mained unchanged. The tube was then heated at 131° for 2 hr. until a reaction accompanied by formation of a liquid and discoloration of the material in the tube appeared complete. Excess hydrogen chloride and the hydrogen sulfide formed in the reaction were allowed to escape from the tube at −78°. The reaction mixture was then separated to give about 1.6 g. (74%) of crude product. Recrystallization from carbon tetrachloride gave pure 2,5-bis(heptafluoropropyl)-1,3,4-thiadiazole, m.p. 30–31°. Infrared spectrum of this material matched those of the two previous preparations.

α,α′-Diaminopimelic Acid Peptides. VIII.[1a] Synthesis of Symmetrical Peptides Containing *meso*-α,α′-Diaminopimelic Acid, D- or L-Alanine, and L-Glutamic Acid[1b,c]

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The synthesis of five tripeptides and three pentapeptides containing *meso*-α,α′-diaminopimelic acid, D- or L-alanine, and L-glutamic acid is described. In these peptides the *meso*-α,α′-diaminopimelic acid residue is symmetrically substituted: (a) at both carboxyl groups (peptides I, II, and III); (b) at both amino groups (peptides IV, V, and VI); and (c) at all four functions (peptides VII and VIII).

Natural peptides containing α,α′-diaminopimelic acid have been found in the peptide–amino sugar polymer forming the essential part of the cell wall of several bacteria and blue-green algae.[3,4]

Their amino acid composition and sequence have been especially well studied in the case of *Escherichia coli*[5] and *Aerobacter cloacae*.[6]

It was shown in the case of the cell wall peptides of these two gram-negative bacteria that the amino and carboxyl functions of *meso*-diaminopimelic acid were nonsymmetrically substituted, this amino acid being linked first to either the α- or the γ-carboxyl function of a D-glutamic acid residue, secondly to the amino function of a D-alanine residue, and thirdly probably also to the carboxyl function of another D-alanine residue.[7]

In order to determine exactly which functions of *meso*-α,α′-diaminopimelic acid are involved in these peptide bonds, it seemed to us particularly desirable to have synthetic model peptides of well-defined structure and configuration. We have recently worked out methods for the preparation of such model peptides. Some of our results have been presented in preliminary communications at the European Peptide Symposia.[8]

The present paper describes the synthesis of five tripeptides and of three pentapeptides. They all include *meso*-α,α′-diaminopimelic acid symmetrically substituted with D- or L-alanine or L-glutamic acid and they were used as model substrates in enzymatic assays with various peptidases.[9] Their structure and configuration are illustrated in Scheme I.

In these syntheses the benzyloxycarbonyl group was used as the protecting group of the amino functions, whereas methyl and benzyl esters were used as temporary protecting groups of the α- and γ-carboxyl functions. The formation of the peptide bond was performed either by the mixed anhydride[10] or by the dicyclohexylcarbodiimide method.[11] Coupling by the azide method gave unsatisfactory results.

Benzyloxycarbonyl groups and benzyl esters were removed by catalytic hydrogenolysis and the methyl esters by saponification in the usual way. In the preparation of pentapeptides III, VII, and VIII a stepwise condensing procedure starting with the C-terminal amino acid was used. The preparation of some of the intermediates used in these syntheses (as well as the preparation of α-L-glutamyl-D-alanine dibenzyl ester hydrochloride) has been reported elsewhere.[1a,12,13]

The rotatory values found for the antipodes of each pair of the enantiomorphic peptides I and II, IV and V, and VII and VIII, which were synthesized in many cases by different procedures, were of the same magnitude and of opposite sign, thus indicating that no appreciable racemization had occurred during their synthesis. Optical purity was of the utmost importance for some of these peptides since they were to be used as enzymatic substrates in the study of the stereospecificity of various peptidases.[9]

The main difficulties encountered in these syntheses were due to (a) the low solubilities of certain derivatives in organic solvent, (b) the formation, during the coupling reactions or during the removal of the protecting groups, of monosubstituted derivatives which

(1) (a) Paper VII: E. Bricas, P. Dezélée, C. Gansser, P. Lefrancier, C. Nicot, and J. van Heijenoort, *Bull. soc. chim. France*, 1813 (1965). (b) A preliminary communication of a part of this work has been published: E. Bricas, C. Nicot, and J. van Heijenoort, *Compt. rend.*, **254**, 1685 (1962). (c) Taken in part from the thesis submitted by C. Nicot to the Université de Paris, Centre d'Orsay, Seine et Oise, France, in partial fulfillment of the requirements for the Doctorat ès Sciences, Sept. 1964.

(2) To whom inquiries should be addressed.

(3) M. R. J. Salton, "The Bacterial Cell Wall," Elsevier Publishing Co., Amsterdam, 1963, pp. 103, 104.

(4) H. Frank, M. Lefort, and H. H. Martin, *Z. Naturforsch.*, **17b**, 262 (1962); *Biochem. Biophys. Res. Commun.*, **7**, 322 (1962).

(5) J. Primosigh, H. Pelzer, D. Maas, and W. Weidel, *Biochim. Biophys. Acta*, **46**, 68 (1961); H. Pelzer, *ibid.*, **63**, 229 (1962).

