Notes

Synthesis and Hypoglycemic Activity of S-Acyl Derivatives of 3-Mercaptopicolinic Acid[1]

Benjamin Blank,* Nicholas W. DiTullio, Linda Deviney, John T. Roberts, and Harry L. Saunders

Division of Research and Development, Smith Kline & French Laboratories, SmithKline Corporation, Philadelphia, Pennsylvania 19101. *Received July 8, 1976*

A series of *S*-alkanoyl and benzoyl derivatives of 3-mercaptopicolinic acid (3-MPA) was prepared and studied for hypoglycemic activity. Three alkanoyl derivatives (propionyl, pivaloyl, and 1-adamantanecarbonyl, **19**–**21**) were prepared with increasing bulk around the thio ester bond. The benzoyl derivatives contained aromatic substituents chosen from a σ–π cluster chart so that the esters prepared had a wide range of electronic and solubility properties. In general, compounds with substituents which increased lipid solubility [*p*-chlorobenzoyl (**4**), *p*-trifluoromethylbenzoyl (**6**), and pivaloyl (**20**)] had the greatest potency at a dose of 300 mg/kg. Hydrolysis rates, measured at pH 6 and 8, indicated that in vivo breakdown to 3-MPA probably did not account for the observed hypoglycemic activity of the esters. **4**, **6**, and **20** were less potent than 3-MPA in comparative dose range studies.

The ability of 3-mercaptopicolinic acid (3-MPA) to inhibit gluconeogenesis and lower blood glucose levels in 48-h fasted rats has been noted[2,3] and associated with a high degree of structural specificity.[2] S-Acetylation and benzoylation of 3-MPA were two of many modifications made that did not lead to inactive structures. The *S*-benzoyl derivative (**1**) of 3-MPA, although quite potent after intraperitoneal administration, was less potent after oral administration.[2] Subsequent testing showed that the variance between the hypoglycemic activity of **1** following intraperitoneal or oral dosing was not as significant as thought originally (see ref 2 and Table I).

Consequently, a series of *S*-benzoyl esters of 3-MPA was prepared and tested for hypoglycemic activity in 48-h fasted rats. In addition, *S*-1-adamantanecarbonyl, pivaloyl, and propionyl derivatives were prepared and evaluated.

The esters (Table I) were prepared under Schotten–Baumann conditions using acid chlorides or mixed anhydrides prepared in situ. Aromatic substituents in the benzoyl esters were chosen so that most of the groups from a cluster chart of σ and π values were represented.[4] Rates of hydrolysis of a representative series of these derivatives were measured (Table II) to determine if the hypoglycemia noted might be caused by in vivo hydrolysis of the esters to 3-MPA.

Discussion

Three esters (**4**, **6**, and **20**) seemed more potent than 3-MPA at a dose of 300 mg/kg po. Subsequent dose range studies refuted these observations (compare the results for **4**, **6**, and **20** at 125 mg/kg with the results for 3-MPA at a comparable dose) (Table I). These findings led us to test succeeding compounds (**12**–**18** and **21**) at a dose of 150 mg/kg.

There was no apparent correlation between the glycemic levels produced and the nature or position of the aromatic substituent or the rates of ester hydrolysis. The most active esters at 300 mg/kg (**1**, **4**, **6**, and **20**) have substituents which enhance lipid solubility but have rates of hydrolysis which cover the gamut from relatively rapid to relatively slow (**4** and **6**, rapid; **1**, intermediate; and **20**, slow). The stability of these esters to acid hydrolysis could not be measured as readily since the esters precipitated from aqueous solutions at pH's below 6. Acid stability of **1** was examined qualitatively by allowing it to stand in 3 N HCl at room temperature for 3.5 h and by refluxing it in 3 N HCl–ethanol for 2.5 h. In both experiments **1** was recovered unchanged as monitored by thin-layer chromatography. For these reasons it seemed unlikely that the hypoglycemia noted with these derivatives was a reflection of their prior conversion to 3-MPA.

