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# SYNTHESIS AND ANTIHYPERTENSIVE ACTIVITY OF N-ACETYLMERCAPTOPROPIONYL-6-(2'-PHENYLETHYL)PIPECOLINIC ACIDS

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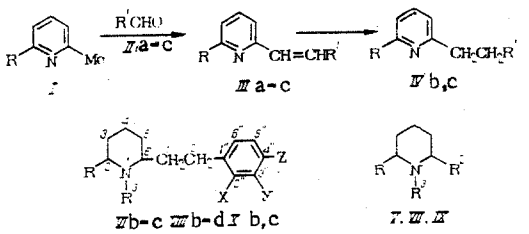
The search for drugs with antihypertensive activity has in recent years been closely associated with the control of the renin-angiotensin and kallikrein-kinin systems, which operate on the vascular tonus, and with the normalizing balances of water and electrolytes in the body [8, 11].

These systems carry out their functions by generating the powerful pressor octapeptide angiotensin II, and the straight-chain peptides possessing higher depressor activity, subsumed under the general title of kinins. The formation of the pressor angiotensin II from angiotensin I, which has little biolical activity, and deactivation of the depressor kinins is effected in the body by the catalytic action of the same enzyme, dipeptidylcarboxypeptidase (DCP). Inhibition of this enzyme prevents the formation of angiotensin II, simultaneously inhibiting the breakdown of kinins, with the overall result of a decrease in arterial pressure (AP).

One of the most effective inhibitors of DCP, which has found practical application in the treatment of malignant and renovascular hypertonia and of hypertonic disease with high AP levels, is N-[(2S)-3-mercapto-2-methylpropionyl]-L-proline, which has received the commercial designation Captopryl.

Our studies with piperidine analogs of Captopryl [5] have shown the desirability of examining N-acetylmercaptopropionyl derivatives of pipecolinic acids as novel antihypertensive drugs. In the course of these studies, it was found that those compounds of this type with a sterically hindered amide function showed the greatest antihypertensive and DCP inhibitory effect. Such steric hindrance is usually achieved in Captopryl type compounds by branching of the side chain of the acylating group. We have shown that it is possible to attain the same effect by introducing screening groups into the heterocyclic framework, rather than into the side chain.

Continuing these studies, we have now synthesized and examined the antihypertensive activity of the hitherto unknown N-acetylmercaptopropionylpipecolinic acids, bearing in the 6-position of the piperidine ring a variety of substituted phenyl and cyclohexylethyl residues.



I, IIIa-c IVb, c, V, VIb, c: R = COOEt; VII, VIIIb-d IX, Xb, c, R = COOH; IIa-c IIIa-c IVb, c: $R^1 = C_6H_2X$-2-Y-3-Z-4; V, VIb, c., VII, VIIIb-d $R^3 = H$; IX, Xb, c: $R^3 = COCH_2CH_2SAc$; V, VII, IX: $R^2 = CH_2CH_2C_6H_{11}$ cyclo, II, III: X = Y = = Z = H (a); II, III, IV, VI, VIII, X: X = Z = Cl, Y = H (b), X = H, Y = Z = = OMe (c); VIIId: X = H, Y = Z = OH.

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TABLE 1. Mass Numbers and Intensities of Characteristic Ions in the Mass Spectra of (III-X) [m/z ($I_{rel}$, %)]

| Compound | M | $[M-COOR]^+$ | $ArCH_2^+$ | $[M-C_2H_4Ar]^{+\bullet}$ | $[M-R'-COOR]^+$ | $[M-COCH_3]^+$ | $[M-SCOCH_3]^+$ | $[M-C_7H_4SCOCH_3]^+$ |
|---|---|---|---|---|---|---|---|---|
| III a | 253 (57) | 180 (35) | | | | | | |
| III b** | 321 (13) | 248 (15) | | | | | | |
| III c | 313 (80) | 240 (20) | | | | | | |
| IV b | 323 (100) | 250 (<1) | 159 (76) | | | | | |
| IV c | 315 (90) | 242 (2) | 151 (100) | | | | | |
| V | 267 (4) | 194 (100) | — | 156 (72) | | | | |
| VI b | 329 (8) | 256 (100) | 159 (33) | 156 (64) | | | | |
| VII | 239 (1) | 194 (20) | — | 128 (100) | | | | |
| VIII b | 301 (<1) | 256 (20) | 159 (25) | 128 (100) | | | | |
| VIII c | 293 (17) | 248 (33) | 151 (41) | 128 (100) | | | | |
| VIII d | 265 (11) | 220 (28) | 123 (42) | 128 (100) | | | | |
| IX | 369 (<1) | 324 (3) | — | 128 (100) | 194 (31) | 326 (2) | 294 (1) | 266 (2) |
| Xb | 431 (3) | 386 (4) | 159 (20) | 128 (100) | 256 (35) | 388 (5) | 356 (7) | 328 (2) |
| X c | 423 (19) | 378 (6) | 151 (39) | 128 (100) | 248 (14) | 380 (4) | 348 (6) | 320 (2) |

*$[M - R' = C_7H_4Ar]^+$ for (IX) and (Xb, c).
**The principal breakdown of (IIIb) is $[M - Cl]^+$.

