B. With Dimethyl Sulfoxide as the Solvent.—To a solution of 100 mg of 6-hydroxyundulatine in 6 ml of dimethyl sulfoxide was added 50 mg of hydroxylamine hydrochloride. The solution was heated on a steam bath for 6 hr. The cooled solution was diluted with water, made basic with ammonium hydroxide, and extracted with chloroform. The chloroform extracts were evaporated under reduced pressure. The residual dimethyl sulfoxide was removed by chromatography on silica gel plates. Removal of the major band (R_f 0.5) allowed the recovery of 78 mg of 6-hydroxyundulatine oxime. Although the material remained amorphous, it was pure by tlc criteria and proved identical with **21** prepared above.

6-Hydroxypowelline (9).—The pure alkaloid afforded colorless prisms from acetone: mp 233–235°; $[\alpha]^{24}D$ −36° (*c* 0.19, MeOH); λ_{max} (95% EtOH) 218 nm (ϵ 17,500), 235 (6800), and 286 (2150); ir ($CHCl_3$) 1510, 1490, 1055, 950 (aromatic methylenedioxy), 3609 (hydroxyl group), 1623 (aromatic methoxyl); nmr ($CDCl_3$) δ 4.02 (s, 3, aromatic methoxyl), 5.38 (s, 1, benzylic proton), 5.88 (2, methylenedioxy), 5.85 and 6.38 (2, olefinic protons), 6.55 (s, 1, aromatic proton).

Anal. Calcd for $C_{17}H_{19}NO_5$: C, 64.34; H, 6.04; N, 4.41. Found: C, 64.17; H, 5.85; N, 4.31.

3,6-*O*,*O*-Diacetylpowelline (11).—By the procedure cited for the preparation of **7**, 100 mg of **9** was converted to 88 mg of crude diacetate. The product was purified by thin layer chromatography and gave 72 mg of 3,6-*O*,*O*-diacetylpowelline after crystallization from acetone: mp 114–117°; $[\alpha]^{24}D$ −26° (*c* 0.20, MeOH); λ_{max} (95% EtOH) 241 nm (ϵ 9020) and 285 (1900); ir ($CHCl_3$) 1738 (C=O), 1625 cm^{-1} (aromatic methoxyl); nmr ($CDCl_3$) δ 2.00 and 2.10 (2 s, 3, CH_3C=O), 3.97 (s, 1, aromatic methoxyl), 5.35 (C_3 methine), 5.90 (q, 2, methylenedioxy), 6.27 (s, 1, benzylic proton), 6.60 (s, 1, aromatic proton).

Anal. Calcd for $C_{21}H_{23}NO_7$: C, 62.83; H, 5.78; N, 3.49. Found: C, 63.02; H, 5.75; N, 3.36.

3-*O*-Acetyl-6-hydroxypowelline (12).—A solution of 50 mg of **11** in 5 ml of dioxane–water (50:50) was allowed to stand at room temperature for 24 hr. The solution was evaporated under reduced pressure, and the crude product was chromatographed on a silica gel plate developed in chloroform–methanol–diethylamine (90:5:5). The major band (R_f 0.6) was recovered as an amorphous material (38 mg): $[\alpha]^{24}D$ −18° (*c* 0.25, MeOH); λ_{max} (95% EtOH) 237 nm (7050) and 287 (1400); ir ($CHCl_3$) 1730 cm^{-1} (C=O); nmr ($CDCl_3$) δ 2.03 (s, 3, CH_3C=O), 4.04 (s, 3, $-OCH_3$), 5.28 (s, 1, benzylic proton), 5.30 (C_3 methine), 5.90 (2, methylenedioxy), 6.59 (s, 1, aromatic proton).

Anal. Calcd for $C_{19}H_{21}NO_6$: C, 63.50; H, 5.89; N, 3.90. Found: C, 63.66; H, 6.01; N, 4.02.

Powelline (13).—A solution of 35 mg of 3-*O*-acetyl-6-hydroxypowelline in 5 ml of thionyl chloride was refluxed for 2 hr on a steam bath and then evaporated to dryness. The residue was combined with 8 ml of dry tetrahydrofuran and 85 mg of lithium aluminum hydride. The solution was refluxed for 12 hr. The cooled solution was hydrolyzed with ethyl acetate followed by water and 25% sodium hydroxide. The tetrahydrofuran solution was filtered, and the filter cake was washed repeatedly with chloroform. The combined chloroform and tetrahydrofuran extracts were evaporated to dryness. The residue was chromatographed on silica gel plates. Removal of the major band (R_f 0.5) gave 22 mg of material which crystallized from acetone, mp 199–201° (lit.[11] mp 200–201°). The compound showed ir and nmr spectra as well as chromatographic characteristics identical with those of powelline.

***N*-Nitroso-6-hydroxypowelline (10, R = H).**—To a solution of 47 mg of **9** in 6 ml of 1.5% aqueous acetic acid was added 50 mg of sodium nitrite. The reaction mixture was allowed to stand at room temperature for 10 hr. *N*-Nitroso-6-hydroxypowelline crystallized from the solution as fine needles. The product (28 mg) was filtered from the solution and recrystallized from acetone–ether: mp 156–159°; $[\alpha]^{24}D$ −14° (*c* 0.17, MeOH); λ_{max} (95% EtOH) 238 nm (ϵ 6040), 289 (1680), and 321 (1720); ir ($CHCl_3$) 1690 (C=O), 1615 cm^{-1} (aromatic methoxyl); nmr ($CDCl_3$) δ 4.12 (s, 3, $-OCH_3$), 6.08 (s, 2, methylenedioxy), 6.07 (s, 2, olefinic protons), 6.85 (s, 1, aromatic proton), 10.4 (s, 1, CHO).

Anal. Calcd for $C_{17}H_{18}N_2O_6$: C, 58.95; H, 5.24; H, 8.09. Found: C, 59.12; H, 5.08; N, 7.92.

