point with an authentic specimen.[20] In an analogous manner, the reaction of *s*-triazine with *p*-nitrophenylhydrazine resulted in *p*-nitrophenylazo-formaldehyde *p*-nitrophenylhydrazone (XIVc); yield 67%. After one recrystallization from benzene, reddish brown crystals, sintering at 94–95° and melting then slowly up to 104–105°, were obtained. They were identical with a sample prepared according to literature.[20]

A mixture of 2,4,6-trimethyl-*s*-triazine (Ia, 1.0 g.) and phenylhydrazine (5.3 g.) after heating for 30 minutes at 150–190° evolved ammonia and then turned deep red. From this crude product isolation of the **N,N'-diphenyl-C-methylformazan**, XVId (phenylazo-acetaldehyde phenylhydrazone) proceeded as described for XVIa. After recrystallization from ethanol the red, glittering needles of XVId (2.3 g.) melted at 122–123°. This melting point was not depressed upon mixing with an authentic sample.[21] With 2,4,6-triphenyl-*s*-triazine (Ib, 5.0 g.) and phenylhydrazine (10.5 g.) the reaction did not proceed to completion, even after two hours at 190°. After cooling the deep red solid obtained could be separated by extraction with boiling ethanol. The insoluble part (4.0 g.) was unreacted Ib, while the desired **N,N'-diphenyl-C-methylformazan**, XVIe, (phenylazo-benzaldehyde phenylhydrazone) crystallized from the concentrated alcoholic extracts (1.4 g.), m.p. 174–175°. The identity of XVIe was established by a mixed melting point with an authentic sample.[22]

(20) M. Busch and W. Wolbring, *J. prakt. Chem.*, [2] **71**, 374 (1905).
(21) E. Bamberger and W. Pemsel, *Ber.*, **36**, 87 (1903).
(22) H. v. Pechmann, *ibid.*, **27**, 1690 (1894).

3-Hydroxy-1,2,4-triazole (XVIII).—A vigorous reaction with evolution of ammonia occurred when a mixture of *s*-triazine (2.9 g.) and semicarbazide (XVII) (8 g.) in a flask was immersed into an oil-bath preheated to 100°. The mixture thereafter was heated for five minutes more at 120° and then allowed to cool. The resulting yellowish resin was extracted with hot ethanol and decolorized with charcoal. After evaporation to dryness the remaining crystalline XVIII (1.7 g., 18.7%) was recrystallized from alcohol, m.p. 234–235°.

3-Mercapto-1,2,4-triazole (XX) was obtained analogously from thio-semicarbazide (XIX) and *s*-triazine at a reaction temperature of 190°. The crude material (63.4%) was recrystallized from water, m.p. 215–216°.

3-Amino-1,2,4-triazole (XXII).—Free aminoguanidine (XXI) was prepared from the sulfate with an equivalent amount of barium hydroxide in aqueous solution, filtering off the precipitated barium sulfate and evaporating the filtrate to dryness *in vacuo*. The remaining crystalline free base is not stable. It soon turned red and must be used immediately. Its reaction with *s*-triazine at 210° as described above yielded 3-amino-1,2,4-triazole (47.4%), m.p. 158–159°, after recrystallization from ethyl acetate.

The compounds XVIII, XX and XXII were identified by mixed melting points with authentic samples prepared according to the literature.[23]

(23) O. Widmann and A. Cleve, *ibid.*, **31**, 379 (1898); M. Freund and C. Meinecke, *ibid.*, **29**, 2484 (1896); J. Thiele and W. Manchot, *Ann.*, **303**, 45 (1898).

COLUMBUS 10, OHIO

[CONTRIBUTION FROM THE CLAYTON FOUNDATION FOR RESEARCH, THE BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS]

Synthesis and Biological Activity of Some 6-(Substituted)-aminopurines

BY CHARLES G. SKINNER, PETE D. GARDNER AND WILLIAM SHIVE

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Thirteen new 6-(substituted)-aminopurines have been synthesized and tested for biological activity in two assay systems. Some compounds which possessed the greatest activity in inhibition of hydra regeneration (*e.g.*, 6-(4-cyclohexylbutylaminopurine)) had relatively little effect on the rate of germination of lettuce seed (Early Curled Simpson); and those compounds (*e.g.*, 6-(α-naphthylmethyl)-aminopurine)) which possessed the greatest seed germination effect had relatively little activity in the hydra regeneration study.