TABLE 2. Ethyl Esters of Substituted 6-Styrylpicolinic Acids

| Compound | Condensation time of (I) with (II), h | Yield, % | mp, °C (solvent for crystallization) | $R_f$ (system) | IR spectrum, $\nu_{max}$, $cm^{-1}$ | GC, ret. time, min | Found, % | | | | Empirical formula | Calculated, % | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | C | H | N | Cl | | C | H | N | Cl |
| III a·HCl | 62 | 25 | 163—5 (acetone) | 0,84 (A) 0,88 (C) | 1740 ($COOC_2H_5$), chl | 2,5 | 66,22 | 5,61 | 4,84 | 12,32 | $C_{16}H_{15}NO_2 \cdot HCl$ | 66,32 | 5,57 | 4,83 | 12,24 |
| III b·HCl | 24 | 95 | 161—3 ethyl acetate/alcohol 2:1) | 0,89 (A) 0,91 (C) | 1745 ($COOC_2H_5$), v/o | 7,17 | 53,50 | 3,93 | 3,82 | 29,64 | $C_{16}H_{13}Cl_3NO_2 \cdot HCl$ | 53,58 | 3,93 | 3,91 | 29,66 |
| III b | | 88 | 93—4 | | | | 59,79 | 4,03 | 4,23 | | $C_{16}H_{13}Cl_2NO_2$ | 59,64 | 4,07 | 4,35 | |
| III c·HCl | 72 | 34 | 196—8 ethyl acetate/alcohol 10:7) | 0,84 (A) 0,82 (B) | 1740 ($COOC_2H_5$), d | 13,5 | 61,80 | 5,73 | 4,18 | 9,93 | $C_{18}H_{19}NO_4 \cdot HCl$ | 61,80 | 5,76 | 4,00 | 10,14 |

Condensation of the readily-available ethyl 6-methylpicolinate (I) [4] with aromatic aldehydes (benzaldehyde (IIa), 2,4-dichlorobenzaldehyde (IIb), and veratraldehyde (IIc)) in the presence of acetic anhydride gave the ethyl 6-styrylpicolinates (IIIa-c). In the case of (IIIa), this method gave better results than when sodium tert-butoxide in DMF was used as the condensing agent as in the preparation of 2-styrylnicotinic acid described in the literature [6], or the use of the well-known method [9] for the preparation of 6-styrylpicolinic acids from 2-picoline N-oxide via 2-styrylpyridine N-oxide and 6-styrylnicotinonitrile.

That the products obtained have the E-configuration was shown in the case of the hydrochloride of ester (IIIc) by its $^1$H NMR spectrum, in which the protons at the double bond correspond to doublets with coupling constants J = 16.3 Hz, which is characteristic of trans-coupling [1].

Catalytic reduction of (IIIb) with hydrogen in the presence of 5% palladium on charcoal proceeds selectively only at the trans-disubstituted double bond of the styryl moiety, to give the ethyl esters of the corresponding substituted 6-(2'-phenylethyl)picolinic acid (IVb, c).

The molecular masses of (IVb) and (IVc) (323* and 315) are two atomic units greater than in the corresponding styrylpicolinate esters (IIIb, c). The mass spectra showed strong peaks for the ions $\overset{+}{C}H_2Ar$ (Table 1), showing unambiguously that the exocyclic double bond had been reduced, with retention of the aromatic rings.

When (IIIa) was reduced catalytically with hydrogen over Adams catalyst, exhaustive hydrogenation occurred to give a 98% yield of ethyl 6-(2'-cyclohexylethyl)pipecolinate (V).

The mass spectrum of (V) showed strong peaks for the ions $[M - COOC_2H_5]^+$ (m/z 194) and $[M - C_8H_{15}]^+$ (m/z 156), corresponding to α-cleavage of the substituents in the piperidine ring.

The mass number of the molecular ion, and the absence of the ion peak $\overset{+}{C}H_2Ph$ indicated that the exocyclic double bond and the pyridine ring, and also the benzene ring had been reduced.

Unexpectedly, (V) was also formed when the ester (IIIc) was reduced similarly over a platinum catalyst, when an excess of hydrogen was used. Use of the calculated amount of hydrogen led to the formation of ethyl 6-[2'-(3", 4"-dimethoxyphenyl)ethyl]pipecolinate (VIc) in 70% yield.

The cis-configuration of esters (V) and (VIb) was established by their $^1$H NMR spectra, in which the protons in positions 2 and 6 of the piperidine give rise respectively to a quartet with $J_{2,3a}$ = 11.5 Hz and $J_{2,3e}$ = 2.7 Hz, and a multiplet with $J_{5a,6}$ = 11 Hz and $J_{5e,6}$ = 2.7 Hz.†

The occurrence of high (>10 Hz) coupling constants for each of these protons shows that they possess the diequatorial orientation [1], i.e., that the compounds obtained have the cis-configuration.

The mass spectra of (VIb) and (VIc) show strong peaks for the ions $[M-COOC_2H_5]^+$, $[M-CH_2CH_2Ar]^+$ and $CH_2Ar$. This breakdown pathway indicates that the pyridine ring is hydrogenated, but the benzene ring is retained.

Hydrolysis of the ester grouping in (V) and (VIb) was carried out in an acidic medium, and in (VIc) with aqueous NaOH to give the acids (VII, VIIIb, and VIIIc).

