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).

DEPARTMENT OF CHEMISTRY JOHN C. SHEEHAN
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
CAMBRIDGE 39, MASSACHUSETTS DAVID A. JOHNSON

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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.

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(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).

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(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).

DEPARTMENT OF BIOLOGY
PRINCETON UNIVERSITY
PRINCETON, N. J. H. S. MASON

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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).

which forms LTPP from TPP and bound LA. The loss of this enzyme is apparently the critical change resulting from mutation of the parent strain. This conclusion is based upon studies of pyruvate oxidation systems, resolved and partially purified by the method of Korkes, *et al.*,[2] from the wild and mutant strains of this organism.

Pyruvate dismutation requires transacetylase, lactic dehydrogenase, orthophosphate, diphosphopyridine nucleotide, coenzyme A, TPP, and two enzyme fractions from the wild strain (designated A_W and B_W)[2] (Table I). However, the enzyme fraction B_W and the cofactor TPP can be replaced by a single substance, LTPP, indicating that the basic pyruvate oxidase system, which is activated by the coenzyme LTPP, is present only in fraction A. The apoöxidase fraction from the mutant strain, A_M, can likewise be activated either by LTPP, or by TPP plus B_W, but *neither* apoöxidase fraction is activated by TPP plus B_M, the fraction from the mutant corresponding to fraction B_W of the wild strain.

TABLE I

PYRUVATE DISMUTATION WITH PURIFIED ENZYME FRACTIONS

Components[a]	μM Products in 90 min. Carbon dioxide	Acetyl phosphate	Lactate
A_W + TPP	0	0	0.2
A_W + B_W + TPP	3.6	3.4	3.7
A_W + TPP + LA	0	0	0.2
A_W + LTPP	4.7	4.4	4.7
A_W + Incubated[b] (B_W + TPP)	3.5	3.3	3.4
A_W + Control[c]	0.1	0.2	0.2
A_M + TPP	0.1	0.1	0.1
A_M + B_W + TPP	3.6	3.3	3.5
A_M + LTPP	4.7	4.4	4.8
A_M + Incubated[b] (B_W + TPP)	3.5	3.2	3.4
A_M + Control[c]	0	0	0.1
A_W + B_M + TPP	0.1	0.1	0.3
A_W + B_M + TPP + LA	0.1	0.1	0.3
A_M + B_M + TPP	0.1	0.1	0.1

[a] Present at following levels: enzyme fractions, 2.0 mg. protein; TPP, 100γ; LA, 10γ; LTPP, 24γ of crude synthetic preparation[1b]; final volume, 2 ml. Supplements and experimental conditions as previously described.[1a] [b] Incubated 90 min. at 25°, boiled 10 min., and supernatant added to A_W or A_M. [c] B_W incubated and boiled prior to contact with TPP.

Incubation of fraction B_W alone with TPP produces a heat stable product, presumably LTPP, which can subsequently activate the apoöxidase of either strain; however, heating fraction B_W prior to its contact with TPP results in an incubation mixture having no coöxidase activity. Fraction B_W must furnish lipoic acid conjugase as well as lipoic acid, presumably bound to the conjugase, or less likely to a contaminating protein, by a union not dissociable by dialysis.

BIOCHEMICAL INSTITUTE AND
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF TEXAS, AND
CLAYTON FOUNDATION FOR RESEARCH
AUSTIN, TEXAS

LESTER J. REED
BETTY G. DEBUSK

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(2) S. Korkes, *et al.*, *J. Biol. Chem.*, **193**, 721 (1951).

VERATROBASINE AND GERALBINE, TWO NEW ALKALOIDS ISOLATED FROM VERATRUM ALBUM[1]

Sir:

After separation of the ester alkaloids, protoveratrine and veralbidine,[2] and the alkamines jervine and rubijervine from the mixture of alkaloids contained in *Veratrum album*, the mother liquor was divided into two fractions, one of which contained the markedly basic alkaloids, and the other the weakly basic alkaloids. From the fraction containing the first group an unknown alkaloid could be crystallized out of a solution in ethyl acetate.[3] This was purified as its hydrochloride which is only slightly soluble in water. After cleavage of the salt with dilute ammonia the pure base was obtained. This we intend to call *veratrobasine*. The new base crystallizes from methanol in large prisms, which turn yellow from 270° upward, and melt at 285–288°, with decomposition. Its optical rotation in pure alcohol is $[\alpha]^{20}D$ −76.6°, and in pyridine $[\alpha]^{20}D$ −126°. When veratrobasine was dissolved in 84% sulfuric acid (2 mg. of the base in 10 cc. acid) an intensely orange fluorescent solution was obtained. The solution kept this color for over 24 hours. *Anal.* Calcd. for $C_{24}H_{37}O_3N$: C, 74.44; H, 9.63; N, 3.62. Found: C, 74.46, 74.38; H, 9.81, 9.57; N, 3.66, 3.85.

In possessing only 24 carbon atoms, the new alkaloid is significantly different from the other alkamines so far obtained from *Veratrum album* and *Veratrum viride*, which all have 27 carbon atoms. Veratrobasine has one $N\text{-}CH_3$ group, the first to be found in the veratrum alkaloids: $N\text{-}CH_3$ calcd. 3.85, found 3.94.

The base also contains two active hydrogen atoms: calcd. 0.508, found 0.51.

The infrared absorption spectrum shows no band typical of ketones; the ultraviolet absorption spectrum, however, shows a definite maximum at 252 mμ (log ε 2.14).

From the fraction containing the weakly basic alkaloids, a further new alkaloid could be crystallized from ethyl acetate. This new alkaloid, which we have called *geralbine*, crystallizes from aqueous acetone in large prisms, and from a mixture of ethyl acetate and ether (1:1) in rectangular plates which melt at 221–223° with slight yellowish discoloration. In contrast to the other alkaloids isolated from *Veratrum*, geralbine exhibited no measurable rotation in pure alcohol, chloroform or pyridine. When dissolved in 84% or pure sulfuric acid a light yellow solution was obtained which had not lost its color after twelve hours. In crystalline form geralbine is fairly stable but when dissolved in alcohol or chloroform, the solution turns yellow within a few hours. *Anal.* Calcd. for $C_{22}H_{33}O_2N$: C, 76.91; H, 9.68; N, 4.07. Found: C, 76.73, 76.78; H, 9.79, 9.78; N, 3.96, 3.98.

Geralbine hydrochloride crystallizes from methanol–ether in fine needles, and melts at 270°. *Anal.* Calcd. for $C_{22}H_{34}O_2NCl$: C, 69.55; H, 9.02; Cl, 9.35. Found: C, 69.96; H, 9.23; Cl, 9.35, 9.37.

(1) Second communication, A. Stoll and E. Seebeck, *Helv. Chim. Acta*, **35**, 1270 (1952).

(2) A. Stoll and E. Seebeck, *Science*, **115**, 678 (1952).

(3) A detailed description of the process used to isolate the two new alkaloids will be published later in *Helv. Chim. Acta*.