**Table I.** Percent Mono- and Dideuteriated Chrysophanol and Emodin After Incubation of Emodin with a Crude Extract from *Pyrenochaeta terrestris* in 50% $D_2O$

| substance recvd | incubn medium | % 1 D/mol | % 2 D/mol |
|---|---|---|---|
| chrysophanol | (a) complete (50% $D_2O$–buffer A) | 38 | 15 |
| emodin | As (a) | 17 | 4 |
| emodin | As (a)—NADPH | 8 | 2 |
| emodin | As (a), boiled extract | 6 | 1 |

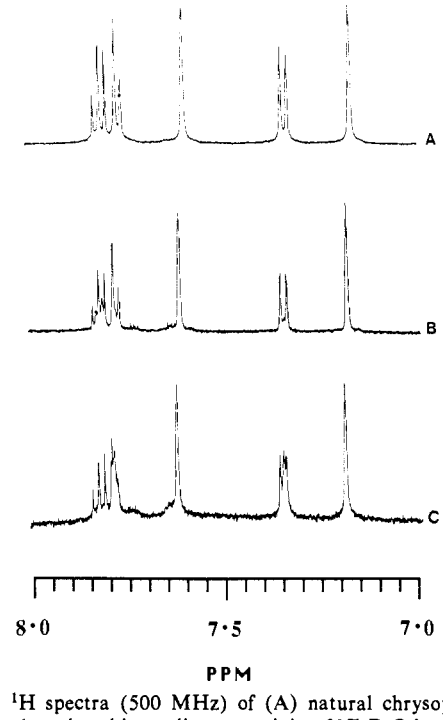
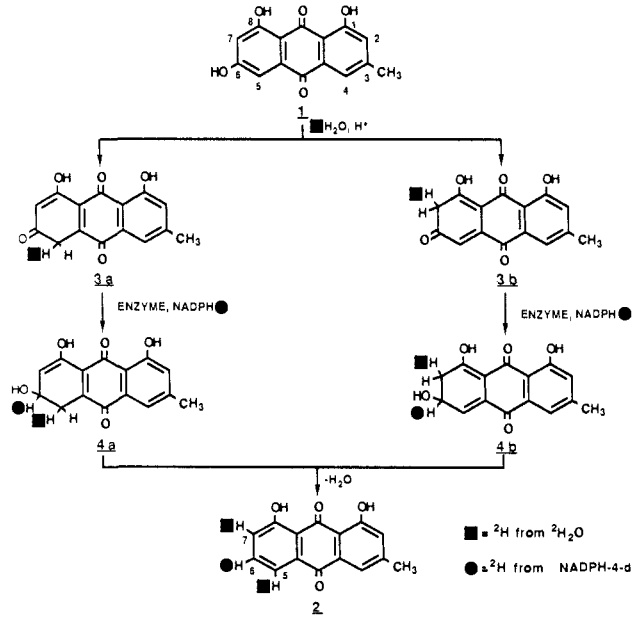



**Figure 1.** $^1H$ spectra (500 MHz) of (A) natural chrysophanol, (B) chrysophanol produced in medium containing 50% $D_2O$ in the presence of NADPH, and (C) chrysophanol produced in medium containing NADPH-*4-d*.

**Scheme I**




cell-free extract in 50% $D_2O$ buffer (Table I). In all cases, significant $^2H$ incorporation took place, but incorporation was highest in the presence of active enzyme (even in the absence of NADPH) indicating enzyme stabilization of the keto tautomers **3a,b**. The presence of 5% $^2H$ enrichment at position 6 could arise from exchange or from production of some NADPH-*4-d* in the medium. The latter mechanism was confirmed by incubation of emodin in the cell-free system containing the coupled enzyme components necessary for the generation of NADPH-*4-d*.[15] Isolation of the resultant chrysophanol and analysis by mass spectrometry showed 40% enrichment with deuterium. Inspection of the 500 MHz NMR spectrum of this specimen (Figure 1C) reveals that regiospecific deuteriation at C-6 has taken place. The sharp triplet (for H-6) at 7.83 $\delta$ is reduced in size by 40%, and the H-5 and H-7 doublets have large singlet components, indicating absence of coupling to H-6 (Scheme I). In one earlier report, NADPH has been shown to be necessary for phenolic reductions,[16] but the emodin–chrysophanol conversion is the first example of reduction of a phenolic substrate at the cell-free level, in which the cofactor NADPH has been shown to serve as the source of hydride at the C-6 position in the final product. It should also be noted that a chemical model for the reduction of 1,3,6,8-tetrahydroxynaphthalene (known to exhibit phenol–keto tautomerism) to the hydroaromatic substance scytalone is available.[17]

