SYNTHESIS AND ANTIHYPERTENSIVE ACTIVITY OF SEVERAL N-ACYL D-PENICILLAMINES

V. G. Granik, S. I. Grizik,
I. F. Fayermark, G. Ya. Shvarts,
N. P. Solov'eva, V. V. Chustyakov,
and M. D. Mashkovskii

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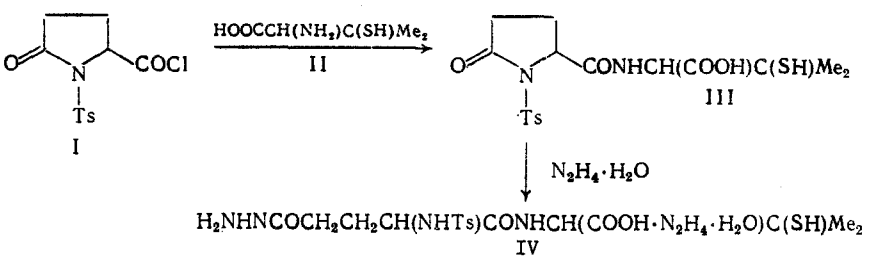
We have recently [1] presented a hypothesis concerning the substantive role of peripheral enzymatic decarboxylation in the inactivation of angiotensin-1-converting enzyme (ACE, kininase II) inhibitors which contain an aminoacid group. To substantiate this hypothesis, it was shown experimentally that the combined administration of captopril or amidomercaptoacids with decarboxylase inhibitors such as isonicotinic hydrazide, cyanoacetate, and other acids led to more potent and prolonged antihypertensive effect than for the compounds alone. On the other hand, it has been established that the presence of hydrazide groups in the absence of carboxyl groups [3] leads to a reduction in antihypertensive effects, which is in agreement with literature data concerning the requirement for a free carboxyl group [4] in inhibitors of ACE.

In order to further substantiate this hypothesis, we undertook to synthesize compounds which combined the groups needed to confer ACE-inhibitory activity [4], such as acylamino, carboxyl, and mercapto groups, with hydrazide moieties capable of playing the role of "internal" decarboxylase inhibitors.

The compounds were derived from condensation of N-tosylpyroglutamyl chloride I [8] and D-penicillamines II substituted with groups (such as amidomercaptoacids) that confer antihypertensive properties [1]. The reaction of compounds I and II in benzene led to the formation of N-(1-tosylpyroglutamyl)-penicillamine III in high yield. The amino group was selectively acylated as shown by NMR and mass spectral data. In the NMR spectrum of compound III in d_6-DMSO, in addition to the methyl and methylene protons and the protons of the benzene ring (see Experimental Section), the SH proton signal is visible at 2.96 ppm (s, 1 proton), as are the methine proton (CH—NH group) appearing as a doublet (with a one-proton integral) centered at 4.47 ppm with a $^3J_{CH,NH}$ = 9.5 Hz and an NH-amide resonance at 8.72 ppm (d, 1 proton). In the mass spectrum of III the molecular ion was not observed. The following intense ions of ratio m/z were observed (intensities in %): 340 (20) $[M-Me_2CS]^+$, 322 (10) $[M-Me_2CS-H_2O]^+$, 238 (17) $[M-Me_2C(SH)-CH(COOH)NHCO]^+$, 155 (80) $[MeC_6H_4SO_2]^+$, 91 (100) $[MeC_6H_4]^+$, 84 (85) $[C_4H_6NO]^+$. The presence in the mass spectrum of peaks attributable to the elimination of the Me_2CS group from the molecular ion demonstrates the N-acylation of II.

The cleavage of the pyrrolidinone ring was carried out by refluxing an alcoholic solution of lactam III with hydrazine hydrate. This reaction led to the formation of compound IV, containing the structural elements described above (SH, COOH, CONH, and $CONHNH_2$), necessary for binding to the ACE and for inhibition of the decarboxylation process. The formation of compound IV was confirmed by elemental analysis and NMR and mass spectrometry (see Experimental Section). In the mass spectrum of IV (similarly to compound III), no molecular ion was observed, but the fragmentation pattern substantiates the proposed structure.



