SYNTHESIS OF ANALOGUES OF THE MELANOCYTE-STIMULATING HORMONE RELEASE-INHIBITING FACTOR CONTAINING 2-OXOIMIDAZOLIDINE-1-CARBOXYLIC ACID

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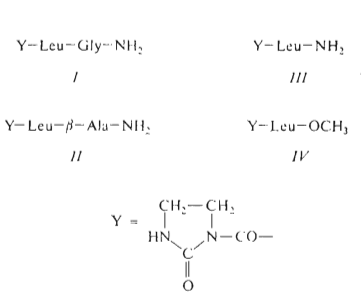
2-Oxoimidazolidine-1-carbonyl-leucyl-glycine amide (*I*), 2-oxoimidazolidine-1-carbonyl-leucyl-β-alanine amide (*II*), and 2-oxoimidazolidine-1-carbonyl-leucine amide (*III*) were synthesized by the acylation of corresponding dipeptide or amino-acid esters with 2-oxoimidazolidine-1-carbonyl chloride and following ammonolysis. Analogues *I* and *II*, containing 2-oxoimidazolidine-1-carboxylic acid instead of proline, exhibited the antiamnestic activity in the doses of 1 mg per kg subcutaneously, *III* was without effect.

The established primary structure of the melanocyte-stimulating hormone release-inhibiting factor (MIF, melanostatin*)[1] and the recognition of its several pharmacological activities, *e.g.* antiparkinsonic action[5], oxotremorine antagonism[6], antidepressant activity[7], morphine dependence facilitation[8], and inhibition of the ACTH release[9] prompted the search for analogous peptides exhibiting a more selective action. Proline residue seems to be the first rank target in these studies because of its known positive effect upon memory and depression[10]. Therefore, analogues modified in position 1 have been preferentially studied, *inter alia* those containing cyclic amino acids nearly isosteric with proline. Thiazolidine-4-carboxylic acid[11] and 5-oxopyrrolidine-2-carboxylic acid (pyroglutamic acid)[12] have been used by other research groups. 2-Oxoimidazolidine-1-carboxylic acid seems to be another structure isosteric with proline. In our present communication we shall describe the synthesis of three analogues of melanostatin having in position 1 this achiral acid. In addition, we have modified also the C-terminus of the peptide backbone. The glycine residue was either replaced by the β-alanine residue or omitted. Following derivatives have been synthesized: 2-Oxoimidazolidine-1-carbonyl-leucyl-glycine amide (*I*), 2-oxoimidazolidine-1-carbonyl-leucyl-leucyl-β-alanine amide (*II*), and 2-oxoimidazolidine-1-carbonyl-leucine amide (*III*).

* Symbols and nomenclature follow the recommendations published by the IUPAC-IUB Commission on Biochemical Nomenclature for amino acids[2,3] and peptide hormones[4]. The chiral amino acids appearing in this paper are of the L-series.

The syntheses of *I* and *II* started from the C-terminal dipeptide esters, *i.e.* from leucyl-glycine ethyl ester[13] and leucyl-β-alanine methyl ester[14], respectively. Acylation by the 2-oxoimidazolidine-1-carbonyl chloride[15] in non-aqueous medium yielded the intermediates – 2-oxoimidazolidine-1-carbonyl-leucyl-glycine ethyl ester and 2-oxoimidazolidine-1-carbonyl-leucyl-β-alanine methyl ester, respectively, which were converted by ammonolysis into the amides *I* and *II*. The derivative *III* was prepared according to the same scheme starting from leucine methyl ester.



Pharmacological activities have been evaluated in the same manner as with melanostatin which, *e.g.*, attenuates puromycin-induced amnesia in mice and increases resistance to extinction of a pole-jumping avoidance response in rats[16,17]. We examined the antiamnestic properties of *I*–*III* in rats using the step-through passive avoidance paradigm[18], the amnesia being induced by general anesthetic halothane. Both *I* and *II* attenuated the effects of amnestic treatment in the dose of 1 mg per kg subcutaneously. *III* was without effect. Details will be published in another paper.

