4-Methylpiperidine Hydrosulfide.—This compound was made by method A in a 70% yield and melted at 29–31°. A similar melting point was obtained using method B.

Anal. Calcd for $C_6H_{15}NS$: S, 23.89. Found: S, 24.06.

2-Phenylpyrrole. A.—2-Phenylpyrrolidine[7] (14.72 g) and sulfur (6.42 g) were heated at 120° for 5 hr and 160° for 0.5 hr. Distillation of the reaction mixture gave two fractions. The first fraction (6.24 g) boiled at 102° (6 mm), melted at 41–44°, and was identical with 2-phenyl-Δ^1-pyrroline.[8] The second fraction boiled at 140–50° (6 mm) and solidified upon standing. Recrystallization from 70% ethanol followed by sublimation gave 2-phenylpyrrole (3.14 g) melting at 128–129° (lit.[9] mp 129–130°).

B.—A mixture of 2-phenyl-Δ^1-pyrroline (45.9 g) and sulfur (11.1 g) was heated at 85° for 7 hr at which time the evolution of hydrogen sulfide had subsided. Distillation at reduced pressure gave starting material (23 g) and 2-phenylpyrrole (8.9 g). The nmr spectrum ($DCCl_3$) showed a singlet at 7.30 (NH), a multiplet at 7.16 for the phenyl hydrogens, doublets at 6.56 and 6.38 for the 5- and 3-pyrrole hydrogens, and a triplet at 6.20 ppm for the 4-pyrrole hydrogen.

Bis(1-methyl-5-phenyl-2-pyrrolyl) Disulfide (IVa).—1-Methyl-2-phenylpyrrolidine[10] (24.5 g) and sulfur (10.6 g) were heated at 125° for 11 hr. The dark, tarry product was dissolved in absolute ethanol (50 ml). Cooling gave a yellow solid melting at 147–51°. Repeated recrystallization from absolute ethanol gave 8.37 g of a yellow, crystalline solid, mp 148–150°. The nmr spectrum ($CDCl_3$) showed a singlet at 3.43 (CH_3N), doublets at 6.23 and 6.53 for 3- and 4-pyrrole hydrogens, and a multiplet at 7.22 ppm for the aromatic hydrogens.

Anal. Calcd for $C_{22}H_{20}N_2S_2$: C, 70.17; H, 5.35; N, 7.44; S, 17.03; mol wt, 376.6. Found: C, 70.47; H, 5.33; N, 7.75; S, 17.24; mol wt, 371 (vapor phase osmometer).

Bis[1-methyl-5-(3-pyridyl)-2-pyrrolyl] Disulfide (IVb).—A mixture of nicotine (25.0 g) and sulfur (9.9 g) was heated at 155° for 4 hr and 170° for 0.5 hr or until the evolution of hydrogen sulfide had ceased. The resulting dark green, viscous liquid was dissolved in absolute ethanol (2 l.) and allowed to cool. The resulting yellow solid after four recrystallizations from alcohol melted at 154–156° (lit.[3] mp 151.5–153.5°), yield 5.9 g. The nmr spectrum ($DCCl_3$) gave a singlet at 3.44 (CH_3), doublets at 6.12 and 6.56 for the 3- and 4-pyrrole hydrogens, respectively, doublet at 8.20 for the 6-pyridine hydrogen, multiplet at 8.36 for the 2-pyridine hydrogen, and a multiplet at 7.24 ppm for the 4- and 5-pyridine hydrogens.

Anal. Calcd for $C_{20}H_{18}N_4S_2$: C, 63.46; H, 4.79; N, 14.80; S, 16.94; mol wt, 378.5. Found: C, 63.69; H, 5.01; N, 14.96; S, 16.76; mol wt, 374 (vapor phase osmometer).

1-Methyl-3-phenylpyrrole.—1-Methyl-3-phenylpyrrolidine[11] (24.6 g) and sulfur (9.8 g) were heated at 105° for 16 hr. The dark tar when poured into hot absolute ethanol (600 ml) gave a dark orange polymeric solid (9.11 g). The alcoholic solution upon evaporation gave a dark oil. Distillation gave the pyrrolidine (2.49 g) and 1-methyl-3-phenylpyrrole (3.6 g), bp 140–144° (4.1 mm), mp 43.4° (from 30% ethanol). The nmr spectrum ($CDCl_3$) showed a singlet at 3.52 (NCH_3), multiplets at 6.95, 6.68, and 7.52 ppm for the 2-pyrrole hydrogen, 4- and 5-pyrrole hydrogens, and aromatic hydrogens, respectively.

Anal. Calcd for $C_{11}H_{11}N$: C, 84.03; H, 7.05; N, 8.91. Found: C, 84.18; H, 7.07; N, 9.07.

1,8-Dimethyl-2,7-di(3-pyridyl)bispyrrolo[2,3-*c*;3′,2′-*g*]-1,2,4,-5-tetrathiocin (V).—A mixture of nicotyrine (9.7 g) and sulfur (3.95 g) was heated at 140° for 4 hr, at which time evolution of hydrogen sulfide had ceased. The hot, tarry mixture was poured into 600 ml of absolute ethanol, and after cooling the liquid was decanted from the tar. This operation was repeated three times using 75% ethanol and finally gave a yellow, flocculent, hydrated solid, mp 125–128°. Recrystallization from absolute ethanol gave 1.06 g of the tetrathiocin (V) melting at 175–176°. The nmr spectrum ($CDCl_3$) gave a singlet at 3.76 (NCH_3), and 6.44 (3-pyrrole hydrogen), doublet at 7.30 for the 4-pyridine hydrogen, and multiplets at 7.66 and 8.56 ppm for the 5- and 2- and 6-pyridine hydrogens, respectively. The infrared spectrum was similar to that of nicotyrine.

Anal. Calcd for $C_{20}H_{16}N_4S_4$: C, 54.51; H, 3.67; N, 12.72; S, 29.11; mol wt, 440.6. Found: C, 54.53; H, 3.67; N, 12.54; S, 29.33; mol wt, 446 (vapor phase osmometer).

Polarographic studies were carried out in acetonitrile containing 0.2 *N* $(C_4H_9)_4NI$ with a mercury pool anode. The tetrathiocin (V) gave two waves at $E_{1/2} = -0.20$ v, $I_d = 5.23$ μa and $E_{1/2} = -0.76$ v, $I_d = 1.23$ μa. The disulfide (IVb) under the same conditions gave $E_{1/2} = -0.36$ v and $I_d = 4.74$ μa. The capillary used had a $m^{2/3}t^{1/6}$ of 1.54 $mg^{2/3}\ sec^{-1/2}$ at 42 cm.

