S-(PYRIMIDIN-2-YL)-L-CYSTEINE: CHEMICAL SYNTHESIS AND BIOSYNTHESIS IN *Escherichia coli**

A. HOLÝ, I. VOTRUBA and K. JOŠT

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

Received June 29th, 1973

Cultivation of bacteria *E. coli* B on a glucose-containing synthetic medium in the presence of 2-mercaptopyrimidine (*I*) resulted in the transformation of compound *I* to S-(pyrimidin-2-yl)-cysteine (*IIa*). Reaction of the sodium salt of compound *I* with 2-chloro-L-alanine led to the completely racemic compound *IIa* while a partially racemic product *IIa* was obtained by reaction with N-benzyloxycarbonyl-O-tosyl-L-serine *p*-nitrobenzyl ester (after the removal of protecting groups). Reaction of N-benzyloxycarbonyl-L-serine methyl, benzyl and *p*-nitrobenzyl ester with compound *I* in the presence of dimethylformamide dineopentylacetal afforded N-benzyloxycarbonyl-S-(pyrimidin-2-yl)-cysteine methyl ester (*IIb*), benzyl ester (*IId*) and *p*-nitrobenzyl ester (*IIc*), resp. The free acid *IIa* was then obtained on removal of protecting groups from the esters *IIb—d*. The degree of racemisation depends on the character of the ester function (methyl $\ll$ benzyl $<$ *p*-nitrobenzyl).

In connection with investigations on the biochemical activity of analogues of pyrimidine bases in the naturally occurring nucleic acid components, we have focussed our attention to the mechanism of the action of 2-mercaptopyrimidine (*I*) which exhibits a marked bacteriostatic activity on *E. coli* B. Thus, the inhibitory effect of compound *I* has been observed to disappear shortly after the addition of compound *I* to the growing culture of *E. coli* B in a mineral medium containing glucose as the exclusive source of carbon. As shown by analysis of the cultivation medium, only a very small smount of compound *I* was present along with additional three UV-absorbing compounds, one of which predominated. The predominating component was isolated from the medium by adsorption on active charcoal and elution with dilute aqueous ammonia, or, by adsorption on a cation exchange resin in the H^+ form followed by elution with dilute aqueous ammonia and purification by paper chromatography.

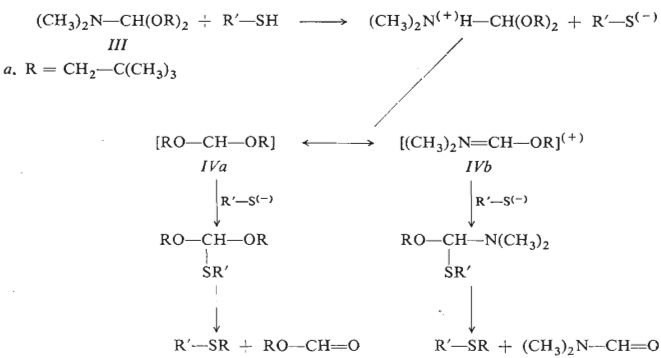
As indicated by the ultraviolet absorption spectrum (λ_{max} 253 nm), the predominant component represents a S-substituted derivative of 2-mercaptopyrimidine. The mass

* Part CLXIII in the series Nucleic Acid Components and their Analogues; Part CLXII: This Journal *38*, 3912 (1973). Part CXIX in the series Amino Acids and Peptides; Part CXVIII: This Journal *39*, 459 (1974). Some results have been reported in a preliminary form elsewhere[1].

spectrum lacks any molecular peak of the component; under the analysis conditions, 2-mercaptopyrimidine represents the single volatile compound present as indicated by the molecular peak and group of signals of fragmentation products, identical with the mass spectrum of compound *I*. The isolated product is of a weakly acidic character but does not contain either an ester-bound phosphoric acid or a sugar moiety. On the other hand, the positive ninhydrin test suggests the occurence of an amino acid. Degradation of the compound with 6M hydrochloric acid at 105°C afforded cystine as the single amino acid; the identification was effected by comparison with an authentic specimen on electrophoresis and an amino-acid analyzer, by the performic acid oxidation to cysteic acid and by the Raney nickel desulfurisation to alanine (both these products were identified by comparison with authentic specimens). Alanine is also the sole ninhydrin-positive product of the direct Raney nickel treatment of the above material. In conclusion, the transformation product of 2-mercaptopyrimidine in bacterial cells possesses the structure of S-(pyrimidin-2-yl)-cysteine (*IIa*).

An unequivocal proof of the structure of the above transformation product was performed by chemical synthesis of S-(pyrimidin-2-yl)-L-cysteine. Three different synthetic routes were used. The first route consists in the reaction of 2-chloro-L-alanine[2] with the sodium salt of 2-mercaptopyrimidine (*I*) in methanol; the resulting methyl ester *IId* was isolated as the hydrochloride and saponified to compound *IIa* in an alkaline medium. The thus-obtained product was completely racemic. When the reaction was performed in aqueous alkali (analogously to the synthesis of the optically pure lanthionine[3] and cystathionine[4]), a mixture of compounds resulted from which we failed to isolate the free *IIa*. The transformation method of O-tosyl serine derivatives and peptides has been also attempted in the synthesis of cysteine[5,6] or seleno-cysteine[7] derivatives. In our case, the reaction of N-benzyloxycarbonyl-O-tosyl-L-serine *p*-nitrobenzyl ester with compound *I* and the subsequent removal of protecting groups afforded a high yield of compound *IIa*, optically active; nevertheless, the optical rotation was lower than that of the product obtained by the following third method, based on a recently reported[8] S-alkylation of 2-mercaptopyridine and related compounds possessing an acid SH group by the action of dimethylformamide dialkylacetals. This reaction, similar to the esterification of carboxylic acids[9] or N-alkylation of heterocyclic bases and nucleosides[10,11] obviously proceeds through the protonated form of the acetal *III* by an attack of the mercapto derivative anion on the cation *IVa* or *IVb* and the subsequent intramolecular alkylation of the intermediary *V* (Scheme 1).

