# Inhibition of Separated Forms of Phosphodiesterases from Pig Coronary Arteries by Uracils and by 7-Substituted Derivatives of 1-Methyl-3-isobutylxanthine[1,2]

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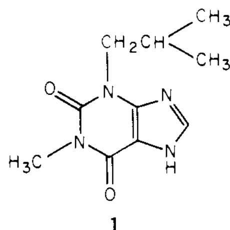
A series of 7-substituted 1-methyl-3-isobutylxanthines was designed in an attempt to increase the specificity of the 1-methyl-3-isobutylxanthine (MIX) structure for one of the two cyclic nucleotide phosphodiesterase peaks isolated by DEAE-cellulose chromatography of the soluble fraction of the intima + 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 $K_2CO_3$. 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-Bzl-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 tissues[3] 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 MIX[1] 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.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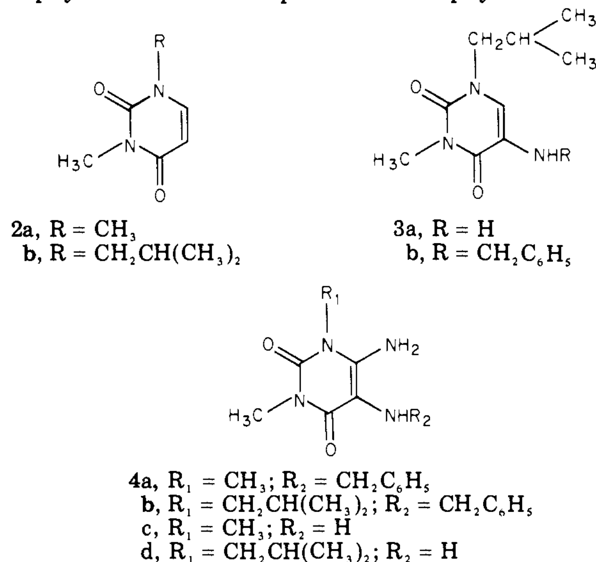
We have separated and partially characterized cyclic nucleotide phosphodiesterase activities from the intima plus media layer of pig coronary arteries.[17] Two fractions of phosphodiesterase activity and a heat-stable, nondialyzable activator were separated by DEAE-cellulose chromatography. Peak I phosphodiesterase 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 $\mu$M) than for cAMP (40–100 $\mu$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 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 $\mu$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 procedures[20] 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


2a, R = $CH_3$
b, R = $CH_2CH(CH_3)_2$

3a, R = H
b, R = $CH_2C_6H_5$

4a, $R_1$ = $CH_3$; $R_2$ = $CH_2C_6H_5$
b, $R_1$ = $CH_2CH(CH_3)_2$; $R_2$ = $CH_2C_6H_5$
c, $R_1$ = $CH_3$; $R_2$ = H
d, $R_1$ = $CH_2CH(CH_3)_2$; $R_2$ = H

