Potential Anticancer Agents.[1] LXXIX. Aromatic Nitrogen Mustards Derived from Cysteine[2]

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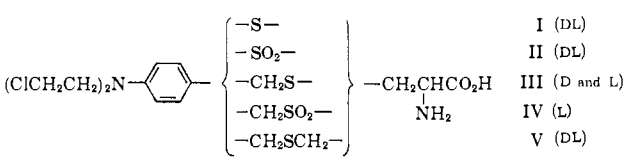
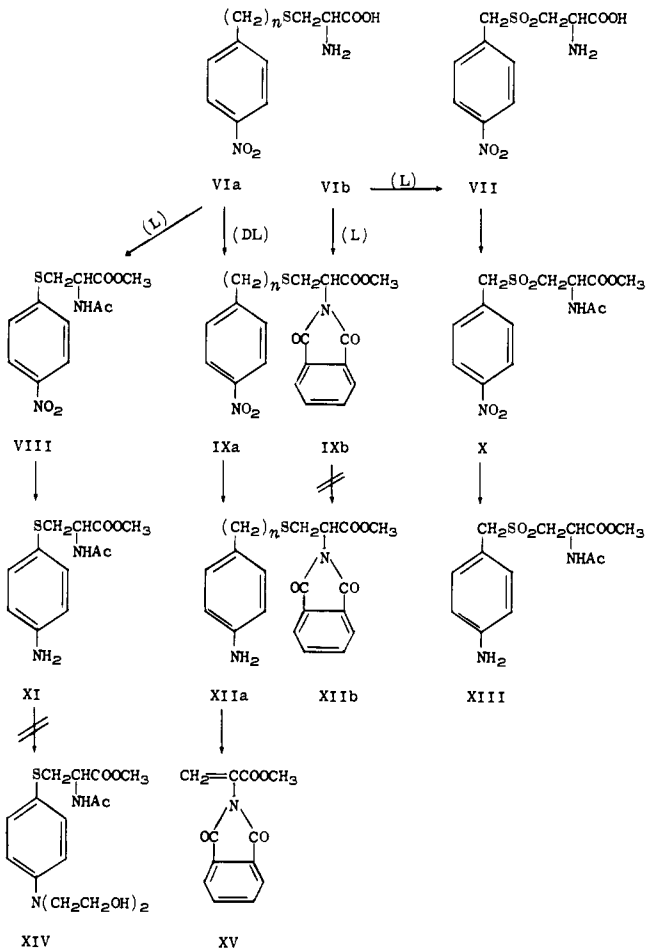
Alkylation of 3-chloro-DL-alanine (XVII) with the thiophenol *p*-mustard XVI afforded the *p*-mustard I of S-phenyl-DL-cysteine, which was oxidized to the sulfone mustard II. The related mustards III, IV, and V were formed by S-alkylation of D- and L-cysteine, L-cysteinesulfinic acid, and DL-homocysteine, respectively, in aqueous acid with benzyl alcohol *p*-mustard (XIX), the preparation of which is described.

The phenylalanine mustards, especially the *meta*- and *para*-isomers,[4] have been studied extensively in animals and in man as anticancer drugs. Their interesting biological properties have created intense interest in the preparation of related compounds in a search for enhanced activity. The nitrogen mustards of S-phenyl- and S-benzylcysteine represent such analogs, with a structural change that may be biologically important; this paper describes the preparation of these and related compounds (I–V).



The standard preparative sequence for aromatic nitrogen mustards generally follows the scheme used in the preparation of *p*-[5a] and *m*-[5b] phenylalanine mustards, namely, appropriate blocking of the functional groups of a nitroarylamino acid, followed by reduction of the nitro group, bishydroxyethylation of the arylamine, conversion to the bis-(chloroethyl)-amine, and removal of the blocking groups. Attempts (shown in Scheme I) to use this route in the preparation of I, III, and IV led to intermediates that were not stable to the final steps of the sequence because of lability of a carbon-sulfur bond.[6] Thus, in the initial attempts to prepare I, transformations of S-phenyl derivatives of cysteine were successful only as far as the protected amines XI and XIIa. A reverse Michael reaction product (XV) was isolated from attempted hydroxyethylation of XIIa. With a series of S-benzylcysteines leading to III, the sequence failed in the reduction of IXb to the amine XIIb; the reduction occurred with some loss of the benzylthio moiety.

