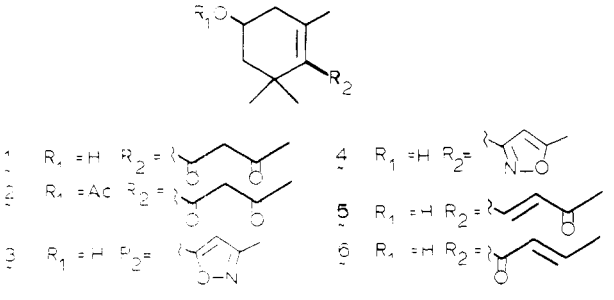
Communications

1-(2,6,6-Trimethyl-4-hydroxycyclohexenyl)-1,3-butanedione, an Extracellular Metabolite from the Dinoflagellate *Prorocentrum minimum*

Summary: Spectral analysis and chemical derivation of a purified component of filtrate extracts have shown that in culture the dinoflagellate *Prorocentrum minimum* excretes 1-(2,6,6-trimethyl-4-hydroxycyclohexenyl)-1,3-butanedione.

Sir: Marine dinoflagellates, along with other unicellular algae, are the primary producers in marine food chains. The production of allelopathic chemicals and mammalian toxins by these phytoplankton is a well-documented biological phenomenon.[1,2] Recent reports have described the structures of the potent neurotoxins, saxitoxin and the gonyautoxins, produced by the red tide dinoflagellates belonging to the genus *Gonyaulax*.[2] The importance of other bioactive dinoflagellate metabolites to marine ecology is obvious; however, very little is known about their chemical structures.

We would like to report the isolation and structure determination of 1-(2,6,6-trimethyl-4-hydroxycyclohexenyl)-1,3-butanedione (1), an extracellular metabolite



produced in laboratory cultures[3] of the dinoflagellate *Prorocentrum minimum*. The organic exudates from 400 L of acidified (pH 2) *P. minimum* culture filtrate were concentrated on Amberlite XAD-2 resin (150 g).[5] Washing the XAD-2 resin with methanol yielded ~60 mg of a crude extract. Repeated silica gel preparative TLC [$CHCl_3$–MeOH (15:1); Et_2O–hexane (2:1)] gave 1 (6 mg) as a colorless oil[6] that was strongly ferric chloride positive and which showed the following: $[\alpha]^{25}_D$ −35° (*c* 0.5, MeOH); UV (MeOH) λ_{max} 287 nm (ϵ 1.1 × 10^4); UV (KOH–MeOH) λ_{max} 299 nm (ϵ 1.9 × 10^4); IR ($CHCl_3$) 3600 (s), 3450 (br), 2600 (br), 1595, 1370, 1215, 1075, 1035, 995, 950 cm^{-1}. The high-resolution mass spectrum of 1 showed a strong parent ion at *m/z* 224.1414, appropriate for a molecular formula of $C_{13}H_{20}O_3$ (calcd *m/z* 224.1412). Fragment ions at *m/z* 209, 206, and 191 indicated facile losses of CH_3 and H_2O. A large peak at *m/z* 85 (25%) corresponding to a composition of $C_4H_5O_2$, in conjunction with the positive ferric chloride test, suggested that the molecule contained a 1,3-butanedione residue. The infrared spectrum, which shows a strong carbonyl absorbance at 1595 cm^{-1}, the ultraviolet spectrum, which shows λ_{max} at 287 nm, and a bathochromic shift in base indicate that the β-diketone system should be further conjugated.

In the ^{13}C NMR (100 MHz, $CDCl_3$), the 1,3-butanedione unit appears as four resonances at δ 189.1, 103.4, 193.4, and 25.8 which correspond very closely to the resonances displayed by the side-chain carbons in benzoylacetone (**7**, Figure 1). A three-proton singlet at δ 2.13 and a sharp one-proton singlet at δ 5.45 in the ^1H NMR (400 MHz, $CDCl_3$) spectra indicate that the β-diketone functionality exists exclusively in one of the two enolized tautomeric forms. One additional olefinic bond is apparent from the

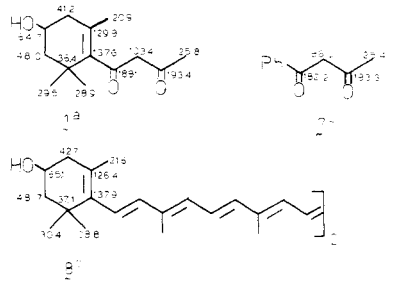


Figure 1. ^{13}C NMR chemical shift data in parts per million relative to Me_4Si: (a) 100 MHz, $CDCl_3$; (b) see ref 7; (c) see ref 8.

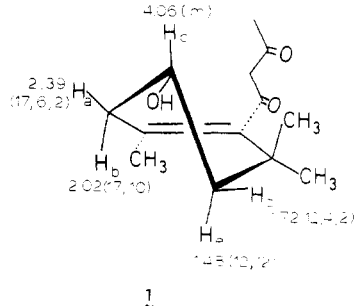


Figure 2. ^1H NMR data (δ, 400 MHz, $CDCl_3$) for the cyclohexene ring protons in 1.[14]

(1) (a) Hasimoto, Y. "Marine Toxins and Other Bioactive Marine Metabolites"; Japan Scientific Societies Press: Tokyo, 1979; p 232. (b) Uchida, T. *Nippon Seitaigaku Kaishi* **1977**, *27*, 1.

