by glpc was shown to contain V and I in the ratio of 2:1. The presence of I in the mixture was checked by infrared bands at 7.68, 9.69, and 13.50 μ.

trans-**2-Phenylcyclopropyl *p*-Tolyl Ketone (VI).**—To a stirred suspension of 300 ml of dry dimethyl sulfoxide and 19.7 g (0.45 mole) of a 54.7% dispersion of sodium hydride in mineral oil under nitrogen was added 100 g (0.45 mole) of trimethylsulfoxonium iodide during 25 min.[9] After 1 hr the mixture was cooled to 22–24° and 95.9 g (0.43 mole) of benzal-*p*-methylacetophenone[18] in 150 ml of dimethyl sulfoxide was added during 45 min. After heating to 50° for 2 hr the cooled mixture was poured on ice and worked up as usual. Distillation afforded 93.4 g of VI as a yellow oil, bp 172–175° (0.3–0.4 mm). Crystallization from hexane yielded 91.0 g (90%) of VI: mp 47–48°; infrared bands at 1.632 μ (*c* 0.405, CCl_4), 6.02, and 9.72. Nmr analysis confirmed the assigned structure.

Anal. Calcd for $C_{17}H_{16}O$: C, 86.4; H, 6.8. Found: C, 86.3; H, 6.9.

The 2,4-dinitrophenylhydrazone, mp 233–235° dec, formed orange plates on crystallization from ethyl acetate.

Anal. Calcd for $C_{23}H_{20}N_4O_4$: C, 66.3; H, 4.8; N, 13.4. Found: C, 66.3; H, 4.8; N, 13.3.

Reactions of VI with PCl_5.—To a suspension of 14.6 g (0.07 mole) of PCl_5 in CH_2Cl_2 was added a solution of 11.8 g (0.05 mole) of VI in 100 ml of CH_2Cl_2. All of the PCl_5 was in solution within 15 min. After 44 hr at 30 ± 2° the solvent was removed under reduced pressure and the residue was treated with ice. The usual work-up yielded 14.2 g of an orange oil shown by glpc analysis to consist of 31% of 1-*p*-tolylnaphthalene (VII) 10% of VI, 21% of several minor unknown substances, and 38% of *trans,trans*-1-chloro-4-phenyl-1-*p*-tolyl-1,3-butadiene (VIII). A small amount of pure VII, mp 50–51.5°, was isolated from this mixture and shown to be identical with a sample of VII prepared in steps from α-tetralone and *p*-tolylmagnesium bromide[5] by mixture melting point, comparison of the ultraviolet spectra, λ_{max}^{hexane} 226 and 290 mμ (ϵ 55,000 and 11.400), and glpc retention time.

Chromatography of a part of the above orange oil over neutral alumina (Woelm grade I) afforded pure VIII as pale yellow plates: mp 112–113°; λ_{max}^{hexane} 237 mμ (ϵ 12,300), 328 (ϵ 54,700), and shoulder at 352 (ϵ 30,800). The nmr spectrum was consistent with the assigned structure.

Anal. Calcd for $C_{17}H_{15}Cl$: C, 80.1; H, 5.9; Cl, 13.9. Found: C, 80.0; H, 6.0; Cl, 13.9.

Reactions of 2,2-Diphenylcyclopropyl Phenyl Ketone (IX) with PCl_5.—To a suspension of 2.92 g (0.014 mole) of PCl_5 in 25 ml of CCl_4 was added 2.98 g (0.01 mole) of IX[19] in 25 ml of CCl_4. After holding at reflux for 4 hr, the solvent was removed under reduced pressure and the residue was treated with ice. After the usual work-up 3.4 g of a yellow oil was obtained which showed three peaks on glpc in the ratio 9:15:76. The first peak was shown to be starting ketone. The second peak (see below) was 1,4-diphenylnaphthalene (X). The third peak was *trans*-1-chloro-1,4,4-triphenyl-1,3-butadiene (XI) which was isolated by trituration of the oil with ethanol to yield 2.1 g of crystals. After two recrystallizations from Skellysolve B, 1.25 g (40%) of pure XI, mp 145–145.5°, $\lambda_{max}^{95\% C_2H_5OH}$ 249 mμ (ϵ 13,300) and 338 mμ (ϵ 38,400),[20] was obtained.

Anal. Calcd for $C_{22}H_{17}Cl$: C, 83.4; H, 5.4; Cl, 11.2. Found: C, 83.4; H, 5.3; Cl, 11.0.

On ozonolysis of 0.3 g of XI in 25 ml of CH_2Cl_2 at −78°, the neutral fraction was treated with 2,4-DPNH reagent. Recrystallization of the solid gave 0.12 g (39%) of orange-red benzophenone 2,4-dinitrophenylhydrazone, mp 244–245° dec. The infrared spectrum was identical with that of an authentic sample.[21]

In a similar experiment except that CH_2Cl_2 was the solvent, on mixing an exothermic reaction occurred for 2 min. The mixture was then allowed to stand at 30° for 18 hr (expt 1, Table II). Chromatography of the resulting oil using Skellysolve F–Skellysolve B, (1:1) afforded a fraction of almost pure X, which on crystallization from ethanol yielded X, mp 136.5–137.5°, alone and mixed with an authentic sample.[22] The ultraviolet spectrum in hexane showed peaks at 232 mμ (ϵ 49,400) and 301 mμ (ϵ 17,200)[23] and the nmr spectrum showed a complex at τ 2.12 (two protons) and 2.59 (14 protons).

No X was formed when a solution of 1,1,4-triphenyl-1-butene-4-one,[17] mp 126–127°, was refluxed in CCl_4 containing HCl and $POCl_3$.

Only a very small amount of compound XII, mp 110–111°, was isolated. We assume that it is the *cis* isomer of XI mainly because of an absorption maximum at 323 mμ (ϵ 43,000). The *trans* isomer XI absorbs at 338 mμ. In the 1,4-diphenyl-1,3-butadiene series, the *trans,trans* isomer absorbs at a longer wavelength than the *trans,cis* isomer.[24]

In addition to the peak at 323 mμ, XII also absorbs at 259 (ϵ 21,000) and at 230 (ϵ 33,000). Thus, our evidence for the structure of XII is incomplete but we did not spend more effort to isolate larger amounts in order to prove the structure.

(18) A. D. Petrov and L. I. Anzus, *Ber.*, **66**, 4320 (1933).

