Synthesis of Ergosta-5,22,24(28)-trienol from Stigmasterol

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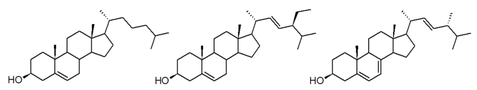
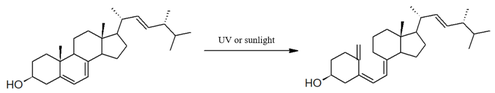
Background



cholesterol stigmasterol ergosterol

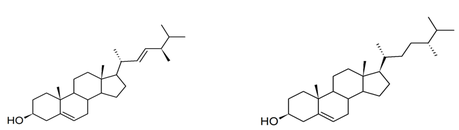
Sterols found in plants (e.g. stigmasterol) and fungi (e.g. ergosterol) are structurally similar to cholesterol but have an extra methyl or ethyl on C-24 of the side chain. Because of their structural similarity to cholesterol, these phytosterols can lower low-density lipoprotein (LDL) cholesterol levels by displacing cholesterol from intestinal micelles.[1] Thus, foods containing phytosterols are thought to have important health benefits. Mushrooms, in particular, have attracted attention for this and are also a rich, natural food source of vitamin D_2. Vitamin D is critical to bone health and has received increased notice for its role in immune function and prevention of some types of cancer and various diseases.[2–5] Vitamin D_2 in mushrooms is produced by exposure to sunlight and other sources of UV light, a direct consequence of their ergosterol content.[6] Therefore, commercially produced mushrooms are sometimes UV treated to increase Vitamin D_2 levels.



Ergosterol Vitamin D_2

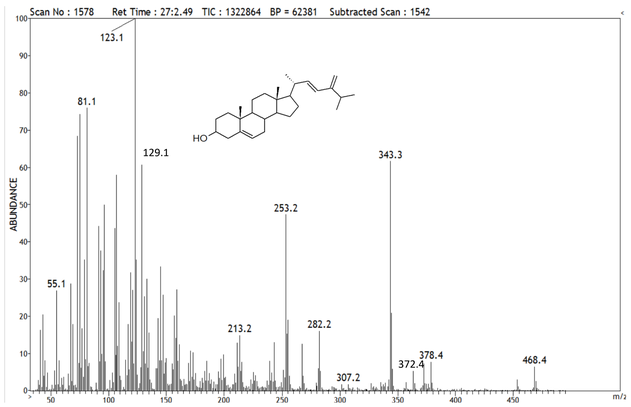
Other mushroom sterols, for example ergosta-5,7-dienol, have recently been shown to undergo photolytic reactions to form previously unrecognized forms of vitamin D (vitamin D_4).[7,8] These D vitamins may turn out to be useful pharmaceutical agents, illustrating the need for more complete knowledge of mushroom sterol profiles.[9]

This research project looked at morel mushrooms (*Morchella esculenta),* which are both highly valued for their delicious flavor and very high vitamin D_2 content.[7] Morels are also interesting because they contain high amounts of brassicasterol and campesterol and relatively little of the usual dominant ergosterol.[7]



GC-MS analysis of TMS-derivatized sterols from dried morels in our lab verified the presence of ergosterol, brassicasterol, and campesterol. More significantly, our work identified several previously unknown sterols in morels including 24-methylenecholesterol and ergosta-7,24(28)-dienol (episterol) based on matching the mass spectra to literature spectra. However, an unidentified compound with a M^+ at *m/z* = 468, indicating a trienol, was also detected. The mass spectrum also showed peaks at *m/z* = 129 ($TMSOC_3H_4^+$), indicating a double bond at C-5, and 123 ($C_9H_{15}^+$), 343 (M – C_9H_{15}, -H_2), and 372 (M – C_7H_{12}) indicating a di-unsaturated side chain (Fig. 1). Thus, this unknown compound has been proposed to be ergosta-5,22,24(28)-trienol (**1**). An intriguing possibility is that an uncommon biosynthesis pathway to brassicasterol, which proceeds through **1** instead of the normal intermediate, campesterol, occurs in morels.

