SELECTIVE OXIDATION OF THE DOUBLE BONDS IN THE 4-PHENYL-1,2,4-TRIAZOLINE-3,5-DIONE DIELS-ALDER ADDUCT OF ERGOSTEROL ACETATE

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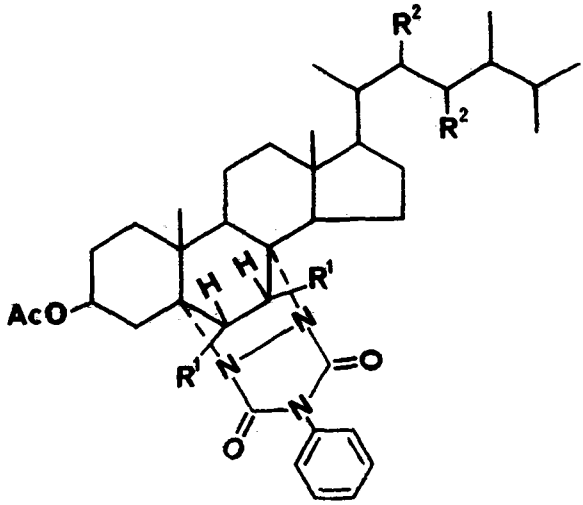
ABSTRACT

Methods for oxidations at the 6(7)- and 22(23)-double bonds in the phenyltriazoline adduct of ergosterol acetate (I) are described. $KMnO_4$ and OsO_4 were found to react with the 6(7)-double bond to yield the 6,7-glycol and osmate ester, respectively. Other reagents (I_2/AgOAc, H_2O_2, *m*-chloroperbenzoic acid, HCO_3H) formed either isomeric epoxides or glycols with the 22(23)-double bond, with the latter two reagents giving their products in quite high yields.

Ozonolysis of the heterocyclic adduct I has been reported (1) to be a convenient means of cleaving the side chain of ergosterol as a prerequisite step in a synthetic route to vitamin D_3 (cholecalciferol). The method, however, suffers from low yields and, in our hands, results in extensive cleavage of the 6(7)-double bond. In order to provide information on potential alternative procedures for ergosterol side chain cleavage, we undertook to test the reactivity of adduct I with various reagents. Reports in the literature (2,3) indicated *m*-chloroperbenzoic acid (*m*CPBA) to be effective at functionalizing the 22(23)-double bond although the product was not crystalline.

$KMnO_4$ was chosen for the initial experiment because of its availability, ease of use, and large size which might hinder its access to the 6(7)-double bond. The heterocyclic dienophile (4-phenyl-1,2,4-triazoline-3,5-dione) most likely adds on the α side of ring B and forces the (C-5)-(C-6)-(C-7)-(C-8) portion somewhat perpendicular to ring C and close to the C-18 and C-19 methyls. This distortion should provide limited accessibility to the ring B double bond and enhance reactions at the 22(23)-double bond of the side chain. Although a 3400 cm^{-1} ir absorption suggested that a glycol formed, the indication



I. R^1, R^1 = R^2, R^2 = double bond. 3β-acetoxy-4'-phenyl-5α, 8α-ergosta-6,22-dieno[5,8-a]1',2',4'-triazolidine-3',5'-dione.

II. R^1=OH; R^2,R^2 = double bond. 3β-acetoxy-6α, 7α-dihydroxy-4'-phenyl-5α, 8α-ergost-22-eno[5,8-a]1',2',4'-triazolidine-3',5'-dione.

III. R^1 = OAc; R^2, R^2 = double bond. 3β, 6α, 7α-triacetoxy-4'-phenyl-5α, 8α-ergost-22-eno[5,8-a]1',2',4'-triazolidine-3',5'-dione.

IV. R^1,R^1 = -O-OsO_2-O-; R^2, R^2 = double bond. 3β-acetoxy-6α, 7α-dihydroxy-4'-phenyl-5α, 8α-ergost-22-eno[5,8-a]1',2',4'-triazolidine-3',5'-dione 6,7-osmate ester.