Consequently, 2-mercaptopyrimidine (*I*) cannot be S-alkylated by the action of dimethylformamide dineopentylacetal[8] (*IIIa*). On the other hand, an easy exchange of the alcoholic group[12,13] may be observed with compounds of the type *III*. Thus in the reaction of 2-mercaptopyrimidine (*I*) with a derivative containing a primary alcoholic group in the presence of compound *IIIa*, there occurs a "transacetalisation"



R = primary alkyl group
R′ = pyrimidin-2-yl

SCHEME 1

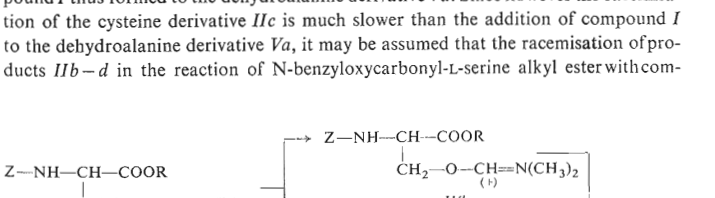
of dimethylformamide dineopentylacetal with the appropriate alcohol followed by alkylation of compound *I* to the corresponding 2-alkylthiopyrimidine[8].

N-Benzyloxycarbonyl-L-serine methyl or *p*-nitrobenzyl ester when treated simultaneously with compound *I* and dimethylformamide dineopentylacetal, afforded N-benzyloxycarbonyl-S-(pyrimidin-2-yl)-L-cysteine methyl (*IIb*) or *p*-nitrobenzyl (*IIc*) ester. The free *IIa* was then obtained in a pure state by removal of the benzyloxycarbonyl group with hydrogen bromide in acetic acid and of the ester groups by alkaline hydrolysis. The specimen obtained from the methyl ester *IIb* exhibits a high optical activity the value of which lies near to that of the naturally occurring material. On the other hand, the product *IIa* obtained from the *p*-nitrobenzyl ester *IIc* is partially racemised. Since the removal of protecting groups was performed under similar conditions in both cases, the different degree of racemisation must be ascribed to the character of the ester group in compound *II*. In this connection, the benzyl ester *IId* was investigated with respect to the extent of racemisation. Compound *IId* was prepared analogously to compounds *IIb* and *IIc*, namely, by reaction of N-benzyloxycarbonyl-L-serine benzyl ester with compounds *I* and *IIIa* under similar conditions. The starting N-benzyloxycarbonyl-L-serine benzyl ester was obtained by reaction of N-benzyloxycarbonyl-L-serine with dimethylformamide dibenzylacetal[9]. Also in this case a partial racemisation has been observed but of a lesser extent than with the *p*-nitrobenzyl ester. The influence of the character of the ester group on racemisation may be thus expressed by the order methyl $\ll$ benzyl $<$ *p*-nitrobenzyl.

The reaction of N-benzyloxycarbonyl-L-serine *p*-nitrobenzyl ester with dimethyl-

formamide dineopentylacetal (*IIIa*) is to a considerable extent accompanied by the formation of N-benzyloxycarbonyldehydroalanine *p*-nitrobenzyl ester (*Va*). In the case of N-benzyloxycarbonyl-L-serine methyl ester, the analogous side reaction occurs only in traces. This side reaction is specific of dimethylformamide acetals and does not take place with the use of other tertiary bases, *e.g.*, triethylamine. It may be assumed that the virtual active intermediate leading to derivatives *V* is represented by a mixed acetal of dimethylformamide with the primary alcoholic group of the serine derivative (of the type *IIIb*) or by some forms of the further intermediates *IVa,b* which can eliminate dimethylformamide or alkyl formate and thus furnish the dehydroalanine derivative (Scheme 2). This process is obviously supported by a simultaneous activation of the α-hydrogen atom by the adjacent alkoxycarbonyl group. The rapid reaction of the dehydroalanine derivative *Va* with the mercaptopyrimidine *I* affords in the presence of the acetal *IIIa* the racemic cysteine derivative *IIc*. In this case, however, the role of the acetal *IIIa* consists merely in the formation of the anion of compound *I* since the reaction *Va* → *IIc* proceeds even in the presence of triethylamine but does not take place in the absence of either of the two components mentioned.

The partial racemisation may also be observed at the stage of the protected cysteine derivative, namely, with compound *IIc*, by the presence of the dineopentylacetal *IIIa*. This process may be explained analogously as above by a primary formation of the dehydroalanine derivative *Va* on removal of the mercaptopyrimidine anion (as a good leaving group) under participation of the activation of the α-hydrogen atom by the *p*-nitrobenzylcarbonyl group and the subsequent rapid readdition of compound *I* thus formed to the dehydroalanine derivative *Va*. Since however the racemisation of the cysteine derivative *IIc* is much slower than the addition of compound *I* to the dehydroalanine derivative *Va*, it may be assumed that the racemisation of products *IIb – d* in the reaction of N-benzyloxycarbonyl-L-serine alkyl ester with com-



Z = benzyloxycarbonyl
R = methyl, *p*-nitrobenzyl

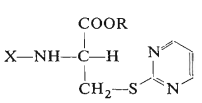
SCHEME 2

pound *I* and *IIIa* occurs mainly on the level of the intermediate of the serine series *IIIb* through the dehydroalanine derivatives *V* and not by elimination and readdition on the level of the products *IIb – d* of the cysteine series.

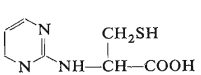
The identity of both the synthetically prepared and the isolated specimens of compound *IIa* was demonstrated on comparison of chromatographical, electrophoretical, and spectroscopical properties. According to the optical rotation, the naturally occurring specimen *IIa* belongs to the L-series as it was also shown by the *in vivo* conversion of [^{14}C]-L-serine to compound *IIa* in a high radiochemical yield[1].

