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A Convenient Synthesis of 2,5-Piperazinediones[1a]

KENNETH D. KOPPLE AND HAGOP G. GHAZARIAN[1b]

Department of Chemistry, Illinois Institute of Technology, Chicago, Illinois 60616

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We have developed a simple and convenient one-step conversion of unblocked dipeptides, or their hydrobromide salts, to cyclic dipeptides (3,6-dialkyl-2,5-piperazinediones). Cyclization occurs on heating in phenol just below the boiling point of phenol; the reaction does not appear to be accompanied by significant side reactions. Cyclic dipeptides prepared in this manner are listed in Tables I and II. As Table II indicates, cyclization in hot phenol is effective in cases where one of the amino acid residues of the dipeptide is potentially sensitive; it has been achieved in excellent yield with L-seryl-L-tyrosine, L-methionyl-L-tyrosine, and glycyl-L-tryptophan, among other peptides. When the reaction is carried out under nitrogen, there is no discoloration of the reaction mixture; only piperazinediones and traces of starting materials are detected by thin layer chromatography of the crude reaction products.

TABLE I
PREPARATIONS OF PREVIOUS REPORTED 2,5-PIPERAZINEDIONES *via* HEATING DIPEPTIDES IN PHENOL

Dipeptide used	Yield, %	Mp, °C Obsd[a]	Reptd
Gly-Val	84	264–265	260–266[b]
Gly-Leu	22	250–251	254–255[c]
Leu-Gly	39	250–251	254–255[c]
Gly-DL-Phe	54	282–283	277–280[d]
Gly-Tyr	78	287–288.5	295[e]
Gly-Trp	91	299.5–300.5	292–303[f]
Gly-His·2HBr	50	244–245[g]	242–243[g]
Leu-Tyr	99	295–296	310[h]

[a] All melting points with partial decomposition; corrected values given. [b] E. Fischer and H. Schiebler, *Ann.*, **363**, 142 (1908). [c] E. Fischer, *Ber.*, **39**, 2914 (1906). [d] E. Fischer and P. Blank, *Ann.*, **354**, 4 (1907). [e] E. Fischer and W. Schrauth, *ibid.*, **354**, 28 (1907). [f] K. Hofmann and S. Lande, *J. Am. Chem. Soc.*, **83**, 2286 (1961). [g] J. Sheehan and D. McGregor, *ibid.*, **84**, 3000 (1962); however, see Experimental Section. [h] E. Fischer, *Ber.*, **37**, 2498 (1904).

Heating a dipeptide in phenol does not result in loss of optical activity. Where the initial dipeptide contains two optically active amino acid residues, racemization, if it occurred, would lead to formation of diastereomeric cyclic dipeptides, but formation of diastereomers is not observed on cyclizing L-leucyl-L-tyrosine and D-leucyl-L-tyrosine by this method; each of these peptides affords a single product, as described in the Experimental Section. Proton magnetic resonance studies, described elsewhere,[2] confirm that the LL dipeptide yields the piperazinedione with the side chains *cis* and the DL isomer yields the ring with the side chains *trans*.

Protonation of the dipeptide terminal amino group by hydrogen bromide does not prevent cyclization; presumably the amine hydrobromide dissociates under the reaction conditions. The crude hydrobromides of both L-leucyl-L-tyrosine (prepared by dissolving the free dipeptide in 30% hydrogen bromide in acetic acid, then precipitating with anhydrous ether) and glycyl-L-histidine (prepared by dissolving benzyloxycarbonylglycyl-L-histidine in 30% hydrogen bromide in acetic acid, then precipitating with anhydrous ether) have successfully been used as starting materials.

A cyclization procedure similar to that reported here, but using molten β-napthol at 135–140°, was reported some years ago;[3] the solvent was removed by ether extraction. Only optically inactive peptides were examined, so that racemization, if any, was undetected. Phenol, however, is undoubtedly a solvent preferable to β-napthol: it has a lower melting point, is readily available in pure form, can be easily removed by sublimation, and is soluble in water, from which many of the piperazinediones can be crystallized.

