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.

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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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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,

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