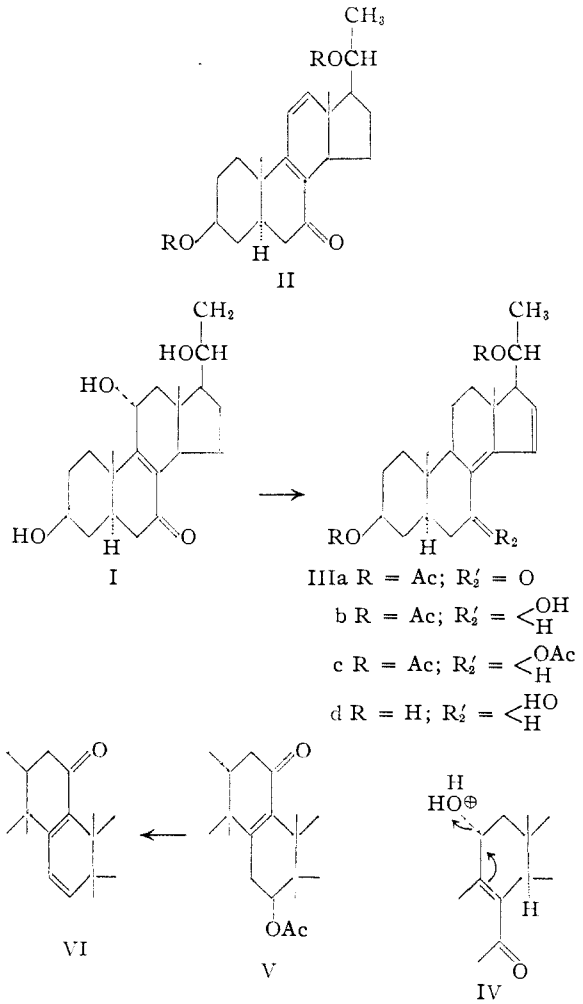
If structure II were correct, then reduction of the 7-keto function[6] should afford a homoannular diene with a characteristic ultraviolet absorption maximum above 260 mμ. When the diacetate of the dehydration product of I was reduced with sodium borohydride, there was obtained an oil with an ultraviolet absorption maximum at 246 mμ. The acetylation product IIIc similarly failed to crystallize but the free triol IIId was obtained as a crystalline solid with an ultraviolet absorption maximum at 247 mμ, log ε 3.90. The position of this maximum completely eliminates a homoannular diene structure II from consideration and makes it appear most likely that the acidic dehydration of the $\Delta^{8}$-11α-ol-7-one leads to a $\Delta^{8(14)15}$-dien-7-one IIIa. The positions of the ultraviolet absorption maxima (298 mμ for IIIa and 247 mμ for IIIb-d) are in excellent agreement with the proposed structures; the relatively low extinction (log ε *ca.* 3.9) is noteworthy. It is generally accepted now[7] that ionic 1,2-elimination in alicyclic systems proceeds most readily when both substituents are polar. Since the 11α-hydroxy group is equatorial and should thus be eliminated toward C-12 only with difficulty, the dehydration probably proceeds as indicated in IV, the initially formed $\Delta^{8(14),9(11)}$-diene rearranging in the acid medium to the thermodynamically more stable linear dienone structure IIIa.




(6) Such a procedure was employed by L. F. Fieser in the structure proof of a similar dienone in the cholesterol series (Abstracts of Ciba Foundation Conference, London, July 7-10, 1952).

(7) *Cf.* D. H. R. Barton and W. J. Rosenfelder, *J. Chem. Soc.*, 1048 (1951).

### Experimental[8]

A mixture of 0.55 g. of $\Delta^{8}$-allopregnene-3β,11α,20β-triol (I)[9] (m.p. 249–251°) was refluxed for one hour with 40 cc. of methanol and 1 cc. of concd. hydrochloric acid and then diluted with water. Filtration afforded 0.48 g. of colorless crystals with m.p. 220–225°, $\lambda_{max}^{EtOH}$ 225 and 298 mμ, log ε 4.20 and 3.77, $\lambda_{max}^{Nujol}$ 1665 cm.$^{-1}$ and free hydroxyl band. The physical constants are in good agreement with those reported earlier,[3] but the yield has been markedly improved. Acetylation produced **$\Delta^{8(14)15}$-allopregnadiene-3β,20β-diol-7-one diacetate** (IIIa) with m.p. 158–160°. $\lambda_{max}^{EtOH}$ 225 and 298 mμ, log ε 4.27 and 3.80, $\lambda_{max}^{CHCl_3}$ 1730 and 1665 cm.$^{-1}$.

