N-Methylated Dioxopiperazines

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The synthesis of several *N*-methylated dioxopiperazines is described. Their ^{1}H n.m.r. and g.l.c. properties are reported.

CYCLIC dipeptides (2,5-dioxopiperazines) are generally high-melting solids, relatively insoluble in organic solvents. The *N*-methyl derivatives reported here are oils or low-melting solids with high solubility in all but the least polar organic solvents. These physical properties greatly facilitate n.m.r. and g.l.c. studies.

Our interest in *N*-methylated dioxopiperazines is twofold. First, gas chromatography of dioxopiperazines[1,2] is of interest in connection with a recently reported pyrolysis procedure for peptide sequence studies.[3] In the application of this procedure to various peptides prior methylation is advantageous.† The *N*-methyl derivatives are more volatile and their diastereoisomeric separation is enhanced. For example, cyclo(alanyl-valyl) and cyclo(alanyl-leucyl) were not separated into diastereoisomers under the conditions described previously,[1] whereas all diastereoisomers in the *N*-methylated series [including *cyclo*-(Ala-Val) and (Ala-Leu)] were widely separated, with retention time ratios in the range 1·20—1·56 (*cis* to *trans*). G.l.c. data are given in the Table.

The second area of interest is connected with n.m.r. studies of peptides containing *N*-methylamino-acids. Such peptides are exceptional owing to the presence of conformers containing *cis* peptide bonds. In order to compare proton chemical shifts of *N*-methyl groups in such peptides, it seemed convenient to consider *N*-methylated dioxopiperazines as models for compounds containing the *cis* peptide bond. The n.m.r. data presented here (see Experimental section) indicate that *N*-methyl chemical shifts for several dioxopiperazines in deuteriochloroform fall within the narrow range δ 2·95—3·02 p.p.m. However, comparison with other peptides (cyclic and acyclic) containing *N*-methylamino-acids has not shown this test to be diagnostic for *cis* peptide bonds, because of the variability of *N*-methyl chemical shifts due to effects of other conformational parameters. This variability is illustrated by recent n.m.r. studies on enniatin B,[4] actinomycin,[5] and synthetic cyclic tetrapeptides.[6]

Two procedures have been employed for *N*-methylation. For preparative purposes, silver oxide and methyl iodide in dimethylformamide were used;[7] yields were variable and moderate. The use of sodium hydride[8] in place of silver oxide was investigated by g.l.c. and appeared to be satisfactory; neither method caused epimerization of *cis*-(L-L) dioxopiperazines. For g.l.c. of diastereoisomeric mixtures, epimerization was effected prior to methylation by the action of hot methanolic sodium methoxide.[1] Cyclo-(L-prolylsarcosyl) was synthesized *via* t-butoxycarbonyl-L-prolylsarcosine methyl ester, and also by the action of methanolic methylamine upon *N*-chloroacetyl-L-proline methyl ester. The latter route was also utilized for the preparation of cyclo(sarcosyl-DL-piperidine-2-carbonyl), required for pyrolysis studies of peptides containing pipecolic acid (piperidine-2-carboxylic acid).[3]

EXPERIMENTAL

The n.m.r. spectra were obtained with a Varian A60 instrument. For g.l.c. a Glowall model 310 instrument (with a flame ionization detector and Honeywell recorder) was used (columns: glass 6 ft × 3·4 mm; A, 5% EGS; B, 1·5% EGSP-Z; C, 3% EGSP-Z, all on 100—120 mesh GasChrom Q; Carrier gas argon, flow rate, 40 ml min^{-1}). Except where otherwise stated, columns A, B, and C were used at 205°, 130°, and 200°, respectively.

General Methylation Procedure.—The dioxopiperazine

† Studies on the pyrolysis–gas chromatography of permethylated peptides will be published elsewhere.

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[2] J. W. Westley, V. A. Close, D. N. Nitecki, and B. Halpern, *Analyt. Chem.*, 1968, **40**, 1888.
[3] A. B. Mauger, *Chem. Comm.*, 1971, 39.
[4] M. Dobler, J. D. Dunitz, and J. Krajewski, *J. Mol. Biol.*, 1969, **42**, 603.
[5] T. A. Victor, F. E. Hruska, C. L. Bell, and S. S. Danyluk, *Tetrahedron Letters*, 1969, 4721.
[6] J. Dale and K. Titlestad, *Chem. Comm.*, 1970, 1403.
[7] B. C. Das, S. D. Géro, and E. Lederer, *Biochem. Biophys. Res. Comm.*, 1967, **29**, 211.
[8] J. R. Coggins and N. L. Benoiton, *Canad. J. Chem.*, 1971, **49**, 1968.

