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CHEMICAL STUDIES ON MALFORMIN—II.*
SYNTHESIS OF CYCLIC PENTAPEPTIDES RELATED TO MALFORMIN A

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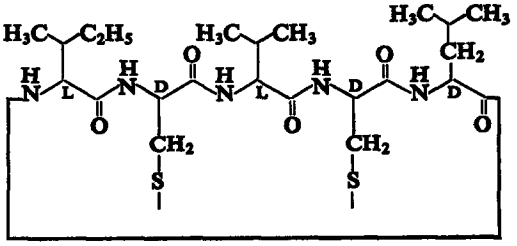
Abstract—Two cyclic pentapeptides related to malformin A, cyclo-D-cysteinyl-L-valyl-D-cysteinyl-D-leucyl-L-isoleucyl and cyclo-L-cysteinyl-L-valyl-L-cysteinyl-L-leucyl-L-isoleucyl, were synthesized by the *p*-nitrophenylester method. During cyclization of the corresponding straight chain *p*-nitrophenyl pentapeptide hydrobromides no doubling reactions occurred, as judged by molecular weight determination employing the isothermal distillation method. Both cyclic pentapeptides were inactive in a biological assay for malformin.

INTRODUCTION

MALFORMIN, produced by the fungus *Aspergillus niger*, has been shown to cause curvatures and malformations of bean plants,[1] and curvatures of corn roots.[2] It was isolated[3] from culture filtrates of *A. niger* and the structure cyclo-L-isoleucyl-D-cysteinyl-L-valyl-D-cysteinyl-D-leucyl (I) was proposed on the basis of degradative studies.[4] The structure surrounding the sulphur was not clarified.

RESULTS AND DISCUSSION

Reduction of malformin A with sodium in liquid ammonia, followed by benzylation[5] with benzyl chloride, produced biologically inactive S,S'-dibenzylmalformin A. This compound could be reduced by sodium in liquid ammonia to give reduced malformin A, which possessed $\frac{1}{10}$ of the biological activity of malformin A itself. This experiment indicated the feasibility of synthesizing reduced malformin A from the corresponding S, S'-dibenzyl cyclic pentapeptide.



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[1] R. W. CURTIS, *Plant Physiol.* **33**, 17 (1958).
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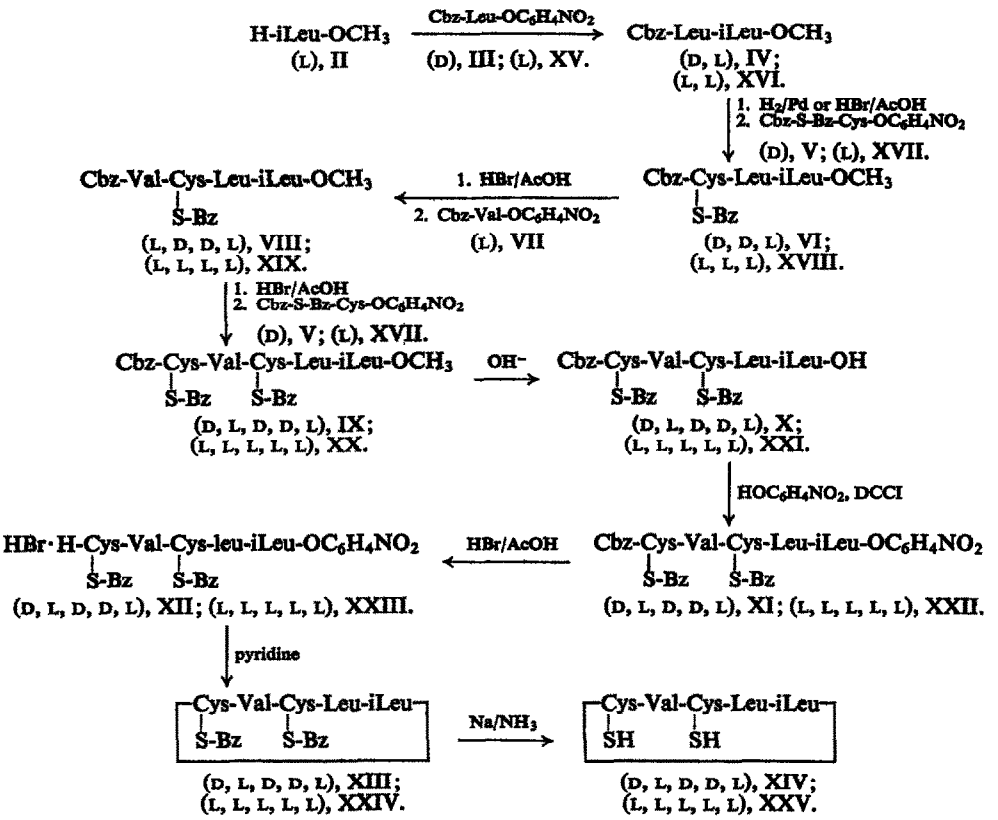
To synthesize the desired straight chain pentapeptide, methods employing the crystalline *p*-nitrophenylester of the appropriately protected amino acids were used. This method was successfully used for the synthesis of oxytocin[6] and lysine vasopressin.[7] *p*-Nitrophenyl carbobenzoxy amino acids were prepared in good yields from the corresponding carbobenzoxy amino acids and esterified with *p*-nitrophenol using dicyclohexylcarbodiimide[6,8] (DCCI) as an acylating agent. The syntheses are summarized in Chart 1.

