containing 10 g (33 mmol) of **22**, 60 ml of $Ac_2O$, and 1.0 ml of concentrated $H_2SO_4$ was heated to 90° for 2 hr. After evaporation of the excess $Ac_2O$ the residue was triturated with cold $H_2O$. The resulting solid was collected by filtration, washed with $H_2O$, and recrystallized to give 9.22 g (81.0%) of **61** as colorless fine prisms.

Compound **62** was prepared in a similar fashion as described above by using $(CF_3CO)_2O$ without $H_2SO_4$.

**Procedure L. Reactions with Isocyanate and Isothiocyanate (66 and 67, Table III).** To a stirred solution of 1.5 g (4.95 mmol) of **22** in 200 ml of 1,2-dichloroethane was added 0.42 g (7.43 mmol) of methyl isocyanate. The mixture was allowed to react for 30 min under ice cooling and then for an additional 1 hr at room temperature. The solvent was evaporated to leave the crude product, which was recrystallized to give 1.3 g (73%) of **66** as colorless prisms.

Compound **67** was prepared in the same manner by using methyl isothiocyanate.

**8-Ethyl-5,8-dihydro-2-[1-(hexahydro-1*H*-1,4-diazepinyl)]-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acid (74).** To a solution of 3.0 g (30 mmol) of hexahydro-1*H*-1,4-diazepine in 30 ml of DMF which had been maintained at 90° was added in portions 3.0 g (11.3 mmol) of **5**. The mixture was heated to 110° for 2 hr and then concentrated to dryness *in vacuo*. The residue was dissolved in 10% AcOH and filtered (charcoal). The filtrate was neutralized with aqueous, saturated $NaHCO_3$ and kept in a refrigerator overnight. The crystals that separated were collected and recrystallized from aqueous 50% EtOH to give 1.2 g (33.4%) of **74** as colorless powder, mp 244–246°, which is slightly hygroscopic. *Anal.* ($C_{15}H_{19}N_5O_3 \cdot \frac{1}{3}H_2O$) C, H, N.

**Microbiological Methods.** *In vitro* antibacterial tests were carried out by the broth-dilution method using nutrient broth[2] for *Staph. aureus* Terajima, *E. coli* K-12, and *P. aeruginosa* Tsuchijima.

*In vivo* antiacterial evaluation for *S. typhimurium* S-9 infection in mice was carried out according to the method of Shimizu, *et al.*[2] The data for *P. aeruginosa* No. 12 infection was obtained in a similar procedure. Groups of ten male mice (ddy strain, 18–20 g) were infected intraperitoneally with *P. aeruginosa* (10–20 $LD_{50}$) suspended in nutrient broth with 4% mucin. The test compounds were suspended in 0.2% sodium carboxymethylcellulose and administered orally at 0 and 6 hr postinfection. Survival rates were determined after 1 week.

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# Purinylhydantoins. Facile Conversion of the Naturally Occurring *N*-(Purin-6-ylcarbamoyl)-L-amino Acids into 3-Purin-6-ylhydantoins and 3-Cyclohexyl-1-(purin-6-ylcarbamoyl)hydantoins†

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The naturally occurring *N*-(purin-6-ylcarbamoyl)-L-threonine (PCT, **1b**), *N*-(purin-6-ylcarbamoyl)glycine (PCG, **1a**), and some of their analogs were converted into novel purine derivatives, the purinylhydantoins. The PCT and PCG underwent intramolecular cyclization in the presence of *N,N'*-dicyclohexylcarbodiimide (DCC) to give the 3-purin-6-ylhydantoins (**2a–c**). The same hydantoins were also obtained when the PCT and PCG were allowed to react through the mixed anhydride formed from cyclohexyl isocyanate or ethyl chloroformate. 1,3-Dicyclohexyl-1-[*N*-(purin-6-ylcarbamoyl)aminoacyl]ureas **3a** and **3c**, by-products obtained from the DCC reaction, were rapidly converted in aqueous NaOH to another type of purinylhydantoins, the 3-cyclohexyl-1-(purin-6-ylcarbamoyl)hydantoins **4a** and **4b**. Compound **4a** when heated in base underwent hydrolysis of the hydantoin ring giving biuret *N*-(cyclohexylcarbamoyl)-*N*-(purin-6-ylcarbamoyl)glycine (**5a**) and *N*-(purin-6-ylcarbamoyl)glycine cyclohexylamide (**6a**). The characterization of these hydantoins was carried out by uv, nmr, and mass spectrometry. The 3-purin-6-ylhydantoins and 3-cyclohexyl-1-(purin-6-ylcarbamoyl)hydantoins showed growth inhibitory activity in the cultured leukemic cells, while the parent amino acid compounds were inactive.

The chemical reactions of the naturally occurring 6-ureidopurines, *N*-(purin-6-ylcarbamoyl)-L-threonine (PCT, **1b**, Scheme I) and *N*-(purin-6-ylcarbamoyl)glycine (PCG, **1a**),[1,2] and their analogs have been of interest to us from two standpoints. Firstly, the 6-ureidopurines derived from amines showed very good cytokinin activity[3,4] as well as a growth inhibitory activity in human leukemic myeloblast cell line (RPMI 6410); however, the analogs derived from the amino acids were devoid of these activities.[2] Thus the compounds of the latter type with the masked carboxyl groups should be of biological interest. Secondly, since the *N*-(purin-6-ylcarbamoyl)-L-threonine is an anticodon adjacent base in tRNA's which respond to the codons beginning with A, reactions of PCT could be very useful in the modification of these tRNA's. This paper describes a facile conversion of naturally occurring *N*-(purin-6-ylcarbamoyl)-L-threonine (PCT, **1b**) and *N*-(purin-6-ylcarbamoyl)glycine (**1a**) into the novel purine derivatives, the 3-purin-6-ylhydantoins **2**. It also discusses the conversion of *N*-(purin-6-ylcarbamoyl)aminoacylureas **3** into another type of hydantoins, 3-cyclohexyl-1-(purin-6-ylcarbamoyl)hydantoins **4**. Structure determination, chemical properties, and biological activities of these hydantoins and ureas are also described.

