A Journal of the Gesellschaft Deutscher Chemiker

Angewandte International Edition GDCh Chemie

www.angewandte.org

Accepted Article

Title: Stereochemical Determination of Tuscolid/Tuscorons and Total Synthesis of Tuscoron D and E: Insights into the Tuscolid/ Tuscoron Rearrangement

Authors: Björn Göricke, Michelle Fernandez Bieber, Kathrin E. Mohr, and Dirk Menche

To be cited as: *Angew. Chem. Int. Ed.* 10.1002/anie.201906166
Angew. Chem. 10.1002/ange.201906166

Link to VoR: http://dx.doi.org/10.1002/anie.201906166
http://dx.doi.org/10.1002/ange.201906166

WILEY-VCH

10.1002/anie.201906166

WILEY-VCH

Stereochemical Determination of Tuscolid/Tuscorons and Total Synthesis of Tuscoron D and E: Insights into the Tuscolid/Tuscoron Rearrangement

Björn Göricke,[a,c] Michelle Fernandez Bieber,[a,c,d] Kathrin E. Mohr,[b] and Dirk Menche*[a]

Abstract: The stereochemistry of the structurally unique myxobacterial polyketides tuscolid/tuscorons was determined by a combination of high field NMR studies, molecular modeling and chemical derivatization and confirmed by a modular total synthesis of tuscorons D and E. Together with the discovery of three novel tuscorons, this study enables detailed insights into the chemically unprecedented tuscolid/tuscoron rearrangement cascade.

Tuscolid (**1**) and tuscorons A and B (**2**, **3**, Figure 1)[1] present structurally unique and stereochemically elaborate polyketides from *Sorangium cellulosum*, which hold a special place among myxobacterial natural products[2] and polyketides[3,4] as they originate from one joint biosynthesis. While tuscolid is characterized by a 22-membered macrolactone ring with an uncommon chiral furan-one bearing a bridgehead double bond,[5] the tuscorons present linear compounds with a related furan-one and polypropionate, however, the appending diene is connected to a different dihydropyran by an sp^2-sp^3 fusion. While an interconversion has been tentatively proposed,[1] proof or details have remained elusive[1] and further studies have been hampered by the lack of full stererochemical knowledge and a missing synthetic approach to resolve the supply issue of these scarce metabolites. Herein, we report full stereochemical assignment of the tuscolid/tuscorons, the discovery of three novel tuscorons (**4**-**6**) and the total synthesis of tuscorons D and E, which unequivocally confirms our stereo-chemical proposal. In combination, these results enable detailed molecular insights into the unprecedented tuscolid/tuscoron rearrangement cascade.

Sorangium cellulosum strain So ce1401 was identified as an efficient tuscolid/tuscoron producing myxobacterium, leading to the discovery of the three novel tuscorons C, D and E (**4**, **5**, **6**, Figure 2). The planar structures of **4** and **5** were assigned by extensive 1D and 2D-NMR analyses. In detail, characteristic ^{1}H,^{1}H-COSY- correlations allowed for identification of the C-12 to

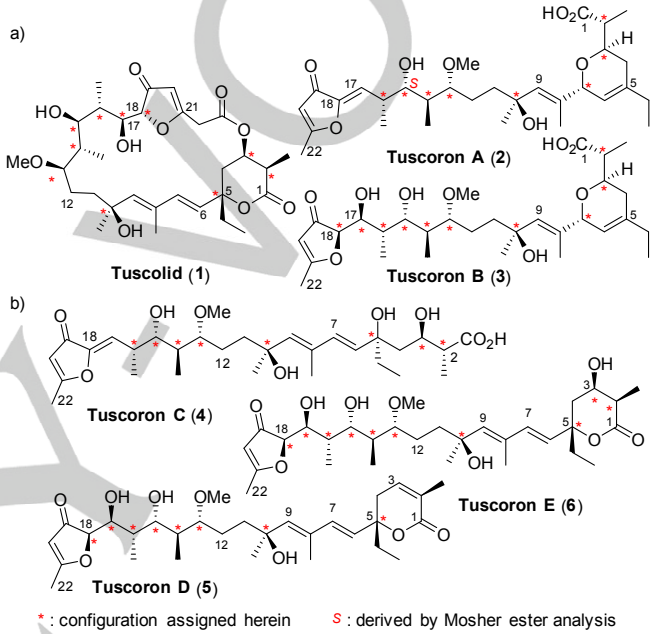


Figure 1. The tuscolid/tuscorons, biosynthetically interconverting polyketides from *Sorangium cellulosum*: a) known representatives and b) novel intermediates of the tuscolid/tuscorons rearrangement reported herein.

