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Sugar-Modified N^6-(3-Methyl-2-butenyl)adenosine Derivatives, N^6-Benzyl Analogs, and Cytokinin-Related Nucleosides Containing Sulfur or Formycin†

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ABSTRACT: Nucleoside analogs of N^6-(3-methyl-2-butenyl)-adenosine (iPA) (**2a**) and their corresponding biologically analogous benzyl derivatives have been prepared. Nonnucleophilic acid acceptors have been found to increase the yield of N^1-alkylation of adenine nucleosides by 3-methyl-2-butenyl bromide. N^6-(3-Methyl-2-butenyl) and N^6-benzyl derivatives of adenosine, 2′-deoxyadenosine, arabinosyladenine, 2′-*O*-methyladenosine, 3′-*O*-methyladenosine, and the corresponding N^7-substituted formycins are reported. In addition, N^6-benzyl-3′-deoxyadenosine and the S^6-(3-methyl-2-butenyl)-substituted 6-mercaptopurine riboside and 6-thioguanosine have been prepared. Reactions involving N^1-alkylation followed by rearrangement to the N^6-substituted compounds, stannous chloride catalyzed sugar methylation by diazomethane, sulfur alkylation, and chloride nucleophilic displacement by amine were employed. Biochemical rationale for the analogs of the tRNA minor component **2a** with respect to its plant cytokinin and mammalian inhibitory activities and preliminary biological results are discussed. Mass spectral fragmentation patterns of these nucleosides are tabulated and discussed.

The isolation of N^6-(3-methyl-2-butenyl)adenosine [N^6-(Δ^2-isopentenyl)adenosine] (iPA)[1] (**2a**) from tRNA (Hall *et al.*, 1966; Robins *et al.*, 1967; Zachau *et al.*, 1966; Biemann *et al.*, 1966; Feldmann *et al.*, 1966) and the demonstration of its plant cytokinin (Skoog *et al.*, 1967) and mammalian inhibitory (Grace *et al.*, 1967; Suk *et al.*, 1970) activities have led to extensive studies on iPA and analogous compounds (Hall, 1970, 1971; Skoog *et al.*, 1967; Leonard *et al.*, 1968, 1969; Fleysher *et al.*, 1969; Fleysher, 1972; Hecht *et al.*, 1971a; and references therein). It has been found that both base and nucleoside analogs of iPA (as well as iPA *per se*) have potent cytokinin activity (Skoog *et al.*, 1967; Leonard *et al.*, 1968, 1969). However, the inhibitory activity of these compounds on mammalian cells has been reported to be significantly dependent upon the presence of the sugar portion of the nucleosides (Grace *et al.*, 1967; Fleysher *et al.*, 1969). iPA has been evaluated pharmacologically (Suk *et al.*, 1970) and degradative pathways in humans determined (Chheda and Mittelman, 1972). Indeed, clinical remission in a case of acute leukemia was reportedly effected by treatment with iPA (Jones *et al.*, 1968) and, as well, iPA acts as an immunosuppressive agent (Hacker and Feldbush, 1969).

Previous studies (Skoog *et al.*, 1967; Leonard *et al.*, 1968, 1969; Myles and Fox, 1968; Fleysher *et al.*, 1969; Fleysher, 1972; and references therein) primarily concentrated on changes in the side chain or the base. Since the effects on mammalian systems seem significantly dependent upon the nucleoside level of metabolic construction, we were interested in compounds modified in the sugar portion or with a stable base–sugar linkage. The cytokinin question had not been solved with 9-methyl-6-benzylaminopurine (Kende and Tavares, 1968) nor 9-methylzeatin (Shaw *et al.*, 1968) since the 9-methyl group is metabolically labile in such plant tissue (Fox *et al.*, 1971). Two recent communications report the isolation of presumed 7-glucosyl derivatives of 6-benzylaminopurine (Deleuze *et al.*, 1972) and zeatin (Parker *et al.*, 1972) after feeding these cytokinins *per se*. This adds further interest and possible logic to base–sugar cleavage for cytokinin activity. We chose N^7-(3-methyl-2-butenyl)formycin (**7a**) as a suitable iPA analog since formycin can substitute for adenosine as a substrate for adenosine deaminase (Fukagawa *et al.*, 1965) and in various other enzymatic reactions (Suhadolnik, 1970). Its C–C ribose–aglycone linkage would be expected to be metabolically stable in most systems.

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[1] Abbreviation used is: iPA, Δ^2-isopentenyladenosine.

Rathbone and Hall (1972) have demonstrated the existence of enzymatic activity in cultured human leukemia cells which cleaves the sugar from iPA (**2a**). They again confirmed that the base has far less inhibitory activity than the nucleoside in this mammalian system. It has been reported (Honjo *et al.*, 1964) that certain 2′-*O*-methyl nucleosides are inert to the action of hydrolase and phosphorylase enzymes. Since 3′-*O*-methyluridine is also apparently inert to the action of uridine phosphorylase (Furukawa *et al.*, 1965), the 2′-*O*-methyl (**3a**) and 3′-*O*-methyl (**3b**) iPA derivatives represent logical candidates to evaluate against this type of biochemically resistant cell (Robins and Naik, 1971a) and as anticytokinins (Hecht *et al.*, 1971b).

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Nmr spectra were recorded on Varian A-60 or HA-100 spectrometers with Me_4Si as internal standard in Me_2SO-d_6–D_2O mixed solvent. Ultraviolet (uv) spectra were recorded on Cary 14 or 15 spectrometers with solutions prepared by diluting a 1-ml sample of an accurately determined stock solution in MeOH to 10 ml with MeOH, 0.1 N HCl, or 0.1 N NaOH (freshly prepared). Optical rotations were determined on a Perkin-Elmer Model 141 polarimeter using a 10-cm 1-ml microcell. Mass spectra were determined by the mass spectrometry laboratory of this department on AEI MS-2 or MS-9 instruments at 70 eV. Sample introduction was *via* a direct probe at temperatures between 150 and 230°. Microanalyses were obtained by the microanalytical laboratory of this department. Thin layer chromatography (tlc) was performed on Eastman Chromatogram sheets (silica gel No. 6060) or on glass plates coated with Merck silica gel GF-254 with sample observation under uv light (2537 Å). Preparative tlc was performed on glass plates coated with Merck silica gel PF-254. The solvent used for tlc was the upper phase of EtOAc–*n*-PrOH–H_2O (4:1:2) unless otherwise stated. Evaporations were effected using a Büchler rotating evaporator under aspirator or mechanical oil pump vacuum at 40° or less. *N*,*N*-Dimethylformamide was dried by distillation from P_2O_5 and Linde 4A Molecular Sieves were dried in an oven at 200°. 9-β-D-Arabinofuranosyladenine (**1b**) was purchased from Pfanstiehl Laboratories. 3-Methyl-2-butenyl bromide was purchased from Chemicals Procurement Laboratories, Inc., and redistilled immediately prior to use. 3-Methyl-2-butenylamine (Semenow *et al.*, 1958) was synthesized from 3-methyl-2-butenyl bromide and potassium phthalimide using the conditions described by Leonard *et al.* (1968) for the corresponding Δ^3 isomer. Filtration by gravity was employed to avoid static charge when hydrocarbon solvents were used.

