Inhibition of Separated Forms of Phosphodiesterases from Pig Coronary Arteries by Uracils and by 7-Substituted Derivatives of 1-Methyl-3-isobutylxanthine^{1,2}

J. E. Garst, G. L. Kramer, Y. J. Wu, and J. N. Wells*

Department of Physiology, School of Medicine, Vanderbilt University, Nashville, Tennessee 37232. Received September 2, 1975

A series of 7-substituted 1-methyl-3-isobutylxanthines was designed in an attempt to increase the specificity of the 1-methyl-3-isobutylxanthine (MIX) structure for one of the two cyclic nucleotide phosphodiesterase peaks isolated by DEAE-cellulose chromatography of the soluble fraction of the intima + media layer of pig coronary arteries. A series of 1,3-dialkyluracils was of low potency as inhibitors of either peak I or peak II. The 7-substituted xanthines were prepared by alkylation of MIX with the corresponding alkyl or aralkyl halide in DMF containing K₂CO₃. These compounds were, in general, much less potent inhibitors of peak II activity than was MIX, but some of them retained the potency of MIX as inhibitors of peak I and, therefore, were relatively specific for inhibition of peak I. 7-B2l-MIX was the most selective compound tested; it was a potent inhibitor of peak I activity but was much less effective as an inhibitor of peak II activity. Substitution of either electron-withdrawing (nitro) or electron-donating (methoxy) groups on the 7-benzyl moiety reduced the effectiveness of the 7-benzyl compounds as inhibitors of peak I. Chlorobenzyl substitution increased the potency slightly over the benzyl but not the selectivity between peaks.

The involvement of adenosine cyclic 3',5'-monophosphate (cAMP) as an intracellular mediator of hormonal action is well established in a variety of tissues³ and an increasing body of evidence suggests a regulatory role for this nucleotide in vascular and other smooth muscle.³⁻⁶ The levels of guanosine cyclic 3',5'-monophosphate (cGMP) are increased by cholinergic and other agents that produce contraction of smooth muscle,⁷⁻¹¹ but the physiological significance of these changing levels of cGMP is unclear. 1-Methyl-3-isobutylxanthine (MIX, 1), a potent inhibitor of cyclic nucleotide phosphodiesterases,^{1,12-14} produces relaxation of smooth muscle^{9,15} but increases the levels of both cAMP and cGMP in these tissues.^{9,16}

The aims of the present study were to (a) study a series of uracils as cyclic nucleotide phosphodiesterase inhibitors in order to further investigate the minimum structural requirements of the xanthine nucleus for inhibition and (b) determine if substitution on the 7 position of MIX would increase the selectivity of MIX¹ for one of the cyclic nucleotide phosphodiesterases of pig coronary arteries. A compound capable of selective inhibition of either cyclic nucleotide phosphodiesterase activity could be a useful tool in defining the roles of cAMP and cGMP and in delineating the relationships between their roles in smooth muscle and other tissues.



We have separated and partially characterized cyclic nucleotide phosphodiesterase activities from the intima plus media layer of pig coronary arteries.¹⁷ Two fractions of phosphodiesterase activity and a heat-stable, nondialyzable activator were separated by DEAE-cellulose chromatography. Peak I phosphodiesterase activity catalyzes the hydrolysis of both cGMP and cAMP and exhibits classical kinetic behavior with both substrates, although it displays a much lower apparent K_m for cGMP (2-4 μ M) than for cAMP (40-100 μ M). Peak I activity is stimulated three- to eightfold by a heat-stable, nondialyzable activator,¹⁷ while peak II activity is unaffected by this material. Peak II activity exhibits apparent negatively

* Address correspondence to this author at the Department of Pharmacology, School of Medicine, Vanderbilt University. cooperative kinetic behavior and is relatively selective for cAMP (the lower apparent K_m value, although difficult to determine accurately from curvilinear plots, is approximately 1 μ M for cAMP). The activity of peak II with cGMP as substrate is too low to permit practical kinetic studies. The two forms of phosphodiesterase from pig coronary arteries (peaks I and II) correspond in at least their kinetic properties and substrate specificities to those found in a number of tissues.¹⁸

We have reported that the imidazole portion of the xanthine nucleus is insufficient to maintain inhibition of phosphodiesterase;¹⁹ that is, when the uracil ring of theophylline is opened, a significant reduction in potency of phosphodiesterase inhibition occurs. We have, therefore, studied a series of uracil derivatives (2, 3, and 4) based upon the structures of both theophylline and MIX to investigate further the minimal structural requirements for inhibition of phosphodiesterase by xanthines. These uracils were prepared by standard procedures²⁰ or were purchased.

