Antimicrobial Activity. The compounds synthesized for this study were assayed for the antimicrobial activity. Clinical isolates of *Candida albicans* (*Ca*), *Escherichia coli* (*Ec*), *Epidermophyton floccosum* (*Ef*), *Microsporum canis* (*Mc*), *Pseudomonas aeruginosa* (*Pa*), *Staphylococcus aureus* (*Sa*), and *Trichophyton mentagrophytes* (*Tm*) were used for this study. In vitro sensitivity of these organisms to this series of imidazo[1,2-*a*]pyrimidines was quantitatively determined by broth dilution assay.[9]

Serial dilutions were prepared in chemically defined medium in a range from 0.4 to 0.005 μmol/ml. The minimal inhibitory concentration (MIC) was recorded as the highest dilution of compound which prevented visible growth of the pathogen. Bacterial and yeast MIC's were read following 24 hr of incubation at 35°. Dermatophyte inhibition was read after 48 hr of incubation at 30°.

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Synthesis of Some Glycoside Analogs and Related Compounds from 9-Amino-6-(methylthio)-9*H*-purine

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Additional information on the anticancer activity of 9-amino-9*H*-purine-6(1*H*)-thione and its derivatives was sought by the synthesis of some 9-(substituted amino)-6-(methylthio)-9*H*-purines in which the 9-substituent contained functional groups capable of either reversible or irreversible binding with an enzymatic site. Condensation of 9-amino-6-(methylthio)-9*H*-purine (1) with some carbonyl compounds followed by hydride reduction of the azomethine linkage in the intermediates leads to the 2-pyrrolylmethyl (**8**), 2,3,4-trihydroxybutyl (**10**), and the 1,5-dihydroxy-2- and -3-pentyl (**11** and **12**) compounds. A 4-hydroxybutyl derivative (**13**) was obtained by alkylation of **18**, the 9-acetyl derivative of 1, with 4-chlorobutyl acetate followed by saponification. The cyclization of **13** and **11** with a sulfonyl chloride gave the 9-pyrrolidin-1-yl (**27**) and the 9-[2-(tosyloxymethyl)pyrrolidin-1-yl] (**28**), respectively. Acylation of 1 with ethyl L-2-pyrrolidine-5-carboxylate and ethyl 1-methyl-5-pyrrolidone-3-carboxylate, respectively, in Me_2SO containing NaH gave the corresponding amides **15** and **17**. Alkylation of **18** with 1-bromo-2-chloroethane and epichlorohydrin gave the *N*-(2-chloroethyl) and *N*-(1,2-epoxy-3-propyl) derivatives **19** and **20**. The chloro group of the chlorobutyl derivative of **18** was displaced with KSCN and NaN_3, respectively, to give the thiocyanate and azido derivatives **23** and **24**. Hydrogenation of the latter gave the amine (**25**), which was acylated with ethyl chloroformate to give the (ethoxycarbonyl)amino compound **26**. None of these compounds showed activity against L1210 leukemia cells implanted ip in mice on a single-dose schedule, suggesting that the activity observed in the simpler 9-aminopurines resulted from cleavage of the hydrazino linkage to give 9*H*-purine-6(1*H*)-thione.

Previously, we reported that 9-amino-9*H*-purine-6(1*H*)-thione and some of its derivatives showed anticancer activity against leukemia L1210 in mice.[1] Although these compounds or their anabolic products might possess intrinsic activity, it is possible that they are degraded to the active agent, 9*H*-purine-6(1*H*)-thione.[2] To provide additional information on this type of compound, one series of 9-(substituted amino)-6-(methylthio)-9*H*-purines was prepared in which the 9-substituent was either a pyrrolylmethyl or a (poly)hydroxyalkyl group, which might lead to tighter binding with an enzymatic site. In another series the 9-substituent contained a reactive center, which might bind irreversibly with an enzymatic site.

