SYNTHESIS OF ANALOGUES OF THE THYREOTROPIN RELEASING FACTOR CONTAINING 2-OXOIMIDAZOLIDINE-1-CARBOXYLIC ACID

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2-Oxoimidazolidine-1-carbonyl-histidyl-proline amide (*I*) and 2-oxoimidazolidine-1-carbonyl--histidyl-phenylalanine amide (*II*) were synthesized by conventional chemical methods. 4,4′-Dimethoxybenzhydryl and 3,3′,4,4′-tetramethoxybenzhydryl groups were used for protection of primary amide functions. Both analogues, *I* and *II*, did not exhibit the increase in thyroxine (radioimmunoassay) when applied to male rats intraperitoneally.

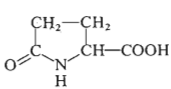
The presence of 5-oxopyrrolidine-2-carboxylic acid (pyroglutamic acid) in the tripeptide pyroglutamyl-histidyl-proline amide (TRF, thyroliberin*) is decisive for the manifestation of the thyreotropic hormone release and it contributes by 50% to the binding energy in the thyroliberin–receptor complex formation[4]. Analogues in which the pyroglutamic acid residue is replaced by another cyclic, potentially isosteric, amino acid exhibit a deep decrease in the thyreotropin releasing activity[4–6]. Surprisingly in another field of medical application of thyroliberin, in depression treatment, the analogue containing a higher homologue of pyroglutamic acid, *i.e.* containing the 6-oxopiperidine-2-carboxylic acid residue in position 1, exhibited a greater activity than the parent compound[7].

In the present paper we will describe the synthesis of two analogues (*I* and *II*) in which the pyroglutamic acid residue (*A*) is replaced by the 2-oxoimidazolidine-1-carbonyl group (*B*). In these analogues the ring β and γ methylene groups are preserved, but the δ-carbonyl oxygen atom and the lactam proton possess a retro orientation. In the analogue *II* the C-terminal proline residue is, in addition, replaced by the phenylalanine residue. The reasoning for the synthesis of the latter sequence, which we can compare with the ACTH 5–7 segment, is assisted by the fact that the behavioural activity of thyroliberin and that of pyroglutamyl-histidyl-phenylalanine amide are equal[8].

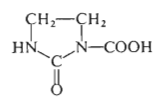
2-Oxoimidazolidine-1-carbonyl-histidyl-proline amide (*I*) and 2-oxoimidazolidine--1-carbonyl-histidyl-phenylalanine amide (*II*) were prepared using classical methods

* Symbols and nomenclature follow the recommendations published by IUPAC-IUB Commision on Biochemical Nomenclature for amino acids[1,2] and peptide hormones[3]. The chiral amino acids appearing in this paper are of the L series.

of peptide chemistry. In both cases the scheme of synthesis was the same as with the natural hormone[9]. For protecting the C-terminal amide group in the analogue *I* we applied the known 4,4′-dimethoxybenzhydryl group[10]. For the similar protection in the analogue *II* we used 3,3′,4,4′-tetramethoxybenzhydryl group which was applied till now only for protection of α-amino and mercapto groups[11,12]. In our hands this group turned out to be applicable for amide protection generally. The key intermediate was the 2-oxoimidazolidine-1-carbonyl-histidine hydrazide (*III*). Its synthesis started from histidine methyl ester which was acylated using the 2-oxoimidazolidine-1-carbonyl chloride[13] in mildly basic aqueous medium to the 2-oxoimidazolidine-1-carbonyl-histidine methyl ester which was converted, without isolation, by hydrazinolysis into the hydrazide *III*. The common intermediate *III* was coupled *via* azide[14] with proline 4,4′-dimethoxybenzhydrylamide[9] and phenylalanine 3,3′,4,4′-tetramethoxybenzhydrylamide (*V*), respectively, yielding the corresponding protected peptides *VI* and *VII*. In accordance with our previous observation pure products separated when the reaction mixture was adjusted to pH 9. The column operations were not necessary for additional purification. The last step, deprotection of *VI* and *VII* using trifluoroacetic acid, afforded trifluoroacetates of compounds *I* and *II* which were converted into acetates using Zerolite FF (acetate cycle). The amide *V* was prepared by hydrogenolysis of the N-benzyloxycarbonyl derivative *IV* resulting from N-benzyloxycarbonylphenylalanine amide and 3,3′,4,4′-tetramethoxybenzhydrole[15].