Compound *IIa* is relatively unstable; it is decomposed in aqueous solutions even at 0°C, especially when it was previously subjected to paper chromatography in acid or alkaline solvent mixtures. The decomposition affords a ninhydrin-negative substance, the electrophoretical and spectrophotometrical properties of which suggest the isomeric structure *VI*, namely, N-(pyrimidin-2-yl)cysteine. The structural proof was performed by synthesis of compound *VI* by reaction of 2-methylthiopyrimidine with cysteine in a weakly alkaline aqueous solution. The elemental analysis, mass spectrum, UV spectrum, and NMR spectrum of the product resulting under the above conditions were in accordance with the structure *VI*; the product was in every respect, including the optical rotation, identical with the specimen isolated by decomposition of compound *IIa*. It may be assumed that compound *VI* is formed from *IIa* by an intramolecular attack of the amino group towards the position 2 of the pyrimidine ring system and by the simultaneous fission of the C—S bond. The synthesis of compound *VI* from cysteine and 2-methylthiopyrimidine was observed to be accompanied by the formation (in addition to traces of compound *IIa*) of a by-product, namely, a ninhydrin-positive compound, isomeric with compounds *IIa* and *VI*. On the basis of experimental data, this by-product was ascribed the structure *VII*. Compound *VII* is obviously formed from compound *VI* by a retro-Dimroth re-

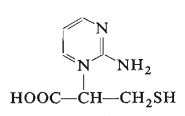
I



IIa; X = H, R = H
IIb; X = $C_6H_5CH_2OCO$, R = CH_3
IIc, X = $C_6H_5CH_2OCO$, R = 4-NO_2—$C_6H_5CH_2$
IId; X = $C_6H_5CH_2OCO$, R = $C_6H_5CH_2$
IIe; X = H, R = CH_3



VI



VII

arrangement which is known to occur with the 2-alkylaminopyrimidine derivatives. The rearrangement consists in a ring opening followed by recyclisation under participation of the exocyclic amino group. Such a formation of compound *VII* would be in accordance with its high optical purity. The reaction is obviously specific of cysteine since glycine or serine or S-benzylcysteine do not react under similar conditions with 2-methylthiopyrimidine at all. On the other hand, in the related S_N2 substitution in the 2-methylthio-4-hydroxypyrimidine and -4-aminopyrimidine series, the aminolysis proceeds regardless the character of the acid[14]. In the present case, an S_N2 attack by the nucleophilic SH group is obviously involved under the primary formation of compound *IIa* which is then converted to compound *VI* by an intramolecular rearrangement and finally, to compound *VII* by the retro-Dimroth rearrangement.

S-(Pyrimidin-2-yl)-L-cysteine constitutes a new type of S-substituted cysteine derivatives. Since compound *IIa* is obviously a detoxication product of mercapto derivatives of the acid character in bacteria and does not exhibit any bacteriostatic effect even in high concentrations, it may be assumed that analogous compounds can be formed in some additional cases.

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block) and are uncorrected. Analytical samples were dried over phosphorus pentoxide at 0·1 Torr. Solutions were taken down on a rotatory evaporator at 35°C/15 Torr. Paper chromatography was performed on paper Whatman No 1 (preparative runs on paper Whatman No 3 MM) in the solvent systems S_1, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2), and S_2, 1-butanol–acetic acid–water(5 : 2 : 3). Paper electrophoresis was carried out at 40 V/cm in the buffer solution E_1, 0·1M triethylammonium hydrogen carbonate (pH 7·5), and at 20 V/cm in the buffer solutions E_2, 1M acetic acid, and E_3, pyridine–glacial acetic acid–water (pH 5·7) for the period of 1 h. Thin-layer chromatography was performed on ready-for-use silica gel foils Silufol UV_{254} (Kavalier Glassworks, Votice, Czechoslovakia) in the solvent system S_3, ethanol–chloroform (1 : 99), and on Kieselgel G (Merck, German Federal Republic) plates in S_4, 2-butanol–90% aqueous formic acid–water (75 : 13·5 : 13·5); S_5, 2-butanol–25% aqueous ammonia–water (85 : 7·5 : 7·5); S_6, 1-butanol–pyridine–glacial acetic acid–water (15 : 10 : 3 : 6); S_7, n-heptane–tert-butyl alcohol–pyridine (5 : 1 : 1); and S_8, benzene–ethanol (50 : 1). Spots were detected by viewing under ultraviolet light (Chromatolite) or by spraying with a 0·1% solution of ninhydrin in ethanol. The UV absorption spectra were measured on a Beckman DM spectrophotometer. The amino acids were analysed on an automatic analyzer (Type 6020, Development Workshops, Czechoslovak Academy of Sciences, Prague).

Isolation of Compound *IIa* after Cultivation of *E. coli* B

To 300 ml of an exponentially growing culture of *E. coli* B on synthetic medium[15] there was added at the stage of $3 . 10^8$ cells per 1 ml 2-mercaptopyrimidine up to the concentration of 50 μg/ml. In the 40th minute, further 2-mercaptopyrimidine was added, again to the concentration of 50 μg/ml of the medium. After the incubation (80 min at 37°C), the bacterial suspension was centrifuged and the medium filtered through the Sartorius membrane filters 0·45 μ, 50 mm

in diameter. The filtrate was adjusted to pH 3·0—3·5 by means of the Dowex 50 X 8 (H^+ form) ion exchange resin, prewashed with water to the loss of the UV-absorption, and the resulting suspension was applied to a column of the same resin (500 ml). The column was then washed with water (3 ml/min) under a continuous measurement of UV-absorption and conductivity. When the corresponding data dropped to the original eluant values, the column was eluted with 2·5% aqueous ammonia under otherwise the same conditions again to the drop to the original eluant values. The ammonia fraction was evaporated to dryness and the residue chromatographed on 3 sheets of paper Whatman No 3 MM in the solvent system S_2. The bands of the product *II* were eluted with 0·5% aqueous ammonia (50 ml), the eluate evaporated, the residue codistilled with methanol, and purified by precipitation from methanol (5 ml) with ether (100 ml). The precipitate was collected by centrifugation, washed with ether, and dried under diminished pressure to afford 25 mg of compound *IIa*, practically homogeneous on chromatography in solvent systems S_1 and S_2, and on electrophoresis in buffer solutions E_1—E_3 (41·7% with respect to the starting compound *I*). M.p. 158—160°C. For $C_7H_9N_3O_2S$ (199·2) calculated: 42·20% C, 4·55% H, 21·10% N, 16·0% S; found: 43·05% C, 4·70% H, 20·81% N, 16·52% S. UV spectrum (water): λ_{max} 253 nm, $A_{250/260}$ 2·92, $A_{280/260}$ 0·46, $A_{290/260}$ 0·37. R_F values: 0·45 (S_1); 0·44 (S_2); compound *I* 0·77 (S_1); 0·60 (S_2). Electrophoretical mobility (with respect to *I*): 0·11 (E_1). Optical rotation: $[\alpha]_D^{25}$ —101° (*c* 0·5, H_2O). In addition to compound *IIa*, the residue of the ammonia eluate contains a lesser amount of further two non-identified compounds: *a*) R_F 0·18 (S_1) and 0·04 (S_2); electrophoretical mobility 1·08 (E_1); ninhydrin-negative; and *b*) R_F 0·70 (S_1) and 0·80 (S_2); mobility 0·84 (E_1); ninhydrin-negative; and a mixture of amino acids containing Ser (8%), Glu (10%), Ala (22%), Val (32%), Ile (18%), Asp + Thr + Gly + Leu + Tyr (10%). The blank cultivation (without the addition of compound *I*) afforded the following mixture of amino acids: 12% Ser, 14% Glu, 21% Ala, 31% Val, 22% Asp + Thr + Gly + Leu + Ile + Tyr + Phe. Total amino acids in the medium containing compound *I*: 10·4 μmol/100 ml (blank: 10·0 μmol : : 100 ml).

