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.

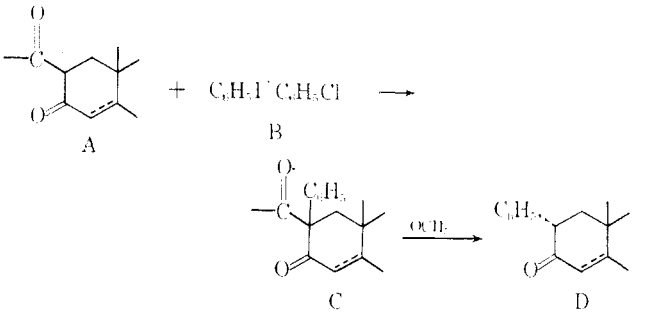
The Preparation of α-Phenylketo Steroids

JOHN F. POLETTO, GEORGE R. ALLEN, JR., AND MARTIN J. WEISS

Organic Chemical Research Section, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York

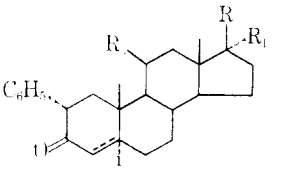
Received September 8, 1966

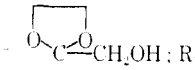
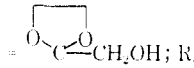
Extensive investigations by Beringer and co-workers[1] have demonstrated that diphenyliodonium salts react with the anion of active methylene compounds to give C-phenyl derivatives. Since numerous researches have shown that α-ethoxalyl and α-hydroxymethyleneketo steroids react with a variety of electrophilic 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.

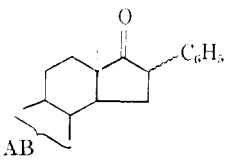


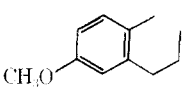
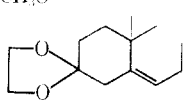
Potassium salts of representative ethoxalyl- and hydroxymethyleneketo steroids (A) were treated with diphenyliodonium chloride (B), and the products (C) were deacylated with methanolic sodium methoxide. In this manner 2α-phenyltestosterone (**1**), 17β-hydroxy-17α-methyl-2α-phenyl-5α-androstan-3-one (**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_1 = H; Δ^1
2. R = OH; R_1 = CH_3; R = H
3. R =  CH_2OH; R_1 = R_1 = H; Δ^1
4. R =  CH_2OH; R_1 = R_2 = OH; Δ^1



5. AB = 
6. AB = 

(1) F. M. Beringer and S. A. Galton, *J. Org. Chem.*, **28**, 3417 (1963), and previous papers.

TABLE I

α-PHENYLKETO STEROIDS PREPARED BY REACTION OF DIPHENYLIODONIUM CHLORIDE WITH α-ACYLKETO STEROIDS

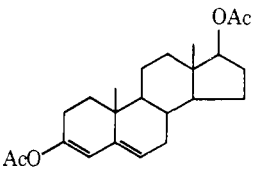
Product	Starting material	Yield, %	Mp, °C[a]	[α]D, deg[b]	Chromatography: Adsorbent or support	Chromatography: Solvent[c]	Formula	C, % Calcd	C, % Found	H, % Calcd	H, % Found
5	16-Ethoxalylestrone methyl ether[d]	8	147–148	+82			$C_{26}H_{28}O_7$	83.29	83.24	7.83	8.00
1	2-Hydroxymethylenetestosterone[e]	32	194–196	+91	Silica	B–E (9:1)	$C_{25}H_{32}O_2$	82.37	81.99	8.85	8.99
2	17β-Hydroxy-2-hydroxymethylene-17α-methyl-5α-androstan-3-one[f]	25	130–132[g] (resolidifies) 157–158	−29	Celite	H–M (1:1)[h,i]	$C_{26}H_{36}O_2$	82.06	82.28	9.54	9.54
6	16-Ethoxalyl-3-ethylenedioxyandrost-5-en-17-one[j]	1	185–187		Silica	B–E (95:5)	$C_{27}H_{34}O_5$	79.76	79.68	8.43	8.55
3	20-Ethylenedioxy-21-hydroxy-2-hydroxymethylenepregn-4-en-3-one[k]	24	210–211[l]	+92	Celite	H–M(1:1[h],[m]	$C_{25}H_{32}O_4$	77.30	76.91	8.50	8.81
4	20-Ethylenedioxy-2-hydroxymethylene-11β,17α,21-trihydroxypregn-4-en-3-one[k]	9	276–279		Florisil	A–E (5:95)	$C_{23}H_{36}O_6$	72.17	72.55	7.94	7.68