C-17 and C-12 to C-18 subunits of **4** and **5** confirmed by C,H-coupling data. HMQC- and HMBC-correlations, together with the double bond equivalents from HRMS analyses, defined the C-7 to C-12 and the C-18 to C-22 subunits of **4** and **5**. The corresponding data for the C-1 to C-9 fragment of **5** suggested a cyclic lactone resembling the analogous subunit of tuscolid, however lacking one H_2O equivalent. For tuscolid C, HMBC-correlations from H-4 and H-6 to C-1 were missing, which together with its molecular formula lead to the assignment of an open chain analog. In combination, these data lead to assignment of the planar structures of tuscorons C and D, which was further corroborated by close spectral similarities with the respective data for tuscorons A and B. For tuscoron E (**6**) 2D-C,H-coupling and ^{13}C-NMR experiments could not be obtained with sufficient quality due to the very low amounts available and its structure could only be derived by comparison with the tuscolid/tuscoron data. In detail, signals for the C-18 to C-22 subunit were very similar to those of tuscolid and tuscoron B, while the C1 to C-5 data resembled those of tuscolid and tuscoron C. Since the remaining signals corresponded to those observed for all tuscolid/tuscorones the planar structure of tuscoron E was tentatively assigned.

[a] B. Göricke, M. F. Bieber, Prof. Dr. D. Menche,
Kekulé-Institut für Organische Chemie und Biochemie
Universität Bonn
Gerhard-Domagk-Str. 1
D-53121 Bonn, Germany
E-mail: dirk.menche@uni-bonn.de

[b] Dr. K. E. Mohr
Helmholtz Centre for Infection Research (HZI): Microbial Drugs
Inhoffenstr. 7
D-38124 Braunschweig, Germany

[c] These authors contributed equally to this work. M.F.B.: structure elucidation, stereochemical determination and significant synthetic studies; B.G.: total synthesis of Tuscoron D and E.

[d] Present address: Molitor Solutions, Germany.

Supporting information for this article is given via a link at the end of the document.

10.1002/anie.201906166

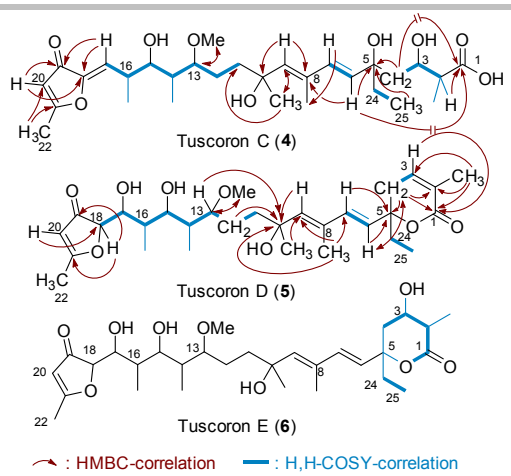


Figure 2. Planar structures of novel tuscorons C, D and E.

