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Synthesis enables a structural revision of the *Mycobacterium tuberculosis*-produced diterpene, edaxadiene†

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Abstract

A stereodivergent synthesis of the [3.3.1] bicyclic core of edaxadiene was completed utilizing a key intramolecular oxidative ketone allylation. Significant discrepancies between the spectroscopic data obtained for the synthetic construct and the natural isolate raised questions about the structural assignment of edaxadiene. A subsequent structural reassignment was validated by completion of a total synthesis of the correct structure of the natural product.

Tuberculosis is a pulmonary disease caused by the pathogen *Mycobacterium tuberculosis*. Roughly two billion people, or one-third of the world's population, are believed to be infected by the bacterium, resulting in over 1.5 million deaths each year.[1] Despite these grave statistics, details about the infectivity and virulence of *M. tuberculosis* are only partially understood.[2] Peters and co-workers recently disclosed their isolation and structural elucidation of a halimane-type diterpenoid, edaxadiene (**1**), a compound produced by *M. tuberculosis*.[3] The genetic operon responsible for the production of edaxadiene is present only in pathogenic strains of mycobacteria.[4] In addition, edaxadiene was demonstrated to be an *in vitro* inhibitor of macrophage maturation.[3] These experimental observations suggest that production of edaxadiene is crucial for the infectivity and virulence of *M. tuberculosis*.

Our group has a growing interest in molecules related to the biology and treatment of tuberculosis.[5] The biological importance as well as the scarcity of edaxadiene (**1**) led us to actively pursue a total synthesis of this intriguing natural product. Our goal was to produce sufficient quantities of this molecule to confirm its structural assignment and enable a search for its biomolecular target.

We recognized that at the heart of this challenge is the synthesis of a [3.3.1] bicyclic core bearing five contiguous stereogenic centers, including two all-carbon quaternary stereogenic centers. In addition, the relative configuration of the C_{13} stereogenic center was not assigned during structural elucidation. Although edaxadiene (**1**) has conservation of the halimane skeleton (Fig. 1), formation of a unique C_7–C_{13} bond locks the conformation of the B-ring and produces a highly unfavorable 1,3-diaxial interaction between the C_{17} methyl group and C_1 methylene that is absent in related diterpenes.[6]

We envisioned that edaxadiene could arise from enone **3** by annulation of the leftmost ring onto the convex face of the [3.3.1] bicycle with subsequent deoxygenation of the C_6 ketone (**4**) to form the C_5–C_6 olefin (Scheme 1). We reasoned that this enone (**3**) could be fashioned

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through an intramolecular ketone allylation of an enone of type **2** to forge the C_7–C_{13} bond. Importantly, we anticipated that variation of olefin geometry and reaction conditions in this cyclization would provide access to both epimers at the unassigned C_{13} stereogenic center.

Our efforts thus began with the development of a synthesis of enone **2**. The thermal Diels–Alder cycloaddition of tiglaldehyde and a modified Rawal diene[7] (**5**) provided silyl enol ether **6** (Scheme 2). Subsequent methylenation of the aldehyde provided alkene **7** in good yield on a large scale (>15 g). Regioselective hydroboration of the terminal olefin with 9-BBN furnished a *B*-alkyl borane that was subjected to a Suzuki–Miyaura cross-coupling reaction[8] with (*Z*)-2-bromo-2-butene (**10a**) or (*E*)-2-bromo-2-butene (**10b**). The geometrical isomers **9a** and **9b** were obtained after deprotection of the enol ether and elimination of oxazolidinone with tetra-*n*-butylammonium fluoride.

