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Allylic oxidation of steroidal olefins by vanadyl acetylacetonate and tert-butyl hydroperoxide

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ABSTRACT

Readily available vanadyl acetylacetonate was found to oxidize the allylic sites of Δ^5 steroidal alcohols without protection of hydroxyl groups. Cholesterol, dehydroepiandrosterone, cholesterol benzoate, cholesterol acetate, pregnenolone, and 5-pregnen-3,20-diene were oxidized to 7-keto products using vanadyl acetylacetonate in one pot reactions at room temperature in the presence of oxygen and water.

1. Introduction

Derivatives of dehydroepiandrosterone (DHEA) oxidation have shown beneficial health related potential [1], such as boosting memory [2] and the resting metabolic rate of overweight adults [3]. Several derivatives of pregnenolone oxidation have also been reported to inhibit undesired cortisone effects [4]. Even 7-ketocholesterol, the major auto-oxidation product of cholesterol, has shown anti-cancer potential [5,6]. With the goal of producing beneficial steroidal derivatives, an innovative approach to a common set of reagents, vanadyl acetylacetonate and tert-butyl hydroperoxide, has been successfully used and is reported here.

Vanadium catalysts are recognized for their epoxidation of olefins [7–14]. The carbon–hydrogen oxidation that occurs simultaneously has mostly been discarded as a side reaction from free radical formation [7,14]. However, carbon–hydrogen oxidation potential was realized when several benzylic carbon were oxidized in high yields using vanadium catalysts [15]. In the present study, we have modified this approach and extended it to steroidal olefins. (We explicitly acknowledge that others have achieved carbon-hydrogen activation before us. Albeit, we are unaware of any studies, such as the one presented in this report, focused on the allylic oxidation of steroidal compounds using vanadium catalysts and TBHP.)

Steroidal oxidations are often proceeded by esterification of the alcohol at the C3 carbon to prevent its oxidation [16]. Attempts to remove the ester, when possible, result in loss of product. However, vanadium catalysts do not significantly oxidize hydroxyl groups past initial complexation [13,17,18]. Without needing a protecting group, oxidation via vanadium removes two entire steps from total synthesis, increasing overall yield. Vanadyl acetylacetonate ($VO(acac)_2$), in particular, is also relatively inexpensive, safe, shelf stable and was found to oxidize several allylic carbons of steroidal olefins in the presence of oxygen and at room temperature using tert-butyl hydroperoxide (TBHP) as the oxidant in benzene solvent.

2. Experimental

2.1. Optimization

Reaction conditions were optimized using cholesterol as a primary substrate. Conversion was qualitatively determined by TLC (silica gel on glass plates) and molybdic acid [19] after eluting with 50:50 ethyl acetate and toluene. Full conversion using minimal concentrations of TBHP and $VO(acac)_2$ was considered optimized.

2.2. General procedure

Substrate, 2.6 mmol (only 1.3 mmol for (**5**), (**12**), (**13**), and (**14**) for lack of solubility), and 0.38 mmol of $VO(acac)_2$ were put into a round bottom flask. The flask was then charged with 25 mL of

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benzene. After dissolving as much substrate as possible, 24 mmol of TBHP were added. The reaction flask was loosely capped to prevent loss of solvent and the reaction was allowed to run for 5 days.

At the end of 5 days, the benzene solvent was removed by evaporation. Residue was then extracted with deionized (DI) water and ethyl ether into a separatory funnel. (If time is available, the precipitate will dissolve into the solution overnight.) The ether layer was retained and the ether was evaporated under reduced pressure at 55 °C.

Once the ether was removed, products (**15**) and (**16**) were dissolved in acetone and recrystallized with DI water. The suspension was chilled for 5 h with an ice bath. Products (**15**) and (**16**) were then filtered out, rinsed with DI water, and dried under reduced pressure with P_2O_5. The filtrate from the first recrystallization of (**16**) should be recrystallized again to extract more product. Beyond work-up of (**15**) and (**16**), we advise against recrystallization for the following products.

After removing the ether, products (**17**–**20**) were separated with column chromatography (silica gel). Product (**17**) was extracted with hexane into the column and eluted using a gradient (0–40% ethyl acetate in hexane). Also, product (**18**) was extracted with 20% ethyl acetate in hexane and eluted with a gradient (20–50% ethyl acetate in hexane). Products (**19**) and (**20**) were extracted into the column with 50:50 ethyl acetate and hexane and were isocratically eluted with 50:50 ethyl acetate and hexane. Fractions were identified by TLC, collected, and dried under reduced pressure with P_2O_5. (We found that, particularly for elution of (**17**), it is better get the products through the column as quickly as possible, without losing separation.)

2.3. Method of purity analysis

^{13}C proton-decoupled NMR was used in conjunction with TLC to confirm the purity of starting materials. For identification of oxidation products, ^{1}H NMR, ^{13}C NMR, and mass spectroscopy (Q-TOF and GCT) were utilized.

2.4. Compounds (**1**–**14**): substrates

2.4.1. Estrone (**1**)

White solid. ^{13}C NMR (150 MHz, $CDCl_3$): δ 153.6, 138.1, 132.2, 126.6, 115.4, 112.9, 50.5, 48.2, 44.0, 38.5, 36.0, 31.6, 29.5, 26.6, 21.6, 14.0. TLC showed one band and spectroscopic data matched expected values [20].