The constitutions of tuscolid and tuscorons A/B had been elucidated by the Höfle group and also a relative stereochemistry within the C-13 to C-17 of **1** and the pyranes of **1** and **2** was tentatively assigned.[1] For full stereochemical determination, optimal NMR resolutions were obtained in CD_3OD and $CDCl_3$, allowing for determination of the relevant $^3J_{H,H}$ coupling constants. These data and ROESY correlations suggested the C-12 to C-21 subunits to be relatively rigid. For tuscolid and tuscoron D, large homonuclear coupling constants between H-14 and H-15 as well as H-16 and H-17 indicated antiperiplanar relationships between these protons (Figure 3a, data for **1** given). Small vicinal couplings between H-13 and H-14, H-15 and H-16, and H-17 and H-18 together with strong NOE correlations between H-11 and H-14, H-15 and H-16, H-17 and H18, and H-15 and OH-17 and C-12 CH_2-protons and H-15 suggested this subunit to reside in conformation **7**. Three further key NOESY correlations, OMe-13 to Me-14, H-14 to Me-16 and Me-14- to H-16, supported the relative assignment, in full agreement with a previous analysis of Höfle for **1**. Following Murata's method[6] assignment of a *syn*-relationship between the adjacent oxygen substituents at C-17 and C-18 was based on small heteronuclear coupling constants observed from H-17 to C-18, and from H-18 to C-16 and C17, in combination with a small coupling constant from H-17 to H-18. This assignment was in agreement with a characteristic low-field shift of H-17 as compared to H-14 and H-15, caused by the carbonyl residing on the same side, subsequently confirmed by modeling, and characteristic coupling constants of synthetic material (*vide infra*). Based on a common biogenesis and close similarity of the spectral data, the stereochemistry of the C-12 to C-21 subunits of all tuscorons was assigned accordingly. In contrast to Höfle,[1] detailed analysis of the $^3J_{H,H}$ coupling couplings observed for the C-1 to C-9 segment of **2** suggested the contribution of two or more rapidly interconverting conformations (Figure 3b). Identical couplings from H-3 to H-4a and H-4b together with a medium value from H-6 to H-7 (3.4 Hz) supported considerable conformational flexibility. With strong NOE-contacts between H-3 and H-9 and H-2 to H-7, two interconverting conformers **8a** and **8b** were proposed and a relative *anti*-configuration between H-3 and H-7 was suggested, which was further supported by the absence of a significant NOE contact between these two protons, leading to a reassignment of this segment.[1] The relative configuration between C-2 and C-3 in turn was based on a large coupling between H- 2 and H-3 and a strong NOE-contact between Me-2 and H4a and H4b. Finally, the the relative configuration of the lactone fragments of **1** and **5** were deduced by strong NOE correlations between H-5 and H-4b, H-6 and H-7, and H-4a to Me-2 in combination with a small coupling from H-5 to H-4b and a large coupling from H-5 to H-4a (**9**, Figure 3c, data for **1** given), leading to an assignment as shown, in agreement with the Höfle proposal.[1]

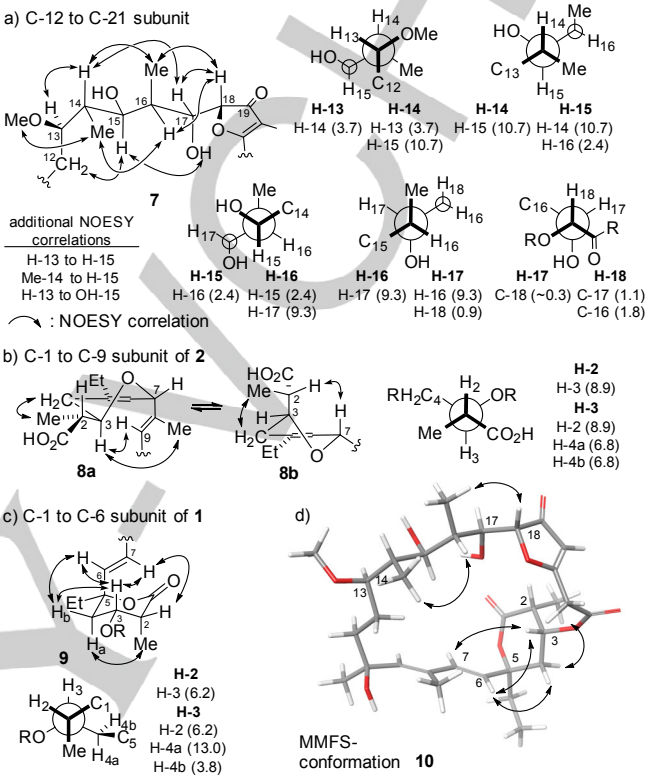
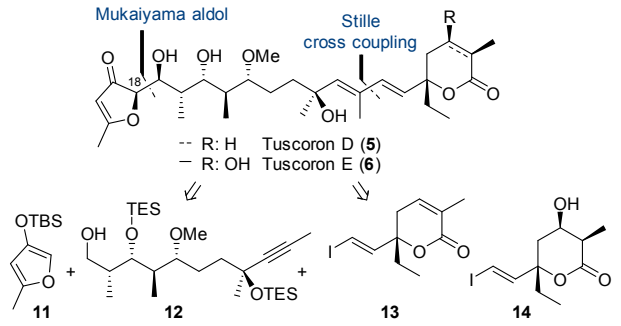


Figure 3. Key data for stereochemical assignment of the tuscolid/tuscorons (data for **1** given in Figures 3a and 3c, except for heteronuclear couplings, given for **6**). Abbreviation: MMFS = Merck molecular force field static.

