creasing the temperature to about 225° and then completing the fusion with a free flame as previously described.[10] The test-tube was cooled to room temperature, 2.25 mmoles of copper chelated *l*-ornithine in a volume of 3.5 cc. was added without attempted isolation of KCNO from the excess K_2CO_4, and the mixture kept at 37° for 5 days. Citrulline-C^{14} was isolated as described above. Yield was 179 mg. (51% based on urea) with m.p. 218–219° dec. Yields of free citrulline as high as 60% based on urea have been obtained. When this synthesis was carried out by heating at 100° for 20 minutes, the yield was usually 10–15% less than that obtained at 37°. The longer reaction period was therefore routinely used for isotopic synthesis.

Synthesis of 1-Homocitrulline·HCl (ε-Carbamyllysine).—The free form of this compound was prepared by Kurtz using the urea fusion method.[8] 365 mg. (2.0 mmoles) of *l*(+)-lysine hydrochloride was chelated with cupric carbonate as described above. Potassium cyanate (178 mg., 2.2 mmoles) was added to the solution in a final volume of 5 cc. and reacted at 37° for 4 days. The crude Cu-homocitrulline was dissolved in 2 cc. of 4 *N* HCl, diluted with 6 cc. of water and treated with H_2S for 10 minutes. The hydrochloride was crystallized from the filtrate by evaporation to dryness and the gradual addition of ethanol-ether with scratching. Yield was 330 mg. (73% based on lysine); m.p. 177–178° dec. (uncor.). Calcd. for $C_7H_{16}N_3O_3Cl$: C, 37.25; N, 18.58; Cl, 15.75. Found: C, 37.50; N, 17.87; Cl, 15.31. Paper chromatography with phenol–water (80:20) revealed a single ninhydrin spot with a R_f of 0.73. In contrast to citrulline and *l*-α-amino-γ-carbamidobutyric acid the hydrochloride of homocitrulline is easily isolated and is much more conveniently recrystallized than the fluffy, amorphous free form.

C^{14} 1-Homocitrulline was synthesized as described for C^{14} citrulline, with isolation as the hydrochloride, on a 1 mM. scale with a yield of 60% based on urea.

Synthesis of *l*-α-Amino-γ-carbamidobutyric Acid.[8]—Seven hundred and sixty-four mg. (4 mmoles) of α,γ-diaminobutyric acid dihydrochloride[11] after chelating with cupric carbonate, reacted with 356 mg. (4.4 mmoles) of potassium cyanate and was converted to the free form as described for citrulline. Yield was 245 mg. (38% based on α,γ-diaminobutyric acid); m.p. 207–208° dec. Since Kurtz reported the m.p. as 225°, an analysis was performed. Calcd. for $C_5H_{11}N_3O_3$: C, 37.30; N, 26.15. Found: C, 37.43; N, 26.18. Paper chromatography with phenol–water (80:20) revealed a single ninhydrin spot with an R_f of 0.58.

C^{14} 1-α-Amino-γ-carbamidobutyric acid was synthesized on a 1 mM. scale as described for C^{14}-citrulline with a 26% yield based on urea.

Enzyme Studies.—Isolated mitochondria from 2 rat livers were frozen in 16 cc. of distilled water and lyophilized. The dried powder (40 mg./cc.) was extracted with 0.02 molar glycylglycine at *p*H 8.0, 0°, and then centrifuged for 15 minutes at 15,000 *g*, −5°. The supernatant was used immediately for either citrullinase or compound X/carbamyl phosphate studies and contained about 1.0 to 2.0 mg. N/cc. The reactions were carried out for 20 minutes, 37°, with constant shaking in closed Warburg type flasks containing 0.6 cc. of 6 *N* KOH in the center well. One hundred μmoles of carrier $NaHCO_3$ was then added to each flask and the reaction was stopped by the addition of 0.5 cc. of 12 *N* H_2SO_4 from the side-arm. Center well bicarbonate, collected by an additional 30 min. of shaking, was precipitated as $BaCO_3$, washed repeatedly and filtered to form infinitely thick planchettes. The planchettes were counted with an end window counter having an efficiency of approximately 5%. An experimentally determined conversion factor was used to calculate citrulline degradation in μM. Details of these procedures will be published elsewhere.[7]

(a) Citrullinase.—Citrullinase activity was measured by arsenolysis of citrulline at *p*H 6.7.[6,7] Each flask contained 250 μ moles of sodium arsenate buffer, 1.0 cc. of mitochondrial extract and 5 μmoles of C^{14}-1(+)-citrulline (0.05 μc./μmole), or 5 μmoles of C^{14}-1-homocitrulline (0.2 μc./μ mole), or 5 μmole of C^{14}-1-α-amino-γ-carbamidobutyric acid (0.2 μc./μmole) in a final volume of 3.0 cc. For inhibition studies varying amounts of non-isotopic *l*(+)-ornithine, 1-homocitrulline or 1-α-amino-γ-carbamidobutyric acid were added to flasks containing 5 μmoles of isotopic citrulline.

