Efficient One-step Synthesis of Diastereoisomeric Cyclic Dipeptides from Amino Acids: Three Diastereoisomers of Cyclo-L-isoleucyl-L-isoleucine

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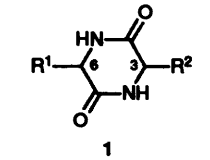
Cyclic dipeptides formed by the self-condensation of amino acids in ethane-1,2-diol comprise readily separable diastereoisomeric mixtures which, in the case of isoleucine, affords three isomers in good yield.

Cyclic dipeptides (2,5-diketopiperazines, **1**) are of wide interest as natural products, potential pharmaceuticals, protein degradation products, models in peptide chemistry and as intermediates facilitating asymmetric synthesis.[1] They can be obtained stereoselectively by cyclization of dipeptide esters,[2,3] but many symmetrically substituted compounds are more conveniently available by heating the corresponding amino acid in a suitable solvent. Ethane-1,2-diol, having both appropriate polarity and boiling temperature, has been a popular choice for amino acid condensations,[4–6] but the stereochemical outcome of this method has been either not addressed, or assigned without evidence. For example, while Machin and Sammes claim the reaction with L-phenylalanine gives the *cis* isomer of the cyclic dipeptide **1a** in 82% yield,[5] Exner and Kostelnik report L-leucine gives exclusively the *trans* isomer of compound **1b**.[6] (The latter result has been questioned



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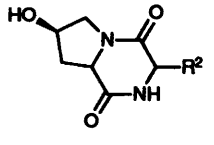
a $R^1 = R^2 = C_6H_5CH_2$
b $R^1 = R^2 = Me_2CHCH_2$
c R^1 =  $R^2 \neq H$
d R^1 =  $R^2 \neq H$



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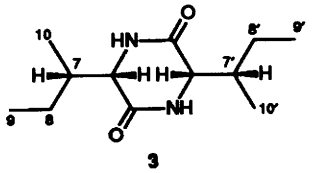
elsewhere.[7]) Although the spontaneous formation of cyclic dipeptides from linear dipeptide and amino acid esters occurs largely with retention of stereochemistry, the well-known propensity of the products to epimerize[8,9] prompts clarification of the position in ethane-1,2-diol.

Such an investigation presents an opportunity also to resolve another apparent inconsistency in the stereochemical analysis of cyclic dipeptides. Compound **1**, where neither R^1 nor R^2 is hydrogen, has only two diastereoisomers, provided there is no chirality in either side chain. This is the case for cyclic dipeptides derived from most of the protein amino acids, and their stereochemical properties have been studied in some detail.[7–10] The amino acids isoleucine, threonine and hydroxyproline, however, would form cyclic dipeptides with additional diastereoisomers because of the (specified) chirality in the side chains of **1c**, **1d** and **2** ($R \neq H$), respectively. But this has been recognised only in the case of hydroxyproline.[10] Reports of work involving cyclic dipeptides containing isoleucine **1c** acknowledge only two diastereoisomers.[11] Particular interest is attached to these compounds also in the contexts of geochronology,[8] microbial metabolites[12] and bitter taste properties.[13]



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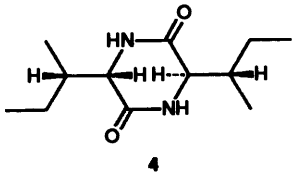

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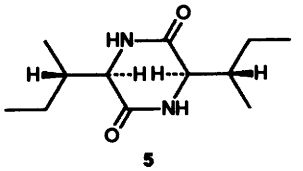

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We now report that the action of heat on a solution of L-isoleucine in ethane-1,2-diol results in the formation of three readily separable diastereoisomers of cycloisoleucylisoleucine **3–5** in good yield. We have found also that the product from similar treatment of L-phenylalanine is a mixture of the two diastereoisomers.