Finally, correlation of these subunits and the centers at C-10 and C-18 was effectuated by molecular modeling (Macromodel: Monte Carlo searches using a generalized water solvent model) of the possible stereochemical permutations. The calculated conformation **10** of tuscolid isomer **1** resulted in a close match of calculated and observed NMR data. No low energy conformers for other C17,C18 configurations were found in accordance with the spectral data, thus confirming this assignment. Finally, the absolute configuration was derived by Mosher ester analysis of the OH-15 of tuscoron A (**2**) (Figure 1). Structure **1** summarizes the assignment of the full relative and absolute configuration for tuscolid. Based on a common biogenesis and close similarity of the spectral data, the stereochemistry of tuscorons A-E (**3**-**6**) was assigned accordingly (all Figure 1).

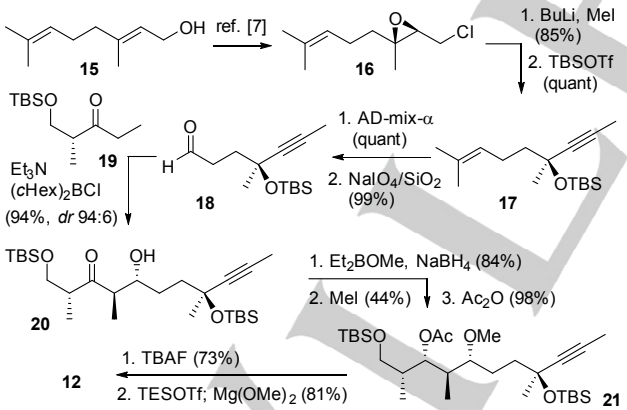
For validation of our proposal, key biosynthetic intermediate tuscoron E was selected together with tuscoron D as synthetic targets. A synthesis of tuscoron E was also required for definite structural proof. Our synthetic approach relied on three building blocks, *i.e.* furanone **11**, polypropionate **12** as well as lactones **13** and **14** for construction of tuscorons D and E, respectively (Scheme 1). For connection, a challenging cross coupling to forge the hindered C-7, C-8 bond and a modular Mukaiyama aldol reaction was planned for attachment of the Western

fragment to enable a stereodivergent access to all stereoisomers for further stereochemical proof.



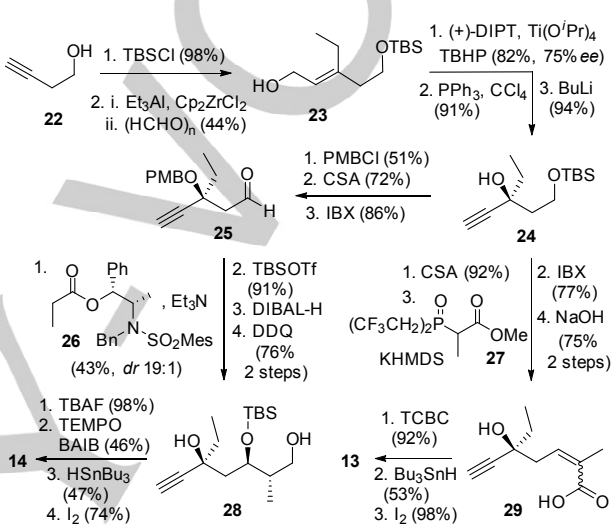
Scheme 1. Retrosynthetic analysis of Tuscoron D and E.

As shown in Scheme 2, synthesis of the central polypropionate fragment **12**, started by early introduction of the C-10 tertiary allylic alcohol. It was generated from geraniol (**15**) by a sequence involving Sharpless epoxidation, Appel reaction, double elimination of resulting chloride **16**, with subsequent methylation of resulting alkyne and TBS protection.[7] Inspired by a related transformation,[8] it proceeded in a reliable fashion giving **17** in high yields (64% from **15**). After quantitative Sharpless dihydroxylation and solid supported periodate cleavage,[9] resulting aldehyde **18** was elongated by an *anti*-aldol coupling with Roche ester derived ketone **19**, giving **20** with high selectivity (*dr* 96:4), purely based on substrate control. After 1,3-*syn* reduction and OH-13 methylation, the hydoxyl at C-15 was protected as an acetate (**21**). Ester migration to the primary during TBAF mediated TBS-deprotection was then effectively used for selective introduction of a TES ether at OH-15, followed by liberation of primary acohol giving **12** in a reliable sequence. Notably, introduction of a TES-ether was required for stereochemical control in the Mukaiyama coupling.