The amino sulfone XIII was prepared as an intermediate toward IV, but when it was found that compound IV was easily available by another approach (*vide infra*), the characterization and further use of XIII were discontinued.



series a, $n = 0$ series b, $n = 1$

SCHEME I

The failure of the conventional routes to I and III made it clear that novel approaches would be required for their syntheses. Methods were sought that would link directly a preformed aryl mustard with the amino acid moiety to form the new carbon-sulfur bond under conditions to which the mustard group was stable. When two molar equivalents of *p*-[bis(2-chloroethyl)-amino]benzenethiol (XVI)[7] were allowed to react

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. SA-43-ph-1892. The opinions expressed in this paper are those of the authors and are not necessarily those of the Cancer Chemotherapy National Service Center. For the preceding paper in this series, see B. R. Baker, W. W. Lee, A. P Martinez, L. O. Ross, and L. Goodman, *J. Org. Chem.*, **27**, 3283 (1962).

(2) A preliminary communication covering some of this work has appeared, R. H. Iwamoto, E. M. Acton, B. R. Baker, and L. Goodman, *Chem. Ind. (London)*, 1404 (1961).

(3) To whom reprint requests should be sent.

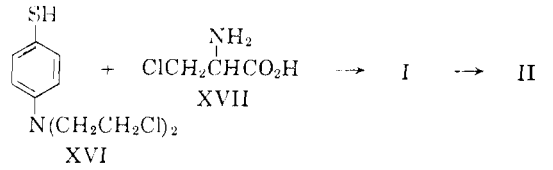
(4) (a) See *Cancer Chemotherapy Reports*, **6**, 61 (1959); (b) M. O. Greene, B. R. Baker, and J. Greenberg, *Cancer Res.*, **20**, 1160 (1960).

(5) (a) F. Bergel and J. A. Stock, *J. Chem. Soc.*, 2409 (1954); (b) H. F. Gram, C. W. Mosher, and B. R. Baker, *J. Am. Chem. Soc.*, **81**, 3103 (1959).

(6) A similar lability was noted in the preparation of some mustards of aromatic thioalkanoic acids; see M. E. Wain, E. M. Acton, B. R. Baker, and L. Goodman, *J. Org. Chem.*, **27**, 2905 (1962).

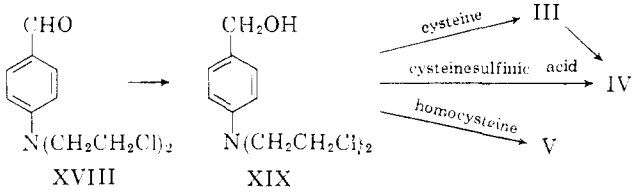
(7) M. H. Benn, L. N. Owen, and A. M. Creighton, *J. Chem. Soc.*, 2800 (1958).

with 3-chloro-DL-alanine (XVII) hydrochloride[8] in refluxing methanol containing three equivalents of potassium hydroxide, 20 to 50% yields of I could be isolated by neutralizing an acid solution of the evaporated reaction mixture.[9] Any deviation from this



stoichiometry caused a drop in yield. Oxidation of I to the sulfone mustard II was accomplished in good yield after 16 hours with a large excess of hydrogen peroxide in glacial acetic acid. The use of less peroxide or a shorter reaction time resulted in a product that was contaminated with a sulfoxide according to the infrared spectrum.

A direct synthesis also was possible in the case of III and was extended to the preparation of IV and V. This depended on reduction of the benzaldehyde mustard XVIII,[10] achieved with a large excess of sodium borohydride in methanol, to form the crystalline benzyl alcohol mustard XIX in excellent yield. It was important to use a large excess of borohydride so that the



reaction was complete within a minimum period. The reaction of XIX with L- or D-cysteine in warm 6 *M* hydrochloric acid under nitrogen for 24 hours afforded the L- and D-forms of III with equal but opposite rotation, indicating that no appreciable racemization had occurred during the condensation. This preparation of III was based on a similar reaction used to prepare the S-thyminylcysteine.[11] That a much higher yield of L-III than D-III was isolated can be attributed to the extensive contamination of the commercial D-cysteine with D-cystine. Under the same reaction conditions, DL-homocysteine and XIX afforded an excellent yield of V, and L-cysteinesulfinic acid and XIX gave the benzyl sulfone mustard IV in high yield. Compound IV could also be prepared from III by oxidation with hydrogen peroxide in glacial acetic acid but the yield was much lower and the product required more purification.

