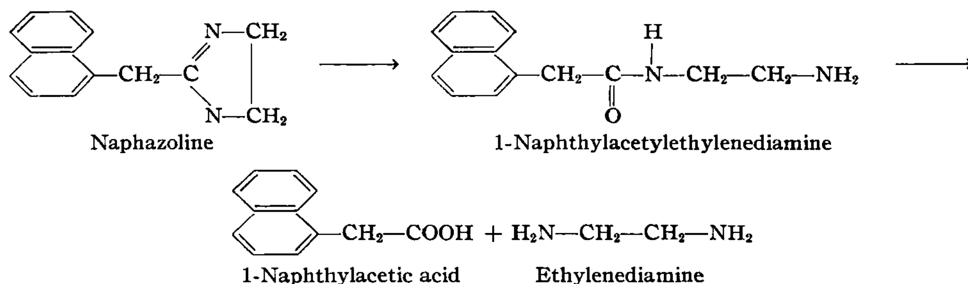
Chromatographic Separation and Analysis of Naphazoline and Its Hydrolytic Degradation Products*

By MICHAEL A. SCHWARTZ†, ROY KURAMOTO‡, and LOUIS MALSPEIS

The probable products of hydrolysis of naphazoline have been isolated. These have been separated by partition chromatography and subsequently analyzed spectrophotometrically. Quantitative recovery of these compounds has been obtained in assays of mixtures of known composition.

In order to study the kinetics of hydrolysis of naphazoline it was necessary to develop a quantitative assay procedure for the probable degradation components in solution after various time intervals.

Chemically naphazoline is 2-(1-naphthylmethyl)-2-imidazoline. The 2-imidazolines, being cyclic amidines, are generally subject to hydrolysis with the formation of 1,2-diamines. It has been shown (1) that naphazoline is quite stable in acid solution even after prolonged heating but it hydrolyzes in water or alkali to yield 1-naphthylacetylethylenediamine. It seemed likely that this compound, having an amide linkage, would undergo further hydrolysis to 1-naphthylacetic acid and ethylenediamine. The postulated overall reaction might therefore be represented as follows:



For purposes of a kinetic study of this system it would be desirable to know at any time interval during the course of the reaction, not only the concentration of residual naphazoline, but also the concentration of 1-naphthylacetylethylenediamine and of either 1-naphthylacetic acid or ethylenediamine.

A survey of the literature revealed several analytical procedures for some of these components (2–5). However, all of these were found to be inadequate since they would not distinguish between two or more of the degradation components.

Attempts at direct spectrophotometric analysis were unsuccessful because of the great similarity in absorption spectra of all the components except ethylenediamine.

Since a method for analysis of the hydrolysis products in the mixture was not available, they were separated by partition chromatography.

EXPERIMENTAL

Materials—Naphazoline hydrochloride,[1] m. p. 255–260°; 1-naphthylacetic acid, Fisher, highest purity, m. p. 131–133°; ethylenediamine, Mathieson, 98–100% pure; silicic acid, Mallinckrodt, chromatography grade; Celite® 545;[2] chloroform, U. S. P.; *n*-heptane; *n*-butanol, Baker analyzed reagent; ethyl acetate, Baker analyzed reagent; buffer solution, *p*H 8.5, 75 ml. 0.2 *M* boric acid plus 25 ml. 0.2 *M* sodium borate.

Equipment.—Beckman Spectrophotometer, Model DU; chromatograph tubes, 400 mm. long, 20 mm. in diameter, constricted at one end into a short delivery tube 7 mm. in diameter. Glass indentations were made near the lower end of the tube to support the column.

Preparation of 1-Naphthylacetylethylenediamine.—This was prepared by a modification of the method used by Miescher, *et al.* (1). Five grams of naphazoline hydrochloride was refluxed with 100 ml. of

* Received June 19, 1956, from the Columbia University College of Pharmacy, New York, N. Y.
† Present address: School of Pharmacy, University of Wisconsin, Madison 6.
‡ Present address: Ciba Pharmaceutical Products, Inc., Summit, N. J.

[1] Supplied by Ciba Pharmaceutical Products, Inc., Summit, N. J.
[2] Supplied by Johns-Manville, Lompoc, Calif.

0.5 *N* sodium hydroxide solution for thirty minutes. The resulting mixture was cooled, made strongly alkaline with concentrated sodium hydroxide solution, and then extracted with chloroform. The chloroform extract was evaporated to dryness leaving a yellowish oil which, upon chilling, gave an off-white colored solid. This was recrystallized several times from 1:1 chloroform-petroleum ether, m. p. 93–94.5°; reported m. p. 90–92° (recrystallized from alcohol-ether).

