Enamine Prodrugs

H. C. CALDWELL, H. J. ADAMS*, R. G. JONES, W. A. MANN, L. W. DITTERT†, C. W. CHONG, and J. V. SWINTOSKY†

Abstract □ Five enamine derivatives of phenylpropanolamine were prepared in a search for novel amine prodrug derivatives. The compounds hydrolyzed *in vitro* at vastly different rates in phosphate buffer at pH 7.4, but the rates were not accelerated by dilute plasma. As expected, these enamines hydrolyzed faster as the pH was lowered. Two compounds were chosen for pharmacologic tests. Acid hydrolysis data suggested that Compound III would hydrolyze *in vivo* to give phenylpropanolamine but that Compound IV would not. Compound III was less toxic and also less potent in the pressor and antitussive tests than phenylpropanolamine (molar basis), giving further support to the suggestion that III was converted *in vivo* to phenylpropanolamine. In contrast, IV was more toxic than phenylpropanolamine (molar basis) but was not active in the pressor test. Thus, IV did not behave as a prodrug of phenylpropanolamine. While the authors did not pharmacologically evaluate Compounds I and II, which hydrolyze faster than III, it is predicted that they would have been more potent than III in these tests. Thus, it has been shown that enamines are potentially useful prodrug derivatives of amines.

Keyphrases □ Phenylpropanolamine, enamine derivatives—amine prodrugs, pharmacological evaluation □ Enamine derivatives of phenylpropanolamine—prodrugs, pharmacological evaluation □ Amine prodrugs—enamine derivatives of phenylpropanolamine □ Prodrugs, amine—enamine derivatives of phenylpropanolamine

There are surprisingly few types of amine prodrug derivatives. Amides (1, 2), Schiff bases (3), and Mannich bases (4) are perhaps the best known. Enamines, or α,β-unsaturated amines, are used extensively as intermediates and as protective groups in selective organic reactions (5–7). Some have been tested as potential medicinal agents (8, 9) but, apparently, none has been evaluated as a prodrug. Enamine structural types vary widely (5), as do their hydrolysis rates (10). The enamine made from pyrrolidine and cholestan-3-one hydrolyzes readily to cholestan-3-one when heated at reflux for 5 min. in 95% ethanol, but the related enamine of stigmastadienone must be heated at reflux for 4 hr. in a sodium acetate–acetic acid buffered solution to regenerate the ketone. Heyl and Herr (10) attributed these hydrolysis differences to the degree of unsaturation. The mechanism for enamine hydrolysis was described recently (11, 12).

For this study, several enamines were made from phenylpropanolamine in a search for derivatives that would hydrolyze at significantly different rates.

EXPERIMENTAL

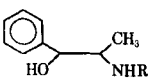
Chemistry—Melting points were determined in capillary tubes on a Thomas-Hoover apparatus and are uncorrected. Reported yields are of pure compounds; most reactions were run once.

Intermediates—Phenylpropanolamine base was purchased, as were the other reactants for Compounds I–V. The five are *N,N*-diethylacetoacetamide, *tert*-butyl acetoacetate, ethyl acetoacetate, diethyl ethoxymethylenemalonate, and diethyl acetonedicarboxylate, respectively.

General Procedure—Molar equivalents of phenylpropanolamine and the other reactants were refluxed in dry benzene for about 2 hr. until the calculated amount of water was collected in a Dean-Stark apparatus; Compound IV produced ethanol instead of water as a by-product. Most of the benzene was removed *in vacuo*, and hexane was added. The cooled solutions provided the products, but two did not crystallize. They were distilled *in vacuo*.

***In Vitro* Hydrolysis Rates**—Half-lives for hydrolysis of the compounds in 0.1 *M* pH 7.4 phosphate buffer, with and without 2% human, 2% dog, or 2% mouse plasma, were determined by spectrophotometric (13) or pH Stat (14) methods as previously described. HCl (0.01 *N*) was the titrant.

Table I—Phenylpropanolamine Enamines



Compound	R	Formula	Molecular Weight	Yield (Pure), %	Melting Point	IR[a] OH,NH	IR[a] C=O	IR[a] C=C	Analysis, % Calc.	Found
I	C(CH3)=CH—CON(C_2H_5)$_2$	$C_{17}H_{26}N_2O_2$	290.4	54	86–87°	3.05	6.20	6.45	C 70.31	70.38
									H 9.02	9.11
									N 9.65	9.81
II	C(CH3)=CH—$CO_2C(CH_3)_3$	$C_{17}H_{25}NO_3$	291.4	27	Oil	2.91	6.10	6.23[b]	C 70.07	69.72
									H 8.65	8.76
									N 4.81	4.83
III	C(CH3)=CH—$CO_2C_2H_5$	$C_{15}H_{21}NO_3$	263.3	96	92.5–93.5°	2.95	6.17	6.25	C 68.42	68.27
									H 8.04	7.96
									N 5.32	5.33
IV	CH=C—($CO_2C_2H_5$)$_2$	$C_{17}H_{23}NO_5$	321.4	48	91–92°	2.92	5.95 6.06	6.25	C 63.53	63.95
									H 7.21	7.25
									N 4.36	4.57
V	C(CH2—$CO_2C_2H_5$)=CH—$CO_2C_2H_5$	$C_{18}H_{25}NO_5$	335.4	41	Oil	2.96	5.81 6.10	6.26[b]	C 64.46	64.45
									H 7.51	7.69
									N 4.18	4.08

[a] Wavelengths in microns; mineral oil mulls unless stated otherwise. [b] Neat.