(6) A. J. Schocher, S. T. Bayley, and R. W. Watson, *Can. J. Microbiol.*, **8**, 89 (1962); D. Jusic, C. Roy, and R. W. Watson, *Can. J. Biochem.*, **42**, 1553 (1964).

(7) W. Weidel and H. Pelzer, *Advan. Enzymol.*, **26**, 193 (1964).

(8) E. Bricas, C. Nicot, and J. van Heijenoort, Abstracts of Papers presented at the 4th European Peptide Symposium, Moscow, 1961 [*Coll. Czech. Chem. Commun.*, **27** 2240 (1962)]; E. Bricas and C. Nicot, Proceedings of the 6th European Peptide Symposium, Athens, 1963, L. Zervas, Ed., Pergamon Press, Oxford, 1965, p. 331; C. Nicot and E. Bricas, Proceedings of the 7th European Peptide Symposium, Budapest, 1964 (*Acta Chim. Acad. Sci. Hung.*, in press).

(9) (a) E. Bricas, C. Nicot, and E. Lederer, *Bull. soc. chim. biol.*, **44**, 1115 (1962); (b) C. Nicot and E. Bricas, *Compt. rend.*, **256**, 1391 (1963).

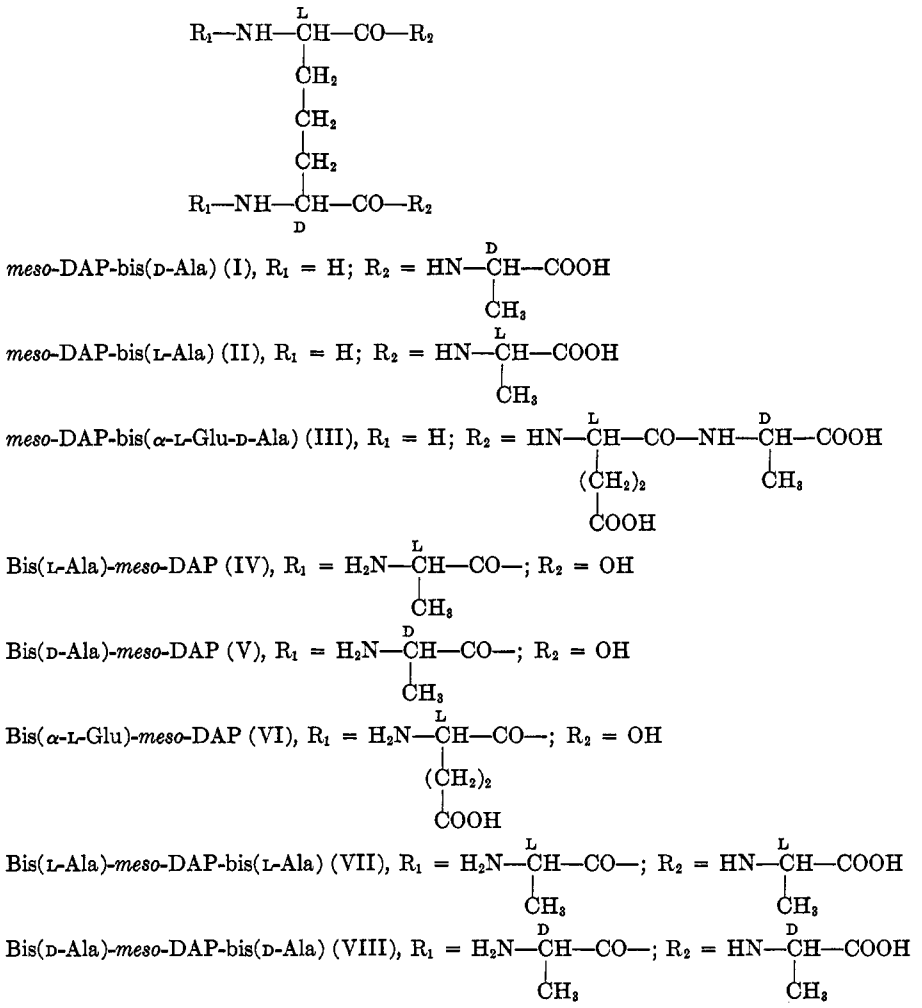
(10) T. Wieland and H. Bernhard, *Ann. Chem.*, **572**, 190 (1951); R. A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951); J. R. Vaughan, *J. Am. Chem. Soc.*, **73**, 3547 (1951).

(11) J. C. Sheehan and G. P. Hess, *ibid.*, **77**, 1067 (1955).

(12) P. Lefrancier, Thèse de Doctorat de 3ème Cycle, Université de Paris, 1963.

(13) P. Lefrancier and E. Bricas, *Bull. soc. chim. France*, in press.

SCHEME I



are often difficult to eliminate, and (c) the formation of gels during the precipitation of these peptides.