Acknowledgment.—The authors wish to thank Mr. T. McIntyre for the polarographic data, the Reilly Tar and Chemical Company for the samples of 4-methylpiperidine and 1-ethylpiperidine, the Houdry Process and Chemical Company for the 1,4-diazabicyclo[2.2.2]-octane, and the Eastman Chemical Products for the 3-azabicyclo[3.2.2]nonane.

(7) E. B. Knott, *J. Chem. Soc.*, 186 (1948).
(8) J. H. Burckhalter and J. H. Short, *J. Org. Chem.*, **23**, 1281 (1958).
(9) H. Rapoport and M. Look, *J. Am. Chem. Soc.*, **75**, 4605 (1953).
(10) R. Lukes, *Chem. Listy*, **27**, 392 (1933).
(11) F. Bergel, N. C. Hindley, A. L. Morrison, and H. Rinderknecht, *J. Chem. Soc.*, 269 (1944).

Sulfur-Containing Polypeptides. V. Studies on N-(2-Hydroxyarylidene) and Enamine Protective Groups[1,2]

RICHARD G. HISKEY AND GEORGE L. SOUTHARD[3]

The Venable Chemical Laboratory, The University of North Carolina, Chapel Hill, North Carolina

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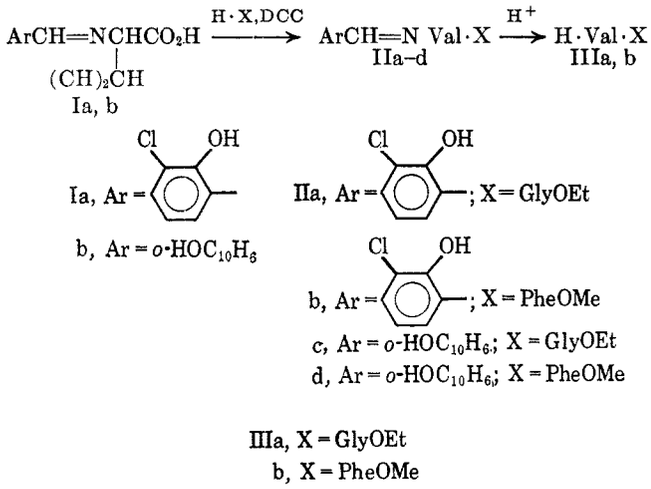
The synthesis of cysteine peptide derivatives using N-salicylidene and N-(1-benzoylisopropenyl) protective groups is described. The N-salicylidene group apparently can only be used with S-tritylcysteine or valine. The N-(1-benzoylisopropenyl) group is readily hydrolyzed and may be useful in specific synthetic situations.

The problem involving the choice of amino and carboxy protective groups compatible with various acid-labile sulfur protective groups for use in the synthesis of cystine peptides has been previously discussed.[1,4] In brief, the most desirable amino protective group would be one which could withstand the conditions of peptide bond formation and could later be removed under conditions mild enough to avoid hydrolysis of acid-labile esters or thioethers. Although the problem of devising such a group has not received a great deal of attention, at least two promising approaches have appeared; these include the proposed use of the N-(2-

(1) Part IV of this series, R. G. Hiskey and J. B. Adams, Jr., *J. Org. Chem.*, **31**, 2178 (1966).
(2) Supported by Grant A-3416 from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, U. S. Public Health Service.
(3) Abstracted in part from a dissertation submitted by G. L. Southard to the University of North Carolina, Chapel Hill, in partial fulfillment of the requirements for the Ph.D. degree, June 1965.
(4) R. G. Hiskey and J. B. Adams, Jr., *J. Am. Chem. Soc.*, **87**, 3969 (1965).

hydroxyarylidene)[5] and enamine[6] protective groups in peptide synthesis.[7] The present report concerns our attempts to utilize the N-(2-hydroxyarylidene) and the N-(1-benzoylisopropenyl) groups in the preparation of protected cysteine derivatives.

N-(2-Hydroxyarylidene) Derivatives.—The previous data of Sheehan and Grenda[5] established that 5-chlorosalicylaldehyde and 2-hydroxy-1-naphthaldehyde could be condensed with L-valine to yield N-(2-hydroxyarylidene)-L-valine (Ia,b) without racemization. The N-protected valine was coupled with ethyl glycinate or methyl L-phenylalaninate to provide the protected dipeptide derivatives (IIa–d); hydrolysis with 1 equiv of acid at 40° afforded ethyl L-valylglycinate (IIIa) and methyl L-valyl-L-phenylalaninate (IIIb). Comparison of these compounds with samples of IIIa,b obtained by routes known to avoid racemization established the optical purity of the esters.

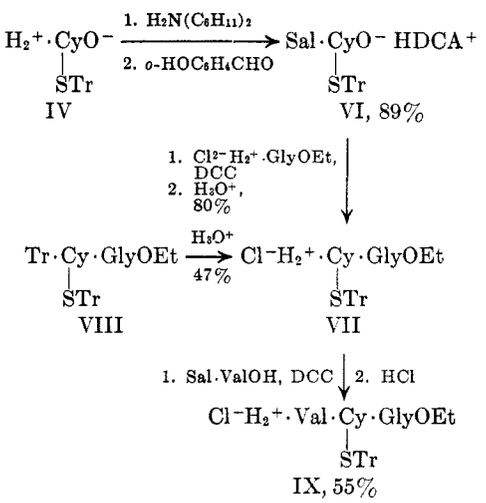


IIIa, X = GlyOEt
b, X = PheOMe

Our initial experiments were concerned with the compatibility of the N-(2-hydroxyarylidene) group and the S-trityl group of cysteine. Although the S-trityl thioethers are readily cleaved by mild acid, the N-(2-hydroxyarylidene) group was expected to be even more acid labile. The condensation of salicylaldehyde with S-trityl-L-cysteine (IV, Scheme I) proceeded smoothly and provided N-salicylidene-S-tritylcysteine (Va) in good yield. However, in contrast to the results obtained with L-valine, complete racemization of IV occurred during the condensation. It is interesting to note that only partial racemization resulted from the formation of N-(2-hydroxy-1-naphthylidene)-S-tritylcysteine (Vb). Presumably this difference is due to the limited solubility of Vb, which precipitates from the reaction mixture as it is formed. When N-salicylidene-L-valine and N-salicylidene-L-leucine were prepared by this procedure no racemization was observed.

