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Total Synthesis and Biological Evaluation of (−)-9-Deoxy-englerin A

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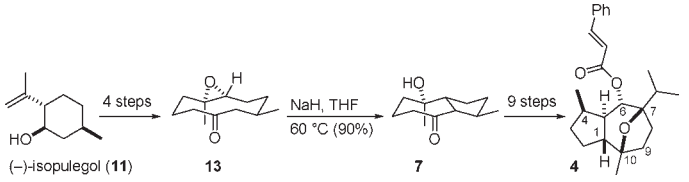
ABSTRACT



An effective total synthesis of (−)-9-deoxy-englerin (4), an analogue of the natural guaiane sesquiterpene englerin A (1), has been achieved. The synthesis features a transannular epoxide opening to construct the 5,7-fused ring system followed by transannular ether formation with mercury(II) trifluoroacetate.

The guaiane sesquiterpene englerin A (**1**) was isolated from the plant extract of *Phyllanthus engleri* by Beutler et al. (Figure 1).[1] This novel guaianolide showed very high selectivity and potency against various cell lines that are involved in renal cancer. As a result of its remarkable activity englerin A (**1**) has attracted significant interest within the synthetic community.[2−4] Recently, the Chen group published the synthesis and biological evaluation of englerin analogues. It turned out that modifications in the cinnamic acid part are tolerated, whereas substantial deviations from the glycolic acid at 9-OH diminish the cytotoxicity.[5] Because other oxygen-brigded guaiane-type sesquiterpenes, like orientalol F[6,7] (**5**), are known, it might be of interest to convert some of them to englerin analogues. In addition, simpler englerin analogues should reveal key structure−activity relationships. We therefore targeted 9-deoxy-englerin (**4**). In this context we also wanted to explore a novel route to the guaianolide core structure.[8,9]

Our retrosynthetic plan for 9-deoxy-englerin (**4**) featured a transannular ether formation leading to azulene

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(2) For total syntheses of englerin A, see: (a) Willot, M.; Radtke, L.; Könning, D.; Fröhlich, R.; Gessner, V. H.; Strohmann, C.; Christmann, M. *Angew. Chem., Int. Ed.* **2009**, *48*, 9105–9108. (b) Zhou, Q.; Chen, X.; Ma, D. *Angew. Chem., Int. Ed.* **2010**, *49*, 3513–3516. (c) Molawi, K.; Delpont, N.; Echavarren, A. M. *Angew. Chem., Int. Ed.* **2010**, *49*, 3517–3519. (d) Nicolaou, K. C.; Kang, Q.; Ng, S. Y.; Chen, D. Y.-K. *J. Am. Chem. Soc.* **2010**, *132*, 8219–8222.
(3) Formal total synthesis: Xu, J.; Caro-Diaz, E. J. E.; Theodorakis, E. A. *Org. Lett.* **2010**, *12*, 3708–3711.
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(8) For a recent review, see: Foley, D. A.; Maguire, A. R. *Tetrahedron* **2010**, *66*, 1131–1175.
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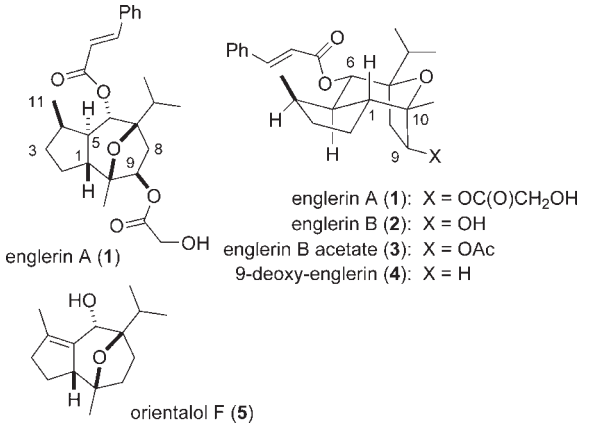
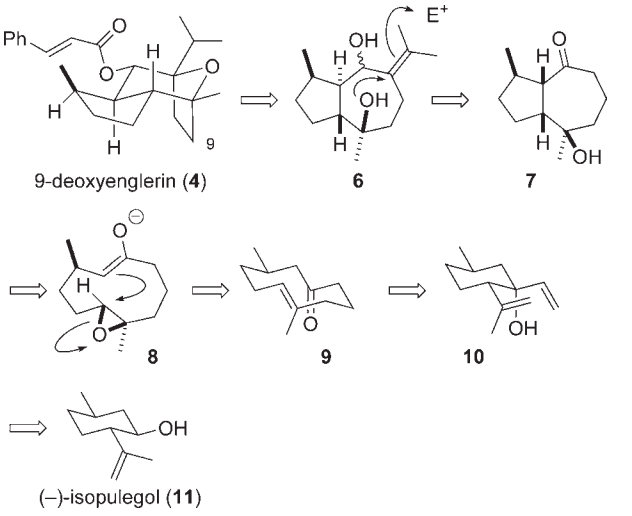


