1107. *Amino-acids and Peptides. Part XVIII.*[1] *Racemisation during the Coupling of Formyl-L-leucine.*

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The coupling of formyl-L-leucine with glycine ethyl ester by the acid azide route, through the *p*-nitrophenyl ester, or by means of dicyclohexylcarbodi-imide, gave peptide of high optical activity, allowing the presence of little or no racemate. The carbonic mixed anhydride method gave some racemate, but markedly less than in the analogous reactions of benzoyl- and acetyl-L-leucine; unexpectedly, the presence of chloride ion during the coupling by this method had no significant effect on the optical activity of the peptide so obtained. Contrary to another report, we find that L-4-isobutyloxazolone reacts with glycine ethyl ester to give nearly completely racemic peptide.

The usefulness of the *N*-formyl protecting group [2,3] has been demonstrated recently during the synthesis of peptides of the polymyxin type [4] and of an eicosapeptide amide related to the corticotropins.[5] There has, however, been differing experience [2,3] with regard to the preservation of optical activity when α-formylamino-acids are coupled, and we have therefore extended our studies of racemisation [6-8] to the coupling of formyl-L-leucine with glycine ethyl ester by some common procedures.

Of the new compounds required for this work, formyl-L-leucine methyl ester could not be crystallised but gave a crystalline hydrazide; formylation of L-leucine *p*-nitrophenyl ester gave a crystalline product. Authentic formyl-L-leucylglycine ethyl ester was prepared by formylation of L-leucylglycine ethyl ester, and the DL-isomer by condensing formyl-DL-leucine with glycine ethyl ester.

In each of our coupling experiments, the crude product was crystalline and analytically pure, and therefore the percentage of racemate could be determined directly from the specific rotation. The results are shown in the Table. The high specific rotations of the products from the acid azide route,[9] from the *p*-nitrophenyl ester,[10] and by the use of dicyclohexylcarbodi-imide,[11] allow the presence of little or no racemate. The carbonic mixed anhydride procedure [12] (with tetrahydrofuran or chloroform as solvent) gave some racemate. Treatment of the *p*-nitrophenyl ester with triethylamine before coupling gave nearly inactive peptide [2(iv)].

From our earlier work [7,8] comparison may be made of the degree of racemisation observed in the coupling of benzoyl-, acetyl-, and formyl-L-leucine with glycine ethyl ester, and two cases are of particular interest. For the carbodi-imide method, with dichloromethane as solvent, the amount of L-peptide found is 54%, *ca.* 70%, and 94%, respectively; for the carbonic mixed anhydride procedure with tetrahydrofuran as solvent, the corresponding figures are 20%, *ca.* 60%, and 86%. With regard to the latter procedure, it is remarkable that the presence of chloride ion (which increases markedly the racemisation during the coupling of the benzoyl and acetyl analogues by various methods [7,8]) has little

[1] Part XVII, Theobald, Williams, and Young, *J.*, 1963, 1927.
[2] Waley, *Chem. and Ind.*, 1953, **72**, 107; Waley and Watson, *Biochem. J.*, 1954, **57**, 529.
[3] Sheehan and Yang, *J. Amer. Chem. Soc.*, 1958, **80**, 1154.
[4] See, *e.g.*, Vogler, Studer, Lergier, and Lanz, *Helv. Chim. Acta*, 1960, **43**, 1751.
[5] Hofmann, Liu, Yajima, N. Yanaihara, C. Yanaihara, and Humes, *J. Amer. Chem. Soc.*, 1962, **84**, 1054.
[6] North and Young, *Chem. and Ind.*, 1955, 1597.
[7] Smart, Young, and Williams, *J.*, 1960, 3902.
[8] Williams and Young, *J.*, 1963, 881.
[9] Curtius, *Ber.*, 1902, **35**, 3226.
[10] Bodanszky, *Acta Chim. Acad. Sci. Hung.*, 1957, **10**, 335.
[11] Sheehan and Hess, *J. Amer. Chem. Soc.*, 1955, **77**, 1067.
[12] Boissonnas, *Helv. Chim. Acta*, 1951, **34**, 874; Vaughan, *J. Amer. Chem. Soc.*, 1951, **73**, 3547; Wieland and Bernhard, *Annalen*, 1951, **572**, 190.

