Table III—Third-Order Rate Constants for the Cinnamoylation of *n*-Propyl Alcohol at 25°

Solvent	ϵ[a]	k_{AN} (anhydride, DMAP)[b]	k_{IN} (chloride, DMAP)[b]	k_{IN} (chloride, NMIM)[b]
Acetonitrile[c]	35.9	2.18	2.18	0.23
Methyl ethyl ketone	18.5	1.50	3.06	0.19
Ethylene dichloride	10.4	2.93	65.1	0.70
Methylene chloride	8.9	2.45	47.0	0.39
Toluene	2.4	3.62	—	6.70

[a] Dielectric constant. [b] (Acylating agent, catalyst); units of rate constants are as given in Tables I and II. [c] Data from Ref. 10.

depends on the ratio K_d^{NHX}/K_d^{IX}, because as reaction occurs the species I^+X^-/I^+ are replaced by NH^+X^-/NH^+:

$$P = \frac{[X^-]}{K_d^{IX}} = \frac{[I^+X^-]}{[I^+]} = \frac{K_d^{NHX}[NH^+X^-]}{K_d^{IX}[NH^+]} \qquad \text{(Eq. 10)}$$

Values of ion-pair dissociation constants are extremely sensitive to the solvent dielectric constant, with typical values (13) of 10^{-16}–10^{-12} at dielectric constants of 2–3 and as high as 10^{-5} at dielectric constants near 6. In solvents more polar than acetonitrile, ion-pair formation is probably kinetically negligible. In a given solvent the ratio of ion-pair dissociation constants for two ion-pairs is controlled largely by the effective ionic sizes.

Solvent Effect on Rates and Mechanism—Table III collects k_{AN} and k_{IN} values for the cinnamoylation of *n*-propyl alcohol in five solvents. The k_{AN} values, describing the general base catalysis, are not very sensitive to the nature of the solvent; this is consistent with earlier data on alcohol acetylation by acetic anhydride catalyzed by *N*-methylimidazole (14)[6]. The k_{IN} values for the acid chloride systems reveal considerable variation with solvent polarity, the less polar solvents tending to give higher rates. In solvents that are very nonpolar k_{IN} may be influenced by ion-pair formation, as discussed in connection with Scheme III; therefore, the k_{IN} in Table III may be a composite reflecting both the ion-pair and the dissociated ion routes.

[6] The titrimetrically determined third-order rate constants in these acetic anhydride systems (14) can be interpreted as k_{AN} in Scheme I.

Since the acid chloride and the anhydride react by different mechanisms, it is dangerous to compare them. Several workers have noted that an anhydride appears to be more reactive than the acid chloride in nonpolar solvents, yet (according to Table III) $k_{IN} > k_{AN}$ for a common catalyst. An explanation of these apparently contradictory observations (9) is that formation of the intermediate from the acid chloride consumes an equimolar amount of catalyst, whereas in the anhydride system this depletion of catalyst concentration does not occur, causing $k_{AN}[N]$ in the anhydride system to be greater than $k_{IN}[N]$ is in the acid chloride system.

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Prodrugs of 6-Thiopurines: Enhanced Delivery Through the Skin

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Abstract □ Soft-alkylated derivatives of 6-mercaptopurine, its riboside, and 2-amino-6-mercaptopurine riboside have been prepared and evaluated to improve the delivery of the thiopurines through the skin. The soft-alkylated derivatives were prepared by the alkylation of the thiopurines with acylheteroalkyl halides under neutral or basic conditions. The penetration of the derivatives through hairless mouse skin was measured using diffusion cells. All of the derivatives underwent extensive degradation during their diffusion through skin so that the parent thiopurine, even in the case of the ribosides, was the major product observed in the receptor phase. The pivaloyloxymethyl derivatives showed the greatest potential for enhancing the penetration of the thiopurines through the skin. Among the 6-mercaptopurine derivatives, VII and XI were the most effective; they delivered 5 and 13 times, respectively, more 6-mercaptopurine than 6-mercaptopurine itself.

Keyphrases □ Prodrugs of 6-thiopurines—soft-alkylated derivatives of 6-mercaptopurine, enhanced delivery through the skin, pivaloyloxymethyl derivatives □ Hairless mouse skin—penetration of prodrugs of 6-thiopurines, pivaloyloxymethyl derivatives. □ Pivaloyloxymethyl derivatives—prodrugs of 6-thiopurines, penetration through hairless mouse skin

Many high-melting drugs are insoluble in water and organic solvents; consequently, they have poor biphasic solubilities and are also poorly absorbed through biological membranes. The thiopurines represent one class of such drugs that exhibit these properties. One approach to increasing the solubilities of the thiopurines, while at the

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Table I—Melting Point and UV Spectra of Selected Thiopurines and their Prodrugs

Compound	mp	UV λ_{max} (log ϵ) in Methanol				
I	>300°[a]		229 (3.88)		322 (4.35)[b]	
II	>360°[a]					
IV	221–222°[a]	217 (4.0)	226 (sh, 3.9)		282 (4.13)	288 (4.13)[c]
Va	222–223°	225 (4.19)			282 (4.13)	288 (4.12)
Vb	273.5–274.5°	225 (4.25)		287 (4.22)	296 (4.29)	
VI	189–190°	215 (4.07)	223 (sh, 4.0)		278 (4.14)	284 (4.12)
VII	87–89°	218 (4.17)	226 (sh, 4.13)		278 (4.31)	283 (4.27)
VIII	135–137°	218 (3.99)	256 (3.56)	279 (sh, 3.96)	287 (4.03)	295 (sh, 3.93)
IX	193.5–195°		227 (3.96)		326 (4.23)	
X	183–188°	218 (3.93)	244 (3.85)	300 (3.57)	337 (4.02)	
XI	Oil				276 (4.3)	282 (4.24)
XII	Oil				312 (4.0)	
XIII	255–256°[d]		236 (4.13)		309 (4.37)	
XIV	221–223°[a]				323 (4.4)	
XV	209–211°[d]		251 (3.92)		317 (4.13)	
XVI	230–231°[a]			335 (4.36)	344 (4.42)	
6-Methylthio-1-methylpurine	Free base unstable				307[b]	
6-Methylthio-3-methylpurine	163–165°[e]		238 (4.05)		312 (4.23)[c]	
6-Methylthio-7-methylpurine	212–213°[f]	220 (4.02)		285 (sh, 4.02)	293 (4.12)	301 (sh, 4.04)[c]
6-Methylthio-9-methylpurine	171–172°[g]	219 (4.10)	227 (sh, 4.04)		284 (4.32)	292 (4.2)[c]
1-Methyl-7-benzylpurine-6-thione	171°[h]		238 (4.01)		324 (4.27)[h]	
1,9-Dimethylpurine-6-thione	250°[b]		233 (3.98)		318 (4.32)[b]	
3,7-Dimethylpurine-6-thione	282–283°[b]		246 (3.77)		341 (4.38)[b]	
3,9-Dimethylpurine-6-thione	276–278°[b]		230 (4.13)		327 (4.24)[b]	
7-Methylpurine-6-thione	306–308°[f]				328 (4.31)[b]	
9-Methylpurine-6-thione	>300°[g]		229 (4.10)		321 (4.42)[b]	
3-Methylpurine-6-thione	>300°[i]		245 (3.96)		338 (4.51)[b]	
1-Methylpurine-6-thione	>300°[h]		234 (3.97)		320 (4.32)[b]	