In the mass spectra of the piperidinecarboxylic acids (VII) and (VIIIb, c) the mode of breakdown is typical of α-substituted pyridines. Comparison of the masses of the molecular ions and of the ions $[M - COOR]^+$ for compounds (VII-VIIIb) with the corresponding spectra for the esters (V) and (VIb) shows that the ester grouping has been hydrolyzed.

Boiling the ester (VIc) with 48% HBr resulted both in hydrolysis of the ethoxycarbonyl group, and almost total hydrolysis of both methoxy-groups to give the dihydroxy-acid (VIIId).

This is shown by the decrease of 28 units, as compared with the spectrum of (VIIIc), both of the molecular ion of (VIIId), and of the ions $ArCH_2^+$ and $[M - COOH]^+$, with retention of the mass numbers of the ion $[M - CH_2CH_2Ar]^+$.

*Here and in Table 1, the mass numbers and peak intensities are given for ions containing $^{35}$Cl.
†The 6-H proton also couples with the protons of the $CH_3$ group of the ethylene bridge, the sum of the coupling constants with these protons being ≈ Hz.

Acetylmercaptopripionylation of (VII) and (VIIIb, c) at the piperidine nitrogen to give (IX) and (Xb, c) was carried out using acetylmercaptopropionyl chloride by a general method analogous to the reactions described in the preceding communications [5].

In the $^1$H NMR spectra of (IX) and (Xb, c) two sets of signals were seen (with an intensity ratio for the protons of the same groups of ~4:1), attributed to the existence of these compounds as a mixture of two conformers with respect to the amide bond.

On the basis of earlier information on acylated 4- and 5-pipecolinecarboxylic acids [3], it is assumed that (IX) and (Xb, c) retain the original cis-configuration.

Molecular ion peaks were seen in the mass spectra of (IX) and (Xb, c) corresponding to the formation of the acetylmercaptopropionyl derivatives. The introduction of a substituent at the nitrogen atom introduces additional routes of: $[M - COCH_3]^+$, $[M - SCOCH_3]^+$, and $[M - C_2H_4SCOCH_3]^+$, but as before, the most intense peaks in the spectra were the characteristic ions discussed above.

## EXPERIMENTAL CHEMICAL

IR spectra* were obtained on a Perkin-Elmer 599 spectrometer (Sweden) in KBr disks (d), Vaseline oil (v/o), chloroform (chl), or dioxane, layer thickness 0.2 mm, $^1$H NMR spectra on a Varian XL-200 (Switzerland), solvent $CDCl_3$, internal standard TMS, and mass spectra on an MAT-112 with direct introduction of the sample into the source. Ionizing electron energy, 70 eV. All the mass spectral data for the compounds are given in Table 1. TLC was carried out on Silufol UV-254 plates in the solvent systems: A, chloroform–methanol–acetic acid (97:3:1); B, ethyl acetate–methanol–formic acid (8:1:1); C, ethanol–ammonia–water (25:4:3); D, chloroform–methanol–ammonia (20:20:1); E, benzene–ethyl acetate (3:2). Visualization for (I, IIIa-c, IVb, c, VIb, c, VIIIb-d, IX, and Xb, c) was by UV, for (V, (VIb, c, VII, and VIIIb-d) by 2% ninhydrin in ethanol and heating to 110°C, and for (IX) and (Xb) with 10% phosphomolybdic acid in ethanol and heating at 110°C for 5 min. GC was carried out on a Khrom-5 apparatus (Czech SSR), flame ionization detector, carrier gas helium, flow rate 40 ml/min, column 100×0.3 cm, stationary phase 5% OV-17 on Chromatone NHDMS (80-100 mesh) at 260°C. Retention times were measured for the bases.

General Method of Preparation of Ethyl Esters of Substituted 6-Styrylpicolinic acids (IIIa-c). A mixture of 100 mmole of ethyl 6-methylpicolinate (I), 300 mmole of the benzaldehyde (IIa-c), and 84 ml of acetic anhydride was boiled, the progress of the reaction being followed by TLC† (IIIa-c, A; IIIc, B) until there was no further change in the amount of (I) in the mixture (the condensation times are given in Table 2), and the mixture was then evaporated under reduced pressure, the residue dissolved in 500 ml of dry ether, decolorized by boiling for 30 min with 2 g of activated charcoal, filtered, and acidified to Congo Red (pH 2.0) with 20% alcoholic HCl. The precipitated hydrochlorides (IIIa-c) were filtered off an recrystallized from a suitable solvent with the addition of alcoholic HCl to pH 2.0 (if no HCl was added, recrystallization resulted in loss of part of the HCl to give mixtures of the hydrochloride and base of (IIIa-c)). In order to obtain the free bases (IIIa-c), the recrystallized hydrochlorides were dissolved in water, basified with 50% aqueous potassium carbonate, and extracted with ether. After drying over magnesium sulfate, the ether solution was evaporated under reduced pressure, and the residue recrystallized from a suitable solvent.

The constants and properties of (IIIa-c) are given in Table 2. $^1$H NMR spectrum of (IIIc): 3-H 8.53 q; 4-H 8.49 t; 5-H 8.26 q; 1'-H, 2'-H 8.02 d, 7.50 d; 2"-H 7.33 d; 5"-H 7.06 d; 6"-H 7.35 q; $CH_3CH_2$ 1.48 t, 4.58 q; 3"-$OCH_3$, 4"-$OCH_3$ 3.90 s, 3.92 s.

