was dried to constant weight *in vacuo*. The resulting powder was very hygroscopic, sintered at 50°, and decomposed at 62°. Fehling's solution was reduced and one major spot was noted on thin layer chromatograms on silica gel developed with BuOH–EtOH–H_2O (visualized by Tollens reagent). *Anal.* ($C_4H_8N_2O_4 \cdot HCl$) C, H; N: calcd, 15.18; found, 15.74.

Hydrogenolysis of 4.—A solution of 184 mg of N-hydroxy compound (**4**) as its hydrochloride in 25 ml of EtOH was hydrogenated at atmospheric pressure with PtO_2 (50 mg). After 6 hr the theoretical uptake was complete, the catalyst was removed, and the filtrate was taken to dryness. The product, dissolved in a few milliliters of H_2O, gave a negative Fehling's test. Electrophoresis performed with authentic samples of isoasparagine, asparagine, and aspartic acid at pH 3.5 (acetate buffer, 500 v, 4 hr) with ninhydrin as an indicator, or paper chromatography with 88% phenol, showed the product to contain but traces of isoasparagine and aspartic acid. The major component, DL-asparagine, precipitated on adjusting the solution to pH 3, had essentially zero rotation, and contained no isoasparagine.

Benzalnitrone of Succinamide.—A solution of 2 g of **2** in 30 ml of 28% aqueous NH_3 was allowed to stand at room temperature for 24 hr. The diamide precipitated (600 mg, 28%) and was collected and recrystallized from 75% EtOH. It melted at 204–205° dec. *Anal.* ($C_{11}H_{13}N_3O_3$) C, H, N.

2-Hydroxylaminosuccinamide Hydrochloride.—A solution of 350 mg of the above nitrone in 10 ml of 1 *N* HCl was concentrated *in vacuo* keeping the bath temperature below 30°. The resulting precipitate was taken up twice in EtOH and precipitated by the addition of ether. The white powder, dried *in vacuo* to constant weight, decomposed at about 80°. Chromatography with BuOH–EtOH–H_2O indicated one component with Tollens reagent at R_f 0.3. *Anal.* ($C_4H_9N_3O_3 \cdot HCl$) C, H, N.

Some 3-Methyl-2-butenylaminopurines and -pyrimidines[1]

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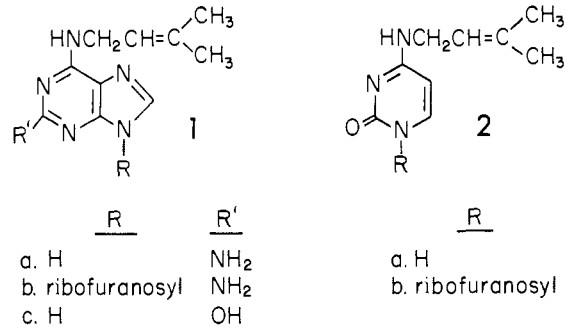
6-(3-Methyl-2-butenylamino)-9-β-D-ribofuranosylpurine has been recently reported as a minor component of yeast and liver soluble ribonucleic acid[2] and of yeast serine transfer ribonucleic acid.[3,4] 6-(3-Methyl-2-butenylamino)purine has been found in extracts of *Corynebacterium fascians*.[5] Both compounds have been synthesized and exhibit a high level of cytokinin activity.[2,6] It was therefore of interest to synthesize other 3-methyl-2-butenylamino pyrimidines and purines and to test them for biological activity.

In all cases the syntheses were carried out by refluxing an excess of γ,γ-dimethylallylamine with the appropriate mercapto or alkylmercaptopurine or -pyrimidine according to the method of Elion, *et al.*[7] The reactions were followed spectrophotometrically in ethanol and were terminated when the absorption maximum of the



starting material (297–315 mμ) was no longer present. Proof of structure of the products **1a–c** and **2a, b** was shown by the similarity of their ultraviolet absorption spectra to the purine N^6- or pyrimidine N^4-monoalkylated parent compounds (Table I). That the double bond in the side chain is in the position shown is demonstrated by the nmr methyl signals at δ 1.75 (DMSO-d_6, TMS).[2,8]

In the leaf senescence test[9] all of the compounds (**1** and **2**) showed about one-tenth the activity reported for 6-furfurylaminopurine (kinetin).[10] None of these compounds exhibited any activity against mouse leukemia L1210 *in vivo*.[11] Compounds **2a** and **2b** were also inactive against Burkitt's cell cultures.[11a] The cytidine analog **2b** was not deaminated by human liver or mouse kidney enzyme systems.[11a]

