Amides and Peptides Derived from 6-Trichloromethylpurine and Amino Acids*

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Under exceedingly mild conditions, 6-trichloromethylpurine reacts with the amino groups of amino acids, peptides, and primary and secondary amines to yield the *N*-purinoyl derivatives of these compounds. Several of these amides have been isolated and characterized. The reaction, which may proceed by two different pathways depending on the *p*H, provides an easy means for the incorporation of the purinoyl group into systems of potential biological interest.

It has been shown recently that 6-trichloromethylpurine (I) undergoes hydrolysis or ammonolysis under exceedingly mild conditions to yield purinoic acid[1] or its amide (Cohen *et al.*, 1962). This observation suggested 6-trichloromethylpurine to be an acylating agent which might prove useful for the incorporation of the purinoyl group into systems of potential biological interest. Accordingly, an evaluation of the reactivity of 6-trichloromethylpurine under various conditions became desirable, and we have studied its reaction with amines and amino acids.

When 6-trichloromethylpurine was added to a solution of an amino acid or aliphatic primary or secondary amine at room temperature and *p*H 8 to 9, and the products of the reaction studied by paper chromatography, the following general observations were made: (1) Complete disappearance of 6-trichloromethylpurine occurred within 3 to 4 hours with most amines involved. (2) Two, and occasionally three new substances were observed; one of these, common to all the amines studied, corresponded to 6-purinoic acid. The other substances, characterized by R_F values and ultraviolet spectral properties that depended on the nature of the amine used, were due to its reaction with 6-trichloromethylpurine. The results of these experiments are shown in Tables I and II. When the reaction mixture was buffered with sodium acetate instead of bicarbonate, 6-trichloromethylpurine could still be detected after 24 hours. Several of these products, identified as the *N*-purinoyl derivatives of the amines or amino acids used, were isolated and further characterized (Table II).

From a preparative standpoint, the success of the reaction varied widely among the various amines used and reflected the ease of isolation of the *N*-purinoyl derivative rather than its relative yield. The presence of excess electrolyte in the reaction mixture prevented crystallization of the product. Purinoic acid was always a by-product and occasionally the major one. A further complication was the tendency of 6-trichloro-

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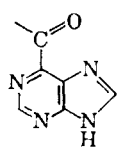
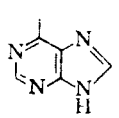
[1] The term "purinoic" acid is new and is intended to replace the less convenient purine-6-carboxylic acid. Accordingly, the group  will be referred to here as purinoyl. Although analogy with the terms benzoic and furoic (from benzene and furane) would require the use of puroic, purinoic is preferable in the interest of phonetics and clarity and is consistent with the term purinyl (see Ward *et al.*, 1961) in referring to  in preference to puryl.

TABLE I

PRODUCTS OF REACTION BETWEEN 6-TRICHLOROMETHYLPURINE AND AMINO ACIDS OR AMINES

Paper chromatograms[a] of aqueous solutions (1 ml) containing products of the reaction between 6-trichloromethylpurine (12 mg, 0.05 mmole) and amino acids[b] or amines (0.1 mmole) in presence of sodium bicarbonate (5%) at room temperature; reaction time 5 to 6 hours.

Amino Acid or Amine	R_F	λ_{max}[c] (mμ)
None	0.16[d]	280
D,L-Aspartic acid	0.49	290
Creatine	0.16	280
L-Cysteine	0.51[e]	290(S), 326, 340(S)
	0.85	268
L-Cystine	0.11	290
	0.35	290
Dimethylamine	0.57	269
Ethanolamine	0.47	290
D,L-Glutamic acid	0.50	290
L-Histidine	0.17	286[f]
Hydrazine	0.52	264, 316
L-Hydroxyproline	0.37	279
D,L-Isoleucine	0.84	290
L-Lysine	0.24	290
	0.39	287
L-Methionine	0.74	272, 294(S)
L-Proline	0.63	276
Sarcosine	0.47	272
Tris(hydroxymethyl)-aminomethane	0.38	288
D,L-Tryptophane	0.90	282, 288
L-Tyrosine	0.85	283, 294(S)
D,L-Valine	0.86	289

