the rapid separation of sodium chloride. The salt was filtered and the filtrate was diluted with 800 cc. of water. The resulting partially cloudy mixture was acidified with hydrochloric acid and the yellow precipitate of 5-chloro-2-pyrimidinethiol collected and dried: 37.5 g. (76%), m. p. 218–223°. After crystallization from methanol the melting point was constant at 221–222°.

Anal.[2] Calcd. for $C_3H_2ClN_2S$: C, 32.8; H, 2.0; S, 21.8. Found: C, 33.0, 33.0; H, 2.3, 2.4; S, 22.1, 22.0.

The yellow color of the compound could not be removed by treatment in alkaline solution with hydrosulfite, zinc dust, charcoal, or a combination of the last two agents.

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[2] Carried out under the direction of Dr. J. A. Kuck.

COMMUNICATIONS TO THE EDITOR

BOUNDARY SPREADING IN SEDIMENTATION VELOCITY EXPERIMENTS

Sir:

The only case in which sedimentation velocity experiments have been successfully used for size distribution analysis is the one in which no significant amount of diffusion occurs during the course of the experiment.[1] This situation does not ordinarily obtain, and in this Communication we present in outline a method to sort out the contributions of heterogeneity and diffusion to the spreading of the sedimentation boundary, thus providing means for the computation of each.

Since the second moments about the mean are additive in a combined distribution composed of independent distributions[2] we may derive the expression

$$\sigma^2 = \sigma_0^2 + 2Dt + \bar{x}^2 \left[p\omega^2 t + \frac{(p\omega^2 t)^3}{3!} + \frac{(p\omega^2 t)^5}{5!} + \ldots \right]^2 \tag{1}$$

Thus, and to a good approximation, we have

$$\frac{\sigma^2 - \sigma_0^2}{2t} = D + \frac{p^2\omega^4}{2}\,\bar{x}^2 t$$

In these equations, σ^2 and σ_0^2 are the second moments of the curve which defines the sedimenting boundary at times $t = t$ and $t = 0$, D is the weight-average diffusion constant, ω is the angular velocity of the ultracentrifuge rotor, p is the standard deviation of the sedimentation constant distribution and $\bar{x}$ may be taken as the distance from the center of rotation to the centroidal ordinate of the boundary. This equation shows that when the apparent diffusion coefficient $(\sigma^2 - \sigma_0^2)/2t$, is plotted against $\bar{x}^2 t$, a straight line is obtained with intercept D and slope $p^2\omega^4/2$. A drift of "diffusion coefficient" with time has long been recognized as a test of homogeneity with respect to sedimentation behavior.[3]

The distribution function, $g(s)$ which gives the relative amount of the molecular species with s_{20} of s is given by

$$g(s) = \frac{\mathrm{d}n}{\mathrm{d}x}\left(\frac{x}{x_0}\right)^2 \frac{x\omega^2 t}{n_1 - n_2}\,\frac{\eta_{20}}{\eta_t} \tag{2}$$

when diffusion is negligible.[4] When diffusion is not negligible, an "apparent distribution" defined in this manner may be extrapolated to infinite time to give the actual distribution of sedimentation constants, since the spreading of the boundary due to differences in s is proportional to $\bar{x}t$, while the spreading due to diffusion is proportional to $t^{1/2}$, as is shown by equation (1).

The method has been applied in the analysis of sedimentation velocity diagrams for pepsin-digested γ-globulin systems from horse anti-diphtheric serum. Additional information has been obtained, not only as regards the actual size distribution in these systems, but also with respect to the mechanism of the enzymatic degradation. A definitive account of these studies will be submitted at a later date.

Grateful acknowledgment is made to the U. S. Public Health Service and to the Wisconsin Alumni Research Foundation.

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[1] *Cf.* for example, W. B. Bridgman, This Journal, **64**, 2349 (1942).

[2] C. E. Weatherburn, "A First Course in Mathematical Statistics," Cambridge University Press, Cambridge, 1946, p. 82.

[3] T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford University Press, Oxford, 1940, p. 287.

[4] R. Signer and H. Gross, *Helv. Chim. Acta*, **17**, 726 (1934).

SYNTHESIS AND ISOLATION OF A CRYSTALLINE SUBSTANCE WITH THE PROPERTIES OF A NEW B VITAMIN

Sir:

A naturally occurring factor active for *Leuconostoc citrovorum* 8081 has been described,[1,2] and a synthetic reaction mixture derived from folic acid has been reported to be active for this organism.[3]

We wish to report the synthesis and isolation

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[1] Sauberlich and Baumann, *J. Biol. Chem.*, **176**, 165 (1948).

[2] Broquist, *et al.*, *Proc. Soc. Exp. Biol. Med.*, **71**, 549 (1949).

[3] Shive, *et al.*, This Journal, **72**, 2818 (1950).

in crystalline form of a substance (I) active for *Leuconostoc citrovorum*, and the chick; furthermore, it competitively reverses the toxicity of 4-aminopteroylglutamic acid (II) for the mouse.

