COMMUNICATIONS TO THE EDITOR

PIPERAZINE DERIVATIVES FROM AMINO ALCOHOLS

Sir:

We have found that certain piperazine derivatives are conveniently prepared from β-hydroxyalkylamines by the catalytic alkylation method of Adkins [Paden and Adkins, THIS JOURNAL, **58,** 2487 (1936); Hill and Adkins, *ibid.*, **60,** 1033 (1938)].

Isopropanolamine in dioxane over copper chromite at 250–275° yields *trans*-2,5-dimethylpiperazine. It was identified as the 1,4-dinitroso derivative, m. p. 174° [Feist, *Ber.*, **30,** 1983 (1897)] and as the 1,4-dibenzoyl derivative, m. p. 228–229° [Bamberger and Einhorn, *ibid.*, **30,** 226 (1897)]. Similarly, diethanolamine yields piperazyl-1,4-*bis*-β-ethanol, m. p. 134–135°. This was identified as the dibenzoate, m. p. 104.5–105° [Pyman, *J. Chem. Soc.*, **93,** 1802 (1908)]. Good yields of diphenylpiperazine, m. p. 163.5–164.5°, were obtained from phenylethanolamine. When mixed with an authentic specimen of diphenylpiperazine the melting point was not changed.

The copper chromite catalyst was partially reduced in most runs. The yields ranged from 20 to 50%. This work is being continued.

CONTRIBUTION FROM THE ORGANIC LABORATORIES OF THE UNIVERSITY OF FLORIDA
GAINESVILLE, FLA.

J. P. BAIN
C. B. POLLARD

RECEIVED JANUARY 16, 1939

ULTRAVIOLET ABSORPTION SPECTRUM OF ANHYDROSARSASAPOGENOIC ACID

Sir:

Dr. F. S. Spring has kindly pointed out in a letter to one of us that the ultraviolet absorption curve for anhydrosarsasapogenoic acid reported in a recent paper with Jacobsen [Fieser and Jacobsen, THIS JOURNAL, **60,** 2753 (1938)] is not, as was stated, indicative of the presence of an α,β-unsaturated ketonic group because, according to the graph, the intensity of the band at 243 mμ is much too low for such a compound (log ε = 0.98). This inconsistency is due to an unfortunate error in reproducing Dr. Webb's curve. The figures for the intensity of absorption inadvertently were reported as log ε values, whereas they actually are values of $\epsilon \times 10^{-4}$. The absorption band thus has both the location and the high intensity characteristic of an α,β-unsaturated ketone. The evidence on this point has been confirmed and extended by an examination of the absorption spectrum of anhydrosarsasapogenoic acid methyl ether acetate in absolute ethyl alcohol. This shows an intense band at 243 mμ (log ε = 4.13) and a secondary band with a maximum at 303 mμ (log ε = 1.86).

CONVERSE MEMORIAL LABORATORY
HARVARD UNIVERSITY
CAMBRIDGE, MASSACHUSETTS

LOUIS F. FIESER
R. NORMAN JONES

RECEIVED JANUARY 21, 1939

THE USE OF PHOSPHOTUNGSTIC ACID IN THE PRELIMINARY REFINING OF EXTRACTS CONTAINING VITAMIN K

Sir:

In this Laboratory, one of the most successful preliminary treatments of hexane extracts of dried alfalfa for the preparation of vitamin K concentrates has been the removal of the green pigments by adsorption on activated magnesium oxide. This procedure, however, is laborious and time consuming, and involves some loss of the vitamin.

It has been found that removal of the green pigments can be accomplished more conveniently by the use of phosphotungstic acid. To one volume of alfalfa hexane extract representing about 5 g. of dried alfalfa per cc., is added one-half volume of redistilled ethyl ether and phosphotungstic acid as a fine powder in the proportion of 0.03 g. per gram of dried alfalfa. The mixture is shaken vigorously until two phases form on standing: an upper, light green layer containing hexane, ether, about one-half of the total solids of the extract, and all of the vitamin K; and a lower, dark, viscous layer of ether, phosphotungstic acid, and the remainder of the solids of the extract. The lower layer is discarded.