Figure 1. Structure of englerin A and the simpler guaianolide orientalol F (**5**).

derivative **6** (Scheme 1). The tetrasubstituted double bond of **6** could possibly be introduced via aldol condensation on the bicyclic ketone **7**. The annulated 5,7-ring system **7** might originate from an intramolecular epoxide opening of a ketone enolate **8** which would be generated from cyclic enone **9**.[10] By considering an oxy-Cope rearrangement[11,12] on dienol **10** to generate cyclodec-5-enone **9**, (−)-isopulegol (**11**) appeared as a suitable starting material.

Starting from commercially available (−)-isopulegol[13] (**11**), vinyl carbinol **10** was prepared by a two-step sequence involving a Corey−Suggs oxidation[14] followed by addition of vinylmagnesium bromide[15] to the corresponding ketone[16] **12** (Scheme 2). Only one diastereomer of the tertiary alcohol was formed, which could be expected from an equatorial attack of the nucleophile on the ketone. Anionic oxy-Cope rearrangement of dienol **10** was achieved with KH in THF at reflux leading to the ten-membered ring **9**.[17] It is appropriate to mention that, up to this step, all compounds could be purified by simple distillation. Several signals in the NMR spectra of cyclodecenone **9** were rather broad, indicating slow movement with regard to the NMR time scale. Subsequent *m*CPBA mediated epoxidation of enone **9** secured epoxide **13** as a single stereoisomer, whose structure was confirmed by X-ray analysis (Scheme 2). This points to efficient

Scheme 1. Retrosynthetic Plan for the Synthesis of 9-Deoxy-englerin (**4**)



macrocyclic control.[18] It seems that the methyl group at the double bond prevents rotation of the double bond which would lead to isomers.

Regioselective enolate formation on keto epoxide **13**, in order to prepare the desired 5,7-fused ring system, was found to be challenging. Thus, when a freshly prepared LDA solution (4.0 equiv) was added dropwise to a solution of ketone **13** at −80 °C a mixture of regioisomers **7** and **14** was obtained (1:4). Higher temperatures (rt) allowed us to increase the ratio of the desired isomer **7** (1:0.7). Furthermore, when NaH was added to a solution of keto epoxide **13** and the resulting suspension was transferred to a preheated (80 °C) oil bath for 1 h only the desired isomer **7** was formed in 90% yield. It seems that the small hydride anion selectively deprotonates 5-H which is perfectly orthogonal to the keto group. This way ketone **7** could be obtained on a 6.0 g scale. The stereochemistry and constitution of **7** were proven on the derived diol **15**. The *cis*-orientation of 1-H, 5-H, and the 4-CH_3 group could be inferred from the NOESY spectrum. The connectivity of the blue bonds in **15** was evident from correlations in the HMBC spectrum. In line with structure **15**, there was a correlation between 1-H and C-4.

The hydroxyketone **7** exists in equilibrium with its cyclic hemiacetal **16** (30:70 ratio, $CDCl_3$, rt) (Scheme 3). It turned out that for further functionalization of the seven-membered ring it was necessary to protect the hydroxyl group of ketone **7**. While silylation preferentially trapped the hemiacetal form, it was found that esterification with pivalic anhydride in the presence of scandium triflate[19] favored the open form, ketone **17**, over the protected hemiacetal **18** (**17**/**18** = 62:38). The two compounds were easily separated by SiO_2 chromatography. Furthermore, ketone **7** could be recycled by transesterification (MeOH, K_2CO_3,

(10) For application of this strategy to the phorbol skeleton, see: Paquette, L. A.; Sauer, D. R.; Edmondson, S. D.; Friedrich, D. *Tetrahedron* **1994**, *50*, 4071–4086.

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(13) (−)-Isopulegol was purchased from Aldrich: 1 kg costs 90 Euro.

