hydrochloric acid. The reaction mixture was warmed on a steam bath for 5 min, cooled, and extracted with chloroform. Crystallization (from chloroform–carbon tetrachloride) of the residue from the chloroform extract afforded 17 mg (72%) of crude flavone. The material was chromatographed over silica gel using chloroform as eluent and finally recrystallized from benzene: yield, 5 mg of yellow prisms; mp and mmp 211–213° with natural hymenoxin. The infrared, ultraviolet, and pmr spectra of the synthetic material were identical with those observed for hymenoxin isolated from *Hymenoxys scaposa*.

**Registry No.**—**1a**, 13509-93-8; **1b**, 13509-94-9; **1c**, 13509-95-0; **1d**, 13509-96-1; **2b**, 13509-97-2.

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## Synthesis of $N^{\alpha}$-Benzoyl-S-2-aminoethyl-L-cysteine Amide Hydrobromide[1]

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In connection with studies on the relative rates of tryptic hydrolysis of arginyl, lysyl, and S-2-aminoethyl-L-cysteinyl (AEC) peptide bonds, the amide of $N^{\alpha}$-benzoyl-S-2-aminoethyl-L-cysteine (**7**) was needed. This paper describes the synthesis of this compound.

The procedure was modeled on Hofmann and Bergmann's synthesis of $N^{\alpha}$-benzoyllysinamide[2] (Scheme I). Cysteine hydrochloride was allowed to react with ethylenimine in aqueous solution to give AEC (**1**) as the hydrochloride. This synthesis of AEC, which is based on the aminoethylation procedure of Raftery and Cole,[3] is superior to previous syntheses of this compound[3-5] in that the AEC is obtained directly in good yield and is free of contaminating salts. The AEC was acylated with benzyloxycarbonyl chloride to give di-(benzyloxycarbonyl)-AEC (**2**) which in turn was converted to the Leuchs' anhydride **3** by treatment with phosphorous pentachloride. Although this anhydride could be isolated in crystalline form, it was convenient to convert it directly to the ester **4b** by treatment with methanol. Following benzoylation of the $\alpha$-amino group, the ester group in **5b** was ammonolyzed to give the amide **6**. The benzyloxycarbonyl group in the latter compound was removed by hydrogen bromide in acetic acid[6] to yield the desired $N^{\alpha}$-benzoyl-AEC amide (**7**) as its hydrobromide salt. All of the reactions proceeded smoothly in good yield (53–93%), and the final product was readily obtained in crystalline form. The procedure also gave ready access to $N^{\alpha}$-benzoyl-AEC ethyl and methyl esters (**8**), which are also substrates for trypsin.

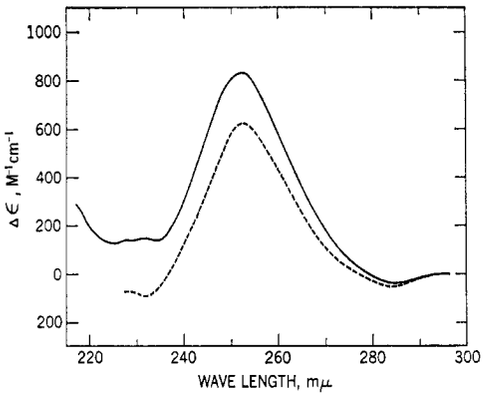


Figure 1.—Difference spectra of $N^{\alpha}$-benzoyl-AEC-amide *vs.* $N^{\alpha}$-benzoyl-AEC (- - - -) and $N_{\alpha}$-benzoyl-AEC ethyl ether *vs.* $N^{\alpha}$-benzoyl-AEC (———), both in pH 9.0, 0.05 *M* sodium borate buffer.

The reaction of benzaldehyde with lysine gives the $N^{\epsilon}$-benzylidine derivative which is useful for the preparation of $\alpha$-substituted compounds of lysine.[7] AEC behaves in a manner similar to lysine in that the benzaldehyde reacted exclusively with the $\omega$-amino group. The resulting $N^{\omega}$-benzylidine derivative **9** was treated with benzoyl chloride in alkali to yield, after acidification, $N^{\alpha}$-benzoyl-AEC (**10**). The latter compound was identical with that prepared by the action of trypsin on $N^{\alpha}$-benzoyl-AEC methyl ester (**8b**).