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

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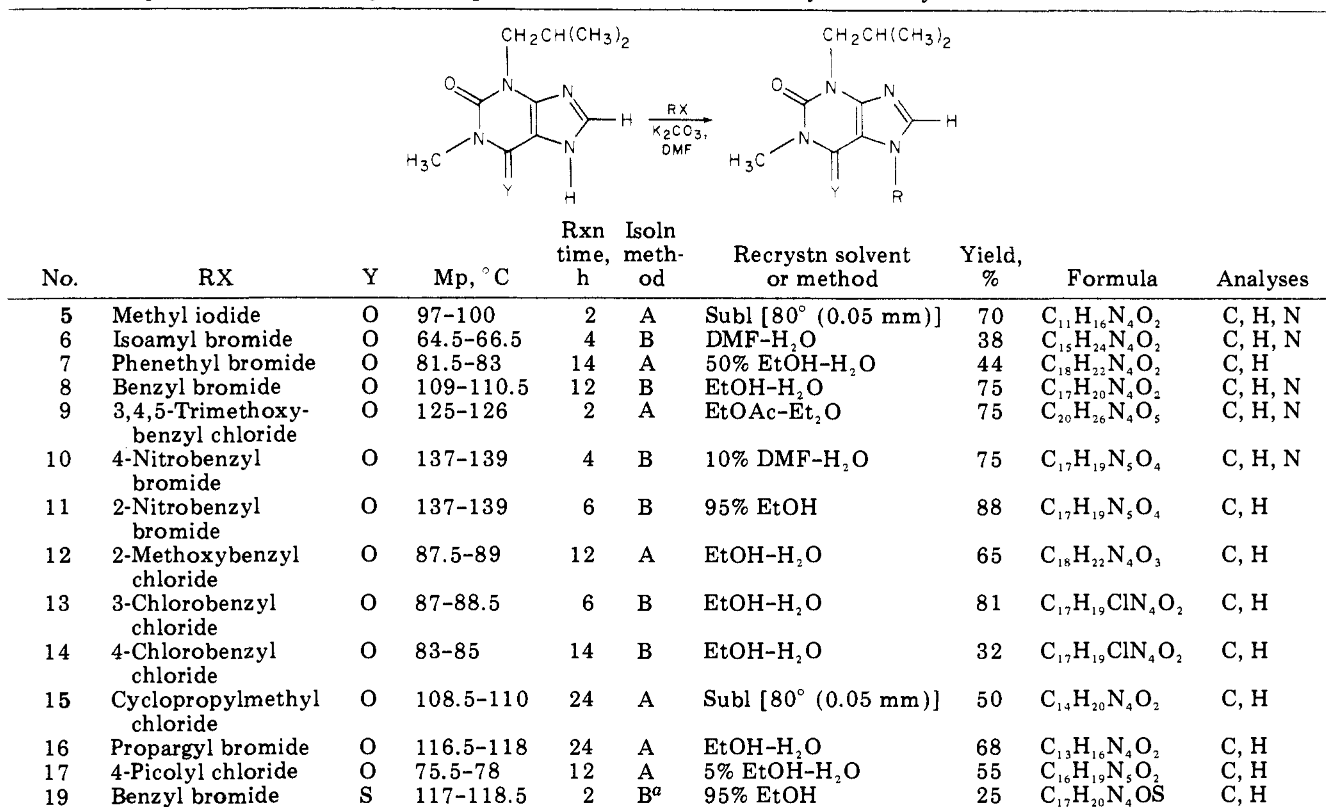
Table I. Preparative Data and Physical Properties of 7-Substituted 1-Methyl-3-isobutylxanthines




| No. | RX | Y | Mp, °C | Rxn time, h | Isoln meth-od | Recrystn solvent or method | Yield, % | Formula | Analyses |
|---|---|---|---|---|---|---|---|---|---|
| 5 | Methyl iodide | O | 97-100 | 2 | A | Subl [80° (0.05 mm)] | 70 | $C_{11}H_{16}N_4O_2$ | C, H, N |
| 6 | Isoamyl bromide | O | 64.5-66.5 | 4 | B | DMF-$H_2O$ | 38 | $C_{15}H_{24}N_4O_2$ | C, H, N |
| 7 | Phenethyl bromide | O | 81.5-83 | 14 | A | 50% EtOH-$H_2O$ | 44 | $C_{18}H_{22}N_4O_2$ | C, H |
| 8 | Benzyl bromide | O | 109-110.5 | 12 | B | EtOH-$H_2O$ | 75 | $C_{17}H_{20}N_4O_2$ | C, H, N |
| 9 | 3,4,5-Trimethoxy-benzyl chloride | O | 125-126 | 2 | A | EtOAc-$Et_2O$ | 75 | $C_{20}H_{26}N_4O_5$ | C, H, N |
| 10 | 4-Nitrobenzyl bromide | O | 137-139 | 4 | B | 10% DMF-$H_2O$ | 75 | $C_{17}H_{19}N_5O_4$ | C, H, N |
| 11 | 2-Nitrobenzyl bromide | O | 137-139 | 6 | B | 95% EtOH | 88 | $C_{17}H_{19}N_5O_4$ | C, H |
| 12 | 2-Methoxybenzyl chloride | O | 87.5-89 | 12 | A | EtOH-$H_2O$ | 65 | $C_{18}H_{22}N_4O_3$ | C, H |
| 13 | 3-Chlorobenzyl chloride | O | 87-88.5 | 6 | B | EtOH-$H_2O$ | 81 | $C_{17}H_{19}ClN_4O_2$ | C, H |
| 14 | 4-Chlorobenzyl chloride | O | 83-85 | 14 | B | EtOH-$H_2O$ | 32 | $C_{17}H_{19}ClN_4O_2$ | C, H |
| 15 | Cyclopropylmethyl chloride | O | 108.5-110 | 24 | A | Subl [80° (0.05 mm)] | 50 | $C_{14}H_{20}N_4O_2$ | C, H |
| 16 | Propargyl bromide | O | 116.5-118 | 24 | A | EtOH-$H_2O$ | 68 | $C_{13}H_{16}N_4O_2$ | C, H |
| 17 | 4-Picolyl chloride | O | 75.5-78 | 12 | A | 5% EtOH-$H_2O$ | 55 | $C_{16}H_{19}N_5O_2$ | C, H |
| 19 | Benzyl bromide | S | 117-118.5 | 2 | B[a] | 95% EtOH | 25 | $C_{17}H_{20}N_4OS$ | C, H |