2.4.2. 4-Cholesten-3-one (**2**)

White solid. ^{13}C NMR (100 MHz, $CDCl_3$): δ 200.0, 172.1, 123.9, 56.2, 56.0, 54.0, 42.5, 39.8, 39.6, 38.8, 38.8, 36.3, 35.9, 35.1, 35.7, 34.1, 33.1, 32.2, 28.3, 28.2, 24.3, 24.0, 23.0, 22.7, 21.2, 18.8, 17.5, 12.1. TLC showed one band and spectroscopic data matched expected values [20].

2.4.3. 1,4-Androstadiene-3,17-dione (**3**)

White solid. ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 186.4, 168.5, 155.5, 127.9, 124.3, 52.5, 50.6, 47.9, 43.6, 35.8, 35.3, 32.7, 32.5, 31.4, 22.3, 22.1, 18.9, 14.0. TLC showed one band and spectroscopic value matched expected values [20].

2.4.4. Cholestan-3-ol (**4**)

White solid. ^{13}C NMR (MHz 100, $CDCl_3$): δ 71.3, 56.5, 56.3, 54.4, 44.9, 42.6, 40.1, 39.5, 38.2, 37.0, 36.2, 35.8, 35.5, 35.5, 32.1, 31.5, 28.8, 28.3, 28.0, 24.2, 23.9, 22.9, 22.6, 21.3, 18.7, 12.3, 12.1. TLC showed one band and spectroscopic data matched expected values [20].

2.4.5. 5-Androgen-3,17-diol (**5**)

5-Androgen-3,17-diol (**5**) was obtained through reduction of DHEA using Luche reduction [21]. Cerium(III) Chloride heptahydrate, 7.5 g, was dissolved in 100 mL of methanol. DHEA, 4.59 g, was then added and dissolved. To the mixture, 1 g of sodium borohydride was slowly added. The solution was stirred overnight with a magnetic stir bar. DI water was added to the mixture to quench the reaction. The reaction mixture was then poured into a separatory funnel. Ethyl ether was added to the separatory funnel to make a second layer. The ether layer was then washed with DI water and collected. TLC revealed only one band from the ether layer. The ether was evaporated and the residue was used as substrate. White solid. ^{13}C NMR (100 MHz, $CDCl_3$): δ 140.9, 121.4, 81.9, 71.7, 51.3, 50.2, 42.3, 37.3, 36.6, 32.0, 31.5, 30.5, 23.5, 20.7, 19.5, 11.0. TLC showed one band.

2.4.6. 7-Dehydrocholesterol (**6**)

White solid. ^{13}C (100 MHz, $CDCl_3$): δ 141.6, 140.0, 119.8, 116.5, 70.6, 56.1, 54.7, 46.4, 43.1, 41.0, 39.7, 39.4, 38.6, 37.2, 36.4, 36.3, 32.2, 28.3, 28.2, 24.1, 23.0, 22.8, 21.3, 19.1, 16.5, 12.0. TLC showed one band and spectroscopic data matched expected values [22].

2.4.7. 7-Cholesten-3-ol (**7**)

7-Cholesten-3-ol (**7**) was obtained through hydrogenation of 7-dehydrocholesterol. 7-Dehydrocholesterol, 15 g, was dissolved in 235 mL of ethyl acetate and 15 mL of glacial acetic acid (a 95:5 volume ratio respectively). The mixture was heated by steam bath and kept warm throughout the hydrogenation process by heat lamps to keep the 7-dehydrocholesterol soluble. Platinum dioxide, 1.5 g, was added to the solution. The solution was then pressurized with H_2 gas (55–60 pounds) for 2 days. Upon completion of the 2 days, the solution was filtered while warm to remove the catalyst. The filtrate was then chilled causing 7-cholesten-3-ol to precipitate. After filtering out the precipitate and rinsing it with DI water, the 7-cholesten-3-ol was dissolved in acetone and recrystallized with DI water. The precipitate was filtered out and rinsed with DI water. 7-Cholesten-3-ol was then dried under reduced pressure. White solid. ^{1}H NMR (250 MHz, $CDCl_3$): δ 5.60, 5.42, 5.18, 3.63, 2.09, 1.81, 1.65, 1.56, 1.37, 1.28, 1.16, 0.92, 0.83, 0.65, 0.58. ^{13}C NMR (400 MHz, $CDCl_3$): δ 139.8, 117.6, 71.3, 56.4, 55.2, 49.6, 43.6, 40.4, 39.8, 39.7, 37.3, 36.4, 36.3, 34.4, 31.6, 29.9, 28.2, 28.2, 24.1, 23.2, 23.0, 22.8, 21.8, 19.1, 13.3, 12.1. ^{1}H NMR showed this starting material to be 74% 7-cholesten-3-ol and 24% 7-dehdrocholesterol. Spectroscopic data matched expected values [23].