Unsymmetrical cyclic dipeptides commonly have been prepared by generating a free dipeptide methyl or ethyl ester from an N-protected dipeptide ester; the blocked ester is obtained by standard peptide synthetic methods and the free dipeptide ester generally cyclizes with very little encouragement. It is likely that a dipeptide ester salt, such as the hydrobromide obtained on removal, say, of an N-carbobenzyloxy group, would also cyclize in hot phenol. This might offer advantages in terms of product purification, since no base would have to be added to free the amino group for reaction. In any event, given the free dipeptides, many of which are commercially available, the use of hot phenol is clearly the cyclization method of choice.

Use of phenol, rather than ethylene glycol[4], as a solvent for the thermal cyclodimerization of amino acids to amino acid anhydrides might also appear advantageous in some cases, because dark by-products, common when glycol is the solvent, are not formed in phenol. However, although DL-phenyalanine does undergo dimerization in phenol, L-tyrosine is too insoluble, and is recovered unchanged after heating for 5 hr at 150°. The phenol method is thus not likely to be general for cyclodimerization of amino acids. On the other hand, all the dipeptides used dissolved in phenol shortly after heating was begun and no failures to cyclize were noted.

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TABLE II

SOME PREVIOUSLY UNREPORTED 2,5-PIPERAZINEDIONES

Amino acid residues[e]	Mp, °C	Yield, %	Molecular formula	Analysis[c] Calcd, % C	H	N	Found, % C	H	N
D-Leu-Tyr	248–249	75	$C_{15}H_{20}N_2O_3$ (1)	65.19	7.30	10.14	65.37	7.33	10.18
Val-Tyr	271–271.5	63[f]	$C_{14}H_{18}N_2O_3$ (2)	64.10	6.92	10.68	64.22	6.68	10.75
Ala-Tyr	286–287	~100	$C_{12}H_{14}N_2O_3$ (3)	61.52	6.02	11.96	61.03	6.19	11.87
Met-Tyr	294–295	89	$C_{14}H_{18}N_2O_3S$ (4)	57.13	6.17	9.52	57.18	6.16	9.60
Ser-Tyr	271.5–272.5	~100	$C_{12}H_{14}N_2O_4$ (5)	57.59	5.64	11.20	57.75	5.72	11.13
Gly-DL-homoPhe[g]	230–232	92	$C_{12}H_{14}N_2O_2$ (6)	66.03	6.47	12.84	66.06	6.50	12.56
Ala-CMTyr[a]	272–274	33[b]	$C_{14}H_{16}N_2O_5$ (7)	57.53	5.52	9.59	57.23	5.49	9.59
Val-CMTyr[a]	232–234	61[b]	$C_{16}H_{20}N_2O_5$ (8)	59.99	6.29	8.75	59.89	6.46	9.06
Leu-CMTyr[a]	217–219	64[b]	$C_{17}H_{24}N_2O_6$[d] (9)	57.94	6.87	7.95	58.37	7.04	8.10
D-Leu-CMTyr[a]	225–226	37[b]	$C_{17}H_{22}N_2O_5$(10)	61.06	6.63	8.38	60.77	6.64	8.63

[a] CMTyr = O-Carboxymethyltyrosyl. [b] Over-all yield of alkylation and hydrolysis steps. [c] Samples were dried 16 hr at 0.05 mm and 100°. Analyses were performed by Micro-Tech Laboratories, Skokie, Ill. [d] Held one molecule of water after drying; cyclic structure indicated by negative ninhydrin reaction. [e] Sequence of the starting dipeptide. [f] Some mechanical losses. [g] homoPhe = α-amino-γ-phenylbutyric acid.

Several of the tyrosine-containing cyclic dipeptides prepared in this work were desired in a water-soluble form and were therefore converted into O-carboxymethyl derivatives. The first step in this procedure was to treat the peptide with sodium ethoxide in cold ethanol to form the phenolate ion. Because the α-hydrogens of the amino acid residues of a diketopiperazine are weakly acidic, racemization occurs under the influence of alkoxide; therefore no large excess of alkoxide was used and temperatures were kept low. The solvent was removed at 0° and the solid sodium salt was transferred to dry dimethylformamide, where it was allowed to react with excess methyl bromoacetate. The resulting methoxycarbonylmethyl derivative was directly saponified in water and precipitated on acidification. Table II includes the tyrosyl peptides to which this process was applied and lists the resulting carboxymethyl compounds. In one case, cyclo(L-tyrosyl-L-alanyl), a trace, perhaps 2–3%, of epimerized product was detected in the proton magnetic resonance spectrum.[5]

