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Abstract

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Keywords

Catalysis, diterpenes, molecular structure, nuclear magnetic resonance, stereoisomerism

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Synthesis of (±)-Nosyberkol (Isotuberculosinol, Revised Structure of Edaxadiene) and (±)-Tuberculosinol

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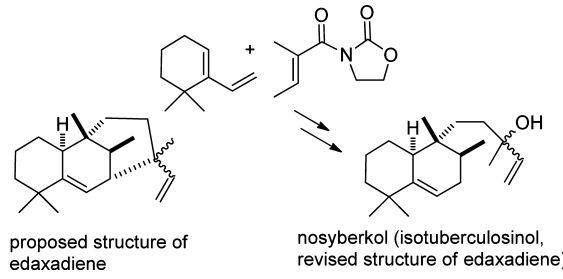
ABSTRACT



Me_2AlCl-catalyzed Diels−Alder reaction of *N*-tigloyloxazolidinone with 6,6-dimethyl-1-vinylcyclohexene selectively provided the exo adduct, which was converted to nosyberkol (isotuberculosinol) and tuberculosinol. The spectral data for nosyberkol are identical with those reported for edaxadiene, whose structure is revised accordingly.

Some of us recently reported the isolation of a biologically active diterpene edaxadiene (**3**) from *Mycobacterium tuberculosis* that is produced from halimadienyl diphosphate (**1**) by the action of the enzyme encoded by Rv3378c (see Scheme 1).[1] Edaxadiene alone arrests maturation of the endocytic phagosomal compartment at an early stage, a result very similar to that observed upon engulfment of *M. tuberculosis* by macrophage cells of the mammalian immune system.[1a] *M. tuberculosis* persists and replicates, causing disease, in such arrested phagosomal organelles, whereas *M. tuberculosis* mutants unable to make edaxadiene exhibit delayed proliferation, at least in macrophage cell culture.[2] Thus, edaxadiene presumably plays an important role early in the *M. tuberculosis* infection process.

Only 250 μg of edaxadiene was isolated from **1** with the use of purified Rv3378c encoded enzyme. Enzymatic dephosphorylation of **1** afforded tuberculosinol (**2**),[3a] which was treated with DCC and $CuCl_2$ in acetone to give 1 mg of edaxadiene in 20% yield. The mass spectrum suggested a molecular formula of $C_{20}H_{32}$ and structure **3** was proposed from analysis of the NMR spectra.[1] However, the formation of **3** would require the insertion of an allylic cation into an allylic C−H bond followed by the loss of a proton. This process is unlikely to occur enzymatically and should not occur by the reaction of **2** with DCC and $CuCl_2$. The ^{13}C NMR spectrum of edaxadiene showed a peak at δ 72.9,

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(1) (a) Mann, F. M.; Xu, M.; Chen, X.; Fulton, D. B.; Russell, D. G.; Peters, R. J. *J. Am. Chem. Soc.* **2009**, *131*, 17526–17527. (b) Mann, F. M.; Prisic, S.; Hu, H.; Xu, M.; Coates, R. M.; Peters, R. J. *J. Biol. Chem.* **2009**, *284*, 23574–23579.

(2) Pethe, K.; Swenson, D. L.; Alonso, S.; Anderson, J.; Wang, C.; Russell, D. G. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 13642–13647.

(3) (a) Nakano, C.; Okamura, T.; Sato, T.; Dairi, T.; Hoshino, T. *Chem. Commun.* **2005**, 1016–1018. (b) Nakano, C.; Sato, T.; Hoshino, T. *Koryo, Terupen oyobi Seiyu Kagaku ni kansuru Toronkai Koen Yoshishu* **2005**, *49*, 247–249. (c) Nakano, C.; Okamura, T.; Sato, T.; Hara, T.; Hoshiono, T.; Dairi, T.; Toyomasu, T.; Sassa, T. *Symp. Chem. Nat. Prod.* **2006**, 19–24. http://ci.nii.ac.jp/naid/110006682624/en.

