oxides were carved from the columns, extracted with 95% ethanol, and filtered. The filtrates, contained the desired derivatives, were concentrated *in vacuo* to dryness, suspended in 1*N* hydrochloric acid, and extracted into ethyl acetate. The ethyl acetate extracts were dried with sodium sulfate and concentrated *in vacuo* to dry solids.

The yellow crystalline solid isolated from the slower of the two bands was recrystallized from 15 ml. of ethanol at −20° to yield 151 mg. of bright yellow prisms. This was shown to be 2,4-dinitrophenyl(+)*S*-methyl-L-cysteine sulfoxide by specific rotation and by infrared, either of which distinguishes the compound from its diastereomer. $[\alpha]_D^{25}$ −111.6 (1,2; *c*, 0.33 in acetic acid). For the synthetic compound, $[\alpha]_D^{25}$ −108.8.

The infrared spectrum (0.96 mg. in 250-mg. potassium bromide pellet) was identical with that of the authentic compound with absorption (μ) at 5.75 (s), 6.15 (s), 6.25 (s), 6.55 (s), 6.65 (shoulder), 7.00 (s), 7.27 (shoulder), 7.45 (s), 7.60 (s), 7.70 (s), 7.98 (m), 8.64 (m), 8.98 (m), 9.38 (m), 9.97 (s), 10.28 (m), 10.40 (w), 10.55 (w), 10.73 (w), 11.96 (w), 12.10 (w), 13.0 (w), 13.40 (w), and 13.95 (w).

Very slow crystallization from ethanol frequently yields the derivative as dense opaque yellow spherules with significant differences in the infrared, particularly in the 10–11-μ region.

Isolation of 2,4-dinitrophenyl(+)S-propyl-L-cysteine sulfoxide. The solid from the band just below the methyl derivative contained, in addition to 2,4-dinitrophenyl-*S*-propyl-cysteine sulfoxide, 2,4-dinitrophenylasparagine and slower-moving unidentified material. Chromatography a second time on a 38 × 765 mm. column containing 500 g. of buffered silicic acid with ethyl acetate yielded three poorly separated zones. Repeated chromatography of the middle zone on a silicic acid column of the same size yielded one band from which 10.7 mg. of yellow prisms was obtained by crystallization from 0.5 ml. of ethyl acetate. The product usually separates first as needles which gradually change to prisms. The compound was identified as 2,4-dinitrophenyl(+)*S*-propyl-L-cysteine sulfoxide by optical rotation and by infrared, $[\alpha]_D^{25}$ −133 (1,0.5; *c*, 0.247 in acetic acid). The synthetic compound has $[\alpha]_D^{25}$ −141.2.

The infrared spectrum in a potassium bromide pellet (0.91 mg./250 mg.) was identical with that of the authentic compound from 3–15 μ with absorption (μ) at 5.15 (m), 5.83 (s), 6.15 (s), 6.29 (s), 6.64 (s), 7.02 (s), 7.45 (s), 7.60 (s), 7.74 (s), shoulder on 7.74, 8.03 (s), shoulder on 8.03, 8.10 (s), 8.63 (m), shoulder on 8.63, 8.76 (m), 8.98 (m), 9.40 (m), 10.25 (s), 10.44 (s), 10.80 (m), 11.60 (w), 11.96 (m), 12.06 (m), 12.30 (m), 13.06 (w), 13.41 (m), 13.70 (w), and 13.90 (m).

Infrared spectra were determined on a Beckman IR-5 recording spectrophotometer.

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ALBANY 10, CALIF.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, THE UNIVERSITY OF TEXAS, M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE]

The Synthesis of *N*-(6-Purinyl)amino Acids. Amino Acids with a Single Reactive Amino Group[1a]

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A group of *N*-(6-purinyl)amino acids has been prepared from the corresponding amino acid and 6-chloropurine in carbonate-bicarbonate buffer. Purification was achieved by chromatography on anion exchange resins with formic acid elution. Instability of some of the compounds in acid solution was the major difficulty encountered.

Adenylosuccinic acid was isolated by Carter and Cohen[2] as the product of the enzymatic reaction of adenosine-5′-phosphate with fumaric acid. The aglycone of this compound, *N*-(6-purinyl)aspartic acid, was synthesized by Carter as part of the proof of structure.[3] Adenylosuccinic acid or the aglycone has subsequently been identified from a number of sources (yeast,[2] *E. coli*, mammalian liver, cod liver, human urine, and *Neurospora*[4]) and is also involved in other biological systems.[5,6]

The present study was undertaken to extend the series of *N*-(6-purinyl)amino acids started by Carter[3] and to make these compounds available in quantities sufficient for antitumor screening. Three methods of synthesis have been proposed.[7,8] Carter[3] utilized the reaction of 6-chloropurine with the free amino acid. In addition to the purine deriv-

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ative of aspartic acid mentioned above, he also prepared similar products from the reaction of 6-chloropurine with glutamic acid, glycine, cysteic acid, histidine, and lysine. The latter products were obtained in small quantities and were characterized with respect to their behaviour on paper chromatography and ultraviolet absorption spectrum. The work of Bendich *et al.*[9] made 6-chloropurine readily available. Carter's method has been used for all the compounds reported here, but with use of a carbonate-bicarbonate buffer to maintain the *p*H during the reaction. This buffer was chosen as acidification—*e.g.* with formic acid—replaces the anion with that to be used in subsequent chromatography.