CHART I



p-Nitrophenyl carbobenzoxy-D-leucinate (III) reacted with methyl L-isoleucinate (II) and the protected dipeptide (IV) was subjected to hydrogenolysis[9] over palladium black to remove the carbobenzoxy group. The free base, methyl D-leucyl-L-isoleucinate, reacted with *p*-nitrophenyl-N-carbobenzoxy-S-benzyl-D-cysteinate (V) to obtain the protected tripeptide (VI). From the tripeptide through the pentapeptide stage, hydrogen bromide in acetic acid[10] was used to remove the carbobenzoxy group because of the presence of sulphur. Thus, the chain was lengthened by successive reactions with the *p*-nitrophenylesters of carbobenzoxy

[6] M. BODANSZKY and V. DU VIGNEAUD, *J. Am. Chem. Soc.* **81**, 5688 (1959).
[7] M. BODANSZKY, J. MEINHOFER and V. DU VIGNEAUD, *J. Am. Chem. Soc.* **82**, 3195 (1960).
[8] D. F. ELLIOT and D. W. RUSSEL, *Biochem. J.* **66**, 49P (1957).
[9] M. BERGMAN and L. ZERVAS, *Ber. Deut. Chem. Ges.* **65**, 1192 (1932).
[10] D. BEN-ISHAI and A. BERGER, *J. Org. Chem.* **17**, 1564 (1952); **19**, 62 (1954).

S-benzyl-D-cysteine, L-valine, and S-benzyl-D-cysteine. The yield from each step, including removal of carbobenzoxy group and peptide formation, varied from 54 to 95%.

Protected pentapeptide (IX) was mildly saponified with sodium hydroxide to obtain the free acid (X), which was esterified with *p*-nitrophenol using dicyclohexylcarbodiimide. *p*-Nitrophenyl protected pentapeptide (XI) was treated with hydrogen bromide in acetic acid to remove the carbobenzoxy group. The hydrobromide (XII) was dissolved in dimethylformamide and subjected to cyclization by slow addition to an excess of hot pyridine. Addition to pyridine was done over a long period to prevent intermolecular reactions. This method was originally used for the synthesis of gramicidin S from the corresponding straight chain *p*-nitrophenyl decapeptide trifluoroacetate by Schwyzer and Sieber.[11] However, they observed double cyclization reactions with a pentapeptide,[12] and especially with tripeptides,[13,14] so as to aggregate two molecules in an anti-parallel fashion and give a dimer. This was not observed in our experiments.

After purification by alumina column chromatography, cyclic compound (XIII) was obtained in 17% yield. It did not react with ninhydrin, decomposed at high temperatures, and was difficultly soluble in numerous solvents. Infrared analysis (Fig. 1) showed no bands at 6·3 and 7·1 μ, regions associated with dipolar ion structures. Determination of molecular weight by the Signer method,[15] using trifluoroacetic acid,[16,17] proved this compound to be a cyclic pentapeptide. Amino acid analysis of the complete acid hydrolysate of (XIII) by the method of Moore and Stein[18] gave a molar ratio of leucine 1·00, isoleucine 0·95, allo-isoleucine 0·05, valine 0·93, benzylcysteine 0·93, half-cystine 0·36 and cysteic acid 0·04. Decomposition of benzylcysteine to cystine and cysteic acid during hydrolysis was confirmed employing benzyl-D-cysteine itself. The total low recovery of the latter three amino acids (combined molar ratio 1·33) could be explained by the unstability of benzylcysteine and cystine during hydrolysis.[3] Trace amounts of allo-isoleucine (5% of isoleucine) indicated that racemization of C-terminal amino acid during cyclization was not significant. Debenzylation of this compound (XIII) with sodium in liquid ammonia produced the sulfhydryl cyclic pentapeptide (XIV).

To avoid errors caused by partial disulfide formation, comparison of the synthetic with the natural compound was made most intensively with the S,S'-dibenzyl compounds. Both natural and synthetic compound had similar decomposition points (over 300° without melting) and i.r. spectra (Fig. 1). Powdered X-ray analysis was not sufficiently characteristic because of their amorphous nature. A significant difference was observed in optical rotation. Specific optical rotation of S,S'-dibenzylmalformin A was +62·7° ($c = 2\%$, trifluoroacetic acid), whereas synthetic compound (XIII) gave a value of +40·2° under similar conditions. The greatest difference observed was in the biological activity of the debenzylated compounds. The synthetic compound (XIV) induced no curvatures of corn roots[2] even at concentrations of 100 ppm, while natural reduced malformin A had optimum activity at 1 ppm. The i.r. spectrum of both reduced compounds was similar (Fig. 2).

It was clear that, although the synthetic and natural compounds had similar physical

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[17] K. MIYAO, *Bull. Agr. Chem. Japan* **24**, 23 (1960).
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properties, they were not identical. If the proposed structure of malformin A (I) is correct, differences between the synthetic and natural compound may be due to stereochemical differences of peptide bonds.