V. R^1,R^1 = double bond; R^2 = OAc (threo). 3β, 22ξ, 23ξ-triacetoxy-4'-phenyl-5α, 8α-ergost-6-eno[5,8-a]1',2',4'-triazolidine-3',5'-dione.

VI. R^1, R^1 = double bond; R^2 = OH (erythro). 3β-acetoxy-22ξ, 23ξ-dihydroxy-4'-phenyl-5α,8α-ergost-6-eno[5,8-a]1',2',4'-triazolidine-3',5'-dione.

VII. R^1,R^1 = double bond; R^2 = OAc (erythro). 3β, 22ξ, 23ξ-triacetoxy-4'-phenyl-5α, 8α-ergost-6-eno[5,8-a]1',2', 4'-triazolidine-3', 5'-dione.

VIII. R^1, R^1 = double bond; R^2, R^2 = O. 3β-acetoxy-22ξ, 23ξ-epoxy-4'-phenyl-5α, 8α-ergost-6-eno[5,8-a]1',2',4'-triazolidine-3', 5'-dione.

that the 6α, 7α-glycol II was the product came from an nmr spectrum which was void of an AB quartet at δ6.21 and 6.40 for the C-6 and C-7 vinylic protons present in adduct I but retained the multiplet at δ5.1 for the C-22 and C-23 vinyl protons.

Further evidence for reaction at C-6 and C-7 was evident from a change in uv data (Table 1). Adduct I displays two fairly intense benzene bands at 215 and 255 nm, the latter enhanced more than usual by interaction between π electrons of the 6(7)-double bond with the phenylurazole ring system. If absorption maxima of each band are compared, an A_{215}/A_{255} ratio of about 3 is found. For glycol II as well as its triacetate III, however, the 255 nm band becomes greatly lessened owing to loss of the π system at C-6 and C-7 and the ratio increases.

Table 1. UV Data for Various Products

Compound	Major λ_{max} (nm)	A_{215}/A_{255}
I	215,255	2.86
II	215	15.4
III	215	18.3
IV	218,250,257,263,285	
V	215,255	2.90
VII	215,255	3.41
VIII	215,255	2.76

A Nuclear Overhauser Effect (NOE) study in which the C-18 and C-19 methyls of triacetate III were irradiated disclosed that the C-6 and C-7 protons were oriented within the proximity of these angular methyls since the AB quartet at δ5.40 and 5.52 was enhanced. Models indicate this occurs only if ring B is in a boat conformation and the glycol system is situated over the phenylurazole ring system. Also, attempts to reverse the Diels-Alder reaction and reobtain the ring B diene by $LiAlH_4$ reduction (1) as had been done with adduct I failed, providing further support for oxidation at the 6(7)-double bond.

Since $KMnO_4$ had provided an unusual and unexpected result, OsO_4 followed by Na_2SO_3 reduction was tried. This reagent behaves similarly to $KMnO_4$ but it is bulkier so that reaction with the 22(23)-double bond might be expected if access to the 6(7)-double bond is indeed poor. An ir spectrum of the material obtained after reduction revealed no hydroxyl absorption. In its nmr spectrum, the AB quartet typical of the C-6 and C-7 protons of adduct I was replaced with one at δ4.73 and 5.08, and the C-22 and C-23 vinyl protons were still evident. In addition, several signals corresponding to pyridine and benzene rings and integrating for 10 protons were visible in the δ7.2-9.0 region. An uv spectrum also had absorption maxima characteristic for pyridine. Efforts to further characterize the product were hampered, however, by ready discoloration and decomposition. From the available spectroscopic data and the behavior of $KMnO_4$, structure IV in which an osmate-pyridine addition complex formed but was not reduced is proposed. Apparently, facile reduction must be blocked by the atoms surrounding the osmium.