Degradation of Compound *IIa* Isolated from the Cultivation Medium

A. *Acid hydrolysis.* A mixture of compound *IIa* (1·05 mg) and 6M-HCl (1 ml) was heated under nitrogen at 110°C for 20 h, evaporated under diminished pressure, the residue dissolved in 2·5 ml of a citrate buffer (pH 2·2), and an aliquot (0·2 ml) applied to the analyser. The hydrolysate was shown to contain 3·46 μmol of Cys as the single amino acid.

B. *Desulfurisation of the hydrolysate of compound* IIa. A portion (0·5 ml) of the above stock solution was diluted with water (5 ml) and refluxed with Raney Ni W 4 (0·5 g) for 3 min. The mixture was filtered while hot, the solid washed with hot water (5 ml), the filtrate and washings combined, and evaporated to dryness under diminished pressure. The residue was dissolved in 0·2 ml of the above buffer (pH 2·2) and the solution applied to the analyser. The mixture was shown to contain alanine as the single amino acid.

C. *Oxidation of the hydrolysate of compound* IIa *with performic acid.* A portion (0·5 ml) of the above stock solution was evaporated to dryness under diminished pressure and the residue oxidised at 20°C with performic acid[16]. The analyser indicated the presence of cysteic acid as the single amino acid.

D. *Desulfurisation of compound* IIa. A mixture of compound *IIa* (0·5 mg), water (5 ml), and Raney Ni W 4 was refluxed for 3 min and processed analogously to paragraph *B*. According to the analysis, the mixture contained alanine as the single amino acid.

N-Benzyloxycarbonyl-L-serine Benzyl Ester

The title compound was prepared[9] from N-benzyloxycarbonyl-L-serine by reaction with dimethylformamide dibenzylacetal; crystallisation from benzene–light petroleum afforded a specimen, m.p. 81–82°C, $[\alpha]_D^{25}$ +2·24° (*c* 0·5, chloroform). Reported[17], m.p. 84–85°C and $[\alpha]_D^{25}$ +5·7° (*c* 0·5, chloroform). Catalytic hydrogenation over 10% palladium on charcoal in acetic acid yielded L-serine, $[\alpha]_D^{25}$ +12·8° (*c* 0·1, 1M-HCl).

N-Benzyloxycarbonyl-O-tosyl-L-serine *p*-Nitrobenzyl Ester

The reported procedure[7] and crystallisation from ethyl acetate afforded the title compound, m.p. 109–110°C and $[\alpha]_D^{25}$ −8·3° (*c* 0·5, dimethylformamide). Reported[7], m.p. 108–110°C and $[\alpha]_D^{25}$ −6·56 (*c* 3·0, dimethylformamide).

N-Benzyloxycarbonyl-S-(pyrimidin-2-yl)-L-cysteine Methyl Ester (*IIb*)

A mixture of the crude N-benzyloxycarbonyl-L-serine methyl ester (prepared analogously to the threonine derivative[18] from 60 mmol of N-benzyloxycarbonyl-L-serine and dried by codistillation with benzene under standard conditions), 2-mercaptopyrimidine (*I*; 9 g; 80 mmol), dimethylformamide dineopentylacetal[12] (*IIIa*; 30 ml), and benzene (300 ml) was refluxed for 7 h under exclusion of atmospheric moisture, cooled down, and the unreacted *I* filtered off. The filtrate was evaporated under diminished pressure and the residue coevaporated with one 300 ml portion of 50% aqueous ethanol and three 100 ml portions of ethanol. The final residue was dissolved in ethanol (25 ml) and the solution was treated portionwise with light petroleum (300 ml) to deposit crystals which were collected with suction, washed with light petroleum, and recrystallised from boiling ethanol (20 ml), light petroleum (80 ml) being added until the mixture is turbid. Yield, 4·5 g (22%) of a chromatographically homogeneous (in S_3) compound *IIb*, m.p. 96–97°C. For $C_{16}H_{17}N_3O_4S$ (347·4) calculated: 55·31% C, 4·93% H, 12·10% N, 9·23% S; found: 55·33% C, 4·94% H, 11·77% N, 8·96% S. Optical rotation: $[\alpha]_D^{25}$ −95·7° (*c* 0·5, dimethylformamide).

N-Benzyloxycarbonyl-S-(pyrimidin-2-yl)-L-cysteine *p*-Nitrobenzyl Ester (*IIc*)

A. *From* N-*benzyloxycarbonyl*-O-*tosyl*-L-*serine* p-*nitrobenzyl ester* (Method *A*). A solution of 2-mercaptopyrimidine (*I*; 0·56 g; 5 mmol) in 1M methanolic sodium methoxide (5 ml) was added to a suspension of 1·32 g (2·5 mmol) of N-benzyloxycarbonyl-O-tosyl-L-serine *p*-nitrobenzyl ester (1·32 g; 2·5 mmol) in methanol (20 ml). The reaction mixture was stirred at room temperature for 16 h under exclusion of atmospheric moisture, neutralised with acetic acid, and evaporated under diminished pressure. The residue was extracted with hot chloroform (50 ml), filtered, and the extract evaporated under diminished pressure. The crude product was purified, by chromatography (in chloroform) on a 40 × 16 × 0·3 cm loose layer of the fluorescent-indicator-containing silica gel (produced by Service Laboratories of this Institute). The band of the product (R_F, 0·30) was eluted with methanol (200 ml), the eluate evaporated, and the residue crystallised from a mixture of ethanol (1 ml) and light petroleum (20 ml) to afford 0·85 g (72·5%) of compound *IIc*, m.p. 76–78°C. For $C_{22}H_{20}N_4O_6S$ (468·4) calculated: 56·41% C, 4·30% H, 11·96% N, 6·85% S; found: 56·62% C, 4·38% H, 12·24% N, 6·92% S. Optical rotation: $[\alpha]_D^{25}$ −32·6° (*c* 1, dimethylformamide).

