(3-Iodobenzoyl)-*n*-pentylhydrazone (3c): mp 109–111 C; from 1:4 THF/petroleum ether (35–60 °C), 69% yield; IR 3200 (NH), 1645 cm^{-1} (C=O). Anal. Calcd for $C_{12}H_{15}IN_2O$: C, 43.65; H, 4.58; N, 8.49. Found: C, 43.78; H, 4.40; N, 8.63.

Doxorubicin (3-iodobenzoyl)hydrazone (4) was prepared by the general method of Cory[15] by condensation of 20 mg (0.035 mmol) of doxorubicin hydrochloride, 9.0 mg (0.035 mmol) of 3-iodobenzoyl hydrazide, and 8 mL of methanol stirred vigorously for 5 days in a light-shielded flask. The reaction mixture was filtered, evaporated to dryness in vacuo, redissolved in 50 mL of 1:4 methanol/acetonitrile, and chilled to precipitate 18.7 mg (65% yield) of dark orange crystals: mp 185–188 °C, of **4**; MS (FAB) M^+ 787 amu. A TLC analysis on Analtech preabsorbant silica plates using 20:10:1 chloroform/methanol/water as eluant and the standard pure reactants and **4** for calibration gave R_f 0.30 for doxorubicin hydrochloride, R_f 0.42 for **2**, and R_f 0.91 for 3-iodobenzoyl hydrazide. Anal. Calcd for $C_{34}H_{34}IN_3O_{11}\cdot HCl\cdot 2H_2O$: C, 47.48; H, 4.57; N, 4.91. Found: C, 47.13; H, 4.26; N, 5.37.

[^{125}I]-3-Iodobenzoyl Hydrazide. A 2-mL pointed vial was charged with 4.50 mCi of $Na^{125}I$ dissolved in 0.45 mL of distilled water, 5.0 mg (21 μmol) of **2**, 0.45 mL of tetrahydrofuran, and a micro stirring bar. The vial was sealed with a fluoropolymer-lined cap and a solution of 3.5 μL of trifluoroacetic acid in 0.1 mL of water injected by syringe through the cap.

The vial was heated in an oil bath at 100 °C for 1 h, treated to a supplemental addition of 9.5 μL of trifluoroacetic acid in 0.1 mL of distilled water, and heated for an additional 1.5 h. The product was cooled and the product isolated by addition of 0.2 mL of saturated aqueous sodium thiosulfate, pH adjustment to 10 with 1 N NaOH and extraction with 3 × 0.2 mL of ethyl acetate. The organic layer was washed once with 0.2 mL of water, dried over $MgSO_4$, and evaporated in a stream of dry nitrogen, and the residue was taken up in 0.1 mL of acetonitrile. Purified (by HPLC, radiation detection), carrier-free ^{125}I-3-iodobenzoyl hydrazide was obtained by collection of the peak eluting at 6.5 min (time at peak maximum) from a 20 cm, C-18 reverse-phase analytical column operated at a flow rate of 1 mL/min with an eluant of 7:3 acetonitrile/water. Retention time (and volume) matched that of authentic [^{127}I]3-iodobenzoyl hydrazide. Radiochemical purity was also confirmed by TLC on silica plates with ethyl acetate eluant whereon the single spot (R_f 0.24) was coincident with that observed for authentic product. A total of 0.54 mCi, 12% radiochemical yield, of [^{125}I]3-iodobenzoyl hydrazide was obtained. The product peak represented 95% of the total radioactivity eluted from the chromatograph.

([^{125}I]-3-Iodobenzoyl)(4-carboxyphenyl)hydrazone (3a). A solution of 0.5 mg of 4-carboxybenzaldehyde, 0.1 mCi of [^{125}I]-3-iodobenzoyl hydrazide, 0.3 mL of methanol, and 1 μL of 0.05 N HCl was refluxed for 3 h, evaporated under nitrogen stream, and the resulting residue taken up in 0.3 mL of water. The aqueous solution was adjusted to pH 10 with 1 N NaOH, extracted with 3 × 0.2 mL of ethyl acetate, and the organic extracts were discarded. The pH of the aqueous phase was then adjusted to 5 with 0.5 N HCl and extracted with 3 × 0.2 mL of ethyl acetate. The product was isolated by preparative TLC (reverse phase C-18, methanol eluant) of the dried ($MgSO_4$) and evaporated organic phase. A single radioactive fraction (R_f 0.73), 58 μCi, 58% radiochemical yield based on [^{125}I]-3-iodobenzoyl hydrazide eluted coincident with authentic starting material.

