Two effective cytochrome P450 (CYP) inhibitors were isolated from tarragon, *Artemisia dracunculus*. Their structures were spectroscopically identified as 2E,4E-undeca-2,4-diene-8,10-diyinoic acid isobutylamide (1) and 2E,4E-undeca-2,4-diene-8,10-diyinoic acid piperidine (2). Both compounds had dose-dependent inhibitory effects on CYP3A4 activity with IC50 values of 10.0 ± 1.3 μM for compound 1 and 3.3 ± 0.2 μM for compound 2, and exhibited mechanism-based inhibition. This is the first reported isolation of effective CYP inhibitors from tarragon (*Artemisia dracunculus*) purchased from a Japanese market.

**Key words:** 2E,4E-undeca-2,4-diene-8,10-diyinoic acid isobutylamide; 2E,4E-undeca-2,4-diene-8,10-diyinoic acid piperidine; cytochrome P450 inhibitor; tarragon; *Artemisia dracunculus*.

Tarragon (estragon), *Artemisia dracunculus*, is a perennial herb with a long history of medicinal and culinary use. In particular, it is a crucial spice for the preparation of aromatic and medicinal plants that either inhibit or exploit CYP enzymes for the treatment of cancer, we evaluated the dry material of tarragon, *A. dracunculus*, purchased in a Japanese market (S&B Foods, Tokyo, Japan) for the compounds active against CYPs. We report here the isolation, identification, and inhibitory activities of two active compounds, 1 and 2, against CYPs 1A2, 2C9, 2D6, and 3A4.

The dry material of *A. dracunculus* (200 g), which was purchased from a Tokyo market, was soaked in 80% acetone (2 L) at room temperature for 24 h. The resulting filtrate was evaporated in vacuo to an aqueous concentrate and then extracted with three equal volumes of ethyl acetate (EtOAc) at pH 3.0 to give an EtOAc extract (21.6 g, IC50 = 30 μg/mL). This EtOAc extract was applied to chromatography in a Sephadex LH-20 column (30 mmφ × 700 mm), using methanol as the eluent. Fifteen 20-mL fractions were collected to give 2.3 g of active fractions 7–10. These active fractions, after evaporation, showed absorption bands, corresponding to the absence of the carbonyl group.

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Tarragon (estragon), *Artemisia dracunculus*, is a perennial herb with a long history of medicinal and culinary use. In particular, it is a crucial spice for French cuisine. The ethanolic extract of *A. dracunculus* has significantly decreased the blood glucose level and improved the insulin level in both genetic and chemical models of hyperglycemia. It had been demonstrated in Caco-2 cells that the extract also had potent inhibitory activity against allergen absorption. It had been demonstrated in Caco-2 cells that the extract also had potent inhibitory activity against allergen absorption.

Cytochrome P450 monoxygenases (CYPs), comprising a large and ubiquitous enzyme family, are heme-containing mixed-function oxidases that play a key role in the hepatic metabolism of lipophilic endogenetic substrates such as hormones, and of ingested hydrophobic xenobiotics such as medicinal chemicals and natural products. CYPs generally convert a large number of exogenous compounds (toxic, carcinogenic, and most pharmaceutical agents) to less toxic compounds. CYPs are heme-containing mixed-function oxidases that play a key role in the hepatic metabolism of lipophilic endogenetic substrates such as hormones, and of ingested hydrophobic xenobiotics such as medicinal chemicals and natural products. CYPs generally convert a large number of exogenous compounds (toxic, carcinogenic, and most pharmaceutical agents) to less toxic compounds.

The co-administration of foods that contain CYP inhibitors and drugs often causes adverse effects (food-drug interaction). Extrahepatic expression of CYPs has also been established. Such CYP families as CYPs 1, 2 and 3 are involved in the metabolism of carcinogenic, pro-carcinogenic, and chemotherapeutic substances, and are found in several extrahepatic tissues. CYPs 17 and 19 are involved in the metabolism required for respectively producing androgens and estrogens, and are located in the testes, ovaries, and adrenals. Food materials with selective inhibitors against specific CYPs are therefore potential candidates for cancer prevention strategies.