Registry No.—**1**, 31128-91-3; **1** (dihydro derivative), 31128-92-4; **1** HCl, 31128-93-5; **2** (6-ethoxy), 31128-94-6; **3**, 31128-95-7; **7**, 31128-96-8; **8**, 31128-97-9; **9**, 31128-98-0; **10** (R = H), 31128-99-1; **10**, (R = Me), 31129-00-7; **11**, 31129-01-8; **12**, 31129-02-9; **14**, 31129-03-0; **15**, 31129-04-1; **17**, 31081-91-1; **17** (*N*,*N*-diacetyl), 31081-92-2; **19** (6-hydroxy), 31129-05-2; **21**, 31129-06-3.

Bufadienolides. 13. Conversion of 3β-Hydroxy-17-oxoandrost-5-ene to 3β-Acetoxy-5β,14α-bufa-20,22-dienolide[1]

GEORGE R. PETTIT* AND JERRY R. DIAS[2]

Department of Chemistry, Arizona State University, Tempe, Arizona 85281

Received March 26, 1971

A synthetic method was developed for converting the readily available dehydroepiandosterone (**1a**) to 3β-acetoxy-5β,14α-bufa-20,22-dienolide (**11**). Important steps in the transformation included condensation of ketone **1b** with diethyl cyanomethylphosphonate to afford olefin **2**. Selective reduction of olefin **2** provided formate **4** which was successively oxidized (**5**) and reduced to ketone **6b**. Application of the Henbest reagent (trimethyl phosphite–chloroiridic acid) to reduction of ketone **6b** provided a convenient pathway to 3β alcohol **7b**. The remaining steps to bufadienolide **11** proceeded *via* intermediates **8**, **9**, and **10**.

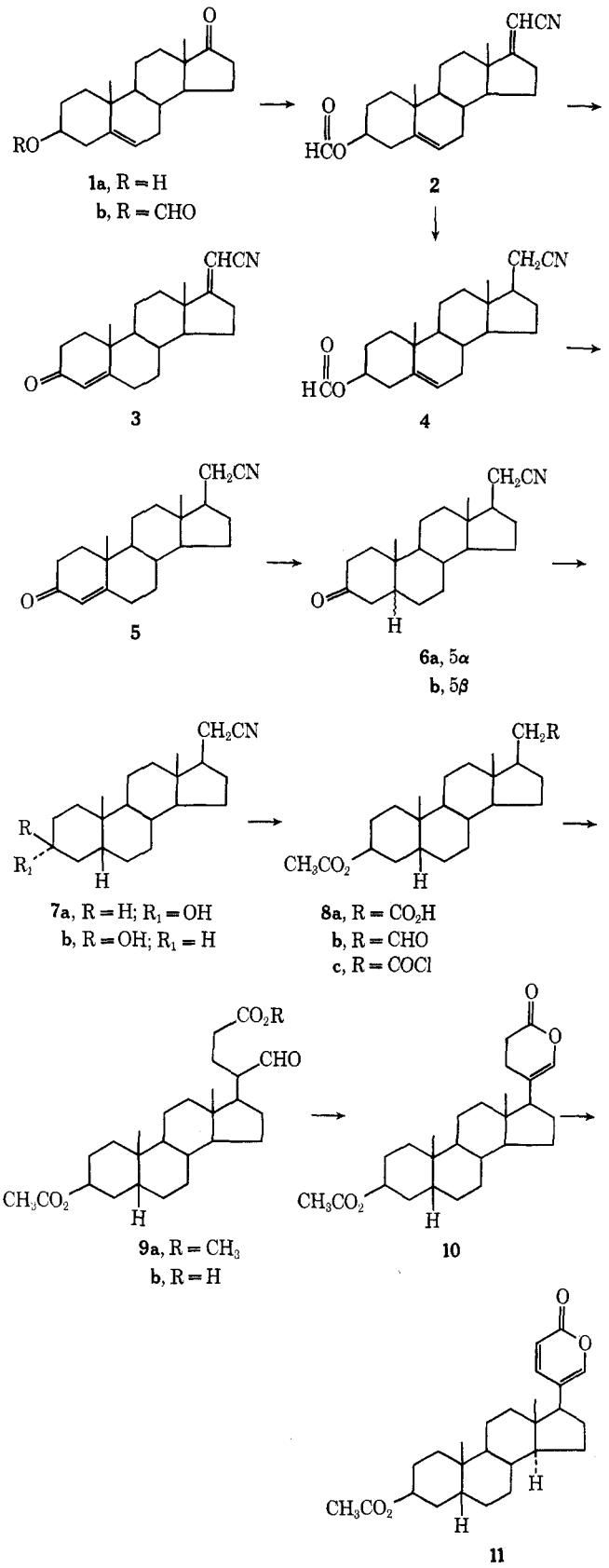
For the dual purposes of making 3β-acetoxy-5β,14α-bufa-20,22-dienolide (**11**) readily available for subsequent conversion to naturally occurring bufadienolides[3] and for comparison purposes a practical total synthesis was required. To meet these objectives the readily available dehydroepiandosterone (**1a**) was selected as starting material. Treatment of alcohol **1a** with acetic–formic anhydride provided formate **1b** in essentially quantitative yield. The formate derivative was employed as protecting group in order to eliminate a saponification step prior to an Oppenauer oxidation[4] envisaged for a later stage in the synthesis. Ketone **1b** was condensed with the carbanion derived from diethyl cyanomethylphosphonate to afford (80%) olefin **2**.

(1) For part 12, see G. R. Pettit, P. Brown, F. Bruschweiler, and L. Houghton, *Chem. Commun.*, 1566 (1971). The present study corresponds to Steroids and Related Natural Products 67; for part 66, refer to G. R. Pettit and J. R. Dias, *J. Org. Chem.*, in press. The investigation described herein was supported by Public Health Service Research Grants CA-10115-04 and CA-11451-02 from the National Cancer Institute. The mass spectrometer was obtained using National Science Foundation Grant GB-4939.

(2) Based in part on a dissertation submitted by J. R. Dias to the Graduate School, Arizona State University, Feb 1970. NIH Predoctoral Fellow, 1968–1970.