Figure 1: Mass Spectrum of Unknown Sterol TMS Ether in Morels



Unfortunately, **1** is a rare compound and the mass spectrum of its TMS ether has not been published. Therefore, the purpose of this research was to synthesize **1** to prove the identity of the unknown compound. We envisioned the synthesis of **1** beginning with stigmasterol, which is converted in four steps using a literature method[12,13] to **5**. Sequential Julia and Wittig reactions followed by hydrolysis of the acetate ester would then afford compound **1** (Scheme 2).

Results

Scheme 1: Synthesis of Modified Julia Reagent

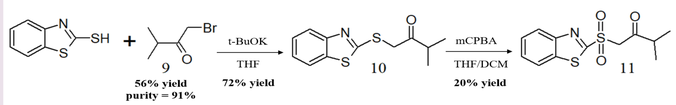
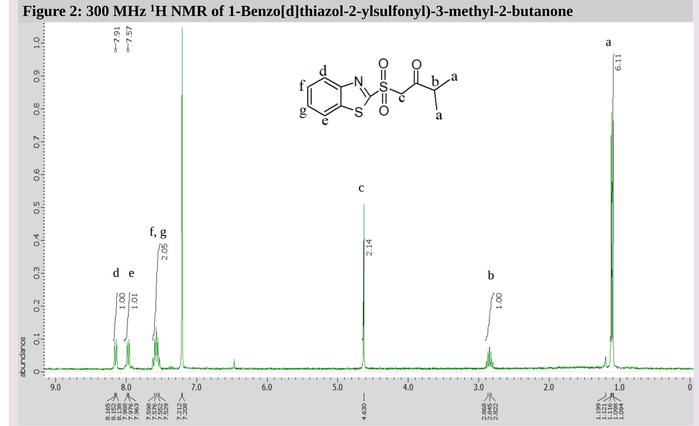


Figure 2: 300 MHz ^{1}H NMR of 1-Benzo[d]thiazol-2-ylsulfonyl)-3-methyl-2-butanone



Scheme 2: Synthesis of Ergosta-5,22,24(28)-trienol

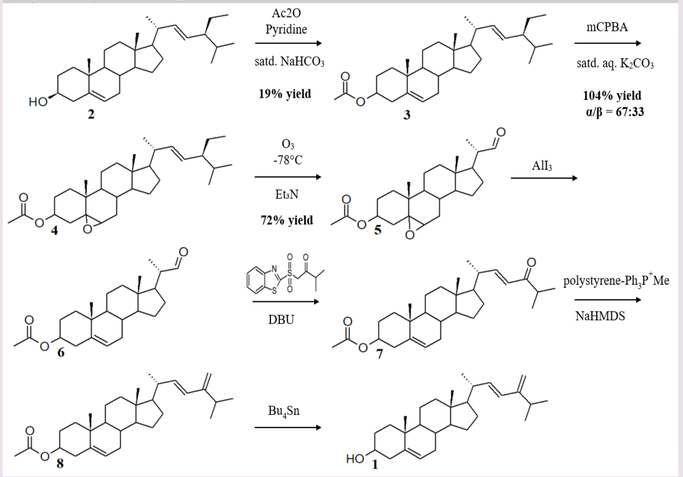
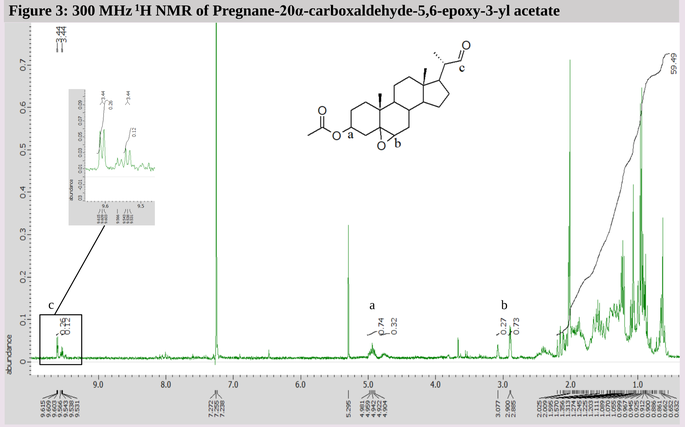