Results and Discussion

The self-condensation of L-isoleucine proceeds cleanly in refluxing ethane-1,2-diol to give cyclic dipeptides with little formation of by-products. Under the reaction conditions, any epimerization might be expected to be limited to the comparatively labile CH groups in the ring, making available only 3 of the 10 possible stereoisomers of **1c**. In confirmation, we have isolated *cis*-(3*S*,6*S*), *trans*- and *cis*-(3*R*,6*R*) isomers of 3,6-bis[(1*S*)-1-methylpropyl]piperazine-2,5-dione, (cyclo-L-isoleucyl-L-isoleucine **3**, cyclo-L-isoleucyl-D-alloisoleucine **4** and cyclo-D-allosioleucyl-D-alloisoleucine **5**, respectively). A sample of cyclo-L-isoleucyl-L-isoleucine was obtained for comparison by stereoselective synthesis, and the other two isomers were

Table 1 Physical characteristics of the three diastereoisomers of cycloisoleucylisoleucine

		3	4	5
Config. seq.: 7,3,6,7′		(*SSSS*)-*cis*	(*SRSS*)-*trans*	(*SRRS*)-*cis*
M.p. (°C)[a]		275–7	280–3	270–3
Rel. capacity factor[b]		12.5	7.1	10.9
ν_{max}(CO)/cm^{-1}		1660	1661	1662
		1670		1675
δ_H/ppm[c]	1,4 (br)	7.92	7.96	7.88
			8.05	
	3,6 (t)	3.75	3.70	3.86
			3.75	
	7,7′ (m)	1.87	1.86–2.00	1.98[d]
	8,8′ (m)	1.19	1.21	1.28[e]
		1.40	1.41	1.43[e]
	9,9′ (t)	0.83	0.83	0.85
			0.86	
	10,10′ (d)	0.92	0.78	0.77
			0.91	
δ_C/ppm[c]	2,5	167.5	167.6	167.9
			167.7	
	3,6	58.5	57.4	57.2
			58.4	
	7,7′	37.8	38.4	37.1
			38.8	
	8,8′	24.4	24.2	24.9
			24.7	
	9,9′	11.9	11.8	11.9
			11.9	
	10,10′	15.0	13.8	14.2
			14.9	

[a] With sublimation. [b] Reversed-phase HPLC (see Experimental section). [c] 400 MHz in [$^2H_6$]DMSO. [d] Quartet. [e] Septet.

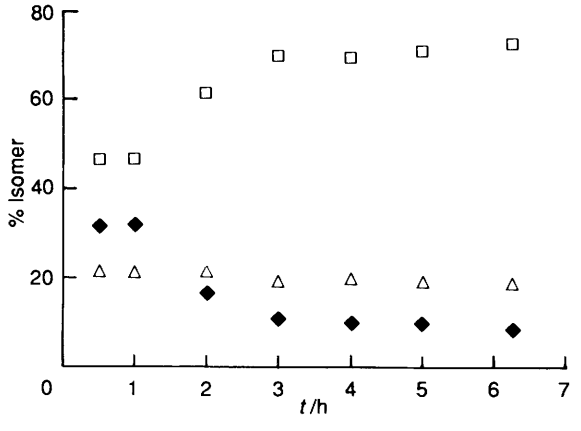

Fig. 1 Distribution of diastereoisomers of 3,6-bis(1-methylpropyl)-piperazine-2,5-dione in the condensation of L-isoleucine in ethane-1,2-diol. □ *trans*; △ *cis*-(3*R*,6*R*); ◆ *cis*-(3*S*,6*S*).

