with acetic acid and filtered. The filtrate after taking up in ether, washing and drying was evaporated and the residue was distilled in an oil pump vacuum at 240°. The distillate, crystallized from dilute alcohol, furnished 0.4 g. of 2,3,5-triphenylpyrrole melting at 141–142°. Identification was made by comparison with an authentic specimen of triphenylpyrrole. Although triphenylpyrrole and triphenylchloropyrrole melt at almost the same temperature, mixtures of the two melt below 130°.


## Summary

This article describes the ring–chain isomerism of 2-hydroxy-2,3,5-triphenylpyrrolenine oxide and the monoxime of phenyldibenzoylethylene, and the behavior of these isomers toward alkylating agents, acetic anhydride and acetyl chloride.


WASHINGTON, D. C. RECEIVED FEBRUARY 1, 1936

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# Physico-Chemical Studies on Lecithin

BY HENRY B. BULL AND VERNON L. FRAMPTON[1]

In spite of the great amount of work on the lecithins, our knowledge concerning this class of compounds is still most unsatisfactory. The difficulty is no doubt due to the very labile character of lecithin. There appears to be no unanimity concerning such a simple property as the isoelectric point and each new communication brings new values differing from all the others. Table I gives some of the values reported in the literature.

TABLE I

| | |
|---|---|
| Price and Lewis[2] (1933) | 2.7 |
| Chain and Kemp[3] (1934) | 6.7 |
| Fujii[4] (1924) | 2.7 |
| Sueyoshi and Kawai[5] (1932) | 4.7 |
| Rona and Deutsch[6] (1926) | 1.75 |
| Remesow[7] (1930) | 2.0–2.8 |

We wish to report in this paper a study of the isoelectric point of mixtures of lecithin and cephalin and a value for the isoelectric point of lecithin obtained by extrapolating to zero concentration of cephalin.

As noted above, Sueyoshi and Kawai[5] found the isoelectric point of lecithin prepared by the method of Sueyoshi[4] to be at a *p*H of 4.7. The method of Sueyoshi depends upon the difference in solubility of lecithin and cephalin in ethyl alcohol as a means of separating these two compounds.

We have modified the method of Sueyoshi somewhat and since we believe the manner of preparation of lecithin is tremendously important we present our modification in detail. As will be shown this method will produce lecithin containing considerable quantities of cephalin.

### Method

All solvents employed were carefully purified. The peroxide normally found in laboratory ether was removed completely. All temperatures higher than 40° were avoided. The material was at no time exposed to air, being always covered with solvent. All filterations were conducted in the dark and in the cold. While not being used the preparation was stored at −15° in the dark and under acetone.

The yolks of the eggs were dehydrated with cold acetone, the acetone drained off and the dehydrated yolks allowed to remain in contact with warm 95% ethyl alcohol for thirty minutes. The residue was then placed in an extractor and extracted with ether until colorless. The alcohol and ether extracts were combined, a small amount of hydroquinone added as anti-oxidant, and the whole evaporated at 40° or below under low pressure to a thick oil. This was extracted with and finally kneaded with small portions of cold acetone in a mortar many times until it assumed a wax-like consistency and no further color was extracted. The preparation at this point should be only faintly yellow. It was dissolved in a minimum quantity of ether and the solution stored at −15° for twelve hours. The precipitate which consisted largely of cerebrosides and sphingomyelin was filtered off in the cold. The filtrate was evaporated greatly under diminished pressure to a viscous liquid which was then poured slowly into a large volume of cold acetone with constant stirring. The white precipitate was collected and the ether treatment repeated. The combined lecithin and cephalin were dissolved in a minimum quantity of warm alcohol and the solution allowed to stand overnight at −15° and filtered at that temperature. This treatment is supposed to remove the cephalin which is considered to be insoluble in alcohol. Actually, as will be shown, the cephalin is only partly removed. The alcohol solution was evaporated under low pressure and poured into cold acetone. This was repeated. About 70 g. of clear nearly colorless wax-like lecithin was obtained from 15 dozen eggs. This material seems to remain unchanged for a long time if stored at −15° in the dark under acetone.

(1) This research was made possible by a generous grant from the Graduate School of the University of Minnesota.

(2) H. B. Bull, *J. Phys. Chem.*, **39**, 577 (1935).

(3) E. Chain and I. Kemp, *Biochem. J.*, **28**, 2052 (1934).

(4) Y. Sueyoshi, *J. Biochem.* (Japan), **13**, 145 (1931).

(5) Y. Sueyoshi and K. Kawai, *ibid.*, **15**, 277 (1932).

(6) H. Fishgold and E. Chain, *Biochem. J.*, **28**, 2044 (1934).

(7) I. Remesow, *Biochem. Z.*, **218**, 86 (1930).

We analyzed several samples of our lecithin and the following is typical.

| | |
|---|---|
| Total nitrogen | 1.69 |
| Amino nitrogen | 0.106 |
| Phosphorus | 3.83 |
| Nitrogen to phosphorus ratio | 1.01 |
| Total fatty acid | 68.9 |
| Cerebrosides | nil |

This analysis indicates the absence of all impurities other than cephalin.