(3) Appropriate application of microbiological and synthetic manipulations to bufadienolide **11** might be expected to result easily in routes to, for example, bufalin and resibufogenin; *cf.* G. R. Pettit, L. E. Houghton, J. C. Knight, and F. Bruschweiler, *J. Org. Chem.*, **35**, 2895 (1970).

(4) H. Ringold, B. Loken, G. Rosenkranz, and F. Sondheimer, *J. Amer. Chem. Soc.*, **78**, 816 (1956).



Originally it was planned to obtain ketone **3** by Oppenauer oxidation (94%) and then in concert reduce both double bonds. However, the mixtures of isomers produced by the reduction step caused this route to be rejected. The alternative, selective reduction of side-chain olefin **2**, was easily achieved using palladium on calcium carbonate as catalyst. Since catalytic reduction at the 17(20) position should occur from the less hindered α side, the configuration of the product at C-17 was *a priori* expected to be β, and this fact was demonstrated in an earlier analogous study.[5] Only one of the two possible geometrical isomers of **2** was obtained and was presumed to be the one where the nitrile group would be projected linearly away from the steroid skeleton. Nitrile **4** was obtained in 86% yield and Oppenauer oxidation led to ketone **5** in 84% conversion.

Generally with palladium-catalyzed hydrogenation of 3-oxo-4-ene steroids the ratio of 5β to 5α product changes with basicity of the reaction environment.[6] In the present case 5% palladium on calcium carbonate, in tetrahydrofuran or acetonitrile,[7] resulted in a 5β to 5α ratio of 2 as estimated from pmr (benzene solution) peak heights. With deuteriochloroform as pmr solvent, identical chemical shifts for the 19-methyl group of each isomer were noted; but with benzene as solvent, a chemical shift difference of 5 Hz was observed.[8] Initial use of *N*-methylpyrrolidine as solvent for the reduction reaction did not improve the 5β to 5α ratio but did give rise to colored side products. The isomer mixtures were separated by column chromatography to afford 34% of the 5α (**6a**, positive Cotton effect) and 55% of the 5β isomer (**6b**, negative Cotton effect). As expected, sodium borohydride reduction of ketone **6b** provided in major yield (74%) equatorial 3α alcohol **7a** accompanied by a small amount (17%) of the required axial alcohol **7b**. While inversion of the 3α-hydroxy group might have been affected by heating the corresponding 3α-tosylate in dimethylformamide,[9] it seemed more efficient to apply the Henbest technique.[10] Reaction of ketone **6b** with chloroiridic acid and trimethyl phosphite resulted in a 73% yield of axial 3β alcohol **7b**.

The remaining approach to bufadienolide **11** was based on a new synthesis of bufadienolides we reported.[5] By this means nitrile **7b** was hydrolyzed (85% yield) and acetylated to provide carboxylic acid **8a**. At this point a substantial but unsuccessful attempt was made to find an alternative to the Rosenmund for conversion of nitrile **7b** to aldehyde **8b**. Attempts to reduce nitrile **7b** directly to aldehyde **6b** by means of Raney nickel with sodium hypophosphite,[11] formic acid,[12] hydrazine,[13] or diisobutylaluminum hydride[14] proved unattractive. Other somewhat less direct methods proceeding *via* carboxylic acid **8a** also proved unsatisfactory. Consequently, carboxylic acid **8a** was converted to acid chloride **8c** and reduced by a Rosenmund reaction to aldehyde **8b** in a manner analogous to that reported earlier.[5] The aldehyde **8b** was thereby obtained in 80% yield and converted *via* the piperidine enamine derivative and

(5) G. R. Pettit, D. C. Fessler, K. D. Paull, P. Hofer, and J. C. Knight, *J. Org. Chem.*, **35**, 1398 (1970).
(6) S. Nishimura, M. Shimahara, and M. Shiota, *ibid.*, **31**, 2394 (1966).
(7) M. Combs, H. Henbest, and W. Jackson, *J. Chem. Soc. C*, 2467 (1967).
(8) For a discussion of the transitory collision π complex assumed to be operative in benzene, see J. V. Hatton and R. E. Richards, *Mol. Phys.*, **5**, 139 (1962).
(9) F. C. Chang and R. Blickenstaff, *J. Amer. Chem. Soc.*, **80**, 2906 (1958).
(10) For example, refer to P. A. Browne and D. N. Kirk, *J. Chem. Soc. C*, 1653 (1969). The reducing species is believed to be a large iridium complex with trimethyl phosphite which transfers hydride to the 3-oxo group from the less hindered α side of 5β steroids.
(11) O. G. Bakeberg and Staskun, *J. Chem. Soc.*, 3961 (1962).
(12) T. van Es and B. Staskun, *ibid.*, 5775 (1965).
(13) W. Zajac and R. Denk, *J. Org. Chem.*, **27**, 3716 (1962).
(14) S. Trofimenko, *ibid.*, **29**, 3046 (1964).

subsequent reaction with methyl acrylate to methyl ester **9a** (46% overall). A number of attempts to substitute ethyl *trans*-β-chloroacrylate in the Michael addition step proved unrewarding. Selective saponification of methyl ester **9a** followed by enol lactonization with *p*-toluenesulfonic acid in refluxing benzene gave (71%) bufenolide **10**. The lactonization reaction seemed readily reversed in the presence of moisture or base. Dehydrogenation of bufenolide **10** to the required bufadienolide **11** was performed using molten sulfur and resulted in 43% conversion.

The experiments described herein suggest that this route to bufadienolides bearing the 3β-hydroxy-5β stereochemistry typical of naturally occurring steroids of this type should provide a sound basis for obtaining these difficultly accessible natural products by total synthesis.