The usual method of preparing hydantoins by heating the *N*-carbamoylamino acids and their esters in concentrated HCl[5,6] failed in the case of PCG and PCT. The reaction of 6-ureidopurine with glyoxal in acidic medium[7] also failed to give the desired hydantoin **2a**. These condi-

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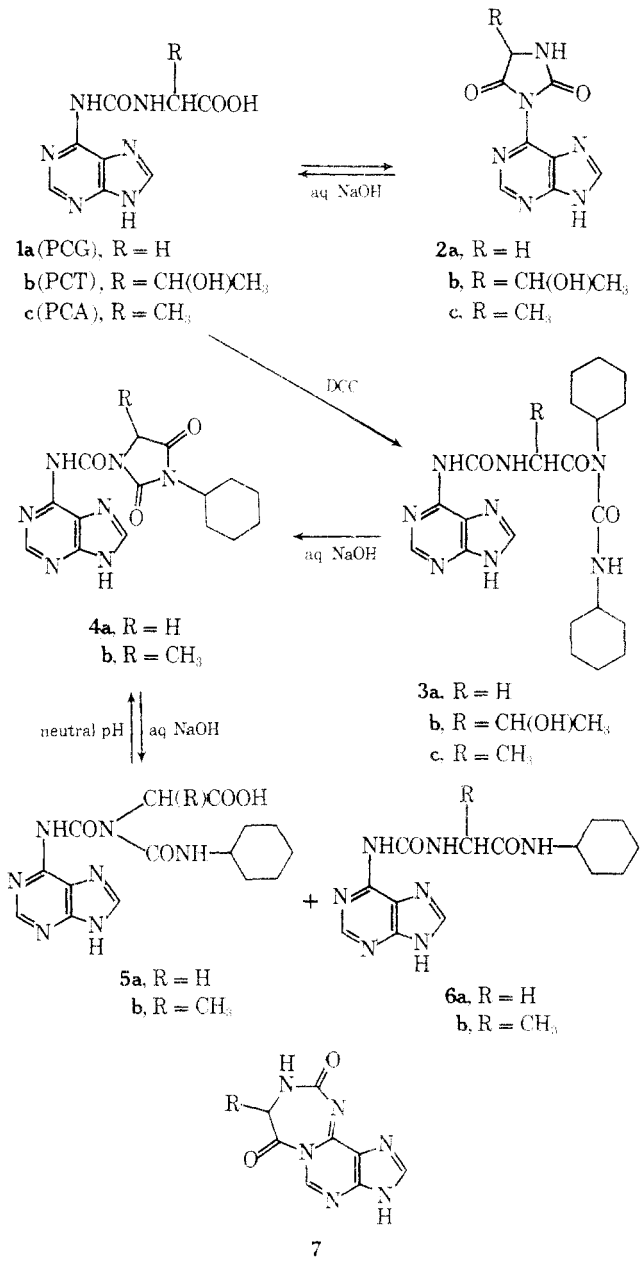
**Scheme I**




tions generally caused the hydrolysis of 6-ureidopurines to adenine. Thus it appeared that the mild procedures were needed for the synthesis of 3-purin-6-ylhydantoins.

The following synthetic methods employed were successful in giving the desired 3-purin-6-ylhydantoins **2** from *N*-(purin-6-ylcarbamoyl)amino acids (Scheme I). In the first method, the reaction of PCG (**1a**) with 2 molar equiv of DCC in pyridine at room temperature gave 3-purin-6-ylhydantoin (**2a**) in 69% yield and 1,3-dicyclohexyl-1-[*N*-(purin-6-ylcarbamoyl)glycyl]urea (**3a**) in 17% yield. To our knowledge this constitutes a first example wherein a hydantoin is prepared from a reaction of carbamoylamino acid mediated through DCC. PCT (**1b**) and *N*-(purin-6-ylcarbamoyl)-L-alanine (PCA, **1c**) underwent similar reaction but gave lower yields of the desired hydantoins **2b** and **2c** and higher yields of the acyl ureas **3b** and **3c**. The formation of the acylurea **3a** takes place through the O → N acyl migration.[8] The substituents on the $\alpha$ carbon of the amino acids appear to interfere with the cyclization, thus resulting in more of the acylurea formation.

In another method, the reaction of PCG with excess of cyclohexyl isocyanate in DMSO at room temperature gave the desired hydantoin **2a** in 67% yield along with some dicyclohexylurea and 9-cyclohexylcarbamoyl-*N*-(purin-6-ylcarbamoyl)glycine cyclohexylamide. This reaction appears to proceed *via* the mixed anhydride of PCG and cyclohexylcarbamic acid.[9] Reaction of PCT and cyclohexyl isocyanate was not satisfactory for the preparation of the hydantoin **2b** because of the side reactions with the OH of threonine. However, PCA (**1c**) reacted smoothly with cyclohexyl isocyanate to give the purinylhydantoin **2c** in 60% yield. Activation of the carboxyl group through the other isocyanates also gave the desired hydantoins. PCG, when reacted with phenyl isocyanate, gave the hydantoin **2a** in 69% yield. In all these cases, carbamoylation also occurred at the $N^9$ position of the purines, giving the purine-9-carboxamides as minor products which were characterized by the degradation studies and by uv spectra.