We have shown previously that MIX (1) shows some selectivity for inhibition of cGMP hydrolysis by crude phosphodiesterase preparations from pig coronary arteries and for peak I phosphodiesterase activity with either substrate.¹ In addition, we have shown that 7-benzyltheophylline is about as potent as theophylline as an



inhibitor of cAMP hydrolysis but is significantly more potent than theophylline as an inhibitor of cGMP hydrolysis by the crude supernatant fraction of pig coronary

Table I. Preparative Data and Physical Properties of 7-Substituted 1-Methyl-3-isobutylxanthines

			CH ₂ N H ₃ C) ₂ H <u>Rx</u> K ₂ CO DMF	$\xrightarrow{\text{CH}_2\text{CH}(\text{CH}_3)_2}_{ }$	4		
			Ŷ	H		Y R			
No.	RX	Y	Mp, $^{\circ}$ C	Rxn time, h	Isoln meth- od	Recrystn solvent or method	Yield, %	Formula	Analyses
5	Methyl iodide	0	97-100	2	A	Subl [80° (0.05 mm)]	70	C ₁₁ H ₁₆ N ₄ O ₂	C, H, N
6	Isoamyl bromide	Ő	64.5-66.5	4	В	DMF-H ₂ O	38	$C_{15}H_{24}N_4O_2$	C, H, N
7	Phenethyl bromide	0	81.5-83	14	A	50% EtOH-H ₂ O	44	$C_{18}H_{22}N_4O_2$	C, H
9	3 4 5-Trimethoxy-	0	125-126	12	Д Д	$EtOA_{c}-EtO$	75 75	$C_{17}H_{20}N_4O_2$	CHN
Ŭ	benzyl chloride	Ŷ	100 100	-	••		10	020112611405	0, 11, 11
10	4-Nitrobenzyl bromide	0	137-139	4	В	10% DMF-H ₂ O	75	$C_{17}H_{19}N_5O_4$	C, H, N
11	2-Nitrobenzyl bromide	0	137-139	6	В	95% EtOH	88	$C_{17}H_{19}N_5O_4$	С, Н
12	2-Methoxybenzyl chloride	0	87.5-89	12	А	EtOH-H ₂ O	65	$C_{_{18}}H_{_{22}}N_{_4}O_{_3}$	С, Н
13	3-Chlorobenzyl chloride	0	87-88.5	6	В	EtOH-H ₂ O	81	$C_{17}H_{19}ClN_4O_2$	С, Н
14	4-Chlorobenzyl chloride	0	83-85	14	В	EtOH-H ₂ O	32	$C_{17}H_{19}ClN_4O_2$	С, Н
15	Cyclopropylmethyl chloride	0	108.5-110	24	Α	Subl $[80^{\circ} (0.05 \text{ mm})]$	50	$C_{_{14}}H_{_{20}}N_{_4}O_{_2}$	С, Н
16	Propargyl bromide	0	116.5 - 118	24	Α	EtOH-H,O	68	$C_{13}H_{16}N_{4}O_{2}$	С, Н
17	4-Picolyl chloride	0	75.5-78	12	Α	5% EtOH-H₂O	55	C ₁₆ H ₁₉ N ₅ O ₂	С, Н
19	Benzyl bromide	s	117-118.5	2	\mathbf{B}^{a}	95% EtOH	25	$C_{17}H_{20}N_4OS$	С, Н

^a The product was treated with 2 equiv of NaOH prior to isolation method B.

arteries.¹⁹ We have, therefore, prepared and studied a series of 7-substituted derivatives of MIX in order to determine if selectivity for inhibition of peak I activity could be achieved. The 7-substituted derivatives of MIX were prepared by treatment of 1 and the appropriate alkyl or aralkyl halide with potassium carbonate in dimethylformamide.² The literature contains numerous reports of alkylations of theophylline to yield 7-substituted products.^{19,24-27} Alkylation of 1-methyl-3-isobutylxanthine (1) under these conditions afforded compounds 5-17 (Table I); the latter were assumed to be 7-substituted derivatives of 1 on the basis of the theophylline precedent and the increased steric hindrance toward alkylation at the 9xanthine nitrogen presented by the bulkier 3-isobutyl substituent. Alkylation of 6-thio-MIX (18)²⁸ using these conditions afforded compound 19, similarly assumed to be 7-substituted. It should be noted, however, that the Sbenzylated compound was probably also formed and apparently underwent base hydrolysis to benzylthiol and the base-soluble MIX (1).28 Questions have been raised by cases²⁶ where theophylline and its analogs have undergone alkylation to afford minor and inseparable, or even predominantly, 9-substituted products. The importance of a correct positional assignment for these potent phosphodiesterase inhibitors prompted an unequivocal preparation of 7-Bzl-MIX (8). Compound 4b was cyclized with formic acid to afford material which gave ir and NMR spectra identical with that of 7-Bzl-MIX (8). Furthermore, a mixture of cyclization and alkylation products failed to depress the melting point from that of either product separately, serving to confirm the structure of the alkylation product as 7-Bzl-MIX (8).

