Organic LETTERS

Letter

pubs.acs.org/OrgLett

Unraveling the Metabolic Pathway in *Leucosceptrum canum* by Isolation of New Defensive Leucosceptroid Degradation Products and Biomimetic Model Synthesis

Shi-Hong Luo,[†,∥] Cedric L. Hugelshofer,[‡,∥] Juan Hua,[†,§] Shu-Xi Jing,[†,§] Chun-Huan Li,[†] Yan Liu,[†] Xiao-Nian Li,[†] Xu Zhao,[†] Thomas Magauer,*[,‡] and Sheng-Hong Li*[,†]

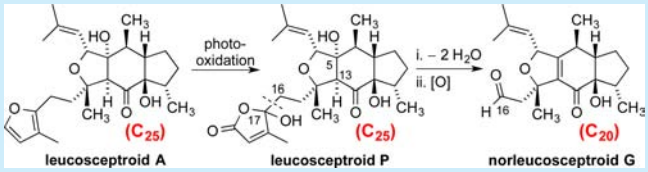
[†]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China

[‡]Department of Chemistry and Pharmacy, Ludwig-Maximilians-University Munich, Butenandstrasse 5-13, 81377 Munich, Germany

[§]Graduate University of Chinese Academy of Sciences, Beijing 100039, People's Republic of China

Supporting Information

ABSTRACT: Seven new leucosceptroid degradation products possessing a C_{20}, C_{21}, or C_{25} framework, norleucosceptroids D−H (**1**−**5**), leucosceptroids P (**6**), and Q (**7**), have been isolated from *Leucosceptrum canum*. Their structures were determined by comprehensive NMR, MS, and single-crystal X-ray diffraction analyses. Discovery of these key intermediates, together with the biomimetic oxidation of a model system, supports the hypothesis that two biosynthetic pathways are operative. Antifeedant activity was observed for compounds **1**−**3**.



Terpenoids, the largest class of natural products with highly diversified chemical structures, have been frequently reported to play important physiological and ecological roles in the natural world.[1] To date, degradation of sesterterpenoids in plants is still largely unknown, while the oxidative degradation of other terpenoids has been documented, although the mechanism underlying these transformations is often poorly understood. Known examples include the apocarotenoids (nortetraterpenoids),[2] antifeedant limonoids (tetranortriterpenoids),[3] schinortriterpenoids (nortriterpenoids),[4] and volatile tetra-norterpenoid degradation products of the diterpenoid (*E*,*E*)-geranyllinalool and the sesquiterpenoid (3*S*)-(*E*)-nerolidol, which were shown to attract enemies of herbivores (indirect defense) in a variety of plant species.[5]

Recently, it was found that the glandular trichomes of two Himalayan−Chinese Labiatae species[6] harbor two unique classes of defensive sesterterpenoids, leucosceptroids and colquhounoids, respectively.[7,8] Subsequently, three intriguing antifeedant C_{20} terpenoids, norleucosceptroids A−C,[9] were isolated from *L. canum* and tentatively classified as pentanorsesterterpenoids. Choudhary and co-workers also reported the isolation of three sesterterpenoids from *L. canum* of Nepalese origin,[10] which are structurally quite different from those discovered in Chinese plants.

Synthetic routes to these natural products were reported by three groups. The core structure of leucosceptroids A−D has been prepared by Horne's group,[11a] an asymmetric total synthesis of leucosceptroid B has been achieved by Liu and co-workers,[11b] and most recently Magauer's group reported the total synthesis of norleucosceptroid A, norleucosceptroid B, and leucosceptroid K.[11c,d]

In order to shed light on the metabolic pathways in *L. canum*, the search for key biosynthetic intermediates of norleucosceptroids A−C has been an ongoing topic of interest. Herein, the isolation, structure elucidation, antifeedant activity, and biogenetic relationship of seven new biosynthetic intermediates including two tetranorsesterterpenoids (**1** and **2**), three pentanorsesterterpenoids (**3**−**5**), and two sesterterpenoids (**6** and **7**) are described. Additionally, the oxidative degradation of the furan appendage using an advanced model system was investigated. The obtained results further support the coexistence of two metabolic pathways in leucosceptroid biosynthesis.

