Arch Pharm Res Vol 27, No 2, 169-172, 2004

Archives of Pharmacal Research
http://apr.psk.or.kr

Phenolic Compounds Obtained from Stems of *Couepia ulei* with the Potential to Induce Quinone Reductase

Dae Sik Jang[1], Eun Jung Park[1], Young-Hwa Kang[1], Jose Schunke Vigo[2], James G. Graham[1], Fernando Cabieses[2], Harry H. S. Fong[1], John M. Pezzuto[1,3], and A. Douglas Kinghorn[1]

[1]Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, U.S.A., [2]Instituto Nacional de Medicina Tradicional (INMETRA), Minesterio de Salud, Jesus Maria, Lima, Peru, and [3]Heine Pharmacy Building, Purdue University, West Lafayette, IN 47907, U.S.A.

(Received December 4, 2003)

Activity-guided fractionation of the EtOAc-soluble extract of the stems of *Couepia ulei*, using a bioassay based on the induction of quinone reductase (QR) in cultured Hepa 1c1c7 mouse hepatoma cells led to the isolation of two active compounds, a new natural product, *erythro*-2,3-bis(4-hydroxy-3-methoxyphenyl)-3-ethoxypropan-1-ol (**1**), and a known compound, evofolin-B (**2**), along with five inactive compounds all of known structure, viz., betulinic acid, oleanolic acid, pomolic acid, (±)-syringaresinol, and ursolic acid. These isolates were identified by analysis of physical and spectral data. Compounds **1** and **2** exhibited QR inducing activity, with observed CD (concentration required to double induction) values of 16.7 and 16.4 μM, respectively.

Key words: *Couepia ulei*, Chrysobalanoideae, Rosaceae, Phenolic compounds, Quinone reductase induction, Cancer chemoprevention

INTRODUCTION

In our ongoing project directed toward the discovery of novel naturally occurring cancer chemopreventive agents (Kinghorn *et al.*, 2003; Pezzuto *et al.*, 1999), the stems of *Couepia ulei* were chosen for more detailed investigation, since the EtOAc-soluble fraction of a MeOH extract significantly induced NADP(H):quinone oxidoreductase (QR) in cultured Hepa 1c1c7 (mouse hepatoma) cells. Induction of phase II enzymes is regarded as an important mechanism of cancer chemoprevention (Maxuitenko *et al.*, 1993; Talalay *et al.*, 1981; Wattenberg, 1997). The genus *Couepia* belongs to the tribe Chrysobalanoideae of the family Rosaceae (Sanduja *et al.*, 1982). Although previous phytochemical investigations on members of the genus *Couepia* have resulted in the isolation of several fatty acids, flavonoids, chromones, and triterpenoids (Sanduja *et al.*, 1982; Sanduja *et al.*, 1983; Spitzer *et al.*, 1991), no phytochemical studies have been carried out to date on *Couepia ulei*.

In the present study, bioassay-guided fractionation of an EtOAc-soluble residue of *Couepia ulei*, using the QR induction assay, led to the isolation and characterization of two active phenolic constituents, a new natural product, *erythro*-2,3-bis(4-hydroxy-3-methoxyphenyl)-3-ethoxypropan-1-ol (**1**), and a known compound, evofolin-B (**2**), along with five inactive compounds of known structure.

MATERIALS AND METHODS

Plant material

The stems of *Couepia ulei* Pilg. were collected in Yarinacocha District, Coronel Portillo Province, Ucayali Department, Peru in July, 1998 by two of us (J. S. V. and J. G. G.). A voucher specimen has been deposited at the Field Museum of Natural History, Chicago, IL (accession no. Graham and Schunke 482).