[a] Aldrich Chemical Co. [b] UV of neutral molecule in water (Ref. 18). [c] B. Pullman, H. Berthod, F. Bergmann, Z. Neiman, H. Weiler-Feilchenfeld and E. D. Bergmann *Tetrahedron*, **26**, 1483 (1970); UV of molecule in ethanol. [d] UV of neutral molecule in water (Ref. 16). [e] Ref. 21. [f] E. Fischer, *Ber.*, 31, 431 (1898). [g] R. K. Robins and H. H. Lin, *J. Am. Chem. Soc.*, **79**, 490 (1957). [h] L. Townsend and R. K. Robins, *J. Org. Chem.*, **27**, 990 (1962). [i] F. Bergmann, G. Levin, A. Kalmus, and H. Kwietny-Govrin, *J. Org. Chem.*, **26**, 1504 (1961); UV of neutral molecule in water.

same time ensuring delivery of only the thiopurine, is to prepare prodrugs of the thiopurines.

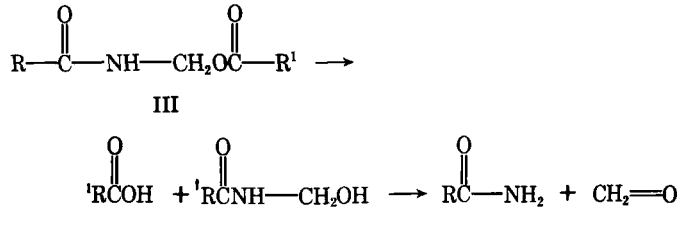
BACKGROUND

Since cancer and psoriasis are both characterized by proliferation of cells in an uncontrolled manner, it is not surprising that a recurrent theme in psoriasis therapy has been the use of the latest cancer chemotherapeutic drugs. In fact, psoriasis has been effectively treated by the systemic administration of cancer chemotherapeutic agents such as methotrexate (1), azaribine (2), 6-mercaptopurine (3), hydroxyurea (4), and mycophenolic acid (5). Unfortunately, these same agents have generally been found to be ineffective when applied topically. However, the real possibility exists that the systemic toxicity of such agents can be either circumvented entirely or at least minimized, and efforts have been continued to find anticancer agents that are effective topically.

Until recently, the reason for the topical ineffectiveness of many anticancer agents had not been investigated. Now, it has been found that the effect of topical antiproliferative drugs on epidermal DNA synthesis in hairless mice correlates well with the clinical effectiveness of the same topical agents in humans (6), but that there is little correlation between the clinical effectiveness of a topical agent and its ability to decrease epidermal DNA synthesis in essential fatty acid-(EFA) deficient mice. Apparently, the compromised epidermal barrier in the EFA-deficient mice allows some agents to penetrate the skin that are not capable of penetrating the skin of psoriatic patients or hairless mice. For instance, methotrexate, which had failed repeatedly in clinical tests as a topical antipsoriasis agent (7) and which was ineffective in decreasing DNA synthesis in the hairless mouse, was effective in decreasing DNA synthesis by 30% in EFA-deficient mice. Similarly, 15% 6-mercaptopurine and 10% thioguanine sodium were inactive clinically in humans and in the hairless mouse but were active in the EFA-deficient mouse. Thus, the reason that a number of the topically administered anticancer agents tried have been found to be ineffective may be simply that they are not absorbed: they do not penetrate the skin in their present form.

It is easy to see why 6-mercaptopurine and thioguanine are not well absorbed topically. Both purines are high-melting solids (Table I) with very low water or lipid solubility. On the other hand, well-absorbed topical drugs exhibit good water and lipid solubilities which allow their facile penetration through the biphasic epidermal barrier. One way to improve the biphasic solubilities of 6-mercaptopurine (I) and thioguanine (II) is to use the prodrug approach. An attractive prodrug strategy in this case is alkylation rather than the more routinely employed strategy of acylation, since chemical precedent suggests that the sulfur or imidazole acylates that would result are too unstable to be practical.

Inspection of the melting points and solubilities of the products obtained from the alkylation of the pyrimidine uracil shows that a correlation exists between a decrease in melting point and an increase in aqueous solubility (8). However, *N*-alkylation is normally not an easily reversible process *in vivo*, so that the biological activity profile of the parent compound is usually drastically changed by such a modification. On the other hand, in the case of uracil (8), and other amides and imide-like molecules (9), the formation of *N*-hydroxymethyl derivatives results not only in a decrease in the melting point and an increase in the water solubility of the prodrug compared with its parent, but also results in an easily reversible derivative. These *N*-hydroxymethyl derivatives are "soft-alkylated" prodrugs[1] of amides, imides, and amines: they are reversed by chemical hydrolysis rather than initially requiring a metabolically activating oxidation step. However, the range of physical properties of hydroxymethyl derivatives is limited by the relatively small number of aldehydes that will form the hydroxyalkyl adducts and by the fact that a hydroxyl group is introduced into the molecule in each case. Thus, with hydroxymethyl derivatives there is a limit to the concomitant increase in lipid solubility that is both possible and essential for efficient penetration of the epidermal membrane by the prodrug. This difficulty has been overcome by several investigators (10–14) who have acylated the hydroxymethyl derivative to give acyloxymethyl derivatives of the general formula III. Cleavage of the acyl group by esterases gives the hydroxymethyl derivative that, in turn, liberates the parent amide or imide. The acyloxymethyl derivatives, then, are also soft-alkylated derivatives which differ from the hydroxymethyl derivatives in that they must first undergo enzymatic hydrolysis before undergoing chemical hydrolysis.