The amide **7**, ester **8**, and free acid **10** all exhibit an absorption maximum at 228 m$\mu$ with a molar extinction coefficient of 1.14–1.19 $\times$ $10^4$ $M^{-1}$ cm$^{-1}$. However, the free acid has an enhanced absorption at 250–260 m$\mu$ which leads to a marked difference spectra between the acid and the amide or the ester in this region. The difference spectra exhibit a peak at 253 m$\mu$ having a $\Delta\epsilon$ of 620 and 830 $M^{-1}$ cm$^{-1}$ for the amide and ester, respectively (Figure 1). Advantage can be taken of this difference in absorption to follow the rate of tryptic cleavage of the amide or ester. It should be noted that the corresponding $N^{\alpha}$-benzoyl derivatives of glycine, lysine, and arginine exhibit similar difference spectra.

### Experimental Section[8]

**S-2-Aminoethyl-L-cysteine Hydrochloride** (**1**).—L-Cysteine hydrochloride (15.7 g, 0.1 mole) was dissolved in 70 ml of water and the solution was cooled in an ice bath. Ethylenimine (5.7 ml, 0.115 mole) was added with stirring along with a few drops of 0.1% phenolphthalein. The solution was then titrated to a slight pink color with more ethylenimine ($\backsim$1 ml) and the stirring was continued for 30 min. At the end of this period, the nitroprusside test for the free SH group was very weak. The reaction mixture was evaporated to one-half of the original volume and an equal volume of ethanol was added. After storage of the mixture at 4° overnight, the solid mass of crystals was collected and recrystallized from water with ethanol to yield 15.1 g (75%): mp 194–195° dec, $[\alpha]^{27}D$ −4.2°, $[\alpha]^{27}_{380}$ −7.6, $[\alpha]^{27}_{230}$ +657°

(1) This work was supported in part by Grant AM-00608 from the National Institutes of Health and Grant GB-5931 from the National Science Foundation.

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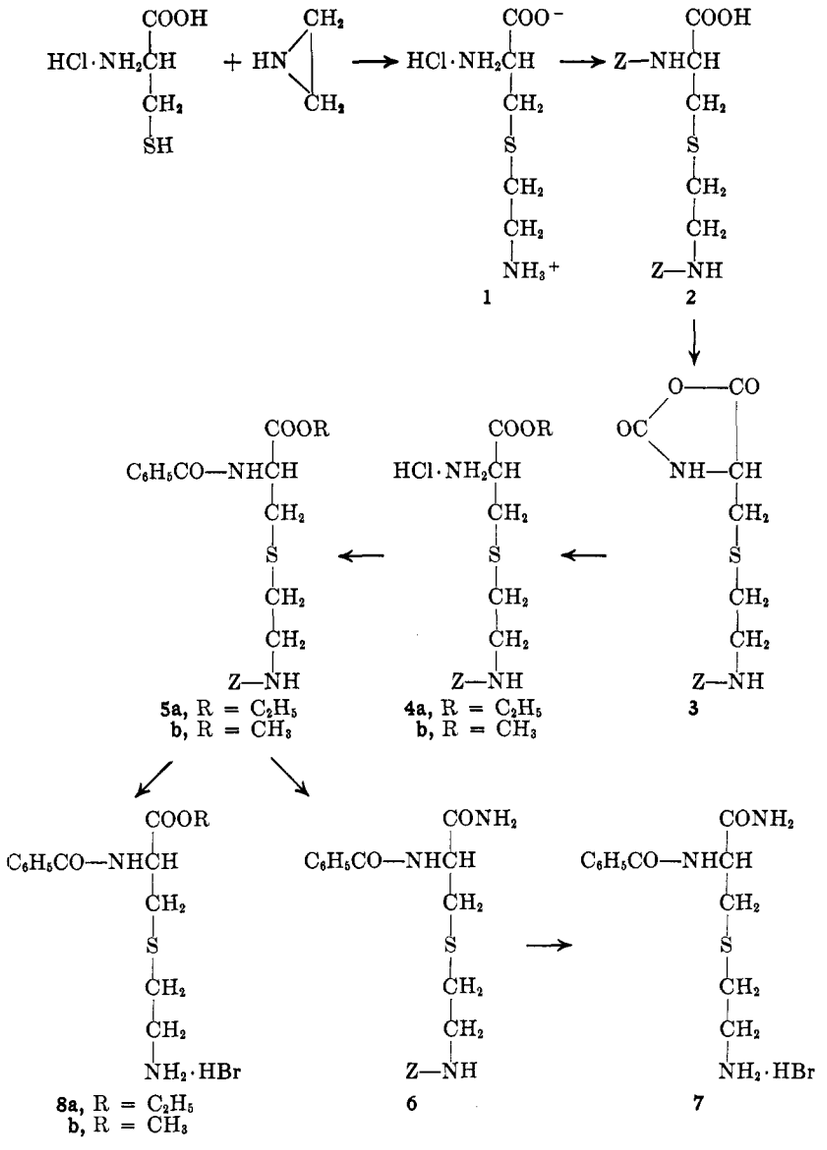
(8) The ultraviolet spectra and the difference spectra were taken on a Cary Model 15 spectrophotometer. The specific rotations were measured on a Cary Model 60 spectropolarimeter. Unless otherwise stated, a Radiometer TTT1c pH-Stat was used whenever the maintenance of the pH of a reaction was required. The elementary analyses were performed by the Chemistry Department, University of California, Berkeley, Calif. Melting points were determined in open capillaries in a Hershberg apparatus and are uncorrected.