Correspondence to: A. Douglas Kinghorn, Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street (M/C 781), Chicago, IL 60612, U.S.A.
Tel: 1-312-996-0914, Fax: 1-312-996-7107
E-mail: kinghorn@uic.edu

General experimental procedures

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were obtained using a Perkin-Elmer 241 polarimeter. UV spectra were recorded with a Beckman DU-7 spectrometer. IR spectra were run on an ATI Mattson Genesis Series FT-IR spectrometer. NMR experiments were conducted on Bruker DPX-300 MHz and Bruker DRX-500 MHz spectrometer with tetramethylsilane (TMS) as internal standard. LREIMS were recorded on a Finnigan MAT 95 instrument operated at 70 eV and HRESIMS on a Hewlett-Packard 5989B mass spectrometer with a 5998A electrospray interface. TLC analysis was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness), with compounds visualized by dipping plates into 10% (v/v) H_2SO_4 reagent (Aldrich, Milwaukee, WI) followed by charring at 110°C for 5-10 min. Silica gel (Merck 60A, 70-230 or 200-400 mesh ASTM) and Sephadex LH-20 (Sigma) were used for column chromatography. Preparative TLC was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness). All solvents used for chromatographic separations were purchased from Fisher Scientific (Fair Lawn, NJ) and distilled before use.

Extraction and isolation

The dried and milled plant material (1.73 kg) was extracted with MeOH (3×8 L) by maceration. The extracts were combined and concentrated *in vacuo* at 40°C. The concentrated extract was suspended in 90% MeOH (1 L) and then partitioned with petroleum ether (3×1 L) to afford a petroleum ether-soluble syrup on drying. Next, the aqueous methanol extract was concentrated and suspended in H_2O (1.5 L) and partitioned again with EtOAc (3×1.5 L) to give an EtOAc-soluble extract and an aqueous residue. The EtOAc-soluble extract (CD value; 15.6 μg/mL, 11 g) was chromatographed over silica gel as stationary phase, using a $CHCl_3$-MeOH gradient (from 1:0 to 1:1 v/v) as mobile phase, to afford 13 pooled fractions (F004-F016). Of these, only fraction F008 showed potent QR-inducing activity (CD value of 4.7 μg/mL). Fraction F008 [eluted with $CHCl_3$-MeOH (24:1 v/v); 240 mg] was chromatographed over silica gel as stationary phase using a petroleum ether-EtOAc gradient (from 4:1 to 0:1 v/v) as mobile phase to afford six subfractions (F017-F022). Further chromatographic separation of subfraction F019 [eluted with petroleum ether-EtOAc (7:3 v/v); 17 mg] was carried out by preparative TLC ($CHCl_3$-MeOH, 19:1 as developing solvent) to afford (±)-syringaresinol (3.4 mg, 0.00020%, R_f = 0.56), mp 170-172°C, $[\alpha]_D^{25}$ 0.0° (*c* 0.1, $CHCl_3$), [lit. mp 174°C, optically inactive in $CHCl_3$ (Nawwar *et al.*, 1982)]. Fraction F020 was passed over a Sephadex LH 20 column, with $CHCl_3$-MeOH (1:3) used as solvent system to give *erythro*-2,3-bis(4-hydroxy-3-methoxyphenyl)-3-ethoxypropan-1-ol (**1**, oil, 2.4 mg, 0.00014%) and evofolin-B (**2**, oil, 2.0 mg, 0.00012%). Betulinic acid (46 mg, 0.0027%), mp >300°C [(lit. mp 295-297°C) (Su *et al.*, 2002)] was obtained as colorless needles by recrystallization in acetone from fraction F005.

Fraction F007 [eluted with $CHCl_3$-MeOH (49:1 v/v); 410 mg], was purified further over a Sephadex LH-20 column, with $CHCl_3$-MeOH (2:3 v/v) used as the solvent system, yielding, in turn, the known compounds oleanolic acid (8.6 mg, 0.00050%), mp >300°C [(lit. mp 299-301°C) (Bhakuni *et al.*, 1988)], ursolic acid (6.2 mg, 0.00036%), mp 241-243°C [(lit. mp 242-244°C) (Hwang *et al.*, 2003)], and pomolic acid (5.4 mg, 0.00031%), mp 294-296°C [(lit. mp 298-300°C) (Kakuno *et al.*, 1992)].

erythro-2,3-Bis(4-hydroxy-3-methoxyphenyl)-3-ethoxypropan-1-ol (1)

