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SYNTHESIS OF METHYL 4-DIHYDROTRISPORATE B, A PROHORMONE OF BLAKESLEA TRISPORA

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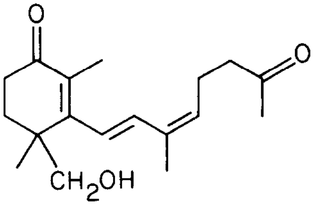
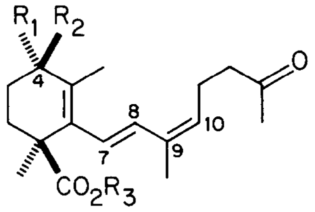
Summary: Methyl 4-dihydrotrisporate, a prohormone from the plus mating type of *Blakeslea trispora*, has been synthesized by a route which confirms its structure and configuration.

The recognition that specific prohormones mediate sexual reproduction in certain Mucoraceous fungi has led to intensive studies on biological aspects of this process.[1] The principal prohormones of the plus and minus mating strains of *Blakeslea trispora* have been identified as trisporol B (1) and the dihydro derivative 2 of methyl trisporate B (3) respectively.[2] A stereoselective synthesis of 1, as well as a practical route to the congeneric trisporic acids (eg 4), have been described.[3] We now disclose a synthesis of 2 which confirms the structural and stereochemical assignments made to this apocarotenoid substance, and which verifies that this prohormone is effective only against minus cultures of *Mucor mucedo*.

