

## TURBINARIC ACID, A CYTOTOXIC SECOSQUALENE CARBOXYLIC ACID FROM THE BROWN ALGA *TURBINARIA ORNATA*

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**ABSTRACT.**—A moderately cytotoxic secosqualene carboxylic acid designated turbinaric acid [**1**] has been isolated from the brown alga *Turbinaria ornata*, and it has been identified to be 4,8,13,17,21-pentamethyl-4,8,12,16,20-docosapentaenoic acid by means of spectral analysis and synthesis.

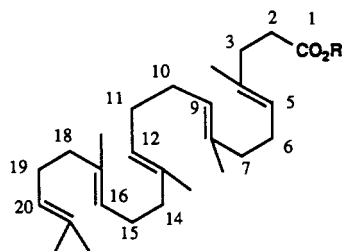
The brown algae of the Sargassaceae are among the most abundant seaweeds growing along the Japanese coast. We have isolated prenylated quinones (1,2), geranylactone-type compounds (3), and a new type of glyceride (4) from the Japanese Sargassaceae algae. In the course of our studies on biologically active substances from marine resources, we have found that a crude extract of the species *Turbinaria ornata* growing in the southern part of Japan exhibited moderate cytotoxicity against mouse melanoma cells. This report deals with the isolation and characterization of the active component.

An Me<sub>2</sub>CO extract of *T. ornata* collected on Okinawa island was separated by chromatography and monitored by cytotoxicity assay. The fractions were further purified by preparative tlc [Merck, Si gel 60, hexane-EtOAc (4:1)], yielding one active fraction. The <sup>1</sup>H-nmr spectrum of this fraction revealed that it was still a mixture of unsaturated fatty acids and an isoprenoid compound; the separation of these was extremely difficult. Finally, methylation of the mixture with CH<sub>2</sub>N<sub>2</sub> followed by medium pressure liquid chromatography [Merck, Lobar Column, RP-2; dioxane-H<sub>2</sub>O (10:3)] furnished the purified components. The pure methyl ester of the isoprenoid carboxylic acid (designated turbinaric acid) was obtained as a colorless oil.

Methyl turbinarate [**2**] shows the molecular ion peak at *m/z* 414 corresponding to C<sub>28</sub>H<sub>48</sub>O<sub>2</sub> in the ms spec-

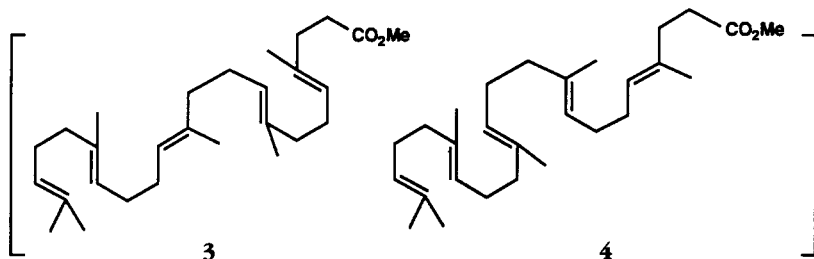
trum. The ir spectrum exhibits an intense absorption band at 1735 and 1250 cm<sup>-1</sup> assignable to an ester group. The mass spectrum indicates the presence of an isoprenoid chain, because it shows fragments at *m/z* 345, 276, and 207 due to a successive loss of three isoprenyl units (C<sub>5</sub>H<sub>9</sub>) from the molecular ion. The <sup>1</sup>H-nmr spectrum (500 MHz, C<sub>6</sub>D<sub>6</sub>) reveals the existence of six olefinic methyls (δ 1.54, 1.62, 1.63, 1.67, 1.75; each 3H, br s), five olefin protons [δ 5.36 (three overlapped t), 5.32 (1H, t), 5.26 (1H, m)], and a methoxy group (δ 3.41). The spectrum taken in CDCl<sub>3</sub> also shows a downfield triplet [δ 2.39 (2H)], which was found to be correlated with a triplet at δ 2.29 (2H) in the H-H COSY spectrum, suggesting the partial structure MeO<sub>2</sub>C-CH<sub>2</sub>-CH<sub>2</sub>-.

The stereochemistry of the four double bonds (at C-4, -8, -12, and -16) was determined to be all *E* from the <sup>13</sup>C-nmr spectrum (CDCl<sub>3</sub>); the chemical shifts of the six methyl groups were δ 15.99, 16.08, 16.11, 16.12, 17.76, and 25.78. The most downfield signal (δ 25.78) is assignable to the methyl group



**1** R = H  
**2** R = Me

which is *cis* to the olefinic proton (at C-20), the other signals being ascribable as the methyls *trans* to the olefinic protons (5). At this stage, structure **2** could be deduced for methyl turbinarate, although there were several other candidates, such as **3** or **4**. In order to eliminate all ambiguity, synthesis of methyl turbinarate [**2**] was carried out (4). The synthesized **2** was identical with the natural **2** in tlc,  $^1\text{H}$ -nmr,  $^{13}\text{C}$ -nmr, and mass spectra.



Turbinaric acid [**1**], 4,8,13,17,21-pentamethyl-4,8,12,16,20-docosapentaenoic acid (6), has been previously obtained as a synthetic product. It is a potent inhibitor against squalene-2,3-epoxide cyclase.

Turbinaric acid [**1**] exhibited cytotoxicity against murine melanoma and human colon carcinoma cells at 26.6  $\mu\text{g}/\text{ml}$  and 12.5  $\mu\text{g}/\text{ml}$ , respectively. Turbinaric acid is a novel type of a seco-squalene carboxylic acid, and this is the first report of the isolation of this acid from natural sources.

