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CYTOTOXIC POLYUNSATURATED FATTY ACID FROM *PEDIASTRUM**

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Key Word Index—*Pediastrum* species; Chlorophyceae; microalga; cytotoxity; fatty acid; hexadecatetraenoic acid.

Abstract—A polyunsaturated fatty acid isolated from a *Pediastrum* species was identified as (4*Z*,7*Z*,10*Z*,13*Z*)-hexadecatetraenoic acid. It inhibited the development of fertilized echinoderm eggs at a concentration of 25 μg/ml.

INTRODUCTION

Recently, microalgae have attracted attention as new sources of pharmaceuticals [1] and metabolites of chemical and biological interest have been isolated especially from blue-green algae [2–4] and dinoflagellates [5, 6]. In the course of our studies on biologically active substances from microalgae, we found that a lipophilic extract of a *Pediastrum* species inhibited the development of fertilized echinoderm eggs [7, 8]. We now describe the isolation an identification of (4*Z*,7*Z*,10*Z*,13*Z*)hexadecatetraenoic acid as the cytotoxic principle.

RESULTS AND DISCUSSION

The ethanol extract of the *Pediastrum* species showed activity in the echinoderm egg assay at a concentration of 100 μg/ml. The extract was partitioned between water and ether. The ether layer was chromatographed successively on Toyo Pearl HW-40 SF and ODS by HPLC to give a colourless oil (6.8 mg, 0.06% fr wt).

The oil exhibited no UV absorption maximum above 210 nm and its IR spectrum indicated the presence of carboxylic acid (1710 cm^{-1}) and *cis*-double bonds (710 cm^{-1}). The 100 MHz ^1H NMR spectrum was very similar to those of polyunsaturated fatty acids and the signals at δ 5.3 (8H, *m*), 2.8 (6H, *br s*), 2.4 (4H, *s*), 2.1 (2H, *m*) and 0.9 (3H, *t*, $J = 7$ Hz) were attributed to double bonds, methylene protons between double bonds, C-2 and C-3 methylene, C-15 methylene and terminal methyl protons, respectively. Decoupling experiments showed the connectivity from C-16 terminal methyl protons to C-14 olefinic protons. Catalytic hydrogenation gave hexadecanoic acid (GC). GC/MS analysis of the methyl ester exhibited properties resembling a polyunsaturated fatty acid: *m/z* 262 $[M]^+$, 231 $[M-OMe]^+$, 193 $[M-MeCH_2CH=CHCH_2]^+$, 188 $[M-CH_2COOMe]^+$ and other peaks characteristic of highly unsaturated fatty acids. The *Z* configurations of the olefin groups were confirmed by the absence of a band at 950 cm^{-1} in the IR spectrum. From these results, the active compound was considered to be an unusual polyunsaturated fatty acid, (4*Z*,7*Z*,10*Z*,13*Z*)hexadecatetraenoic acid.

The acid inhibited the development of starfish (*Asterina pectinifera*) embryos at 25 μg/ml; the affected embryos failed to develop beyond the one cell stage. However, the compound did not show antimicrobial activity against eight species of microorganisms tested.

In addition to the present finding, several reports have already been published on the isolation of polyunsaturated fatty acids from microalgae and on macroalgae as the principles of ichthyotoxicity [9], allelopathy [1, 10], and antibiotic activity [11, 12], suggesting that further work is needed to clarify the role of fatty acids in microalgae.