N^6-(*3-Methyl-2-butenyl*)*adenosine* [*N*^{6}-(Δ^2-*Isopentenyl*)*adenosine*] (*iPA*) (**2a**). To a solution of 0.80 g (0.003 mol) of **1a** in 14 ml of dry dimethylformamide was added 0.66 g (0.0044 mol) of 3-methyl-2-butenyl bromide and 1.0 g of $BaCO_3$. The mixture was stirred for 37 hr while protected from light and moisture. Tlc indicated that N^1-alkylation was about 90% complete. The mixture was filtered using a Celite pad and the filter cake was washed with dimethylformamide. The combined filtrate was evaporated to a small volume and this solution was treated with 30 ml of Me_2NH–MeOH (1:1) at room temperature for 2 hr. Tlc indicated approximately 80% of **2a** and 20% of **1a** plus minor trace spots. The solution was evaporated to a light yellow oil which was partitioned between EtOAc and H_2O. The combined organic phase was dried over Na_2SO_4, filtered, and evaporated. The residue was coevaporated with MeOH to give 0.74 g (74%) of **2a** as a colorless solid. Recrystallization of this material from 4 ml of MeCN–EtOH (3:1) gave 0.64 g (63%) of **2a** as fine needles, mp 135–136°. A small sample was subjected to preparative tlc to remove trace impurities and crystallized from the same solvent mixture to give needles of **2a**: mp 147–149°; $[\alpha]_D^{23}$ −65.4° (*c* 1, MeOH), −71.5° (*c* 0.14, EtOH), $[\alpha]_{546}^{23}$ −78.1° (*c* 1, MeOH), −85.0° (*c* 0.14, EtOH); uv max (MeOH) 268 nm (ε 18,100).

Anal. Calcd for $C_{15}H_{21}N_5O_4$: C, 53.72; H, 6.31; N, 20.89. Found: C, 53.79; H, 6.44; N, 20.68.

N^6-*Benzyladenosine* (**2b**). To a solution of 1.0 g (0.0038 mol) of **1a** in 20 ml of dry dimethylformamide was added 1.92 g (0.011 mol) of benzyl bromide. The solution was stirred for 48 hr at 40° while protected from moisture. Solvent was evaporated to about 1 ml (40°, vacuum pump) and this was added dropwise to 100 ml of dry acetone with vigorous stirring. Dry Et_2O (200 ml) was added and the precipitate was collected by centrifugation. It was dissolved in 25 ml of MeOH and 25 ml of Me_2NH–MeOH (1:1) was added. This solution was stirred for 4 hr, evaporated, and coevaporated with additional MeOH. The residue was dissolved in 25 ml of warm MeOH, H_2O (150 ml) was added, the solution was extracted with EtOAc, and the combined organic phase was dried over Na_2SO_4. Drying agent was filtered, the filtrate evaporated, and the residue coevaporated twice with EtOH and then an additional time to a volume where crystallization began. This mixture was allowed to stand at 4° for 18 hr to give 0.88 g (67%) of colorless granules of **2b**, mp 167.5–168°. A sample for analysis was recrystallized from EtOH to give crystals, mp 186–187° with partial melting at 169–170°, followed by resolidification (lit. Fleysher *et al.*, 1969, mp 183°): $[\alpha]_D^{28}$ −65.1° (*c* 1, MeOH); uv max (MeOH) 270 (ε 20,800), 266.5 nm (sh, ε 20,500); nmr δ 3.50–3.70 (br s, 2, $H_{5'}$, $H_{5''}$), 4.08 (t, 1, $H_{4'}$), 4.21 (m, 1, $H_{3'}$) 4.61 (t, $J_{2'-1'}$ = 6.0 Hz, 1, $H_{2'}$), 4.74 (s, 2, *N*^{6}-CH_2Ph), 5.93 (d, $J_{1'-2'}$ = 6.0 Hz, 1, $H_{1'}$), 7.33 (br s, 5, phenyl), 8.26 (s, 1, H_2), 8.38 (s, 1, H_8).

Anal. Calcd for $C_{17}H_{19}N_5O_4$: C, 57.13; H, 5.36; N, 19.60. Found: C, 56.98; H, 5.40; N, 19.85.

*6-N-(3-Methyl-2-butenyl)amino-9-β-*D*-arabinofuranosylpurine* (*N*^{6}-(Δ^2-*Isopentenyl*)-*AraA*) (**2c**). Reaction of 0.52 g (0.002 mol) of **1b** and 0.45 g (0.003 mol) of 3-methyl-2-butenyl bromide in 10 ml of dry dimethylformamide in the presence of 2 g of molecular sieves proceeded analogously to conversion of **1a** → **2a** described above. Rearrangement to the N^6 isomer was effected using NH_4OH solution under reflux (Robins *et al.*, 1967). The resulting syrup was crystallized from *i*-PrOH to give 0.35 g (54%) of off-white material, mp 158–160°. Recrystallization of this product from *i*-PrOH–MeOH (7:3) gave colorless flakes of **2c**, mp 160–161.5°. A sample for analysis was recrystallized from CH_3CN–EtOH to eliminate *i*-PrOH of solvation. Pure **2c** had mp 161.5–162°; $[\alpha]_D^{28}$ +1.9° (*c* 1, MeOH); uv max (MeOH) 266.5 nm (ε 18,100); nmr δ 1.68 (s, 6, $C(CH_3)_2$), 3.6–3.8 (br m, 3, $H_{5'}$, $H_{5''}$, $H_{4'}$), 4.00–4.30 (br m, 4, $H_{3'}$, $H_{2'}$, *N*^{6}-CH_2CH), 5.24 (br t, *J* = 7.0 Hz, 1, -CH=), 6.27 (d, $J_{1'-2''}$ = 4.0 Hz, 1, $H_{1'}$), 8.20 (s, 2, H_2, H_8).

Anal. Calcd for $C_{15}H_{21}N_5O_4$: C, 53.72; H, 6.31; N, 20.89. Found: C, 53.91; H, 6.01; N, 20.61.

*6-N-Benzylamino-9-β-*D*-arabinofuranosylpurine* (*N*^{6}-*Benzyl-AraA*) (**2d**). A solution of 1.87 g (0.007 mol) of **1b** and 3.13 g (0.021 mol) of benzyl bromide in 30 ml of dried dimethylformamide was converted to **2d** in a similar manner to

that described above for the conversion of **1a** → **2b**. Crystallization of the solid obtained from EtOH gave 1.72 g (69%) of colorless needles which were recrystallized from EtOH to give pure **2d**: mp 198.5–199.5°; $[\alpha]_D^{28}$ +2.8° (*c* 1, MeOH); uv max (MeOH) 267 nm (ε 20,000); nmr δ 3.72 (br s, 2, $H_{5'}$, $H_{5''}$), 3.86 (q, 1, $H_{4'}$), 4.11–4.31 (br m, 2, $H_{2'}$, $H_{3'}$), 4.76 (s, 2, N^6-CH_2Ph), 6.36 (d, $J_{1'-2'}$ = 4.5 Hz, 1, $H_{1'}$), 7.35 (br s, 5, phenyl), 8.27 (s, 1, H_2), 8.33 (s, 1, H_8).

Anal. Calcd for $C_{17}H_{19}N_5O_4$: C, 57.13; H, 5.36; N, 19.60. Found: C, 57.39; H, 5.49; N, 19.51.