Colorless oil: $[\alpha]_D^{25}$ 0.0° (*c* 0.1, MeOH); IR ν_{max} NaCl cm^{-1}: 3349, 2923, 2853, 1601, 1513, 1458, 1372, 1272, 1120, 753; UV λ_{max} MeOH nm (log ε): 281 (3.42); ^{1}H-NMR (CD_3OD, 500 MHz): δ 6.68 (1H, d, *J* = 1.7 Hz, H-2"), 6.67 (1H, d, *J* = 8.0 Hz, H-5'), 6.58 (1H, dd, *J* = 8.0 and 1.7 Hz, H-6'), 6.55 (1H, dd, *J* = 8.0 and 1.7 Hz, H-6"), 6.46 (1H, d, *J* = 1.7 Hz, H-2'), 6.45 (1H, d, *J* = 8.0 Hz, H-5"), 4.59 (1H, d, *J* = 5.2 Hz, H-3), 3.87 (1H, dd, *J* = 10.6 and 7.1 Hz, H-1a), 3.74 (3H, s, CH_3-3'), 3.65 (1H, dd, *J* = 10.6 and 5.2 Hz, H-1b), 3.63 (3H, s, CH_3-3"), 3.34 (1H, dd, *J* = 9.3 and 7.0 Hz, H-1'"a), 3.26 (overlapped, H-1'"b), 2.81 (1H, m, H-2), 1.10 (3H, t, *J* = 7.0 Hz, H-2'"); ^{13}C-NMR (CD_3OD, 125 MHz): δ 148.6 (C-3"), 148.3 (C-3'), 146.7 (C-4'), 146.2 (C-4"), 134.0 (C-1'), 132.3 (C-1"), 123.4 (C-6"), 121.1 (C-6'), 115.5 (C-5' and C-5"), 114.8 (C-2"), 111.8 (C-2'), 82.8 (C-3), 65.2 (C-1'"), 64.5 (C-1), 56.6 (C-2), 56.4 (CH_3-3'), 56.2 (CH_3-3"), 15.6 (C-2'"); HRESIMS *m/z*: 347.1439 ([M-H]$^+$, calcd for $C_{19}H_{23}O_6$, 347.1495).

Evofolin-B (2)

Yellowish oil: $[\alpha]_D^{25}$ 12.8° (*c* 0.1, $CHCl_3$) {lit. $[\alpha]_D$ 14.3° (*c* 0.021, MeOH) (Wu *et al.*, 1995)}; LREIMS *m/z* (rel. int.): 318 ([M]$^+$, 2), 300 (3), 288 (3), 167 (2), 151 (100), 123 (6). ^{1}H- and ^{13}C-NMR data were in agreement with reported values (Wu *et al.*, 1995).

Quinone reductase induction assay with cultured mouse hepatoma cells

For the evaluation of plant extracts, fractions, and pure isolates as inducers of quinone reductase (QR), cultured mouse Hepa 1c1c7 cells (supplied by Dr. J. P. Whitlock, Jr., Stanford University, Stanford, CA) were used as described previously (Gerhäuser *et al.*, 1997; Jang *et al.*, 2002).

RESULTS AND DISCUSSION

The new natural product, *erythro*-2,3-bis(4-hydroxy-3-methoxyphenyl)-3-ethoxypropan-1-ol (**1**) and the known compounds, evofolin-B (**2**) (Wu *et al.*, 1995) (Fig. 1), betulinic acid (Mahato and Kundu, 1994), oleanolic acid (Mahato and Kundu, 1994), pomolic acid (Kakuno *et al.*, 1992; Cheng and Cao, 1992), (±)-syringaresinol (Nawwar *et al.*, 1982), and ursolic acid (Takeoka *et al.*, 2000) were isolated from an EtOAc-soluble fraction of the stems of *Couepia ulei* by bioassay-guided fractionation, using the QR induction assay as a monitor. The structures of the known compounds were identified by physical and spectroscopic data (mp, $[\alpha]_D$, MS, ^{1}H- and ^{13}C-NMR) measurement and by comparison with published values. The amount of **2** obtained in this study was insufficient to determine its stereochemistry at C-2.