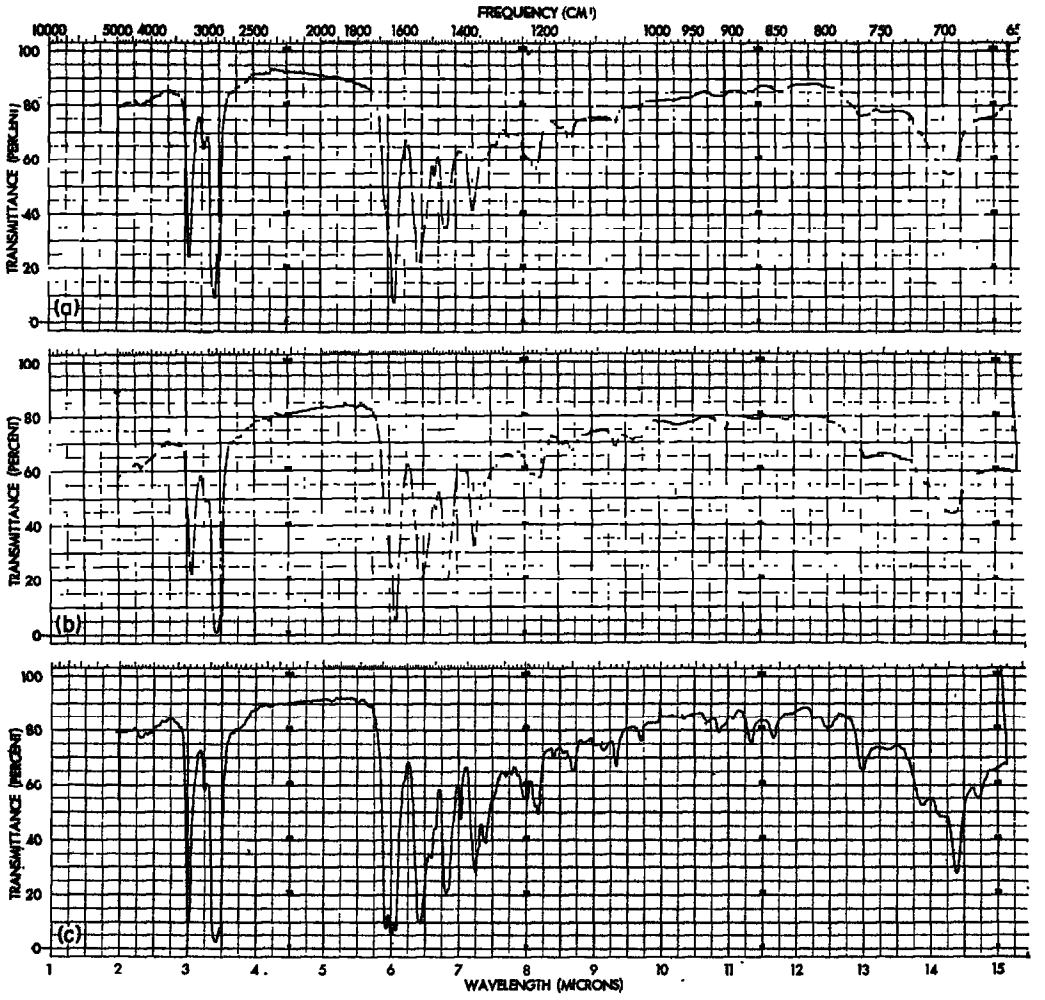

FIG. 1. INFRARED ABSORPTION SPECTRA.

(a) S,S'—dibenzylmalformin A,
(b) Cyclo-S-benzyl-D-cysteinyl-L-valyl-S-benzyl-D-cysteinyl-D-leucyl-L-isoleucyl (XIII), and
(c) Cyclo-S-benzyl-L-cysteinyl-L-valyl-S-benzyl-L-cysteinyl-L-leucyl-L-isoleucyl (XXIV).

An all L-cyclic pentapeptide with the same amino acid sequence was synthesized in the same manner. Yield of the cyclization reaction was 25%, and the cyclization product (XXIV) was shown to be mostly pentapeptide by molecular weight determination. The benzyl cyclic pentapeptide had a lower decomposition point (286–290° with melting) than that of the D and L compound, as well as a significantly different i.r. spectrum (Fig. 1). The debenzylated

free sulfhydryl compound (XXV) was also inactive in a malformin bioassay employing corn roots, even at concentrations of 100 ppm.