Further examples of this type of deoxygenation process are under study at the cell-free level in the expectation that the *absence* of a phenolic hydroxyl (e.g., in sterigmatocystin) may frequently signal operation of post-aromatic (rather than precyclic)[4-6] reduction–dehydration as demonstrated in this study, especially for polycyclic phenols.

**Acknowledgment.** We thank the R. A. Welch Foundation [Grants No. D-1043 (J.A.A.) and A-0943 (A.I.S.)] and N.I.H. for support of this research and Dr. R. D. Stipanovic for helpful discussions.

(15) Crude extract[8] (80 mL, 2.7 mg/mL protein) was brought to 0.75% in protamine sulfate and centrifuged. Ammonium sulfate (32 g, 63% saturation) was added to the supernatant. After centrifugation, the pellet was dialyzed against buffer A of ref 8. A solution containing 5.4 mmol of 2-propanol-*d8*, 80 units of *Thermoanaerobium brockii* alcohol dehydrogenase (Sigma A8278), and 42 $\mu$mol of $NADP^+$ in buffer A in a total volume of 7.5 mL was incubated for 1.0 h at 35 °C. $FeCl_2$, ATP, and emodin (same concentrations as in ref 8), ammonium sulfate fraction (48 mg), and buffer A were added to give a final volume of 22.5 mL. The mixture was incubated at 25 °C for 30 h. Chrysophanol was purified by preparative TLC in the usual manner[8] (yield 100 $\mu$g).

(16) Wheeler, M. H. *Exp. Mycol.* **1982**, *6*, 171.

(17) Bycroft, B. W.; Cashyap, M. M.; Leong, T. K. *J. Chem. Soc., Chem. Commun.* **1974**, 443.

## Total Synthesis of (-)-Botryococcene

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The broadly distributed, unicellular green alga *Botryococcus braunii* (Kützing) produces a large number of linear and monocyclic irregular triterpenes ("botryococcenes") that constitute as much as 90% of the dry weight of the organism.[1] The geochemical significance of this prolific hydrocarbon source has been noted,[2] and considerable effort has been expended on its cultivation for commerical purposes.[3] The most abundant member of this

(1) Maxwell, J. R.; Douglas, A. G.; Eglinton, G.; McCormick, A. *Phytochemistry* **1968**, *7*, 2157. More recent studies (Metzger, P.; Berkaloff, C.; Casadevall, E.; Coute, A. *Phytochemistry* **1985**, *24*, 2305) have shown that most wild strains of *B. braunii* consist of two closely related races, designated A and B. Although the two races have no detectable morphological differences, botryococcene metabolites are strictly characteristic of the B race, the A race metabolites being straight-chain odd-numbered hydrocarbons derived from oleic acid.

(2) Review: Cane, R. F. *Trans. R. Soc. S. Aust.* **1977**, *101*, 153.

(3) (a) Destordeur, M. *Ann. Mines Belg.* **1985**, *3*, 137. (b) Casadevall, E.; Dif, D.; Largeau, C.; Gudin, C.; Chaumont, D.; Desanti, O. *Biotechnol. Bioeng.* **1985**, *27*, 286.