To elucidate the food factors in aromatic and medicinal plants that either inhibit or exploit CYP enzymes for the treatment of cancer, we evaluated the dry material of tarragon, *A. dracunculus*, purchased in a Japanese market (S&B Foods, Tokyo, Japan) for the compounds active against CYPs. We report here the isolation, identification, and inhibitory activities of two active compounds, 1 and 2, against CYPs 1A2, 2C9, 2D6, and 3A4.

The dry material of *A. dracunculus* (200 g), which was purchased from a Tokyo market, was soaked in 80% acetone (2 L) at room temperature for 24 h. The resulting filtrate was evaporated in vacuo to an aqueous concentrate and then extracted with three equal volumes of ethyl acetate (EtOAc) at pH 3.0 to give an EtOAc extract (21.6 g, IC50 = 30 μg/mL). This EtOAc extract was applied to chromatography in a Sephadex LH-20 column (30 mmφ × 700 mm), using methanol as the eluent. Fifteen 20-mL fractions were collected to give 2.3 g of active fractions 7–10. These active fractions were chromatographed by preparative HPLC (Capcell Pak C18 UG120 column, 15 mmφ × 250 mm, Shiseido, Tokyo, Japan), using 0.05% TFA-CH3CN (6:4) at a flow rate of 8.8 mL/min with detection at 254 nm, to gave compounds 1 (tR = 27.4 min) and 2 (tR = 32.9 min). Further purification was performed by thin-layer chromatography (PLC silica gel 60 F254 Art. no. 13794, Merck, Darmstadt, Germany), developed by using a solvent system of chloroform-methanol (9:1), to afford compounds 1 (Rf = 0.66, 18.2 mg) and 2 (Rf = 0.75, 17.3 mg).

Active compound 1 was obtained as a colorless amorphous powder with the molecular formula C15H18NO, based on high-resolution electrospray ionization mass spectra (HRESI-MS, pos.), m/z 252.13546 (M + Na)+ (calcd. as 252.13643 for C15H16NONa). The infrared spectrum of 1 showed absorption bands, νmax
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Fig. 1. Chemical Structures of Active Compounds 1 and 2.

(ATR)cm⁻¹: 3422, 3294, 2936, 2855, 2223, 1621, 1590, 1440, 1257, and 997. The ¹H-nuclear magnetic resonance (NMR, CDCl₃, 400 MHz) spectra of 1 exhibited 19 protons, δH: 0.93 (6H, d, J = 6.6 Hz, 3'-H₆, 4'-H₆), 1.81 (1H, m 2'-H), 2.00 (1H, s, 11-H), 2.39 (2H, brs, 6-H₂), 2.39 (2H, brs, 7-H₂), 3.16 (2H, t, J = 6.6 Hz, 1'-H₂), 5.70 (1H, brt, NH), 5.83 (1H, d, J = 14.8 Hz, 2-H), 6.05 (1H, m, 5-H), 6.20 (1H, dd, J = 10.7 and 15.1 Hz, 4-H), 7.18 (1H, dd, J = 10.7 and 15.1 Hz, 3-H), and the ¹³C-NMR data (CDCl₃, 100 MHz) gave 14 peaks, δC: 18.8 (C-7), 20.1 (C-3’ and C-4’), 28.6 (C-2’), 31.3 (C-6), 40.7 (C-1’), 65.1 (C-9 or C-10), 65.5 (C-9 or C-10), 68.2 (C-11), 76.9 (C-8), 123.2 (C-2’), 129.8 (C-4’), 139.0 (C-5’), 140.4 (C-3’), 166.2 (C-1’). The structure was further elucidated by interpreting the heteronuclear multiple-quantum coherence (HMQC) and heteronuclear multiple-bond coherence (HMBC) data. The structure was consistent with the literature (Fig. 1).¹⁰

