chloric acid. The precipitate was filtered off and identified as 1-hydroxy-2-naphthoic acid (m.p. and mixture m.p. 190°) and the green color with alcoholic ferric chloride solution).

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Synthesis of Some α- and β-(6-Purinylthio)carboxylic Acids

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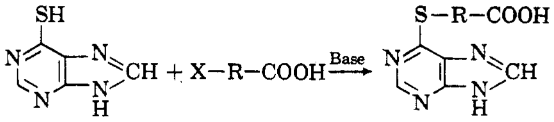
A number of 6-alkyl- and 6-(ω-phenylalkyl)-thiopurines have been found to possess biological activity (in several assay systems) comparable to the corresponding 6-(substituted)aminopurine analogs.[1,2] The unexpected biological activity of the thiopurine derivatives led to the preparation of α-(6-purinylthio)succinic acid[1] as an analog of *N*-(6-purinyl)aspartic acid.[3] Subsequently, the thiosuccinic acid derivative was found to promote growth of etiolated bean leaf disks either in the presence or absence of light.[4] In an effort to extend this latter study, additional purinylthiocarboxylic acid derivatives were prepared and their biological activity on bean leaf expansion will be reported elsewhere.

TABLE I

α- AND β-(6-PURINYLTHIO)CARBOXYLIC ACIDS



Halogen Acid Used	Reaction Conditions	Yield, %	M.p., °C.	Empirical Formula	Analysis N (Calcd.)	Analysis N (Found)
α-Bromoacetic	NaOH, 25°	93[a]	235–260(dec.)	$C_7H_6N_4O_2S$[c]	26.65	26.81
α-Bromopropionic	Triethylamine, 25°	59[a]	199–203	$C_8H_8N_4O_2S$	24.99	24.91
β-Bromopropionic	NaOH, 90°	55[a]	219–220	$C_8H_8N_4O_2S$	24.99	25.01
α-Bromobutyric	Triethylamine, 25°	32[a]	208–209	$C_9H_{10}N_4O_2S$	23.52	23.21
α-Bromovaleric	NaOH, 25°	45[b]	199–205	$C_{10}H_{12}N_4O_2S$	22.21	22.29
α-Bromocaproic	NaOH, 90°	64[b]	178–183	$C_{11}H_{14}N_4O_2S$	21.04	21.06
β-Bromocaproic	Triethylamine, 25°	46[b]	182–184	$C_{11}H_{14}N_4O_2S$[d]	—	—

[a] Recrystallized from water. [b] Recrystallized from ethanol-water. [c] *Anal.* Calcd.: C, 39.99; H, 2.88. Found: C, 40.15; H, 3.25. [d] *Anal.* Calcd.: C, 49.61; H, 5.30. Found: C, 49.33; H, 5.09.

The desired compounds were synthesized by a condensation of the appropriate α or β-halocarboxylic acid with 6-mercaptopurine under alkaline conditions, using either an equivalent amount of dilute sodium hydroxide or an excess of triethylamine as indicated in Table I. Although the reactants did condense slowly at room temperature in most instances, several of the reactions were carried out in a glass-lined steel bomb heated to about 90°. The sodium hydroxide condensation mixtures, upon acidification, yielded a precipitate which was normally dissolved in alkali and reprecipitated with acid to yield a purified product. In the triethylamine condensation, the reaction mixture was reduced to dryness *in vacuo* in order to remove the excess amine, prior to crystallization.

As in the case of α-(6-purinylthio)succinic acid, the various purinylthiocarboxylic acids indicated in Table I showed little or no response in several biological systems which did respond to 6-alkylthio- and 6-alkylaminopurines. α-(6-Purinylthio)acetic acid at concentration levels as high as 1 mg./ml. did not inhibit hydra tentacle regeneration.[5,6] The purinylthiocarboxylic acids also did not possess either inhibitory or stimulatory effects on a pteridine-inhibited *Lactobacillus arabinosus* at concentration levels up to 40 γ/disk.[7] The presence of a carboxylic acid moiety in the alkyl group of 6-alkylthiopurines appears to cause a loss of biological activity of the compounds in many of these test systems. A decrease in biological activity was also

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[1] C. G. Skinner, W. Shive, R. G. Ham, D. C. Fitzgerald, Jr., and R. E. Eakin, *J. Am. Chem. Soc.*, **78,** 5097 (1956).

[2] C. G. Skinner, J. R. Claybrook, F. D. Talbert, and W. Shive, *Plant Physiol.*, **32,** 117 (1957).

[3] C. E. Carter and L. H. Cohen, *J. Am. Chem. Soc.*, **77,** 499 (1955).

[4] R. A. Scott, Jr., and J. L. Liverman, *Science*, **126,** 122 (1957).

[5] R. G. Ham, D. C. Fitzgerald, Jr., and R. E. Eakin, *J. Exp. Zool.*, **133,** 559 (1956).

[6] R. G. Ham, Ph.D. dissertation, University of Texas, Austin, June 1957.