Biological Results.—Of the transplanted tumors in which the mustards I–V have been tested, Walker Carcinoma 256 was the most susceptible to inhibition. Compound IV caused complete inhibition of this implanted tumor in rats[12] at a single dose of 50 mg./kg. and was non-toxic at 500 mg./kg. Compounds L-III and V were both less effective (complete inhibition at 125 mg./kg.) and more toxic (death at 500 mg./kg.). Compound I caused complete inhibition at 250 mg./kg., but the sulfone II had no effect at that dose; both were non-toxic at 500 mg./kg. The L-form of *p*-phenylalanine mustard, in comparison, at 1 mg./kg. caused complete inhibition of the tumor[13] but was toxic at 9 mg./kg. Though active at a higher dose level, compound IV on this basis may be equal or superior to L-*p*-phenylalanine mustard in therapeutic index. Preliminary results with[14] Carcinoma 755, Sarcoma 180, and Leukemia L-1210 in mice showed some life extension in treatment of Leukemia L-1210 with III and V, and some inhibition of Carcinoma 755 occurred with V. Further tests in all these systems are in progress.

As is pointed out by Ross[15] in his recent monograph, the pair of compounds, I and II, may have interesting biological implications. It could be predicted that the alkylating activity of I should be much greater than that of II, which contains the strongly electron-withdrawing sulfone substituent *para* to the mustard group. If normal tissue contained an oxidative enzyme that was lacking in tumor tissue, compound I might be detoxified in normal tissue by oxidation to II, while retaining its effect on the tumor tissue.

Experimental[16]

Paper Chromatography.—All compounds described were, when purified, homogeneous to paper chromatography by the descending technique in system A: 1-butanol–acetic acid–water (5/2/3) on Whatman No. 1 paper; or system B: benzene–methanol–water (2/6/1) on Schleicher and Schuell acetylated paper No. 2496. Spots were detected by visual observation under ultraviolet light (for the aromatic compounds) and then (for the free amino acids) by spraying with ninhydrin reagent. Values expressed by R_{Cy} are compared with cysteine, where $R_{Cy} = R_f$ compound/R_f cysteine.

3-{*p*-[Bis(2-chloroethyl)amino]phenylthio}-DL-alanine (I).—A stirred solution, under nitrogen, of 5.0 g. (0.020 mole) of *p*-[bis(2-chloroethyl)-amino]benzenethiol (XVI)[7] in 160 ml. of absolute methanol (previously purged with nitrogen) was treated with 1.60 g. (0.010 mole) of 3-chloro-DL-alanine (XVII) hydrochloride[8,17] and, immediately thereafter, with 30.0 ml. of 1 *M* methanolic potassium hydroxide. The resulting solution was refluxed for 45 min., during which time a yellow gum separated. After cooling, the supernatant was decanted and concentrated *in vacuo* at 40°. A cold 6 *M* hydrochloric acid (60 ml.) solution of the semisolid residue was diluted with 100 ml. of water, extracted with two 50-ml. portions of chloroform, and adjusted to pH 5–6 at 5° with 5 *M* ammonium hydroxide. The resultant precipitate after 2 hr. was washed with water and then with ether-benzene (2/1). The dried product, m.p. 157–165° dec.,[18] weighed 1.57 g. (50%), with R_{Cy} 2.3 in system A, R_f 0.78 in system B (R_f 0.0 for XVI). An analytical sample (38%), identical in melting point and infrared spectrum, was obtained by reprecipitation from a 6 *M* hydrochloric acid solution with 5 *M* am-

(8) J. L. Wood and L. Van Middlesworth, *J. Biol. Chem.*, **179**, 529 (1949).

(9) A somewhat related reaction, that of *o*-[bis(2-chloroethyl)amino]-benzyl chloride and diethyl acetamidomalonate in the presence of sodium ethoxide, was used in the preparation of *o*-phenylalanine mustard. See T. A. Connors, W. C. J. Ross, and J. G. Wilson, *J. Chem. Soc.*, 2994 (1960).

