material in spectral and chemical properties but could not be induced to yield more crystals.

Bis(1-*n*-butyl-3-ethyldiaziridinyl-2-)phosphinic Chloride (Va).—To a solution of IIIc (10 g, 0.0785 mole) and triethylamine (15 g, 0.146 mole) in toluene (300 ml), cooled to −15°, was added $POCl_3$ (6 g, 3.7 ml, 0.04 mole) in toluene (30 ml). After 3 days at room temperature with exclusion of air, the precipitated triethylamine hydrochloride was filtered (10 g, 94%), and the solvent was evaporated *in vacuo*. The residue, a very hygroscopic, oily substance, was completely soluble in pentane. In 50% aqueous ethanol, it was immediately hydrolyzed (presumably to Vb) with lowering of the pH to 4 and quantitative liberation of 1 equiv of chloride ion (based on the molecular weight calculated for Va), as determined by titration with $AgNO_3$. Attempts to isolate Vb from the aqueous solution led to partially polymerized syrupy material. Iodometric determination of the diaziridine groups[9] also gave results in agreement with structure Va; $\nu_{max}^{CHCl_3}$ cm^{-1}) 3000 (s) (CH), 1460 (s) (CH_2), 1480 (m) (CH_3), 1290 (sh) (P=O), 1250–1210 (s) (CN), 1060 (w), 975–920 (w), 720 (s). Due to the instability of the compound, an elemental analysis was not performed.

Hydrolysis Rate Studies.—The compound (0.1 g) was dissolved in a mixture of ethanol (10 ml) and 2 *N* aqueous H_2SO_4 (10 ml), and the solution was kept in a thermostat at 22°. After the specified period, 2 ml of a 20% aqueous KI was added to the sample, and the iodine was titrated after 10 min, then again after 1 hr, with 0.1 *N* $Na_2S_2O_3$.

Effects on the Activity of DNA as "Template" in a DNA-Dependent RNA–Polymerase System.[15]—A solution of calf thymus DNA, 1.5 mg/ml in 0.1 *M* phosphate buffer, pH 5.3, was incubated with 15 μmoles of each of the compounds at 37° for 16 and 60 hr. The DNA was precipitated by the addition of ethanol and isolated by centrifugation. Control samples of DNA (incubated in the same buffer, without the compounds) were prepared in an identical manner. The "template" activities of the treated and control DNA samples were compared in a DNA-dependent RNA–polymerase system, using a modification[16] of the assay described by Nakamoto, *et al.*[14] The reaction mixture, 0.5 ml, contained varying amounts of DNA, RNA–polymerase (15–30 units),[14] 0.4 μmole each of the triphosphates of uridine, cytidine, and guanosine, 0.2 μmole of 8-^{14}C-adenosine triphosphate (2.5×10^3 counts/min), 0.8 μmole of spermidine phosphate, 1.25 μmoles of $MnCl_2$, and 50 μmoles of Tris buffer, pH 7.5. After incubation with agitation for 30 min at 30°, the reaction was terminated by immersion in ice and addition of 0.1 ml of 50% trichloroacetic acid (TCA). Carrier ribonucleic acid, 0.1 ml (2.0 mg/ml), was added, and the final volume was brought to 1.0 ml by addition of 5% TCA. The solids were collected by centrifugation, washed twice with 5% TCA, and dissolved in formic acid. Aliquots were plated on stainless steel planchets, diluted with water, and dried, and the radioactivity was determined in a gas-flow counter. Incorporation of ^{14}C-ATP (millimicromoles) was plotted *vs.* the concentration of DNA (micrograms per tube, on the basis of optical density at 260 mμ)[19] to give the comparative-activity curves shown in Figure 3.

Acknowledgment.—The authors wish to express their thanks to Mr. James Baker and Mrs. Catherine Kavai for their technical assistance in this work.

