volution assay.[13] In this connection it should be noted that the 20-ketal derivatives of a variety of glucocorticoids have substantial activity in a thymus involution assay.[14] 2α-Phenyldeoxycorticosterone had no effect on sodium or potassium excretion or retention.[15]

Experimental Section

Melting points were determined in an open capillary tube on a Mel-Temp apparatus and are corrected. Ultraviolet spectra are for methanol solutions unless stated otherwise, and infrared spectra were determined in pressed KBr disks. Nmr spectra were determined with a Varian A-60 spectrometer using tetramethylsilane as an internal standard; $CDCl_3$ was used as the solvent.

16-Ethoxalylestrone 3-Methyl Ether.[16]—To a solution of 1.14 g (4 mmoles) of 3-methoxy-1,3,5-estratrien-17-one (estrone methyl ether) in 120 ml of anhydrous benzene was added 1.8 ml of ethyl oxalate and 0.42 g of a 50% NaH–oil dispersion. The reaction was primed by the addition of a few drops of ethanol and the mixture was stirred under nitrogen for 16 hr. The yellow suspension was extracted several times with cold 1% aqueous KOH solution, and the extracts were added to aqueous 30% NaH_2PO_4 solution. This mixture in turn was extracted with several portions of chloroform until these extracts no longer gave a positive enol test. The combined chloroform extracts were washed with water, dried, and evaporated. The residue was crystallized from ether to give 1.16 g of a white solid (strong positive enol test), mp 140–146°. A sample recrystallized from acetone–hexane had mp 141–145°; $[\alpha]D$ +63.4°; λ_{max} 285 mμ (ε 9800 in acid), 298 mμ (ε 11,600 in methanol), 302 mμ (ε 20,600 in base); λ_{max} 5.74, 5.96, 6.21 μ.

Anal. Calcd for $C_{23}H_{28}O_5$: C, 71.85; H, 7.34. Found: C, 71.62; H, 7.46.

Reaction of the α-Acylketo Steroids with Diphenyliodonium Chloride.—The following preparation of 2α-phenyltestosterone illustrates the general procedure. To a solution prepared by the interaction of 248 mg (6.33 mg-atoms) of potassium with 50 ml of dry *t*-butyl alcohol was added 2.00 g (6.33 mmoles) of 2-hydroxymethylenetestosterone. Within a few minutes 2.00 g (6.33 mmoles) of diphenyliodonium chloride was added, and the stirred suspension was heated at reflux temperature for 24 hr. The solvent was partially removed, and the reaction mixture was diluted with water, acidified with concentrated HCl, and extracted with methylene chloride. The extract was washed with saline, dried, and evaporated. The residue was dissolved in 50 ml of methanol and, under nitrogen, 3 ml of 1 *N* methanolic sodium methoxide was added. This solution was refluxed for 1 hr. The solution was cooled, neutralized with acetic acid and, after partial removal of solvent, diluted with water and extracted with CH_2Cl_2. The extract was washed with saline, dried, and evaporated. Chromatography of the residue gave 2α-phenyltestosterone (1) as white crystals, mp 194–196°. The characterization of this material and the other substances prepared in a similar manner is given in Table I.

With the exception of one example, the α-acyl-α-arylketo steroids were amorphous materials. However, **16ξ-ethoxalyl-16ξ-phenylestrone 3-methyl ether** was obtained as white crystals from methylene chloride–ether; mp 190–192°; λ_{max} 5.70, 5.80, 6.20, 6.31, 7.80, 7.92, 8.09, 14.25 μ.

Anal. Calcd for $C_{29}H_{32}O_5$: C, 75.63; H, 7.00. Found: C, 75.15; H, 7.06.

2α-Phenyltestosterone propionate was prepared in 82% yield by acylation of 2α-phenyltestosterone with pyridine–propionic anhydride. Recrystallization of the product from ether gave white crystals: mp 158–159°; $[\alpha]D$ +72.5°; λ_{max} 240 mμ (ε 16,200); λ_{max} 5.77, 5.90, 6.13, 14.35 μ.

Anal. Calcd for $C_{28}H_{36}O_3$: C, 79.96; H, 8.63. Found: C, 79.66; H, 8.89.

2α-Phenyldeoxycorticosterone.—20-Ethylenedioxy-21-hydroxy-2α-phenylpregn-4-en-20-one (200 mg) was hydrolyzed with 8% H_2SO_4 in methanol. The product was recrystallized from methylene chloride–petroleum ether (bp 30–60°) to give 127 mg (67%) of white crystals: mp 191–195°; $[\alpha]D$ +168°; λ_{max} 241 mμ (ε 16,500); λ_{max} 5.86, 5.98, 6.16, 14.30 μ.

Anal. Calcd for $C_{25}H_{34}O_4$: C, 79.76; H, 8.43. Found: C, 79.89; H, 8.60.

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(13) G. Tonelli, L. Thibault, and I. Ringler, *Endocrinology*, **79**, 463 (1966).

(14) W. S. Allen, H. M. Kissman, S. Mauer, I. Ringler, and M. J. Weiss, *J. Med. Pharm. Chem.*, **5**, 133 (1962).