Ethyl 6-[2'-(2",4"-Dichlorophenyl)ethyl]picolinate (IVb). To a solution of 7.8 g (21.7 mmole) of (IIIb) hydrochloride in 500 ml of anhydrous ethanol was added 9 ml of 20% alcoholic HCl (to pH 2 to Congo Red), and hydrogenated over 0.9 g of 5% palladium on charcoal catalyst at 20°C and a pressure of 20-30 cm of water, until (IIIb) was no longer present in the mass spectrum. The catalyst was filtered off, washed with alcohol (3×50 ml), and the filtrate evaporated under reduced pressure. The residue was dissolved in 65 ml of water, the solution basified with 5 ml of 50% aqueous potassium carbonate, and extracted with ether (6×200 ml). After drying over magnesium sulfate, the ether extract was evaporated under reduced pressure,

*The IR spectra were obtained and interpreted by N. M. Rubtsov, and GC by V. A. Kuzovkin, to whom the authors express their sincere thanks.

†The $R_f$ of ethyl 6-methylpicolinate in system A was 0.74, and system B, 0.66.

and the residue crystallized from 50 ml of dry ether to give 6.11 g (87%) of (IVb), mp 65-67°C, as a colorless, crystalline powder, soluble in alcohols, chloroform, warm ether, benzene, ethyl acetate, and insoluble in water. $R_f$ 0.89 (A), 0.91 (C). GC: ret. time 3.33 min. IR spectrum, $\nu_{max}$, $cm^{-1}$: 1745 ($COOC_2H_5$) (v/o). Found, %: C 58.99; H 4.75; Cl 21.48; N 4.22. $C_{16}H_{15}Cl_2NO_2$. Calculated, %: C 59.27; H 4.67; Cl 21.87; N 4.32.

Ethyl 6-[2'-(3",4"-Dimethoxyphenyl)ethyl]picolinate (IVc). To a suspension of 7.8 g (22.3 mmole) of (IIIc) hydrochloride in 900 ml of anhydrous ethanol was added 9 ml of 20% ethanolic HCl, and the mixture hydrogenated over 0.9 g of 5% palladium on charcoal catalyst at 20°C and a pressure of 20-30 cm of water, until (IIIc) was no longer seen on TLC (system A, B). The catalyst was filtered off, washed with alcohol (2×100 ml), and the filtrate evaporated. The residue was crystallized from 325 ml of ethyl acetate with the addition of 3 ml of 20% ethanolic HCl to give 5.37 g (78%) of (IVc·HCl) as yellow crystals mp 136-138°C, soluble in alcohols, chloroform, water, hot ethyl acetate and benzene, but insoluble in acetone and ether. $R_f$ 0.78 (A), 0.76 (B), 0.89 (C). GC: ret. time 5.0 min, IR spectrum, $\nu_{max}$, $cm^{-1}$: 1740 ($COOC_2H_5$) v/o). Found; %: Cl 10.51; N 3.92. $C_{13}H_{21}NO_4 \cdot HCl$. Calculated %: Cl 10.08; N 3.98.

Ethyl 6-(2'-cyclohexylethyl)pipecolinate (V). To a solution of 6.9 g (23.8 mmole) of (IIIa) hydrochloride in 600 ml of anhydrous ethanol was added 6 ml of 20% ethanolic HCl and 0.6 g of Adams platinum catalyst. Hydrogenation was carried out at 20°C and a water pressure of 20-30 cm until no more hydrogen was taken up. The catalyst was filtered off and washed three times with 20 ml of anhydrous alcohol. The filtrate was evaporated under reduced pressure, and the residue of (V·HCl), 7.08 g (98%), mp 154-156°C, was recrystallized from 70 ml of a mixture of ethyl acetate and ethanol (4:3) to give colorless crystals, mp 161-162°C, soluble in alcohol, hot water (1:10), and chloroform, and insoluble in ether, benzene, acetone, and ethyl acetate. $R_f$ 0.71 (C). GC: ret. time 0.5 min. IR spectrum of (V·HCl), $\nu_{max}$, $cm^{-1}$: 2760-2400 ($\rangle NH_2^+$), 1745 ($COOC_2H_5$) (v/o). $^1H$ NMR spectrum, 2-H, 3.34 q; 6-H, 2.48 m; 3,4,5-$H_2$, $C_6H_{11}$, 018-2.1; 6-$CH_2$, 2'-$CH_2$, ∿1.68; $CH_3CH_2$, 1.26 t, 4.19 q. Found, %: C 63.46; 63.32; H 9.9; 10.20; Cl 11.52; 11.23; N 4.35, 4.24. $C_{16}H_{29}NO_2 \cdot HCl$. Calculated, %: C 63.24; H 9.95; Cl 11.67; N 4.61.