Racemization could be avoided by utilizing the N,N-dicyclohexylamine salt of IV in the condensation with salicylaldehyde. The product, N-salicylidene-S-trityl-L-cysteine N,N-dicyclohexylamine salt (VI), was hydrolyzed to IV using ethanolic hydrochloric acid; the specific rotation of IV was unchanged during this transformation. In addition, VI was coupled with ethyl glycinate hydrochloride, using N,N-dicyclohexylcarbodiimide (DCC), to provide ethyl S-trityl-L-cysteinylglycinate (VII). A sample of VII prepared by the procedure of Velluz, *et al.*,[8] from ethyl N,S-dityrityl-L-cysteinylglycinate (VIII) was identical with that obtained *via* VI. N-Salicylidene-L-valine could be coupled to VII using DCC; hydrolysis of the Schiff base protective group provided ethyl L-valyl-*S*-trityl-L-cysteinylglycinate hydrochloride (IX) in 55% yield.

SCHEME I



Despite the rather low over-all yields obtained with the N-salicylidene group and the experimental difficulties involved in utilizing a group of great acid lability, the combination of the N-salicylidine and S-trityl groups appeared promising. However, additional experiments using other α-amino acids indicated another serious disadvantage associated with this protective group. When the N,N-dicyclohexylamine salts of N-salicylidene-L-alanine, -L-leucine, and -L-tyrosine, or glycine were allowed to react with ethyl glycinate hydrochloride in the presence of DCC, no dipeptide derivative was obtained. Recovered reactants were obtained in every case; presumably, the difficulty involved the attack of the amine component on the carbon–nitrogen double bond of the protective group rather than nucleophilic addition to the active ester group. Thus, the use of the N-salicylidene residue appears to be limited to valine and S-tritylcysteine.

N-(1-Benzoylisopropenyl) Derivatives.—Dane, *et al.*,[6] have investigated the chemical reactivity of several adducts formed from the potassium salts of DL-amino acids and β-dicarbonyl compounds. For example, treatment of potassium N-(1-benzoylisopropenyl)glycinate (X) with chloroacetonitrile provided the cyanomethyl ester XI. Aminolysis of XI with ethyl glycinate yielded ethyl N-(1-benzoylisopropenyl)glycylglycinate (XII); mild hydrolysis of XII provided ethyl glycylglycinate hydrochloride (XIII). Derivatives such as X could also be coupled to acid salts of amino acid esters using DCC. (See Scheme II.)

In our experiments the use of a modified procedure provided the potassium salts of N-(1-benzoylisopropenyl)amino acids that were not contaminated with unreacted amino acid. Since benzoylacetone was found

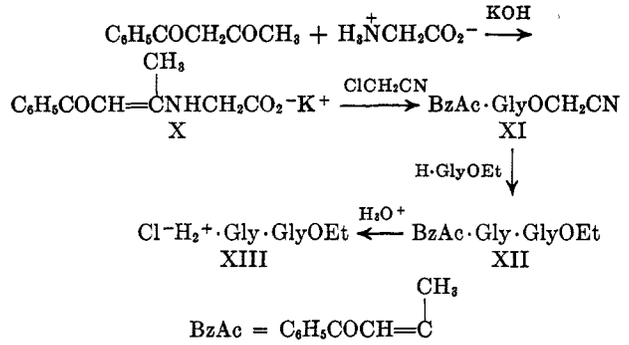
(5) J. C. Sheehan and V. J. Grenda, *J. Am. Chem. Soc.*, **84**, 2417 (1962).

(6) E. Dane, F. Drees, P. Konrad, and T. Dockner, *Angew. Chem.*, **74**, 873 (1962); E. Dane, F. Drees, and P. Konrad, Belgium Patent, 616 (1962).

(7) Since these reports appeared, L. Zervas, D. Borovas, and E. Gazis [*J. Am. Chem. Soc.*, **85**, 3660 (1963)] have reported the use of the *o*-nitrophenylsulfenyl group as an acid-sensitive N-protective group.

(8) G. Amiard, R. Heymes, and L. Velluz, *Bull. Soc. Chim.*, France, 698 (1956).

SCHEME II

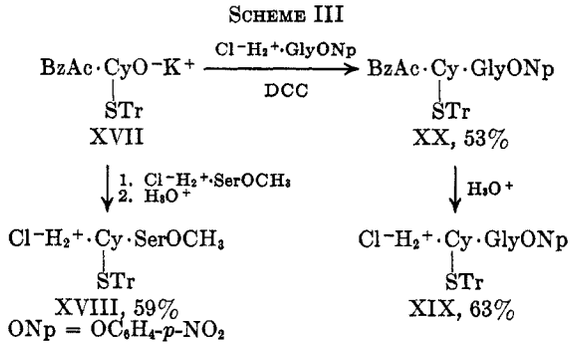


to yield more crystalline derivatives than acetylacetone, it was employed in subsequent experiments. Although no attempts were made to establish the structure of the salts, they are assigned the enamine form X on the basis of earlier studies by Dudek and Dudek.[9] The lability of these materials proved to be a serious disadvantage for their practical utility; satisfactory elemental analyses could not be obtained and the derivatives readily decomposed on storage.

The coupling of X with ethyl L-tyrosinate hydrochloride, using DCC, provided the protected dipeptide derivative, XIVa, in only 50% yield; hydrolysis of XIVa provided 71% of ethyl glycyl-L-tyrosinate hydrochloride (XVa). A similar sequence using potassium N-(1-benzoylisopropenyl)-L-alaninate (XVI) afforded 46% of XIVb and 69% of ethyl L-alanyl-L-tyrosinate hydrochloride (XVb) after hydrolysis. The poor yields of XIVa,b can probably be attributed to low solubility of the protected dipeptide derivatives since some of the dipeptide precipitated with the N,N-dicyclohexylurea produced in the reaction.

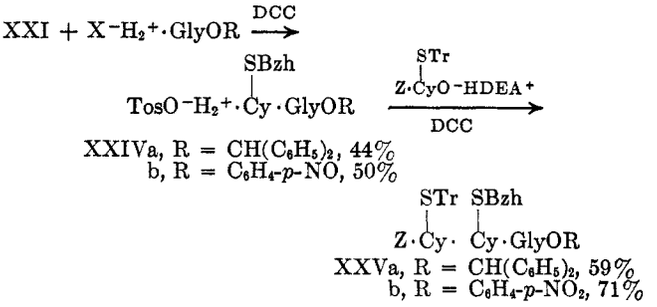
The applicability of the enamine group to the synthesis of cysteine peptide derivatives was studied in the following situations. The selective removal of the N-(1-benzoylisopropenyl) group from S-trityl-L-cysteine derivatives was demonstrated by the synthesis of methyl S-trityl-L-cysteinylserinate hydrochloride (XVIII) and *p*-nitrophenyl S-trityl-L-cysteinylglycinate hydrochloride (XIX) from potassium N-(1-benzoylisopropenyl)-S-trityl-L-cysteinate (XVII) (Scheme III).

