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]

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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*.

TABLE I

PHYSICAL CONSTANTS OF *t*-BOC-DIPEPTIDE ESTERS[a]

Compound	Mp, °C	$[\alpha]^{25}D$, deg	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N	Yield, %
t-Boc-L-phe-L-phe-OMe	114–115	−13.8	$C_{24}H_{30}N_2O_5$	67.58	7.09	6.57	67.59	7.28	6.43	71
t-Boc-L-phe-D-phe-OMe	127–130	2.44[b]					67.73	7.11	6.51	82
t-Boc-L-phe-L-ala-OMe	98–99	−18.0	$C_{18}H_{26}N_2O_5$	61.70	7.48	8.00	61.50	7.32	7.95	59
t-Boc-L-phe-D-ala-OMe	63–64	−21.1					61.64	7.50	7.97	50
t-Boc-L-leu-L-phe-OMe	78–79	−27.6	$C_{21}H_{32}N_2O_5$	64.26	8.22	7.14	64.32	8.20	7.26	51
t-Boc-L-leu-D-phe-OMe	95–96	−16.0					64.24	8.24	7.18	71
t-Boc-L-leu-L-leu-OMe	132–133	−50.4	$C_{18}H_{34}N_2O_5$	60.30	9.56	7.82	60.60	9.54	7.75	62
t-Boc-L-leu-D-leu-OMe	108	−2.2					60.31	9.55	7.81	59
t-Boc-L-val-L-leu-OMe	126–128	−53.2	$C_{17}H_{32}N_2O_5$	59.28	9.37	8.13	59.16	9.23	8.10	55
t-Boc-L-val-D-leu-OMe	78	6.0					59.37	9.46	8.09	65
t-Boc-L-val-L-ala-OMe	133–134	−47.9	$C_{14}H_{26}N_2O_5$	55.61	8.67	9.27	55.37	8.58	9.56	37
t-Boc-L-val-D-ala-OMe	91–92	8.0					55.57	8.66	9.16	53
t-Boc-L-pro-L-leu-OMe	80–81	−78.7	$C_{17}H_{30}N_2O_5$	59.62	8.83	8.18	59.51	8.96	8.31	80
t-Boc-L-pro-D-leu-OMe	126–127	−23.8					59.55	8.88	8.05	92
t-Boc-L-thr-ε-cbz-L-lys-OBz	82–83	−22.7	$C_{30}H_{41}N_3O_8$	63.03	7.23	7.35	63.05	7.14	7.61	87

[a] Melting points are uncorrected and the optical rotations were measured in methanol (*c* 1) unless otherwise specified. Analyses were performed by the Microanalytical Service, Chemistry Department, Stanford University. [b] *c* 5.

TABLE II

PHYSICAL CONSTANTS OF 2,5-DIKETOPIPERAZINES[a]