Experimental Section

Cyclization of Dipeptides in Phenol.—The dipeptides used were obtained from Mann Research Laboratories or Cyclo Chemical Corp., with the exception of N-glycyl-DL-α-amino-γ-phenylbutyric acid, the preparation of which is described later, and glycyl-L-histidyl dihydrobromide, which was obtained by treatment of benzyloxycarbonylglycyl-L-histidine with hydrogen bromide in acetic acid. The cyclization procedure used was the same in all cases. Two examples are given below. The cyclic dipeptides reported in Tables I and II were crystallized from water or ethanol–water except for cyclo(glycyl-L-histidyl). Corrected melting points, analytical data, and literature citations appear in the tables.

cyclo(L- (and D-) Leucyl-L-tyrosyl).—L-Leucyl-L-tyrosine (0.24 g) was mixed with 10 g of phenol and heated under nitrogen, with magnetic stirring, in an oil bath held at 140–150°. The dipeptide dissolved in the molten phenol within a few minutes, but heating was continued for 1 hr. Phenol was then removed by sublimation under vacuum at room temperature and the residue was crystallized from ethanol–water to give 0.224 g (99%) of chromatographically homogeneous cyclo(L-leucyl-L-tyrosyl), mp 292–293°. A second crystallization raised the mp to 295–296°.

In a similar manner was prepared cyclo(D-leucyl-L-tyrosyl), mp 248–249°. The two diastereomeric cyclic dipeptides separated on thin layer chromatography on silica when 2-butanol, chloroform–methanol–acetic acid (70:10:5),[6] or 2-propyl ether–chloroform–acetic acid (6:3:1)[6] were used as solvents. The DL isomer had the higher R_F value in each solvent system. A hypochlorite–iodide spraying sequence[7] was used to detect the peptides and each crude cyclization product was shown to be free of its diastereomer.

cyclo(Glycyl-L-histidyl).—Glycyl-L-histidine dihydrobromide (0.60 g) was mixed with 10 g of phenol and heated for 5 hr, with stirring, under nitrogen at 140–150°. Solution of the peptide occurred within a few minutes. Most of the phenol was removed by sublimation under vacuum at room temperature, but a significant quantity remained bound to the residue. The residue was triturated with several portions of anhydrous ether; it was thereby converted into a colorless crystalline material that was crystallized once from water–acetone, to yield 0.21 g (50%) of a chromatographically homogeneous product, mp 244–245° dec. This material contained bromide ion, although its melting point was coincident with that reported for free cyclo(glycyl-L-histidyl).[8] It was converted into the free base by passage, in aqueous solution, through a column of BioRad AG3 X4 resin (hydroxide form). The free base was crystallized from acetone–water and had mp 269–271° dec.

Anal. Calcd for $C_8H_{10}N_4O_2$: C, 49.48; H, 5.19; N, 28.85. Found: C, 49.37; H, 5.40; N, 28.71.

Glycyl-L-histidine monohydrochloride monohydrate (Cyclo Chemical Corp.) yielded, on cyclization, a hydrochloride, mp 264–265° dec, that was converted into a free base identical with that prepared from the bromide.

N-Glycyl-DL-α-amino-γ-phenylbutyric Acid (Glycyl-DL-homophenylalanine).—α-Amino-γ-phenylbutyric acid (Cyclo Chemical Corp., 4.9 g, 0.027 mole) in 50 ml of 4 *N* sodium hydroxide was stirred vigorously at 0° during addition of 9.3 g (0.082 mole) of choloroacetyl chloride. Addition of the acid chloride required 1 hr. The reaction mixture was then acidified to pH 1.7 and stored at 0–5° overnight to allow solidification of the precipitated N-chloroacetyl compound, mp 115–116°.