			Crude product						
			Yield			L-Isomer †	Found (%) ‡		
Method [a]		Solvent	(%)	M. p.	$[\alpha]_D$ *	(%)	C	H	N
1. Acid azide	(i)	Ether	89	67—69°	−60° (*c* 2·2)	100	54·25	8·1	11·6
	(ii)	,,	84	65—69	−60 (*c* 0·3)	100	54·4	8·3	11·2
	(iii)	,,	84	65—69	−58 (*c* 2·5)	97	54·3	8·3	11·2
2. *p*-Nitrophenyl ester	(i)	EtOAc	85	69	−60 (*c* 2·1)	100	54·45	8·3	11·35
	(ii)	,,	96	69·5	−59 (*c* 2·0)	98	53·45	8·2	11·3
	(iii)	,,	75	69·5	−60 (*c* 1·8)	100	54·0	8·0	11·5
	(iv)	,,	62	64·5—65·5	−2 (*c* 0·9)	3	54·6	8·4	11·0
3. Carbodi-imide	(i)	CH_2Cl_2	70	63—66	−57 (*c* 2·2)	95	54·4	8·5	11·05
	(ii)	,,	88	62—64	−56 (*c* 2·0)	93	54·2	8·3	11·0
4. Carbonic mixed anhydride	(i)	THF §	77	57—58	−53 (*c* 0·6)	88	54·0	8·2	11·0
	(ii)	,,	86	58—59	−52 (*c* 0·7)	87	54·4	8·1	11·0
	(iii)	,,	79	62—63	−50 (*c* 2·3)	83	53·8	8·1	11·6
	(iv)	$CHCl_3$	70	63—65	−49 (*c* 0·6)	82	54·2	8·6	11·1
	(v)	,,	67	59—63	−43 (*c* 1·7)	72	53·9	8·3	11·05
	(vi)	,,	61	60—62	−46 (*c* 3·0)	77	53·9	8·2	11·6
	(vii)	THF–$CHCl_3$	79	63—64	−53 (*c* 2·4)	88	53·9	8·15	11·4
	(viii)	,,	70	62—64	−51 (*c* 2·9)	85	53·95	8·4	11·0

[a] Differences in procedure are recorded in the Experimental section.

* At 18—23° in EtOH. † Excluding L-isomer present as racemate. ‡ Calc. for formyl-leucylglycine ethyl ester, $C_{11}H_{20}N_2O_4$: C, 54·1; H, 8·25; N, 11·5%. § Tetrahydrofuran.

effect on the optical activity of the product [4(iv)—(viii)]. In the last experiment benzyltrimethylamminonium chloride was also present. Interpretations of these facts, which are consistent with the common view that such racemisation proceeds through an oxazolone,[13] will be considered together with other results during a general discussion in a subsequent paper of this series, but one point must be made here. Siemion and Nowak [14] prepared L-4-isobutyloxazolone by the action of dicyclohexylcarbodi-imide on formyl-L-leucine (an excellent route to such oxazolones), and reported that with glycine ethyl ester this compound gave formyl-L-leucylglycine ethyl ester of m. p. 113—115°, $[\alpha]_D$ −7·1° in ethanol. If fully active peptide were obtained from this reaction, then the oxazolone could not be an intermediate in racemisation during coupling. In the course of our work we have prepared this peptide by a variety of methods and find m. p. 69·5°, $[\alpha]_D^{20}$ −60° for the L-isomer and m. p. 65—66° for the racemate. We have repeated the preparation of the oxazolone (we prefer tetrahydrofuran to ethyl acetate as solvent in the reaction) and obtained a product of $[\alpha]_D^{25}$ −76° in ethyl acetate (Siemion and Nowak give $[\alpha]_D$ −46·4°, solvent unspecified); the rotation of the solution falls slowly with time and some racemisation may have occurred during isolation. With glycine ethyl ester the oxazolone gave formyl-leucylglycine ethyl ester (which was isolated without recrystallisation in order to avoid preferential removal of stereoisomer) of m. p. 61—62·5°, $[\alpha]_D^{21}$ −3·6 in ethanol, and with cyclohexylamine it gave inactive amide. The identity of the compound isolated by Siemion and Nowak is uncertain; although formyl-DL-leucine has m. p. 115—116° (corr.),[15] the nitrogen analysis is not in agreement. Analogously, 2-methyl-L-4-isobutyloxazolone reacts with glycine ethyl ester to give nearly racemic peptide, although Siemion and Nowak report that with DL-norleucine methyl ester [14] and with hydrazine hydrate [16] it yields optically active products. It is clear that our results allow the possibility that racemisation may proceed through such oxazolones.