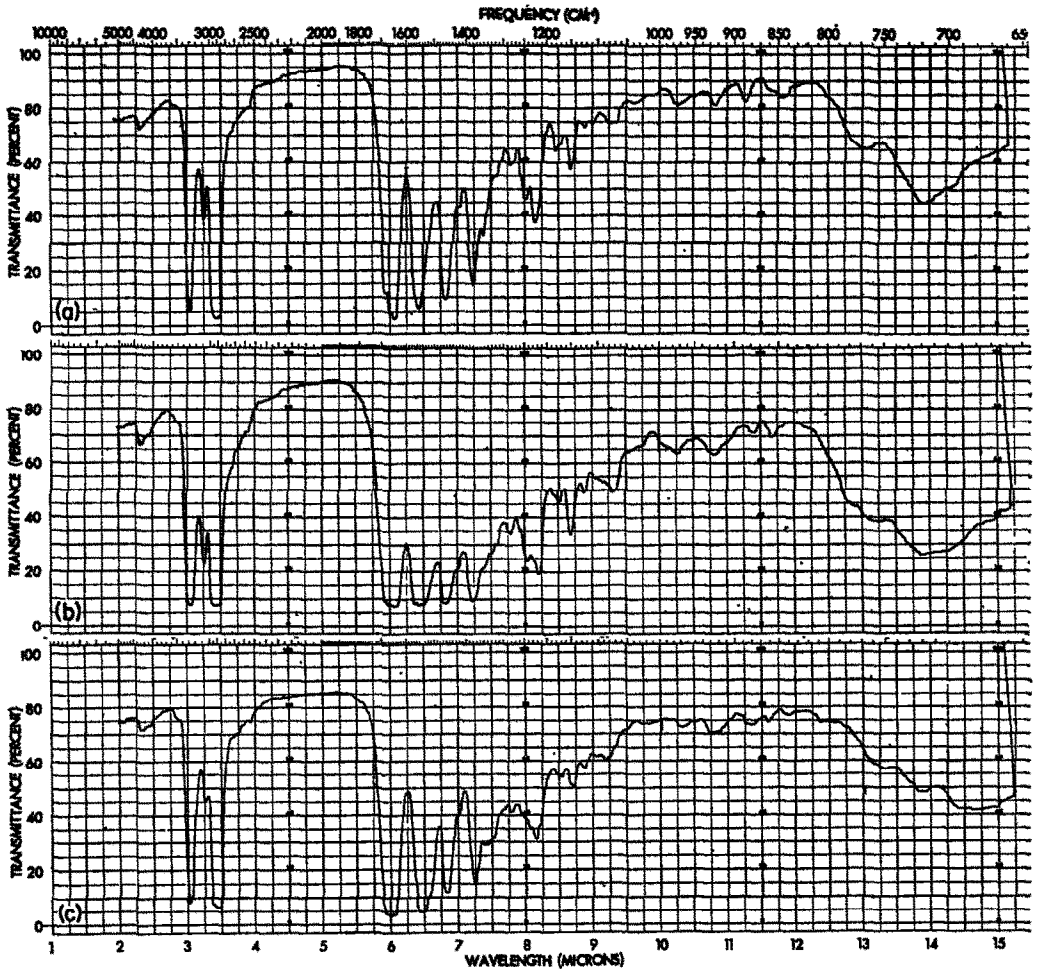

FIG. 2. INFRARED ABSORPTION SPECTRA.
(a) Reduced malformin A,
(b) Cyclo-D-cysteinyl-L-valyl-D-cysteinyl-D-leucyl-L-isoleucyl (XIV), and
(c) Cyclo-L-cysteinyl-L-valyl-L-cysteinyl-L-leucyl-L-isoleucyl (XXV).

EXPERIMENTAL

S,S'-Dibenzylmalformin A (Natural)

Malformin A, 200 mg, was suspended in liquid ammonia, 100 ml, and treated with sodium, 60 mg, in four 15-mg portions. Malformin dissolved and a blue color lasted for 15 min. Benzylchloride, 0·8 ml, was added and the solution stirred for 20 min. Ammonium chloride, 150 mg, was added and the resulting clear solution stirred for 10 min. Ammonia was evaporated, first under atmospheric pressure and finally *in vacuo*. The white residue was washed with water, 40 ml, ethanol, 40 ml, and ether, 20 ml; wt. 170 mg (63%), colorless powder.

A sample was recrystallized from dimethylformamide, d.p. over 300° without melting, $[\alpha]_D^{24} = +62{\cdot}7°$ ($c = 2$, trifluoroacetic acid). (Found: C, 62·20; H, 7·80; N, 10·24; S, 9·25. $C_{37}H_{53}N_5O_5S_2$ required: C, 62·43; H, 7·53; N, 9·84; S, 9·01%.) No biological activity was detected by corn root assay at concentrations as high as 100 ppm.

Reduced Malformin A (Natural)

S,S'-Dibenzylmalformin A, 40 mg, was suspended in liquid ammonia, 20 ml, and treated with sodium in capillary tubes until the compound was dissolved. Blue coloration was discharged by addition of excess ammonium chloride. The clear solution was evaporated in a nitrogen stream. The white residue was washed with 2% acetic acid, 40 ml, ethanol, 40 ml, ether, 20 ml, and dried over KOH and $CaCl_2$ *in vacuo*. Weight 25 mg (84%), colorless powder, d.p. over 300° without melting. (Found: C, 51·68; H, 7·55; N, 12·93; S, 12·07. $C_{23}H_{41}N_5O_5S_2$ required: C, 51·88; H, 7·71; N, 13·16; S, 12·06%.) The same compound was obtained by direct reduction of malformin A by sodium in liquid ammonia.

Methyl L-Isoleucinate Hydrochloride (II)

L-Isoleucine, 48 g, was esterified by methanolic hydrogen chloride. The product was recrystallized from acetone and ether; wt. 38·6 g (59%), colorless crystals, m.p. 95–98° (Ref. 19, 98–100°), $[\alpha]_D^{25} = +26{\cdot}3°$ ($c = 2{\cdot}051$ in water) (Ref. 19 $[\alpha]_D^{23} = +26{\cdot}6°$).