(11) D. W. Adamson, *J. Chem. Soc.*, 1564 (1939).

(b) Compound X[12] and Carbamyl Phosphate[13] Formation.—It has been demonstrated that the carbamyl group of citrulline is transferred to aspartate *via* compound X and carbamyl phosphate to form ureidosuccinate.[7] This reaction is dependent on the presence of ATP, Mg^{++} and acetylglutamate (or carbamylglutamate) and has a *p*H optimum of 8.0. In the absence of aspartate the reaction can be followed by the production of "acid labile CO_2" from citrulline at *p*H 8.0 and dependent upon the presence of acetylglutamate. Each flask contained 10 μmoles of acetylglutamate, 20 μmoles of ATP, 40 μmoles of Mg^{++}, 250 μmoles of glycylglycine buffer *p*H 8.0, 1.0 cc. of mitochondrial extract and 5 μmoles of C^{14}-1(+)-citrulline (0.1 μc./μmole), or 5 μmoles of C^{14}-1-homocitrulline (0.2 μc./μmole), or 5 μmoles of C^{14}-1-α-amino-γ-carbamidobutyric acid (0.2 μc./μmole) in a final volume of 3.0 cc. Inhibition studies were carried out as above.

Table I summarizes the enzyme studies. Homocitrulline and α-amino-γ-carbamidobutyric acid were completely inactive as substrates for these two enzymes and were weak inhibitors of citrullinase only. Ornithine caused a 50% inhibition of citrullinase in equimolar concentration, but exhibits an inhibition index of about 0.05 for compound X/carbamyl phosphate formation.

TABLE I

ENZYME DATA ON CITRULLINE AND TWO ANALOGS

		Citrullinase[b]		Compound X/carbamyl phosphate[b]	
		C.p.m.	μmole	C.p.m.	μmole
C^{14}-Homocitrulline		0	0	0	0
C^{14}ABC[a]		0	0	0	0
C^{14}-Citrulline		1320	1.37	895	0.45
+ Homocitrulline	50 μmole	790	0.82	970	.49
+ ACB[a]	50 μmole	700	.73	660	.33
+ Ornithine	5 μmole	575	.60		
	0.1 μmole			730	.37
	.2 μmole			555	.28
	.3 μmole			275	.14

[a] ABC indicates α-amino-γ-carbamidobutyric acid. [b] Results are given in counts/min. and the calculated μ mole of the C^{14} precursor this represents.

(12) S. Grisolia and P. P. Cohen, Jr., *J. Biol. Chem.*, **198**, 561 (1952).

(13) M. E. Jones, L. Spector and F. Lipmann, THIS JOURNAL, **77**, 819 (1955).

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Synthesis of Some 6-(Substituted)-Aminopurines

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Adenine and certain of its derivatives have been found to increase the number of buds from which gametophores develop in moss (*Tortella caespitosa*).[1] The structural relationship of kinetin,[2] 6-(2-furfuryl)-aminopurine (isolated from autoclaved sperm desoxyribonucleic acid as a cell division factor for tobacco callus tissue) to these compounds which increase budding in moss, led to the preparation and testing of kinetin and a group of its analogs in this Laboratory.

All of the analogs were synthesized by the general procedure of Elion, Burgi and Hitchings[3] whereby 6-methylmercaptopurine is condensed with the appropriate amine at elevated temperatures, as indicated in Table I.

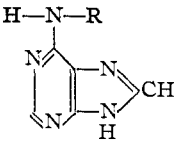
(1) R. E. Eakin and B. S. Gorton, personal communication.

(2) C. O. Miller, *et al.*, THIS JOURNAL, **77**, 2662 (1955).

(3) G. B. Elion, E. Burgi and G. H. Hitchings, *ibid.*, **74**, 411 (1952).

TABLE I

SYNTHESIS OF 6-(SUBSTITUTED)-AMINOPURINES



R	Formula	Wt. 6-methyl mercapto-purine, mg.	Wt. amine, mg.	Time of run,[a] hr.	Yield, %	M.p., °C. dec.[b]	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
2-Furfuryl	$C_{10}H_9N_5O$	1500	3000	12	62	269–270[c]	55.81	56.02	4.22	4.46	32.54	32.98
Benzyl	$C_{12}H_{11}N_5$	200	400	13	66	229	63.98	64.28	4.92	4.77	31.09	31.20
2-Pyridylmethyl	$C_{11}H_{10}N_6$	400	1000	8	45	257	58.39	58.79	4.46	4.12	37.15	37.39
3-Pyridylmethyl	$C_{11}H_{10}N_6$	500	1000	7	58	259	58.39	58.70	4.46	4.43	37.15	37.20
4-Pyridylmethyl	$C_{11}H_{10}N_6$	500	1000	10	50	265–266	58.39	57.82	4.46	4.41	37.15	36.83
2-Thenyl	$C_{10}H_9N_5S$	500	1500	12	52	250	51.93	51.88	3.92	3.89	30.28	30.73

[a] All runs were conducted at a temperature of 130°. [b] Melting points determined on a Fisher melting point block. [c] C. O. Miller, *et al.*, THIS JOURNAL, **77**, 2662 (1955), reported m.p. 266–267°.