6-N-(3-Methyl-2-butenyl)amino-9-(2-deoxy-β-D-erythro-pentofuranosyl)purine (2′-Deoxy-N^6-(Δ²-isopentenyl)adenosine) (**2e**). A solution of 5.02 g (0.020 mol) of dried **1c** and 4.47 g (0.030 mol) of 3-methyl-2-butenyl bromide in 100 ml of dried dimethylformamide was treated as described above in the conversion of **1a** → **2a** using 25 g of dried molecular sieves as acid acceptor. $CHCl_3$ was used as the extraction solvent after rearrangement to the N^6 isomer. The crude syrup obtained was purified by chromatography on a column (2.3 × 74 cm, 300 g) of Woelm alumina (deactivated to grade III). The column was eluted with $CHCl_3$–MeOH (50:1). Fractions containing **2e** were combined, evaporated, and dissolved in MeOH–H_2O (1:3). This solution was extracted with Skellysolve B and then several times with EtOAc. The Skellysolve B extract contained colored impurities and was discarded. The latter EtOAc extracts contained pure **2e** and were set aside. The first two EtOAc extracts contained **2e** contaminated with minor impurities and were combined, evaporated, and subjected to the above extraction procedure. Two repetitions of this process using EtOAc as extraction solvent afforded EtOAc solutions containing only **2e**. These fractions were combined, evaporated, and coevaporated with EtOH and then benzene to afford a syrup which was dissolved in a minimal quantity of benzene containing EtOH to affect solution and freeze-dried at −78°. After 15 hr 1.75 g of crystalline solid remained in the flask. This product was recrystallized from acetone–cyclohexane in three crops to give 1.33 g (23%) of colorless needles of **2e**: mp 106–109°; $[\alpha]_D^{28}$ −19.5° (*c* 1, MeOH); uv max (MeOH) 268 nm (ε 18,900); nmr δ 1.71 (s, 6, $C(CH_3)_2$), 2.33 (m, 1, $H_{2'}$), 2.60–2.90 (m, 1, $H_{2''}$), 3.55–3.71 (br s, 2, $H_{5'}$ $H_{5''}$), 3.95 (m, 1, $H_{4'}$), 4.12 (br d, *J* = 6.0 Hz, 2, N^6-CH_2CH), 4.46 (m, 1, $H_{3'}$), 5.30 (br, t, *J* = 6.0 Hz, 1, –CH=), 6.36 (t, $J_{1'-2',2''}$ = 7.0 Hz, 1, $H_{1'}$), 8.22 (s, 1, H_2), 8.32 (s, 1, H_8).

Anal. Calcd for $C_{15}H_{21}N_5O_3$: C, 56.41; H, 6.63; N, 21.93. Found: C, 56.27; H, 6.93; N, 21.59.

6-N-Benzylamino-9-(2-deoxy-β-D-erythro-pentofuranosyl)purine (N^6-Benzyl-2′-deoxyadenosine) (**2f**). A solution of 1.76 g (0.007 mol) of dried **1c** and 2.52 g (0.014 mol) of benzyl bromide in 35 ml of dry dimethylformamide was allowed to react to produce **2f** in a similar manner to that described above in the conversion of **1a** → **2b** except that no attempt was made to isolate the N^1 isomer and $CHCl_3$ was used as the extraction solvent. The crude syrup of **2f** was purified by chromatography on a column (1.7 × 60 cm, 150 g) of Woelm alumina (deactivated to grade III). The column was packed and developed with $CHCl_3$–MeOH (40:1) and the fractions containing product were combined and evaporated to give 1.29 g (58%) of **2f** which was crystallized from EtOH to yield 0.94 g (42%) of crystalline product: mp 175.5–176.5°; $[\alpha]_D^{28}$ −19.3° (*c* 1, MeOH); uv max (MeOH) 270 nm (ε 21,800), 267 (sh, ε 21,600); nmr δ 2.30 (m of 8, 1, $H_{2'}$), 260–2.80 (m, 1, $H_{2''}$), 3.62 (m, 2, $H_{5'}$, $H_{5''}$), 3.94 (q, 1, $H_{4'}$), 4.45 (m, 1, $H_{3'}$), 4.78 (s, 2, N^6-CH_2Ph), 6.38 (t, $J_{1'-2',2''}$ = 7.0 Hz, 1, $H_{1'}$), 7.30 (m, 5, phenyl), 8.23 (s, 1, H_2), 8.37 (s, 1, H_8).

Anal. Calcd for $C_{17}H_{19}N_5O_3$: C, 59.85; H, 5.61; N, 20.55. Found: C, 60.15; H, 5.84; N, 20.80.

6-N-Benzylamino-9-(3-deoxy-β-D-erythro-pentofuranosyl)purine (N^6-Benzyl-3′-deoxyadenosine) (**2g**). A solution of 0.91 g (0.0036 mol) of **1d** (Robins *et al.*, 1972) and 1.25 g (0.0072 mol) of benzyl bromide in 25 ml of dried dimethylformamide was converted to **2g** as described above in the conversion of **1a** → **2b**. The EtOAc solution resulting from the extraction was evaporated to the point of crystallization and then allowed to stand at 4° for 15 hr. Filtration gave 0.75 g (65%) of colorless granules of **2g** which was recrystallized from EtOH to give pure **2g**: mp 185–186°; $[\alpha]_D^{28}$ −49.1° (*c* 1, MeOH); uv max (MeOH) 271 nm (ε 21,000), 267 (sh, ε 20,900); nmr δ 1.80–2.10 (m, 1, $H_{3'}$), 2.12–2.45 (m, 1, $H_{3''}$), 3.45–3.85 (m, 2, $H_{5'}$, $H_{5''}$), 4.40 (m, 1, $H_{4'}$), 4.61 (m, 1, $H_{2'}$), 4.78 (br s, 2, N^6-CH_2Ph), 5.90 (d, $J_{1'-2'}$ = 3.0 Hz, 1, $H_{1'}$), 7.29 (m, 5, phenyl), 8.22 (s, 1, H_2), 8.37 (s, 1, H_8).

Anal. Calcd for $C_{17}H_{19}N_5O_3$: C, 59.81; H, 5.61; N, 20.52. Found: C, 59.97; H, 5.83; N, 20.27.

N^6-(3-Methyl-2-butenyl)-2′-O-methyladenosine (**3a**) *and N^6-(3-Methyl-2-butenyl)-3′-O-methyladenosine* (**3b**). To a stirred suspension of 4.50 g (0.0133 mol) of **2a** in 150 ml of a 10^{-3} M solution of $SnCl_2 \cdot 2H_2O$ in MeOH (Robins and Naik, 1971b) was slowly added a stock solution (Khwaja and Robins, 1966) of diazomethane in 1,2-dimethoxyethane (glyme) until the solution became clear and a faint yellow color persisted. Tlc ($CHCl_3$–MeOH, 95:5, silica gel) showed complete monomethylation. The solution was evaporated to leave a pale yellow solid foam which was dissolved in 30 ml of glyme–H_2O (1:1) by warming on the steam bath. Upon cooling this solution, 2.76 g (59%) of the 3′-isomer **3b** containing traces of the 2′-isomer **3a** crystallized (mp 109–111°) and was filtered. This material was boiled with acetone and filtered hot using a Celite pad. The filter cake was washed well with boiling acetone. The combined filtrate was concentrated to about 20 ml and allowed to cool at 4°. The product which crystallized was recrystallized from 10 ml of acetone to give 1.82 g (39%) of colorless granules of **3b**, mp 112.5–113.5° (powdered and dried at 78° (0.1 mm) for 24 hr over P_2O_5 and Paraffin wax). The mother liquor from crystallization was evaporated and the resulting solid foam was dissolved in 5 ml of EtOH–H_2O (30:70). This solution was applied to a column (3.3 × 120 cm, 1000 ml) of Dowex 1-X2 (OH^-) (200–400 mesh). The column was packed and developed with 30% EtOH–H_2O and 20-ml fractions were collected at the rate of 1 ml/min. Fractions 110–189 were combined, evaporated, and coevaporated with $CHCl_3$ to give 1.44 g (31%) of **3a** as a solid foam. This material was dissolved in 15 ml of MeOH and 60 ml of H_2O and this solution was extracted with Skellysolve B, Et_2O, and then EtOAc. The Skellysolve B extracts were discarded and the Et_2O and EtOAc extracts were combined and washed with H_2O. The organic phase now contained no visible slower migrating products on tlc and was set aside. The combined aqueous extracts were washed with EtOAc and this combined organic phase was backwashed with H_2O and then added to the above EtOAc extracts. This combined solution was evaporated and the residue was dissolved in 5 ml of MeOH and 20 ml of H_2O. This solution was extracted several times with Et_2O. The first Et_2O extract was contaminated with a trace of faster migrating material on tlc. The additional Et_2O extracts were combined, dried over Na_2SO_4, filtered, evaporated, and coevaporated with benzene. A benzene solution (20 ml) of the oily residue was

freeze-dried to give 0.48 g of chromatographically pure **3a**: mp 47–60°, complete at 85°; $[\alpha]_D^{28}$ −68.9° (*c* 1, $CHCl_3$); uv max (MeOH) 268 nm (ε 18,400); nmr δ 1.71 (s, 6, $C(CH_3)_2$), 3.33 (s, 3, OCH_3), 3.70 (br s, 2, $H_{5'}$, $H_{5''}$), 4.02 (m, 1, $H_{4'}$), 4.14 (br, 2, N^6-CH_2CH), 4.40 (br s, 2, $H_{2'}$, $H_{3'}$), 5.31 (br t, J = 6.0 Hz, 1, –CH═), 6.03 (d, $J_{1-2'}$ = 5.0 Hz, 1, $H_{1'}$), 8.25 (s, 1, H_2), 8.38 (s, 1, H_8).