Carbobenzoxy Amino Acids

Amino acids were dissolved in equimolar volumes of 4 N sodium hydroxide and treated with carbobenzoxy chloride[20] in the usual manner. Details of preparation and properties of the individual carbobenzoxy amino acids are summarized in Table 1.

TABLE 1. CARBOBENZOXY AMINO ACIDS

Carbobenzoxy amino acids	Yield (%)	m.p. (° C)
D-Leucine	90	oil
L-Leucine	100	oil
S-Benzyl-D-cysteine*	94	90–95
S-Benzyl-L-cysteine*	84	90–95 (93–95)[21]
L-Valine	91	56–61 (57–59)[22]

* Prepared from corresponding cystine by reduction and benzylation.[23]

p-Nitrophenyl Esters of Carbobenzoxy Amino Acids

Methods of Bodanszky and duVigneaud[6] were employed. The reaction period was lengthened to overnight. All products were crystalline. Yields and properties of the nitrophenyl esters are summarized in Table 2.

[19] E. SMITH, D. H. SPACKMAN and W. J. POLGLASE, *J. Biol. Chem.* **199**, 803 (1952).
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[22] J. R. VAUGHAN and J. A. EICHLER, *J. Am. Chem. Soc.* **75**, 5556 (1953).
[23] J. L. WOOD and V. DU VIGNEAUD, *J. Biol. Chem.* **130**, 109 (1939).

TABLE 2. *p*-NITROPHENYL ESTERS OF CARBOBENZOXY AMINO ACIDS

Carbobenzoxy *p*-nitrophenyl amino acids	Yield (%)	m.p. (°C)	$[\alpha]_D^{25}$*
D-Leucine (III)	89	95–96·5	+33·7°
L-Leucine (XV)	88	93–95·5 (95)[6]	−31·8° (−33·5°)[6]
S-Benzyl-D-cysteine (V)	67	91–94	+42·5°
S-Benzyl-L-cysteine (XVII)	84	83–93 (93–94)[6]	−40·8°(−43°)[6]
L-Valine (VII)	59	66–67 (63)[24]	−23·7°

* $c = 2$, dimethylformamide.

*Methyl Carbobenzoxy-*D*-leucyl-*L*-isoleucinate (IV)*

II, 43·7 g, was dissolved in chloroform, 300 ml, and triethylamine, 31·9 ml, and III, 81·2 g, were added. The mixture was kept at 27° overnight, concentrated *in vacuo* to yield a yellowish syrup, and dissolved in ethyl acetate, 400 ml, and water, 100 ml. The ethyl acetate layer was washed with 1 N ammonia, 100 ml each of seven times, water, 100 ml each of three times, 1 N hydrochloric acid, 100 ml each of two times, and finally with water, 50 ml each of four times. The ethyl acetate was dried over sodium sulfate and concentrated *in vacuo* until a crystalline mass precipitated. The crystals were washed with ether: ligroin (1 :2); wt. 60·7 g, colorless crystals, m.p. 80–82°. From the washing 18·5 g of crystals were recovered; yield 95%. For analysis, small quantities were recrystallized from 50% ethanol; m.p. 80–82°, $[\alpha]_D^{25} = +6{\cdot}18$ ($c = 7{\cdot}36$, dimethylformamide). (Found: C, 64·40; H, 8·11, N, 7·19. $C_{21}H_{32}N_2O_5$ required: C, 64·26; H, 8·22; N, 7·11%.)

*Methyl Carbobenzoxy-*L*-leucyl-*L*-isoleucinate (XVI)*

Preparation of XVI was the same as for the D and L peptide (IV). From 12·7 g II and 23·2 g XV, 23·2 g of colorless crystals were obtained; yield 98%, m.p. 64–65°, $[\alpha]_D^{25} = -5{\cdot}91°$ ($c = 6{\cdot}06$, dimethylformamide). (Found: C, 64·05; H, 8·02; N, 7·13. $C_{21}H_{32}N_2O_5$ required: C, 64·26; H, 8·22; N, 7·11%.)

*Methyl N-carbobenzoxy-S-benzyl-*D*-cysteinyl-*D*-leucyl-*L*-isoleucinate (VI)*

IV, 27·5 g, was dissolved in methanol and palladium black,[25] freshly prepared from 4·2 g palladium chloride, was added. Hydrogen was passed through as the mixture was stirred. After 48 hr CO_2 was no longer evolved. The catalyst was filtered off and the solvent evaporated *in vacuo*, leaving a ninhydrin positive, slightly yellow oil, 21 g, which was dissolved in ethyl acetate, 140 ml. Compound V, 30·29 g, and 14 drops triethylamine were added. After 16 hr at 37° protected tripeptide ester began to crystallize; after 48 hr at 37° a crystalline slurry was obtained. After the slurry was chilled in a refrigerator the crystals were filtered off, washed three times with cold ethyl acetate, and dried, wt. 18·2 g, colorless needles, m.p. 134–135°. From the mother liquor an additional 3·4 g were isolated (yield 54%). For analysis a small sample was recrystallized from aqueous ethanol; m.p. 136–137°, $[\alpha]_D^{25} = +55{\cdot}9°$ ($c = 3{\cdot}26$, $CHCl_3$). Found: N, 7·32; S, 5·55. $C_{31}H_{43}N_3O_6S$ required: N, 7·17; S, 5·47%.)