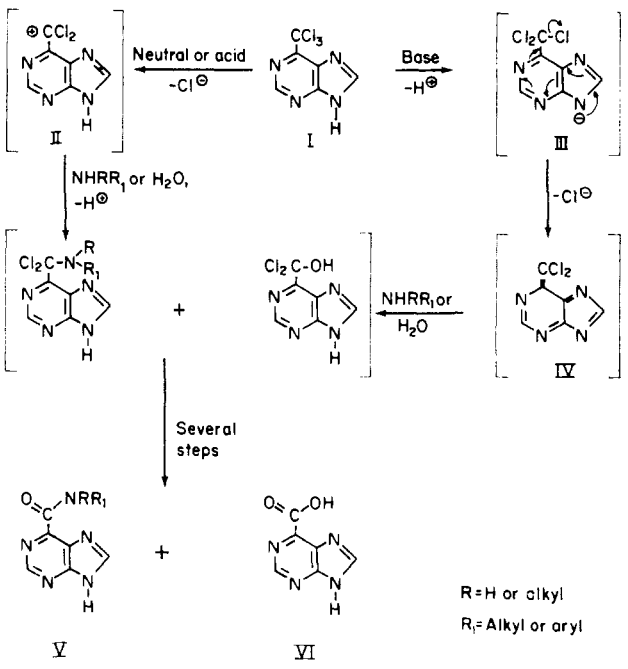
[a] Developed with butanol–formic acid–water (77:10:13 v/v) by the ascending technique, and viewed under ultraviolet light. [b] The other amino acids were similarly treated. The R_F values and spectral properties of their respective reaction products are further listed in Table II of this paper. [c] In 0.1 M phosphate buffer, *p*H 6.2. (S) = shoulder. [d] The chromatograms of all amino acids or amines listed in this table show this spot, with varying degrees of intensity. [e] Further resolved by the system isopropanol-HCl-water (170:41:49) into two spots: R_F 0.33, λ_{max} 288; R_F 0.43, λ_{max} 290(S), 325, 340(S) mμ. [f] Further resolved by the system isopropanol-HCl-water into two spots: R_F 0.30, λ_{max} 280; R_F 0.44, λ_{max} 290 mμ.

methylpurine to yield a polycondensation product when treated with base. This product, having the same composition as purinoic acid, differs from it in physical properties and may be a hydrated purinoyl polymer. At *p*H values 9 to 10, which would be required for the acylation of the more basic secondary amines and imino acids (pK_a *ca.* 10), polymer formation becomes more serious. With the much weaker aromatic amines such as *p*-aminobenzoic acid (pK_{a1} = 2.3; pK_{a2} = 4.9) or aniline (pK_a = 4.6), acylation proceeded at *p*H 5 to 7, even in the absence of added base. Aniline, however, yielded an amidine, *N*,*N*′-diphenylpurinylamidine, in its reaction with 6-trichloromethylpurine. An extreme case is provided by the exceedingly weak base, *p*-nitroaniline (pK_a = 1.1), which could be acylated even in 50% acetic acid solution. The reactions in neutral or acidic media proceeded at a slower rate, and application of heat, though not necessary, proved advantageous.

The reaction of 6-trichloromethylpurine with human and bovine serum albumins proceeded readily at *p*H 10 and room temperature and led to the incorporation of about 25 purinoyl residues into 1 mole of protein. The resulting conjugated antigen stimulated, in rabbits, the formation of antibody with purine specificity. Details of this immunochemical study are reported elsewhere (Butler *et al.*, 1962).

Results and Discussion

In the absence of quantitative kinetic data, any proposed mechanism for the reaction of 6-trichloromethylpurine with amines is necessarily speculative. The results suggest the existence of two possible pathways given in Scheme 1. In acidic or neutral media,



Scheme 1

the first step of the reaction may involve formation of an intermediate carbonium ion (II) by slow heterolysis of a carbon-chlorine bond, followed by rapid coordination of the carbonium ion and an amine nitrogen or a water molecule to yield, in several steps, an *N*-purinoylamine (V) or purinoic acid (VI). Analogy with the known hydrolysis of benzotrichloride (Olivier and Weber, 1934; Hughes, 1941; Hine and Lee, 1951),

