Flavonoids from Koelreuteria henryi and Other Sources as Protein-Tyrosine Kinase Inhibitors

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FLAVONOIDS FROM *KOELREUTERIA HENRYI* AND OTHER SOURCES AS PROTEIN-TYROSINE KINASE INHIBITORS

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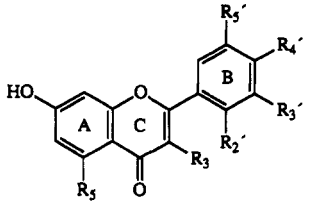
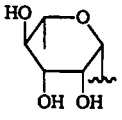
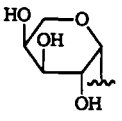
ABSTRACT.—The EtOH extract of *Koelreuteria henryi* was investigated in a search for natural products with potential protein-tryrosine kinase (PTK) inhibitory activity. The PTK inhibitory activity of the crude fractions was determined by measuring their inhibition of $p56^{lck}$ partially purified from bovine thymus using angiotensin I as a substrate. Analysis of those fractions that exhibited significant activity led to the isolation of kaempferol and quercetin, in addition to two kaempferol glycosides, kaempferol-O^3-α-rhamnopyranoside [**1**] and kaempferol-O^3-α-arabinopyranoside [**2**]. This study represents the first report on the isolation of flavonols and their PTK inhibitory activities from the genus *Koelreuteria*. Eight other flavonoids were also examined to study the role of the hydroxy groups on the B ring on PTK inhibitory activity.

The genus *Koelreuteria* (Sapindaceae) includes eight species, most of which grow in China. *Koelreuteria henryi* Dummer (*Koelreuteria formosana* Hyata), is known as the "Flame Golden Tree" (1–3). No systematic study has been reported on its chemical constituents. Overall, little is known about the secondary metabolites of the whole genus. Triterpenoid glycosides from *Koelreuteria paniculata* (4), and cyanolipids from *Koelreuteria apiculata* (5) and *K. paniculata* (6–8) were reported. In this report, we describe the first study of the bioactive constituents from *K. henryi*.

Protein-tyrosine kinases (PTKs) are involved in signal transduction for cell growth and transformation (9–12) and thus become potential targets for agents for controlling growth of transformed cells. As part of our continuing research on the discovery of novel antitumor agents from plants, we have designed an efficient PTK bioassay for directing the isolation of PTK inhibitors from plants (12,13). A bioassay-directed fractionation of the crude extract of *K. henryi* has led to the discovery of kaempferol and quercetin as potent inhibitors of the PTK $p56^{lck}$. Two additional less potent inhibitors, kaempferol-O^3-α-rhamnopyranoside [**1**] and kaempferol-O^3-α-arabinopyranoside [**2**], were also isolated for structure-activity studies. A comparison of their inhibitory activities (Table 1) showed that glycosylation of the 3-hydroxyl group of a flavonol markedly decreased the activity (kaempferol vs. **1** or **2**), which is consistent with previous findings on the inhibition of the p40 protein-tyrosine kinase (14). In order further to probe the essential role of the hydroxyl groups on the B ring, the inhibitory activities of several other natural flavonols were examined (Table 1). These results indicated that: (a) the 4′-hydroxyl is critical for activity (kaempferol vs. galangin); (b) replacement of the free 4′-hydroxyl group with a methoxyl group significantly reduces the activity (kaempferol vs. kaempferide); (c) movement of the 4′-hydroxyl group of kaempferol to the 2′-hydroxyl group (datiscetin) decreases the activity; (d) addition of a hydroxyl group ortho to the 4′-hydroxyl group of kaempferol (quercetin) does not alter the potency, whereas addition of a meta hydroxyl group (morin) clearly reduces the activity; (e) the presence of a 2′-hydroxyl group in flavonols may hamper the binding with the enzyme active site (datiscetin vs. kaempferol or morin vs. quercetin); (f) addition of two hydroxyl groups ortho to the 4′- hydroxyl group of kaempferol retains similar activity (kaempferol vs. myricetin); and (g) removal of the 5-hydroxyl or 3-hydroxyl group of kaempferol does not change the potency

TABLE 1. Inhibition of Protein-Tyrosine Kinase Activity of p56^{lck} by Flavonoids.