Experimental Section

Acetic–formic anhydride was prepared by slowly adding 1 vol. of 100% formic acid to 2 vol. of acetic anhydride at 0°, heating at 50° for 15 min, and cooling to 0°. Acetonitrile (from phosphorus pentoxide), diethyl cyanomethylphosphonate (Aldrich Chemical Co.), piperidine, and trimethyl phosphite were redistilled. Acetonitrile was stored over molecular sieves (4A), and piperidine was stored over potassium hydroxide pellets. Sodium hydride, dispersed in mineral oil (53% NaH), was obtained from Metals Hydrides (Ventron Corp.). Palladium (5%) on calcium carbonate and palladium (5%) on barium sulfate were obtained from Engelhard Industries, Newark, N. J. Chloroiridic acid (brown and deliquescent, $H_2IrCl_6 \cdot 6H_2O$) was obtained in a sealed ampoule from Alfa Inorganics, Beverly, Mass. Methyl acrylate was obtained from Celanese Corp. of America, New York N. Y., and stored over molecular sieves in a refrigerator.

Unless otherwise noted, column chromatography was performed using E. Merck (Darmstadt, Germany) silica gel (0.2–0.5 mm). Preparative and other thin layer chromatograms were prepared employing silica gel HF_{254} (E. Merck). Benzene–ethyl acetate (5:1) was generally used as solvent phase for tlc and development was effected with either iodine vapor or by heating with a spray solution composed of 2% ceric sulfate in 2 *N* sulfuric acid. The preparative thin layer plates were viewed under ultraviolet light. All solvent extracts of aqueous solutions were dried over anhydrous sodium sulfate. Solvents were removed using a rotating evaporator at a bath temperature of 50–60°.

Elemental microanalyses were performed by the laboratories of Dr. A. Bernhardt, 5251 Elbach uber Engelskirchen, West Germany, and each specimen corresponded to a single spot on a thin layer chromatogram. Melting points were determined using a Kofler apparatus. Spectral data was provided by Dr. P. Brown, Miss K. Reimer, and Mr. R. Scott employing instruments referred to in the experimental part of ref 3. Except where indicated otherwise, infared spectra were determined in potassium bromide and pmr spectra in deuteriochloroform solution.

3β-Formyloxy-17-oxo-5-androstene (1b).—To a solution (ice bath) of 3β-hydroxy-17-oxoandrost-5-ene (**1a**, 11.4 g) in pyridine (35 ml) was added (dropwise) acetic–formic anhydride (25 ml). After stirring at room temperature for 0.5 hr, the mixture was poured into water (500 ml) and refrigerated for 5 hr. Filtration led to 12.5 g (99%) of fluffy solid: mp 147–148°; ν_{max} 2980, 1740 (C-17 carbonyl), 1700 and 1175 K (formate carbonyl, shoulder at 1670); pmr δ 8.09 (s, 1, formate proton), 5.47 (d, 1, C-6), 4.75 (broad, 1, C-3), 2.47 and 2.33 (m, 1, C-16), 1.08 (s, 3, C-19), and 0.9 (s, 3, C-18); RD (absolute ethyl alcohol, *c* 0.106) at 25–26° $[\alpha]_{589}$ − 3.4°, $[\alpha]_{520}$ 0.000, $[\alpha]_{500}$ +2.5°, $[\alpha]_{400}$ +64°, $[\alpha]_{350}$ +290°, $[\alpha]_{310}$ +1695° (peak), $[\alpha]_{296}$ 0.000, $[\alpha]_{285}$ −1954°, $[\alpha]_{270}$ −2689° (trough), $[\alpha]_{250}$ −2340° (positive Cotton effect).

Anal. Calcd for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.71; H, 8.77.

3β-Formyloxy-17-cyanomethylandrost-5,17(20)-diene (2).—Diethyl cyanomethylphosphonate (9.0 g) in tetrahydrofuran (75 ml) was added (dropwise) to a stirred suspension of sodium hydride (2.3 g) in tetrahydrofuran (150 ml) at 0° (ice bath). To the resulting clear yellow solution was added 3β-formyloxy-17-oxo-5-androstene (9.3 g) in tetrahydrofuran (75 ml). After standing at room temperature for 26 hr, the solution was concentrated (to 100 ml). Water (500 ml) was added and the mixture was refrigerated for 5 hr. The solid was collected and reformylated [pyridine (25 ml), acetic–formic anhydride (6 ml)]. Recrystallization of the product (8.0 g, 80%) from ethyl acetate–ligroin yielded an analytical sample: mp 181–185° (plates); ν_{max} 2960, 2210 (weak C≡N stretch), 1720 and 1180 (formate), 1635 (weak C=CCN stretch), and 940 (weak C=CH bend); pmr δ 5.44 (broad, 1, C-6), 5.14 (t, 1, J = 2 Hz, C-20 vinyl proton coupled with C-16 allylic protons), 4.72 (broad, 1, C-3 proton), 2.47 (m, 6, C-4, C-7, and C-16 allylic protons), 1.07 (s, 3, 19-methyl), and 1.00 (s, 3, 18-methyl); RD (dioxane, *c* 0.313) $[\alpha]_{650}$ −35°, $[\alpha]_{589}$ −48°, $[\alpha]_{500}$ −67°, $[\alpha]_{400}$ −134°, $[\alpha]_{300}$ −350°, $[\alpha]_{250}$ −910° (plain curve).

Anal. Calcd for $C_{22}H_{29}NO_2$: C, 77.84; H, 8.61; O, 9.43; N, 4.13. Found: C, 77.81; H, 8.86; O, 9.29; N, 4.04.