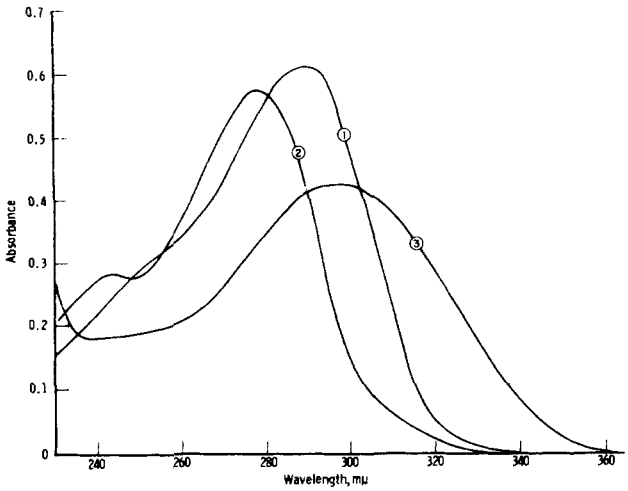


FIG. 1.—Ultraviolet absorption spectrum of *N*-purinoylglycine, 0.615 mmole/liter, in (1) 0.1 M phosphate buffer, *p*H 6.2; (2) 0.1 N HCl; (3) 0.1 N NaOH.

which proceeds at a rate independent of base concentration, lends support to the present hypothesis.

In the *p*H range 7 to 9 required for the acylation of most aliphatic amines, 6-trichloromethylpurine exists in part as its anion (III) (pK_a = 7.93) (Cohen *et al.*, 1962). Analogy with the behavior of the anion derived from chloroform (Hine, 1951; Hine and Dowell, 1954) or trichloroacetic acid (Parham and Schweizer, 1959), and the unlikelihood of stable separation of opposite charges in a conjugated structure, strongly suggest that the anion (III) would lose chloride more readily than the neutral species. Valency requirements do not permit the extension of the analogy of carbene formation to 6-trichloromethylpurine. The process does imply the hypothetical formation of a highly reactive, uncharged intermediate, IV, which contains a strongly electrophilic exocyclic carbon atom[2] (Scheme 1). In agreement with this assumption is the observation that 6-trichloromethylpurine is fairly stable in solution at *p*H values below 7, but undergoes rapid hydrolysis to purinoic acid at higher *p*H (Cohen *et al.*, 1962).

The subsequent reactions may not be identical for all amines; amidine formation from aniline suggests *N*-phenylpurinimidyl chloride, PuC(Cl)=NR [Pu = purin-6-yl; R = phenyl], as a possible intermediate. This suggestion finds support in the analogous formation of *N*,*N*-diphenylbenzamidine from *N*-phenylbenzimidyl chloride and aniline (Shriner and Newman, 1944). However, the possibility of such a mechanism is precluded in the case of secondary amines.

Almost all the *N*-purinoyl derivatives of aliphatic primary amines and amino acids have very similar ultraviolet absorption spectra as typified by that for glycine (Fig. 1). The derivatives of secondary amines and imino acids exhibit different spectra, with maxima shifted to lower wave lengths, in agreement with observations made on the *N*-methyl and *N*,*N*-dimethylamide of purinoic acid (Giner-Sorolla and Bendich, 1958). The chromophores of the cysteine derivatives may have an entirely different structure.

Because of the possible use of 6-trichloromethylpurine for the study of N-terminal end-groups in proteins, the stability of *N*-purinoylglycine as a model compound was studied by following the spectral changes of its

[2] A further point of resemblance between chloroform and 6-trichloromethylpurine is the Reimer-Tiemann reaction with phenol, which will be described elsewhere.