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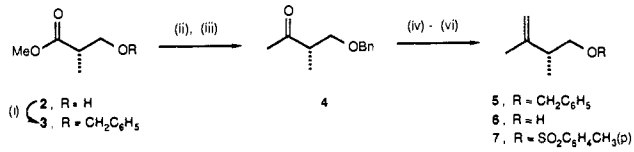
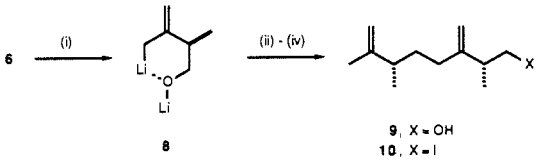
**Scheme I**[a]



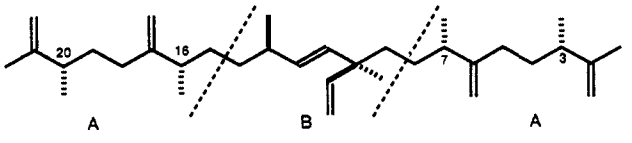

[a] (i) $Cl_3CC(=NH)OCH_2C_6H_5$, $CF_3SO_3H$, cyclohexane: $CH_2Cl_2$ (2:1), room temperature, 18 h, 97%; (ii) LiOH, MeOH, room temperature, 6 h, 99%; (iii) MeLi (2 equiv), $Et_2O$, room temperature, 4 h, 70%; (iv) $Ph_3PCH_2$, THF, 0 °C, 1 h, then room temperature, 1 h, 68%; (v) Li, $NH_3$, −78 °C, 2 min, 63%; (vi) *p*-TsCl, py, 0 °C, 18 h, 98%.

**Scheme II**[a]




[a] (i) *t*-BuOK–BuLi (2 equiv), hexane, 0 °C, 2 h; (ii) **7**, −78 °C → room temperature, 3 h, 80%; (iii) *p*-TsCl, py, 0 °C, 18 h, 99%; (iv) NaI, $CH_3COCH_3$, Δ, 12 h, 82%.

family of terpenoid hydrocarbons is botryococcene (**1**),[4] the absolute configuration of which was recently established as shown.[5] We now describe the total synthesis of **1** by a convergent route that fully confirms our stereochemical assignment.

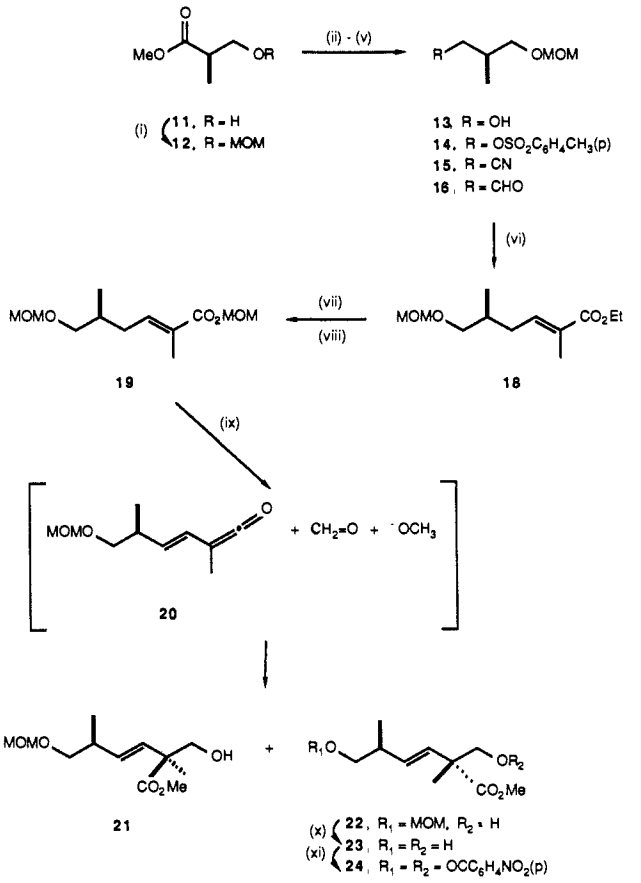



**1**

The matched configurations at C-3, -7, -16, and -20 of **1** suggested a synthetic plan that connected segment A to each end of the central unit B. Cognizant of the possibility that other members of the botryococcene series may not exhibit the stereochemical regularity of **1**, however, we sought a flexible strategy that could readily accommodate variations at all six stereogenic centers.[6] This requirement led to our selection of S (**2**) and R (**11**) enantiomers of methyl 3-hydroxy-2-methylpropionate as starting materials for segments A and B, respectively.