(19) F. J. Impastato and H. M. Walborsky, *J. Am. Chem. Soc.*, **84**, 4838 (1962).

(20) Compare with spectrum for 1,1,4-triphenyl-1,3-butadiene.[18]

(21) R. L. Shriner, R. C. Fuson, and D. Y. Curtin ["The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1956, p 318] reported mp 239°.

(22) We thank Professor E. Bergmann, Hebrew University, Israel, for an authentic sample of X. C. Dufraisse and R. Briou [*Bull. Soc. Chim. France*, [5] **5**, 502 (1938)] reported mp 135–136° for X.

(23) I. Gillet [*ibid.*, 1141 (1950)] gives λ_{max} at 302 mμ (ϵ 14,100) and end absorption with a cutoff at 250 mμ.

(24) J. H. Pinckard, B. Wille, and L. Zechmeister [*J. Am. Chem. Soc.*, **70** 1938 (1948)] gave λ_{max} 328 mμ (ϵ 55,500) for the *trans,trans* isomer and λ_{max} 315 mμ (ϵ 30,000) for the *trans,cis* isomer.

Sulfur-Containing Polypeptides. IV. Synthetic Routes to Cysteine Peptides[1,2]

RICHARD G. HISKEY AND JOHN B. ADAMS, JR.[3,4]

Venable Chemical Laboratory, The University of North Carolina, Chapel Hill, North Carolina 27515

Received November 23, 1965

Procedures for the incorporation of protected cysteine residues into peptide chains have been investigated and applied to the synthesis of two model hexapeptide derivatives. Methods for the selective removal of various protective groups have also been devised.

In several previous reports a number of aspects of the general problem associated with the synthesis of polypeptides cross-linked or looped at known positions by cystine residues have been considered. These have included studies on various sulfur,[1,5,6] nitrogen,[7] and carboxylic acid[7] protective groups and methods for the stepwise formation of two disulfide bonds within the same molecule.[8] Another important segment of

(1) Part III of this series: R. G. Hiskey, T. Mizoguchi, and T. Inui, *J. Org. Chem.*, **31**, 1192 (1966).

(2) Supported by Grant A-3416 from the Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, U. S. Public Health Service.

(3) Abstracted in part from a dissertation by J. B. Adams, Jr., submitted to the University of North Carolina, Chapel Hill, in partial fulfillment of the requirements for the Ph.D. degree, June 1965.

(4) Shell Chemical Corp. Fellow, 1963–1964.

(5) R. G. Hiskey and W. P. Tucker, *J. Am. Chem. Soc.*, **84**, 4794 (1962).

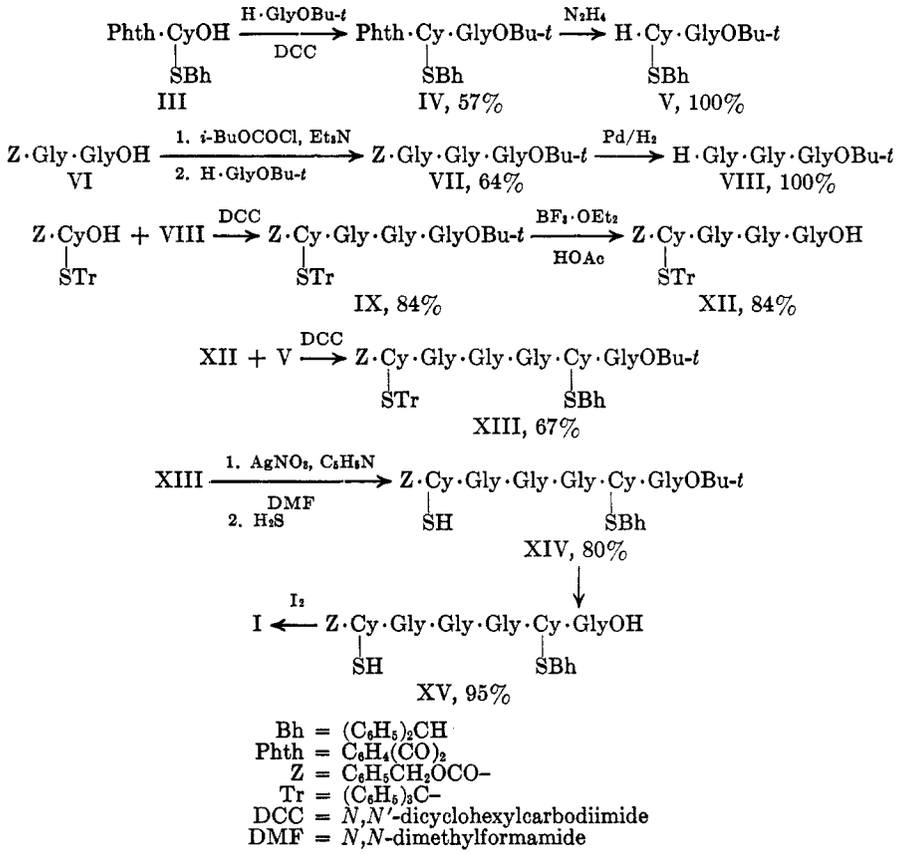
(6) R. G. Hiskey, T. Mizoguchi, and H. Igeta, *J. Org. Chem.*, **31**, 1188 (1966).

(7) R. G. Hiskey and J. B. Adams, Jr., *J. Am. Chem. Soc.*, **87**, 3969 (1965).

(8) R. G. Hiskey and D. N. Harpp, *ibid.*, **87**, 3965 (1965).

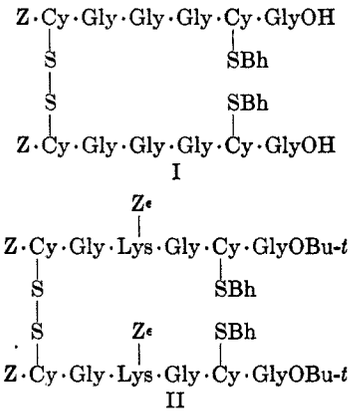
CHART I

PROCEDURE FOR THE SYNTHESIS OF I



Bh = $(C_6H_5)_2CH$
Phth = $C_6H_4(CO)_2$
Z = $C_6H_5CH_2OCO$–
Tr = $(C_6H_5)_3C$–
DCC = *N,N'*-dicyclohexylcarbodiimide
DMF = *N,N*-dimethylformamide

the problem which remains to be evaluated involves the development of methods for the introduction of suitably protected cysteine residues into a peptide chain. The present data concerns the initial portion of our studies on this phase of the over-all problem and describes methods for the preparation of two model compounds, bis(*N*-carbobenzoxy-L-cysteinylglycylglycylglycyl-*S*-benzhydryl-L-cysteinylglycine) (I) and bis(*t*-butyl *N*-carbobenzoxy-L-cysteinylglycyl-N^{ϵ}-carbobenzoxy-L-lysylglycyl-*S*-benzhydryl-L-cysteinylglycinate) (II).