For compound **1**, a molecular formula of $C_{21}H_{30}O_4$ was deduced from the high-resolution (HR) EI-MS (*m*/*z* 346.2148 $[M]^+$, calcd 346.2144) and the IR spectrum indicated the presence of two carbonyl groups (1736 and 1709 cm^{-1}). Analysis of the NMR spectra (Tables S1 and S2 and Figures S2−S7 in the Supporting Information) revealed various similarities with those of leucosceptroids B[7] (**9**) and E[8b] (**12**), although the furan or lactone moieties in the C14 side chains were absent and instead a carboxylic acid group was present in **1**. Consideration of all the spectroscopic data suggested that **1** is a tetranorsesterterpenoid lacking C11 and C5 oxygenation. Eventually, a single crystal of **1** was obtained from a mixture of MeOH/water (8:1), and X-ray crystallographic analysis unambiguously established the complete structure of **1** which was named norleucosceptroid D (Figure 1).

Received: November 6, 2014
Published: December 4, 2014

dx.doi.org/10.1021/ol503230s | *Org. Lett.* 2014, 16, 6416−6419

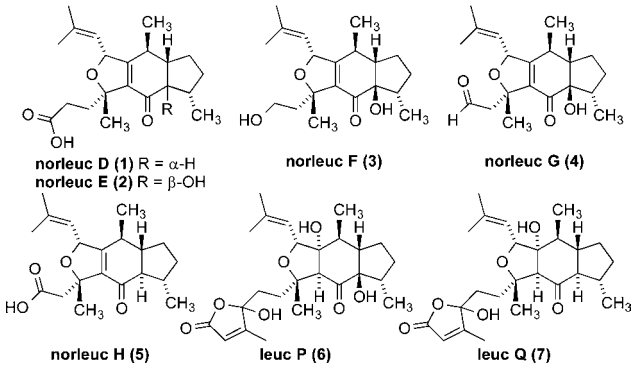


Figure 1. Chemical structures of norleucosceptroids D–H (**1**–**5**), leucosceptroids P (**6**), and Q (**7**).

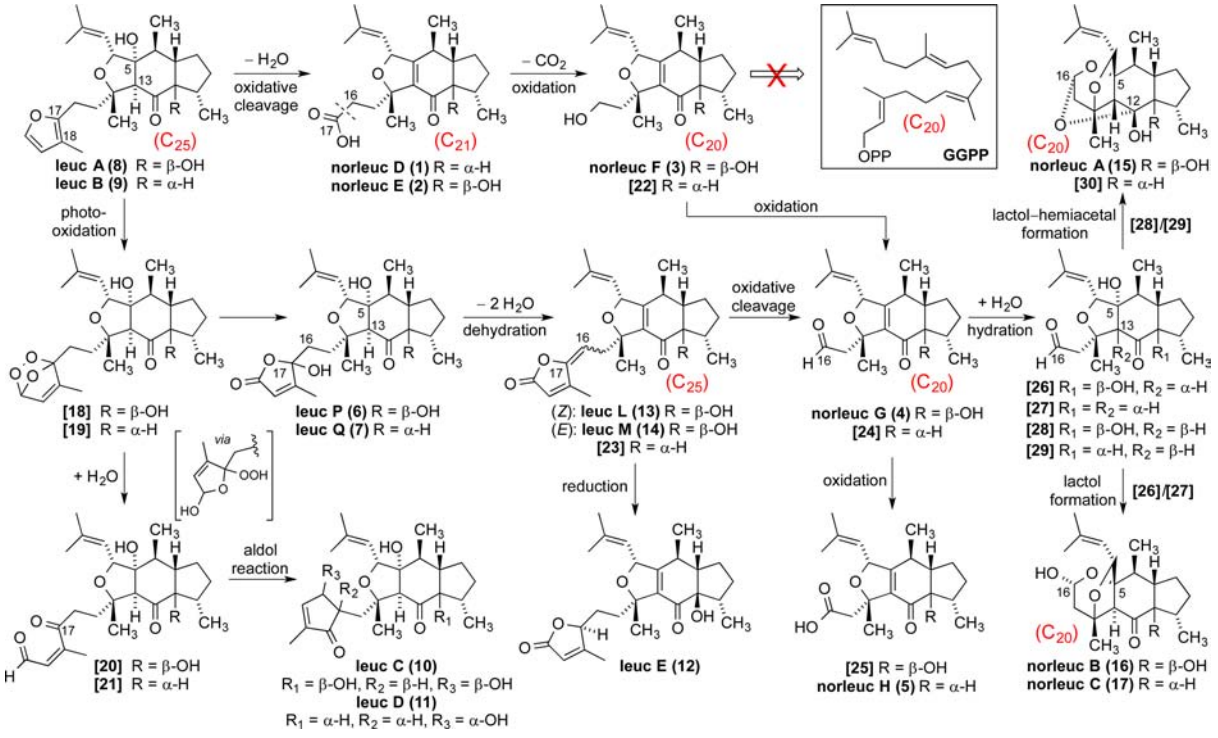
In an analogous fashion, comprehensive NMR, MS, and IR analysis together with comparison of spectroscopic data of previously isolated parent leucosceptroids allowed compounds **2**–**7** to be characterized as shown in Figure 1, and they were named norleucosceptroids E–H (**2**–**5**), leucosceptroids P (**6**), and Q (**7**). A detailed description of the isolation and structure elucidation of all these compounds is provided in the Supporting Information of this report.

