TABLE II

GROWTH-PROMOTING ACTIVITIES OF HYDROGENATED COMPOUNDS

		Transmission, %		
	μg./10 ml.	Hydrogenated N^{10}-methyl PGA	Hydrogenated aminopterin	Hydrogenated A-methopterin
For *S. faecalis* R.	0.1	100	53	100
	1.0	100	36	100
	10.0	68	25	100
	100.0	66	26	100
For *L. citrovorum 8081*	0.1	90	91	91
	1.0	92	90	93
	10.0	90	77	94
	100.0	78	33	90

The hydrogenated materials were also tested as growth factors for *Streptococcus faecalis* R. and *Leuconostoc citrovorum 8081*. For the former organism, the same basal medium was used as was used for the inhibition studies but no folic acid was added. For the latter organism, the basal medium and technique of Sauberlich[8] were used with the exceptions that no supplementary glycine and alanine were used and a Lumitron colorimeter with a 660 mμ filter was employed. Turbidity was determined after 17 hours. Data are given in Table II. It is quite likely that the growth-promoting activity is due to an impurity in the original compound as suggested by Weygand.

Acknowledgment.—The authors wish to thank Lederle Laboratories for supplying the folic acid, N^{10}-methylpteroylglutamic acid, aminopterin and A-methopterin used in these experiments.

(8) H. E. Sauberlich, *J. Biol. Chem.*, **181**, 467 (1949).

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COMMUNICATIONS TO THE EDITOR

A NEW METHOD FOR THE PREPARATION OF THIO ACIDS AND APPLICATION TO PEPTIDE CHEMISTRY

Sir:

Although Pawlewski[1] demonstrated that thio acids were very active acylating agents, the methods of preparation which have been available heretofore[2] have not been suitable for making the acylaminothio acids which could be useful in peptide synthesis. By passing hydrogen sulfide into a solution of the mixed anhydrides,[3,4,5] RCOO-$COOC_2H_6$, in methylene chloride with an equivalent of triethylamine at −20° and warming to room temperature, we have obtained the thio acids, RCOSH.

In this manner we have prepared, in addition to thioacetic and thiobenzoic acids, *p*-phenylthiobenzoic acid, 88% yield (from the carboxylic acid), m.p. 90–92° (*Anal.* Calcd. for $C_{13}H_{10}OS$: C, 72.89; H, 4.71; S, 14.94. Found: C, 72.86; H, 4.83; S, 15.09); thiohippuric acid, 70% yield, m.p. 98–100° (*Anal.* Calcd. for $C_9H_9NO_2S$: C, 55.39; H, 4.65; N, 7.18; S, 16.40. Found: C, 55.30; H, 4.69; N, 6.79; S, 15.99); phthaloylthioglycine, 45% yield, m.p. 114–116° (*Anal.* Calcd. for $C_{10}H_7NO_3S$: C, 54.30; H, 3.19; S, 14.47. Found: C, 54.52; H, 3.32; S, 14.21).

When thiohippuric acid was warmed to 90–110° in dimethylformamide with *d,l*-alanine in a nitrogen atmosphere, hydrogen sulfide was rapidly evolved and there was obtained a 70% yield of hippuryl-alanine, m.p. 200–201.5°[6] and giving the correct elemental analysis.

Upon treatment of thiohippuric acid with Raney nickel which had been deactivated over acetone[7] there was obtained in one experiment, a 30% yield of hippuraldehyde,[8] isolated as the 2,4-dinitrophenylhydrazone, m.p. 200–202° (*Anal.* Calcd. for $C_{15}H_{13}N_5O_5$: C, 52.48; H, 3.82; N, 20.40. Found: C, 52.63; H, 3.78; N, 20.18).

(1) Br. Pawlewski, *Ber.*, **31**, 661 (1898); **34**, 657 (1901); **35**, 110 (1902).

(2) R. Connor, "Organic Sulfur Compounds," p. 835 in Gilman's "Organic Chemistry," Vol. I, Second Edition, John Wiley and Sons, Inc., New York, N. Y., 1943; S. Sunner and T. Nilson, *Svensk. Kem. Tid.*, **54**, 163 (1942) [*C. A.*, **38**, 3249 (1944)]; B. Tchoubar and Letellier-Dupre, *Bull. soc. chim. France*, 792 (1947).