SCHEME I[a]




[a] Z = $C_6H_5CH_2OCO$.

(*c* 0.3, water); lit.[4a] mp 194–195°, $[\alpha]^{25}D$ −7.0° (*c* 1, 1 *N* HCl), $[\alpha]^{20}D$ −8.1 (*c* 4, water); lit.[4b] mp 192–192.5°, $[\alpha]^{25}D$ +7.2° (*c* 1, water); lit.[4c] mp 194–195°, $[\alpha]^{22}D$ −4.4 ± 0.3° (*c* 4, water); lit.[5] mp 195°, $[\alpha]^{16}D$ −4.4° (*c* 3, water). There has been some disagreement in the literature about the specific rotation. Our results agree with those of Lindley[5] and Blaha, *et al.*[4c]

*Anal.* Calcd for $C_5H_{13}N_2O_2SCl$ (200.7): C, 29.92; H, 6.52; N, 13.96; S, 15.97. Found: C, 29.69; H, 6.39; N, 14.11; S, 15.70.

**$N^{\alpha}$,$N^{\omega}$-Dibenzyloxycarboxyl-S-2-aminoethyl-L-cysteine (2).**—Twenty grams of AEC (1, 0.1 mole) was dissolved in 150 ml of cold 2 *N* NaOH and treated in an ice bath under vigorous stirring with 55 ml of benzyloxycarbonyl chloride (0.3 mole) together with 130 ml of 4 *N* NaOH added dropwise from two separatory funnels over a period of 30 min. The mixture was stirred vigorously for another hour and acidified; then the product was extracted into ether. It was then extracted into 600 ml of 0.4 *M* $KHCO_3$ and transferred after acidification to fresh ether again. The syrup which remained upon evaporation of the solvent was dissolved in a small volume of ethyl acetate, and the product was crystallized by addition of petroleum ether (bp 30–60°) to yield 33.7 g (78%): mp 92–93°, $[\alpha]^{27}D$ −7.5, $[\alpha]^{27}_{300}$ −28° (*c* 1.6, ethyl acetate).

*Anal.* Calcd for $C_{21}H_{24}N_2SO_6$ (432.5): C, 58.31; H, 5.59; N, 6.48; S, 7.41. Found: C, 58.06; H, 5.63; N, 6.42. S, 7.15.