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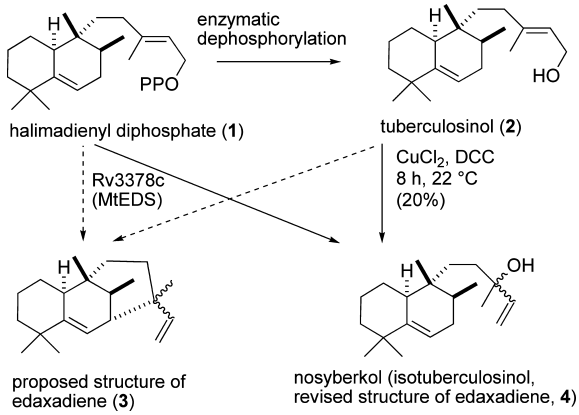
which suggested the presence of a tertiary alcohol in edaxadiene, which could readily lose H_2O to give the high mass peak corresponding to $C_{20}H_{32}$. The ^1H NMR spectrum of edaxadiene shows doubling of peaks for the vinyl protons indicating the presence of a 1:1 mixture of diastereomers.

Scheme 1. Conversion of Halimadienyl Diphosphate (**1**) to Nosyberkol (Isotuberculosinol, Revised Structure of Edaxadiene, **4**)

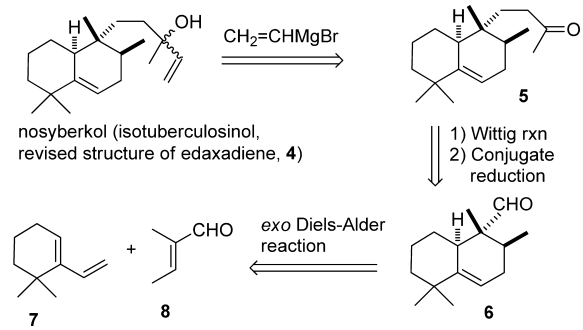


This analysis suggested that the structure of edaxadiene is a mixture of stereoisomers of the tertiary alcohol nosyberkol (**4**), which can be easily formed by hydrolysis of **1** with allylic rearrangement. Nosyberkol (**4**) was isolated by Kashman from the Nosy be Islands (Madagascar) sponge *Raspailia* sp. in 2004 as a single stereoisomer.[4] In 2005, Nakano reported the isolation of a 1:1 mixture of tuberculosinol (**2**) and **4** (as a 3:1 mixture of stereoisomers), which he called isotuberculosinol, by treatment of **1** with Rv3378c encoded enzyme.[3b,c] The ^1H and ^{13}C NMR spectra of edaxadiene in C_6D_6 are similar to those reported for nosyberkol in $CDCl_3$, but the different solvents used preclude a definitive comparison. Only partial data were reported by Nakano for isotuberculosinol.[3b,c] We therefore set out to synthesize nosyberkol (isotuberculosinol, **4**) to establish that it is identical to edaxadiene and to provide a ready source of material for further biological evaluation.

Our retrosynthetic analysis is shown in Scheme 2. Nosyberkol (isotuberculosinol, **4**) should be available as a mixture of isomers by addition of vinylmagnesium bromide to ketone **5**. A Wittig reaction on aldehyde **6** should give an enone that will be reduced to ketone **5** with Li/NH_3. An *exo* Diels−Alder reaction of diene **7**[5] and tiglic aldehyde (**8**) could give the required bicyclic aldehyde **6**.

Unfortunately, the Lewis acid-catalyzed Diels−Alder reaction of diene **7** with tiglic aldehyde (**8**) is known to give the expected, but undesired, *endo* Diels−Alder adduct **9** with 91−96% selectivity (see Scheme 3).[6] On the other hand, heating diene **7** in excess methyl tiglate (**10**) at 170 °C for

Scheme 2. Retrosynthesis of Nosyberkol (Isotuberculosinol, Revised Structure of Edaxadiene)



1 week afforded 74% of Diels−Alder adducts with 60% selectivity for the *exo* adduct **12**.[7] Most significantly, Danishefsky reported that the $EtAlCl_2$-catalyzed reaction of diene **7** with vinyl ketone **13** provided exclusively the desired *exo* Diels−Alder adduct **14**, which was converted to mamanuthaquinone.[8] Steric interactions between the aryl group of dienophile **13** and the methyl substituents on the cyclohexene of diene **7** retard the *endo* Diels−Alder reaction, whereas the methyl groups on the dienophile are small enough so that steric interactions with the methyl group of **7** have no effect on the *exo* Diels−Alder reaction. Steric interactions are still significant with the smaller methyl ester of **10**, which gave 60% of the *exo* adduct **12**, whereas the aldehyde of **8** is small enough that the undesired *endo* adduct **9** is formed almost exclusively.