2.4.8. 24,25-dihydrolanosterol (**8**)

24,25-dihydrolanosterol (**8**) was obtained through hydrogenation of a lanosterol/24,25-dihydrolanosterol (40:60 respectively) mixture that was commercially obtained. The mixture's identity and purity were confirmed by ^{1}H NMR, ^{13}C NMR, and TLC. Lanosterol mixture, 10 g, was dissolved in 300 mL of glacial acetic acid. To the solution, 1.75 g of a platinum dioxide and platinum black (46:54 respectively) mixture was added. The lanosterol mixture was warmed by steam bath and kept warm during the hydrogenation process with heat lamps. To the mixture, 55–60 pounds of H_2 gas were applied for 17 h. The mixture was then filtered while warm to remove the catalyst. Recrystallization occurred by cooling the reaction solution and adding DI water. The precipitate was filtered out and rinsed with water. It was then dissolved in acetone and recrystallized with DI water. The precipitate was filtered out and allowed to dry using P_2O_5 under reduced pressure. White solid. ^{1}H (400 MHz, $CDCl_3$): 3.25, 2.07, 1.72, 1.52, 1.36, 1.27, 1.00, 0.89, 0.82, 0.70, 0.58. ^{13}C (62.5 MHz, $CDCl_3$): 134.4, 130.9, 125.3, 79.0, 50.4, 79.8, 44.5, 38.9, 37.0, 35.6, 31.0, 30.9, 29.7, 28.0, 27.8, 26.5, 25.8, 24.3, 21.0, 19.2, 18.3, 17.7, 15.8, 15.5. TLC showed one band.

*2.4.9. Cholesteryl benzoate (**9**)*

Cholesteryl benzoate (**9**) was obtained by slowly adding 100 mL of benzoyl chloride to 50 g of cholesterol dissolved in 200 mL of pyridine at 40 °C for 3.5 h. That mixture was poured into chilled DI water. Cholesteryl benzoate precipitated and was filtered out. It was rinsed with DI water and dried. The precipitate was dissolved in warm chloroform. That solution was put into a freezer for 5 h, during which time the cholesteryl benzoate precipitated. The precipitate was filtered, rinsed with methanol, and dried. White solid. ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 166.2, 139.8, 132.9, 131.0, 129.7, 128.4, 123.0, 74.8, 56.9, 56.3, 50.2, 42.5, 39.8, 38.7, 37.3, 36.2, 32.1, 28.3, 24.2, 23.2, 21.5, 19.6, 12.3. TLC showed one band and spectroscopic data matched expected values [24].

*2.4.10. Cholesteryl acetate (**10**)*

Cholesteryl acetate (**10**) was obtained by slowly adding 12 g of acetic anhydride to 10 g of cholesterol dissolved in 200 mL of pyridine at 0 °C. The reaction was allowed to warm to room temperature after 3 h and was left overnight with magnetic stirring. To quench the reaction, DI water was added, also causing the cholesteryl acetate to precipitate. The precipitate was filtered out, rinsed with DI water, and dried. After drying, the solids were dissolved in acetone and recrystallized by adding DI water. The precipitate was filtered out, rinsed with DI water, and dried. White solid. ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 170.5, 139.7, 122.7, 74.0, 56.7, 56.1, 50.0, 42.3, 39.5, 36.6, 35.8, 31.9, 31.9, 28.0, 24.3, 22.8, 22.6, 19.3, 18.7, 11.9. TLC showed one band and spectroscopic data matched expected values [20].

*2.4.11. Cholesterol (**11**)*

White solid. ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 140.8, 121.7, 71.7, 56.8, 56.2, 50.2, 42.3, 42.2, 39.8, 39.5, 37.3, 36.5, 36.2, 35.8, 31.9, 31.6, 28.3, 28.0, 24.3, 23.9, 22.8, 22.6, 21.1, 19.4, 18.7, 11.9. TLC showed one band and spectroscopic data matched expected values [20]. R_f = 0.85 (ethyl Acetate/Toluene = 50/50).

*2.4.12. Pregnenolone (**12**)*

White solid. ^{13}C NMR (100 MHz, $CDCl_3$): δ 209.8, 140.9, 121.2, 71.5, 63.7, 56.9, 49.9, 44.0, 42.2, 38.8, 37.3, 36.5, 31.8, 31.7, 31.4, 31.5, 24.5, 22.8, 21.1, 19.4, 13.2. TLC showed one band and spectroscopic data matched expected values [20].

*2.4.13. Dehydroepiandrosterone (**13**)*

White solid. ^{13}C NMR (100 MHz, $CDCl_3$): δ 141.2, 120.7, 71.3, 51.7, 50.2, 47.5, 42.1, 37.2, 35.8, 31.5, 21.9, 20.3, 19.4, 13.5. TLC showed one band and spectroscopic data matched expected values [20].