Benzylation of **2** with benzyl trichloroacetimidate[7] gave **3**, which was saponified and converted to ketone **4** with methyllithium (see Scheme I). A Wittig reaction of **4** afforded **5**, from which the benzyl group was removed to yield **6**.[8] The latter was converted quantitatively to its sulfonate **7**. Treatment of **6** with potassium *tert*-butoxide followed by *n*-butyllithium in hexane ("Schlosser base")[9] resulted in carbon deprotonation exclusively at the allylic methyl site, as judged by formation of a single MPTA ester with Mosher's reagent,[10] to produce a dianionic species formulated as **8** (see Scheme II). This highly nucleophilic synthon effected smooth displacement of tosylate **7** to furnish **9** as a single diastereomer, from which iodide **10** was prepared as the progenitor for segment A.

**Scheme III**[a]




[a] (i) $ClCH_2OCH_3$, $(i\text{-}Pr)_2NEt$, $CH_2Cl_2$, room temperature, 3 h, 99%; (ii) $LiAlH_4$, $Et_2O$, room temperature, 4 h, 86%; (iii) *p*-TsCl, py, 0 °C, 18 h, 96%; (iv) KCN, DMSO, room temperature, 18 h, 97%; (v) $(i\text{-}Bu)_2AlH$, $Et_2O$, room temperature, 4 h, 90%; (vi) $Ph_3PC(CH_3)CO_2Et$ (**17**), $PhCH_3$, Δ, 6 h, 77%; (vii) KOH, MeOH, Δ, 2 h, 77%; (viii) $ClCH_2OCH_3$, $(i\text{-}Pr)_2NEt$, $CH_2Cl_2$, room temperature, 4 h, 99%; (ix) 1 equiv of $(i\text{-}Pr)_2NLi$–HMPA, THF, −78 °C, 3 h, then room temperature 4 h, 43%; (x) concentrated HCl (catalyst), MeOH, Δ, 0.5 h, 90%; (xi) 2 equiv of $p\text{-}O_2NC_6H_4COCl$, 2 equiv of DMAP, py, $CH_2Cl_2$, room temperature 18 h, 78%.

Synthesis of the B subunit of **1** began with protection of **11** as its methoxy methyl (MOM) ether **12**, and the latter was reduced to **13** (see Scheme III). This alcohol was homologated via tosylate **14** and nitrile **15** to aldehyde **16**, which underwent a Wittig reaction with phosphorane **17** to furnish *E* αβ-unsaturated ester **18**.[11] Saponification of **18**, followed by reaction with chloromethyl methyl ether, gave MOM ester **19**.

Schultz and Berger have shown that αβ-unsaturated MOM esters undergo fragmentation–recombination in the presence of LDA–HMPA complex to give an α-hydroxymethyl-βγ-unsaturated methyl ester.[12] Application of this protocol to **19** led, via ketene **20**, to a 1:1.5 mixture of **21** and **22**. After separation (μ-Porasil, hexane–ethyl acetate, 2:3), acidic methanolysis of major diastereomer **22** gave diol **23** which afforded a crystalline bis *p*-nitrobenzoate **24**. X-ray crystallographic analysis of this substance established the stereostructure shown.

