Hindered Amines in Peptide Synthesis. Synthesis of 7-Glycine-oxytocin

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OUR main purpose was to test the applicability of a hindered amine in peptide synthesis. Removal of amino-protecting groups usually results in a salt of the *N*-deprotected peptide. For the subsequent acylation the amino-group is set free by addition of a tertiary amine, the nature of which affects the extent of racemization during acylation,[1,2] but Bodanszky and Bodanszky[3] have shown that racemization *via* azlactones can be only prevented by extreme steric hindrance in the tertiary amine (*e.g.* tribenzylamine). A lesser hindrance such as in ethyldi-isopropylamine was almost without effect, but this base efficiently protects against loss of optical purity when racemization proceeds through β-elimination. In entirely stepwise syntheses[4,5] with amino-acids carrying urethan-type protecting groups, no azlactone intermediates can be expected, but racemization through β-elimination still has to be considered, especially if the sequence to be synthesized contains serine or cysteine residues. In preliminary experiments, ethyldi-isopropylamine was more promising than tribenzylamine, and its applicability to the synthesis of 7-glycine-oxytocin was tested.

7-Glycine-oxytocin was selected partly because the influence on biological activity of the amino-acid in position 7 of oxytocin is not yet known. Furthermore, proline and glycine play similar roles in the structure of proteins; both occur at the ends of helical stretches and lead to bending of a chain. Thus, while the proline residue probably lends a definite conformation to oxytocin, a glycine moiety in the same position would at least allow the molecule to take up a similar conformation, particularly if the geometry of the hypothetical receptor site provides for this. While our synthetic work was in progress, a paper on 7-glycine-lysine-vasopressin by Kolc, Zaoral, and Sorm[6] suggested similar considerations.

The synthesis of 7-glycine-oxytocin, closely followed the stepwise preparation[4] of oxytocin except that instead of converting the protected *C*-terminal tripeptide ester into the corresponding amide, the build-up of the chain started directly with the amide of the *C*-terminal amino-acid. Properties of the final product and of the intermediates in the synthesis of the hormone analogue are summarized in the Table.

Although the risk of racemization through β-elimination was present only during the introduction of the two *S*-benzylcysteine residues, ethyldi-isopropylamine was used in all acylations. The application of ethyldi-isopropylamine instead of triethylamine caused no difficulty in the coupling steps or in the isolation of the products. This preparation of 7-glycine-oxytocin shows that this hindered base can be applied in practical peptide synthesis.

The 7-glycine analogue of oxytocin exhibits the uterine-contracting ability of oxytocin; its potency[7] is about 330 units/mg. In the assay for anti-diuretic activity[8] only about 0·01 unit/mg. potency is found; thus 7-glycine-oxytocin seems to have an increased selectivity in its hormonal spectrum.

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TABLE

Compound[a]	M.p. (uncorr.)	$[\alpha]_D^{28}$	Found (calc.) (%) C	H	N	S	NH_3	Asp	Glu	Gly	Cys	i-Leu	Leu	Tyr	S-Bzl-Cys	R_F
(I)		−14·7 *c* 0·9 N-HOAc	49·4 (49·7)	6·5 (6·5)	17·6 (17·4)	6·6 (6·6)	3·0	1·0	1·1	2·0	2·0	1·0	1·0	1·0		0·40[b] 0·60[c]
(II)	103–104°	−8·5 *c*1, DMF														
(III)	113–115	−15·3 *c*1, DMF	56·9 (57·1)	7·0 (6·9)	14·8 (14·8)		1·0			2·0			0·95			
(IV)	182	−35·5 *c*1, DMF	56·9 (57·0)	6·6 (6·5)	12·2 (11·9)	5·7 (5·4)	1·0			2·2			1·0		1·0	0·59[d]
(V)	221–222	−43·1 *c*1, 80% HOAc	56·2 (56·0)	6·4 (6·3)	13·4 (13·3)	4·7 (4·7)	2·1	1·1		1·8			1·0		1·0	0·46[d]
(VI)	234–236 (decomp.)	−47·6 *c*1, 80% HOAc	54·3 (54·5)	6·5 (6·3)	15·6 (15·5)	4·0 (3·9)	2·8	1·0	1·1	2·0			0·9		0·95	0·29[d]
(VII)	252 (decomp.)	−48·3 *c*1, 80% HOAc	55·3 (55·7)	6·7 (6·7)	15·1 (15·1)	3·6 (3·5)	2·9	1·0	1·0	2·1		0·9	1·0			0·40[d]
(VIII)	258–260 (decomp.)	−32·1 *c*1, DMF	59·9 (60·0)	6·7 (6·6)	13·2 (13·1)	2·8 (2·7)		1·0	1·0	2·3		0·9	0·9		0·9	0·56[d]
(IX)	257—259 (decomp.)	−47·6 *c*1, DMF	58·1 (58·0)	6·6 (6·4)	13·2 (13·1)	5·1 (5·0)	2·9	1·1	1·1	2·1		1·0	1·0	0·95	2·1	

[a] (I) 7G-lycine-oxytocin, (II) Benzyloxycarbonyl-L-leucylglycinamide, (III) Benzyloxycarbonylglycyl-L-leucyl-glycinamide, (IV) *N*-Benzyloxycarbonyl-*S*-benzyl-L-cysteinylglycyl-L-leucyl-glycinamide, (V) Benzyloxycarbonyl-L-asparaginyl-*S*-benzyl-L-cysteinylcyl-L-leucyl-glycinamide, (VI) Benzyloxycarbonyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinylglycyl-L-leucylglycinamine, (VII) Benzyloxycarbonyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinylglycyl-L-leucylglycinamide, (VIII) *N*-Benzyloxycarbonyl-*O*-benzyl-L-tyrosyl-L-isoleucyl-L-glytaminyl-L-asparaginyl-*S*-benzyl-L-cysteinylglycyl-L-leucylglycinamide, (IX) *N*-Benzyloxycarbonyl-*S*-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinylglycyl-L-leucylglycinamide.

[b] T.l.c. on pre-coated silica gel plates; developed with butanol–acetic acid–water (4 : 1 : 5); spot revealed with ninhydrin.

[c] Developed with butanol–pyridine–acetic acid–water (30 : 20 : 6 : 24); spot revealed with ninhydrin.

[d] Hydrobromide of the *N*-deprotected peptide. T.l.c. developed with butanol–acetic–acid, water (4 : 1 : 1); spot revealed with ninhydrin.

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[1] G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Amer. Chem. Soc.*, **1966**, **88**, 1339.
[2] S. Sakakibara, and M. Itoh, *Bull. Chem. Soc., Japan*, 1967, **40**, 656.
[3] M. Bodanszky and A. Bodanszky, *Chem. Comm.*, 1967, 519.
[4] M. Bodanszky and V. du Vigneaud, *J. Amer. Chem. Soc.*, 1959, **81**, 5688.
[5] M. Bodanszky, *Ann. New York Acad. Sci.*, 1960, **88**, 655.
[6] J. Kolc, M. Zaoral, and F. Sorm, *Coll. Czech. Chem. Comm.*, 1967, **32**, 2667.
[7] R. A. Munsick, *Endocrinology*, 1960, **66**, 451.
[8] W. H. Sawyer, *Endocrinology*, 1958, **63**, 694.