All the *meso*-α,α′-diaminopimelic symmetrical peptides synthesized yielded a reddish purple ninhydrin-positive spot on paper chromatography run in the Rhuland–Work solvent system,[14] whereas free diaminopimelic acid, as well as certain nonsymmetrical peptides and derivatives in which diaminopimelic acid has only one carboxyl function substituted, gave, under the same conditions, a characteristic green color.[15]

Furthermore the two enantiomorphic tripeptides IV and V had the same R_f value in the Rhuland–Work solvent system, whereas tripeptide II had in the same system a slightly higher R_f value than its antipode I (Table I). A similar difference of R_f value between two antipodes in this solvent system has already been established for the LL and DD forms of the diaminopimelic acid.[14]

TABLE I

R_f VALUES OF THE *meso*-DAP SYMMETRICAL PEPTIDES[a]

Peptides	Solvent system A[b]	Solvent system B[c]
meso-DAP-bis(D-Ala) (I)	0.15	1.7
meso-DAP-bis(L-Ala) (II)	0.15	2
Bis(L-Ala)-*meso*-DAP (IV)	0.11	1.8
Bis(D-Ala)-*meso*-DAP (V)	0.11	1.8
Bis(L-Ala)-*meso*-DAP-bis(L-Ala) (VII)	0.14	3
Bis(D-Ala)-*meso*-DAP-bis(D-Ala) (VIII)	0.14	3
Bis(α-L-Glu)-*meso*-DAP (VI)	0.07	1.1
meso-DAP-bis(α-L-Glu-D-Ala) (III)	0.09	3
meso-DAP and DD-DAP	0.08	1
LL-DAP	0.08	1.2

[a] Whatman No. I, descending technique. [b] 1-Butanol–pyridine–acetic acid–water (30:20:6:24) [S. G. Waley and J. Watson, *J. Chem. Soc.*, 475 (1953)]. [c] Methanol–pyridine–10 *N* hydrochloric acid–water[14] (80:10:2.5:17.5). With the latter system R_f values are expressed as multiples of distances traveled by a *meso*-DAP marker.

(14) L. E. Rhuland, E. Work, R. F. Denman, and D. S. Hoare, *J. Am. Chem. Soc.*, **77**, 4844 (1955).

Furthermore the two enantiomorphic tripeptides IV and V had the same R_f value in the Rhuland–Work solvent system, whereas tripeptide II had in the same system a slightly higher R_f value than its antipode I (Table I). A similar difference of R_f value between two antipodes in this solvent system has already been established for the LL and DD forms of the diaminopimelic acid.[14]

Experimental Section[16]

The melting points were determined in capillary tubes and are not corrected. Elemental analyses were carried out by the microanalytical laboratory of the Institut de Chimie des Substances Naturelles du C.N.R.S., Gif-sur-Yvette (Seine et Oise). Prior to analyses the peptide derivatives were dried under high vacuum at 80° or at room temperature in the case of some free peptides. The preparation of optically pure *meso*-DAP used in these syntheses has been reported in a previous paper.[1a] For the coupling reactions anhydrous reagents and dry freshly distilled solvents were used. Unless stated otherwise solvents were evaporated under vacuum at a bath temperature of 30–35° in a rotatory evaporator and dimethylformamide under high vacuum.

N,N′-Bis(benzyloxycarbonyl)-*meso*-diaminopimelylbis(D-alanine benzyl ester) (Ia). A. By the Mixed Anhydride Procedure.—A mixed anhydride was prepared in the usual manner from

(15) E. Bricas and C. Nicot, *J. Chromatog.*, **13**, 273 (1964).

(16) The following abbreviations are used: *meso*-DAP = *meso*-α,α′-diaminopimelic acid, Z = benzyloxycarbonyl, OMe = methyl ester, OBZL = benzyl ester, DCCI = dicyclohexylcarbodiimide, DCHU = dicyclohexylurea, DMF = dimethylformamide, and THF = tetrahydrofuran.

bis-Z-*meso*-DAP[1a] (1.4 g. 3 mmoles) in dry, peroxide-free THF (8 ml.), triethylamine (0.86 ml., 6 mmoles), and redistilled isobutyl chlorocarbonate (0.79 ml., 6 mmoles) with cooling to −10°. After 15 min. at this temperature an ice-cooled solution of D-alanine benzyl ester in THF [prepared from 2.74 g. (7.8 mmoles) of D-alanine benzyl ester *p*-toluenesulfonate according to the Hillmann procedure[17]] was added slowly. The mixture was stirred overnight at 4° and the solvent was evaporated. The residue was dissolved in chloroform and the solution was washed successively with several portions of 1 *N* hydrochloric acid, water, 7% potassium bicarbonate, and water and dried over magnesium sulfate. Evaporation of the chloroform gave an oily residue which was dissolved in hot dry ethanol. The gelatinous precipitate formed after cooling was collected by filtration and dried: yield 1.42 g. (60%), m.p. 145–148° with softening at 143°. After a new precipitation from the same solvent, the product had m.p. 147.5–149°, $[\alpha]^{19}D$ +13.4 ± 2° (*c* 1.4, DMF).

Anal. Calcd. for $C_{43}H_{48}N_4O_{10}$ (780.8): C, 66.14; H, 6.19; N, 7.17. Found: C, 65.87; H, 6.22; N, 7.21.

B. By the Carbodiimide Procedure.—This procedure is described in detail for the preparation of the antipodic derivative (IIa). The yield after precipitation from ethanol was 2.65 g. (85%), m.p. 147–149° with softening at 146°. After another precipitation from the same solvent, the product had m.p. 150–151°. The product was then dissolved in hot THF and crystallized after several weeks in the cold room: m.p. 166.5–167.5°, $[\alpha]^{16}D$ +14.9 ± 1° (*c* 2, DMF).