After exploring multiple methods for constructing the C_7–C_{13} bond to provide a [3.3.1] bicycle we found that oxidative cyclization of **9a** with a bimetallic system of manganese(III) acetate and copper(II) acetate in a mixture of acetic acid and benzene (3:1) provided the volatile [3.3.1] bicycle **12a** in moderate yield as a single stereoisomer (Scheme 3).[9] This cyclization most likely proceeds through the intermediacy of an A(1,3) minimized intermediate (**11a**) to provide **12a**. Utilizing the same reaction conditions enone **9b** underwent cyclization to provide **12b**, the opposite C_{13} epimer, albeit with a lower degree of diaster-eoselectivity. We were pleased to find that we could gain access to both C_{13} epimers by controlling the geometry of the starting trisubstituted olefin.

It was at this point in our efforts that we began to note discrepancies between the reported ^{13}C shifts of the [3.3.1] core of edaxadiene and our synthetic constructs. To address our scepticism of the proposed structure, we converted the diastereomeric bicycles **12a,b** into dienes **14a,b**, bearing the full [3.3.1] bicyclic core of structure **1**. Thus, stepwise reduction of each enone to the saturated alcohol (**13a** and **13b**) was followed by dehydration to the corresponding alkene (**14a** and **14b**) with Martin sulfurane in refluxing benzene.[10] The unusually high temperature requirement for the dehydration is attributed to the equatorial disposition of the substrate alcohols. Although the high volatility and non-polarity of these compounds complicated their isolation, we were able to obtain sufficient quantities of each C_{13} epimer for characterization *via* semi-preparative gas chromatography. The spectroscopic data for each C_{13} epimer differed dramatically from the reported spectra of edaxadiene; most notably, the reported ^{13}C-NMR chemical shift at C_{13} was substantially different ($\Delta\delta$ of >30 ppm) from the ^{13}C chemical shifts observed for bicycles **14a,b** (Fig. 2).

These significant differences between the ^{13}C-NMR data of the isolated **1** and our synthetic bicycles (**14a** and **14b**) led to our reevaluation of the reported spectroscopic data for **1**. Our consideration of this data led us to propose compound **15** (Fig. 3) as the actual structure of edaxadiene. This proposal is consistent with the ^{13}C-NMR data, most notably the deshielded nature of C_{13}. Observation of the parent $[M - H_2O]^+$ ion by EI mass spectrometry is presumably due to the facile fragmentation of the tertiary allylic alcohol at C_{13} of **15**. We further suspect that edaxadiene, as reported, is likely not a pure compound, but rather a diastereoisomeric mixture at the remote C_{13} stereogenic center, as indicated by fine doubling of the olefinic protons in the ^1H spectrum of **1**. Our confidence in this reassignment was bolstered by a comparison of this structure to known terpenes in the literature, which indicated that this was a previously identified halimane diterpene, nosyberkol (**15**), isolated in 2004 from extracts of the Red Sea sponge *Raspailia* sp.[11] Furthermore nosyberkol (**15**, also referred to as isotuberculosinol) was previously speculated to be the product of the Rv3378c enzyme produced by *M. tuberculosis.*[12]

This proposed structural reassignment led us to develop a synthesis of nosyberkol (**15**) for structural verification.[13] Our efforts began with an *exo*-selective Diels–Alder cycloaddition[14] of diene **17** (available in two steps from 2,2-dimethylcyclohex-anone)[15] and ethyl tiglate to give the desired cycloadduct as an inseparable mixture of diastereoisomers (2:1) (Scheme 4). Subsequent reduction of the esters and separation of the primary alcohols provided the desired C_{10} epimer.[16] Aldehyde **18** was obtained in good yield using the conditions of Parikh and Doering.[17] Our attempts to perform a homologation of **18** through reaction with a phosphonate or phosphonium ylide led to no product formation; however, an aldol condensation with the sodium enolate of acetone and conjugate reduction of the resultant enone with Wilkinson's catalyst[18] allowed the desired homologation to ketone **20**. Our synthesis of nosyberkol was completed by the addition of vinylmagnesium bromide to the ketone to provide the desired compound as a 1.5:1 diastereomeric mixture at the tertiary allylic alcohol-bearing carbon. The spectroscopic data obtained for synthetic nosyberkol (**15**) were identical with those reported for both natural nosyberkol and edaxadiene (Fig. 2, see ESI[†]).[19]