The choice of *N*-(6-purinyl)amino acids as possible antimetabolites for cancer chemotherapy is speculative, although 6-substituted purines are known to inhibit some tumor cells.[10] Since our original communication[1a] two other groups have studied amino acid substituted purines. Lettré and Ballweg[11] have reported the preparation of the ω-purinyl derivative of a number of diamino acids, and also the *N*-(6-purinyl)derivatives of tryptophan and histidine. These authors also report the preparation of the 6-purinyl derivatives of a number of amines related to the natural amino acids. An extensive discussion of some of this work with a consideration of antitumor activity in tissue culture is presented in a report by Lettré.[12]

Ballio and Di Vittorio[13] have reported the synthesis of six of the compounds described below: namely, the *N*-(6-purinyl) derivative of glycine, alanine, β-alanine, α-aminobutyric acid, aspartic acid, and glutamic acid. The properties reported by these authors for the foregoing compounds are in good agreement with those reported below with minor exceptions as noted. While these workers dried their compounds over phosphorus pentoxide at temperature of 25° to 78° and obtained a material that analyzed as the monohydrate, our samples have been dried at 100° for forty-eight hours and often had analyses corresponding to the hemihydrate or dry product. The syntheses of Ballio and Di Vittorio[13] were carried out on a 100- to 200-mg. scale and the products were isolated by a chromatography quite similar to that which we have used. However, for those products which precipitate at *p*H 3 (see Experimental) the chromatographic purification fails when applied to larger quantities which we used, as the product precipitates in the column during formic acid elution.

The ultraviolet absorption spectra for all derivatives reported below were characteristically similar. Ballio and Di Vittorio[13] have published typical examples of the spectra of this type of compound. In basic solutions the spectra resemble those of adenine and hypoxanthine.[14]

Antitumor testing[1a] of the compounds described in the present report is not yet complete. All derivatives tested were tolerated in doses as high as 250 mg./kg. body weight/day. Of the compounds screened to date, only *N*-(6-purinyl)-L-glutamic acid has shown antitumor activity. This compound was active against Carcinoma 755, a mouse mammary tumor, as tested in the National Institutes of Health primary screening program. Tumor growth inhibition was a function of the log of the dose, being 7% at 31 mg./kg./day, and 91% at 250 mg./kg./day, with two intermediate dose levels tested.

The *N*-(6-purinyl)-DL-valine derivative gave a two-fold increase in the rate of tumor growth with carcinoma 755 when tested at dose levels ranging from 12 to 100 mg./kg./day. The response showed no dose dependence over this range.

EXPERIMENTAL[15]

The general procedure was as follows. The amino acid—*e.g.*, 0.032 mole—was dissolved in 25 ml. of water, the *p*H adjusted to 9, and 0.016 to 0.04 mole of sodium carbonate added. Warming after addition of the sodium carbonate dissolved the less soluble amino acids. The 6-chloropurine (0.016 mole) was added and the solution refluxed for 3 hr. If the *p*H exceeded 10.5 during the reaction a yellow by-product was obtained which was virtually insoluble in dilute alkali, acid, ethanol, or acetone. (Analysis of this material recrystallized from boiling ethyl acetate, m.p. 320°: C, 37.79; H, 3.05; N, 28.71.) As this material appeared to be the same regardless of the amino acid used as judged by melting point it is probably derived from a side-reaction of the 6-chloropurine.

At the end of the reflux period the *p*H of the solutions had usually decreased to about 8 to 8.8. Insoluble material, if present, were filtered. An estimate of the yield in the reaction may be obtained from the ultraviolet absorption at 270 mμ at *p*H 7. Such an estimate is invariably high by 5–10% in crude mixtures. The *p*H was adjusted to 3 with 88% formic acid. (In those cases where the amino was insoluble at the isoelectric point, part of the unchanged amino acid was removed by precipitation at *p*H near 6.) In a number of instances the purine-substituted amino acid precipitated as the acid form at *p*H 3–4.5 and was triturated with ether to remove formic acid and used directly for recrystallization from water or reprecipitation after redissolving at *p*H 8.0. In those instances where a precipitate was not obtained at *p*H 3–4.5, the mixture was passed over a Dowex-2-X8

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column (200–400 mesh) in the formate form (5.5 cm. × 4.5 cm. dia., 100 meq. capacity). The column was prepared according to the procedure of Hurlbert *et al.*[16] Unchanged amino acid, 6-chloropurine, and hypoxanthine were washed from the column with 0.1*M* formic acid at a flow rate of 150 to 180 ml./hr. (For syntheses involving acidic amino acids, 0.3*M* formic acid was used at this point, but subsequent elution of the product required such strong acid that decomposition was obtained; thus other methods were also applied for purification of the derivative of glutamic, aspartic, or cysteic acid.) All the amino acid and unchanged purine was washed from the column, as determined by a negative ninhydrin reaction[17] and no ultraviolet absorption at 270 mμ. (This usually required five column volumes.) Elution of the purinyl amino acid was then carried out with 0.3*M* or 0.6*M* formic acid. *N*-(6-Purinyl)-glutamic or *N*-(6-purinyl)aspartic acid required 2*N* formic acid, and *N*-(6-purinyl)cysteic acid required 4*N* formic acid for efficient elution. Elution was followed by ultraviolet absorption. The eluate containing the product was concentrated in a flash evaporator at a temperature of 35° or less, excess formic acid washed from the oily residue with ether, and the product recrystallized from water. The chromatography and concentration to dryness were carried out as rapidly as possible since all the desired compounds decompose in strong acid.

Purity of the products was checked by paper chromatography using the ammonium acetate–ethanol solvent of Carter[3] for one dimensional chromatograms and a two dimensional chromatogram with *n*-butyl alcohol–formic acid–water (15:3:2 parts by volume) as the first solvent and either the ammonia–isopropyl alcohol solvent of Carter[3] or a 2,4-lutidine-water (3:2 parts by volume) as the second solvent. In the case of the threonine, leucine and isoleucine derivatives, analytical samples were obtained by continuous flow electrophoresis in a *p*H 7.5, 0.01*M* ammonium acetate buffer at 1000 volts and 75 m.a., using a Spinco Model CP apparatus. The product was followed by its ultraviolet absorption. The appropriate tubes (toward the anode side) were pooled, concentrated, and lyophilized to remove most of the ammonium acetate, and finally recrystallized from water after adjusting the *p*H to 3 with formic acid.