Experimental Section

Acylation with Acid Chlorides 1–14 and 19–21. These conversions were carried out in aqueous bicarbonate as described for **1** and the acetyl derivative of 3-MPA.[2]

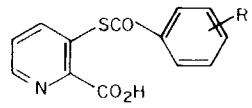
Acylation with Mixed Anhydrides 15–18. A suspension of the benzoic acid (0.015 mol) in 21 mL of Me_2CO and 4 mL of H_2O was cooled and to it was added 3 mL of Et_3N in 7 mL of Me_2CO followed by 2.7 mL of isobutyroyl chloride in 7.5 mL of Me_2CO. This mixture was stirred with cooling for 40 min and added to a stirred solution of 2.3 g (0.015 mol) of 3-MPA in 50 mL of 5% $NaHCO_3$ containing 4 g of solid $NaHCO_3$. The reaction mixture was stirred overnight at room temperature under N_2, diluted with H_2O, and acidified with dilute HCl. The solid was collected, washed with H_2O, dried, and recrystallized.

Biochemistry. Hypoglycemic activity was measured in 48-h fasted male rats weighing ca. 200 g. On the morning of the test day, a zero time tail-vein sample was obtained, followed by the oral administration of the test compound suspended in 0.5% tragacanth or dissolved in aqueous bicarbonate at a dose of 150 (**12**–**18**, **21**, and 3-MPA) or 300 mg/kg (**1**–**11** and **20**). In comparative dose range studies with 3-MPA, the compounds were administered at doses of 62.5, 125, and 250 mg/kg. Only the 125-mg dose from these studies is shown for **4**, **6**, **20**, and 3-MPA in Table I. A similar group of animals receiving only the vehicle served as controls. Tail-vein samples were obtained at 1, 2, and 4 h after drug administration. Glucose determinations and the significance of the values reported in Table I have been described previously.[2,3] Tolbutamide, after an oral dose of 200 mg/kg, lowered blood glucose levels in this test system 28% at 1 h, 47% at 2 h, and 48% at 4 h after treatment. These values were significant at the $p \leq 0.001$ level.

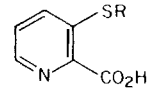
Hydrolysis Studies. The analytical technique used for measuring the concentration of 3-MPA produced during the hydrolyses was that described by Ellman.[5] The reagent 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) reacts with mercaptans in a mercaptan–disulfide interchange reaction producing 2-nitro-5-mercaptobenzoic acid which absorbs at 410 nm in the visible spectrum, is stable, and gives solutions that adhere to Beer's law over a wide concentration range. These parameters were studied in separate experiments which verified the validity of the method.

Buffers were flushed with N_2 for 15 min prior to use. Accurately weighed samples of about 10 mg of the esters were dissolved in 100 mL of pH 6 buffer and pH 8 buffer and placed in 125-mL

