Natural Product Synthesis

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Synthesis of Strophasterol A Guided by a Proposed Biosynthesis and Innate Reactivity

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Dedicated to Professor K. C. Nicolaou on the occasion of his 70th birthday

Abstract: *The synthesis of strophasterol A, a moderator of endoplasmatic reticulum (ER) stress in Alzheimer's disease, and the first member of a structurally unprecedented class of secosterols, was achieved through the implementation of a key step of its proposed biosynthesis and two C–H oxidations. Analysis of the innate reactivity of the intermediates enabled the identification of a novel way to prepare an α-chloro-γ-hydroxy-δ-keto enone, as well as its vinylogous α-ketol rearrangement to a δ-keto carboxylic acid.*

Neurodegenerative diseases significantly contribute to the global burden of disease: more than 40 million people suffer from dementia worldwide, and Alzheimer's disease (AD) is the most frequent cause. AD is characterized by progressive memory loss that may be associated with behavioral changes; patients may also experience language disorders, impairment of visuospatial skills, and executive dysfunction. The neuropathological hallmarks of AD are amyloid plaques and neurofibrillary tangles.[1] The major risk factor for AD is ageing.[2] In addition, genetic predisposition (e.g., the $APOE_4$ allele) and lifestyle factors like obesity and physical inactivity significantly increase the risk of developing AD. Only 5% of AD cases are inherited or result from mutations in the genes encoding the amyloid precursor protein (APP), presenilin 1 (PS1), or presenilin 2 (PS2) for early-onset AD, or TREM2 and CD33 for late-onset AD.[3] The presenilins (6–8 transmembrane proteins) are mainly localized to the endoplasmic reticulum, where they are part of the γ-secretase protein complex that cleaves APP to generate Aβ peptides. The resulting amyloidogenic peptides can damage neurons by inducing membrane-associated oxidative stress, thereby rendering neurons vulnerable to Ca^{2+} overload, particularly under conditions of impaired mitochondrial energy production.[4] The elevated intracellular Ca^{2+} levels also contribute to tau hyperphosphorylation and the formation of neurofibrillary tangles. It has been shown in APP/PS1 transgenic mice that ER stress triggered by Aβ promotes neurotoxicity through increased mitochondrial cholesterol influx.[5]

Strophasterols A–D (**1A**–**D**; Figure 1), four secosterols with an unprecedented carbon skeleton, have recently been isolated from the mushroom *Stropharia rugosoannulata*, which is abundant in the northern temperate zone.[6] While the structures of the former two (strophasterols A and B, **1A** and **1B**) have been unequivocally determined by single-crystal X-ray diffraction,[7] the structures of strophasterols C and D (**1C** and **1D**) remain uncertain with regard to their relative and absolute configuration. A biosynthetic hypothesis has been put forward entailing either a retro-aldol/epoxide-opening cascade, or (and as depicted in Scheme 1) radical cleavage of the C14–C15 bond in a potential precursor **2**, followed by reductive radical cyclization onto C22 of the Δ22,23 double bond (see structure **3**). This biosynthetic proposal was further supported by isolation of the corresponding alcohol of **2** from the same mushroom.[8] Strophasterol A (**1A**) has been shown to reduce ER stress; some other structurally related natural products that might or might not represent biosynthetic precursors to the strophasterols are known to possess comparable, although weaker, activities.[8] Strophasterol A may therefore present an opportunity for the treatment of AD on the molecular level through stabilization of the age- and disease-related processes that impose oxidative and proteotoxic stress on the ER.

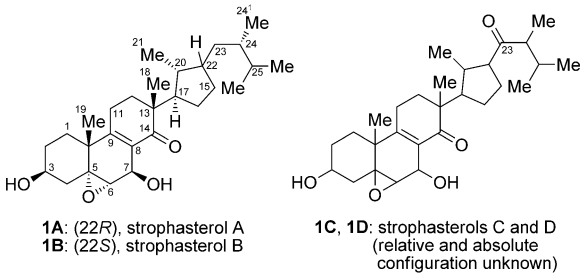

Figure 1. Molecular structures of strophasterols A–D (**1A**–**D**).