Ethyl6-[2'-(2",4"-dichlorophenyl)ethyl]pipecolinate (VIb). To a solution of 5.84 g (18 mmole) of (IVb) in 300 ml of anhydrous ethanol was added 20 ml of 20% alcoholic HCl (to pH 2.0 to Congo Red), and the mixture hydrogenated over 0.4 g of Adams platinum catalyst at 20°C and a pressure of 20-30 cm of water until the theoretical amount of hydrogen had been taken up (1322 ml) for the reduction of the pyridine ring to piperidine. The catalyst was filtered off, washed with hot alcohol (75°C, 3×50 ml), and the filtrate evaporated under reduced pressure. The residue was crystallized from 90 ml of a mixture of ethyl acetate and ethanol (5:1) with charcoal to give 4.58 g (69%) of (VIb·HCl), mp 157-160°C, colorless crystals, soluble in chloroform, methanol, hot ethanol and propan-2-ol, and water, insoluble in ether, acetone, ethyl acetate, and benzene. $R_f$ 0.48 (A), 0.91 (C). GC: ret. time 2.5 min. IR spectrum, $\nu_{max}$, $cm^{-1}$: 2700-2400 ($\rangle NH_2^+$), 1745 ($COOC_2H_5$) (v/o). Found, %: C 52.09; H 6.08; Cl 28.99; N 3.66. $C_{16}H_{21}Cl_2NO_2 \cdot HCl$. Calculated, %: C 52.40; H 6.05; Cl 29.0; N 3.82.

Ethyl 6-[2'-(3",4"-dimethoxyphenyl)ethyl]pipecolinate (VIc) was obtained from (IVc) hydrochloride as described for the synthesis of (VIb), yield 70%, mp 211-213°C (from a mixture of ethyl acetate and ethanol, 1:1). Colorless crystals, soluble in methanol, hot ethanol and propan-2-ol, and water, insoluble in ether, acetone, ethyl acetate, and benzene. $R_f$ 0.73 (C). GC: ret. time 3.67 min. IR spectrum, $\nu_{max}$, $cm^{-1}$: 2660-2380 ($\rangle NH_2^+$), 1745 ($COOC_2H_5$) (v/o). $^1H$ MMR spectrum: 2-H, 3.31 q; 6-H, 2.57 m; 3,4,5-$H_2$, 1.05-2.05; 1'-$H_2$, ∿1.72; 2'-$H_2$, ∿2.63; 2"-H, 6.73 d; 5"-H, 6.77 d; 6"-H, 6.74 d; $CH_3CH_2$, 1.24 t, 4.19 q; 3"-$OCH_3$, 4"-$OCH_3$, 3.86 s, 3.87 s. Found, %: C 60.52; H 7.93; Cl 9.89; N 3.72. $C_{18}H_{27}NO_4 \cdot HCl$. Calculated, %: C 60.41; H 7.89; Cl 9.91; N 3.91.

Reduction of Ethyl 6-(3',4'-Dimethoxystyryl)picolinate (IIIc) over a Platinum Catalyst with Excess Hydrogen. A solution of 7 g (20 mmole) of (IIIc) hydrochloride in 600 ml of anhydrous ethanol was treated with 6 ml of ethanolic HCl, and hydrogenated over 0.6 g of Adams catalyst at 20°C and a pressure of 20-30 cm of water until no more hydrogen was taken up.

When the reaction was complete, the mixture, according to GC, consisted of 46.8% of (V) and 35.1% of (VIc). The catalyst was filtered off, washed with hot (75°C) ethanol (3×50 ml), and the filtrate evaporated under reduced pressure. The residue was crystallized from 110 ml of a mixture of ethanol and ethyl acetate (3:4) to give 1.73 g (24.2%, calculated on (IIIc)) of (VIc·HCl), mp 211-213°C. This material gave no depression of melting point on admixture with (VIb·HCl) obtained from (IVc), and had an identical IR spectrum.

The mother liquors were evaporated, and the residue crystallized from water to give 1.30 g (24.3%, calculated on (IIIc)) of (V) hydrochloride, mp 161-162°C. The compound gave no depression of melting point on admixture with (V·HCl) obtained from (IIIa), and had the same IR spectrum.

6-(2'-Cyclohexylethyl)pipecolinic Acid (VII). A solution of 2 g (6.6 mmole) of (V) hydrochloride in 50 ml of 17% HCl was boiled for 5 h. As boiling proceeded, more solid separated. This was filtered off, and 1.8 g of the 6-(2'-cyclohexylethyl)pipecolinic acid hydrochloride thus obtained was crystllized from 76 ml of 1 N HCl. Yield of (VII·HCl), 1.59 g (87.5%). Colorless crystals, mp 262-263°C, soluble in methanol, sparingly soluble in hot water, ethanol, benzene, and chloroform, and insoluble in ether, acetone, and ethyl acetate. Found, %: C 60.68; H 9.74; Cl 12.83; N 5.49. $C_{14}H_{25}NO_2 \cdot HCl$. Calculated, %: C 60.95; H 9.50; Cl 12.86; N 5.08.

To a solution of 1.5 g (5.45 mM) of the acid (VII·HCl) in 30 ml of hot water was added 54.3 ml of 1 N NaOH. The reaction mass was kept for 10 min at 97°C and cooled to 20°C. The solid which was separated was filtered off washed with water (2 × 11.5 ml). Of the free acid XII, 1./10 g (77%) was obtained mp 252-253°C as colorless crystals, poorly soluble in water, soluble in methanol, sparingly soluble in ethyl alcohol, and insoluble in other standard organic soluents. $R_f$ 0.54 (B), 0.77 (C), 0.76 (D). IR spectrum, $\nu_{max}$, $cm^{-1}$: 2740-2440 ($\rangle NH_2^+$), 1585 (COO) (v/o). Found, %: C 70.50; H 10.68; N 5.64. $C_{14}H_{25}NO_2$. Calculated, %: C 70.25; H 10.53; N 5.85.