In another variation of the mixed anhydride method, when PCG was allowed to react with ethyl chloroformate at −10°, it gave the desired hydantoin **2a** in 35% yield. It is suggested that the presumed intermediate, mixed anhydride,[10] undergoes intramolecular cyclization to give the hydantoin **2a**. Alternatively, the reactions of $N^6$-glycyladenine[11] with ethyl chloroformate and *p*-nitrophenyl chloroformate in pyridine failed to give the desired hydantoin **2a**. The reaction of $N^6$-chloroacetyladenine[12] with silver cyanate resulted in the formation of purple-colored unidentifiable products; no hydantoin could be detected.

Attempts to establish the structure of 3-purin-6-ylhydantoin (**2a**) through unequivocal synthesis by displacement of 6-chloropurine and 6-iodopurine with K, Na, and Tl salts of hydantoin were unsuccessful. The structures of 3-purin-6-ylhydantoins **2a–c** were assigned on the basis of chemical and spectral properties in addition to the correct elemental analyses. In a high-resolution mass spectrum compound **2a** gave the required molecular ion at *m/e* 218.0551 (base peak) as well as the other explainable fragments as shown in Scheme II. In these hydantoin forming reactions, alternatively the cyclization could occur at the $N^1$ position to form the strained seven-membered ring compound **7** or at the $N^7$ position to give a compound with an eight-membered ring. The ir spectra were particularly useful in supporting structure **2a** rather than **7**. The ureido carbonyl absorption of PCG at 1670 cm$^{-1}$ disappeared in the ir spectrum of compound **2a** and two new absorptions appeared at 1800 and 1730 cm$^{-1}$ which are characteristic of the hydantoin ring.[6,13] These absorptions were in a good agreement with those of $N^6$-phthaloyladenine[14] (1790 and 1730 cm$^{-1}$), 3-phenylhydantoin (1800 and 1710 cm$^{-1}$), and hydantoin (1775 and 1700 cm$^{-1}$) (Table I).

**Scheme II.** Fragmentation Pattern of 3-Purin-6-ylhydantoin (2a)

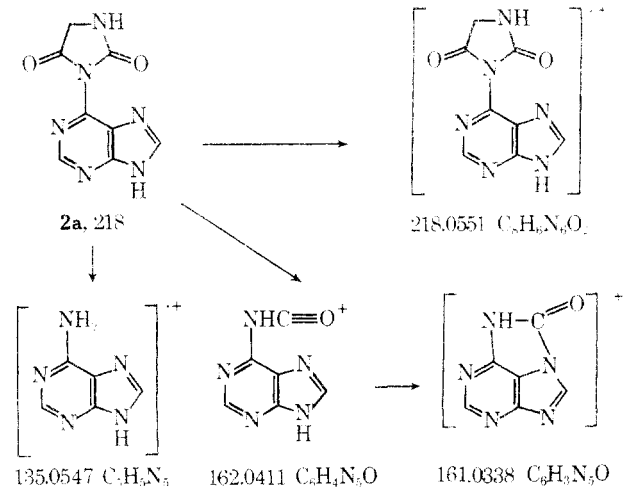


**Table I.** Spectral Data

| Compd | Uv max, nm ($\epsilon \times 10^{-3}$) | | | Nmr, δ, ppm | | | | Ir, $cm^{-1}$, ureido C=O or hydantoin C=O |
|---|---|---|---|---|---|---|---|---|
| | 0.1 *N* HCl | $H_2O$ | 0.1 *N* NaOH | 2-H | 8-H | Δδ,[a] Hz | $CH_2$ | |
| **1a** | 277 (18.6) | 269 (17.4), 276 (16.9) | 278 (16.2) | 8.56 | 8.43 | 7.5 | 4.03 (d), $J$ = 5.5 Hz | 1670 |
| 1b | 277 (20.6) | 269 (19.2), 276 (18.9) | 278 (18.1) | 8.59 | 8.45 | 7.5 | | 1710 |
| 1c | 277 (21.4) | 269 (20.0), 276 (19.7) | 277 (18.5) | 8.62 | 8.50 | 7.5 | | 1680 |
| 2a | 270 (10.3) | 270 (9.8) | 277 (16.5) | 9.1 | 8.84 | 15.0 | 4.32 (d), $J$ = 1 Hz | 1800, 1770, 1730 |
| 2b | 271 (11.5) | 270 (10.7) | 276 (13.6) | 8.89 | 8.64 | 15.0 | | 1780, 1730 |
| 2c | 270 (9.6) | 270 (9.4) | 277 (15.7) | 8.89 | 8.64 | 15.0 | | 1775, 1725 |
| 3a | 275 (22.3) | 268 (20.5), 274 (19.6) | 285 (14.8) | 8.66 | 8.52 | 7.5 | 4.20 (d), $J$ = 5 Hz | 1680 |
| 3b | 275 (19.2) | 268 (18.1), 274 (17.0) | 276 (15.4) | 8.52 | 8.42 | 6.0 | | 1680 |
| 3c | 275 (22.1) | 268 (20.5), 275 (19.6) | 293 (15.5) | 8.58 | 8.42 | 6.5 | | 1680 |
| 4a | 250 sh[b] (9.2), 273 (19.4), 280 (18.8) | 250 sh (8.6), 273 (17.3), 277 (17.5), 288 sh (10.5) | 294 (15.3) | 8.80 | 8.62 | 11.0 | 4.40 (s) | 1785, 1730, 1710 |
| 4b | 251 sh[b] (8.7), 273 (19.2), 280 (18.6) | 252 sh (8.2), 274 (16.8), 278 (17.1), 289 sh (10.0) | 296 (15.1) | 8.83 | 8.66 | 10.5 | | 1775, 1715 |
| 5a[c] | 274, 281, 311 | 273, 278, 288 sh,[b] 310 | 294 | | | | | |
| 5b[c] | 272, 308 | 273, 277, 287 sh, 305 | 296 | | | | | |
| **6a** | 277 (20.4) | 268 (19.0), 275 (18.2) | 277 (17.3) | 8.52 | 8.41 | 6.5 | 3.89 (d), $J$ = 5 Hz | 1660 |
| 6b | 276 (22.2) | 268 (19.8), 274 (19.0) | 277 (18.0) | 8.54 | 8.42 | 7.0 | | 1665 |
| Hydantoin | | | | | | | 3.92 (d), $J$ = 1 Hz | 1775, 1700 |
| 3-Phenylhydantoin | | | | | | | 4.08 (d), $J$ = 0.6 Hz | 1800, 1765, 1710 |
| 6-Phthaloyladenine | 276 (16.5) | 276 (15.2) | 287 (13.4) | 9.22 | 8.92 | 18.0 | | 1790, 1730 |
| *N*-(Purin-6-ylcarbamoyl)dimethylamine | 280 (21.5) | 255 sh[b] (8.6), 271 (17.3), 277.5 (18.6), 286 sh[b] (12.0) | 278 (14.9) | 8.58 | 8.41 | 10.0 | | 1667 |