Table II—Hydrolysis Half-Lives for Phenylpropanolamine Enamines (37°)[a]

Compound	Half-Lives in pH 7.4 Phosphate Buffer —with and without 2% v/v Human Plasma— Plasma, min.	Buffer, min.	Half-Lives in pH 7.4 Phosphate Buffer —with Other Plasmas— Plasma	$t_{1/2}$, min.	—Half-Lives at Other pH's— Buffer pH	$t_{1/2}$, min.
I	3.4[b]	4.0	2% Mouse	3.0	6	1.1
					6.4	1.5
					7	2.5
					8.4	4.6
II	10.25	9.75	2% Dog	10	6.4	4.0
		9.35[c]	2% Mouse	8.5	8.4	11.5
					9.5	36.6[c]
III	40	36	2% Mouse	32	5	0.6[d], 0.6[c]
					6	5
					8	35
IV	Not hydrolyzed	Not hydrolyzed	2% Mouse	Not hydrolyzed	4	Not cleaved
					5.5	32
V	>160	160	—	—	6	72

[a] Values are single determinations based on phenylpropanolamine appearance. [b] Values determined on pH Stat (with 0.01 *N* HCl as titrant) except where indicated. [c] UV determination. [d] $t_{1/2}$ with 0.5% pepsin at pH 5, 0.6 min.

Table III—Acute Oral Toxicity in Mice

Compound	Dose, mg./kg. as Base	Mortality, %	LD_{50} and 95% Fieller Confidence Limits, mg./kg. as Base	LD_{50}, moles/kg.
Phenylpropanol-amine hydrochloride	750	0	1443 (1185–1853)	9.5×10^{-3}
	1100	20		
	1520	60		
	2350	100		
III	1000	0	5105 (3243–[a])	19×10^{-3}
	1587	10		
	2519	20		
	5038	50		
IV	1000	10	2227 (1596–3145)	6.9×10^{-3}
	1587	20		
	2519	70		
	3998	80		
Controls	—	0	—	—

[a] Because of the nature of the data, an upper confidence limit could not be calculated.

Acute Toxicity in Mice—Male mice[1], weighing 15–25 g., were divided into groups of 10 and dosed orally with vehicle or with drug suspended or dissolved in 0.5% tragacanth. Dose volumes varied from about 10 to 40 ml./kg. After being dosed, animals were observed at intervals for several hours for overt effects and fatalities. Survivors were housed in groups according to compound and dose level and were checked once daily for 7 days to determine whether additional fatalities occurred. Studies were terminated on the 7th day postdrug, and LD_{50}'s and potency ratios were calculated according to the minimum logit chi square method of Berkson (15).

Pressor Activity in Unanesthetized Rats—Sexually mature male and female rats[2] were used. Animals were anesthetized with ether, and the right carotid arteries were cannulated with a piece of heparin-filled polyethylene tubing. After the animals recovered from the ether, blood pressure was recorded by means of a transducer complex[3] attached to a direct writing optical oscillograph[3]. Control readings were taken until blood pressure and heart rate stabilized. Test compounds were administered orally as suspensions or solutions in 0.5% tragacanth at a dose volume of 10 ml./kg. Control animals received 10 ml./kg. of vehicle. Blood pressure recordings were taken at 5, 15, 30, 60, 90, and 120 min. postdrug. At least two animals received vehicle and served as controls in each study, and three or four animals were used at each dose level of test compound.

Antitussive Activity—Antitussive activity was evaluated in dogs according to the method described by Tedeschi *et al.* (16). Four sexually mature mongrel dogs, weighing between 9 and 15 kg., were used. Drugs were administered orally in gelatin capsules. Animals were tested prior to drug administration to establish control values and then at hourly intervals until the cough response returned to control level.

[1] Carworth Farms.
[2] Charles River.
[3] Sanborn.

RESULTS AND DISCUSSION

Synthesis and Physical Properties—Synthesis did not present any unusual problems, but two compounds resisted all attempts at crystallization. They were obtained as pure oils upon distillation. Molecular weights, yields, melting points, elemental analyses, and IR assignments are shown in Table I.