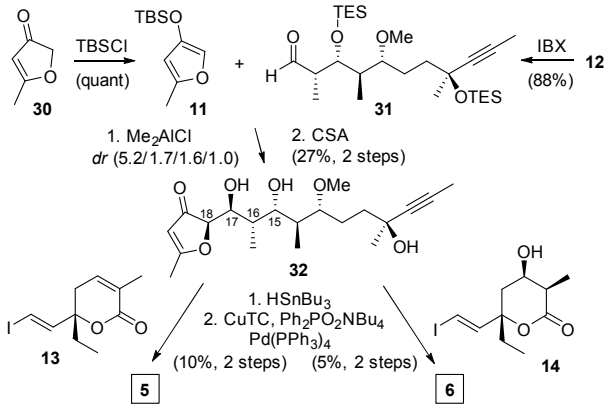
Scheme 2. Synthesis of central polypropionate **12**. Abbreviations: TBSOTf = *tert*-butyldimethylsilyl trifluoromethanesulfonate, AD-mix-α = asymmetric dihydroxylation mixture alpha, $(cHex)_2BCl$ = dicyclohexylboron chloride, TBAF = tetra-*n*-butyl ammonium fluoride, TESOTf = triethylsilyl trifluoromethanesulfonate.

Joint synthesis of Eastern fragments **13** and **14** was accomplished by a diversification of common precursor **24** (Scheme 3). Zirconium induced carboalumination[10–12] of commercial **22** with addition of formaldehyde gave allylic alcohol **23** and the tertiary alcohol was obtained by an analogous sequence as above. Notably, innovative use of Et_3Al in the initial zirconium-carboalumination presents an extension of this method. For tuscoron D fragment **13**, **24** was deprotected, oxidized and elongated by a Still-Gennari-olefination[13] with phosphonate **27**.[14] After saponification, both resulting diastereomers **29** (*E*/*Z* = 2.1:1) were cyclized by a Yamaguchi protocol, involving a dynamic *E*/*Z* isomerization.[15] Finally, iodide **13** was obtained by hydrostannylation and tin-iodine exchange. For tuscoron E fragment **14** joint intermediate **24** was protected and oxidized to aldehyde **25**, which was homologated by a Masamune *anti*-aldol[16] reaction with **26** proceeding with high selectivity (*dr* 19:1) towards **28**,[17] after auxiliary removal and protecting group manipulations. Finally, oxidative lactonization[18] and hydrostannylation/ tin-iodine exchange gave building block **14**.



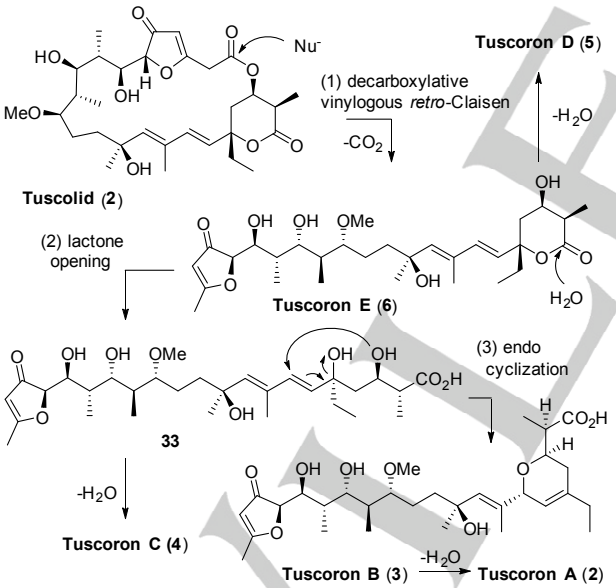
Scheme 3. Synthesis of Eastern building blocks. Abbreviations: TBS = *tert*-butyldimethylsilyl, (+)-DIPT = (+)-diisopropyl L-tartrate, PMB = *p*-methoxybenzyl, CSA = campher sulfonic acid, IBX = 2-iodoxybenzoic acid, DIBAL-H = diisobutylaluminum hydride, DDQ = 2,3-dichloro-5,6-dicyano-*p*-benzoquinone, TEMPO = 2,2,6,6-tetramethyl-1-piperidinyloxy, BAIB = bisacetoxyiodobenzene, TCBC = 2,4,6-trichlorobenzoyl chloride.