(3 mmol) was dissolved in dimethylformamide (20 ml). Silver oxide (2·78 g, 12 mmol) and methyl iodide (3·41 g, 24 mmol) were added and the suspension was stirred for 18 h at room temperature and filtered through Celite. The filtrate was evaporated *in vacuo* and the residue, dissolved in ethyl acetate (50 ml), was washed with 5% potassium cyanide solution (20 ml) and saturated sodium chloride solution (2 × 20 ml), dried ($MgSO_4$), and evaporated. The residue was sublimed or distilled (short-path) at 120—140° and 1 mmHg.

suspension of methyl DL-piperidine-2-carboxylate hydrochloride (2·40 g) in dry ether (100 ml) containing triethylamine (2·73 g). The mixture was stirred overnight and filtered, and the filtrate was washed with N-hydrochloric acid, aqueous sodium hydrogen carbonate, and water, dried ($MgSO_4$), and evaporated. A sample of the residual oil was distilled (short-path) at 130° and 1 mmHg to furnish *methyl N-chloroacetyl-DL-piperidine-2-carboxylate* as an oil (Found: C, 49·4; H, 6·3; N, 6·1. $C_9H_{14}ClNO_3$ requires C, 49·2; H, 6·4; N, 6·4%). The bulk of the product was

Elemental analyses and g.l.c. retention times of dioxopiperazines

		Analysis (%)			Required (%)			Retention times *				
Dioxopiperazines	Formula	C	H	N	C	H	N	t_A	t'_A	t_B	t'_B	t_C
cyclo-(Sar-L-MeLeu)	$C_{10}H_{18}N_2O_2$	60·55	9·3	13·9	60·6	9·15	14·15	13·2		21·6		
cyclo-(Sar-L-MeVal)	$C_9H_{16}N_2O_2$	58·85	8·85	15·35	58·65	8·75	15·2	12·1		17·0		4·9
cyclo-(L-MeAla-L-MeVal)	$C_{10}H_{18}N_2O_2$	60·8	9·1	14·0	60·6	9·15	14·15	10·3	7·9	15·9	11·1	
cyclo-(L-MeAla-L-MeLeu)	$C_{11}H_{20}N_2O_2$	62·1	9·8	12·8	62·25	9·5	13·2	10·4	8·7	18·2	14·5	
cyclo-(L-MeVal-L-MeLeu)	$C_{12}H_{24}N_2O_2$	65·2	9·8	11·4	64·95	10·05	11·65	10·0	7·5	21·3	14·4	
cyclo-(Sar-L-Pro)	$C_8H_{14}N_2O_2$	57·15	7·2	16·3	57·15	7·2	16·65					12·8
cyclo-(Sar-L-Pip) †	$C_9H_{12}N_2O_2$	59·15	7·65	15·4	59·3	7·75	15·35					13·1

* t_A, t_B, t_C refer to retention times (minutes) on columns A, B, and C respectively; t' refers to the corresponding *trans*-diastereoisomer. For Sar-MeAla,[9] t_A = 12·5, t_B = 14·5. † Pip = piperidine-2-carboxylic acid.

Cyclo(sarcosyl-N-methyl-L-leucyl) was obtained as an oil (29%), δ ($CDCl_3$) 0·96 (6H, d, *J* 5·4 Hz, leucyl Me), 1·5—2·0 (3H, m, leucyl β- and γ-H), 2·97 (6H, s, NMe), 3·88 (1H, t, *J* 6·2 Hz, leucyl α-H), and 3·80 and 4·12 (ABq, 2H, *J* 17·6 Hz, sarcosyl α-H).

Cyclo(sarcosyl-N-methyl-L-valyl) was obtained as needles (18%), m.p. 122—124°, δ ($CDCl_3$) 0·97 and 1·10 (6H, 2d, *J* 7·0 Hz, valyl Me), 2·0—2·6 (1H, m, valyl β-H), 2·98 and 3·02 (3H, s, NMe), 3·75 (1H, d, *J* 4·1 Hz, valyl α-H), and 3·84 and 4·13 (2H, ABq, *J* 17·8 Hz, sarcosyl α-H).

Cyclo-(N-methyl-L-alanyl-N-methyl-L-valyl) was obtained as needles (38%), m.p. 140—142°, δ ($CDCl_3$) 1·01 and 1·14 (6H, 2d, *J* 6·8 Hz, valyl Me), 1·56 (3H, d, *J* 7·0 Hz, alanyl Me), 1·9—2·5 (1H, m, valyl β-H), 2·95 and 2·99 (6H, 2s, NMe), 3·75 (1H, d, *J* 4·6 Hz, valyl α-H), and 3·96 (1H, q, *J* 7·0 Hz, alanyl α-H).

Cyclo-(N-methyl-L-alanyl-N-methyl-L-leucyl) was obtained as an oil (32%), δ ($CDCl_3$) 0·98 and 1·02 (6H, 2d, *J* 6·0 Hz, leucyl Me), 1·52 (3H, d, *J* 7·0 Hz, alanyl Me), 1·5—2·1 (3H, m, leucyl β- and γ-H), 2·96 (6H, 2s, NMe), 3·87 (1H, t, *J* 7·0 Hz, leucyl α-H), and 3·93 (1H, q, *J* 7·0 Hz, alanyl α-H).