Given the unprecedented structures and promising biological features of the strophasterol class of natural products, along with the cloud of uncertainty that shrouds the structures of two members of this family (strophasterols C and D, **1C** and **1D**), a program to gain synthetic access to this class of natural products as well as to designed analogues was recently initiated in our laboratory. We here report the first synthesis of strophasterol A (**1A**), which explores and exploits the innate reactivity of the intermediates through C–H oxidation and also sheds some light on the proposed biosynthesis of the strophasterol class of natural products.

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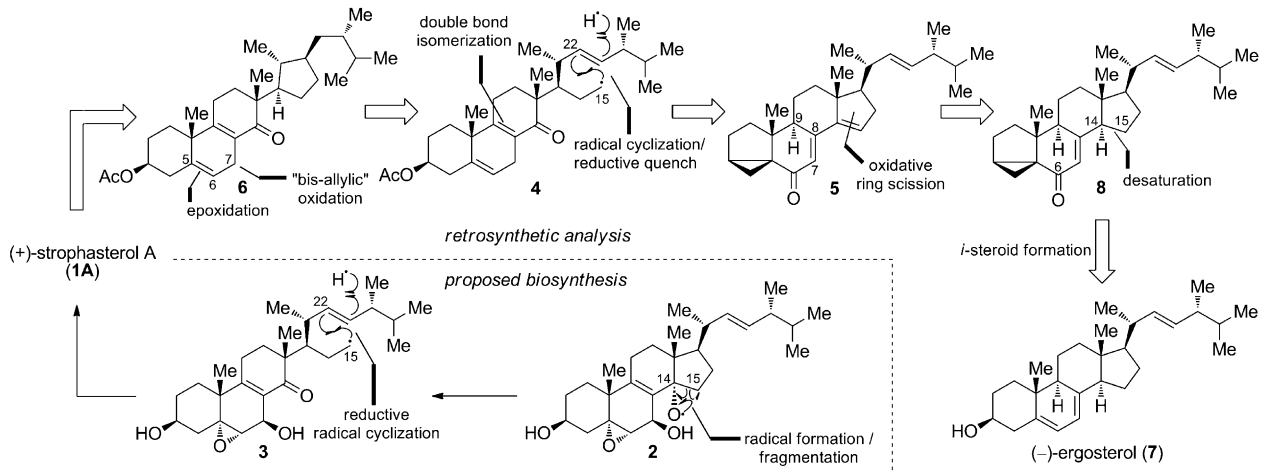
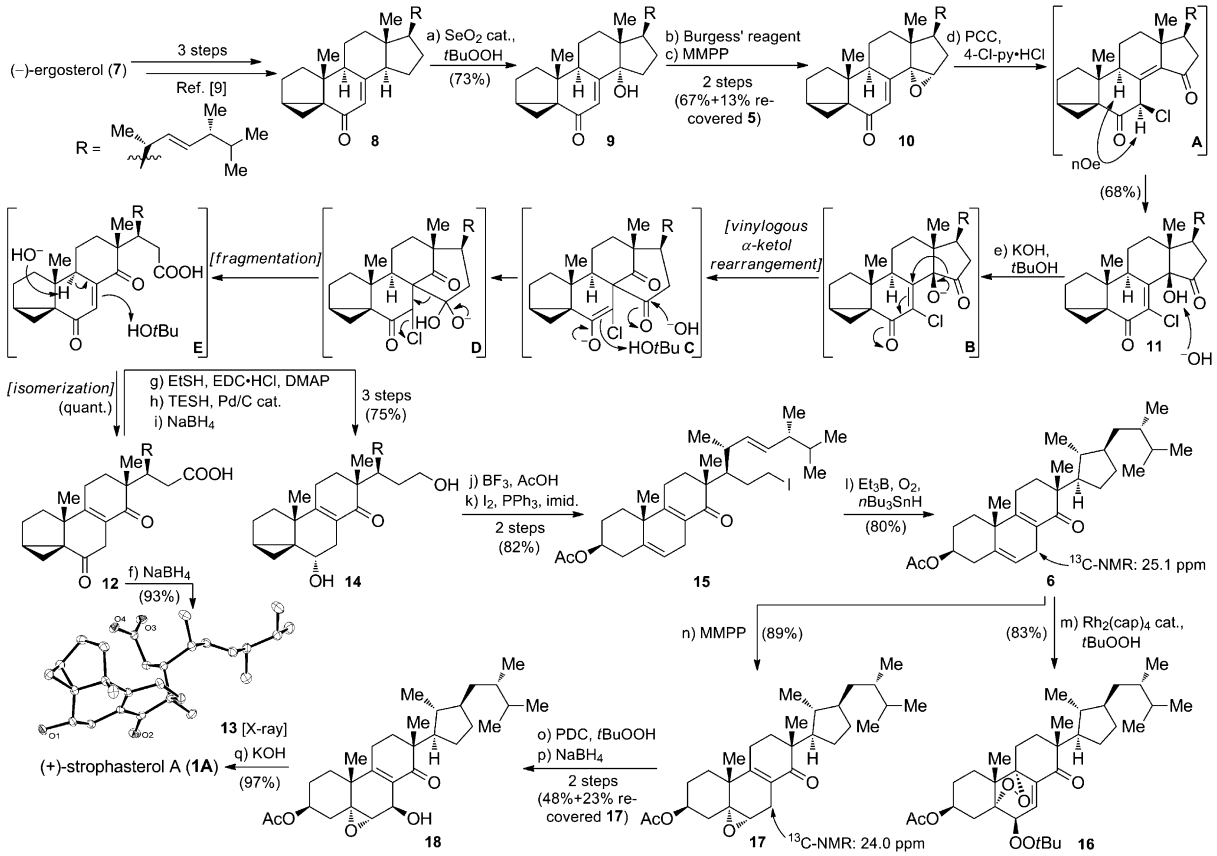