[a] Products were recrystallized from ether–petroleum ether (bp 30–60°) unless noted otherwise. [b] For 1–2% solution in chloroform. [c] A = acetone, B = benzene, E = ether, H = heptane, M = methanol. [d] This work. [e] F. L. Weisenborn, D. C. Remy, and T. L. Jacobs, *J. Am. Chem. Soc.*, **76**, 552 (1954). [f] H. J. Ringold, E. Batres, O. Halpern, and E. Necoechea, *ibid.*, **81**, 427 (1959). [g] Recrystallized from ethyl acetate–heptane. [h] Upper phase. [i] For a description of this technique see M. J. Weiss, R. E. Schaub, G. R. Allen, Jr., J. F. Poletto, C. Pidacks, R. B. Conrow, and C. J. Coscia, *Tetrahedron*, **20**, 357 (1964). The eluate was collected in 100-ml fractions and aliquots of each fraction were chromatographed on silica gel plates using a benzene–acetone–water (2:1:2) system; these chromatograms were developed with phosphomolybdic acid. The product was found in fractions 35–47 (V_m/V_s = 2.76). [j] H. M. Kissman, A. S. Hoffman, and M. J. Weiss, *J. Org. Chem.*, **26**, 973 (1961). [k] H. M. Kissman, A. S. Hoffman, and M. J. Weiss, *ibid.*, **27**, 3168 (1962). [l] Recrystallized from methanol. [m] Product eluted at peak hold-back volume 5.5 (V_m/V_s = 3.13).

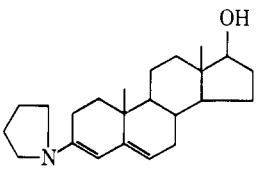
14.4 μ; aryl proton resonances at δ 7.16–7.34). The α configuration for the 2-phenyl substituent in **2** was assigned by consideration of the nmr spectrum, which revealed a single-proton resonance at δ 3.62 coupled with the 1β-proton (J_{ae} = 6.5 cps) and the 1α-proton (J_{aa} = 13.0 cps). These values for the coupling constants are in excellent accord with those reported for 2α-acetoxycholestan-3-one (J_{ae} = 6.6 cps, J_{aa} = 13.1 cps).[2] A similar resonance (J_{ae} = 5.5 cps, J_{aa} = 13.0 cps) is observed for **1**, but is obscured in the spectra of **3** and **4** by the ketal methylene proton resonances. However, an identical resonance was observed in 2α-phenyldeoxycorticosterone, obtained by acid hydrolysis of ketal **3**. The difference noted in J_{ae} for the saturated derivative **2** and the unsaturated derivatives is most probably a reflection of the change in the dihedral angle between the 2β- and 1β-protons imposed by introduction of the double bond into ring A. The values (−6 to +80) observed for the molecular rotation differences (ΔMD) between the 2-phenyl derivatives and the corresponding 2-hydrogen compounds also support the α configuration for the substituent.[3] An assignment of configuration to the 16-phenyl derivatives **5** and **6** was not possible. However, the nmr spectrum of **5** indicated the isolation of one epimer, inasmuch as a single sharp resonance was observed for the 18-protons (δ 0.93).[8]

2α-Phenyltestosterone (**1**) was converted into the propionate ester, a derivative of greater interest for assay as a possible anabolic agent. In this connection, an attempt to prepare the 2α-phenyl derivative of dihydrotestosterone by reduction of **1** with lithium in liquid ammonia failed, possibly as a result of phenyl substituent involvement.