To preclude reaction at the 6(7)-double bond, I_2/AgOAc was selected next because of its well-known mechanistic pathway to give cis-glycols on the side opposite to that acted upon by $KMnO_4$ and OsO_4. Since the opposite side of the 6(7)-double bond is blocked by the C-18 and C-19 methyls, the iodonium ion formed initially on the α side cannot be subjected to backside attack by an acetate ion. Reaction, though, is possible at the 22(23)-double bond. After the I_2/AgOAc reaction, an ir spectrum of the product V demonstrated that acetylation had occurred, as indicated by the strong absorption at 1730 cm^{-1}. An nmr spectrum confirmed the projected side chain reaction had taken place, because the AB quartet for the C-6 and C-7 vinylic protons was still visible and the

multiplet at δ5.2 for the C-22 and C-23 protons was replaced by one at δ5.0. In addition, three separate singlets for acetate methyls were seen. The uv absorbance ratio (Table 1) remained at about 3. Hplc analysis of triacetate V indicated a 4:3 ratio of isomers. Although definite studies on which particular isomer prevailed were not carried out, the 22S, 23S, 24R stereochemistry is favored, based upon previous work on the reaction of reagents of this type with the 22(23)-double bond (4).

To further extend the double bond selectivity studies, adduct I was also treated with performic acid which mechanistically results in trans glycols. The product was the 22, 23-glycol VI, the structure of which was confirmed by ir and nmr spectra. It was then acetylated to facilitate isolation and purification. The triacetate VII had appropriate ir, nmr, and uv spectral data verifying a product with an oxidized 22(23)-double bond. Hplc analysis revealed a 2:1 isomer ratio in keeping with previous observations (4).

Lastly, two separate reagents, mCPBA and H_2O_2/HOAc, were used with adduct I since they are epoxidation reagents. Although previous workers (2,3) had secured only a glass from the mCPBA reaction, we obtained the crystalline 22,23-epoxide VIII. Ir and uv data confirmed epoxide formation at C-22 and C-23. Its nmr spectrum had the expected C-6 and C-7 AB quartet as well as multiplets at δ2.34 and 2.61 for the C-22 and C-23 protons on the oxirane ring. With H_2O_2/HOAc, the identical epoxide VIII was obtained albeit in lower yield. An isomer ratio of 4:3 was indicated by hplc.

Several interesting points can be made about the regiospecificity, oxidation site, and/or reaction mechanisms. In instances where a three-

membered cyclic intermediate (i.e., I_2/AgOAc or HCO_3H reactions) or product (i.e., epoxide VIII) is involved, oxidation occurs at the 22(23)-double bond. Molecular models indicate that formation of a three-membered ring on C-6 and C-7 presents considerable strain, whereas one on C-22 and C-23 does not.

In the I_2/AgOAc reaction, the three-membered iodonium ion may accommodate the increased strain at C-6 and C-7 because of its greater overall size; however, iodonium ion formation is reversible and the glycol reaction is completed only when the acetate ion reacts on the backside. The C-18 and C-19 methyls, though, block the backside approach of the acetate anion, so completion of the reaction cannot take place.

The $KMnO_4$ and OsO_4 regiospecific preference for the 6(7)-double bond may be explained by release of ring strain through addition of a metal atom and two oxygen atoms in a five-membered transition state. Also, attraction between these reagents and the phenyltriazolidine system might account for the preference of these reagents toward this portion of the steroid.

It should be noted that yields for these reactions at C-22 and C-23, particularly with mCPBA and HCO_3H, are quite good which may provide an alternative synthesis of side chain modified steroids containing a ring B diene system.

EXPERIMENTAL

Melting points (mp) were determined on a Fisher-Johns apparatus and are uncorrected. Ir spectra were obtained using a Sargent-Welch 3-200 spectrometer. Nmr spectra were recorded with a IBM NR80 spectrometer on samples in $CDCl_3$ with tetramethylsilane as an internal standard. Uv spectra were secured with a Cary 14 instrument.