Although the absence of stereoselection in the Schultz reaction of **19** was disappointing, a ready means was at hand for utilization of both **21** and **22** in our plan (see Scheme IV). First, **21**, protected as the bis ether **25**, was reduced to **26**. Then **22**, after protection as silyl ether **27**, was reduced to **28** and converted to MOM ether **29**. Removal of the silyl group gave **26**, completing an effective inversion at the quaternary carbon of **22** and thereby making the synthesis of B fully stereoconvergent. Oxidation of

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(5) White, J. D.; Somers, T. C.; Reddy, G. N. *J. Am. Chem. Soc.* **1986**, *108*, 5352.
(6) Botryococcenes have been subclassified into two principal groups, designated n- and m-metabolites. Normal or "*n*-botryococcenes" are close relatives of **1** bearing fewer methyl substituents, whereas modified or "*m*-botryococcenes" are characterized by anomalous methylation patterns and/or cyclization. The structure of braunicene, a member of the latter group, will be the subject of a forthcoming publication (Zheng, H.; Poulter, C. D.; Wolf, F. R.; Somers, T. C.; White, J. D. *J. Am. Chem. Soc.*, in press).
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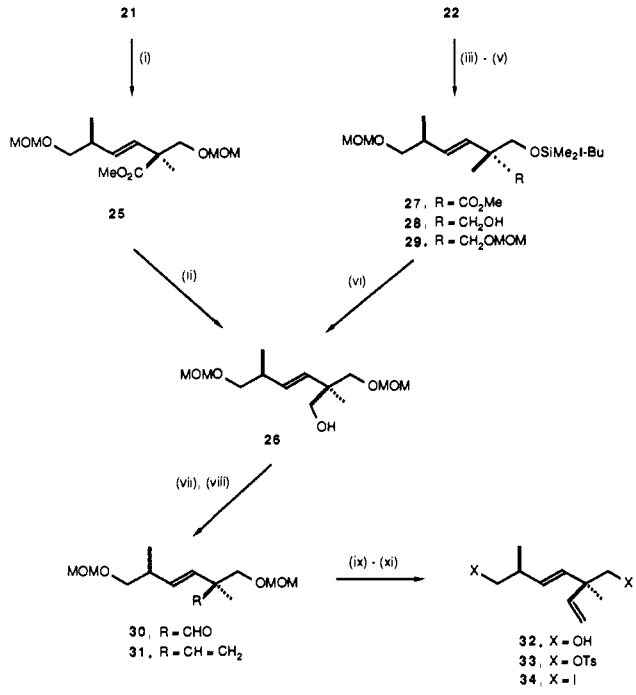
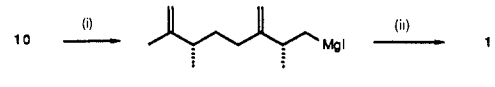
(12) Schultz, A. G.; Berger, M. H. *J. Org. Chem.* **1976**, *41*, 585.

**Scheme IV**[a]




[a] (i) $ClCH_2OCH_3$, $(i\text{-}Pr)_2NEt$, $CH_2Cl_2$, room temeprature, 4 h, 98%; (ii) $LiAlH_4$, $Et_2O$, room temperature, 4 h, 98%; (iii) $t\text{-}BuMe_2SiCl$, imidazole, DMF, room temperature, 18 h, 93%; (iv) $(i\text{-}Bu)_2AlH$, $Et_2O$, room temperature, 4 h, 70%; (v) $ClCH_2OCH_3$, $(i\text{-}Pr)_2NEt$, $CH_2Cl_2$, room temperature, 4 h, 79%; (vi) $Bu_4NF$, THF, room temperature, 2 h, 73%; (vii) $(COCl)_2$, DMSO, $CH_2Cl_2$, -78 °C, 1 h, then $Et_3N$, 1 h, 60%; (viii) $Ph_3PCH_2$, THF, 0 °C, 1 h, then room temperature, 1 h, 70%; (ix) concentrated HCl catalyst, MeOH, Δ, 1 h, 99%; (x) p-TsCl, py, 0 °C, 24 h, then room temperature, 24 h, 74%; (xi) NaI, $CH_3CO\text{-}CH_3$, Δ, 18 h, then in 2-butanone, Δ, 48 h, 63%.

**Scheme V**[a]




[a] (i) Mg, THF, Δ, 6 h; (ii) CuI, THF, then **34**, room temperautre, 5 days, 33-42%.