Anal. Found: C, 66.31; H, 6.29; N, 7.28.

N,N′-Bis(benzyloxycarbonyl)-*meso*-diaminopimelylbis(L-alanine benzyl ester) (IIa).—To a solution of bis-Z-*meso*-DAP (1.84 g., 4 mmoles) in distilled acetonitrile cooled to −5°, a solution of L-alanine benzyl ester in acetonitrile [from 3.65 g. (10.4 mmoles) of L-alanine benzyl ester *p*-toluenesulfonate] and 2.14 g. (10 mmoles) of DCCI were added. The reaction mixture was stirred for 2 hr. at −5° and overnight at 4°. A few drops of glacial acetic acid were added. The precipitate, which was collected and washed with acetonitrile, was found to be a mixture of the peptide derivative and of the DCHU. The combined filtrate and washings were evaporated under vacuum. The residue was dissolved in chloroform and the solution was washed with 1 *N* hydrochloric acid, water, 7% potassium bicarbonate, and water and dried over magnesium sulfate. After evaporation of the solvent the residue was dissolved together with the first precipitate in a minimum amount of hot DMF. After the solution was cool, a first crop of DCHU was obtained and concentrating the solution gave a second one [total yield of DCHU was 2.1 g. (90%), m.p. 231–232°]. The filtrate was evaporated under high vacuum and the residue was dissolved in hot absolute ethanol. The gelatinous precipitate formed was collected and dried: yield 2.47 g. (79%), m.p. 145–149° with softening at 138°. After another precipitation from the same solvent the product had m.p. 147–152°. Dissolved in hot THF the product crystallized after several weeks in the cold room: yield 1.98 g. (63.5%), m.p. 166–167.5°, $[\alpha]^{18}D$ −14.3 ± 1° (*c* 2, DMF).

Anal. Found: C, 65.88; H, 6.26; N, 7.25.

N,N′-Bis(benzyloxycarbonyl)-*meso*-diaminopimelylbis(L-alanine methyl ester) (IIb). A. By the Carbodiimide Procedure.—The same procedure as the one described above for the preparation of compound IIa was followed. The coupling reaction was accomplished with 7.87 g. (17.2 mmoles) of bis-Z-*meso*-DAP, 5.28 g. (37.8 mmoles) of L-alanine methyl ester hydrochloride and 7.8 g. (37.8 mmoles) of DCCI. The residue obtained after elimination of the DCHU (yield 85%) and evaporation of the solvent was triturated with hot methanol. A first crop of crystals was collected: 2.4 g. (25%), m.p. 216–217.5°. Two further crops with lower melting points were obtained. The total yield was 35%. After recrystallization from DMF, the product had m.p. 220–221.5°, $[\alpha]^{18}D$ −14.3 ± 1° (*c* 1.2, DMF).

Anal. Calcd. for $C_{31}H_{40}N_4O_{10}$ (628.6): C, 59.23; H, 6.41; N, 8.91. Found: C, 59.14; H, 6.42; N, 8.71.

B. By the Mixed Anhydride Procedure.—The same procedure as the one described above for the preparation of compound Ia was followed. The coupling reaction was accomplished by the action of the mixed anhydride of bis-Z-*meso*-DAP (3.9 g., 8.5 mmoles) and ethyl carbonic acid with L-alanine methyl ester (prepared from 3 g. of its hydrochloride). After evaporation of the solvent the residue was triturated with hot chloroform. The product was very slightly soluble in this solvent. The gel-like suspension thus obtained was filtered and washed copiously with chloroform and dried: yield 3.7 g. (70%), m.p. 221.5–223° with softening at 218°. The chloroform solution was washed successively with several portions of 1 *N* hydrochloric acid, water, 7% potassium bicarbonate, and water, and dried over magnesium sulfate. The residue obtained after evaporation of the solvent was crystallized from purified Methyl Cellosolve: yield 0.63 g., m.p. 221–223.5° (total yield 82%). After a further recrystallization from the same solvent, the product had m.p. 222–224° with softening at 218°.

Anal. Found: C, 59.39; H, 6.46; N, 8.90.

N,N′-Bis(benzyloxycarbonyl)-*meso*-diaminopimelylbis(D-alanine methyl ester) (Ib).—This compound was prepared by the mixed anhydride procedure as described above for the preparation of the enantiomorphic compound IIb: yield 60%, m.p. 222–225°, $[\alpha]^{19}D$ +13.4 ± 1.5° (*c* 2, DMF).

N,N′-Bis(benzyloxycarbonyl)-*meso*-diaminopimelylbis(L-alanine) (IIc).—To a solution of 820 mg. (1.05 mmoles) of bis-Z-*meso*-DAP-bis(L-Ala OBZL) (IIa) in 30 ml. of purified dioxane, 0.625 ml. of 4 *N* sodium hydroxide (2.5 mmoles) was added. After 2 hr. at 20° the solution was diluted with 70 ml. of water. The solution was concentrated under vacuum and the chilled aqueous solution was acidified with 6 *N* hydrochloric acid. The gel-like precipitate formed was filtered and washed with water: yield 587 mg. (93%). After a crystallization from absolute ethanol, the product had m.p. 188–189°, $[\alpha]^{20}D$ −5 ± 1° (*c* 2, DMF).