Results and Discussion

The uracil moiety alone appears insufficient to maintain the potent inhibition of coronary artery cyclic nucleotide phosphodiesterases that is seen with MIX. The only uracils that, at 10^{-4} M, inhibited either peak I or II phosphodiesterase activities by more than 10% at 1 μ M substrate were 3b, 4a, and 4b. Compound 3b (10^{-4} M) inhibited the hydrolysis of cGMP and cAMP by peak I by 10 and 17%, respectively, and inhibited peak II hydrolysis of cAMP by only 6%. Compound 4a (10-4 M) inhibited the hydrolysis of cGMP and cAMP by peak I by 17 and 26%, respectively, and hydrolysis of cAMP by peak II by 15%. Compound 4b (10⁻⁴ M), which resembles 7-Bzl-MIX more closely than any of the other uracils studied, inhibited hydrolysis of 1 μ M cAMP and cGMP by peak I by 37 and 30%, respectively, and inhibited hydrolysis of 1 μ M cAMP by peak II by only 9%. These data, along with similar findings for various imidazole derivatives, 19,29 indicate that the intact xanthine nucleus or at least a larger heterocyclic ring system than uracil or imidazole is necessary to effectively compete with the cyclic nucleotides for the hydrolytic site of phosphodiesterase.

MIX (1) is one of the most potent inhibitors of cyclic nucleotide phosphodiesterase that has been reported; it is 30–60 times more potent than theophylline as an inhibitor of pig coronary artery phosphodiesterases when either cAMP or cGMP is used as substrate.¹ Similar differences in potency have been reported for MIX and theophylline as inhibitors of cAMP phosphodiesterase activities from other tissues.^{12–14} MIX has also been shown to be relatively selective for peak I phosphodiesterase with either cyclic nucleotide as substrate.¹

Various 7-substituted MIX derivatives (Table II, 5–17) were prepared in an attempt to determine if these substituents would increase the inhibitory potency and/or selectivity of the MIX molecule. The most logical and demanding assessment of selectivity in this system is to compare the potency of a compound as an inhibitor of cGMP hydrolysis by peak I with the compound's potency as an inhibitor of cAMP hydrolysis by peak II, since these are the high-affinity substrates for the respective enzyme

Table II.	Inhibition of	f Peak I and	II Cyclic	Nucleotide	Phosphodiesterase	Activities l	by 7-8	Substituted
1-Methyl-	3-isobutylxan	nthines						

			Peal		
No.	7-Subst	6-Subst	cGMP	cAMP	Peak II, cAMP
1	Н	0	6.3 ± 0.7 (6)	3.9 ± 0.3 (6)	$15 \pm 2(3)$
18	Н	S	$7.1 \pm 0.6 (4)$	$5.5 \pm 0.2 (4)$	$6.8 \pm 2(3)$
5	Methyl	0	$65 \pm 6(3)$	$55 \pm 2(4)$	$126 \pm 14(3)$
6	Isoamyl	0	$16 \pm 2(5)$	$19 \pm 4(4)$	>100 ^c
7	Phenethyl	0	$28 \pm 3(3)$	$17 \pm 1(3)$	>100 ^c
8	Benzyl	0	$4.4 \pm 0.7(7)$	$3.6 \pm 0.8(7)$	$108 \pm 14 (4)$
19	Benzyl	S	$3.3 \pm 0.1 (4)$	$2.7 \pm 0.1 (4)$	$28 \pm 6 (3)$
9	3.4.5-Trimethoxybenzyl	0	46 ± 5 (3)	$35 \pm 4(3)$	>100 ^c
10	4-Nitrobenzyl	0	$34 \pm 8(3)$	27 ± 3 (3)	>100 ^c
11	2-Nitrobenzyl	0	$60 \pm 15(3)$	$41 \pm 1(3)$	$142 \pm 4 (3)$
12	2-Methoxybenzyl	0	26 ± 2 (3)	$19 \pm 4(3)$	75 ± 5 (3)
13	3-Chlorobenzyl	0	$2.1 \pm 0.2(3)$	$1.3 \pm 0.1 (3)$	$48 \pm 2(3)$
14	4-Chlorobenzyl	0	$2.1 \pm 0.2 (3)$	2.3 ± 0.3 (3)	$43 \pm 11(3)$
15	Cyclopropylmethyl	0	$6.1 \pm 0.6 (3)$	$3.7 \pm 0.2 (3)$	$20 \pm 3(3)$
16	Propargyl	0	36 ± 1 (3)	28 ± 2 (3)	65 ± 3 (3)
17	4-Picolyl	0	$146 \pm 10(3)$	$105 \pm 27(3)$	>300 ^c