B. *From* N-*benzyloxycarbonyl*-L-*serine* p-*nitrobenzyl ester* (Method B). A mixture of N-benzyloxycarbonyl-L-serine *p*-nitrobenzyl ester[19] (3·75 g; 10 mmol), 2-mercaptopyrimidine (*I*; 1·5 g; 13·4 mmol), dimethylformamide dineopentylacetal (*IIIa*; 5 ml), and benzene (100 ml) was re-

fluxed for 5 h under exclusion of atmospheric moisture, filtered, the filtrate evaporated to dryness under diminished pressure, and the residue codistilled with one 100 ml portion of 50% aqueous ethanol and with three 50 ml portions of ethanol. The final residue was crystallised from hot ethanol (20 ml), ether being added to the solution until turbid. Yield, 3·0 g (64%) of compound *IIc* homogeneous on chromatography in the solvent system S_3; m.p. 105—106°C. Found: 55·98% C, 4·35% H, 11·96% N, 7·05% S. Optical rotation: $[\alpha]_D^{25}$ −12·8° (*c* 0·5, dimethylformamide). UV spectrum (methanol): λ_{max} 248 nm (ε_{248} 22600), λ_{min} 225 nm (ε_{225} 6850). NMR spectrum: 3·56 (dd, 1 H), C_β—H (J_{gem} = 14, J_{vic} = 7); 3·73 (dd, 1 H) C_β—H; 4·80 (br q, 1 H) C_α—H ($J_{a,b}$ = 7, $J_{a,b'}$ = 4·5, $J_{a,NH}$ = 7·5), 5·09 (s, 2 H) $C_6H_5C\underline{H}_2$—O—; 5·26 (s, 2 H) *p*-nitrophenyl—$C\underline{H}_2$—; 6·17 (d, 1 H) NH ($J_{NH,aH}$ = 7·5); 6·95 (t, 1 H) $C_{(5)}$—H ($J_{5,4} = J_{5,6}$ = 5·0); 7·30 (s, 5 H) + 7·47 (d) + 8·15 (d, 4 H) aromatic protons; 8·42 (d, 2 H) $C_{(4)}$—H + $C_{(6)}$—H.

C. *From* N-*benzyloxycarbonyldehydroalanine* p-*nitrobenzyl ester*. A mixture of compound *Va* (100 mg; 0·28 mmol), 2-mercaptopyrimidine (*I*; 100 mg; 0·89 mmol), dimethylformamide dineopentylacetal (*IIIa*; 0·4 ml), and benzene (5 ml) was refluxed for 30 min (as shown by thin-layer chromatography in S_3, the reaction was quantitative), evaporated under diminished pressure, and the residue chromatographed on one layer of loose silica gel in chloroform. The band of the product *IIc* was eluted with methanol, the eluate evaporated, and the residue crystallised from a mixture of ethanol and light petroleum. Yield, 100 mg (76%) of compound *IIc*, m.p. 104—105°C, $[\alpha]_D^{25}$ 0° (*c* 1, dimethylformamide).

N-Benzyloxycarbonyl-S-(pyrimidin-2-yl)-L-cysteine Benzyl Ester (*IId*)

A mixture of N-benzyloxycarbonyl-L-serine benzyl ester (1·65 g; 5 mmol), 2-mercaptopyrimidine (*I*; 0·75 g; 6·7 mmol), dimethylformamide dineopentylacetal (*IIIa*; 2·5 ml), and benzene (50 ml) was refluxed for 4 h under exclusion of atmospheric moisture (as shown by thin-layer chromatography in the solvent system S_3, the reaction was quantitative after 3 h). The mixture was evaporated under diminished pressure, the residue codistilled with 50% aqueous ethanol (50 ml) and ethanol (100 ml), and chromatographed on one layer of loose silica gel in 1 : 9 ethyl acetate–benzene. The band of the product (R_F, 0·42) was eluted with methanol (200 ml), the eluate evaporated, and the residue crystallised from a mixture of ethanol and light petroleum. Yield, 1·4 g (66%) of the benzyl ester *IId*, $[\alpha]_D^{25}$ −35·0° (*c* 0·5, dimethylformamide). NMR spectrum: 3·66 (dd, 2 H) C_β—H_2; 4·76 (br q, 1 H) C_α—H; 5·05 (s, 2 H) $C_6H_5C\underline{H}_2$; 5·14 (*s*, 2 H) $C_6H_5C\underline{H}_2$; 6·04 (br d, 1 H) NH ($J_{NH,CH}$ = 7·3); 6·92 (t, 1 H) $C_{(5)}$—H ($J_{5,6}$ = 5·0); 7·29 (br s, 10 H) aromatic protons; 8·43 (d, 2 H) $C_{(4)}$—H + $C_{(6)}$—H ($J_{4,5} = J_{6,4}$ = 5·0). For $C_{22}H_{21}N_3O_4S$ (423·5) calculated: 62·38% C, 5·00% H, 9·92% N, 7·56% S; found: 61·81% C, 4·74% H, 9·54% N, 7·08% S.

S-(Pyrimidin-2-yl)-L-cysteine (*IIa*)

A. *From* 2-*chloro*-L-*alanine methyl ester*. To a solution of 2-mercaptopyrimidine (*I*, 2·8 g; 25 mmol) in 0·5M methanolic sodium methoxide (50 ml) there was added with stirring 2-chloro-L-alanine methyl ester hydrochloride[2] (4·0 g; 23 mmol) followed by 1M methanolic sodium methoxide (25 ml). The reaction mixture was stirred at room temperature for 3·5 h (after 2 h, the reaction was almost quantitative, as indicated by electrophoresis in buffer solutions E_1 and E_2), neutralised with acetic acid, filtered, and the material on the filter washed with methanol (50 ml). The filtrate and washings were combined and evaporated to dryness under diminished pressure. The residue was stirred in ethanol (50 ml) for 10 min, the undissolved portion filtered off, and washed with ethanol (20 ml). The combined filtrate and washings were concentrated to the volume of 25 ml and the concentrate was treated successively with 7·5M hydrogen chloride in ethanol

