Methyl 2,3-Diacetamido-2,3-dideoxy-α-L-ribopyranoside (12).—To a solution of 108 mg of methyl 2,3-diamino-2,3-dideoxy-α-L-ribopyranoside (**10**) in 1 ml of water was added 0.5 ml of acetic anhydride. The mixture was stirred for 5 min and evaporated to dryness *in vacuo* to give 148 mg of crude product as a white solid. Purification was carried out by trituration with two 1-ml portions of methanol to give 109 mg of white solid, mp >275°, which was homogeneous on tlc with R_f 0.2 using ethyl acetate–methanol (9:1); λ_{max}^{Nujol} 2.83–3.2 (NH), 6.05, 6.15 (C=O), 6.35–6.45 μ (secondary amide).

Anal. Calcd for $C_{10}H_{18}N_2O_5 \cdot 1/3\ H_2O$: C, 47.7; H, 7.40; N, 11.1. Found: C, 47.5; H, 7.23; N, 10.9.

Ethylthio 2,3-Diacetamido-2,3-dideoxy-L-riboside (14).—A solution of 100 mg of 12 in 1.5 ml of concentrated hydrochloric acid and 1.5 ml of ethanethiol was stirred at 0–5° for 20 hr and neutralized with ammonia. The aqueous solution was extracted with chloroform and the aqueous phase was evaporated to dryness *in vacuo*. The dry residue was extracted with several 2–3 ml portions of chloroform. The chloroform extracts were evaporated to dryness *in vacuo* and the solid residue was recrystallized from methanol–ether to give 12 mg of product as a white solid: mp 275° dec; λ_{max}^{Nujol} 3.0 (NH), 6.05 (C=O), 6.5 μ (secondary amide). The nmr spectrum in D_2O showed one ethyl group and two N-acetates.

Anal. Calcd for $C_{11}H_{20}N_2O_4S \cdot {}^3/_4H_2O$: C, 45.6; H, 7.43; N, 9.70. Found: C, 45.9; H, 7.20; N, 9.52.

Registry No.—**4**, 20453-03-6; **5**, 20452-98-6; **6**, 20452-99-7; **7**, 20453-00-3; **8**, 20453-04-7; **9**, 20453-05-8; **10**, 20452-95-3; **11** (2HCl), 20452-96-4; **12**, 20452-97-5; **14**, 20453-06-9.

The Condensation of Glyoxylic Acid with 5α-Androstanolone

MANUEL DEBONO, R. MICHAEL MOLLOY, AND L. E. PATTERSON

The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana

Received December 17, 1968

The condensation of 5α-androstanolone with glyoxylic acid gave steroids with an α-hydroxyacetic acid side chain at C-2. This side chain then reacts further with the C-3 carbonyl group to give ring-A-fused lactols. The chemistry of these lactols was studied, and the lactols were used to synthesize the novel ring-A-fused pyridazone **6**. When the condensation reaction was carried out in refluxing methanol, the C-2 *trans*-ylidene acetic acid derivative was isolated. The chemistry of these acids was studied, and their derivatives were used for synthesis of ring-A-fused γ-lactones.

Recent interest in the synthesis of ring-A-fused heterocyclic steroids has been prompted by the discovery of the unique biological properties of the steroidal pyrazoles.[1]

The goal of the present study was the synthesis of a 3-keto steroidal intermediate bearing a two-carbon side chain at C-2. This could be subsequently converted to fused steroidal heterocyclic systems possessing six-membered rings bearing two heteroatoms or five-membered rings bearing one heteroatom. This synthesis was accomplished by a modification of the procedure outlined by Newman, *et al.*, and also by Kurath and Cole, for the synthesis of 17-keto-16-*trans*-ylidene acetic acid steroids.[2] Condensation of 5α-androstanolone with glyoxylic acid in aqueous methanol and sodium hydroxide at room temperature gave hydroxyketo acid **1**, which could readily be lactolized to give the methoxylactol **2** when treated with methanolic HCl (Scheme I). An analogous condensation has recently been employed by Pettit[3] for synthesis of the isocardenolide side chain from 20-keto steroids.