^a I_{s_0} value is defined as the concentration (μ M) of the agent required to produce 50% inhibition of the hydrolysis of 1 μ M substrate. Values are mean ± SEM of the number of duplicate determinations with different enzyme preparations given in parentheses. ^b Peak I activity was measured in the presence of an optimal amount of activator (15 μ g of protein).¹⁷ ^c % inhibition at 10⁻⁴ M concentration: 6, 36 ± 7 (3); 7, 41 ± 3 (4); 9, 31 ± 4 (4); 10, 44 ± 1 (3); 17, 30 ± 4 (3).

preparations. Substitution of the 7-hydrogen of MIX by methyl (5) dramatically reduced potency without increasing selectivity. The only MIX derivative studied (6) that had a purely aliphatic 7-substituent larger than methyl was more potent than 7-Me-MIX as an inhibitor of either peak I or II phosphodiesterase activity. Since methyl and isoamyl substituents have approximately the same electronic effects (methylamine and isoamylamine have identical pK_a values³⁰), the increased potency of 7-isoamyl-MIX appeared dependent on the chain length or hydrophobic effects rather than on electronic effects involving the xanthine ring.

7-Bzl-MIX (8) was found to be 20–30 times more potent as an inhibitor of peak I than of peak II phosphodiesterase activity (Table II), in contrast to the threefold selectivity exhibited by MIX.¹ This selectivity was not at the expense of potency since 7-Bzl-MIX (8) was as potent as MIX as an inhibitor of peak I activity.

In this regard, during their study of hydrophobic binding in thymidine phosphorylase, Baker and Rzeszotarski³¹ found that the 3-chlorobenzyl moiety was more capable of hydrophobic binding than either of its 2- or 4-chlorobenzyl isomers or the unsubstituted benzyl group. In the present work it was found that 7-(3-ClBzl)-MIX (13) and 7-(4-ClBzl)-MIX (14) were slightly more potent than 7-Bzl-MIX as inhibitors of peak I activity; however, neither agent was more selective than 7-Bzl-MIX because 13 and 14 were also more potent than 7-Bzl-MIX as inhibitors of peak II activity.

Extension of the chain length (e.g., 7-PhEt-MIX) or addition of nitro or methoxy groups to the benzyl ring resulted in diminished potency and/or selectivity. Substitution of other electron-rich substituents such as cyclopropylmethyl (15) or propargyl (16) in the 7 position of MIX, while resulting in compounds more potent than 7-Me-MIX, did not give rise to the combination of high potency against peak I and low potency against peak II that was found with 7-Bzl-MIX (8).

Therefore, it appears that for a combination of potency and selectivity in this 7-substituted MIX series a range of size larger than methyl, but not larger than benzyl, is necessary. An aromatic 7-substituent is not mandatory but seems, in the light of this small series, to be optimum for selectivity. 6-Thiotheophylline has been shown to be more potent than theophylline as a dilator of dog coronary arteries.³² We have, therefore, prepared 6-thio-MIX^{28,33} and 6thio-7-Bzl-MIX in order to determine if additional selectivity could be attained with the somewhat bulkier thio functionality which might also influence the freedom of rotation of the 7-substituent. The two 6-thio derivatives studied (18 and 19) had approximately the same potency as MIX and 7-Bzl-MIX as inhibitors of peak I phosphodiesterase activity. However, 6-thio-MIX was more potent and 6-thio-7-Bzl-MIX was only slightly less potent than MIX as an inhibitor of peak II activity. That is, the substitution of a benzyl group onto the 7 position of 6thio-MIX did not reduce activity against peak II as dramatically as the corresponding substitution on MIX.

