Natural Product Synthesis

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Total Synthesis of (−)-Episilvestrol and (−)-Silvestrol**

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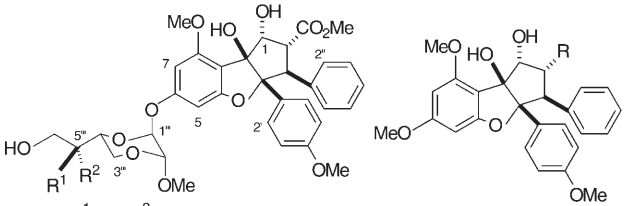
Extracts of the bark of the woody South-East Asian shrub *Aglaia leptantha*, Miq. (Meliaceae) show potent cytotoxic activity, which is attributed to two new molecules **1** and **2**.[1]



1 R^1 = H; R^2 = OH; Silvestrol
2 R^1 = OH; R^2 = H; Episilvestrol

3 R = CO_2Me; Methyl rocaglate
4 R = $CONH_2$; Rocaglamide

Compounds **1** and **2** are diastereoisomers (epimers at 5‴) that contain a common cyclopenta[*b*]benzofuran core,[2] found in the related *Aglaia* metabolites aglafolin[3] (methyl rocaglate, **3**)[4–6] and rocaglamide (**4**),[6,7] as well as a novel, unprecedented 1,4-dioxanyloxy pseudosugar substituent. In addition, two metabolites, named silvestrol and episilvestrol were isolated from *Aglaia foveolata* Pannell by Kinghorn and coworkers and found to be identical to **1** and **2**, respectively.[8] The structure of silvestrol (**1**) has been determined by NMR spectroscopy and X-ray analysis of a derivative which served to confirm the relative and absolute configuration of this compound.

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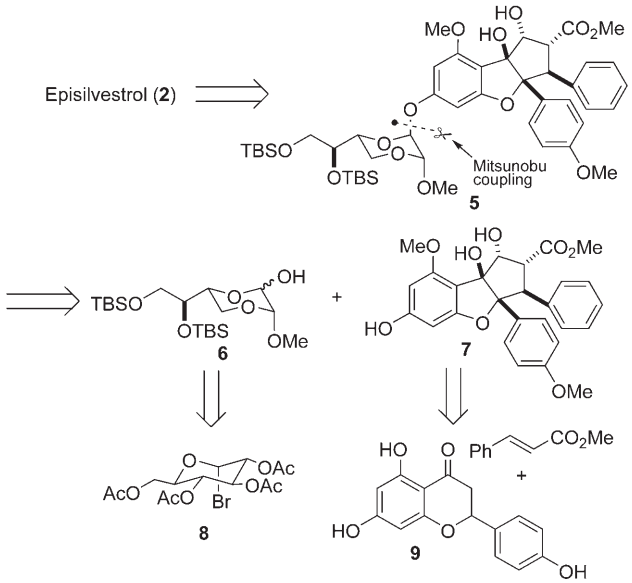
Both silvestrol (**1**) and episilvestrol (**2**) show comparable potent cytotoxic activity against several human tumor cell lines including lung, prostate, and breast cancer with IC_{50} values ranging from 1 to 7 nM.[1,8] Recently, it has been demonstrated that the cytotoxicity induced by silvestrol (**1**) in human prostate cancer (LNCaP) cells is associated with a block in the cell cycle at the G2/M checkpoint.[9] Specifically, compound **1** alters the expression of genes regulating apoptosis and cell cycle in a way that is not dependent on human tumor protein 53 (p53). Interestingly, compounds that possess only the parent cyclopenta[*b*]benzofuran, such as **3** and **4**, are significantly less active, suggesting that the presence of the 1,4-dioxanyloxy group is critical for activity.[1] The in vivo activity of **1** has also been demonstrated. Administration of silvestrol (**1**) by intraperitoneal injection (3 mg kg^{-1}) to athymic mice with human prostate cell xenografts reduced tumor weights by about 60% after 29 days, while the body weight of the mouse was unaffected.[1] Unfortunately, the paucity of both **1** and **2** from the natural source (0.01% isolated yield) precludes isolation as a sufficient supply of these important compounds. Therefore, an efficient chemical synthesis is an attractive alternative for accessing quantities of **1** or **2** as well as analogues for further biological evaluation. We now report the total synthesis of (−)-episilvestrol (**2**) and (−)-silvestrol (**1**) from common simple precursors which could address the supply problem.[10]

Our retrosynthesis of episilvestrol (**2**; Scheme 1) is inspired, in part, by the proposed biosynthesis of the two



Scheme 1. Retrosynthetic analysis of episilvestrol (**2**). TBS = *tert*-butyldimethylsilyl.

