PAPAIN-CATALYZED SYNTHESIS OF PHENYLHYDRAZIDES OF N-ACYLAMINO ACIDS*

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Received March 2nd, 1984

Phenylhydrazides of N^{α}-acyl derivatives of all coded amino acids, except proline, were prepared by papain (E.C. 3.4.22.2)-catalyzed synthesis. Comparison of yields affords information about the suitability of the amino acid in position P_1 in papain-catalyzed syntheses of peptides.

The use of phenylhydrazide group for protection of the α-carboxyl of amino acids* in the peptide synthesis has been so far only sporadical[2-4]. Only recently, this group has been employed mainly in enzyme-catalyzed peptide syntheses[5-7]. Phenylhydrazide group of the amino acid, whose amino group is engaged in the arising bond, enhances the affinity towards the catalytic site of the enzyme, lowers the solubility of the product affecting thus favourably the chemical equilibrium, is stable in mildly acidic as well as alkaline media and first of all it is resistant against undesired enzymatic cleavage during the synthesis. The facile oxidative removal[4-6,8,9] of this protecting group enables further synthesis of the peptide chain. It can be also oxidized to phenyldiimide[3,10-12] which can be used in a further acylation.

Beside by classical organic synthesis[13,14], phenylhydrazides of N-acylamino acids were prepared mainly by papain-catalyzed reaction[3,4,8,10,11,15-18]. Our comparative study concerns the preparation of phenylhydrazides of all coded amino acids using papain. This enzyme acts as an almost universal condensation reagent which reacts without racemization and side-reactions, enables synthesis of phenylhydrazides without protection of functional groups in the amino acid side chain and reacts selectively with the α-carboxy group in dicarboxylic acids.

The empirically chosen reaction conditions were the same for all the amino acids studied (except the amount of dimethylformamide required for dissolving the starting derivatives). The α-amino group was protected with benzyloxycarbonyl or tert-butyloxycarbonyl group. Fluorenylmethyloxycarbonyl derivatives of Ile, Gln and Asn

* Part CLXXXVII in the series Amino Acids and Peptides; Part CLXXXVI: This Journal *49*, 2012 (1984).

* The nomenclature and symbols obey the published recommendation[1]. All the chiral amino acids used in this work belong to the L-series.

did not react. The results of the reactions, together with data of the products are given in Table I.

Table I shows the effect of structure of the amino acid in position P_1 (ref.[19]). The enzyme is highly specific towards X-benzylcysteine and tryptophan; there is marked difference between glutamic acid and glutamine as compared with aspartic acid and asparagine. The low yield in the case of basic amino acids can be explained by high solubility of the products in the reaction mixture which hinders an equilibrium shift towards the desired direction. On the contrary, the cleavage of synthetic substrates with an arginine or lysine moiety in position P_1 is very rapid[20].

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. The mixtures were evaporated on a rotatory evaporator at bath temperature 30°C, dimethylformamide-containing mixtures at the same temperature at 150 Pa. Thin-layer chromatography was carried out on Silufol plates (Kavalier, Czechoslovakia) in the following systems: 2-butanol–98% formic acid–water (75 : 13·5 : 11·5) (S1), 2-butanol–25% aqueous ammonia–water (85 : 7·5 : 7·5) (S2), 1-butanol–acetic acid–water (4 : 1 : 1) (S3), 1-butanol–pyridine–acetic acid–water (15 : 10 : 3 : 6) (S4). Spots were detected by the chlorination method. Optical rotations were measured on a Perkin–Elmer 141 MCA polarimeter. High performance liquid chromatography was performed on a Spectra Physics SP 8700 instrument with an SP 8400 UV-detector and SP 4100 integrator. Preparative chromatography was done on a 25 × 1·27 cm column of Separon SIX C—18 (Laboratorní přístroje, Prague), flow rate 360 ml/h, detection at 220 nm; for analytical purposes a 15 × × 0·32 cm column filled with reversed phase of the same origin was used; mobile phase methanol–0·05% trifluoroacetic acid (1 : 1), flow rate 30 ml/h.