III

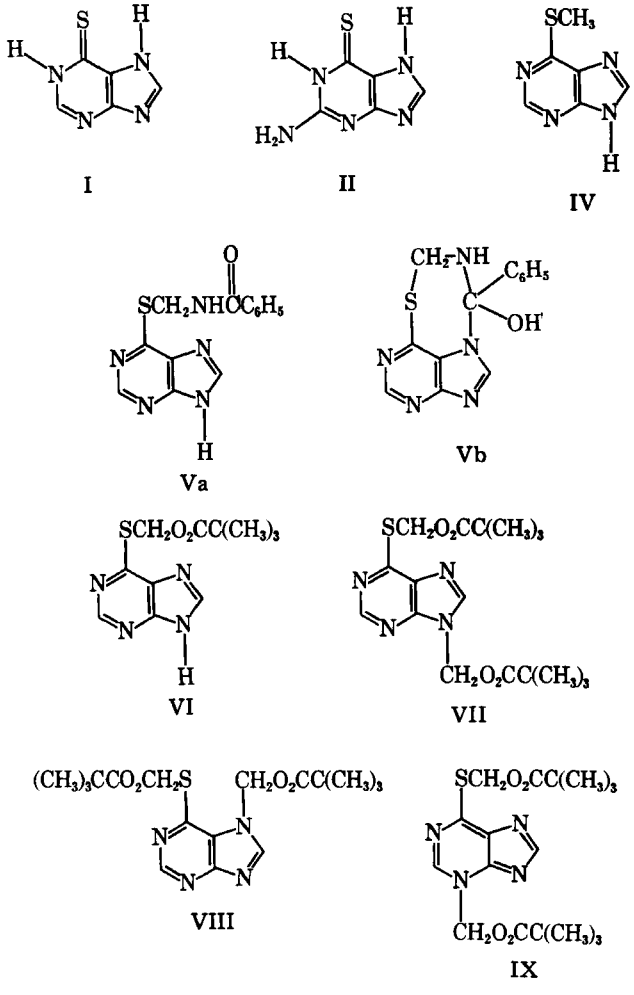
[1] These soft-alkylated derivatives are not soft drugs, although other soft-alkylated compounds are soft drugs. For example see N. Bodor, J. Kaminski, and S. Selk, *J. Med. Chem.*, **23**, 469 (1980).

Table II—NMR Spectra of Prodrugs [a]

Compound	(C2)H	(C8)H	SCH_2X	NCH_2X
Va	8.77	8.55	5.85 (d, J = 6Hz) [b]	
Vb	8.90	8.57	5.85 (d, J = 6Hz) [c]	
VI	8.83	8.30	6.06	
VII	8.80	8.23	6.03	6.17
VIII	8.90	8.27	6.03	6.17
IX	8.53	8.08	6.06	6.40
X	8.50	8.23		6.37, 6.63
XI	8.71	8.01	5.90	
XII		7.80	5.90	

[a] Chemical shifts in δ in $CDCl_3$. [b] Run in DMSO-d_6 + $CDCl_3$ (1:5). [c] Run in DMSO-d_6.

The advantage of this type of modification can be illustrated by the case of theophylline (12) in which prodrugs with increased water or lipid solubility or with partition coefficients of ~1 were obtained by varying the acyl group R^1 in III above. Selected derivatives in this series were also found to penetrate the epidermal barrier better than theophylline and to revert to theophylline *in vivo*. In addition, these same derivatives were found to decrease the synthesis of DNA in hairless mice after their topical administration. Thus, it is possible to reduce the melting point and increase not only the biphasic solubility but also the ability of amide and imide drugs to penetrate the epidermal barrier by acyloxyalkylation.



It is clear from the data listed in Table I that alkylation on nitrogen in 6-mercaptopurine does not drastically decrease the melting point of the 6-mercaptopurine derivative. This is probably because I and its 7- and 9-alkylated derivatives exist primarily in the thioamide form (15). On the other hand, S-alkylation drastically reduces the melting point of the 6-mercaptopurine derivative, either by itself or more dramatically in combination with N-alkylation; there are similar trends in the melting points of the alkylated 2-amino-6-mercaptopurine (II) series. Therefore, as a first step in determining whether the lack of clinical activity exhibited by I and II was due to their inability to penetrate the epidermis, a number of S-acylheteroalkylated derivatives of I and XVI were synthesized and their physical properties examined. The preliminary results of that work are reported here.

RESULTS AND DISCUSSION

There have not been any previous reports on the synthesis of S-acylheteroalkyl derivatives of heterocyclic thione amides such as I or II. However, the alkylations were relatively straightforward. Representatives of two types of acylheteroalkyl derivatives were prepared for I. The first type was the S-acylaminomethyl derivative V. Compound V was prepared from the reaction of chloromethylbenzamide with I in dimethylsulfoxide. Under these conditions, alkylations of I with other alkylating agents have been shown to take place exclusively on sulfur (18). In addition, the elemental analysis, and the NMR and mass spectra were consistent only with a monoalkylated derivative. Among the possible monoalkylated derivatives, the UV spectrum (Table I) was consistent only with the S-alkylated derivative (compare Va with IV).

The only unusual feature of this reaction was the formation of two monoalkylated products, which have been designated as Va and Vb. Compound Va differs significantly from Vb in its TLC, melting point, solubility, and UV and mass spectra. The NMR spectra (Table II) of the two compounds are identical except for the difference in the position of the (C2)H absorptions, which is comparable to the difference exhibited by the (C2)H absorptions in the imidazole alkylated tautomers VII and VIII. The UV spectral difference between Va and Vb is also similar to the difference between 6-methylthio-7- and 6-methylthio-9-methylpurine and between VII and VIII. In each case the S^6,7-disubstituted product exhibits a UV maximum at a longer wavelength than that of the S^6,9-disubstituted product. However, although the spectral data suggest that Va and Vb are simply 9-H and 7-H tautomers, the separation and isolation of the two tautomers should be impossible because the energy difference between the 7-H (Vb) and 9-H (Va) tautomers is only about 3.5 kcal (19). On the other hand, the 7-H tautomer can form a cyclic adduct, which is shown as Vb[2]. There is chemical precedent for the formation of similar cyclols from amide–amide adducts in small peptides (20). In this case the adduct stabilizes the less stable 7-H tautomer of V as Vb.

Only one representative of the S-acylaminoalkyl-type derivative was studied in diffusion cell experiments. Although the melting points of both derivatives (Va and Vb) were lower than that of I, only the lower melting derivative Va was studied. Derivative Va did not enhance the diffusion of I through the skin (Table III). Apparently the amide N—H group of this type of derivative introduces too much polarity into the derivative to provide much assistance to the diffusion of 6-mercaptopurine.