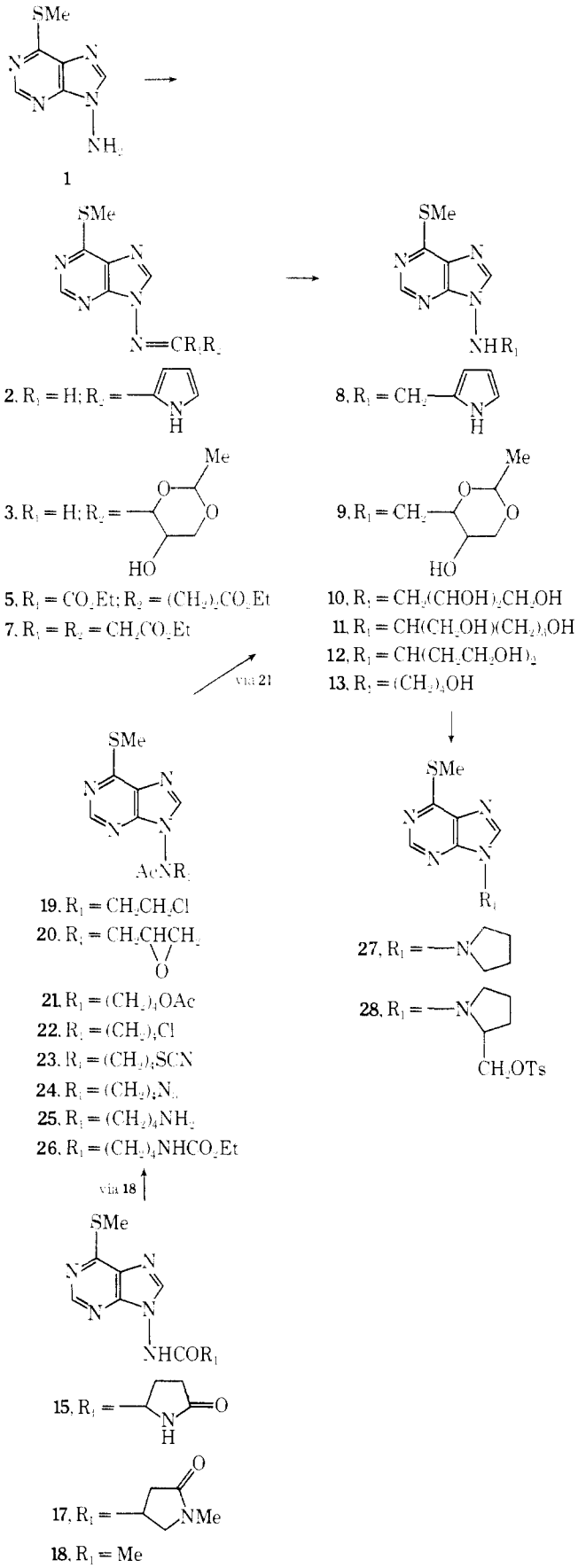
The condensation of 1 and pyrrole-2-carboxaldehyde gave **2**, which was treated with $NaBH_4$ to reduce the –N=CH– linkage to give **8**. Similarly, **9** was prepared by the condensation of 1 with 2,4-*O*-ethylidene-D-erythrose[3] to give **3** followed by reduction of this product with $NaBH_4$. Hydrolysis of the ethylidene blocking group of **9** with hot 60% aqueous HOAc gave the 2,3,4-trihydroxybutyl compound **10**. The dihydroxypentyl derivatives **11** and **12** were prepared by the condensation of 1 with diethyl 2-oxoglutarate (**4**)[4] and 3-oxoglutarate (**6**),[5] respectively, to give **5** and **7** followed by reduction of both the –N=C= linkage and the ester moieties of these intermediates with $NaBH_4$. Of interest is the observation that **5** is cleaved in Me_2SO-d_6, but not $CDCl_3$, to 6-(methylthio)-9*H*-purine (^1H NMR). Further, reduction of the azomethine linkage of **5** gave a compound (**11**) that was stable in Me_2SO-d_6 (^1H NMR). The 4-hydroxybutyl compound **13** was prepared by saponification of **21** (see below).