The synthesis, and some biological activities, of several 6-(substituted)-purines has recently been reported from these laboratories.[1–4] In an effort to examine the relationship between biological activity and structure of the substituent group in the 6-positions of the purine nucleus, additional compounds have been prepared and are reported in this paper in conjunction with their biological activity in two assay systems.

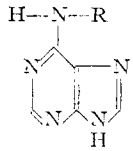
6-(2-Furfuryl)-aminopurine (kinetin) has received considerable attention since it was reported by Miller, *et al.*,[5,6] to be a cell division factor in tobacco "wound" callus tissue. More recently, Miller has reported a stimulation of lettuce seed germination with kinetin, 6-benzylaminopurine and 6-hexylaminopurine,[7] and we have also observed such effects with various 6-(substituted)-thiopurine derivatives.[8] Liverman has observed an effect with both kinetin and several other adenine analogs in a leaf disk expansion type assay,[9] and the effects of some fifty 6-(substituted)-purines have been studied by Eakin, *et al.*, in these laboratories for their effect upon moss budding[10] and hydra tentacle regeneration.[2–4] More recently, a bacteriological assay for these purine analogs has been developed.[11] While it appears that no overall statement may be made with respect to most active compound(s) in the separate assay systems, a particular homolog is frequently the most active in more than one of the biological assays.

In the present study, differences in activity of some new 6-(substituted)aminopurines (Table I) in two different assay systems have been observed.

(1) C. G. Skinner and William Shive, THIS JOURNAL, **77**, 6692 (1955).
(2) R. G. Ham, R. E. Eakin, C. G. Skinner and W. Shive, *ibid.*, **78**, 264 (1956).
(3) C. G. Skinner, R. G. Ham, D. C. Fitzgerald, R. E. Eakin and W. Shive, *ibid.*, **78**, 5097 (1956).
(4) C. G. Skinner, R. G. Ham, D. C. Fitzgerald, R. E. Eakin and W. Shive, *J. Org. Chem.*, **21**, 1330 (1956).
(5) C. O. Miller, F. Skoog, M. H. von Saltza and F. M. Strong, THIS JOURNAL, **77**, 1392 (1955).
(6) C. O. Miller, F. Skoog, F. S. Okumura, M. H. Von Saltza and F. M. Strong, *ibid.*, **77**, 2662 (1955).
(7) C. O. Miller, *Plant Phys.*, **31**, 318 (1956).
(8) C. G. Skinner, J. R. Claybrook, F. D. Talbert and W. Shive, *Arch. Biochem. and Biophys.*, **65**, 567 (1956).
(9) R. A. Scott, Jr., and J. L. Liverman, *Plant Phys.*, **31**, 321 (1956).
(10) B. S. Gorton, C. G. Skinner and R. E. Eakin, *Arch. Biochem. and Biophys.*, **66**, 493 (1957).
(11) E. M. Lansford, Jr., C. G. Skinner and W. Shive, *Arch. Biochem. and Biophys.*, accepted for publication, 1957.