*2.4.14. 5-Pregnen-3,20-diol (**14**)*

5-Pregnen-3,20-diol (**14**) was obtained through reduction of pregnenolone using Luche reduction [21]. Cerium(III) Chloride heptahydrate, 7.5 g, was dissolved in 100 mL of methanol. Pregnenolone, 5 g, was then added and dissolved. To the solution, 1 g of sodium borohydride was added. The solution was stirred overnight with a magnetic stir bar. DI water was then added to quench the reaction. The reaction mixture was poured into a separatory funnel and ethyl ether was added forming a second layer. The ether layer was washed with DI water and collected. TLC revealed only one band from the ether layer, which was subsequently evaporated leaving 5-pregnen-3,20-diol. White solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.28 (s, 1H), 4.64 (s, 1H), 4.12 (m, 1H), 3.50 (m, 1H), 3.27 (m, 1H), 2.52 (s, 1H), 2.14 (m, 3H), 2.00–0.50 (comp, 25H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 140.8, 121.6, 71.7, 70.6, 70.4, 58.4, 58.4, 56.6, 56.2, 50.1, 50.1, 42.2, 41.6, 39.9, 38.8, 37.3, 36.5, 31.9, 31.7, 31.6, 31.5, 25.8, 25.6, 24.2, 23.5, 20.9, 20.8, 19.4, 12.4, 12.4.

*2.5. Compounds (**15**–**20**): products*

*2.5.1. 7-Ketocholesteryl benzoate (**15**)*

White solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (d, 2H, J = 7.3 Hz), 7.57 (t, 1H, J = 7.4 Hz), 7.46 (t, 3H, J = 7.6 Hz), 5.75 (m, 1H), 4.98 (m, 1H), 2.75–0.97 (comp, 28H), 0.94 (d, 3H, J = 6.5 Hz), 0.87 (dd, 6H, J = 6.7 Hz, J = 1.8 Hz), 0.70 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 202.1, 165.9, 164.0, 133.1, 130.3, 128.4, 127.0, 72.9, 54.8, 50.0, 49.8, 45.5, 43.1, 39.5, 38.4, 37.9, 36.2, 35.7, 28.6, 28.0, 26.3, 23.9, 22.9, 22.6, 18.9, 17.3, 12.0. HRMS (Q-TOF, ESI+) m/z: [M+1] calculated for $C_{34}H_{49}O_3$ 505.3682; found 383.3228. MS (GCT) m/z: 504.4295, 502.0367, 382.3056, 269.2161, 218.9884, 174.1001, 161.1125, 105.0313, 77.0457. (1.28 g, 98%) Spectroscopic data matched expected values [24].

*2.5.2. 7-Ketocholesteryl acetate (**16**)*

White solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.66 (s, 1H), 4.74–4.64 (m, 1H), 2.57–0.94 (comp, 32H), 0.90 (d, 3H, J = 6.4 Hz), 0.86–0.82 (m, 6H), 0.66 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 201.9, 170.2, 163.9, 126.7, 72.2, 54.8, 49.9, 49.8, 45.4, 43.1, 39.5, 38.7, 38.3, 37.7, 36.2, 36.0, 35.7, 28.5, 28.0, 27.3, 26.3, 23.8, 22.8, 22.6, 21.3, 21.2, 18.6, 17.2, 12.0. MS (Q-TOF, ESI+) m/z: [M+1] calculated for $C_{29}H_{47}O_3$ 443.3525; found 885.6874, 443.3505, 383.3316, MS (GCT) m/z: 382.1558, 173.9955. (0.96 g, 83%) Spectroscopic data matched expected values [25].

*2.5.3. 7-Ketocholesterol (**17**)*

White solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.70 (d, 1H, J = 1.9 Hz), 3.74–3.62 (m, 1H), 2.56–0.96 (comp, 30H), 0.93 (d, 3H, J = 6.6 Hz), 0.87 (dd, 6H, J = 6.6 Hz, J = 2.0 Hz), 0.69 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 202.8, 166.0, 126.0, 70.4, 54.9, 50.1, 50.0, 45.5, 43.2, 41.9, 39.6, 38.8, 38.4, 36.5, 36.3, 35.8, 31.1, 28.7, 28.1, 26.4, 24.0, 23.0, 22.7, 21.3, 19.0, 17.4, 12.1. MS (Q-TOF, ESI+) m/z: [M+1] calculated for $C_{27}H_{43}O_3$ 401.3420; found 401.3335. MS (GCT) m/z: 400.3997, 346.3411, 161.1247, 81.0792. R_f = 0.60 (ethyl acetate/-toluene = 50/50). (0.47 g, 45%) Spectroscopic data matched expected values [25].

*2.5.4. 5-Pregnen-3-ol-7,20-dione (**18**)*

White solid. ^{1}H NMR (400 MHz, Deuterated DMSO): δ 5.70 (s, 1H), 3.72–3.62 (m, 1H), 2.59–0.50 (comp, 28H). ^{13}C NMR (100 MHz, Deuterated DMSO): δ 210.3, 201.8, 166.0, 126.2, 70.7, 62.5, 50.2, 45.6, 42.1, 38.5, 36.6, 32.0, 26.8, 23.8, 21.3, 17.5, 13.7. MS (Q-TOF, ESI+) m/z: [M+1] calculated for $C_{29}H_{31}O_3$ 331.2273; found 331.2200. MS (GCT) m/z: 329.9635, 245.1098, 161.1338, 91.0515, 79.0365. (0.20 g, 23%) Spectroscopic data matched expected values [25].