Experimental

M.p.s were taken on a Kofler block. Infrared spectra were recorded on a Perkin-Elmer model 21 spectrophotometer. Optical rotations were recorded on an Ericcson automatic

[13] Williams and Young, Peptides: Proc. Fifth European Symposium, Oxford, 1962, ed. G. T. Young, Pergamon Press, Oxford, 1963.
[14] Siemion and Nowak, *Roczniki Chem.*, 1961, **35**, 979.
[15] Fischer and Warburg, *Ber.*, 1905, **38**, 3997.
[16] Nowak and Siemion, *Roczniki Chem.*, 1961, **35**, 153.

polarimeter. Solutions in organic solvents were dried over $MgSO_4$; light petroleum was of b. p. 60—80°; evaporation was by water-pump unless otherwise stated.

Formyl-L-leucylglycine Ethyl Ester.—L-Leucylglycine ethyl ester acetate (syrup; obtained by catalytic hydrogenation of 7·0 g. of benzyloxycarbonyl-L-leucylglycine ethyl ester [17] in the presence of acetic acid) was dissolved in 98% formic acid (60 ml.), the solution was cooled to 5°, and acetic anhydride (20 ml.) was run in slowly with stirring. After 6 hr. the solution gave only a weak ninhydrin test, ice–water (23 ml.) was then added, and the solution was evaporated to dryness. The residue was extracted into ethyl acetate (100 ml.), and the solution was washed with 2N-hydrochloric acid, 2N-sodium carbonate, and then water, dried, and evaporated to leave a syrup which crystallised under light petroleum containing a little ether. The *peptide* was recrystallised from ethyl acetate–cyclohexane, giving needles (3·4 g., 70%), m. p. 69·5°, $[\alpha]_D^{20}$ −60° (*c* 0·5 in EtOH) (Found: C, 54·3; H, 8·2; N, 11·1. $C_{11}H_{20}N_2O_4$ requires C, 54·1; H, 8·25; N, 11·5%). A sample was hydrolysed in 6N-hydrochloric acid at 100° for 7·5 hr.; the hydrochloric acid was removed by repeated evaporation of water, and the residue then had $[\alpha]_D^{21}$ +14·9° (calc. as leucine) in 6N-HCl.

Formyl-DL-leucylglycine Ethyl Ester.—Formyl-DL-leucine [15] was coupled with glycine ethyl ester through the mixed anhydride formed with ethyl chloroformate in tetrahydrofuran solution. The *peptide* (77% yield) was recrystallised from ethyl acetate–cyclohexane to give needles of m. p. 65—66° (Found: C, 54·3; H, 8·4; N, 11·4%).

Formyl-L-leucine Methyl Ester.—L-Leucine methyl ester hydrochloride (13·1 g.) was dissolved in 98% formic acid (152 ml.) and fused sodium acetate (5·34 g.) was added. The solution was cooled to 0° and acetic anhydride (51 ml.) was added dropwise with stirring. After 2·5 hr. the solution was evaporated to dryness and the product was taken up in ethyl acetate; the solution was washed with 2N-hydrochloric acid and water, and then dried. Evaporation gave the *ester* as a liquid (8·8 g., 79%) which distilled at 95—96°/0·5 mm., and had n_D^{20} 1·4525 (Found: C, 55·9; H, 9·0; N, 8·0. $C_8H_{15}NO_3$ requires C, 55·5; H, 8·7; N, 8·1%).