Figure 3: 300 MHz ^{1}H NMR of Pregnane-20*α*-carboxaldehyde-5,6-epoxy-3-yl acetate



Procedure

1-Bromo-3-methyl-2-butanone (9)[10]

To a 0-5 °C solution of 3-methyl-2-butanone (15 g, 174 mmol) in methanol (105 mL), bromine (28 g, 174 mmol) was added, forming a dark red color. The solution was stirred for 1.5 h during which the reaction temp was allowed to reach 20 °C. Once the red color faded, water (52 mL) was added and the solution was stirred at room temp overnight. Water (160 mL) was added and the solution washed with DCM (4 x 85 mL). The organic layers were combined and washed with aq 10% potassium carbonate (35 mL) and water (2 x 35 mL), then dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo*, yielding the crude product (29.31 g, 102%), which was distilled under reduced pressure to give a light brown liquid product (16.03 g, 56%). The ^{1}H NMR showed the desired **9** along with the 3-bromo isomeric product in a 91:9 ratio. ^{1}H NMR ($CDCl_3$) 3.98 (s, 2H), 2.99-2.92 (m, 1H), 1.15 (d, J= 6.9 Hz, 6H), APCI-MS, *m/z* = 164.9, 166.9 (M + H).

1-Benzo[d]thiazol-2-ylthio)-3-methylbutan-2-one (10)[11]

A suspension of 2-mercaptobenzothiazole (7.31 g, 44 mmol) and t-BuOK (5.40 g, 48 mmol) in THF (60 mL) was refluxed for 30 min. Over 10 min, **9** (8 g, 48 mmol) was added dropwise. The solution was refluxed for 5 h, then cooled to room temp. After water (45 mL) was added, the solution was extracted using DCM (3 x 45 mL) then dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo* to give the crude product (11.28 g, 93%). The crude (5 g) was purified by silica column chromatography (ethyl acetate: hexane = 1:3) to give a **10** as a light brown solid (3.84 g, 72%): mp 37-41°C; ^{1}H NMR ($CDCl_3$) 7.88 (d, J= 8.3 Hz, 1H), 7.73 (d, J= 8.3 Hz, 1H), 7.42 (t, J= 16.5 Hz, 1H), 7.30 (t, J= 16.2, 1H). 4.45 (s, 2H), 3.0-2.9 (m, 1H), 1.21 (d, J= 6.9, 6H); EI-MS, *m/z* = 219, 176, 149, 121, 108, 71, 63. APCI-MS, *m/z* = 252.1 (M + H).

1-Benzo[d]thiazol-2-ylsulfonyl)-3-methyl-2-butanone (11)[11]

To a solution of **10** (1.136 g, 4.5 mmol) in THF (7 mL) was added *m*-CPBA (1.93 g, 11 mmol) in DCM (12 mL) dropwise at room temp. After the reaction was complete, as detected by TLC, sodium bicarbonate (10 mL) was added. The mixture was extracted with ethyl acetate (3 x 25 mL), dried over anhydrous Na_2SO_4, and the solvent removed *in vacuo* to give the crude product (1.5 g, 117%), which was recrystallized (methanol) to afford **11** as a crystalline white solid (0.25 g, 19.5%): mp 104-108°C; IR 2972, 2921, 2875, 2360, 1718 cm-1; ^{1}H NMR ($CDCl_3$) 8.15 (d, J= 7.9 Hz, 1H), 7.98 (d, J= 7.6 Hz, 1 H), 7.60-7.53 (m, 2H), 4.63 (s, 2H), 2.87-2.82 (m, 1H), 1.11 (d, J= 6.9 Hz, 6H); EI-MS, m/z = 219, 177, 162, 148, 136, 109, 69, 63, 43; APCI-MS, *m/z* = 283.70 (M + H).