The final product for all the syntheses reported below was a white powder or colorless crystals. In a number of instances a yellow-colored impurity proved very troublesome to remove. In such cases recrystallization from aqueous Methyl Cellosolve has proven most effective, and is probably the solvent of choice for recrystallization. The utility of this solvent was not discovered until many of the compounds had been prepared.

All samples were dried *in vacuo* at 100° over phosphorus pentoxide for 48 hr. prior to analysis. With less drying time the amount of water retained by the molecule appeared quite variable and microanalyses were not reproducible.

Ultraviolet absorption spectra were measured with a Beckman DK-2 recording spectrophotometer using solutions of 4×10^{-5} *M* in the following buffers: 0.1*M* glycine, *p*H 2.95; 0.1*M* phosphate, *p*H 7.0; and 0.1*M* carbonate, *p*H 10.1. Molar extinction coefficients were estimated from the spectra thus obtained.

Optical rotation was measured using 1 or 2% solutions, freshly prepared, in 3*N* hydrochloric acid or in 0.1*M* phosphate at *p*H 6.4 at 24°, 2 dm. tubes, sodium light source.

N-(6-Purinyl)-L-alanine. L-Alanine, 5.82 g. (0.068 mole), and 4.2 g. sodium carbonate (0.032 mole), were dissolved in 20 ml. of water, *p*H 9.6; 6-chloropurine, 5 g. (0.032 mole), was added and the solution refluxed for 3 hr. After cooling the *p*H was 8.75. Adjusting to *p*H 3.5 with formic acid precipitated 6.93 g. of crude product (105% of theoretical), which was filtered and washed with cold water. The crude product was worked up in 1-g. batches. (Heating for direct recrystallization from water decomposed the product as this compound proved very labile in acid.) The crude product, 1 g., was dissolved in 25 ml. of water by adjusting the *p*H to 9.0 with sodium hydroxide, 0.4 ml. of concd. hydrochloric acid was added followed by 50 ml. of Methyl Cellosolve to precipitate 0.80 g. (84% of theoretical) of the desired product, m.p. 228° dec. with effervescence. (Ballio and Di Vittorio[18] reported their product turned brown at 185° and melted at 235–237° dec.) The ultraviolet absorption spectra had, at *p*H 10.1, λ_{max} 270 mμ (log ε 4.21); at *p*H 7.0, λ_{max} 267 mμ (log ε 4.23); at *p*H 2.95, λ_{max} 273 mμ (log ε 4.21). In 3*N* hydrochloric acid the compound decomposed too rapidly to measure optical rotation; at *p*H 6.4, $[\alpha]_D^{24}$ +61.7°, *c* = 2.

Anal. Calcd. for $C_8H_9O_2N_5 \cdot {}^1/_2\ H_2O$: C, 44.44; H, 4.66; N, 32.40. Found: C, 45.12; H, 4.64; N, 31.33.

N-(6-Purinyl)-β-alanine. β-Alanine, 2.91 g. (0.032 mole), 6-chloropurine, 2.5 g. (0.016 mole), and sodium carbonate, 2.1 g. (0.018 mole), were refluxed in 25 ml. of water for 3.5 hr. The solution was then adjusted to *p*H 3 with formic acid to precipitate 3.3 g. (98% yield) of the crude product. Recrystallization from 290 ml. of boiling water after treatment with charcoal gave 3.13 g. of product which contained a trace of an impurity. Recrystallization once more gave 2.82 g. of chromatographically pure material; over-all yield, 95%; m.p., 246° dec. with effervescence. (Ballio and Di Vittorio[18] reported their product decomposed progressively between 200 and 230°.) The ultraviolet absorption spectra had, at *p*H 10.1, λ_{max} 272 mμ (log ε 4.22); at *p*H 7.0, λ_{max} 267 mμ (log ε 4.24); at *p*H 2.95, λ_{max} 271 mμ (log ε 4.21).

Anal. Calcd. for $C_8H_9O_2N_5 \cdot H_2O$: C, 42.66; H, 4.92; N, 31.10. Found: C, 42.54; H, 4.93; N, 31.17.

N-(6-Purinyl)-DL-α-aminobutyric acid. DL-α-Aminobutyric acid, 3.0 g. (0.032 mole), 6-chloropurine, 2.5 g. (0.016 mole), and sodium carbonate, 2.1 g. (0.018 mole), were mixed in 25 ml. of water and refluxed for 3 hr. The solution was adjusted to *p*H 3.5; a heavy precipitate formed at once; yield, 3.49 g. (97%) of crude product which decomposed with effervesence at 188°. The crude product was dissolved in 130 ml. of hot water, treated with approximately 100 mg. of charcoal and filtered hot. The filtrate crystallized at once; yield 2.22 g. (63%), 219–220° dec., with effervescence. The ultraviolet absorption spectrum had, at *p*H 10.1, λ_{max} 272 mμ (log ε 4.21); at *p*H 7.0, λ_{max} 268 mμ (log ε 4.22); at *p*H 2.95 λ_{max} 274 mμ (log ε 4.20).

Anal. Calcd. for $C_9H_{11}O_2N_5 \cdot {}^1/_2\ H_2O$: C, 46.95; H, 5.25; N, 30.42. Found: C, 47.64; H, 5.17; N, 30.46.