The second type of acylheteroalkyl derivative of I and II is the pivaloyloxymethyl derivative. The pivaloyloxymethyl group has been used previously as a protecting and reaction-steering group in some alkylation reactions of adenine examined by Rasmussen and Leonard (21). However, this is the first report of the pivaloyloxymethyl group being used as a protecting group for sulfur. In this study it was possible to condense I with chloromethyl pivalate in dimethylsulfoxide to give the monoalkylated derivative VI, albeit in very low yield especially compared with the good yields of V that were obtained above. The structure of VI followed from its UV and NMR spectra which were consistent with monoalkylation on sulfur[3]. Derivative VI was not tested in the diffusion cells because other, lower-melting representatives of this class of acylheteroalkylated I were more attractive candidates.

It was also possible to prepare dialkylated derivatives of I. Compound I was condensed with excess chloromethyl pivalate in the presence of base in dichloromethane to give a mixture of dialkylated products based on the NMR spectrum of the crude reaction product. Although chemical precedent (18) suggests that the S-alkylated product is formed as an intermediate, such as intermediate (VI) is much more soluble in dichloromethane than I and is more available than I itself for further reactions with chloromethyl pivalate. Thus, VI was not obtained as a product under these reaction conditions even when only one equivalent of chloromethyl pivalate was used; instead, unreacted I and the same mixture of the dialkylated products were obtained. Methyl iodide did not react with I in dichloromethane in the presence of triethylamine. On the other hand, chloromethylbenzamide did react to give two high-melting dialkylated products of undetermined structure. Their diffusion through skin was not studied because of their melting points.

[2] Based on mass spectral considerations, K. B. Sloan and Alice Ng, unpublished results.

[3] The UV absorption maxima for the 1-, 3-, 7-, or 9-monomethyl derivatives are all at much longer wavelengths (see Table I).

Table III—Diffusion of Prodrugs of Thiopurines Through Hairless Mouse Skin

Compound	% Prodrug Remaining in Donor Phase after 48 hr, ± *SD*	Drug in Receptor Phase 6-Mercaptopurine after 48 hr, m*M* ± *SD*	Drug in Receptor Phase as Intact Prodrug after 48 hr, m*M* ± *SD*
I		$2.6 \times 10^{-3} \pm 4.9 \times 10^{-4}$	
Va	96.5 ± 1.09	$0.3 \times 10^{-3} \pm 1.1 \times 10^{-4}$	0
VII	75.1 ± 3.0	$13 \times 10^{-3} \pm 2 \times 10^{-3}$	0
IX	89.7 ± 2.8	$3.9 \times 10^{-3} \pm 1.3 \times 10^{-3}$	$0.37 \times 10^{-3} \pm 1.3 \times 10^{-4}$
XI	61.9 ± 8.8	$33 \times 10^{-3} \pm 1 \times 10^{-2}$	$0.35 \times 10^{-3} \pm 2.1 \times 10^{-4}$
XII	38.9 ± 10.5		
XIII	92.5 ± 6.4	$2.15 \times 10^{-3} \pm 1.1 \times 10^{-3}$	$0.2 \times 10^{-3} \pm 1 \times 10^{-4}$
XIV	91.7 ± 0.33	$1.5 \times 10^{-3} \pm 3 \times 10^{-4}$	0

The structures of the dipivaloyloxymethylated products were determined by a combination of NMR and UV spectroscopy. The dialkylated products VII and VIII were assigned the S^6,9- and S^6,7-structures because their NMR spectra contained both SCH_2O and NCH_2O absorptions (Table II), and because their UV spectra were very similar to those of the corresponding S^6,9- and S^6,7-dimethyl derivatives of I (Table I);[4] the UV spectra are characteristic of the respective dialkyl substitution patterns in I.

The dialkylated product IX was assigned the S^6,3-dialkylated structure because its NMR spectrum contained the SCH_2O and NCH_2O groups and its UV spectrum contained a UV maximum at 326 nm. The only other possible sulfur, nitrogen-dialkylated I candidate which exhibits similar UV spectral properties to IX is the S^6,1-dialkylated structure. However, such structures are very unstable as the free base, while IX has been observed to be stable at room temperature for 2 years. The formation of IX is not surprising in view of the fact that the alkylation of I with methyl tosylate under neutral conditions gives S^6,3-dimethylmercaptopurine (22). Furthermore, since the reactions between triethylamine and chloromethyl pivalate and between triethylamine and VI were found to be incomplete reactions, there was ample opportunity for VI, rather than its ion, to react with chloromethyl pivalate.

The fourth product (X) that was isolated from the reaction mixture was also a dialkylated product, but its NMR spectrum lacked the SCH_2O absorption. Therefore, it was not an *S,N*-dialkylated product but rather a *N,N*-dialkylated product. The various possible *N,N*-dialkylated products arise by alkylation of a *S,N*-dialkylated product followed by *S*-dealkylation. In the case of the 3,7- or 3,9-dialkylated products, *S*-dealkylation only takes place by thio-hydrolysis and would not occur spontaneously under the above reaction conditions. On the other hand, 1,7- or 1,9-dialkylated products have been shown to form spontaneously from S^6,1,9- and S^6,1,7-trialkylated intermediates during the alkylation of 1-methyl-6-mercaptopurine (18) with methyl iodide or benzyl chloride, respectively. Thus, X is probably a 1,7- or 1,9-dialkylated product based on its NMR and UV spectra. To check the possibility that X was formed from further alkylation of the major product VII, VII was allowed to react for 2 days with chloromethyl pivalate either in excess or with triethylamine in excess; no reaction at all occurred. Since the only reactions leading to 1,7- or 1,9-dialkylated products start with 1-alkyl-6-mercaptopurine, it appears likely that in addition to initial *S*-alkylation of I some small amount of 1-alkylation also takes place under these reaction conditions and ultimately leads to the formation of X.

Compounds XI and XII were also prepared under basic conditions but in acetone using potassium carbonate as the base. The riboside hydroxyl groups in these cases were converted to their acetate esters to protect them from possible side reactions with chloromethyl pivalate. The structures of XI and XII follow from their UV and NMR spectra. The UV spectrum of XI is very similar to that of VII, which is characteristic of the S^6,9-substitution pattern of dialkylated 6-mercaptopurine. Both XI and XII exhibited an absorption at δ 5.90 in their NMR spectra, which is characteristic of the SCH_2O group.