[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 9-xanthine 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 S-benzylated compound was probably also formed and apparently underwent base hydrolysis to benzylthiol and the base-soluble MIX (1).[28] Questions have been raised by cases[26] 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 $\mu$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 $\mu$M cAMP and cGMP by peak I by 37 and 30%, respectively, and inhibited hydrolysis of 1 $\mu$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 Peak I and II Cyclic Nucleotide Phosphodiesterase Activities by 7-Substituted 1-Methyl-3-isobutylxanthines

| | | | $I_{50}$ values[a] | | |
|---|---|---|---|---|---|
| | | | Peak I[b] | | |
| No. | 7-Subst | 6-Subst | cGMP | cAMP | Peak II, cAMP |
| **1** | H | O | 6.3 ± 0.7 (6) | 3.9 ± 0.3 (6) | 15 ± 2 (3) |
| 18 | H | S | 7.1 ± 0.6 (4) | 5.5 ± 0.2 (4) | 6.8 ± 2 (3) |
| **5** | Methyl | O | 65 ± 6 (3) | 55 ± 2 (4) | 126 ± 14 (3) |
| 6 | Isoamyl | O | 16 ± 2 (5) | 19 ± 4 (4) | >100[c] |
| 7 | Phenethyl | O | 28 ± 3 (3) | 17 ± 1 (3) | >100[c] |
| 8 | Benzyl | O | 4.4 ± 0.7 (7) | 3.6 ± 0.8 (7) | 108 ± 14 (4) |
| **19** | Benzyl | S | 3.3 ± 0.1 (4) | 2.7 ± 0.1 (4) | 28 ± 6 (3) |
| **9** | 3,4,5-Trimethoxybenzyl | O | 46 ± 5 (3) | 35 ± 4 (3) | >100[c] |
| 10 | 4-Nitrobenzyl | O | 34 ± 8 (3) | 27 ± 3 (3) | >100[c] |
| 11 | 2-Nitrobenzyl | O | 60 ± 15 (3) | 41 ± 1 (3) | 142 ± 4 (3) |
| 12 | 2-Methoxybenzyl | O | 26 ± 2 (3) | 19 ± 4 (3) | 75 ± 5 (3) |
| **13** | 3-Chlorobenzyl | O | 2.1 ± 0.2 (3) | 1.3 ± 0.1 (3) | 48 ± 2 (3) |
| 14 | 4-Chlorobenzyl | O | 2.1 ± 0.2 (3) | 2.3 ± 0.3 (3) | 43 ± 11 (3) |
| 15 | Cyclopropylmethyl | O | 6.1 ± 0.6 (3) | 3.7 ± 0.2 (3) | 20 ± 3 (3) |
| **16** | Propargyl | O | 36 ± 1 (3) | 28 ± 2 (3) | 65 ± 3 (3) |
| **17** | 4-Picolyl | O | 146 ± 10 (3) | 105 ± 27 (3) | >300[c] |