3-Oxo-17-cyanomethylandrost-4,17(20)-diene (3).—Before adding aluminum isopropoxide (4.1 g in 17 ml of xylene), 20 ml of solvent was distilled from a solution of 3β-formyloxy-17-cyanomethyl-androst-5,17(20)-diene (3.4 g) in xylene (110 ml)–cyclohexanone (41 ml). After heating (at reflux) for 1.5 hr, water (75 ml) was added, and cyclohexanone was removed by steam distillation. The cool solution was decanted and solid was extracted with acetone and then chloroform. Evaporation of solvent yielded a yellow oil which was chromatographed on a column of silica gel (100 g of 0.2–0.5 mm). Elution with ligroin–benzene gave 2.9 g (94%) of needles: mp 158–165°; ν_{max} 2960, 2210 (weak C≡N stretch), 1670 (conjugated C=O stretch), 1635 (weak C=CC≡N stretch), 1610 (weak C=C—CO stretch), and 950 (weak C=CH bend); pmr δ 5.74 (s, 1, C-4 vinyl proton), 5.14 (t, 1, J = 2 cps, C-20 vinyl proton coupled with C-16 allylic protons), 2.5 (m, 6, C-2, C-6, and C-16 protons), 1.23 (s, 3, 19-methyl), and 1.03 (s, 3, 18-methyl); RD (dioxane, *c* 0.195) $[\alpha]_{650}$ +110°, $[\alpha]_{589}$ +130°, $[\alpha]_{400}$ +320°, $[\alpha]_{350}$ +1000°, $[\alpha]_{300}$ +2600°, $[\alpha]_{260}$ +6100° (irregular curve).

Anal. Calcd for $C_{21}H_{27}NO$: C, 81.51; H, 8.79; O, 5.17; N, 4.53. Found: C, 81.32; H, 8.97; O, 5.06; N, 4.65.

3β-Formyloxy-17β-cyanomethyl-5-androstene (4).—A solution of 3β-formyloxy1-7-cyanomethyl-androst-5,17(20)-diene (22.3 g in five aliquots) in tetrahydrofuran (1150 ml) containing 5% palladium on calcium carbonate (22.3 g) was hydrogenated for 48 hr at room temperature and 1-atm pressure (730 Torr); 1650 ml of hydrogen was absorbed (theory 1650 ml). The catalyst was removed (filtration), and the filtrate was concentrated. Chromatography of the yellow residue in ligroin–benzene on a column of silica gel yielded 19.2 g (86%) of product: mp 147.5–150° (prisms from ethyl acetate); ν_{max} 2960, 2250 (weak C≡N stretch), 1720 and 1180 (formate), 1450, 1370 (medium), 1180 (CO stretch), and 950 (medium C=CH bend); pmr δ 5.41 (broad, 1, 6 H), 4.76 (hump, 1, 3α proton), 2.33 (m, 6, C-4, C-7, and C-20 protons), 1.05 (s, 3, 19-methyl), and 0.66 (s, 3, 18-methyl).

Anal. Calcd for $C_{22}H_{31}NO_2$: C, 77.37; H, 9.15; N, 4.10. Found: C,77.46; H, 9.27; N, 4.28.

3-Oxo-17-cyanomethyl-4-androstene (5).—The oxidation procedure employed to obtain ketone **3** was repeated employing a solution of 3β-formyloxy-17β-cyanomethyl-androst-5-ene (13.4 g) in xylene (300 ml, 40 ml of solvent was distilled)–cyclohexanone (120 ml) and aluminum isopropoxide (15 g in 35 ml of xylene). Slow evaporation of an ethyl acetate extract afforded cubic crystals which were collected and washed with ligroin to provide 10.3 g (84%) of product: mp 146.5–149.5°; ν_{max} 2960, 2250 (weak C≡N stretch), 1380 (medium), and 865 (medium C=CH bend); pmr δ 5.74 (s, 1, C-4 vinyl proton), 2.27 (m, 6, C-2, C-6, and C-20 protons), 1.20 (s, 3, 19-methyl), and 0.70 (s, 3, 18-methyl).

Anal. Calcd for $C_{21}H_{29}NO$: C, 80.98; H, 9.38; N, 4.50; O, 5.14. Found: C, 80.95; H, 9.58; N, 4.59; O, 4.88.

3-Oxo-17β-cyanomethyl-5α-androstane (6a) and 3-Oxo-17β-cyanomethyl-5β-androstane (6b).—A solution of 3-oxo-17β-cyanomethyl-androst-4-ene (7.6 g) in acetonitrile containing a suspension of 5% palladium on calcium carbonate (7.6 g) was hydrogenated for 10 hr. The catalyst was removed, washing well with acetonitrile, and the combined filtrate was concentrated. The residue was chromatographed on a column of silica gel (700 g). Consecutive elution with ligroin (bp 60–90°), benzene

(until most of the product was eluted), and finally with benzene–ethyl acetate (20:1) gave in the first fractions 5α product **6a**, in the middle fractions a mixture of 5α and 5β isomers, and in the final fractions 5β product **6b**. The middle fractions were rechromatographed and by this means was obtained 2.6 g (34%) of the 5α isomer (**6a**): mp 190–192 (needles from ligroin–benzene); ν_{max} 2960, 2260 (weak C≡N stretch), 1710 (strong C=O stretch); pmr δ (benzene) 2.00 (m, 4), 0.58 (s, 3, 19-methyl), and 0.23 (s, 3, 18-methyl); mass spectrum M^+ 313 (100%), $M^+ - 15$ (44%); RD (dioxane, *c* 1.45) $[\alpha]_{589}$ +22°, $[\alpha]_{450}$ +56°, $[\alpha]_{350}$ +178°, $[\alpha]_{312}$ +656° (peak), $[\alpha]_{292}$ 0.0°, $[\alpha]_{285}$ −352°, $[\alpha]_{268}$ −678° (trough), $[\alpha]_{250}$ −505° (positive Cotton effect). The 5β isomer (**6b**) weighed 4.2 g (55%): mp 142–144° (plates from ligroin–benzene); ν_{max} 2960, 2260, 1710, fingerprint region distinctly different from that of the 5α isomer; pmr δ (benzene) 2.0 (m, 4, C-2 and C-4 protons), 0.71 (s, 3, 19-methyl), and 0.28 (s, 3, 18-methyl); mass spectrum M^+ 313 (100%), $M^+ - 15$ (15%); RD (dioxane, *c* 1.48) $[\alpha]_{589}$ +19°, $[\alpha]_{450}$ +31°, $[\alpha]_{370}$ +36° (hump), $[\alpha]_{325}$ 0.0°, $[\alpha]_{311}$ −128° (trough), $[\alpha]_{298}$ 0.0°, $[\alpha]_{290}$ +199° $[\alpha]_{267}$ +411° (peak) (negative Cotton effect).