Diketopiperazine	Mp, °C	$[\alpha]D$, deg[b]	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N	Yield, %	Mp, °C ($[\alpha]$,[c] deg)
c-L-Phe-L-phe	308–310	−100 (*c* 0.2)	$C_{18}H_{18}O_2N_2$	73.45	6.16	9.52	73.24	6.36	9.51	90	315–316 (−107)[d]
c-L-Phe-D-phe	289–290	...					73.12	6.18	9.52	94	289–291[d]
c-L-Phe-L-ala	290–291	67.1 (*c* 1.4)	$C_{12}H_{14}O_2N_2$	66.03	6.47	12.84	65.90	6.42	12.92	50	271 (66.3)[e]
c-L-Phe-D-ala	264–265	95.8 (*c* 1.4)					65.97	6.43	12.91	69	...
c-L-Leu-L-phe	263–264	32.8 (*c* 0.8)	$C_{15}H_{20}O_2N_2$	69.20	7.74	10.76	68.68	7.82	10.91	73	...
c-L-Leu-D-phe	253–256	−89.8 (*c* 1)					69.10	7.87	10.95	66	...
c-L-Leu-L-leu	275–276	−44.6 (*c* 1)	$C_{12}H_{22}O_2N_2$	63.68	9.80	12.38	63.57	9.75	12.45	79	270–271 (−42.8)[f]
c-L-Leu-D-leu	275–277	...					63.81	9.85	12.29	66	287–289[g]
c-L-Val-L-leu	246–248	−47.3 (*c* 1)	$C_{11}H_{20}O_2N_2$	62.23	9.50	13.20	62.02	9.44	13.06	75	282 (−46.5)[h]
c-L-Val-D-leu	242–244	5.2 (*c* 1)					62.11	9.42	13.19	90	...
c-L-Val-L-ala	264–265	−27.0 (*c* 1)	$C_8H_{14}O_2N_2$	56.45	8.29	16.46	56.49	8.17	16.50	62	268–270 (−29.3)[h]
c-L-Val-D-ala	272–273	24.4 (*c* 1)					56.23	8.35	16.63	54	...
c-L-Leu-L-pro	157–158	−133 (*c* 1, ethanol)	$C_{11}H_{18}O_2N_2$	62.83	8.63	13.32	62.97	8.72	13.50	53	160 (−143.4)[i]
c-L-Thr-ε-cbz-L-lys	164–165	−44.8 (*c* 1, methanol)	$C_{18}H_{25}O_5N_3$	59.49	6.93	11.56	59.56	6.94	11.76	82	...
c-L-Leu-L-try	265–268	48.0 (*c* 0.4)	$C_{17}H_{21}O_2N_3$	68.20	7.07	14.04	68.02	7.12	13.98		...
c-Gly-L-phe	268	97.8 (*c* 1)	$C_{11}H_{12}O_2N_2$	64.69	5.92	13.72	64.65	5.96	13.82	69	265.5 (100.5)[j]

[a] Melting points are uncorrected and the rotations were determined in acetic acid unless otherwise specified. [b] At 25–28°. [c] Rotations measured with sodium light in solvents specified in third column. [d] Z. J. Vejdelek, *Collection Czech. Chem. Commun.*, **15**, 929 (1950). [e] F. K. Beilstein, "Handbuch der organischen Chemie," Vol. 24, 1927, p 207, E-11. [f] See ref 5. [g] E. Fischer and A. Koelker, *Ann.*, **354**, 39 (1907). [h] E. Fischer and H. Scheibler, *ibid.*, **363**, 136 (1908). [i] E. Fischer and A. Reif, *ibid.*, **363**, 126 (1908). [j] E. Fischer and H. Schoeller, *Ann.*, **357**, 22 (1907).

TABLE III

GAS CHROMATOGRAPHIC SEPARATION OF DIASTEREOISOMERIC TFA-DIPEPTIDE METHYL ESTERS

Peptide	Separation temp, °C	Retention times of diastereoisomers, min: LD	Retention times of diastereoisomers, min: LL
Leu-phe	235° (a)	13.1	11.6
Pro-leu	235° (a)	6.7	7.1
Leu-leu	235° (a)	16.5	15.0
Phe-phe	235° (a)	33.7	31.1
Val-ala	180° (b)	13.0	14.5

[a] Aerograph 705: 15 ft × 0.25 in. QF-1 on 60/80 DCMS treated Chromosorb W, with N_2 flow 67 ml/min. [b] Aerograph 1200: 150 ft × 0.02 in. Carbowax 20M, capillary column, with N_2 flow 10 ml/min.

TABLE IV

R_f VALUES OF DIASTEREOISOMERIC DIKETOPIPERAZINES

2,5-Diketopiperazine	Solvent system	R_f value: LL	R_f value: LD
c-Phe-phe	a	0.38	0.65
c-Phe-ala	a	0.17	0.34
c-Leu-phe	a	0.33	0.60
c-Leu-leu	a	0.55	0.74
c-Val-leu	a	0.54	0.72
c-Val-ala	a	0.31	0.44
c-Leu-pro	a	0.42	
c-Thr-ε-cbz-lys	b	0.66	
c-Leu-try	a	0.31	

L-val-D-leu-OMe, L-phe-L-ala-OMe, and L-phe-D-ala-OMe and almost complete racemization for the reaction with L-leu-L-leu-OMe, L-val-L-ala-OMe, and L-val-D-ala-OMe.