Compound 2 was identified as a colorless amorphous powder with the molecular formula C₁₆H₁₉NO, HRESI-MS (pos.), m/z 264.13722 (M + Na)⁺ (calcld. as 262.13643 for C₁₆H₁₈NO). The structure was elucidated by interpreting the 1D- and 2D-NMR spectra. Based on these findings, compound 2 was identified as 2E,4E-undeca-2,4-diene-8,10-dionoic acid isobutylamide, consistent with the literature (Fig. 1).¹⁰

Compounds 1 and/or 2 had previously been isolated from Achillea and Echinaacea species.¹¹⁻¹⁵ Compound 1 isolated from Echinaacea has recently been reported as a CYP3A4 inhibitor.¹⁶

The inhibitory effects of compounds 1 and 2 on isoforms of CYP1A2, CYP2C9, CYP2D6, and CYP3A4 were investigated. The determined inhibitory effects of compounds 1 and 2 were based on the formation of the fluorescent metabolite (3-cyano-7-hydroxycoumarin, CHC) from the Vivid CYP Blue substrate (7-ethyloxy methoxy-3-cyano coumarin, EOMCC or 7-benzyloxy methoxy-3-cyano coumarin, BOMCC) by each CYP isoform in black 96-well microtititer plates (Sumitomo Bakelite, Tokyo, Japan). EOMCC was the substrate used for testing the inhibitory activity of CYP1A2 or CYP2D6, and BOMCC was used for testing that of CYP3A4 or CYP2C9. The positive controls were safrone for CYP1A2, dicumarol for CYP2C9, cimetidine for CYP2D6, and erythromycin for CYP3A4. An adequate concentration of compound 1 or 2, or a positive control was dissolved in methanol. Briefly, 40 µL of the obtained solution was added to 50 µL of an enzyme/NADPH-CYP reductase/glucose 6-phosphate/glucose 6-phosphate dehydrogenase mixture in a 96-well microtiter plate, and pre-incubated for 20 min at 25 °C. The reaction was initiated by adding 10 µL of the substrate/NADP⁺ (starter) mixture for 15 min at 25 °C. The reaction was terminated by adding 10 µL of each stop solution (30 µM of α-naphthoflavone for CYP1A2, 100 µM of sulfaphenazole for CYP2C9, 10 µM of quinidine for CYP2D6, and 30 µM of ketoconazole for CYP3A4). The CYP1A2 and CYP2D6 activities were assayed by monitoring the metabolism of EOMCC to CHC. CYP2C9 and CYP3A4 activity was assayed by monitoring the metabolism of BOMCC to CHC. Fluorescence was monitored with a fluorescence plate reader at an excitation wavelength of 409 nm and an emission wavelength of 460 nm. Activity was measured as the rate of fluorescent metabolite production over the course of the reaction. The IC₅₀ values were calculated by linear interpolation.¹⁷ Compounds 1 and 2 inhibited the CYP3A4 enzyme activity in vitro. Both compounds showed a dose-dependent inhibitory effect on the CYP3A4 activity with IC₅₀ values of 10.0 ± 1.3 µM for compound 1 and 3.3 ± 0.2 µM for compound 2 (Table 1). Compound 2 had no more inhibitory strength than the positive control, erythromycin (IC₅₀ = 2.1 ± 0.2 µM). We next investigated the inhibitory effects of compounds 1 and 2 on CYP1A2, CYP2C9, and CYP2D6. The IC₅₀ values for these compounds toward CYP1A2, CYP2C9, and CYP2D6 are shown in Table 1. Their inhibitory profiles towards CYP1A2 were similar to those towards CYP3A4, revealing compound 2 to also have the more potent inhibitory effect. Three positive controls, cimetidine, dicumarol, and safrone, also had inhibitory effects on the respective CYP enzymes (Table 1).