([^{125}I]-3-Iodobenzoyl)(4-(dimethylamino)phenyl)-hydrazone (3b) was prepared by reaction of 0.1 mCi of [^{125}I]-3-iodobenzoyl hydrazide, 0.5 mg of 4-(dimethylamino)benzaldehyde, 0.3 mL of methanol, and 1 μL of 0.05 N HCl over 3-h reflux. The cooled solution was adjusted to pH 10 with 1 N NaOH, extracted with 3 × 0.2 mL of ethyl acetate, and the dried ($MgSO_4$), evaporated extracts purified by preparative TLC (silica plates, 1:1 ethyl acetate/methylene chloride) to give 72 μCi of a single radioactive component (R_f 0.61) coincident in elution with authentic unlabeled material. Radiochemical yield was 72% calculated on [^{125}I]-3-iodobenzoyl hydrazide.

([^{125}I]-3-Iodobenzoyl)-*n*-pentylhydrazone (3c) was prepared from *n*-pentanal in a manner identical with that described above for **3b**. Preparative TLC afforded 65 μCi (65% radiochemical yield from the [^{125}I]-3-iodobenzoyl hydrazide) of **3c** migrating as a single radioactive spot coincident with authentic nonradioactive material (silica plate, diethyl ether eluant).

Dioxorubicin ([^{125}I]-3-iodobenzoyl)hydrazone ([^{125}I]-4) was prepared by 5 days of vigorous agitation in a light shielded pointed reactor vial of 0.10 mCi of [^{125}I]-3-iodobenzoyl hydrazide, 1.0 mg of doxorubicin hydrochloride, and 0.3 mL of methanol. Purification of the evaporated reaction mixture was accomplished on preparative TLC using 20:10:1 chloroform/methanol/water to yield 68 μCi, radiochemical yield 68% from I-125 3-iodobenzoyl hydrazide, with R_f 0.39, coincident with authentic **4**.

Acknowledgment. This study was supported by grants from the Milheim Foundation, the Elsa U. Pardee Foundation, and DHHS (No. CA31245).

Registry No. **1**, 31822-06-7; **2**, 96096-01-4; **3a**, 96096-02-5; **3b**, 96096-03-6; **3c**, 96096-04-7; **4**, 96096-05-8; **4** (^{125}I), 96096-07-0; $CH(OEt)_3$, 122-51-0; *m*-$H_2NC_6H_4\overline{C=NN=CHO}$. 5378-35-8; *m*-$IC_6H_4CONHNH_2$, 39115-94-1; *m*-$^{125}IC_6H_4CONHNH_2$, 96096-06-9; *p*-$CO_2HC_6H_4CHO$, 619-66-9; *p*-$Me_2NC_6H_4CHO$, 100-10-7; $CH_3(CH_2)_4CHO$, 66-25-1; pyrrolidine, 123-75-1; doxorubicin hydrochloride, 25316-40-9.

A Novel Approach to Cardenolides

Wolfram Harnisch,[1] Enrico Morera, and Giorgio Ortar*

Centro di Studio per la Chimica del Farmaco del C.N.R., Istituto di Chimica Farmaceutica dell' Universitâ, 00185 Roma, Italy

Received October 17, 1984

Most of the work that has been carried out over past years aimed at cardenolide synthesis has utilized pregnan-20-one derivatives as starting materials.[2]

Recent availability of efficient microbiological methods for the production of 17-oxo steroids[3] has, however, revived interest in the conversion of these intermediates into cardenolides.[4]

In this note we report a simple four-step synthesis of the cardenolides **6a** and **6b** from 3β-hydroxy-5α-androstan-17-one acetate (**1a**) and its 5-epimer **1b**, respectively.

