An Anti-Estrogenic Lignan Glycoside, Tracheloside, from Seeds of *Carthamus tinctorius*

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The lignan glycoside, tracheloside, was isolated from seeds of *Carthamus tinctorius* (Compositae) as an anti-estrogenic principle against cultured Ishikawa cells by employing a bioassay-linked HPLC-ELSD method. Tracheloside significantly decreased the activity of alkaline phosphatase (AP), an estrogen-inducible marker enzyme, with an IC\(_{50}\) value of 0.31 \(\mu\)g/ml, a level of inhibition comparable to that of tamoxifen (IC\(_{50}\) = 0.43 \(\mu\)g/ml).

Key words: *Carthamus tinctorius*; tracheloside; anti-estrogenic activity; Ishikawa cell; bioassay-linked HPLC-ELSD

Interest in estrogen analogs as antagonists has increased tremendously because of the clinical application of anti-estrogens for treating breast cancer and their possible chemopreventive effects on women who are at high risk for developing breast cancer. Indeed, tamoxifen, a non-steroidal anti-estrogen, is extensively utilized in the prevention and treatment of breast cancer in women. However, the effectiveness of tamoxifen in treating breast cancer has been limited, because of the possible increased risk of endometrial carcinoma.\(^1\)

Therefore, the search for novel anti-estrogens without side effects, especially those from natural origins, has been extensive. As part of our current project to discover novel plant-derived anti-estrogens as chemopreventive agents, we utilized Ishikawa cells. Ishikawa cells are estrogen responsive human endometrial adenocarcinoma cells, and the alkaline phosphatase (AP) activity in these cells is markedly stimulated by estrogens.\(^2\) We report here on the isolation of the active component from *C. tinctorius* seeds and the biological potential of its anti-estrogenic activity by using a bioassay-linked HPLC-ELSD method.

Seeds of *Carthamus tinctorius* L. (Compositae) were purchased from the Kyung-Dong Market, Seoul, Korea in March 2001 and were identified by Emeritus Prof. D. S. Han of Seoul National University, Korea. A voucher specimen of this plant (NP-03-011) has been deposited at the Herbarium of the College of Pharmacy, Seoul National University, Korea. Milled seeds of *C. tinctorius* (2.0 kg) were extracted with MeOH. After filtering and evaporating the solvent, the resulting extract was suspended in water, and then partitioned between EtOAc and \(n\)-BuOH to afford dried EtOAc-soluble (8 g) and \(n\)-BuOH-soluble residues (6.5 g). Each of the residues was tested against Ishikawa cells, and the EtOAc-soluble fraction was found to be active (Inhibition at 4 \(\mu\)g/ml = 92%). Fifty microliters of the EtOAc-soluble residue (20 mg/ml MeOH) was injected into an ODS column (Kanto Mightysil, RP-18, 5 \(\mu\)m, 4 \(\times\) 250 mm), and the column was eluted with 1% acetic acid in CH\(_3\)CN/1% acetic acid in H\(_2\)O (30/70, 0 min \(\rightarrow\) 100/0, 15 min \(\rightarrow\) 100/0, 30 min) at a rate of 1 ml/min. The eluent from the column was introduced through a micro-splitter valve into ELSD and a fraction collector in the ratio of 1:9, respectively. The eluent collected in a 96-well plate was dried and used for determining the anti-estrogenic activity. Figure 1 displays the HPLC-ELSD chromatogram (A) and the bioactivity profile (B) of the EtOAc-soluble fraction of *C. tinctorius* seeds. The activity profile indicates a retention time for the component exhibiting strong AP inhibitory activity of around 11 min, and the HPLC-ELSD chromatogram shows a peak corresponding to the active component. On this basis, the active component, tracheloside, was isolated by using prep-TLC.