[a] Difference in chemical shift for 2- and 8-protons. [b] sh = shoulder. [c] Uv spectra of this compound were obtained on a material extracted from a tlc band.

The nmr spectrum further supported the structure of the purinylhydantoin **2a**. The chemical shifts for $C_2$ and $C_8$ protons of compound **2a** were assigned at δ 9.1 and 8.84, respectively, on the basis of nmr spectra of the deuterated 6-methylureidopurine-8-$d_1$. These assignments were in good agreement with those of $N^6$-phthaloyladenine (δ 9.22 and 8.92). Particularly, the differences (Δδ) in these two chemical shifts are very close to each other: 15 Hz for **2a** and 18 Hz for $N^6$-phthaloyladenine. The $N^1$-cyclized compound **7** should have caused a greater Δδ separation in these chemical shifts since the $N^1$-substituted adenines are known to have Δδ of 26 Hz.[15] Furthermore, a doublet at δ 3.92 ($J$ = 1 Hz) of hydantoin by the coupling between the N-1 and C-5 proton is also present in the spectra of both **2a** at δ 4.32 ($J$ = 1 Hz) and 3-phenylhydantoin at δ 4.08 ($J$ = 0.6 Hz).

The uv spectra of these novel purinylhydantoins are quite different from the parent compounds PCG (**1a**) and PCT (**1b**). In a neutral aqueous solution (Figure 1), the hydantoin **2a** does not show the two peaks of PCG, but instead exhibits a single maximum at 270 nm with half of the extinction coefficient of PCG. In aqueous NaOH, 3-purin-6-ylhydantoin (**2a**) underwent an instantaneous reversion to the parent compound PCG (**1a**) (see Figure 1). In neutral aqueous solution (pH 5) and in acidic medium (pH 1) this hydantoin is stable for 10 hr at least.

Characterization of the *N*-(purin-6-ylcarbamoyl)aminoacylureas **3a–c** was carried out by elemental analyses and uv and mass spectral data. The uv spectra of these compounds were similar to those of the 6-ureidopurines (Table I). In a mass spectrum the compounds gave the fragment ions corresponding to dicyclohexylurea$^+$ ($m/e$ 224), purine–NH–CO$^+$ (162), adenine$^+$ (135), and cyclohexyl isocyanate$^+$ (125). The nmr spectra also supported the structure of these compounds.

When the acylurea **3a** was treated with aqueous NaOH,

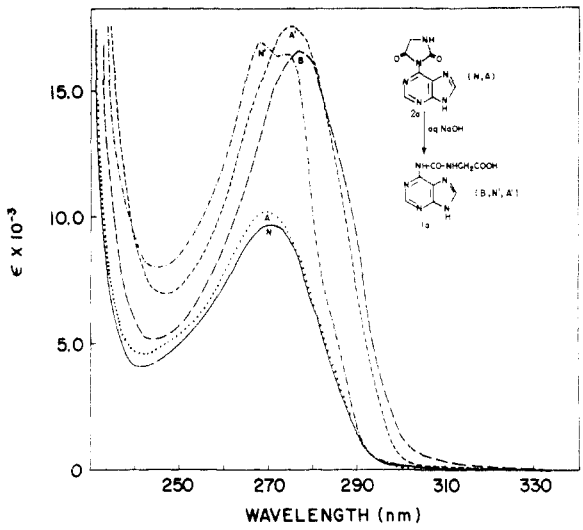



**Figure 1.** Quantitative uv spectra of 3-purin-6-ylhydantoin (**2a**): N (—), $H_2O$; A (···), 0.1 *N* HCl; B (--), 0.1 *N* NaOH; N′ (-·-), neutralized B with AcOH; A′ (---), acidified N′ to pH 1.5 with concentrated HCl. The curves marked N′, A′, and B are identical with the uv spectra of *N*-(purin-6-ylcarbamoyl)glycine (**1a**).