*2.5.5. 3-Hydroxyandrost-5-ene-7,17-dione (**19**)*

White solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.75 (s, 1H), 3.75–3.64 (m, 1H), 2.87–2.77 (m, 1H), 2.60–0.80 (comp, 23H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 201.1, 166.1, 126.0, 70.3, 50.1, 47.9, 45.7, 44.3, 41.9, 38.4, 36.3, 35.7, 31.1, 30.7, 24.2, 20.6, 17.5, 13.8. MS (Q-TOF, ESI+) m/z: [M+1] calculated for $C_{19}H_{27}O_3$ 303.1960; found 303.1958. MS (GCT) m/z: 301.8061, 161.534, 91.0481, 79.0190 (0.14 g, 19%).

*2.5.6. 5-Pregnen-3, 20-diol-7-one (**20**)*

White solid. ^{1}H NMR (400 MHz, Deuterated DMSO): δ 5.58 (s, 1H), 4.90 (d, 1H, J = 4.4 Hz), 4.15–4.10 (m, 1H), 3.54–3.25 (m, 2H), 2.49 (m, 5H), 2.42–0.95 (comp, 18H), 0.67 (s, 3H), 0.60 (s, 1H) ^{13}C NMR(100 MHz, Deuterated DMSO): δ 201.7, 167.4, 125.3, 69.5, 68.9, 56.8, 50.1, 45.2, 43.1, 38.5, 36.4, 31.5, 26.9, 24.4, 21.1, 17.4, 12.5. MS (Q-TOF, ESI+) m/z: [M+1] calculated for $C_{21}H_{33}O_3$ 333.2430; found 333.2350. MS (GCT) m/z: 332.1760, 161.0795, 91.0423 (0.11 g, 19%).

2.6. Use of other vanadium complexes to produce 7-ketocholesterol from cholesterol

For each reaction, 1 g (2.6 mmol) of cholesterol, was dissolved in 25 mL of benzene with 0.38 mmol of one of the following vanadium catalysts: bis(cyclopentadienyl)vanadium (IV) dichloride (0.1 g), vanadium (IV) oxide sulfate hydrate (0.06 g), vanadium (III) acetylacetonate (0.13 g), vanadium (IV) carbide (0.02 g), tris(triphenylsiloxy)vanadium (V) oxide (0.34 g), and vanadium (V) oxytriisopropoxide (0.09 g). To these, 3.09 g (24 mmol) of 70% TBHP was added. (0.85 M TBHP overall) Results were analyzed through TLC and Q-TOF of neat product. This data is presented in the Supplementary material section with further details.

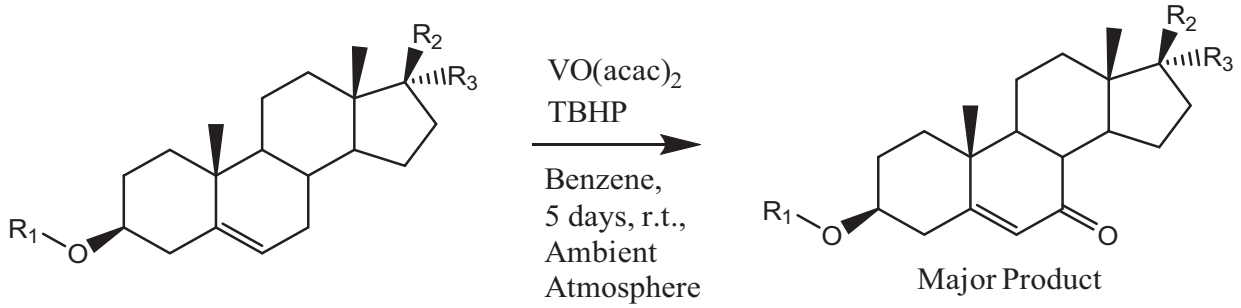
3. Results/discussion

3.1. Optimization

The Δ^5 steroids are particularly useful substrates due to the selective oxidation of the allylic C7 carbon over the more sterically hindered C4 carbon [26]. There is also an energetic benefit of C7 oxidation over C4 oxidation [25]. Therefore, optimization of this system centered around cholesterol, an easily obtainable Δ^5 steroid precursor. Cholesterol, 1 g (2.6 mmol), dissolved in 25 mL of benzene, was almost completely oxidized using 3.09 g (24 mmol) of 70% TBHP (0.85 M TBHP overall) and 0.1 g (0.38 mmol) $VO(acac)_2$. When the overall concentration of TBHP was below 0.85 M, conversion diminished while higher concentrations seemed to have no gained effect. Lower concentrations of $VO(acac)_2$ also led to lower conversion percentages. Only slight oxidation of cholesterol occurred when $VO(acac)_2$ was not present. Nor did any significant oxidation occur when TBHP was substituted with hydrogen peroxide or tert-butyl peroxide.

Cholesterol oxidation occurred in benzene, 20% methanol/80% benzene, and chloroform. However, use of chloroform as a solvent was abandoned because of its potential reaction with TBHP to form phosgene [27]. The addition of methanol lowered the conversion of the cholesterol, making pure benzene the preferred solvent. Several solvents in which the reaction did not significantly occur include tert-butyl alcohol, pyridine, acetonitrile, 75% acetonitrile/25% chloroform, and methanol. The addition of heat (50 °C) caused the solution to become murky and too viscous. Addition of acetic acid was also detrimental to overall conversion. Adding 1 mL of toluene also decreased the overall conversion of cholesterol.