The above diacetate IIIa (0.175 g.) in 5 cc. of methanol and 1 cc. of dioxane was allowed to stand at room temperature for one hour with 0.01 g. of sodium borohydride. Dilution with water, extraction with ether, washing, drying and evaporation afforded 0.175 g. of an oil, IIIb, $\lambda_{max}^{EtOH}$ 246 mμ, log ε 3.85. Acetylation similarly produced an oil $\lambda_{max}^{EtOH}$ 246 mμ, log ε 3.90, but saponification of the triacetate (IIIc) with methanolic potassium hydroxide (2 hours refluxing) followed by recrystallization from acetone led to colorless crystals of **$\Delta^{8(14)15}$-allopregnadiene-3β,7,20β-triol** (IIId) with m.p. 198–200°, $\lambda_{max}^{EtOH}$ 247 mμ, log ε 3.90.

*Anal.* Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.90; H, 10.17.

(8) Melting points are uncorrected and were taken on the Fisher block. Ultraviolet absorption spectra were determined in absolute ethanol solution; infrared spectra were measured with a Baird double beam infrared spectrometer.

(9) G. Stork, J. Romo, G. Rosenkranz and C. Djerassi, THIS JOURNAL, **73**, 3546 (1951).

DEPARTMENT OF CHEMISTRY
WAYNE UNIVERSITY
DETROIT 1, MICHIGAN

## Fractionation of an Enzyme by Foaming[1]

BY MORRIS LONDON, MARTIN COHEN AND PERRY B. HUDSON[2]

RECEIVED NOVEMBER 8, 1952

In this Laboratory a 15-fold purification of prostatic acid phosphatase[3] was obtained from an enzyme fraction already 300-fold purified on a wet tissue weight basis, by foaming off inactive protein. Several recent papers[4,5,6] have dealt with the foaming power and foam stability of protein solutions. Some authors have attempted to use these properties for the analysis of protein mixtures,[7] and two other investigators[8,9] used the method of foaming for preparing more or less pure proteins from a mixture of proteins.

This is a report on a series of experiments carried out on jackbean urease (Arlco brand) with the view of ascertaining the effect of various conditions on

(1) This research was supported by grants from the American Cancer Society, and the Damon Runyon Memorial Fund.

(2) Damon Runyon Memorial Fellow.

(3) The purification of this enzyme is the subject of another paper in preparation.

(4) H. Kimizuka and T. Sasaki, *Bull. Chem. Soc. Japan*, **24**, 230 (1951).

(5) F. Schütz, *Trans. Faraday Soc.*, **42**, 437 (1946).

(6) Wo. Ostwald and A. Siehr, *Kolloid-Z.*, **76**, 33 (1936).

(7) D. Peters, *ibid.*, **125**, 157 (1952).

(8) A. Doguon, "Surface Chemistry," Butterworth, London, 1949, page 253.

(9) F. Schütz, R. Bader and M. Stacey, *Nature*, **154**, 183 (1944).

the concentration of one of the components of a protein mixture (*viz.*, urease) in the foam.

The apparatus used consisted either of gas washing bottles (Corning) or of graduated cylinders fitted with fritted glass discs. The gas was supplied from a bottle partially filled with chips of Dry Ice (in the *p*H range investigated, carbon dioxide does not affect the *p*H appreciably). The foam was led from the top of the apparatus by glass tubing and a rubber hose, and collected in graduated tubes so that series of 2 to 3 ml. foam fractions could be collected.

The original material, the foam fractions ("froth") and the residue ("frothate") were analyzed for nitrogen after sulfuric acid digestion by direct nesslerization, and for urease by Sumner and Graham's method.[10] The ratio, urease/total nitrogen, was defined as "purity number." The ratio, purity number of fraction/purity number of original material, was defined as "purification."

The effect of protein concentration on the accumulation of urease in the froth was found by a series of experiments where solutions of varying protein concentrations were foamed under identical conditions. A distinct optimum, from the point of view of both purification and recovery, was found at a concentration of 0.16% (Table I).

TABLE I

EFFECT OF PROTEIN CONCENTRATION ON THE ACCUMULATION OF UREASE IN FROTH FRACTIONS[a]

| Concn. % prepn.[b] | Av. purifn. (froth fractions only) | Max. purifn. (best fraction) | Activity recovd. in all fractions, % |
|---|---|---|---|
| 0.125 | 4.1 (2)[c] | 4.1 | 52 |
| .160 | 7.2 (3) | 9.7 | >100[d] |
| .300 | 2.3 (4) | 3.0 | 77 |
| .500 | 1.9 (7) | 2.5 | 72 |

[a] The jackbean urease was dissolved in 0.2 *M* acetate buffer *p*H 5.0. The volume foamed was 100 ml. The foaming was carried out in a gas washing bottle (Corning No. 31760, 250 ml.) with a "coarse" disk. [b] Urease preparation as weighed out was approximately 50% protein. [c] Number of froth fractions. [d] 71–78% of all urease recovered was found in the froth fractions and maximum purification, the best fraction, was usually found in a central froth fraction.