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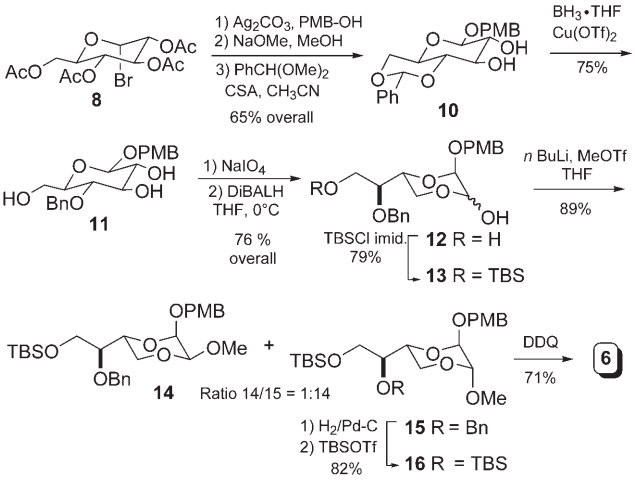
key fragments: the cyclopentabenzofuran core[2,11] and the 1,4-dioxanyloxy fragment.[12] Episilvestrol (**2**) could be produced by deprotection of precursor **5**, which in turn could be secured by a modified Mitsunobu-type[13] coupling between the hemiacetal **6** and core phenol **7**. Compound **6** could arise from the known D-glucose derivative **8** by following a route based on a biosynthetic proposal suggested by us.[12] The cyclopentabenzofuran core **7** could be secured from commercially available (±)-4′,5,7-trihydroxyflavanone (naringenin, **9**) and methyl cinnamate by adapting the elegant oxidopyrillium [3+2] cycloaddition approach to these systems as reported by Porco and co-workers.[5,6]

The synthesis of the dioxane fragment **6** (Scheme 2) is based on our approach to a model system.[12] Glycosylation of commercially available bromide **8** with *p*-methoxybenzyl

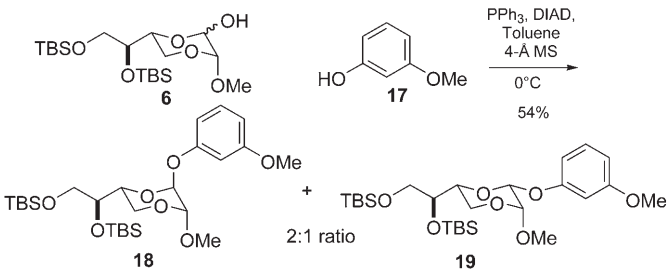


Scheme 2. Synthesis of 1,4-dioxane fragment **6**. PMB = *p*-methoxybenzyl, Bn = benzyl, CSA = 10-camphorsulfonic acid, DiBAL-H = diisobutylaluminum hydride, OTf = trifluoromethanesulfonate, DDQ = dichlorodicyanobenzoquinone.

alcohol mediated by silver carbonate followed by methanolysis of the acetate groups and benzylidene formation gave acetal **10**[14] in a reasonable overall yield. Exposure of **10** to BH_3·THF in the presence of $Cu(OTf)_2$[15] effected highly selective C–O6 benzylidene cleavage to afford the O4-protected sugar **11**. Periodate cleavage[16] followed by immediate selective reduction of the resultant aldehyde-lactol afforded lactol as an approximately 1:3 mixture of anomers.

Selective protection of the primary alcohol as the TBS ether and subsequent methylation of the lactol mixture using butyllithium and methyl triflate[17] gave the desired axial product **15** in high yield, along with a small amount of equatorial ketal **14**. The observed selectivity can be explained by rapid alkylation of the intermediate axial alkoxide at faster rate than the corresponding equatorial alkoxide. A protecting-group exchange was then carried out to swap the benzyl ether for a TBS group to provide **16**. DDQ-induced removal of the PMB group gave the lactols **6** as a roughly 1:1 mixture.