It seems unlikely that the selectivity demonstrated with these compounds arises as an artifact from the influence of Me₂SO on the enzyme systems being studied. 3%Me₂SO does inhibit peak I and peak II activity by 15–20%, but the data for MIX (Table II) and papaverine (data not shown) in the presence of Me₂SO are indistinguishable from our previous data for the inhibition of these enzyme preparations by these agents in the absence of Me₂SO. We also have shown that MIX and 7-Bzl-MIX (8), in the presence of 3% EtOH (data not shown) instead of 3%Me₂SO, have inhibitory potencies that are identical with those reported in Table II.

We are currently examining the effects of substitution in other positions of MIX, with regard to selectivity for one of the coronary artery phosphodiesterases and to determine if the conformation of the 7-benzyl substituent is a significant determinant of selectivity. We should point out that the agents reported here may not be specific enough for one cyclic nucleotide phosphodiesterase activity to allow selective pharmacological manipulation of cAMP or cGMP levels, since in the pig coronary artery at least, total cAMP phosphodiesterase activity at 1 μ M substrate is approximately the same in peak I as in peak II and, therefore, regulation of either activity could potentially affect cAMP levels. We believe, however, that the data reported here do indicate that it will be possible to find agents that can selectively inhibit one of the multiple forms of phosphodiesterase. If the relative abundance of the various phosphodiesterases differs from one tissue to another, tissue selectivity should be possible. Others have reported tissue selectivity with certain cyclic nucleotide phosphodiesterase inhibitors.³⁴

Experimental Section

Melting points were determined in open glass capillary tubes using a Laboratory Devices Mel-Temp and are reported uncorrected. Infrared spectra were obtained using a Perkin-Elmer Model 257. Nuclear magnetic resonance spectra were recorded on a Jeol Model JNM-MH-100 spectrometer using tetramethylsilane as an internal standard. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. In all cases spectral data were consistent with the proposed structures and elemental analyses were within $\pm 0.4\%$ of the calculated value.

Phosphodiesterase Assay. The compounds were assayed for inhibitory activity against the separated forms of phosphodiesterase from pig coronary arteries. Enzyme preparation and assay procedures have been reported.^{1,17} Assays were performed with $1 \,\mu M$ substrate at 30° for 30 min at enzyme dilutions which gave 10-20% hydrolysis of substrate in the absence of inhibitor. MIX $(10^{-5} \text{ and } 10^{-4} \text{ M})$ was included in each experiment to assure that the enzyme preparation was responding in a normal manner. The compounds were dissolved in 30% Me₂SO and then 25 µl of this solution was added to the assay tube (final volume was $250 \ \mu$ l). All activities (including control and no-enzyme blank) were measured in the presence of 3% Me2SO and product accumulation was linear for at least 30 min under the conditions of the assay. None of the compounds altered the efficacy of the nucleotidase step or subsequent steps in the assay. Concentrations of the compounds which inhibited by 50% the hydrolysis of 1 μ M substrate (150) were determined from concentration-percent inhibition curves, utilizing concentrations of the compounds from 10^{-7} to 10^{-4} M (or 10^{-7} - 10^{-3} M if the compound was sufficiently soluble). The more potent xanthines were competitive inhibitors of peak I phosphodiesterase but K_i values are not reported since the purpose of this study was to compare inhibition of the separated forms, and the apparently negatively cooperative behavior of peak II complicated the determination of K_i values with this enzyme.¹ The presence or absence of the activator and/or calcium ion did not affect the activity of the compounds studied.

Synthesis. 1-Isobutyl-3-methyluracil (2b). To a cooled solution of 1-isobutyluracil³⁵ (3.2 g, 19 mmol) and NaOH (1.26 g, 32 mmol) in 12 ml of H₂O was added dimethyl sulfate (4.3 g, 34 mmol) dropwise with stirring. The resulting mixture was allowed to warm to room temperature and then heated to boiling. After cooling (0°), the mixture was extracted several times with CH₂Cl₂. The combined extracts were dried (MgSO₄), and the solvent was removed in vacuo to give clear, colorless oil: 3.2 g (92%). An analytical sample was obtained by distillation in vacuo: bp 125-127° (0.1 mm). Anal. (C₉H₁₄N₂O₂) C, H, N.

1-Isobutyl-3-methyl-5-aminouracil (3a). 1-Isobutyl-3methyl-5-nitrouracil was prepared from 1-isobutyl-3-methyluracil by the method of Brown, Hoerger, and Mason³⁶ (76%, mp 92–93°, EtOH) and used without further characterization. Reduction of 1-isobutyl-3-methyl-5-nitrouracil by the method of Besly and Goldberg³⁷ gave an oily product which was extracted from the reaction mixture with CH₂Cl₂. The CH₂Cl₂ solution was dried (MgSO₄) and then evaporated in vacuo. The residual solid was recrystallized from anhydrous Et₂O: 67%; mp 77–78°. Anal. (C₉H₁₅N₃O₂) C, H.