Table 1
Δ^5 Steroidal alcohols and their 7-keto products



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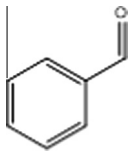
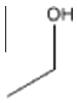
R_1	R_2	R_3	Substrate	Product	Yield %[a]
	C_8H_{17}	H	(9)	(15)	98
	C_8H_{17}	H	(10)	(16)	83
H	C_8H_{17}	H	(11)	(17)	45
H		H	(12)	(18)	24
H	$R_2 + R_3$	Ketone	(13)	(19)	19
H		H	(14)	(20)	26

[a] Isolated.

The optimal amounts of reagents at room temperature, were proposed to be 2.6 mmol substrate, 25 mL benzene, 0.38 mmol $VO(acac)_2$, and 24 mmol (0.85 M) TBHP for 5 days. For 5-androgen-3,17-diol (**5**), pregnenolone (**12**), DHEA (**13**), and 5-pregnen-3,20-diol (**14**), only 1.3 mmol were used due to solubility issues. Five days was determined by monitoring the progress of the reaction through TLC. Of note, this reaction was upscaled 5 and 10 times with no difference in yields using multiples of these amounts of reagents.

3.2. Oxidation

Fourteen steroidal substrates were tested under optimal conditions. Five steroids showed no significant oxidation: estrone (**1**), 4-cholestan-3-one (**2**), 1,4-androstadiene-3,17-dione (**3**), cholestan-3-ol (**4**), and 5-androgen-3,17-diol (**5**). The only Δ^5 to not show high conversion was 5-androgen-3,17-diol, which was probably due to lack of solubility. Most important of this set, cholestan-3-ol did not show significant oxidation, if any at all, confirming that this reaction does not oxidize hydroxyl groups, at least not of cholesterol. Steroidal $\Delta^{8(9)}$ and Δ^7 olefins, 7-dehydrocholesterol (**6**), 7-cholesten-3-ol (**7**) and 24,25-dihydrolanosterol (**8**), had high conversion percentages, but gave complex mixtures of oxidation products. This was expected given the multiple allylic sites of (**7**) and (**8**) and 7-dehydrocholesterol is well-known to form numerous oxidation products [28].

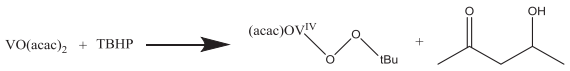
Several Δ^5 steroids showed susceptibility to oxidation at the allylic C7 carbon. These steroids were cholesteryl benzoate (**9**), cholesteryl acetate (**10**), cholesterol (**11**), pregnenolone (**12**), DHEA (**13**), and 5-pregnen-3,20-diol (**14**), shown in Table 1. They yielded as major products, 7-ketocholesterol benzoate (**15**), 7-ketocholesterol acetate (**16**), 7-ketocholesterol (**17**), 5-pregnen-3-ol-7,20-dione (**18**), 3-hydroxyandrost-5-ene-7,17-dione (**19**), and 5-pregen-3,20-diol-7-one (**20**) respectively.

Table 1 shows a correlation between the isolated yields and the lipophilic natures of these steroids. Two large drops in yield can be seen going from the esters to the hydroxyl group, (**16**–**17**), and from side chain to no side chain, (**17**–**18**). The large drop in yields between the esters and the other compounds is partly due to a simpler work-up. Additionally, the differences in isolated yields between (**18**) through (**19**) can be attributed to work-up inefficiency. It is not certain why these yield variances appear otherwise.

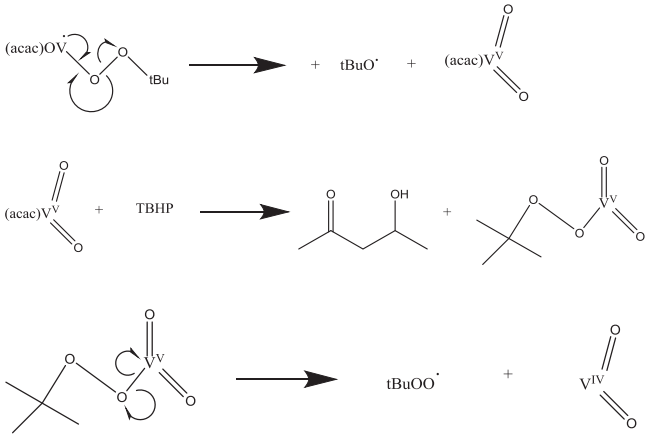
3.3. Mechanism

Three probable mechanisms for this oxidative reaction are through free radical formation, singlet oxygen formation [29], and formation of a reactive vanadium complex [15]. Because singlet oxygen and radicals have short life times and the reaction could last at least 5 days, a longer lasting vanadyl peroxide complex seemed logical. However, vanadyl peroxide complexes are thought to lead to epoxides rather than carbon–hydrogen oxidations [7,12,13]. There was the possibility of radicals [7] and singlet oxygen [29] continuously being generated. Although a singlet oxygen mechanism seemed possible given auto-oxidation of cholesterol, free radical oxidation was more prominent in the literature surveyed [7,14]. Therefore, Scheme 1 was proposed as the primary mechanism based on radical formation. (All three mechanisms may contribute.)