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Aminomethylation and Hydroxymethylation of Purine-6(1H)-thione and 6-Alkylthiopurines[1a]

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An extensive investigation of the anticancer activity of S-substituted derivatives of purine-6(1H)-thione would be of great interest because numerous investigators[2] have prepared alkylthiopurines and found that they showed activity against Adenocarcinoma 755[3] and Sarcoma 180[4] comparable to that of purine-6(1H)-thione. Grillot, *et al.*,[5] had noted earlier that Mannich reactions involving thiophenol as well as substituted thiophenols gave S-substituted thiophenols instead of C-alkylated products. This observation prompted us to study the reaction of purine-6(1H)-thione with aqueous formaldehyde and a secondary amine such as piperidine or morpholine. These reactions gave good yields of products, with sharp melting points, which gave one spot on thin layer chromatograms. The ultraviolet spectra of these compounds showed λ_{max} 328 mμ (ϵ_{max} 21,400) for the morpholine derivative (**11**) and λ_{max} 327 mμ (ϵ_{max} 18,000) for the piperidino derivative (**12**). These ultraviolet spectra lead us to believe that substitution was not occurring at the thiono group of purine-6(1H)-thione. Burckhalter and Dill[6] treated theophylline with aqueous formaldehyde and secondary amines to give 7-(dialkylaminomethyl)theophyllines (caffeine derivatives). However, the ultraviolet spectra of the products obtained from purine-6(1H)-thione did not show the bathochromic shift expected for a 7-substituted purine.[7] The fact that the products showed absorptions very similar to purine-6(1H)-thione, λ_{max} 328 mμ (ϵ_{max} 16,800), indicated that substitution was occurring in either the 1, 8, or 9 positions. Bredereck and co-workers[8] found that caffeine was hydroxymethylated in the 8 position by aqueous formaldehyde.

Compound **13** was obtained when morpholinomethylpurine-6(1H)-thione in aqueous ethyl alcohol containing sodium hydroxide was treated with *n*-propyl bromide at 45°. Compound **13** is identical with the product formed from 6-(*n*-propylthio)purine, aqueous formaldehyde, and morpholine. When **13** was treated with dimedon in ethyl alcohol at room temperature, a solid formed which proved to be the dimedon–formaldehyde adduct. Evaporation of the filtrate, which remained after the adduct was collected, gave 6-(*n*-propylthio)purine. This evidence, in combination with the ultraviolet spectra, thin layer, and paper chromatographic data, indicates that purine-6(1H)-thione undergoes the Mannich reaction at the 9 position.

The failure of 9-cyclopentyl-9H-purine-6(1H)-thione to morpholinomethylate when treated with aqueous formaldehyde and morpholine under the usual condi-

(1) (a) This work was supported in part by a Research Grant from the Michigan Cancer Foundation and a National Cancer Institute Grant, CA-06140, U. S. Public Health Service, and was presented at the 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 12–17, 1965, p 8P. (b) Abstracted in part from the thesis of C. P. Bryant, submitted in partial fulfillment of the Master's of Arts Degree. (c) To whom inquiries should be addressed.

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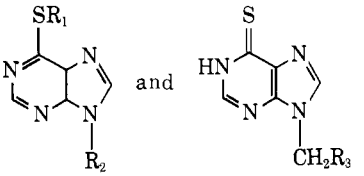
TABLE I