The cyclization of **13** to the known 9-pyrrolidin-1-yl derivative **27** was effected in dioxane with methanesulfonyl chloride in the presence of Et_3N.[1] The isolation of the mesyloxy intermediate in this reaction was unsuccessful. Similarly, treatment of **11** in pyridine with excess *p*-toluenesulfonyl chloride gave the 9-[2-(tosyloxymethyl)pyrrolidin-1-yl] compound, **28** (Scheme I).

For the preparation of some acyl derivatives, the mild conditions of Singh were used.[6] Reaction of 1 with ethyl L-2-pyrrolidone-5-carboxylate (**14**)[7] and ethyl 1-methyl-5-pyrrolidone-3-carboxylate (**16**), respectively, in Me_2SO containing NaH gave **15** and **17**.

The synthesis of the compounds containing a reactive center was effected by alkylation of the known 9-acetamido compound **18**.[1] Treatment of the latter with 1-bromo-2-chloroethane, epichlorohydrin, and 4-chlorobutyl acetate, respectively, in a dipolar aprotic solvent in the presence of

Scheme I



a base gave **19–21**. Replacement of the chloro group of **19** by reaction with KSCN and ethylenimine was attempted, but no pure products were isolated from these reactions. In contrast, reaction of the chlorobutyl compound **22**[1] with KSCN gave the thiocyanate **23**. Support for this structure, rather than the isomeric isothiocyanate, was provided by the infrared spectrum.[8] Similarly, treatment of **22** with NaN_3 gave the azidobutyl compound **24**. Hydrogenation of **24** in the presence of Raney nickel gave the corresponding amine **25**, which was treated with ethyl chloroformate to give **26**. The uv spectra of these compounds are similar with that of **1**, which in pH 7 buffer showed peaks at 286 and 292 nm.

Compounds **2**, **3**, **8–13**, **15**, **17**, **19–21**, **23**, **24**, **26**, and **28** showed no significant cytotoxicity in the KB cell culture screen.[9] Also, the same compounds with the exception of **12**, **13**, and **21**, which were not tested, showed no activity against L1210 leukemic cells implanted ip in mice on a single-dose schedule[9] suggesting that the activity observed in the simpler 9-aminopurine compounds[1] probably resulted from cleavage of the hydrazino linkage to give 9*H*-purine-6(1*H*)-thione.

Experimental Section

Melting points were determined on a Kofler Heizbank or, when indicated, on a Mel-Temp apparatus. The infrared spectra were determined in pressed KBr disks with a Perkin-Elmer Model 521 spectrophotometer, and the mass spectra were determined with a Hitachi Perkin-Elmer RMU-6D-3 spectrometer.

6-(Methylthio)-9-[(2-pyrrolylmethylene)amino]-9*H*-purine (2). A solution of 1 (1.0 g) and pyrrole-2-carboxaldehyde (0.55 g) in benzene (100 ml) containing *p*-toluenesulfonic acid (10 mg) was refluxed under a Dean-Stark water trap for 4 hr. The reaction mixture was cooled to precipitate the product, which was collected by filtration and dried in vacuo over P_2O_5: yield 1.3 g (91%); mp 246° dec. Anal. ($C_{11}H_{10}N_6S$) C, H, N.

2,4-*O*-Ethylidine-*N*-[6-(methylthio)-9*H*-purin-9-yl]-erythrosimine (3) was prepared by a similar method from 1 (6.0 g), 2,4-*O*-ethylidene-D-erythrose (5.3 g),[3] and *p*-toluenesulfonic acid (0.2 g) by refluxing for 6.5 hr: yield 6.7 g (65%); mp 228°. Anal. ($C_{12}H_{15}N_5O_3S$) C, H, N.

Diethyl 2-Oxoglutarate (4). A suspension of 2-oxoglutaric acid (25 g) in $SOCl_2$ (100 ml) was refluxed for 3 hr, and the resulting solution was evaporated to dryness. This residue was dissolved in EtOH, and the solution was distilled: yield 20 g (58%); bp 142–145° (13.7–14.0 mm) [lit.[4] bp 144° (13 mm)]. Anal. ($C_9H_{14}O_5$) C, H.