TABLE II
N-PURINOYL DERIVATIVES OF AMINO ACIDS AND AMINES

Amino Acid or Amine	Method of Preparation	Crystallization Solvent	Yield (%)	Elemental Analysis						
				Calcd. for	C	H	N	Found C	H	N
D,L-α-Alanine	B	H_2O	25	$C_9H_9O_3N_5$	45.9	3.8	29.8	45.8	3.7	30.2
β-Alanine	A	H_2O	67	$C_9H_9O_3N_5$	45.9	3.8	29.8	46.0	4.2	29.6
D,L-Alanyl-D,L-alanine	B	H_2O-methanol	58	$C_{12}H_{14}O_4N_6$	47.1	4.6	27.5	47.1	5.3	27.5
p-Aminobenzoic acid	See text			$C_{13}H_9O_3N_5$	55.0	3.2	24.7	55.0	3.6	24.6
γ-Aminobutyric acid	A	H_2O	58	$C_{10}H_{11}O_3N_5$	48.0	4.4	28.1	47.9	3.6	28.1
ε-Aminocaproic acid	A	Methanol	18	$C_{12}H_{15}O_3N_5$	51.9	5.4	25.3	52.0	6.3	24.7
L-Asparagine[d]	A	H_2O	31	$C_{10}H_{10}O_4N_6 \cdot {}^1/_2H_2O$	41.7	3.8	29.3	41.6	3.6	29.6
n-Butylamine	A	H_2O	18	$C_{10}H_{13}ON_5$	55.3	5.9	32.0	55.1	5.6	31.9
D,L-Glutamine	A	See text	48	$C_{11}H_{12}O_4N_6 \cdot H_2O$	42.6	4.5	27.4	42.7	4.6	27.4
Glycine	A, C, see text	H_2O	33, 42	$C_8H_7O_3N_5$	43.5	3.2	31.7	43.1	3.2	31.0
Glycylglycine	A	H_2O	83	$C_{10}H_{10}O_4N_6$	43.2	3.6	30.2	43.3	3.6	30.0
Glycylglycylglycine	A	See text	71	$C_{12}H_{13}O_5N_7$	43.0	4.0	29.3	43.1	4.4	28.9
Glycyl-D,L-serine	B	H_2O-ethanol	62	$C_{11}H_{12}O_5N_6$	42.8	3.9	27.3	42.9	4.5	27.2
L-Leucine	See text		18	$C_{12}H_{15}O_3N_5$	52.0	5.4	25.3	51.9	5.7	25.4
D,L-Phenylalanine	A	H_2O-methanol	39	$C_{15}H_{13}O_3N_5$	57.9	4.2	22.5	57.5	4.1	22.5
L-Phenylalanine	See text		20	$C_{15}H_{13}O_3N_5$	57.9	4.2	22.5	57.6	4.5	21.4
Piperidine	B, see text	Ethanol-ether	26	$C_{11}H_{14}ON_5$	56.8	6.0	30.2	57.1	5.9	30.1
D,L-Serine	C	Ethanol	24	$C_9H_9O_4N_5$	43.0	3.6	27.9	42.8	4.3	27.6
Sulfanilic acid	See text[f]		80	$C_{12}H_9O_4N_5S \cdot H_2O$	42.7	3.4	20.8	42.8	4.2	20.5
D,L-Threonine	C	H_2O	23	$C_{10}H_{12}O_4N_5$	45.1	4.5	26.3	45.6	4.8	26.4

[a] (S) = shoulder. [b] Data from ascending chromatograms developed in butanol–formic acid–water (77:10:13 v/v); viewed under ultraviolet light. [c] 0.1 M phosphate buffer. [d] $[\alpha]_D^{25}$ (4 N HCl) = +4.7°. [e] In isopropanol-ammonia-water (85:1.3:15). [f] Calculated: S, 9.5; found: S, 9.5.

solutions in acid and base. A dilute solution in 1 N hydrochloric acid displayed no spectral changes over a period of 15 days at room temperature (20–25°). Significant spectral changes occurred only after 3 hours of reflux with the acid, and the compound was completely degraded after 6 hours to unidentified products. Purinoic acid undergoes degradation much more rapidly, and gives rise first to purine as a result of decarboxylation. A complex mixture of products may arise from the acid degradation of the latter (Gordon *et al.*, 1957). Table III shows the spectral and chromatographic changes accompanying these degradations.

Basic hydrolysis proceeds rapidly. A solution of *N*-purinoylglycine in 1 N sodium hydroxide exhibited no significant spectral changes during the first hour at room temperature, but hydrolysis to purinoic acid was almost complete after 48 hours (Fig. 2).

Potentiometric titration of a number of *N*-purinoyl amino acids revealed two dissociations in the *p*H range 2 to 12 (Table IV). The first of these is assigned to the carboxylic group, which, for the glycine derivative, has a pK_{a1} value of 3.70, comparable to the pK_a = 3.60 for acetylglycine. Therefore, the effect of the electron-deficient purine ring system is not transmitted to the carboxylic group. The second dissociation (pK_{a2} = 9.15), that of the imidazole moiety, is slightly depressed in comparison with that reported for purinoic acid *N*-methylamide (8.9) (Giner-Sorolla and Bendich, 1958) or purine-6-carboxaldehyde (8.8) (Giner-Sorolla *et al.*, 1959). Thus, a feeble interaction between the two dissociations may be possible. The most pronounced effect of such an interaction is exhibited by purinoic acid itself (pK_{a1} = 2.9 ± 0.1, pK_{a2} = 9.75 ± 0.05), where a hydrogen bridge between a carboxylic oxygen and the nitrogen at position 7 would strongly favor the monoanionic species at the expense of the second dissociation. A similar intramolecular hydrogen bonding was implicated to account for the reduced acidity of 4-benzotriazole (Miller and Wagner, 1954) and may also be operative in several purines structurally related to purinoic acid (Doerr *et al.*, 1961).