(3) R. A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951); T. Wieland and H. Bernhard, *Ann.*, **572**, 190 (1951); J. R. Vaughan and R. L. Osato, THIS JOURNAL, **74**, 676 (1952).

(4) T. Wieland, W. Schäfer and E. Bokelmann, *Ann.*, **573**, 99 (1951), prepared $RCOSC_6H_5$ by addition of C_6H_5SH to the mixed anhydride.

(5) H. Adkins and Q. E. Thompson, THIS JOURNAL, **71**, 2242 (1949), prepared thiobenzoic acid by passing H_2S into dibenzoyl sulfide in pyridine.

(6) T. Curtius and E. Lambotte, *J. prakt. Chem.*, [2] **70**, 114 (1904).

(7) G. B. Spero, A. V. McIntosh and R. H. Levin, THIS JOURNAL, **70**, 1907 (1948).

(8) J. Bougault, E. Cattelain and P. Chabrier, *Bull. soc. chim.*, [5] **5**, 1699 (1938), have reported the conversion of thioacetic acid to acetaldehyde.

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THE SYNTHESIS AND REACTIONS OF N-ACYL THIOL AMINO ACIDS

Sir:

Recent evidence that enzymatic acylations involve thiolacid derivatives as activated intermediates[1] has stimulated interest in similar thiol analogs of amino acids as possible participants in the physiological synthesis of peptides. By two

(1) For example, acetyl coenzyme A is considered to be a key intermediate in biological acetylations; F. Lynen, E. Reichert and L. Rueff, *Ann.*, **574**, 1 (1951); T. C. Chou and F. Lipmann, *J. Biol. Chem.*, **196**, 89 (1952).

methods, we have prepared N-acylated thiol amino acids and have found them to be active acylating agents[2] for amines and amino acid derivatives under mild conditions.

Interaction of phenaceturic acid, triethylamine and ethyl chlorocarbonate[3,4] in methylene chloride solution at $-10°$, followed by treatment with excess hydrogen sulfide, led to a 72% yield of thiolphenaceturic acid,[5] m. p. 116.5–118.0° (dec.). *Anal.* Calcd. for $C_{10}H_{11}NO_2S$: C, 57.39; H, 5.30; N, 6.69. Found: C, 57.45; H, 5.33; N, 6.75. A solution of thiophenaceturic acid and aniline in 50–50 ethanol–phosphate buffer of *p*H 7.5 (0.1 *M*) deposited 78% of phenaceturanilide, m.p. 163–164°, in 18 hours at room temperature.

Phthaloylthioglycine was prepared by a similar procedure in 61% yield, m.p. 116.5–118.0°. *Anal.* Calcd. for $C_{10}H_7NO_3S$: C, 54.29; H, 3.19; N, 6.33. Found: C, 54.49; H, 3.33; N, 6.36. Treatment of phthaloylglycyl chloride with sodium hydrosulfide in dimethylformamide solution also afforded phthaloylthioglycine in good yield.

A solution in methylene chloride of phthaloylthiolglycine, glycine methyl ester hydrochloride and triethylamine reacted at room temperature to give phthaloylglycylglycine methyl ester. The addition of iodine-potassium iodide[6] to an aqueous solution (0–5°) of phthaloylthiolglycine and glycine methyl ester hydrochloride containing excess sodium bicarbonate produced an immediate precipitate of the peptide derivative.

(2) Thiolacetic acid has been reported previously to react readily with amines, B. Pawlewski, *Ber.*, **31**, 661 (1898).

(3) R. A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951).

(4) J. R. Vaughan and R. L. Osato, THIS JOURNAL, **74**, 676 (1952).

(5) A similar method has been used to prepare N-acyl thiol amino acid esters; T. Wieland, W. Schäfer and E. Bokelmann, *Ann.*, **573**, 99 (1951).

(6) G. Alliger, G. E. P. Smith, Jr., E. L. Carr and H. P. Stevens, *J. Org. Chem.*, **14**, 962 (1949).

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THE BETA-LUCIFERIN OF *CYPRIDINA*[1]

Sir:

The production of light by the marine ostracod crustacean, *Cypridina hilgendorfii*, is a result of interaction between oxygen, the enzyme luciferase, and either of two amorphous substances of unknown nature which have been designated α- and β-luciferin.[2,3,4] We wish to report evidence that these luciferins are chromopolypeptides.