Diethyl 2-[*N*-[6-(Methylthio)-9*H*-purin-9-yl]imino]glutarate (5). A suspension of 1 (1.0 g) and **4** (1.1 ml) in C_6H_6 (100 ml) containing *p*-toluenesulfonic acid (10 mg) was refluxed under a Dean-Stark water trap for 4 hr. After filtration the clear yellow filtrate was evaporated to dryness, and the resulting residue was extracted with Et_2O. The extract was evaporated to dryness to give crude **5** as a waxy solid: yield 1.8 g (89%). A portion of this sample (0.80 g) was recrystallized from hexane: yield 0.26 g; mp 95° premelting. Anal. ($C_{15}H_{19}N_5O_4S$) C, H, N.

An additional amount of oily product (0.22 g) was obtained from the hexane filtrate.

6-(Methylthio)-9-[(2-pyrrolylmethyl)amino]-9*H*-purine (8). A suspension of **2** (1.0 g) and $NaBH_4$ (0.5 g) in ethanol (150 ml) was stirred at room temperature for 48 hr. After 24 hr an additional amount of $NaBH_4$ (0.5 g) was added. The practically clear solution was heated at 60° for 1 hr, acidified to pH 6 (paper) with dilute HCl, and filtered. The filtrate was evaporated to dryness, and the resulting residue was recrystallized from MeCN: yield 0.70 g (69%); mp 172°. Anal. ($C_{11}H_{12}N_6S$) C, H, N.

1-Deoxy-2,4-*O*-ethylidene-1-[6-(methylthio)-9*H*-purin-9-ylamino]erythritol (9) was prepared by a similar method from **3** (3.3 g) and $NaBH_4$ (2.4 g) in refluxing EtOH (500 ml) for 1 hr: yield 2.1 g (63% from C_6H_6); mp 158–160°. Anal. ($C_{12}H_{17}N_5O_3S$) C, H, N.

Concentration of the C_6H_6 filtrate gave an additional 0.4 g (12%) of crude **9**: mp 150°. The total yield was 75%.

6-(Methylthio)-9-[(2,3,4-trihydroxybutyl)amino]-9*H*-purine (10). A solution of **9** (500 mg) in 60% aqueous HOAc was refluxed for 1.5 hr and evaporated to dryness in vacuo. This residue was extracted with hot EtOAc (40 ml), and the extract was concentrated slowly to precipitate a crude hygroscopic sample of **10**: yield 102 mg (22%). Further concentration of the filtrate gave a precipitate of pure **10**: yield 118 mg (26%); mp 127°. Anal. ($C_{10}H_{15}N_5O_3S$) C, H, N.

The EtOAc filtrate from above was evaporated to dryness to give mainly a mixture of **9** and **10**: yield 178 mg.

9-[(1,5-Dihydroxy-2-pentyl)amino]-6-(methylthio)-9*H*-purine (11). To a solution of crude **5** (1.7 g) in EtOH (170 ml) was added a suspension of $NaBH_4$ (1.1 g) in EtOH (90 ml) over a period of 30 min. The resulting solution was stirred at room temperature for 24 hr and acidified to pH 6 (paper) with dilute HCl. After filtration the filtrate was evaporated to dryness, the residue was extracted with hot MeCN, and the extract was evaporated to give an oily product: yield 1.2 g. This sample was extracted by stirring with portions of Et_2O over a period of 48 hr. The remaining residue was dissolved in EtOH and treated with about 1 equiv of ethanolic HCl to give a precipitate of the hygroscopic HCl salt: yield 0.64 g. For analysis this sample was recrystallized from EtOH: yield 0.32 g; mp 162–164° (Mel-Temp); M^+ 283. Anal. ($C_{11}H_{17}N_5O_2S \cdot HCl \cdot 0.33H_2O$) C, H, N.