Anal. Calcd. for $C_{29}H_{36}N_4O_{10}$ (600.6): C, 57.98; H, 6.04; N, 9.33. Found: C, 57.55; H, 6.10; N, 9.36.

N,N′-Bis(benzyloxycarbonyl)-*meso*-diaminopimelylbis(D-alanine) (Ic).—This compound was prepared by the same procedure as described above for the preparation of the enantiomorphic compound IIc: m.p. 189.5–191°, $[\alpha]^{19}D$ +6 ± 1.5° (*c* 2, DMF).

***meso*-Diaminopimelylbis(D-alanine) (I).**—Bis-Z-*meso*-DAP-bis(D-Ala OBZL) (**Ia**) (300 mg., 0.38 mmole) was dissolved in 20 ml. of 80% aqueous acetic acid and hydrogenolyzed with 5% palladized carbon catalyst in a stream of hydrogen. After 2.5 hr. the hydrogenation was complete and the catalyst was removed. The filtrate was taken to dryness and the residue was redissolved in acetone; this procedure was repeated several times. Finally the residue was dissolved in a minimum amount of water and the free peptide was precipitated with absolute ethanol. A slightly hygroscopic white powder was collected by centrifugation: yield 123 mg. (98%), m.p. >300°, $[\alpha]^{20}D$ +47.7 ± 1° (*c* 2, 0.1 *N* hydrochloric acid). A sample for analysis was dried at room temperature for 18 hr. and at 110° for 3 hr.

Anal. Calcd. for $C_{13}H_{24}N_4O_6$ (332.25): C, 46.98; H, 7.28; N, 16.87. Found: C, 46.80; H, 7.40; N, 16.90.

***meso*-Diaminopimelylbis(L-alanine) (II).**—This compound was prepared by the same procedure as described above for the preparation of the enantiomorphic compound I: yield 89%, $[\alpha]^{18}D$ −48.2 ± 1° (*c* 2, 0.1 *N* hydrochloric acid).

Anal. Found: C, 47.1; H, 7.47; N, 16.66.

N,N′-Bis(benzyloxycarbonyl)-*meso*-diaminopimelylbis(γ-benzyl-L-glutamyl-D-alanine benzyl ester) (IIIa).—To a cooled solution of 1.9 g. (4.1 mmoles) of bis-Z-*meso*-DAP in acetonitrile a solution of γ-benzyl-L-glutamyl-D-alanine benzyl ester was added (prepared from 4.6 g. of its hydrochloride[18] according to the Hillmann procedure[17]). To this cooled solution 1.7 g. of DCCI (10.7 mmoles) was added. After the solution was stirred for 20 hr. at 4°, 0.5 ml. of acetic acid was added, a precipitate was collected, and the filtrate was put aside. The precipitate was dissolved in hot DMF and the DCHU precipitated after a night at 4° and was collected: 1.98 g. (80%), m.p. 232–234°. The filtrate was combined with the first filtrate and the mixture was taken to dryness. The residue was dissolved in ethyl acetate and the solution was washed with 1 *N* hydrochloric acid, water, 3% sodium bicarbonate, water, and dried over magnesium sulfate. After evaporation of the solvent, the crystalline residue was recrystallized from THF: yield 4 g. (80%), m.p. 148° with softening at 145°, $[\alpha]^{25}D$ +1.2 ± 1° (*c* 1.65, DMF).

Anal. Calcd. for $C_{67}H_{75}N_6O_{16}$ (1219.8): C, 65.93; H, 6.19; N, 6.88. Found: C, 66.01; H, 6.29; N, 7.08.

(17) G. Hillmann, *Z. Naturforsch.*, **1**, 682 (1946).

(18) Optically pure γ-benzyl-L-glutamyl-D-alanine benzyl ester hydrochloride, m.p. 109–110°, $[\alpha]^{22}D$ +52.3 ± 1° (*c* 3, H_2O), was obtained by deformylation of the N-formyl-γ-benzyl-L-glutamyl-D-alanine benzyl ester,[18,18] according to the procedure of Waley and Watson.[19]

(19) S. G. Waley and J. Watson, *Biochem. J.*, **57**, 529 (1954).

meso-**Diaminopimelylbis(α-L-glutamyl-D-alanine) (III).**—The above tetrabenzyl ester derivative IIIa (2.5 g.) was dissolved in 10 ml. of acetic acid. Hydrogenolysis was carried out with palladium black catalyst in a stream of hydrogen. After 5 hr. hydrogenation was complete. Concentration under vacuum of the filtrate gave a residue which was dried over sodium hydroxide pellets. The free peptide was crystallized by the addition of ethanol to a solution of the above residue in a minimum amount of water. The crystalline precipitate was collected by centrifugation and dried: yield 1.09 g. (98%), m.p. >300°, $[\alpha]^{25}D$ +4.7 ± 1° (*c* 1.65, 1 *N* hydrochloric acid).

Anal. Calcd. for $C_{23}H_{39}N_6O_{12}$ (591.6): C, 46.69; H, 6.64; N, 14.19. Found: C, 46.59; H, 6.50; N, 13.82.