Anal. Calcd for $C_{16}H_{23}N_5O_4$: C, 55.00; H, 6.63; N, 20.05. Found: C, 54.98; H, 6.84; N, 19.86.

Fractions 445–595 were combined, evaporated (vacuum pump 40°), and coevaporated with EtOH and then $CHCl_3$ to give 0.28 g of **3b** as a solid foam. This material was treated with boiling acetone and filtered hot using a Celite pad. The filter cake was washed with EtOH and the combined filtrate was evaporated to a solid residue which was crystallized from 2 ml of acetone to give 0.13 g (3%) of **3b** (total yield of pure crystalline **3b**, 1.95 g (42%)): mp 113–114°; $[\alpha]_D^{28}$ −76.0° (*c* 1, MeOH); uv max (MeOH) 268 nm (ε 19,700); nmr δ 1.68, 1.70 (s, s, 6, $C(CH_3)_2$), 3.43 (s, 3, OCH_3), 3.64 (t, 2, $H_{5'}$, $H_{5''}$), 3.88 (q, 1, $H_{4'}$), 4.02–4.22 (m, 3, $H_{3'}$, N^6-CH_2CH), 4.77 (t, 1, $H_{2'}$), 5.44 (br t, J = 6.5 Hz, 1, –CH═), 5.92 (d, $J_{1'-2'}$ = 6.5 Hz, 1, $H_{1'}$), 8.28 (s, 1, H_2), 8.41 (s, 1, H_8).

Anal. Calcd for $C_{16}H_{23}N_5O_4$: C, 55.00; H, 6.63; N, 20.05. Found: C, 54.71; H, 6.78; N, 20.00.

N^6-Benzyl-2′-O-methyladenosine (**3c**) *and N^6-Benzyl-3′-O-methyladenosine* (**3d**). A stirred suspension of 7.5 g (0.020 mol) of **2b** was converted to a mixture of **3c** and **3d** as described above for the conversion of **2a** → **3a** and **3b**. Fractions containing **3c** were combined and evaporated to give 2.76 g (36%) of a pale yellow solid foam: nmr δ 3.33 (s, 3, OCH_3), 3.70 (m, 2, $H_{5'}$, $H_{5''}$), 4.02 (m, 1, $H_{4'}$), 4.42 (m, 2, $H_{2'}$, $H_{3'}$), 4.80 (br s, 2, N^6-CH_2Ph), 6.07 (d, $J_{1'-2'}$ = 5.0 Hz, 1, $H_{1'}$), 7.38 (m, 5, phenyl), 8.29 (s, 1, H_2), 8.47 (s, 1, H_8). This material (0.0074 mol) was dissolved in 6.5 ml of EtOH and 16 ml of acetone and treated with a solution of 0.27 g (0.0074 mol) of HCl gas in 3.2 ml of EtOH and 16 ml of acetone. An additional 65 ml of acetone was added and the mixture was allowed to stand at 4° for 15 hr to yield 2.37 g (28%) of crystalline **3c** hydrochloride, mp 129–132°. A sample for analysis was recrystallized from dry EtOH–Et_2O containing 0.37 equiv of HCl to give crystals with mp 147.5–149.5°: $[\alpha]_D^{28}$ −44.1° (*c* 1, MeOH); uv max (MeOH) 271 nm (ε 20,100), 267 (sh, ε 20,000), (NaOH) 269.5 (ε 20,000).

Anal. Calcd for $C_{18}H_{21}N_5O_4 \cdot HCl$: C, 53.00; H, 5.44; N, 17.17; Cl, 8.69. Found: C, 52.89; H, 5.73; N, 16.89; Cl, 8.70.

The following 74 fractions were combined and evaporated to give 0.45 g (6%) of a mixture of **3c** and **3d** as a solid foam. Elution was continued and the concentration of EtOH in H_2O was gradually increased to 90%. Fractions containing **3d** were combined and evaporated to give 4.50 g (58%) of a solid foam which was crystallized from 32 ml of acetone to yield 3.68 g (47%) of colorless granules of **3d**: mp 141–142.5° (dried for 15 hr at 60° (0.1 mm) over P_2O_5 and Paraffin wax); $[\alpha]_D^{28}$ −44.1° (*c* 1, MeOH); uv max (MeOH) 271.5 nm (ε 20,300); nmr δ 3.47 (s, 3, OCH_3), 3.68 (m, 2, $H_{5'}$, $H_{5''}$), 3.91 (t, 1, $H_{4'}$), 4.12 (q, 1, $H_{3'}$), 4.79 (m, 3, N^6-CH_2Ph, $H_{2'}$), 5.92 (d, $J_{1'-2'}$ = 6.0 Hz, 1, $H_{1'}$), 7.30 (m, 5, phenyl), 8.23 (s, 1, H_2), 8.38 (s, 1, H_8).

Anal. Calcd for $C_{18}H_{21}N_5O_4$: C, 58.21; H, 5.70; N, 18.86. Found: C, 58.14; H, 5.63; N, 19.16.

6-(3-Methyl-2-butenyl)thio-9-β-D-ribofuranosylpurine [*S^6-(Δ^2-Isopentenyl)mercaptopurine Ribonucleoside*] (**5a**). To a solution of 1.50 g (0.0053 mol) of **4a** in 15 ml of dry dimethylformamide was added 1 g of anhydrous K_2CO_3 and 0.82 g (0.0055 mol) of 3-methyl-2-butenyl bromide and the mixture was stirred for 1 hr at room temperature while protected from moisture. The mixture was partitioned between H_2O and EtOAc. The combined organic phase was dried over Na_2SO_4, filtered, evaporated to a small volume, and partitioned between $CHCl_3$ and H_2O. The combined organic phase was dried over $MgSO_4$, filtered using a Celite pad, and evaporated to a volume of 30 ml. This solution was added dropwise to 400 ml of vigorously stirred Skellysolve B (freshly distilled). The resulting precipitate was filtered by gravity and dried at 0.1 mm at room temperature to give 1.56 g (82%) of colorless solid **5a**: mp 72–76°; $[\alpha]_D^{28}$ −52.7° (*c* 1, MeOH); uv max (MeOH) 292 nm (ε 20,900), 287 (sh, ε 20,400), (HCl) 296 (ε 18,300), (NaOH) 295 (ε 18,700); nmr δ 1.72, 1.76 (s, s, 6, $C(CH_3)_2$), 3.69 (m, 2, $H_{5'}$, $H_{5''}$), 3.97–4.03 (m, 3, S-CH_2CH, $H_{4'}$), 4.24 (t, 1, $H_{3'}$), 4.63 (t, 1, $H_{2'}$), 5.37 (br t, J = 7.0 Hz, 1, –CH═), 6.02 (d, $J_{1'-2'}$ = 5.5 Hz, 1, $H_{1'}$), 8.67, 8.73 (s, s, 2, H_2, H_8).