9-[(1,5-Dihydroxy-3-pentyl)amino]-6-(methylthio)-9*H*-purine (12). A mixture of 1 (0.75 g), diethyl 3-oxoglutarate (1.1 g) (**6**),[5] and *p*-toluenesulfonic acid (10 mg) in C_6H_6 (100 ml) was refluxed under a Dean-Stark water trap for 4 hr and filtered. The yellow filtrate was evaporated to dryness, and the semisolid residue of **7** (1.6 g) was dissolved in EtOH (180 ml) and treated with a suspension of $NaBH_4$ (1.0 g) in EtOH (100 ml) over a period of 35 min. After 18 hr the reaction mixture was acidified to pH 6 (paper) with dilute HCl, and the precipitate was removed by filtration. The filtrate was evaporated to dryness, and the resulting residue was extracted with hot MeCN (85 ml). Evaporation of the extract to dryness gave a mixture of **12** and the corresponding intermediate diethyl ester (M^+ 367). This sample was retreated with $NaBH_4$ as described above to give crude **12**: yield 1.0 g. This residue was triturated with 5 *M* ethanolic HCl (5 ml) followed by dissolving the resulting solid in hot EtOH. On cooling, the precipitate of **1** was removed by filtration, the filtrate was evaporated to dryness, and the hygroscopic residue was washed with Et_2O. The resulting residue was eluted from a silica gel H column with $CHCl_3$ (200 ml, discarded) followed by 9:1 $CHCl_3$–MeOH (40 ml). The last 20 ml of the $CHCl_3$–MeOH mixture was evaporated to dryness, the residue was dissolved in $CHCl_3$, and the solution was filtered (Celite). The filtrate was evaporated to dryness and the product was triturated with ethanolic HCl and Et_2O to give the HCl salt as a hydroscopic orange solid: yield 82 mg; mp 138–141° dec (Mel-Temp); M^+ 283. Anal. ($C_{11}H_{17}N_5O_2S \cdot 1.3HCl \cdot 0.25C_2H_6O$) C, H, N.

9-[(4-Hydroxybutyl)amino]-6-(methylthio)-9*H*-purine (13). A solution of **21** (328 mg) in 0.1 *N* NaOH (20 ml) was stirred at room temperature for 72 hr and extracted with $CHCl_3$ (2 × 100 ml portions). The combined extracts were washed with H_2O, dried ($MgSO_4$), and evaporated to dryness in vacuo: yield 159 mg (65%); mp 170° with presoftening (Mel-Temp); M^+ 253. The mass spectrum indicated the presence of a trace amount of an acetyl derivative of **21** (M^+ 295). Anal. ($C_{10}H_{15}N_5OS$) C, H, N.

Ethyl L-2-Pyrrolidone-5-carboxylate (14). A mixture of L-2-pyrrolidone-5-carboxylic acid (10 g) and $SOCl_2$ (35 ml) was heated at 50° for 20 min, and the resulting solution was evaporated to a small volume in vacuo. The residue was cooled in a water bath and dissolved in EtOH, and after 1 hr the solution was distilled. The fraction containing the product crystallized on cooling in an ice bath: yield 8.0 g (66%); bp 149–150° (2.5 mm) [lit.[7] bp 161° (3 mm)]; mp 48°; $[\alpha]^{25}D$ +3.8 ± 0.2° (*c* 6.26, EtOH). Anal. ($C_7H_{11}NO_3$) C, H, N.

***N*-[6-(Methylthio)-9*H*-purin-9-yl]-2-oxo-L-5-pyrrolidinecarboxamide (15).** In a flask equipped with an air condenser (drying tube), a solution of **1** (5.0 g) and **14** (4.6 g) in Me_2SO (100 ml) was treated with NaH (2.5 g, 57% dispersed in mineral oil). The resulting dark foamy reaction mixture was stirred at room temperature for 18 hr and evaporated to dryness in vacuo. The residue was washed with Et_2O, and the resulting solid was dissolved in H_2O (100 ml) and adjusted to pH 6 (paper) with HOAc. This solution was evaporated to dryness, the residue was extracted with boiling EtOH (250 ml), and the extract was concentrated to one-fourth volume. The solid that precipitated was removed by filtration, and the filtrate was concentrated (~35 ml) to give a heavy precipitate: yield 4.7 g. Recrystallization of this sample from EtOH gave the analytical sample: yield 2.9 g (36%); mp >260°. Anal. ($C_{11}H_{12}N_6O_2S$) C, H, N.