The conversion of the cardenolide **6b** into digitoxigenin (**6c**) has already been described.[2c]

Compounds **1a** and **1b** have been transformed into 17-enol trifluoromethanesulfonates (enol triflates) **2a** and **2b** with triflic anhydride and 2,6-di-*tert*-butyl-4-methylpyridine according to the procedure of Stang[5] in 55% and 46% yields, respectively.

(1) On leave from Pharmazeutisches Institut, ETH Zentrum, CH-8092 Zürich.

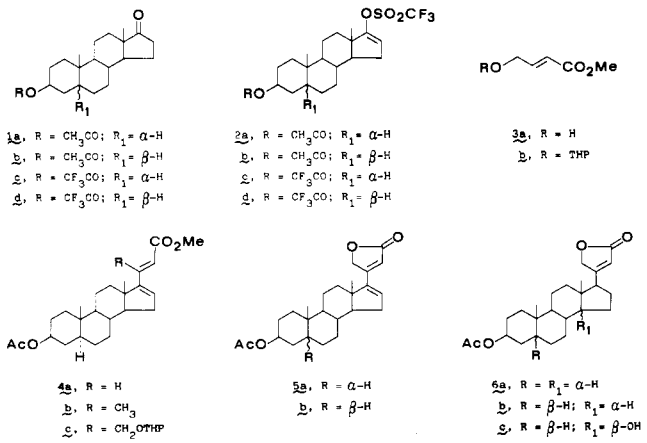
(2) For some recent syntheses from 20-oxo steroids, see: (a) Welzel, P.; Stein, H.; Milkova, T. *Liebigs Ann. Chem.* **1982**, 2119. (b) Nickisch, K.; Kose, W.; Bohlmann, F. *Chem. Ber.* **1980**, *113*, 2038. (c) Donovan, S. F.; Avery, M. A.; McMurry, J. E. *Tetrahedron Lett.* **1979**, 3287. (d) Lenz, G. R.; Schulz, J. A. *J. Org. Chem.* **1978**, *43*, 2334. For reviews on cardenolides, see: Gorovitz, M. B.; Abubakirov, N. K. *Khim. Prir. Soedin.* **1978**, 283. Thomas, T.; Boutagy, J.; Gelbert, A. *J. Pharm. Sci.* **1974**, *63*, 1648. Rao, Y. S. *Chem. Rev.* **1976**, *76*, 625.

(3) Wovcha, M. G.; Antosz, F. J.; Knigt, J. C.; Kominek, L. A.; Pyke, T. R. *Biochim. Biophys. Acta* **1978**, *531*, 308. Eder, U.; Sauer, G.; Haffer, G.; Neef, G.; Wiechert, R.; Weber, A.; Popper, A.; Kennecke, M.; Mueller, R. Ger. Offen. 2534911, 1977; *Chem. Abstr.* **1977**, *86*, 187659n.

(4) (a) Kabat, M. M.; Kurek, A.; Wicha, J. *J. Org. Chem.* **1983**, *48*, 4248. (b) Wicha, J.; Kabat, M. M. *J. Chem. Soc. Chem. Commun.* **1983**, 985. (c) Sen, A.; Jaggi, F. J.; Tsai, T. Y. R.; Wiesner, K. *J. Chem. Soc., Chem. Commun.* **1982**, 1213. (d) Wiesner, K.; Tsai, T. Y. R.; Jaggi, F. J.; Tsai, C. S. J.; Gray, G. D. *Helv. Chim. Acta* **1982**, *65*, 2049. (e) Kurek, A.; Gumulka, M.; Wicha, J. *J. Chem. Soc., Chem. Commun.* **1981**, 25.

(5) Stang, P. J.; Treptow, W. *Synthesis* **1980**, 283. Stang, P. J.; Fisk, T. E. *Synthesis* **1979**, 438. For a review on enol triflates, see: Stang, P. J.; Hanack, M.; Subramanian, L. R. *Synthesis* **1982**, 85.