SCHEME III



An indication that a minimum amount of racemization is associated with the preparation and coupling of the potassium salt of N-(1-benzoylisopropenyl)-S-benzhydryl-L-cysteine (XXI) was obtained by the synthesis of ethyl N-carbobenzoxy-S-trityl-L-cysteinyl-S-benzhydryl-L-cysteinylglycinate (XIII, Scheme IV). The tripeptide derivatives prepared *via* either XII or the *p*-nitrophenyl ester method exhibited identical physical properties.

The N-(1-benzoylisopropenyl) group could be selectively hydrolyzed in the presence of a benzhydryl ester; however, the yield of dipeptide ester hydrochloride XXIVa was low. Although the N-(1-benzoylisopropenyl) group may be adaptable to certain synthetic situations, the extreme lability detracts considerably from the general utility of this group.



Experimental Section[10]

S-Trityl-L-cysteine (IV) was prepared by the procedure of Zervas and Photaki,[11] mp 167–168°, $[\alpha]^{20}D$ 108° (*c* 1.45, 0.04 *N* ethanolic HCl).

N-Salicylidene-S-tritylcysteine (Va).—A suspension of S-trityl-L-cysteine (5.0 g, 0.0138 mole) in 150 ml of ethanol was treated with 1.88 g (0.0154 mole) of salicylaldehyde. The suspension was stirred for 20 hr at room temperature and filtered; the product was washed with ether. The N-salicylidene derivative was obtained as 5.77 g (90%) of yellow solid, mp 141° dec, $[\alpha]^{20}D$ −5.5° (*c* 0.6, THF).

Anal. Calcd for $C_{29}H_{25}NO_3S$: C, 74.49; H, 5.39; N, 3.0; S, 6.86. Found: C, 76.64; H, 5.41; N, 3.04; S, 6.94.

Despite numerous determinations the carbon analyses of the noncrystalline Schiff bases invariably gave high results. The yield of the N-salicylidene derivative was increased to 97% using THF as the reaction solvent, $[\alpha]^{20}D$ −4.3° (*c* 1, THF).

Hydrolysis of N-Salicylidene-S-trityl-L-cysteine.—To 1.0 g (2.4 mmoles) of Va in 50 ml of 80% ethanol was added 3 ml of 1 *N* hydrochloric acid. The solution was warmed (below 50°) until the color disappeared, the solvent was removed *in vacuo*, and the residue was triturated with ether. The solid residue was dissolved in 10 ml of ethanol and treated with 0.3 ml of triethylamine. The solvent was removed *in vacuo* and the residue was washed with ether, water, and hot acetone. The S-tritylcysteine was crystallized from the acetone solution: 0.654 g (84%), mp 164–165° dec, $[\alpha]^{20}D$ +1.0° (*c* 1.45, 0.042 *N* ethanolic HCl). The starting S-trityl-L-cysteine had the following specific rotation: $[\alpha]^{20}D$ 107° (*c* 1.45, 0.042 *N* ethanolic HCl), lit.[11] mp 164–165° dec, $[\alpha]^{20}D$ 108° (*c* 1.45, 0.0042 *N* ethanolic HCl).

N-Salicylidene-S-trityl-L-cysteine N,N-Dicyclohexylamine Salt (VI).—To 10.0 g (0.0276 mole) of S-trityl-L-cysteine in 300 ml of THF was added 5.0 g (0.0276 mole) of N,N-dicyclohexylamine. The stirred solution was treated with 3.65 g (0.042 mole) of salicylaldehyde. After 3 hr the solution was filtered; the filtrate was evaporated to dryness. Trituration of the residue with pentane–ether (1:1) provided a yellow solid which was recrystallized from a small volume of methylene chloride–ether to yield 15.96 g (89%) of VI, mp 150–151° dec, $[\alpha]^{20}D$ −41.3 (*c* 1, THF).

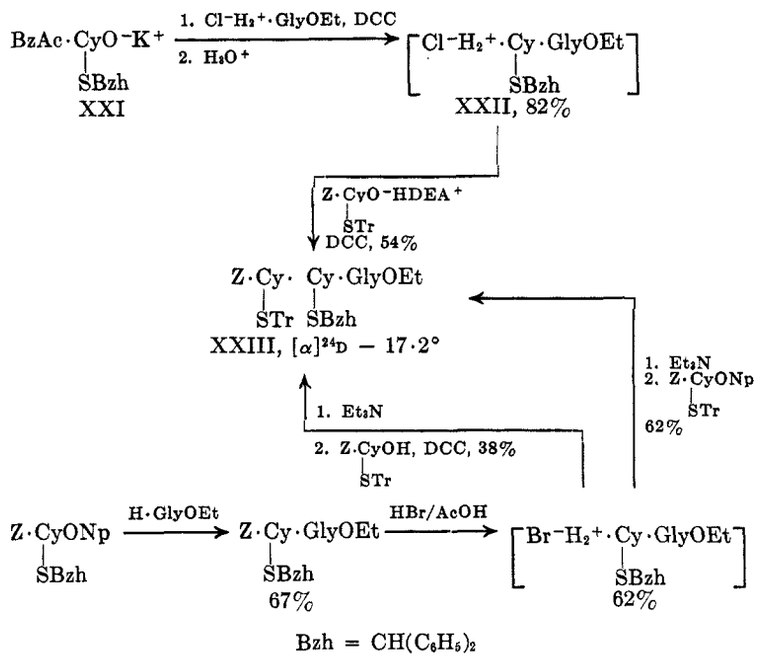
Anal. Calcd for $C_{41}H_{48}N_2O_3S$: C, 75.89; H, 7.46; N, 4.32; S, 4.94. Found: C, 75.81; H, 7.38; N, 4.62; S, 4.83.

(9) G. O. Dudek and E. P. Dudek, *J. Am. Chem. Soc.*, **86**, 4283 (1964).

(10) Elemental analyses were performed by Triangle Laboratories, Carrboro, N. C., and Micro-Tech Laboratories, Skokie, Ill. Amino acids were obtained from Mann Research Laboratories, Inc. Optical rotations were determined on a Rudolph Model 200 polarimeter equipped with a Model 80 photoelectric attachment. Thin layer chromatograms (tlc) were carried out on silica gel G; paper chromatograms were carried out on Whatman No. 14. Solvent systems employed were *n*-butyl alcohol–water–acetic acid (4:5:1), system A, or chloroform–benzene–ethanol (12:12:1), system B.

(11) L. Zervas and I. Photaki, *J. Am. Chem. Soc.*, **84**, 3887 (1962).