N,N′-Bis(benzyloxycarbonyl-L-alanyl)-*meso*-diaminopimelic Dimethyl Ester (IVa).—This derivative was prepared by the same procedure as described above for the preparation of the compound Ia. The coupling reaction was accomplished by the reaction of the mixed anhydride of benzyloxycarbonyl-L-alanine (2.95 g., 13.2 mmoles) and isobutyl carbonic acid, with the *meso*-diaminopimelic dimethyl ester [prepared from 1.75 g. (6 mmoles) of its dihydrochloride[18]]. The residue was crystallized from ethyl acetate: m.p. 129–132°, yield 2.72 g. (73%). After recrystallization from the same solvent, the product had m.p. 134–136°, $[\alpha]^{17}D$ +7.5 ± 1° (*c* 2, DMF).

Anal. Calcd. for $C_{31}H_{40}N_4O_{10}$ (628.66): C, 59.22; H, 6.41; N, 8.91. Found: C, 59.16; H, 6.46; N, 9.05.

N,N′-Bis(benzyloxycarbonyl-D-alanyl)-*meso*-diaminopimelic Dimethyl Ester (Va).—This derivative was prepared by the same procedure as described above for the enantiomorphic compound IVa: yield 74%, m.p. 135.5–137.5°, $[\alpha]^{15}D$ −6.9 ± 1° (*c* 2, DMF).

Anal. Found: C, 59.49; H, 6.57; N, 9.06.

N,N′-Bis(benzyloxycarbonyl-D-alanyl)-*meso*-diaminopimelic Acid (Vb).—This derivative was obtained by saponification of compound Va by the same procedure as the one described for the preparation of compound IIc. In this case methanol was used instead of dioxane. The yield was 2.32 g. (86%), m.p. 141–144°. After crystallization from ethyl acetate a product (A) melting at 85–110° was formed. After recrystallization from the same solvent a product (B) melting at 146.5–148° was obtained. By a new recrystallization of the product B in the same solvent, a product (A) melting at 90–110° was again obtained, $[\alpha]^{14}D$ −11.3 ± 1° (*c* 2, DMF).

Anal. Calcd. for $C_{29}H_{36}N_4O_{10}$ (600.6): C, 57.99; H, 6.04; N, 9.33. Found for product A: C, 57.64; H, 6.05; N, 9.56. Found for product B: C, 57.83; H, 6.0; N, 9.54.

It is therefore extremely likely that products A and B are two different crystalline forms of the same product.

N,N′-Bis(benzyloxycarbonyl-L-alanyl)-*meso*-diaminopimelic Acid (IVb).—This derivative was obtained by saponification of compound IVa by the same procedure as was used for the preparation of the enantiomorphic compound Vb. Again two forms A and B were obtained as in the case of Vb. The yield was 82.5%: m.p. 103–111° for the form A and 147–148.5° for the form B, $[\alpha]^{16}D$ +10.4 ± 1° (*c* 2, DMF).

Anal. Found: C, 57.78; H, 6.02; N, 9.40.

meso-**Diaminopimelylbis(L- or D-alanine methyl ester) Di-*p*-toluenesulfonate (IId and Id).**—Bis-Z-*meso*-DAP-bis(L- or D-Ala OMe) [IIb or Ib, 628 mg. (1 mmole)] was dissolved in a mixture of acetic acid and absolute methanol and 413 mg. (2.4 mmoles) of anhydrous *p*-toluenesulfonic acid[20] was added. Hydrogenolysis was carried out with 5% palladized carbon catalyst in a stream of hydrogen. Concentration under vacuum of the filtrate and washings gave an oily residue which solidified when dried in the desiccator. The products were very hygroscopic and were used directly for the preparation of **VIIa** and **VIIIa**, respectively.

N,N′-Bis(benzyloxycarbonyl-γ-benzyl-L-glutamyl)-*meso*-diaminopimelic Acid Dibenzyl Ester (VIa). A. By the Carbodiimide Procedure.—To a solution of 1.48 g. (4 mmoles) of N-benzyloxycarbonyl-L-glutamic acid γ-benzyl ester[21] in distilled acetonitrile cooled to 0°, a solution of *meso*-diaminopimelic acid dibenzyl ester in 10 ml. of acetonitrile [prepared from 1.4 g. (2 mmoles) of its benzenesulfonate[18]] and 0.9 g. (4.4 mmoles) of DCCI were added. The reaction mixture was stirred for 4 hr. at room temperature and worked out as described above for the preparation of compound IIa; 0.93 g. (94%) of DCHU (m.p. 232–324°) was collected. The residue after evaporation of the solvent was crystallized from ethyl acetate: 1.47 g. (65%), m.p. 115–122° with softening at 109°. A second crop was obtained after addition of hexane on the filtrate: 200 mg., m.p. 104–110°. After a recrystallization of the first crop of crystals from ethyl acetate, the product had m.p. 116–120° with softening at 113° and, after a further recrystallization from acetonitrile, m.p. 119–123° with softening at 116°, $[\alpha]^{18}D$ −1 ± 1° (*c* 2, DMF).

Anal. Calcd. for $C_{61}H_{64}N_4O_{14}$ (1077.1): C, 68.01; H, 5.98; N, 5.20. Found: C, 67.98; H, 6.04; N, 5.43.