0022-3263/85/1950-1990$01.50/0



Improved values (71% and 76% overall yields, respectively) could be obtained by using trifluoroacetates **1c** and **1d** in the triflating step, then removing the trifluoroacetate group with potassium carbonate, and finally reacetylating the resulting alcohols.

It may be of interest that the triflation reaction on 3β-methoxy-5α-androstan-17-one gave distinctly lower yields of the corresponding 17-enol triflate (38%). Thus, the trifluoroacetate group appears to be, as far as we investigated, a valuable protecting group of the alcoholic function in the preparation of enol triflates from keto alcohols.

Enol triflate **2a** reacted with (*E*)-3-(methoxycarbonyl)-2-propenyl tetrahydropyranyl ether (**3b**) in the presence of tributylamine and a palladium acetate–triphenylphosphine catalyst to give the coupling product **4c** in 40% yield.

No reaction occurred when the unprotected hydroxybutenoate **3a** was used. Equally ineffective were attempts to couple the enol triflate **2a** with 2(5*H*)-furanone, directly.

The palladium-catalyzed vinylation of enol triflates has been recently described by us.[6] Among other examples, enol triflate **2a** has been found to afford the conjugated ester **4a** in 82% yield, when coupled with methyl acrylate.

An inevitable decrease in the yield with increasing size of substituents around the double bond of the α,β-unsaturated ester was thus evident.

Indeed, the palladium-catalyzed reaction of compound **2a** with methyl crotonate afforded the ester **4b** in an intermediate yield (55%).

The depicted configuration of the 20(22)-double bond in the esters **4b,c** was expected on the basis of the stereochemical outcome of related palladium-catalyzed vinylations of vinyl halides[7] and was indirectly confirmed by the clean acid-catalyzed conversion of compound **4c** to the carda-16,20(22)-dienolide **5a** (90% yield). Moreover, compound **4b** showed a downfield shift for the 21-Me group (2.28 ppm) which correlates well with the anisotropic effect of the cis 23-carboxylate group.[8]

Experimentally, we found it more advantageous to perform the cyclization step directly on the mixture containing compound **4c** because of the difficulties experienced in separating it from unreacted hydroxybutenoate derivative **3b** by conventional chromatography. In this way, a 40% yield of the cardenolide **5a** was obtained.

Repetition of the procedure on the enol triflate **2b** afforded the cardenolide **5b** in 37% overall yield.

Hydrogenation of compounds **5a** and **5b** with 5% palladium on charcoal in ethyl acetate was stereoselective and led to the target substances **6a** and **6b**, respectively, in nearly quantitative yield.

The cardenolides **5a**, **6a**, and **6b**, exhibited melting points and optical rotations in agreement with those reported in literature[9] and showed together with product **5b** spectral properties consistent with the proposed structures.

In conclusion, although yields are only moderate, palladium-catalyzed coupling of 17-enol triflates with appropriate, α,β-unsaturated esters represent a simple route to cardenolides and cardenolide analogues.[8]

Experimental Section

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured at 20 °C with a Schmidt-Haensch Polartronic D polarimeter (1-dm cell) in 1% $CHCl_3$ solutions. The UV spectra were recorded in EtOH solutions, IR spectra as KBr disks, and ^1H NMR spectra in $CDCl_3$ solutions, with Me_4Si as internal standard.

General Procedure for Preparation of 17-Enol Triflates 2a–d. 3β-Acetoxy-5α-androst-16-en-17-yl Triflate (2a). Utilizing the procedure of Stang et al.,[5] a solution of **1a** (1.66 g, 5 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (1.44 g, 7 mmol) in dry dichloromethane (25 mL) was treated with triflic anhydride (0.98 mL, 6 mmol) at 0 °C and stirred at room temperature for 4 h. The solvent was removed in vacuo and the residue combined with ether. The solid was filtered off and washed with additional ether. The ethereal solution was washed with 2 N HCl, and then water until neutral, dried (Na_2SO_4), and evaporated. Chromatography of the residue (2.39 g) on silica gel (72 g) with hexane–dichloromethane (1:1) as eluent gave 1.27 g (55%) of **2a**: mp 82–83 °C (from methanol); $[\alpha]_D$ +7°; IR 1731 (C=O), 1632 (C=C), 1422, 1205, 1146 (OSO_2) cm^{-1}; ^1H NMR δ 0.83 (3 H, s, 10-Me), 0.93 (3 H, s, 13-Me), 1.99 (3 H, s, 3 β-OAc), 4.7 (1 H, m, 3α-H), 5.58 (1 H, m, C-16 H).