Anal. Calcd for $C_{15}H_{20}N_4O_4S$: C, 51.20; H, 5.71; N, 15.90; S, 9.09. Found: C, 50.91; H, 5.66; N, 16.05; S, 8.83.

2-Amino-6-(3-methyl-2-butenyl)thio-9-β-D-ribofuranosylpurine (**5b**). A solution of 1.50 g (0.005 mol) of **4b** in 15 ml of dry dimethylformamide was treated with 1.5 g of anhydrous K_2CO_3 and 0.76 g (0.0051 mol) of 3-methyl-2-butenyl bromide as described above in the preparation of **5a**. Further portions of organic solvents were required to complete extraction of the more insoluble **5b**. The precipitated product (1.85 g, 85%) was redissolved in 30 ml of $CHCl_3$ and reprecipitated into 400 ml of freshly distilled Skellysolve B to give 1.65 g (76%) of **5b** as a powder: mp 87–92°; $[\alpha]_D^{28}$ −45.1° (*c* 1, MeOH); uv max (MeOH) 248 nm (ε 14,600), 303 (ε 13,300), (HCl) 250 (ε 9200), 327 (ε 11,700), (NaOH) 248 (ε 12,200), 313.5 (ε 13,300); nmr δ 1.75 (s, 6, $C(CH_3)_2$), 3.64 (m, 2, $H_{5'}$, $H_{5''}$), 3.90–4.00 (m, 3, S-CH_2, $H_{4'}$), 4.17 (t, 1, $H_{3'}$), 4.33 (t, 1, $H_{2'}$), 5.37 (br t, J = 7.5 Hz, 1, –CH═), 5.82 (d, $J_{1'-2'}$ = 6.0 Hz, 1, $H_{1'}$), 8.17 (s, 1, H_8).

Anal. Calcd for $C_{15}H_{21}N_5O_4S$: C, 49.05; H, 5.76; N, 19.09; S, 8.74. Found: C, 49.27; H, 5.52; N, 19.04; S, 8.49.

7-N-(3-Methyl-2-butenyl)amino-3-β-D-ribofuranosylpyrazolo-[4,3-d]pyrimidine [*N^7-(Δ^2-Isopentenyl)formycin*] (**7a**). A solution of 0.80 g (0.0028 mol) of **6** (Long *et al.*, 1971) in 2.7 g (0.032 mol) of 3-methyl-2-butenylamine was stirred for 2.5 hr at room temperature while protected from moisture and then poured into 25 ml of ice-cold 1 N NaOH. This solution was extracted with Et_2O and the organic phase discarded. Ice was added to the aqueous phase and it was cautiously neutralized to pH 6 (pHydrion paper) and extracted with EtOAc. This combined organic phase was dried over Na_2SO_4, filtered, and evaporated. The colorless solid residue was dissolved in MeOH and filtered using a Celite pad. The filtrate was evaporated to give 0.73 g (77%) of **7a** as a solid foam which was treated with 20 ml of acetone and allowed to stand overnight. The product had crystallized and two crops of **7a**, 0.51 g (55%), mp 192–193°, and 0.09 g (9%), mp 165–173°, were obtained. A sample for analysis was recrystallized from acetone–EtOH (2:1) to give colorless crystals of **7a**: mp 193.5–195° (lit. mp 120–122°, Hecht *et al.*, 1971a); $[\alpha]_D^{28}$ −55.6° (*c* 1, MeOH); uv max (MeOH) 296, 238.5 nm (ε 16,000, 6,300), shoulders 307, 288 (ε 11,700, 14,000), (NaOH) 300, 243.5 (ε 12,200, 13,200), (HCl) 311, 301, 239 (ε 16,500, 17,300, 6,600); nmr δ 1.78 (s, 6, $C(CH_3)_2$), 3.62 (m, 2, $H_{5'}$, $H_{5''}$), 3.97 (m, 1, $H_{4'}$), 4.13 (m, 3, $H_{3'}$, N^7-CH_2CH═), 4.46 (t, 1, $H_{2'}$), 4.98 (d, $J_{1'-2'}$ = 7.0 Hz, 1, $H_{1'}$), 5.33 (br t, J = 7.0 Hz, 1, N^7-CH_2CH═), 8.33 (s, 1, H_5).

Anal. Calcd for $C_{15}H_{21}N_5O_4$: C, 53.72; H, 6.31; N, 20.89. Found: C, 53.44; H, 6.25; N, 21.09.

7-N-Benzylamino-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine (N⁷-Benzylformycin) (**7b**). A solution of 0.50 g (0.0018 mol) of **6** (Long *et al.*, 1971) in 5 ml of benzylamine was reacted and subjected to the same procedure as **7a**. Evaporation of the dried EtOAc solution and coevaporation of the residue with H_2O and then with EtOH gave 0.52 g (83%) of crude **7b** as a yellow solid foam. This material was dissolved in a minimum volume of MeOH and applied to three preparative chromatography plates (18 × 18 cm) which were developed and the appropriate major bands were removed and extracted with four 60-ml portions of MeOH. The extracts were combined, filtered, and evaporated to dryness, and the residue was dissolved in $CHCl_3$ containing a small amount of EtOH. This cloudy solution was filtered using a Celite pad and the clear filtrate was evaporated to give 0.44 g (70%) of **7b** as a colorless solid foam: nmr δ 3.65 (m, 2, $H_{5'}$, $H_{5''}$), 3.97 (m, 1, $H_{4'}$), 4.15 (m, 1, $H_{3'}$), 4.53 (t, 1, $H_{2'}$), 4.81 (s, 2, N^7-CH_2Ph), 5.03 (d, $J_{1'-2'}$ = 7.0 Hz, 1, $H_{1'}$), 7.38 (br s, 5, Ph), 8.24 (s, 1, H_5). This material was dissolved in 1 ml of 2 N HCl in EtOH and 0.34 g (50%) of **7b**·HCl crystallized as colorless granules, mp 206–209° (some decomposition). A sample for analysis was recrystallized from EtOH–MeOH (containing 2.0 equiv of HCl) to give fine needles of **7b**·HCl: mp 206–212° (some decomposition); $[\alpha]_D^{24}$ −28.8° (*c* 0.5, MeOH); uv max (MeOH) 307, 297, 289 (sh), and 239 nm (ε 11,900, 14,800, 13,000, and 6,800), (HCl) 301 and 291 (ε 15,500 and 16,100), (NaOH) 305 and 243 (ε 10,700 and 12,500).

Anal. Calcd for $C_{17}H_{19}N_5O_4$·HCl: C, 51.84; H, 5.12; N, 17.79; Cl, 9.00. Found: C, 51.92; H, 5.31; N, 17.49; Cl, 9.21.

Results

N^6-(3-Methyl-2-butenyl)adenosine (iPA) (**2a**) was first synthesized by Leonard and coworkers (Leonard *et al.*, 1966) by N^1-alkylation of adenosine with 3-methyl-2-butenyl bromide followed by Dimroth rearrangement to the N^6 isomer. The yield of the alkylation step was observed to be about 50%. In agreement with Leonard *et al.* (1966) and Martin and Reese (1968), we observed that the alkylation step proceeded to about 50–60% and then ceased. Shimizu and Miyaki (1970) reported migration of 3-methyl-2-butenyl and benzyl groups presumably by reversal of the alkylation reaction by bromide (present in the hydrobromide salt) followed by realkylation. However, we found that a dimethylformamide solution of N^1-(3-methyl-2-butenyl)adenosine (isolated by preparative tlc) did not observably reverse to adenosine at room temperature even in the presence of a 2 mol ratio of added hydrogen bromide.

iPA synthesized by this alkylation–rearrangement procedure invariably contained a small amount of material which was not removed completely by recrystallization, migrated faster than iPA in chromatographic systems, and had mol wt = 403 (mass spectroscopy). This corresponds to iPA − H + isopentenyl (335 − 1 + 69). If sufficient HBr is generated to protonate about 50% of the adenosine molecules by the time that alkylation has occurred to 50%, the adenosine N^1-hydrobromide would be inaccessible to alkylation and addition of further quantities of the alkenyl bromide would have no effect (as is observed). Therefore, nonnucleophilic acid acceptors were added to the reaction mixture and, indeed, 80–90% alkylation of adenosine to the N^1 isomer occurred (see Experimental Section).