B. By the Mixed Anhydride Procedure.—The same procedure as the one described above for the preparation of compound Ia was followed. The coupling reaction was accomplished by the action of the mixed anhydride of Z-L-Glu γ-OBZL (1.56 g., 4.2 mmoles) and isobutyl carbonic acid with *meso*-DAP diOBZL in THF [prepared from 1.4 g. (2 mmoles) of its benzenesulfonate[18]] and the reaction mixture was worked up as described above for the preparation of compound Ia. After recrystallization from ethyl acetate 1.9 g. (88%) of the tetraester derivative was obtained: m.p. 118–124°. After a further recrystallization from acetonitrile, the product had m.p. 119–123°, $[\alpha]^{22}D$ −1.5 ± 1° (*c* 2, DMF).

N,N′-Bis(α-glutamyl)-*meso*-diaminopimelic Acid (VI).—The tetrabenzyl ester derivative (VIa) (500 mg., 0.46 mmole) was dissolved in 15 ml. of acetic acid and 2 ml. of water. The hydrogenolysis was carried out with 5% palladized carbon catalyst in a stream of hydrogen. After elimination of the catalyst, filtrate and washings were taken to dryness and the residue was dissolved in a minimum amount of water. The free peptide was precipitated with absolute ethanol: yield 208 mg. (90%), m.p. >300°, $[\alpha]^{22}D$ +39.2 ± 1.5° (*c* 2, 0.1 *N* hydrochloric acid). A sample for analysis was dried at room temperature for 3 hr.

Anal. Calcd. for $C_{17}H_{28}N_4O_{10}\cdot 2.5H_2O$ (493.4): C, 41.37; H, 6.75; N, 11.35. Found: C, 41.60; H, 7.05; N, 11.11.

N,N′-Bis(benzyloxycarbonyl-L-alanyl)-*meso*-diaminopimelylbis(L-alanine methyl ester) (VIIa).—Z-L-Alanine (535 mg., 2.4 mmoles) was dissolved in 5 ml. of THF and, after addition of 0.4 ml. of triethylamine and cooling to −10°, 0.23 ml. of ethyl chlorocarbonate was added. After 15 min. at this temperature a cooled solution of *meso*-DAP-bis(L-Ala OMe) in THF was added. [The solution of the free ester was obtained according to the Hillmann procedure[17] from di-*p*-toluenesulfonate (IId) which had been prepared by hydrogenolysis of 628 mg. (1 mmole) of the bis-Z-*meso*-DAP-bis(L-Ala OMe) (IIb) in the presence of toluenesulfonic acid.] The mixture was stirred 2 hr. at −10° and left overnight at 4°. After evaporation of the solvent the residue was triturated with hot chloroform. A fraction insoluble in chloroform was collected: 570 mg. (74%), m.p. 224–226.5° with softening at 222°. A second fraction was obtained from the chloroform solution: 50 mg., m.p. 205–212°. After the first fraction was dissolved in hot Methyl Cellosolve and cooled, a gel-like precipitate was obtained: m.p. 224.5–226.5°, $[\alpha]^{22}D$ −8.4 ± 1° (*c* 2.25, DMF).

Anal. Calcd. for $C_{37}H_{50}N_6O_{12}$ (770.8): C, 57.64; H, 6.53; N, 10.90. Found: C, 57.71; H, 6.88; N, 10.96.

N,N′-Bis(benzyloxycarbonyl-D-alanyl)-*meso*-diaminopimelylbis(D-alanine methyl ester) (VIIIa).—This derivative was obtained by the same procedure as the one described for the preparation of the enantiomorphic compound VIIa: yield 79%, m.p. 224–227°, $[\alpha]^{19}D$ +9.5 ± 1° (*c* 2, DMF).

N,N′-Bis(benzyloxycarbonyl-L-alanyl)-*meso*-diaminopimelylbis(L-alanine) (VIIb).—To a solution of 230 mg. (0.3 mmole) of the above-described dimethyl ester (VIIa) in 10 ml. of purified Methyl Cellosolve, 0.35 ml. of 2 *N* sodium hydroxide was added and the mixture was left at room temperature for 2 hr. After addition of water the solution was concentrated under vacuum to a volume of 5 ml. and after cooling was acidified with 6 *N* hydrochloric acid. The precipitate formed was collected, washed with water, and dried: yield 190 mg. (85%), m.p. 192.5–196.5°. The product was successively precipitated from absolute ethanol and from a mixture of Methyl Cellosolve and ether: m.p. 194–197.5°, $[\alpha]^{22}D$ −1 ± 1° (*c* 2, DMF).

Anal. Calcd. for $C_{35}H_{46}N_6O_{12}$ (742.7): C, 56.59; H, 6.24; N, 11.31. Found: C, 56.21; H, 6.33; N, 11.30.

N,N′-Bis(benzyloxycarbonyl-D-alanyl)-*meso*-diaminopimelylbis(D-alanine) (VIIIb).—This derivative was prepared by saponi-

(20) Anhydrous *p*-toluenesulfonic acid was prepared by dissolving the monohydrate in hot carbon tetrachloride and by concentrating the solution to a third of its initial volume. The crystals formed were collected and dried.

(21) W. E. Hanby, S. G. Waley, and J. Watson, *J. Chem. Soc.*, 3229 (1950).

fication of the compound VIIIa: yield 94%, m.p. 195.5–198.5°, $[\alpha]^{20}D$ +2.1 ± 1° (*c* 2, DMF).