(5 ml) and then with ether (100 ml), to deposit the hydrochloride of compound *IIa* which was collected with suction, washed with ether, and dried under diminished pressure. Yield, 3·5 g of the crude hydrochloride of compound *IIa*. R_F values: 0·42 (in S_4), 0·60 (in S_5), 0·61 (in S_6), 0·10 (in S_7). Electrophoretical mobilities: 0·88 (His) in E_2; 1·04 (His) in E_3. The hydrochloride was dissolved in methanol (30 ml), the solution treated with 1M-NaOH (30 ml), the whole kept at room temperature for 20 min, and applied to a column of Dowex 50 (H^+ cycle) ion exchange resin (250 ml). The column was washed with cold water and the product eluted with cold 10% aqueous pyridine. The eluates were taken down, the residue triturated with ether, the solid collected with suction, washed with ether (1·7 g of the crude product, m.p. 100—110°C), and recrystallised twice from a mixture of 80% aqueous ethanol and ether. Yield, 0·75 g of compound *IIa*, m.p. 146—148°C (recrystallisation of the analytical sample from the same solvent mixture did not change the melting point value), $[\alpha]_D^{25}$ ±0° (*c* 1·33, 0·2M-HCl). R_F values: 0·36 (in S_4), 0·09 (in S_5), 0·42 (in S_6), 0·10 (in S_7). Electrophoretical mobilities: 0·43 (Gly) in E_2; 1·00 (Gly) in E_3. For $C_7H_9N_3O_2S$ (199·2) calculated: 42·20% C, 4·55% H, 21·10% N; found: 42·15% C, 4·54% H, 20·86% N.

B. *From compound* IIb. N-Benzyloxycarbonyl-S-(pyrimidin-2-yl)cysteine methyl ester (*IIb*; 0·70 g; 2 mmol) was dissolved in 35% hydrogen bromide in acetic acid (10 ml). The solution began to deposit crystals after several minutes at room temperature. Ether was added to the mixture after 10 min, the hydrobromide collected with suction, and washed with ether. R_F values: 0·42 (in S_4), 0·60 (in S_5), 0·61 (in S_6), 0·10 (in S_7). Electrophoretical mobilities: 0·88 (His) in E_2; 1·04 (His) in E_3. The hydrobromide was dried, dissolved in a mixture of methanol (10 ml) and 1M-NaOH (8 ml), the solution kept at room temperature for 2 h, and the methanol evaporated. The remaining aqueous solution was applied to a column of Dowex 50 (H^+ cycle) ion exchange resin (50 ml), the column washed with water, and the product eluted with 10% aqueous pyridine. The eluate was evaporated and the residue crystallised twice from a mixture 80% aqueous ethanol–ether to afford 0·25 g (62·7%) of compound *IIa*, m.p. 152—155°C; after an analogous recrystallisation, the analytical sample melted at 152—154°C. Optical rotation: $[\alpha]_D^{25}$ −65 ± 0·5° (*c* 1·48, 0·2M-HCl) and −116° (*c* 0·5, water). R_F values: 0·37 (in S_4), 0·09 (in S_5), 0·41 (in S_6), 0·00 (in S_7). Electrophoretical mobilities: 0·43 (Gly) in E_2; 1·00 (Gly) in E_3. Found: 41·89% C, 4·78% H, 20·92% N, 15·96% S.

C. *From compound* IIc (prepared by Method *B*). To a solution of compound *IIc* (2·5 g; 5·3 mmol) in acetic acid (10 ml) there was added 36% hydrogen bromide in acetic acid (20 ml) and the mixture was kept at room temperature for 30 min to deposit the hydrobromide (the deposition occurred in 15 min). The mixture was then poured into ether (300 ml) under stirring, the solid collected with suction, washed with ether, and dried over sodium hydroxide under diminished pressure to afford 2·6 g of the hydrobromide. R_F values: 0·55 (in S_4), 0·65 (in S_5), 0·71 (in S_6), 0·09 (in S_7). Electrophoretical mobilities: 0·70 (His) in E_2; 0·67 (His) in E_3. The hydrobromide (1·25 g) was dissolved in a mixture of methanol (10 ml) and 1M-NaOH (9 ml), the solution kept at room temperature for 20 min, and the methanol evaporated. The remaining aqueous solution was processed analogously to paragraphs *A* and *B* on a column of Dowex 50 (H^+ cycle) ion exchange resin (100 ml). Crystallisation from the solvent mixture 80% aqueous ethanol–ether afforded 0·40 g (67%) of the product, m.p. 145—147°C, undepressed on admixture with the specimen of compound *IIa* prepared in paragraph B. R_F values: 0·37 (in S_4), 0·09 (in S_5), 0·42 (in S_6), 0·00 (in S_7). Electrophoretical mobilities: 0·43 (Gly) in E_2; 1·00 (Gly) in E_3. Optical rotation: $[\alpha]_D$ −2·4° (*c* 0·5, 0·2M-HCl). UV spectrum (H_2O): λ_{max} 244 nm (ε_{max} 12 400), λ_{infl} 280 nm, ε_{260} 3 500, $A_{250/260}$ 2·9, $A_{280/260}$ 0·47 ,$A_{290/260}$ 0·36; (pH 2): λ_{max} 244 nm (ε_{max} 12 400), ε_{260} 3 700, λ_{infl} 280 nm, $A_{250/260}$ 2·7, $A_{280/260}$ 0·59, $A_{290,260}$ 0·47; (pH 12): λ_{max} 249 nm (ε_{max} 17 200), ε_{260} 6 900, λ_{infl} 285 nm, $A_{250/260}$ 1·83 $A_{280/260}$ 0·25, $A_{290/260}$ 0·21.