Use of pure 3-methyl-2-butenylamine in reaction with 6-chloropurine ribonucleoside (Robins *et al.*, 1967, and the present work) precludes the formation of the polypentenyl products (*i.e.*, no mol wt = 403 and analogous products in mass spectra) and thus these products probably have not contributed significantly to biological effects.

A further note on iPA *per se* concerns its optical rotation. Various samples prepared by the procedure of Leonard *et al.* (1966), Martin and Reese (1968), and our modification gave $[\alpha]_D$ values near −70° and $[\alpha]_{546}$ near −85° in ethanol which are significantly different from the reported values of $[\alpha]_D^{28}$ −103° (*c* 0.14, EtOH) (Leonard *et al.*, 1966) and $[\alpha]_{546}^{25}$ −97° (*c* 0.07, EtOH) (Robins *et al.*, 1967). A carefully purified sample of iPA which had correct elemental analyses and no higher molecular weight impurities had $[\alpha]_D^{23}$ −71.5° (*c* 0.14, absolute EtOH), $[\alpha]_D^{23}$ −70.0° (*c* 0.07, absolute EtOH), $[\alpha]_{546}^{23}$ −85.0° (*c* 0.14, absolute EtOH), and $[\alpha]_{546}^{23}$ −85.8° (*c* 0.07, absolute EtOH). These values are consistent with values obtained in 95% EtOH and also with values obtained on a "drug" sample of iPA (kindly provided by Dr. G. B. Chheda, Roswell Park Memorial Institute, Buffalo, N. Y.) within ±1.5°.

Preparation of biologically analogous (Skoog *et al.*, 1967) and chemically stable benzyl analogs of iPA was undertaken also. Alkylation of adenine nucleosides with benzyl bromide proceeds readily to about 90% in the absence of any acid acceptor. A general alkylation procedure (Jones and Robins, 1963; Fleysher *et al.*, 1969) coupled with the mild methanolic dimethylamine rearrangement conditions of Martin and Reese (1968) gave 6-benzylaminopurine ribonucleoside (**2b**) in 67% yield.

Analogous reactions using **1b** gave the corresponding N^6-(3-methyl-2-butenyl)-Ara A (**2c**) and N^6-benzyl-Ara A (**2d**). Again, 4A molecular sieves were effective in the alkenyl bromide reaction but were unnecessary with benzyl bromide. After this work was completed, a patent appeared describing **2c** prepared by a base–sugar coupling procedure (Vorbrueggen *et al.*, 1971).

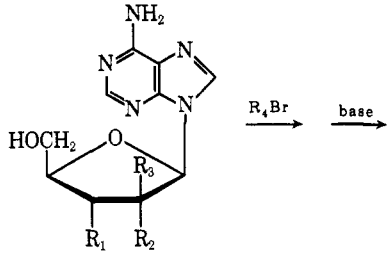
Martin and Reese (1968) and Brookes *et al.* (1968) treated 2′-deoxyadenosine with 3-methyl-2-butenyl bromide and benzyl bromide, respectively, and obtained only the alkylated adenine aglycone salts. Sivadjian *et al.* (1969) have reported the enzymatic transfer of 2-deoxy-D-*erythro*-pentose (2-deoxyribose) to N^6-(3-methyl-2-butenyl)adenine and 6-benzylaminopurine to presumably give the 2′-deoxynucleosides, but no characterization of products was given. Leonard *et al.* (1969) have reported 2′-deoxy-iPA (**2e**), characterized as a hygroscopic solid hydrate.

Alkylation of 2′-deoxyadenosine in the presence of 4A molecular sieves folowed by rearrangement to the N^6 isomer and purification from several minor products gave crystalline **2e**. Analogous reaction using benzyl bromide (no sieves necessary) gave **2f**. The synthesis of N^6-benzyl-3′-deoxyadenosine (N^6-benzylcordycepin) (**2g**) was effected similarly.

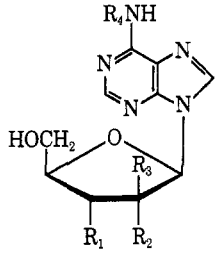
iPA (**2a**) and N^6-benzyladenosine (**2b**) were quantitatively monomethylated with diazomethane in the presence of stannous chloride (Robins and Naik, 1971b) (see Reaction Scheme). The resulting mixtures of 2′-*O*- and 3′-*O*-methyl isomers **3a** and **3b** and **3c** and **3d**, respectively, were readily separated by anion exchange chromatography (Dekker, 1965). These methylations gave 3′-*O*-methyl:2′-*O*-methyl isomer ratios of about 60:40 as observed with analogous methylation of adenosine (Robins and Naik, 1971b).

Sulfur alkylation of 6-mercaptopurine ribonucleoside (**4a**) and 6-thioguanosine (**4b**) proceeded rapidly to completion

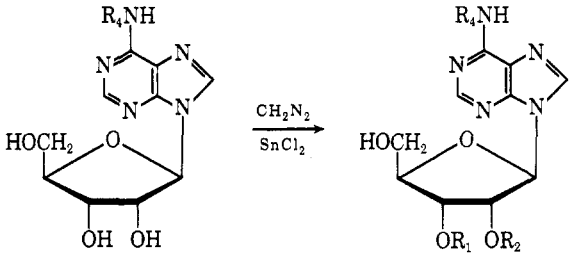
REACTION SCHEME



1a, $R_1 = R_2 = OH$; $R_3 = H$
b, $R_1 = R_3 = OH$; $R_2 = H$
c, $R_1 = OH$; $R_2 = R_3 = H$
d, $R_1 = R_3 = H$; $R_2 = OH$

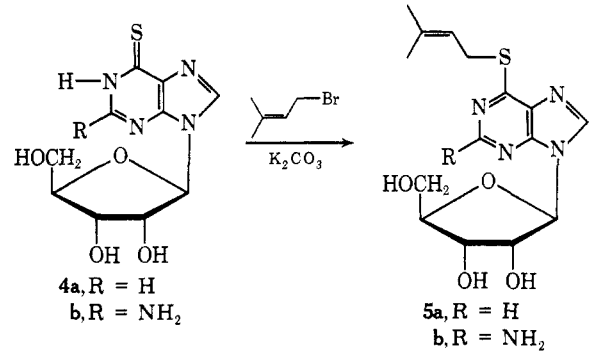


2a, $R_1 = R_2 = OH$; $R_3 = H$; R_4 = iP
b, $R_1 = R_2 = OH$; $R_3 = H$; R_4 = Bzl
c, $R_1 = R_3 = OH$; $R_2 = H$; R_4 = iP
d, $R_1 = R_3 = OH$; $R_2 = H$; R_4 = Bzl
e, $R_1 = OH$; $R_2 = R_3 = H$; R_4 = iP
f, $R_1 = OH$; $R_2 = R_3 = H$; R_4 = Bzl
g, $R_1 = R_3 = H$; $R_2 = OH$; R_4 = Bzl



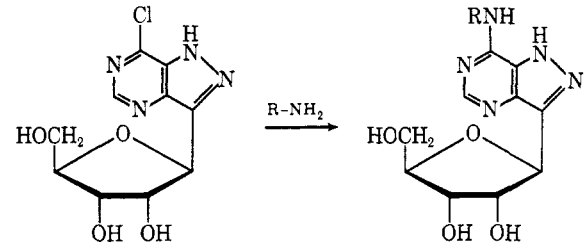
2a, R_4 = iP
b, R_4 = Bzl

3a, $R_1 = H$; $R_2 = CH_3$; R_4 = iP
b, $R_1 = CH_3$; $R_2 = H$; R_4 = iP
c, $R_1 = H$; $R_2 = CH_3$; R_4 = Bzl
d, $R_1 = CH_3$; $R_2 = H$; R_4 = Bzl



4a, R = H
b, R = NH_2

5a, R = H
b, R = NH_2



6

7a, R = iP
b, R = Bzl

iP = $(CH_3)_2C{=}CHCH_2-$
Bzl = $C_6H_5CH_2-$

in dry dimethylformamide in the presence of anhydrous potassium carbonate (Johnston *et al.*, 1958) to give the corresponding 3-methyl-2-butenyl thionucleosides **5a** and **5b**, respectively.