6-[2'-(2",4"-Dichlorophenyl)ethyl]pipecolinic Acid (VIIIb). A suspension of 4 g (10.91 mmole) of (VIb) hydrochloride in 100 ml of 18% HCl was boiled for 5 h, then acetic acid (50 ml) was added until the solid had dissolved completely, and boiling continued for a further 3 h until all the (VIb) had been consumed according to TLC (C). The mixture was evaporated to a volume of ∿50 ml, cooled to 0-2°C, and the solid which separated was filtered off to give 3.24 g (88%) of (VIIIb·HCl), mp 245-246°C. Colorless crystals, soluble in alcohols and water, insoluble in ether, chloroform, acetone, ethyl acetate, and benzene. Found, %: C 49.74; H 5.45; N 4.10. $C_{14}H_{17}Cl_2NO_2 \cdot HCl$. Calculated, %: C 49.65 H 5.36; N 4.14.

To a solution of the acid (VIIIb·HCl) in 400 ml of water was added 8.1 ml of 1 N NaOH, to pH 8.0. The mixture was kept for 10 min at 97°C, and cooled to 0°C. The solid which separated was filtered off, to give 2.45 g (78%) of hydrated crystals of the free acid (VIIIb), mp 241-242°C as colorless crystals, soluble in hot alcohols and water, insoluble in benzene, ether, acetone, ethyl acetate, and chloroform. $R_f$ 0.67 (C), IR spectrum, $\nu_{max}$, $cm^{-1}$: 2700-2500 ($\rangle NH_2^+$), 1610 ($COO^-$) (v/o). Found, %: $H_2O$ 10.72, $C_{14}H_{17}Cl_2NO_2 \cdot 2H_2O$. Calculated, %: $H_2O$ 10.64.

After drying the dihydrate in vacuo over $P_2O_5$, the (VIIIb) had mp 232-234°C. Found, %: C 55.52; H 5.41; Cl 23.59; N 4.66, $C_{14}H_{17}Cl_2NO_2$. Calculated, %: C 55.64; H 5.67; Cl 23.46; N 4.64.

6-[2'-(3",4"-Dimethoxyphenyl)ethyl]pipecolinic Acid (VIIIc). (VIc) hydrochloride (4 g, 11.2 mmole) was dissolved with heating in 264 ml of 0.1 N NaOH (26.4 mmole), and the solution boiled for 4 h until the (VIc) had been consumed according to TLC (C). Excess NaOH (pH 10.0) was neutralized with 15 ml of 1 N HCl to pH 6.0. The mixture was evaporated, the residue diluted with 25 ml of dry acetone, and the NaCl filtered off. This operation was repeated three times more. The combined acetone filtrates were evaporated to dryness, and the residue repeatedly dissolved in 6 ml of methanol and precipitated with 60 ml of dry ether, to give 2.57 g (78%) of (VIIIc), mp 102-104°C, colorless crystals, soluble in alcohols, acetone, chloroform, and water, sparingly soluble in hot ethyl acetate and benzene, insoluble in ether and heptane. $R_f$ 0.58(C), 0.65 (D). IR spectrum $\nu_{max}$, $cm^{-1}$: 2740-2480 ($\rangle NH_2^+$), 1590 ($COO^-$)(chl). Found, %: N 4.72. $C_{16}H_{23}NO_4$. Calculated, %: N 4.76.

(VIIIc·HCl) was obtained as colorless crystals, mp 232.5-233.5°C, soluble in alcohols and chloroform, sparingly soluble in water, and insoluble in ethyl acetate, benzene, and acetone. Found, %: Cl 10.91; N 4.17. $C_{16}H_{23}NO_4 \cdot HCl$. Calculated, %: Cl 10.75; N 4.25.

6-[2'-(3",4"-Dihydroxyphenyl)ethyl]pipecolonic Acid (VIIId). The hydrochloride of (VIIIc) (2.18 g, 6.09 mmole) was diluted with 25 ml of water, and 1.5 ml of 50% potassium carbonate solution added. The mixture was extracted with chloroform (6×80 ml), the extract dried over magnesium sulfate, and evaporated under reduced pressure. The residue was dissolved with heating in 20 ml of 48% HBr, and the mixture boiled for 7 h, completion of the reaction being established by TLC (C). Hydrobromic acid was removed under reduced pressure, and residual acid removed by the addition of two portions of water (10 ml each) followed by distillation under reduced pressure. Traces of moisture were then removed by adding 10 ml of benzene, followed by distillation under reduced pressure. The residue was dissolved in 50 ml of anhydrous ethanol, boiled for 30 min, filtered, and the alcohol removed under reduced pressure to give 1.89 g (89%) of (VIIId·HBr), soluble in alcohols and acetone, sparingly soluble in water and benzene, insoluble in ether, ethyl acetate, and chloroform. $R_f$ 0.53 (C). IR spectrum, $\nu_{max}$, $cm^{-1}$: 2540-2400 ($>N^+H_2$), 3660-3420 (OH), 1580 (COO-), 1400 (COOH) (dioxane). Found, %: N 3.73; Br 23.20. $C_{14}H_{19}NO_4 \cdot HBr$. Calculated, %: N 4.05; Br 23.08.

