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

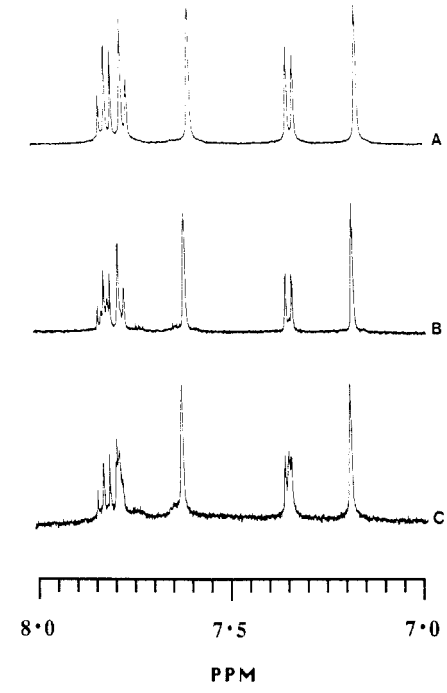
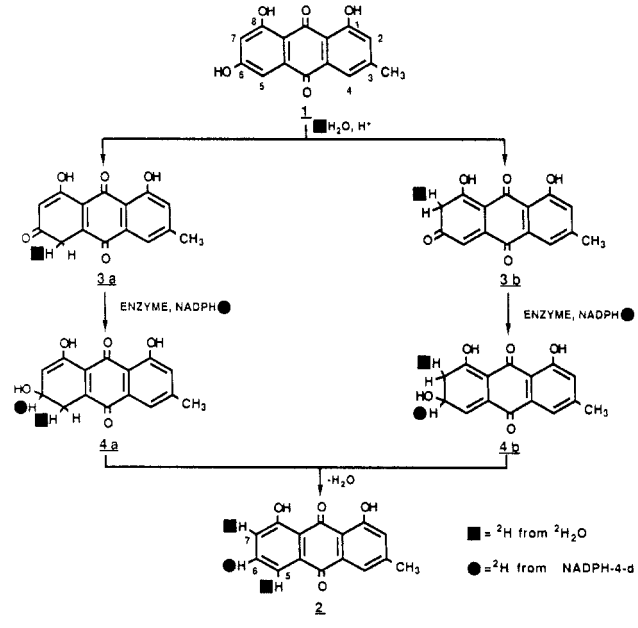


Figure 1. ^1H spectra (500 MHz) of (A) natural chrysophanol, (B) chrysophanol produced in medium containing 50% D_2O in the presence of NADPH, and (C) chrysophanol produced in medium containing NADPH-*4-d*.

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 δ 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 μmol 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 μg).

(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

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(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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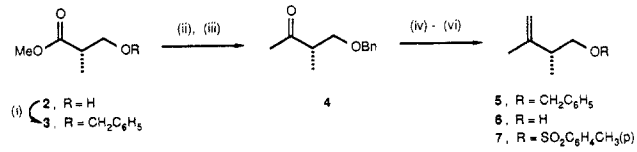
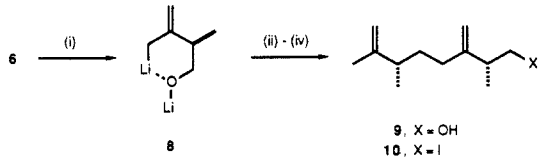
Scheme I[a]



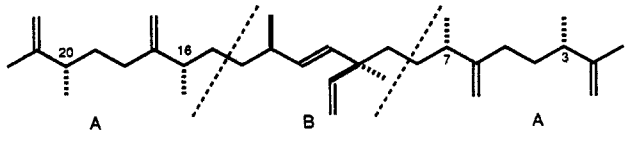
[a] (i) $Cl_3CC(=NH)OCH_2C_6H_5$, CF_3SO_3H, cyclohexane: CH_2Cl_2 (2:1), room temperature, 18 h, 97%; (ii) LiOH, MeOH, room temperature, 6 h, 99%; (iii) MeLi (2 equiv), Et_2O, room temperature, 4 h, 70%; (iv) Ph_3PCH_2, THF, 0 °C, 1 h, then room temperature, 1 h, 68%; (v) Li, NH_3, −78 °C, 2 min, 63%;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