Stigmasterol acetate (3)[12]

Acetic anhydride (36 mL, 0.39 mol) was added to a stirred solution of **2** (2.5 g, 6 mmol) in absolute pyridine (72 mL) containing catalytic amounts of DMAP. The resulting solution was stirred at room temp for 0.5 h then treated with $NaHCO_3$(sat) at 0 °C. The solution was extracted with ethyl acetate (3 x 50 mL) and the organic layers were combined, washed with water (60 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give crude **3** with significant contamination by pyridine as determined by ^{1}H NMR. Thus, the crude was dissolved in ethyl acetate (150 mL) and washed with HCl (0.5 M) and water twice more. After drying and removal of the solvent, **3** was obtained as a pale yellow solid (0.52 g, 19%): ^{1}H NMR ($CDCl_3$) 5.15 (dd, J= 15.1, 8.3 Hz, 1H), 5.01 (dd, J= 15.0, 8.4 Hz, 1H) 4.65-4.53 (m, 1H) 2.31 (d, J= 7.6 Hz, 2H), 2.03 (s, 3H).

α and β Epoxides of stigmasterol acetate (4)[13]

To a 0 °C solution of **3** (0.52 g, 1.1 mmol) in DCM (35 mL) was added *m*-CPBA (0.23 g, 1.3 mmol). After 4 h at 0°C, the reaction was diluted with satd. aq. K_2CO_3 (135 mL) and extracted with DCM (3 x 100 mL). The combined organic layers were dry over anhydrous Na_2SO_4 and concentrated *in vacuo* to give a 3:1 mixture of diastereomers (0.56 g, 104%): ^{1}H NMR ($CDCl_3$) 5.10 (dd, J= 15.3, 8.4 Hz, 1H), 5.03-4.70 (m, H) 3.07 (d, J= 2.1 Hz, 0.33H), 2.88 (d, J = 4.1 Hz, 0.68H), 2.03 (s, 3H).

Pregnane-20*α*-carboxaldehyde-5,6-epoxy-3-yl acetate (5)[13]

A -78 °C solution of **4** (0.35 g, 0.74 mmol) in 1:1 DCM/MeOH (12 mL) was treated with a gaseous stream of ozone (1 g/h) from an A2Z Ozone MP 1000 110V Multi-Purpose Ozone Generator for 1.5 h. The solution was purged with oxygen (5 min). Triethylamine (0.21 mL, 1.5 mmol) was added and stirred for several days at room temp. The solution was extracted with DCM (50 mL), washed with HCl (3 x 30 mL) and water (30 mL) then dried over Na_2SO_4 and concentrated *in vacuo* to afford a sticky, clear substance (0.21 g, 72%): ^{1}H NMR ($CDCl_3$) 9.61 (d, J= 3.44 Hz, 0.26H), 9.54 (d, J= 3.44 Hz, 0.12H), 5.01-4.70 (m, 1H), 3.08 (d, 0.27H), 2.89 (d, J= 4.5 Hz, 0.73H).