Without purification, the N-chloroacetyl compound (6.8 g) was pulverized and stirred 72 hr with 170 ml of 30% aqueous ammonia. The resulting solution was evaporated to dryness at reduced pressure, taken up in water, and again evaporated to dryness. The process was repeated twice more to yield a product that gave a negative test with Nessler's reagent. This was crystallized twice from water–acetone to give glycyl-DL-homophenylalanine, mp 226–228°, in 56% over-all yield, based on homophenylalanine (3.63 g of dipeptide).

An analytical sample, mp 228–230°, was obtained by recrystallization from water and was dried at 0.01 mm and 100° overnight. Acid hydrolysis of a portion of this sample yielded only glycine and α-amino-γ-phenylbutyric acid, as detected by thin layer chromatography.

Anal. Calcd for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.83; N, 11.86. Found: C, 60.68; H, 7.10; N, 11.96.

(5) There is a difference of about 1 ppm in the position of the methyl resonances of the diastereomeric forms. The LL (*cis*) form has the higher field resonance.[3]

(6) We are indebted to Dr. Danute E. Nitecki, Department of Genetics, Stanford University, for results obtained with these two solvent systems.

(7) D. E. Nitecki and J. W. Goodman, *Biochemistry*, **5**, 665 (1966).

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O-Carboxymethyl-L-tyrosyl Derivatives.—The procedure used is exemplified by the preparation of cyclo(O-carboxymethyl-L-tyrosyl-L-leucyl). To 0.544 g (0.00197 mole) of cyclo(L-leu-L-tyr) in 15 ml of absolute ethanol at 0° was added 4.6 ml of 0.52 *N* sodium ethoxide in absolute ethanol (0.0024 mole). The mixture was stirred at 0° for 1 hr, then evaporated to dryness at 0° and freed of residual solvent under vacuum. To the dried residue was added 15 ml of dried dimethylformamide and to this stirred mixture was added 0.3 ml (about 0.5 g, 0.003 mole) of methyl bromoacetate. Complete solution shortly resulted. The solution was stirred at room temperature overnight; a crystalline precipitate appeared after 1 hr.

The solvent and excess bromo ester were distilled off at reduced pressure and the residue was crushed and suspended in 80 ml of 25% methanol in water. Sodium hydroxide solution, 1.0 *N*, was added to the stirred suspension, held at about 35°, at a rate sufficient to keep the pH of the mixture at 11.5. When the ester had completely hydrolyzed, there was complete solution. The product was recovered by acidification to pH 2. The resulting crystalline precipitate was crystallized from 25% methanol-water: yield, 0.424 g (64%); mp 217-219°. Elementary analysis is g ven in Table II.

Registry No.—**1**, 15266-78-1; **2**, 15266-79-2; **3**, 15266-80-5; **4**, 15285-83-3; **5**, 15266-81-6; **6**, 15266-82-7; **7**, 15266-83-8; **8**, 15266-84-9; **9**, 15266-85-0; **10**, 15266-86-1; cyclo(L-leucyl-L-tyrosyl), 15266-87-2; cyclo(glycyl-L-histidyl), 15266-88-3; N-glycyl-DL-α-amino-α-phenylbutyric acid, 15266-72-5; N-chloroacetyl-DL-α-amino-α-phenylbutyric acid, 15266-73-6.

A Simple Route to Sterically Pure Diketopiperazines[1]

D. E. NITECKI, B. HALPERN, AND J. W. WESTLEY

Department of Genetics, Stanford University School of Medicine, Palo Alto, California 94304

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In connection with another study, we have recently prepared a number of formate salts of dipeptide methyl esters by deblocking the respective *t*-butyloxycarbonyl derivatives with formic acid.[2] The behavior of these salts during melting point determinations (the formates resolidified on further heating) suggested a facile conversion to another compound at elevated temperatures. A thin layer chromatogram (tlc) of the reaction product derived from L-phe-L-phe-OMe formate showed it to be a less polar, ninhydrin negative material which was identified as L-phe-L-phe-diketopiperazine. We have since shown that many formates of dipeptide methyl esters can be converted into their diketopiperazine derivatives by boiling the salts in a neutral solvent for several hours. Under these conditions the formic acid is readily removed from its salts by azeotropic distillation and the conversion to the cyclic compound is accomplished in good yields. By using sterically pure dipeptide derivatives,[3] it was shown by tlc and a chlorination technique for detection[4] that the cyclization step proceeds without racemization. For reference purposes, we also prepared the diketopiperazines by the Fischer method,[5] which involves the action of excess ammonia on depeptide methyl esters. A tlc examination of the reaction mixtures always showed the presence of some racemic material (5–40%). This is mostly due to the exposure of unreacted dipeptide ester to the base during the extended reaction periods (1–5 days), since treatment of sterically pure diketopiperazines with methanolic ammonia for several days showed little or no racemization.