(2) Shimizu, Y. In "Marine Natural Products, Chemical and Biological Perspectives"; Scheuer, P. J., Ed.; Academic Press: New York, 1978; Vol. 1, Chapter 1.

(3) A unialgal culture of *Prorocentrum minimum* was obtained from the Pacific Northeast Culture Collection, UBC. Initially 1-L axenic cultures of *P. minimum* were grown in charcoal-treated seawater which was autoclaved prior to addition of sterile ES[4] enrichment. Cells were grown on a 16:8 light/dark cycle at 18 °C under 60 μE m^{-2} s^{-1} illumination to senescence (~14 days). Cells were removed by membrane filtration, and the acidified filtrate (pH 2) was either extracted with $CHCl_3$ or XAD-2 resin. Both methods gave 1 in similar yields. Sea-water media blanks and extracts of the cell pellets failed to show 1. Large-scale unialgal cultures were subsequently grown in 200-L polyethylene drums by using membrane-filtered (0.45 μm) seawater containing ES enrichment. Cells were removed via continuous centrifugation, and the acidified filtrate was extracted with XAD-2 resin. Complete details on culturing *P. minimum* will be published elsewhere.

(4) Provasoli, L. In "Cultures and Collection of Algae", Proceedings of the U.S.–Japanese Conference at Hakone, Sept. 1966; Watanabe, A.; and Hattori, A., Eds.; *Jap. Soc. Plant Physiol.* **1966**, 63–75.

(5) XAD-2 resin is a copolymer of styrene and divinylbenzene produced by Mallinckrodt Inc.

(6) The oil solidified in the freezer but we have been unable to crystallize it from solution.

(7) Spectra number 357 in "^{13}C Data Bank"; Bruker Physik: 1976, Vol. 1.

(8) Englert, G. *Helv. Chim. Acta* **1975**, *58*, 2367.

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two resonances at δ 129.9 and 137.6 in the ^{13}C NMR, leading to the conclusion that the molecule must also contain one ring. The striking correspondence between the carbon resonances in **1** and those assigned to terminal ring carbons in zeaxanthin (**8**, Figure 1) strongly suggest that the 1,3-butadione group in **1** must be attached to a 2,6,6-trimethyl-4-hydroxycyclohexenyl substituent. Examination of the ^1H NMR (Figure 2) confirms this structural hypothesis and provides evidence concerning the conformation of the cyclohexene ring. Thus the geminal methyl groups at C6 appear as two singlets at δ 1.08 (3 H) and 1.19 (3 H) while the olefinic methyl attached to C2 is a sharp singlet at δ 1.69 (3 H). All four methylene protons on C3 and C5 are clearly resolved at 400 MHz. A 2-Hz W coupling between H_a and H_d and an observed J_{bc} = 10 Hz and J_{ce} = 12 Hz suggest that the hydroxyl group on C4 must be pseudoequatorial as shown (Figure 2).

Acetylation of **1** (Ac_2O, pyridine, room temperature, 24 h) gave the monoacetoxy derivative **2** (60% yield):[9] mass spectrum, m/z 266.1521 (M^+), calcd for $C_{15}H_{22}O_4$ m/z 266.1518; ^1H NMR ($CDCl_3$, 270 MHz) δ 2.03 (s, 3 H, CH_3CO_2), 5.07 (m, 1 H, AcOCH<).[10] Reaction of β-diketone **1** with NH_2OH·HCl (refluxing EtOH, NaOAc, 3 h) gave a quantitative yield of a single isoxazole derivative which is one of the two regioisomers **3** or **4**: mass spectrum, m/z 221.1414 (M^+), calcd for $C_{13}H_{19}NO_2$ m/z 221.1415; ^1H NMR ($CDCl_3$, 270 MHz) δ 0.95 (s, 3 H), 1.12 (s, 3 H), 1.57 (s, 3 H), 2.31 (s, 3 H), 1.51 (m, unresolved, 1 H), 1.84 (ddd, J = 12, 2, 3 Hz, 1 H), 2.09 (dd, J = 17.5, 10 Hz, 1 H), 2.44 (ddd, J = 17.5, 6, 2 Hz, 1 H), 3.58 (br, 1 H), 4.10 (m, 1 H), 5.84 (s, 1 H).

The β-diketone **1** is related to 3-hydroxy-β-ionone (**5**)[11] and 3-hydroxy-β-damascone (**6**),[12] flavoring constituents of Burley tobacco. It is generally believed that metabolites containing this carbon skeleton are degradation products of carotenoids.[13] Zeaxanthin or a related algal carotenoid could conceivably lead to **1**.

The β-diketone **1** shows in vitro antibiotic activity against *Staphylococcus aureus*, and it has the potential of being an excellent trace-metal chelator. We are currently exploring the biological significance of these properties.

