal gold, cephalin shows, therefore, a closer resemblance to gelatin than to the proteins of the blood and of the egg-white, which precipitate gold, unless in the presence of electrolytes.[8]

(5) W. Pauli and E. Valkó, "Elektrochemie der Kolloide," J. Springer, Wien, 1929.

(6) K. Singer, *Biochem. Z.*, **179**, 432 (1926).

(7) E. Keeser, *ibid.*, **154**, 321 (1924).

(8) M. Spiegel-Adolf, *ibid.*, **180**, 395 (1927).

Cephalin and Proteins.—In a preliminary test it was ascertained that, like egg lecithin, lecithin made from human brain flocculates with electrodialyzed serum-albumin and pseudoglobulin. Similar tests were made with fresh and six-day old solutions of cephalin and with freshly electrodialyzed samples of serum-albumin and pseudoglobulin. The latter were prepared from horse serum by fractionation with ammonium sulfate. In all experiments the cephalin concentration was kept constant at 1%, while the protein was varied. The following results could be obtained: Neither serum-albumin in concentrations up to 1%, nor pseudoglobulin in concentrations up to 2.5% cause any precipitation in fresh samples of cephalin. Fresh samples of lecithin, on the other hand, were flocculated to an increasing extent by serum-albumin from 0.4% upward, while pseudoglobulin became effective at a concentration of 0.2%. In aged samples of cephalin no apparent change was called forth by serum-albumin, although 1–2.5% pseudoglobulin caused an increasing cloudiness. An aged egg lecithin sol is far more sensitive to pseudoglobulin than is a fresh sample,[4] and in aged samples of egg lecithin sol as well as in more recent preparations pseudoglobulin is more effective than is serum-albumin.

Even an interferometer did not show a change in the degree of dispersion of a cephalin sol upon the addition of serum-albumin, such as has been found in lecithin–protein mixtures when flocculation had been prevented by a low amount of salt.[4] A 0.25% cephalin solution gave an interferometer reading of 75 units, a 0.25% serum-albumin sol a reading of 112 units and a sol containing 0.25% cephalin and 0.25% serum-albumin a reading of 189 interferometer units, this latter being within experimental error of the calculated 187 which should have been obtained had the values been additive.

The behavior of cephalin as a protective colloid toward colloidal gold shows features in common with the action of gelatin. Gelatin not only prevents precipitation of colloids by electrolytes but also gives protection against coagulating effects from physical agents. Some years ago Spiegel-Adolf[9] showed that gelatin prevents the coagulating effect of shaking on proteins. It has been since observed by the author[10] that no apparent heat coagulation takes place in the presence of certain amounts of gelatin, if the temperature is just sufficient to initiate heat coagulation in an unprotected serum-albumin solution. The action of gelatin on proteins has tentatively been called protective. Though certain electrolytes are able to raise the coagulation temperature of proteins, they seem to act rather through an increase in electric charge and in viscosity of the proteins themselves.

In experiments with cephalin, with serum-albumin and with pseudoglobulin, a similar protective effect of cephalin was found. The results were not altered by the use of dialyzed cephalin solutions. Cephalin prevented heat coagulation in serum-albumin of twice its own concentration, and failed to do so when the relation of cephalin to serum-albumin was increased to 1:3. In all these experiments the lipoid–protein mixtures and the controls were heated for fifteen minutes in a boiling water-bath.

(9) M. Spiegel-Adolf, "Die Globuline," Th. Steinkopff, Dresden, 1930, p. 377.

(10) Yet unpublished.

Cephalin and Lecithin.—The above-mentioned differences in the behavior of cephalin and lecithin can be used as tests for the protective power of cephalin on lecithin against the precipitating effect of protein. Lecithin of human brain was used in these experiments. In spite of the fact that cephalin sols mixed with lecithin sols show added values in the interferometer and therefore do not indicate a change of dispersion, nevertheless the influence of cephalin on lecithin becomes very marked when protein is present.

Cephalin apparently protects lecithin against the precipitating effect both of serum-albumin and of pseudoglobulin. In a 0.6% serum-albumin sol containing 0.5% of lecithin there was an immediate precipitation when 0.025% or less of cephalin was present. If, however, the cephalin concentration equaled 0.05% or more, no precipitation of the albumin–lecithin system took place. The importance of using cephalin-free samples of lecithin for physico-chemical tests is evident. These findings may on one hand explain the discrepancies in the literature about the flocculating effect of proteins on lecithin sols. On the other hand they show that there exists a mechanism beside the action of neutral salts[4] that is able to prevent a flocculation of lecithin by proteins.