The most highly purified preparations of the luciferins are oxygen-sensitive orange-yellow resins from which no bioluminescent substance can be sublimed and from which it has not been possible to obtain crystalline fractions.[4] At 65° in high vacuum, α-luciferin is converted to B-luciferin; the transformation is reversed in dilute acid. The infrared spectrum of films of β-luciferin lacks fine structure but contains strong absorptions at 3250, 2825, 1680, 1625, and 1510 cm.$^{-1}$, which collectively indicate the amide bond as it occurs in peptides[5,6,7] or in cyclic ureides.[8,9] Accordingly, an attempt was made to degrade β-luciferin by hydrolysis and, although 0.5 *N* hydrochloric acid does not attack the molecule appreciably at 100°, de-oxygenated 4 *N* acid slowly degrades it at 125° with loss of activity. β-Luciferin does not give a ninhydrin test but the product of its hydrolysis contains a number of ninhydrin-positive substances. These have been presumptively identified by two-dimensional paper chromatography as the amino acids: glycine, threonine, proline, lysine, aspartic acid, glutamic acid, and leucine, isoleucine, or phenylalanine. The hydrolysate contains an unidentified ninhydrin-positive substance and a yellow pigment readily separable from the amino acid fraction. In addition, when β-luciferin is chromatographed on paper with either hydrogen-saturated *n*-butanol or *i*-amyl alcohol (R_f 0.8 and 0.65, respectively, determined by the position of light-emitting areas after wetting the chromatogram with luciferase) the N-chloroamide test,[10] the retention test,[11] and hydrolysis of eluted substance show that the position of luciferin activity coincides with the position of a polypeptide. Our preparations of β-luciferin contain in addition polypeptide (R_f 0 in both solvents) which does not possess luminescent activity in the presence of luciferase but which may be related to luciferin since it and the active polypeptide have identical amino acid compositions. Only the active polypeptide is yellow and accordingly belongs to the class of pigmented polypeptides hitherto encountered in *Actinomyces*.[12,13] Such substances thus occur in higher organisms, and the bioluminescent reaction between *Cypridina* luciferin and luciferase is a naturally-occurring phase in the metabolism of these compounds.

(1) This investigation was supported by a grant from the Research Corporation, New York.

(2) E. N. Harvey, "Living Light," Princeton University Press, Princeton, N. J., 1940; "Bioluminescence," Academic Press, New York, N. Y.

(3) R. S. Anderson, *J. Gen. Physiol.*, **19**, 301 (1935).

(4) H. S. Mason and E. F. Davis, *J. Biol. Chem.*, **197**, 41 (1952).

(5) A. Elliot and E. J. Ambrose, *Nature*, **165**, 921 (1950).

(6) I. M. Klotz and P. Griswold, *Science*, **109**, 309 (1949).

(7) I. M. Klotz, P. Griswold and D. M. Gruen, THIS JOURNAL, **71**, 1615 (1949).

(8) E. R. Blout and M. J. Fields, *J. Biol. Chem.*, **178**, 335 (1949).

(9) E. R. Blout and M. J. Fields, THIS JOURNAL, **72**, 479 (1950).

(10) H. N. Rydon and P. W. G. Smith, *Nature*, **169**, 922 (1952).

(11) F. A. Robinson, K. L. A. Fehr and W. Dickinson, *Biochem. J.*, **51**, 298 (1952).

(12) S. A. Waksman and M. Tishler, *J. Biol. Chem.*, **142**, 519 (1942).

(13) H. Lehr and J. Berger, *Arch. Biochem.*, **23**, 503 (1949).

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LIPOIC ACID–CONJUGASE

Sir:

Lipothiamide pyrophosphate (LTPP), the amide of lipoic acid (LA) and thiamin pyrophosphate (TPP), is required for the oxidative decarboxylation of pyruvate and α-ketoglutarate by cell-free extracts of an *Escherichia coli* mutant.[1a] It has now been demonstrated that cell-free extracts of wild-type *E. coli* contain an enzyme, lipoic acid conjugase,

(1) (a) L. J. Reed and B. G. DeBusk, THIS JOURNAL, **74**, 3964 (1952); (b) **74**, 3457 (1952).