[24] B. ISELIN, W. RITTEL, P. SIEBER and R. SCHWYZER, *Helv. Chim. Acta* **40**, 386 (1957).
[25] R. WILLSTÄTTER and E. WALDSCHMIDT-LEITZ, *Ber. Deut. Chem. Ges.* **54**, 113 (1921).

Methyl N-carbobenzoxy-S-benzyl-L-cysteinyl-L-leucyl-L-isoleucinate (XVIII)

XVI, 18·9 g, was dissolved in acetic acid, 25 ml, and acetic acid saturated with HBr, 50 ml, was added. After 1 hr at room temperature, dry ether, 1 l., and ligroin, 1 l., were added. After standing overnight at 4°, the precipitated oil was washed with ether five times and dried over KOH and $CaCl_2$ *in vacuo*, leaving a yellowish oil, 17·6 g. The hydrobromide thus obtained was dissolved in chloroform, 220 ml, and triethylamine, 7·5 ml, and compound XVII, 24 g, were added. After six days at 37° the mixture was dried *in vacuo*. The residue was dissolved in ethyl acetate, 300 ml, and washed with water, two times, 0·5 N ammonia, seven times, water, three times, 1 N HCl, two times, and water, three times. Ethyl acetate was dried over $MgSO_4$ and dried *in vacuo*, leaving a white residue which was crystallized from aqueous ethanol. Weight 31·12 g, white crystals, m.p. 127–145°. Because these crystals were contaminated with N-carbobenzoxy-S-benzyl-L-cysteinamide, they were dissolved in a minimum of ethyl acetate, adsorbed to an alumina column, and eluted with acetone. The first fractions were dried and recrystallized from aqueous ethanol to give the final product. Weight 21·1 g (yield 84%), colorless crystals, m.p. 146–148°, $[\alpha]_D^{25} = -26{\cdot}6°$ ($c = 3{\cdot}15$, $CHCl_3$). (Found: C, 63·71; H, 7·64; N, 7·28; S, 5·70. $C_{31}H_{43}N_3O_6S$ required: C, 63·56; H, 7·39; N, 7·17; S, 5·47%.)

Methyl Carbobenzoxy-L-valyl-S-benzyl-D-cysteinyl-D-leucyl-L-isoleucinate (VIII)

VI, 21·4 g, was suspended in acetic acid, 30 ml, and treated with acetic acid saturated with HBr, 60 ml. After 70 min at room temperature, ether, 1·2 l., and ligroin, 0·5 l., were added. The mixture stood for 3 hr at 4°. An oily precipitate was washed with ether several times and dried over KOH and $CaCl_2$ *in vacuo*, leaving a yellowish solid, 20·6 g. The solid was dissolved in chloroform, 200 ml, and treated with triethylamine, 9·2 ml, and compound VII, 14·8 g. After six days at 37° the solvent was removed *in vacuo* and the residue extracted with ethyl acetate, 400 ml, which was concentrated to a small volume. Crystallization occurred when the mixture was triturated. The crystals were filtered off and washed with ethyl acetate and ether. Weight 3·9 g, m.p. 183–185°. Additional crystals, 10·5 g, were obtained from the mother liquor after washing six times with 0·5 N ammonia, three times with 1 N hydrochloric acid and three times with water (yield, 57%). For analysis, a small sample was recrystallized from aqueous ethanol; colorless needles, m.p. 187·5–188°, $[\alpha]_D^{23} = +29{\cdot}3°$ ($c = 2{\cdot}47$, dimethylformamide). (Found: N, 8·04; S, 4·83. $C_{36}H_{52}N_4O_7S$ required: N, 8·18; S, 4·68%.)

Methyl Carbobenzoxy-L-valyl-S-benzyl-L-cysteinyl-L-leucyl-L-isoleucinate (XIX)

Procedures described above were followed. From 21·1 g of compound XVIII and 13·3 g of compound VII, 18·2 g (74%) of XIX were obtained; m.p. 192–194°. A small portion was recrystallized from aqueous ethanol, m.p. 194–195·5°, $[\alpha]_D^{23} = -27{\cdot}1°$ ($c = 2{\cdot}12$, dimethylformamide). (Found: N, 8·21; S, 4·54. $C_{36}H_{52}N_4O_7S$ required: N, 8·18; S, 4·68%.)

Methyl N-carbobenzoxy-S-benzyl-D-cysteinyl-L-valyl-S-benzyl-D-cysteinyl-D-leucyl-L-isoleucinate (IX)

VIII, 14·8 g, was suspended in acetic acid, 20 ml, and treated with acetic acid saturated with HBr, 40 ml. After 90 min at room temperature, dry ether, 650 ml, and ligroin, 350 ml, were added. After standing overnight at 4° the oily precipitate was washed with ether and dried over KOH and $CaCl_2$ for 3 hr, wt. 14·9 g. The brownish solid was dissolved in chloroform, 100 ml, to which was added triethylamine, 6·6 ml, and V, 10·07 g. After four days at 37° the mixture was dried *in vacuo*. The yellowish residue was washed with ethyl acetate, 300

ml, and 50% ethanol, 250 ml, leaving a white product, m.p. 192–196°, which was recrystallized from ethanol–chloroform, wt. 11·0 g, white micro-crystals, m.p. 193–197°. 1·6 g of the same compound were recovered from the ethyl acetate after washing with 0·5 N ammonia, 1 N HCl, and water and following alumina column chromatography (solvent, ethyl acetate:chloroform = 5:1). Yield, 12·5 g (66%). For analysis, a small quantity was recrystallized from aqueous ethanol and chloroform–ethyl acetate, m.p. 197–199·5°, $[\alpha]_D^{23} = +39{\cdot}8$ ($c = 2{\cdot}87$, dimethylformamide). (Found: C, 62·70; H, 7·17; N, 7·84; S, 7·27. $C_{46}H_{63}N_5O_8S_2$ required: C, 62·91; H, 7·23; N, 7·98; S, 7·29%.)