(10) R. H. Wiley and G. Irick, *J. Org. Chem.*, **26**, 593 (1961).

(11) R. E. Cline, R. M. Fink, and K. Fink, *J. Am. Chem. Soc.*, **81**, 2521 (1959).

(12) These tests were performed at Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, London, S.W. 3, England, and we wish to thank Mr. J. L. Everett for supplying the data.

(13) L. A. Elson and A. Haddow; F. Bergel and J. A. Stock, *Biochem. Pharmacol.*, in press.

(14) These tests were performed at Stanford Research Institute by Dr. Jean Scholler and staff under contract to the Cancer Chemotherapy National Service Center.

(15) W. C. J. Ross, "Biological Alkylating Agents," Butterworths, London, 1962, p. 179.

(16) Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Infrared spectra were determined, as pure liquids or as solids in Nujol mull, for all compounds described.

(17) The precursor, XVII methyl ester hydrochloride, was prepared according to P. A. Plattner, A. Boller, H. Frick, A. Fürst, B. Hegedüs, H. Kirchensteiner, S. Majnoni, R. Schläpfer, and H. Spiegelberg, *Helv. Chim. Acta*, **40**, 1531 (1957).

(18) These rather broad melting points with decomposition could vary by several degrees, depending on rate and duration of heating.

monium hydroxide. The yields sometimes fell to 20% for no apparent reason.

Anal. Calcd. for $C_{13}H_{18}Cl_2N_2O_2S$: C, 46.3; H, 5.38; Cl, 21.0; S, 9.53. Found: C, 46.2; H, 5.19; Cl, 20.9; S, 9.40.

3-{*p*-[Bis(2-chloroethyl)amino]phenylsulfonyl}-DL-alanine (II).—A suspension of 0.486 g. (1.44 mmoles) of I in 20 ml. of glacial acetic acid was treated with 4.0 ml. (39 mmoles) of 30% hydrogen peroxide. The resulting solution was stored for 16 hr. at room temperature and then stirred with a little 5% palladium–charcoal for 10 min. to decompose the excess peroxide. The filtered solution was diluted with 70 ml. of water, adjusted to pH 5 with 5 *M* ammonium hydroxide, and chilled overnight while the product precipitated. The yield of II, filtered and washed with cold water, 50% ethereal ethanol, and ether, was 0.396 g. (74%), m.p. 146–151° dec.,[18] R_{Cy} 1.9 in system A. Characteristic sulfone bands appeared at 7.7 and 8.8 μ in the infrared, but there was no band at 9.7 μ due to sulfoxide contamination. The analytical sample, m.p. 148–159° dec.,[18] was obtained in 39% overall yield from a solution (clarified by filtration) of the initial product in glacial acetic acid (15 ml.) containing a few drops of 6 *M* hydrochloric acid; the filtrate was diluted with 75 ml. of water, adjusted to pH 5 with 5 *M* ammonium hydroxide, chilled for 2 hr., and the precipitate collected.

Anal. Calcd. for $C_{13}H_{18}Cl_2N_2O_4S$: C, 42.3; H, 4.91; Cl, 19.2; S, 8.68. Found: C, 42.6; H, 4.75; Cl, 19.0; S, 8.84.

p-[Bis(2-chloroethyl)amino]benzyl Alcohol (XIX).—A fresh solution of sodium borohydride was prepared by adding 5.8 g. (0.15 mole) in portions to 100 ml. of absolute methanol at 5°, avoiding excessive effervescence. This solution was stirred and treated, during 25–30 min., with a solution of *p*-[bis(2-chloroethyl)amino]benzaldehyde (XVIII)[10] (12.0 g., 0.048 mole) in 200 ml. of absolute methanol, maintaining the temperature at 25–30°. Stirring was continued for 1.5 hr. and the solution was poured slowly with stirring onto 500 ml. of ice and water, whereupon the product crystallized. The mixture was adjusted to *p*H 5 with 1 *M* hydrochloric acid, stirred for 0.5 hr., and filtered. The product, washed with water and dried *in vacuo* at 25°, weighed 10.5 g. (87% yield), m.p. 51–52°, R_f 0.34 in system B (R_f 0.19 for XVIII). Loss of carbonyl absorption at 5.99 μ characteristic of XVIII was observed in the infrared. Recrystallization from an ethyl ether–petroleum ether mixture afforded material of analytical purity (70–75% yield), m.p. 52–53°. The product, when pure and stored at 5°, showed no deterioration within 1 year.