Tlc separations were carried out on silica gel HF_{254} supplied by EM Reagents. Hplc analyses were performed with a Waters 6000A chromatograph equipped with a Model 720 Systems controller on a μPorasil column. Organic extracts were dried over anhyd $MgSO_4$.

Potassium Permanganate Reaction with Adduct I. Diels-Alder adduct I (1) (250 mg) was dissolved in acetone (5.2 mL) containing piperidine (0.2 mL) and stirred. The temperature was reduced to -5°C, $KMnO_4$ (100 mg) was introduced, and the solution was stirred for 1 hr. HOAc (0.03 mL) in acetone (0.4 mL) was then added and stirring was continued at 2°C for 4 hr. The reaction mixture was filtered, diluted with water, and extracted 3 times with $CHCl_3$. The combined organic extracts were washed with 10% HCl, water, satd $NaHCO_3$ solution, and then dried. Separation of the residue by preparative tlc with 40% ethyl acetate in hexane yielded 100 mg of starting material and 100 mg of glycol II. Recrystallization of the glycol from ethyl acetate-hexane gave crystals with mp 163-166°C, ν_{max}(KBr) 3400, 1745, 1730, 1690 cm^{-1}; nmr δ0.85(s, 18-CH_3), 0.98(s, 19-CH_3), 2.00(s, CH_3CO), 5.21(m, CH=CH), 7.42(s, C_6H_5). Anal: Calcd for $C_{38}H_{53}N_3O_6 \cdot H_2O$: C, 68.54; H, 8.33; N, 6.31. Found: C, 68.22, 68.09; H, 8.43, 8.43; N, 6.21, 6.05%.

Triacetate III. Glycol II was acetylated with acetic anhydride-pyridine in the usual manner to yield triacetate III, mp 203-205°C; ν_{max}(KBr) 1730, 1690 cm^{-1}; nmr δ0.85(18- and 19-CH_3), 1.98, 2.05, 2.08(3 x CH_3CO); 5.19(m, CH=CH), 5.40 and 5.42(AB, J_{AB} 7.8Hz, -OCH-CHO-), 7.44 (s, C_6H_5-). Anal: Calcd for $C_{42}H_{57}N_3O_8$: C, 68.92; H, 7.85; N, 5.74. Found: C, 68.67; H, 7.91; N, 5.79%.

Osmate Ester IV. A solution of OsO_4(250 mg) in anhyd benzene (50 mL) containing pyridine (4 mL) was added to adduct I (627 mg) in benzene (25 mL), and the reaction was stirred in the dark for 5 days. A solution of sodium thiosulfate (1.7 g) in water (5 mL) was introduced, and stirring was continued for 2 days. The reaction mixture was extracted with ethyl acetate, and the organic layer was then washed with water and dried. Crystals obtained from ethyl acetate-hexane were dissolved in ethyl acetate and decolorized by Norite A. Further recrystallizations gave the osmate ester IV, mp 135-138°C (dec); ν_{max}(KBr) 1745, 1730, 1690, 1610, 1500 cm^{-1}; nmr δ0.80(s, 18-CH_3), 0.98(s, 19-CH_3), 1.93(s, CH_3CO), 4.73 and 5.08(AB, J_{AB} 7.5Hz,-OCH-CHO-,2H), 5.18(m, CH=CH,2H), 5.42(m, CH-OAc,1H), 7.37, 7.74 and 8.82(m, C_6H_5N and C_6H_5,10H).

Attempts to dry this compound resulted in decomposition which precluded C, H, N analyses. The ester was immobile on tlc in several solvents, and no starting material was evident.