SCHEME IV



Bzh = $CH(C_6H_5)_2$

Hydrolysis of N-Salicylidene-S-trityl-L-cysteine N,N-Dicyclohexylamine Salt.—To 2.0 g (3.08 mmoles) of VI in 50 ml of 60% ethanol was added 8.0 ml of 1 *N* hydrochloric acid. The solution was warmed until the color disappeared and then evaporated to dryness. The residue was washed with ether, suspended in 15 ml of ethanol, and treated with 0.71 g (7 mmoles) of triethylamine. The solvent was removed *in vacuo* and the residue was washed sequentially with ether, water, and hot acetone. The product was obtained as 0.522 g (46.5%) of white solid, mp 167° dec, $[\alpha]^{20}D$ 93.0° (*c* 0.45, 0.04 *N* ethanolic HCl). The sample of S-trityl-L-cysteine used as starting material had a specific rotation of $[\alpha]^{20}D$ 93.0°.

N-(2-Hydroxy-1-naphthylidene)-S-trityl-L-cysteine (Vb).—To a suspension of 2 g (5.52 mmoles) of S-trityl-L-cysteine in 200 ml of an ethanol–methanol mixture (95:5) was added 1.05 g (6.08 mmoles) of 2-hydroxy-1-naphthaldehyde. Solution occurred on stirring and crystallization began immediately. The solid was washed with ether to yield 2.46 g (87%), mp 154.5–155°, $[\alpha]^{20}D$ −23° (*c* 0.11, EtOH).

Anal. Calcd for $C_{33}H_{27}NO_3S$: C, 76.87; H, 5.26; N, 2.72; S, 6.22. Found: C, 76.46; H, 5.31; N, 2.78; S, 6.22.

Hydrolysis of Vb.—A suspension of 2.06 g (4 mmoles) of Vc in 100 ml of 80% ethanol was added 5 ml of 1 *N* hydrochloric acid and the solution was warmed. Work-up in the usual manner provided 0.362 g (25%) of S-trityl-L-cysteine, mp 166–168° dec, $[\alpha]^{20}D$ 85° (*c* 1.4, 0.04 *N* ethanolic HCl).

Ethyl S-Trityl-L-cysteinylglycinate Hydrochloride (VII).—To a solution of 10.0 g (15.4 mmoles) of VI in 100 ml of methylene chloride was added 2.15 g (15.4 mmoles) of ethyl glycinate hydrochloride and 3.175 g (15.4 mmoles) of DCC. The mixture was stirred at 0° and allowed to warm to room temperature over 10 hr. The reaction mixture was filtered and evaporated, and the residue was dissolved in ethyl acetate. The solution was washed with 20-ml portions of 5% sodium bicarbonate, water, 1 *N* hydrochloric acid, and water, and dried. The solvent was removed *in vacuo* to leave a yellow powder, mp 81–83°. The residue was dissolved in 160 ml of ethanol and diluted with 40 ml of water and 20 ml of 1 *N* hydrochloric acid. The solution was warmed to 50° for a few minutes; the ethanol was removed *in vacuo*, replaced with 2-propanol, and evaporated. Repetition of this process provided a white solid which was crystallized from a benzene–hexane mixture to give 4.08 g (54.8%) of dipeptide: $[\alpha]^{20}D$ 28.4° (*c* 2, EtOH); tlc homogeneous (iodine, ninhydrin); paper chromatography, one spot R_f 0.94 (system A). This substance was identical in all respects with a sample of the authentic dipeptide obtained below.

Ethyl S-Trityl-L-cysteinylglycinate Hydrochloride (VII) *via* N,S-Ditrityl-L-cysteine (VIII).—A 47% yield of dipeptide was obtained *via* the N,S-ditrityl-L-cysteine procedure,[8] $[\alpha]^{20}D$ 31.6° (*c* 2, EtOH); paper chromatography, one spot R_f 0.94.

N-Salicylidene-L-valine.—A suspension of 3.0 g (26 mmoles) of L-valine and 4.64 g (38 mmoles) of salicylaldehyde in 700 ml of ethanol and 50 ml of methanol was stirred for 20 hr. The unreacted L-valine was filtered; the filtrate was evaporated to dryness and worked up in the usual manner to provide 4.84 g (86%) of the Schiff base, mp 124–125°.

Anal. Calcd for $C_{12}H_{16}NO_3$: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.41; H, 7.09; N, 6.75.

A 1.0-g (4.52 mmoles) sample of N-salicylidene-L-valine in 50 ml of 60% methanol was hydrolyzed by warming with 4.6 ml of 1 *N* hydrochloric acid. Work-up of the reaction mixture and crystallization of the material from aqueous ethanol provided 0.4 g (75%) of L-valine, $[\alpha]^{20}D$ 25.2° (*c* 2, 6 *N* HCl). The L-valine used for the Schiff base had a specific rotation of $[\alpha]^{20}D$ 27.4° (*c* 2, 6 *N* HCl).

N-(2-Hydroxy-1-naphthylidene)-L-leucine.—To 2.23 g (0.017 mole) of L-leucine in 480 ml of ethanol and 35 ml of methanol was added 4.41 g (0.025 mole) of 2-hydroxynaphthaldehyde. The reaction mixture was stirred 20 hr and worked up in the usual manner to provide 2.81 g (58%) of the Schiff base, mp 162° dec, $[\alpha]^{20}D$ −84.4° (*c* 1, EtOH).

Anal. Calcd for $C_{17}H_{19}NO_3$: C, 71.56; H, 6.71; N, 4.91. Found: C, 71.35; H, 6.81; N, 5.03.

Hydrolysis of 1.43 g (5 mmoles) of the Schiff base was performed in the usual manner to provide 0.23 g (35%) of L-leucine, $[\alpha]^{20}D$ 12.7° (*c* 2, 5 *N* HCl). The specific rotation of the L-leucine used for Schiff base preparation was $[\alpha]^{20}D$ 12.8° (*c* 2, 5 *N* HCl).

Ethyl L-Valyl-S-trityl-L-cysteinylglycinate Hydrochloride (IX).—To a suspension of 0.90 (4.1 mmoles) of N-salicylidene-L-valine in 60 ml of methylene chloride at 0° was added 0.842 g (4.1 mmoles) of DCC followed immediately by a precooled solution of 2.0 g (4.1 mmoles) of VII and 0.412 g (4.1 mmoles) of triethylamine in 10 ml of chloroform. The mixture was stirred at 0° and allowed to warm to room temperature over 11 hr. The reaction mixture was filtered and evaporated, and the residue was dissolved in ethyl acetate. The solution was washed with 10 ml each of 5% sodium bicarbonate, water, 1 *N* hydrochloric acid, and water, and dried. The solvent was removed *in vacuo* and replaced with THF. Hydrolysis was conducted with 20 ml of water and 6 ml of 1 *N* hydrochloric acid; the solution was warmed to 50°, the THF was replaced with 2-propanol, and the water was removed by azeotropic distillation *in vacuo*. The residue was crystallized from a 2-propanol–petroleum ether (bp 30–60°) to give 1.29 g (55%) of tripeptide IX: mp 130–131°; $[\alpha]^{20}D$ 12.9° (*c* 1, EtOH); tlc homogeneous; paper chromatography (system A), one spot R_f 0.93. Quantitative amino acid analysis of the substance gave the following ratios of amino acids: valine, 1.12; cysteic acid, 1.08; glycine, 1.16.