Mobility measurements were made in a microelectrophoretic cell described by Bull[2] on a fresh suspension of fresh lecithin at a constant ionic strength of 0.01 in a NaCl–HCl buffer. The suspension of lecithin was readily made by stirring a small piece of lecithin into the buffer with a stirring rod. For the denser suspensions the lecithin was suspended with the aid of a mechanical glass stirrer. Measurements of the electrophoretic speed were made on individual emulsion droplets of the suspended lecithin. The variation in the speeds from droplet to droplet at a given *p*H was remarkably small. Four determinations of speeds were made at 0.211 distance from the top and a like number 0.211 distance from the bottom and these averaged for the final value. The isoelectric point was considered to be the point of zero mobility and was arrived at by interpolation of a smooth curve of the speeds plotted against *p*H. At least six determinations were made over a wide *p*H range.

We investigated the isoelectric point at various stages of purification and obtained the following results:

| | |
|---|---|
| After removal of fats with acetone | 2.85 |
| After removal of material insoluble in ether (cerebrosides and sphingomyelin) | 2.70 |
| After removal of material insoluble in ethyl alcohol (large part of the cephalin) | 4.00 |

Apparently the presence of cephalin lowers the isoelectric point greatly. This is to be expected since the basic group of cephalin is weak while the acid group is quite strong. It is very difficult to remove the cephalin from lecithin. We dissolved a sample of lecithin in a small volume of ether and poured the solution into boiling acetone. A small amount of lecithin precipitated and was separated out. As the solution cooled to −15° several samples of lecithin were thus obtained. The amino nitrogen and the isoelectric point of each sample was determined with the following results:

| | | | | |
|---|---|---|---|---|
| $NH_2$ nitrogen | 0.42 | 0.38 | 0.25 | 0.06 |
| Isoelectric point | 3.70 | 3.90 | 4.10 | 5.90 |

These data are plotted in Fig. 1 and when extrapolated to zero concentration of cephalin will yield an isoelectric point of 6.4, which is in good agreement with that of Chain and Kemp.[3] The marked dependence of the *p*H of the isoelectric point of lecithin on the presence of cephalin probably also explains the results of Sueyoshi and Kawai;[5] they were, no doubt, dealing with a lecithin which contained about 0.25% amino nitrogen.

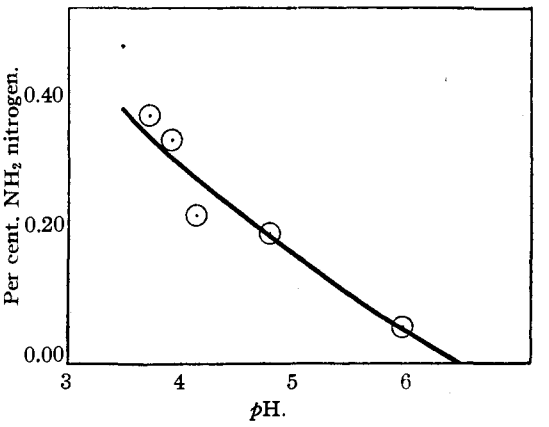

Fig. 1.

The lecithin and cephalin mixture is no doubt made up of many lecithins and cephalins. The alpha and beta forms as well as the various acids present give rise to a number of isomers. These isomers probably have wide differences in solubility in acetone. There is then no doubt considerable overlapping in the solubilities of the lecithins and cephalins and a separation based on a differential solubility in acetone will be only partial. Lecithin and cephalin mixture which has been exhaustively extracted with acetone appears to have more or less a constant composition of 0.42% $NH_2$ nitrogen with an isoelectric point of 3.73. Repeated extraction with acetone failed to alter this isoelectric point. Fishgold and Chain[6] expressed as their belief that isoelectric points lower than 7.0 were due to the presence of fatty acids which had been split off from the glycerol. That this is not true in our case is shown by the fact that this exhaustive treatment failed to alter its isoelectric point and as the fatty acids from our lecithin are very soluble in acetone, the exhaustive treatment with acetone would certainly have removed the free fatty acids.

In agreement with Sueyoshi, the isoelectric point of an aqueous suspension of lecithin was found to drop upon standing. This is shown in Fig. 2.