Reaction of Adduct I with Iodine-Silver Acetate. A mixture of adduct I (360 mg), AgOAc(220 mg), I_2(157 mg), and HOAc (3 mL) was stirred for 30 min. Aq. HOAc (96%, 0.3 mL) was introduced, and the mixture was heated at 90-95°C for 3 hr. The cooled reaction mixture was diluted with a satd NaCl solution and extracted with ethyl acetate. The organic layer was washed with a satd $NaHCO_3$ solution, water, and then dried. Crystallization of the residue from ethyl acetate-hexane gave 160 mg (37%) of the 3,22,23-triacetate V, mp 137-138°C; ν_{max}(KBr) 1750, 1730, 1700, 1240 cm^{-1}; nmr δ0.82(s, 18-CH_3), 0.98(s, 19-CH_3), 2.01, 2.06, and 2.09(s, 3 x CH_3CO), 5.02(m,-CH(OAc)-CH(OAc)-), 5.42(m, CHOAc). Anal: Calcd for $C_{42}H_{57}N_3O_3$: N, 5.74. Found: N, 5.69%.

Hplc indicated two isomers present in a 4:3 ratio.

Performic Acid Oxidation of Diels-Alder Adduct I. Adduct I (310 mg) was dissolved in HCO_2H (2 mL) containing H_2O_2 (30%; 0.2 mL), and the solution was stirred for 16 hr. It was then added to water (5 mL) at reflux temperature, cooled, and filtered. The precipitate was dissolved in methanol (6 mL) containing $NaHCO_3$ (120 mg) and water (0.5 mL) and stirred for 12 hr. After dilution with water the reaction mixture was extracted with ethyl acetate. Crude glycol VI, which had ν_{max}(KBr) 3450, 1750, 1710, 1695 cm^{-1} and nmr δ0.82, 0.94, 2.03, and 7.41, was isolated.

The crude material VI was acetylated with acetic anhydride-pyridine in the usual manner to yield the triacetate VII. Crystallization from ethyl acetate-hexane gave 300 mg (82%) of VII with mp 154-157°C; ν_{max} 1750, 1730, 1700 cm^{-1}; nmr δ0.80(s, 18-CH_3), 0.96(s, 19-CH_3), 1.99(s, 3 x CH_3CO), 5.12(m, CH(OAc)-CH(OAc)-), 5.41(m, CHOAc), 6.20 and 6.40 (AB, J_{AB} 8.5Hz), 7.38(br s, C_6H_5-). Anal: Calcd for $C_{42}H_{57}N_3O_8$: C, 68.92; H, 7.85; N, 5.74. Found: C, 68.72; H, 7.87; N, 5.58%.

Hplc analysis indicated two isomers in a 2:1 ratio.

Epoxide VIII Formation. (a) H_2O_2 (9.56M, 0.09 mL) was added to a stirred solution of adduct I (550 mg) in glacial HOAc (20 mL) at 0°C. The solution was stirred for 4 days at ambient temperature, diluted with water, and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with a satd $NaHCO_3$ solution, water, and then dried. Recrystallization of the residue gave 260 mg (46%) of the 22,23-epoxide VIII, mp 133-135°C; ν_{max}(KBr) 1745, 1730, 1695 cm^{-1}; nmr δ0.80(s, 18-CH_3), 0.99(s, 19-CH_3), 2.01(s, CH_3CO), 2.34 and 2.61 (m, C-22 and C-23 H), 6.20 and 6.40 (AB, J_{AB} 8.5Hz, C-6 and C-7 H), 7.40(s, C_6H_5-). Anal: Calcd for $C_{38}H_{51}N_3O_5$: C, 72.47; H, 8.16; N, 6.67. Found: C, 72.63; H, 8.38; N, 6.38%.

Hplc indicated a 4:3 ratio of isomers.

(b) Adduct I (280 mg), m-CPBA (80 mg) and dried CH_2Cl_2 (10 mL) were stirred together for 16 hr at room temperature. The reaction solution was washed with a 10% Na_2SO_3 solution, water, and a satd $NaHCO_3$ solution. Preparative tlc (40% ethyl acetate in hexane) gave 270 mg (94%) of epoxide VIII, mp 132-134°C, identical in all respects to the material obtained above.

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