Hecht *et al.* (1971a) reported the synthesis of 7-*N*-(3-methyl-2-butenyl)amino-3-β-D-ribofuranosylpyrazolo[4,3-*d*]-pyrimidine [N^7-(3-methyl-2-butenyl)formycin] (**7a**) by alkylation of formycin followed by rearrangement to the N^7 isomer. These authors reported that **7a** was obtained in "low yield" and record mp 120–122°. We had previously studied this reaction and found that at least five spots (visible under uv light on tlc) are observed in the alkylation solution in the presence or absence of molecular sieves. It is one of the minor intensity spots which disappears upon rearrangement in base and a minor spot corresponding to **7a** appears.

We, therefore, treated 7-chloro-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidine (**6**) (Long *et al.*, 1971) with 3-methyl-2-butenylamine to give **7a** in 64% yield, mp 193.5–195°. Analogous reaction of **6** with benzylamine gave N^7-benzylformycin (**7b**).

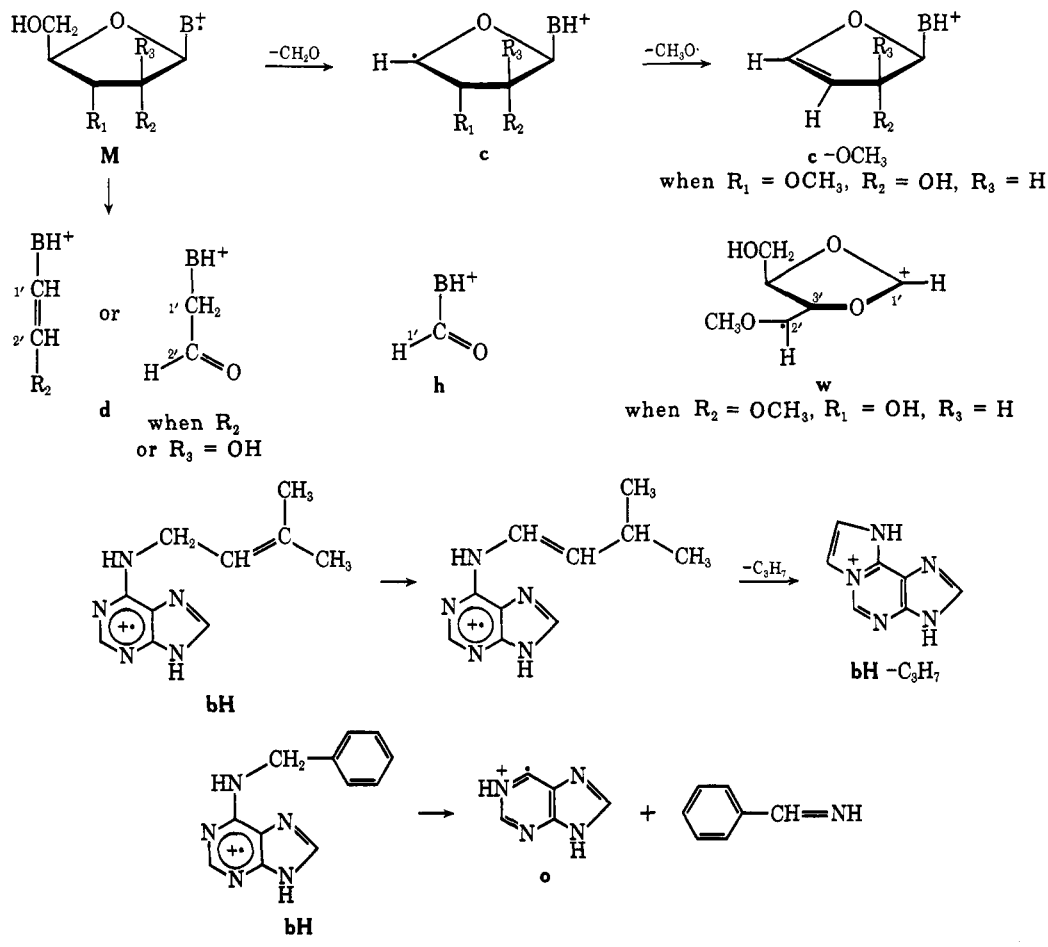
Discussion

The diverse and pronounced biological activity of N^6-(3-methyl-2-butenyl)adenosine (**2a**) is of considerable curren-interest (for reviews see: Hall, 1970, 1971; Skoog and Armt strong, 1970; and references therein). The importance of the nucleoside level of construction has again been emphasized in a mammalian system (Rathbone and Hall, 1972) and nucleoside–base cleavage for plant cytokinin activity seems probable (Hecht *et al.*, 1971a,b).

Yields of approximately 90% in the alkylation of adenine nucleosides by 3-methyl-2-butenyl bromide can now be realized (see Experimental Section) by employing nonnucleophilic acid acceptors. This route was used to synthesize **2a**, **2c**, and **2e**. Similar reactions using benzyl bromide gave **2b**, **2d**, **2f**, and **2g**. Stannous chloride catalyzed methylation of iPA (**2a**) and 6-benzylaminopurine ribonucleoside (**2b**) with diazomethane (Robins and Naik, 1971b) gave **3a**–**3d**. Alkylation of 6-mercaptopurine ribonucleoside (**4a**) and 6-thioguanosine (**4b**) with 3-methyl-2-butenyl bromide gave **5a** and **5b**, respectively. The C–C linked nucleosides **7a** and **7b** were prepared by nucleophilic displacement of chloride from 7-chloro-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidine (**6**) (Long *et al.*, 1971) by the appropriate amine after it was observed that direct alkylation of formycin gave a complex mixture of products.

Mass spectroscopy is a valuable tool for characterizing these compounds and was particularly useful for distinguishing the 2′-*O*- and 3′-*O*-methyl isomers. McCloskey and coworkers have reported characteristic fragmentations of the sugar residue (Biemann and McCloskey, 1962; Shaw *et al.*, 1970). Sugar cleavage to ion **d** gives *m/e* **b** + 58 for the 2′-*O*-methyl derivatives and *m/e* **b** + 28 for the 2′-deoxy derivatives. Ion **w**, which is also characteristic of 2′-*O*-methylation, occurs at *m/e* 147 (Shaw *et al.* 1970). An ion that occurred exclusively in the spectra of the 3′-*O*-methyl compounds **3b** and **3d** had *m/e* **M** − 61. Accurate mass deter-

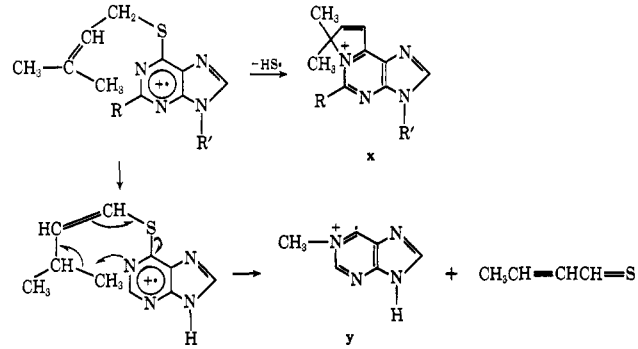
FRAGMENTATION SCHEME



mination is consistent with the molecular formula **c** − OCH_3 (see Fragmentation Scheme).