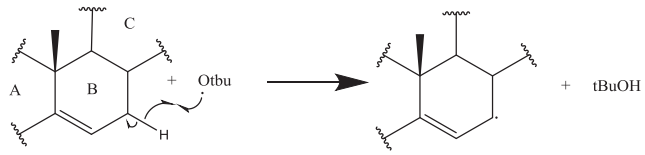
Step 1: Substitution of acac Ligand, Ref. [7].



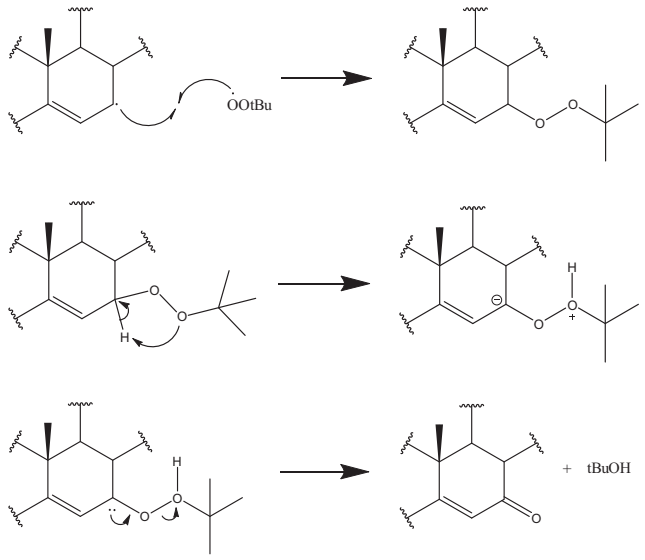
Step 2: Radical Initiation and Vanadium Oxidation, Ref. [7].



Step 3: Propagation and termination.



Upon adding TBHP to the reaction mixture, a reddish color was observed, indicating the presence of a vanadium (IV) peroxide complex [9,12]. Precipitate formed on the glass of the reaction flask indicating substitution of the acetylacetonate ligand by the peroxide in step 1 [29]. In step 2, homolytic cleavage occurs at the



Scheme 1. Proposed reaction mechanism.