EXPERIMENTAL

Algal sample and culture conditions. The freshwater chlorophyte *Pediastrum* sp. was collected at Lake Kitaura in Ibaraki Prefecture, Japan in August 1985 and was isolated in a unialgal state by the procedure of ref. [13]. Unialgal mass culture of the chlorophyte was carried out in 5 l glass bottles containing 4 l modified C medium (CB medium): $Ca(NO_3)_2$ 15 mg, KNO_3 10 mg, $MgSO_4 \cdot 7H_2O$ 4 mg, β-Na_2glycerophosphate 5 mg, thiamin·HCl 1 μg, biotin 0.01 μg, vitamin B_{12} 0.01 μg, PIV metals 0.3 ml (dist H_2O 100 ml, $FeCl_3 \cdot 6H_2O$ 19.6 mg, $MnCl_2 \cdot 4H_2O$ 3.6 mg, $ZnCl_2$ 1.05 mg, $CoCl_2 \cdot 6H_2O$ 0.4 mg, $Na_2Mo_4 \cdot 2H_2O$ 0.25 mg, $Na_2EDTA \cdot 2H_2O$ 100 mg), bicine 50 mg, dist H_2O 99.7 ml. The medium was adjusted to pH 7.5 with 6 M HCl and autoclaved. After inoculation, the cultures were grown with aeration at 25° under cool white fluorescent light at 80 μE/m^2/sec on a 14:10 hr, light:dark cycle. When cultures reached the stationary phase in two or three weeks, cells were harvested by continuous flow centrifugation at 7000 rpm. The yield was 0.5 g/l (fr. wt). Packed cells were kept in a freezer at −20° until used.

Extraction and isolation. Harvested cells (11.4 g, fr. wt) were homogenized and extracted with 200 ml EtOH. After filtration, the residue was twice extracted in the same manner. The combined extracts were concd under red. pres. and partitioned between H_2O and Et_2O. The lipophilic layer (273 mg) was subjected to CC on Toyo Pearl HW-40 SF (100 × 3 cm). Active fractions were finally purified by HPLC (YMC-ODS AM-324, 300 × 10 mm) with 85% MeOH to give a fatty acid (6.8 mg, 0.06% fr. wt).

Analysis. UV and IR spectra were recorded in $CHCl_3$. 100 MHz ^1H NMR spectra were recorded in $CDCl_3$. GC/MS were obtained with a fused silica capillary column SS-10 (50 m × 0.25 mm) at 200°. GC analysis was performed under the same

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conditions using He as carrier gas at 60 ml/min with a split ratio of 100:1

Esterification. The fatty acid (1 mg) was esterified with 0.5 ml of BF_3–MeOH complex (BF_3; *ca* 14%).

Catalytic hydrogenation. A soln of the fatty acid (1 mg) in MeOH was stirred with 10% Pd-C under H_2 for 1 hr at room temp, filtered and evapd to obtain the sample for GC analysis.

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TWO LACTONIC COMPOUNDS, LANCIFOLIDE AND ISOLANCIFOLIDE, FROM *ACTINODAPHNE LANCIFOLIA*

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Key Word Index—*Actinodaphne lancifolia*; Lauraceae; leaves; wood; C_{15}-lancifolide; lancifolide; isolancifolide; γ-lactone; furan compounds.

Abstract—Two lactonic compounds, lancifolide and isolancifolide, were isolated from *Actinodaphne lancifolia*. Their structures were elucidated on the basis of spectral and chemical evidence.

INTRODUCTION

Actinodaphne lancifolia (Japanese name 'Kagonoki') is an evergreen tree of the family Lauraceae distributed in the southern part of Japan. So far, there are a few reports [1, 2] on the chemical components of the plant but many terpenes and four furans (sesquirosefuran, longifolin, 5-methyl furfural, and 8-[2'(3'-methyl)furanyl]-2,6-dimethyl-2,6-octadiene-4-one) have been isolated from the essential oil of mesocarps, seeds, roots and leaves. In this paper, we describe the isolation and structural elucidation of two novel lactonic compounds, lancifolide (**1**) and isolancifolide (**2**) from the plant.

RESULTS AND DISCUSSION

Lancifolide (**1**), colourless oil, $C_{15}H_{24}O_3$, exhibited OH (3400 cm^{-1}) and α,β-unsaturated-γ-lactone (1680 and 1780 cm^{-1}) bands in its IR spectrum. From analysis of ^1H NMR and UV spectra, **1** has the same β-hydroxy-γ-methylene-α,β'-unsaturated-γ-lactone structure as that of obtusilactone (**3**) [3]. The structure of γ-lactone segment was also determined as follows. On selective hydrogenation using $Rh(Ph_3P)_3Cl$ in benzene, **1** afforded a mixture of **4a** and **4b**, which, without further separation, was treated with acetic anhydride in pyridine to yield the unstable compound **5**. On the other hand, when **1** was