(14) Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, *16*, 2647–2650.

(15) In the case of freshly prepared vinylmagnesium bromide the reaction proceeded spot to spot, while the use of a commercially available Grignard reagent gave only traces of product **10**.

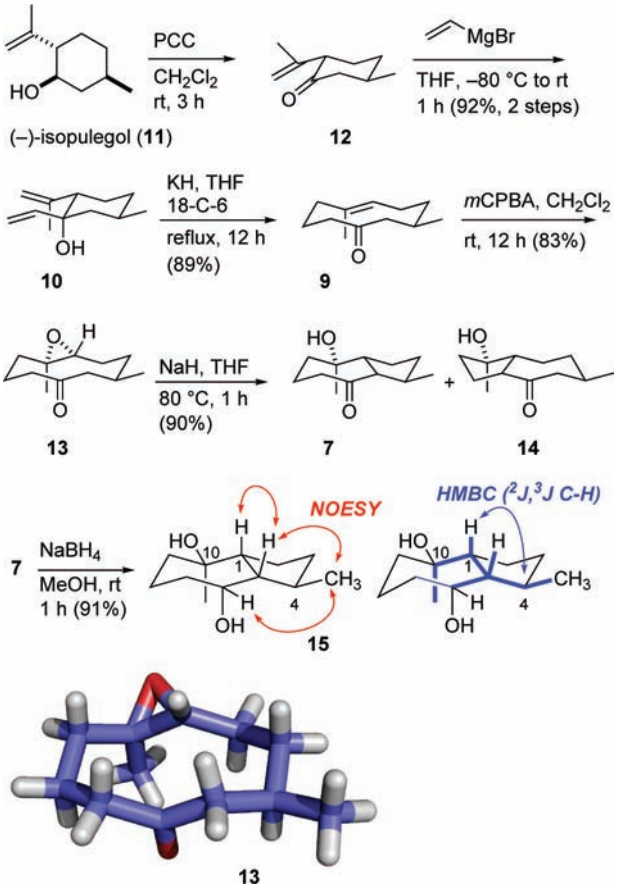
(16) Moreira, J. A.; Corrêa, A. G. *Tetrahedron: Asymmetry* **2003**, *14*, 3787–3795.

(17) We found that the use of catalytic amounts (0.1 equiv) of 18-crown-6 ether increased the rate and the yield up to 89%. Without 18-crown-6 ether the rearrangement took 16 h and gave 71% yield of **9**.

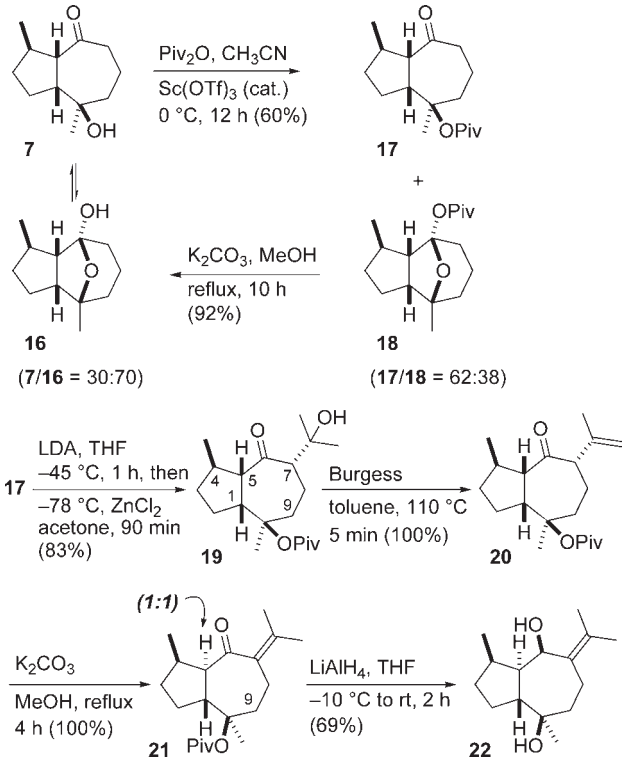
(18) Still, W. C.; Galynker, I. *Tetrahedron* **1981**, *37*, 3981–3996.

(19) Ishihara, K.; Kubota, M.; Kurihara, H.; Yamamoto, H. *J. Org. Chem.* **1996**, *61*, 4560–4567.