We were also intrigued by the reported biomimetic conversion of tuberculosinol[20] (**16**) (Fig. 3) into edaxadiene (**15**) by treatment with a mixture of copper(II) chloride and *N*,*N*′-dicyclohexyl-carbodiimide (DCC).[3] To examine this conversion we pursued a short synthesis of tuberculosinol from aldehyde **18**. Thus methylenation of the aldehyde provided diene **19** in good yield. Subsequent regioselective hydroboration with 9-BBN and a palladium-mediated cross-coupling with (*E*)-3-iodobut-2-en-1-ol[21] (**21**) (Scheme 4) provided tuberculosinol (**16**). On exposure to catalytic copper(II) chloride tuberculosinol (**16**) was converted into nosyberkol (**15**) (Scheme 4). We found that addition of DCC is unnecessary for this transformation and propose that this is a Lewis acid-mediated allylic transposition.[22] In addition, we observed elimination of the allylic alcohol to provide a mixture of dehydrated products (34% as an *E*/*Z* mixture at the C_{12}–C_{13} olefin); however, formation of the proposed structure **1** was never observed.

In conclusion, a rapid and stereodivergent synthesis of the core of the originally proposed structure of edaxadiene (**1**) raised questions regarding its assignment. Re-evaluation of the spectral data led to the proposal that edaxadiene is actually nosyberkol (**15**), a known diterpene previously isolated from *Raspailia* sp. Furthermore, an independent synthesis of nosyberkol has unambiguously established this structural revision.[23] This synthesis of nosyberkol should provide sufficient quantities of this compound to elucidate its role in the infectivity and virulence of *M. tuberculosis.*

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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[†]Electronic supplementary information (ESI) available: Experimental procedures and spectroscopic data; interview with Erik Sorensen. See DOI: 10.1039/c0sc00284d

Notes and references

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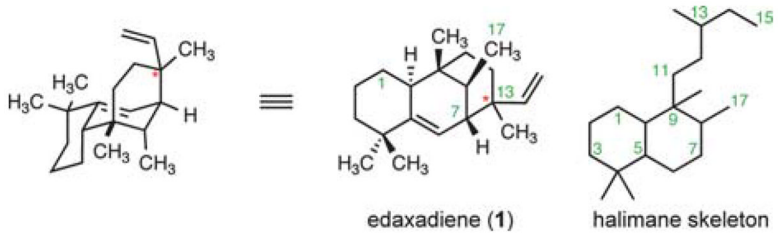


Fig. 1.
Proposed structure of edaxadiene. Stereochemistry is undefined at the starred (*) carbon.

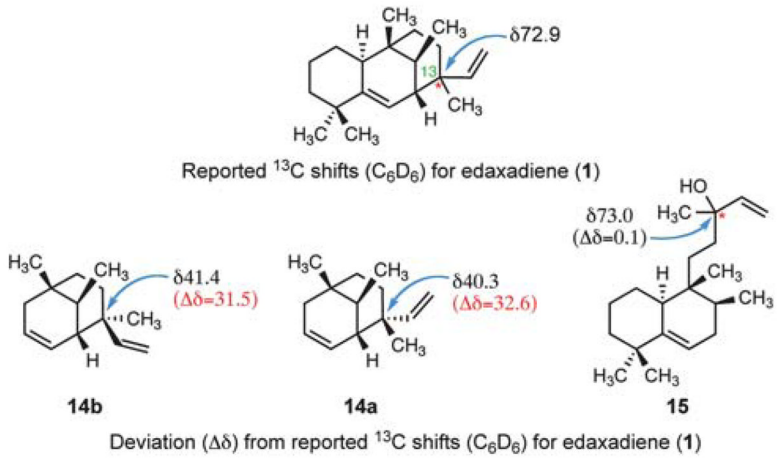


Fig. 2.