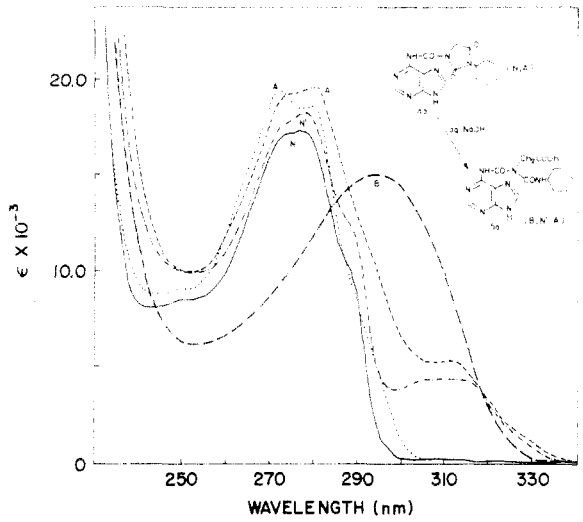
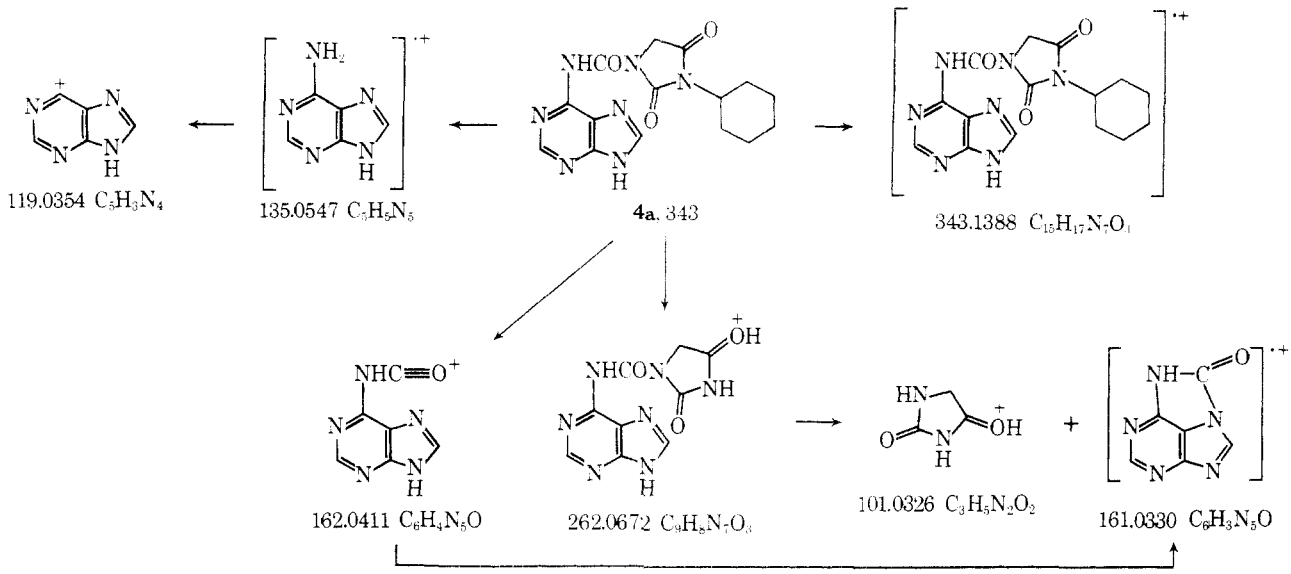



**Figure 2.** Qualitative uv spectra of 3-cyclohexyl-1-(purin-6-ylcarbamoyl)hydantoin (**4a**): N (—), $H_2O$; A (···), 0.1 *N* HCl; B (--), 0.1 *N* NaOH; N′ (-·-), neutralized B with AcOH; A′ (---), acidified N′ to pH 1.5 with concentrated HCl. The curves marked N′, A′, and B are presumably uv spectra of **5a**. The uv spectra on a material (**5a**) isolated by tlc of the NaOH (0.1 *N*) hydrolysate were identical with the B, N′, and A′. The peak at 310 nm appears to be characteristic of the biurets, **5a** and **5b**.

a new hydantoin derivative, 3-cyclohexyl-1-(purin-6-ylcarbamoyl)hydantoin (**4a**), was obtained. This cyclization most probably occurs through the attack of the glycyl nitrogen on the carbonyl of dicyclohexylureido moiety. A similar type of cyclization has been observed, wherein an *N*-(*N*-carbobenzoxyglycyl)-*N,N*′-dicyclohexylurea was converted to 3-cyclohexylhydantoin.[6] The acylurea **3c** derived from *N*-(purin-6-ylcarbamoyl)-L-alanine underwent a similar reaction as compound **3a**, giving the 3-cyclohexyl-1-(purin-6-ylcarbamoyl)-5-methylhydantoin (**4b**). The *N*-acylurea **3b** derived from PCT, however, rapidly hydrolyzed to adenine in 1 *N* NaOH. Here the OH group of the threonine moiety probably participates in hydrolytic reaction giving adenine and the oxazolidine derivative.[16]