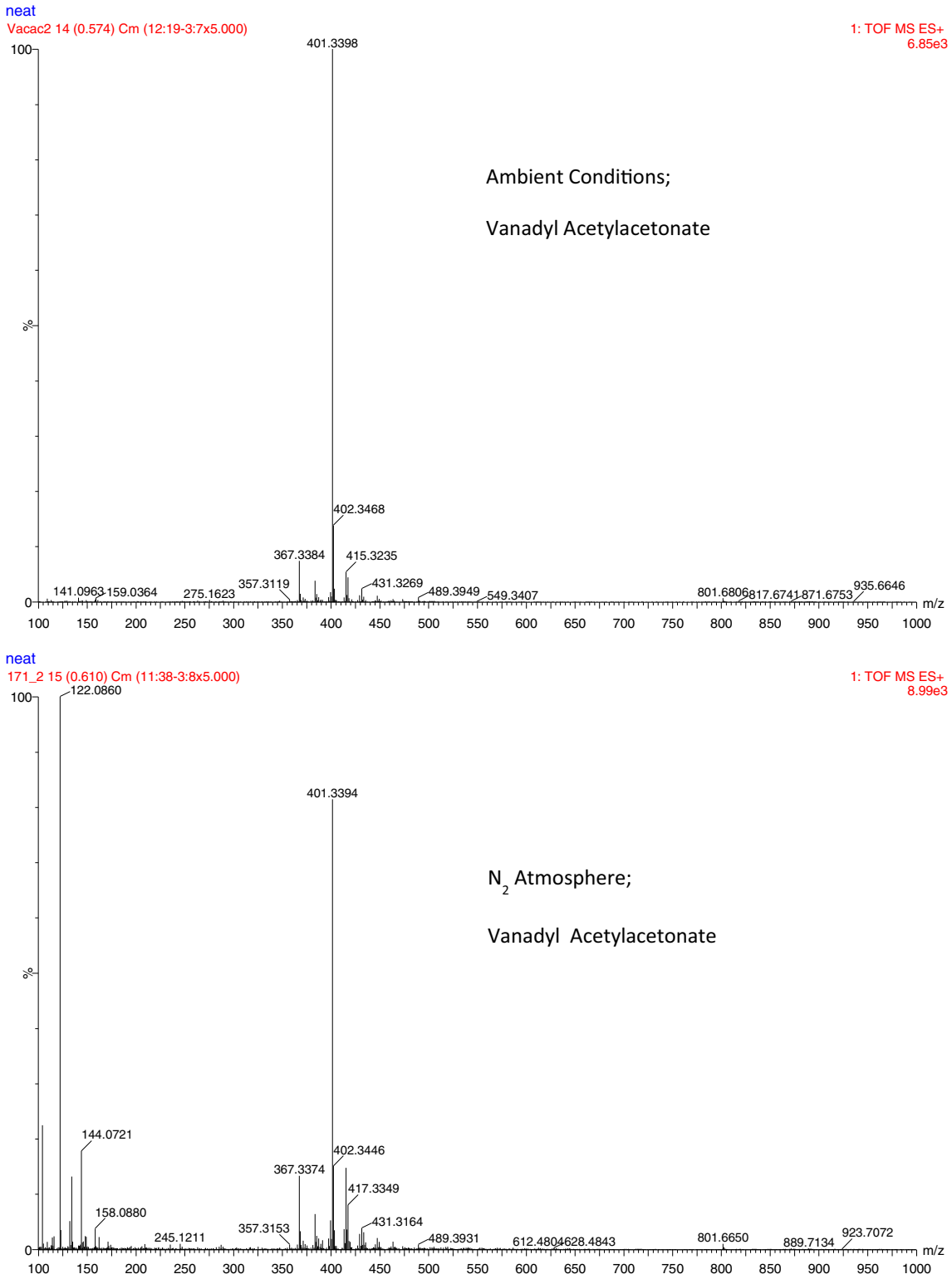


Fig. 1. Mass spectra of neat product from the reaction conducted with optimal conditions, with cholesterol as the substrate, under ambient and nitrogen environments. These spectra were taken with positive ion mode ESI Q-TOF MS. *M*/*z* 401 coincides with 7-ketocholesterol. TLC was used in conjunction to show that the starting material was completely converted and not able to contribute to the *m*/*z* 401 peak.

oxygen–oxygen bond of the vanadium (IV) peroxide complex ligand forming a tert-butoxide radical, which then abstracts a hydrogen from the allylic carbon in step 3. A vanadium (V) species is produced from the homolytic cleavage of the peroxide, which is consistent with the observation of yellow colored solvent at the end of the reaction [9]. In turn, the remaining acetylacetonate ligand is also substituted by TBHP. A peroxyl radical is emitted from the vanadium (V) complex. Respective of the small molar amount of vanadyl compound used, it is necessary that the vanadium (V) species is regenerated from excess TBHP. We believe the $V^{V}O_2(OOtBu)$ to be regenerated from $V^{IV}O_2$ and TBHP and that $V^{IV}O_2$ is the actual catalyst. In step 3, the enone product is formed

through either subsequent hydrogen abstraction at the allylic site in conjunction with termination via peroxyl radical and rearrangement or deprotonation leading to rearrangement as shown in Scheme 1.

Although not shown, molecular oxygen is likely used as a source of oxygen. This is evident because much lower ion counts of product appear in mass chromatograms of neat product when the reaction is conducted under nitrogen atmosphere as opposed to ambient conditions. Additionally, there is an increase in side product formation shown in the mass spectrum of the neat product from the reaction conducted under a nitrogen atmosphere compared to the spectrum from the ambient atmosphere. (Fig. 1) This suggests that the presence of oxygen is also beneficial for the reaction in that it terminates radical side polymerization and product formation.

3.4. Other vanadium catalysts

We also wanted to find out whether other vanadium compounds would oxidize cholesterol to produce 7-ketocholesterol. Given that the ligands were merely being substituted as per Scheme 1, different vanadium complexes were predicted to also work well regardless of their ligands. Bis(cyclopentadienyl)-vanadium (IV) dichloride, vanadium (IV) oxide sulfate hydrate, vanadium (III) acetylacetonate, vanadium (IV) carbide, vanadium tris(triphenylsiloxy)vanadium (V) oxide, and vanadium (V) oxytriisopropoxide all worked as substitutes for $VO(acac)_2$ under the same reaction conditions.

Indeed, all of the vanadium catalysts were usable for oxidation, albeit to various degrees. Comparison of neat product on direct injection Q-TOF chromatograms (using mass selection software), in conjunction with TLC, showed that the vanadium (V) species gave the highest yield of 7-keto product while vanadium (III) acetylacetonate gave the lowest. Among the vanadium (IV) species, $VO(acac)_2$ gave the highest yield. It could be said that all metal-TBHP reactions should yield the same results given that the metal catalysts does not, to a large degree, interact with the substrate directly. However, that is not considering that different catalyst emit different radicals and ligands which can form side products rather than the desired product.

3.5. Replicability

The Q-TOF data also gave us an idea of the replicability of this reaction. Oxysterol yields consist of two phases, oxidation and isolation. Isolation is by far the more difficult and most precarious of the two. Thus, Q-TOF analysis of neat product allowed us to gain a more variable-free analysis. To test the value of this method, its precision and accuracy, we ran three trials with $VO(acac)_2$ several weeks apart with Q-TOF maintenance performed in between and through comparison of Q-TOF ion counts, found the 7-ketocholesterol yield of the reaction to be very consistent, showing both our reaction and Q-TOF method to be reproducible. Being reproducible established that the Q-TOF analysis had precision. Furthermore, the data was analyzed two separate ways with similar results also indicating that to a relative degree, there was accuracy. (The Q-TOF data is presented in the Supplementary material.)

4. Conclusion

Vanadyl acetylacetonate can be used as a pre-catalyst to oxidize steroidal alcohols under ambient conditions. Given the tolerance of this reaction, it should be found useful in the oxidation of other complex natural products.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2015.06.005.

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