Scheme 1. Retrosynthetic analysis and proposed biosynthesis of strophasterol A (**1A**).

Our retrosynthetic analysis of strophasterol A (see Scheme 1) is based on the proposed biosynthetic pathway and involves a sequence of oxidative ring scission and radical ring formation (with the latter following a 5-*exo*-trig pathway, see structure **4**) as a logical means to convert an androstane skeleton (like **5**) into the desired 14,15-secosterol skeleton **6**. Diene **6** can act as a viable intermediate to strophasterol A if two oxidation-state adjustments can be realized: 1) oxidation of the "bis-allylic" C7 position, and 2) epoxidation of the electron-rich Δ5,6 double bond. The androstane precursor **5**, on the other hand, was retrosynthetically connected to the readily obtainable ergosterol (**7**) as a starting point of our synthetic efforts through transposition of reactivity towards C−H oxidation in the C14 position through conversion of the homoallylic alcohol in **7** into *i*-steroid **8**.

Our synthesis of strophasterol A (**1A**) is shown in Scheme 2 and commenced with the conversion of ergosterol (**7**) into *i*-steroid enone **8** by employing a reported three-step procedure.[9] In this approach, 1) mesylation of the C3 hydroxyl moiety, followed by 2) aqueous basic treatment, and 3) oxidation of the so-obtained labile allylic alcohol to enone **8** furnished decagram quantities for probing of the first coveted C−H oxidation. Gratifyingly, treatment of **8** with substoichiometric amounts of SeO_2 and stoichiometric *tert*-butyl hydroperoxide enabled its clean conversion into γ-hydroxy enone **9** in 73% yield. Elimination of the newly installed hydroxy moiety to furnish dienone **5** (see Scheme 1) was accompanied by isomerization of the Δ7,8 double bond to the 8,9 position. While initially, this reactivity seemed desirable (since it installed the correct Δ8,9 double bond found in the strophasterols), the so-obtained intermediate turned out to be a dead-end for our synthetic efforts: Extensive experimentation failed to identify a suitable means for the selective oxidation (either by dihydroxylation or epoxidation) of the Δ14,15 double bond without epoxidizing the more reactive Δ8,9 double bond or dihydroxylating the Δ22,23 double bond. We thus turned our attention to the elimination step again and discovered a two-step approach that proceeded without isomerization of the Δ8,9 double bond: 1) Burgess elimination[10] and 2) selective epoxidation (d.r. = 25:1) of the so-obtained Δ14,15 double bond using magnesium bis(monoperoxyphthalate) (MMPP, 67% yield over the two steps, 13% recovered dienone **5**).