When pteroylglutamic acid (PGA) or its N^{10}-formyl derivative was reduced catalytically over platinum in formic acid at 0 to 30°, two moles of hydrogen were absorbed. Isolation of the crystalline substance (I) was accomplished by adsorption of impurities on Magnesol at *p*H 7, adsorption of activity on Darco G-60 at *p*H 4, elution, fractional crystallization of the barium salt, and finally chromatographic separation on Magnesol columns. *Anal.* Calcd. for $C_{20}H_{21}N_7O_7Ba \cdot 5H_2O$: C, 34.4; H, 4.47; N, 14.0; Ba, 19.7; CHO, 4.15. Found: C, 34.7; H, 4.31; N, 14.1; Ba, 20.2; CHO, 3.80. Calcd. for $C_{20}H_{23}N_7O_7 \cdot 3H_2O$: C, 45.5; H, 5.54; N, 18.6; CHO, 5.50; H_2O, 10.3. Found: C, 45.2; H, 5.67; N, 18.8; CHO, 5.07; H_2O, 11.2. In 0.1 *N* sodium hydroxide solution I (10 mg./l.) exhibited a maximum at 282 mμ (%T = 27.0) and a minimum at 243 mμ (%T = 75.3). Although stable in solution at neutral to mildly alkaline *p*H under aerobic conditions, I rapidly changes at *p*H 2 with loss of activity for *Leuconostoc citrovorum*, but retains PGA-like activity for *Streptococcus faecalis* R and *Lactobacillus casei*. The primary product of anaerobic acid treatment of I appeared to be a labile derivative of tetrahydropteroylglutamic acid.

In microbiological assays of I (barium salt $\cdot 5H_2O$), about 0.15 mγ to 0.20 mγ corresponded to one "unit."[1] Thymidine was not added to the basal culture medium although this addition has been reported to increase the sensitivity of the assay.[4]

In mice I prevented the lethal effects of 4-aminopteroylglutamic acid.[5] Doses were injected three times weekly.[5] With 10 γ of II the average survival time was 6.8 days; with 20 γ, 5.0 days. With 10 γ of II and 15 γ of I injected simultaneously, 9 out of 11 mice survived the 14-day assay period. Average gain was 0.3 g. With 10 γ of II and 30 γ of I, all survived and the gain was 5.3 g.; with 20 γ of II and 30 γ of I, 10 out of 11 survived and the loss was 2.1 g. With 20 γ of II and 60 γ of I all survived and the gain was 4.3 g. The toxic action of 10 γ of II has been shown[6] not to be diminished by 20 γ of PGA.

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(4) Shive, Paper presented at 117th Meeting, American Chemical Society, Houston, Texas, March, 1950.

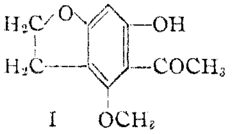
(5) Franklin, *et al.*, *Proc. Soc. Exp. Biol. Med.*, **67**, 398 (1948).

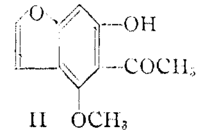
(6) Broquist, *et al.*, *J. Biol. Chem.*, **185**, 399 (1950).

COUMARONE DEHYDROGENATION WITH N-BROMOSUCCINIMIDE

Sir:

In the course of studies on the synthesis of the furochromones khellin, visnagin and related compounds, we have found that the dehydrogenation of certain coumaranes to coumarones, typified by the conversion of dihydrovisnaginone (I) to visnaginone (II), can be carried out by a halogenation-dehydrohalogenation process involving the use of N-bromosuccinimide.


I


II

Since Horning and Reisner[1] have recently described unsuccessful attempts to use this reagent for the dehydrogenation of dihydrofurocoumarins, we wish to describe our findings.

The treatment of the acetate of I with N-bromosuccinimide and a trace of benzoyl peroxide in carbon tetrachloride yielded an oily product which when treated successively with dimethylaniline and alcoholic alkali afforded crude visnaginone (II). After purification there was obtained 59% of pure visnaginone, m. p. and mixed m. p. 108–109°. The synthetic material showed the same color reactions (ferric chloride, concentrated sulfuric acid) as the natural substance,[2] and was converted into an acetate, m. p. 63.5–65.5° which did not depress the melting point of a sample of the acetate prepared from natural visnaginone (calcd. for $C_{13}H_{12}O_5$: C, 62.90; H, 4.87; found, C, 63.07; H, 5.12).

This "dehydrogenation" procedure has been applied to some related compounds; the details will be reported in forthcoming papers.

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(1) Horning and Reisner, THIS JOURNAL, **72**, 1514 (1950).

(2) Späth and Gruber, *Ber.*, **74B**, 1492 (1941).

(3) Visiting Fellow; present address, University of Manchester, Manchester, England.

(4) S. B. Penick and Co. Fellow, 1949–50.

(5) The authors gratefully acknowledge the financial assistance of the Smith, Kline and French Laboratories and S. B. Penick and Co.

REARRANGEMENTS INVOLVING 2-THENYLMAGNESIUM CHLORIDE

Sir:

When an ethereal solution of 2-thenyl chloride, at once a β-halo-sulfide and an isolog of benzyl chloride, was passed over amalgamated magnesium turnings in the "cyclic reactor,"[1] the Grignard reagent was obtained in 92% yield; hydrolysis of this solution gave an 86% yield of 2-methylthiophene (b. p. 111–113°). When the

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(1) Rowlands, Greenlee and Boord, Abstracts of Papers, American Chemical Society meeting, Philadelphia, April 9 to 13, 1950, p. 8L.