Anal. Calcd for $C_{31}H_{38}ClN_3O_4S$: C, 63.74; H, 6.56; N, 7.19; S, 5.49. Found: C, 64.03; H, 6.42; N, 6.96; S, 5.70.

General Procedure for Preparation of the Potassium Salts of N-(1-Benzoylisopropenyl)amino Acids.—A hot solution containing 23.9 mmoles of amino acid in 10 ml of 2.4 *N* methanolic potassium hydroxide and 3 ml of water was treated with a hot solution containing 3.9 g (24 mmoles) of benzoylacetone in 20 ml of ethanol. The solution was concentrated on the steam bath and diluted with 2-propanol (50–70 ml), and the process was repeated. The residue was dissolved in 2-propanol and allowed to crystallize. The melting points of the crystalline salts were not characteristic; thus, the salts were coupled without further characterization.

Ethyl N-(1-Benzoylisopropenyl)glycyl-L-tyrosinate (XIVa).—A mixture of 0.98 g (4.4 mmoles) of ethyl L-tyrosinate hydrochloride and 1.1 g (4.4 mmoles) of X in 20 ml of chloroform at 0° was treated with 0.82 g (4 mmoles) of DCC in 10 ml of chloroform. Stirring was continued for 1 hr at 0° and 21 hr at room temperature. The filtrate from the reaction mixture was washed with two 10-ml portions of water and the chloroform solution was dried and evaporated. The residue was recrystallized from an ethyl acetate–*n*-pentane mixture to provide 0.82 g (50%) of white powder, mp 95–97°. Additional recrystallization from the same solvent raised the melting point to 114–115°.

Anal. Calcd for $C_{23}H_{26}N_2O_5$: C, 67.30; H, 6.39; N, 6.83. Found: C, 67.72; H, 6.62; N, 7.11.

Ethyl Glycyl-L-tyrosinate Hydrochloride (XVa).—To 0.41 g (1 mmole) of XIVa in 15 ml of ethanol was added 1.5 ml of 1 *N* hydrochloric acid. The solution was allowed to stand for 10 min at room temperature and evaporated *in vacuo*. The residue was washed with ether and crystallized from ethanol–ether to yield 0.21 g (71%) of XVa: mp 239°, $[\alpha]^{20}D$ 17.6° (*c* 2, H_2O); lit.[12] mp 245°, $[\alpha]^{20}D$ 17.1° (*c* 2, H_2O).[13] Amino acid analysis indicated the expected ratio of amino acids.

Anal. Calcd for $C_{13}H_{19}ClN_2O_4$: C, 51.57; H, 6.33; N, 9.25. Found: C, 51.12; H, 6.45; N, 9.29.

Ethyl-N-(1-Benzoylisopropenyl)-L-alanyl-L-tyrosinate (XIVb).—A mixture of 2.18 g (8 mmoles) of potassium N-(1-benzoylisopropenyl)-L-alaninate (XVI) and 1.97 g (8 mmoles) of ethyl L-tyrosinate hydrochloride in 80 ml of cold chloroform was treated with 1.65 g (8 mmoles) of DCC. Stirring and work-up in the manner previously described provided 1.57 g (46%) of white needles from acetone, mp 198–200°.

Anal. Calcd for $C_{24}H_{28}N_2O_5$: C, 67.91; H, 6.65; N, 6.60. Found: C, 67.96; H, 7.15; N, 6.84.

Ethyl L-Alanyl-L-tyrosinate Hydrochloride Monohydrate (XVb).—To 0.29 g (0.689 mmole) of XIVb in 10 ml of acetone was added 1 ml of 1 *N* hydrochloric acid. The mixture was worked up in the usual manner and crystallized from ethanol–ether containing a trace of *n*-pentane. The product was obtained as 0.15 g (69%) of white powder: mp 146–147°; $[\alpha]^{20}D$ 10.5° (*c* 2, H_2O); paper chromatography, single spot (ninhydrin) R_f 0.69.

Anal. Calcd for $C_{14}H_{21}ClN_2O_4 \cdot H_2O$: C, 50.22; H, 6.92; N, 8.37. Found: C, 50.46; H, 6.68; N, 8.73.

Methyl S-Trityl-L-cysteinyl-L-serinate Hydrochloride (XVIII).—A cold suspension of 10.9 g (20 mmoles) of IV and 3.1 g (20 mmoles) of methyl L-serinate hydrochloride was treated with 4.12 g (20 mmoles) of DCC, stirred for 1 hr, and filtered. The filtrate was evaporated; the residue was dissolved in 35 ml of 6 *N* hydrochloric acid. After 5 min the solvent was removed and the residue was extracted with ether and 90 ml of 0.25 *N* sodium hydroxide. The ether layer was washed with water, dried, and treated with 15 ml of 1.4 *N* hydrogen chloride in ethyl acetate. Evaporation and crystallization from benzene–ether afforded 5.9 g (59%) of XVII as a white powder: tlc homogeneous (ethanol–chloroform, 1:9); paper chromatography, one spot (ninhydrin, system A).

Anal. Calcd for $C_{26}H_{29}ClN_2O_4S$: C, 62.33; H, 5.83; Cl, 7.08; N, 5.59; S, 6.40. Found: C, 61.50; H, 6.25; Cl, 7.29; N, 5.41; S, 6.23.

***p*-Nitrophenyl N-(1-Benzoylisopropenyl-S-trityl-L-cysteinylglycinate (XX).**—A suspension of 1.09 g (2 mmoles) of XVII and 0.56 g (2 mmoles) of *p*-nitrophenylglycinate hydrobromide in 20 ml of chloroform was treated with 0.41 g (2 mmoles) of DCC. The suspension was stirred at 0° for 1 hr and at room temperature for 20 hr. The reaction mixture was filtered and evaporated, and the residue was dissolved in ethyl acetate. The solution was washed with water, dried, and evaporated. The residue precipitated from 2-propanol to yield 0.73 g (53%) of white powder, mp 103–106°, $[\alpha]^{25}D$ −27.5° (*c* 3, $CHCl_3$).

Anal. Calcd for $C_{40}H_{35}N_3O_6S$: C, 70.06; H, 5.14; N, 6.13; S, 4.68. Found: C, 70.26; H, 5.15; N, 6.06; S, 4.83.