The results from the diffusion studies with the *S*-acylheteroalkyl derivatives are shown in Table III. It is obvious that all of the *S*-alkylated prodrugs of I (except for Va) enhance the delivery of I to the receptor phase, and that in any comparison among prodrugs the lower melting prodrug is the one that produces the higher level of 6-mercaptopurine in the receptor phase (compare for instance VII and IX). Compounds VII, XI, and XII appear to be the most attractive candidates for screening in the hairless mouse model (6).

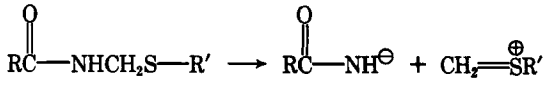
[4] The 1,9-, 1,7-, 3,7-, 3,9-, S^6,1-, and S^6,3-dimethyl derivatives all exhibit UV absorption maxima at much longer wavelengths and between the S^6,7- and S^6,9-derivatives the derivative exhibiting the shorter wavelength UV maximum is the S^6,9-dimethyl derivative.

The results from the diffusion studies also illustrate the advantage that soft alkylation enjoys over the more traditional approach, *i.e.*, esterification, to prodrug modification of the physical chemical properties of drugs. The triacetyl riboside of I (XIII), although marginally more effective than the riboside itself (XIV), is <7% as effective as the soft-alkylated prodrug based on the triacetylated riboside (XI). Thus, to significantly improve the delivery of I through the skin, it is imperative that the thione amide group be alkylated in a transient manner.

The prodrugs deliver very little if any of the intact prodrug to the receptor phase. This does not appear to be due to degradation of the prodrug in the receptor phase after diffusion of the intact prodrug, because even the earliest samples taken of the receptor phase (3 hr) show the same ratio of prodrug to 6-mercaptopurine, and the prodrugs are stable at pH 7.4 for that length of time (Table IV). The fact that primarily 6-mercaptopurine (I) and 2-amino-6-mercaptopurine (II) are found in the receptor phase after the absorption of the alkylated forms of I, its riboside, and the riboside of II, is a consequence of using fresh skin with metabolic systems that were still active and of the sensitivity of the nucleosides to nucleoside phosphorylase (23).

In the case of XII, it was impossible to determine quantitatively the amount of 2-amino-6-mercaptopurine or XII in the receptor phase. The major product appeared to be 2-amino-6-mercaptopurine (II), but there were a number of other unidentified products also formed which interfered with its analysis. Based only on the very small amount of XII remaining in the donor phase, it seems that XII is a very efficient prodrug form for the delivery of II or XVI.

It is interesting that the *S*-benzamidomethyl derivative Va hydrolyzed completely during its diffusion through the skin. Although the stability of *S*-benzamidomethyl cysteine (24) has not been reported, *S*-acetamidomethyl cysteine is reportedly completely stable at pH 13 and 1 (25). Apparently, amidomethyl derivatives of thioamides such as I are much more labile than those of aliphatic thiol groups. The mechanism for the hydrolysis of *S*-acetamidomethyl or *S*-benzamidomethyl groups is not known, but it may be similar to the mechanism proposed for the hydrolysis of amidomethylamines (26) which involves a unimolecular N—C cleavage as the rate-determining step. The relatively short $t_{1/2}$ in buffer which increases[5] with increasing methanol concentration (decreasing dielectric constant) suggests that such an interpretation has merit.



Based on the solubility data in Table IV, the prodrugs that were completely soluble in isopropyl myristate at the concentration at which they were applied were the prodrugs that gave the greatest absorption. Thus, since flux is directly proportional to concentration of the drug in the applied phase (27), it is possible, and even probable, that the reason that I and Va were not absorbed more completely was primarily that they were not soluble enough in the vehicle and, hence, not available for absorption while VII, XI, and XII were available. The prodrug approach in this case then may be considered (*a*) as a quasi-formulation approach to enhancing delivery which overcomes the poor solubility of the drug in a commonly used component of formulations or (*b*) as actually increasing the diffusivity of the prodrug in the skin. There is not enough information available to decide between these two explanations. However, this should not detract from the practical result that more I is delivered by using these *S*-acyloxymethyl prodrugs than by using I, XIII, or XIV.

There does not appear to be any linear correlation between the lipo-

[5] K. B. Sloan and Alice Ng, unpublished results.

Table IV—Physicochemical Characteristics of Thiopurines and Selected Prodrugs

Compound	Solubility, m*M*			log k' Methanol–Water 50:50	$t_{1/2}$ (hr) for Loss of Intact Prodrug at pH 7.4
	pH 7.4 Buffer	Isopropyl Myristate	Chloroform		
I	0.2	−0[a]	0.004	−0.336	
II				−1.074	
Va	0.003	0.42		0.820	0.53
VII		>10.0		1.659	
IX		0.74		1.142	
XI	0.02	>10.0	>2.0	1.260	31.2
XII	0.02	>10.0	>2.0	0.877	30.3
XIII	7.8	0.16	>2.0	0.735	25.6
XIV		0.01		−0.513	196

[a] Could not be measured.

philic index log k' (28) generated from HPLC data and the absorption of the prodrugs.

Finally, from a practical point of view, since both VII and XI primarily deliver I, which is subsequently converted to the corresponding riboside phosphate by hypoxanthine–guanine phosphoribosyltransferase (29) *in vivo*, and the riboside XIV cannot be converted to its phosphate directly, the greater cost of the starting material (XIV compared with I) for XI balances the greater efficiency of delivery of I by XI. Thus, depending on the relative activity of VII and XI in inhibiting DNA synthesis in the hairless mouse model, VII may be the more practical prodrug form of I in spite of the fact that it actually enhances the delivery of I less than XI.

EXPERIMENTAL[6]

Synthesis of 6-Benzamidomethylmercaptopurine (Va)(Vb)—To a dimethylsulfoxide solution (4 ml) containing 0.80 g (0.0047 mole) of 6-mercaptopurine hydrate was added 0.90 g (0.0053 mole) of freshly prepared chloromethylbenzamide. The solution was stirred at room temperature overnight to give a suspension which was dissolved in 10 ml of chloroform, and then stirred with 1 ml of triethylamine for 30 min. The solution was diluted to 100 ml with dichloromethane and was then extracted with 50 ml of water. The water layer was quickly separated, because a precipitate formed in the dichloromethane layer immediately after the water wash. The dichloromethane suspension was filtered. The residue was dried to give 0.60 g (mp 205–211°, 45% yield) of Vb as white crystals. A 0.21-g sample of Vb was crystallized from methanol to give an analytical sample of Vb that was identical with the crude product by TLC, NMR, and IR spectroscopy: 0.19 g, mp 273.5–274.5°, IR (KBr) 1640 and 1660 cm^{-1} (s) (C=O); TLC (silica gel, ether–methanol, 10:3) R_f 0.52 (6-mercaptopurine R_f 0.47); ^1H NMR (DMSO-d_6) δ 9.77 (t, 1, J = 6 Hz, N—*H*), 8.0–7.73 (m, 2, aromatic *H*), 7.7–7.33 (s, 3, aromatic *H*), 5.47 (s, 1, imidazole N—*H*).