N-(6-Purinyl)-L-aspartic acid. L-Aspartic acid, 1.2 g. (0.009 mole), was dissolved in 35 ml. of water, the *p*H adjusted to 9.5 with potassium hydroxide, 6-chloropurine added, 2 g. (0.013 mole), and the solution refluxed for 3 hr. The resulting solution was stirred into 1 l. of absolute ethanol and stored overnight in a refrigerator. The supernate was decanted, the residue filtered and redissolved in 160 ml. of water. The *p*H was adjusted to 6.9 and the solution applied to a Dowex-2 column in the formate form, washed with 0.3*M* formic acid to remove unchanged amino acid, and the product eluted with 0.6*M* formic acid. The eluate was concentrated in a flash evaporator at 35°. Formic acid was removed by washing with ether, the product dissolved in a minimum volume of water, and lyophilized; Yield, 0.74 g. (32.4%); m.p., 139° dec. with effervescence. Attempts at further recrystallization from water or aqueous Methyl Cellosolve gave decomposition of the product. The ultraviolet absorption spectrum had, at *p*H 10.1, λ_{max} 271 mμ (log ε 4.26); at *p*H 7.0, λ_{max} 268 mμ (log ε 4.28); at *p*H 2.95, λ_{max} 273 mμ (log ε 4.26). $[\alpha]_D^{24}$ + 27.7, 3*N* hydrochloric acid $[\alpha]_D^{24}$ +61.4°, *p*H 6.4; *c* = 1.

Anal. Calcd. for $C_9H_9O_4N_5 \cdot {}^1/_2\ H_2O$: C, 41.51; H, 3.84; N, 26.90. Found: C, 42.30; H, 4.00; N, 26.78.

The product could also be precipitated from the original reaction mixture as the copper salt, which was decomposed with hydrogen sulfide. This product, obtained in yields of 60 to 90%, was always contaminated with unchanged amino

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acid (5 to 15% as estimated by the quantitative ninhydrin reaction[17]).

N-(6-Purinyl)-L-cysteic acid. L-Cysteic acid monohydrate, 6.05 g. (0.036 mole), and 5.0 g. of 6-chloropurine (0.032 mole) were dissolved in 20 ml. of water and the *p*H adjusted to 9.5 with 30% potassium hydroxide. The solution was refluxed for 1.3 hr. Refluxing was discontinued when the reaction mixture began turning a dark yellow; final *p*H 4.9. The *p*H was raised to 7.0 with potassium hydroxide and 107 ml. of 0.3*M* cupric acetate was added. The copper salt was allowed to precipitate overnight in the cold. The resulting gelatinous precipitate was collected on a Buchner funnel, suspended in 100 ml. of water, and the copper salt decomposed by bubbling hydrogen sulfide through the solution while stirred with a magnetic stirrer. The cupric sulfide was removed by centrifugation and remaining hydrogen sulfide swept out of the supernate with a stream of nitrogen over a period of 2 hr. The solution must be kept cool at this point or the product will decompose. (Reprecipitation of a portion of the product as the copper or cadmium salt did not improve the product, as judged by paper chromatography after decomposition of the salt with hydrogen sulfide.) The supernate was freeze-dried to obtain the crude product as a pale yellow material which was very hygroscopic. The ultraviolet absorption prior to freeze-drying indicated a 51% yield (approximately 4.7 g.) of the desired product. Portions of this sample were tried with various purification procedures involving adsorption and elution from weak or strong base anion exchange resins. Only small portions may be worked up with sufficient speed to avoid decomposition in solution because of the highly acidic nature of the product itself.

The crude product, 250 mg., was dissolved in 1.5 ml. of water and 20 ml. Methyl Cellosolve was added at once, to precipitate 131 mg. (52% yield) of the desired product as a white powder; m.p., darkened at 278°, decomposed at 288–289° with effervescence. The ultraviolet absorption spectra had, at *p*H 10.1, λ_{max} 270 mμ (log ε 4.18); at *p*H 7.0, λ_{max} 267 mμ (log ε 4.18); at *p*H 2.95, λ_{max} 275 mμ (log ε 4.18). $[\alpha]_D^{24}$ −27.4°, 3*N* hydrochloric acid; $[\alpha]_D^{24}$ −8.6, *p*H 6.4; $c = 1$.

Anal. Calcd. for $C_8H_9O_5N_5S$: C, 33.46; H, 3.16; N, 24.39; S, 10.85. Found: C, 33.52; H, 3.20; N, 24.28; S, 10.71.

N-(6-Purinyl)-DL-ethionine. DL-Ethionine, 10.6 g. (0.065 mole), in 50 ml. of water was adjusted to *p*H 9 with sodium hydroxide; 5 g. of 6-chloropurine (0.032 mole) and sodium carbonate, 4.2 g. (0.036 mole), were added to bring the *p*H to 9.7. The solution was refluxed for 3 hr. and cooled (final *p*H 8.6). The mixture was adjusted to *p*H 3 with formic acid. The crude product precipitated at once, 8.62 g. (95%). This was recrystallized from hot water (approximately 75 mg./ml.) in 77% yield (6.63 g.). Three-fold concentration of the filtrate with reduced pressure gave a second crop, an additional 15% yield. Both products decomposed at 218–219° with evolution of gas. The absorption spectra had, at *p*H 10.1, λ_{max} 271 mμ (log ε 4.23); at *p*H 7.0, λ_{max} 268 mμ (log ε 4.25); at *p*H 2.95, λ_{max} 275 mμ (log ε 4.24).

Anal. Calcd. for $C_{11}H_{15}O_2N_5S \cdot H_2O$: C, 44.14; H, 5.73; N, 23.4 ; S, 10.69. Found: C, 44.25; H, 5.50; N, 23.20; S, 10.61.