Fragment fusion was initiated by a Mukaiyama aldol reaction between ether **11** and aldehyde **31**, which were obtained by TBS protection of furanone **30**[19] and IBX oxidation of polypropionate **12** (Scheme 4). After considerable experimentation, useful selectivity towards desired isomer **32** was obtained with Me_2AlCl in non-polar DCM, presumably by enforcing an OTES-chelation the aldehyde **31**, together with minimization of steric interactions of the α- and β-center,[20] and favorable dipole-dipole interactions.[21] TES-deprotection of sensitive **32** proved delicate, giving decomposition under various methods. Finally, traces of CSA in DCM targeted triol **32** in acceptable yield, considering its lability. Comparison of the spectral data of all isomers with the natural tuscorons revealed only for the major isomer a close match, while considerable deviations were observed for all other isomers were observed.[22] Finally, hydrostannylation and cross coupling with either **13** or **14**, by a protocol by Fürstner,[23] gave tuscorons D (**5**) and E (**6**). All spectroscopic data (^{1}H-NMR, ^{13}C-NMR, MS) and CD spectra of authentic and synthetic material of **5** were in agreement, thus confirming their architectures, including that of key biosynthetic intermediate **6** and also verified the stereochemical proposals of all tuscolid/tuscorons.



Scheme 4. Completion of the total synthesis of tuscoron D and E. Abbreviation: CuTC = Copper(I) thiophene-2-carboxylate.

These results allow for a detailed formulation of the tuscolid/tuscorone rearrangement. In contrast to a tentative previous hypothesis a concerted action ca be ruled out,[1] and a stepwise mechanism has to be formulated. Also, full stereochemical knowledge is now available. The chemicall unprecedented cascade is initiated by a decarboxylative macrolactone opening followed by a vinylogous retro-Claisen condensation towards tuscoron E (Scheme 5). The δ-lactone may then be cleaved to give **33**, in agreement with the discovery of dehydrated tuscoron C. Finally, an intramolecular S_N2'-type *endo*-cyclisation would close the dihydropyran to tuscoron B (**3**). Tuscorons A and D (**2**, **5**), in turn are derived by water elimination from tuscorons B and E.



Scheme 5. Proposed tuscolid/ tuscoron rearrangement.

In summary, full stereochemical assignment of the tuscolid/tuscorons has been accomplished by a combination of high field NMR studies, modeling and chemical derivatization. This proposal was unambiguously confirmed by a joint total synthesis of tuscorons D and E by a versatile strategy, which proceeds in 18 steps from geraniol. Key steps involve highly stereoselective aldol reactions, a challenging, modular Mukaiyama coupling on highly elaborate fragments and a late-stage highly hindered and protective group free Stille cross coupling. This first total synthesis of the tuscolid/tuscoron family should be applicable also for synthesis of other members of this class and enable biological studies of these scarce metabolits. Furthermore, this study enables detailed insights into the biosynthetically and chemically unprecedented tuscolid/tuscoron rearrangement cascade, which should be further investigated.

Acknowledgements

Funding by the DFG (ME 2756/7-1) is gratefully acknowledged. We thank Andreas J. Schneider for excellent HPLC-support, Hendrik Schnepel for modeling support, Jutta Niggemann (HZI) for preparation of an extract and the fermentation service of the HZI.

Keywords: total synthesis • polyketides • rearrangements • natural products • myxobacteria