TABLE I

6-(SUBSTITUTED)-AMINOPURINES



R	Reaction conditions: Time, hr.	Temp., °C.	Solvent of recrystn.	Yield, %	M.p., °C. dec.	Empirical formula	Analyses, % Calculated: Carbon	Hydrogen	Found: Carbon	Hydrogen
$C_6H_5(CH_2)_7$-	16	140	H_2O (H^+)	54	112–113	$C_{18}H_{23}N_5$	69.87	7.49	69.99	7.56
$C_6H_5(CH_2)_{11}$-	15	130	EtOH–H_2O	33	[a]	$C_{22}H_{31}N_5$	72.29	8.55	72.34	8.51
α-$C_{10}H_7(CH_2)$-	18	130	EtOH	48	258–259	$C_{16}H_{13}N_5$	69.80	4.76	70.02	4.61
α-$C_{10}H_7(CH_2)_5$-	19	130	EtOH	56	158–160	$C_{20}H_{21}N_5$	72.48	6.39	72.70	6.46
$C_6H_5O(CH_2)_2$-	18	140	EtOH	50	246–248	$C_{13}H_{13}N_5O$	61.16	5.13	60.86	5.23
$C_6H_5O(CH_2)_4$-	16	130	EtOH	35	156–158	$C_{15}H_{17}N_5O$	63.58	6.05	63.75	5.96
$C_6H_5OCHCH_2$- (CH$_3$)	19	140	EtOH–ether	35	172–173	$C_{14}H_{15}N_5O$	62.43	5.61	62.79	5.57
$C_6H_{11}CH_2$-	18	140	Ether	75	219–222	$C_{12}H_{17}N_5$	62.31	7.41	62.74	7.71
$C_6H_{11}(CH_2)_2$-	19	140	Ether	90	243	$C_{13}H_{19}N_5$	63.64	7.81	63.36	7.48
$C_6H_{11}(CH_2)_3$-	17	140	EtOH	42	189–190	$C_{14}H_{21}N_5$	64.83	8.16	65.02	8.23
$C_6H_{11}(CH_2)_4$-	18	130	EtOH–H_2O	63	187–188	$C_{15}H_{23}N_5$	65.90	8.47	65.62	8.14
$C_6H_{11}(CH_2)_5$-	17	130	EtOH–H_2O (H^+)	65	163–164	$C_{16}H_{25}N_5$	66.86	8.77	66.92	8.50
$C_6H_{11}(CH_2)_6$-	16	130	EtOH–H_2O (H^+)	60	140–141	$C_{17}H_{27}N_5$	67.73	9.03	67.26	9.26

[a] This compound was a wax-like solid.

It is apparent from Table II that some compounds which possessed the greatest activity in inhibition of hydra regeneration (*e.g.*, 6-(4-cyclohexylbutyl)-aminopurine) had relatively little effect on the rate of germination of lettuce seed. Also, those compounds which possessed the greater seed germination effect (*e.g.*, 6-(α-naphthylmethyl)-aminopurine) had relatively little effect in the hydra regeneration study.

The preparations of these purine derivatives were accomplished through the general procedure of Elion, Burgi and Hitchings[12] which involves a thermal reaction between an amine and 6-methylthiopurine. Data concerning these compounds are reported in Table I. The intermediate amines usually were obtained through the corresponding nitrile by catalytic reduction. In an alternate procedure, the corresponding acid amide was reduced with lithium aluminum hydride.

In several instances, the acid required to prepare the desired amine was not available. These acids were prepared through the condensation of the appropriate aldehyde with ethyl ethylidenemalonate followed by reduction, hydrolysis and decarboxylation. Certain of the later condensation procedures did not yield the desired product. For example, the attempted condensation of 2-hydroxyl-1-naphthaldehyde with ethyl ethylidenemalonate failed, and an 81% recovery of starting material was obtained.

Experimental[13,14]

Starting Materials.—6-Methylthiopurine was prepared by the method of Elion, Burgi and Hitchings[12] from 6-mercaptopurine. The previously reported amines were synthesized by the catalytic reduction of the corresponding nitrile over Raney nickel in an alcohol–ammonium hydroxide solution: α-naphthylmethylamine, b.p. 153° (11 mm.)[15]; 2-phenoxyethylamine, b.p. 127° (27 mm.)[16]; 4-phenoxybutylamine, b.p. 145° (17 mm.), $n^{25}D$ 1.5235.[17]

Biological Testing.—The biological technique used to obtain the hydra regeneration data has been reported by Ham, Fitzgerald and Eakin.[18] The stimulation of lettuce seed germination was determined on a field variety of seed, Early Curled Simpson. The seeds were pre-soaked in the purine solutions for eight hours and then allowed to germinate in the dark at 30° on wet filter paper in covered Petri dishes. The percentage germination data recorded in Table II is based on 100 or more seeds per assay and the experimental technique will be presented elsewhere.[19]

7-Phenylheptylamine.—The amide of 7-phenylenanthic acid was prepared by a previously reported method[20] and used as the crude material, m.p. 82–85°.