D. *From compound* IIc (*prepared by Method* A). N-Benzyloxycarbonyl-S-(pyrimidin-2-yl)cysteine *p*-nitrobenzyl ester (0·55 g) was decarbobenzoxylated with hydrogen bromide in acetic acid analogously to paragraph *C*. The thus-obtained hydrobromide was dissolved in a mixture of methanol (10 ml) and 1M-NaOH (5 ml), the whole kept at room temperature for 20 min, and the methanol evaporated. The remaining aqueous solution was applied to a column of Dowex 50 (H^+ cycle) ion exchange resin (40 ml), the column washed with water, and the product eluted with 10% aqueous pyridine. The eluate was evaporated and the residue crystallised from the solvent aqueous ethanol–ether to afford 0·20 g (85%) of compound *IIa*, m.p. 144—146°C, homogeneous on chromatography and electrophoresis and identical with specimens obtained in paragraphs *A* to *C*. The analytical sample was recrystallised in the same manner; m.p. 151—153°C; $[\alpha]_D^{25}$ —15·9° (*c* 0·5, 0·2M-HCl) and —25·8° (*c* 0·5 water). Found: 42·23% C, 4·52% H, 20·80% N.

E. *From compound* IId. The decarbobenzoxylation of compound *IId* (205 mg) was performed with hydrogen bromide in acetic acid analogously to paragraph *C*. The alkaline hydrolysis afforded 92 mg (95%) of the crude product which was crystallised from a mixture of aqueous methanol and ether to yield 62 mg (65%) of compound *IIa*, m.p. 150—152°C, identical on chromatography and electrophoresis with specimens obtained in paragraphs *A* to *C*. Optical rotation: $[\alpha]_D^{25}$ —64·1° (*c* 0·21; water).

N-Benzyloxycarbonyldehydroalanine *p*-Nitrobenzyl Ester (*Va*)

A mixture of N-benzyloxycarbonyl-L-serine *p*-nitrobenzyl ester[18] (1·0 g; 2·7 mmol), dimethylformamide dineopentylacetal (1·3 ml), and benzene (25 ml) was refluxed for 4 h under exclusion of atmospheric moisture (calcium chloride tube), evaporated under diminished pressure, the residue codistilled with 50% aqueous ethanol (50 ml), and finally chromatographed on two 40 × 16 × 0·3 cm loose layers of the fluorescent-indicator-containing silica gel in the solvent system 75 : 25 benzene–ethyl acetate. The UV-absorbing bands were eluted with ethyl acetate and the eluates evaporated. The residue of the R_F 0·26 band was crystallised from ethyl acetate and light petroleum to afford 0·50 g (50%) of the starting compound; $[\alpha]_D^{25}$ 0° (*c* 1·0, dimethylformamide). The residue of the R_F 0·36 band was crystallised from ethanol to afford 0·10 g (100%) of compound *Va*, m.p. 94—95°C, $[\alpha]_D^{25}$ 0° (*c* 0·5, dimethylformamide). For $C_{18}H_{16}N_2O_6$ (356·3) calculated: 60·67% C, 4·52% H, 7·86% N; found: 60·67% C, 4·88% H, 8·35% N. NMR spectrum: 5·17 (s, 2 H) $C_6H_5C\underline{H}_2O$—; 5·34 (s, 2 H) $C\underline{H}_2$-*p*-nitrophenyl; 5·87 (dd, 1 H, $J_{NH,CH} = J_{NH,CaH} < 5$, $J_{CH,CH} = 0·5$; 6·33 (br s, 1 H) both $C\underline{H}_2{=}C\diagdown$; 7·21 (br, s, 1 H) NH; 7·36 (s, 5 H) phenyl; 7·52 and 8·22 (2 × 2 H, d) *p*-nitrophenyl.

N-Benzyloxycarbonyldehydroalanine Methyl Ester (*Vb*)

A mixture of N-benzyloxycarbonyl-L-serine methyl ester (1·26 g; 5 mmol), dimethylformamide dineopentylacetal (3 ml), and benzene (33 ml) was refluxed for 6 h under exclusion of atmospheric moisture (calcium chloride tube), evaporated, the residue codistilled with 50% aqueous ethanol (50 ml), and finally chromatographed on one layer of loose silica gel in chloroform. The R_F 0·33 band was eluted with methanol and the eluate evaporated under diminished pressure to afford 1·0 g (79%) of the starting methyl ester. The R_F 0·90 band was processed analogously to afford 0·01 g (2·5%) of compound *Vb*. Mass spectrum: mol. peak 235 ($C_{12}H_{13}NO_4$). NMR spectrum: 3·81 (s, 3 H) —$COOC\underline{H}_3$; 5·18 (s, 2 H) $C_6H_5C\underline{H}_2O$—; 5·79 (br d, 1 H) and 6·25 (br s, 1 H) $C\underline{H}_2{=}C\diagdown$; 7·31 (br s, 1 H) NH; 7·36 (s, 5 H) phenyl.

Racemisation of Compound *IIc*

A. *By reaction with dimethylformamide dineopentylacetal.* A mixture of compound *IIc* (0·70 g), dimethylformamide dineopentylacetal (1 ml), and benzene (20 ml) was refluxed for 8 h under exclusion of atmospheric moisture (calcium chloride tube) and evaporated under diminished pressure. The residue was chromatographed on one layer of loose silica gel in the solvent system 8 : 2 benzene–ethyl acetate and the band of compound *IIc* (R_F 0·47) was eluted with methanol. The eluate was evaporated under diminished pressure and the residue crystallised from ethanol and cyclohexane to afford 0·45 g (64%) of compound *IIc*, $[\alpha]_D^{25}$ −12·8° (*c* 1, dimethylformamide).

B. *By reaction with triethylamine.* The reaction was performed analogously to paragraph *A*, dimethylformamide dineopentylacetal being replaced by triethylamine (2·5 ml), to afford 74% of the recovered *IIc*, $[\alpha]_D^{25}$ −8·6° (*c* 1, dimethylformamide).

As shown by thin-layer chromatography on silica gel, both reaction mixtures contained only trace amounts of compound *Va*.