Although a number of elegant syntheses of complex polypeptides containing two or more cysteine residues have been reported,[9] these approaches have usually involved the simultaneous removal of all *S*-protective groups and random oxidation of the resulting thiols. When the stepwise formation of several disulfide bonds is desired the protective group requirements become much more severe. Thus our approaches have been guided by the following points: (a) amino and carboxy protecting groups should be compatible with the *S*-trityl and/or benzhydryl thioethers (or in some circumstances the *S*-benzoyl or carbobenzoxy groups); (b) synthetic procedures must proceed in good yield and be adaptable to large scale; (c) the conditions and reagents used in various steps must not racemize or otherwise destroy other functional groups (*e.g.*, another disulfide bond) that could be present.

Our initial considerations involved the choice of groups to be utilized in the *C*-terminal cysteine residue. The optimum derivative would be one with groups of different acid lability on the amino, carboxy, and thiol groups. A number of possible group combinations were examined;[10] in each case the starting material could be obtained but could not be converted to the anticipated product. In view of these rather disappointing results the problem was circumvented by the use of a *t*-butyl glycinate residue in the *C*-terminal portion of the molecule. The *C*-terminal portion, *t*-butyl *S*-benzhydryl-L-cysteinylglycinate (V) was prepared as outlined in Chart I from *N*-phthaloyl-*S*-benzhydryl-L-cysteine (III) and *t*-butyl glycinate. The cysteine derivative, III, was obtained in 77% overall yield by the direct *S*-alkylation of cysteine[11] fol-

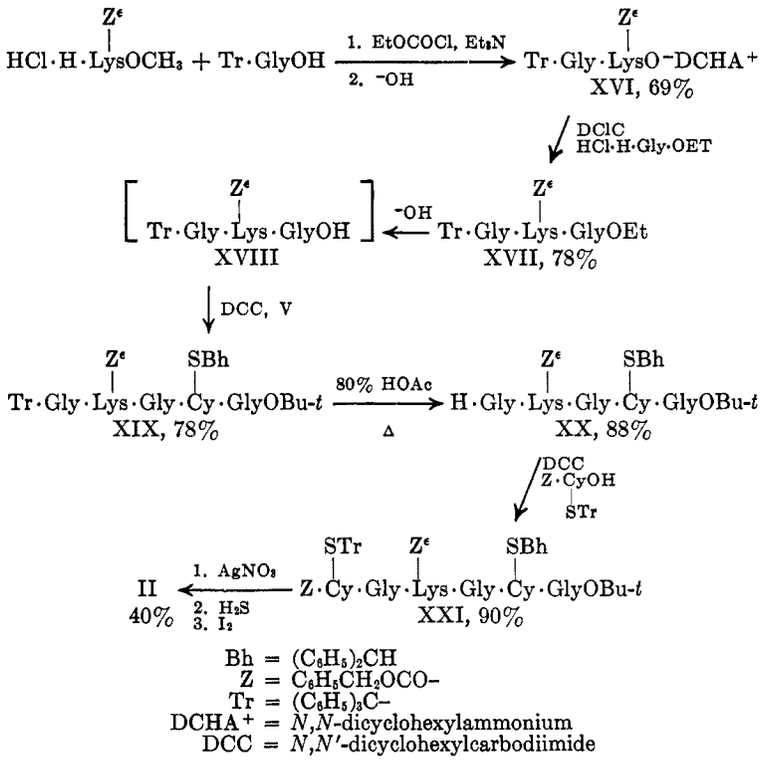
(9) (a) P. G. Katsoyannis, A. Tometsko, and K. Fukuda, *J. Am. Chem. Soc.*, **85**, 2863 (1963); J. Meienhofer, E. Schnabel, H. Bremer, O. Brinkhoff, R. Zabel, W. Sroka, H. Klostermeyer, D. Brandenburg, T. Okuda, and H. Zahn, *Z. Naturforsch.*, **18B**, 1120 (1963). J. Meienhofer [*Chimia* (Aarau), **16**, 385 (1962)] has reviewed the synthesis of oxytocin and vasopressin analogs.

(10) J. B. Adams, Jr., Ph.D. Dissertation, University of North Carolina, Chapel Hill, June 1965.

(11) R. G. Hiskey and J. B. Adams, Jr., *J. Org. Chem.*, **30**, 1390 (1965).

CHART II

SYNTHETIC PROCEDURE FOR THE SYNTHESIS OF II



lowed by direct fusion with phthalic anhydride or *via* *N*-ethoxycarbonylphthalimide.[12] These procedures could not be successfully applied to *S*-trityl-L-cysteine. The most convenient method for the preparation of *t*-butyl glycinate also involved the *N*-phthaloyl group.[13] Although the coupling reaction to generate IV proceeded in good yield on a small scale with either *N,N'*-dicyclohexylcarbodiimide or isobutyl chloroformate, the former reagent gave better yields on a large scale. The *p*-nitrophenyl and cyanomethyl esters of III were also prepared, although the latter compound was completely racemized. The removal of the *N*-phthaloyl group from IV provided V in quantitative yield.

The remaining portion of I was prepared from *N*-carbobenzoxyglycylglycine (VI) *via* VIII and IX. An alternate path which involved *N*-phthaloylglycylglycine (X) provided the tripeptide XI when coupled with *t*-butyl glycinate. The use of isobutyl chloroformate provided a somewhat higher yield of XI (43%) than did ethyl chloroformate (22%); DCC gave only the *N*-

Phth·Gly·GlyOH → Phth·Gly·Gly·GlyOBu-*t*
X XI

acylurea derivative of X. In view of these results the sequence indicated in Chart I was employed.

With the availability of reasonable quantities of IX, the selective removal of the *t*-butyl ester was considered. The results of a number of experiments directed to this end are given in Table I. The only acceptable conditions found involve the use of a 40–70% molar excess of boron trifluoride in acetic acid. Although these conditions will undoubtedly be useful for related peptides, results in this laboratory suggest that the optimum conditions for hydrolysis of *t*-butyl esters vary widely and depend on the particular substrate. The synthesis of the blocked hexapeptide, XIII, was completed by coupling of XII with the *C*-terminal dipeptide, V.