Cyclo-N-methyl-L-valyl-N-methyl-L-leucyl) was obtained as needles (48%), m.p. 75—77°, δ ($CDCl_3$) 0·98 and 1·02 (6H, 2d, *J* 6·0 Hz, leucyl Me), 1·05 and 1·12 (6H, 2d, *J* 6·6 Hz, valyl Me), 1·4—2·2 (4H, m, β- and γ-H), 2·95 and 3·00 (6H, 2s, NMe), 3·62 (1H, d, *J* 6·0 Hz, valyl α-H), and 3·83 (1H, dd, *J* 5·0 and 7·8 Hz, leucyl α-H).

N-Chloroacetyl-L-proline Methyl Ester.—Chloroacetyl chloride (4·48 g) was added dropwise during 15 min to a stirred, ice-cooled solution of freshly distilled L-proline methyl ester (3·90 g) and triethylamine (3 ml) in dry ether (100 ml). The mixture was stirred overnight and filtered and the filtrate was washed with N-hydrochloric acid, aqueous sodium hydrogen carbonate, and water, dried ($MgSO_4$), and evaporated. The residual oil was distilled at 1 mmHg and the fraction of b.p. 108—112° collected as an *oil* (41%) (Found: C, 46·9; H, 6·15; N, 6·85. $C_8H_{11}ClNO_3$ requires C, 46·7; H, 5·9; N, 6·8%).

Cyclo(sarcosyl-DL-piperidine-2-carbonyl).— Chloroacetyl chloride (1·68 g) was added dropwise to a stirred, ice-cooled dissolved in saturated methanolic methylamine (100 ml). After 18 h the solution was evaporated and the residue dissolved in ethyl acetate; the solution was washed with water, dried ($MgSO_4$), and evaporated. The product was sublimed at 120° and 1 mmHg to give white needles (87%), m.p. 84—86°.

t-Butoxycarbonyl-L-prolylsarcosine Methyl Ester.—t-Butoxycarbonyl-L-proline (306 mg), sarcosine methyl ester hydrochloride (208 mg), and triethylamine (0·22 ml) in acetonitrile (5 ml) were stirred together, and dicyclohexylcarbodi-imide (380 mg) in acetonitrile (3 ml) was added. After 18 h the solution was evaporated, the residue dissolved in ethyl acetate (20 ml), and the solution filtered. The filtrate was washed with 0·2N-hydrochloric acid, aqueous sodium hydrogen carbonate, and water, dried (Na_2SO_4), and evaporated. The residual syrup (393 mg) was chromatographed on a column (20 × 2 cm) of silica gel (Merck; 0·05—0·20 mm) and fractions (5 ml) were collected. Elution was commenced with 3 : 1 chloroform–ethyl acetate and changed to 1 : 1 after fraction 15. The product was located by g.l.c. (single peak on column A at 180°) in fractions 23—34, which were evaporated. The residual *syrup* was distilled (short-path) at 155° and 1 mmHg (yield 330 mg) (Found: C, 55·95; H, 7·95; N, 9·45. $C_{14}H_{24}N_2O_5$ requires C, 56·0; H, 8·05; N, 9·35%).

Cyclo(sarcosyl-L-prolyl).— t-Butoxycarbonyl-L-prolylsarcosine methyl ester (386 mg) was dissolved in 30% hydrogen bromide–acetic acid (5 ml). After 1 h at room temperature, ether (50 ml) was added, the mixture was stirred, and the ether layer was decanted. The gummy residue was washed with fresh portions (2 × 30 ml) of ether and dissolved in methanolic ammonia (25 ml). After 24 h the solution was evaporated and the residue extracted with benzene (50 ml); the extract was filtered and evaporated. The residue was chromatographed on a column (14 × 1·5 cm) of silica gel (Merck; 0·05—0·20 mm) in ethyl acetate–methanol (9 : 1). Fractions (5 ml) were collected and the product was located by g.l.c. in fractions 12—22. Distillation (short-path) at 140° and 1 mmHg gave an oil (280 mg).

[9] P. A. Levene, H. S. Simms, and M. H. Pfaltz, *J. Biol. Chem.*, 1926, **70**, 253.

The same product was also prepared by the reaction of *N*-chloroacetyl-L-proline methyl ester with methanolic methylamine.

Small-scale Methylation of Dioxopiperazines for G.l.c.— This was effected either as already described, or as follows (with similar results). The dioxopiperazine (5 mg) was dissolved in dimethylformamide (0·3 ml) and methyl iodide (25 μl) was added, followed by sodium hydride (6 mg). The mixture was stirred at room temperature for 3 h and filtered. A sample was injected into the gas chromatograph. Cyclo(valylvalyl) (L-L and D-L) and cyclo(glycyl-L-alanyl) were methylated in this way. Gas chromatography was effected with argon as carrier gas at 40 ml min^{-1}; see Table for retention times.

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