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Registry No. (-)-**1**, 72726-22-8; **2**, 72726-23-9.

(9) The other products were an inseparable mixture of the isomeric diacetates.

(10) The remainder of the ^1H NMR of **2** is complicated by the existence of all three tautomeric forms.

(11) Fujimori, T.; Kasuga, R.; Naguchi, M.; Kaneko, H. *Agric. Biol. Chem.* **1974**, *38*, 891. 3-Hydroxy-β-ionone is numbered according to the carotenoid convention which differs from the IUPAC nomenclature for cyclohexenes.

(12) Kaneko, H.; Harada, M. *Agric. Biol. Chem.* **1972**, *36*, 168.

(13) Isoe, S.; Hyeon, S. B.; Sakan, T. *Tetrahedron Lett.* **1969**, 279.

(14) All signals could be decoupled at 270 MHz.

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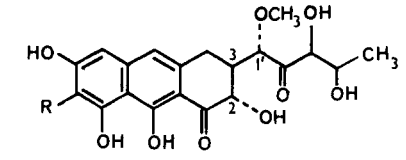
Stereocontrolled Synthesis of a Model Aureolic Acid Aglycone via Diels-Alder Reaction of an Unsaturated Sugar

Summary: The model aureolic acid aglycone **20** has been prepared in five steps from readily available starting materials. The first step, a Diels–Alder reaction between cyanobenzocyclobutene **3**, as its ring-opened *o*-quinone methide, and glucal derivative **8**, is both stereoselective and regioselective. The last step, a modified permanganate oxidation of an unsaturated nitrile to an aromatic acyloin, is also stereoselective.

Sir: The aureolic acid class of antitumor antibiotics is comprised of three subgroups. One group, the olivomycins, is a family of antibiotics isolated from *Actinomycetes olivoreticuli* with the polyoxygenated anthracene aglycone **1**, called olivin. The aglycone is linked to a disaccharide



1 R = H

2 R = CH_3

at C6 and a trisaccharide at C2.[1] Similarly, the chromomycins and the mithramycins are antibiotics from *Streptomyces* which have the aglycone **2** which is a 7-methylolivin.[2]

The only descriptions of synthetic studies in the aureolic acid series come from the Weinreb group.[3] Among several problems there remains open the question of establishing the correct stereochemical relationships at carbons 1′, 2, and 3, and it is our response to this aspect of the aureolic acid challenge that we now describe. Our retrosynthetic analysis suggested that a Diels–Alder addition of a naphthoquinone methide and an unsaturated sugar (as its cyclic enol ether) would afford a convergent synthesis of the aureolic acid group. The sugar would need to be derived from D-fucose (6-deoxygalactose) in order to correspond to the natural product configuration. For our initial studies we chose a readily available benzoquinone methide precursor.

Although precedents for the addition of an enol ether to a quinone methide and for the Diels–Alder reaction of sugars both existed, the regio- and stereoselectivity of our proposed reaction had to be tested.[4,5] Thus, thermolysis of cyanobenzocyclobutene **3** and dihydropyran **4** afforded a 4:1 mixture of tricyclics **5** and **6** in 70% yield. Each product could be ring opened to the same bicyclic **7**, thus demonstrating that they indeed were exo–endo epimers at the cyano group with the necessary regiochemistry for

(1) (a) Berlin, Y. A.; Esipov, S. E.; Kolosov, M. N.; Shemyakin, M. M.; *Tetrahedron Lett.* **1966**, 1431–6, 1643–7. (b) Bakhaeva, G. P.; Berlin, Y. A.; Chuprinova, O. A.; Kolosov, M. N.; Peck, G. Y.; Pietrovich, L. A.; Shemyakin, M. M.; Vasina, I. V. *Chem. Commun.* **1967**, 10–11. (c) Skarbek, J. O.; Brady, L. R. *Lloydia* **1975**, *38*, 369–77. (d) Remers, W. A. "The Chemistry of Antitumor Antibiotics"; Wiley: New York, 1979; pp 133–75. The last reference is a comprehensive review of the field.

(2) (a) Miyamoto, M.; Morita, K.; Kawamatsu, Y.; Noguchi, S.; Marumoto, R.; Tanaka, K.; Tatsuoka, S.; Nakanishi, K.; Nakadaira, Y.; Bhacca, N. S. *Tetrahedron Lett.* **1964**, 2355–65. (b) Bakhaeva, G. P.; Berlin, Y. A.; Boldyreva, E. F.; Chuprevna, O. A.; Kolosov, M. N.; Soifer, V. S.; Vasiljova, T. E.; Yartseva, I. V. *Ibid.* **1968**, 3595–8. (c) Berlin, Y. A.; Kolosov, M. N.; Pietrovich, L. A. *Ibid.* **1970**, 1329–31.

(3) (a) Hatch, R. P.; Shringapure, J.; Weinreb, S. M. *J. Org. Chem.* **1978**, *43*, 4172–4177. (b) Dodd, J. H.; Weinreb, S. M. *Tetrahedron Lett.* **1979**, 3593–3596.

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(5) Jurczak, J.; Tkacz, M. *Synthesis* **1979**, 42–4.

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