Titration curves of a 1.1% aqueous suspension of a lecithin–cephalin mixture (containing 0.42% amino nitrogen) were made. A glass electrode was used in conjunction with a saturated calomel half-cell. Very few indeed of the available acid or basic groups are titratable. Only 3% of the basic groups have bound hydrogen ions and this

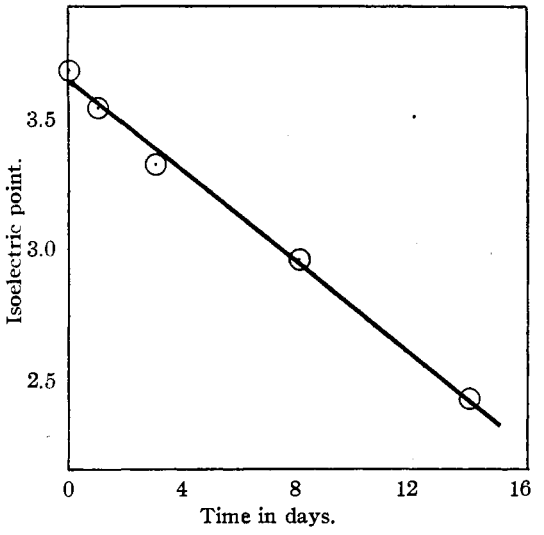

Fig. 2.

apparently represents a constant value. The acid groups open up slightly more until 11.7% of a monobasic acid is available. While it is of course impossible to evaluate the dissociation constants of lecithin with such data, they do indicate that the lecithin–cephalin mixture has a somewhat more acid than basic reaction which is in keeping with the electrophoretic measurement of an isoelectric point of 3.73.

## Summary

1. Lecithin–cephalin mixtures have been prepared from eggs.

2. The importance of the effect of cephalin upon the isoelectric point has been emphasized and the isoelectric point has been plotted as a function of the amino nitrogen content. The curve has been extrapolated to zero amino nitrogen content and yields an isoelectric point of 6.4 for lecithin.

3. The change of the isoelectric point with time has been studied.

4. Titration curves for lecithin suspensions are reported.

ST. PAUL, MINNESOTA RECEIVED DECEMBER 30, 1935

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[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF WESLEYAN AND HARVARD UNIVERSITIES]

# Semicarbazones of Certain Ketones

BY HERBERT S. RHINESMITH

Certain ketones of the ethyl, methyl and propyl series have been prepared in order that the melting points of their semicarbazones might serve to identify certain degradation products obtained during work now under way in the Hall Laboratory.

## Experimental

**5-Carbmethoxyheptanone-2.**—Thirty-four grams of ethyl ethylmalonate was dissolved in 50 ml. of absolute ethyl alcohol and mixed with 12.0 g. of methyl vinyl ketone.[1] Addition of 5 drops of 10% sodium ethoxide caused a vigorous reaction, and after standing one hour at room temperature the solution was acidified and extracted with ether. The latter, dried over sodium sulfate and distilled, yielded 18.5 g. of the dibasic ester, 5,5-dicarbethoxyheptanone-2.

*Anal.* Calcd. for $C_{13}H_{22}O_5$: C, 60.5; H, 8.5. Found: C, 60.8; H, 8.8.

*Anal.* Semicarbazone. Calcd. for $C_{14}H_{25}O_5N_3$: C, 53.3; H, 7.9. Found: C, 52.9; H, 8.2.

This ester upon saponification with cold, dilute alcoholic potassium hydroxide yielded the acid ester, 5-carboxy-5-carbethoxyheptanone-2, as a colorless oil.

*Anal.* Calcd. for $C_{11}H_{18}O_5$: C, 57.4; H, 7.8. Found: C, 57.4; H, 8.2.

*Anal.* Semicarbazone. Calcd. for $C_{12}H_{21}O_5N_3$: C, 50.2; H, 7.3. Found: C, 50.2; H, 7.6.

On treating the dibasic ester with hot, concentrated alcoholic potassium hydroxide, the potassium salt of the dibasic acid separated in tiny white flakes. Subsequent acidification and extraction with ether yielded a pale yellow oil which in turn evolved carbon dioxide at 130°, to form the monobasic acid, 5-carboxyheptanone-2.

*Anal.* Calcd. for $C_8H_{14}O$: C, 60.8; H, 8.9. Found: C, 60.5; H, 9.3.

*Anal.* Semicarbazone. Calcd. for $C_9H_{17}O_3N_3$: C, 50.2; H, 7.9. Found: C, 50.2; H, 8.4.

This acid readily formed the desired methyl ester upon standing for four hours at 0° in a solution of absolute methyl alcohol saturated with dry hydrogen chloride.

*Anal.* Calcd. for $C_9H_{16}O_3$: C, 62.8; H, 9.3. Found: C, 63.0; H, 9.6.

*Anal.* Semicarbazone. Calcd. for $C_{10}H_{20}O_3N_3$: C, 52.4; H, 8.3. Found: C, 52.5; H, 8.9.

**5-Ethylheptanone-3.**—Phosphorous tribromide (51 g.) was added dropwise to 57 g. of 2-ethylbutanol-1 and the solution was warmed for one hour. The oil layer on exhaustive fractional distillation yielded 52 g. of crude 1-bromo-2-ethylbutane, b. p. 144–145° and $n^{20}D$ 1.4498. Of this, 47 g. refluxed for twelve hours with 42 g. of sodium

(1) Obtained through the courtesy of W. H. Carothers, du Pont Experimental Station, Wilmington, Delaware.