Cephalin and Cholesterol.—Finally the effect of cephalin on cholesterol sol was studied. In order to have comparable data for human lecithin all experiments were made with this substance. The sol of cholesterol (Merck) was made according to the method of Keeser.[7] After filtration the milky solution was fairly stable. In preliminary tests the reactions of the cholesterol sol toward neutral salts, serum-albumin and pseudoglobulin were investigated. The observations were in harmony with experiences of former authors.[11] In series of experiments it was shown that cephalin as well as lecithin is able to protect cholesterol sol against flocculation by salts or proteins. In a system containing 0.5% cholesterol and 0.1 cc. of a normal potassium chloride, cephalin in 0.0016% nearly inhibited the flocculation of the cholesterol. When the cephalin concentration was 0.0024% or greater, the flocculation was completely inhibited. Flocculation was likewise inhibited in a similar system when lecithin in concentration of 0.02% or more was used instead of cephalin.

In a system containing 0.5% of cholesterol and 0.05% serum-albumin, cephalin in concentration of 0.01% or more inhibited flocculation, whereas definite flocculation took place in a similar system, when lecithin was present to the extent of 0.05%; but no flocculation of the cholesterol–protein system took place when the lecithin concentration was raised to 0.1%.

These findings may have to be considered in all cases in which the role of protective colloids has been thought important for the stability of cholesterol, *e. g.*, in the formation of stones under pathologic conditions.[12]

No theory has been formulated as yet as to the nature of the special arrangement in the cephalin molecule to which the protective power of the lipoid is due. Some evidence for such formulation might be obtained, however, by the results of the following experiments. It was ascertained that the protective power of cephalin on colloidal gold is practically unchanged after the solution has undergone boiling. This is in agreement with the results reported on the protective power of cephalin against the heat coagulation of protein and proves the heat stability of the protective power of cephalin.

On the contrary, the protective power of cephalin on cholesterol is practically completely destroyed by intensive irradiation of cephalin with ultraviolet light (quartz mercury lamp Hanovia, 220 v. d. c., four hours at 20 cm. distance, 0.25% cephalin sol). Thus, for example, a 0.5% cholesterol sol containing 0.1 cc. of normal potassium chloride was not protected, even when a quantity of irradiated cephalin as great as 0.8% was added to the system.

The irradiated cephalin sol does not show gold sol flocculation as observed in proteins under similar conditions,[13] nor hemolysis of erythrocytes as has been described by Fabre and Simonnet[14] in irradiated lecithin, through the formation of a lysolecithin-like substance. At all events, according to Magistris[15] a sample of lysocephalin free from lysolecithin is not hemolytic. As in proteins[16] heat and short wave light have a different effect on cephalin. An analysis of the effects of irradiation may, therefore, be helpful for the understanding of the protective power of cephalin.

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Summary

1. The protective power of cephalin (made from human brains) was tested on colloidal gold, proteins, lecithin and cholesterol.

2. Cephalin protects colloidal gold against flocculation by neutral salts 3–4 times more efficiently than does brain lecithin. The protective power of egg lecithin is still smaller.

3. Contrary to lecithin, cephalin is not flocculated by serum-albumin, and only in aged samples of cephalin is cloudiness called forth by high pseudoglobulin concentrations.

4. One part cephalin is able to prevent heat coagulation of 2 parts serum-albumin in aqueous solution.

5. One part cephalin protects 10 parts lecithin efficiently against precipitation by proteins.

6. Cephalin and lecithin are both able to protect cholesterol sol against precipitation by salts or proteins. Cephalin is ten times more effective than lecithin.

7. The protective power of cephalin for colloidal gold is unimpaired by boiling but is destroyed (tested on cholesterol sols) by an intensive irradiation of the cephalin by ultraviolet light.

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(11) R. Stern, *Arch. exp. Path. u. Pharm.*, **112**, 129 (1926).

(12) M. Spiegel-Adolf, *Klin. Wochenschr.*, **5**, 28 (1926).

(13) M. Spiegel-Adolf, *Biochem. J.*, **28**, 372 (1934).

(14) R. Fabre and H. Simonnet, *Bull. soc. chim. biol.*, **10**, 1306 (1928).

(15) H. Magistris, *Biochem. Z.*, **210**, 85 (1929).

(16) M. Spiegel-Adolf, *ibid.*, **186**, 181 (1927).