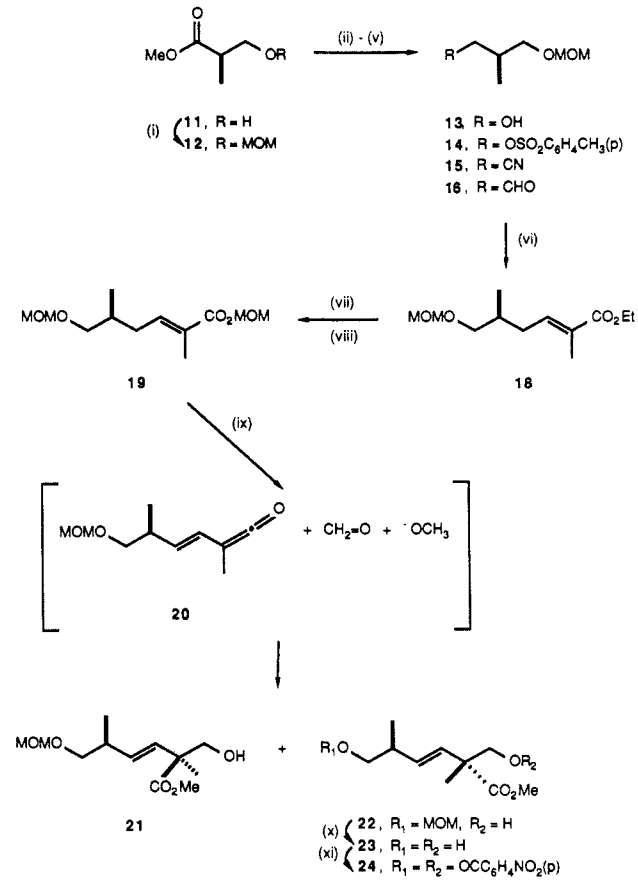


1

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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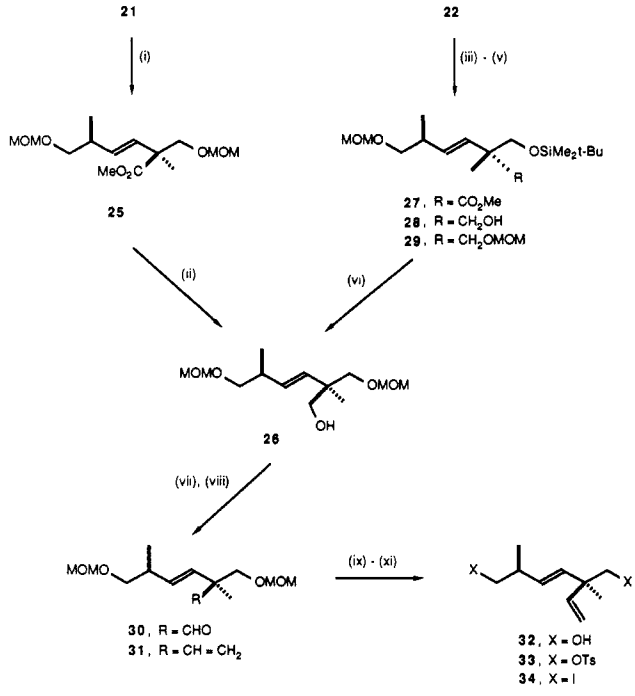
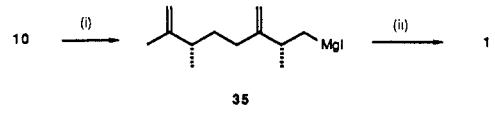
Scheme IV[a]



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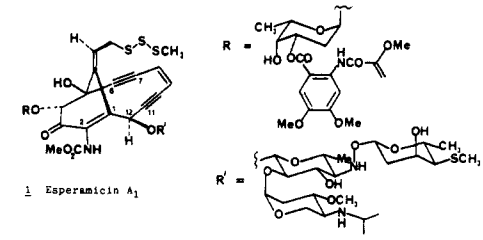
Very recently two groups reported the extraordinary structures of a new class of extremely potent antitumor antibiotics, of which esperamicin A_1 **1** has the common aglycone bicyclo[7.3.1]diynene system.[1] Co-occurring with these metabolites is an inactive



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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