Discrepancies in ^{13}C-NMR shifts that indicate the structure of edaxadiene has been incorrectly assigned.

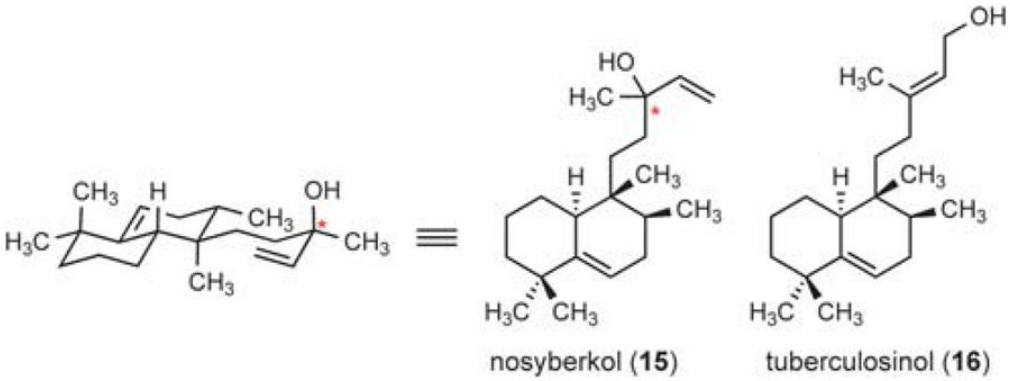
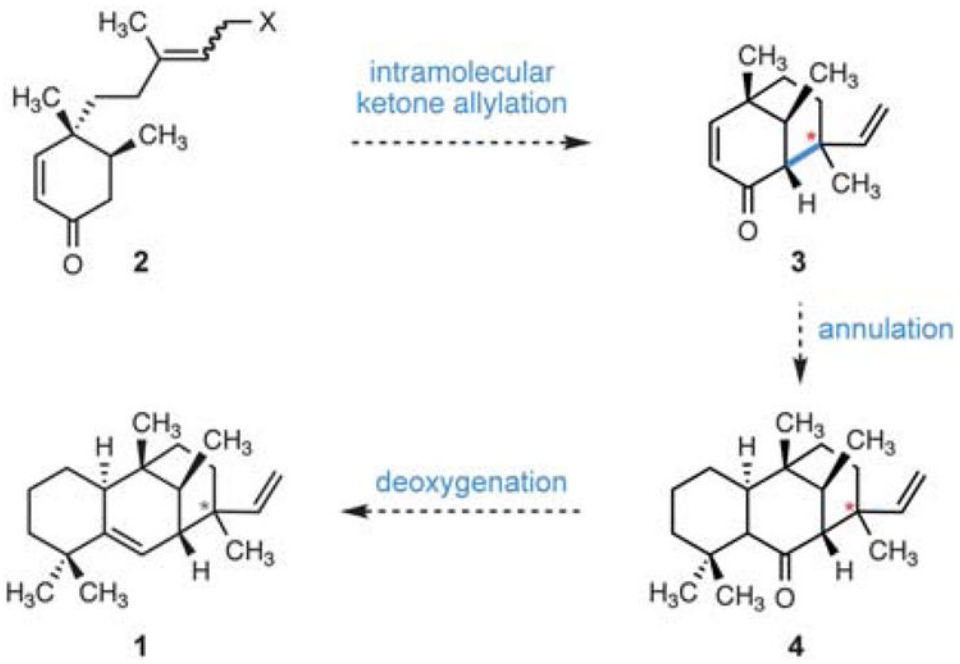
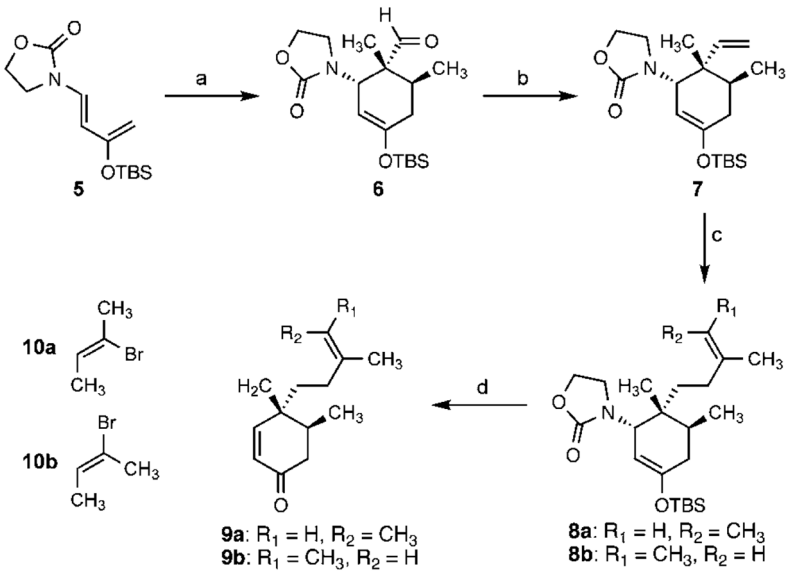


