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A New Cytotoxic Compound from a Water Extract of Corn

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A new cytotoxic compound 2 was isolated from a water extract of corn germ. Structural elucidation by spectroscopic data and chemical evidence fully substantiated 2 to be 11(*E*)-10-oxo-11-octadecen-13-olide. Compound 2 exhibited considerably strong cytotoxic activity against various cell lines with IC_{50} of 0.9–2.8 μg/ml.

Key words: corn; cytotoxic activity; lypoxygenase; 11(*E*)-10-oxo-11-octadecen-13-olide; macrolide

In the course of screening for new bioactive substances from grain, we have previously isolated an antitumoral compound **1** from a water extract of corn.[1–3] Compound **1** is known to be derived from linoleic acid contained in corn.[4–6] Linoleic acid in corn was first converted into linoleic acid hydroperoxide by corn lypoxygenase,[4,5] this hydroperoxide being was further transformed into an unstable fatty acid allene oxide by corn hydroperoxide dehydrase.[6] Finally, hydrolysis of the allene oxide occurred spontaneously to afford a ketol form of fatty acid.[6] In this pathway, the enzymatic or auto-oxidation of unsaturated fatty acids results in the formation of **1** in plants.

During the search for an effective method to obtain **1** in a high yield from the corn germ, we discovered a new antitumoral compound **2**, 11(*E*)-10-oxo-11-octadecen-13-olide, which had stronger cytotoxic activity than that of **1**.[7]

In this communication, we describe the isolation and structural elucidation of **2**. The isolation of **2** was guided by its cytotoxic activity against P388 mouseleukemia cells and was carried out by the procedure outlined in Fig. 1. Final purification of the bioactive compound was performed by HPLC to afford **2** as a white powder, mp 51°C, $[\alpha]_D^{20} = 0.00$ ($c = 1.00$, MeOH).[8]

Figure 2 shows an evaluation of the *in vitro* cytotoxic activity of **1** and **2** against various cell lines.[9] Compound **2** exhibited cytotoxic activity against tumorial cell lines P388, Ehrlich, B16, KB, S180, and Meth A with IC_{50} values in the range of 0.9–2.8 μg/ml. The cytotoxic activity of **2**

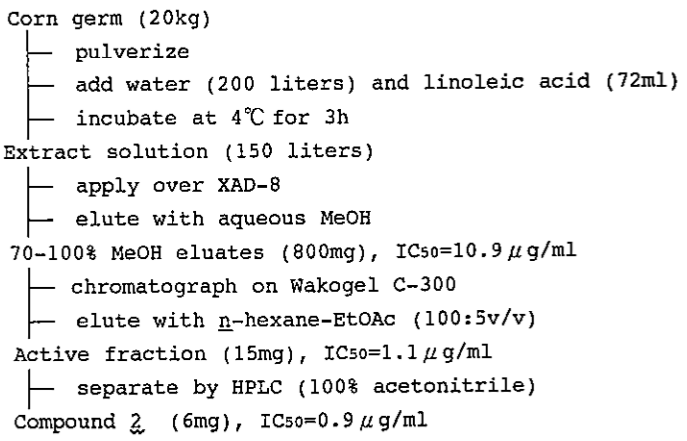


Fig. 1. Purification Procedure for Compound **2**.
IC_{50} is the concentration (μg/ml) which caused 50% inhibition of the cell growth of P388.

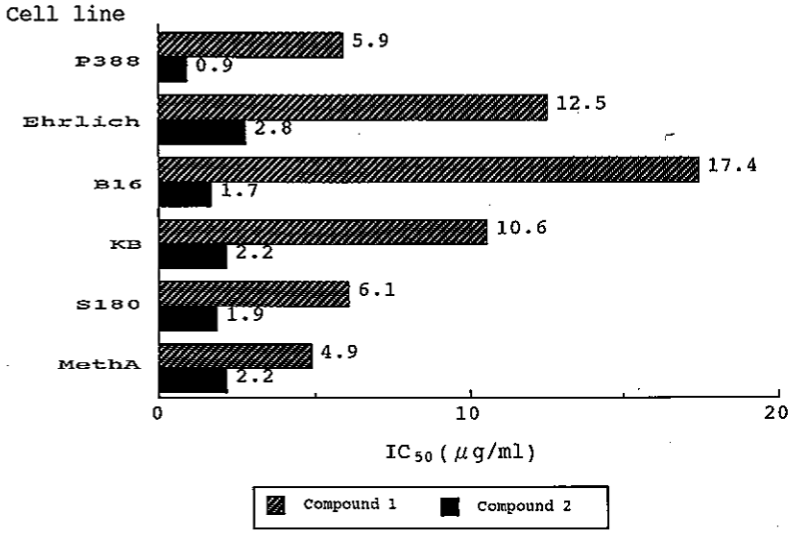
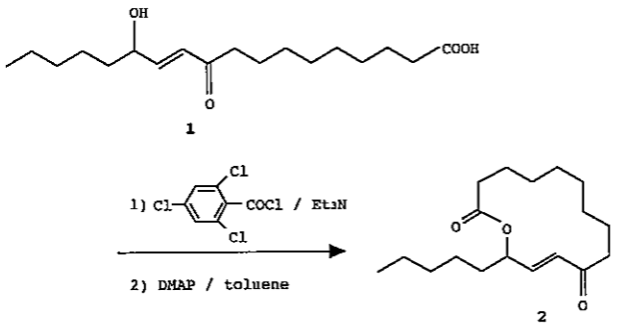


Fig. 2. Cytotoxic Activity of Compounds **1** and **2** against Various Cell Lines.