Anal. Calcd. for $C_{11}H_{15}Cl_2NO$: C, 53.2; H, 6.04; Cl, 28.6. Found: C, 53.2; H, 6.04; Cl, 28.7.

3-{*p*-[Bis(2-chloroethyl)amino]benzylthio}-L-alanine (L-III).—A solution of 1.24 g. (5.00 mmoles) of XIX in 20 ml. of 6 *M* hydrochloric acid was treated with 0.88 g. (5.0 mmoles) of L-cysteine hydrochloride monohydrate and warmed at 50–55° under a nitrogen atmosphere for 24 hr. The clear solution was cooled to 5° and neutralized (pH 6–7) with 5 *M* ammonium hydroxide. The white precipitate was collected on a filter, ground to a powder, and washed with water and ether. Traces of L-cystine, present in some runs (R_{Cy} 0.42), could be removed by filtering a solution in absolute methanol at 30–40° and evaporating the filtrate *in vacuo;* the residue was dissolved in 1 *M* hydrochloric acid solution and the product precipitated by addition of 5 *M* ammonium hydroxide to pH 6–7, yielding 1.2 g. (68%) of solid, m.p. 159–160° dec.,[18] R_{Cy} 2.2 in system A (XIX was at solvent front), $[\alpha]^{26}D$ −4.8° ± 0.2 (*c* 2 in glacial acetic acid).

Anal. Calcd. for $C_{14}H_{20}Cl_2N_2O_2S$: C, 47.9; H, 5.74; Cl, 20.2; N, 7.97; S, 9.13. Found: C, 47.9; H, 5.79; Cl, 20.2; N, 8.11; S, 8.93.

3-{*p*-[Bis(2-chloroethyl)amino]benzylthio}-D-alanine (D-III) was obtained from D-cysteine hydrochloride monohydrate[19a] in 45% yield as described for L-III, m.p. 158–162° dec.,[18] $[\alpha]^{26}D$ +5.0° ± 0.2 (*c* 2 in glacial acetic acid). The infrared spectrum and paper chromatographic data were identical with those of L-III.

Anal. Found: C, 48.1; H, 5.88; Cl, 20.2; N, 7.91; S, 8.90.

3-{*p*-[Bis(2-chloroethyl)amino]benzylsulfonyl}-L-alanine (L-IV). A. From XIX.—After 18 hr. as for L-III, L-cysteinesulfinic acid[19b] (0.155 g., 1.00 mmole) and 0.248 g. (1.00 mmole) of XIX in 2.5 ml. of 6 *M* hydrochloric acid afforded 0.343 g. (93%) of the sulfone, m.p. 148–151° dec.,[18] R_{Cy} 1.9 in system A (pink ninhydrin color), $[\alpha]^{26}D$ +11.2° ± 0.2 (*c* 1 in 6 *M* hydrochloric acid). A sample for analysis, m.p. 149–152° dec.,[18] was reprecipitated from 6 *M* hydrochloric acid solution with 5 *M* ammonium hydroxide. Characteristic sulfone bands in the infrared were found at 7.7 and 8.9 μ.

Anal. Calcd. for $C_{14}H_{20}Cl_2N_2O_4S$: C, 43.9; H, 5.26; Cl, 18.5; S, 8.46. Found: C, 44.1; H, 5.27; Cl, 18.5; S, 8.54.

B. From III.—A solution of 1.00 g. (2.85 moles) of L-III in 20 ml. of glacial acetic acid and 3.0 ml. of 30% hydrogen peroxide was treated as described for II for 24 hr. The reaction solution, decanted from the dark oil that separated, on neutralization afforded 0.35 g. (32%) of L-IV, which was chromatographically homogeneous (R_{Cy} 1.9 in system A). A sample for analysis, m.p. 146–149° dec.,[18] was prepared by two reprecipitations; it was nearly identical to L-IV, prepared from XIX, as shown by infrared spectrum.

Anal. Found: C, 44.3; H, 5.36; Cl, 18.8; S, 8.12.