**$N^{\omega}$-Benzyloxycarbonyl-$N^{\alpha}$-carboyl-S-2-aminoethyl-L-cysteine Anhydride (3).**—To 150 ml of etheral solution of the di(benzyloxycarbonyl) derivative (2, 33.7 g, 0.078 mole) at 0° was added 22 g of powdered $PCl_5$ and the mixture was stirred for 30 min, at which time most of the material had dissolved. After the mixture had been filtered through sintered glass, the solvent was removed under vacuum at 40° with precautions to exclude moisture. Ethyl acetate was added and evaporated to leave a colorless syrup. This syrup is the most convenient form for conversion to the esters as described below owing to its good solubility in acidic alcohols. The compound crystallized slowly as rosettes from ethyl acetate–petroleum ether during storage for several days at 4° to yield 20 g (79%), mp 95°, $[\alpha]^{27}D$ −50.2° (*c* 0.22, ethyl acetate).

*Anal.* Calcd for $C_{14}H_{16}N_2SO_5$ (324.4): C, 51.84; H, 4.97; N, 8.64; S, 9.88. Found: C, 51.90; H, 5.24; N, 8.62; S, 9.87.

**$N^{\omega}$-Benzyloxycarbonyl-S-2-aminoethyl-L-cysteine Ethyl or Methyl Ester Hydrochloride (4). A. Ethyl Ester 4a.**—Carboxyl anhydride (3, 24 g, 0.074 mole) in the form of a syrup was dissolved in 150 ml of 1 *N* ethanolic HCl and the solution was warmed up to 50° for several minutes, whereupon $CO_2$ gas evolution was observed. The resultant solution was then left standing at room temperature overnight. The ester hydrochloride was crystallized from ethanol upon the addition of a small volume of anhydrous ether to yield 17.4 g (65%): mp 140°, $[\alpha]^{27}D$ +4.9°, $[\alpha]^{27}_{235}$ +1150° (*c* 1, ethanol).

*Anal.* Calcd for $C_{15}H_{23}N_2SClO_4$ (362.9): C, 49.64; H, 6.39; N, 7.72; S, 8.83. Found: C, 50.03; H, 6.44; N, 7.44; S, 8.54.

**B. Methyl Ester 4b.**—By a procedure similar to that used on **4a**, the methyl ester **4b** was obtained in 72% yield upon treatment of the anhydride with methanol, mp 117–118°, $[\alpha]^{27}D$ +3.2°, $[\alpha]^{27}_{235}$ +1240° (*c* 0.63, methanol).

*Anal.* Calcd for $C_{14}H_{21}N_2SClO_4$ (348.9): C, 48.20; H, 6.07; N, 8.03; S, 9.19. Found: C, 48.23; H, 6.23; N, 8.16; S, 9.13.

**C. Methyl Ester 4b from $N^{\omega}$-Benzyloxycarbonyl-S-2-aminoethyl-L-cysteine.**—Twelve grams of $N^{\omega}$-benzyloxycarbonyl-S-2-aminoethyl-L-cysteine (0.04 mole), prepared according to the procedure of Lindley[5] (mp 212–213°), was esterified in 90 ml of anhydrous methanol plus 60 ml of 2.5 *N* methanolic HCl at room temperature overnight. After removing the solvent under vacuum, the product was crystallized from acetone with ether. The crystallized compound weighed 13 g (93%), mp 117°.

**$N^{\alpha}$-Benzoyl-$N^{\omega}$-benzyloxycarbonyl-S-2-aminoethyl-L-cysteine Ethyl or Methyl Ester (5). A. Ethyl Ester 5a.**—The hydrochloride (**4a**, 7.4 g, 0.0204 mole) was suspended in a mixture of ethyl acetate (100 ml) and ether (100 ml). A solution of 3.1 g of $K_2CO_3$ (0.0225 mole) in 180 ml of water was added with stirring. The organic layer containing the free base was separated and treated with 2.8 ml (0.024 mole) of benzoyl chloride and a solution of $K_2CO_3$ in water (2.9 g in 120 ml) with stirring for 30 min. Several drops of pyridine were added. The product in the organic layer was washed with 0.1 *N* HCl, 2% $KHCO_3$, and water. After the organic layer had been dried over anhydrous $Na_2SO_4$, the solvent was removed under vacuum. The product was crystallized from acetone with petroleum ether to yield 4.62 g (53%), mp 94–96°, $[\alpha]^{27}D$ −37.8° (*c* 1, ethanol).