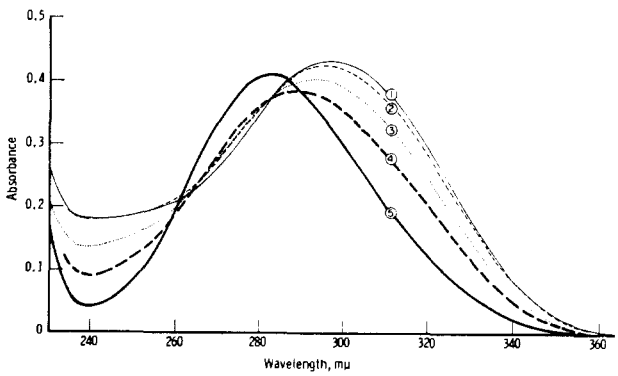


FIG. 2.—Ultraviolet absorption spectra of solutions of N-purinoylglycine (0.615 mmole/liter) in 1 N NaOH determined at (1) 0 time; (2) 30 minutes; (3) 6 hours; (4) 24 hours; (5) 48 hours. 6-Purinoic acid shows an ultraviolet maximum of 279 mμ at *p*H 11: Mackay and Hitchings (1956).

EXPERIMENTAL

Melting points were determined with a Thomas-Hoover apparatus. The R_F values are for ascending chromatograms on Whatman No. 1 paper, with *n*-butanol (77%), formic acid (10%), and water (13%) (v/v), at room temperature.

The spectrophotometric measurements were made with a Cary model 11 recording spectrophotometer.

Chromatographic Study of the Products of the Reaction Between 6-Trichloromethylpurine and Amines.—6-Trichloromethylpurine (I) (Cohen *et al.*, 1962) (12 mg) was added to a solution of an amine or amino acid (0.1 mmole) in 5% aqueous sodium bicarbonate solution (1 ml). The mixture was slowly shaken at room temperature for 5 to 6 hours. A sample of the reaction mixture was then subjected to paper chromatography

M.p. or dec. point, °C	R_F[b]	λ_{max} (mμ) ($A_M \cdot 10^{-3}$)[a] 0.1 N HCl	*p*H 6.2[c]	0.1 N NaOH
260–262 (dec.)	0.67	243(S) (4.50), 280 (9.34)	290 (9.55)	295 (7.03)
290–292 (dec.)	0.53	243(S) (4.00), 279 (8.75)	290 (8.90)	294 (6.10)
255–257 (dec.)	0.64	245(S) (5.37), 282 (12.10)	290 (12.10)	297 (8.56)
>320	0.38[e]	253 (10.05), 290(S) (11.20), 310 (12.20)	243 (8.95), 304 (12.20)	243 (7.68), 286 (11.50), 324(S) (8.31)
233–235 (dec.)	0.59	243(S) (4.67), 279 (10.70)	289 (10.60)	292 (7.27)
202–203 (dec.)	0.72	243(S) (4.46), 280 (10.60)	289 (10.70)	294 (7.21)
248–249 (dec.)	0.27	243(S) (4.66), 282 (8.43)	290 (9.57)	297 (6.86)
190–191	0.88	243(S) (4.62), 280 (10.95)	289 (10.70)	293 (7.40)
235–236 (dec.)	0.33	244(S) (5.34), 278 (11.20)	290 (10.65)	297 (7.31)
282–285 (dec.)	0.48	243(S) (4.72), 278 (9.41)	290 (10.10)	297 (6.98)
290–295 (dec.)	0.28	244(S) (4.40), 281 (9.50)	290 (9.82)	296 (6.83)
283–285 (dec.)	0.21	244(S) (4.60), 281 (9.87)	290 (9.80)	296 (7.05)
240–242 (dec.)	0.27	244(S) (4.56), 281 (9.76)	290 (9.86)	297 (6.98)
170–172 (dec.)	0.86	245(S) (5.00), 282 (10.85)	290 (11.50)	296 (7.80)
260–265 (dec.)	0.85	245(S) (4.76), 282 (10.00)	291 (10.87)	298 (7.70)
168–170 (dec.)	0.83	243(S) (4.92), 283 (9.90)	291 (10.80)	297 (7.23)
204–205	0.74	267 (9.30)	268 (9.40)	276 (8.36)
195–196 (dec.)	0.42	244(S) (4.57), 282 (9.40)	290 (9.50)	297 (6.91)
>320	0.08	233 (13.30), 289 (12.25), 302 (12.15)	296 (16.40), 233 (12.50)	285 (14.00)
236–237 (dec.)	0.60	244(S) (4.42), 283 (9.14)	291 (9.73)	298 (6.72)

and the dried chromatogram was examined in ultraviolet light. The position of the absorbing spots was determined, and then these were eluted with 0.1 M phosphate buffer of *p*H 6.2 and the absorption spectrum of the resulting solution was determined. The results are shown in Tables I and II.