Anal. Calcd for $C_{22}H_{31}F_3O_5S$ (464.5): C, 56.88; H, 6.73. Found: C, 56.84; H, 6.77.

The following enol triflates were prepared and isolated in a similar manner from the corresponding ketones **1b**,[4a] **1c**,[10] and **1d**.[10]

3β-Acetoxy-5β-androst-16-en-17-yl triflate (2b, 46%): mp 91–92 °C (from methanol); $[\alpha]_D$ +18°; IR 1723 (C=O), 1627 (C=C), 1421, 1210, 1147 (OSO_2) cm^{-1}; ^1H NMR δ 0.93 (3 H, s, 13-Me), 0.97 (3 H, s, 10-Me), 2.01 (3 H, s, 3β-OAc), 5.11 (1 H, m, 3α-H), 5.60 (1 H, m, C-16 H).

Anal. Calcd for $C_{22}H_{31}F_3O_5S$ (464.5): C, 56.88; H, 6.73. Found: C, 56.92; H, 6.87.

3β-(Trifluoroacetoxy)-5α-androst-16-en-17-yl triflate (2c, 74%): mp 121–121.5 °C (from hexane); $[\alpha]_D$ +8°; IR 1787 (C=O), 1623 (C=C), 1404, 1225, 1162 (OSO_2) cm^{-1}; ^1H NMR δ 0.87 (3 H, s, 10-Me), 0.94 (3 H, s, 13-Me), 4.9 (1 H, m, 3α-H), 5.58 (1 H, m, C-16 H).

Anal. Calcd for $C_{22}H_{28}F_6O_5S$ (518.5): C, 50.96; H, 5.44. Found: C, 51.04; H, 5.56.

3β-(Trifluoroacetoxy)-5β-androst-16-en-17-yl triflate (2d, 80%): mp 105.5–106.5 °C (from hexane); $[\alpha]_D$ +16°; IR 1783 (C=O), 1626 (C=C), 1418, 1208, 1146 (OSO_2) cm^{-1}; ^1H NMR δ 0.95 (3 H, s, 13-Me), 1.00 (3 H, s, 10-Me), 5.33 (1 H, m, 3α-H), 5.60 (1 H, m, C-16 H).

Anal. Calcd for $C_{22}H_{28}F_6O_5S$ (518.5): C, 50.96; H, 5.44. Found: C, 50.99; H, 5.38.

Compound **2c** (166 mg, 0.32 mmol) was dissolved in CH_2Cl_2 (2 mL) and MeOH (8 mL), K_2CO_3 (220 mg, 1.6 mmol) in 0.7 mL of H_2O was added, and the mixture was stirred vigorously for 0.5 h at room temperature. The 3β-hydroxy derivative, isolated with CH_2Cl_2, was acetylated in pyridine (0.64 mL) with acetic anhydride (0.32 mL) to give **2a** (142 mg, 96% overall yield). In the same fashion, **2b** was obtained from **2d** in 95% yield.

(6) Cacchi, S.; Morera, E.; Ortar, G. *Tetrahedron Lett.* **1984**, *25*, 2271.
(7) Heck, R. F. *Org. React. (N.Y.)* **1982**, *27*, 345.
(8) Theil, F.; Lindig, C.; Repke, K. *J. Prakt. Chem.* **1980**, *322*, 1012.
(9) (a) Ruzicka, L.; Plattner, P. A.; Heusser, H. *Helv. Chim. Acta* **1946**, *29*, 473. (b) Ruzicka, L.; Plattner, P. A.; Fürst, A. *Helv. Chim. Acta* **1942**, *25*, 79. (c) Hauser, E.; Linde, H.; Meyer, K. *Helv. Chim. Acta* **1966**, *49*, 1212.
(10) Prepared from the corresponding alcohols by treatment with pyridine–trifluoroacetic anhydride.