Structure determination of the 3-cyclohexyl-1-(purin-6-ylcarbamoyl)hydantoins **4a** and **4b** was carried out by elemental analyses and ir, ur, nmr, and mass spectrometry. The characteristic ir absorptions for the hydantoin ring were also observed at 1785, 1730, and 1710 $cm^{-1}$ in the ir spectra of **4a** and **4b**. The comparison of the shape and maxima of the uv spectra of the hydantoins **4a** and **4b** (Figure 2) with the model compound, *N*-(purin-6-ylcarbamoyl)dimethylamine,[2] revealed that **4a** and **4b** were the 6-ureidopurines wherein the $\alpha$ nitrogen (derived from an amino acid) was fully substituted. In a high-resolution mass spectrum of **4a** the strong molecular ion at *m/e* 343.1388 was observed (Scheme III). The fragment ion observed at *m/e* 262.0672 can be represented as protonated 1-(purin-6-ylcarbamoyl)hydantoin$^+$ ($C_9H_8N_7O_3$, calcd 262.0688) after losing cyclohexene$^+$ from $M^+$. This ion further fragments to ions at *m/e* 161.0330 (purine−6-NCO$^+$) and *m/e* 101.0326 (protonated hydantoin$^+$, $C_3H_5N_2O_5$, calcd 101.0351). The base peak ion was found at *m/e* 162.0411 (purine−6-NH−CO$^+$). The nmr spectrum of **4a** further supports the structure, in which a singlet at $\delta$ 4.40 of C-5 protons of the hydantoin ring indi-

**Scheme III.** Fragmentation Pattern of 3-Cyclohexyl-1-(purin-6-ylcarbamoyl)hydantoin (**4a**)



cates that there is no proton at N-1 of the hydantoin ring.

In the base treatment of the *N*-acylurea **3a**, there was obtained another substance in addition to the hydantoin **4a**. To this material is assigned a tentative structure of biuret **5a** on the following grounds. The hydantoin **4a** when treated with a large excess of aqueous NaOH at room temperature gave compound **5a** as could be monitored by tlc and uv (see Figure 2). When the alkaline solution of hydantoin **4a** was heated for 1 min at 100°, it gave *N*-(purin-6-ylcarbamoyl)glycinecyclohexylamine (**6a**) and adenine as minor products in addition to the major yield of biuret **5a**. When compound **5a** was allowed to stand in neutral aqueous solution, it converted back to the parent hydantoin **4a** (50% in 2 hr). Compound **5a** has slow mobility in a silica gel tlc in solvent A, is quite soluble in $H_2O$, and is retained on AG1-X8 (formate). This behavior is in agreement with structure **5a** which has a free COOH group. The uv spectra are also quite similar to those of *N*-(purin-6-ylcarbamoyl)dimethylamine indicating again that the $\alpha$ nitrogen (derived from amino acid) is fully substituted (Table I and Figure 2). Hydrolysis of the hydantoin **4b** under the same conditions gave a mixture of the starting material, *N*-(purin-6-ylcarbamoyl)-L-alanine cyclohexylamide (**6b**), adenine, and a trace of the biuret **5b**. The extended time periods for hydrolysis led to the formation of adenine as a major product. Compounds **6a** and **6b** were characterized by the chromatographic and the uv spectral comparison with the authentic samples prepared from a reaction of ethyl purine-6-carbamate[1] with glycine cyclohexylamide and L-alanine cyclohexylamide.

**Biological Activity Studies.** The hydantoins **2a–c** were treated for growth inhibitory activity toward mammalian cells in axenic culture derived from normal (Nc 37) and leukemic (RPMI 6410) buffy coats and mouse leukemic cells L1210 (Table II). They were marginally active at the concentration of $1 \times 10^{-4}$ *M;* especially compound **3a** was more active indicating that the conversion of the COOH groups of PCT, PCG, and other analogs[2] into hydantoin derivatives changed their growth inhibitory activities. The hydantoins **2a–c** were tested for their cytokinin activity measured in terms of fresh weight yields of cytokinin-dependent tobacco callus tissue.[17] The compounds were dissolved in DMSO in serial dilution and the incubation mixtures were cultured for 35 days at room temperature in the dark. Compound **2b** was slightly more active than the parent compound PCT (**1b**). However, the hydantoins **2a** and **2c** did not show improvement in cytokinin activity as compared to their parent amino acid compounds **1a** and **1c**. Since these purinylhydantoins are not stable for a prolonged period of time required in the cytokinin assay, it remains to be seen if more stable derivatives of these *N*-(purin-6-ylcarbamoyl)-L-amino acids would be more active.

Since these are the first known purin-6-ylhydantoins, eventually we hope to evaluate these compounds in the antiviral and other appropriate screens. From the biochemical standpoint, formation of hydantoin from PCT and PCG under the mild conditions certainly deserves an exploration at a polynucleotide level.

## Experimental Section‡

**3-Purin-6-ylhydantoin (2a). Method A.** A mixture of 2.36 g (10 mmol) of PCG (**1a**), 4.12 g (20 mmol) of DCC, and 700 ml of anhydrous pyridine was stirred at room temperature for 3 days. The white solid was then collected on a filter and washed with pyridine, EtOH, and ether (1.05 g). After evaporating the filtrate, the residue was then triturated with 200 ml of hot EtOH and additional product collected: 0.453 g; total yield, 1.503 g (69.0%). The filtrate was used for isolation of the *N*-acylurea **2a**. The analytical sample was prepared by crystallization of the above product from DMSO–dioxane (1:3): mp 280–290° dec; mass spectrum *m/e* (rel %) 219 (13, $M^+ + 1$), 218 (100, $M^+$), 162 (87), 161 (70), 135 (26), and 119 (57). 3-(Purin-6-yl)-5-(1-hydroxyethyl)hydantoin (**2b**) and 3-(purin-6-yl)-5-methylhydantoin (**2c**) were prepared by the above method.