The structure of **2** was deduced from its infrared spectrum[4] (1760 cm^{-1}) and from the properties of its diacetoxy derivative (**3**) obtained by refluxing **2** with acetic anhydride containing sodium acetate. The infrared spectrum showed that the lactol carbonyl group was unaltered by these acetylation conditions. Since no enol lactonization was observed under these conditions, it was concluded that the methoxyl group was at C-3 rather than on the side chain.[5] The position of the C-2′ hydroxyl group was confirmed by the observation that the doublet in the nmr of **2** at δ 4.85 shifted to δ 5.85 upon acetylation. The nmr spectrum of **3** (see Figure 1) showed two acetoxy groups as singlets at δ 2.06 and 2.16; the C-2 proton was observed as a quintet at δ 2.74 (J = 5 Hz). The proton at C-2′ was observed as a doublet at δ 5.83 (J = 5 Hz), and the methoxyl group and 17α proton gave signals at δ 3.37 and 4.62, respectively.

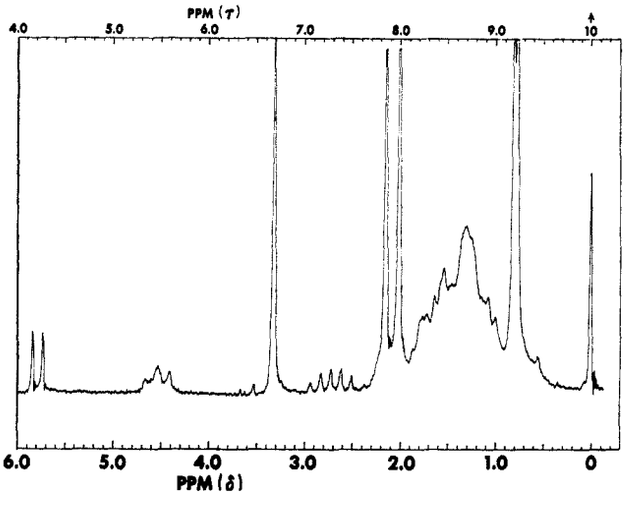


Figure 1.—Nuclear magnetic resonance spectrum of lactol diacetate **3**.

The stereochemistry of ring fusion of the lactol to ring A and its orientation can be deduced from the nmr spectrum of **3** and through the use of conformational analysis. It is well established that γ-lactones prefer a

(1) (a) R. O. Clinton, A. J. Manson, F. W. Stonner, A. L. Beyler, G. O. Potts, and A. Arnold, *J. Amer. Chem. Soc.*, **81**, 1513 (1959); (b) D. K. Phillips and A. J. Manson, *J. Org. Chem.*, **28**, 2886 (1963), and references cited therein. (c) R. Hirschmann, P. Buchschacher, N. G. Steinberg, J. H. Fried, R. Ellis, G. J. Kent, and M. Tishler, *J. Amer. Chem. Soc.*, **86**, 1520 (1964), and references cited therein.

(2) (a) P. Kurath and W. Cole, *J. Org. Chem.*, **26**, 1939 (1961); (b) M. S. Newman, W. C. Sagar, and C. C. Cochrane, *ibid.*, **23**, 1832 (1958).

(3) G. R. Pettit, G. L. Dunn, and B. Green, *Chem. Ind. (London)*, 1265 (1964).

(4) R. S. Rasmussen and R. R. Brattain, *J. Amer. Chem. Soc.*, **71**, 1073 (1949).

(5) M. S. Newman and C. A. VanderWerf, *ibid.*, **67**, 233 (1945).

cis fusion to six-membered rings[6] and that large groups prefer the 2α orientation (equatorial) in the 5α-androstane system.[7] Applying these two facts to the lactol diacetate **3**, it is evident that the most favorable ring fusion is at the 2α–3α positions with the methoxyl group situated at 3β-equatorial position. Dreiding models indicate that the methoxyl group severely hinders the β side of the lactol ring, thereby favoring the orientation of the 2′-acetoxyl function toward the α side. That this preference exists can be deduced from the similarity of the coupling between the C-2′–C-2 protons (J = 6.5 Hz, dihedral angle *ca.* 30°) with those reported by Williamson and Johnson in their study of the conformation effects on coupling constants in various α-acetoxycholestanones.[8]