*Methyl N-carbobenzoxy-*S*-benzyl-*L*-cysteinyl-*L*-valyl-*S*-benzyl-*L*-cysteinyl-*L*-leucyl-*L*-isoleucinate* (*XX*)

Procedures used were the same as those described above. From 22·5 g XIX and 15·5 g XVII, 18·13 g (62%) recrystallized micro-crystals of XX were obtained, m.p. 221–224°, $[\alpha]_D^{23} = -27{\cdot}4°$ ($c = 2{\cdot}70$, dimethylformamide). (Found: N, 7·93; S, 7·50. $C_{46}H_{63}N_5O_8S_2$ required: N, 7·98; S, 7·29%.)

*N-carbobenzoxy-*S*-benzyl-D-cysteinyl-L-valyl-S-benzyl-D-cysteinyl-D-leucyl-L-isoleucine* (*X*)

Compound IX, 7·04 g, was dissolved in dioxane, 120 ml, and treated with 1 N sodium hydroxide, 9·6 ml, water, 6·4 ml, and methanol, 25 ml. After standing 3 hr at 27°, water, 1 l., was added. The turbid solution was acidified with 1 N hydrochloric acid, 10 ml, and refrigerated overnight. The precipitate was filtered, washed with water (white powder, 6·56 g), dissolved in acetone and subjected to alumina chromatography. Unsaponified compound, 2·5 g, was eluted from the column with acetone, while saponified product was eluted with 80% acetone and recrystallized twice from aqueous ethanol (white powder, 1·8 g, yield 40%, m.p. 185–195°). (Found: C, 62·44; H, 7·14. $C_{45}H_{61}N_5O_8S_2$ required: C, 62·54; H, 7·12%.)

*N-carbobenzoxy-*S*-benzyl-L-cysteinyl-L-valyl-S-benzyl-L-cysteinyl-L-leucyl-L-isoleucine* (*XXI*)

XX, 6·63 g, was saponified as described above. By alumina chromatography, unsaponified material (XX), 2·36 g, and saponified material (XXI) (2·26 g after recrystallization from aqueous ethanol, yield 55%, white powder, m.p. 197–201°) were recovered. (Found: C, 62·34; H, 7·03. $C_{45}H_{61}N_5O_8S_2$ required: C, 62·54; H, 7·12%.)

p-*Nitrophenyl N-carbobenzoxy-*S*-benzyl-D-cysteinyl-L-valyl-S-benzyl-D-cysteinyl-D-leucyl-L-isoleucinate* (*XI*)

X, 2·36 g, was dissolved in dimethylformamide, 15 ml, and ethyl acetate, 75 ml, cooled in an ice bath, and treated with *p*-nitrophenol, 460 mg, and dicyclohexylcarbodiimide, 570 mg. After 1 hr in the ice bath the solution was kept at room temperature overnight. Crystals of dicyclohexylurea were filtered off, 470 mg. The filtrate was dried *in vacuo*. The syrupy residue was crystallized from absolute ethanol, wt. 1·44 g (54%), white powder, m.p. 135–170°. (Found: N, 8·50; S, 6·79. $C_{51}H_{64}N_6O_{10}S_2$ required: N, 8·53; S, 6·51%.) *p*-Nitrophenolate liberated from this compound by dissolving in ethanol:NaOH (1:1, 1×10^{-5} M) was estimated spectrophotometrically[11] at 400 mμ. By comparison with a standard curve derived from *p*-nitrophenyl carbobenzoxy-D-leucine, purity was found to be 81%.

p-*Nitrophenyl N-carbobenzoxy-*S*-benzyl-L-cysteinyl-L-valyl-S-benzyl-L-cysteinyl-L-leucyl-L-isoleucinate* (*XXII*)

XXI, 3·02 g, was treated with *p*-nitrophenol, 580 mg, and dicyclohexylcarbodiimide, 720 mg, as described above. The compound was recrystallized twice from absolute ethanol.