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Discussion

Synthesis of the modified Julia reagent commenced with the bromination of 3-methyl-2-butanone using molecular bromine (Scheme 1). The desired **9** was obtained with good yield (102%). However, the isomer 3-bromo-3-methyl-2-butanone (9%) was observed in the ^{1}H NMR spectrum. Distillation to remove the minor isomer was unsuccessful but lowered our yield (56%). The bromoketone mixture was reacted with 2-mercaptobenzothiazole and t-BuOK in THF to afford **10** with a good crude yield (93%). Purification using silica column chromatography (ethyl acetate:hexane = 1:3) gave a pure product (72%) as determined by ^{1}H NMR. Oxidation with mCPBA gave the desired sulfone, **11,** in a crude yield of 117%. Recrystallization afforded a white powder (17%) with good purity according to ^{1}H NMR. GC-MS analysis also showed good purity but was puzzling because it showed an apparent M^+ at *m/z* = 219. Rather than M+, this corresponds to a loss of SO_2 (64 g/mol) from the molecular ion (*m/z* = 283) indicating an intriguing rearrangement of the product in the mass spectrometer. APCI mass spectrometry confirmed the molecular weight as 283 showing M+H at *m/z* = 284.

The synthesis of **1** began with successful acetylation of stigmasterol **2** by acetic anhydride to form stigmasterol acetate **3** (Scheme 2). Although the yield (19%) was disappointing, the ^{1}H-NMR spectrum closely matched the lit spectrum aside from the presence of ethyl acetate impurity. Epoxidation of **3** using *m*-CPBA resulted in a yield of 104%, with water as the most likely impurity inflating the yield. Additionally, both α and β isomers were observed in the ^{1}H NMR spectrum in a ratio of 0.67:0.33 in agreement with the literature ratio (0.72:0.28).

The most recent synthesis step completed was the ozonolysis of compound **4**. Due to safety concerns, we deviated from the literature procedure, which called for concentration *in vacuo* after O_3 addition, followed by re-dissolution in H_2O/AcOH and treatment with zinc. Rather, a work-up with triethylamine (2 equiv) was used. For O_3 addition, we used an inexpensive multi-purpose ozone generator (rated at 1 g/h and purchased from Amazon) connected to an O_2 tank. To test the capability of this O_3 generator, a solution of 1-octene in 1:1 DCM/MeOH was treated at -78 °C with a gaseous stream of ozone for 1.5 h, then successfully treated with triethylamine to give heptanal and formaldehyde as indicated by ^{1}H NMR. This procedure was then used for the attempted ozonolysis of **4**. The product showed two aldehyde doublets (9.61, 9.52 ppm) with the same coupling constant (3.4 Hz) and the predicted 2:1 α/β ratio in the ^{1}H NMR spectrum (Figure 3). In addition, the side chain double bond was fully reacted as indicated by the disappearance of the doublets of doublets at 5 ppm. Thus, the ozonolysis went to completion. Unfortunately, for reasons we have yet to determine, the integration values of the aldehyde doublets (0.26, 0.12) are small compared to the other peaks and there appear to be additional smaller aldehyde peaks. It is possible that the triethylamine work-up caused epimerization at the α-carbon of the aldehyde, something we were not anticipating, leading to the extra aldehyde peaks observed. Therefore, the next step of this research will be to look further into what is causing the small aldehyde peaks and repeating the ozonolysis with a different reductive work-up reagent, e.g. dimethylsulfide or zinc..

Conclusions

Synthesis of the modified Julia reagent was successfully completed in three steps in which **9**, **10**, and **11** were produced with good purity and decent yield (56%, 72%, 20%).

The first two steps in the synthesis of **1**, acetylation of stigmasterol then epoxidation using mCPBA, were accomplished with yields of 19% and 104%. Following epoxidation, both α and β isomers were observed in a ratio of 0.67:0.33.

Currently, we are looking further into the results of our ozonolysis reaction to determine why the aldehyde peaks are small compared to the other peaks. We may repeat the ozonolysis again with an alternative work-up.

After synthesizing **5** with good purity and yield, we will remove the epoxide using AlI_3 because of its potential to react with the nucleophilic Julia reagent (**11**).

We anticipate that sequential Julia and Wittig reactions followed by hydrolysis of the acetate ester will afford **1**.

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