Finally we have used the cyclization reaction in conjunction with the tlc separation of diastereoisomeric diketopiperazines as a test for the optical purity of *t*-boc-dipeptide esters. This technique may be useful for the determination of steric purity of dipeptides which do not yield volatile trifluoracetyl derivatives suitable for gas chromatographic analysis.

Experimental Section

Preparation of *t*-Boc-Dipeptide Esters.—The *t*-boc-amino acid (2 mmoles) was dissolved in methylene chloride (10 ml) at 0° and the amino acid methyl ester hydrochloride (2 mmoles) and triethylamine (0.28 ml, 2 mmoles) were added. After addition of N-ethyl-N′-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.384 g, 2 mmoles), the solution was stored at −5° overnight. The reaction mixture was then washed with water, citric acid (1 *N*), sodium bicarbonate, water, and the solution evaporated to dryness. The crude peptide was then recrystallized from an appropriate solvent. Over-all yields and physical data of the *t*-boc-dipeptide esters are given in Table I.

Preparation of 2,5-Diketopiperazines.—The *t*-boc-dipeptide methyl ester (200 mg) was dissolved in formic acid (20 ml, 98%) and the solution kept at room temperature for 2 hr.[2] After removal of the excess formic acid *in vacuo* (<30°), the residue containing the crude dipeptide ester formate was dissolved in *sec*-butyl alcohol (10–40 ml) and toluene (5–10 ml). The solution was boiled for 2–3 hr and the solvent level maintained by addition of fresh butanol. In some cases the diketopiperazine began to crystallize out of the hot reaction mixture. After concentrating the solution to 5–10 ml and cooling to 0° the products were filtered off and recrystallized from a suitable solvent. The overall yields and physical constants of the diketopiperazines are given in Table II.

Steric Analyses of Dipeptide Methyl Ester Formates.—The dipeptide ester formate (1 mg) was dissolved in methanol (0.5 ml) and methyl trifluoroacetate (0.2 ml) and triethylamine (0.1 ml) was added. After 3 hr ethyl acetate (10 ml) was added and the solution washed with dilute acid, sodium bicarbonate, and water. The dried organic layer was then concentrated to 0.5 ml and a portion (0.2 μl) injected into the gas chromatograph (see Table III).

Steric Analyses of Diketopiperazines.—The steric purity of the reaction mixtures and the final products were established by tlc on silica gel plates using the solvent system (a) isopropyl ether–chloroform–acetic acid (6:3:1) or system (b) chloroform–methanol–acetic acid (14:2:1). The chromatograms were developed by the chlorination technique[4] (Table IV). For analytical purposes the cyclization of 1–5 mg of a dipeptide was followed by ninhydrin. After completion (1–5 hr) the reaction mixture was concentrated and 1 drop of the residue examined for the presence of the other diastereoisomer by tlc.

Preparation of Diketopiperazines by Fischer's Method.[5-9]—A solution of each of the dipeptide methyl ester formates (100 mg) in methanol (10 ml), which had previously been saturated with dry ammonia at 0°, was stored in a glass-stoppered flask at room temperature for 1–5 days. The course of the cyclization was followed by tlc isopropyl ether–chloroform–acetic acid (6:3:1) and the diastereoisomers were detected by the chlorination procedure.[4] In all cases some racemization was observed; this varied from <5% for the cyclization of L-phe-L-phe-OMe, L-phe-D-phe-OMe, L-leu-D-phe-OMe, L-leu-L-phe-OMe, and L-leu-D-leu-OMe to appreciable amounts for L-val-L-leu-OMe,

(1) This investigation was supported by NASA Grant No. NsG 81-60.
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(6) See Table II, footnote *j*.
(7) See Table II, footnote *g*.
(8) See Table II, footnote *h*.
(9) See Table II, footnote *f*.