## EXPERIMENTAL

**GENERAL EXPERIMENTAL PROCEDURES.**—The ir spectra were recorded on a 215 Hitachi grating infrared spectrophotometer.  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr spectra were taken on a Bruker AM-500 spectrometer. Gc-ms spectra were measured on a Hitachi RMU-6M spectrometer, using a glass column (1% OV-1; 0.5 cm = 100 cm). Absorbant for cc and tlc was Merck Si gel 60.

**PLANT MATERIAL.**—*T. ornata* (40 kg) was collected at Henoko Beach, Okinawa in August 1987. A voucher specimen is preserved at the Experimental Fishery Station at Okinawa (Itoman).

**EXTRACTION AND ISOLATION.**—The algal

bodies of *T. ornata* were soaked in  $\text{Me}_2\text{CO}$  immediately after collection. The extract was concentrated, the residue (100 g) was mixed with  $\text{H}_2\text{O}$  (1 liter), and the mixture was extracted with hexane (2 liters),  $\text{CH}_2\text{Cl}_2$  (3 liters), and  $\text{EtOAc}$  (2 liters). The hexane solution was concentrated into an oily residue (14.1 g), which was chromatographed on Si gel (Merck, Kieselgel 60; 500 g) using gradient portions of hexane- $\text{EtOAc}$ . The fraction (1 g) eluted with the solvent system Hexane- $\text{EtOAc}$  (95:5) exhibited intense  $^1\text{H}$ -nmr signals around  $\delta$  1.6 due to olefinic methyls. One half of this fraction was further purified by Si gel chromatography (Kieselgel 60; hexane/ $\text{EtOAc}$ ) to

afford a cytotoxic fraction (135 mg), which was found from its  $^1\text{H}$ -nmr spectrum to be a mixture of a terpenoid carboxylic acid and unsaturated fatty acids. The mixture was treated with excess  $\text{CH}_2\text{N}_2$  in  $\text{Et}_2\text{O}$ , and a 10-mg portion of the methylation product was separated by medium pressure liquid chromatography [Merck, Lobar RP-2 (1 cm  $\times$  24 cm)] using dioxane- $\text{H}_2\text{O}$  (5:1), yielding pure **2** (6 mg).

**METHYL 4,8,13,17,21-PENTAMETHYL-4,8,12,16,20-DOCOSAPENTAENOATE [2] (METHYL TURBINARATE).**—Ir  $\nu$  max ( $\text{CHCl}_3$ ) 1735, 1250, 1130, 1070  $\text{cm}^{-1}$ ;  $^1\text{H}$  nmr (500 MHz,  $\text{C}_6\text{D}_6$ )  $\delta$  1.54 (3H, s), 1.62 (3H, s), 1.63 (3H, s), 1.67 (3H, s), 1.68 (3H, s), 1.75 (3H, s), 2.15 (10H, m), 2.25 (5H, m), 2.35 (5H, m), 3.41 (3H, s), 5.26 (1H, m), 5.32 (1H, t,  $J = 6.3$  Hz), 5.36 (3H, t,  $J = 6.3$  Hz);  $^{13}\text{C}$  nmr (125 MHz,  $\text{C}_6\text{D}_6$ )  $\delta$  16.27 (q), 16.49 (q), 16.51 (q), 16.55 (q), 18.11 (q), 26.22 (q), 27.39 (t), 27.49 (t), 27.62 (t), 29.12 (t;  $2 \times \text{C}$ ), 33.54 (t), 35.40 (t), 40.40 (t), 40.60 (t;  $2 \times \text{C}$ ), 51.32 (q), 125.20 (d), 125.23 (d), 125.33 (d), 125.75 (d;  $2 \times \text{C}$ ), 131.48 (s), 133.97 (s), 135.36 (s), 135.39 (s), 135.59 (s), 173.36 (s); ms  $m/z$  [ $\text{M}$ ] $^+$  414, 399, 383, 345, 276, 207.

**SYNTHESIS OF METHYL TURBINARATE [2].**—The procedure was exactly the same as reported previously (4). The starting squalene was purchased from Tokyo Kasei and used without purification. Synthesized turbinaric acid [**1**] was used in the cytotoxicity bioassay.

## EXPERIMENTAL

We are grateful to Bristol Meyers Research Institute, Tokyo for cytotoxicity bioassay.

## LITERATURE CITED

1. T. Kusumi, Y. Shibata, M. Ishitsuka, T. Kinoshita, and H. Kakisawa, *Chem. Lett.*, 277 (1979).
2. M. Ishitsuka, T. Kusumi, Y. Nomura, T. Konno, and H. Kakisawa, *Chem. Lett.*, 1269 (1979).
3. T. Kusumi, M. Ishitsuka, Y. Nomura, T. Konno, and H. Kakisawa, *Chem. Lett.*, 1181 (1979).
4. T. Kusumi, M. Ishitsuka, T. Iwashita, H. Naoki, T. Konno, and H. Kakisawa, *Chem. Lett.*, 1393 (1981).
5. L.F. Johnson and W.C. Jankowski, "Carbon-13 NMR Spectra: A Collection of Assigned, Codes, and Indexed Spectra," Wiley-Interscience Publication, John Wiley & Sons, New York, 1972, p. 497.
6. E.E. van Tamelen and T.J. Curphey, *Tetrahedron Lett.*, 121 (1962).

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