The compatibility of the *S*-trityl, *S*-benzhydryl, and *t*-butyl ester groups is indicated by the deblocking

TABLE I

CLEAVAGE STUDIES ON *t*-BUTYL *N*-CARBOBENZOXY-*S*-TRITYL-L-CYSTEINYLGLYCYLGLYCYLGLYCINATE (IX)

Cleavage conditions	Time, min	Result, mtlc, system A
a	10	No cleavage
a	30	Partial cleavage[b]
a	60	Partial cleavage[b]
c, room temp	16 hr	No cleavage
c, reflux	15	Mixture[d]
CH_3OH–6 *N* HCl (3:1 or 2:1), 40–45°	15	No cleavage
Satd HCl in HOAc, room temp or warm	5–30	Mixture
p-TosOH in C_6H_6, reflux	30	Mixture
CF_3CO_2H, 28°	15	Mixture
HOAc (50 ml), $BF_3 \cdot OEt_2$ (3 ml, 0.024 mole),[e] 25°	60	Cleavage[f,g]
HOAc (50 ml), $BF_3 \cdot OEt_2$ (0.6 ml, 0.0047 mole),[e] 25°	60	Partial cleavage[h]
HOAc (5 ml), $BF_3 \cdot OET_2$ (0.2 ml, 0.0016 mole),[i] steam bath	10	Mixture[i]

[a] Acetone–5 *N* hydrochloric acid (4:1), room temperature. [b] Faint spot due to XII, mainly ester. [c] Methanol–5 *N* hydrochloric acid (4:1). [d] Products other than XII formed. [e] 0.014 mole of ester. [f] When *N*-carbobenzoxyglycine was stored in HOAc–$BF_3 \cdot OEt_2$ (1:1) for 10 hr a ninhydrin test was negative. [g] XII isolated in 84% yield. [h] XII isolated in 9% yield. [i] Product mainly XII contaminated by another component of lower R_f value. [j] 0.0014 mole of ester.

(12) G. H. L. Nefkens, G. I. Tesser, R. J. F. Nivard, *Rec. Trav. Chim.*, **79**, 688 (1960).

(13) The *N*-phthaloyl derivative is more easily adaptable to large-scale synthesis than *t*-butyl *N*-carbobenzoxyglycinate. The use of the oxalate salt of *t*-butyl glycinate simplified isolation of the substance. When barium chloride was used to remove the oxalic acid, complete cleavage of the *t*-butyl ester occurred; hence, barium hydroxide was employed.

experiments on XIII (the *N*-carbobenzoxy group of XIII cannot be removed without cleavage of the *S*-trityl and *t*-butyl ester groups). Treatment of XIII with silver nitrate[14] provided XIV in 80% yield; hydrolysis of the ester with boron trifluoride afforded the acid XV, which could be oxidized to I. When mercury(II) acetate was substituted for silver nitrate the yield of XIV was much lower. Although this reagent was found to be superior in removal of *S*-trityl groups from various tripeptides,[6] the low solubility of XIII apparently reduces the effectiveness of mercury(II) acetate in this situation.

The synthesis of II (Chart II) proceeded in similar over-all yield. The protected tripeptide acid, XVIII, was obtained as an amorphous solid which could not be crystallized. However this material was cleanly coupled to V to provide the fully protected pentapeptide, XIX. Of particular interest was the selective hydrolysis of the *N*-trityl group in the presence of the *t*-butyl ester XIX; this was readily accomplished in warm 80% acetic acid. Coupling of crude XX with *N*-carbobenzoxy-*S*-trityl-L-cysteine provided XXI, which was deblocked in low yield with silver nitrate–pyridine in DMF; oxidation of the resulting thiol gave II. Both reaction sequences (Chart I, II) are easily adaptable to large scale and provide useful models for studies involving the reactivity of disulfide bonds, cross-linking two peptide chains and looping a single chain. The results of these investigations will be reported separately.

Experimental Section[15]

A. Bis(*N*-carbobenzoxy-L-cysteinylglycylglycylglycyl-*S*-benzhydryl-L-cysteinylglycine) (I). *t*-Butyl Glycinate Oxalate Salt.—A solution of 39.5 g (0.151 mole) of *t*-butyl *N*-carbobenzoxyglycinate in methanol was hydrogenated with 4 g of 10% palladium-on-charcoal catalyst at 50 psi. The filtered solution was treated with a methanolic solution of oxalic acid and the salt precipitated with ether. The product, mp 146.5–147°, was obtained in 92% yield: lit.[16] mp 156°. Paper chromatography (system A) indicated one spot, R_f 0.61.

Anal. Calcd for $C_5H_{15}NO_6$: C, 43.44; H, 6.84; N, 6.34. Found: C, 43.27; H, 6.89; N, 6.62.

***N*-Phthaloyl-*S*-benzhydryl-L-cysteine (III).**—A mixture of 28.7 g (0.1 mole) of powdered *S*-benzhydryl-L-cysteine,[11] 11 g (0.1 mole) of sodium carbonate, and 150 ml of water was stirred and treated with 150 ml of DMF and 24.4 g (0.12 mole) of powdered *N*-ethoxycarbonylphthalimide.[12] The mixture was stirred for 40 min, diluted with 1500 ml of water, acidified with concentrated hydrochloric acid to pH 1–2, and filtered. The solid was washed with water and dissolved in hot ethyl acetate. The organic layer was washed with hot saturated sodium chloride solution, dried, and concentrated. The mixture was cooled and the precipitated solid separated. The product was washed with petroleum ether (bp 30–60°) to give 35.3 g (85%) of white solid, mp 203–204°. Recrystallization from acetone–petroleum ether raised the melting point to 205–206°: $[\alpha]^{24}D$ −149° (*c* 0.675, EtOAc); mtlc (system A) homogeneous.

Anal. Calcd for $C_{24}H_{23}N_3O_4S$: C, 69.04; H, 4.59; N, 3.36; S, 7.68. Found: C, 69.22; H, 4.75; N, 3.33; S, 7.90.