N,N'-Bis(D-alanyl)-*meso*-diaminopimelylbis(L-alanine) (VII).—Bis(Z-L-Ala)-*meso*-DAP-bis(L-Ala) (VIIb) (180 mg., 0.25 mmole) was dissolved in aqueous methanol and a few drops of acetic acid. Hydrogenolysis was carried out with 5% palladized carbon catalyst in a stream of hydrogen. After 2 hr. the hydrogenation was complete and the catalyst was removed; filtrate and washings were concentrated under vacuum. The residue was redissolved in acetone and after evaporation was dissolved in a minimum amount of water and the free peptide was precipitated with ethanol. A gel-like precipitate was collected and dried: yield 93 mg. (81%) of hygroscopic white powder. After total hydrolysis of the product by 6 *N* hydrochloric acid (18 hr. at 110°) the amount of DAP and Ala was determined by a method previously described[9] and 0.036 μmole of DAP and 0.150 μmole of Ala were found, thus confirming the molecular ratio 1:4 for the constituents of this pentapeptide: m.p. >300°, $[\alpha]^{22}D$ −16.7 ± 1° (*c* 1.35, 0.1 *N* hydrochloric acid). A sample for analysis was dried at room temperature for 20 hr.

Anal. Calcd. for $C_{19}H_{34}N_6O_8 \cdot 2H_2O$ (510.5): C, 44.69; H, 7.50; N, 16.46. Found: C, 44.64; H, 7.62; N, 16.0.

N,N'-Bis(D-alanyl)-*meso*-diaminopimelylbis(D-alanine) (VIII).—This pentapeptide was prepared by the same procedure as the one described for the preparation of the enantiomorphic compound VII: yield 80%, m.p. >300°, $[\alpha]^{19}D$ +17.7 ± 1° (*c* 1.3, 0.1 *N* hydrochloric acid).

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Oxidation of Aromatic Acids. V. Preparation of Salicylic Acids from Benzoic Acids

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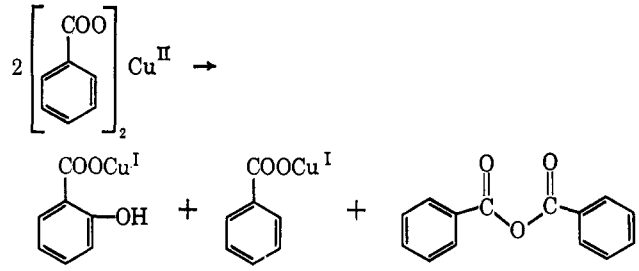
Salicylic acid was produced from benzoic acid by heating the cupric salt of the latter in a variety of aprotic media. Cupric ion was the oxidizing agent. This reaction has also been successfully used with a variety of substituted benzoic acids with at least one vacant *ortho* position. The use of air for the oxidation of benzoic acid to salicylic acid is discussed.

A previous paper of this series[2] has described a method for the preparation of salicylic acids from the corresponding benzoic acid by the thermal rearrangement of the basic cupric salt. This procedure has limited practical value for syntheses because of the necessity to prepare the basic salt and because of the low conversions to product. Free acid must also be rigorously excluded from the system to prevent the destruction of the basic salt.

On the other hand, the normal cupric salt can be prepared with ease by the reaction between the oxide, hydroxide, or carbonate of copper with benzoic acid. The thermal rearrangement of cupric benzoate in benzoic acid solution, in the presence of air and water, is being used for the large-scale industrial production of phenol.[3] Studies concerning the mechanism of this reaction have suggested that salicylic acid was a transient intermediate which rapidly decarboxylated during the oxidation of benzoic acid to phenol.[4] An interpretation of recent kinetic work on the mechanism of this reaction has suggested a bimolecular reaction between the salicylate anion and a proton.[5] Conditions were very favorable for this since the solvent, benzoic acid, provided a large source of protons and the catalysts and promoters facilitated the formation of salicylate anion.

If the thermal rearrangement of cupric benzoate were carried out in an aprotic medium, and if the decarboxylation mechanism suggested is valid, the proposed salicylic acid intermediate would be denied a proton and could not decarboxylate. When this theory was tested experimentally, salicylic acid was isolated as a major product of the reaction. Therefore, a new route is available for the preparation of salicylic acid, starting with benzoic acid. This reaction appears to be general and can also be utilized for the convenient preparation of a number of substituted salicylic acid derivatives.



Results

Salicylic acids were produced simply by heating a suspension of the cupric salt of the aromatic carboxylic acid in an aprotic media. The starting material was conveniently prepared *in situ* by the addition of basic cupric carbonate to a solution of the aromatic acid in the reaction medium. The thermal rearrangement appeared to occur as the cupric salt dissolved. The characteristic blue or blue-green color disappeared and was replaced by a solid with a dull copper to gold cast. The latter was a mixture of the cuprous salts of the starting acid and the salicylic acid produced by the thermal rearrangement. The results are summarized in Table I.

A variety of aprotic media was tested. Aliphatic hydrocarbons appeared to be inert. A high-boiling mineral oil which was a mobile liquid at room temperature was convenient because the reaction could be carried to completion at atmospheric pressure. Cyclo-

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