DL-2-Amino-4-{*p*-[bis(2-chloroethyl)amino]benzylthio}butyric Acid (DL-V).—Using the method for L-III, 0.338 g. (2.50 mmoles) of DL- homocysteine and 0.620 g. (2.50 mmoles) of XIX in 5 ml. of 6 *M* hydrochloric acid afforded 0.73 g. (78%) of product, chromatographically homogeneous in system A, R_{Cy} 2.2 (R_{Cy} 0.84 for homocysteine). A sample for analysis was obtained by reprecipitation, first from 6 *M* hydrochloric acid solution with 5 *M* ammonium hydroxide at pH 6, and then from a solution in hot methanol which was clarified by filtration, diluted with water, and cooled. The amorphous solid melted gradually, turning yellow, from 110–150°.

Anal. Calcd. for $C_{15}H_{22}Cl_2N_2O_2S$: C, 49.3; H, 6.07; Cl, 19.4; S, 8.78. Found: C, 49.8; H, 5.87; Cl, 19.3; S, 8.77.

Esterification.—N-Acetyl and N-phthalyl cysteine derivatives were converted to methyl esters by treatment with an equal weight of acetyl chloride in 10 vol. of methanol at reflux for 30 min. The esters IXa and X crystallized on cooling; IXb and VIII crystallized on concentrating the reaction mixture to one-half the volume. All were recrystallized from methanol.

DL-α-[(*p*-Nitrophenylthio)methyl]-1,3-dioxo-2-isoindolineacetic Acid.—S-*p*-Nitrophenyl-DL-cysteine (DL-VIa)[20] was fused with phthalic anhydride[21] at 160–165° for 45 min. The solid that formed on cooling was triturated with benzene and collected on a filter. Recrystallization from boiling benzene containing charcoal afforded 61% of product, m.p. 169–171°, R_f 0.93 in system A.

Anal. Calcd. for $C_{17}H_{12}N_2O_6S$: C, 54.8; H, 3.25; S, 8.61. Found: C, 55.0; H, 3.34; S, 8.38.

The **methyl ester (IXa)** was obtained in 87% yield, m.p. 116–117°, R_f 0.28 in system B, R_f 0.85 (0.24 for the free acid) in water saturated 1-butanol on Whatman No. 1 paper.

Anal. Calcd. for $C_{18}H_{14}N_2O_6S$: C, 55.9; H, 3.65; S, 8.30. Found: C, 55.9; H, 3.69; S, 8.38.

L-α-[(*p*-Nitrobenzylthio)methyl]-1,3-dioxo-2-isoindolineacetic Acid.—S-*p*-Nitrobenzyl-L-cysteine (L-VIb),[22] m.p. 190–191° (lit. 196°,[23] 223°[22]), with phthalic anhydride[21] at 160–170° for 1.5 hr.[24] as with DL-VIa afforded 70% of product, m.p. 82–84°, R_f 0.92 in system A. Elemental analysis even after drying *in vacuo* at 64° showed solvation with benzene.

Anal. Calcd. for $C_{18}H_{14}N_2O_6S \cdot C_6H_6$: C, 62.1; H, 4.33; S, 6.90. Found: C, 62.2; H, 4.08; S, 6.64.

The **methyl ester (L-IXb)** was obtained in 36% yield, m.p. 111–112°, R_f 0.28 in system B.

Anal. Calcd. for $C_{19}H_{16}N_2O_6S$: C, 57.0; H, 4.03; S, 8.01. Found: C, 57.1; H, 3.94; S, 8.02.

DL-α-[(*p*-Aminophenylthio)methyl]-1,3-dioxo-2-isoindolineacetic Acid Methyl Ester (XIIa).—A solution of 2.91 g. (7.59 mmoles) of IXa and ammonium chloride (0.84 g., 12 mmoles) in 375 ml. of methanol-water (80/20) was treated with 7.05 g. (0.108 g.-atom) of zinc dust. The mixture was stirred under reflux for 4 hr. and filtered through a pad of Celite, which then was washed with 50 ml. of boiling methanol. The combined filtrates were concen-

(19) (a) Nutritional Biochemicals Corp., Cleveland 28, Ohio; a large amount of D-cystine was present; (b) Cyclo Chemical Corp., Los Angeles 1, Calif.