Anal. Calcd for $C_{21}H_{31}NO$: C, 80.46; H, 9.97; N, 4.47; O, 5.10. Found for **6b**: C, 80.46; H, 10.02; N, 4.51; O, 5.01.

3α-Hydroxy-17β-cyanomethyl-5β-androstane (7a).—A solution composed of 3-oxo-17β-cyanomethyl-5β-androstane (6.2 g), methanol (200 ml), acetonitrile (40 ml), and sodium borohydride (1.2 g) was stirred for 0.5 hr. Water was added and the precipitated solid was chromatographed on a column of alumina (400 g of Baker pH 7.6). Elution with benzene–ethyl acetate (10:1) gave 1.1 g (17%) of the 3β-ol (**7b**): mp 144.5–146° (needles from benzene–ligroin); ν_{max} 3450 (strong OH stretch), 2940 (strong CH stretch), 2240 (weak C≡N stretch), and 1020 K (medium CO stretch); pmr δ 4.12 (peak, 1, C-3 equatorial H), 2.17 (m, 2, C-20 proton), 1.50 (methylene envelope), 1.48 (s, superimposed upon the methylene envelope), 0.97 (s, 3, 19-methyl), and 0.61 (s, 3, 18-methyl). A second group of fractions eluted with the same solvent yielded 4.6 g (74%) of the 3α-ol (**7a**): mp 135–138° (needles from benzene–ligroin, mp 142–144.5°); ν_{max} 3450 (strong OH stretch), 2940 (strong CH stretch), 2240 (weak C≡N stretch), and 1040 K (medium CO stretch); pmr δ 3.60 (heptet, J = 5 Hz, 1, C-3 axial H), 2.22 (m, 2, C-20 proton), 1.92 (s, removed by D_2O), 0.93 (s, 3, 19-methyl), and 37 (s, 3, 18-methyl).

Anal. Calcd for $C_{21}H_{33}NO$: C, 79.95; H, 10.54; N, 4.44; O, 5.07. Found for **7a**: C, 79.88; H, 10.82; N, 4.36; O, 4.94.

3β-Hydroxy-17β-cyanomethyl-5β-androstane (7b).—A solution composed of 3-oxo-17β-cyanomethyl-5β-androstane (4.3 g), chloroiridic acid (0.20 g), trimethyl phosphite (8.8 ml), and aqueous 2-propanol (120 ml containing 10% H_2O) was heated at reflux for 60 hr. Water (250 ml) was added and the mixture was extracted with ether (150 ml in three portions). Solvent was evaporated and the residue was chromatographed on a column of silica gel (50 g) to yield 3.2 g (73%) of needles, mp 143–148° (from ligroin–benzene), upon elution with benzene–ethyl acetate (100:1). In addition, 0.21 g (5%) of 3α-hydroxy-17β-cyanomethyl-5β-androstane was isolated.

3β-Acetoxy-5β-pregnan-21-oic Acid (8a).—A solution prepared from 3β-hydroxy-17β-cyanomethyl-5β-androstane (2.5 g), dioxane (60 ml), methanol (75 ml), water (25 ml), and potassium hydroxide (25 g) was heated at reflux for 64 hr. After diluting with water (350 ml), acidifying with concentrated hydrochloric acid, and cooling, the solid was collected. The dried (*in vacuo*), cream-colored solid was heated in pyridine (26 ml)–acetic anhydride (13 ml) for 40 min. Water (350 ml) and concentrated hydrochloric acid (26 ml) were added, and the mixture was extracted with ether (240 ml in three portions). The ethereal phase was washed with water (400 ml in two portions) and saturated sodium chloride solution. The residue obtained by removing solvent was chromatographed on a column of silica gel (150 g of 0.2–0.5 mm). Elution with benzene–ethyl acetate yielded 2.6 g (85%) of acid **8**: mp 163–165° (prisms from ligroin, bp 60–110°); ν_{max} 3450 (medium, tails), 2940, 1730, and 1250 K (acetate), 1690 (acid); pmr δ 7.50 (broad, 1, eliminated by D_2O), 5.10 (peak, 1, 3α proton), 2.07 (s, 3, 3-acetate), 0.98 (s, 3, 19-methyl), and 0.60 (s, 3, 18-methyl).

Anal. Calcd for $C_{23}H_{36}O_4$: C, 73.36; H, 9.64. Found: C, 73.21; H, 9.54.

3β-Acetoxy-5β-pregnan-21-al (8b).—A solution of 3β-acetoxy-5β-pregnan-21-oic acid (1.8 g) in benzene (45 ml) containing oxalyl chloride (3.3 ml) was allowed to remain (protected from moisture) at room temperature for 7.5 hr. Solvent and excess oxalyl chloride were removed and the amber residue was dissolved in toluene (30 ml). To the solution was added 5% palladium on barium sulfate and hydrogen was passed through the refluxing mixture over 4.5 hr. The catalyst was collected, the solvent evaporated, and the residue chromatographed on a column of silica gel (60 g). Upon elution with ligroin–ethyl acetate (40:1) 1.4 g (80%) of aldehyde **8b**, mp 76–83°, was obtained: ν_{max} 2950, 2720 (weak aldehyde CH stretch), 1730 and 1250 (acetate), 1715 K (aldehyde CO stretch); pmr δ 9.00 (d, J = 2 Hz, 1, aldehyde), 5.12 (peak, 1, 3α proton), 2.33 (m, 2, C-20 protons), 2.07 (s, 3, acetate), 85 (methylene envelope), 1.00 (s, 3, 19-methyl), and 0.14 (s, 3, 18-methyl).

Anal. Calcd for $C_{23}H_{36}O_3$: C, 76.62; H, 10.07. Found: C, 77.07; H, 10.39.