An additional amount of very crude **15** (1.3 g) was obtained from the EtOH filtrate.

Ethyl 1-methyl-5-pyrrolidone-3-carboxylate (16) was prepared by a method similar to the preparation of **14** from 1-methyl-5-pyrrolidone-3-carboxylic acid (4.1 g). The crude **16** was isolated as an oil and used without further purification: yield 5.0 g; M^+ 171.

1-Methyl-*N*-[6-(methylthio)-9*H*-purin-9-yl]-2-oxo-4-pyrrolidinecarboxamide (17) was prepared by a method similar to the preparation of **15** from **1** (0.50 g), **16** (0.50 g), and NaH (0.23 g, 57% dispersed in mineral oil) in Me_2SO (10 ml). After a reaction time of 66 hr, the isolated residue was recrystallized from MeCN–hexane: yield 0.15 g (18%); mp 184–185°. Anal. ($C_{12}H_{14}N_6O_2S$) C, H, N.

***N*-(2-Chloroethyl)-*N*-[6-(methylthio)-9*H*-purin-9-yl]acetamide (19).** A solution of **18** (5.0 g)[1] in DMF (50 ml) containing 1-bromo-2-chloroethane (2.4 ml) and anhydrous K_2CO_3 (3.7 g) was stirred at room temperature for 18 hr and diluted with H_2O (350 ml). The solution was extracted with Et_2O (3 × 500 ml portions), and the combined extracts were washed with H_2O (2 × 100 ml portions), dried ($MgSO_4$), and evaporated to dryness in vacuo. The dried residue was triturated with Et_2O (50 ml) and allowed to stand for 20 hr. The Et_2O layer was decanted from a dark oily residue, which was washed with an additional amount of Et_2O (50 ml). The combined Et_2O extracts were evaporated to dryness, and the resulting oil was dried in vacuo over P_2O_5 at 56°: yield 3.9 g (61%); M^+ 285, 287. Anal. ($C_{10}H_{12}ClN_5OS$) H, N; C: calcd, 42.03; found, 42.47.

***N*-(1,2-Epoxy-3-propyl)-*N*-[6-(methylthio)-9*H*-purin-9-yl]acetamide (20).** A solution of **18** (4.0 g)[1] in Me_2SO (60 ml) containing K_2CO_3 (2.5 g) and epichlorohydrin (1.5 ml) was stirred at room temperature for 18 hr and diluted with H_2O (360 ml). The solution was extracted with $CHCl_3$ (3 × 400 ml), and the combined extracts were washed first with 10% $NaHCO_3$ (3 × 200 ml) and then H_2O (2 × 200 ml). The $CHCl_3$ solution was dried ($MgSO_4$) and evaporated to dryness in vacuo to give the product as a hydroscopic pink solid: yield 2.0 g (40%). This sample underwent softening from about 95°, but the melting point was indefinite: M^+ 279. Anal. ($C_{11}H_{13}N_5O_2S \cdot 0.83H_2O$) C, H, N.

4-[*N*-Acetyl-*N*-[6-(methylthio)-9*H*-purin-9-yl]amino]butyl Acetate (21). To a solution of **18** (3.4 g)[1] in DMAC (68 ml) containing NaH [0.7 g, prepared by washing 50% NaH (1.4 g) dispersed in mineral oil with petroleum ether] was added 4-chlorobutyl acetate (6.8 ml), and the whole mixture was stirred at room temperature for 18 hr. The mixture was evaporated to dryness in vacuo, and the resulting residue was extracted with Et_2O (3 × 200 ml portions). The combined extracts were washed with H_2O, dried ($MgSO_4$), and evaporated to dryness in vacuo. The resulting oil was dried in vacuo over P_2O_5 at 56°: yield 0.40 g (8%); M^+ 337. Anal. ($C_{14}H_{19}N_5O_3S \cdot 0.66H_2O$) C, H, N.

The residue remaining from the Et_2O extraction was dissolved in H_2O, and the resulting solution was acidified to precipitate unreacted **18**: yield 1.8 g (53% recovery).