A slurry of 10.0 g. of the amide in 150 ml. of anhydrous ether was added slowly with stirring to a suspension of 5.0 g. of lithium aluminum hydride in 100 ml. of ether. After addition was complete the mixture was stirred for an additional 10 hours. Upon the addition of dilute hydrochloric acid three layers resulted. The center one, presumably containing the hydrated amine-salt, was separated and made basic by the addition of dilute sodium hydroxide. The free amine was isolated by ether extraction and distilled to give 6.17 g. of colorless liquid, b.p. 157–160° (740 mm.). This amine did not form a solid picrate; however, a solid hydrochloride salt formed readily but was extremely hygroscopic. A sample of the amine which was redistilled for analysis boiled entirely at 157° (740 mm.).

Anal. Calcd. for $C_{13}H_2N$: N, 7.32. Found: N, 7.45.

11-Phenylundecylamine.—A sample of 50 g. of 11-phenylundecanoic acid (Eastman Organic Chemicals) was converted to the acid chloride *via* thionyl chloride, without purification, and transformed to 11-phenylundecylamide with concen-

(12) G. B. Elion, E. Burgi and G. H. Hitchings, THIS JOURNAL, **74**, 411 (1952).

(13) All melting points were made on a Fisher–Johns melting point block and are uncorrected.

(14) The analyses were done by Mr. J. R. Claybrook of the Biochemical Institute, The University of Texas, Austin, Texas, and by Drs. Weiler and Strauss, 164 Banbury Road, Oxford, England.

(15) Reported b.p. 155° (12 mm.); J. v. Braun, G. Blessing and F. Zobul, *Ber.*, **56**, 1996 (1923).

(16) Reported b.p. 115° (12 mm.); J. v. Braun, *Ber.*, **70**, 979 (1937).

(17) Reported b.p. 146–148° (17 mm.); C. S. Marvel and A. L. Tanenbaum, THIS JOURNAL, **44**, 2649 (1922).

(18) R. G. Ham, D. C. Fitzgerald, Jr., and R. E. Eakin, *J. Exp. Zool.*, **133**, 559 (1956). We are indebted to them for the data presented in Table II on the hydra tentacle regeneration effects of these compounds.

(19) C. G. Skinner, J. R. Claybrook, F. D. Talbert and W. Shive, *Plant Phys.*, 1957, accepted for publication.

(20) P. D. Gardner, W. J. Horton, G. Thompson and R. R. Twelves THIS JOURNAL, **74**, 5527 (1952) (in p. 87-89°).

TABLE II

BIOLOGICAL ACTIVITY OF SOME 6-(SUBSTITUTED)-AMINOPURINES

6-(Substituted)-aminopurine	Lettuce seeds germinated after 48 hr., %[a] 1 γ/ml.	10 γ/ml.	Inhibition of hydra-regeneration[b] Minimum concn. for full inhibition, μ-mole/ml.	Activity in terms of adenine equal to 1
Water blank control	.. (7.4) ..		...	..
7-Phenylheptyl-	5.6	1.4	0.001	5000
11-Phenylundecyl-	11.0	19.2	.008	625
α-Naphthylmethyl-	68.6	67.8	.04	125
2-α-Naphthylethyl-	9.0	2.7	.003	1700
5-α-Naphthylpentyl-	9.0	8.7	.004	1250
2-Phenoxyethyl-	59.0	63.8	.08	60
4-Phenoxybutyl-	7.9	6.3	.009	550
2-Phenoxypropyl-	30.6	65.2	.07	70
Cyclohexylmethyl-	74.4	57.9	.05	100
2-Cyclohexylethyl-	47.9	57.2	.02	250
3-Cyclohexylpropyl-	10.6	13.7	.004	1250
4-Cyclohexylbutyl-	9.6	11.5	.0009	5500
5-Cyclohexylpentyl-	10.0	16.2	.002	2500
6-Cyclohexylhexyl-	10.5	16.0	.002	2500

[a] The seeds were pre-soaked in purine solutions of the noted concentrations for 8 hours, drained, and allowed to germinate on wet filter paper in the dark at 30°. [b] These data were furnished by Mr. R. G. Ham and Dr. R. E. Eakin of the Biochemical Institute, The University of Texas, by a previously reported technique, ref. 18.

trated ammonium hydroxide. The reaction mixture tended to gel and only 16 g. of crystalline material was easily obtained. This product was dried over phosphorus pentoxide under vacuum, m.p. 42–44°.

Anal. Calcd. for $C_{17}H_{27}NO$: N, 5.36. Found: N, 5.64.