The anti-estrogenic activity was evaluated by using Ishikawa cells as described previously.\(^3\) Briefly, Ishikawa cells provided by R. B. Hochberg (Yale University, New Haven, CT, USA) were incubated overnight with an estrogen-free DMEM/F12 medium in 96-well plates (5 \(\times\) 10\(^4\)/well). To determine the anti-estrogenic activity, the cells were incubated with 1 nM estradiol (E\(_2\)) and a test compound in a total volume of 200 \(\mu\)l of medium/well at 37 \(^\circ\)C for 4 days. The enzyme
activity was measured by reading at 405 nm the liberation of p-nitrophenol with a microplate reader at 15-s intervals to give a total of 16–20 readings. The maximum slope of the lines generated from the kinetic data was calculated by using a computer program. The percentage induction for determining the estrogenic activity was calculated as [(slope for sample − slope for cells)/(slope for estrogen − slope for cells)] × 100. To determine the anti-estrogenic activity, the percentage induction was determined as [(slope for sample − slope for cells)/(slope for DMSO − slope for cells)] × 100. The data represent the average of triplicate determinations. Tracheloside alone did not significantly induce AP activity in the absence of estradiol (E2). However, when the cells were treated with 1 nM E2, the AP activity was increased by approximately 10-fold, and the induction was markedly inhibited by tracheloside in a dose-dependent manner (Fig. 3). The IC₅₀ value for tracheloside was 0.31 µg/ml, comparable to that of tamoxifen (IC₅₀ = 0.43 µg/ml). Tracheloside had no effect on the cell viability at any of the concentrations tested, as judged by parallel studies of cytotoxicity with a sulforhodamine B assay (data not shown).⁴)

A variety of plant chemicals that bind to the estrogen receptor have been identified and may exert estrogenic-like action by various mechanisms.⁵) Representative of these phytoestrogens are the isoflavonoids and diphenolic compounds contained in the bean subfamily of Leguminosae. However, the potency of most of these phytoestrogens is low compared to such endogenous or synthetic steroid estrogens as 17β-estradiol or ethinylestradiol, and these phytoestrogens therefore sometimes exhibit antagonistic actions against estrogens. Epidemiological data have suggested that the consumption of phytoestrogens may have beneficial effects on breast and prostate cancer which are both influenced by hormones.⁶¹ Tracheloside, a lignan glycoside, is known to occur abundantly in the Trachelospermum species. Lignan glycosides are generally known to be phytoestrogens or cytotoxic agents.⁷¹ Tracheloside is structurally similar to the well-known lignan phytoestrogen, arctiin, in which side-chain C-2 of a five-membered ring is changed from a hydrogen to a hydroxyl group (Fig. 2), and thus tracheloside can also be considered to function as a phytoestrogen. It is noteworthy that arctiin is transformed to estrogenic and anti-estrogenic substances by human intestinal bacteria,⁸) suggesting that tracheloside might also be metabolized by human intestinal bacteria in the same manner. There is therefore the possibility that an intake of tracheloside via the diet or a herbal medicine such as safflower seeds might show estrogenic action in the body, contrary to the effect of its constituent, tracheloside. In addition, tracheloside and...
arctiin are known to be respectively transformed into the aglycones, trachelogenin and arctigenin by gastric juice and intestinal bacteria in rats. Further studies on the in vivo metabolism of tracheloside will be needed to clarify the estrogen-related effect of tracheloside in vivo.

There have been few reports to date concerning the anti-estrogenic properties of lignans, and some have been to be reported inactive in anti-estrogenic tests. Therefore, this result for the anti-estrogenic activity of tracheloside in Ishikawa cells will be helpful to our understanding of the hormone-like action of lignans and will provide informative data concerning the effect of safflower seeds as a herbal medicine, although further studies using in vivo models are needed. The LC-ELSD method used in this investigation in conjunction with the Ishikawa cell system permitted the anti-estrogenic potential of tracheloside from seeds of C. tinctorius to be evaluated by a rapid and simple procedure.

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References