Table Physico-chemical Properties of Compounds 1 and 2

Compound	1	2
Appearance	White powder	White powder
mp	64°C	51°C
$[\alpha]_D^{20}$	0.00 (c=1.00, MeOH)	0.00 (c=1.00, MeOH)
EI-MS (m/z)	294 ($M^+ - H_2O$)	294 (M^+)
Molecular formula	$C_{18}H_{30}O_3$	$C_{18}H_{32}O_4$
Elemental analysis		
Calcd.	C, 73.43; H, 10.27%	C, 69.19; H, 10.32%
Found	C, 73.39; H, 10.32%	C, 69.16; H, 10:38%
UV ν_{max} (EtOH) nm (ε)	210 (8500)	210 (7500)
IR ν_{max} (KBr) cm^{-1}	3475, 2926, 2852, 1691, 1627, 1471, 1443, 1412, 1080	2935, 2860, 1735, 1691, 1668, 1627, 1464, 1337, 1251
Solubility	Soluble: DMSO, MeOH, EtOAc Insoluble: H_2O	Soluble: DMSO, MeOH, EtOAc Insoluble: H_2O
^{1}H-NMR ($CDCl_3$)	12.02 (1H, br., s)	
	6.88 (1H, dd, J=15.9 Hz, J=4.6 Hz)	6.72 (1H, dd, J=6.4 Hz, J=15.8 Hz)
	6.25 (1H, dd, J=15.9 Hz, J=1.5 Hz)	6.44 (1H, dd, J=15.8 Hz, J=1.0 Hz)
	5.11 (1H, br., s)	5.48 (1H, m)
	4.21 (1H, br., s)	
	2.62 (2H, t, J=7.3 Hz)	2.65–2.26 (4H, m)
	2.58 (1H, m)	1.76–1.58 (6H, m)
	1.58–1.32 (20H, m)	1.38–1.21 (14H, m)
	0.94 (3H, t, J=6.7 Hz)	0.89 (3H, t, J=6.7 Hz)
^{13}C-NMR ($CDCl_3$)	203.4, 177.6, 150.8, 128.9, 71.7, 41.1, 37.6, 34.9, 32.8, 30.3, 30.2, 30.1, 26.1, 26.0, 25.4, 23.6, 14.4	201.8, 173.0, 143.3, 128.8, 72.1, 41.3, 34.4, 33.5, 31.5, 26.4, 26.2, 25.8, 25.4, 25.3, 24.8, 24.5, 22.5, 14.0



Scheme Lactonization of Compound 1.

was 10.2 (B16) to 2.2 (Meth A) times stronger than that of **1**.

The structural elucidation of **2** was substantiated by comparing its physico-chemical properties with those of **1** as shown in Table and by its chemical synthesis from **1**. The molecular formula of **2** was determined to be $C_{18}H_{30}O_3$ by both EI-MS (m/z 294 M^+) and an elemental analysis. The UV spectrum of **2** (λ_{max} 210 nm, ε_{max} 7500) showed very similar absorption to that of **1** (λ_{max} 210 nm, ε_{max} 8500), while the IR spectrum of **2** exhibited no characteristic absorption at 3650–3200 cm^{-1} based on a hydroxyl group. The ^{1}H-NMR spectrum of **2** was also very similar to that of **1** except for signals attributable to a carboxylic proton (δ_H 12.0 (br., s)) and an alcoholic hydroxyl proton (δ_H 4.21 (br., s)), which were not present in **2**. These observations indicate that ring closure had occurred between the carboxylic acid moiety and the C-13 hydroxyl group of **1** to yield a macrolide **2**. Further verification of the structure of **2** was provided by the lactonization of **1** as depicted in Scheme. As expected, lactonization of **1** could be achieved by applying the procedure of Yamaguchi *et al.*[10] and furnished desired compound **2** in a 70.2% yield, whose spectroscopic data agreed quite closely with those of the natural product in all respects (IR, ^{1}H-NMR, ^{13}C-NMR, and MS). These results confirmed that the structure of compound **2** was (±)-11(*E*)-10-oxo-11-octadecen-13-olide. More recently, we have succeeded in synthesizing the optical isomer of **2**.[8] A bioassay of the relationship between the cytotoxic activity and stereochemistry will be published elsewhere.

