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. Expil. 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.

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2-Pyrimidinethiols

BY ROGER A. MATHES

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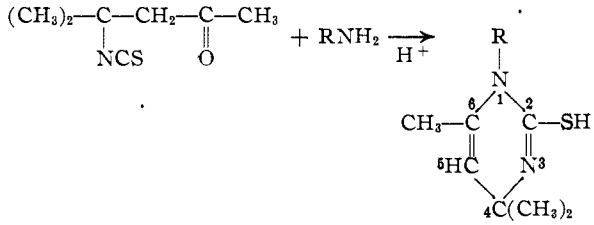
The preparation of 2-mercapto-1-substituted-4,4,6-trimethyl-1H,4H-pyrimidines, obtained by reaction of 2-methyl-2-isothiocyano-4-pentanone with amines[1] and with amino acids,[2] was described in two previous papers. This series of pyrimidines has now been extended to include further examples derived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

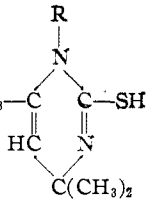
(2) R. A. Mathes and F. D. Stewart, *ibid.*, **72**, 1879 (1950).

(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.

TABLE I

2-MERCAPTO-1-SUBSTITUTED-4,4,6-TRIMETHYL-1H,4H-PYRIMIDINES



Compd.[a]	R	Derived from	M.p., °C.[b]	Yield, %[c]	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
1	Amino	Hydrazine	209–210	80	$C_7H_{13}N_3S$	49.09	49.11	7.65	7.70	24.54	24.57
2	*p*-Aminophenyl	Phenylhydrazine	170–171	95	$C_{13}H_{17}N_3S$	63.12	63.33	6.93	6.93	16.99	16.73
3	Methyl[d]	Methylamine	86–87	..	$C_8H_{14}N_2S$	56.43	56.47	8.29	8.17	16.46	16.42
4	Ethyl	Ethylamine	148–149	78	$C_9H_{16}N_2S$	58.65	58.75	8.75	8.87	15.20	15.13
5	*n*-Butyl	*n*-Butylamine	113–114	70	$C_{11}H_{20}N_2S$	62.21	62.28	9.50	9.57	13.19	13.11
6	Allyl[e]	Allylamine	129–130[f]	..	$C_{10}H_{16}N_2S$	61.18	61.10	8.22	8.28	14.27	14.11
7	3-Isopropoxypropyl	3-Isopropoxypropylamine	84–85	50	$C_{13}H_{24}N_2OS$	60.89	60.77	9.43	9.54	10.93	10.87
8	*p*-Nitrophenyl	*p*-Nitroaniline	201	75	$C_{13}H_{14}N_3O_2S$	56.30	56.41	5.45	5.53	15.15	14.70
9	2,4-Dichlorophenyl	2,4-Dichloroaniline	203–204	78	$C_{13}H_{14}Cl_2N_2S$	51.83	51.88	4.68	4.72	9.30	8.99
10	*o*-Mercaptophenyl	*o*-Aminobenzenethiol	172–173	79	$C_{13}H_{16}N_2S_2$	59.05	59.19	6.10	6.16	10.60	10.60
11	*p*-Hydroxyphenyl	*p*-Aminophenol	200	86	$C_{13}H_{16}N_2OS$	62.88	62.74	6.50	6.56	11.29	11.19
12	*p*-Anisyl	*p*-Anisidine	189	41	$C_{14}H_{18}N_2OS$	64.09	64.15	6.91	6.98	10.68	10.62
13	*p*-Acetylphenyl	*p*-Aminoacetophenone	189	78	$C_{15}H_{18}N_2OS$	65.66	65.65	6.61	6.46	10.21	10.11
14	Benzyl	Benzylamine	181–182	87	$C_{14}H_{18}N_2S$	68.25	68.35	7.36	7.40	11.37	11.20
15	Furfuryl	Furfurylamine	126–127	86	$C_{12}H_{16}N_2OS$	60.98	61.03	6.83	6.73	11.86	11.79
16	*p*-(α-Phenylisopropyl)-phenyl	*p*-(α-Phenylisopropyl)-aniline	173–175	76	$C_{22}H_{26}N_2S$	75.38	75.55	7.48	7.36	7.99	7.93