(*E*)-3-(Methoxycarbonyl)-2-propenyl Tetrahydropyranyl Ether (3b). A solution of **3a**[11] (2.32 g, 20 mmol) and dihydropyran (1.82 mL, 20 mmol) containing 1 drop of concentrated HCl was stirred for 2 h at room temperature. Ether was added, and the organic phase was washed with saturated $NaHCO_3$ and water and dried (Na_2SO_4). Evaporation of the solvent and bulb-to-bulb distillation of the residue (0.1 mmHg, 80–85 °C, oven temperature) furnished 3.35 g (84%) of **3b**: UV λ_{max} 207 nm (ϵ 10900); ^{1}H NMR δ 1.3–2.0 (6 H, m, THP), 3.3–4.0 (2 H, m, THP), 3.73 (3 H, s, CO_2Me), 4.13 and 4.43 (2 H, ddd, J = 16.5, 4.5, and 1.5 Hz, C-1 H_2), 4.67 (1 H, m, THP), 6.13 (1 H, dt, J = 16.5 and 1.5 Hz, C-3 H), 7.03 (1 H, dt, J = 16.5 and 4.5 Hz, C-2 H).

Anal. Calcd for $C_{10}H_{16}O_4$ (200.2): C, 59.98; H, 8.05. Found: C, 60.00; H, 8.15.

Methyl (*Z*)-3β-Acetoxy-21-[(tetrahydropyranyl)oxy]-24-nor-5α-chola-16,20(22)-dien-23-oate (4c). A mixture of **2a** (0.46 g, 1 mmol), **3b** (0.30 g, 1.5 mmol), tributylamine (0.48 mL, 2 mmol), palladium acetate (11 mg, 0.05 mmol), and triphenylphosphine (26 mg, 0.10 mmol) in DMF (4 mL) was stirred at 80 °C for 24 h, under nitrogen. The reaction was then diluted with water, extracted with ether, washed with 2 N HCl and then water until neutral, dried (Na_2SO_4), and evaporated. Chromatography of the residue (0.63 g) on deactivated (grade II) Woelm neutral alumina (32 g) using benzene as eluent and further purification of the main fractions (0.27 g) on silica gel (14 g) with benzene–ethyl acetate (97:3) as eluent gave 204 mg (40%) of **4c**: mp 105–106 °C (from hexane); $[\alpha]_D$ −6°; UV λ_{max} 279 nm (ϵ 12900); IR 1727 (C=O), 1613 (C=C) cm^{-1}; ^{1}H NMR δ 0.83 (3 H, s, 10-Me), 0.93 (3 H, s, 13-Me), 1.98 (3 H, s, 3β-OAc), 3.4–4.1 (2 H, m, THP), 3.69 (3 H, s, CO_2Me), 4.5–5.0 (4 H, m, THP, C-21 H_2, and 3α-H), 6.00 (1 H, s, C-22 H), 6.28 (1 H, m, C-16 H).

Anal. Calcd for $C_{31}H_{46}O_6$ (514.7): C, 72.34; H, 9.01. Found: C, 72.28; H, 9.13.

3β-Hydroxy-5α,14α-carda-16,20(22)-dienolide Acetate (5a). A mixture of **4c** (103 mg, 0.2 mmol) and Dowex-50W × 8 resin (200–400 mesh, H^+ form, 100 mg) in CH_2Cl_2 (1 mL) and MeOH (4 mL) was stirred at 45–50 °C for 2 h. Filtration of the resin and evaporation of the solvent furnished 72 mg (90%) of almost pure **5a**: mp 235.5–237 °C (from methanol); $[\alpha]_D$ +29° (lit.[9a] mp 235–238 °C; $[\alpha]_D$ +37°); UV λ_{max} 272 nm (ϵ 16800); IR 1783, 1744 (lactone C=O), 1730 (acetate C=O), 1614 (C=C) cm^{-1}; ^{1}H NMR δ 0.85 (3 H, s, 10-Me), 0.92 (3 H, s, 13-Me), 2.00 (3 H, s, 3β-OAc), 4.7 (1 H, m, 3α-H), 4.95 (2 H, br s, C-21 H_2), 5.97 (1 H, br s, C-22 H), 6.18 (1 H, m, C-16 H).