9-SUBSTITUTED 6-ALKYLTHIOPURINES AND PURINE-6(1H)-THIONES



No.	R_1	R_2	R_3	Mp,[a] °C	Method[b]	Yield, %	Recrystn[c] solvent	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, %[d] C	Found, %[d] H	Found, %[d] N
1	CH_3	CH_2OH	...	160–161	A	83	D	$C_7H_8N_4OS$	42.84	4.11	28.55	42.85	4.04	28.22
2	C_2H_5	CH_2OH	...	122–123	A	69	D	$C_8H_{10}N_4OS$	45.70	4.79	26.65	45.81	4.92	26.49
3	n-C_3H_7	CH_2OH	...	123–125.5	A	88	D	$C_9H_{12}N_4OS$	48.20	5.39	24.98	48.41	5.43	25.11
4	n-C_4H_9	CH_2OH	...	83–84	A	78	E	$C_{10}H_{14}N_4OS$	50.40	5.92	23.51	50.44	5.78	23.47
5	n-C_5H_{11}	CH_2OH	...	110–111	A	94	D	$C_{11}H_{16}N_4OS$	52.35	6.39	22.21	52.51	6.55	22.04
6	c-C_5H_9	CH_2OH	...	139–140	A	96	D	$C_{11}H_{14}N_4OS$	52.78	5.64	22.38	52.98	5.94	22.12
7	n-C_6H_{13}	CH_2OH	...	82–88	A	83	F	$C_{12}N_{18}N_4OS$	54.11	6.81	21.04	54.32	7.03	20.81
8	n-C_7H_{15}	CH_2OH	...	91–92	A	89	F	$C_{13}H_{20}N_4OS$	55.68	7.19	19.98	55.87	7.27	20.27
9	n-C_8H_{17}	CH_2OH	...	76–78	A	92	F	$C_{14}H_{22}N_4OS$	57.11	7.53	19.05	57.32	7.70	19.01
10	n-$C_{10}H_{21}$	CH_2OH	...	86–88	A	87	G	$C_{16}H_{26}N_4OS$	59.59	8.13	17.37	59.60	8.18	17.41
11	...	...		230 dec	B	87	H	$C_{10}H_{12}N_6OS$	47.79	5.21	27.87	47.52	5.26	27.69
12	...	...		207–208	B	80	H	$C_{11}H_{16}N_6S$	52.98	6.06	28.09	52.91	6.13	27.96
13	n-C_3H_7	CH_2N 	...	74–75	B	73	I	$C_{13}H_{19}N_5OS$	53.22	6.53	23.87	53.40	6.62	23.64
14	n-C_3H_7	$CH_2O_2CNHC_6H_5$	...	163–164	C	70	G	$C_{16}H_{17}N_5O_2S$	55.96	4.99	20.40	56.15	4.93	20.12
15	n-C_3H_7	$CH_2O_2CNHC_4H_9$-n	...	88–89	C	89	J	$C_{14}H_{21}N_5O_2S$	51.99	6.55	21.66	51.94	6.70	21.53

[a] The melting points were taken in open glass capillaries in a stirred silicone oil bath. [b] See Experimental Section for general methods used. [c] D = ethyl acetate, E = ethyl, F = ligroin (bp 85–110°), G = benzene, H = 95% ethyl alcohol, I = ethyl acetate–petroleum ether (bp 30–60°), J = ethyl ether–petroleum ether (bp 30–60°). [d] Analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

tions would seem to further indicate that substitution is not occurring at the 6 or 8 position of purine-6(1H)-thione. Several attempts to hydroxymethylate purine-6(1H)-thione, under a variety of conditions, failed. However, a series of 6-(alkylthio)purines[9] were treated with aqueous formaldehyde and sodium carbonate to give (hydroxymethyl)-6-alkylthiopurines. The 6-alkylthiopurines studied in this investigation gave infrared spectra showing an absorption band at 3.25 μ[10] (NH) and they all gave ultraviolet spectra which were very similar, λ_{max} 288–292 mμ (ϵ_{max} 17,900–22,000). The hydroxymethylated compounds did not show an absorption at 3.25 μ and gave ultraviolet spectra, λ_{max} 288–292 mμ (ϵ_{max} 17,200–22,200), which indicated the hydroxymethyl group is on the 9 position in all these compounds. Thin layer and paper chromatography gave one spot for each of these compounds. The 6-(alkylthio)-9-(hydroxymethyl)-9H-purines, where the alkyl group may vary from methyl to decyl, are reasonably stable and yield carbamates when treated with aryl or alkyl isocyanates in anhydrous benzene heated under reflux. Nmr studies substantiated the conclusion that substitution had occurred in the 9 position of purine-6(1H)-thione in the hydroxy- and aminomethylation reactions. These studies were carried out on some selected derivatives of purine-6(1H)-thione in perdeuterated dimethyl sulfoxide. The nmr spectra of **3** and **11–14** (Table I), purine-6(1H)-thione hydrate, 9-butylpurine-6(1H)-thione, 6-(propylthio)-purine, and 8-(methylthiopurine-6(1H)-thione[9] were determined. All the spectra were consistent with the assigned structures. Of particular importance were two singlets for the 2- and 8-hydrogens at τ 1.39–1.82 (2 H) and 1.22–1.68 (8 H) which were present in the spectra of all the compounds except 8-(methylthio)purine-6(1H)-thione which showed a singlet at τ 1.82 (2 H). The assignments agree with those made by Bullock and Jardetzky and others.[11] All the compounds except **13** exchanged hydrogen or hydrogens when D_2O was added to the spectral samples. The correct number of hydrogens was exchanged for each of the compounds studied.