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EXPERIMENTAL (CHEMISTRY)

Mass spectra were obtained on a Varian MAT-112 using direct introduction of the sample into the ionization chamber. The temperature of the ionization chamber was 180°C. The energy of the ionizing electron was 70 eV. NMR spectra were obtained using a Varian XL-200, with TMS as an internal standard. Melting points were obtained using a Boetius hot stage. The elemental compositions found corresponded to the calculated values.

N-(1-Carboxy-2-methyl-2-mercaptopropyl) Amide of Pyroglutamic Acid (III). A mixture of D-penicillamine (2.08 g, 14 mmole) and N-tosylpyroglutamyl chloride (4.22 g, 14 mmole) [8] in 50 ml benzene was refluxed for 5 and cooled; the precipitate was filtered out and dried in a desiccator to yield 4.67 g (82%) of compound III. mp 225-226°C (ethanol). $C_{17}H_{22}N_2O_6S_2$. NMR (d_6-DMSO, δ, ppm): 1.41 ($2CH_3$, s, 6H), 2.41 (p-CH_3, s, 3H), 1.9-2.2 (β-CH_2, γ-CH_2, m, 4H), 2.96 (SH, s, 1H), 4.47 (NH-CH, d, 1H, $^3J_{CH,NH}$ = 9.5 Hz), 5.08 (α-CH, m, 1H), 8.72 (NH-CH, d, 1H), 7.38 and 7.80 (C_6H_4, d, 2H each).

Hydrazine Hydrate Salt of N-(α-(N'-Tosylamino)-γ-carbohydrazidobutyryl)-penicillamine (IV). A mixture of amide III (2.3 g, 5.5 mmole) and hydrazine hydrate (5.55 g, 0.11 mole) in 50 ml absolute alcohol was refluxed for 4 h and filtered; the filtrate was evaporated, the residue triturated in absolute ether, and the solid filtered out and dried in a desiccator to yield 2.04 g (75%) compound IV. mp 128-138°C (2-propanol–absolute ether 20:1). $C_{17}H_{32}N_6O_7S_2$. Mass spectrum: m/z (relative intensity %): 337 (7) $[M-H_2O-CH_3C_6H_4]^+$, 238 (13) $[M-N_2H_4-Me_2C(SH)CH(COOH)NHCO]^+$, 155 (50) $[MeC_6H_4SO_2]^+$, 91 (100) $[MeC_6H_4]^+$. NMR (d_6-DMSO, 50°C, δ, ppm): 1.18 and 1.25 ($2CH_3$, s, 3H each), 1.70 and 2.05 (β- and γ-CH_2, m, 2H each), 4.05 (α-CH, t, 1H), 4.15 (HOOCCHNH, d, $^3J_{CH,NH}$ = 6 Hz, 1H), 7.30 and 7.62 (C_6H_4, d, 2H each), 7.91 (NHCHCOOH, d, 1H).

EXPERIMENTAL (PHARMACOLOGY)

Compounds III and IV were examined for characteristic indications of ACE inhibitory activity, antihypertensive activity, potentiation of bradykininaction, and influences of the pressor effect of angiotensin-I.

The effect of compounds III and IV on arterial pressure (AP) were studied using non-narcotized female rats of 250-270 g mass with model renovascular hypertension, induced by the application of a Nichrome wire spiral to the right renal artery [5], by the methods of direct recording (with the animals in a free condition) and indirect recording. For direct recording of AP in rats in a free state a polyethylene catheter (external diameter 0.61 mm) was implanted 24 h before the experiment in the carotid artery of the animals, the end of which was conducted beneath the skin and fixed to the nape of the neck [6].

Measurements of AP in the carotid artery was carried out using a "Statham P236B Gould" transducer (USA), through a 551-A, HSE pressure unit (FRG) with a "Watanabe Mark III" recorder (Japan).

Compounds III and IV were administered internally in doses of 10 mg/kg; the recording was continued 2 h from the time of administration.