[a] Compounds 2 and 13 were recrystallized from benzene; 3,7 from hexane; all others from ethanol. [b] Melting points are for analytical samples and are uncorrected. [c] Yields are based on crude products. [d] The intermediate, 1-(1,1-dimethyl-3-oxobutyl)-3-methyl-2-thiourea, obtained in 93% yield, and melting at 161° (with decomposition) after recrystallization from hexane, was the initial product. *Anal.* Calcd. for $C_8H_{16}N_2OS$: C, 51.03; H, 8.57; N, 14.88. Found: C, 50.64; H, 8.48; N, 14.64. [e] The intermediate, 1-(1,1-dimethyl-3-oxobutyl)-3-allyl-2-thiourea, obtained in 83% yield, and melting at 138° after recrystallization from alcohol, was the initial product. *Anal.* Calcd. for $C_{10}H_{18}N_2OS$: C, 56.04; H, 8.46; N, 13.07. Found: C, 56.01; H, 8.39; N, 13.06. W. Traube and H. Lorenz, *Ber.*, **32**, 3156 (1899), reported a melting point of 138°. [f] W. Traube and H. Lorenz, *ibid.*, **32**, 3156 (1899), reported a melting point of 130°.

Experimental

2-Mercapto-1-substituted-4,4,6-trimethyl-1H,4H-pyrimidine. Typical Preparation.—Ethylamine (45 g., 1 mole) was added as a 25% aqueous solution,[5] over a period of 15 minutes, to a vigorously agitated mixture of 157 g. (1 mole) of 2-methyl-2-isothiocyano-4-pentanone, 300 ml. of water and 5 ml. of hydrochloric acid. The reaction mixture was heated to reflux and after cooling to room temperature, the product which precipitated was filtered, washed with water and dried.

In two instances (Table I, compounds 3 and 6) the products initially obtained were the intermediate thioureas. By heating these intermediates with an excess of 25% sulfuric acid, ring closure was effected to give the corresponding pyrimidines.

Acknowledgment.—The analyses of all compounds were made by J. R. Kubik and A. K. Kuder.

(5) Water dilution is advantageous in controlling the reaction rate when employing water soluble amines and hydrazines.

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Crystalline Sodium Lactobionate Monohydrate

BY GEORGE E. N. NELSON AND FRANK H. STODOLA

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The study and utilization of the bionic acids have been hampered by the lack of salts which can be readily purified by crystallization. After many attempts Isbell[1] was able to prepare crystalline calcium lactobionate, but unfortunately its gelling tendency made it of little use in purification. Recently we succeeded in preparing crystalline sodium lactobionate and find it to be readily obtainable in the pure state as a monohydrate which filters easily and is stable to heat.

(1) H. S. Isbell, *Bur. Stand. J. Res.*, **11**, 713 (1933).

Experimental

Calcium lactobionate, prepared by the oxidation of lactose by *Pseudomonas graveolens*,[2] was converted to the sodium salt by reaction with the calculated amount of sodium oxalate. The filtered solution was brought to incipient cloudiness by the addition of alcohol and then stirred with seed crystals. More alcohol was then added and the crystals, which filtered rapidly, were washed successively with 70% alcohol, absolute alcohol and ether. For analysis, this product was recrystallized as follows: One gram was dissolved in the minimum amount of water (2.5 ml.) at room temperature. After addition of more water (0.5 ml.) 3 ml. of 95% alcohol was added gradually. Seed crystals were added and the solution stirred vigorously for several minutes. On standing at room temperature, clusters of bars crystallized out. After 1 day at room temperature and another day in the refrigerator, the crystals (810 mg.) were filtered off and washed as described above. After drying in a vacuum desiccator (20 mm., 25°) for a day, the compound lost no further weight at 78° (1 mm.); it was then analyzed.

Anal. Calcd. for $C_{12}H_{21}O_{12}Na \cdot H_2O$: C, 36.18; H, 5.82; Na, 5.77; H_2O, 4.51. Found: C, 36.1; H, 5.81; Na, 5.92; H_2O (Karl Fischer Method), 4.7.

A sample dried to constant weight at 140° (1 mm.) lost only 0.3% of its weight.

(2) F. H. Stodola and L. B. Lockwood, *J. Biol. Chem.*, **171**, 213 (1947).