Registry No.—*t*-Boc-L-phe-L-phe-OMe, 13122-89-9; *t*-Boc-L-phe-D-phe-OMe, 15215-74-4; *t*-Boc-L-phe-L-ala-OMe, 15136-29-5; *t*-Boc-L-phe-D-ala-OMe, 15136-30-8; *t*-Boc-L-leu-L-phe-OMe, 5874-73-7; *t*-Boc-L-leu-D-phe-OMe, 15136-32-0; *t*-Boc-L-leu-L-leu-OMe, 15136-12-6; *t*-Boc-L-leu-D-leu-OMe, 15136-13-7; *t*-Boc-L-val-L-leu-OMe, 15215-73-3; *t*-Boc-L-val-D-leu-OMe, 15136-14-8; *t*-Boc-L-val-L-ala-OMe, 15275-65-7;

t-Boc-L-val-P-ala-OMe, 15136-15-9; *t*-Boc-L-pro-L-leu-OMe, 15136-16-0; *t*-Boc-L-pro-D-leu-OMe, 15136-17-1; *t*-Boc-L-thr-E-cbz-L-eys-OBz, 15180-24-2; cyclo-L-phe-L-phe, 5,254-61-5; cyclo-L-phe-D-phe, 15136-18-2; cyclo-L-phe-L-ala, 15180-22-0; cyclo-L-phe-D-ala, 15136-19-3; cyclo-L-leu-L-phe, 7280-77-5; cyclo-L-leu-D-phe, 13620-18-3; cyclo-L-leu-L-leu, 952-45-4; cyclo-L-leu-D-leu, 15136-23-9; cyclo-L-val-L-leu, 15136-24-0; cyclo-L-val-D-leu, 15136-25-1; cyclo-L-val-L-ala, 15136-26-2; cyclo-L-val-D-ala, 15136-27-3; cyclo-L-leu-L-pro, 2873-36-1; cyclo-L-thr-ε-cbz-L-lys, 15180-23-1; cyclo-L-leu-L-try, 15136-34-2; cyclo-gly-L-phe, 10125-07-2.

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Di(cyclopropanecarbonyl)furoxan[1]

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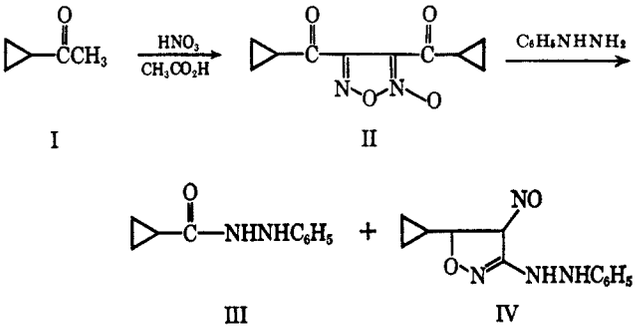
The conversion of aromatic methyl ketones by a mixture of nitric and glacial acetic acids into diaroylfuroxans has been reported.[2] In similar studies with thiophene derivatives, bis(3-thianaphthenoyl)furoxan[3] and di(2-thenoyl)furoxan[4] have been prepared. Dipicolinoyl- and di(6-acetylpicolinoyl)furoxans[5] have been isolated in studies of pyridine derivatives.

We have now been successful in extending this reaction to methyl cyclopropyl ketone, thus broadening the scope of a well-known reaction and lending hope for the synthesis of other alicyclic and possibly aliphatic substituted furoxans. Di(cyclopropanecarbonyl)furoxan (II) was isolated in 45% yield as a pale yellow liquid, bp 63–64° (0.005 mm). The infrared spectra has bands characteristic of furoxan.[6] The nmr spectra indicated that the cyclopropane ring remains unchanged (δ 1.35, methylene protons; δ 2.65, methine protons, areas ratio of 4:1). However, attempts made to distil at higher temperature only result with decomposition and polymerization.