Weight 1·59 g (47%), white micro-crystals, m.p. 187–194°. (Found: N, 8·80; S, 6·81. $C_{51}H_{64}N_6O_{10}S_2$ required: N, 8·53; S, 6·51%.) By photometric determination of *p*-nitrophenol, 91% purity was estimated.

p-Nitrophenyl S-*benzyl*-D-*cysteinyl*-L-*valyl*-S-*benzyl*-D-*cysteinyl*-D-*leucyl*-L-*isoleucinate Hydrobromide* (*XII*)

XI, 1·36 g, was treated with 5 ml 2·8 N HBr in acetic acid. After 80 min at room temperature (occasional shaking) the mixture was treated with dry ether, 80 ml, ligroin, 30 ml, and chilled. The precipitate was washed with ether six times and dried over KOH and $CaCl_2$ *in vacuo*. Weight 1·32 g, yellowish powder. Only one ninhydrin positive compound, R_f 0·95, was demonstrated by paper chromatography using butanol:acetic acid:water (4:1:5).

p-Nitrophenyl S-*benzyl*-L-*cysteinyl*-L-*valyl*-S-*benzyl*-L-*cysteinyl*-L-*leucyl*-L-*isoleucinate Hydrobromide* (*XXIII*)

XXII, 1·23 g, was treated as described above with 2·8 N HBr in acetic acid. Weight of hydrobromide, 1·25 g, yellowish powder. Only one ninhydrin positive compound was detected by paper chromatography.

Cyclo-S-*benzyl*-D-*cysteinyl*-L-*valyl*-S-*benzyl*-D-*cysteinyl*-D-*leucyl*-L-*isoleucyl* (*XIII*)

XII, 1·32 g, was dissolved in dimethylformamide, 50 ml, containing acetic acid, 0·3 ml. The solution was added dropwise, with stirring, into anhydrous pyridine, 300 ml, at 88° over a 16-hr period. The reaction mixture was heated an additional 3 hr, concentrated to dryness *in vacuo*, leaving a brownish residue which was extracted three times with 200-ml aliquots of ether. Each ether extract was tested for liberated *p*-nitrophenol by addition of 1 N sodium hydroxide. The first and second extract produced a yellow color, whereas the third extract was almost colorless. The residue also produced only a weak color with NaOH and gave a weak ninhydrin reaction.

The residue was washed with ethanol to remove a brownish material. The slightly brownish powder, 350 mg (36%), was dissolved in dimethylsulfoxide, 15 ml, and ethyl acetate, 90 ml, passed through a basic alumina column prepared as an ethyl acetate slurry, and eluted with ethyl acetate, 200 ml. All eluates were combined and concentrated to near dryness *in vacuo*. An excess of ethyl acetate was added, resulting in a colorless precipitate, which was filtered, and washed with ethyl acetate, aqueous ethanol, ethanol, acetone, and ether. Weight 160 mg (17%), colorless powder, d.p. over 300° (without melting), $[\alpha]_D^{24} = +40{\cdot}2$ ($c = 2$, trifluoroacetic acid). (Found: C, 62·19; H, 7·47; N, 9·65; S, 8·78. $C_{37}H_{53}N_5O_5S_2$ required: C, 62·43; H, 7·53; N, 9·84; S, 9·01%.)

The final product did not react with ninhydrin, and a saturated solution in methyl cellosolve had absorption at 260 mμ due to the benzyl group. Molecular weight was determined by the Signer method using trifluoroacetic acid as a solvent and recrystallized azobenzene as a standard. An approx. 0·05 M solution, calculated on the basis of a monomer, was used. Under evacuated, isothermal (27°) conditions, with constant shaking, equilibrium was reached in 10–15 days. (Found: M.W. 680, 657. $C_{37}H_{53}N_5O_5S_2$ required: M.W. 712.)

Cyclo-S-*benzyl*-L-*cysteinyl*-L-*valyl*-S-*benzyl*-L-*cysteinyl*-L-*leucyl*-L-*isoleucyl* (*XXIV*)

Compound XXIII, 1·05 g, was dissolved in dimethylformamide, 50 ml. Acetic acid, 0·25 ml, was added and the solution added dropwise, with stirring, into anhydrous pyridine at 90° over a period of 9 hr. The reaction mixture was heated an additional 3 hr. The residue, after

extraction with ether, produced neither a yellow color with 1 N sodium hydroxide nor did it react with ninhydrin. The residue, purified as described above, yielded 190 mg (25%) of colorless powder. M.p. 286–290°, $[\alpha]_D^{23} = -111°$ ($c = 1{\cdot}5$, trifluoroacetic acid). (Found: C, 62·25; H, 7·71; N, 9·67; S, 8·94. $C_{37}H_{53}N_5O_5S_2$ required: C, 62·43; H, 7·53; N, 9·84; S, 9·01%.) The molecular weight was determined as described above. (Found: 742. $C_{37}H_{53}N_5O_5S_2$ required: 712.)

Cyclo-D-*cysteinyl*-L-*valyl*-D-*cysteinyl*-D-*leucyl*-L-*isoleucyl* (*XIV*)

XIII, 40 mg, was debenzylated by sodium in liquid ammonia as described for the preparation of reduced malformin A. 25 mg (84%) of white powder were obtained, d.p. over 300° without melting. (Found: C, 50·58; H, 7·54; N, 12·41; S, 11·41. $C_{23}H_{41}N_5O_5S_2$ required: C, 51·88; H, 7·71; N, 13·16; S, 12·06%.)

Cyclo-L-*cysteinyl*-L-*valyl*-L-*cysteinyl*-L-*leucyl*-L-*isoleucyl* (*XXV*)

XXIV, 70 mg, was debenzylated as described above. 46 mg (53%) of a white powder were obtained, d.p. over 270°. (Found: C, 51·67; H, 7·83; N, 12·96; S, 11·84. $C_{23}H_{41}N_5O_5S_2$ required: C, 51·88; H, 7·71; N, 13·16; S, 12·06.)

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