Compound **1** was obtained as colorless oil and gave a protonated molecular ion at *m/z* 347.1439 $[M-1]^-$ by negative HRESIMS, consistent with an elemental formula of $C_{19}H_{23}O_6$. Assignments of the resonances of all of the hydrogen and carbon atoms in the molecule were made by application of one- and two-dimensional NMR experiments (^{1}H-NMR, ^{13}C-NMR, DEPT, COSY, HMQC, and HMBC). The ^{1}H-NMR spectrum of **1** showed resonances for two sets of ABX-type signals [δ 6.46 (1H, d, *J* = 1.7 Hz), 6.67 (1H, d, *J* = 8.0 Hz), and 6.58 (1H, dd, *J* = 8.0, 1.7 Hz); 6.68 (1H, d, *J* = 1.7 Hz), 6.45 (1H, d, *J* = 8.0 Hz), and 6.55 (1H, dd, *J* = 8.0, 1.7 Hz)], indicating that compound **1** has two guaiacyl groups (Yoshikawa *et al.*, 1998). The ^{1}H-NMR spectrum of **1** also showed resonances for ABMX-type, ethoxyl signals, and methoxyl signals [δ 4.59 (1H, d, *J* = 5.2 Hz), 3.87 (1H, dd, *J* = 10.6, 7.1 Hz), 3.65 (1H, dd, *J* = 10.6, 5.2 Hz), and 2.81 (1H, m); 3.34 (1H, dd, *J* = 9.3, 7.0 Hz), 3.26 (overlapped), and 1.10 (3H, t, *J* = 5.2 Hz); 3.74 (3H, s) and 3.63 (3H, s)] in the aliphatic region. The ^{13}C-NMR and DEPT experiments with **1** revealed 12 aromatic signals, with four (δ 148.6, 148.3, 146.7, and 146.2) bearing oxygen atoms, as well as five aliphatic signals, with three of these (δ 82.8, 65.2, and 64.5) bearing oxygen atoms and two methoxyl signals (δ 56.4 and 56.2). The HMBC experiment revealed long-range coupling from H-2 (δ 2.81) to C-1 (δ 64.5), C-3 (δ 82.8), C-1' (δ 134.0), C-1" (δ 132.3), C-2" (δ 114.8), and C-6" (δ 123.4); and from H-3 (δ 4.59) to C-1 (δ 64.5), C-2 (δ 56.6), C-1' (δ 134.0), C-2' (δ 111.8), C-6' (δ 121.1), C-1" (δ 132.3), and C-1"' (δ 65.2). On the basis of these spectral data, the structure of **1** was assigned as 2,3-bis(4-hydroxy-3-methoxyphenyl)-3-ethoxypropan-1-ol. Comparison of the coupling constant between H-2 and H-3 ($J_{2,3}$ = 5.2 Hz) with that ($J_{2,3}$ = 8.8 Hz) of its *threo* isomer (Lee *et al.*, 2002) provided evidence for the determination of the *erythro* configuration of the C-2 and C-3 substituents. Thus, the structure of **1** was elucidated as *erythro*-2,3-bis(4-hydroxy-3-methoxyphenyl)-3-ethoxypropan-1-ol.

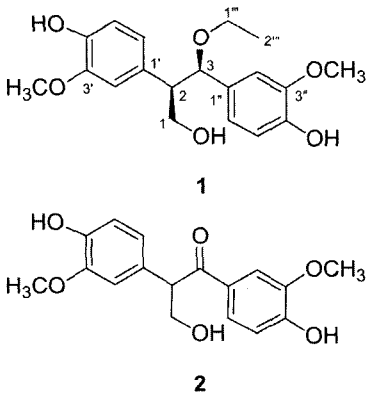

Fig. 1. Structures of quinone reductase inducers isolated from *C. ulei*.