Table I. *S*-Acyl Derivatives of 3-MPA



1-18



3-MPA, 19-21

No.	R	Mp, °C	Recrystn solvent[a]	% yield	Formula[b]	Hypoglycemic act. in 48-h fasted rats[c] 1 h	2 h	4 h
1	H	151-152	A	72	$C_{13}H_9NO_3S$	−27[d]	−42[d]	−56[d]
2	*m*-Cl	140	B	33	$C_{13}H_8ClNO_3S$	−27[d]	−32[d]	−42[d]
3	*o*-Cl	235-236	C-A	35		−35[d]	−39[d]	−40[d]
4	*p*-Cl	173-176	B-D	25		−43[d]	−34[f]	−57[e]
						−10 (−12)[g,h]	−23[g] (−34)[e,h]	−7 (−40)[d,h]
5	*m*-CF_3	116	C-A	40	$C_{14}H_8F_3NO_3S$	−35[d]	−38[e]	−45[e]
6	*p*-CF_3	147	C-A	40		−26[d]	−54[d]	−75[d]
						−24[d] (−28)[e,h]	−35[d] (−41)[e,h]	−1 (−32)[g,h]
7	*m*-OCH_3	119-120	B	53	$C_{14}H_{11}NO_4S$	−15[g]	−24[e]	−30[e]
8	*p*-OCH_3	133-135	B	64		−16[g]	−25[e]	−19[f]
9	*p*-CH_3	147-148	B	63	$C_{14}H_{11}NO_3S$	−21[d]	−16[g]	−4
10	*p*-F	142-144	B	61	$C_{13}H_8FNO_3S$	−29[e]	−47[e]	−44[e]
11	*p*-NO_2	150-151	B	36	$C_{13}H_8N_2O_5S$	0	0	12
12	*p*-CN	168-170	B	35	$C_{14}H_8N_2O_3S$	−18[e]	−16[g]	0
13	*p*-CO_2Me	169-171	E	28	$C_{15}H_{11}NO_5S$	−17[g]	−20[e]	1
14	*p*-SO_2NH_2	153-155[i]	F-G	33	$C_{13}H_{10}N_2O_5S^j$	−20[d]	−16[e]	3
15	*p*-$CONH_2$	218-220[i]	H-I	18	$C_{14}H_{16}N_2O_4S^{j,k}$	−22[f]	−6	−25[f]
16	*p*-NHAc	207-209[i]	H-I	61	$C_{15}H_{12}N_2O_4S$	−18[d]	−14[e]	−7
17	*p*-$NHCOC_6H_5$	183-185	E	50	$C_{20}H_{14}N_2O_4S$	−20[d]	−20[d]	−4
18	*p*-NMe_2	174-176[i]	E	44	$C_{15}H_{14}N_2O_3S$	−12[g]	−2	−8
3-MPA	H					−32[d]	−44[d]	−56[d]
19	COC_2H_5	90-92	C-G	44	$C_9H_9NO_3S$	−22[e,l]	−52[d,l]	−55[d,l]
20	$COC(CH_3)_3$	95-96	C-G	55	$C_{11}H_{13}NO_3S$	−20[d]	−43[d]	−70[d]
						−6 (−21)[e,h]	−3 (−42)[d,h]	−3 (−52)[d,h]
21	$COC_{10}H_{15}$	157-158	B	25	$C_{17}H_{19}NO_3S$	−14[g]	−3	−9

[a] The abbreviations have the following meanings: A, CCl_4; B, MeCN; C, $CHCl_3$; D, Et_2O; E, THF, F, EtOAc; G, petroleum ether (bp 40-60 °C); H, DMF; I, H_2O. [b] Analyses (C, H, and N) for compounds listed in this table were within ±0.4% of the theoretical values unless otherwise noted. Melting points were determined in a Thomas-Hoover melting point apparatus and are uncorrected. [c] Results are expressed as the percent difference between the mean change in control and treated groups after a dose of 300 mg/kg po for 1-11 and 20 and 150 mg/kg po for 12-18, 21, and 3-MPA. 19 was tested at a dose of 250 mg/kg po. [d] $p \leq 0.001$. [e] $p \leq 0.01$. [f] Not significant. [g] $p \leq 0.05$. [h] Results at 125 mg/kg po. Values in parentheses are for a comparable dose of 3-MPA. Assays were carried out concurrently. [i] With decomposition. [j] 0.25 mol of H_2O. [k] S: calcd, 10.45; found, 9.70. [l] Results are for a dose of 250 mg/kg po.

Table II. Rates of Hydrolysis[a]

No.	pH 6 $t_{1/2}$, h	pH 6 K, h^{-1}	pH 8 $t_{1/2}$, h	pH 8 K, h^{-1}
1	60.1	0.0115	21.1	0.0329
2	14.8	0.0468	4.7	0.1480
3	75.8	0.0091	27.2	0.0255
4	26.6	0.0261	7.9	0.0879
5	10.0	0.0695	3.9	0.1790
6	10.1	0.0688	3.0	0.2310
7	63.7	0.0109	11.9	0.0583
8	182.0	0.0038	43.9	0.0158
9	80.1	0.0087	25.6	0.0271
10	111.0	0.0062	12.1	0.0575
11	4.7[a]	0.1470	1.4	0.5130
20	115.0	0.0060	61.1	0.0114

[a] The rates of hydrolysis studied conform to a pseudo-first-order reaction law, with the exception of 11 at pH 6. It appears that 11 reaches a state of equilibrium at about 10 h. Because of this exception the analysis was repeated and the same hydrolysis rate was obtained. At equilibrium there remained 24.3 mol % of ester.