***t*-Butyl *N*-Phthaloyl-*S*-benzhydryl-L-cysteinylglycinate (IV).**—A mixture of 47.5 g (0.114 mole) of III and 14.9 g (0.114 mole) of *t*-butyl glycinate in DMF was treated with 23.5 g (0.114 mole) of DCC at −10°. The reaction mixture was stirred for 30 min, diluted with DMF, and stirred for 1 hr. The mixture was filtered; the product was precipitated with water and dissolved in hot ethyl acetate. The organic layer was washed with hot saturated sodium chloride solution, dried, and concentrated to 600 ml. Cooling and slow addition of petroleum ether provided 30.2 g (57%) of white solid: mp 188–190°; $[\alpha]^{24}D$ −102° (*c* 0.5, acetone).

Anal. Calcd for $C_{30}H_{30}N_2O_5S$: C, 67.90; H, 5.70; N, 5.28; S, 6.04. Found: C, 67.67; H, 5.83; N, 5.35; S, 6.25.

***t*-Butyl *S*-Benzhydryl-L-cysteinylglycinate (V).**—To a suspension of 26.53 g (0.05 mole) of IV in 400 ml of refluxing methanol was added 6 ml (0.12 mole) of 85% hydrazine hydrate. The mixture was refluxed for 1 hr after solution was complete. The solution was evaporated *in vacuo* and treated with 500 ml of 6% potassium carbonate solution and 500 ml of ethyl acetate. The clear layers were separated; the organic layer was dried and evaporated to a white solid, 20 g (100%). Precipitation from a methylene chloride–hexane solvent gave a white powder: $[\alpha]^{19}D$ −17.8° (*c* 1.34, DMF); mtlc (system A) homogeneous; paper chromatography (system A) homogeneous, R_f 0.95.

Anal. Calcd for $C_{22}H_{28}N_2O_3S$: C, 65.97; H, 7.05; N, 6.99; S, 8.01. Found: C, 65.91; H, 7.20; N, 7.03; S, 8.07.

Ethyl *N*-Carbobenzoxyglycylglycinate was prepared by coupling *N*-carbobenzoxyglycine with ethyl glycinate hydrochloride in the presence of triethylamine and DCC in chloroform solution. Recrystallization from ethyl acetate–petroleum ether provided the ester in 83% yield: mp 76–78°; lit.[17] mp 77.5°.

N-Carbobenzoxyglycylglycine (VI) was prepared by saponification of the ethyl ester.[18] The acid was obtained in 58% yield: mp 177–178°; lit.[18] mp 178°.

***t*-Butyl *N*-Carbobenzoxyglycylglycylglycylglycine (VII).**—To a stirred solution of 60.9 g (0.229 mole) of VI and 31.8 ml (0.229 mole) of triethylamine in 500 ml of chloroform at −40°, was added 30.2 ml (0.229 mole) of isobutyl chloroformate at a rate which maintained the temperature below −20°. The mixture was stirred for 20 min at −10 to −15°, cooled to −40°, and treated with 50.6 g (0.229 mole) of *t*-butyl glycinate oxalate salt and 63.6 ml (0.458 mole) of triethylamine. The solution was stirred for 7 hr (−10 to +12°), evaporated and the residue treated with water and ethyl acetate. The organic layer was washed with water, saturated sodium chloride solution, and dried. Addition of petroleum ether caused crystallization. The solid was recrystallized from ethyl acetate–petroleum ether to give 55.2 g (64%) of VII, mp 127–128°.

Anal. Calcd for $C_{18}H_{25}N_3O_8$: C, 56.98; H, 6.64; N, 11.08. Found: C, 57.16; H, 6.50; N, 11.38.

***t*-Butyl Glycylglycylglycinate (VIII).**—A solution of 55.2 g (0.146 mole) of VII in ethanol was hydrogenated for 21 hr with 4 g of 10% palladium-on-charcoal catalyst. The filtered solution was evaporated *in vacuo* to afford an oil which was coupled directly to *N*-carbobenzoxy-*S*-trityl-L-cysteine.

***t*-Butyl *N*-Carbobenzoxy-*S*-trityl-L-cysteinylglycylglycylglycinate (IX).**—To a cooled solution of 72.7 g (0.146 mole) of *N*-carbobenzoxy-*S*-trityl-L-cysteine[6] and 0.146 mole of VIII (obtained above) was added 30.1 g (0.146 mole) of DCC; the mixture was stirred for 30 min, diluted with ethyl acetate, stirred, heated to boiling, and filtered. The solid was resuspended in hot ethyl acetate and filtered, and the combined filtrates were washed with 1 *N* hydrochloric acid, dilute sodium chloride solution, saturated sodium bicarbonate solution, and saturated sodium chloride solution. The organic layer was dried and evaporated to a white solid which was recrystallized from acetone–petroleum ether to yield 88.4 g (84%) of IX: mp 176–178°; $[\alpha]^{26}D$ +23.2° (*c* 0.626, acetone); mtlc (system A) one spot, at origin (system B), homogeneous. Amino acid analysis of a deblocked, performic acid oxidized hydrolysate of IX[19] indicated a ratio of cysteic acid to glycine of 1:2.9.

Anal. Calcd for $C_{40}H_{44}N_4O_7S$: C, 66.28; H, 6.12; N, 7.73; S, 4.42. Found: C, 66.18; H, 6.26; N, 7.69; S, 4.14.

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

(15) Elemental analyses were performed by Triangle Chemical Laboratories, Carrboro, N. C., and Micro-Tech Laboratory, Skokie, Ill. Optical rotations were taken with a Ruldolph polarimeter Model 80 equipped with a Model 200 photoelectric attachment. Amino acids were of the L configuration and were obtained from the Mann Research Laboratories, N. Y. Chromatography procedures employed *n*-butyl alcohol–acetic acid–water (4:1:5), system A and benzene–chloroform–ethanol (12:12:1), system B. Thin layer chromatography was performed on microscope slides (mtlc) with silica gel G or silica gel GF_{254}. Paper chromatography was ascending and conducted on Whatman no. 1 paper.

(16) A. Vollmar and M. S. Dunn, *J. Org. Chem.*, **25**, 387 (1960).

(17) D. W. Clayton, J. A. Farrington, G. W. Kenner, and J. M. Turner, *J. Chem. Soc.*, 1398 (1957).

(18) S. Goldschmidt and H. Lautenschlager, *Ann.*, **580**, 68 (1953).