3β-Acetoxy-5β,14α-buf-20(21)-enolide (10).—A mixture composed of 3β-acetoxy-5β-pregnan-21-al (1.0 g), toluene (14 ml), piperidine (0.7 ml), and molecular sieves (4A) was allowed to stand at room temperature for 6.5 hr. The solvent was removed and traces of piperidine were removed by addition and evaporation of toluene (25 ml). The enamine as an amber oil was heated (reflux) with acetonitrile (12 ml) and methyl acrylate (0.32 ml) under nitrogen for 61 hr. Acetic acid (0.3 ml) and water (4 ml) were added and heating at reflux was continued for a further 2 hr under nitrogen. The cool mixture was diluted with ether (30 ml), and the ethereal phase was washed with saturated sodium chloride solution (60 ml in three portions). Solvent was removed and the yellow solid was chromatographed on silica gel (60 g). Elution with ligroin (bp 30–60°)–ethyl acetate (40:1) afforded 0.43 g (42%) of recovered aldehyde **8b**, and further elution with ligroin–ethyl acetate (10:1) yielded 0.56 g (46%) of an epimeric mixture of methyl ester **9** as a solid: ν_{max} 2940, 1730 (broad), and 1250, and 1020 K; pmr δ 9.60 (m, 1, aldehyde proton), 5.17 (s, 1, 3α proton), 3.75 (s, 3, methyl ester), 2.10 (s, 3, acetate), 1.00 (broad, s, 3, 19-methyl), and 0.70 (d, 3, 18-methyls of epimeric mixture).

A solution of methyl 3β-acetoxy-21-oxo-5β-(20*R*,*S*)-cholan-24-oate (**9a**, 0.65 g) in methanol (6 ml)–tetrahydrofuran (15 ml)–0.4 *M* sodium carbonate was stirred (under nitrogen) at room temperature for 2 hr. A 3 *N* hydrochloric acid solution (6 ml) was added and stirring was continued for 0.5 hr. The mixture was extracted with ether (160 ml in four portions). The oil obtained upon evaporating solvent was dried *in vacuo*. The solid (**9b**) which resulted gave the following pmr data: δ 9.95 (m, 0.3), 9.64 (0.7), 5.15 (peak, 1, 3α proton), 3.75 (s, 3, acetate), 0.98 (broad, s, 3, 19-methyl), and 0.73 (s, 1) and 0.68 (s, 2, 18-methyl).

The solid carboxylic acid (9b) was dissolved in benzene (70 ml) containing toluenesulfonic acid (0.1 g) and the solution was heated at reflux for 27 hr (under nitrogen) using a Dean–Stark trap containing molecular sieves (4A). The product was immediately chromatographed on a column of silica gel (25 g). Elution with benzene (1000 ml) followed by benzene–ethyl acetate (50:1) led to 0.43 g (71%) of bufenolide **10**: mp 131–132° (large orthorhombic crystals from ligroin); ν_{max} 2960, 1770, 1730, 1650, 1260, 1130, and 1020 K; pmr δ 388 (s, 1, C-21), 6.49 (peak, 1, 3α proton), 2.50 (m, 4, C-22 and C-23 protons), 3.75 (s, 3, acetate), 1.00 (s, 3, 19-methyl), and 0.60 (s, 3, 18-methyl).

Anal. Calcd for $C_{26}H_{38}O_4$: C, 75.32; H, 9.24. Found: C, 75.24; H, 9.24.

3β-Acetoxy-5β,14α-bufa-20,22-dienolide (11).—An intimate mixture of 3β-acetoxy-5β,14α-buf-20(21)-enolide (0.23 g) and sulfur (0.23 g) was heated at 212–217° (nitrogen atmosphere) for 0.5 hr; the initial reaction evolved hydrogen sulfide. The cool brown mixture was dissolved in carbon disulfide and chromatographed on silica gel (40 g). Elution with benzene–ether (10:1) afforded 98 mg (43%) of product (**11**) as a brown oil which gave one spot on tlc. Recrystallization from methanol yielded tetragonal crystals: mp 111.5–113.5°; ν_{max} ($CHCl_3$) 2970, 1730 (broad), 1640, 1550, 1270, and 1030 K; pmr δ 7.38 (m, 2, C-21 and C-23), 6.38 (d, 1, J = 10.5 Hz, C-22), 5.17 (peak, 1, 3α proton), 3.75 (s, 3, acetate), 1.00 (s, 3, 19-methyl), and 0.54 (s, 3, 18-methyl); λ_{max} (EtOH) 300 mμ (log ε 3.76); mass spectrum M^+ 412 (28%), 370 (8%), 352 (100%), 337 (44%).

Anal. Calcd for $C_{26}H_{36}O_4$: C, 75.69; H, 8.80. Found: C, 75.41; H, 9.06.

While the specimen of bufadienolide **11** prepared as described above was pure by thin layer chromatography and proton magnetic resonance evidence, a tenacious deep straw discoloration remained evident. The color was eventually completely re-

moved by consecutive preparative thin layer chromatography, treatment with activated carbon, and again preparative layer chromatography. The product so obtained was colorless but no change in the physical measurements cited above was noted.

Registry No.—**1a**, 53-43-0; **1b**, 29163-23-3; **2**, 31020-62-9; **3**, 31020-63-0; **4**, 31020-64-1; **5**, 31020-65-2; **6a**, 31020-66-3; **6b**, 31020-67-4; **7a**, 31020-68-5; **7b**, 31020-69-6; **8a**, 31020-70-9; **8b**, 31107-24-1; (20*R*)-**9a**, 31020-71-0; (20*S*)-**9a**, 31020-72-1; **9b**, 31107-25-2; **10**, 31020-73-2; **11**, 14414-50-7.