[7] E. M. Lansford, Jr., C. G. Skinner, and W. Shive, *Arch. Biochem. Biophys.*, **73,** 191 (1958).

observed in the hydra tentacle regeneration assay when a hydrophilic group was present in the alkyl side chain of certain 6-(substituted)aminopurines.[1]

A slight but definite stimulatory effect on the rate of germination was observed when lettuce seed (Early Curled Simpson) were presoaked in 10 γ/ml. solutions of α-(6-purinylthio)butyric acid and the corresponding valeric acid derivative. The butyric acid analog gave approximately a 250% increase in the number of seeds germinating after 72 hours in the dark at 30°, while the valeric acid derivative gave an 80% increase, as compared with seed presoaked in water alone. These values represent only moderate stimulations of seed germination in comparison with the more active 6-(substituted)purines.[2,8,9] It is interesting to note that the most active 6-alkylthiopurines with respect to stimulation of seed germination were those derivatives containing four to six carbon atoms in the substituent group, and correspondingly, the more active purinylthiocarboxylic acids are those which contain groups of similar size.

EXPERIMENTAL[10]

Biological assay techniques. The assay procedures used were the same as those previously reported in the study of similar 6-(substituted)purine compounds for stimulation of lettuce seed germination,[2] inhibition of hydra tentacle regeneration,[5] and microbiological assays.[7]

α- and β-(6-Purinylthio)carboxylic acids. These compounds were prepared by an alkaline-catalyzed condensation between the appropriate haloacid and 6-mercaptopurine, as indicated in Table I, following a procedure which has previously been reported.[1]

An alternate condensation procedure involved using an excess of triethylamine as the condensing agent and allowing the reaction to proceed at room temperature. The course of these reactions was followed by observing the decrease in ultraviolet absorption at 328 mμ and the appearance of an absorption band at 282–289 mμ. The former λ_{max} is associated with 6-mercaptopurine, and the latter absorption is indicative of a 6-(substituted)thiopurine.[11] The reaction mixtures were then taken to dryness *in vacuo* to remove the excess amine, and the residues were crystallized as indicated in Table I. Attempts to increase the rate and yield of the reactions by heating in the presence of triethylamine resulted in varying amounts of decomposition products.

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[8] C. G. Skinner, J. R. Claybrook, F. D. Talbert, and W. Shive, *Arch. Biochem. Biophys.*, **65**, 567 (1956).

[9] C. G. Skinner, P. D. Gardner, and W. Shive, *J. Am. Chem. Soc.*, **79**, 2843 (1957).

[10] All melting data were taken on a Fisher-Johns micro hot stage and the temperatures are uncorrected. The ultraviolet absorption spectra were determined on a Beckman model DK-2 recording spectrophotometer using a 10 γ/ml. solution of the appropriate compounds dissolved in 95% ethyl alcohol.

[11] C. G. Skinner, R. G. Ham, O. C. Fitzgerald, Jr., R. E. Eakin, and W. Shive, *J. Org. Chem.*, **21**, 1330 (1956).

Reactions of Nitrate Esters. V.[1] Decomposition of Primary Nitrates in Perfluorinated Acids

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We wish to report the occurrence of an unusual decomposition reaction of simple primary aliphatic nitrates, which can be achieved by merely dissolving the esters in trifluoroacetic acid (TFA) and allowing the solutions to stand at room temperature for 24 hours or less. The main products obtained from the decomposition are nitric oxide and the carboxylic acid having the same number carbon atoms as the ester. The reaction is catalyzed by ultraviolet light, although it does proceed even in the dark. Atmospheric oxygen appears to have no effect. A similar reaction occurs in other perfluorinated acids but not in 100% acetic or 100% sulfuric acid.

In addition to the main decomposition products, which are produced in yields of the order of 50%, there are obtained traces of solid acidic materials, and significant amounts (*ca.* 50%) of the alkyl trifluoroacetates. The latter are presumably formed either by an ordinary metathesis, or by a complex ionization similar to that reported for solutions of alkyl nitrates in sulfuric acid.[2]

Most of the work thus far has been with *n*-butyl nitrate, and typical procedures are described in the Experimental portion. Ethyl and *n*-propyl nitrates react in the same way with TFA. The butyl nitrate decomposition proceeds in perfluorobutyric or perfluorohexanoic acid in the same manner as in TFA. The reactions of secondary mononitrates with TFA have not been investigated, but 2,3-dinitroxybutane has been found to react readily, giving oxides of nitrogen and diacetyl.

The rate of disappearance of butyl nitrate has been followed polarographically. Attempts to follow the reaction by observing changes in the ultraviolet spectrum led to ambiguous results, due to the fact that ultraviolet light catalyzes the reaction, and to the fact that reaction products absorb in the same spectral region (*ca.* 255 mμ) as does the nitrate ester. The absorbance in this region remains essentially constant for a period of several hours, after which it increases rapidly. The length of this apparent induction period varies with concentration and with the duration of exposure to ultraviolet light in the spectrophotometer. The existence of an apparent induction period seems to be due to a compensating effect of increasing absorbance by reaction products and decreasing absorbance (at the same wave length) by butyl nitrate, since no

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[1] Previous paper: R. T. Merrow, *J. Am. Chem. Soc.*, **78**, 1297 (1956).

[2] L. P. Kuhn, *J. Am. Chem. Soc.*, **69**, 1974 (1947).