Experimental

Materials. Corn germ was purchased from Hiyobaku Co. (Hyogo, Japan), and linoleic acid was from Wako Pure Chemicals (Osaka, Japan). RPMI 1640 medium and Eagle's minimum essential medium (Eagle MEM) were from Nissui Seiyaku Co. (Tokyo, Japan). Fetal bovine serum (FBS) was obtained from Cansera Co. (Ontario, Canada), and trypsin–EDTA (0.05% trypsin–0.53 mM EDTA–Na) was from Gibco Co. (New York, U.S.A.).

Instrumental analyses. UV spectra were recorded with a Hitachi Model 150-20 spectrophotometer, and IR spectra were recorded in a KBr tablet with a Perkin-Elmer Model 1760X FT-IR instrument. ^{1}H- and ^{13}C-NMR spectra were obtained with a JEOL Model JNM-GX270 NMR instrument in $CDCl_3$ with tetramethylsilane as an internal standard. Mass spectra (EI-MS) were recorded at 70 eV with a JEOL Model JMS-AX500 MS instrument. Melting point (mp) values were measured with Laboratory Devices model MEL-TEMP II apparatus.

Bioassay. The cytotoxity of the antitumoral factor against various cultured cells was assayed by the cell count method. In order to examine the cytotoxic effect on cell growth, growing cells (5×10^4) were suspended in a dish (35 × 10 mm, Beckton Dickenson, Lincoln, U.S.A.) which contained 2.0 ml of RPMI 1640 medium or Eagle MEM supplemented with 10% FBS. Each reaction mixture was incubated for 3 h (P388, S180, sv-fH, and FM3A) or for 24 h (MethA, B16, and KB) at 37°C in an incubator with a 5% CO_2 atmosphere. Each test sample was initially dissolved in DMSO and diluted 2"-fold with the experimental growth medium, in which the final DMSO concentration was less than 0.5%. After adding the sample, the reaction mixture was incubated for 72 h at 37°C in the 5% CO_2 atmosphere. After this incubation, the viable cell numbers were counted by a Burker–Turk type of hemocytometer after staining with 0.1%

trypan blue. In the case of the adherent cell culture, the culture medium in the dish was removed, and the cells were washed with PBS. To the cells in the dish was then added 0.2 ml of 0.05% trypsin–0.53 mM EDTA, and the cells incubated at 37°C for 5 min. Phosphate-buffered saline (PBS, 0.8 ml) was then added to the cell suspension while stirring. The viable cell numbers were counted as already described. The evaluation is expressed as an average of duplicated assays, the value for IC_{50} being determined by the concentration required to inhibit 50% of cell growth of the control.[8]

Isolation of compounds **1** *and* **2**. Pulverized corn germ (20 kg) was extracted with 200 liters of water at room temperature. To the extract was added 72 ml of linoleic acid, and the mixture was incubated at 4°C for 3 h. The reaction mixture (150 liters) was chromatographed over Amberlite in an XAD-8 column (10 × 50 cm, Organo Co., Tokyo, Japan). The non-adsorbed species were washed out with water, eluted stepwise with 30–70% aqueous methanol and then with 100% methanol. Each fraction was evaporated under reduced pressure. The residue from the 70–100% methanol fractions (800 mg) was chromatographed in a silica gel column developed with *n*-hexane–ethylacetate (100 : 5, v/v). The bioactive fraction (15 mg) was further purified by HPLC under the following conditions: column, Cosmosil 5C18-AR (20 i.d. × 200 mm, Nacalai Tesque Co., Tokyo, Japan); detection, 210 nm; mobile phase, 100% acetonitrile; flow rate, 3.0 ml/min. Compound **2** was obtained as white powder (6 mg), mp 51°C, $[\alpha]_D^{20} = 0.00$ ($c = 1.00$, MeOH).

Synthesis of **2** (*lactonization of compound* **1**). Macrolactonization of **1** could be achieved by applying the procedure of Yamaguchi *et al.*[9] To a chilled solution (0°C) of **1** (200 mg, 0.640 mmol) in anhydrous tetrahydrofuran (7.5 ml) were added 2,4,6-trichlorobenzoylchloride (0.201 ml, 1.28 mmol) and triethylamine (0.197 ml, 1.41 mmol), and the mixture was stirred for 3 h at ambient temperature. The resulting precipitate was removed by filtration, and the filtrate was diluted with anhydrous toluene (340 ml). The solution was added dropwise to a solution of DMAP (782 mg, 6.40 mmol) in anhydrous toluene (56 ml) at 110°C for 4 h while stirring. After additionally stirring for 30 min, the reaction mixture was cooled to room temperature, washed with an aqueous solution of citric acid and dried over Na_2SO_4. Evaporation of the solvent afforded crude **2**, which was subsequently applied to silica gel column chromatography. Elution of the column with *n*-hexane–ethyl acetate (100 : 5) gave 132 mg of **2** as white powder, mp 52°C, $[\alpha]_D^{20} = 0.00$ ($c = 1.00$, MeOH) in a 70.2% yield, whose IR, ^{1}H-NMR, ^{13}C-NMR, and MS data were identical with those of the natural product.

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