A tigloyl dienophile was needed that (1) has a large substituent that would sterically retard the *endo* Diels−Alder reaction as observed by Danishefsky with the aroyl group of **13** and (2) could be converted to aldehyde **6** after the Diels−Alder reaction. The two methyl groups on the dienophile decrease its reactivity as shown by the harsh conditions needed for the thermal Diels−Alder reaction with methyl tiglate (**10**). Although *exo* adduct **14** was isolated in 85% yield by Danishefsky, the yield dropped to 43% without 1 equiv of THF and to 0% with other Lewis acids. A substituent was also needed that would enhance the reactivity of the dienophile.

N-Tigloylisoxazolidinone (**15**)[9] seemed well suited for this purpose. Evans developed asymmetric Diels−Alder reactions with chiral acryloyl and crotonyl oxazolidinones catalyzed by 2 equiv of Et_2AlCl.[10] These reactions are not very

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(5) (a) Knapp, S.; Sharma, S. *J. Org. Chem.* **1985**, *50*, 4996–4998. (b) Tanis, S. P.; Abdallah, Y. M. *Synth. Commun.* **1986**, *16*, 251–259.

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(7) de Miranda, D. S.; da Conceição, G. J. A.; Zukerman-Schpector, J.; Guerrero, M. A.; Schuchardt, U.; Pinto, A. C.; Rezende, C. M.; Marsaioli, A. J. *J. Braz. Chem. Soc.* **2001**, *12*, 391–402.

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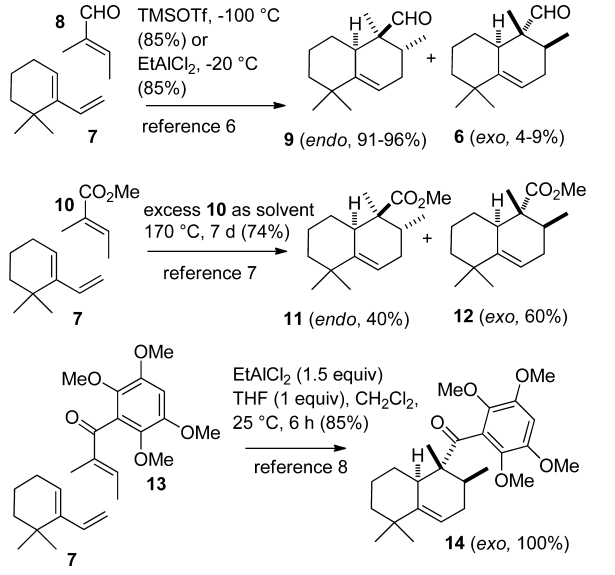
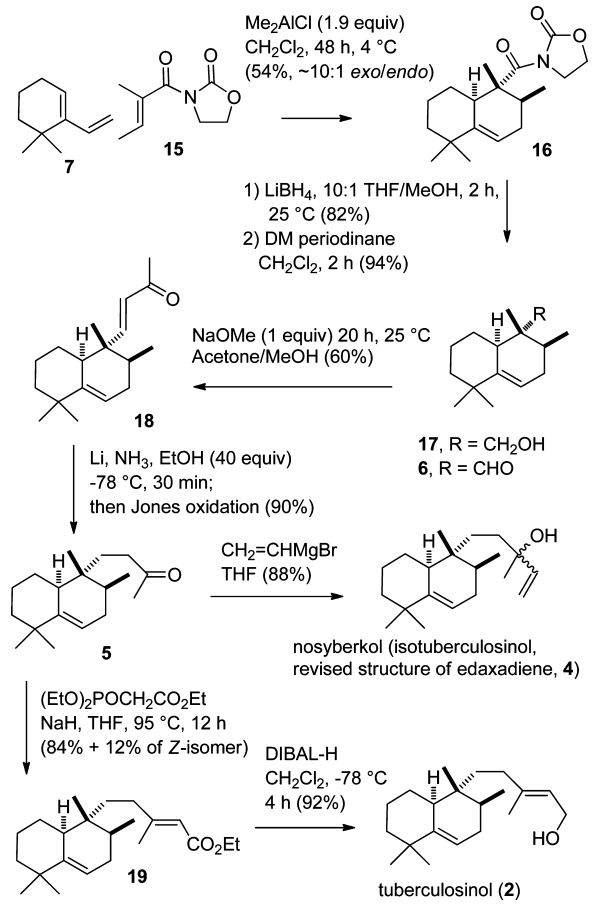
(10) Evans, D. A.; Chapman, K. T.; Bisaha, J. *J. Am. Chem. Soc.* **1988**, *110*, 1238–1256.