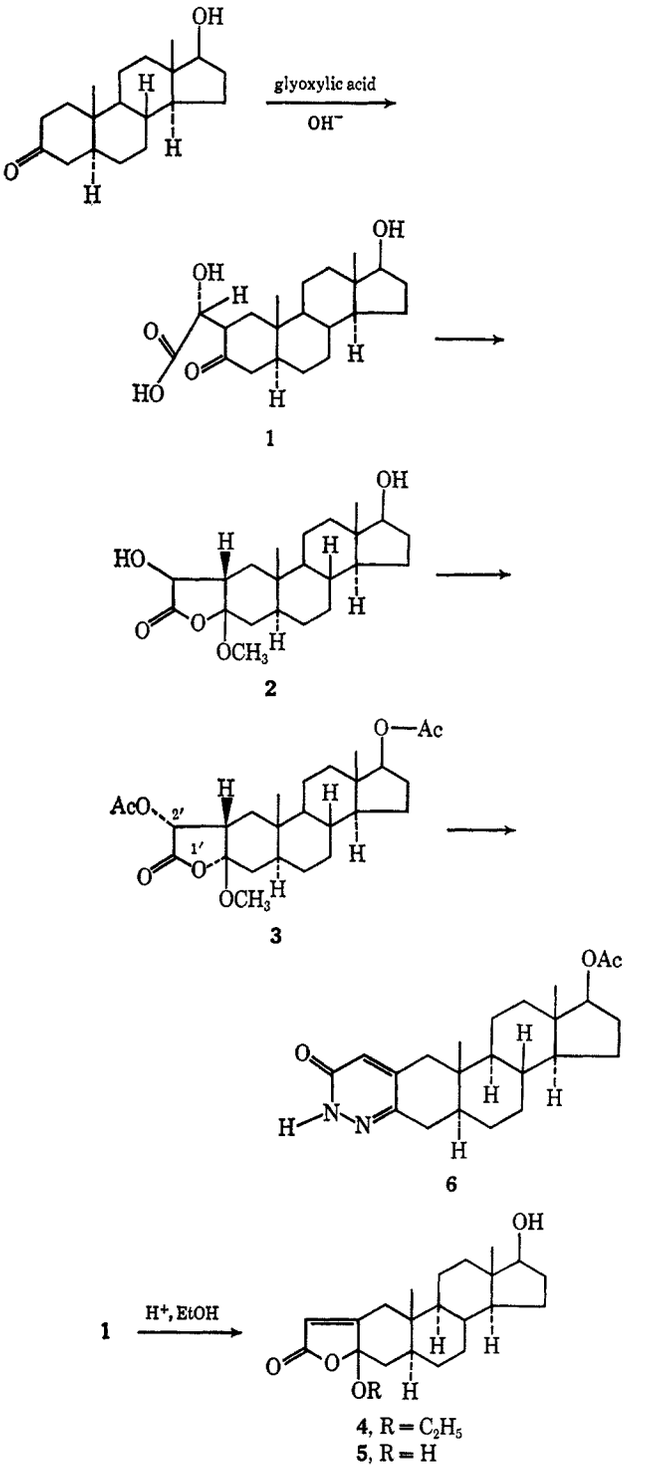
Further chemical proof of the structure of **2** was obtained by isolation of the α,β-unsaturated ethoxylactol **4** when **2** was refluxed in acidic ethanol. Recrystallization of **4** from moist acetone gave the less soluble lactol **5**.

The methoxylactol acetate **3** was transformed to the corresponding ring-A-fused pyridazone **6** by refluxing in ethanolic hydrazine. The nmr spectrum of **6** showed one aromatic proton at δ 6.72, while infrared (1660 cm^{-1}) and ultraviolet spectra (293 mμ) are typical of substituted pyridazines.[9]

The condensation of glyoxylic acid with androstanolone in refluxing aqueous methanolic base gave exclusively 17α-hydroxy-3-oxo-5α-androstan-$\Delta^{2,\alpha}$-acetic acid (**7**) (Scheme II) in 85% yield. The uv spectrum showed an absorption maximum at 240 mμ and an nmr signal at δ 6.28 for the olefinic proton. These properties are in general agreement with those reported by Kurath and Cole for the 16-*trans*-ylidene acetic acid 17-keto steroids.[2a,10] The exclusive formation of the *trans*-ylidene keto acid by this reaction is in full accord with the observation of Zimmerman and Ahramjian that *threo*- and *erythro*-3-hydroxy-2,3-diphenylpropionic acid each give exclusively *trans*-α-phenylcinnamic acid in better than 99% yields.[11]