Finally, we would note the inability to detect products in the attempted reaction of diphenyliodonium chloride with enol acetate **7** and enamine **8**.[9]



7



8

Biology.—In general, it appears that introduction of the phenyl group negates the biological effects of the parent hormones. Thus, the testosterone derivatives **1** and **2**, as well as the propionate ester of **1**, showed no significant androgenic or anabolic activity when assayed (500-μg oral dose) by the ventral prostate–levator ani procedure.[10] 16ξ-Phenylestrone 3-methyl ether (**5**) was less active at a total dose of 1000 μg than estrone was at a total dose of 3 μg in an estrogen assay.[11] Also, **5** showed no significant hypocholesterolemic effect when administered to the rat at 0.005% of the diet.[12] 2α-Phenylhydrocortisone 20-ketal (**4**) was inactive following a single subcutaneous injection of 900 μg/rat in an antiphlogistic–thymus in-

(2) K. L. Williamson and W. S. Johnson, *J. Am. Chem. Soc.*, **83**, 4623 (1961). For the epimeric 2β-acetoxycholestan-3-one, J_{ae} = 7.4 cps and J_{aa} = 9.5 cps.

(3) These values are in general agreement with the effect on molecular rotation caused by substitution of halogen,[4] hydroxyl,[5] acetoxy,[5] methyl,[6] and cyano[7] groups at the 2α position.

(4) B. Ellis and V. Petrow, *J. Chem. Soc.*, 1179 (1956).

(5) G. Rosenkranz, O. Mancera, and F. Sondheimer, *J. Am. Chem. Soc.*, **77**, 145 (1955).

(6) H. J. Ringold and G. Rosenkranz, *J. Org. Chem.*, **21**, 1333 (1956).

(7) H. M. Kissman, A. S. Hoffman, and M. J. Weiss, *ibid.*, **27**, 3168 (1962).

(8) The nmr spectra of the 16-nitro and -cyano derivatives of estrone methyl ether show double resonances for the 18-protons, each of which is shifted downfield from the position (0.88 ppm) of this resonance in the spectrum of estrone methyl ether: R. E. Schaub, H. M. Kissman, and M. J. Weiss, *ibid.*, **29**, 2775 (1964).

(9) The reaction of 1-(N-morpholinyl)cyclohexene with diphenyliodonium chloride is reported to give a low yield of 2-phenylcyclohexanone: M. E. Kuehne, *J. Am. Chem. Soc.*, **84**, 837 (1962).

(10) This assay is a modification of that reported by L. G. Hershberger, E. G. Shipley, and R. K. Meyer, *Proc. Soc. Exptl. Biol. Med.*, **83**, 175 (1953).

(11) R. I. Dorfman and A. S. Dorfman, *Endocrinology*, **55**, 65 (1954).

(12) For a description of this assay see S. Gordon, E. W. Cantrall, W. P. Cekleniak, H. J. Albers, S. Mauer, S. M. Stolar, and S. Bernstein, *Steroids*, **4**, 267 (1964).

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 $C_{27}H_{34}O_4$: C, 79.76; H, 8.43. Found: C, 79.89; H, 8.60.

Acknowledgments.—We are indebted to Messrs. L. Brancone, W. Fulmor, C. Pidacks, and their staffs for the microanalyses, spectral data, and partition chromatography, respectively. We also are grateful to Drs. S. Gordon, S. Mauer, G. Tonelli, and their associates for the biological assays.

(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

W. C. J. Ross

Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, London, S.W. 3, England

Received September 13, 1966

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 ethyleneimine, 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) $\quad$ 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.