Scheme 3. Diels-Alder Reactions of Diene **7** with Dienophiles **8**, **10**, and **13**



Scheme 4. Synthesis of Nosyberkol (Isotuberculosinol, Revised Structure of Edaxadiene, **4**) and Tuberculosinol (**2**)



selective with methacryloyl oxazolidinones and have not been investigated with tigloyl oxazolidinones. The unsubstituted oxazolidinone **15** was chosen to minimize steric interactions between the α-methyl group and the oxazolidinone and because asymmetric induction seemed unlikely in an *exo* Diels−Alder reaction in which a chiral oxazolidinone would be far away from the diene. Reaction of diene **7** and oxazolidinone **15** (1 equiv) with 1.9 equiv of Me_2AlCl in CH_2Cl_2 at 4 °C for 2 days afforded a difficultly separable ∼10:1 mixture of the desired *exo* Diels−Alder adduct **16** and the *endo* adduct in 54% yield (see Scheme 4).[11] We also obtained *N*-2,3-dimethylbutanoyloxazolidinone[12] (20%), which was formed by conjugate addition of a methyl group to the dienophile. The ∼10:1 selectivity for the *exo* isomer was not quite as good as that observed by Danishefsky with dienophile **13**, but was a significant improvement over the 3:2 ratio obtained in the thermal Diels−Alder reaction with methyl tiglate (**10**).

Reduction of acyloxazolidinone **16** proceeded uneventfully with $LiBH_4$ in 10:1 THF/MeOH for 2 h at 25 °C to give alcohol **17** in 82% yield. Oxidation of **17** with Dess−Martin periodinane in CH_2Cl_2 for 2 h at 25 °C provided the desired *exo* aldehyde **6** in 94% yield. A similar sequence on a 9:1 mixture of **16** and the undesired *endo* isomer afforded a 9:1 mixture of aldehyde **6** and the undesired *endo* aldehyde **9**, which has spectral data identical with those previously reported.[6]

The equatorial aldehyde of **6** is very hindered by the flanking methyl group and cyclohexane methylene group. Reaction of **6** with diethyl 2-oxopropylphosphonate, LiCl, and *i*-Pr_2EtN in CH_3CN (Roush−Masamune conditions)[13] did not proceed at 25 °C. At reflux, enone **18** was formed in 15−20% yield, but the yield could not be improved because the Horner−Emmons−Wittig reagent decomposed at a comparable rate. Other Wittig reaction conditions gave even lower yields of **18**. Fortunately, aldehyde **6** cannot undergo aldol dimerization and is stable in base so **6** was converted to **18** in 60% yield by treating it with 1 equiv of NaOMe in acetone for 20 h at 25 °C to effect a mixed aldol reaction.[14]

The β-position of enone **18** is very hindered for the reasons discussed above for the aldehyde carbonyl of **6**. Nickel boride reduction of **18** to give **5** was unsuccessful.[15] Reduction with Li in NH_3/THF/*t*-BuOH (10 equiv) gave a mixture of ketone **5**, the corresponding saturated alcohol, and what may be the unsaturated pinacol reduction product.[16] Jones oxidation of this mixture afforded a 3:1 mixture of the desired saturated

(11) Use of other Lewis acids or thermal conditions gave significantly poorer results with **7** and the more readily available analogous diene 5,5-dimethyl-1-ethenylcyclohexene.