It was similarly found that an optimum *p*H of the foaming medium exists. This optimum was found to be close to the isoelectric point of urease for various protein concentrations (Table II). However, since so many variables affect this process, it is not proposed to draw any conclusions from this fact before other protein mixtures are investigated.

TABLE II

EFFECT OF THE *p*H OF THE FOAMING MEDIUM ON THE ACCUMULATION OF UREASE IN FROTH FRACTIONS[a]

| *p*H | Av. purifcn. (froth fractions) | Max. purifcn. (best fraction) | Activity recovd. in all fractions, % |
|---|---|---|---|
| 4.6 | 3.9 | 5.8 | 86 |
| 5.0 | 7.2 | 9.7 | >100 |
| 5.2 | 3.9 | 3.9 | 55 |

[a] 0.16% jackbean urease was dissolved in 0.2 *M* acetate buffer. Other conditions as in Table I.

(10) J. B. Sumner and H. Graham, *Proc. Soc. Exptl. Biol. Med.*, **22**, 504 (1925). However, the material was incubated at 37° for 15 minutes and the urease was diluted with 1% bovine albumin dissolved in 0.2 *M* acetate buffer *p*H 5.0. The incubate was directly nesslerized.

The porosity of the foaming disc was also found to affect purification and recovery (Table III).

TABLE III

EFFECT OF THE POROSITY OF THE FOAMER ON THE ACCUMULATION OF UREASE IN FROTH FRACTIONS[a]

| Disc designation (Corning) | Av. max. pore size (Corning), μ | Av. purifcn. (froth) | Max. purifcn. | Activity recovd. in all fractions, % |
|---|---|---|---|---|
| Extra coarse | 160 | 2.6 | 2.7 | 47 |
| Coarse | 40 | 2.1 | 3.5 | 61 |
| Medium | 14 | 4.9 | 7.2 | 100 |

[a] 300 ml. of 0.16% jackbean urease dissolved in 0.2 *M* acetate buffer *p*H 5.0 were foamed in cylinders with liquid height of 12 cm. and foam height of 35 cm.

**Acknowledgment.**—The authors express gratitude to Dr. J. M. Reiner for advice and encouragement received during this work.

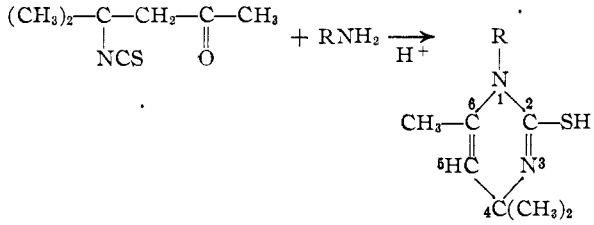
DEPARTMENTS OF BIOCHEMISTRY AND UROLOGY AND FRANCIS DELAFIELD HOSPITAL
COLLEGE OF PHYSICIANS AND SURGEONS
COLUMBIA UNIVERSITY, NEW YORK, N. Y.

## 2-Pyrimidinethiols

BY ROGER A. MATHES

RECEIVED NOVEMBER 24, 1952

The preparation of 2-mercapto-1-substituted-4,4,6-trimethyl-1H,4H-pyrimidines, obtained by reaction of 2-methyl-2-isothiocyano-4-pentanone with amines[1] and with amino acids,[2] was described in two previous papers. This series of pyrimidines has now been extended to include further examples derived from other types of amines and from hydrazines.[3]




The preparation[1] of "2-methyl-2-thiocyano-4-pentanone" used in the synthesis of 2-pyrimidinethiols was described previously. A further examination of this compound including both its chemical reactions and infrared absorption affords quite conclusive evidence that it is 2-methyl-2-isothiocyano-4-pentanone. Infrared absorption spectra measurements showed a band at about 4.9 μ, the characteristic broad band attributed to the isothiocyano group. 2-Methyl-2-isothiocyano-4-pentanone in its reaction with amines to form pyrimidines, which can be considered as cyclic thioureas, conforms to the well known reaction of isothiocyanates with amines to give thioureas. In a qualitative test for isothiocyanates,[4] 2-methyl-2-isothiocyano-4-pentanone when shaken with ammoniacal silver nitrate in aqueous alcohol gives silver sulfide readily.

(1) R. A. Mathes, F. D. Stewart and F. Swedish, Jr., THIS JOURNAL, **70**, 1452 (1948).

(2) R. A. Mathes and F. D. Stewart, *ibid.*, **72**, 1879 (1950).

(3) R. A. Mathes and F. D. Stewart, U. S. Patent 2,535,858 (Dec. 26, 1950).

(4) S. P. Mulliken, "Identification of Pure Organic Compounds," Vol. IV, J. Wiley and Sons, Inc., New York, N. Y., 1922, p. 17.