The upper layer is washed repeatedly with approximately 50% ethanol and then with water. The ethyl ether is removed by distilling under reduced pressure and the remaining solution chilled at 0° for at least twenty-four hours. Solids which precipitate out are removed by filtering at 0° and discarded. From this point, the

procedures formerly employed, namely, repeated chilling, filtering and concentrating in hexane solution and then in ethanol solution are continued. Finally, the concentrate may be fractionated by distillation under very low pressure and the vitamin isolated in the form of the colorless, highly active fraction as already described [*J. Biol. Chem.*, **120,** 635 (1937)].

In Table I are given assay results illustrating the relative vitamin K activities of crude extracts of alfalfa and of the same extracts after the preliminary treatment with phosphotungstic acid. Results with two distillates obtained under very low pressure subsequent to the phosphotungstic acid treatment are also given. The fact that the average blood clotting times of chicks were not greater after the crude extract had been treated with phosphotungstic acid is ample evidence that in this step the losses of vitamin K are very slight, if any. Further advantages of this step are speed and applicability to concentrated solutions. Assays were conducted according to a procedure given elsewhere [*Biochem. J.*, **32,** 1897 (1938)].

TABLE I

AVERAGE CLOTTING TIMES OF CHICKS FED CERTAIN SOURCES OF VITAMIN K

Source of vitamin K	Amount per kilogram of diet[a]	Average blood clotting time, minutes
Hexane extract	10 cc.	3.9
of alfalfa	12 cc.	4.0
Phosphotungstic	≃10 cc.	3.5
acid treated	≃12 cc.	2.4
extract	≃12 cc.	4.1
Molecular	20 mg.	5.4
distillate	20 mg.	4.0

[a] Standard solution representing 1 g. of dried alfalfa per cc.

We wish to acknowledge technical assistance obtained through the WPA under Project A. P. No. 465-03-3-209. We are also indebted to Philip J. Grant, who developed the use of phosphotungstic acid as a precipitant for the green pigment fraction.

DIVISION OF POULTRY HUSBANDRY
COLLEGE OF AGRICULTURE
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA
A. A. KLOSE
H. J. ALMQUIST

RECEIVED DECEMBER 19, 1938

THE MOLECULAR WEIGHT OF THE DIPHTHERIA TOXIN PROTEIN

Sir:

In connection with an investigation of the biological and chemical aspects of the flocculation reaction involving diphtheria toxin and antitoxin now under way in our respective laboratories, we have had occasion to study the sedimentation, diffusion and electrophoresis behavior of the purified toxin [method of preparation, Pappenheimer, *J. Biol. Chem.*, **120,** 543 (1937)]. By these criteria, the toxin is found to behave as a homogeneous substance.

Sedimentation velocity determinations have been made in the standard oil turbine ultracentrifuge of Svedberg by using the refractive index method to locate the position of the boundaries at finite times during the experiment. For a 1% solution of the diphtheria toxin in $M/15$ phosphate buffer at pH 6.9 and 0.17 M sodium chloride, the scale displacement–distance curves give as sedimentation constant, corrected to the basis of a process taking place in pure water at 20°, $s_{20} = 4.6 \times 10^{-13}$ cm./sec./dyne.

Diffusion experiments with a similar solution, except that the protein concentration was reduced to 0.4%, were made by observing, again by the refractive index method, the blurring of a boundary formed and held stationary in the "U" tube of a Tiselius electrophoresis apparatus. The boundary was moved to the middle of an upper section with the compensation mechanism. The diffusion constant was calculated from the area and maximum height of the usual scale displacement–distance diagram and corrected to a process occurring in water at 20° to give as provisional value $D_{20} = 6.2 \times 10^{-7}$ cm.^{2}/sec.

From the sedimentation constant, s, and diffusion constant, D, the molecular weight is obtained

$$M = \frac{RTs}{D(1 - V\rho)} \cong 72{,}000$$

In the absence of direct determination the toxin protein is assumed to have partial specific volume, $V = 0.75$.

When subjected to a potential gradient in the Tiselius electrophoresis apparatus, containing a 1% solution of the toxin in borate–phosphate buffer at pH = 7 and ionic strength = 0.02, the protein migrates essentially as a single component. However, when the schlieren diaphragm is nearly closed, a trace of faster moving material may be observed. The latter is probably identical with a small amount of inactive protein known to be present.

Electrodialyzed antitoxic pseudoglobulin in 1% solution in $M/15$ phosphate buffer at pH 6.9 and 0.17 M NaCl shows a sedimentation behavior not