Effect of Base on 6-Trichloromethylpurine.—A suspension of I (0.5 g) in water (20 ml) was treated with 10 N sodium hydroxide (1 ml). After 8 hours at room temperature the solution displayed a deep brown coloration but was clear. Acidification with glacial acetic acid caused the formation of a dark brown colloidal precipitate (0.15 g) which was collected by filtration, washed with water, and dried to constant weight; m.p. above 320°; λ_{max} (water) 330 mμ, with shoulders at 278, 317, and 347 mμ.

Anal. Calcd. for $C_6H_4O_2N_4$: C, 43.8; H, 2.4; N, 34.1. Found: C, 43.3; H, 2.6; N, 34.0.

Migration of this substance as the sodium salt on paper chromatograms was negligible. (Systems used: Butanol – formic acid – water; isopropanol – hydrochloric acid – water; isopropanol – ammonia – water). The spot on paper was strongly fluorescent under ultraviolet light.

The Preparation of N-Purinoyl Derivatives of Amino Acids and Amines

Method A

A mixture of the specified quantities of 6-trichloromethylpurine (I), amine or amino acid, and sodium bicarbonate in water was stirred at room temperature for 4 to 5 hours. The resulting yellow to brown homogenous solution was acidified to *p*H 1 with concentrated hydrochloric acid. The resulting *N*-purinoyl derivative precipitated immediately or crystallized after a few hours at room temperature. The following examples of compounds were prepared by this method; others are listed in Table II.

N-Purinoyl-L-phenylalanine. The clear, brown solution obtained from 6-trichloromethylpurine (2.4 g), L-phenylalanine (1.5 g), sodium bicarbonate (3.5 g), and water (100 ml) was treated with glacial acetic acid. The gummy precipitate was separated by decantation and extracted with hot acetone (300 ml). Evaporation of this solvent left a fluffy material which was extracted with tetrahydrofuran and recrystallized from this solvent, after treatment with charcoal. $[\alpha]_D^{25}$ Methanol + 67°.

N-Purinoyl-L-leucine, 0.5 g (18%), was prepared from 6-trichloromethylpurine (2.4 g), L-leucine (1 g), sodium bicarbonate (3.5 g), and water (100 ml). The precipitated material was extracted with acetone, the solvent was evaporated, and the sirupy residue was shaken with anhydrous ether to induce crystallization. The product was finally recrystallized from cold tetrahydrofuran. $[\alpha]_D^{25}$ Methanol + 39°.

(N-Purinoylglycyl)glycylglycine, 1 g (71%), was prepared from I (1 g), glycylglycylglycine (1 g), and sodium bicarbonate (3 g) in water (100 ml), and was purified by treating its solution in dilute aqueous ammonia with charcoal and reacidification.

N-Purinoyl-D,L-glutamine, 1.5 g (48%), was obtained as a stable hydrate from 6-trichloromethylpurine (2.4 g), D,L-glutamine (2.8 g), and sodium bicarbonate (4 g) in water (250 ml), and was purified by dissolving it in dilute aqueous ammonia, treatment with charcoal, filtration, and acidification.

Method B

Reaction was carried out as in Method A, but no precipitate was formed on acidification. The acidified solution was brought completely to dryness in a flash evaporator. The solid residue was scraped off the sides of the flask and was shaken with water (10 ml) for 2 to 3 minutes and the insoluble material collected by filtration.

N-Purinoylglycine, 0.75 g (33%), was prepared from 6-trichloromethylpurine (2.4 g), glycine (2.3 g), and

TABLE III
SPECTRAL AND CHROMATOGRAPHIC CHANGES ACCOMPANYING HYDROCHLORIC ACID TREATMENT OF *N*-PURINOYLGLYCINE (66 MG/10 ML 1 N HCl) AND PURINOIC ACID (50 MG/10 ML 1 N HCl)