A



B

Biological activity was estimated on Wistar strain male rats (groups of 10 animals). The intraperitoneal application of analogues *I* and *II*, respectively, (50 μg in 5 ml of physiological solution) did not exhibit after 3 h any significant change of the thyroxine level in blood. The results of other pharmacological tests will be published separately.

EXPERIMENTAL

Melting points were determined on a Kofler block. Samples for elemental analyses were dried for several hours over phosphorus pentoxide at 70 Pa and 105°C; compounds melting below 120°C were dried at room temperature. Optical rotations were measured on the Perkin–Elmer

photoelectric polarimeter in methanol, *c* 0·2. Thin-layer chromatography was carried out on silica gel plates (Kieselgel G, Merck) in the solvent systems 1-butanol–acetic acid–water 4 : 1 : 1 (S1) and 1-butanol–acetic acid-pyridine–water 15 : 3 : 10 : 6 (S2). Analytical electrophoresis was performed in a moist chamber on Whatman 3 MM paper in the pyridine–acetic acid buffer of pH 5·7 at 23 V cm^{-1} for 50 min. The solutions were taken down under diminished pressure on a rotatory evaporator.

2-Oxoimidazolidine-1-carbonyl-histidine Hydrazide (*III*)

To the solution of histidine methyl ester dihydrochloride (2·42 g, 10 mmol) in water (40 ml) sodium hydrogen carbonate (4·0 g) and 1-butanol (40 ml) were added. The mixture was treated, after cooling to 0°C, with 2-oxoimidazolidine-1-carbonyl chloride (1·64 g, 11 mmol) in three portions within 20 min, stirred for further 30 min at room temperature and separated. The aqueous layer was extracted twice with 1-butanol, the combined organic layers were evaporated and the residue dissolved in methanol (20 ml). 80% Hydrazine hydrate (1·25 ml) was added to this solution. After standing for 2 days the mixture was taken down and the residue crystallized from methanol (10 ml); 1·2 g (59%) of *III*, m.p. 208—211°C. The sample for analysis was crystallized from 2-propanol, the change in the melting point value was not observed, $[\alpha]_D^{20}$ —27·2° (*c* 0·2, dimethylformamide). For $C_{10}H_{15}N_7O_3 + 1/2\,H_2O$ (209·3) calculated: 41·38% C, 4·56% H, 33·78% N; found: 41·15% C, 5·26% H, 34·03% N.

N-Benzyloxycarbonylphenylalanine 3,3′4,4′-Tetramethoxybenzhydrylamide (*IV*)

3,3′4,4′-Tetramethoxybenzhydrole (6·1 g, 20 mmol) and concentrated sulphuric acid (0·2 ml) were added to the solution of the N-benzyloxycarbonylphenylalanine amide (6·0 g, 20 mmol) in acetic acid (40 ml). The product which separated after 2 days standing at room temperature was filtered off, washed with acetic acid and water, dried and crystallized from ethyl acetate (100 ml) and light petroleum (150 ml). The yield was 9·1 g (77%), m.p. 196—197°C. The melting point of the sample for analysis was not changed after crystallization from ethanol, $[\alpha]_D^{20}$ +3·8° (*c* 0·2, dimethylformamide). For $C_{34}H_{36}N_2O_7$ (584·7) calculated: 69·85% C, 6·21% H, 4·79% N; found: 69·73% C, 6·21% H, 4·82% N.

Phenylalanine 3,3′,4,4′-Tetramethoxybenzhydrylamide (*V*)

The solution of *IV* (5·85 g, 10 mmol) in dimethylformamide (100 ml) was hydrogenated in the presence of palladium black for 7 h, filtrated and diluted with water (200 ml). After 2 h of standing at room temperature the product was filtered off, washed with water and dried; 3·7 g (76%) of *V* were collected with the melting point of 110—112°C which was not changed after recrystallization from the aqueous dimethylformamide, $[\alpha]_D^{20}$ —10·1° (*c* 0·2, dimethylformamide). For $C_{26}H_{30}N_2O_5 + H_2O$ (468·6) calculated: 66·65% C, 6·88% H, 5·98% N; found: 66·25% C, 6·69% H, 5·98% N.