A metabolite of terminal acetylene formed by CYP covalently binds to a nitrogen atom of the CYP heme group, which is irreversibly inactivated through the formation of a heme adduct; for example, oxidative metabolites of arylacetylenes by CYP form irreversible heme alkylation products with iron porphyrin.¹⁸ Compounds 1 and 2 possess terminal acetylene structures in their diynic moieties. These compounds may produce irreversible heme alkylation products, because no type II spectra, which appeared in association with the formation of a coordination linkage to the iron atom in iron porphyrin, of the mixture of CYP3A4 and compound 1 or 2 were apparent.¹⁹ This type of inhibition is considered as being “mechanism-based.” 17α-Ethyny-lestradiol, an orally active semi-synthetic steroid estrogen with a terminal acetylene moiety, inactivates CYP3A4 through its metabolites with mechanism-based inhibition by the CYP enzyme.²⁰ Time-dependent CYP inhibition with NADPH-CYP reductase and a coenzyme (NADPH) is one of the key factors involved in mechanism-based inhibition. After pre-incubating for 20 min, using CYP3A4 and the reductase with the coenzyme (an NADPH-CYP reductase/glucose 6-phosphate/glucose 6-phosphate dehydrogenase mixture and NADP⁺), compounds 1 and 2 showed respective IC₅₀ values of 10.0 ± 1.3 and 3.3 ± 0.2 µM with respect to the reference activity. In the absence of the starter (NADP⁺), the inhibitory activities of all the tested
incubation, were steady at around their IC$_{50}$ (with pre-incubation for 30 min) at 10.0 $\pm$ 83 m 30 min) at 3.3 $\pm$ 0.3 m compounds, including compounds 1 and 2 with pre-incubation, were steady at around their IC$_{50}$ values (Fig. 2A). In the presence of the starter, however, the inhibitory effect of compound 1 increased from 19.6 $\pm$ 3.9% (without pre-incubation) to 63.5 $\pm$ 3.7% (with pre-incubation for 30 min) at 10.0 $\mu$M, whereas that of compound 2 increased from 29.8 $\pm$ 3.6% (without pre-incubation) to 83.9 $\pm$ 3.5% (with pre-incubation for 30 min) at 3.3 $\mu$M. The known mechanism-based inhibitors, erythromycin and 17α-ethynylestradiol, used as the positive controls showed similar behavior; in contrast, a known competitive inhibitor (ketoconazole) showed no time-dependent inhibition (Fig. 2B). The dose and pre-incubation time-dependent manner of compounds 1 and 2 suggest that they were potent mechanism-based inhibitors.

Food-drug interactions can be critically important, and interactions between the bioactive components in herbs and CYPs are generally recognized. The inhibition of CYP3A4, CYP1A2, CYP2C9, and CYP2D6 by bioactive components in foods containing herbs changes the bioavailability of clinically used drugs. CYP3A4 is the most abundant drug-metabolizing enzyme in the human liver and gastrointestinal tract, and the major enzyme responsible for the metabolism of pharmacologic agents. The consequence is that patients taking medicines must be aware of possible interactions between the drug being taken and the foods they are consuming, including foods containing CYP enzyme inhibitors.

The findings of the present study prompt us to report for the first time that compounds 1 and 2 exhibited strong CYP inhibitory effects in vitro. These findings suggest that routine heavy consumption of tarragon has potential negative effects in numerous food-drug interactions. According to the CYP isozymes that can selectively inhibit depending on the difference in structure of alkylamide, tarragon could also become a significant food material for preventing cancer through its inhibition of the metabolic activation of carcinogens.

**Acknowledgments**

We appreciate the helpful suggestions and advice contributed by Dr. H. Koshino (RIKEN). We thank Dr. Y. Q. Ye for measuring the high-resolution mass spectra. This work was performed as a part of the Advanced Research Project of Tokyo University of Agriculture.

**References**

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