N-(6-Purinyl)-L-glutamic acid. L-Glutamic acid, 9.56 g. (0.065 mole), and sodium carbonate (12.1 g., 0.1 mole) were dissolved in 50 ml. of water, 6-chloropurine (5.0 g., 0.032 mole) was added, and the solution refluxed 3 hr. After cooling the *p*H was adjusted to 3 with formic acid. The solution was reduced to 3 ml. on a flash evaporator at 35°. A precipitate formed overnight in the cold (10 g.), which contained 31% unchanged glutamic acid, estimated by the ninhydrin reaction,[17] and 85% of the desired product as estimated by the ultraviolet absorption. This crude material was purified in two ways: (Method 1) Crude product (1 g.) was leached with 50 ml. of water, stirred for 6 hr., and cooled overnight. The insoluble portion, 0.54 g. (64%), was the desired product. An additional 0.17 g. (20%) of product was obtained by concentrating the filtrate to 25 ml. Recrystallization from boiling water gave analytically pure material, m.p. 204° dec. with effervescence. Attempted further recrystallization decomposed the product. (Method 2) The crude product (3.5 g.) was dissolved in 50 ml. of water, the *p*H adjusted to 6.8 with sodium hydroxide, and the solution added to a Dowex-2 formate column. Ninhydrin positive material was washed from the column with 0.3*M* formic acid. The desired product was eluted with 1*M* formic acid. The fractions showing absorption in the ultraviolet were quickly pooled and dried in a flash evaporator. The product was dissolved in 275 ml. of warm ethanol and ether was added until the solution became cloudy; 1 g. of product (40% based on ultraviolet absorption from the column) precipitated in the cold. This fraction contained a trace of a fluorescent impurity detected by paper chromatography. The supernate was concentrated and lyophilized to yield 1.5 g. (60%) of the desired product, m.p. 211° dec. with effervescence. The ultraviolet absorption had, at *p*H 10.1, λ_{max} 272 mμ (log ε 4.21); at *p*H 7.0, λ_{max} 268 mμ (log ε 4.23); at *p*H 2.95, λ_{max} 273 mμ (log ε 4.22). $[\alpha]_D^{24}$ −11.2°, 3*N* hydrochloric acid; $[\alpha]_D^{24}$ +27.9°, *p*H 6.4; $c = 1$.

Anal. Calcd. for $C_{10}H_{11}O_4N_5 \cdot 1/2\ H_2O$: C, 43.79; H, 4.41; N, 25.54. Found: C, 43.61; H, 4.44; N, 25.24.

N-(6-Purinyl)-D-glutamic acid. D-Glutamic acid (4.78 g., 0.032 mole), and sodium carbonate (6.87 g., 0.05 mole), were dissolved in 35 ml. of water, *p*H 9.2. To this was added 2.5 g. (0.016 mole) of 6-chloropurine. The solution was refluxed for 3 hr. and cooled (final *p*H 9.9); yield, 79%, estimated by ultraviolet absorption. The *p*H was adjusted to 3 and the solution taken to dryness. One-third of the total mixture was leached with 7 ml. of water to remove salt and free amino acid. The residue, containing principally the desired product, was dissolved in 300 ml. of water (*p*H 4.5) and passed over an Amberlite CG-50 cation exchange column (3 × 3 cm., acid form). A yellow-colored impurity was retained by the column and the product washed off with 500 ml. of water. The eluate contained 98% of the ultraviolet absorbing material applied to the column. The combined fractions were concentrated to 10 ml. to obtain 0.19 g. of the desired product, which was used for further characterization. The filtrate was dried by lyophilization to obtain 1.54 g. of impure product (30% free amino acid contaminant). The purified material decomposed at 209° with effervescence. The ultraviolet absorption had, at *p*H 10.1, λ_{max} 271 mμ (log ε 4.21); at *p*H 7.0, λ_{max} 268 mμ (log ε 4.23); at *p*H 2.95, λ_{max} 273 mμ (log ε 4.22). $[\alpha]_D^{24}$ +15.3°, 3*N* hydrochloric acid; $[\alpha]_D^{24}$ −27.3°, *p*H 6.4.

Anal. Calcd. for $C_{10}H_{11}O_4N_5 \cdot 1/2\ H_2O$: C, 43.79; H, 4.41; N, 25.54. Found: C, 44.15; H, 4.24; N, 25.60.

N-(6-Purinyl)glycine. Glycine, 4.86 g. (0.064 mole), 6-chloropurine, 5.0 g. (0.032 mole), and sodium carbonate, 4.2 g. (0.036 mole) were combined in 50 ml. of water and refluxed. After the first hour a precipitate began to form which became so heavy that the refluxing was ended after 2 hr. The final *p*H was 9.1. An additional 50 ml. of water was added and the *p*H adjusted to 3 with formic acid. The precipitate was stirred for 0.5 hr. prior to filtration; crude yield, 6.15 g. (98%). The product was recrystallized, in approximately 2-g. batches, from 1 l. of boiling water. A second recrystallization gave a product chromatographically pure, 4.68 g. (76%). In another experiment using one-half the above quantities but omitting the sodium carbonate, the initial *p*H was adjusted to 9.5 with sodium hydroxide. After 3 hr. of refluxing the *p*H had fallen to 4. At *p*H 3 a crude product (59% yield) was precipitated. This product was yellow. This suggests an advantage to maintaining the *p*H near 9 during reaction. The recrystallized product from either set of reaction conditions did not melt below 325° but browned slowly above 265°. The absorption spectra had, at *p*H 10.1, λ_{max} 272 mμ, (log ε 4.23); at *p*H 7.0, λ_{max} 267 mμ (log ε 4.24); at *p*H 2.95, λ_{max} 271 mμ (log ε 4.23).

Anal. Calcd. for $C_7H_7O_2N_5 \cdot 1/2\ H_2O$: C, 41.58; H, 3.99; N, 34.65. Found: C, 42.21; H, 4.04; N, 34.14.