Anal.—Calc. for $C_{13}H_{11}N_5OS$: C, 54.74; H, 3.88; N, 24.56. Found: C, 55.03; H, 3.88; N, 23.99.

The dichloromethane filtrate from the above isolation of Vb was concentrated to give a yellow wax which was crystallized from methanol and dichloromethane to give 0.38 g (mp 206–208°, 29% yield) of Va as yellow crystals. A 65-mg sample of Va was recrystallized from 20 ml of chloroform to give 50 mg (*foamed at* 150°; resolidified at 175°; mp 222–223°) of Va as white crystals which was identical with the crude Va by TLC, NMR, and IR spectroscopy: IR (KBr) 1660, 1650, and 1640 cm^{-1} (s) (C=O); ^1H NMR ($CDCl_3$-DMSO-d_6, 5:1) δ 8.0–7.73 (m, 2, aromatic *H*) and 7.7–7.33 (s, 3, aromatic *H*).

Anal.—Calc. for $C_{13}H_{11}N_5OS$·0.4 $CHCl_3$: C, 48.31, H, 3.45, N, 21.02 Found: C, 47.97; H, 3.05; N, 20.98.

When the chloroform solvate was heated to 200° for 2 min then cooled, the light-yellow solid (mp 215–218°) that was obtained exhibited only one spot upon TLC analysis and the same UV spectrum as the solvate.

Anal.—Calc. for $C_{13}H_{11}N_5OS$: C, 54.74; H, 3.89; N, 24.56. Found: C, 54.34; H, 3.66; N, 24.33.

6-Pivaloyloxymethylmercaptopurine (VI))—A dimethylsulfoxide solution (3 ml) containing 0.85 g (0.0057 mole) of chloromethyl pivalate was allowed to react with 0.63 g (0.00375 mole) of 6-mercaptopurine hydrate at room temperature overnight. The reaction mixture was processed as above. However, not all of the precipitate in the reaction mixture was soluble in the dichloromethane, and when that suspension was filtered 0.35 g (mp >270°, 56% recovery) of 6-mercaptopurine was obtained as a yellow solid. After the filtration, the dichloromethane solution was washed with water, but a precipitate did not form in the dichloromethane solution as above. The residue from the dichloromethane solution was chromatographed on silica AR CC-7 using ether as the eluent. An oil was obtained which was crystallized from hexane–dichloromethane to give a total of 25 mg (mp 179–182°, 2.5% yield, 5.7% conversion) of VI as white fibrous crystals: IR (KBr) 1740 cm^{-1} (s) (C=O); ^1H NMR ($CDCl_3$) δ 1.2 [s, 9, (CH_3)$_3$C]; TLC (silica gel, ether) R_f 0.12. The 25 mg which had been used for the NMR sample was recrystallized from dichloromethane–hexane to give 11 mg (mp 189–190°) of an analytical sample of VI, which was identical with the crude product by IR spectroscopy and TLC.

Anal.—Calc. for $C_{11}H_{14}N_4O_2S$: C, 49.61; H, 5.30; N, 21.04. Found: C, 49.53; H, 5.34; N, 20.99.

Reaction of Chloromethyl Pivalate with 6-Mercaptopurine Hydrate: The Preparation of VII, VIII, IX, and X—To 2.0 g (0.0118 mole) of 6-mercaptopurine hydrate suspended in 10 ml of dichloromethane and 6 ml of triethylamine was added 5.28 g (0.035 mole) of chloromethyl pivalate. The reaction was stirred at room temperature overnight to give a suspension which was diluted to 100 ml with dichloromethane to give a clear-yellow solution. The solution was washed with water (50 ml), 10% concentrated HCl (50 ml), and water (50 ml) and then was dried over sodium sulfate and concentrated. The residue was triturated with 50 ml of ether and allowed to stand overnight. The suspension was filtered to give 0.40 g of IX as a yellow solid. This material was recrystallized from dichloromethane–ether (2:8 ml) to give 0.35 g (mp 193.5–195.0°, 8% yield) of IX as yellow crystals: IR (KBr) 1735 cm^{-1} (s) (C=O); ^1H NMR ($CDCl_3$) δ 1.2 [s, 18, (CH_3)$_3$C]; TLC (silica gel, ether) R_f 0.0.

Anal.—Calc. for $C_{17}H_{24}N_4O_4S$: C, 53.66; H, 6.36; N, 14.73. Found: C, 53.68; H, 6.30; N, 14.80.

The ether filtrate described above was chromatographed on silica AR CC-7 using ether as the eluent to give three fractions. The first fraction was crystallized from petroleum ether (bp 37–42°)-heptane (20:15 ml) to give 2.05 g (mp 87–89°, 45% yield) of VII as white crystals: IR (KBr) 1735 cm^{-1} (s) (C=O); ^1H NMR ($CDCl_3$) δ 1.18 [s, 18, (CH_3)$_3$C]; TLC (silica gel, ether) R_f 0.52.

Anal.—Calc. for $C_{17}H_{24}N_4O_4S$: C, 53.66; H, 6.36; N, 14.73. Found: C, 53.88; H, 6.51; N, 14.80.

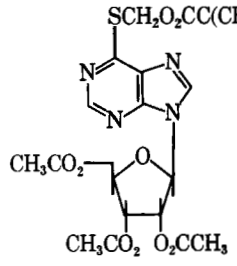
The second fraction was crystallized from ether to give 17 mg (mp 135–137°, 0.3% yield) of VIII as yellow crystals: IR (KBr) 1735 cm^{-1} (s) (C=O); ^1H NMR ($CDCl_3$) δ 1.18 [s, 18, (CH_3)$_3$C]; TLC (silica gel, ether) R_f 0.25.

Anal.—Calc. for $C_{17}H_{24}N_4O_4S$: C, 53.66, H, 6.36, N, 14.73. Found: C, 53.48; H, 6.41; N, 14.67.