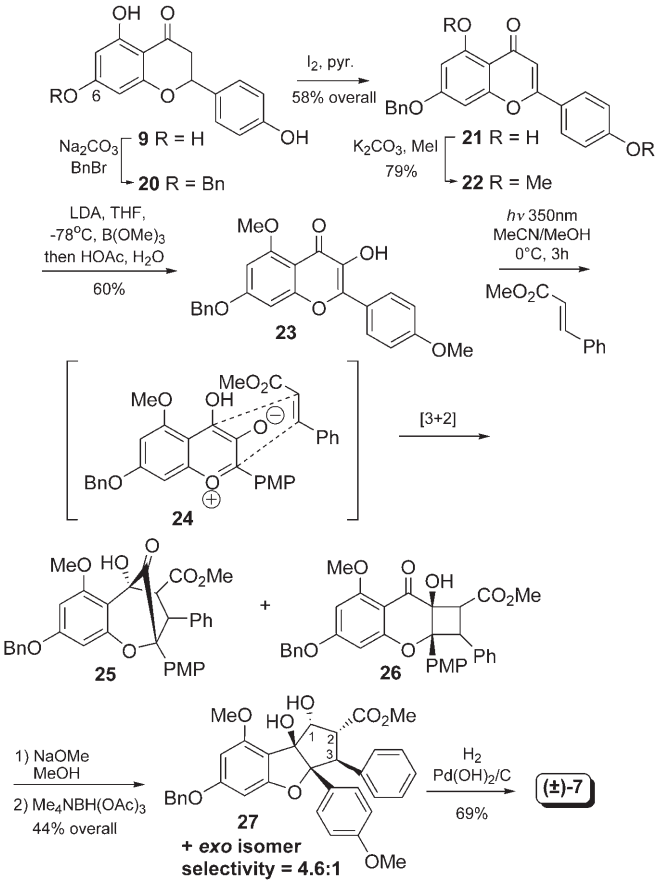
After some experimentation, we found that the Misunobu reaction as utilized by Roush and Lin for the synthesis of *O*-aryl glycosides[18] could be applied to couple the model phenol **17** and lactols **6** (Scheme 3). The addition of 4-Å molecular



Scheme 3. Model Mitsunobu coupling. DIAD = diisopropylazodicarboxylate.

sieves was critical to the success of this reaction, and the adducts **18** and **19** were obtained in 54% yield favoring the desired axial isomer **18**. With the success of this coupling in a model system, we next prepared the aromatic core **7** required for the production of **2**.

The synthesis of the required cyclopentabenzofuran core **7** was achieved as detailed in Scheme 4. Selective O6 benzylation of (±)-naringenin (**9**) using Na_2CO_3 as base afforded ether **20**, which was oxidized to the flavone **21** upon exposure to iodine in pyridine. Methylation provided the ether **22**, which could be converted into the cycloaddition precursor **23** through C3 deprotonation with LDA[19] and



Scheme 4. Synthesis of the cyclopentabenzofuran core (±)-**7**. Bn = benzyl, LDA = lithium diisopropylamide, pyr. = pyridine, PMP = *p*-methoxyphenyl.

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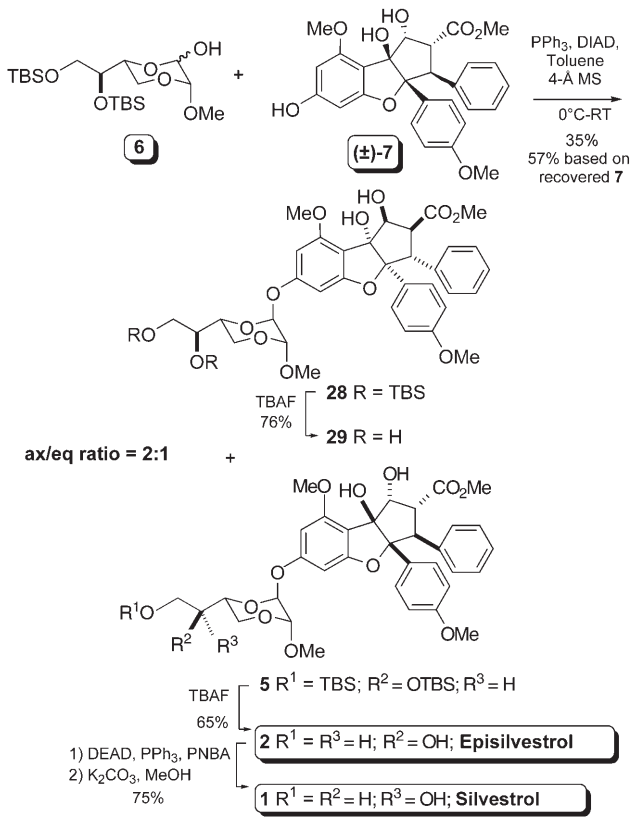
quenching of the trimethylborate followed by oxidative workup. Irradiation[20] of the 3-hydroxyflavone **23** (350 nm, 450-W medium-pressure Hg lamp) generated the dipole **24**, which underwent cycloaddition[5] with methyl cinammate to give a complex mixture of the adduct **25** along with the cyclobutane **26**, presumably as a result of an alternative [2+2] cycloaddition[21] or α-ketol rearrangement. Treatment of this mixture with base, in analogy to that reported by Porco and co-workers,[5] induced α-ketol rearrangement to a thermodynamically preferred β-ketoester, and subsequent selective *anti* reduction[22] afforded the desired cyclopentabenzofuran **27** along with the corresponding *exo* isomer in a ratio of 4.6:1, respectively. The stereochemistry of the major *endo* isomer was assigned on the basis of its ^{1}H NMR spectrum, which displayed signals at $\delta = 5.03$ ppm (1H, d, $J = 6$ Hz, H1), 4.31 ppm (1H, d, $J = 14$ Hz, H3), and 3.90 ppm (1H, dd, $J =$ 14, 6 Hz, H2) suggesting a 1,2-*cis*, 2,3-*trans* arrangement. These H1–H3 signals matched those observed for episilvestrol (**2**).[8] Hydrogenolysis of **27** gave the desired core **7** as a racemate. This short sequence provided useful quantities of (±)-**7** ready for coupling to the dioxane fragment.