**Method B.** To a solution of 472 mg (2 mmol) of PCG (**1a**) in 5 ml of anhydrous DMSO was added 0.5 ml (excess) of cyclohexyl isocyanate and the mixture was stirred at room temperature for 3 hr. The precipitated 1,3-dicyclohexlurea was removed by filtration, and the filtrate was evaporated to dryness. The residue was triturated with hot EtOH (50 ml) and the insoluble solid was collected on a filter and washed with EtOH: 223 mg. The additional product was obtained from the filtrate: 71 mg; total yield, 294 mg (67.6%). The product was further purified by crystallization from DMSO–dioxane (1:3): mp 280–290° dec. The compound was also prepared in 67.8% yield from a reaction of PCG and phenyl isocyanate.

3-(Purin-6-yl)-5-methylhydantoin (**2c**) was also prepared by this method.

**Method C.** A solution of 472 mg (2.0 mmol) of PCG in 10 ml of DMSO was diluted with 20 ml of anhydrous pyridine and the mixture was cooled to −10°. To this cold mixture was dropwise added 0.6 ml (excess) of ethyl chloroformate and stirred at room temperature overnight. The mixture was then refluxed for 1 hr and evaporated to dryness. The residual pyridine was azeotroped with toluene (10 ml). The residue was triturated with 20 ml of hot EtOH and filtered. The solid product was washed with EtOH and dried: yield, 152 mg (35%); mp 280–290° dec. Samples of 3-purin-6-ylhydantoin prepared by methods A, B, and C were identical with one another in ir, uv, and nmr spectra.

**1,3-Dicyclohexyl-1-[*N*-(purin-6-ylcarbamoyl)glycyl]urea (3a). Method D.** The filtrate from the preparation of **2a** in method A was concentrated to about 100 ml and the precipitated dicyclohexylurea was removed by filtration. The filtrate was mixed with 100 g of silica gel (100–200 mesh) and dried *in vacuo*. The silica gel bound material was packed on the top to a silica gel column (dry packed, 100–200 mesh, 5 × 80 cm) and the column was eluted with solvent A. The fractions between 400 and 1040 ml were evaporated to dryness and the residue was dissolved in 50 ml of hot EtOH. After cooling at room temperature for 2 hr, the additional dicyclohexylurea was removed by filtration and the filtrate was cooled at 0° overnight. The white crystals were collected on a filter and washed with cold EtOH (yield, 435 mg). The additional product (330 mg) was obtained from the filtrate: total yield, 765 mg (17.3%); mp 214–216° (softens) and >300° dec; mass spectrum *m/e* (rel %)§ 264 (>1) 224 (>1, dicyclohexylurea·$^+$), 219 (>1), 192 (1, purine−CO−$NHCH_3$·$^+$), 182 (>1), 162 (>1), 161 (5), 143 (3), 135 (5), 125 (4, cyclohexyl isocyanate·$^+$), 108 (4), 101 (58), 97 (45), 82 (71), 69 (53), and 67 (100).

**3-Cyclohexyl-1-(purin-6-ylcarbamoyl)hydantoin (4a). Method E.** A suspension of 400 mg (0.9 mmol) of **3a** in 300 ml of 2 *N* NaOH was stirred at room temperature for 3 hr and filtered. The filtrate was neutralized to pH 6.0 with concentrated HCl. After evaporating to dryness, the residue was extracted with 500 ml of boiling EtOH and the ethanolic extract was evaporated. The residue was dissolved in 50 ml of $H_2O$ and left at room temperature for 2 hr, followed by cooling at 0–5° overnight. The amorphous solid was collected on a filter and washed with $H_2O$. This was further purified by dissolving in a minimal amount of DMSO, followed by precipitating with EtOH: yield, 81 mg (26%); mp >300° dec; mass spectrum *m/e* (rel %) 344 (24, $M^+ + 1$), 343 (45, $M^+$), 263 (12), 262 (44), 218 (1), 182 (2), 163 (40), 162 (100), 161 (40), 135 (20), 119 (40), and 101 (42). Compound **4b** was prepared in 35% yield by an analogous method and a trace of biuret **5b** was formed in this reaction.

***N*-(Cyclohexylcarbamoyl)-*N*-(purin-6-ylcarbamoyl)glycine (5a). Method F.** Compound **4a** (35 mg, *ca.* 0.1 mmol) was heated in 20 ml of 0.1 *N* NaOH at 100° for 1 min and neutralized to pH 6.0 with concentrated HCl. After evaporating to dryness, the resi-

‡ Melting points, ir, uv, mass, and nmr ($Me_4Si$ as an internal reference) spectra, and tlc were recorded as reported previously.[1,2] When running the mass spectra, the source temperature was at 300–350° and the probe temperature was 200–250°. The mass spectra of compounds **2a** and **4a** were obtained on Varian-Mat CH5 double-focusing high-resolution mass spectrophotometer of Upjohn Co. The following solvent systems were used for the column and thin-layer chromatography: solvent A, EtOAc–*n*-PrOH–$H_2O$ (4:1:2); solvent B, EtOAc–2-ethoxyethanol–16% $HCO_2H$ (4:1:2).

§ The relative intensities appear to be dependent on the probe temperature.