Anal.—Calcd. for $C_{14}H_{16}N_2O$: C, 73.65; H, 7.06; N, 12.27. Found:[3] C, 73.43; H, 7.14; N, 12.08.

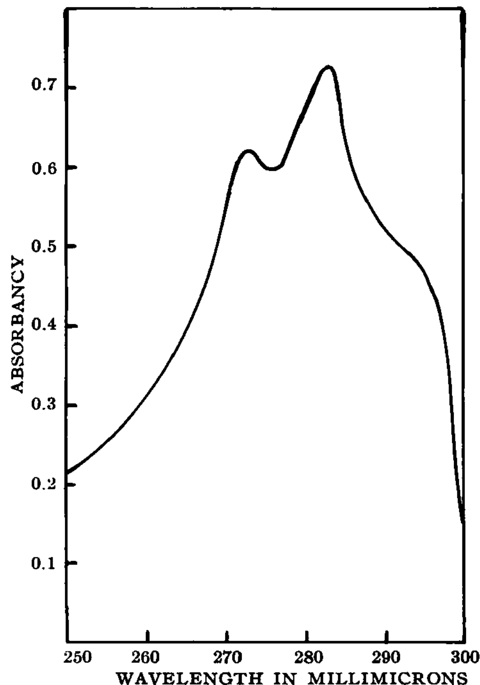


Fig. 1.—Ultraviolet absorption spectrum of 1-naphthylacetic acid in chloroform (0.020 Gm./L.).

The hydrochloride was prepared by dissolving the base in a small amount of chloroform and bubbling in anhydrous hydrogen chloride gas, which produced an oily liquid. As the chloroform was evaporated off, an off-white colored solid precipitated out. This was recrystallized from alcohol-anhydrous ether, m. p. 142–148°; reported m. p. 146–148°.

Anal.—Calcd. for $C_{14}H_{17}N_2OCl$: C, 63.16; H, 6.47; N, 10.58. Found:[3] C, 62.54; H, 6.67; N, 10.26.

Isolation of 1-Naphthylacetic Acid as a Hydrolysis Product.—Five grams of naphazoline hydrochloride was refluxed with 50 ml. of 1 *N* sodium hydroxide solution for two hours. This was cooled and acidified with hydrochloric acid giving a flocculent white precipitate which was filtered off,

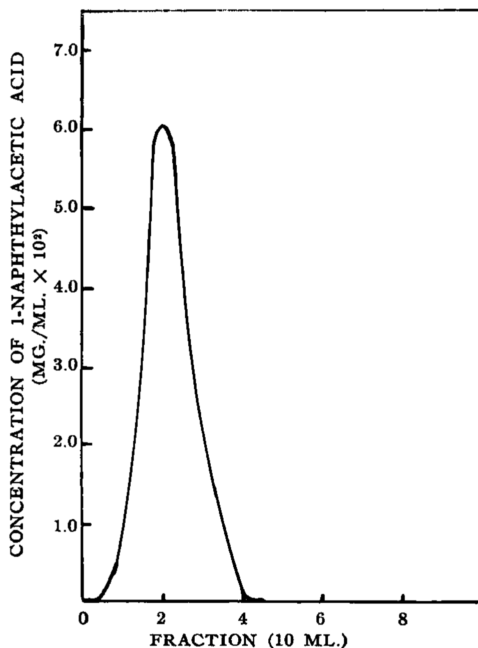


Fig. 2.—Chromatogram showing elution of 1-naphthylacetic acid from a silicic acid partition column.

washed with cold water, and recrystallized from hot water, m. p. 133–134°; reported m. p. of 1-naphthylacetic acid is 131–134°. A mixed melting point taken with a pure sample of 1-naphthylacetic acid was found to be 131–133°. Neutral equivalent found: 193.2; calcd. 186.2.

Partition Chromatography with Silicic Acid

Preparation of Column.—Fifteen grams of silicic acid was hydrated with 15 ml. of water in a 400-ml. beaker and sufficient chloroform added to make a thin slurry. The lower orifice of the chromatograph tube was stoppered, a plug of glass wool placed in the column, and a small amount of chloroform added. The slurry was then transferred to the tube in small portions, each portion being packed down tightly with a close-fitting glass plunger. A layer of solvent was always kept above the column to avoid entrapment of air.

Analysis.—Two grams of silicic acid was hydrated with exactly 2 ml. of the sample solution in a 30-ml. beaker. Chloroform was added to make a thin slurry and this was transferred to the column quantitatively. The beaker was rinsed with two 5-ml. portions of chloroform and the rinsings added to the column. The solvent was allowed to pass into the column before more solvent was added to elute 1-naphthylacetic acid. The first 100 ml. of eluate was collected in a volumetric flask and the concentration of 1-naphthylacetic acid determined spectrophotometrically.