The acid **7** was reduced to the 3β-hydroxy acid **8** with sodium borohydride. The resulting acid showed no tendency to lactonize, which is in accord with the *trans* orientation of the side chain. Acetylation of **8** gave the diacetoxy acid **9**. In the nmr spectrum of **9** an olefinic proton signal is situated at δ 5.88 and a doublet at δ 4.2 (1 H, J = 15 Hz) which was assigned to the equatorial proton at C-1. This rigid orientation of the *trans*-ylidene side chain at C-2 allows the deshielding plane of the carboxyl group to approach C-1 closely, thereby shifting the equatorial proton downfield.[12] This interpretation was bolstered further by the use of double resonance techniques. Irradiation at precisely 105 Hz upfield from the δ 4.2 signal resulted in the collapse of this broadened doublet to a broad singlet. Conversely, irradiation at δ 4.2 caused a singlet at δ 1.94 to appear through the methylene envelope. The nmr signal at δ 5.25 for the C-3 proton has a band width at half-height

SCHEME I



(6) (a) J. Klein, *J. Amer. Chem. Soc.*, **81**, 3611 (1959). (b) W. S. Johnson, V. J. Bauer, J. L. Margrave, M. A. Frisch, L. H. Dreger, and W. N. Hubbard, *ibid.*, **83**, 66 (1961).

(7) (a) J. A. Hogg, F. H. Lincoln, R. W. Jackson, and W. P. Schneider, *ibid.*, **77**, 6401 (1955); (b) H. J. Ringold and G. Rosenkranz, *J. Org. Chem.*, **21**, 1333 (1956).

(8) K. L. Williamson and W. S. Johnson, *J. Amer. Chem. Soc.*, **83**, 4623 (1961); the Williamson–Johnson data show that protons on carbon bearing both an acetoxy and a carbonyl group will couple with protons on adjacent carbon atoms having dihedral angles in the vicinity of 30° to produce a coupling constant of about 6.6 Hz.

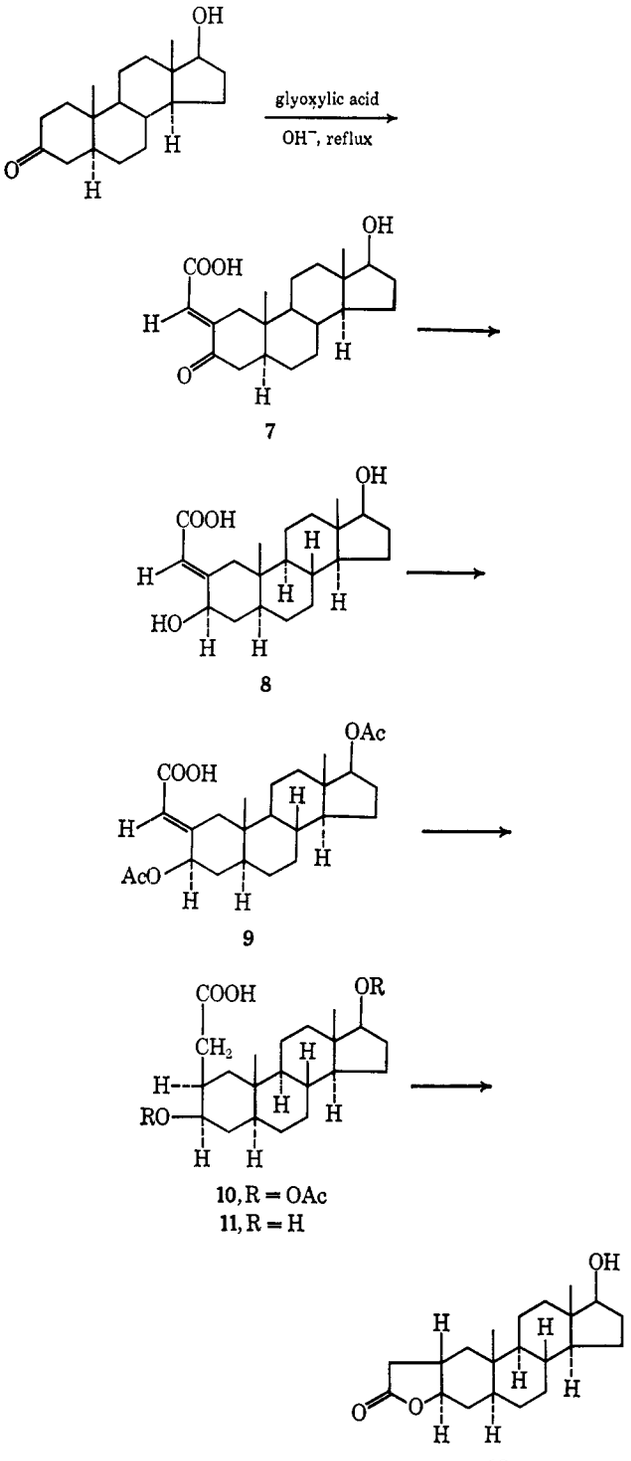
(9) T. L. Jacobs in "Heterocyclic Compounds," Vol. 6, R. C. Elderfield, Ed., John Wiley & Sons, Inc., New York, N. Y., 1957, p 120.