N-(6-Purinyl)-L-isoleucine. L-Isoleucine (allo-free), 4.26 g. (0.032 mole), in 25 ml. of water was adjusted to *p*H 9 with sodium hydroxide; 0.018 mole of sodium carbonate (final *p*H

9.4), and 6-chloropurine, 2.5 g. (0.016 mole) were added. The solution was refluxed for 3 hr., cooled, and the *p*H adjusted to 3.0 with formic acid. Precipitation began at *p*H 3.8; yield, 4.6 g. (98%) of crude white product containing traces of free amino acid. The product redissolved very slowly in hot water; longer heating turned the material yellow. An analytically pure sample was obtained by continuous flow electrophoresis with *p*H 7.5, 0.01*M* ammonium acetate buffer. From 1.0 g. of crude product, the desired fraction was dried *in vacuo* after electrophoresis, dissolved in a minimum volume of water, and precipitated at *p*H 3 to recover 0.5 g. (50%); m.p., 232° dec. with effervescence. The absorption spectra had, at *p*H 10.1, λ_{max} 272 mμ (log ε 4.25); at *p*H 7.0, λ_{max} 269 mμ (log ε 4.25); at *p*H 2.95, λ_{max} 275 mμ (log ε 4.23). $[\alpha]_D^{24}$ +16.0°, 3*N* hydrochloric acid; $[\alpha]_D^{24}$ +52.0°, *p*H 6.4; *c* = 1.

Anal. Calcd. for $C_{11}H_{15}O_2N_5$: C, 53.00; H, 6.07; N, 28.10. Found: C, 53.03; H, 6.20; N, 28.06.

*N-(6-Purinyl)-*L*-leucine.* L-Leucine, 4.24 g. (0.032 mole), was dissolved in 25 ml. of water by adjusting to *p*H 9 with sodium hydroxide; 2.1 g. (0.018 mole) of sodium carbonate (final *p*H 9.4), and 6-chloropurine (2.5 g., 0.016 mole) were added. The mixture was refluxed for 3 hr., cooled, and adjusted to *p*H 3. At *p*H 4.0 precipitation began but the material redissolved at *p*H 3.0. The *p*H was raised to 3.75, where precipitation was maximal, and the solution placed overnight in the cold; yield, 3.73 g. (93.5%) crude product, containing traces of leucine and a material that fluoresced under ultraviolet light after paper chromatography. The material redissolved slowly in hot water and on longer heating turned yellow. An analytically pure sample was obtained by continuous flow electrophoresis of 0.89 g. of crude product in *p*H 7.5 ammonium acetate buffer (0.01*M*). The desired fraction was dried *in vacuo*, dissolved in a minimum volume of water and precipitated at *p*H 3.7; yield, 0.21 g. (24%). (An undetermined portion was lost due to failure of the electrophoresis equipment during the run.), m.p., 227° dec. with evolution of gas. The absorption spectra had, at *p*H 10.1, λ_{max} 272 mμ (log ε 4.26); at *p*H 7.0, λ_{max} 268 mμ (log ε 4.27); at *p*H 2.95, λ_{max} 275 mμ (log ε 4.25); $[\alpha]_D^{24}$ −32.1°, 3*N* hydrochloric acid; $[\alpha]_D^{24}$ +27.8°, *p*H 6.4; *c* = 1.

Anal. Calcd. for $C_{11}H_{15}O_2N_5$: C, 53.00; H, 6.07; N, 28.10. Found: C, 53.09; H, 5.59; N, 27.49.

*Nα-(6-Purinyl)-Nε-tosyl-*L*-lysine.* Nε-Tosyl-L-lysine (I) was prepared by the method of Roeske *et al.*[18] To 1.95 g. of I (0.006 mole) in 25 ml. of water, adjusted to *p*H 9.0, were added 1.0 g. of sodium carbonate (0.008 mole) and 1.0 g. (0.006 mole) of 6-chloropurine, and the mixture was refluxed for 3 hr. The *p*H was adjusted to 3 with formic acid to precipitate 2.04 g. of crude product overnight in the cold (yield, 79%), m.p. 220–221° dec., with effervescence. The crude product (0.96 g.) was dissolved in 1.3 l. of hot water, treated with charcoal, and cooled. Overnight, 0.44 g. (46%) of the desired product was obtained; concentration of the filtrate gave an additional 0.22 g. (total 69%). Both products melted at 224–225° dec. with effervescence. The absorption spectra had, at *p*H 10.1, λ_{max} 272 mμ (log ε 4.22); at *p*H 7.0, λ_{max} 268 mμ (log ε 4.23); at *p*H 2.95, λ_{max} 273 mμ (log ε 4.18); $[\alpha]_D^{24}$ −11.9°, 3*N* hydrochloric acid; $[\alpha]_D^{24}$ + 11.4°, *p*H 6.4; *c* = 1.

Anal. Calcd. for $C_{18}H_{22}O_4N_6S$: C, 51.67; H, 5.30; N, 20.09; S, 7.64. Found: C, 51.58; H, 5.41; N, 20.04; S, 7.48.

*N-(6-Purinyl)-*DL*-methionine.* DL-Methionine, 9.2 g. (0.065 mole) in 50 ml. of water was adjusted to *p*H 9 with sodium hydroxide; 0.08 mole of sodium carbonate (final *p*H 9.7) and 5 g. (0.032 mole) of 6-chloropurine were added. The mixture was refluxed for 3.5 hr., cooled (final *p*H 8.5), and the *p*H adjusted to 3 with formic acid. The product precipitated at once (8.46 g., 97% yield). This was dissolved in 420 ml. of hot water, treated with approximately 200 mg. of charcoal, filtered, and 6.54 g. of product (77%) recovered; m.p., 233–236° dec., with effervescence. The product was recrystallized once more from hot water; yield 5.74 g. (86%), m.p., 227–228° dec. with effervescence. The ultraviolet absorption spectra had, at *p*H 10.1, λ_{max} 272 mμ (log ε 4.22); at *p*H 7.0, λ_{max} 268 mμ (log ε 4.24); at *p*H 2.95, λ_{max} 275 mμ (log ε 4.18).

Anal. Calcd. for $C_{10}H_{13}O_2N_5S$: C, 44.94; H, 4.90; N, 26.21; S, 11.97. Found: C, 45.10; H, 4.99; N, 26.17; S, 12.02.