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. Standard working up of a reaction mixture implies evaporation under diminished pressure, solution of the residue in ethyl acetate, washing the solution with 1M-HCl, water, 5% aqueous sodium hydrogen carbonate solution, and water, drying with sodium sulphate, and taken down under diminished pressure on a rotatory evaporator.

2-Oxoimidazolidine-1-carbonyl-leucine Methyl Ester (*IV*)

The solution of leucine methyl ester hydrochloride (5·46 g, 30 mmol) in dichloromethane (150 ml) was mixed with N-ethylpiperidine (9·6 ml, 67·5 mmol), cooled down to —10°C and treated with 2-oxoimidazolidine-1-carbonyl chloride (5·64 g, 37·5 mmol, in 3 portions within 30 min). The

mixture was stirred at room temperature for further 30 min and worked up. The residue was crystallized from ethyl acetate (15 ml) and light petroleum (150 ml), the yield was 3·6 g (47%) of *IV*, m.p. 64—65°C. The sample for analysis was crystallized once again from the same solvent mixture, no change in the melting point value was observed; $[\alpha]_D^{20}$ —19·7. For $C_{11}H_{19}N_3O_4$ (257·3) calculated: 51·35% C, 7·44% H, 16·35% N; found: 51·27% C, 7·61% H, 16·23% N.

2-Oxoimidazolidine-1-carbonyl-leucyl-glycine Amide (*I*)

The solution of leucyl-glycine ethyl ester hydrobromide, prepared from the corresponding N-benzyloxycarbonyl derivative (3·5 g, 10 mmol) by deprotection using 35% HBr in acetic acid, in dichloromethane (50 ml) was mixed with N-ethylpiperidine (2·8 ml, 20 mmol), cooled down to —10°C and treated with 2-oxoimidazolidine-1-carbonyl chloride (1·49 g, 10 mmol). The mixture was stirred for further 30 min at room temperature and worked up. The residue was disolved in methanol (5 ml), the solution was treated with a solution of ammonia in methanol (17%, 5 ml), and evaporated after standing for 2 days at room temperature. The residue yielded after crystallization from 2-propanol 1·45 g (48% calculated on the starting protected dipeptide) of *I*, m.p. 159—162°C. The sample for analysis was crystallized from 2-propanol, m.p. 176 to 177°C, $[\alpha]_D^{20}$ +4·2°. For $C_{12}H_{21}N_5O_4$ (299·3) calculated: 48·15% C, 7·07% H, 23·40% N; found: 48·49% C, 7·32% H, 23·60% N.

2-Oxoimidazolidine-1-carbonyl-leucyl-β-alanine Amide (*II*)

Using the same procedure as for *I*, the amide *II* was prepared in the yield of 42% and m.p. 176 to 179°C. The analytical sample exhibited m.p. 181—183°C (2-propanol) and $[\alpha]_D^{20}$ —4·0°. For $C_{13}H_{23}N_5O_4$ (313·4) calculated: 49·83% C, 7·40% H, 22·35% N; found: 49·58% C, 7·33% H, 21·66% N.

2-Oxoimidazolidine-1-carbonyl-leucine Amide (*III*)

To the solution of *IV* (1·3 g, 5 mmol) in methanol (5 ml) a solution of ammonia in methanol (17%, 5 ml) was added. The mixture was left for 2 days at room temperature, taken to dryness and the residue was crystallized from a methanol-diethyl ether mixture, the yield of this crop was 0·83 g, m.p. 151—153°C. The sample for analysis was crystallized from the same solvent mixture, m.p. 177—180°C, $[\alpha]_D^{20}$ + 5·8°. For $C_{10}H_{18}N_4O_3$ (242·3) calculated: 49·58% C, 7·49% H, 23·12% N; found: 49·00% C, 7·20% H, 23·14% N.

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