*Anal.* Calcd for $C_{22}H_{26}N_2SO_5$ (430.5): C, 61.37; H, 6.09; N, 6.50; S, 7.45. Found: C, 61.11; H, 5.88; N, 6.32; S, 7.15.

**B. Methyl Ester 5b.**—Similar treatment of the methyl ester hydrochloride **4b** gave a syrup which failed to crystallize but which could be converted to the crystalline amide as described below.

**$N^{\alpha}$-Benzoyl-$N^{\omega}$-benzyloxycarbonyl-S-2-aminoethyl-L-cysteinamide (6).**—The syrupy methyl ester **5b**, prepared by benzoylation of 13 g (0.037 mole) of **4b**, was dissolved in 200 ml of anhydrous methanol, and the solution was saturated with anhydrous ammonia at 0°. After the solution had been kept overnight at room temperature, the product was obtained by evaporating the solvent and was recrystallized from methanol with ether plus a small amount of petroleum ether to yield 5.0 g (about 60% for each step; 34% over-all), mp 117–119°, $[\alpha]^{27}D$ −41.2° (*c* 0.23, methanol).

*Anal.* Calcd for $C_{20}H_{23}N_3SO_4$ (401.5): C, 59.83; H, 5.77; N, 10.46; S, 7.98. Found: C, 59.18; H, 5.77; N, 10.41; S, 8.04.

**$N^{\alpha}$-Benzoyl-S-2-aminoethyl-L-cysteinamide Hydrobromide (7).**—Two grams (0.005 mole) of the benzyloxycarbonyl compound (**6**) was treated with 12 ml of 30% HBr in glacial acetic acid at room temperature.[6] After 45 min, when the evolution of $CO_2$ had ceased, ten volumes of dry ether was added to precipitate the hydrobromide. The product was crystallized from methanol with ether to yield 1.2 g (69%): mp 182–184°, $[\alpha]^{27}D$ −22°, $[\alpha]^{27}_{300}$ −175° (*c* 1.23 water).

*Anal.* Calcd for $C_{12}H_{18}N_3SBrO_2$ (348.3): C, 41.18; H, 5.20; N, 12.07; S, 9.20; Br, 22.94. Found: C, 40.88; H, 5.16; N, 12.08; S, 8.92; Br, 22.82.

**$N^{\alpha}$-Benzoyl-S-2-aminoethyl-L-cysteine Ethyl or Methyl Ester Hydrobromide (8). A. Ethyl Ester 8a.**—The benzyloxycarbonyl compound (**5a**, 4.3 g, 0.01 mole) was treated with 11.2 g of 30% HBr in glacial acetic acid.[6] The evolution of carbon dioxide ceased after about 15 min, at which time dry ether (120 ml) was added to precipitate the ester hydrobromide as an oily material. This ester resisted crystallization from all solvents tried. It was precipitated from ethanolic solution by dropping into a large volume of dry ether and dried under high vacuum to give a white, glassy, hygroscopic solid: 2.62 g (69.5%): mp 62–65°, $[\alpha]^{27}D$ −49.5°, $[\alpha]^{27}_{300}$ −378° (*c* 1.5, water).

*Anal.* Calcd for $C_{14}H_{21}N_2SBrO_3$ (377.3): C, 44.56; H, 5.61; N, 7.42; S, 8.49. Found: C, 44.31; H, 5.53; N, 7.22; S, 8.24.

**B. Methyl Ester 8b.**—Removal of the benzyloxycarbonyl group by similar treatment of the syrupy methyl ester **5b** gave rise to another noncrystalline compound. The latter was converted to the crystalline free acid by treatment with trypsin (see below).