Formyl-L-leucylhydrazide.—To formyl-L-leucine methyl ester (1·9 g.) in ethanol (12 ml.) was added 50% hydrazine hydrate (4·4 g.). After 42 hr. the solution was evaporated to dryness, leaving a white solid which was washed with ether (1·7 g., 90%) and then recrystallised from ethyl acetate, giving the *hydrazide*, m. p. 144—145°, $[\alpha]_D^{21}$ −36° (*c* 0·9 in dimethylformamide) (Found: C, 48·55; H, 8·65; N, 24·8. $C_7H_{15}N_3O_2$ requires C, 48·5; H, 8·7; N, 24·3%).

Formyl-L-leucine p-*Nitrophenyl Ester.*—L-Leucine *p*-nitrophenyl ester hydrobromide (2·4 g.) [18] was formylated as described above for the methyl ester, giving a pale yellow syrup (1·5 g.) which crystallised under ether–light petroleum. Recrystallisation from ether–light petroleum gave the *ester* as colourless needles, m. p. 60—61°, $[\alpha]_D^{21}$ −90° (*c* 1·1 in EtOH) (Found: C, 55·5; H, 5·8; N, 9·8. $C_{13}H_{16}N_2O_5$ requires C, 55·7; H, 5·75; N, 10·0%). A portion was hydrolysed at 100° with 0·5N-hydrochloric acid for 80 min. The acid was removed by evaporation; the residue was washed with dry ether, and then had $[\alpha]_D^{21}$ +13·8° (calc. as leucine) in 6N-HCl.

Investigation of Racemisation during Coupling.—Formyl-L-leucine was prepared by the general method of Sheehan and Yang [3] and had m. p. 142—144°, $[\alpha]_D^{20}$ −18·3° (*c* 0·6 in EtOH). The methods of coupling and working-up were analogous to those described for the coupling of benzoyl-L-leucine,[8] except that, owing to the greater solubility of formyl-leucylglycine ethyl ester in water, the volume used for washing was kept low. The product crystallised under light petroleum at −12°; in some cases addition of a little ether was helpful. The results of these experiments are in the Table.

The following notes apply to the experiments numbered as in the Table. In all experiments 0·01 mole each of formyl-L-leucine (or its appropriate derivative) and distilled glycine ethyl ester was used except where otherwise stated. (2, i—iii) 0·0041 mole of *p*-nitrophenyl ester was used. (2, iv) 0·0026 mole of *p*-nitrophenyl ester was used; an equivalent amount of triethylamine was added to the solution of this ester, and the glycine ethyl ester was added next day. (4, iv—vi) Glycine ethyl ester hydrochloride (0·012 mole) and triethylamine (0·01 mole) were used instead of free glycine ester. (4, vii) The mixed anhydride was formed in tetrahydrofuran (20 ml.), and chloroform (35 ml.) was then added, followed by the glycine ester. (4, viii) The coupling was carried out as in (4, vii), except that 0·02 mole of benzyltrimethylammonium chloride (dissolved in the chloroform) was added before the glycine ester.

Formyl-L-leucylglycine.—The corresponding ethyl ester was dissolved in N-sodium hydroxide;

[17] Vaughan and Osato, *J. Amer. Chem. Soc.*, 1952, **74**, 676.
[18] Goodman and Stueben, *J. Amer. Chem. Soc.*, 1959, **81**, 3980.

after 1 hr. the solution was brought to pH 3 by the addition of Amberlite CG-120(H^+), and was then freeze-dried. A few drops of dilute hydrochloric acid were added to the gummy residue, and evaporation gave the *acid* as a white solid, which was recrystallised from ethyl acetate and then had m. p. 150°, $[\alpha]_D^{20}$ −56° (*c* 0·5 in EtOH) (Found: C, 50·25; H, 7·6; N, 12·95. $C_9H_{16}N_2O_4$ requires C, 50·0; H, 7·5; N, 13·0%).