Preparation of Phenylhydrazides of N^{α}-Acylamino Acids

The given N^{α}-benzyloxycarbonyl- or N^{α}-tert-butyloxycarbonylamino acid (1 mmol) was dissolved in a 0·2 mol l^{-1} acetate buffer, pH 4·8; in cases of a sparingly soluble derivative, dimethylformamide (1—3 ml) was added so as the final volume of the reaction mixture was 10 ml. After addition of ethylenediaminetetraacetic acid (3 mg), phenyl hydrazine (100 μl) and cysteine hydrochloride (10 mg), the mixture was adjusted (if needed) to pH 4·8. Papain (52 mg) was added and the mixture was incubated for 24 h at 38°C. In most cases the product began to precipitate already during the first hours of incubation. The incubated mixture was extracted with ethyl acetate, washed with 1 mol l^{-1} hydrochloric acid (in cases of compounds containing tert-butyloxycarbonyl protecting group the washing was done with a HSO_4^- buffer), 0·5M-$NaHCO_3$, dried over sodium sulfate and taken down. The residue was triturated with light petroleum and the product was weighed and crystallized. In the experiment with benzyloxycarbonylglutamine the precipitate was filtered, washed with 1M-HCl, 0·5M-$NaHCO_3$ and water, weighed and crystallized.

In case of benzyloxycarbonylglutamic and benzyloxycarbonylaspartic acid the precipitate was filtered, washed with 1M-HCl and water and dissolved in ethyl acetate. The organic solution was washed with 1M-HCl, water, dried over sodium sulfate and the solvent was evaporated. The residue was triturated with light petroleum, weighed and further crystallized. All the above-mentioned derivatives were homogeneous in the thin-layer as well as high performance liquid chromatography.

TABLE I

Yields, physical properties and analytical data for the prepared phenylhydrazides of N-acylamino acids

X—$N_2H_2C_6H_5$ yield, %	Solvent	M.p., °C $[\alpha]_D$, deg[a]	Formula (mol.wt.)	Calculated/Found		
				% C	% H	% N
Z-Gly	ethanol	141·5[b]	$C_{16}H_{17}N_3O_3$	64·21	5·73	14·04
48		—	(299·3)	63·91	5·82	14·13
Z-Ala	ethyl acetate–	152—153[c]	$C_{17}H_{19}N_3O_3$	65·15	6·11	13·41
68	–light petroleum	−25·5[d]	(313·4)	65·26	5·87	13·40
Z-Leu	light petroleum	138·5—139·5	$C_{20}H_{25}N_3O_3$	67·58	7·09	11·82
74		−24·0	(355·4)	67·65	7·16	11·84
Boc-Leu	ethyl acetate–	131—132[e]	$C_{17}H_{27}N_3O_3$	63·53	8·47	13·07
60	–light petroleum	−38·8	(321·4)	63·48	8·80	12·79
Boc-Ile	ethyl acetate	155[f]	$C_{17}H_{27}N_3O_3$	63·53	8·47	13·07
30		−33·3[g]	(321·4)	63·49	8·67	13·01
Boc-Phe	light petroleum	67—69[h]	$C_{20}H_{25}N_3O_3$	67·57	7·09	11·82
68		−28·7	(355·5)	67·51	7·10	11·70
Boc-Tyr	ethyl acetate–	168—170	$C_{20}H_{25}N_3O_4$.	63·14	6·89	11·04
71	–light petroleum	−20·6	.0·5 H_2O (380·5)	63·62	6·54	10·85
Boc-Val	ethyl acetate–	138—139	$C_{16}H_{25}N_3O_3$	62·52	8·20	13·67
50	–light petroleum	−36·0	(307·4)	62·82	8·22	13·90
Z-Ser	methanol	172	$C_{17}H_{19}N_3O_4$	61·98	5·81	12·76
55		−22·1	(329·4)	62·05	5·84	12·88
Boc-Thr	ether–	83	$C_{15}H_{23}N_3O_4$	58·23	7·49	13·58
45	–light petroleum	−24·8	(309·4)	58·66	7·32	13·34
Boc-Met	ethyl acetate	117·5	$C_{16}H_{25}N_3O_3S$	56·61	7·42	12·38
82		−25·1	(339·5)	56·62	7·41	12·81
Z-Gln	methanol	227	$C_{19}H_{22}N_4O_4$	61·61	5·98	15·13
77		− 7·6	(370·4)	61·29	5·68	15·13
Z-Glu	ethyl acetate	182—183	$C_{19}H_{21}N_3O_5$	61·45	5·77	11·31
71		−25·7	(371·4)	61·01	5·82	11·12
Boc-Asn	ethyl acetate	185	$C_{15}H_{22}N_4O_4$	55·88	6·88	17·38
33		−28·6	(322·4)	55·92	6·97	17·14
Z-Asp	methanol–ether	163—164	$C_{18}H_{19}N_3O_5$	60·49	5·35	11·76
34		−23·8	(357·4)	60·76	5·15	12·11
Boc-Trp	ethyl acetate–	167[i]	$C_{22}H_{26}N_4O_3$	66·98	6·64	14·20
93	–light petroleum	−13·3	(394·5)	66·67	6·53	14·25