During the structure elucidation of the novel leucosceptroids **1**–**7**, a biogenetic relationship between the previously isolated C_{25} sesterterpenoids and C_{20} terpenoids from *L. canum* was recognized (Scheme 1). It is likely that the carboxylic acids norleucosceptroids D (**1**) and E (**2**) directly originate from leucosceptroids A (**8**) and B (**9**), which both are major sesterterpenoids in *L. canum*, by 5,13-dehydration and oxidative cleavage of the furan moiety (scission of the C17/C18 bond). Subsequent decarboxylation and oxidation[12] of tetranorsesterterpenoids **1** and **2** affords the pentanorsesterterpenoids norleucosceptroid F (**3**) and **22**. Oxidation of the latter compounds yields norleucosceptroid G (**4**) and aldehyde **24**, which then may be further oxidized to norleucosceptroid H (**5**) and carboxylic acid **25**.

An alternative degradation pathway of leucosceptroids A (**8**) and B (**9**) is hypothesized to proceed through peroxides **18** and **19**, which arise from the [4 + 2] cycloaddition of singlet oxygen with the furan moiety.[13] A subsequent Kornblum–DeLaMare-type rearrangement[14] of **18** and **19** yields the hydroxy lactones leucosceptroids P (**6**) and Q (**7**), which upon 5,13- and 16,17-dehydration are converted to leucosceptroids L (**13**) and M (**14**) and **23**. While reduction of the C16/C17 bond furnishes leucosceptroid E (**12**), an oxidative cleavage of this bond affords the same aldehydes **4** and **24** as from the $C_{25} \rightarrow C_{21} \rightarrow C_{20}$ decarboxylative pathway. Through hydration of the C5/C13 bond, norleucosceptroid G (**4**) and **24** are then converted to a mixture of intermediates **26**–**29**.[9] While **28** and **29** with the AB-*cis*-BC-*trans* fusion may spontaneously convert to the thermodynamic epimers **26** and **27**, respectively, intramolecular hemiacetal formation can alternatively preserve this conformation from epimerization to the AB-*cis*-BC-*cis* system. Biosynthetically, norleucosceptroids B (**16**) and C (**17**) thus arise from intermediates **26** and **27** through lactol formation between 5-OH and 16-CHO. Similarly, lactol formation between 5-OH and 16-CHO in intermediates **28** and **29**, followed by hemiacetal formation between 16-OH and 12-C=O affords norleucosceptroid A (**15**) and **30**.