Fragmentation of the Δ^2-isopentenyl side chain gives rise to a characteristic set of ions (Shannon and Letham, 1966). The isopentenyl nucleosides (**2a**, **2c**, **2e**, **3a**, **3b**) show this series of ions which in iPA (**2a**) occur at *m/e* 188 (**bH** − CH_3, 58% relative intensity), 160 (**bH** − C_3H_7, 100%), 148 (**bH** − C_4H_7, 16%), 135 (**bH** − C_5H_8, 57%), and 41 ($C_3H_5^+$, 32%) in addition to ions arising directly from side-chain fragmentation from the molecular ion at *m/e* M − 15 (M − CH_3) and M − 43 (M − C_3H_7) (see Fragmentation Scheme and Table I).

The mass spectra of the 6-thio analogs **5a** and **5b** are similar to that of 6-(3-methyl-2-butenyl)thiopurine (Hecht, 1971). Extrusion of an HS· radical to give ion **x** occurs from both the molecular ion and the **bH** ion. Ion **y**, which corresponds to formal loss of C_4H_6S from **bH**, is apparently significant only after loss of the sugar. Hecht (1971) has suggested this ion as the 6-methylpurine isomer which would involve a rather complex single step or multiple step decomposition. He quotes evidence for a single step fragmentation in the observation of a peak at *m/e* 85 (C_4H_6S), which we also observe as a low intensity ion. A cyclic process evolving the 1-methyl isomer (ion **y**) plus thiocrotonaldehyde represents a plausible pathway (see Fragmentation Scheme. Remaining significant ions correspond to the usual sugar and side-chain fragmentation patterns. For example, the spectrum of **5a** has peaks at *m/e* 352 (M, 13%), 263 (**d**, 2.4%), 249 (**h**, 1.8%), 220 (**bH**, 22%), 152 (**bH** − C_5H_8, 44%), and 41 ($C_3H_5^+$, 100%).



R	R′	x, *m/e* (Rel Intensity (%))	y *m/e* (Rel Intensity (%))
5a H	$C_5H_9O_4$	319 (5.7)	
H	H	187 (84)	134 (38)
5b NH_2	$C_5H_9O_4$	334 (12)	
NH_2	H	202 (100)	149 (21)

The spectrum of N^7-(3-methyl-2-butenyl)formycin (**7a**) has been reported (Hecht, 1971). Spectra of both **7a** and **7b** have high intensities of ion **h** due to the stability of the C–C "glycosidic" bond (Townsend and Robins, 1969). This stability is also evidenced by significant ions at **d** − C_5H_8 and **h** − C_5H_8 in the spectrum of **7a** in which side-chain loss from these "glycoside" linked fragments occurs. An ion at *m/e* M − 36 (M − $2H_2O$) is present in the spectrum of **7b** but not in **7a** and, thus, this loss is not general for formycin derivatives (Townsend and Robins, 1969). The spectrum of **7a** also has peaks at *m/e* 69 ($C_5H_9^+$, 30%) and 41 ($C_3H_5^+$, 100%) (see Table I).

Mass spectra of the benzyl derivatives **2b**, **2d**, **2f**, **2g**, **3c**, **3d**, and **7b** have a series of characteristic ions analogous to

TABLE I

Compd	*m/e* (Relative Intensity (%))								
	M	M − CH_3	c	M − C_3H_7	d	h	bH	bH − C_3H_7	Others
2a	335 (71)	320 (9.3)	305 (2.3)	292 (36)	246 (18)	232 (27)	203 (69)	160 (100)	
2c	335 (36)	320 (4.1)	305 (0.8)	292 (12)	246 (12)	232 (33)	203 (59)	160 (100)	
2e	319 (29)	304 (1.3)	289 (2.7)	276 (2.1)	230 (4.2)	232 (24)	203 (50)	160 (100)	
3a	349 (100)	334 (7.6)	319 (11)	306 (15)	260 (56)	232 (13)	203 (68)	160 (91)	w, 146 (10)
3b	349 (68)	334 (9.0)	319 (2.8)	306 (13)	246 (13)	232 (16)	203 (71)	160 (100)	c − OCH_3, 288 (18)
7a[a]	335 (8.8)	320 (2.3)	305 (0.2)	292 (1.8)	246 (8.1)	232 (42)			d − C_5H_8, 178 (9.1)
									h − C_5H_8, 164 (45)

[a] The base peak of this spectrum was at *m/e* 41.

TABLE II

Compd	*m/e* (Relative Intensity (%))						
	M	c	d	h	bH	o	Others
2b	357 (28)	327 (3.0)	268 (15)	254 (29)	225 (100)	120 (18)	
2d	357 (35)	327 (2.3)	268 (14)	254 (54)	225 (100)	120 (11)	
2f	341 (27)	311 (4.2)	252 (12)	254 (32)	225 (100)	120 (17)	
2g	341 (18)	311 (3.8)	268 (39)	254 (25)	225 (100)	120 (16)	
3c[a]	371 (35)	341 (10)	282 (40)	254 (21)	225 (100)	120 (14)	w, 146 (15)
3d[b]	371 (11)	341 (1.2)	268 (4.6)	254 (15)	225 (10)	120 (15)	c − OCH_3, 310 (4.1)
7b[b]	357 (9.5)		268 (10)	254 (53)	225 (1.1)		

[a] The spectrum of this compound was obtained on the hydrochloride, which vaporizes in the spectrometer as the free nucleoside. [b] The base peak of this spectrum was at *m/e* 91.

those found with 6-benzylaminopurine (Shannon and Letham, 1966) (see Table II). For example, the spectrum of N^6-benzyladenosine (**2b**) has peaks at *m/e* 148 (**bH** − C_6H_5, 6.6%), 106 ($C_6H_5CH{=}NH_2^+$, 46%), and 91 ($C_7H_7^+$, tropyllium ion, 51%). The benzyl derivatives also give rise to ion **o** (Shaw *et al.*, 1970) by extrusion of benzylamine from ion $\mathbf{bH}^+$ with accompanying transfer of a hydrogen to the purine ring (see Fragmentation Scheme). The formycin derivative **7b** has the tropyllium ion as its mass spectral base peak, *m/e* 91 ($C_7H_7^+$, 100%).

This study presents a number of new and biochemically interesting Δ^2-isopentenyl and benzyl nucleosides which are available in practical quantities for cytokinin, tRNA incorporation (Burrows *et al.*, 1971), and other biochemical studies. It is of interest to note that adenosine aminohydrolase slowly deaminates **7a** and **7b** to formycin B (Robins and Trip, 1973[2]). In a human leukemia cell line, the N^6-benzyl derivatives **2b**, **2d**, **2f**, and **3c** cause 50% inhibition of growth in the 10^{-5} M range whereas the N^6-(3-methyl-2-butenyl) derivatives **2c**, **2e**, and **3a** are less active. iPA *per se* is most inhibitory at about 10^{-6} M and the two sulfur analogs **5a** and **5b** are inhibitory (Dr. A. Bloch, personal communication). It is highly interesting that any modification of the sugar moiety of **2a** or changing the purine base drastically reduces the effect (*i.e.*, **2c**, **2e**, **3a**, **3b**, and **7a** are required at $>10^{-4}$ M for 50% inhibition). Further biochemical studies, cytokinin, and inhibition results will be reported separately.

[2] Robins, M. J., and Trip, E. M. (1973), unpublished results.

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