(19) We are indebted to Mr. G. W. Davis and Mrs. M. Pendergraft for this determination.

t-Butyl *N*-phthaloylglycylglycylglycinate (XI) was prepared from *N*-phthaloylglycylglycine[20] and *t*-butyl glycinate *via* the mixed anhydride method in chloroform. When ethyl chloroformate was used the yield of pure XI was 22%; with isobutyl chloroformate, *t*-butyl glycinate oxalate salt, and 2 equiv of triethylamine, the yield was 43%. The tripeptide derivative was recrystallized from ethyl acetate: mp 186–186.5°.

Anal. Calcd for $C_{18}H_{21}N_3O_6$: C, 57.59; H, 5.64; N, 11.19. Found: C, 57.45; H, 5.40; N, 11.17.

When DCC was employed as the coupling agent in DMF solution only the *N*-acylurea derivative of X was obtained: mp 268–269°.

Anal. Calcd for $C_{25}H_{32}N_4O_5$: C, 64.0; H, 6.9; N, 11.9. Found: C, 64.2; H, 7.4; N, 12.1.

N-Carbobenzoxy-*S*-trityl-L-cysteinylglycylglycylglycine (XII).—A solution containing 10.00 g (0.0138 mole) of ester XI in 50 ml of acetic acid was treated with 3 ml (0.0236 mole) of boron trifluoride–diethylether complex. The solution was stored under dry nitrogen at room temperature for 1 hr and the solvent was removed by lyophilization to yield an orange solid. The material was dissolved in ethyl acetate and the solution was washed with saturated sodium chloride solution. A concentrated aqueous solution containing 2.32 g (0.0276 mole) of sodium bicarbonate was added to the organic extract. Solid sodium chloride was added to separate the layers and the ethyl acetate was decanted. The aqueous suspension was washed with ethyl acetate several times by decantation and acidified with 1 *N* HCl, and the product was extracted into ethyl acetate. The organic layer was washed with water, saturated sodium chloride solution, dried, and evaporated to a solid foam. The solid was dissolved in benzene and lyophilized to yield 7.67 g (84%) of white powder. The crude acid could not be crystallized and was used directly in the coupling reaction.

Anal. Calcd for $C_{36}H_{36}N_4O_7S$: C, 64.65; H, 5.43; N, 8.38; S, 4.79. Found: C, 63.88; H, 5.76; N, 8.02; S, 4.84.

t-Butyl *N*-Carbobenzoxy-*S*-trityl-L-cysteinylglycylglycylglycyl-*S*-benzhydryl-L-cysteinylglycinate (XIII).—A mixture of 20.06 g (0.03 mole) of XII and 12.06 g (0.03 mole) of V in 39 ml of methylene chloride was warmed to accomplish solution, cooled to −10°, and treated with 6.19 g (0.03 mole) of DCC in 6 ml of methylene chloride. The mixture was stirred at −10° until solidification occurred and kept at −10° for 1.5 hr and at room temperature for 1 hr. The mixture was diluted with DMF, filtered, and the methylene chloride removed *in vacuo* from the filtrate. The residue was diluted with 1800 ml of water, filtered, and the solid reprecipitated from DMF by the addition of water. The solid was boiled with 800 ml of acetone for several minutes and cooled for 2 hr. The mixture was filtered to give 21.0 g (67%) of the hexapeptide XIII: mp 213.5–214° dec, $[\alpha]^{19}D$ −11.2 (*c* 0.569, DMF). A second crop of less pure hexapeptide was obtained from the acetone filtrate by addition of water.

Anal. Calcd for $C_{58}H_{62}N_6O_9S_2$: C, 66.26; H, 5.94; N, 8.00; S, 6.10. Found: C, 65.91; H, 6.01; N, 8.00; S, 6.29.

t-Butyl *N*-Carbobenzoxy-L-cysteinylglycylglycylglycyl-*S*-benzhydryl-L-cysteinylglycinate (XIV).—To a solution of 10.51 g (0.01 mole) of XIII in 100 ml of DMF was added a solution of 1.70 g (0.01 mole) of silver nitrate and 0.805 ml (0.01 mole) of pyridine in 50 ml of ethanol. The solution was stored in the dark for 1 hr and diluted with 250 ml of methanol; the precipitated silver mercaptide was removed by filtration. This was washed with methanol, suspended in 100 ml of DMF, and treated with hydrogen sulfide for 15 min. The mixture was filtered and the product was precipitated by addition of ether to the filtrate. The precipitate was washed with ether and dried to give 6.45 g (80%) of XIV: mp *ca.* 202° dec; $[\alpha]^{19}D$ −22.6° (*c* 1.66, DMF); the material gave a positive sodium nitroprusside test; mtlc (system A) homogeneous.

Anal. Calcd for $C_{39}H_{48}N_6O_9S_2$: C, 57.90; H, 5.98; N, 10.39; S, 7.93. Found: C, 57.91; H, 6.30; N, 10.12; S, 8.04.

The use of mercury(II) acetate in refluxing ethanol–ethyl acetate gave incomplete reaction, probably due to the limited solubility of the hexapeptide in this medium.

N-Carbobenzoxy-L-cysteinylglycylglycylglycyl-*S*-benzhydryl-L-cysteinylglycine (XV).—To a solution of 8.09 g (0.01 mole) of XIV in 100 ml of acetic acid was added 5 ml (0.0675 mole) of ethyl mercaptan and 15 ml (0.118 mole) of boron trifluoride–diethylether complex. The flask was swept with dry nitrogen and kept at 25° for 15 min. The acid was precipitated by addition of excess ether; reprecipitation by the same procedure from DMF gave 7.12 g (95%) of the hexapeptide XV: $[\alpha]D^{19}$ −43.8° (*c* 0.774, DMF); nitroprusside positive; mtlc homogeneous (system A).

Anal. Calcd for $C_{35}H_{40}N_6O_9S_2$: C, 55.83; H, 5.35; N, 11.16; S, 8.51. Found: C, 55.48; H, 5.44; N, 10.86; S, 8.63.

The same substance could be obtained in lower yield by treatment of the ester with a saturated solution of hydrogen chloride in acetic acid.

Bis(*N*-carbobenzoxy-L-cysteinylglycylglycylglycyl-*S*-benzhydryl-L-cysteinylglycine (I).—To a solution of 0.753 g (0.001 mole) of XV in 1 ml of DMF was added a solution containing 0.0011 mole of iodine in DMF. After 3 min I was precipitated by the addition of excess ether in a quantitative yield: mp 197–198° dec; $[\alpha]^{19}D$ −80° (*c* 0.135, DMF); mtlc (system A) revealed a single spot at the origin. The product was nitroprusside negative.