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(14) For related aldol reactions, see: (a) Kumar, K.; Wang, S. S.; Sukenik, C. N. *J. Org. Chem.* **1984**, *49*, 665–670. (b) Cockerill, G. S.; Kocienski, P.; Treadgold, R. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2101–2108. (c) Parry, A. D.; Neill, S. J.; Horgan, R. *Phytochemistry* **1990**, *29*, 1033–1039. (d) Yamamoto, T.; Shimada, A.; Ohmoto, T.; Matsuda, H.; Ogura, M.; Kanisawa, T. *Flavour Fragrance J.* **2008**, *19*, 121–133.

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(16) Caine, D. *Org. React.* **1976**, *23*, 1–258.

ketone **5** and unsaturated ketone **18**, which was presumably regenerated by oxidative cleavage of the pinacol product. Formation of pinacol byproducts is not usually a major side reaction in the reduction of conjugated enones with lithium in ammonia, but steric hindrance at the β-position of **18** will retard protonation of the radical anion so that dimerization at the unhindered carbonyl carbon to give the pinacol product is a significant side reaction. This problem was solved by using more of the less hindered alcohol EtOH. Reduction with Li in NH_3/THF/EtOH (40 equiv) at −78 °C, followed by oxidation of the mixture of saturated ketone and alcohol gave saturated ketone **5** in 90% yield. Addition of ketone **5** to vinylmagnesium bromide in THF at 0 °C provided nosyberkol (isotuberculosinol, **4**) in 88% yield as a 2:1 mixture of stereoisomers.

The 400 MHz ^{1}H and 100 MHz ^{13}C NMR spectra in $CDCl_3$ of synthetic **4** correspond well to those of nosyberkol. Some carbons appear as closely spaced doublets in synthetic **4**. The presence of stereoisomers is detectable in the ^{1}H NMR spectrum only by the two methyl doublets. The major isomer absorbs downfield (δ 0.790 vs 0.783). Because the data for the two isomers are so similar, it cannot be determined whether nosyberkol corresponds to the major or minor synthetic isomer.

Both the retention time and fragmentation pattern in the GCMS of synthetic **4** and edaxadiene are identical. The 700 MHz ^{1}H and 175/100 MHz ^{13}C NMR spectra in C_6D_6 of synthetic **4** (2:1 mixture of isomers) and edaxadiene (1:1 mixture of isomers) are identical except for differences due to the differing isomer ratios. Therefore, the structure of edaxadiene should be revised from **3** to that of nosyberkol (isotuberculosinol, **4**). The vinyl protons of the two diastereomers are slightly separated at 700 MHz. The methyl doublets are better separated in C_6D_6 (major at δ 0.839 vs minor at δ 0.813) than in $CDCl_3$. The major methyl doublet of the 3:1 mixture of isotuberculosinol isomers obtained by Nakano absorbs downfield in C_6D_6 indicating that Nakano's major isomer is identical to the major synthetic isomer.[3c]

Reaction of saturated ketone **5** with triethyl phosphonoacetate and NaH in THF at 95 °C[17] afforded ethyl enoate **19**[18] in 84% yield and the *Z*-isomer in 12% yield. Reduction of **19** with DIBAL-H in CH_2Cl_2 afforded tuberculosinol (**2**) in 92% yield. The spectral data are identical with those reported for the natural product.[3a]

In conclusion, we have revised the structure of edaxadiene from **3** to nosyberkol (isotuberculosinol, **4**). The biological activity previously reported for edaxadiene by some of us[1] is still valid, but should be ascribed to nosyberkol (**4**, isotuberculosinol). The efficient routes to isotuberculosinol (**4**) (six steps, 19% overall yield) and tuberculosinol (**2**) (seven steps, 17% overall yield) make these compounds readily available for further biological evaluation.

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Supporting Information Available: Complete experimental procedures, comparison of the spectral data of synthetic and natural isobuterculosinol (**4**) and tuberculosinol (**2**), and copies of ^{1}H and ^{13}C NMR spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Ester **19** was prepared by an unrelated route as an intermediate in the synthesis of agelasimines A and B: Ohba, M.; Kawase, N.; Fujii, T. *J. Am. Chem. Soc.* **1996**, *118*, 8250–8257.