General Method of Preparation of N-Acetylmercaptopropionyl-6-substituted Pipecolinic Acids (IX, Xb, Xc). To a solution (or a suspension in the case of VIIIb) of 6.7 mmole of (VII) or (VIIIb, c) in 16-35 ml of dimethylacetamide was added slowly with stirring 8.1 mmole of β-acetylthiopropionyl chloride [7]. The temperature of the mixture rose to 28-30°C. When the temperature had fallen spontaneously to 20°C, N-methylmorpholine (13.4 mmole) was added. A solid separated, and the temperature rose to 30°C. The mixture was allowed to stand for 3-4 h with stirring at ambient temperature, the progress of the reaction being followed by TLC (IX — B, Xb — C, Xc — C, D). The solid was filtered off, washed with ethyl acetate (3×5 ml), and the filtrate evaporated to dryness. The solid was dissolved in the minimum amount of benzene, and applied to a column (700×20 mm) with a mass of silica gel (L 40/100 μm) fifty times that of the residue, suspended in benzene. Elution was carried out with a mixture of benzene and ethyl acetate, the proportion of ethyl acetate being gradually increased from 100-1 to 100-20, the progress of chromatographic separation being followed by TLC (IX, Xc) and mass spectrometry (Xb). There were obtained β-acetylthiopropionic acid* (29-40% on the β-acetylpropionyl chloride used), followed by a mixture of products in which the N-acetylmercaptopropionyl-6-substituted pipecolinic acid (IX or (Xb, c)) preodminated. These were purified either via their cyclohexylamine (DCHA) salts (IX and Xb), or by repeated column chromatography under pressure (Xc).

A solution of 1 mmole of (IX) or (Xb) in 2.5 volumes of ethyl acetate was treated with 0.9 mmole of DCHA, followed by dilution with 20 volumes of dry ether. The solid which separated was isolated and crystallized from a suitable solvent. The cyclohexylamine salt (1 mmole), 7.5 ml of 0.12 N sulfuric acid (0.75 mmole), and 15 ml of ethyl acetate were then shaken for 10 min in a separatory funnel, the ethyl acetate layer separated, the aqueous layer again extracted with ethyl acetate (6 × 20 ml), and the combined ethyl acetate extracts washed with saturated sodium chloride (2 × 10 ml), dried over magnesium sulfate, and evaporated to give (IX) and (Xb).

Purification of (Xc) was effected by wetting 143 g of silica gel (L 40/100 μm) with 300 ml of a mixture of benzene and ethyl acetate (3:2), and air was removed under reduced pressure in order to fill the pores of the sorbent with solvent. The resulting suspension was applied to a column (500 × 50 mm), and compacted under pressure to remove the layer of liquid above the surface of the sorbent. There was then applied to the column 2.97 g of the purified acid (Xc) in 10 ml of a mixture of benzene and ethyl acetate (3:2), and the column washed through with 1000 ml of a mixture of benzene and ethyl acetate (3:2) at a rate of 1 ml/sec. There was eluted 1.4 g of the acid (Xc).

Acids (IX), (Xb), and (Xc) are souble in alcohols, benzene, ethyl acetate, chloroform, and ether, sparingly soluble in acetone, and insoluble in water. The constants and properties of (IX), (Xb), and (Xc) are given in Table 3.

## EXPERIMENTAL PHARMACOLOGICAL

The antihypertensive activity of (IX), (Xb), and (Xc) was examined in female rats weighing 180-200 g with model birenal vascular hypertension, induced by prior application of a spiral (internal diameter 0.2 mm) made of nichrome wire to the left renal artery [2]. The AP was measured in non-narcotized animals by a bloodless method in the caudal artery using a

*The $R_f$ value of β-acetylthiopropionic acid in system A is 0.8, B 0.54, C 0.66, and D 0.73.

TABLE 3. N-Acetylmercaptopropionyl-6-substituted Pipecolinic Acids, with Their Physicochemical Properties, Antihypertensive Activity, and Acute Toxicity