The ultraviolet absorption spectrum of 1-naphthylacetic acid in chloroform is shown in Fig. 1. The compound exhibits maximum absorption at 283 mμ with $E(1\%, 1\text{ cm.})$ equal to 360.

Figure 2 shows the elution of 1-naphthylacetic

[3] The authors wish to acknowledge the valuable assistance given by Mr. Dorfman and his group at Ciba Pharmaceutical Products, Inc., for performing these analyses.

acid from the silicic acid partition column. The compound is eluted within the first 60 ml. of eluate while naphazoline and 1-naphthylacetylethylenediamine are held on the column.

Partition Chromatography with Celite 545

Preparation of Column.—Twenty grams of Celite 545 was covered with 175 ml. of 65:35 *n*-heptane-chloroform (v/v) in a 400-ml. beaker. To this, 15 ml. of *p*H 8.5 borate buffer solution was added dropwise with constant stirring to form a uniform slurry. This was then transferred to the chromatograph tube in the same manner as described in the preparation of the silicic acid column. A layer of solvent was always kept above the column to avoid entrapment of air.

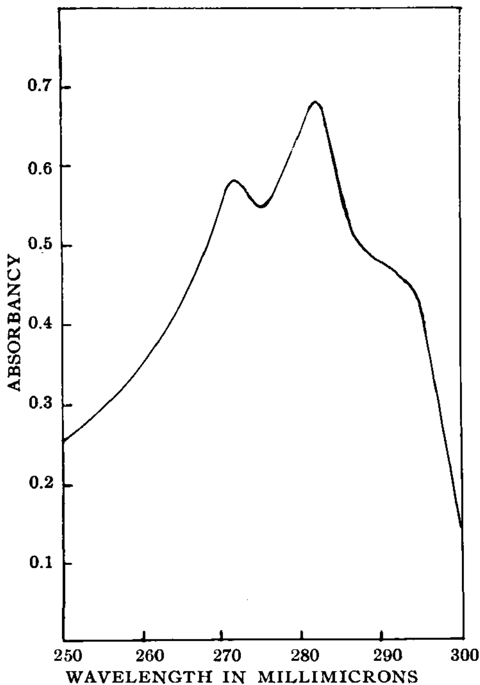


Fig. 3.—Ultraviolet absorption spectrum of naphazoline in 65:35 *n*-heptane-chloroform (v/v) (0.020 Gm./L.).

Analysis.—When all of the organic solvent had just entered the column, exactly 2 ml. of sample solution was pipetted on to the column. Then 65:35 *n*-heptane-chloroform (v/v) was added as eluant as soon as the sample passed into the column. The first 70 ml. of eluate was rejected. The next 125 ml., containing the naphazoline, was collected in 100-ml. and 25-ml. volumetric flasks and analyzed. The eluant was then changed to 100% chloroform and the first 10 ml. of eluate was rejected. The next 100 ml., containing the 1-naphthylacetylethylenediamine was collected in a volumetric flask and analyzed. The concentration of solute in the collected portions was determined spectrophotometrically at the wavelength of maximum absorption of solute.

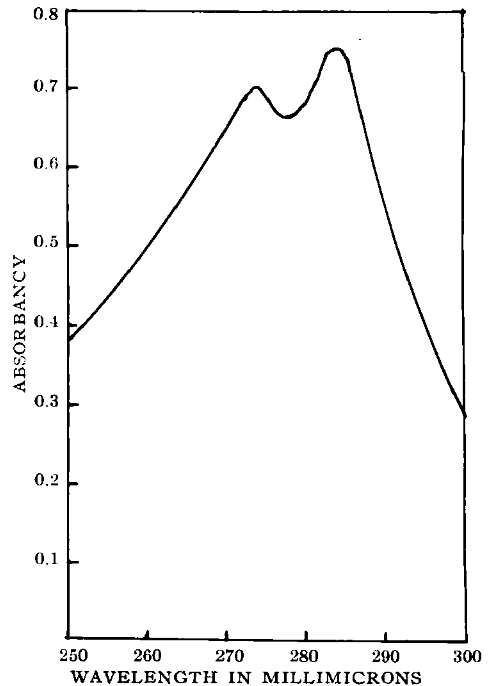


Fig. 4.—Ultraviolet absorption spectrum of 1-naphthylacetylethylenediamine in chloroform (0.020 Gm./L.).