Scheme 2. Ring Expansion via Oxy-Cope Rearrangement followed by Transannular Enolate Alkylation Yielding Octahydroazulenone **7**



Scheme 3. Synthesis of Dihydroxy Alkene **22** via Condensation of Ketone **17** with Acetone



reflux) of ketal ester **18**. The synthesis continued with the aldol reaction of ketone **17** with acetone which yielded hydroxyketone **19** as a single diastereomer.[20] The stereochemistry of the aldol reaction followed from a 5-H/7-H cross peak in the NOESY spectrum of **19**. Subsequent dehydration with the Burgess reagent[21] ($MeO_2CN^-SO_2NEt_3^+$) provided alkene **20** in 83% yield over two steps. By the use of K_2CO_3 in methanol under reflux, migration of the double bond was observed with further epimerization at C-5 (*trans*/*cis* = 1:1).[22] The two stereoisomers were separated by SiO_2 column chromatography, and the wrong isomer was reintroduced in the epimerization reaction again. Interestingly, the pivalic ester remained untouched under the isomerization conditions. This is in contrast to pivalic ketal ester **18** which underwent smooth deprotection under the same conditions. Reductive cleavage of pivalic ester **21** with $LiAlH_4$ led to reduction of the keto group as well, providing diol **22** in good overall yield.

With dihydroxy alkene **22** in hand we tested various conditions in order to promote the transannular cyclization. Acid-induced cyclization ($CHCl_3$; silica gel, $CHCl_3$; TFA, CH_2Cl_2[23]) failed as well as reaction with PhSeCl followed by Bu_3SnH/AIBN.[24] After careful experimentation we found that reaction of alkenediol **22** with $Hg(O_2CCF_3)_2$ and reduction of the resulting organomercurial intermediate with $NaBH_4$ provided oxygen-bridged compound **23** in 95% yield (Scheme 4). Crystallization of **23** from hexane provided crystals suitable for X-ray analysis (Scheme 4). Unfortunately, esterification of **23** with cinnamic acid under Mitsunobu conditions was unsuccessful. Also, inversion of the stereochemistry at C-6 by Mitsunobu reaction with stronger acids did not work. Therefore, inversion by an oxidation/reduction sequence was tried. Oxidation of alcohol **23** with Dess-Martin periodinane provided ketone **24** in 89% yield. However, its reduction with various hydride reagents ($LiAlH_4$, $NaBH_4$, L-selectride, DIBAL-H) provided again alcohol

(20) For a related aldol reaction, see: Gijsen, H. J. M.; Wijnberg, J. B. P. A.; Stork, G. A.; De Groot, A. *Tetrahedron* **1991**, *47*, 4409–4416.

(21) Burgess, E. M.; Penton, H. R.; Taylor, E. A. *J. Org. Chem.* **1973**, *38*, 26–31.

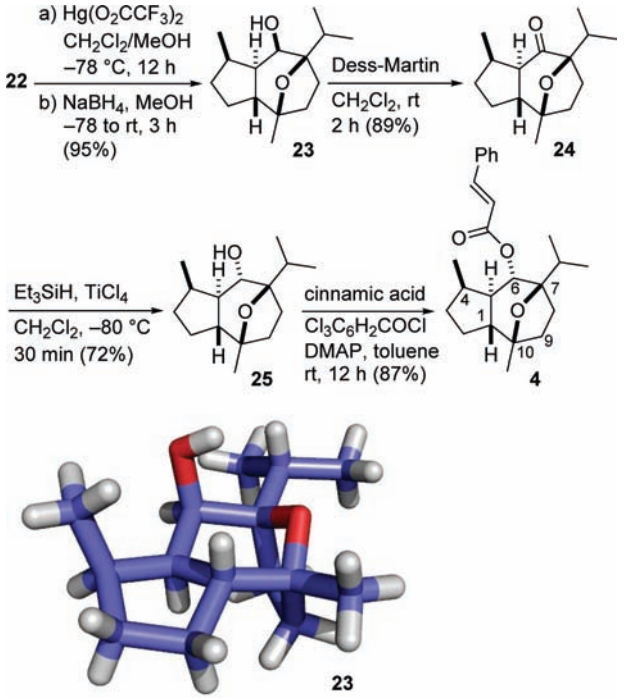
(22) For epimerization reactions on *cis*-fused 5,7-ring systems, see: (a) Lange, G. L.; Gottardo, C.; Merica, A. *J. Org. Chem.* **1999**, *64*, 6738–6744. (b) House, H. O.; Nomura, G. S.; VanDerveer, D. *J. Org. Chem.* **1986**, *51*, 2416–2423. (c) House, H. O.; Gaa, P. C.; Lee, J. H. C.; VanDerveer, D. *J. Org. Chem.* **1983**, *48*, 1670–1678.