***p*-Nitrophenyl S-Trityl-L-cysteinylglycinate Hydrochloride (XIX).**—To 0.46 g (0.66 mmole) of XX in 15 ml of acetone was added 1 ml of water, and the solution was saturated with carbon dioxide for 20 min. The acetone was evaporated, and the residue was dissolved in 40 ml of ethyl acetate–ether (1:1). The solution was washed with water and dried. The solution was treated with 2.5 ml of 1.39 *N* hydrogen chloride in ethyl acetate and the precipitated powder (XIX) was collected, 0.24 g (63%), $[\alpha]^{23}D$ 39.0° (*c* 3.2, EtOH), lit.[11] $[\alpha]^{27}D$ 39.6° (*c* 3, EtOH).

Ethyl S-Benzhydryl-L-cysteinylglycinate Hydrochloride (XXII).—A mixture of 2.06 g (4.4 mmoles) of XXI and 0.61 g (4.4 mmoles) of ethyl glycinate hydrochloride in 30 ml of chloroform at 0° was treated with 0.91 g (4.4 mmoles of DCC). The mixture was stirred for 25 hr, filtered, and evaporated. The residue was dissolved in ethyl acetate, washed with water, and evaporated. The residue was dissolved in 10 ml of acetone and hydrolyzed with 5 ml of 1 *N* hydrochloric acid. Evaporation provided 1.21 g (82%) of XXII as an amorphous powder: tlc homogeneous (ethyl acetate, system A); paper chromatography, one spot (ninhydrin, system A). The material was used directly in the following reaction.

Ethyl N-Carbobenzoxy-S-trityl-L-cysteinyl-S-benzhydryl-L-cysteinylglycinate (XXIII) *via* XXII.—A cold mixture of 1.59 g (2.79 mmoles) of the N,N-diethylamine salt of N-carbobenzoxy-S-trityl-L-cysteine[14] and 1.2 g (2.79 mmoles) of XXII in 20 ml of chloroform was treated with 0.58 g (2.79 mmoles) of DCC. After 22 hr the reaction mixture was filtered and evaporated to dryness. The residue was dissolved in 40 ml of ethyl acetate and washed with 10-ml portions of 5% sodium bicarbonate, water, 1 *N* hydrochloric acid, and water, and dried. The solvent was removed *in vacuo* and the residue was recrystallized from ethyl acetate to give 1.27 g (54%) of XXIII, mp 184–184.5°, $[\alpha]^{24}D$ −17.2° (*c* 1, DMF). Mixture melting points with authentic samples obtained by the following procedures were not depressed.

***p*-Nitrophenyl N-Carbobenzoxy-S-benzhydryl-L-cysteinate.**—To a cold solution of 5.0 g (0.012 mole) of N-carbobenzoxy-S-benzhydryl-L-cysteine[14] and 1.66 g (0.012 mole) of *p*-nitrophenol, was added 2.45 g (0.012 mole) of DCC. The mixture was stirred at 0° for 1 hr and at room temperature for 4 hr. Addition of ether and filtration provided 4.68 g (72%) of white solid, mp 96–97°, $[\alpha]^{24}D$ −19.9° (*c* 0.782, EtOAc).[15]

Anal. Calcd for $C_{30}H_{26}N_2O_6S$: C, 66.38; H, 4.82; N, 5.15; S, 5.95. Found: C, 66.27; H, 4.94; N, 5.33; S, 6.13.

Ethyl N-Carbobenzoxy-S-benzhydryl-L-cysteinylglycinate.—A suspension of 2.32 g (0.017 mole) of ethyl glycinate hydrochloride and 1.73 g (0.017 mole) of triethylamine in 55 ml of chloroform was treated with 9.3 g (0.017 mole) of *p*-nitrophenyl N-carbobenzoxy-S-benzhydryl-L-cysteinate. The mixture was let stand for 24 hr at room temperature, the solvent was removed *in vacuo*, and the residue was dissolved in ethyl acetate. The solution was washed with water, 1 *N* hydrochloric acid, and water, and then dried. Removal of the ethyl acetate and crystallization from ethanol gave 5.9 g (67%) of the protected dipeptide derivative, mp 117–118°, lit.[11] mp 117–118°.

Ethyl N-Carbobenzoxy-S-trityl-L-cysteinyl-S-benzhydryl-L-cysteinylglycinate (XXIII).—To a 54-ml aliquot of 2 *N* hydrogen bromide in acetic acid was added 4.17 g (7.6 mmoles) of ethyl N-carbobenzoxy-S-benzhydryl-L-cysteinylglycinate. After 30 min the crude hydrobromide was precipitated by the addition of 900 ml of ether and 100 ml of petroleum ether to yield 2.35 g (62%) of the hydrobromide derivative as a hygroscopic solid, paper chromatography, one spot (ninhydrin) R_f 0.83 (system A).

A solution containing 2.25 g (4.7 mmoles) of the crude hydrobromide in 10 ml of N,N-dimethylformamide was treated with 0.48 g of triethylamine and the suspension was filtered. The filtrate was treated with 2.36 g (4.74 mmoles) of N-carbobenzoxy-S-trityl-L-cysteine followed by 0.98 g (4.74 mmoles) of DCC, stirred overnight, and filtered. The product was precipitated

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(15) This preparation was originally performed by Dr. J. B. Adams, Jr.

by the addition of water, dissolved in ethyl acetate, and washed with 15-ml portions of 1 *N* sodium bicarbonate, water, 1 *N* hydrochloric acid, and water. The solution was filtered through sodium sulfate, evaporated, and crystallized from ethyl acetate–ether to yield 1.52 g, mp 178.5–181° (37.5%) of XXIII. Recrystallization from ethyl acetate raised the melting point to 184–185°, $[\alpha]^{24}D$ −17.2° (*c* 1, DMF).

Anal. Calcd for $C_{50}H_{49}N_3O_6S_2$: C, 70.73; H, 5.86; N, 4.91; S, 7.67. Found: C, 70.48; H, 5.79; N, 4.93; S, 7.53.

The tripeptide derivative (XXIII) was also prepared in 62% yield from the reaction of *p*-nitrophenyl N-carbobenzoxy-S-trityl-L-cysteinate with the crude hydrobromide derivative. The sample of XXIII obtained by this procedure melted at 183.5–185°, $[\alpha]^{24}D$ −17.2° (*c* 1, DMF). A mixture melting point with XXIII obtained *via* the DCC method was not depressed.