*N-(6-Purinyl)-*DL*-norleucine.* DL-Norleucine, 4.24 g. (0.032 mole), was suspended in 25 ml. of water and the *p*H adjusted to 9.2 with sodium hydroxide; 2.1 g. (0.018 mole) of sodium carbonate raised the *p*H to 9.8. To this, 2.5 g. (0.016 mole) of 6-chloropurine was added. The amino acid did not dissolve until refluxing began; the mixture was refluxed for 3 hr. The solution was cooled (*p*H 9.4); a precipitate (norleucine) formed and was removed. The mixture was adjusted to *p*H 3 to precipitate 4.07 g. (100%) of crude product contaminated with amino acid. The crude product was dissolved in 1.8 l. of hot water, treated with charcoal, and 2.29 g. of crystalline product (56%) was obtained on cooling. (An additional 0.89 g. (22%) was obtained after chromatography of the supernate on Dowex-2.) Recrystallization from water gave an analytically pure sample, m.p., 239–240° dec. with effervescence. The ultraviolet absorption spectra had, at *p*H 10.1, λ_{max} 272 mμ (log ε 4.21); at *p*H 7.0, λ_{max} 268 mμ (log ε 4.26); at *p*H 2.95, λ_{max} 275 mμ (log ε 4.20).

Anal. Calcd. for $C_{11}H_{15}O_2N_5 \cdot {}^1/_2\ H_2O$: C, 51.15; H, 6.24; N, 27.12. Found: C, 51.88; H, 6.05; N, 27.66.

*N-(6-Purinyl)-*DL*-phenylalanine.* DL-Phenylalanine, 10.7 g (0.064 mole), was suspended in 50 ml. of water, adjusting the *p*H to 9.0 with sodium hydroxide; 4.2 g. (0.036 mole) of sodium carbonate (giving a *p*H of 9.4), and 6-chloropurine (5.0 g., 0.032 mole) were added. The mixture was refluxed for 3 hr. The mixture was cooled and adjusted to *p*H 4.5 with formic acid. A sticky, yellow-green precipitate formed. After standing, the supernate was decanted, and the precipitate converted to a powder by trituration with ether; yield, 6.12 g. (67%) of crude product. The supernate was adjusted to *p*H 3 and a second precipitate formed on standing overnight in the cold; yield, 4.90 g. of which approximately 3.2 g. was unchanged phenylalanine. (The ultraviolet absorption spectrum of the original reaction mixture indicated a yield of 87.6%; 95% of this quantity was obtained in the two precipitates.) Analytically pure material was obtained by one recrystallization from hot water with either of the precipitates using small batches to avoid prolonged heating during solution. Too much heating of the acid form of the product gave decomposition to a yellow material. For recrystallization, from 3 to 4 mg. of crude product per ml. of water gave approximately 70% recovery of pure product; m.p., 166° dec. with effervescence. A second recrystallization in a number of instances gave a product that decomposed at 160–162° with evolution of gas. No difference could be detected chromatographically after one or two recrystallizations. The ultraviolet absorption spectra had, at *p*H 10.1, λ_{max} 272 mμ (log ε 4.18); at *p*H 7.0, λ_{max} 268 mμ (log ε 4.23); at *p*H 2.95, λ_{max} 275 mμ (log ε 4.17).

Anal. Calcd. for $C_{14}H_{13}O_2N_5$: C, 59.35; H, 4.63; N, 24.72. Found: C, 59.26; H, 4.74; N, 24.61.

*N-(6-Purinyl)-*L*-proline.* L-Proline, 3.74 g. (0.032 mole) and 2.0 g. of sodium carbonate (0.016 mole) were dissolved in 30 ml. of water to give a *p*H of 9.95; 6-chloropurine, 2.5 g. (0.016 mole), was added and the solution refluxed for 3 hr., final *p*H, 8.0. The solution was adjusted to *p*H 3.5 with formic acid to remove bicarbonate; then the *p*H was raised to 6.5 with sodium hydroxide and the solution diluted to 200 ml. with water. The solution was applied to a Dowex 2-formate column, and the unchanged amino acid, 6-chloropurine and a by-product which had a yellow fluorescence under ultraviolet light were eluted from the column with 0.1 *M* formic acid. The desired product was then eluted with 0.6 *M* formic acid. After removal of formic acid and freeze-drying, 3.03 g. (80%) of product was obtained; m.p., 173° dec. with effervescence. The ultraviolet absorption spectra

(18) R. Roeske, F. H. C. Stewart, R. J. Stedman, and V. du Vigneaud, *J. Am. Chem. Soc.*, **78**, 5883 (1956).

had, at *p*H 10.1, λ_{max} 279 mμ (log ε 4.22); at *p*H 7.0, λ_{max} 270 mμ (log ε 4.24); at *p*H 2.95, λ_{max} 278 mμ (log ε 4.16). $[\alpha]^{24}_{D}$ −123.3°, 3*N* hydrochloric acid; $[\alpha]^{24}_{D}$ −88.3°, *p*H 6.4; *c* = 1.

Anal. Calcd. for $C_{14}H_{11}O_2N_5$: C, 51.49; H, 4.75; N, 30.03. Found: C, 51.10; H, 5.00; N, 29.43.

*N-(6-Purinyl)-*DL*-serine.* DL-Serine, 3.42 g. (0.032 mole) with 2.1 g. (0.018 mole) of sodium carbonate in 25 ml. of water gave *p*H 9.2; 6-chloropurine (2.5 g., 0.016 mole) was added and the solution refluxed for 3 hr. After cooling the final *p*H was 8.8. The solution was adjusted to *p*H 3 with formic acid and cooled overnight; 2.88 g. (79.6%) of the crude product was obtained. This was recrystallized from hot water (650 ml.), yield, 2.3 g. (80%). A second recrystallization from 250 ml. of boiling water gave 1.82 g. of product (79%); m.p. 219–221° dec. with effervescence. The ultraviolet absorption spectra had, at *p*H 10.1, λ_{max} 270 mμ (log ε 4.19); at *p*H 7.0, λ_{max} 267 mμ (log ε 4.21); at *p*H 2.95, λ_{max} 274 mμ (log ε 4.19).