(23) For example, see: Nicolaou, K. C.; Li, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 7086–7090.

(24) For a recent example, see: Pedrosa, R.; Andrés, C.; Mendiguchía, P.; Nieto, J. *J. Org. Chem.* **2006**, *71*, 8854–8863.

(25) For carbonyl reductions using silanes in the presence of a Lewis acid, see: (a) Doyle, M. P.; McOsker, C. C.; Ball, N.; West, C. T. *J. Org. Chem.* **1977**, *42*, 1922–1928. (b) Parks, D. J.; Blackwell, J. M.; Piers, W. E. *J. Org. Chem.* **2000**, *65*, 3090–3098. (c) Larson, G. L.; Fry, J. L. *Org. React.* **2008**, *71*, 1–737.

Scheme 4. Synthesis of 9-Deoxy-englerin (**4**) via Hg^{2+}-Mediated Cyclization



23 as a single stereoisomer. Reduction with SmI_2 was not successful either. Finally, we discovered that the reaction of ketone **24** with Et_3SiH and $TiCl_4$ proceeded with exclusive formation of the desired alcohol **25** in 72% yield.[25] Esterification of **25** with cinnamic acid under Yamaguchi conditions provided (−)-9-deoxy-englerin (**4**) in good yield.

(−)-Englerin A[26] (**1**), (−)-9-deoxy-englerin (**4**), and ketone **24** were tested against cancer cell lines, using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[27] The results are summerized in Table 1. As expected, englerin A (**1**) was quite active with the renal cancer cell line. In general **1** was more active than **4** with the other cell lines as well, pointing to the importance of the glycolate at C-9. However, the difference in activity between **1** and **4** is much less pronounced with the other cell types. The inhibition curve of **1** with the A-498 cells is actually rather shallow, and the curves of **1** and **4** meet at 37 μg mL^{-1} (100% growth inhibition). A distinct phenotype could not be seen with either **1** or **4** which would provide hints regarding the mode of action.

Table 1. Cytotocicity (IC_{50} in μM) of Englerin A (**1**) and Analogue **4** against Selected Cell Lines[a,b]

compound	L-929	A-498	KB-3-1	MCF-7	HUVEC
1	29	0.4	12	18	4.3
4	65	35	41	41	9.8

[a] L-929 (mouse fibroblasts), A-498 (renal cancer cell line), KB-3-1 (HeLa cells), MCF-7 (breast cancer cells) HUVEC (primary endothel cells). [b] Compound **24** was essentially inactive (IC_{50} > 170 μM).

In summary, a novel strategy toward the guaianolide structure has been developed. Starting from (−)-isopulegol (**11**) a ring expansion via an oxy-Cope rearrangement gave rise to ten-membered 1,5-enone **9**. After epoxidation to epoxy ketone **13**, a transannular epoxide opening with the ketone enolate furnished the 5,7-annulated ring system **7**. Functionalization of the seven-membered ring via an aldol reaction with acetone led to alkenediol **22**. The ether bridge could be created by a Hg^{2+}-mediated cyclization. A final esterification of alcohol **25** with cinnamic acid delivered (−)-9-deoxy-englerin (**4**). The route to **4** proceeded in 13.4% overall yield with a longest linear sequence of 15 steps. Biological studies on **4** confirmed the role of the glycolic acid at C-9.

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Supporting Information Available. Experimental procedures and characterization for all new compounds reported and copies of NMR spectra for important intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

(26) A sample of englerin A was purchased from AppliChem GmbH, Darmstadt, Germany.

(27) For example, see: (a) Vintonyak, V. V.; Calà, M.; Lay, F.; Kunze, B.; Sasse, F.; Maier, M. E. *Chem.—Eur. J.* **2008**, *14*, 3709–3720. (b) Cramer, N.; Helbig, S.; Baro, A.; Laschat, S.; Diestel, R.; Sasse, F.; Mathieu, D.; Richter, C.; Kummerloewe, G.; Luy, B.; Schwalbe, H. *ChemBioChem* **2008**, *9*, 2474–2486.