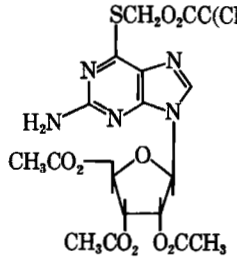
The third fraction was also crystallized from ether to give 43 mg (mp 183–188°, 1% yield) of an unknown compound X: IR (KBr) 1720 and 1745 cm^{-1} (s) (C=O); ^1H NMR ($CDCl_3$) δ 1.18 [s, 18, (CH_3)$_3$C]; TLC (silica gel, ether) R_f 0.21.

Anal.—Calc. for $C_{17}H_{24}N_4O_4S$: C, 53.66; H, 6.36; N, 14.73. Found: C, 53.60; H, 6.36; N, 14.69.

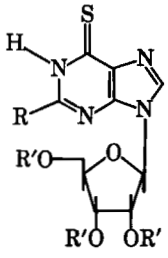
[6] TLC were run on Brinkman Polygram Sil G/UV 254; ether or ether–methanol mixtures. Melting points (uncorrected) were taken with a Thomas-Hoover Capillary apparatus. NMR spectra were recorded on a Varian T-60. IR spectra were obtained on a Beckman Accu Lab 4 infrared spectrophotometer. Microanalyses were obtained by Midwest Microlab, Ltd., Indianapolis, Ind. or by Atlantic Microlab, Atlanta Ga. The benzamide, chloromethyl pivalate, formaldehyde, acetyl chloride, and thionyl chloride were obtained from Aldrich. The 6-mercaptopurine, 6-mercaptopurine-9-riboside, 6-mercapto-2-aminopurine, 6-mercapto-2-aminopurine riboside, and 6-methylmercaptopurine were obtained from Sigma. The bulk solvents were obtained from Mallinckrodt. The hairless mice (SKH-hr-1) were obtained from the Temple University Skin and Cancer Hospital and the diffusion cells were obtained from Kercso Engineering Consultants., Palo Alto, Calif. The HPLC system was a Water Associates instrument using a Chrompack C_{18} column.



XI



XII



XIII: R = H; R′ = $COCH_3$
XIV: R = H; R′ = H
XV: R = NH_2; R′ = $COCH_3$
XVI: R = NH_2; R′ = H

Preparation of 9-β-D-Ribofuranosyl-6-pivaloyloxymethylthio-9H-purine 2′,3′,5′-triacetate (XI)—Chloromethyl pivalate (112 mg) in acetone (10 ml) was allowed to react with sodium iodide (135 mg) in the dark for 40 min. The solution was decanted into a solution of 9-β-D-ribofuranosyl-6-thio-9H-purine 2′,3′,5′-triacetate (16) (254 mg, 0.00062 mole) in 30 ml of acetone. Potassium carbonate (1.0 g) was added to the resulting solution and the mixture was stirred in the dark. When the reaction was complete by TLC[7], the reaction mixture was filtered and the filtrate was evaporated. The residue was dissolved in ethyl acetate, and the solution was washed with water (three times), dried over sodium sulfate, and the solvent removed to yield a residue (350 mg, contaminated with traces of pivaloyloxymethyl halides) which was dissolved in ethyl acetate and filtered through silica gel (12.5 g). Compound XI was obtained as a pale white waxy solid (296 mg 91%) and showed one spot on TLC: IR (neat) 1755, cm^{-1} (s) (C=O); ^1H NMR ($CDCl_3$)δ 1.10 [9, s, $(CH_3)_3C$], 2.03 (3, s, CH_3CO_2), 2.06 (3, s, CH_3CO_2), 2.15 (3, s, CH_3CO_2), 4.43 (3, $CH_2OAc + 4'H$), 5.70 (1, m, 3′H), 6.0 (1, 6, 2′H), 6.25 (1, d, 1′H); TLC (silica gel, ethyl acetate–chloroform, 1:1) R_f 0.32; exact mass calculated for $C_{22}H_{28}N_4O_9S$ (m/z) 524.1575 (M^+): Found, 524.1561 (m/z).

Preparation of 9-β-D-Ribofuranosyl-2-amino-6-pivaloxymethylthio-9H-purine 2′,3′,5′-triacetate (XII)—To a solution of chloromethyl pivalate (106 mg) in acetone (15 ml) was added sodium iodide (130 mg). The suspension was stirred for 35 min while protected from light. The supernatant was decanted from the precipitated sodium chloride and added to a solution of thioguanosine triacetate (16) (250 mg, 0.00059 mole) in acetone (25 ml). Potassium carbonate (1.0 g) was added to the reaction mixture, stirring was continued for 69 hr, and then the suspended solid was filtered and washed with acetone. The filtrate was evaporated to dryness. The residue was dissolved in ether, and the resulting solution washed with water (three times), dried over sodium sulfate, and then the solvent was evaporated. The residue was purified by chromatography on silica gel (12.5 g, chloroform–ethyl acetate 1:1), to give the pivaloyloxymethyl derivative XII (82 mg, 57.4%) as a foam which showed one spot on TLC: IR (film) 3380 and 3490 cm^{-1} (s) (NH) 1738 cm^{-1} (s) (C=O); ^1H NMR ($CDCl_3$) δ 1.15 [9, s, $(CH_3)_3C$] 2.05 (3, s, CH_3CO_2), 2.08 (3, s, CH_3CO_2), 2.13 (3, s, CH_3CO_2), 4.41 (3, s, $CH_2OAc + 4'H$), 5.20 (2, s, NH_2), 5.80 (1, broad, 3′H), 5.96 (1, t, 2′H), 6.0 (1, d, 1′H); exact mass calculated for $C_{22}H_{29}N_5O_9S$, 539.1683 (m/z): Found, 539.1680 (m/z); TLC (silica gel[3], ethyl acetate–chloroform, 1:1) R_f 0.32.

Diffusion Cell Studies—Full thickness dorsal skin of 12- to 14-week-old female hairless mice was used. The mice were sacrificed by snapping the spinal cord. The excised skin was gently scrapped to remove fat and visceral debris and then gently secured over the diffusion cell with a rubber gasket. The diffusion cells themselves have been previously described (17). The receptor side of the cell (43 ml) was filled with pH 7.4 isotonic phosphate buffer containing 100-ppm gentamicin, which was stirred magnetically. The compounds were applied as 0.01 *M* solutions or suspensions in 500 μl of isopropyl myristate to the donor side of skin. The cells were kept at 32° and 1-ml samples were removed at 3, 9, 24, and 48 hr; 1 ml of buffer was added to the receptor after each sample was withdrawn. Samples were frozen immediately after they were taken and stored at 0° until analyzed. Controlled studies showed that using this procedure, no change in the sample composition took place during the cold storage time. After 48 hr the donor side was washed with 25 ml of methanol, and the methanol and buffer samples were analyzed for the prodrug and its parent drug by high-performance liquid chromatography (HPLC). The HPLC analysis was performed at 25° using mixtures of methanol and distilled water as the mobile phase and a flow rate of 1–2 ml/min. The results for each compound studied are for four diffusion cells.