Furthermore, the existence of leucosceptroids C (**10**) and D (**11**) also strongly suggests that photooxidation of leucosceptroids A (**8**) and B (**9**) initiates the partial metabolic degradation

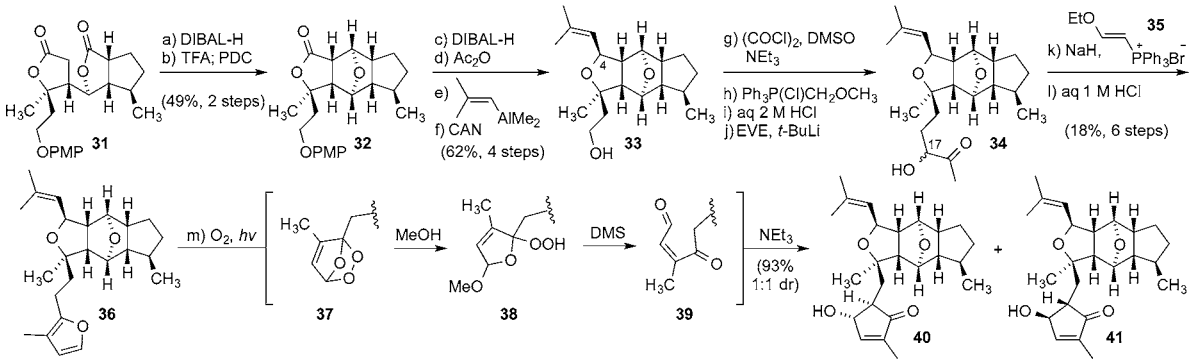
Scheme 1. Proposed Metabolic Pathway of Leucosceptroids A (8) and B (9)[a]



[a]For clarity, numbers in parentheses are used for isolated natural products, while numbers in square brackets correspond to intermediates or suspected natural products.

dx.doi.org/10.1021/ol503230s | *Org. Lett.* 2014, 16, 6416–6419

Scheme 2. Model System Synthesis and Biomimetic Furan Photooxidation[a]



[a]DIBAL-H = diisobutylaluminum hydride, TFA = trifluoroacetic acid, PDC = pyridinium dichromate, CAN = ceric ammonium nitrate, DMSO = dimethyl sulfoxide, EVE = ethyl vinyl ether, DMS = dimethyl sulfide.

of these sesterterpenoids. In lieu of the proposed rearrangement of peroxides **18** and **19** to **6** and **7**, respectively, it is envisaged that also nucleophilic addition of water takes place, affording keto aldehydes **20** and **21** via expulsion of hydrogenperoxide from a hemiacetal intermediate. An intramolecular aldol cyclization of the enolized 17-ketone with the aldehyde group in **20** and **21** then gives rise to leucosceptroids C (**10**) and D (**11**).

Although further intermediates remain to be discovered, it is likely that two parallel biosynthetic pathways exist in *L. canum*, leading to the 11-hydroxylated and 11-deoxygenated sesterterpenoids or norsesterterpenoids, respectively. The isolation of intermediates **1**−**7** provides direct evidence that the C_{20} terpenoids, norleucosceptroids A−C (**15**−**17**), are, in fact, sesterterpenoid degradation products and should be classified as pentanorsesterterpenoids rather than diterpenoids which are directly biosynthesized from the universal precursor geranylgeranyl diphosphate (GGPP).