Anal. Calcd. for $C_8H_9O_3N_5$: C, 43.05; H, 4.06; N, 31.38. Found: C, 43.12; H, 4.07; N, 31.32.

*N-(6-Purinyl)-*DL*-threonine.* DL-Threonine, 3.88 g. (0.032 mole), and sodium carbonate, 2.01 g. (0.016 mole), were dissolved in 25 ml. of water and 6-chloropurine was added, 2.5 g. (0.016 mole). The solution was refluxed for 3 hr. No precipitate was obtained after adjusting to *p*H 3 with formic acid. The solution was readjusted to *p*H 6.8 with sodium hydroxide, the solution diluted 200 ml. and applied to a Dowex-2 formate column. The column was washed with 0.1*M* formic acid until all ninhydrin positive material was removed (650 ml.). The product was gradually eluted with 0.3*M* formic acid (650 ml.) and finally 0.6*M* formic acid (300 ml.) to elute remaining product. The 0.3 and 0.6*M* eluates were combined and taken to dryness in a rotary evaporator. An additional 50 ml. of water was added and the sample taken to dryness; this was repeated three more times to remove most of the formic acid. The product was washed to a lyophilizing flask. After freeze-drying, 3.0 g. (89%) of pure product was obtained; m.p. 130° dec. with effervescence. After drying at 100° for 48 hr., the m.p. was 161° dec. with effervescence. The material with the lower melting point had an analysis corresponding to the monohydrate after drying over phosphorus pentoxide *in vacuo* for 48 hr. at room temperature; it was dried at 100° for 48 hr. and the analysis shown below was obtained. The ultraviolet absorption spectra had, at *p*H 10.1, λ_{max} 271 mμ (log ε 4.19); at *p*H 7.0, λ_{max} 267 mμ (log ε 4.23); at *p*H 2.95, λ_{max} 275 mμ (log ε 4.19).

Anal. Calcd. for $C_9H_{11}O_3N_5$: C, 45.57; H, 4.67; N, 29.53. Found: C, 45.63; H, 4.79; N, 29.47.

*N-(6-Purinyl)-*DL*-valine.* DL-Valine, 3.8 g. (0.032 mole) was suspended in 50 ml. of water and the *p*H adjusted to 9 with sodium hydroxide. Sodium carbonate, 2.1 g. (0.018 mole), giving *p*H 9.7, and 6-chloropurine, 2.5 g. (0.016 mole), were added. The mixture was refluxed for 3 hr. and cooled; final *p*H, 8.35. The *p*H was adjusted to 3 with formic acid; a precipitate formed at once. After standing overnight in the cold, 2.74 g. of crude product was collected (73% yield); 2.3 g. was recrystallized from hot water, giving 1.07 g. (46%) of the desired product; m.p. 225–226° dec. with effervescence. The ultraviolet absorption spectra had, at *p*H 10.1, λ_{max} 273 mμ (log ε 4.20); at *p*H 7.0, λ_{max} 269 mμ (log ε 4.26); at *p*H 2.95, λ_{max} 275 mμ (log ε 4.18).

Anal. Calcd. for $C_{10}H_{13}O_2N_5$: C, 51.05; H, 5.57; N, 29.77. Found: C, 50.83; H, 5.80; N, 29.51.

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[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

Thyroformic Acids. II

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The syntheses of methoxylated analogs of 3,5,3′,5′-tetraiodothyroformic acid are reported, as well as some novel side reactions encountered in several of the syntheses.

The first paper of this series[1] dealt with the chemistry of thyroformic acids in which at least some of the iodines of the parent substance, tetraiodothyroformic acid, were replaced with methyl groups. The present report is a continuation of this study in which the replacing group is an alkoxyl function. In general the iodine substitution is in the benzoic acid ring and the alkoxyl substitution is in the phenoxy ring.

The method of synthesis employed parallels that of the literature.[2] This involved, as a general rule, the condensation of a sulfonate ester of ethyl 3,5-dinitro-4-hydroxybenzoate (II) with a phenol (I) in pyridine solution and subsequent conversion of the nitro groups into amino and then into iodo substituents as indicated in Fig. 1. In the condensations to make III in which tosyl chloride was employed and the reaction mixture was heated to boiling, brown fumes were eliminated, and the product was difficult to purify. Although the actual nature of this side reaction was not investigated, the generation of nitrous acid fumes suggested that the phenol I was also replacing one of the nitro groups. This is analogous to the experience of Loudon and McCapra[3] with 2,4-dinitro-6-benzoyl-2′-hydroxydiphenyl ether, which at high temperature in pyridine forms a dibenzodioxadiene. The condensations with either tosyl or mesyl chloride were therefore heated only to steam-bath temperature. The products obtained in the couplings are listed in Table I in the Experimental section. All the products were purified easily except the one from 2,6-dimethoxyhydroquinone. Initially,

(1) E. Van Heyningen, *J. Org. Chem.*, **26**, 3850 (1961).

(2)(a) E. T. Borrows, J. C. Clayton, and B. A. Hems, *J. Chem. Soc.*, S185, S199 (1949); E. T. Borrows, J. C. Clayton, B. A. Hems, and A. G. Long, *J. Chem. Soc.*, S190 (1949). (b) R. I. Meltzer, D. M. Lustgarten, and A. Fischmann, *J. Org. Chem.*, **22**, 1577 (1957).

(3) J. D. Loudon and F. McCapra, *J. Chem. Soc.*, 1899 (1959).