(15) This assay is based on the response of adrenalectomized male rats to a single subcutaneous 16-μg dose as measured after a 6-hr urine collection.

(16) We thank Dr. H. M. Kissman and Mrs. A. S. Hoffman for permission to publish this experiment.

Isobutyl N-Chloroethyl-N-nitrosocarbamate

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1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) is very effective against the lymphoid leukemia, L1210, inoculated subcutaneously or intracerebrally.[1] Its outstanding activity against intracerebral L1210 is probably connected with its high lipoid solubility. Since it seemed likely that the compound owed its effectiveness to the formation of 1-chloro-2-diazoethane *in vivo* and that the chloroethyl group attached to the non-nitrosated nitrogen atom was merely conferring lipoid solubility, it was decided to synthesize an analog with similar properties.

Isobutyl N-chloroethylcarbamate (I), obtained by the action of isobutyl chloroformate on ethylenimine, was readily nitrosated by treatment with sodium nitrite in formic acid solution to give the required isobutyl N-chloroethyl-N-nitrosocarbamate (II).

i-$C_4H_9OCONHCH_2CH_2Cl$ (I) *i*-$C_4H_9OCON(NO)CH_2CH_2Cl$ (II)

The nitroso derivative II was inactive against the Walker 256 tumor when given as six daily injections of 10 mg/kg ip in arachis oil starting on the day following implantation. It showed only marginal activity against subcutaneously inoculated L1210 lymphoid leukemia (survival time as compared with untreated controls = 110, 120, 100%), when given as five daily injections of 1.75, 3.5, and 7 mg/kg, respectively, starting on the day following inoculation;[2] the LD_{50} in the host mouse was 7 mg/kg.

The lack of activity could be due to the greater lability of the ester linkage in II as compared with the amide linkage in BCNU. This was not unexpected, but the hope that selective hydrolysis would occur in normal cells was apparently not realized.

(1) F. M. Schabel, Jr., T. P. Johnson, G. S. McCaleb, J. A. Montgomery, W. R. Laster, and H. E. Skipper, *Cancer Res.*, **23**, 725 (1963); M. A. Chirigos, S. R. Humphreys, and A. Goldin, *Cancer Chemotherapy Rept.*, **49**, 15 (1965).

(2) The protocol for the carcinostatic assay against the Walker tumor is given by T. A. Connors, B. C. V. Mitchley, V. M. Rosenauer, and W. C. J. Ross, *Biochem. Pharmacol.*, **13**, 395 (1964), and that for the L1210 assay is essentially as given in *Cancer Chemotherapy Rept.*, **1**, 42 (1959), C_{57}/DBA2 hybrid mice being used as hosts.

Experimental Section

Isobutyl N-Chloroethylcarbamate.—Ethyleneimine (7 g) in dry chloroform (20 ml) was added during 2 hr to a stirred solution of isobutyl chloroformate (21.5 g) in chloroform (150 ml) kept at 0–2°. The solution was then warmed to 30° and kept at this temperature for 3 hr. After removing the solvent under reduced pressure at 30° the residual oil was extracted with two 250-ml portions of petroleum ether (bp 30–40°). Distillation of the material contained in this extract afforded an oil, bp 76° (0.04 mm), yield 20 g.

Anal. Calcd for $C_7H_{14}ClNO_2$: C, 46.8; H, 7.9; Cl, 19.7; N, 7.8. Found: C, 46.8; H, 7.9; Cl, 19.2; N, 7.9.

Isobutyl N-Chloroethyl-N-nitrosocarbamate.—Sodium nitrite (20 g) in water (125 ml) was added during 1 hr to a stirred solution of the above carbamate (18 g) in formic acid (125 ml) keeping the temprature below 5°. After standing for 4 hr at 5°, the solution was diluted with water (125 ml) and extracted with two 250-ml portions of petroleum ether (bp 60–80°). On concentrating and distilling the dried ($CaCl_2$) solution, an oil, bp 74° (0.02 mm), was obtained, yield 15 g. It showed strong absorption at 1400 cm^{-1} (NO).

Anal. Calcd for $C_7H_{13}ClN_2O_3$: C, 40.3; H, 6.3; Cl, 17.0; N, 13.4. Found: C, 40.4; H, 6.6; Cl, 17.3; N, 13.0.

Abolition of Immunosuppressive Activity of 6-Mercaptopurine and Thioguanine by 8-Phenyl Substitution[1a]

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Purine derivatives are the most promising immunosuppressive agents tested to date.[2] Their activity has been ascribed to the purine moiety which is capable of blocking the interconversion of nucleotides, particularly from inosinic acid to adenylic acid, thus interfering with the synthesis of nucleic acids.[3] Although many studies on immunosuppressive activity have been carried out with 6-mercaptopurine (6-MP) and its S-substituted derivatives, 2-amino-6-mercaptopurine (TG) seems to be the only ring substituted 6-MP reported.[2c-e] The present investigation details the synthesis of 2-amino-6-mercapto-8-phenylpurine (8-PTG) and the experimental data on the immunosuppressive studies of 8-PTG and of 6-mercapto-8-phenylpurine (8-PMP) which we synthesized previously.[4]

2,4-Diamino-6-hydroxy-5-benzamidopyrimidine, which was prepared by benzoylation of 2,4,5-triamino-6-hydroxypyrimidine in alkaline solution,[5] was dehydrocyclized with polyphosphoric acid[4] to the hydroxypurine. Treatment of the hydroxypurine with phosphorus oxychloride in the presence of N,N-diethylaniline afforded 2-amino-6-chloro-8-phenylpurine. Subsequent thiation of the chloro compound with thiourea in absolute ethanol gave a nearly quantitative yield of 8-PTG.