(10) The olefinic proton for 17-keto-16-*trans*-ylidene acetic acid Δ^{5}-androstan-3β-ol was observed at δ 6.42 and absorbed at 244 mμ (ε 10,000) in the ultraviolet region. The shifts in these properties in going from a 16- to a 2-ylidene side chain reflect the higher degree of planarity achieved between this grouping and the carbonyl function when attached to a five-membered ring. This effect is also reflected in the greater deshielding of the olefinic proton by the carbonyl group in the latter case.

(11) H. E. Zimmerman and L. Ahramjian, *J. Amer. Chem. Soc.*, **81**, 2086 (1959).

(12) Recent studies on the structure of cassaic acid have similarly demonstrated deshielding of methylene groups adjacent to ylidene acetic acid groupings: (a) R. L. Clarke, S. J. Daum, P. E. Shaw, and R. K. Kullnig, *ibid.*, **88**, 5865 (1966); (b) H. Hauth, D. Stauffacher, P. Niklaus, and A. Melera, *Helv. Chim. Acta*, **48**, 1087 (1965).

SCHEME II



of 17 Hz, indicating that the acetate group at C-3 has the equatorial 3β orientation.[13]

The dihydroxy acid **8** was acetylated to **9**, and the latter was hydrogenated smoothly to the fully saturated diacetoxy acid **10**. Hydrogenation caused the δ 4.2 nmr signal to disappear. Hydrolysis of **10** gave the acid **11**, which was readily lactonized to the fused γ-lactone **12**. On the grounds of greater steric hindrance of the ring-A β face, the main hydrogenation product is assumed to have the C-2 side chain in the 2β position.

(13) (a) J. C. Orr, M. L. Franco, A. D. Cross, and F. Sondheimer, *Steroids*, **3**, 1 (1964); (b) N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, Calif., 1964, p 79.

The successful syntheses of the fused pyridazone **6** and the lactone **12** demonstrate the usefulness of the condensation of glyoxylic acid with keto steroids for the synthesis of heterocyclic steroid derivatives. The further utility of this method is currently under study.

Experimental Section

Infrared spectra were determined on a Beckman IR-7 spectrophotometer. Nuclear magnetic resonance spectra were obtained with a Varian Model HR-60 spectrometer with internal tetramethylsilane. Ultraviolet spectra were obtained with a Cary Model 15 spectrophotometer. Melting points were determined on a Mel-Temp apparatus and are uncorrected.

Condensation of 5α-Androstanolone with Glyoxylic Acid. Method A.—A slurry of 10 g (0.034 mol) of 5α-androstanolone in 200 ml of 1:1 aqueous MeOH was treated with 7 g of 40% aqueous glyoxylic acid solution and 2.72 g of NaOH. Stirring was continued at room temperature for 18 hr, and the reaction mixture was diluted with 600 ml of H_2O. Ether extraction of this solution gave 1 g of unreacted starting ketone. The alkaline layer was carefully acidified with acetic acid to pH 5–6 and extracted three times with Et_2O, and the extracts were dried over anhydrous $MgSO_4$. Evaporation of the solvent *in vacuo* gave a foam. The foam crystallized from MeOH–H_2O to give 17β-hydroxy-5α-androstan-2α-(α-hydroxyacetic acid)-3-one (**1**) (6 g): mp 190–191°; ir (Nujol) 1710 cm^{-1}; $[\alpha]^{20}D$ 32.9° (*c* 1, EtOH).