Table I. Induction of quinone reductase (QR) by compounds **1** and **2** from *C. ulei*

Compound	QR[a]		
	CD (μM)	IC_{50} (μM)	CI
1	16.7	>57.5	>3.4
2	16.4	>62.9	>3.8
sulforaphane[b]	0.43	11.0	25.0

[a] CD, concentration required to double QR activity; IC_{50}, concentration inhibiting cell growth by 50%; CI, Chemoprevention Index (= IC_{50}/CD). Compounds with CD values of <10 μg/mL were considered active.
[b] Sulforaphane was used as a positive control (Gerhäuser *et al.*, 1997).

Compound **1** was previously obtained as its triacetate from *Ficus beecheyana* (Lee *et al.*, 2002). This is the first report of the isolation of **1** from natural source, and of its physical and spectroscopic parameters.

All isolates were tested for their potential to induce QR activity in Hepa 1c1c7 cells (Table I). Of these compounds, only compounds **1** and **2** modulated QR activity, with observed CD (concentration required to double induction) values of 16.7 and 16.4 μM, respectively. Evofolin-B (**2**) was also isolated recently as a potent QR inducer from *Sida acuta* by our group (Jang *et al.*, 2003).

ACKNOWLEDGEMENTS

We thank Dr. J. A. (Art) Anderson, Research Resources Center, University of Illinois at Chicago, and Dr. K. Fagerquist, Mass Spectrometry Facility, Department of Chemistry, University of Minnesota, Minneapolis, MN, for the mass spectral data. We are grateful to the Research Resources Center, UIC, for providing spectroscopic equipment, and to Dr. R. A. Kleps of the Research Resources Center, UIC, for facilitating the running of the 500 MHz NMR instrument. This research was supported

by program project P01 CA48112, funded by the National Cancer Institute, NIH, Bethesda, MD.

REFERENCES

Bhakuni, R. S., Shukla, Y. N., and Thakur, R. S., New triterpenoid and aliphatic esters from *Cornus capitata*. *Fitoterapia*, 59, 45-46 (1988).

Cheng, D. and Cao, X., Pomolic acid derivatives from the root of *Sanguisorba officinalis*. *Phytochemistry*, 31, 1317-1320 (1992).

Gerhäuser, C., You, M., Liu, J., Moriarty, R. M., Hawthorne, M., Mehta, R. G., Moon, R. C., and Pezzuto, J. M., Cancer chemopreventive potential of sulforamate, a novel analogue of suforaphane that induces phase 2 drug-metabolizing enzymes. *Cancer Res.*, 57, 272-278 (1997).

Hwang, B. Y., Chai, H.-B., Kardono, L. B. S., Riswan, S., Farnsworth, N. R., Cordell, G. A., Pezzuto, J. M., and Kinghorn, A. D., Cytotoxic triterpenes from the twigs of *Celtis philippinensis*. *Phytochemistry*, 62, 197-201 (2003).

Jang, D. S., Park, E. J., Hawthorne, M. E., Vigo, J. S., Graham, J. G., Cabieses, F., Santarsiero, B. D., Mesecar, A. D., Fong, H. H. S., Mehta, R. G., Pezzuto, J. M., and Kinghorn, A. D., Constituents of *Musa* x *paradisiaca* cultivar with the potential to induce the phase II enzyme, quinone reductase. *J. Agric. Food Chem.*, 50, 6330-6334 (2002).

Jang, D. S., Park, E. J., Kang, Y.-H., Su, B.-N., Hawthorne, M. E., Vigo, J. S., Graham, J. G., Cabieses, F., Fong, H. H. S., Mehta, R. G., Pezzuto, J. M., and Kinghorn, A. D., Compounds obtained from *Sida acuta* with the potential to induce quinone reductase and to inhibit 7,12-dimethylbenz[*a*]anthracene-induced preneoplastic lesions in a mouse mammary organ culture model. *Arch. Pharm. Res.*, 26, 585-590 (2003).

Kakuno, T., Yoshikawa, K., and Arihara, S., Triterpenoid saponins from *Ilex crenata* fruit. *Phytochemistry*, 31, 3553-3557 (1992).