1-Isobutyl-3-methyl-5-benzylaminouracil (3b). To a solution of 1-isobutyl-3-methyl-5-aminouracil (3.5 g, 15 mmol) in 25 ml of warm H₂O was added freshly distilled benzaldehyde (2.5 g, 24 mmol) dropwise with stirring according to the method of Nubel and Pfleiderer.³⁸ The reaction mixture was extracted with CH₂Cl₂. The CH₂Cl₂ solution was dried (MgSO₄) and then evaporated in vacuo to give the crude Schiff base which was used without further purification and characterization. A solution of the above Schiff base (0.5 g, 1.7 mmol) in 25 ml of absolute MeOH was stirred with 5% Pd/C with cooling at 0°. A solution of NaBH4 (0.2 g, 5 mmol) in 10 ml of absolute MeOH was added and the mixture was stirred for 15 min at which time a second portion of NaBH4 in 10 ml of absolute MeOH was added and the mixture was stirred for another 15 min. The catalyst was removed by filtration and 50 ml of H₂O was added to destroy the borohydride.

The resulting mixture was extracted several times with benzene and the combined extracts were evaporated in vacuo. The residue was dried by the addition and evaporation in vacuo of 3×10 ml of absolute EtOH to give an oil which crystallized on standing and was recrystallized from Et₂O-hexane: bp 175–176° (0.1 mm); mp 72–73.5°. Anal. (C₁₆H₂₁N₃O₂) C, H.

1,3-Dimethyl-5-benzylamino-6-aminouracil (4a). A mixture of 1,3-dimethyl-5-bromo-6-aminouracil³⁹ (1.0 g, 4.3 mmol) and benzylamine (1.0 g, 9.4 mmol) in 2 ml of DMF was heated at 100° for 2 h. To the resulting mixture was added 50 ml of H₂O. Heating was continued for a short time and then the mixture was allowed to cool. The solid was separated by filtration and air-dried: 0.5 g (45%). An analytical sample was obtained by recrystallization from EtOAc: mp 183° dec. Anal. (C₁₃H₁₆N₄O₂) C, H, N.

1-Isobutyl-3-methyl-5-benzylamino-6-aminouracil Hydrochloride (4b). 1-Isobutyl-3-methyl-5-bromo-6-aminouracil was prepared from 1-isobutyl-3-methyl-6-aminouracil by the method of Schroeder⁴⁰ (81%, mp 164-165°, acetone) and used without further characterization. A mixture of 1-isobutyl-3methyl-5-bromo-6-aminouracil (2.2 g, 8 mmol) and benzylamine (1.9 g, 18 mmol) in 4 ml of DMF was heated at 100° for 2 h. To the resulting mixture was added 50 ml of H₂O and heating was continued for a short time. The mixture was allowed to cool and extracted several times with Et2O. The extracts were combined and dried over MgSO₄. Anhydrous Et₂O was added to increase the volume to 50 ml and the HCl salt was precipitated with dry HCl. The resulting solid was separated by filtration and air-dried: 1.2 g (29%). An analytical sample was obtained by recrystallization from anhydrous MeOH: mp 222° dec. Anal. (C16-H₂₃N₄O₂Cl) C, H.

7-Alkylation of 1-Methyl-3-isobutylxanthines (or 6-Thioxanthines). General Procedures. To a stirred slurry of the xanthine (2.25 mmol) and dry K_2CO_3 (0.35 g) in 4 ml of dry DMF was added 2.25 mmol of alkyl halide (22.5 mmol for 15 and 16). Stirring was continued at 35° for the length of time noted for each individual preparation at which time the reaction mixture was either (a) poured into water (10 ml of H₂O for each milliliter of DMF) or (b) evaporated in vacuo to remove the volatile materials. Products were isolated as described below.

Method A. The H_2O -DMF mixture was evaporated and the solid was extracted with hot EtOAc. The EtOAc was evaporated to dryness in vacuo. The products were then purified by recrystallization from the solvent indicated or sublimed.

Method B. The H_2O -DMF suspension was cooled overnight at 4° to induce complete crystallization. These crystals were then purified by recrystallization from the solvent indicated.