A New, General Synthesis of 2-, 8-, and 9-Substituted Adenines[1]

EDWARD C. TAYLOR,* GEORGE P. BEARDSLEY,[2] AND YOSHIFUMI MAKI

Department of Chemistry, Princeton University, Princeton, New Jersey 08540, and the Gifu College of Pharmacy, Mitahora, Gifu, 501-21, Japan

Received April 6, 1971

A new synthesis of adenine derivatives is described which involves the initial conversion of a 4,6-diamino-5-nitrosopyrimidine to a 7-aminofurazano[3,4-*d*]pyrimidine (**2**) by lead tetraacetate oxidation, introduction of the eventual adenine 9 and 8 substituents by reaction of **2** with an alkylamine followed by acylation (**3** → **4**), and reductive cleavage of the furazan ring to give an intermediate **5** which recyclizes to the desired adenine derivative **6**. All reactions proceed under mild conditions, and all substituents are introduced unambiguously.

The classical and still most widely employed synthetic route to adenine and adenine derivatives, compounds of ubiquitous natural occurrence and extreme biological importance, is that of Traube involving cyclization of a 4,5,6-triaminopyrimidine with reagents such as formamide and other carboxamides, triethyl orthoformate–acetic anhydride (or diethoxymethyl acetate), carboxylic acids, carbon disulfide, and sodium dithioformate.[3] This approach possesses several intrinsic disadvantages in that (a) reaction conditions required for ring closure are often severe and (b) in its application to 9-substituted adenines, an inevitable ambiguity arises as to the direction of ring closure.[4-6]

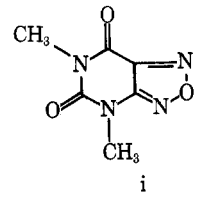
The most commonly employed alternate route involves aminolysis of a purine derivative carrying a 6-chloro, thio, methylthio, methylsulfinyl, methylsulfonyl, or sulfonate substituent, but here as well severe reaction conditions are often required and the requisite purine intermediates are tedious to prepare.[7] 9-Substituted adenines may also be prepared by alkylation, but, since both 7- and 9-alkylated derivatives are often obtained, this process is also inherently ambiguous.[8]

We describe in this paper a new approach to the synthesis of adenines which is widely applicable and which possesses two important special features: (a) all reactions proceed under mild conditions and (b) it introduces all substituents, including the critical 9 substituent, unambiguously.

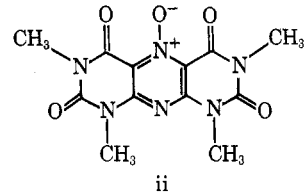
This new route to adenines involves the following steps: (a) oxidation of a 4,6-diamino-5-nitrosopyrimidine (**1**) with lead tetraacetate to a 7-aminofurazano-[3,4-*d*]pyrimidine (**2**); (b) reaction of **2** with an alkylamine to give a 7-alkylaminofurazano[3,4-*d*]pyrimidine (**3**); (c) formylation, acylation, or aroylation of the exocyclic alkylamino group to give **4**; and (d) reductive cleavage of the furazan ring, which is followed by spontaneous recyclization to the desired adenine (**6**). In this manner, the eventual 9 substituent is determined by an appropriate choice of the alkylamine used in step b, and the eventual 8 substituent is determined by the choice of reagent employed in step c. The eventual 2 substituent is determined in the usual manner at the initial stage of pyrimidine synthesis. The furazan ring serves to protect both the potential 6-amino group and 7-nitrogen atom while allowing the unequivocal introduction of both the eventual 9-nitrogen and 8-carbon atoms, together with their desired substituents in the target adenine. In addition, it imparts to the system a remarkable reactivity (see discussion below) which allows step b to proceed under very mild conditions.

Preparation of 7-Aminofurazano[3,4-*d*]pyrimidines (2). Step a.—We have found that a wide variety of 2-substituted 4,6-diamino-5-nitrosopyrimidines (**1**) are converted smoothly and at room temperature to 7-aminofurazano[3,4-*d*]pyrimidines (**2**) upon treatment in acetic acid solution with 1 equiv of lead tetraacetate.[9] The requisite 5-nitrosopyrimidines are readily available *via* thermal cyclization of amidine salts of isonitrosomalononitrile[10] or by direct nitrosation of the corresponding 4,6-diaminopyrimidines.[11]

Proof of the structure of **2** rests upon nmr and mass spectral data, elemental analyses, and subsequent chem-



i



ii

(1) We acknowledge with gratitude financial support for this work from Eli Lilly and Co. and from the National Science Foundation and the Japan Society for the Promotion of Science, Office of International Programs, U. S.-Japan Committee on Scientific Cooperation, Grants No. GF-390 and 5R012.

(2) NSF Predoctoral Fellow, 1968–1971.

(3) For an excellent review of these methods, see R. K. Robins, in "Heterocyclic Compounds," Vol. 8, R. C. Elderfield, Ed., Wiley, New York, N. Y., 1967, pp 142–144.

(4) G. A. Howard, B. Lythgoe, and A. R. Todd, *J. Chem. Soc.*, 556 (1945), and preceding papers in this series.

(5) R. Hull, *ibid.*, 2746 (1958).

(6) H. C. Koppel, D. E. O'Brien, and R. K. Robins, *J. Amer. Chem. Soc.*, **81**, 3046 (1959).

(7) For numerous examples see ref 3, p 319.

(8) J. Baddiley, B. Lythgoe, and A. R. Todd, *J. Chem. Soc.*, 318 (1944); for additional examples see ref 3, p 372.

(9) Extrapolation of this oxidative procedure to 1,3-dimethyl-5-nitroso-6-aminouracil was found to give a modest (19%) yield of 4,6-dimethyl-5,7-(4*H*,6*H*)-furazano[3,4-*d*]pyrimidinedione (i); the major product, however, was 1,3,6,8-tetramethyl-2,4,5,7-(1*H*,3*H*,6*H*,8*H*)-pyrimido[5,4-*g*]pteridinetetrone 5-*N*-oxide (ii). Details of this surprising reaction will be given in a separate paper.

(10) E. C. Taylor, O. Vogl, and C. C. Cheng, *J. Amer. Chem. Soc.*, **81**, 2442 (1959).

(11) D. J. Brown, "The Pyrimidines," Interscience, New York, N. Y., 1962.