4-[*N*-[6-(Methylthio)purin-9-yl]acetamido]butyl Thiocyanate (23). A mixture of a solution of **22** (1.0 g)[1] in dioxane (20 ml) and a solution of KNCS (1.0 g) in H_2O (5 ml) was refluxed for 74 hr and filtered. The filtrate was evaporated to dryness, and the residue was extracted by stirring with Et_2O for 18 hr. The extract was evaporated to dryness, and the resulting oil was triturated with Et_2O while cooling in Dry Ice: yield 0.40 g (37%); mp ~80° with presoftening; M^+ 336; $\bar{\nu}_{max}$ 2155 cm^{-1} (–SCN).[3] Anal. ($C_{13}H_{16}N_6OS_2$) C, N; H: calcd, 4.79; found 5.25.

***N*-(4-Azidobutyl)-*N*-[6-(methylthio)-9*H*-purin-9-yl]acetamide (24).** To a solution of **22** (4.0 g)[1] in dioxane (90 ml) was added a solution of NaN_3 (4.0 g) in H_2O (23 ml), and the whole mixture was refluxed for 40 hr. The solution was evaporated to dryness and the resulting dried residue was extracted with Et_2O. The extract was filtered (Celite) and the filtrate was evaporated to dryness. The resulting oil was washed with hexane (2 × 200 ml portions) and dried in vacuo over P_2O_5: yield 2.3 g (56.5%); M^+ 320; $\bar{\nu}_{max}$ 2095 cm^{-1} (N_3). Anal. ($C_{12}H_{16}N_8OS$) C, H, N.

***N*-[[4-(Ethoxycarbonyl)amino]butyl]-*N*-[6-(methylthio)-9*H*-purin-9-yl]acetamide (26).** A solution of **24** (1.8 g) in EtOH (120 ml) was hydrogenated in the presence of Raney nickel (4.3 g wet, washed with H_2O and EtOH) at room temperature and atmospheric pressure for 18 hr and filtered (Celite) under N_2. The filtrate was evaporated to dryness to give crude **25** as an oil: yield 1.5 g (91%); M^+ 294. The infrared spectrum of this sample showed the absence of an azido absorption band. This oil was dissolved in dioxane (150 ml) and treated with ethyl chloroformate (1.5 ml) and Et_3N (1.5 ml). After 70 hr the mixture was evaporated to dryness and the residue was extracted with Et_2O. The extract was cooled in Dry Ice to precipitate the product, which is a semisolid at room temperature: yield 0.40 g (19% from **24**); M^+ 366. For analyses a portion of this sample was dried in vacuo over P_2O_5 at 56° for 2 hr. Anal. ($C_{15}H_{22}N_6O_3S$) C, H, N.

The Et_2O filtrate from above was evaporated to dryness to give

crude **26**: yield 1.0 g; M^+ 366.

6-(Methylthio)-9-[2-(tosyloxymethyl)pyrrolidin-1-yl]-9*H*-purine (28). A solution of 11 (0.55 g) in anhydrous pyridine (14 ml) containing *p*-toluenesulfonyl chloride (0.75 g) was stirred at room temperature for 18 hr. The reaction mixture was diluted with H_2O (125 ml) and extracted with $CHCl_3$ (3 × 50 ml). After drying ($MgSO_4$), the combined extracts were evaporated to dryness and the resulting residue was triturated with Et_2O: yield 0.37 g (45%); mp 137°. Anal. ($C_{18}H_{21}N_5O_3S_2$) C, H, N.

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Synthesis of 6α-Methyldigitoxigenin 3-Acetate

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In order to determine the influence of a 6α-methyl group activity, the 6α-methyl derivative of digitoxigenin 3-acetate **14** was prepared and pharmacologically tested in comparison with digitoxigenen 3-acetate. The synthesis of 6α-methyldigitoxigenin 3-acetate (**14**) was performed starting from 21-hydroxy-4-pregnene-3,20-dione (**1**). According to the cardiac activity determined on guinea-pig isolated heart and by slow infusion in the cat, the 6α-methyldigitoxigenin 3-acetate (**14**) is not more active than digitoxigenin 3-acetate.