Anal. Calcd for $C_{21}H_{32}O_5$: C, 69.10; H, 9.09. Found: C, 68.92; H, 8.84.

A methanolic solution of **1** (3 g in 75 ml of MeOH) was treated with a catalytic amount of methanolic HCl (1 ml of 10% methanolic HCl per 25 ml of methanolic solution of **1**), and the solvent was allowed to evaporate slowly under a slight vacuum and cooling. When the first indication of crystallization occurred, the solution was placed in the refrigerator. A white solid, 3ξ-methoxy-17β-hydroxy-5α-androstan-2α-(α-hydroxyacetic acid)-3-one-lactol (**2**) (2 g) was isolated and recrystallized from MeOH: mp 255–260°; ir (Nujol) 1760 cm^{-1}; $[\alpha]^{20}D$ 85.5° (*c* 1.17, DMF).

Anal. Calcd for $C_{22}H_{34}O_4 \cdot H_2O$: C, 66.66; H, 9.08. Found: C, 66.62; H, 9.08.

The gross structure of **2** was further verified by conversion to the crystalline diacetoxymethoxylactol **3**, as described below.

Method B.—The procedure outlined by Kurath and Cole for the condensation of glyoxylic acid with 17-keto steroids[2a] was used, except that the reaction mixture was not refluxed after the 18-hr room temperature stirring period. Instead, the mixture was gradually heated to 50° over a 20-min period. The products and yields were comparable with those obtained by method A. The simplicity of using glyoxylic acid directly instead of generating it *in situ* makes method A more attractive.

Acetylation of Methoxylactol 2.—A solution containing 2 g (0.0053 mol) of **2** in 50 ml of Ac_2O was refluxed under nitrogen for 1.5 hr. At this time, 0.5 g of anhydrous NaOAc was added to the brilliant yellow solution; refluxing under nitrogen was continued for 3 hr. The solution was cooled and evaporated to dryness *in vacuo*, and the residue was extracted with Et_2O and filtered. The Et_2O was removed *in vacuo*, and the residual oil was dissolved in Me_2CO to give the solid **3** (0.5 g): mp 171–174° (EtOH); $[\alpha]^{25}D$ – 12.6° (*c* 0.350, $CHCl_3$); ir ($CHCl_3$) 1760 cm^{-1} (lactone); for nmr ($CDCl_3$) see Figure 1.

Anal. Calcd for $C_{26}H_{38}O_7$: C, 67.59; H, 8.29. Found: C, 67.57; H, 8.28.

17β-Acetoxy-6′-(1′H)-oxo-5α-androstano[3,2-*c*]pyridazine (6).—A solution of 0.5 g (0.0011 mol) of **3** in EtOH (50 ml) was treated with 0.5 ml of 85% hydrazine hydrate and refluxed for 3 hr. The solvent was evaporated *in vacuo*, and the residue was recrystallized from absolute EtOH (0.38 g): mp 295–299°; $[\alpha]^{20}D$ 38.5° (*c* 0.52, $CHCl_3$).

Anal. Calcd for $C_{23}H_{32}N_2O_3$: C, 71.84; H, 8.39; N, 7.29. Found: C, 71.55; H, 8.65; N, 7.27.

The ultraviolet spectrum, λ_{max} (EtOH) 295 mμ (ϵ 2062), was typical of pyridazones.[9]

3ϵ-Ethoxy-17β-hydroxy-5′(2′H)-oxo-5α-androstano[3,2-*b*]furan (4).—A solution containing 0.5 g (0.013 mol) of the hydroxy acid **1** in 50 ml of EtOH was acidified with 5 drops of ethanolic HCl and refluxed overnight. The product was chromatographed on 30 g of Florisil with Et_2O. Elution with this solvent gave 200 mg of **4** as a foam which crystallized from Me_2CO–hexane: mp

169–171°; ir ($CHCl_3$) 1770, 1670 cm^{-1}; uv λ_{max} (EtOH) 215 mμ (ε 13,700); $[\alpha]^{20}D$ −73.8° (*c* 1.35, $CHCl_3$).