***In Vitro* Hydrolysis Behavior**—Hydrolysis half-life data are summarized in Table II. The enamines cleaved at different rates in phosphate buffer at pH 7.4: I and II hydrolyzed rapidly, III hydrolyzed slowly, V hydrolyzed very slowly, and IV did not hydrolyze. Hydrolysis was not accelerated by adding dilute plasma to

Table IV—Pressor Activity in Unanesthetized Rats

Compound	Dose, mg./kg. as Base	Maximum Effect, % Increase over Predrug Control Level
Phenylpropanolamine hydrochloride	50	27
III	218	10
	436	32
	871	24
IV	500	No effect
	1000	No effect

Table V—Cough Inhibition in Dogs

Compound	Dose, mg./kg. as Base	Mean Average and Inhibition, hr. Postdrug 1	2	3	4	5	6	7	Maximum Mean Percent Inhibition	ED_{50} and 95% Fieller Confidence Limits, mg./kg.	Molar ED_{50}
Phenylpropanolamine hydrochloride	5	21	16	28	5	—	—	—	37		
	10	35	47	55	46	35	12	—	57	7.8 (4.8–11.8)	5.2×10^{-5}
	15	52	64	64	56	51	30	—	70		
III	10	15	18	15	18	4	—	—	21		
	26	30	39	35	43	36	18	—	48	25.8 (17.8–38.7)	9.8×10^{-5}
	52	23	45	64	54	53	57	47	74		

the buffer. As expected, the compounds generally cleaved faster as the pH was lowered. Two of the compounds, III and IV, were chosen for pharmacologic tests. The acid hydrolysis data suggested that III would hydrolyze *in vivo* to give phenylpropanolamine but that IV would not hydrolyze.

Acute Toxicity—The acute oral toxicity data are recorded in Table III. On a weight basis, phenylpropanolamine is 3 times more toxic than III and 1.5 times more toxic than IV. But LD_{50}'s should be compared in moles per kilogram because 1 mole of each prodrug will release 1 mole of phenylpropanolamine regardless of the molecular weight of the prodrug. Thus, when toxicities are compared on this realistic basis, IV is slightly more toxic than phenylpropanolamine while phenylpropanolamine is twice as toxic as III. This suggests that the true molar toxicity of intact IV is greater than that of phenylpropanolamine and that III is not absorbed and converted rapidly to phenylpropanolamine because, if it were, the molar toxicities of III and phenylpropanolamine would be equivalent. These data do not, however, rule out the possibility that IV is cleaved to phenylpropanloamine.

Pressor Activity—Pressor activity data are cited in Table IV. The oral administration of 50 mg./kg. of phenylpropanolamine elicited a pressor response that reached a peak at about 5 min. postdrug and returned to control level by 60 min. postdrug. Oral administration of III at doses of 218, 436, and 871 mg./kg. elicited pressor responses accompanied by compensatory bradycardias. The maximum responses to the two higher doses were about equal in magnitude, but the duration of the pressor response was greater with the highest dose. The time of peak effect was about 15 min. postdrug with III, and all three doses produced pressor responses that remained above predrug control levels for at least 120 min. postdrug. No pressor response was elicited with doses of 500 and 1000 mg./kg. of IV. Thus, III showed the expected pressor activity of a phenylpropanolamine prodrug. Because IV was inactive, it was presumed that it was not cleaved and, therefore, that IV is not a prodrug of phenylpropanolamine.

Antitussive Activity—Compound IV was not tested because it did not show pressor activity. Compound III was active in the antitussive test. It was about one-half as potent as phenylpropanolamine on a molar basis. Equipotent doses of these compounds produced the same incidence of peripheral vasodilation, mydriasis, and piloerection. Thus, by these test parameters, III appears to be converted to phenylpropanolamine after oral dosing in dogs.

CONCLUSION

A series of phenylpropanolamine enamines were found to hydrolyze at significantly different rates in phosphate buffer at pH 7.4. Hydrolysis was not accelerated when dilute plasma was added to the system. As expected, the compounds cleaved faster as the pH of the test system was lowered.

Two compounds were chosen for pharmacologic tests. From hydrolysis data, it was expected that III would hydrolyze *in vivo* to give phenylpropanolamine but that IV would not hydrolyze. Acute toxicity data in mice suggest that the conversion of III to phenylpropanolamine is not rapid because III has only about one-half the molar toxicity of phenylpropanolamine. Tests for pressor activity in rats showed that III produced pressor activity, presumably after conversion to phenylpropanolamine, but was less potent than phenylpropanolamine. These data support the suggestion that the conversion of III to phenylpropanolamine is not rapid. Because IV was inactive in this test, it was concluded that it was not hydrolyzed *in vivo* to phenylpropanolamine and, therefore, that it was not a prodrug of phenylpropanolamine; it was not studied further.

In the antitussive test in dogs, III was about one-half as potent on a molar basis as phenylpropanolamine. Thus, these data also support the suggestion that the conversion of III to phenylpropanolamine is not rapid. The data from three tests in three species suggest that III is indeed hydrolyzed *in vivo* to phenylpropanolamine. In contrast, the pressor activity data suggest that IV is not hydrolyzed *in vivo* to phenylpropanolamine.

In summary, enamines are potentially useful prodrug derivatives.

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* Present address: Astra Pharmaceutical Products, Inc., Worcester, MA 01606

† Present address: College of Pharmacy, University of Kentucky, Lexington, KY 40506