A suspension of 12.0 g. of 11-phenylundecylamide in 250 ml. of ether was added, with efficient stirring, to a suspension of 5.0 g. of lithium aluminum hydride in 200 ml. of dry ether at such a rate that reflux was not too vigorous. After standing overnight, ethyl acetate was added dropwise to the well-stirred mixture until the vigorous reaction subsided. A few milliliters of water was then added dropwise, followed by enough 10% hydrochloric acid solution to break the emulsion. The ether phase was separated, and was found to contain the amine hydrochloride which was converted to the free amine by drying over sodium hydroxide for several days. After removal of the solvent, the residue was distilled under reduced pressure, b.p. 145–149° (2 mm.), to yield 10.0 g. of product, $n^{25}D$ 1.4970.

Anal. Calcd. for $C_{17}H_{29}N$: N, 5.66. Found: N, 5.59.

ω-Cyclohexylalkylamines.—Methyl-(I), ethyl-(II), propyl-(III), butyl-(IV), pentyl-(V) and hexyl-(VI): Compounds I, II, III, IV and VI were prepared by hydrogenation, under 50 lb./sq. in. pressure of hydrogen, of a mixture containing 1 part of the corresponding nitrile,[21] 1 part of ammonium hydroxide and 3 parts of 95% ethyl alcohol in the presence of Raney nickel catalyst. After the theoretical amount of hydrogen had been consumed, the reaction mixture was filtered and the catalyst washed with alcohol. The solvent was then removed, and the residue was distilled under reduced pressure.

Compound V was prepared by the interaction of 5-cyclohexylvaleramide (18.3 g.) and lithium aluminum hydride (11.1 g.) in the presence of ether (500 ml.); and the reaction product was isolated in the same manner as that previously described for this reduction technique.

I, b.p. 37° (4 mm.), $n^{20}D$ 1.4641[22]; II, b.p. 48–50° (2.8 mm.), $n^{25}D$ 1.4645[23]; III, b.p. 60–26° (1.2 mm.), $n^{25}D$ 1.4640, 44% yield. *Anal.* Calcd. for $C_9H_{19}N$: N, 9.92. Found: N, 9.97. IV, b.p. 73° (1 mm.); $n^{25}D$ 1.4651, 75% yield. *Anal.* Calcd. for $C_{10}H_{21}N$: N, 9.02. Found: N, 9.12. V, b.p. 108–113° (6 mm.), $n^{25}D$ 1.4659, 60% yield. *Anal.* Calcd. for $C_{11}H_{23}N$: N, 8.27. Found: N, 8.17. VI, 104–112° (3 mm.), $n^{25}D$ 1.4641, 69% yield. *Anal.* Calcd. for $C_{12}H_{25}N$: N, 7.64. Found: N, 7.99.

2-Carboxyl-5-(1-naphthyl)-2,4-pentadienoic Acid.—A solution was prepared comprised of 15.6 g. of 1-naphthaldehyde and 27.9 g. of ethyl ethylidenemalonate[24] and treated with a solution prepared from 21.7 g. of potassium hydroxide (85%) and 150 ml. of methanol. The flask and contents were cooled as required to maintain the temperature below 30°. After 20 hr. at 30° the solution was acidified by the addition of 100 ml. of concd. hydrochloric acid in 150 ml. of water (with cooling). The resulting semi-solid precipitate was collected by suction filtration and triturated with hot methanol. The suspension was cooled to 0° and filtered to give 13.4 g. of yellow solid, m.p. 224–226° dec. A sample repeatedly recrystallized from butyrolactone–water or from acetic acid gave m.p. 232–233° dec.

Anal. Calcd. for $C_{16}H_{12}O_4$: C, 71.63; H, 4.51. Found: C, 71.60; H, 4.53.

2-Carboxyl-5-(1-naphthyl)-valeric Acid.—A solution of 16.3 g. of the above acid was slurried with 200 ml. of glacial acetic acid. One-half gram of 5% palladium–charcoal was added and the mixture was shaken under 2 atm. pressure of hydrogen until the theoretical absorption of gas occurred. Isolation of the product in the usual manner and recrystallization from ethyl acetate–petroleum ether (60–68°) gave 13.95 g. of colorless solid (2 crops), m.p. 153–155° dec.