**26** under Swern conditions afforded aldehyde **30** which was transformed to **31** in a Wittig reaction. This diene was unmasked to yield **32**, and the latter was converted to diiodide **34** via its bis tosylate **33**.

The union of 2 equiv of **10** with **34** was investigated under a variety of conditions, and, although coupling could be effected rapidly at the sterically less encumbered terminus of **34**, the neopentyl iodide proved to be extremely sluggish in its reactivity. Eventually, it was found that preparation of Grignard reagent **35**, followed by treatment with anhydrous cuprous iodide, afforded an alkylcopper species[13] that underwent slow reaction with **34** to give botryococcene (**1**) (see Scheme V). The synthetic material was identical with the natural hydrocarbon in all respects, including optical rotation. This first synthesis of a member of the botryococcene family, together with the stereochemical investigation completed earlier,[5] sets the stage for biogenetic and other studies of this intriguing class of terpenoids.

**Acknowledgment.** We are indebted to Professor Arthur G. Schultz for providing unpublished information and to Professor Douglas A. Keszler for assistance with the X-ray crystal structure determination. Finanical support for this research was provided by the National Science Foundation (CHE-8619029) and by the Petroleum Research Fund, administered by the American Chemical Society, through a Summer Research Fellowship to G.O.S. Funds for the purchase of a Bruker AM 400 NMR spectrometer and a Rigaku X-ray diffractometer were provided by the National Science Foundation.

**Supplementary Material Available:** Spectral data are available for compounds **1**, **3-7**, **9**, **10**, **12-15**, **18**, **19**, **21**, **22**, **24-31**, and **33** (8 pages). Ordering information is given on any current masthead page.

(13) Tamura, M.; Kochi, J. *J. Am. Chem. Soc.* **1971**, *93*, 1485.

## A Model for the Proposed Mechanism of Action of the Potent Antitumor Antibiotic Esperamicin $A_1$

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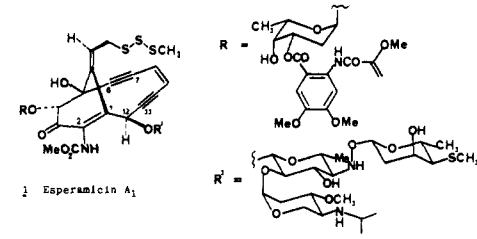
Very recently two groups reported the extraordinary structures of a new class of extremely potent antitumor antibiotics, of which esperamicin $A_1$ **1** has the common aglycone bicyclo[7.3.1]diynene system.[1] Co-occurring with these metabolites is an inactive




compound, esperamicin X **2**.[2] It was speculated that the mode of biological action of **1** involves nucleophilic attack on the central sulfur atom and thiol addition to the α,β-unsaturated carbonyl group to give the putative intermediate **3** (see Scheme I). It was suggested that the change of hybridization at C-1 from $sp^2$ to $sp^3$, in effect, pulls together the ends of the diynene C-6 and C-11 to allow cyclization of the diynene **3** into the 1,4-diyl(*p*-benzyne) **4**. This diradical can abstract a hydrogen atom from the sugar phosphate backbone of DNA and result in strand scission. While **3** can cyclize to the [3.3.1]system **4**, esperamicin **1** cannot, since the transition state would be prohibitively high due to the bridgehead double bond at C-1. Consequently, the triggering thiol addition at C-1 does more than reduce the distance between C-6 and C-11, it allows access to a reasonable kinetic pathway to **4**. The 1,4-diyl process has a parallel in the earlier work of Bergman,[3] who showed that the prototype diynene **5** could be converted into benzene and 1,4-dichlorobenzene when exposed to 1,4-cyclohexadiene and $CCl_4$, respectively. The conditions (195 °C) hardly parallel the mild conditions (room temperature to 37 °C) speculated for the conversion of **3** into **4**. The $\Delta G^{\ddagger}$ for the conversion of **5** into benzene via the 1,4-diyl is approximately 32 kcal $mol^{-1}$.

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