Indirect measurements of AP in non-narcotized rats was carried out with a photoelectric transducer (IITC, USA) in the tail artery of the animals. The signal was recorded on a "Mod-45" unit through a "Mod-29" calculator produced by the same company. At the time of measurement the animals were kept in a chamber at 28°C [7].

Compounds III and IV were administered internally in doses of 10 mg/kg, and measurements were taken up to the time of administration (original level) and 15, 30 min, 1, 2, 3, 5, and 24 h after administration.

The effects of compounds III and IV on the pressor effect of angiotensin-I (AI, 5 μg/kg, internally) and the depressor effect of bradykinin (BK, 1 μg/kg, internally) were studied using narcotized (1 g/kg intraperitoneal urethane) rats of both sexes and of mass 230-250 g. Carotid artery AP was determined using the above-mentioned procedure for direct recording. Compounds III and IV were given through a venous catheter in doses of 0.1, 0.5 and 1.0 mg/kg as extemporal solutions.

The effects of compounds III and IV on the spasmogenic effect of BK was examined using isolated ilium segments from guinea pigs of both sexes (weight 300-350 g). The contractions of the segments, which were kept in a thermostatic bath (37°C) designed for isolated organs (content 20 ml), in aerated Tyrode's solution, were recorded in an isotonic regime with an

TABLE 1. Effects of Compounds III and IV on AP in Non-narcotized Rats with Model Renovascular Hypertension (indirect recording) in Comparison with Captopril (number of animals in each group 6)

Compound (10 mg/kg internal)	AP (original) mm Hg	AP and ΔAP (mm Hg) after compound administration						
		after 15 min	after 30 min	after 1 h	after 2 h	after 3 h	after 5 h	after 24 h
III	145±4,5	142±4,0	138±3,0	136±3,0 Δ = 9	127±4,5* Δ = 18	126±6,4* Δ = 19	134±7,5 Δ = 12	140±5,0
IV	155±8,0	149±9,5	139±6,2 Δ = 16	134±4,0* Δ = 21	133±5,3* Δ = 22	120±6,5* Δ = 35	120±4,5* Δ = 35	148±4,9
Captopril	155±6,5	145±8,6	135±5,0* Δ = 20	125±6,5* Δ = 30	125±7,5* Δ = 30	115±5,4* Δ = 40	118±6,3* Δ = 38	152±5,6

*Confidence level $p < 0.05$.

HSE mechanoelectric transducer (FRG) and a "Multicorder 6602" recorder (Watanabe, Japan). The final concentration of BK was 10^{-7} g/ml, and the duration of contact of the test object with the experimental compounds was 3 min.

Captopril (Squibb, USA) was used as a control compound in all experiments.

Statistical analysis of the data included calculation of mean and standard error; the Student's t criteria were used in comparison of the means.

Data concerning the effect of compounds III and IV on AP in renovascularized rats using indirect recording in the tail artery are presented in Table 1.

As is apparent from Table 1, compound IV, containing an "internal hydrazide," exhibited greater potency and duration of effect than compound III in assays of antihypertensive activity, but did not exceed the effect of captopril, in rats with model renovascular hypertension. Compound III produced a decrease in AP, starting with the second hour after administration (maximum decrease 20 mm Hg), but the duration of action did not exceed 3 h, whereas compound IV produced a decrease in AP starting with the first hour after administration, with the maximal decrease in AP appearing in the third to fifth hour after administration with a maximum decrease of 35 mm Hg (compared to 40 mm Hg decrease for captopril). In other words the intensity and duration of the antihypertensive effect of compound IV exceeded that of compound III by a factor of 2.

In direct recording of AP in rats in a free condition, compound IV at the same dose (10 mg/kg internal) elicited a definite decrease in AP relative to the initial level at 30 min after administration. The maximal decrease in AP was 25-30 mm Hg, and the duration of the effect was observed to be greater than 2 h. Captopril was more active in these experiments; the decrease in AP was found to be 40-45 mm Hg after administering doses of 10 mg/kg. Compound III was found to be inferior to compound IV and captopril in activity.