Anal. Calcd. for $C_{16}H_{16}O_4$: C, 70.57; H, 5.92. Found: C, 70.51; H, 6.07.

5-(1-Naphthyl)-valeric Acid.—An 11.50-g. sample of the above dicarboxylic acid was heated at 190–200° (1 atm. pressure) until CO_2 evolution ceased. The cooled liquid crystallized upon trituration with petroleum ether and was recrystallized from ethyl–petroleum ether (60–68°) to give 8.50 g. of nearly colorless solid, m.p. 88–89°.

Anal. Calcd. for $C_{15}H_{16}O_2$: C, 78.91; H, 7.07. Found: C, 78.65; H, 7.12.

The amide of the above substituted valeric acid was prepared by treating 7.57 g. with 20 ml. of thionyl chloride at 45° for 30 minutes (until gas evolution ceased). Excess thionyl chloride was removed using a water aspirator and warming on a steam-cone. The residue was poured slowly, with stirring, into 60 ml. of cold concentrated ammonium hydroxide. The solid thus formed was recrystallized from aqueous methanol or an ethyl acetate–petroleum ether mixture, to give 6.52 g. of colorless amide, m.p. 127–128°.

Anal. Calcd. for $C_{15}H_{17}ON$: C, 79.26; H, 7.54. Found: C, 79.50; H, 7.36.

5-(1-Naphthyl)-pentylamine.—The amine was prepared by slowly adding a solution of 5.27 g. of the amide described above, dissolved in 100 ml. of dry ether, to a suspension of 2.28 g. of lithium aluminum hydride in 50 ml. of ether. The mixture was stirred for 20 hr. at room temperature and processed as before. Upon acidification three layers resulted, the amine salt being in the middle one. It was separated, made basic with dilute sodium hydroxide and extracted with ether. Distillation of the residue gave 3.40 g. of the amine as colorless liquid, b.p. 151–153° (1.0 mm.), $n^{25}D$ 1.5857.

Anal. Calcd. for $C_{15}H_{19}N$: N, 6.57. Found: N, 6.87.

2-Phenoxypropylamine.—This compound was prepared by reduction of the corresponding nitrile over Raney nickel catalyst and 50 lb./sq. in. hydrogen gas pressure, using the procedure previously described; b.p. 125–126° (22 mm.), $n^{20}D$ 1.5207.

Anal. Calcd. for $C_9H_{13}NO$: N, 9.27. Found: N, 9.29.

Attempted Condensation of 2-Hydroxy-1-naphthaldehyde with Ethyl Ethylidenemalonate.—Treatment of 20.0 g. of 2-hydroxyl-1-naphthaldehyde with 35.7 g. of ethyl ethylidenemalonate and 27.8 g. of 85% potassium hydroxide for a 20-hour period at 30° followed by the processing technique described above for 2-carboxyl-5-(1-naphthyl)-2,4-pentadienoic acid gave 16.3 g. (81%) of recovered starting material.

(21) The intermediates to form the various nitriles have previously been reported by P. L. Pickard and C. W. Young, THIS JOURNAL, **73**, 42 (1951).

(22) Reported values, b.p. 163.5°, $n^{18}D$ 1.4646; O. Wallach, *Ann.*, **353**, 297 (1907).

(23) Reported values, b.p. 85° (25 mm.), $n^{20}D$ 1.4656; B. L. Zenitz, E. B. Macks and M. L. Moore, THIS JOURNAL, **69**, 1117 (1947).

(24) F. R. Goss, C. K. Ingold and J. F. Thorpe, *J. Chem. Soc.*, **123**, 3342 (1923).

2-Carboxyl-5-(2-thenyl)-penta-2,4-dienoic Acid.—This substance was prepared by the procedure described above in which 19.5 g. of 2-thiophenaldehyde, 48.5 g. of the ester, 37.8 g. of potassium hydroxide and 200 ml. of methanol was used. Acidification gave the product as a yellow solid which, after recrystallization from acetic acid, melted at 211–212° dec. and weighed 11.1 g.

Anal. Calcd. for $C_{10}H_8O_4S$: C, 53.56; H, 3.60. Found: C, 53.91; H, 3.60.

Reduction of this substance to the corresponding saturated derivative could not be effected under 2 atmospheres of hydrogen pressure using platinum as a catalyst.