Reflux Period (Hr.)	Purinoic Acid		*N*-Purinoylglycine	
	λ_{max} (mμ)[a]	R_F (λ_{max}, mμ)[b]	λ_{max} (mμ)[a]	R_F (λ_{max}, mμ)[b]
0	280	0.17 (280)	290	0.45 (290)
1	263	0.17 (280)	290	0.42 (290)
	290–325(S)	0.52 (263)[c]		
3	268	0.16 (280)	290	0.23[d]
		0.54 (263)[c]		0.38 (290)
6			(very broad)	0.26[e] (285, 310S)
				0.45[e] (275)
				0.61[e] (263, 341)

[a] Ultraviolet absorption spectrum of solution, at neutral *p*H, after the specified reflux period. (S) = shoulder. [b] Paper chromatograms (ascending, butanol–formic acid–water, 70:10:13 v/v) of neutralized solution after specified reflux period. [c] Data are the same for purine. [d] Very faint. [e] Developed with isopropanol–hydrochloric acid–water (170:41:49 v/v).

TABLE IV
APPARENT pK_a VALUES[a] OF PURINOIC ACID DERIVATIVES IN WATER AT 16 ± 0.5°

Compound	Quantity Used[b] (mg)	pK_{a_1}	pK_{a_2}
Purinoic acid	48.1	2.9 ± 0.1	9.75 ± 0.05
N-Purinoyl-methylamine[c]		(*ca.* 1)[d]	8.9
Purine-6-carboxaldehyde[e]		(2.4 ± 0.1)[d]	8.8
N-Purinoylglycine	96.6	3.70 ± 0.10	9.15 ± 0.05
N-Purinoyl-α-D,L-alanine	89.0	4.00 ± 0.01	8.90 ± 0.05
N-Purinoyl-β-alanine	53.6	4.5 ± 0.10	9.05 ± 0.10
ε-(*N*-purinoylamino)caproic acid	79.3	5.00 ± 0.10	9.15 ± 0.05
(*N*-Purinoylglycyl)glycine	99.0	3.60 ± 0.10	8.80 ± 0.05
N-Purinoyl-D,L-glutamine	81.2	3.5 ± 0.10	9.00 ± 0.05
N-Purinoyl-L-leucine[f]	78.8	3.50 ± 0.10	9.05 ± 0.05

[a] Determined by potentiometric titration according to Parke and Davis (1954). [b] Dissolved in 10.0 ml 0.1 N KOH, then titrated with 2 N HCl. [c] Data from Giner-Sorolla and Bendich (1958). [d] Cationic dissociation. [e] Data from Giner-Sorolla *et al.* (1959). [f] Evidence of cationic dissociation, pK_a *ca.* 2.5.

sodium bicarbonate (4 g) in water (250 ml), and was purified by recrystallization from boiling water.

In another experiment, the clear solution obtained from 6-trichloromethylpurine (2 g), glycine (2 g), and sodium bicarbonate (4 g) in water (100 ml) was acidified with glacial acetic acid and was concentrated under reduced pressure to *ca.* 20 ml. The sodium salt of *N*-purinoylglycine, 0.4 g (18%), crystallized after 4 to 5 days at room temperature and was purified by recrystallization from cold aqueous methanol; m.p. above 350°.

Anal. Calcd. for $C_8H_6O_3N_5Na \cdot H_2O$: C, 36.5; H, 3.1; N, 26.8. Found: C, 36.9; H, 3.2; N, 26.8.

N-Purinoylpiperidine was prepared from 6-trichloromethylpurine (2.4 g), piperidine (2.5 g), and sodium bicarbonate (5 g) in water (250 ml) as outlined in Method B, except that the dry residue was extracted with hot acetone (500 ml). The concentrated acetone extracts deposited a non-ultraviolet-absorbing material (piperidine salt?) which was discarded. Further concentration of the mother liquor and dilution with ether gave the purinoyl derivative. It was purified by recrystallization from ethanol-ether.

METHOD C

The reaction was carried out as in Method A, then the specified quantity of Dowex-50 (H-form, 50% moisture) was added and the mixture stirred for 30 minutes. It was then filtered, and the resin was extracted with boiling water (200 ml) and filtered again. The combined filtrates were brought to dryness and the residue, which contained the purinoyl derivative, was subjected to recrystallization. This method was applied to the following:

N-Purinoylglycine was prepared from 6-trichloromethylpurine (2.4 g), glycine (1.5 g), and sodium bicarbonate (8.5 g) in water (100 ml), and was treated with Dowex-50 (30 g). It was recrystallized from hot water and proved to be identical with the product described earlier.