Initial experiments for oxidative cleavage of this epoxide (using, for example, H_5IO_6, $NaIO_4$, or $Pb(OAc)_4$) produced low yields of keto aldehyde and extensive decomposition, likely due to sluggish reactivity of the intermediary *trans*-diol. A step-wise cleavage, beginning with the oxidation of epoxide **10** into an α-hydroxy ketone, was envisioned instead. Attempts using pyridinium chlorochromate provided no desired product but appreciable amounts of a different product, the structure of which was identified as α-chloro-γ-hydroxy-δ-keto enone **11** by 2D-NMR spectroscopy. The *cis* junction of the CD ring was assigned based on Zürcher's prediction model for distorted steroid systems.[11] In terms of mechanism, we assume an initial vinylogous epoxide opening by chloride (from PCC) to generate **11** from **10**. This mechanistic interpretation was further supported by isolation and structural elucidation of the fleeting intermediate **A**, which was competent under the same reaction conditions to form product **11**. The β-Cl stereochemistry of **A** was assigned based on nOe spectroscopic analysis (cross-peak between α-H7 and α-H9). In an attempt to further increase the yield of **11**, pyridine·HCl derivatives as a source of chloride were tested as additives, with 4-chloro-pyridine·HCl giving the highest yield of **11** (68%). The unusual molecular structure of **11** prompted our interest in investigating its reactivity. Under basic conditions (KOH in *t*BuOH), we observed the quantitative transformation of **11** to give a single product. NMR spectroscopy and mass spectrometry analysis strongly supported its structural assignment as keto acid **12**. Further proof was then obtained through reduction of the C6 ketone in **12** with $NaBH_4$ to give a single diastereoisomer, single-crystal X-ray diffraction analysis[12] of which revealed the molecular structure to be that of 6α-hydroxy keto acid **13**, thus indirectly confirming the correct assignment of **12**. Mechanistically, we propose that deprotonation of the tertiary alcohol in **11** initiates a vinylogous α-ketol rearrangement (see **B**→**C**), rupturing the C14−C15 bond and forming a new but fleeting C8−C15 bond in the process. A hydrate formed from the C15



Scheme 2. Synthesis of strophasterol A (**1A**). Reagents and conditions: a) SeO_2 (0.5 equiv), *t*BuOOH (4.0 equiv), CH_2Cl_2, 60 °C (sealed tube), 17 h, 73 %; b) 1-methoxy-*N*-triethylammoniosulfonyl-methanimidate (1.4 equiv), PhMe, 60 °C, 16 h, 93 %; c) MMPP·6 H_2O (80 %, 1.1 equiv), MeOH, 25 °C, 24 h, 72 % (d.r. = 25:1) + 13 % recovered **5**; d) PCC (4.0 equiv), 4-chloro-pyridine·HCl (6.0 equiv), $MgSO_4$ (7.2 equiv), CH_2Cl_2, 25 °C, 17 h, 68 %; e) KOH (5.0 equiv), *t*BuOH, 50 °C, 20 min, quant.; f) $NaBH_4$ (10 equiv), MeOH, 0 °C, 2 h, 93 %; g) EtSH (4.0 equiv), EDC·HCl (1.5 equiv), DMAP (0.1 equiv), CH_2Cl_2, 0→25 °C, 16 h; h) TESH (4.0 equiv), Pd (10 % on C, 0.1 equiv), acetone, 25 °C, 1.5 h; i) $NaBH_4$ (5.0 equiv), MeOH, 0 °C, 30 min, 75 % for three steps; j) BF_3·Et_2O (80 equiv), Et_2O/AcOH (2:1), 25 °C, 15 min, 91 %; k) I_2 (1.5 equiv), PPh_3 (1.4 equiv), imid. (1.5 equiv), Et_2O/MeCN (5:3), 0 °C, 45 min, 90 %; l) Et_3B (1.0 M in hexanes, 1.5 equiv), nBu_3SnH (2.2 equiv), O_2, PhMe, 0 °C, 4 h, 80 %; m) $Rh_2(cap)_4$ (0.1 equiv), *t*BuOOH (70 % aq., 5.0 equiv), 1,2-dichloroethane, 25 °C, 3 h, 83 %; n) MMPP·6 H_2O (80 %, 1.1 equiv), CH_2Cl_2/H_2O (150:1), 25 °C, 24 h, 89 % (d.r. > 50:1); o) PDC (10 equiv), *t*BuOOH (5.5 M in decane, 50 equiv), Celite®, C_6H_6, 25 °C, 24 h; p) $NaBH_4$ (10 equiv), MeOH, −15 °C, 20 min, 48 % (d.r. = 5:1) + 23 % recovered **17**; q) KOH (5 % in MeOH, 65 equiv), 25 °C, 20 min, 97 %. DMAP = 4-dimethylaminopyridine; EDC = *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide; imid. = imidazole; MMPP = magnesium bis(monoperoxyphthalate); nOe = nuclear Overhauser effect; PCC = pyridinium chlorochromate; PDC = pyridinium dichromate; $Rh_2(cap)_4$ = dirhodium tetracaprolactamate; TESH = triethyl silane.