Modification of the mercapto group of 6-MP produced 6-alkyl-, 6-aryl-, 6-purinyl-, and 6-imidazolyl mercaptopurines[6] which retained or, in some cases, showed an enhancement of the immunosuppressive activity.[2c-e,6] The only ring-substituted compound tested, TG, was found to be as potent as 6-MP but was more toxic.[2c,d,7] The 8-phenyl substituted 6-MP and TG in our present studies showed no immunosuppressive property as measured by the inhibition of antibody production in mice. The activity of the S-substituted 6-MP is considered to be unsheathed only after *in vivo* hydrolysis or reduction with liberation of the free mercapto group.[8] By this hypothesis, 8-PMP and 8-PTG should retain at least some of the activity because of the intact free mercapto group. The inactivity of 8-PMP and 8-PTG seems to show that a free 8 position is also essential for immunosuppressive activity.

All the active immune suppressors are also antitumor agents even though the reverse is not always true.[2c] This indicates a certain duality of action which is displayed by some of the chemical agents, including 6-MP and TG. Various 8-substituted purines showed some degree of inhibition toward experimental animal tumors although most of them are only moderately active.[6,9] Our present observation of the abolition of immunosuppressive activity of 6-MP and TG by 8-phenyl substitution is noteworthy. However, both 8-PMP and 8-PTG showed moderate inhibitory activity in the KB-line cell culture, ID_{50} at 30 and 20 μg/ml, respectively.[10] These details will be reported with other antineoplastic data elsewhere. Substitutions on different positions in the 6-MP nucleus would be of interest for studies on the chemical structure–biological activity relationship and the pharmacodynamics of the immunosuppressive processes. Judicious choice of substitutions could also lead to derivatives of 6-MP and other purines, some of which might be of significant biological importance.

Experimental Section[11]

2-Amino-6-hydroxy-8-phenylpurine.—To a mixture of 3 g of dry 2,4-diamino-6-hydroxy-5-benzamidopyrimidine[6] and 25 g of P_2O_5, cooled to 0°, was added 18 ml of 85% H_3PO_4. The mixture was then heated to 160–170° and stirred for 1.5 hr. By this time, the slowly dissolving amidopyrimidine had gone into solution. After cooling to room temperature, the thick syrup was poured with vigorous stirring onto crushed ice. The precipitate was allowed to stand at 4° for 18 hr and was then filtered and washed thoroughly with water and ether. The crude product was recrystallized from 2.7 l. of 2 *N* HCl to give 2.7 g (64%) of orange

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(2) (a) R. Schwartz, J. Stack, and W. Dameshek, *Proc. Soc. Exptl. Biol. Med.*, **99**, 164 (1958); R. Schwartz, A. Eisner, and W. Dameshek, *J. Clin. Invest.*, **38**, 1394 (1959); (b) J. Sterzl, *Nature*, **189**, 1022 (1961); (c) H. C. Nathan, S. Bieber, G. B. Elion, and G. H. Hitchings, *Proc. Soc. Exptl. Biol. Med.*, **107**, 796 (1961); (d) A. W. Frisch and G. H. Davies, *J. Immunol.*, **88**, 269 (1962); (e) F. D. Moore, *Science*, **144**, 388 (1964).

(3) M. C. Berenbaum, *Brit. Med. Bull.*, **21**, 140 (1965).

(4) S.-C. J. Fu, E. Chinoporos, and H. Terzian, *J. Org. Chem.*, **30**, 1916 (1965).

(5) W. Wilson, *J. Chem. Soc.*, 1157 (1948).

(6) D. A. Clarke, G. B. Elion, G. H. Hitchings, and C. C. Stock, *Cancer Res.*, **18**, 445 (1958); G. H. Hitchings and G. B. Elion, U. S. Patents, 3,056,785 (Oct 2, 1962), 3,149,111 (Sept 15, 1964), 3,315,754 (June 2, 1964).

(7) G. H. Hitchings and G. B. Elion, U. S. Patent, 3,132,144 (May 5, 1964).

(8) G. B. Elion, S. Callahan, S. Bieber, G. H. Hitchings, and R. W. Rundles, *Cancer Chemotherapy Rept.*, **No. 14**, 93 (1961).

(9) R. K. Robins, *J. Med. Chem.*, **7**, 186 (1964).

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(11) The elemental analyses were performed by Dr. C. K. Fitz, P. O. Box 115, Needham Heights, Mass. The infrared spectra were measured in potassium bromide disks, using a Perkin-Elmer 137B spectrophotometer.