Fig. 3.
Structures of nosyberkol and tuberculosinol. Stereochemistry is unassigned at the starred (*) carbon.



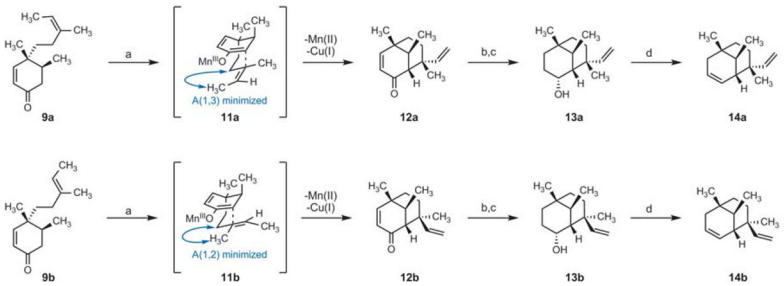
Scheme 1.

Proposed synthesis of **1** *via* an intramolecular ketone allylation. Stereochemistry is undefined at the starred (*) carbon.



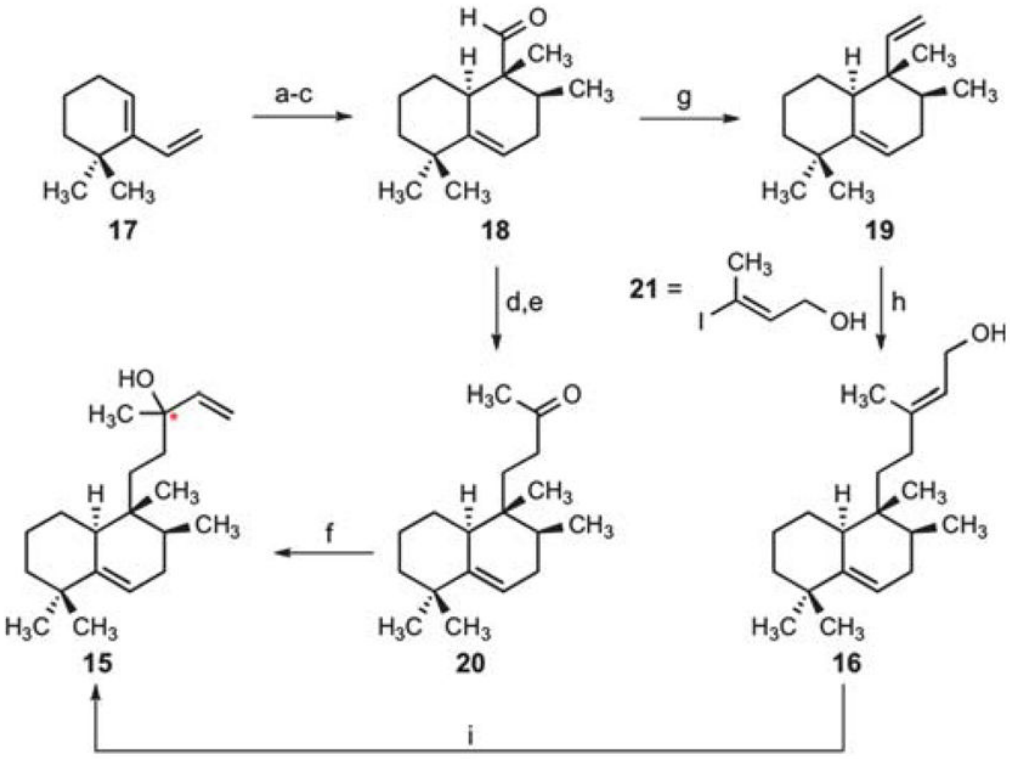
Scheme 2.