Erylenmeyer flasks (reaction vessels). Aliquots of 5 mL were withdrawn from each reaction vessel and placed in 25-mL volumetric flasks (zero time). The reaction vessels were then immediately placed in a 37 °C H_2O bath and N_2 was bubbled through the solutions throughout the experiment. At selected time intervals, 5-mL aliquots were withdrawn, reacted with 2 mL of DTNB solution, and diluted with 25 mL of pH 7.5 buffer. The absorbance at 410 nm was recorded in 1-cm cells against a reagent blank (used to balance the spectrophotometer, a Cary Model 118).

Since the analytical method measured the growth of a reaction product rather than the decay of the ester under study, the concentration of intact ester at each sampling time was calculated. This concentration was proportional to the difference ΔA between the absorbance observed and that calculated for complete hydrolysis. First-order rate constants and half-lives were calculated by computer using a nonlinear least-squares fit to the first-order rate equation

$$C_t = C_0 \cdot \exp(-kt)$$

$$t_{1/2} = \ln 2/k$$

At pH 6, the rates of hydrolysis of all the esters studied, except 11, conformed to a pseudo-first-order reaction law (Table II). It appeared that 11 reached a state of equilibrium at about 10 h. Because of this exception the analysis was repeated and the same hydrolysis rate was obtained. At equilibrium there remained 24.3 mol % of ester. As expected, the hydrolysis rates for this series of compounds were more rapid at pH 8 than at pH 6.

Acknowledgment. We are indebted to Messrs. A. F. Zappala and A. Post and Dr. W. W. Holl of our Analytical and Physical Chemistry Section for the hydrolysis studies and calculations. We also thank Miss E. Reich and staff for elemental analyses.

References and Notes

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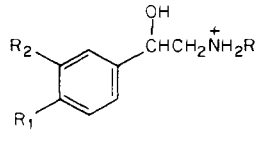
Relative Concentrations of Zwitterionic and Uncharged Species in Catecholamines and the Effect of N-Substituents

C. Robin Ganellin

The Research Institute, Smith Kline & French Laboratories Limited, Welwyn Garden City, Hertfordshire, England.
Received July 12, 1976

The relative concentrations of zwitterionic and uncharged species for the series of N-substituted catecholamines (I, $R_1 = R_2 = OH$; R = H, Me, Et, *i*-Pr, *t*-Bu) are derived from the pK_a data published in 1962 by Sinistri and Villa. The concentration ratios, represented by the tautomeric equilibrium constant K_t, show a definite trend and are respectively 1.8, 4.3, 4.7, 4.7, and 7.1. These values suggest that any mechanism of action involving proton transfer, which might transform the zwitterion into the uncharged form, would be most favorable for norepinephrine and least favorable for the *t*-Bu derivative.

Knowledge of the chemistry of catecholamines is fundamental to understanding the molecular mechanisms of their biological actions.[1] Catecholamines exist as equilibrium mixtures of different ionic species and conformers and there have been various studies to determine proton dissociation constants[2] and conformational preference.[3] The effects of *N*-alkyl substitution are of particular interest; in the simple series classified by Ahlquist,[4] viz. norepinephrine, epinephrine, and isoproterenol (1, R_1 =



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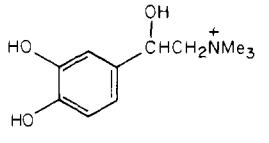

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R_2 = OH; R = H, Me, *i*-Pr), isoproterenol is the least active at α-receptors but is the most active at β-receptors, and the chemical basis for selectivity imparted by the isopropyl substituent continues to intrigue medicinal chemists.[1b,e,f,5,6] Various molecular models for the receptor site interaction have been reviewed by Brittain et al.[5] There is, however, no proven mechanism of drug action and it is worthwhile to continue to examine features of catecholamine chemistry attributable to the *N*-alkyl groups.

The author wishes to draw attention to a subtle effect of the alkyl group derived from proton dissociation. Determination of catecholamine proton acidities by potentiometric titration affords two stoichiometric pK_a values (e.g., for the epinephrine cation Lewis reported[2c] values of 8.7 and 9.9) but assignment of proton ionization to particular sites in the molecule is problematical. Making comparison with simpler molecules such as catechol (pK_a = 9.4) and phenylethanolamine (pK_a = 8.9) some authors attributed the lower pK_a to the $-NH_2{}^+R$ group[2a,d] and the higher pK_a to the phenolic OH.[2i,l] However, using a UV