*Formyl-*DL*-leucylglycine.*—This *acid* was prepared similarly, and had m. p. 159—161° (Found: C, 50·4; H, 7·5; N, 12·6%).

Attempted Isolation of Racemate from Partially Racemised Peptide.—A synthetic mixture of formyl-L-leucylglycine ethyl ester with 10% of DL-isomer was fractionally crystallised from ethyl acetate–cyclohexane; the first fractions were pure L-peptide. A similar mixture of the corresponding acids gave only L-acid in the first fractions from ethyl acetate.

L-4-*Isobutyloxazolone.*—This was prepared as described by Siemion and Nowak,[14] except that tetrahydrofuran was the solvent. The best product (a syrup) had $[\alpha]_D^{25}$ −76° (*c* 2·0 in EtOAc); the same solution had $[\alpha]_D^{25}$ −72° after 7·5 hr., $\nu_{max.}$ 2890, 1830s, 1639s, 1120, and 1032 $cm.^{-1}$ (in CH_2Cl_2) (Found: C, 59·4; H, 7·9. Calc. for $C_7H_{11}NO_2$: C, 59·5; H, 7·9%).

Reaction of L-4-*Isobutyloxazolone.*—(*a*) *With glycine ethyl ester.* The oxazolone (0·374 g.) was dissolved in dried benzene (5 ml.), and distilled glycine ethyl ester (0·26 ml.) was added dropwise. After 18 hr. the solvent was removed and the residue was taken up in ethyl acetate; the usual procedure (without recrystallisation) gave formyl-leucylglycine ethyl ester (0·44 g., 69%), m. p. 61—62·5°, $[\alpha]_D^{21}$ −3·6° (*c* 1 in EtOH) (Found: C, 53·9; H, 8·3; N, 11·4%).

(*b*) *With cyclohexylamine.* The oxazolone (0·207 g.) was dissolved in benzene (5 ml.), and cyclohexylamine (0·15 g.) was added dropwise. After 18 hr. the solvent was removed, and the *amide* was recrystallised from methanol–water, giving needles (0·23 g., 66%), m. p. 127—128°, $[\alpha]_D^{20}$ 0° (*c* 1·4 in EtOH) (Found: C, 65·0; H, 10·1; N, 11·75. $C_{13}H_{24}N_2O_2$ requires C, 65·0; H, 10·1; N, 11·7%).

*Formyl-*L*-leucine* N*-Cyclohexylamide.*—Formyl-L-leucine *p*-nitrophenyl ester (0·54 g.) in ethyl acetate reacted with cyclohexylamine (0·22 ml.) to give *amide* which was recrystallised from methanol–water; it had m. p. 154°, $[\alpha]_D^{18}$ −47° (*c* 1·5 in EtOH) (Found: C, 65·0; H, 9·9; N, 11·1%).

*Reaction of 2-Methyl-*L*-4-isobutyloxazolone with Glycine Ethyl Ester* (with Miss. I. ANTONOVICS). —The oxazolone, prepared as for L-4-isobutyloxazolone (above), had $[\alpha]_D^{20}$ −124·5° (*c* 1·1 in EtOAc); Nowak and Siemion [16] record $[\alpha]_D$ −136·6° (solvent not specified). Reaction (as described above for 4-isobutyloxazolone) with glycine ethyl ester gave acetyl-leucylglycine ethyl ester (83%) of m. p. 119—121·5°, $[\alpha]_D^{20}$ −2·8° (*c* 1·0 in EtOH); authentic L-peptide [7] has m. p. 99—100°, $[\alpha]_D^{24}$ −55·0° (*c* 1 in EtOH), and DL peptide [7] has m. p. 120—120·5°.

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