TABLE I
(*Continued*)

X—$N_2H_2C_6H_5$ yield, %	Solvent	M.p., °C $[\alpha]_D$, deg[a]	Formula (mol.wt.)	Calculated/Found % C	% H	% N
Z-Cys(Bzl)	ethyl acetate	141	$C_{24}H_{25}N_3O_3S$	66·18	5·78	9·65
90		−16·8	(435·6)	65·80	5·55	10·10
Z-His	methanol–ether	178–179	$C_{20}H_{21}N_5O_3$	63·31	5·58	18·46
21		−21·8	(379·4)	63·10	5·74	18·89
Boc-Lys	ether	108	$C_{17}H_{28}N_4O_3$	50·65	6·48	12·44
27[j]		− 5·8	$.CF_3COOH$	50·42	6·78	12·81
			(450·5)			
Z-Arg	ethyl acetate	150–151	$C_{20}H_{26}N_6O_3.0{\cdot}5\ H_2O$	50·67	5·41	16·12
25[j]		− 6·8	$.CF_3COOH$	50·32	5·43	16·00
			(521·5)			

[a] In methanol *c* 0·3–0·5 g/100 ml; [b] 142–144°C (ref.[18]); [c] 153–155°C (ref.[18]); [d] $[\alpha]_D$ −31·9° (*c* 2·0, pyridine) (ref.[18]); [e] 134–135°C (ref.[11]); [f] 152–154°C (ref.[7]); [g] $[\alpha]_D$ −21·2°C (*c* 2·0, dimethylformamide) (ref.[7]); [h] 134–136°C (ref.[11]), the reason of the difference from our value is not known, perhaps it is a printing error; [i] 168–169°C (ref.[11]); [j] other work-up procedure.

In case of benzyloxycarbonylhistidine the mixture was made alkaline with 1M-NaOH and extracted with ethyl acetate. The organic solution was washed with 0·5N-$NaHCO_3$ and water. After drying and removal of the solvent, the residue was several times triturated with light petroleum, decanted, crystallized from ethyl acetate and light petroleum, weighed and recrystallized; R_F 0·42 (S1), 0·36 (S2), 0·21 (S3), 0·71 (S4); $k' = 2{\cdot}90$.

α-Phenylhydrazides of benzyloxycarbonylarginine and tert-butyloxycarbonyllysine were, after evaporation of the reaction medium, isolated by HPLC using methanol–0·05% trifluoroacetic acid (9 : 11 and 7 : 13, respectively) as the mobile phase. For Z-Arg R_F 0·45 (S1), 0·03 (S2), 0·33 (S3), 0·64 (S4), $k' = 3{\cdot}22$; for Boc-Lys R_F 0·39 (S1), 0·08 (S2), 0·30 (S3), 0·63 (S3), $k' = 2{\cdot}72$.

We are indebted to Mrs Z. Ledvinová for optical rotation measurements. The elemental analyses were carried out in the Analytical Department of this Institute (Dr J. Horáček, Head).

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Translated by M. Tichý.