In experiments on narcotized rats, compound III in doses of 0.1 and 0.5 mg/kg (internal administration) did not show any effect on the intensity or duration of the depressor effect of BK or on the increase in AP elicited by administration of AI. On increasing the dose to 1 mg/kg (internal) compound III produced a strengthening of the hypotensive response to an administration of BK (1 μg/kg internal) of 25%, and diminished by 20% the pressor reaction to AI. Compound IV in doses of 0.1 mg/kg similarly did not show any influence on the depressor effect of BK; however, increasing the dose to 0.5 and 1 mg/kg (internal) led to the appearance of a BK-potentiating effect: the increase of the hypotensive reaction from a dose of 0.5 mg/kg amounted to 30%, and doses of 1 mg/kg produced an increase of 100% relative to the original level. Compound IV in doses of 0.1 and 0.5 mg/kg, similarly to compound III, had no effect on the pressor reaction of AP to administration of AI, but in doses of 1 mg/kg it produced a 50% decrease in the pressor reaction to AI relative to the original level; the duration of the effect was found to be 50-60 min. Captopril was found in these experiments to be more effective than either compound III or IV, producing a strengthening of the depressor effect of BK by 60% relative to the initial level, and an inhibition of the pressor reaction to AI of 80% in doses of 0.1 mg/kg (internal).

It was shown using isolated guinea pig ilium segments that compounds III, IV, and Captopril exhibited a BK-potentiating effect of approximately the same degree. The EC_{200}, that is,

the concentration of compound that increased the spasmogenic effect of BK by 100% relative to the original level, was found to be 10^{-6} g/ml for compound III and captopril, and 2×10^{-6} g/ml for compound IV.

The results of these experiments show that compounds III and IV show properties characteristic of ACE (kininase II) inhibitors: the presence of a BK-potentiating effect in vitro and in vivo, diminishment of the pressor reaction of AP on administration of angiotensin-I, and an antihypertensive effect.

Hydrazide IV in experiments in vivo exceeded compound III by a factor of 2 in all biological activities (approaching in several instances that of captopril) that can be associated with the inhibitory influence of the hydrazide group on the process of enzymatic decarboxylation.

The data obtained confirm the possibility of increasing the intensity and duration of effect of inhibitors of ACE that contain carboxyl groups by decreasing the rate of enzymatic decarboxylation, and suggest that the search for compounds of this type that contain mercapto-, carboxyl, and amido groups also include compounds with hydrazide moieties.

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SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF FURO-1,4-DIHYDROPYRIDINES

I. P. Skrastin'sh, V. V. Kastron,
R. O. Vitolin', G. Ya. Dubur,
M. I. Stivrinya, and K. A. Kaidaka

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Interest has recently arisen in furo-1,4-dihydropyridines following the detection of good inotropic activity in compounds of this type [3, 9, 12]. Unlike 1,4-dihydropyridines, which are antogonists for calcium ions, furo-1,4-dihydropyridines facilitate the entry of these ions into the cell [4].

Previously reported methods of synthesis of furo-1,4-dihydropyridines [5, 9, 13] are multistage, and require the use of complex reactants. It has recently been shown [6] that pyridinium bromide perbromide reacts with 1,4-dihydropyridines to give furo-1,4-dihydropyridines. We have examined the effects of some other brominating agents on 2,6-dimethyl-3,5-dimethoxycarbonyl-4-(2'-nitrophenyl)-1,4-dihydropyridine (nifedipin, fenigidin) [1]. It was found that these reactions also give furo-1,4-dihydropyridines, and that the best yields were obtained with N-bromosuccinimide (NBS). (See scheme on next page.)

In order to identify new furo-1,4-dihydropyridines with cardiotropic activity, a number of its derivatives were obtained by reacting the 1,4-dihydropyridines (Ia-i) with NBS in chloroform.

The resulting furo-1,4-dihydropyridines (IIa-i) were crystalline solids, stable in the solid state and in solution. Oxidation of (IIb, d, e, g) with 3 N nitric acid gave good yields of the furopyridines (IIIb, d, e, g).

Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR.

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