Anal. Calcd for $C_{23}H_{34}O_4$: C, 73.76; H, 9.5. Found: C, 73.85; H, 9.23.

Allowing this material to stand in moist Me_2CO caused gradual precipitation of the crystalline product **5**: mp 246–250°; $[\alpha]^{25}D$ −123.4° (*c* 0.95); ir (Nujol) 1750 cm^{-1}; uv λ_{max} (EtOH) 217 mμ (ε 13,350).

Anal. Calcd for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.57; H, 8.76.

17β-Hydroxy-3-oxo-5α-androstan-$\Delta^{2,\alpha}$-acetic Acid (7).—A solution of 5.4 g (0.02 mol) of 5α-androstanolone in 100 ml of MeOH and 100 ml of H_2O was treated with 1.6 g of NaOH followed by 7.4 g of commercial 40% glyoxylic acid solution (Eastman Kodak) in 50 ml of MeOH. The milky suspension cleared upon addition of the glyoxylic acid and gradually a gelatinous precipitate formed. The mixture was refluxed for 3 hr. The cooled solution was diluted with H_2O and extracted with Et_2O to remove unreacted starting material. The aqueous layer was acidified to pH 5 with a glacial AcOH and the residual Et_2O was removed by bubbling in a nitrogen stream. A white granular precipitate formed rapidly after most of the residual Et_2O had been removed. The precipitate (5 g) was collected and recrystallized: mp 221–223° dec (MeOH–H_2O); uv λ_{max} (EtOH) 240 (ε 10,000) and 230 mμ (shoulder). The nmr spectrum showed olefinic absorption at δ 6.30; $[\alpha]^{25}D$ 114.3° (*c* 1.05, EtOH).

Anal. Calcd for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.90; H, 8.90.

Sodium Borohydride Reduction of 7.—A solution of **7** (0.5 g) in 15 ml of absolute MeOH was cooled to 0° and treated with 15 ml of an aqueous solution of 0.3 g of $NaBH_4$. The ice bath was removed after 30 min, and stirring was continued for 30 min at room temperature. The reaction was refluxed for 30 min and then cooled. After the dropwise addition of 25 ml of 25% NaOH solution, the solvent was removed *in vacuo* and the residual solid was slurried with H_2O. Acidification of the alkaline mixture gave a white precipitate of **8** which was crystallized from MeOH (0.35 g): mp 278–282° dec; uv λ_{max} (EtOH) 220 mμ (ε 20,000); $[\alpha]^{25}D$ −3.6° (*c* 0.684, $CHCl_3$); nmr (DMSO) δ 5.88 (olefinic proton).

Anal. Calcd for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.02; H, 9.53.

This dihydroxy acid was further characterized by conversion into its diacetate by dissolving 0.3 g in 30 ml of 1:1 pyridine–Ac_2O and allowing the solution to stand overnight at room temperature. Water (20 ml) was added very carefully with cooling, and the solution was heated for 2 hr on a steam bath and added to 100 ml of ice–water. The precipitate **9** was collected and recrystallized from MeOH (0.25 g): mp 206–210°; $[\alpha]^{25}D$ −2.90° (*c* 0.831, $CHCl_3$); uv λ_{max} (EtOH) 222 mμ (ε 20,000).

Anal. Calcd for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.41; H, 8.45.

3β,17β-Dihydroxy-5α-androstan-2β-acetic Acid 3,17-Diacetate (10).—A solution of 10.0 g of **9** in 250 ml of HOAc containing 1.0 g of PtO_2 was hydrogenated in a Parr shaker until the rate of hydrogenation diminished to a low level. The product was isolated, and its uv spectrum showed 55% starting material still remaining. This material was diluted with HOAc (250 ml), fresh catalyst was added (0.6 g), and the mixture was hydrogenated at atmospheric pressure until the level of starting material was reduced to 10–15% (assayed by uv). The product was isolated as an oil, dissolved in 3:1 petroleum ether (30–60°)–Et_2O, and chilled. An amorphous solid was precipitated (2.33 g) which was recrystallized from Me_2CO–petroleum ether to give a crystalline solid **10**, mp 220–224°, which was identical with 3β,17β-dihydroxy-5α-androstan-2β-acetic acid 3,17-diacetate obtained by us in another study.[14] The mother liquors were concentrated further to give an amorphous solid, mp 103–107° (7.3 g). When a sample of the latter was hydrolyzed and lactonized (see accompanying procedure for preparation of **12**), it was judged to be a mixture of epimers at C-2. The 2β isomer was present in approximately 60–70% as indicated by the nmr signal of the C-19 methyl group; the chemical shifts of the two epimers occur at 54 and 58 cps, with the latter signal predominating slightly. Attempts at purifying this mixture further were not successful.