(20) L. Goodman, L. O. Ross, and B. R. Baker, *J. Org. Chem.*, **23**, 1251 (1958).

(21) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," John Wiley and Sons, Inc., New York, N. Y., 1961, Vol. 2, p. 901.

(22) C. Berse, R. Boucher, and L. Piché, *J. Org. Chem.*, **22**, 805 (1957); ref. 21, p. 1080.

(23) H. G. Bray, S. P. James, and W. V. Thorpe, *Biochem. J.*, **70**, 570 (1958).

(24) The effect of these conditions on the integrity of the optical center was not ascertained; *cf.* K. Balenović and B. Gašpert, *Chem. Ind. (London)*, 115 (1957).

trated *in vacuo*, the residual sirup was dissolved in 1 *M* hydrochloric acid (150 ml.), and the solution was clarified by filtration and neutralized (pH 5) with saturated aqueous sodium acetate. The resulting oil was extracted into dichloromethane (100 ml.) and recovered from the dried (magnesium sulfate) extracts by concentration to form a glass (2.00 g., 75%), R_f 0.43 in system B.

The **p-toluenesulfonic acid salt** was prepared by adding 0.70 g. (1.9 mmoles) of XIIa in 15 ml. of absolute methanol to 0.61 g. (3.2 mmoles) of *p*-toluenesulfonic acid monohydrate in 15 ml. of methanol with stirring. After 30 min., 400 ml. of ether was added, the mixture was chilled at 5° overnight, and 0.44 g. (37%) of white crystals, m.p. 214–230°, was collected on a filter. Recrystallization from acetonitrile (200 ml.) afforded 0.27 g. (25%) of analytically pure solid, m.p. 237–239°.

Anal. Calcd. for $C_{25}H_{25}NO_7S_2$: C, 56.7; H, 4.75; S, 12.1. Found: C, 56.6; H, 5.00; S, 11.8.

Methyl α-Phthalimidoacrylate (XV) from XIIa.—According to conventional hydroxyethylation procedure, 0.50 g. (1.4 mmoles) of free XIIa in 25 ml. of 50% aqueous acetic acid was treated with 1.5 ml. of liquid ethylene oxide. The stoppered solution was stored for 2 days at room temperature, adjusted to *p*H 5-6 with saturated aqueous sodium acetate, and extracted with three 25-ml. portions of dichloromethane. The combined extracts, when dried and concentrated *in vacuo*, afforded 0.34 g. of semisolid residue. Recrystallization from an ethyl acetate–petroleum ether mixture afforded 0.080 g. (25% yield of XV), m.p. 103–107°, R_f 0.50 in system B. The infrared spectra of both the crude and the purified product showed strong bands at 6.1 μ (—C=C—) and 11.3 μ (=CH_2); expected bands near 2.9 (OH), 6.25 (aryl), 9.5 (C—OH), and 12.1 μ (*p*-disubstituted benzene) were missing. Broad absorption near 5.75 μ (C=O) and a band at 7.19 μ (N-phthalyl) remained as in XIIa.

Anal. Calcd. for $C_{12}H_9NO_4$: C, 62.4; H, 3.89; N, 6.26. Found: C, 62.5; H, 4.09; N, 6.05.

3-(*p*-Nitrophenylthio)-L-alanine (L-VIa).—A suspension of 1-bromo-4-nitrobenzene (13.1 g., 0.065 mole) in 220 ml. of 95% ethanol was treated with a solution of L-cysteine hydrochloride (8.16 g., 0.052 mole) and sodium bicarbonate (12.6 g., 0.15 mole) in 40 ml. of water. The mixture, under a nitrogen atmosphere, was refluxed for 5 hr., cooled, and filtered from salts. The filtrate was concentrated *in vacuo* to one-fourth the volume, and diluted with 250 ml. of water. Unchanged bromonitrobenzene was removed by filtration, the filtrate (pH 8) was extracted with ether (150 ml.), and the aqueous layer was neutralized (pH 6–7) with 5 *M* ammonium hydroxide. A yellow solid (3.10 g.) precipitated on chilling. The combined product (4.52 g., 40% yield) after collecting a second crop was recrystallized from hot water (200 ml.) containing charcoal, affording 3.24 g. (28% yield), m.p. 170–172° dec. (lit.[20] 175° for the racemate), $[\alpha]^{26}D$ +4.3° ± 0.1 (*c* 1 in 6 *M* hydrochloric acid), R_f 0.70 in system A (R_{Cy} 1.30) and 0.66 in system B, identical with DL-VIa in both systems.