| Compound | Yield, % | mp of dicyclohexylamine salt, °C (crystallization solvent) | $R_f$ (system) | IR spectrum, $\nu_{max}$, $cm^{-1}$ (chl) | $^1H$ NMR spectrum,* δ, ppm | Found, % C | Found, % H | Found, % N | Found, % S | Empirical formula | Calculated, % C | Calculated, % H | Calculated, % N | Calculated, % S | Antihypertensive activity in rats with birenal hypertension: dose, mg/kg (internal) | Antihypertensive activity in rats with birenal hypertension: reduction in AP, mm Hg | $LD_{50}$ in mice, mg/kg (internal) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IX | 46 | | 0,62 (B)<br>0,91 (D) | 3530 (OH); 3460—3080 (polyassociates); 1715, 1685, 1640, (CON<, COS, COOH) | 2-H 5.15; 6-H 3,84; 3, 4, 5-$H_2$, $C_6H_{11}$ 0,8—2,1; 1'-$H_2$, 2'-$H_2$: ~1,68; $CH_2CO$ ~2,85; $CH_2S$: ~3,20 $COCH_3$: 2,34 | 62,12 | 8,49 | 3,51 | | $C_{19}H_{31}NO_4S$ | 61,75 | 8,46 | 3,79 | | 50<br>100 | 11<br>27 | 1000 |
| IX DCHA | | 172—4,5 (ethyl-acetate) | | | | 67,62 | 9,78 | 5,15 | 5,67 | $C_{19}H_{31}NO_4S \cdot C_{12}H_{23}N$ | 67,59 | 9,88 | 5,09 | 5,82 | | | |
| Xb | 39,9 | | 0,73 (E)<br>0,55 (C) | 3300—3040 (polyassociates); 1710, 1685, 1610 (CON<, COS, COOH) | 2-H 5,22; 6-H 3,94; 3, 4, 5-$H_2$ 1,5—1,8; 1'-$H_2$ ~ 1,90; 2'-$H_2$: ~2,70; 3"-H 7,35; 5", 6"-H ~ 7,15; $CH_2CO$: ~2,60; $CH_2S$ ~3,15; $COCH_3$: 2,33 | 52,89 | 5,51 | 2,98 | 7,14** | $C_{19}H_{23}Cl_2NO_4S$ | 52,78 | 5,36 | 3,24 | 7,42 | 50<br>100 | 43<br>— | 1000 |
| Xb DCHA | | 186,5 -7 (isopro-panol) | | | | 60,82 | 7,68 | 4,13 | 5,33*** | $C_{19}H_{23}Cl_2NO_4S \cdot C_{12}H_{23}N$ | 60,67 | 7,56 | 4,57 | 5,23 | | | |
| Xc | 46 | | 0,4 (C)<br>0,53 (D)<br>0,33 (E) | 3530 (OH); 3460—3080 (polyassociates); 1715, 1685, 1610 (CON<, COS, COOH) | 2-H 5,24; 6-H 3,90; 3, 4, 5-$H_2$; 1,5—1,9; 1'-$H_2$ ~ 1,85; 2'-$H_2$: ~2,60; 2", 5", 6": 7,4—7,6; $CH_2CO$: ~2,60; $CH_2S$ ~3,12; 3"-$OCH_3$, 4"-$OCH_3$ 3,88, 3,89 $COCH_3$ 2,32 | 59,38 | 6,95 | 3,50 | 7,32<br>7,70 | $C_{21}H_{29}NO_6S$ | 59,55 | 6,90 | 3,31 | 7,57 | 50<br>100 | 16<br>— | 1000 |
| Xc DCHA | | 175—8 (isopro-panol) | | | | 65,54 | 8,68 | 4,71 | 5,48 | $C_{21}H_{29}NO_6S \cdot C_{12}H_{23}N$ | 65,5 | 8,67 | 4,63 | 5,30 | | | |
| Captopryl | | | | | | | | | | | | | | | 10 | 52 | 2500 |

*The signals were broadened as a result of exchange between the amide conformers. The signals given are for the preferred conformer.

**Found, %: Cl 16.02. Calculated, %: Cl 16.40.

***Found, %: Cl 11.63. Calculated, %: Cl 11.55.

semiautomatic instrument supplied by IITC Inc (USA). The compounds, dissolved in distilled water, were administered internally to the animals in doses of 50 and 100 mg/kg. The animals in the control groups received the same volume of distilled water.

The standard used was Captopryl in a dose of 10 mg/kg (internally).

The acute toxicities of the compounds were determined in female mice weighing 16-17 g by the intragastric route. The $LD_{50}$ values were calculated as described in [10]. The animals were observed for a period of 48 h.

The results shown in Table 3 show that (IX), (Xb), and (Xc) are all of low toxicity, and reduce the AP in rats with birenal renovascular hypertension. The radical in the 6-position of the piperidine ring has a considerable influence on antihypertensive activity. For example, the clearest reduction in AP was obtained with (Xc), which has the dichlorophenyl residue. The presence of a cyclohexyl residue reduces the degree of activity (compound IX).

The test compounds were inferior to Captopryl in activity, but they were also somewhat less toxic than the latter. The duration of the reduction in AP brought about by (Xc) was around 60 min when given in a dose of 50 mg/kg, and 150 min in a dose of 100 mg/kg.

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# SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF 5-SUBSTITUTED COUMARANS


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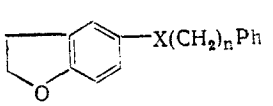
012.1


Certain 6-(ω-phenylacyl)chromans have anti-inflammatory activity [2] and 6-(amino-ω-phenylalkyl)chromans — a local anesthetic one [3]. Therefore, as our aim was to search for new drugs, we synthesized and studied coumarin analogs of the above compounds.

5-(ω-Phenylacyl)coumarans (Ia-d) were synthesized by acylating coumaran with acid chlorides in the presence of anhydrous $AlCl_3$, while 5-(α-amino-ω-phenylalkyl)coumarans (IIIa-d) were obtained by reducing oximes (IIa-d), synthesized by the reaction of ketones Ia-d with $H_2NOH \cdot HCl$ in a pyridine solution, by sodium in a butanol solution



Ia-d; IIa-d; IIIa-d

a-d X = CO; IIa-d: X = —CH=NOH; IIIa-d X = $CHNH_2$; a: n = 0, b: n = 1, c: n = 2, d: n = 3.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