[a] $I_{50}$ value is defined as the concentration ($\mu$M) of the agent required to produce 50% inhibition of the hydrolysis of 1 $\mu$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 $\mu$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 p$K_a$ values[30]), 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 Rzeszotarski[31] 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 6-thio-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 6-thio-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 $Me_2SO$ on the enzyme systems being studied. 3% $Me_2SO$ 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 $Me_2SO$ are indistinguishable from our previous data for the inhibition of these enzyme preparations by these agents in the absence of $Me_2SO$. We also have shown that MIX and 7-Bzl-MIX (**8**), in the presence of 3% EtOH (data not shown) instead of 3% $Me_2SO$, 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 $\mu$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 ±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 ($10^{-5}$ and $10^{-4}$ M) was included in each experiment to assure that the enzyme preparation was responding in a normal manner. The compounds were dissolved in 30% $Me_2SO$ and then 25 $\mu$l 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% $Me_2SO$ 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 $\mu$M substrate ($I_{50}$) 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-isobutyluracil[35] (3.2 g, 19 mmol) and NaOH (1.26 g, 32 mmol) in 12 ml of $H_2O$ 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 $CH_2Cl_2$. The combined extracts were dried ($MgSO_4$), 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. ($C_9H_{14}N_2O_2$) C, H, N.

**1-Isobutyl-3-methyl-5-aminouracil (3a).** 1-Isobutyl-3-methyl-5-nitrouracil was prepared from 1-isobutyl-3-methyluracil by the method of Brown, Hoerger, and Mason[36] (76%, mp 92–93°, EtOH) and used without further characterization. Reduction of 1-isobutyl-3-methyl-5-nitrouracil by the method of Besly and Goldberg[37] gave an oily product which was extracted from the reaction mixture with $CH_2Cl_2$. The $CH_2Cl_2$ solution was dried ($MgSO_4$) and then evaporated in vacuo. The residual solid was recrystallized from anhydrous $Et_2O$: 67%; mp 77–78°. Anal. ($C_9H_{15}N_3O_2$) 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 $H_2O$ 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 $CH_2Cl_2$. The $CH_2Cl_2$ solution was dried ($MgSO_4$) 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 $NaBH_4$ (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 $NaBH_4$ 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 $H_2O$ 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 $Et_2O$–hexane: bp 175–176° (0.1 mm); mp 72–73.5°. Anal. ($C_{16}H_{21}N_3O_2$) C, H.

**1,3-Dimethyl-5-benzylamino-6-aminouracil (4a).** A mixture of 1,3-dimethyl-5-bromo-6-aminouracil[39] (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 $H_2O$. 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. ($C_{13}H_{16}N_4O_2$) 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 Schroeder[40] (81%, mp 164–165°, acetone) and used without further characterization. A mixture of 1-isobutyl-3-methyl-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 $H_2O$ and heating was continued for a short time. The mixture was allowed to cool and extracted several times with $Et_2O$. The extracts were combined and dried over $MgSO_4$. Anhydrous $Et_2O$ 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. ($C_{16}H_{23}N_4O_2Cl$) 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 $H_2O$ 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–$H_2O$: 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.

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

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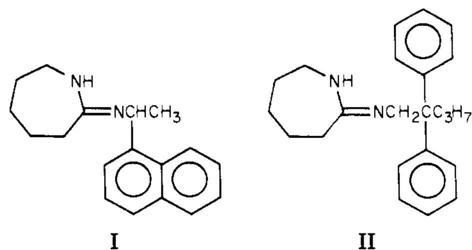
*N*-[1-(*p*-Phenoxyphenyl)ethyl]hexahydro-2*H*-azepin-2-imine hydrochloride (**10**) and *N*-[1-(2-dibenzothienyl)-ethyl]hexahydro-2*H*-azepin-2-imine hydrochloride (**22**) were found to inhibit in vitro aggregation of human blood platelets induced by ADP with minimal release of procoagulant platelet 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-2*H*-azepin-2-imine hydrochloride (I) and *N*-(2,2-diphenylpentyl)hexahydro-2*H*-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-