N-Acetyl-3-(*p*-nitrophenylthio)-L-alanine.—A stirred suspension of 5.5 g. (0.024 mole) of S-*p*-nitrophenyl-L-cysteine (L-VIa) in 250 ml. of water at 70° was treated with 40 ml. of acetic anhydride. The temperature rose to 80°; the resultant clear solution was stirred 1 hr. while it was allowed to cool to room temperature. It then was stored at 5° for 4 hr. Yellow crystals (4.73 g.) separated; concentration of the mother liquor to one-third the volume afforded a second crop (0.80 g.). After two recrystallizations of the combined product from boiling water, the yield was 4.61 g. (67%), m.p. 160–162° (lit.[25] 156–158°), R_f 1.00 in system A.

The **methyl ester (VIII)** crystallized from the reaction mixture (72% yield after collecting a second crop), m.p. 159–161°, R_f 0.26 in system B, $[\alpha]^{25}D$ +3.9 ± 0.05° (*c* 1 in 2-methoxyethanol).

Anal. Calcd. for $C_{12}H_{14}N_2O_5S$: C, 48.3; H, 4.74; S, 10.8. Found: C, 48.0; H, 4.87; S, 11.0.

N-Acetyl-3-(*p*-aminophenylthio)-L-alanine Methyl Ester (XI). The nitro compound VIII was reduced by the method used for preparation of XIIa. The crude residual sirup was partitioned between dichloromethane and 1 *M* hydrochloric acid. The acid layer, treated as for XIIa, afforded the free amine as a solid (90%), m.p. 93–96°. Recrystallization from benzene afforded an analytically pure sample, m.p. 94–96° (48% yield), R_f 0.45 in system B, $[\alpha]^{26}D$ +103.6 ± 1.5° (*c* 1 in chloroform).

3-(*p*-Nitrobenzylsulfonyl)-L-alanine (VII).—A stirred suspension of L-VIb[22] (10 g., 0.043 mole) in 150 ml. of glacial acetic acid was treated with 35 ml. of 30% hydrogen peroxide and, after 16 hr., with another 50 ml. After the first 15 min., VIb had dissolved and the deposition of VII had begun. After 40 hr., the product (69%, m.p. 180–183° dec.[18]) was filtered from the chilled mixture and washed with water. Recrystallization from boiling water (250 ml./1 g.) afforded 51%, m.p. 185–189° dec.,[18] $[\alpha]^{26}D$ −0.7° ± 0.01 (*c* 1 in 50% aqueous acetic acid), R_f 0.48 in system A (R_{Cy} 1.3), λ_{max}^{Nujol} 7.7 and 8.8 μ (–SO_2–).

Anal. Calcd. for $C_{10}H_{12}N_2O_6S$: C, 41.7; H, 4.20; S, 11.1. Found: C, 41.7; H, 4.51; S, 11.3.

N-Acetyl-3-(*p*-nitrobenzylsulfonyl)-L-alanine.—Acetylation of VII by the method applied to L-VIa, using triple the amounts of water and acetic anhydride, afforded 74% of crude amide, isolated in 2 crops (m.p. 117–122° and 120–123°) by step-wise concentration of the clear reaction solution to one-sixth the volume. The combined product was recrystallized (63% yield) from boiling water. An analytical sample, m.p. 130–132°, R_f 0.71 in system A and 0.74 in system B, was dried at 64° *in vacuo* but was the monohydrate.

Anal. Calcd. for $C_{12}H_{14}N_2O_7S \cdot H_2O$: C, 41.4; H, 4.63; N, 8.04. Found: C, 41.8; H, 4.52; N, 8.04.

The **methyl ester (X)** was obtained in 68% yield, m.p. 198–200°, $[\alpha]^{26}D$ −24.6° ± 0.06 (*c* 1 in pyridine), R_f 0.24 in system B.

Anal. Calcd. for $C_{13}H_{16}N_2O_7S$: C, 45.4; H, 4.68; N, 8.13; S, 9.31. Found: C, 45.4; H, 4.71; N, 8.15; S, 9.39.

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