Compound	R_3	R_5	$R_{2'}$	$R_{3'}$	$R_{4'}$	$R_{5'}$	IC_{50} (μg/ml)
kaempferol	OH	OH	H	H	OH	H	8×10^{0}
quercetin	OH	OH	H	OH	OH	H	8×10^{0}
1		OH	H	H	OH	H	4×10^{2}
2		OH	H	H	OH	H	2×10^{2}
galangin	OH	OH	H	H	H	H	3×10^{1}
kaempferide	OH	OH	H	H	OMe	H	3×10^{1}
datiscetin	OH	OH	OH	H	H	H	6×10^{1}
morin	OH	OH	OH	H	OH	H	5×10^{1}
myricetin	OH	OH	H	OH	OH	OH	1×10^{1}
resokaempferol	OH	H	H	H	OH	H	5×10^{0}
apigenin	H	OH	H	H	OH	H	4×10^{0}
luteolin	H	OH	H	OH	OH	H	4×10^{0}

(kaempferol vs. resokaempferol or apigenin).

In summary, the above results provide important information on the structural requirement for the inhibitory interaction with the p56^{lck} protein-tyrosine kinase. This information can be used to design more specific inhibitors for studying the function of specific protein-tyrosine kinases in the control of tumor cell growth. In addition, flavonoids have been shown to occur in many plants; thus it is desirable to design specific dereplication methods to distinguish these ubiquitous compounds from other structurally unique inhibitors in plants.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were taken on a Varian VXR-500S spectrometer. Fabms were measured on a Kratos MS-50 spectrometer. Ir spectra were obtained on a Beckman DU-7 spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Melting points were recorded on a Mel-temp apparatus and uncorrected. The p56^{lck} protein-tyrosine kinase inhibition assay was described previously (12–14). Emodin was routinely used as a reference compound (IC_{50} 10±5 μg/ml) for this assay. Morin, myricetin, and apigenin were purchased from Sigma Chemical Company. Galangin, kaempferide, datiscetin, resokaempferol, and luteolin were purchased from Indofine Chemical Company (Somerville, New Jersey). The purity of these compounds was examined by ^{1}H nmr.

PLANT MATERIAL.—Leaves and twigs of *K. henryi* were collected from Taiwan in July 1989 by Mr. W.-l. Chu, Taiwan Forestry Research Institute, Heng-Chun Branch Station, where a voucher sample was deposited.

EXTRACTION AND ISOLATION.—The dried pulverized leaves and twigs (5.8 kg) were ex-

tracted by maceration with 95% EtOH three times. The EtOH extract (F001, 522 g) was partitioned between CH_2Cl_2-H_2O (1:1) to give the H_2O residue (F002, 337 g) and the CH_2Cl_2 residue (F003, 213 g). F002 (50 g) was dissolved in 90% H_2O in MeOH and then extracted with CH_2Cl_2, EtOAc, and *n*-BuOH successively to give F004, F005, and F006, respectively; the aqueous fraction was freeze-dried as F007. Screening of fractions F004–F007 for PTK inhibitory activity indicated that F005 was the most active fraction. F005 was then chromatographed on a small Si gel column into two fractions, F008 and F009, using EtOAc as eluent with an increasing MeOH gradient. The PTK inhibitory activity of F008 was found to exceed that of F009. F008 was further chromatographed on another Si gel column, using EtOAc as eluent with an increasing MeOH gradient, into eight fractions (F010–F017). PTK inhibitory activity was found to reside in fractions F010–F015. Preparative Si gel tlc separation of F010 has led to the isolation of kaempferol and quercetin. Compound **1** was isolated from F012, while compound **2** was isolated from F013.

KAEMPFEROL-O^3-α-RHAMNOSIDE [**1**] (AFZELIN).—Yellow crystals: mp 171–173° (MeOH) [lit. (15) 174–176°]; $[\alpha]_D$ −145° (*c*=0.1, EtOH) [lit. (15) −116° (*c*=0.5, MeOH)]. The structure identification was made on the basis of detailed analysis of ir, uv, fabms, and ^{1}H- and ^{13}C-nmr data.

KAEMPFEROL-O^3-α-ARABINOSIDE [**2**].—Yellow crystals: mp 251–253° (MeOH) [lit. (15) 255–258°]; $[\alpha]_D$ −49° (*c*=0.1, MeOH) [lit. (15) −43.8° (*c*=0.32, MeOH)]. The structure identification was made on the basis of detailed analysis of ir, uv, fabms, and ^{1}H- and ^{13}C-nmr data.

KAEMPFEROL.—Yellow crystals: mp 276–278° (MeOH) [lit. (15) 278–280° (MeOH)]; ^{1}H nmr was analyzed.

QUERCETIN.—Yellow needles: mp. 300° (MeOH) [lit. (15) 300° (MeOH)]; ^{1}H nmr was analyzed.

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