distinguished by their NMR spectra. The latter, and some other physical properties, are compared in Table 1. Most notable is the doubling of the NMR signals for the *trans*-(*SSRS*) isomer, and the sensitivity of the ^{1}H chemical shift of the 10-Me group to the configuration of the side chain. In addition, some chemical shifts of the *trans* isomer are displaced from their (*S*,*S*) and (*S*,*R*) counterparts in the *cis* isomers. For example, while the δ_H values at 10- and 10′-H seem to be wholly determined by side chain configuration, those at 3- and 6-H and the δ_C values at C-7 and -7′, indicate the outcome of a less localised structural influence such as a change in ring conformation. Studies with similar compounds suggest this may be expected. Thus, *trans* isomers of both cyclovalylvaline **1** ($R^1 = R^2$ = 1-methylethyl) and cycloleucylleucine **1** ($R^1 = R^2$ = 2-methylpropyl) have centrosymmetric planar or chair conformations, while the *cis* isomers adopt planar axial and flagpole-boat conformations, respectively.[7] The latter have been attributed to hydrophobic stabilization at the expense of steric crowding, and similar interactions may explain the surprising sensitivity to structure of affinity for C-18 bonded silica in reversed-phase HPLC (Table 1). Both observations illustrate the significant influence this effect can exert on the behaviour of small molecules as well as, in its more familiar cummulated form, on the structure of proteins.[14]

While a sample of cyclophenylalanylphenylalanine **1a** obtained by self-condensation of L-phenylalanine in ethane-1,2-diol[5] has an IR spectrum closely similar to that of the *cis* isomer obtained stereoselectively,[15] it is resolved in reversed-phase HPLC into two peaks of similar area and response characteristics, the more retained corresponding with the *cis* isomer. Thus, epimeric equilibration appears to be a general outcome of this procedure, the time course of which, in the case of cycloisoleucylisoleucine, is illustrated in Fig. 1.

Experimental

Cyclo-L-isoleucyl-L-isoleucine[3] and cyclo-L-phenylalanyl-L-phenylalanine[15] were synthesised by known procedures.

Self-condensation of L-*Isoleucine and Separation of Diastereoisomeric Cyclic Dipeptides.*—A mixture of L-isoleucine (5 g, 3.8 mmol) and dry ethane-1,2-diol (10 cm^3) was allowed to reflux for 24 h. After cooling, the off-white solid deposited was recrystallized from ethanol, yielding a mixture of diastereoisomers of cycloisoleucylisoleucine (2.8 g). A sample (0.4 g) dissolved in chloroform (50 cm^3) was fractionated on a silica column (150 g) with 2 dm^3 0–5% methanol–chloroform gradient giving, in order of elution and after solvent evaporation, the cleanly separated *trans*, **4** *cis*-(3*R*,6*R*) **5** and *cis*-(3*S*,6*S*) **3** isomers of 3,6-bis[(1*S*)-1-methylpropyl]piperazine-2,5-dione as white crystals (0.2, 0.06 and 0.05 g, respectively) (Found: C, 63.4; H, 9.8; N, 12.3; C, 63.3; H, 9.7; N, 11.9 and C, 63.8; H, 9.8; N, 12.0, respectively. Calc. for $C_{12}H_{22}N_2O_2$: C, 63.7; H, 9.8; N, 12.4%). A similar size sample was also fractionated by preparative normal-phase HPLC of a

saturated solution in dichloromethane, with similar recovery [stationary phase (SP), 15 μm SiO_2, 215 × 18 mm; mobile phase (MP), CH_2Cl_2; flow rate (FR) 25 cm^3 min^{-1}; λ 238 nm; 2 cm^3 sample loop].

The condensation reaction was monitored by reversed-phase HPLC [SP, 15 μm C-18 end-capped SiO_2, 250 × 5 mm; MP, H_2O–MeCN (8:2); FR 1 cm^3 min^{-1}; λ 245 nm; sample, 0.01 cm^3 of reaction mixture diluted × 100 with ethanol].

Self-condensation of L-*Phenylalanine.*—The cyclic dipeptide was isolated according to Machin and Sammes' method[5] and analysed by reversed-phase HPLC [SP, FR, λ and sample as above; MP, 0.05 mol dm^{-3} KH_2PO_4 (pH 2.6)–MeCN (6:4)]. UV response characteristics for each of the two peaks were compared using a Poly 9065 diode array detector.

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