The procedure was repeated on the residue (0.56 g) of the palladium-catalyzed coupling, directly. The new residue (0.47 g) was chromatographed on silica gel (23 g); elution with benzene–ethyl acetate (97:3) afforded 158 mg (40%) of **5a**.

Repetition of the coupling–cyclization sequence on **2b** in the same conditions as used for **2a** gave 147 mg (37%) of **3β-hydroxy-5β,14α-carda-16,20(22)-dienolide acetate (5b)**: mp 227–229 °C (from acetone–hexane); $[\alpha]_D$ +46°; UV λ_{max} 272 nm (ϵ 17800); IR 1785 (lactone C=O), 1741 (lactone and acetate C=O), 1610 (C=C) cm^{-1}; ^{1}H NMR δ 0.92 (3 H, s, 13-Me), 1.00 (3 H, s, 10-Me), 2.02 (3 H, s, 3β-OAc), 4.93 (2 H, br s, C-21 H_2), 5.10 (1 H, m, 3α-H), 5.97 (1 H, br s, C-22 H), 6.17 (1 H, m, C-16 H).

Anal. Calcd for $C_{25}H_{34}O_4$ (398.5): C, 75.34; H, 8.60. Found: C, 75.21; H, 8.50.

3β-Hydroxy-5α,14α-card-20(22)-enolide Acetate (6a). **5a** (100 mg) in ethyl acetate (20 mL) was hydrogenated over 5% palladium on charcoal (20 mg) at room temperature and atmospheric pressure for 3 h. Filtration and evaporation gave 100 mg (99%) of **6a**: mp 191–192.5 °C (from methanol); $[\alpha]_D$ −1° (lit.[9b] mp 193–194 °C; $[\alpha]_D$ −1°); UV λ_{max} 219 nm (ϵ 12600); IR 1785, 1741 (lactone C=O), 1739 (acetate C=O), 1622 (C=C) cm^{-1}; ^{1}H NMR δ 0.60 (3 H, s, 13-Me), 0.82 (3 H, s, 10-Me), 2.00 (3 H, s, 3β-OAc), 4.7 (1 H, m, 3α-H), 4.65 and 4.87 (2 H, AB q, J = 18 Hz, C-21 H_2), 5.85 (br s, 1 H, C-22 H).

Hydrogenation of **5b** in the same conditions gave **3β-hydroxy-5β,14α-card-20(22)-enolide acetate (6b)**: mp 194–195 °C (from acetone–hexane); $[\alpha]_D$ +15° (lit.[9c] mp 195–197 °C; $[\alpha]_D$ +12°); UV λ_{max} 219 nm (ϵ 13800); IR 1791, 1754 (lactone C=O), 1727 (acetate C=O), 1631 (C=C) cm^{-1}; ^{1}H NMR δ 0.60 (3 H, s, 13-Me), 0.95 (3 H, s, 10-Me), 2.02 (3 H, s, 3β-OAc), 4.63 and 4.85 (2 H, AB q, J = 18 Hz, C-21 H_2), 5.10 (1 H, m, 3α-H), 5.83 (1 H, br s, C-22 H).