1-Methyl-3-isobutyl-7-benzylxanthine (8). Alternate Preparation. A mixture of the hydrochloride salt of 1-isobutyl-3-methyl-5-benzylamino-6-aminouracil (0.4 g, 1.2 mmol) and formic acid (5.6 g, 120 mmol) was heated at reflux for 1 h. Formic acid was removed in vacuo. To the residue was added 10 ml of 10% NaOH and the mixture was heated at reflux for 15 min. To the heated mixture was added 95% EtOH until all solid had dissolved. The resulting solution was allowed to cool and the precipitate collected by filtration and recrystallized from EtOH-H2O: 0.33 g (82%); mp 111-111.5°; the mixture melting point with material 8 prepared by method B was not depressed and spectral data were identical with those of 8 prepared by method B.

Acknowledgment. This work was supported by NIH Grants GM 21220, HL 13996, GM 16811, AM 07462, and AM 17560 and by the Vanderbilt University Diabetes-Endocrinology Center Grant AM 17026. We thank Mrs. Susan Mitchel for her expert technical help.

References and Notes

- J. N. Wells, Y. J. Wu, C. E. Baird, and J. G. Hardman, Mol. Pharmacol., 11, 775 (1975) (paper 1).
- (2) A preliminary account of this work was presented at the 169th National Meeting of the American Chemical Society, Philadelphia, Pa., 1975, MEDI 50.
- (3) G. A. Robison, R. W. Butcher, and E. W. Sutherland, "Cyclic AMP", Academic Press, New York and London, 1971.
- (4) R. Anderson, L. Lundholm, E. Mohme-Lundholm, and K.

Nilsson, Adv. Cyclic Nucleotide Res., 1, 213 (1972).

- (5) H. P. Bar, Adv. Cyclic Nucleotide Res., 4, 195 (1974).
- (6) L. Triner, G. G. Nahas, Y. Vulliemoz, N. I. A. Overweg, M. Verosky, D. V. Habif, and S. H. Ngai, Ann. N.Y. Acad. Sci., 185, 458 (1971).
- (7) T. P. Lee, J. F. Kuo, and P. Greengard, Proc. Natl. Acad. Sci. U.S.A., 69, 3287 (1972).
- G. Schultz, J. G. Hardman, and E. W. Sutherland in "Asthma, Physiology, Immunopharmacology and Treatment", K. F. Austen and L. M. Lichtenstein, Ed., Academic Press, New York, N.Y., 1973, p 123.
 G. Schultz, J. G. Hardman, K. Schultz, J. W. Davis, and E.
- (9) G. Schultz, J. G. Hardman, K. Schultz, J. W. Davis, and E. W. Sutherland, Proc. Natl. Acad. Sci. U.S.A., 70, 1721 (1973).
- (10) G. Schultz, J. G. Hardman, K. Schultz, C. E. Baird, and E. W. Sutherland, Proc. Natl. Acad. Sci. U.S.A., 70, 3889 (1973).
- (11) N. D. Goldberg, R. F. O'Dea, and M. K. Haddox, Adv. Cyclic Nucleotide Res., 3, 155 (1973).
- (12) J. A. Beavo, N. L. Rodgers, O. B. Crofford, C. E. Baird, J. G. Hardman, E. W. Sutherland, and E. V. Newman, *Ann. N.Y. Acad. Sci.*, 185, 129 (1971).
- (13) J. A. Beavo, N. L. Rodgers, O. B. Crofford, J. G. Hardman, E. W. Sutherland, and E. V. Newman, *Mol. Pharmacol.*, 6, 597 (1970).
- (14) A. Peytremann, W. E. Nicholson, G. W. Liddle, J. G. Hardman, and E. W. Sutherland, *Endocrinology*, 92, 525 (1973).
- (15) C. A. Sutherland, G. Schultz, J. G. Hardman, and E. W. Sutherland, Fed. Proc., Fed. Am. Soc. Exp. Biol., 32, 773 (1973).
- (16) C. A. Sutherland and J. G. Hardman, unpublished observation.
- (17) J. N. Wells, C. E. Baird, Y. J. Wu, and J. G. Hardman, Biochim. Biophys. Acta, 384, 430 (1975).
- (18) W. J. Thompson and M. M. Appleman, J. Biol. Chem., 246, 3145 (1971).
- (19) R. Buchman, P. F. Heinstein, and J. N. Wells, J. Med. Chem., 17, 1168 (1974).
- (20) Compound 2a has been reported.²¹ Compounds 3a and 4d have been prepared previously²² but physical data have not been reported. Ir and NMR spectral data were consistent

with structures. Structures were further confirmed by conversion to the known xanthine. 3a has mp 185.5–188°. 4d has mp 178–185° dec. Compound 4c is available commercially.²³

