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EMODIN, A PROTEIN TYROSINE KINASE INHIBITOR FROM *POLYGONUM CUSPIDATUM*

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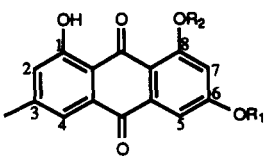
ABSTRACT.—Bioassay-directed fractionation of a Chinese medicinal plant, *Polygonum cuspidatum* (Polygonaceae), has led to the discovery of an anthraquinone, emodin [**1**], as a strong inhibitor of a protein tyrosine kinase ($p56^{lck}$) partially purified from bovine thymus. Comparison of the IC_{50} values of emodin for protein tyrosine kinase inhibitory activity with physcion [**2**] and emodin-O^8-D-glucoside [**3**], also isolated from the same plant, reveal the importance of the hydroxyl groups at C-6 and C-8 for the observed activity.

Protein tyrosine kinases (PTKs) are a group of enzymes that catalyze the transfer of phosphate from ATP to the hydroxyl of tyrosine on many essential proteins which, in turn, play important roles in the regulation of cell growth and transformation (1–3). PTKs are therefore potential targets for modulating cancer cell growth. The development of specific inhibitors of PTK may uncover potential anticancer agents and pharmacological probes to define the physiological roles of PTKs.

A PTK bioassay was therefore designed using the tyrosine-containing peptide angiotensin I as a substrate for the measurement of the PTK activity of $p56^{lck}$ partially purified from bovine thymus. A bioassay-directed fractionation of the roots of *Polygonum cuspidatum* was undertaken to isolate inhibitors of PTK. The dried roots of *P. cuspidatum* (Polygonaceae) have been used for the treatment of suppurative dermatitis, gonorrhea, favus, athletes foot, and hyperlipemia in Chinese and Japanese traditional medicine (4–6). Anthraquinones, naphthoquinones, stilbenes, and other phenolic compounds (5–8) have been isolated from the roots of *P. cuspidatum*. None of these phytochemical studies have been bioassay-directed toward the evaluation of PTK inhibitors.



1 $R_1=R_2=H$
2 $R_1=Me$, $R_2=H$
3 $R_1=H$, $R_2=\beta$-glc

The bioassay-directed fractionation of extracts from the roots of *P. cuspidatum* led to the discovery of emodin [**1**] as a potent inhibitor of the PTK $p56^{lck}$. Two additional derivatives of emodin, physcion [**2**] and emodin-O^8-D-glucoside [**3**], were also isolated for comparative studies. As shown in Table 1, emodin possessed the strongest inhibitory activity of the three anthraquinones isolated from this plant. Substitution of the 6-OH with an -OMe [**2**] or the 8-OH with glucose [**3**] completely abolished inhibitory activity ($IC_{50}>800$ μg/ml), indicating that the presence of free OH groups at C-6 and C-8 is important for the observed activity of emodin.

TABLE 1. Protein Tyrosine Kinase Activity of the Anthraquinones **1–3**.

Compound	IC_{50} (μg/ml)
1	5
2	>800
3	>800

An analysis of the kinetics of PTK inhibition indicated that emodin was a competitive inhibitor ($K_i = 13$ μM) of $p56^{lck}$ with respect to ATP ($K_m = 15$ μM), and was noncompetitive with re-

spect to the tyrosine-containing peptide substrate (Figure 1). These results indicated that emodin inhibited the activity of $p56^{lck}$ by preventing the binding of ATP.

Emodin has been reported as an anticancer agent against lymphocytic leukemia in mice (9), and has also shown cytotoxicity and inhibiton of precursor incorporation into DNA and RNA in HL-60 human leukemic cells (10).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined on a Fisher-Johns melting point apparatus and were uncorrected. Ir spectra were obtained on a Perkin-Elmer 1600 series FTIR. Uv spectra were taken on a Beckman DU-7 spectrophotometer. ^{1}H-nmr spectra were obtained on a Varian VXR-500S spectrophotometer using DMSO-d_6 as a solvent and referencing to TMS. Mass spectra were measured on a Finnigan 4000 spectrophotometer. An authentic sample of emodin was obtained from Sigma Chemical Company.