Methyl (*E*)-3β-Acetoxy-24-nor-5α-chola-16,20(22)-dien-23-oate (4b). A mixture of **2a** (0.46 g, 1 mmol), methyl crotonate (0.21 mL, 2 mmol), tributylamine (0.48 mL, 2 mmol), palladium acetate (11 mg, 0.05 mmol), and triphenylphosphine (26 mg, 0.10 mmol) in DMF (4 mL) was stirred at 80 °C for 9 h. Residue from usual workup (0.42 g) was chromatographed on silica gel (32 g) with benzene–ethyl acetate (99:1) as eluent to afford 228 mg (55%) of **4b**: mp 136–137 °C (from methanol); $[\alpha]_D$ −4°; UV λ_{max} 275 nm (ϵ 15100); IR 1739 (C=O), 1612 (C=C) cm^{-1}; ^{1}H NMR δ 0.83 (3 H, s, 10-Me), 0.92 (3 H, s, 13-Me), 1.98 (3 H, s, 3β-OAc), 2.28 (3 H, s, 20-Me), 3.68 (3 H, s, CO_2Me), 4.7 (1 H, m, 3α-H), 5.93 (1 H, br s, C-22 H), 6.13 (1 H, m, C-16 H).

Anal. Calcd for $C_{26}H_{38}O_4$ (414.6): C, 75.32; H, 9.24. Found: C, 75.39; H, 9.30.

Registry No. **1a**, 1239-31-2; **1b**, 4820-41-1; **1c**, 3959-78-2; **1d**, 7557-85-9; **2a**, 91934-55-3; **2b**, 96150-02-6; **2c**, 96095-92-0; **2c** (R = H, R_1 = α-H), 96095-93-1; **2d**, 96095-94-2; **3a**, 29576-13-4; **3b**, 96095-95-3; **4b**, 96095-96-4; **4c**, 96095-97-5; **5a**, 96095-98-6; **5b**, 96150-03-7; **6a**, 3697-94-7; **6b**, 6564-57-4; triflic anhydride, 358-23-6; methyl (*E*)-crotonate, 623-43-8.

(11) Rambaud, R. *Bull. Soc. Chim. Fr.* **1934**, *1*, 1317.

Enantioselective Oxidation of 1,2-Diols to L-α-Hydroxy Acids Using Coimmobilized Alcohol and Aldehyde Dehydrogenases as Catalysts[1]

Chi-Huey Wong* and Jose R. Matos[2]

Department of Chemistry, Texas A&M University, College Station, Texas 77843

Received September 20, 1984

We have described before the biochemical aspects of enzyme catalysis with respect to the stereospecificity of the oxidation of 1,2-diols and α-amino alcohols catalyzed by alcohol dehydrogenases and the rationalization of the stereospecificity based on a cubic-space active-site section model.[3] Although both yeast and horse liver alcohol dehydrogenases were found to have the same enantioselectivity in the oxidations, the yeast enzyme was unstable and less active than the horse liver enzyme. In connection with our interest in developing practical enzymatic procedures for use in synthetic organic chemistry, we here report the preparation of several enantiomerically pure L-α-hydroxy acids from racemic 1,2-diols based on the stereospecificity of the oxidations observed,[3] in a process using coimmobilized alcohol dehydrogenase/aldehyde dehydrogenase as catalysts (Scheme I). As indicated in the scheme, the enzyme horse liver alcohol dehydrogenase (HLADH) catalyzes the enantioselective oxidation of a number of racemic 1,2-diols to L-α-hydroxy aldehydes which are further converted to L-α-hydroxy acids catalyzed by aldehyde dehydrogenase (AldDH). Both enzymatic reactions require NAD as a cofactor; a cofactor regeneration system is therefore incorporated into the synthetic scheme.

The α-hydroxy acids **3a–h** prepared here are useful in synthetic organic chemistry. L-α-Halolactic acid, for example, can be converted to L-glycidic acid for use as a synthon.[4] 3-Amino-2-hydroxypropionic acid has the

(1) This research was supported by the National Science Foundation Grant CHE-8318217 and the Robert A. Welch Foundation Grant A-1004. Part of this research was presented at the ACS National Meeting in Philadelphia on August 28, 1984.

(2) NSF Predoctoral Fellow.

(3) Matos, J. R.; Smith, M. B.; Wong, C.-H. *Bioorg. Chem.*, in press.

(4) Hirschbein, B. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1982**, *104*, 4458–4460.

0022-3263/85/1950-1992$01.50/0