2-Carboxyl-5-(2-furyl)-penta-2,4-dienoic Acid.—Application of the above-described reaction conditions to a mixture of 43.0 g. of potassium hydroxide, 250 ml. of methanol, 19.2 g. of furfural, and 55.8 g. of the ester gave 9.53 g. of red-brown solid, m.p. 197–198° dec. Further purification afforded a tan sample having the same melting point. This substance absorbed four mole-equivalents of hydrogen (platinum, ethanol) at 30° and 2 atmospheres pressure.

Anal. Calcd. for $C_{10}H_8O_5$: C, 57.69; H, 3.87. Found: C, 58.29; H, 4.00.

Subsequent reactions with this material proceeded anomalously in several respects and will be described in a later report.

6-(Substituted)-aminopurines.—The appropriate amines were condensed by the same general procedure whereby one part of 6-methylthiopurine was mixed with 2 to 5 parts of the corresponding amine, and the reaction mixture was sealed in a micro Carius tube and heated at 130 to 140° for 15 to 18 hours. At the conclusion of the heating period, the cooled bomb was opened carefully with an oxygen torch. The liberated methylmercaptan was immediately evident as a foul smelling by-product. In several instances a precipitate was present at this stage which could be washed with cold alcohol, dried, and analyzed directly. In other experiments the solvent had first to be removed under reduced pressure to yield a mass of crystals which, after treating with charcoal, and cooling, yielded the desired product. The yields varied considerably among the different amines; however, this may have been due more to solubility factors than to extent of the reaction, since the more soluble products were difficult to isolate in the small scale experiments that were conducted. Specific reaction conditions are itemized in Table I for each of the amines studied. The extent of purity of the reaction product could be ascertained readily by determining its ultraviolet absorption spectrum. 6-Methylthiopurine has a characteristic double peak at about 283 and 290 mμ (10 γ/ml. in 95% ethanol), which is absent in the 6-(substituted)-aminopurines. The 6-(substituted)-aminopurine spectra were almost identical in possessing a λ_{max} from about 267–271 mμ when examined at concentrations of 10 γ/ml. in 95% ethyl alcohol.

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[Contribution from the Chemical Laboratory of Iowa State College and the Chemistry Department and the Oceanographic Institute of The Florida State University]

Recoverability of Phenylthiohydantoins from Amino Acids[1]

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Factors in the reaction of phenyl isothiocyanate with amino acids and amino acid residues in the analysis of peptides have been studied. Some of the same phenomena which interfere with quantitation of the extractive procedure contribute to the validity of the subtractive procedure. Use of an excess of phenyl isothiocyanate enhances the decomposition of amino acids to other substances.

In the development of methods of peptide analysis the need for convenient determinations of residue sequence and of characterization has been apparent for well over a decade.[4] The characteristics of an ideal N-terminal reagent for sequence assignment were prescribed in 1945.[4] Many of the attributes of such a reagent were found in fluorodinitrobenzene (FDNB) which has been successfully used by Sanger,[5] du Vigneaud,[6] Craig[7] and others.

In the development of more highly quantitative methods, of stepwise methods, and of procedures for characterization, a reagent of choice,[8] however, appears to be phenyl isothiocyanate[9] (PTC). Direct identification of the extracted phenylthiohydantoin (PTH) formed by fission of the reaction product of phenyl isothiocyanate with protein or peptide nevertheless poses procedural difficulties. Recoveries are usually low[10] (typically 20–40%), although higher recoveries have been attained.[11]

Probably the greatest error in the direct extraction procedure is introduced by destruction of the phenylthiohydantoin by the same mineral acid used for its formation. Such an effect was evident in the earliest experiments with phenyl isothiocyanate in this Laboratory.[12] This picture of simultaneous formation and destruction of PTH has been mentioned,[8] confirmed in detail[10,11] and is in accord with the known behavior of hydantoins.[13] The probability that destruction of PTH was occurring in the presence of mineral acid first led, indeed, to use of a subtractive method. The subtractive method consists of amino acid assays of an hydrolyzate followed by subsequent hydrolyses and assays after the terminal residues are successively removed. If the PTH formed decom-

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(2) Chemistry Department and Oceanographic Institute, The Florida State University, Tallahassee.

(3) In part from the M. S. thesis of William Serat, 1953.

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