In order to experimentally evaluate the proposed degradation pathway, which is initiated by photooxygenation, a model system was developed. With this objective in mind, known dilactone **31**, available in gram quantities,[11c] was converted to lactone **32** by 2-fold reduction, followed by an intramolecular dilactol aldol-type condensation−oxidation sequence (Scheme 2). DIBAL-H reduction of **32** and acetylation of the resulting lactol afforded an intermediate acetyl acetal as a mixture of diastereomers at C4 (dr = ∼2:1). Treatment of this crude product with the mixed organoaluminum reagent, dimethyl-(2-methyl-1-propenyl)aluminum (prepared from aluminum trichloride, methylmagnesium bromide, and 2-methyl-1-propenylmagnesium bromide; see the Supporting Information for details), furnished alcohol **33** as a single diastereomer after cleavage of the *p*-methoxyphenyl ether (62% yield over four steps). Next, Swern oxidation[15] of **33** followed by Wittig−Levine reaction[16] of the so-obtained aldehyde with (methoxymethyl)triphenylphosphonium chloride was carried out, yielding the corresponding C_1−extended aldehyde after acidic hydrolysis. Addition of lithiated ethyl vinyl ether to the latter compound provided α-hydroxy ketone **34** as an inconsequential mixture of diastereomers at C17 (dr = ∼1:1). Alkoxide formation in **34** with sodium hydride followed by addition of Wittig salt **35**[17] furnished an intermediate ethoxy acetal, which underwent facile elimination under acidic conditions to afford furan **36**, which constituted the model system of leucosceptroids A (**8**) and B (**9**).

This system was then used to mimic the putative conversion of leucosceptroids A (**8**) and B (**9**) to the hydroxycyclopentenone leucosceptroids C (**10**) and D (**11**) (vide supra), respectively, in a biomimetic manner. Thus, irradiation of an oxygen-sparged solution of furan **36** in methanol containing Rose Bengal as a photosensitizer with light at −78 °C led to rapid consumption of the starting material (<5 min), affording a more polar product, as seen by thin-layer chromatographic analysis. Addition of dimethyl sulfide (DMS) to the putative hydroperoxide **38**, arising from nucleophilic addition of methanol to *endo*-peroxide **37**,[18] followed by warming of the reaction mixture to 23 °C and addition of triethylamine to induce the intramolecular aldol reaction in keto aldehyde **39**, cleanly furnished hydroxy cyclopentenones **40** and **41** as a 1:1 mixture of diastereomers (93% combined yield). It is proposed that, in nature, the conversion from *endo*-peroxide **37** to the hydroxy cyclopentenones would occur via nucleophilic addition of water (instead of methanol) followed by expulsion of hydrogen peroxide to afford keto aldehyde **39** (in the laboratory mimic, peroxide **38** is first reduced with DMS and methanol then serves as the leaving group). The final cyclization step in a natural environment could be catalyzed by an aldolase which is capable of controlling the stereochemical outcome of this aldol reaction.[19] Although the model furan **36** is certainly simplified compared to leucosceptroids A (**8**) and B (**9**), the experimental outcome corroborates the hypothesis that photooxidation occurs readily and might be the first step in the metabolic pathway of the defensive leucosceptroid sesterterpenoids.

Finally, norleucosceptroids D−F (**1**−**3**) were evaluated for their antifeedant activity against a generalist insect, cotton bollworm (*H. armigera*), as previously described.[7] Significant antifeedant activity for **1**−**3** was observed with EC_{50} values of 3.81, 10.86, and 7.35 μg/cm^2, respectively (neem oil: EC_{50} = 2.63 μg/cm^2), suggesting that these leucosceptrane sesterterpenoid degradation products could also be involved in the plant defense against insect enemies.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, plant material, crystallographic data, physical-chemical properties, key HMBC correlations and NMR spectra of **1**−**7**. Synthetic procedures, analytical data, and NMR spectra of the biomimetic model synthesis. This

dx.doi.org/10.1021/ol503230s | *Org. Lett.* 2014, 16, 6416−6419

material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: thomas.magauer@lmu.de.
*E-mail: shli@mail.kib.ac.cn.

Author Contributions

[‖]These authors contributed equally to this work.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was supported financially by the NSFC−Yunnan Joint Funds (U1202263), the National Basic Research Program of China (973 Program) on Biological Control of Key Crop Pathogenic Nematodes (2013CB127505), the National Natural Science Foundation of China (31100222 and 31070320), and the "Hundred Talents Program" of the Chinese Academy of Sciences. Financial support from the FCI (Liebig Fellowship to T.M. and Kekulé Mobility Fellowship to C.L.H) and the DFG (Emmy-Noether Fellowship to T.M.) is gratefully acknowledged. We also thank Dr. Kevin Mellem (Midasyn, Inc.) for helpful discussions during the preparation of this manuscript.