Further transformations have confirmed the furoxan structure. On treatment with phenylhydrazine, the yellow liquid II is transformed into a colorless benzoyl β-phenylhydrazine and yellow 3(β-phenylhydrazino)-4-nitroso-5-phenylisoxazole.[7] One of the two isolated products has been established as cyclopropanecarboxylic acid 2-phenylhydrazide (III) on the basis of the melting point of an admixture with an authentic sample which was prepared by the reaction of ethyl cyclopropylcarboxylate with phenylhydrazine. By analogy, the other product is assigned the structure of 3-(β-phenylhydrazino)-4-nitroso-5-cyclopropylisoxazole (IV) (Scheme I).

SCHEME I



Treatment of II with a 1:1 ratio of 2,4-dinitrophenylhydrazine in methanol gave the mono-2,4-dinitrophenylhydrazone derivative; a 1:2 ratio of II and 2,4-dinitrophenylhydrazine in ethanol gave the bis(2,4-dinitrophenylhydrazone).

Alkaline hydrolysis of compound II resulted in nearly quantitative transformation of 1 mole of the furoxan to 2 moles of cyclopropanecarboxylic acid which was identified by conversion into the corresponding amide.

Experimental Section[8]

Di(cyclopropanecarbonyl)furoxan (II).—To 8.4 g (0.1 mole) of cyclopropyl methyl ketone in 10 ml of glacial acetic acid at 50–55° (external heating with a water bath) was added with stirring in one portion 13 ml of 69% nitric acid (*d* 1.42) dissolved in 10 ml of glacial acetic acid. Immediately, 0.2 g of sodium nitrite was added. Stirring was continued until the temperature reached 80°; then the water bath was removed. After the exothermic reaction subsided, the reaction mixture was then added to 250 ml of ice water which caused an oil to separate. After extraction with three portions of 150 ml of ether, the combined ether extracts were washed with a small amount of cold water, then washed with 5% sodium carbonate solution until the aqueous phase was yellow. Finally, the ether fraction was washed again with cold water and dried over anhydrous sodium sulfate. Evaporation of the ether to dryness under vacuum gave a yellow oil which was heated at 50–55° for 96 hr under 0.1-mm pressure to remove starting materials and volatile impurities. Yellow oil (5 g, 45%) was isolated as crude material. The pure sample was obtained by distillation at 63–64° (0.005 mm), $n^{25}D$ 1.5360.

Anal. Calcd for $C_{10}H_{10}O_4N_2$ (222): C, 54.05; H, 4.54; N, 12.61. Found (osometric in benzene): C, 53.67; H, 4.83; N, 12.51; mol wt, 220.

Infrared absorption (cm^{-1}, neat) was found at 695 s, 720 m, 790 m, 815 m, 870 s, 950 s, 990 s, 1035 s, 1060 s, 1100 s, 1160 m, 1200 s, 1215 w, 1300 s, 1390 s, 1400 s, 1430 s, 1610 s, 1700 s, and 3005 m.

Reaction of Di(cyclopropanecarbonyl)furoxan (II) with Phenylhydrazine.—Compound II (1 g, 0.45 mmole) was suspended in 5 ml of phenylhydrazine in a small flask and shaken until an exothermic reaction began. This was accompanied by evolution of the gas. The flask was allowed to cool slowly to room temperature. The reaction mixture was then poured into a large volume of water. After decanting the water layer, the residue was fractionally crystallized from ethanol to yield two fractions. One fraction of colorless needles, 0.7 g (0.4 mmole, 44%), mp 188–189°, was identified as cyclopropylcarboxylic acid phenylhydrazide (III) by mixture melting point with an authentic sample with no depression.

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(8) (a) All the melting points and boiling points are uncorrected. (b) The di(cyclopropanecarbonyl)furoxan is particularly irritating and may cause severe rash with certain individuals.