Many methyl derivatives of steroid hormones and of adrenocortical steroids were prepared and pharmacologically tested; some of them (6α-, 16α-, or 16β-methyl derivatives) were far more active than the nonmethylated parent compounds (perhaps influencing the metabolism of the drugs) and are today widely used in human therapy as progestative or antirheumatic compounds.[1,2]

On the contrary, although cardenolides have been used for more than two centuries in human therapy as cardioactive compounds, no methyl or other alkyl derivatives of digitoxigenin have yet been prepared and pharmacologically tested. This may be due to some difficulties in the synthesis of such methyl derivatives of cardenolides. Here we report the synthesis of the 6α-methyl derivative of digitoxigenin 3-acetate **14**.

Chemistry. The 21-hydroxy-6α-methyl-4-pregnene-3,20-dione (**3**, previously obtained starting from 3β-hydroxy-5-pregnen-20-one with many steps and with low yield[3]) was obtained by us[4] in five steps starting from easily disposable 21-hydroxy-4-pregnene-3,20-dione (**1**). In order to synthetize the 6α-methyldigitoxigenin 3-acetate (**14**), involving 12 biological–chemical steps, it was extremely important to produce easily the starting compound **3**. The 21-hydroxy-4-pregnene-3,20-dione (**1**) was treated with ethylene glycol and pyridine hydrochloride giving the 3,20-diethyleneketal derivative which was transformed with monoperphthalic acid into the 5α,6α-epoxy compound **2**. Compound **2** was treated with methylmagnesium iodide giving the 6β-methyl-5α-hydroxy intermediate (trans-diaxial opening of the oxirane ring[1]) which was hydrolized with oxalic acid into the 5α,21-dihydroxy-6β-methylpregnane-3,20-dione; this last compound was treated with hydrochloric acid giving (by means of water elimination from the 5α-hydroxyl group and isomerization of the methyl group from the 6β-axial into the 6α-equatorial position) compound **3**, which was identical (ir, uv, TLC, melting point, NMR) with the compound obtained by another synthetic approach.[3]

Compound **3**, when incubated with *Mucor griseo-cyanus* ATCC 1207 a(+), gave a 60% yield of the 14α-hydroxy derivative[5] which was acetylated to give the 21-acetate **4**. Compound **4** was catalytically reduced to the 5β-dihydro derivative **5**; the 5β configuration was assigned to compound **5** because catalytic hydrogenation of 3-keto-4-pregnene compounds gave, as the chief products, the 5β-dihydro derivatives,[6-11] because in compound **4** the 6α-methyl group may furthermore hinder the approach of the catalyst from the rear α-side of the steroid nucleus and because compound **5** showed a single positive Cotton effect at 310 nm (the same of the 6α-nonmethylated compound 14α,21-dihydroxy-5β-pregnane-3,20-dione 21-acetate of known configuration[11]) (see Scheme I).

Compound **5** was dehydrated with $KHSO_4$ and acetic anhydride to give the 14-dehydro derivative **6**; the 14,15 positions were assigned to the double bond because compound **7** was catalytically reduced with platinum in methanol at room temperature and a pressure of 1 atm to the 21-hydroxy-6α-methyl-5β-pregnene-3,20-dione, identical with the product obtained by the same reduction and in the same conditions as compound **3** (the $\Delta^{8(14)}$-steroids are resistant to hydrogenation[1]).

Compound **6** was hydrolyzed to the 21-alcohol **7** which, on treatment with methanesulfonyl chloride in acetone–pyridine, gave the 21-mesilate **8**. Treatment of the 21-mesilate **8** with the monoethyl ester potassium salt of malonic acid in dimethylformamide gave the 21-ethylmalonyl derivative **9** which was cyclized with sodium methoxide in methanol and then was decarboxylated with *p*-toluenesulfonic acid to give the butenolide **10**. To compound **10** the 17β configuration was assigned because between compounds **10** and **6** there is a difference in the molecular opti-