2-Oxoimidazolidine-1-carbonyl-histidyl-proline 4,4′-Dimethoxybenzhydrylamide (*VI*)

To the solution of *III* (620 mg, 2·2 mmol) in dimethylformamide (40 ml) and concentrated hydrochloric acid (0·8 ml) the solution of sodium nitrite (155 mg) in water (0·62 ml) was added at —30°C. The reaction mixture was neutralized, after 10 min standing at —30°C, with N-ethylpiperidine to pH 6·9 and added to the solution of proline 4,4′-dimethoxybenzhydrylamide

oxalate (860 mg, 2 mmol) in dimethylformamide (20 ml) neutralized with N-ethylpiperidine (0·56 ml). After 12 h standing at 0°C the mixture was evaporated, the residue mixed with water (20 ml) and the pH of the solution was adjusted to 9 with 0·1M-NaOH. The mixture was left aside for 12 h at 0°C, the separated material was triturated three times with the saturated aqueous solution of sodium hydrogen carbonate, dried azeotropically with an ethanol–benzene mixture and crystallized from 2-propanol (15 ml) and diethyl ether (50 ml). The crystals were collected after 2 h standing, washed with diethyl ether and dried in a desiccator over sodium hydroxide. The yield was 410 mg (35%) of the product which was homogeneous in thin-layer chromatography and paper electrophoresis, exhibited a positive reaction with the Pauly reagent and was ninhydrin-negative, m.p. 145—148°C. The sample for analysis was crystallized on the same way, m.p. 147—150°C, $[\alpha]_D^{20}$ —46·8° (*c* 0·2, methanol); R_F 0·25 (S1), 0·64 (S2). For $C_{30}H_{35}N_7O_6$ + + 2 H_2O (625·7) calculated: 57·59% C, 6·28% H, 15·67% N; found: 56·91% C, 5·86% H, 15·39% N.

2-Oxoimidazolidine-1-carbonyl-histidyl-phenylalanine 3,3′,4,4′-Tetramethoxybenzhydrylamide (*VII*)

The crude *VII* was prepared from *III* and *V via* azide synthesis. The solid resulting after evaporation of the reaction mixture was crystallized three times from 50% aqueous 2-propanol (10 ml). This purification yielded 290 mg (20%) of *VII* which was chromatographically and electrophoretically homogeneous. The sample for analysis was crystallized from 2-propanole and diethyl ether, m.p. 124—126°C; R_F 0·22 (S1), 0·54 (S2). For $C_{36}H_{41}N_7O_8$ + 1/2 H_2O (708·8) calculated: 61·01% C, 5·97% H, 13·83% N; found: 60·72% C, 5·95% H, 13·40% N.

2-Oxoimidazolidine-1-carbonyl-histidyl-proline Amide Acetate (*I*)

The solution of *VI* (390 mg, 0·62 mmol) in trifluoroacetic acid (4·1 ml) and anisole (0·41 ml) yielded after 10 min boiling and after precipitation with diethyl ether a voluminous solid which was filtered off, washed with diethyl ether, dried in a desiccator over sodium hydroxide for 2 h, and dissolved in water (10 ml). The solution was stirred for 2 h with Zerolite FF (acetate cycle, about 2 ml) and then filtered through a column of the same ion-exchanger. The conversion was finished by elution with 1% aqueous acetic acid. The combined eluates were evaporated, the residue dried azeotropically with ethanol–benzene and precipitated from the solution in methanol (2 ml) with diethyl ether (50 ml). 135 mg (50%) of a homogeneous product were collected which was ninhydrin-negative and exhibited the positive reaction with Pauly reagent, $[\alpha]_D^{20}$ —50·8° (*c* 0·26, water); R_F 0·11 (S1), 0·43 (S2). For $C_{15}H_{21}N_7O_4$ + $C_2H_4O_2$ + H_2O (441·5) calculated: 46·25% C, 6·16% H, 22·21% N; found: 46·56% C, 5·93% H, 22·56% N.

2-Oxoimidazolidine-1-carbonyl-histidyl-phenylalanine Amide Acetate (*II*)

The compound *II* was prepared from *VII* in a similar way as described for *I*. The product (70% yield) was homogeneous, ninhydrin-negative and positive in reaction with the Pauly reagent, $[\alpha]_D^{20}$ —37·7° (*c* 0·05, 50% methanol), R_F 0·14 (S1), 0·51 (S2). For $C_{19}H_{23}N_7O_4$ + $C_2H_4O_2$ + + H_2O (493·6) calculated: 51·10% C, 5·92% H, 19·86% N; found: 51·00% C, 5·44%H , 20·53% N.

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