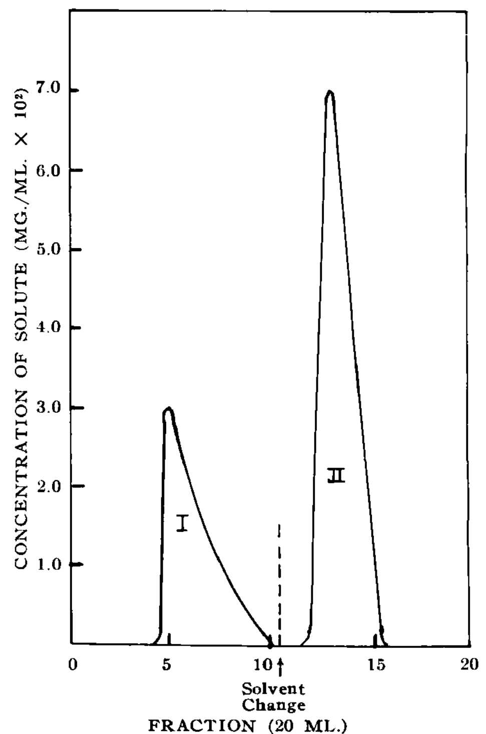


Fig. 5.—Chromatogram showing elution of naphazoline (I) and 1-naphthylacetylethylenediamine (II) from Celite 545 partition column.

The ultraviolet absorption spectrum of naphazoline in 65:35 *n*-heptane-chloroform (v/v) is shown in Fig. 3. The compound shows maximum absorption at 282 mμ with $E(1\%, 1 \text{ cm.})$ equal to 340. The ultraviolet absorption spectrum of 1-naphthylacetylethylenediamine in chloroform is shown in Fig. 4. This shows maximum absorption at 284 mμ $E(1\%, 1 \text{ cm.})$ equal to 314.

Since ethylenediamine does not absorb in this region it was not considered in the separation.

Figure 5 shows the elution of naphazoline and 1-naphthylacetylethylenediamine from the Celite 545 partition column.

RESULTS

Several solutions containing known amounts of the degradation products were prepared and analyzed by the procedures described. The results are shown in Table I.

TABLE I

Sample	Component[a]	Amount in Sample, mg.	Amount Found, mg.	Recovery, %
1	A	2.000	2.025	101.3
	C	1.000	0.993	99.3
2	A	1.707	1.713	100.4
	C	1.000	0.989	98.9
3	A	1.000	0.990	99.0
	B	1.000	0.973	97.3
	C	1.000	0.985	98.5
4	A	1.707	1.660	97.3
	B	2.000	2.066	103.3
	C	2.000	2.002	100.1

[a] Components are designated as: A, naphazoline; B, 1-naphthylacetylethylenediamine; C, 1-naphthylacetic acid.

It is apparent from the data in Table I that the precision of the method is within ± 3%.

SUMMARY AND CONCLUSIONS

1. Naphazoline hydrolyzes, upon heating in neutral or alkaline solution, to 1-naphthylacetylethylenediamine, which probably, upon further heating, breaks down to 1-naphthylacetic acid and ethylenediamine.

2. A two-step procedure for separating the components of the hydrolysate by means of partition chromatography has been developed. By partition with a 15-Gm. silicic acid column, containing 15 ml. of water as the internal phase, and using chloroform as the eluant, 1-naphthylacetic acid was separated from the other degradation components and analyzed spectrophotometrically.

3. Naphazoline and 1-naphthylacetylethylenediamine were separated by partition chromatography with a 20-Gm. Celite 545 column containing 15 ml. of *p*H 8.5 borate buffer solution as the internal phase. Naphazoline was eluted first using 65:35 *n*-heptane-chloroform as eluant. The solvent was then changed to 100% chloroform to quickly elute the 1-naphthylacetylethylenediamine. The concentration of solute in these eluates was determined spectrophotometrically.

Quantitative recovery of the degradation products was obtained in assays of mixtures of known composition using the methods described.

REFERENCES

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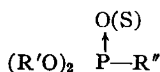
A Note on Chemical Constitution and Biological Activity of Some Derivatives of Thiophosphoric Acid*

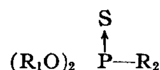
By M. L. BORKE and E. R. KIRCH

DURING RECENT YEARS certain organophosphorus compounds have come into use as insecticides and fungicides, while others are classified as "nerve gases." Their general formula may be represented as follows:



The phosphoryl (P→O) or the thiophosphoryl (P→S) group present in all these compounds seems to be responsible for their affinity toward certain enzymes and particularly toward acetylcholinesterase (AChase).

In the course of an investigation of some derivatives of thiophosphoric acid, two series of compounds having the general formula



were prepared in this laboratory. In one of the series $R_1 = C_6H_5$, while in the second series $R_1 = p\text{-}C_6H_4CH_3$. In both series R_2 was an amine (aniline, *ortho-*, *meta-* or *para*-toluidine,

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