**$N^{\omega}$-Benzylidine-S-2-aminoethyl-L-cysteine (9).**—Five grams of AEC (**1**, 0.025 mole) was dissolved in 25 ml of ice-cold 1 *N* LiOH. To this solution, 2.75 ml of benzaldehyde (0.027 mole) was added under vigorous stirring. The product started to separate as thin plates after about 10 min of reaction. The whole mixture was kept at 4° for several hours and the product was filtered and washed with water followed by ethanol to yield 4.95 g (78.5%), mp 168–169°, $[\alpha]^{27}D$ −27.2° (*c* 0.53, 0.1 *N* NaOH).

*Anal.* Calcd for $C_{12}H_{16}N_2O_2S$ (252.3): C, 57.12; H, 6.39; N, 11.10; S, 12.70. Found: C, 57.26; H, 6.23; N, 11.29; S, 12.58.

**$N^{\alpha}$-Benzoyl-S-2-aminoethyl-L-cysteine (10).**—The benzylidine derivative (**9**, 4.5 g, 0.018 mole) was benzoylated in the presence of an equivalent amount of 1 *N* NaOH at 0° with benzoyl chloride. During the reaction, it was necessary to maintain a weak basic condition with added NaOH in order to avoid the dissociation of benzaldehyde from the $\omega$-$NH_2$ group. After benzoylation was complete, the benzylidine group was removed by acidification of the reaction mixture to pH 1 and warming at 55° for several minutes. The mixture was then washed four times with ether and neutralized to pH 6.2. Evaporation of the solvent to one-third of its original volume followed by refrigeration resulted in the formation of needlelike crystals. The material was recrystallized from water to yield 1.1 g (23%), mp 219–220° dec, $[\alpha]^{27}D$ −58.6° (*c* 0.67, 1 *N* HCl).

*Anal.* Calcd for $C_{12}H_{16}N_2SO_3$ (268.3): C, 53.71; H, 6.01; N, 10.44; S, 11.94. Found: C, 53.64; H, 6.11; N, 10.64; S, 11.75.

To establish that the benzoyl group was on the $\alpha$-$NH_2$ rather than $\omega$-$NH_2$ group, the product was allowed to react at pH 2.5 with excess ninhydrin.[9] Since there was no $CO_2$ formation from the product **10** in contrast to S-2-amnoethyl-L-cysteine (**1**) and DL-valine, used as controls, it could be concluded that the compound was an $\alpha$-benzoyl derivative. This same observation serves to prove that **9** is an $\omega$- rather than an $\alpha$-benzylidine derivative.

Compoud **10** was also prepared by tryptic hydrolysis of $N^{\alpha}$-benzoyl-S-2-aminoethyl-L-cysteine methyl ester (**8b**) at pH 8.0 in aqueous solution (enzyme–substrate, 1:3000, by weight). After the completion of the reaction, as indicated by no more comsumption of NaOH by the reaction mixture, the solvent was evaporated under vacuum. The product was taken up with a few milliliters of absolute ethanol, precipitated with ether, and finally crystallized from water as described above, mp 209–211°.

*Anal.* Calcd for $C_{12}H_{16}N_2SO_3$ (268.3): C, 53.71; H, 6.01; N, 10.44; S, 11.94. Found: C, 52.61; H, 6.19; N, 10.25; S, 10.96.

**Registry No.**—**1**, 4099-35-8; **2**, 13618-73-0; **3**, 13618-74-1; **4a**, 13618-75-2; **4b**, 13618-76-3; **5a**, 13618-77-4; **6**, 13639-91-3; **7**, 13618-78-5; **8a**, 13618-79-6; **9**, 13618-80-9; **10**, 13619-05-1.

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## A Simple Method for the Synthesis of Inosine, 2-Alkylinosine, and Xanthosine from 5-Amino-1-β-D-ribofuranosyl-4-imidazolecarboxamide[1]

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Although a number of papers have been reported on the synthesis of purines from imidazole derivatives, there are few reports[2] in which purines were prepared by the ring closure of the pyrimidine starting from

(1) This paper has been presented at the 86th Annual Meeting of the Pharmaceutical Society of Japan, Oct 22, 1966, Sendai, Japan.

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