One of the compounds listed in Table I was screened under the auspices of the Cancer Chemotherapy National Service Center for antitumor activity and showed the activities given in Table II. The other compounds

TABLE II

ANTITUMOR SCREENING DATA OF 9-MORPHOLINOMETHYL-6(1H)-PURINETHIONE (**12**)[a]

Dose, mg/kg	No. of survivors	Animal wt dif T − C, g	Tumor wt (g) or survival (days) T/C	Stage index % T/C
		Lymphoid Leukemia L-1210		
100	6/6	−1.8	16.3/9.2	177
100	6/6	−2.9	11.8/8.4	140
		Friend Virus Leukemia		
100	10/10	−2.6	0/953	0
100	10/10	−5.3	0/1025	0
100	10/10	−3.4	0/767	0
100	10/10	−6.1	0/833	0

[a] *Cancer Chemotherapy Rept.*, **25**, 1 (1962). A compound is active against solid Friend virus leukemia if the average T/C $\leqq$ 42% in three confirmation tests, and against lymphoid leukemia L1210 if T/C $\geqq$ 125% in a confirmation test.

(9) Authentic samples of a homologous series of 6-(alkylthio)purines, purine-6(1H)-thione, 9-butylpurine-6(1H)-thione, and 8-methylthiopurine-6(1H)-thione were furnished by Dr. Harry B. Wood, Chief, Drug Development Branch, Cancer Chemotherapy National Service Center, Bethesda 14, Md.

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listed in Table I are being evaluated for their anticancer activity.

Experimental Section

The alkylthiopurines and purine-6(1H)-thione monohydrate were obtained from the Cancer Chemotherapy National Service Center and were used without further purification. Infrared spectra were determined on KBr pellets of the purines on a Beckman IR-8 spectrophotometer. The ultraviolet spectra were determined on approximately 10^{-4} *M* solutions of the purines in 95% ethyl alcohol on a Cary 14 spectrophotometer. The nmr spectra were determined in perdeuterated DMSO using Me_4Si as an internal standard on a Varian A-60 spectrometer obtained by Grant No. Pe17069 from the National Science Foundation. The descending paper chromatograms which were run on Whatman No. 1 paper with water-saturated butanol as carrier in an NH_3 atmosphere gave one spot. Thin layer chromatograms on silica gel G (Darmstadt) with methanol and with ethyl acetate gave one spot.

Method A. 6-(Alkylthio)-9-hydroxymethyl-9H-purines.—To 10 ml of aqueous 37% formaldehyde, was added 0.010 mole of an alkylthiopurine. After all the material had dissolved, the reaction mixture was heated to 35°. Then 0.005 mole of $Na_2CO_3 \cdot H_2O$ and 7 ml of water were added. The reaction mixture was allowed to stand overnight, and the solid which formed was collected by vacuum filtration and dried. See Table I for physical and chemical properties of these compounds.

Method B.—9-(Morpholinomethyl)- or 9-(Piperidinomethyl)-9H-purines.—Morpholine or piperidine (0.060 mole) was added to a suspension of 0.030 mole of purine-6(1H)-thione in 50 ml of absolute ethanol. The suspension was stirred for 15 min and 2.5 ml of aqueous 37% formaldehyde was added. The reaction mixture was stirred overnight, and the solid which formed was collected by vacuum filtration and air dried. See Table I for chemical and physical properties.