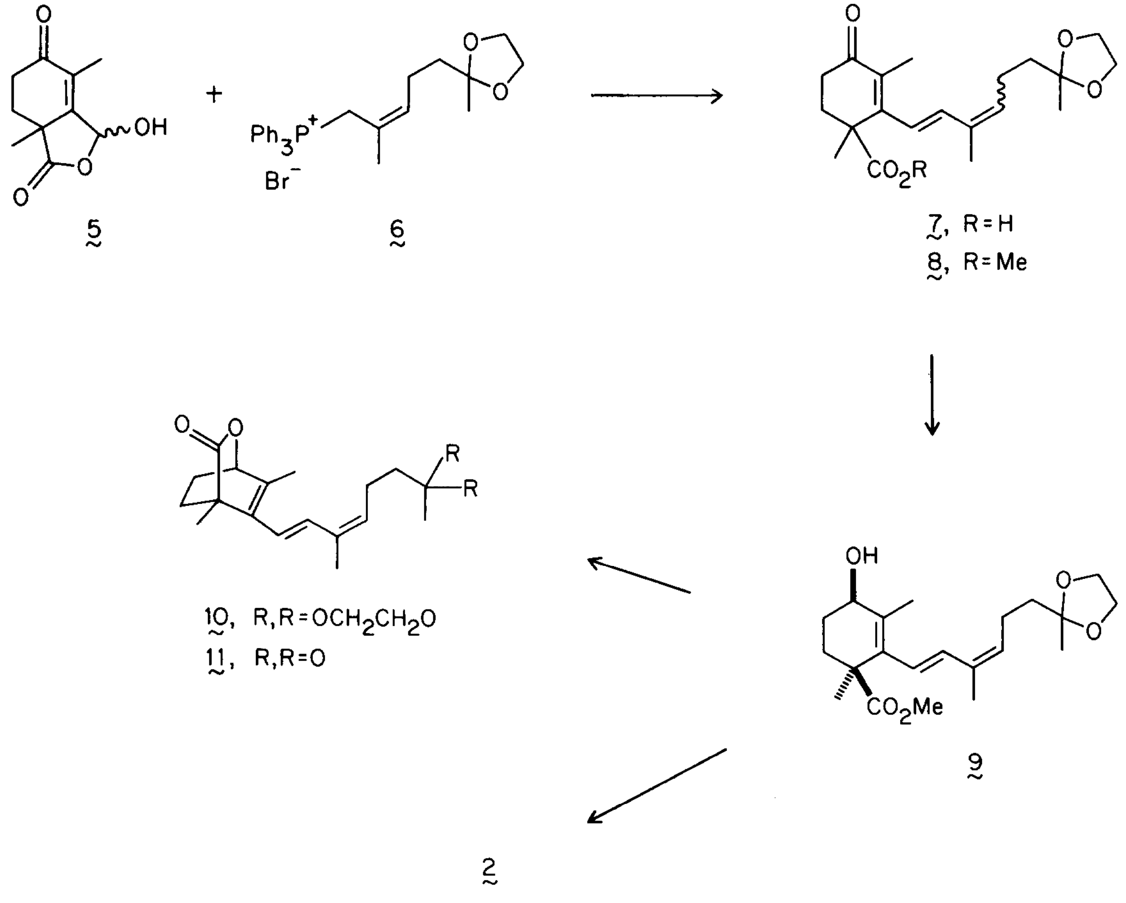


1



2, R_1=H, R_2=OH, R_3=Me
3, R_1,R_2=O, R_3=Me
4, R_1,R_2=O, R_3=H

The lactol 5 was prepared from α-methyltetronic acid[4] as described previously.[5] In an earlier study of the Wittig reaction of 5 with the ylide derived from Z phosphonium bromide 6,[3] it was found that a 1:1 mixture of the trisporic acid derivatives 7E and 7Z was produced in fair yield. Subsequently, we discovered that *in situ* conversion of 5 to its sodium carboxylate (NaH, THF-HMPA, 0°C), followed by addition of this solution to the ylide from 6 (BuLi, hexane, -40°C), resulted in a rapid Wittig reaction affording a 69% yield of keto acid 7Z, with less than 20% of its undesired E isomer. Without separation, this mixture was esterified with ethereal diazomethane to give a quantitative yield of 8E and 8Z; ν_{max} (neat liquid) 1730 (ester) 1665 cm^{-1} (ketone). Although the E and Z esters can be readily distinguished in this mixture by their characteristic vinyl proton signals in the NMR spectrum (see Table I),



5

6

7, R=H

8, R=Me

9

10, R,R=OCH_2CH_2O

11, R,R=O

2

Table I. Selected Proton NMR Resonances of Methyl 4-Dihydrotrisporate B and Related Structures

Compound	Chemical Shift (ppm) and Coupling Constant (Hz)			
	H_4	H_7	H_8	H_{10}
7Z		6.36 (d,J=17)	6.75 (d,J=17)	5.54 (t,J=7)
7E		6.20 (d,J=16)	6.22 (d,J=16)	5.60 (t,J=7)
8Z		6.33 (d,J=17)	6.76 (d,J=17)	5.57 (t,J=7)
8E		6.22 (d,J=16)	6.29 (d,J=16)	5.62 (t,J=7)
9Z	4.01 (m)	6.11 (d,J=16)	6.39 (d,J=16)	5.33 (t,J=7)
9E	4.00 (broad)	5.96 (2H,s)		5.40 (t,J=7)
2	4.08 (broad)	6.09 (d,J=16)	6.28 (d,J=16)	5.68 (m)
11	5.88 (t,J=3)	5.96 (d,J=16)	6.61 (d,J=16)	5.44 (t,J=7)

their sensitivity to photooxidation and isomerization made separation by chromatography at this point impractical. Consequently, the combined esters 8 were reduced directly with sodium borohydride in a 1:1 mixture of glyme and t-butyl alcohol. After chromatography on silica gel (Activity II, hexane-ethyl acetate 3:2 as eluent), the hydroxy ester 9 was obtained in 29% yield (based on lactone 5), along with a small amount of its E isomer.[6] The assignment of configuration at C-4 in 9 is deduced from its facile conversion, in the presence of potassium t-butoxide, to bicyclic lactone 10. The latter shows a carbonyl band at 1755 cm^{-1} but no hydroxyl absorption in its infrared spectrum. This transformation unambiguously specifies a cis orientation of hydroxyl and ester groups in 9. The assignment of 9Z geometry to this product is based on a comparison of vinyl proton chemical shifts with those of related substances of known configuration, as shown in Table I.

Hydrolysis of ketal **9** was accomplished with 80% acetic acid (room temp, 4·5 h) which afforded, after chromatography on silica gel, methyl dihydrotrisporate (**2**) in 46% yield. A similar hydrolysis of **10** gave the keto lactone **11** in 90% yield. The spectral properties of (±)-**2** are in excellent agreement with those reported for the P^+ prohormone from *B. trispora*.[2] In addition, it was shown that synthetic **2** induced extensive zygophore formation in minus cultures of *Mucor mucedo* at dose levels as low as 5 μg. No response was observed when **2** was administered to plus cultures of this organism at > 50 μg dosage levels, thus confirming that **2** is a mating-type specific prohormone of this organism.

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(6) Satisfactory analytical and spectroscopic data were obtained for all new compounds.

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