Diels–Alder-mediated synthesis of enones **9a,b**. Conditions: (a) tiglaldehyde, toluene, 120 °C, 80%; (b) Ph_3PCH_3Br, *n*-BuLi, THF, 0°C, 67%; (c) **8a**: 9-BBN, THF, reflux; then 10 mol% $PdCl_2$(dppf), aq. K_3PO_4, **10a**, DMF, 50 °C; **8b**: 9-BBN, THF, reflux; then 10 mol% $PdCl_2$(dppf), aq. K_3PO_4, **10b**, DMF, 50 °C; (d) **9a**: TBAF, THF, 86% (2 steps); **9b**: TBAF, THF, 100% (2 steps). 9-BBN = 9-bor-abicyclo(3.3.1)nonane; dppf =1,1′-bis(diphenylphosphino)ferrocene; TBAF = tetra-*n*-butylammonium fluoride.



Scheme 3.

Synthesis of the [3.3.1] bicyclic core of **1** *via* a stereodivergent intramolecular oxidative ketone allylation. Conditions: (a) $Mn(OAc)_3{\cdot}2H_2O$, $Cu(OAc)_2{\cdot}H_2O$, 80–100 °C, AcOH–benzene (1:3), **12a**: 57%, **12b**: 51% (1.4:1 dr); (b) $NaBH_4$, MeOH; (c) $LiAlH_4$, Et_2O, 0 °C, **13a**: 87% (2 steps), **13b**: 73% (2 steps); (d) Martin sulfurane, benzene, 80 °C, **14a**: 23%, **14b**: 34%.



Scheme 4.

Syntheses of nosyberkol and tuberculosinol *via* an *exo*-selective Diels–Alder reaction. Conditions: (a) ethyl tiglate, neat, 160 °C, 71% (2:1 *exo:endo*); (b) $LiAlH_4$, THF, 40 °C, 56% (+24% *endo* isomer); (c) SO_3·pyridine, NEt_3, CH_2Cl_2–DMSO, 0 °C, 86%; (d) acetone, NaHMDS, THF, −78 → 23 °C, 87%; (e) 10 mol% $Rh(PPh_3)_3Cl$, $HSiEt_3$, CH_2Cl_2, 40 °C, 83%; (f) vinylmagnesium bromide, THF, 0 °C, 93% (1.5:1 dr at the starred (*) carbon); (g) Ph_3PCH_3Br, KHMDS, 0 °C, THF, 91%; (h) 9-BBN, THF, 80 °C; then 10 mol% $PdCl_2$(dppf), Ph_3As, Cs_2CO_3, **21**, DMF, 73%; (i) 20 mol% $CuCl_2$, acetone, 20% (dr = 1:1 at the starred (*) carbon). 9-BBN =9-borabicyclo(3.3.1)nonane; dppf = 1,1′-bis(diphenylphosphino)ferrocene.