Experimental Section

Melting points were taken on a Mel-Temp apparatus and are corrected. Spectra were determined using a Cary Model 15 spectrophotometer. Analyses were done by Spang Microanalytical Laboratory, Ann Arbor, Mich., and Galbraith Laboratories, Inc., Knoxville, Tenn. Where analyses are indicated only by the symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

2-Amino-6-(3-methyl-2-butenylamino)purine (1a).—2-Amino-6-methylthiopurine[12] (1.25 g, 6.9 mmoles) and γ,γ-dimethylallylamine[13] (5.0 g, 59 mmoles) were refluxed for 30 hr. A precipitate which formed on cooling was washed with petroleum ether (30–60°) to remove the excess amine and was dissolved in 95% EtOH. After the addition of concentrated HCl, 1.18 g (67%) of **1a** hydrochloride was obtained. A sample for analysis was recrystallized from EtOH after treating with charcoal; mp 250–251° dec. *Anal.* ($C_{10}H_{14}N_6 \cdot HCl$) C, H, N, Cl.

2-Amino-6-(3-methyl-2-butenylamino)-9-β-D-ribofuranosylpurine (1b).—6-Ethylthioguanosine[14] (2.0 g, 6.1 mmoles) and γ,γ-dimethylallylamine (6.0 g, 71 mmoles) were refluxed for 60 hr. After the addition of petroleum ether (30–60°), a dark oil separated. The oil was triturated several times with petroleum ether to remove any excess amine and was then dissolved in 95% EtOH, treated with charcoal, and filtered. The crystals which formed were washed with cold EtOH and dried (yield 1.31 g, 48%). A sample for analysis was recrystallized from EtOH, mp 93–97°. *Anal.* ($C_{15}H_{22}N_6O_4 \cdot EtOH$) C, H, N.

2-Hydroxy-6-(3-methyl-2-butenylamino)purine (1c).—2-Hydroxy-6-methylthiopurine[15] (0.42 g, 2.3 mmoles), γ,γ-dimethylallylamine (4.0 g, 47 mmoles), and EtOH (5.0 ml) were refluxed for 90 min. The crystals which formed on cooling were washed with EtOH, dried, dissolved in 1 *N* NaOH, and reprecipitated

(1) (a) This investigation was supported in part by National Cancer Institute Grant CA 08748. (b) The side chains of these compounds have also been referred to as γ,γ-dimethylallylamino or Δ^2-isopentenyl groups.

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TABLE I
ULTRAVIOLET ABSORPTION

Compd	0.1 N HCl λmax, mμ (ε × 10⁻³)	0.1 N HCl λmin, mμ (ε × 10⁻³)	pH 7 λmax, mμ (ε × 10⁻³)	pH 7 λmin, mμ (ε × 10⁻³)	0.1 N NaOH λmax, mμ (ε × 10⁻³)	0.1 N NaOH λmin, mμ (ε × 10⁻³)
1a	280 (14.3)	261 (9.0)	281 (13.2)	261 (7.3)	286 (13.1)	253 (4.8)
	247.5 (12.6)	237.5 (11.3)	248 (7.9)	242 (7.6)		
1b	291 (12.9)	271 (8.8)	281 (15.5)	243 (6.8)	281 (15.5)	243 (6.8)
	254 (12.1)	238 (8.0)	263 sh (10.9)		263 sh (10.9)	
1c	287 (17.3)	250 (1.9)			285 (15.9)	256 (3.7)
2a	278 (12.4)	241 (2.5)	267.5 (9.2)	248 (6.9)	282 (8.1)	253 (3.7)
			232 (8.5)		230 sh (8.3)	
2b	282.5 (15.8)	243 (2.9)	271 (13.0)	247.5 (9.2)	271 (13.0)	247.5 (9.2)
			239 (9.6)	228 (9.1)	239 (9.6)	228 (9.1)

by neutralization with glacial AcOH. The precipitate was washed with H_2O and dried to give 300 mg (55%) of **1c** monohydrate. An analytical sample was prepared by recrystallization from 1 *N* NaOH by acidification with AcOH; mp >300° dec. *Anal.* ($C_{10}H_{13}N_5O \cdot H_2O$) C, N; H: calcd, 6.37; found, 5.92.