Solubility Studies—The lipophilic indices ($\log k' = \log [(t_r - t_0)/t_0]$, where t_r is the retention time and t_0 is the elution time of solvent) for the prodrugs were determined by HPLC using mixtures of methanol and distilled water on Chrompack C_{18} column at 25°. There was no effect of pH 3–10 of the mobile phase on the retention time of the prodrugs Va, VII, IX, XI, and XII; of pH < 7.5 on the retention time of XIII; and of pH < 4 on the retention time of I and XIV. A plot of log *k′ versus* percent methanol was linear in each case over four methanol–water concentrations. The values for log *k′* are given in Table IV along with some miscellaneous solubility data. These solubilities were obtained by suspending an excess of the drug or prodrug in the appropriate solvent and sonicating the suspension for 20 min. The suspension was then quickly equilibrated at 37° and centrifuged. The supernatant was then filtered, diluted with more of the solvent, and analyzed by HPLC using the above conditions.

Stability Studies—The rates of degradation of the thiopurines and their prodrugs were determined by adding 50 μl of a 0.025 m*M* methanol solution of the compound to 10 ml of a pH 7.4 phosphate buffer solution at 37°. The solutions were shaken vigorously and then stirred magnetically during sampling. Samples were stored at 0° until analyzed. Analysis was accomplished by HPLC using the above conditions.

[7] Analtech silica gel GHLF.

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Sustained Release of Theophylline from Hydroxypropylcellulose Tablets

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Abstract □ Compressed tablets were prepared from theophylline and hydroxypropylcellulose. Effects of the viscosity grades of the polymer, the mixing ratios of two polymers with different viscosity grades, and the polymer contents in the tablets on release patterns of theophylline were examined *in vitro*. Release rate was decreased with increasing viscosity designation and polymer contents in the tablets. In salivary level profiles of theophylline following oral administration of sustained-release tablets to five human volunteers, a low but sustained level was noted indicating sustained release of the drug from the tablets *in vivo*.

Keyphrases □ Theophylline—hydroxypropylcellulose tablets □ Hydroxypropylcellulose—viscosity grades, mixing ratio, contents □ Compressed tablets—sustained release *in vitro*, oral administration □ Salivary levels—reverse-phase high-performance liquid chromatography

Since most of the commercially available water-soluble cellulose derivatives (1) are considered to be stable against microbial attack and safe when ingested orally, it appeared to be worthwhile to evaluate them as suitable materials for sustained-release preparations.

Although several studies have reported the sustained release of drugs from compressed hydrophilic matrices prepared from cellulose derivatives (2–8), few have examined the relationship between release rates *in vitro* and drug concentration profiles in body fluids (7).

Following the examination of representative viscosity grade polymers of methylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, and hydroxypropylcellulose, hydroxypropylcellulose was selected for further studies, since it exhibited release patterns suitable for a sustained-release preparation.

In the present study, modification of the release rate of theophylline from compressed hydroxypropylcellulose tablets was examined by changing viscosity grades of the polymer, mixing ratios of two polymers with different viscosity grades, and changing polymer contents in the tablets. Theophylline was used as a representative drug, since sustained-release formulations are desirable because of the short elimination half-life in humans, especially in children (9). To evaluate body fluid level profiles of theophylline in volunteers, saliva levels were measured, since correlation of serum and saliva theophylline concentrations after administration of a sustained-release preparation has been reported (10).

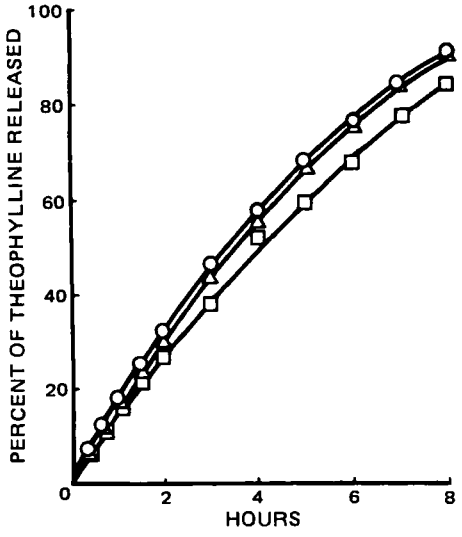

Figure 1—*Release profiles of theophylline from tablets prepared from 1:1 mixture of theophylline (250 mg) and hydroxyproylcellulose (1:1 mixture of low- and medium-viscosity grades) by compressing the drug–polymer mixture for 5 sec (○), 30 sec (△), or 120 sec (□). Average of three determinations.*

EXPERIMENTAL

Materials—Three viscosity grades of hydroxypropylcellulose[1] were used. Theophylline (anhydrous) and 7-(2-hydroxyethyl)theophylline were used as supplied [2]; and all other chemicals were of reagent grade.

Preparation of Tablets—Flat-faced tablets (500 mg, 13-mm diameter, and ~3-mm thickness) were prepared by compressing mixtures of theophylline and hydroxypropylcellulose directly under 180 kg/cm^2 for 30 sec using a potassium bromide tablet die and a hydraulic press[3].

To examine the effect of compression pressure on drug release, pressures of 60, 180, or 540 kg/cm^2 were applied to the drug–polymer mixture for 30 sec. To examine the effect of compression periods on drug release, a compression pressure of 180 kg/cm^2 was applied to the drug–polymer mixture for 5, 30, or 120 sec.

Release Studies—A tablet was suspended by means of a polyethylene net in a 200-ml release medium in a wide-mouthed bottle. Since the release rate of theophylline from hydroxypropylcellulose tablets is not very dependent on the pH values of the medium, a 0.2% NaCl solution, ad-

[1] HPC-L, M, and H from Nihon Soda Co., Tokyo. Viscosity ranges of 2% aqueous solutions at 20° are 6–10, 150–400 and 1000–4000 cps, respectively.
[2] Tokyo Kasei Kogyo Co., Tokyo.
[3] Shimadzu potassium bromide press, Shimadzu Manufacturing Co., Kyoto.

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