Anal. Calcd for $C_{70}H_{78}N_{12}O_{18}S_4$: C, 55.91; H, 5.23; N, 11.18; S, 8.53. Found: C, 55.56; H, 5.48; N, 10.61; S, 8.54.

B. Bis(*t*-Butyl *N*-carbobenzoxy-L-cystinylglycyl-N^ϵ-carbobenzoxy-L-lysylglycyl-*S*-benzhydryl-L-cysteinylglycinate). *N*-Trityglycyl-N^ϵ-carbobenzoxy-L-lysine *N*,*N*-Dicyclohexylammonium Salt (XVI).—*N*-Tritylglycyl-N^ϵ-carbobenzoxy-L-lysine was prepared from 47.6 g (0.15 mole) of *N*-tritylglycine, 49.6 g, (0.15 mole) of methyl N^ϵ-carbobenzoxy-L-lysinate hydrochloride,[21] and 14.4 ml (0.15 mole) of ethyl chloroformate.[22] The crude *N*-trityl acid was treated with 29.4 ml (0.15 mole) of *N*,*N*-dicyclohexylamine. Recrystallization of the salt from ethyl acetate provided 78.9 g (69%) of white solid (XVI): mp 152–154°, $[\alpha]^{28}D$ +12.4° (*c* 1.98, $CHCl_3$).

Anal. Calcd for $C_{47}H_{60}N_4O_5$: C, 74.18; H, 7.95; N, 7.36. Found: C, 74.29; H, 7.86; N, 7.50.

The salt was converted to the free acid by stirring an aqueous ethanol solution of the salt with 1 equiv of Dowex 50WX-8 (H^+): $[\alpha]^{24}D$ +12.6° (*c* 2.0, EtOH), lit.[22] $[\alpha]^{21}D$ +4.1° (*c* 2.0, MeOH).

Ethyl *N*-Trityglycyl-N^ϵ-carbobenzoxy-L-lysylglycinate (XVII).—A mixture of 57.99 g (0.0762 mole) of XVI and 10.64 g (0.0762 mole) of ethyl glycinate hydrochloride in chloroform was cooled in ice and treated with 17.29 g (a 10% excess) of DCC. The mixture was stirred for 6 hr, evaporated *in vacuo*, and the residue was treated with ethyl acetate. The suspension was filtered and the filtrate was washed with 0.5 *N* hydrochloric acid, sodium chloride solution, saturated sodium bicarbonate solution, and sodium chloride solution. The extract was dried and evaporated to an oil, which was dissolved in acetone, filtered, and diluted with petroleum ether and ether. The tripeptide derivative was obtained as 39.3 g (78%) of white solid, XVII: mp 138–139°; $[\alpha]^{24}D$ −3.5° (*c* 1.18, $CHCl_3$); mtlc (system A) homogeneous.

Anal. Calcd for $C_{39}H_{44}N_4O_6$: C, 70.46; H, 6.67; N, 8.43. Found: C, 70.50; H, 7.15; N, 8.54.

t-Butyl *N*-Trityglycyl-N^ϵ-carbobenzoxy-L-lysylglycyl-*S*-benzhydryl-L-cysteinylglycinate (XIX). A. Alkaline Hydrolysis of XVII.—A mixture of 32.24 g (0.05 mole) of XVII, 75 ml of dioxane, and 75 ml of 0.914 *N* sodium hydroxide was stirred for 1.5 hr, diluted with water, acidified with 5 *N* hydrochloric acid to pH 1–2 and extracted with ethyl acetate. The aqueous layer was reextracted with ethyl acetate and the combined extracts were washed with sodium chloride solution and dried. The solution (ninhydrin negative) was evaporated to a solid foam XVIII, which could not be crystallized. The foam was homogeneous on mtlc (system A).

B. Coupling with *t*-Butyl *S*-benzhydryl-L-cysteinylglycinate (V).—A mixture of 31.8 g (0.05 mole) of XVIII obtained in A and 20 g (0.05 mole) of *t*-butyl *S*-benzhydryl-L-cysteinylglycinate (V) in 57 ml of methylene chloride was cooled to −10° and treated with 10.3 g (0.05 mole) of DCC. The mixture was stirred until it solidified (*ca.* 12 min); after 1 hr, 11 ml of methylene chloride was added, and the mixture filtered. The filtrate was washed with water, 0.25 *N* hydrochloric acid, water, saturated sodium bicarbonate solution, and water. The organic solution was

(20) J. C. Sheehan and V. S. Frank, *J. Am. Chem. Soc.*, **71**, 1856 (1949).

(21) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 2, John Wiley and Sons, Inc., New York, N. Y., 1961, p 1057.

(22) R. A. Boissonnas, S. Guttman, R. L. Huguenin, P. A. Jaquenoud, and E. Sandrin, *Helv. Chim. Acta*, **41**, 1867 (1958).

evaporated; the residue was dissolved in hot acetone and precipitated with *n*-hexane. The pentapeptide XIX was obtained as a white solid: 39.6 g (78%); mp 130–134°; $[\alpha]^{19}D$ +12.8° (*c* 2.13, DMF); mtlc (system A) homogeneous.

Anal. Calcd for $C_{59}H_{66}N_6O_8S$: S, 69.52; H, 6.53; N, 8.25; S, 3.15. Found: C, 69.54; H, 6.39; N, 8.41; S, 3.28.

***t*-Butyl *N*-Carbobenzoxy-*S*-trityl-L-cysteinylglycyl-N^{ϵ}-carbobenzoxy-L-lysylglycyl-*S*-benzhydryl-L-cysteinylglycinate (XXI).**

A. Generation of XX.—A solution of 10.88 g (0.0107 mole) of *N*-trityl ester XIX in 120 ml of acetic acid and 30 ml of water was heated for 2–3 min on a steam bath, diluted with 750 ml of water, evaporated *in vacuo*, and the residue was partitioned between ethyl acetate and saturated sodium bicarbonate solution. The organic layer was washed with hot saturated sodium chloride solution, dried, and the free base XX was precipitated as a gelatinous solid, 6.86 g (88%): mtlc (system A) homogeneous (iodine, ninhydrin, ultraviolet).

Treatment of XIX with a methanol–5 *N* hydrochloric acid (4:1) solution for 10–20 min at room temperature or at reflux for 1 min gave a product which was homogeneous (mtlc, ninhydrin, system A).