Benzhydryl S-Benzhydryl-L-cysteinylglycinate Tosylate (XXIVa).—A mixture of 2.35 g (5 mmoles) of XXI and 2.07 g (5 mmoles) of benzhydrylglycinate tosylate in 30 ml of chloroform was treated with 1.03 g (5 mmoles) of DCC. The reaction mixture was stirred at 0° for 1 hr and at room temperature for 15 hr. The filtered reaction mixture was dissolved in 30 ml of acetone, filtered, and treated with 5.4 ml of 1 *N* hydrochloric acid. After 15 min the solvent was evaporated and the residue was treated with 20 ml of 0.25 *N* sodium hydroxide followed by 50 ml of ether. The ether layer was washed with water, dried, and treated with 0.95 g (5 mmoles) of *p*-toluenesulfonic acid. The ether was removed and the residue was crystallized from a methylene chloride–ether mixture to give 1.48 g (44%) of XXIV as a white powder: mp 152–153°; $[\alpha]^{20}D$ 16.6° (*c* 1, DMF); paper chromatography, one spot (ninhydrin) R_f 0.96 (system A).

Anal. Calcd for $C_{38}H_{38}N_2O_6S_2$: C, 66.84; H, 5.61; N, 4.10; S, 9.39. Found: C, 66.63; H, 5.88; N, 4.27; S, 9.24.

Benzhydryl N-Carbobenzoxy-S-trityl-L-cysteinyl-S-benzhydryl-L-cysteinylglycinate (XXVa).—A mixture of 1.14 g (2 mmoles) of N-carbobenzoxy-S-trityl-L-cysteine N,N-diethylamine salt and 1.33 g (2 mmoles) of XXIVa in 20 ml of chloroform was treated with 0.41 g (2 mmoles) of DCC. The reaction mixture was stirred at 0° for 1 hr and at room temperature for 22 hr. The filtered solution was washed with 5-ml portions of 5% sodium bicarbonate, water, 1 *N* hydrochloric acid, and water, and dried. Removal of the solvent and crystallization of the residue from ethyl acetate gave 1.15 g (59%) of XXVa, mp 184–185°, $[\alpha]^{20}D$ −27.8° (*c* 1, DMF).

Anal. Calcd for $C_{60}H_{55}N_3O_6S_2$: C, 73.67; H, 5.67; N, 4.30; S, 6.56. Found: C, 73.85; H, 5.87; N, 4.49; S, 6.54.

***p*-Nitrophenyl S-Benzhydryl-L-cysteinylglycinate Tosylate Salt (XXIVb).**—A mixture containing 6.12 g (13.2 mmoles) of XXI and 3.66 g of *p*-nitrophenylglycinate hydrobromide in 60 ml of chloroform was treated with 2.72 g (13.2 mmoles) of DCC; the mixture was stirred at 0° for 1 hr and at room temperature for 14 hr. The filtrate from the reaction mixture was evaporated and the residue was dissolved in 40 ml of acetone. The solution was treated with 1.44 g (13.2 mmoles) of *p*-toluenesulfonic acid monohydrate and the product XXIVb was collected: 4.2 g (50%); mp 167–168°; $[\alpha]^{20}D$ 21.5° (*c* 1, DMF); paper chromatography, one spot (ultraviolet analysis, ninhydrin) R_f 0.92 (system A).

Anal. Calcd for $C_{31}H_{31}N_3O_8S_2$: C, 58.38; H, 4.90; N, 6.59; S, 10.06. Found: C, 58.29; H, 4.87; N, 6.48; S, 10.26.

***p*-Nitrophenyl N-Carbobenzoxy-S-trityl-L-cysteinyl-S-benzhydryl-L-cysteinylglycinate (XXVb).**—A solution containing 1.14 g (2 mmoles) of N-carbobenzoxy-S-trityl-L-cysteine and 1.27 g (2 mmoles) of XXIVb in 20 ml of chloroform was treated with 0.41 g (2 mmoles) of DCC. The mixture was stirred for 1 hr at 0° and at room temperature for 17 hr. The filtered reaction mixture was washed with 5-ml portions of 5% sodium bicarbonate, water, 1 *N* hydrochloric acid, and water, and dried. Removal of the chloroform and crystallization of the residue from ethyl acetate provide 1.13 g (71%) of XXVb, mp 187–190°, tlc homogeneous (system B), $[\alpha]^{20}D$ −11.1° (*c* 1, DMF).

Anal. Calcd for $C_{54}H_{48}N_4O_8S_2$: C, 68.62; H, 5.12; N, 5.93; S, 6.79. Found: C, 68.76; H, 5.65; N, 6.02; S, 6.89.

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The Reaction of Methanesulfenyl Chloride with Alkoxides and Alcohols. Preparation of Aliphatic Sulfenate and Sulfinate Esters

T. L. MOORE AND D. E. O'CONNOR

The Procter and Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio 45239

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Methanesulfenyl chloride reacts with an equimolar or greater ratio of lithium *n*-pentyloxide, 4-methyl-2-pentyloxide, or *t*-butoxide in 1,2-dimethoxyethane at −40 to −60° to give the corresponding methanesulfenate esters, CH_3SOR, in 40–75% yields. Keys to the successful isolation of the sulfenate esters are use of the alkoxide rather than alcohol and the strict avoidance of an excess of sulfenyl chloride. When a 0.5 *M* excess of methanesulfenyl chloride is employed with lithium *n*-pentyloxide or 4-methyl-2-pentyloxide, the corresponding sulfinate esters, $CH_3\overset{\overset{O}{\uparrow}}{S}OR$, are formed in good yield. However, reaction with lithium *t*-butoxide gives the sulfenate ester, even with an excess of methanesulfenyl chloride. The methanesulfenate esters derived from primary and secondary alcohols are converted by air, or most oxidizing agents, to the corresponding sulfinate esters. These results are interpreted in terms of competition between sulfenate ester and alkoxide or alcohol for methanesulfenyl chloride.

Sulfenate esters, RSOR′, in which R is aromatic[1] or halogenated alkyl[2] are well known. However, we are aware of only two references to isolation of a sulfenate ester in which R and R′ are simple aliphatic groups—the preparation of **1** by the route indicated in eq 1[3]

$$C_2H_5SSCN + NaOC_2H_5 \longrightarrow \underset{\mathbf{1}}{C_2H_5SOC_2H_5} + NaSCN \quad (1)$$

$$(t\text{-}C_4H_9S\text{-})_2Hg + 2I_2 \longrightarrow HgI_2 + 2t\text{-}C_4H_9SI \xrightarrow{C_2H_5O^-} \underset{\mathbf{2}}{2t\text{-}C_4H_9SOC_2H_5} \quad (2)$$

and of **2** by the route of eq 2.[4] A more recent attempt by Douglass to prepare methyl methanesulfenate by reaction of methanesulfenyl chloride with methanol

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