N-(Pyrimidin-2-yl)-L-cysteine (*VI*)

A mixture of 2-methylthiopyridine[8] (1·0 g; 8 mmol), L-cysteine hydrochloride monohydrate (2·8 g; 16 mmol), sodium carbonate (1·4 g 14 mmol), and water (20 ml) was refluxed for 6 h, cooled down, diluted with water (20 ml), washed with two 20 ml portions of ether, the aqueous phase neutralised with concd. hydrochloric acid, applied to a column of Dowex 50 × 8 (H^+ cycle) ion exchange resin (100 ml), and the column eluted with water to the drop of UV-absorption and conductivity. The elution was then continued with 2·5% aqueous ammonia, the UV-absorbing fraction evaporated, and the residue applied to a 80 × 4 cm column of DEAE-cellulose (HCO_3^-; Cellex D, standard capacity). The elution (rate, 3 ml/min; fractions were taken in 10 min intervals) with the use of a linear gradient of triethylammonium hydrogen carbonate (pH 7·5) with 2 l of water in the mixing chamber and 2 l of the 0·3M buffer solution in the reservoir. The 0·17—0·25M fraction containing compound *VI* was evaporated and the residue chromatographed on 4 sheets paper Whatman No 3 MM in the solvent system S_1. The UV-absorbing bands of compound *VI* (R_F, 0·54) were eluted with dilute (1 : 1000) aqueous ammonia (20 ml), the eluate evaporated under diminished pressure, the residue redissolved in water (100 ml, the solution adjusted with hydrochloric acid to pH 3·5, and treated portionwise with active charcoal (prewashed with 1M-HCl and water, and dried at 110°C) until the supernatant extinction dropped to 0·5 at 235 nm. The charcoal was collected by centrifugation, washed with three 100 ml portions of water, eluted with an 1% solution of ammonia in 50% aqueous methanol, the eluate filtered through Cellite, the filtrate evaporated under diminished pressure, and the residue purified by precipitation from methanol (5 ml) with ether (200 ml). The precipitate was collected by centrifugation, washed with ether, and dried under diminished pressure. Yield, 246 mg of compound *VI*, chromatographically (R_F values: 0·54 in S_1; 0·75 in S_2) and electrophoretically (buffer solutions E_2 and E_3) homogeneous and ninhydrin-negative. For $C_7H_9N_3O_2S$ (199·2) calculated: 42·20% C, 4·55% H, 21·10% N, 16·09% S; found: 42·35% C, 4·51% H, 21·30% N, 15·73% S. Optical rotation: $[\alpha]_D^{25}$ −57·9° (*c* 0·5, water). UV spectrum (water): λ_{max} 235 nm (ε_{235} 13000), 303 nm (ε_{303} 1900). Mass spectrum: mol. peak 199 ($C_7H_9N_3O_2S$), 122, 106, 96, 95 (m.p.). NMR spectrum (hexadeuteriodimethyl sulfoxide): 3·00—3·70 (br m, 2 H) β-CH_2; 6·12 (br m) SH + α-CH; 6·52 (t, 1 H) $C_{(5)}$—H; 6·79 (br d, 1 H) 2-NH ($J_{NH,CH}$ = 6); 8·25 (d, 2 H) $C_{(4)}$—H + $C_{(6)}$—H ($J_{4,5} = J_{5,6}$ = 4·5). In addition to compound *VI*, the reaction mixture contained as contaminants compound *IIa* and a ninhydrin-positive product (R_F values: 0·41 in S_1; 0·38 in S_2. Electrophoretical mobilities: $0·26_{His}$ in E_2; $-0·67_{His}$ in E_3) with identical spectral properties as compound *VI*. Optical rotation: $[\alpha]_D^{25}$ −189·5° (*c* 1, water). Mass spectrum:

mol. peak 199, 152 (M—CH_2SH), 122, 120, 95, 79. These properties are in accordance with structure *VII*.

Identification of Compound *VI* Formed by Rearrangement of Compound *IIa* (isolated from the cultivation medium)

An aqueous solution of compound *IIa* ($c = 3{\cdot}5 \times 10^{-3}$M; 5 ml) was kept at 4°C for 3 days, evaporated under diminished pressure, and the residue subjected to electrophoresis on two 30 cm wide strips of paper Whatman No 3 MM in the buffer solution E_1 at 15 V/cm (1 h). The bands of compound *VI* were eluted with water (20 ml) and the concentration of the eluate determined spectrophotometrically at 235 nm (pH 7). Yield, 27·5% of compound *VI*, identical with an authentic specimen (*vide supra*) on chromatography (in S_1 and S_2), electrophoresis (in E_1-E_3), and UV spectrum. Optical rotation: $[\alpha]_D^{25}$ −52·1° (*c* 0·1, water).

The authors wish to thank Dr M. Buděšínský for measurement and interpretation of NMR spectra, Dr A. Trka for mass spectra, Mrs Z. Ledvinová for optical rotations, and Mrs H. Farkašová for the analysis of amino acids. Elemental analyses were carried out in the Analytical Department (Dr J. Horáček, Head) of this Institute by Mrs V. Rusová, Mrs E. Šípová, and Mr V. Štěrba. The excellent technical assistance of Mrs B. Kopecká and Mr J. Oupický is gratefully acknowledged.

REFERENCES

1. Votruba I., Holý A., Jošt K.: FEBS Letters *22*, 287 (1972).
2. Fischer E., Raske K.: Ber. *40*, 3717 (1907).
3. Brown G. B., du Vigneaud V.: J. Biol. Chem. *137*, 611 (1941).
4. Brown G. B., du Vigneaud V.: J. Biol. Chem. *140*, 767 (1941).
5. Photaki I., Bardakos V.: J. Am. Chem. Soc. *87*, 3489 (1965).
6. Zioudrou C., Wilchek M., Patchornik I.: Biochemistry *4*, 1811 (1965).
7. Theodoropoulos D., Schwartz I. L., Walter R.: Biochemistry *6*, 3927 (1967).
8. Holý A.: Tetrahedron Letters *1972*, 585.
9. Brechbühler H., Büchi H., Hatz E., Schreiber J., Eschenmoser A.: Helv. Chim. Acta *48*, 1746 (1965).
10. Žemlička J.: This Journal *28*, 1060 (1963).
11. Žemlička J.: This Journal *35*, 3572 (1970).
12. Arnold Z., Kornilov M.: This Journal *29*, 645 (1964).
13. Meerwein H., Florian W., Schön N., Stopp G.: Ann. *641*, 1 (1961).
14. Hoffmann S., Schubert H., Nitsche K.: Z. Chem. *12*, 21 (1972).
15. Škoda J., Hess V., Šorm F.: This Journal *22*, 1330 (1957).
16. Hirs C. H. W.: J. Biol. Chem. *219*, 611 (1956).
17. Baer E., Maurukas J.: J. Biol. Chem. *212*, 25 (1955).
18. Jones J. K. N., Millington J. P., Perry M. B.: Can. J. Chem. *40*, 2229 (1962).
19. Wünsch E., Jentsch J.: Chem. Ber. *97*, 2490 (1964).

Translated by J. Pliml.