Kinghorn, A. D., Su, B.-N., Lee, D., Gu, J.-Q., and Pezzuto, J. M., Cancer chemopreventive agents discovered by activity-guided fractionation: an update. *Curr. Org. Chem.*, 7, 213-226 (2003).

Lee, T.-H., Kuo, Y.-C., Wang, G.-J., Kuo, Y.-H., Chang, C.-I., Lu, C.-K., and Lee, C.-K., Five new phenolics from the roots of *Ficus beecheyana*. *J. Nat. Prod.*, 65, 1497-1500 (2002).

Mahato, B. S. and Kundu, P. A., ^{13}C-NMR spectra of pentacyclic triterpenoids- A compilation and some salient features. *Phytochemistry*, 37, 1517-1575 (1994).

Maxuitenko, Y. Y., MacMillan, D. L., Kensler, T. W., and Roebuck, B. D., Evaluations of the post-initiation effects of oltipraz on aflatoxin B1-induced preneoplastic foci in a rat model of hepatic tumorigenesis. *Carcinogenesis*, 14, 2423-2425 (1993).

Nawwar, M. A. M., Buddrus, J., and Bauer, H., Dimeric phenolic constituents from the roots of *Tamarix nilotica*. *Phytochemistry*, 21, 1755-1758 (1982).

Pezzuto, J. M., Song, L. L., Lee, S. K., Shamon, L. A., Mata-Greenwood, E., Jang, M., Jeong, H.-J., Pisha, E., Mehta, R. G., and Kinghorn, A. D., Bioassay methods useful for activity-guided isolation of natural product cancer chemopreventive agents. In Hostettmann, K., Gupta, M. P., and Marston, A. (Eds.). *Chemistry, Biological and Pharmacological Properties of Medicinal Plants from the Americas*. Harwood Academic Publishers, Amsterdam, pp. 81-110 (1999).

Sanduja, R., Alam, M., and Euler, K. L., Constituents of *Couepia paraensis*. *J. Nat. Prod.*, 46, 149 (1983).

Sanduja, R., Euler, K. L., Alam, M., Korp, J. D., and Bernal, I., Isolation and crystal structure of 5-hydroxy-2,8-dimethyl-6,7-dimethoxybenzopyran-4-one from *Couepia paraensis*. *Phytochemistry*, 21, 1457-1453 (1982).

Spitzer, V., Marx, F., Maia, J. G. S., and Pfeilsticker, K., Occurrence of conjugated fatty acids in the seed oil of *Couepia longipendula* (Chrysobalanaceae). *J. Am. Oil Chem. Soc.*, 68, 440-442 (1991).

Su, B.-N., Cuendet, M., Farnsworth, N. R., Fong, H. H. S., Pezzuto, J. M., and Kinghorn, A. D., Activity-guided fractionation of the seeds of *Ziziphus jujuba* using a cyclooxygenase-2 inhibitory assay. *Planta Med.*, 68, 1125-1128 (2002).

Talalay, P., De Long, M. J., and Prochaska, H. J., Molecular mechanisms in protection against carcinogenesis. In Cory, J. G., and Szentivanyi, A. (Eds.). *Cancer Biology and Therapeutics*. Plenum Press, New York, NY, pp. 197-216 (1981).

Takeoka, G., Dao, L., Teranish, R., Wong, R., Flessa, S., Harden, L., and Edwards, R., Identification of three triterpenoids in almond hulls. *J. Agric. Food Chem.*, 48, 3437-3439 (2000).

Wattenberg, L. W., An overview of chemoprevention: current status and future prospects. *Proc. Soc. Exp. Biol. Med.*, 216, 133-141 (1997).

Wu, T.-S., Teh, J.-H., and Wu, P.-L., The heartwood constituents of *Tetradium glabrifolium*. *Phytochemistry*, 40, 121-124 (1995).

Yoshikawa, K., Mimura, N., and Arihara, S., Isolation and absolute structures of enantiomeric 1,2-bis(4-hydroxy-3-methoxyphenyl)-1,3-propanediol 1-*O*-glucosides from the bark of *Hovenia trichocarpa*. *J. Nat. Prod.*, 61, 1137-1139 (1998).