The pivotal Mitsunobu coupling between **6** and (±)-**7** proceeded with selectivity for the desired axial diastereoisomers in reasonable yield based on recovered core **7** (Scheme 5). A higher selectivity for the axial isomers (≈6:1 ax/eq ratio) was obtained when the coupling was run at 0 °C and not warmed to room temperature, however, the conversion was lower (14%, 49% based on recovered **7**). As expected, the combination of racemic core **7** with optically pure **6** afforded two major axial diastereoisomers **28** and **5** in equal amounts which were inseparable by conventional flash chromatography. Subsequent normal-phase HPLC (5 μm silica, 250 mm × 10 mm, 2 mL min^{-1} with 50% EtOAc/petroleum ether eluent) cleanly separated the isomers **5** ($R_t =$ 10.10 min) and **28** ($R_t = 10.48$ min). It was difficult at this stage to determine which was the correct isomer required for the production of **2**, however, deprotection the slower eluting compound with TBAF afforded diastereoisomer **29** while deprotection of the isomer with the shorter retention time on HPLC, namely compound **5**, then afforded (−)-episilvestrol (**2**).



Scheme 5. Completion of the total synthesis of episilvestrol (**2**) and silvestrol (**1**). TBAF = tetra-*n*-butylammonium fluoride, DEAD = diethylazodicarboxylate, PNBA = *p*-nitrobenzoic acid.

Synthetic episilvestrol (**2**) ($[\alpha]_D^{25} = -91.3$, $c = 0.03$, $CHCl_3$) was identical to natural **2**[1,8] (lit.[8] $[\alpha]_D^{20} = -94.5$, $c = 0.43$, $CHCl_3$) in all respects (^{1}H and ^{13}C NMR, IR, and mass spectra), while isomer **26** ($[\alpha]_D^{24} = -23.9$, $c = 0.09$, $CHCl_3$) displayed slightly different physical data. In addition, synthetic episilvestrol (**2**) displayed anticancer activity against human colon cancer cells LIM 1215 ($IC_{50} = 2.0$ nM) comparable to that reported for the natural product, whilst compound **29** was less active by at least one order of magnitude ($IC_{50} = 56$ nM). Thus, it appears that the stereochemistry of the core has some influence on the cytotoxicity. Finally, conversion of episilvestrol (**2**) into silvestrol (**1**) was easily achieved by a selective "double Mitsunobu" reaction, which resulted in inversion at 5‴ (Scheme 5).[10]

In conclusion, we have achieved a total synthesis of episilvestrol (**2**; 21 steps in total; longest linear sequence of 13 steps) from readily available precursors through a route inspired by the possible biogenesis of these compounds. In addition, episilvestrol (**2**) was converted into silvestrol (**1**). With the development of an asymmetric approach to the cyclopentabenzofuran core,[6] the efficient synthesis of **2** and analogues which are not available from the natural products themselves is possible.

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