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

Disciplines
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Comments

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

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ABSTRACT

Me₂AlCl-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). 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. 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. 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), which was treated with DCC and CuCl₂ in acetone to give 1 mg of edaxadiene in 20% yield. The mass spectrum suggested a molecular formula of C₂₀H₃₂ and structure 3 was proposed from analysis of the NMR spectra. 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 CuCl₂. The ¹³C NMR spectrum of edaxadiene showed a peak at δ 72.9.
which suggested the presence of a tertiary alcohol in edaxadiene, which could readily lose H₂O to give the high mass peak corresponding to C₂₀H₃₂. The ³¹H NMR spectrum of edaxadiene shows doubling of peaks for the vinyl protons indicating the presence of a 1:1 mixture of diastereomers.

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.² In 2005, Nakano reported the isolation of a 1:1 mixture of tuberculinosinol and 4 (as a 3:1 mixture of stereoisomers), which he called isoteruberculinosinol, by treatment of 1 with Rv3378c encoded enzyme.³b,c The ¹H and ¹³C NMR spectra of edaxadiene in CDCl₃ are similar to those reported for nosyberkol in CDCl₃, but the different solvents used preclude a definitive comparison. Only partial data were reported by Tanis, S. P.; Abdallah, Y. M. Raspailia (4) is a mixture of stereoisomers of the tertiary alcohol nosyberkol (5) and tiglic aldehyde (6). A Wittig reaction on aldehyde of 5 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 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 85% of the exo adduct 12 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 Et₂AlCl.¹⁰ These reactions are not very

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

![Diagram](image)

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

![Diagram](image)

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selective with methacryloyl oxazolidinones and have not been investigated with tigloyl oxazolidinones. The unsubstituted oxazolidinone 15 was chosen to minimize steric interactions between the $R$-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$_2$AlCl in CH$_2$Cl$_2$ 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 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$_2$Cl$_2$ provided the desired 18 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$_2$EtN in CH$_3$CN (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 $\beta$-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 NaH/THF/i-ButOH (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

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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)

(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-ethenylocyclohexene.

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 $\beta$-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 $-78^\circ$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 $^\circ$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$_6$D$_6$ 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$_6$D$_6$ (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$_6$D$_6$ indicating that Nakano’s major isomer is identical to the major synthetic isomer. Sc

Reaction of saturated ketone 5 with triethyl phosphonoacetate and NaH in THF at 95 $^\circ$C\textsuperscript{17} afforded ethyl enoate 19 in 84% yield and the Z-isomer in 12% yield. Reduction of 19 with DIBAL-H in CH$_2$Cl$_2$ afforded tuberculosinol (2) in 92% yield. The spectral data are identical with those reported for the natural product.\textsuperscript{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\textsuperscript{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 isotuberculosinol (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.