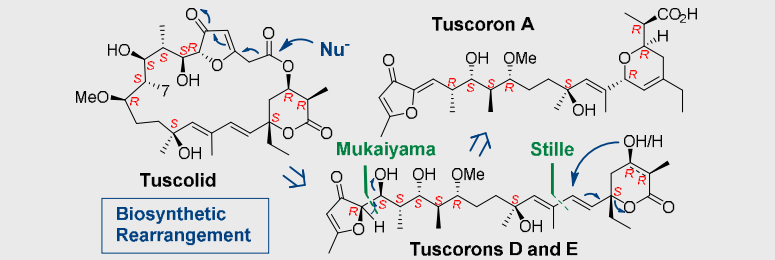
[1] J. Niggemann, M. Herrmann, K. Gerth, H. Irschik, H. Reichenbach, G. Höfle, *Eur. J. Org. Chem.* **2004**, 487.
[2] K. J. Weissman, R. Müller, *Nat. Prod. Rep.* **2010**, *27*, 1276.
[3] E. J. N. Helfrich, J. Piel, *Nat. Prod. Rep.* **2016**, *33*, 231.
[4] C. Hertweck, *Angew. Chem. Int. Ed.* **2008**, *48*, 4688.
[5] J. Y. W. Mak, R. H. Pouwer, C. M. Williams, C. M. *Angew. Chem. Int. Ed.* **2014**, *53*, 13664.
[6] a) N. Matsumori, D. Kaneno, M. Murata, H. Nakamura, K. Tachibana, *J. Org. Chem.* **1999**, *64*, 866. b) D. Menche *Nat. Prod. Rep.* **2008**, *25*, 905-918; heteronuclear coupling data were obtained for tuscoron D in CH_2Cl_2 due to optimal resolution.
[7] J. S. Yadav, P. K. Deshpande, G. V. M. Sharma, *Tetrahedron* **1990**, *46*, 7033.
[8] D. K. Mohapatra, C. Pramanik, M. S. Chorghade, M. K. Gurjar, *Eur. J. Org. Chem.* **2007**, 5059.
[9] Y. L. Zhong, T. K. M. Shing, *J. Org. Chem.* **1997**, *62*, 2622.
[10] E. Negishi, C. L. Rand, K. P. Jadhav, *J. Am. Chem. Soc.* **1981**, *46*, 5041.
[11] E. Negishi, D. Y. Kondakov, D. Choueiry, K. Kasai, T. Takahashi, *J. Am. Chem. Soc.* **1996**, *118*, 9577.
[12] T. Murakami, R. Hirono, K. Furusawa, *Tetrahedron* **2005**, *61*, 9233.
[13] W. C. Still, C. Gennari, *Tetrahedron Lett.* **1983**, *24*, 4405.
[14] C. Patois, P. Savignac, E. About-Jaudet, N. Collignon, *Synth. Commun.* **1991**, *21*, 2391.
[15] M. Ono, X. Y. Zhao, Y. Shida, H. Akita, *Tetrahedron* **2007**, *63*, 10140.
[16] A. Abiko, J.-F. Liu, S. Masamune, S. *J. Am. Chem. Soc.* **1997**, *119*, 2586.
[17] Small amounts of minor diastereomers originating from the Sharpless or the aldol coupling were removed at this stage. _The absolute configuration at C3 was assigned by Mosher ester analysis, while the relative configuration within the Eastern fragment was confirmed by NOE data, see SI section.
[18] T. M. Hansen, G. J. Florence, P. Lugo-Mas, J. Chen, C. J. Forsyth, *Tetrahedron Lett.* **2003**, *44*, 57.
[19] J. L. Shamshina, T. S. Snowden, *Tetrahedron Lett.* **2007**, *48*, 3767.
[20] D. A. Evans, B. D. Allison, M. G. Yang, C. E. Masse, *J. Am. Chem. Soc.* **2001**, *123*, 10840.
[21] J. D. Winkler, K. Oh, S. M. Asselin, *Org. Lett.* **2005**, *7*, 387.
[22] Configurational assignment at C17 was based on the acetonide method. S. D. Rychnovsky, B. Rogers, G. Yang, *J. Org. Chem.* **1993**, *58*, 3511, while the relative C17C18 configuration was confirmed by characteristic coupling constants, *i.e.* 3.7 and 3.7 Hz for the two *syn*-diastereomers and 7.3 and 7.9 Hz for the *anti*-isomers and the expected selectivity of the aldol coupling: see Ref. [20]. The main product resulted from the expected selectivity
[23] A. Fürstner, J. Funel, M. Tremblay, L. C. Bouchez, C. Nevado, M. Waser, J. Ackerstaff, C. C. Stimson. *Chem. Commun.* **2008**, 2873.

10.1002/anie.201906166

WILEY-VCH

Entry for the Table of Contents

COMMUNICATION



Insights into an unprecedented rearrangement: stereochemical determination, total synthesis and discovery of novel tuscorons reveal mechanistic insights into the biosynthetic tuscolid/tuscoron reaction cascade.

*Björn Göricke, Michelle Fernandez Bieber, Kathrin E. Mohr, and Dirk Menche**

Page No. – Page No.

Stereochemical Determination of Tuscolid/Tuscorons and Total Synthesis of Tuscoron D and E: Insights into the Tuscolid/Tuscoron Rearrangement