Some of the biological activities of these compounds are summarized in Table II.[4] At low concentrations (0.01 mg. per l.) not only kinetin but also the benzyl and thenyl derivatives stimulate budding in moss. At higher concentrations (1 mg. per l.) the pyridylmethyl derivatives also stimulate budding, but only the 2-pyridylmethyl compound attains activity comparable to kinetin. At the higher concentration the thenyl derivative becomes inhibitory.

TABLE II

SOME BIOLOGICAL EFFECTS OF 6-(SUBSTITUTED)-AMINOPURINES

Compound	Stimulation of budding of moss[a]	Concn. for 50% inhibition of length of main axis in tomato root cuttings, mg./liter × 10^3
Control	1	..
6-(2-Furfuryl)-aminopurine	20	6
6-Benzylaminopurine	25	6[b]
6-(2-Pyridylmethyl)-aminopurine	1	6[c]
6-(3-Pyridylmethyl)-aminopurine	1	..
6-(4-Pyridylmethyl)-aminopurine	1 (?)	..
6-(2-Thenyl)-aminopurine	20	5[d]

[a] These data were determined at a concentration of 1×10^{-2} mg./liter, and are reported relative to a control equal to *one*. [b] This compound also stimulated the growth in length of the ten basal laterals at 10^{-5} mg./liter. [c] This compound also stimulated the number of laterals at 10^{-4} mg./liter. [d] This compound also stimulated the growth in length of the ten basal laterals at 10^{-6} to 10^{-8} mg./liter.

Preliminary data[5] on the 2-furfuryl, benzyl and 2-thenyl derivatives show an inhibition of growth on the main axis of excised tomato roots (*Lycopersicon esculentum*. Mill.) at concentrations of 5 to 6×10^{-3} mg./liter, whereas the 2-pyridylmethyl derivative is not toxic at this level. Furthermore, the 2-pyridylmethyl derivative increased the growth in length of the main axis at 10^{-4} mg./liter. Also, the benzyl and 2-thenyl compounds stimulate the growth in length of the ten basal laterals, while the 2-pyridylmethyl derivative stimulates the number of laterals at 10^{-5} and 10^{-4} mg./liter, respectively.

From these data, it appears that a number of different 6-(substituted)-aminopurines have potent effects on plant growth.

(4) The authors are indebted to Dr. R. E. Eakin and Mr. B. S. Gorton for the results on moss growth, the details of which will be published separately, and to Dr. W. G. Boll for preliminary data on growth of tomato root cuttings.

(5) W. G. Boll, personal communication.

Experimental

6-Methylmercaptopurine.—The method of Elion and Burge[6] was used except that the course of the reaction appeared somewhat different. Three grams of 6-mercaptopurine[7] was suspended in one equivalent of 2 *N* sodium hydroxide plus 15 ml. of water; and to this well-stirred mixture was added one equivalent of methyl iodide in 9 portions over a period of one hour. The *p*H changed from about 9 to 7, and the mixture set to a semi-solid mass. About 40 ml. of water was then added, and the solid dissolved by warming to yield a solution of *p*H 5, from which 3.4 g. of product separated, m.p. 220° dec.

2-, 3- and 4-Pyridylmethylamines.—These amines were prepared through the catalytic reduction of the corresponding nitrile by the procedure of Kolloff and Hunter.[8]

2-Thenylamine.—This compound was prepared by the method of Hartough[9] through the interaction of thiophene, formaldehyde and ammonium chloride; b.p. 95–99° (28 mm.), $n^{20}D$ 1.5643, reported[9] $n^{20}D$ 1.5615.

6-(Substituted)-aminopurines.—All of these compounds were prepared and recovered in approximately the same manner. One equivalent of 6-methylmercaptopurine and two to three equivalents of the corresponding amine were sealed in a micro Carius tube and heated at about 130° for a given number of hours as noted in Table I. The cooled bomb, in every case, yielded a white to light yellow crystalline mass which was washed with ice-cold ethanol and recrystallized from ethanol or ethanol-water. The final product was dried over P_2O_5 at 120° under vacuum for several hours.

(6) G. B. Elion and E. Burgi, THIS JOURNAL, **74**, 413 (1952).

(7) Burroughs Wellcome and Co., Tuckahoe, N. Y.

(8) H. G. Kolloff and T. H. Hunter, THIS JOURNAL, **63**, 491 (1941).

(9) H. D. Hartough, "Thiophene and Derivatives," Interscience Publishers, Inc., New York, N. Y., 1952, p. 510.

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p-Phenylazophenylsemicarbazones of Trioses and Biologically Related Compounds

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The derivatives of *p*-phenylazophenylsemicarbazide with carbonyl compounds of biological origin are of interest in that they may offer a means of isolation and identification of the latter substances and