2-Hydroxy-4-(3-methyl-2-butenylamino)pyrimidine (2a).—4-Methylthiouracil[16] (1.5 g, 10.5 mmoles), γ,γ-dimethylallylamine (5.0 g, 59 mmoles), and EtOH (5.0 ml) were refluxed for 2.5 hr. The precipitate which formed on cooling was washed with petroleum ether (30–60°) and recrystallized from 95% EtOH and dilute H_2SO_4 to give 1.82 g (95%) of **2a**·0.5H_2SO_4. A sample for analysis was recrystallized twice from EtOH–H_2O and dried; mp 229–230°. *Anal.* ($C_9H_{13}N_3O \cdot 0.5H_2SO_4$) C, H, N.

2-Hydroxy-4-(3-methyl-2-butenylamino)-1-β-D-ribofuranosylpyrimidine (2b).—1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-4-thiouracil[17] (2.0 g, 4.3 mmoles) was refluxed with γ,γ-dimethylallylamine (10 g, 118 mmoles) for 90 min. The excess amine was removed under vacuum to give a brown oil. The oil was dissolved in 10 ml of EtOH and 300 ml of Et_2O was added. The pale tan oil which separated was shown by thin layer chromatography (silica gel; GF_{254}, $CHCl_3$–MeOH 3:1) to be essentially free of N-(3-methyl-2-butenyl)benzamide. Final separation was carried out on a silica gel column (2.5 × 18 cm, Fisher silica gel 100–200 mesh, grade 923) by elution with $CHCl_3$–MeOH 3:1. The solvent was removed under vacuum to give an amorphous and somewhat unstable product. *Anal.* ($C_{14}H_{21}N_3O_5$) H, N; C: calcd, 54.01; found, 53.02.

A tetrabenzoyl derivative of **2b** was prepared by heating 100 mg of **2b** with an excess of benzoyl chloride in pyridine at 60° until tlc (silica gel (GF_{254}, C_6H_6–Et_2O 2:1) showed a single spot at R_f ~0.50. The product was poured over ice and gave a light tan oil. The oil was triturated with H_2O and allowed to stand for a few days. The needles which formed were recrystallized twice from EtOH; mp 193–194°. *Anal.* ($C_{32}H_{37}N_3O_9$) C, H, N.

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Nucleosides. XLVII. Syntheses of Some N⁴-Substituted Derivatives of 1-β-D-Arabinofuranosylcytosine and -5-fluorocytosine[1]

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The clinical usefulness of 1-β-D-arabinofuranosylcytosine (**4**) in the treatment of acute leukemias and lymphomas has been demonstrated,[2a] but various pharmacological studies[2b,c] have shown that this drug is rapidly deaminated to the inactive nucleoside, 1-β-D-arabinofuranosyluracil (**1**). It has been shown previously that alkylation of the exocyclic amino function of cytosine nucleosides, such as 2′-deoxy-5-fluorocytidine produced a marked decrease of the susceptibility of such compounds to deamination by bacterial deaminases.[3] It was further shown[4] that **4** also serves as a substrate for bacterial deaminases. The 5-fluoro analog (**4b**, R″ = H)[5] was also effective against several mouse leukemias, but this analog, too, is degraded by human liver or mouse kidney deoxycytidine deaminases to **1b**. R = H.[6] These results[6,7] suggested the synthesis of N⁴-substituted derivatives of **4a** and **4b** as potential chemotherapeutic agents and/or as deoxycytidine deaminase inhibitors. The present report describes the synthesis of several of these N⁴-substituted derivatives selected for biological evaluation.

The syntheses of N⁴-substituted arabinosylcytosine nucleosides (**4**) were accomplished by the thiation process[5,8] which involved thiation of a suitably protected nucleoside (**1**) with phosphorus pentasulfide in pyridine to give the 4-thiones (**2**) followed by alkylation to the 4-methylthio analogs (**3**). Treatment of **3** with various nucleophiles (*e.g.*, NH_2OH, NH_2NH_2, CH_3NH_2) gave the desired products (**4**) (Scheme I).

Though the synthesis of **2b** (R = $COCH_3$) had been reported,[5] its preparation by thiation of **1b** was rather difficult and several retreatments with P_2S_5 were necessary to drive the reaction to completion. Moreover, during the extended thiation-reaction time, some of the acetyl-protecting groups were apparently lost and **2b** could not be obtained in pure form. Although crude **2b**, thus obtained, was satisfactory for subsequent conversions, it was hoped that benzoyl blocking groups

(1) This investigation was supported in part by funds from the National Cancer Institute (Grant CA 08748).

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