3β,17β-Dihydroxy-5α-androstan-2β-acetic Acid (11).—A solution of **10** (2 g, 0.46 mmol) was dissolved in 270 ml of H_2O and 30 ml of 2 *N* methanolic KOH. After standing for 4 hr at room temperature, the reaction mixture was concentrated to half volume and extracted with three 100-ml portions of Et_2O. The extract was dried over $MgSO_4$ and evaporated to give 43 mg of starting material. The basic layer was acidified to pH 2 and extracted with Et_2O. The dried extract was evaporated to give 1.51 g of **11** as a white solid which was recrystallized from Me_2CO–petroleum ether: mp 177–180° (resolidifies, melts at 202–204°).

Anal. Calcd for $C_{21}H_{34}O_4$: C, 71.96; H, 9.78. Found: C, 72.06; H, 9.71.

Lactonization of 3β,17β-Dihydroxy-5α-androstan-2β-acetic Acid (11).—A solution of 0.68 g of **11** in 75 ml of C_6H_6 and 50 mg of *p*-toluenesulfonic acid monohydrate was refluxed for 4 hr until tlc showed no starting material remaining. The solution was cooled and more C_6H_6 was added. After washing with saturated $NaHCO_3$ solution and drying over $MgSO_4$, the solvent was evaporated to dryness and the resulting oil was crystallized from MeOH to give lactone **12** as a white solid (0.7 g): mp 199–201° (MeOH); ir ($CHCl_3$) 1750 cm^{-1}.

Anal. Calcd for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.58; H, 9.54.

Registry No.—Glyoxylic acid, 298-12-4.

Acknowledgment.—The authors are indebted to Drs. E. Farkas and R. T. Rapala for their timely advice and criticisms during this work, as well as to the members of the physicochemical and microanalytical sections of the Lilly Laboratories for technical support.

(14) M. Debono and R. M. Molloy, *J. Org. Chem.*, in press.

N-Pyruvoylanthranilic Acid. Evidence against a Cyclol Structure[1]

PAUL F. WEGFAHRT AND HENRY RAPOPORT

Department of Chemistry, University of California at Berkeley, Berkeley, California 94720

Received February 20, 1969

N-Pyruvoylanthranilic acid and several of its derivatives, metabolites of a number of microorganisms, were examined for the presence of a cyclol form in solution. The nmr spectra in several solvents failed to provide evidence for such a form, although a hemiketal was observed in protic solvents. Selective exchange with $H_2{}^{18}O$ at the ketone carbonyl, followed by cyclization to 1-acetyl-3-methylene-4,1-benzoxazepine-2,5-dione and subsequent ^{18}O analysis, showed complete retention of the isotope label. The previously[2] postulated cyclol intermediate, invoked to explain the nmr spectrum and formation of the benzoxazepine, is inconsistent with this evidence. An alternate mechanism for the formation of benzoxazepine is proposed.

Recent investigations of bacterial and mold metabolites have uncovered several derivatives of N-pyruvoylanthranilic acid (**1a**). In *Aerobacter aerogenes*, **1a** has been proposed as an intermediate in the biosynthesis of anthranilic acid.[3] Fermentations of *Pencillium chrysogenum* and *P. notatum* were found to produce N-pyruvoylanthranilamide (**1b**).[4] A related compound, 2-(3-hydroxy-3-phenylpyruvoylamino)-N-methylben-

(1) Supported in part by the U. S. Army Research Office, Durham, N. C.
(2) F. Lingens and B. Sprössler, *Ann.*, **702**, 169 (1967).
(3) C. Ratledge, *Nature*, **203**, 428 (1964).
(4) P. J. Suter and W. B. Turner, *J. Chem. Soc., C*, 2240 (1967).