- (21) T. B. Johnson and S. H. Clapp, J. Biol. Chem., 5, 49 (1908).
- (22) V. Papesch and E. F. Schroeder, J. Org. Chem., 16, 1879 (1951).
- (23) Available from the Aldrich Chemical Co., Inc., Milwaukee, Wis.
- (24) Reaction of theophylline with benzyl bromide and K₂CO₃ in dimethylformamide gave excellent yields of 7-benzyltheophylline, mp 156.5-158° (lit.²⁷ mp 154-155°).
- (25) R. Zelnick, M. Pesson, and M. Polonovski, Bull. Soc. Chim. Fr., 888 (1956).
- (26) J. A. Montgomery and K. Hewson, J. Org. Chem., 26, 4469 (1961).
- (27) G. P. Hager, J. C. Krantz, Jr., and J. B. Harmon, J. Am. Pharm. Assoc., 42, 36 (1953).
- (28) K. R. H. Wooldridge and R. Slack, J. Chem. Soc., 1863 (1962).
- (29) R. B. Meyer, Jr., D. A. Shuman, R. K. Robins, J. P. Miller, and L. N. Simon, J. Med. Chem., 16, 1319 (1973).
- (30) "Handbook of Chemistry and Physics", Chemical Rubber Publishing Co., Cleveland, Ohio, 1964, p D-76.
- (31) B. R. Baker and W. Rzeszotarski, J. Med. Chem., 11, 639 (1968).
- (32) A. K. Armitage, J. Boswood, and B. J. Large, Br. J. Pharmacol., 17, 196 (1961).
- (33) A. K. Armitage, J. Boswood, and B. J. Large, Br. J. Pharmacol., 16, 59 (1961).
- (34) C. G. Smith, Adv. Enzyme Regul., 12, 187 (1974), and references cited therein.
- (35) L. Doub, U. Krolls, J. M. Vandenbelt, and M. W. Fisher, J. Med. Chem., 13, 242 (1970).
- (36) D. J. Brown, E. Hoerger, and S. F. Mason, J. Chem. Soc., 211 (1955).
- (37) D. M. Besly and A. A. Goldberg, J. Chem. Soc., 4997 (1957).
- (38) G. Nubel and W. Pfleiderer, Chem. Ber., 98, 1060 (1965).
- (39) V. Papesch and E. F. Schroeder (G. D. Searle and Co.), U.S. Patent 2,602,795 (1952); Chem. Abstr., 47, 4920f (1953).
- (40) E. F. Schroeder (G. D. Searle and Co.), U.S. Patent 2,731,465 (1956); Chem. Abstr., 51, 1257e (1957).

Substituted α -Methylbenzyl and Tricyclic Arylalkyl Lactamimides as Inhibitors of Blood Platelet Aggregation

J. Martin Grisar,* George P. Claxton, and Robert D. MacKenzie

Merrell-National Laboratories, Division of Richardson-Merrell Inc., Cincinnati, Ohio 45215. Received August 28, 1975

N-[1-(p-Phenoxyphenyl)ethyl]hexahydro-2H-azepin-2-imine hydrochloride (10) and N-[1-(2-dibenzothienyl)ethyl]hexahydro-2H-azepin-2-imine hydrochloride (22) were found to inhibit in vitro aggregation of human blood platelets induced by ADP with minimal release of procoagulant platelet factor 3. The compounds were selected from a series of substituted α -methylbenzyl and tricyclic arylalkyl lactamimides that were free of hypoglycemic and diuretic effects. Compounds 10 and 22, as well as N-[1-(1-naphthyl)ethyl]hexahydro-2H-azepin-2-imine hydrochloride (I) and N-(2,2-diphenylpentyl)hexahydro-2H-azepin-2-imine hydrochloride (II), were evaluated for effects on ADP-induced platelet aggregation after repeated oral administration to guinea pigs. Compound II (RMI 12,366A) showed in vivo activity in this system 2 h after the last of four daily doses of 100 mg/kg po.

Naphthylalkyl lactamimides, especially I (RMI 7822A), were found earlier to inhibit adenosine diphosphate (ADP) and collagen-induced aggregation of human blood platelets.¹ Later it was found that I also has hypoglycemic and diuretic properties in rats.² We therefore set out to find lactamimides that inhibit platelet aggregation without causing hypoglycemia or diuresis. Compound II (RMI 12,366A), which was reported earlier,² meets these requirements. In this paper, we report preparation and evaluation of a series of substituted α -methylbenzyl lactamimides III, in which the substituent R is large and imparts lipophylic properties to the molecules, and tricyclic



arylalkyl lactamimides IV. Compounds of this type inhibit aggregation of platelets but are free of hypoglycemic ef-