B. Coupling of XX with *N*-Carbobenzoxy-*S*-trityl-L-cysteine.—A solution of 7.05 g (0.0142 mole) of *N*-carbobenzoxy-*S*-trityl-L-cysteine[5] and 10.96 g (0.0142 mole) of XX in 20 ml of DMF was cooled to −10° and treated with 2.92 g (0.0142 mole) of DCC in 3 ml of DMF. The mixture was stirred for 9.5 hr, diluted with DMF, and filtered. The filtrate (*ca.* 125 ml) was diluted with 575 ml of water, cooled, and filtered to give 16 g (90%) of hexapeptide XXI: mtlc homogeneous (iodine, ultraviolet, system A). The peptide was dissolved in hot acetone containing a little DMF, the solution brought to the cloud point with *n*-hexane, and diluted to twice its volume with ether. The mixture was cooled, filtered, and the solid was washed with ether to provide 14.2 g (89% recovery) of XXI: mp 189–191°, $[\alpha]^{19}D$ −7.4° (*c* 0.19, DMF).

Anal. Calcd for $C_{70}H_{77}N_7O_{11}S_2$: C, 66.91; H, 6.18; N, 7.80; S, 5.10. Found: C, 67.08; H, 6.24; N, 7.78; S, 5.29.

Bis(*t*-Butyl *N*-Carbobenzoxy-L-cysteinylglycyl-N^{ϵ}-Carbobenzoxy-L-lysylglycyl-*S*-benzhydryl-L-cysteinylglycinate) (II).—To a solution of 1.26 g (0.001 mole) of XXI in 10 ml of DMF was added a solution of 0.17 g (0.001 mole) of silver nitrate and 0.081 ml (0.001 mole) of pyridine in 10 ml of ethanol. The reaction mixture was stored in the dark for 1 hr, treated with 50 ml of ether, and filtered. The gel was washed with methanol and ether, dried, and suspended in DMF. The suspension was treated with hydrogen sulfide for 15 min, warmed, and filtered. The thiol precipitated on addition of ether and petroleum ether; the solid was washed with ether to provide 0.4 g (40%) of thiol. The nitroprusside test was positive; mtlc (system A) homogeneous.

Air oxidation of the thiol was conducted in 10 ml of DMF containing 2 drops of triethylamine but was incomplete (positive nitroprusside test) after 2 days. Treatment of the solution with an ether solution of iodine provided the disulfide; after 0.5 min, II was precipitated by addition of ether and purified by two reprecipitations from DMF. The melting point was 192–202° dec; nitroprusside negative; mtlc homogeneous (system A); $[\alpha]^{19}D$ −60.2° (*c* 0.997, DMF).

Anal. Calcd for $C_{102}H_{124}N_{14}O_{22}S_4$: C, 60.46; H, 6.16; N, 9.68; S, 6.33. Found: C, 59.88; H, 6.35; N, 9.78; S, 6.62.

The Synthesis and Stereochemistry of 1,2,3,4,4a,11b-Hexahydro-9,10,11-trimethoxydibenzo[*b*,*d*]thiepin-7(6H)-one[1]

F. J. LOTSPEICH AND S. KARICKHOFF

Department of Biochemistry, West Virginia University Medical Center, Morgantown, West Virginia

Received October 14, 1965

The addition of mercaptoacetic acid to 1-(2′,3′,4′-trimethoxyphenyl)cyclohexene under free-radical conditions yielded the *cis* acid IV. The resulting acid was converted to the acid chloride and cyclized with aluminum chloride to *cis*-1,2,3,4,4a,11b-hexahydro-9,10,11-trimethoxydibenzo[*b*,*d*]thiepin-7(6H)-one (VI). The corresponding *trans* compound XII was prepared by displacement of the *trans*-tosylate of 2-(2′,3′,4′-trimethoxyphenyl)cyclohexanol by the potassium salt of mercaptoacetic acid and subsequent cyclization of the acid chloride with aluminum chloride. Nucleophilic addition of mercaptoacetic acid to 1-(2′,3′,4′-trimethoxyphenyl)cyclohexene could not be accomplished.

Although the antimitotic activity of the rather complex molecule colchicine has been known for many years its application in the treatment of cancer has been limited by its general toxicity. Some success has been achieved in synthesizing derivatives of colchicine having less toxicity but retaining a high antimitotic activity. The need for more effective antitumor agents with lower toxicity has prompted the investigation of the titled compounds.

Our approach to the preparation of the titled compounds is illustrated in Scheme I.

1-(2′,3′,4′-Trimethoxyphenyl)cyclohexanol (II) was prepared according to the procedure of Ginsburg and Pappo[2] which involved the condensation of 2,3,4-trimethoxyphenyllithium with cyclohexanone. The yield of II was considerably improved by using 1-bromo-2,3,4-trimethoxybenzene in place of 1,2,3-trimethoxybenzene. The method of Gutsche and Fleming[3] employing the Grignard in diethyl ether was unsuccessful in our hands. However, in later experiments a 50% yield of III was realized when tetrahydrofuran was used as a solvent and the product was distilled.

The tertiary alcohol II was dehydrated to 1-(2′,3′,4′-trimethoxyphenyl)cyclohexene with oxalic acid in boiling toluene.[2] Addition of mercaptoacetic acid in the presence of benzoyl peroxide to 1-(2′,3′,4′-trimethoxyphenyl)cyclohexene was extremely slow at 25°. This was in contrast to the findings with styrene[4] and 1-methylcyclohexene[5] where the free-radical addition of mercaptoacetic acid was rapid. Initial attempts to crystallize the oil resulting from the above reaction using various solvents were unsuccessful and identification of the acid was initially achieved by conversion to the crystalline sulfone ester. The oil did slowly crystallize on standing at room temperature and the resulting crystals could then be further purified by crystallization.

The conversion of acid IV to the cyclic keto sulfide VI was attempted using polyphosphoric acid and hydrogen fluoride since these reagents had been used successfully

(1) Presented in part at the 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1965. The interpretation of the results in this paper which was presented at the American Chemical Society meeting has been revised to explain additional experimental evidence.

(2) D. Ginsburg and R. Pappo, *J. Am. Chem. Soc.*, **75**, 1094 (1953).

(3) C. D. Gutsche and F. A. Fleming, *ibid.*, **76**, 1771 (1954).

(4) B. Holmberg, *J. Prakt. Chem.*, **141**, 93 (1934).

(5) J. I. Cunneen, *J. Chem. Soc.*, **36** (1947).