N-Purinoyl-D,L-serine. The solution obtained from 6-trichloromethylpurine (1.2 g), D,L-serine (1 g), and sodium bicarbonate (2 g) in water (50 ml) was treated with Dowex-50 (10 g). The residue was taken up in ethanol (150 ml), filtered, and concentrated to about 10–20 ml. The purinoyl derivative, 0.3 g (24%), crystallized after 48 hours and was recrystallized from cold ethanol.

N-Purinoyl-D,L-threonine, 0.3 g (23%), was obtained from 6-trichloromethylpurine (1.2 g), D,L-threonine (1.2 g), and sodium bicarbonate (2 g) in water (100 ml) after treatment with Dowex-50 (10 g).

Reaction with Aniline. (*a*) A solution of 6-trichloromethylpurine (0.6 g) and aniline (0.5 g) in methanol (3 ml) was added to a solution of sodium bicarbonate (1 g) in water (25 ml). The mixture was stirred at room temperature for 4 hours and the precipitate *N,N'*-diphenyl-purinylamidine, 0.3 g (38%), was collected by filtration and recrystallized from aqueous ethanol; m.p. 245–6°.

Anal. Calcd. for $C_{18}H_{14}N_6$: C, 68.7; H, 45.; N, 26.8. Found: C, 68.3; H, 4.3; N, 26.9.

(*b*) A solution of 6-trichloromethylpurine (1.2 g) and aniline (2 g) in methanol (10 ml) was diluted with water (40 ml) and refluxed for 2 hours. The precipi-

tated material, 0.3 g (19%), was identical with the compound obtained by method (*a*).

The ultraviolet absorption spectrum displayed the following features: λ_{max},mμ ($A_M \cdot 10^{-3}$), in 0.1 N HCl, 304 (11.38); at *p*H 6.2, 270 (12.37); in 0.1 N NaOH, 262 (14.96).

Reaction with p-nitroaniline. (*a*) A solution of 6-trichloromethylpurine (0.6 g) and *p*-nitroaniline (0.3 g) in tetrahydrofuran (25 ml) was added to a solution of sodium bicarbonate (1 g) in water (25 ml) and the mixture was stirred at room temperature for 4 hours. The precipitated *N*-purinoyl derivative (0.4 g) (56%) was collected by filtration and purified by washing with water, methanol, and ether; m.p. above 340°.

Anal. Calcd. for $C_{12}H_8O_3N_6$: C, 50.6; H, 2.8; N, 29.6. Found: C, 50.2; H, 3.4; N, 29.7.

(*b*) A solution of 6-trichloromethylpurine (0.6 g), *p*-nitroaniline (0.8 g), and anhydrous sodium acetate (2 g) in glacial acetic acid (10 ml) and water (10 ml) was refluxed for 1 hour. The precipitated material (0.3 g, 42%) was identical to the compound obtained by method (*a*).

The ultraviolet absorption spectrum displayed the following properties: λ_{max},mμ ($A_M \cdot 10^{-3}$), in 0.1 N HCl, 328 (10.00), 290(S) (7.58); at *p*H 6.2, 328 (9.91); in 0.1 N NaOH, 351 (13.60), 282 (11.78).

Reaction with p-Aminobenzoic Acid. A mixture of 6-trichloromethylpurine (1.4 g), *p*-aminobenzoic acid (1.4 g), and sodium acetate (4 g) in water (200 ml) was stirred and its *p*H adjusted to 7–8 with 10 N sodium hydroxide. After 4 hours, the *p*H dropped to 5–6. The brown precipitate (0.5 g, 35%) that had formed overnight was collected, washed with water, methanol, and ether, and dried to constant weight.

In a modification of this procedure, a stirred mixture of 6-trichloromethylpurine (1.2 g), *p*-aminobenzoic acid (1.4 g), and sodium acetate (5 g) in water (100 ml) was refluxed for 1 hour. The brown precipitate, 0.7 g (49%), was treated as above.

Reaction with Sulfanilic Acid. A solution of 6-trichloromethylpurine (1.2 g) in methanol (10 ml) was added to a solution of sulfanilic acid monohydrate (1 g) and sodium bicarbonate (3 g) in water (50 ml). The mixture was stirred for 3 hours at room temperature and the thick creamy precipitate of the sodium salt of *N*-purinoylsulfanilic acid was separated by filtration and redissolved in hot water (100 ml) and the free acid, 0.4 g (40%), precipitated by the addition of hydrochloric acid. An additional crop of 0.4 g (40%), which was not as pure, was recovered by acidifying the original mother liquor; the product was recrystallized from boiling water.

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