PLANT MATERIAL.—The roots of *P. cuspidatum* were collected by Dr. C.-T. Chang (Union Chemical Laboratory, Industrial Technology Research Institute, Hsinchu, Taiwan) in Taiwan. The authentic specimen was identified

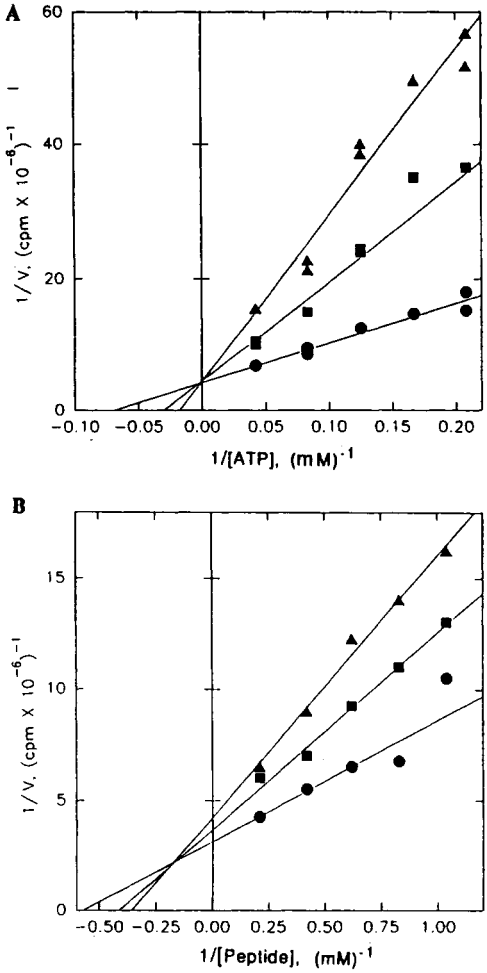


FIGURE 1. Inhibiton of $p56^{lck}$ by emodin [**1**]. A, effect of increasing concentrations of ATP on the inhibiton of $p56^{lck}$ by emodin [0 (●), 6 (■) or 12 (▲) μg/ml]. B, effect of increasing concentrations of angiotensin I (peptide) on the inhibition of $p56^{lck}$ by emodin [0 (●), 6 (■) or 12 (▲) μg/ml].

by the Taiwan Forestry Herbarium (Taiwan Forestry Research Institute, Taipei, Taiwan) where a voucher specimen is deposited.

BIOASSAY-GUIDED ISOLATION OF ANTHRAQUINONES.—The dried ground roots of *P. cuspidatum* (4.8 kg) were extracted with 95% EtOH at room temperature. After vacuum evaporation of EtOH the residue (600 g) was partitioned between CH_2Cl_2 and H_2O (1:1). The H_2O fraction was lyophilized to produce a residue (220 g), which was inactive in the protein tyrosine kinase assay. The residue of the CH_2Cl_2 extract (130 g) was further partitioned between hexane and 90% aqueous MeOH (1:1). The MeOH fraction (95 g) exhibited strong inhibitory activity (IC_{50} = <80 μg/ml).

The active 90% MeOH extract was chromatographed on a Si gel flash column. Elution with $CHCl_3$/MeOH (5–50%) afforded seven fractions (A–G). Emodin (30 mg) was obtained by recrystallization of a portion of the residue (35 mg) obtained after concentrating the active fraction B (IC_{50} = <80 μg/ml). Physcion [**2**] and emodin-O^8-D-glucoside [**3**] were obtained from fractions A and F, respectively, by repeated recrystallizations, and used for structure-activity relationship studies.

Emodin [**1**] was identified by comparison of its mp and ^1H nmr with an authentic sample and also by a comparison with the literature (11).

Physcion [**2**] and emodin-O^8-D-glucoside [**3**] were identified by comparing their mp and spectral data to those reported in the literature (12).

$P56^{lck}$ was partially purified from bovine thymus through the butyl-agarose chromatography step and was assayed by the procedures described previously using as substrates angiotensin I and [γ-^{32}P]ATP (13). All assays contained 8% DMSO, which was used as a solvent for all inhibitory compounds.

ACKNOWLEDGMENTS

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