REFERENCES

(1) Gershenzon, J.; Dudareva, N. *Nat. Chem. Biol.* **2007**, *3*, 408−414.

(2) (a) Walter, M. H.; Floss, D. S.; Strack, D. *Planta* **2010**, *232*, 1−17. (b) Simkin, A. J.; Schwartz, S. H.; Auldridge, M.; Taylor, M. G.; Klee, H. J. *Plant J.* **2004**, *40*, 882−892. (c) Schwartz, S. H.; Qin, X.; Zeevaart, J. A. D. *Plant Physiol.* **2003**, *131*, 1591−1601. (d) Matusova, R.; Rani, K.; Verstappen, F. W. A.; Franssen, M. C. R.; Beale, M. H.; Bouwmeester, H. J. *Plant Physiol* **2005**, *139*, 920−934. (e) Ilg, A.; Beyer, P.; Al-Babili, S. *FEBS J.* **2009**, *276*, 736−747.

(3) (a) Tan, Q. G.; Luo, X. D. *Chem. Rev.* **2011**, *111*, 7437−7522. (b) Gopalakrishnan, G.; Singh, N. D. P.; Kasinath, V. *Molecules* **2002**, *7*, 112−118. (c) Jarvis, A. P.; Johnson, S.; Morgan, E. D.; Simmonds, M. S. J.; Blaney, W. M. *J. Chem. Ecol.* **1997**, *23*, 2841−2860.

(4) Xiao, W. L.; Li, R. T.; Huang, S. X.; Pu, J. X.; Sun, H. D. *Nat. Prod. Rep.* **2008**, *25*, 871−891.

(5) (a) Herde, M.; Gärtner, K.; Köllner, T. G.; Fode, B.; Boland, W.; Gershenzon, J.; Gatz, C.; Tholl, D. *Plant Cell* **2008**, *20*, 1152−1168. (b) Kappers, I. F.; Aharoni, A.; van Herpen, T. W. J. M.; Luckerhoff, L. L. P.; Dicke, M.; Bouwmeester, H. J. *Science* **2005**, *309*, 2070−2072.

(6) Luo, S. H.; Liu, Y.; Hua, J.; Niu, X. M.; Jing, S. X.; Zhao, X.; Schneider, B.; Gershenzon, J.; Li, S. H. *Org. Lett.* **2012**, *14*, 4146−4149.

(7) Luo, S. H.; Luo, Q.; Niu, X. M.; Xie, M. J.; Zhao, X.; Schneider, B.; Gershenzon, J.; Li, S. H. *Angew. Chem., Int. Ed.* **2010**, *49*, 4471−4475.

(8) (a) Luo, S. H.; Weng, L. H.; Xie, M. J.; Li, X. N.; Hua, J.; Zhao, X.; Li, S. H. *Org. Lett.* **2011**, *13*, 1864−1867. (b) Luo, S. H.; Hua, J.; Niu, X. M.; Liu, Y.; Li, C. H.; Zhou, Y. Y.; Jing, S. X.; Zhao, X.; Li, S. H. *Phytochemistry* **2013**, *86*, 29−35. (c) Luo, S. H.; Hua, J.; Li, C. H.; Liu, Y.; Li, X. N.; Zhao, X.; Li, S. H. *Tetrahedron Lett.* **2013**, *54*, 235−237. (d) Li, C.-H.; Jing, S.-X.; Luo, S.-H.; Shi, W.; Hua, J.; Liu, Y.; Li, X.-N.; Schneider, B.; Gershenzon, J.; Li, S.-H. *Org. Lett.* **2013**, *15*, 1694−1697.

(9) Luo, S. H.; Hua, J.; Li, C. H.; Jing, S. X.; Liu, Y.; Li, X. N.; Zhao, X.; Li, S. H. *Org. Lett.* **2012**, *14*, 5768−5771.