Method C.—An aryl isocyanate (0.010 mole) was added to 0.010 mole of 9-(hydroxymethyl)-6-alkylthiopyrine in 50 ml of anhydrous benzene. The reaction mixture was heated under reflux overnight and then cooled to 10°, and the resulting solid was collected by vacuum filtration. The aryl carbamate was recrystallized from benzene.

In the case of the alkyl carbamate esters of 6-(alkylthio)-9-(hydroxymethyl)purines, 0.010 mole of the purine derivative was suspended in 15 ml of the alkyl isocyanate, and two drops of pyridine was added. The reaction mixture was stirred until solution had been effected, and stirring was continued for 2 hr. Then 15 ml of petroleum ether (bp 30–60°) was added, and the solution was cooled in an ice bath to give a solid which was collected by vacuum filtration. It was recrystallized from ether–petroleum ether. See Table I for chemical and physical properties.

Acknowledgment.—We wish to thank Dr. Harry B. Wood and Mr. Robert Ing of the Cancer Chemotherapy National Service Center for their assistance and the Department of Chemistry, Washington State University, for making their research facilities available to us during the summer of 1965.

The Preparation of α-Phenylketo Steroids

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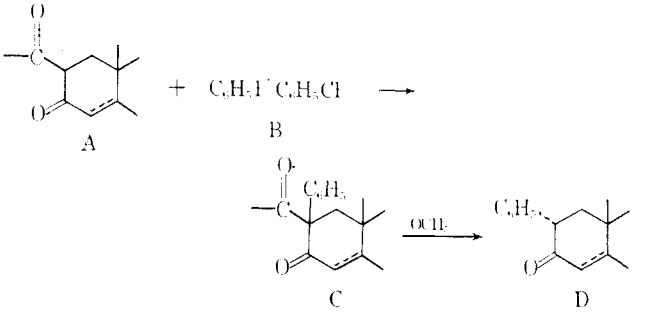
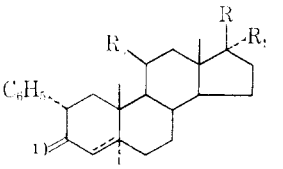
Extensive investigations by Beringer and co-workers[1] have demonstrated that diphenyliodonium salts react with the anion of active methylene compounds to give C-phenyl derivatives. Since numerous researches have shown that α-ethoxalyl and α-hydroxymethyleneketo steroids react with a variety of electrophilic reagents, we have investigated the reaction of these active methylene compounds with diphenyliodonium chloride as a method for the preparation of representative phenyl-substituted steroids. Moreover, inasmuch as centers of high electron density are available in enol acetate and enamine derivatives, we have also studied the interaction of diphenyliodonium chloride with these systems in the steroid series.

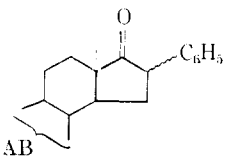


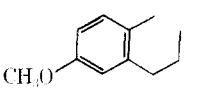
Potassium salts of representative ethoxalyl- and hydroxymethyleneketo steroids (A) were treated with diphenyliodonium chloride (B), and the products (C) were deacylated with methanolic sodium methoxide. In this manner 2α-phenyltestosterone (**1**), 17β-hydroxy-17α-methyl-2α-phenyl-5α-androstan-3-one (**2**), 2α-phenyldeoxycorticosterone 20-ethylene ketal (**3**), 2α-phenylhydrocortisone 20-ethylene ketal (**4**), 16ξ-phenylestrone methyl ether (**5**), and 3-ethylenedioxy-16ξ-phenylandrost-5-en-17-one (**6**) were prepared. The characterization of these substances is given in Table I. The yield of phenyl derivatives afforded by this procedure was erratic, ranging from 1–32%. However, no effort was made to determine the optimum yield.

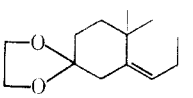
The introduction of the phenyl substituent was indicated by analyses and spectral data (λ_{max} 14.3



1. R = OH; R_2 = R_3 = H; Δ¹
2. R = OH; R_1 = CH_3; R = H
3. R =  CH_2OH; R_1 = R_3 = H; Δ¹
4. R =  CH_2OH; R_2 = R_3 = OH; Δ¹



5. AB = 

6. AB = 

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