**Table II.** Physical and Biological Data

| Compd | Method | Yield, % | Mp, C[a] | Formula[b] | Tlc ($R_f \times 100$) in solvent[c] A | Tlc ($R_f \times 100$) in solvent[c] B | Biological act., mammalian cells[d] Nc 37 | 6410 | L1210 |
|---|---|---|---|---|---|---|---|---|---|
| 2a | A | 69.0 | 280–290 dec | $C_8H_6N_6O_2$ | 39 | 51 | - | ± | ± |
| | B | 67.6 | 280–290 dec | | 39 | 51 | | | |
| | C | 35 | 280–290 dec | | 39 | 51 | | | |
| 2b | A | 12 | 170–180 ef, 188–198 dec | $C_{10}H_{10}N_6O_3$ | 37 | 55 | ± | NA | ± |
| 2c | A | 19.5 | 238–240 | $C_9H_8N_6O_2$ | 47 | 61 | ± | NA | ± |
| | B | 59.7 | 238–240 | | 47 | 61 | | | |
| 3a | D | 17.3 | 214–216 s, >300 dec | $C_{21}H_{30}N_8O_3$ | 73 | 84 | NA | NA | ++ |
| 3b | D | 22.3 | 150–160 s, 162–164 | $C_{23}H_{34}N_8O_4$ | 77 | 85 | ± | NA | ± |
| 3c | D | 71.7 | 197–198 | $C_{22}H_{32}N_8O_3$ | 80 | 88 | NA | NA | NA |
| 4a | E | 26 | >300 dec | $C_{15}H_{17}N_7O_3$ | 69 | 83 | ± | ± | ± |
| 4b | E | 35.3 | 210–220 s, >300 dec | $C_{18}H_{19}N_7O_3$ | 74 | 85 | - | NA | NA |
| 5a | F | See footnote e | | $C_{15}H_{19}N_7O_4$ | 8.2 | 3.4 | | | |
| 5b | E | See footnote f | | $C_{16}H_{21}N_7O_4$ | 4.0 | 3.0 | | | |
| 6a | G | 52.6 | 265–270 s, >300 dec | $C_{14}H_{13}N_7O_2$ | 55 | 73 | NA | NA | + |
| 6b | G | 50.0 | 230–240 s, >300 dec | $C_{15}H_{21}N_7O_2$ | 63 | 80 | ± | ± | ± |

[a] dec = melts with decomposition; ef = effervescence; s = softens. [b] All compounds were analyzed for C, H, and N except for **5a** and **5b**. The analytical results were within ±0.4% of the theoretical values. The compounds prepared by different methods were identical with one another in their uv and ir spectra and the chromatographic mobilities. [c] Tlc was carried out on Bakerflex silica gel 1B-F (J. T. Baker) using the following solvent systems: A, EtOAc–*n*-PrOH–$H_2O$ (4:1:2); B, EtOAc–2-ethoxyethanol–16% $HCO_2H$ (4:1:2). [d] The notation represents the viable cell number relative to the controls after 72 hr of incubation: ++, 30–60%; +, 60–80%; ±, 80–90%; NA 90–110%. [e] Monitoring of the reaction mixture showed the quantitative conversion. [f] A trace of **5b** was formed, isolated by tlc and characterized by uv spectra.

due was triturated with 5 ml of EtOH. The solid product **5a** was collected on a filter. This was contaminated with NaCl. Attempts to isolate this material (**5a**) in a pure form were unsuccessful because of its instability. The yield on tlc appeared to be almost quantitative.

**Conversion of Biuret 5a into Hydantoin 4a.** A neutral solution of 100 mg of biuret **5a** in 15 ml of water was allowed to stand at room temperature for a period of 2 hr. Monitoring of the reaction mixture on a tlc revealed that the compound was converted about 50% to the hydantoin **4a**. The isolation of a tlc band and comparison of the uv spectra (Figure 2) and chromatographic mobilities of this material confirmed that it was the hydantoin **4a**.

***N*-(Purin-6-ylcarbamoyl)glycine Cyclohexylamide (6a). Method G.** A stirred mixture of 1.04 g (5 mmol) of ethyl purine-6-carbamate[1] and 1.42 g (10 mmol) of glycine cyclohexylamide[6] in 50 ml of anhydrous pyridine was heated in a glass bomb at 120° for 6 hr and cooled at room temperature overnight. The white solid was filtered and washed with EtOH. The crude product was dissolved in 1 *N* NaOH and the filtered solution was acidified to pH 2.0 with concentrated HCl. The resulting white solid was collected on a filter and washed with $H_2O$, EtOH, and ether: yield, 835 mg (52.6%). The analytical sample was recrystallized from DMSO–EtOH (1:3) mp 265–270° (softens), >300° dec; mass spectrum *m/e* (rel %) 199 (1), 184 (2), 182 (1), 162 (8), 161 (85), 139 (16), 135 (77), 119 (19), 108 (32), and 101 (100).

**3-Phenylhydantoin.** This compound was prepared by refluxing *N*-phenylcarbamoylglycine[5] with concentrated HCl for 3 hr: mp 155–156°; mass spectrum *m/e* (rel %) 177 (9, $M^+ + 1$), 176 (60, $M^+$), 120 (37), 119 (100, $C_6H_5NCO^+$), 91 (40), and 77 (30, $C_6H_5^+$). *Anal.* ($C_9H_8N_2O_2$) C, H, N.

***N*⁶-Phthaloyladenine.**[14] This compound was prepared by fusion of adenine with phthalic anhydride at 150° for 1 hr, followed by crystallization from EtOH: mp 269–270°; mass spectrum *m/e* (rel %) 266 (22, $M^+ + 1$), 265 (100, $M^+$), 237 (11, $M^+ - CO$), 209 (10, $M^+ - 2CO$), 194 (6), 158 (4), 130 (8), 119 (14, purine$^+$), 104 (56, $C_6H_5CO^+$), and 76 (69). *Anal.* ($C_{13}H_7N_5O_2$) C, H, N.

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