ketone (see **C**→**D**) may then rearrange in a semi-benzilic acid fashion under cleavage of the C8–C15 bond (to form a carboxylic acid at C15) and loss of chloride to give ene-1,4-dione **E**. Isomerization of the Δ7,8 double bond of the latter to the 8,9 position was already earlier proven to be a facile process and would lead to observed product **12**. Notably, the overall process is redox neutral.

With gram amounts of **12** in hand, we turned our attention to the proposed biomimetic radical cyclization. Reduction of the carboxylic acid in **12** by employing the Fukuyama protocol[13] under concomitant reduction of the C6 carbonyl group (1. EtSH, EDC·HCl, DMAP; 2. TESH, Pd/C cat.; 3. $NaBH_4$) gave diol **14** in 75 % yield for the three steps. Unmasking of the *i*-steroid (BF_3, HOAc)[14] furnished a homoallylic acetate, the primary alcohol of which was then converted into iodide **15** (I_2, PPh_3, imid.; 82 % yield for the two steps). Gratifyingly, iodide **15** lent itself readily to the coveted radical cyclization (Et_3B, O_2, nBu_3SnH)[15] and gave the desired cyclopentane **6** in a convincing yield of 80 %. With only two oxidation-state adjustments remaining for completion of the synthesis, we investigated the propensity of **6** to undergo oxidation at C7. Against our original rationale of "bis-allylic" C7 being the most reactive center to undergo C–H oxidation preferentially, all attempts to introduce oxygen in this position met with failure. In particular, 1,3-endoperoxide **16** (stereochemistry at C6 assigned tentatively on the grounds of nOe spectroscopic evidence) was found to be the main product under a variety of conditions.[16] We hence attempted epoxidation of the Δ5,6 double bond to selectively generate compound **17** (MMPP, 89 % yield, d.r. > 50:1). The ^{13}C-NMR shift comparison of C7 between olefin epoxide **17** and diene **6** revealed only a small difference of $\Delta\delta = -1.1$ ppm, which indicates that they are comparably reactive. Endoperoxide formation was not possible for **17** anymore, though. Indeed,

this reactivity assignment proved correct, with pyridinium dichromate/*t*BuOOH[17] successfully providing the desired, but fleeting, 7-keto compound.[18] It proved crucial to immediately reduce this species and convert it into the stable and isolable alcohol **18** ($NaBH_4$, 48% yield for the two steps, d.r. = 5:1, 23% recovered **17**). Finally, deacetylation (KOH in MeOH, 97% yield) gave strophasterol A (**1A**) in 15 steps and an overall yield of 6% from known compound **8**.[19]

The described synthesis renders strophasterol A readily available for thorough biological investigations and opens the way for the synthesis of the whole strophasterol class of natural products, as well as for analogue design, synthesis, and biological evaluation. It also provides the foundation for method development and reactivity studies of α-chloro-γ-hydroxy-δ-keto enones in vinylogous α-ketol rearrangements. Further research in these areas is currently in progress in our laboratory.

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Keywords: α-ketol rearrangement · Alzheimer's disease · C–H activation · strophasterol A · total synthesis

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[19] All physicochemical and spectroscopic properties matched reported data (see Ref. [6]).

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