(10) (a) Choudhary, M. I.; Ranjit, R.; Atta-ur-Rahman; Shrestha, T. M.; Yasin, A.; Parvez, M. *J. Org. Chem.* **2004**, *69*, 2906−2609. (b) Choudhary, M. I.; Ranjit, R.; Atta-ur-Rahman; Hussain, S.; Devkota, K. P.; Shrestha, T. M.; Parvez, M. *Org. Lett.* **2004**, *6*, 4139−4142.

(11) (a) Xie, J.; Ma, Y. L.; Horne, D. A. *J. Org. Chem.* **2011**, *76*, 6169−6176. (b) Huang, X.; Song, L. Q.; Zhu, G. L.; Liu, B. *Angew. Chem., Int. Ed.* **2013**, *52*, 952−955. (c) Hugelshofer, C. L.; Magauer, T. *Angew. Chem., Int. Ed.* **2014**, *53*, 11351−11355. (d) During the preparation of this manuscript, a synthesis of leucosceptroid A and B was reported: Guo, S.; Liu, J.; Ma, D. *Angew. Chem. Int. Ed.* **2014**, DOI: 10.1002/anie.201410134.

(12) A sequence of decarboxylation followed by oxidation has also been suggested in the biogenetic relationships of other terpenes; see: (a) Li, S. H.; Wang, J.; Niu, X. M.; Shen, Y. H.; Zhang, H. J.; Sun, H. D.; Li, M. L.; Tian, Q. E.; Lu, Y.; Cao, P.; Zheng, Q. T. *Org. Lett.* **2004**, *6*, 4327−4330. (b) Zou, J.; Du, X.; Pang, G.; Shi, Y. M.; Wang, W. G.; Zhan, R.; Kong, L. M.; Li, X. N.; Li, Y.; Pu, J. X.; Sun, H. D. *Org. Lett.* **2012**, *14*, 3210−3213.

(13) For selected references discussing biomimetic furan photooxygenations, see: (a) Gollnick, K.; Griesbeck, A. *Tetrahedron* **1985**, *41*, 2057−2068. (b) Carté, B.; Kernan, M. R.; Barrabee, E. B.; Faulkner, D. J.; Matsumoto, G. K.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 3528−3532. (c) Vassilikogiannakis, G.; Stratakis, M. *Angew. Chem., Int. Ed.* **2003**, *42*, 5465−5468. (d) Margaros, I.; Montagnon, T.; Tofi, M.; Pavlakos, E.; Vassilikogiannakis, G. *Tetrahedron* **2006**, *62*, 5308−5317. (e) Montagnon, T.; Tofi, M.; Vassilikogiannakis, G. *Acc. Chem. Res.* **2008**, *41*, 1001−1011. (f) Kalaitzakis, D.; Triantafyllakis, M.; Alexopoulou, I.; Sofiadis, M.; Vassilikogiannakis, G. *Angew. Chem., Int. Ed.* **2014**, *53*, 13201−13205.

(14) Kornblum, N.; DeLaMare, H. E. *J. Am. Chem. Soc.* **1951**, *73*, 880−881.

(15) Omura, K.; Swern, D. *Tetrahedron* **1978**, *34*, 1651−1660.

(16) Levine, S. G. *J. Am. Chem. Soc.* **1958**, *80*, 6150−6151.

(17) Bestmann, H. J.; Roth, K.; Ettlinger, M. *Chem. Ber.* **1982**, *115*, 161−171.

(18) Nucleophilic addition of MeOH to the *endo*-peroxide **37** should be faster than a Kornblum−DeLaMare rearrangement.

(19) For two selected reviews on aldolases in context with synthetic organic chemistry, see: (a) Dean, S. M.; Greenberg, W. A.; Wong, C. H. *Adv. Synth. Catal.* **2007**, *349*, 1308−1320. (b) Bolt, A.; Berry, A.; Nelson, A. *Arch. Biochem. Biophys.* **2008**, *474*, 318−330.