JOURNAL OF NATURAL PRODUCTS

Subscriber access provided by ISTANBUL TEKNIK UNIV

Cytotoxic Cyclolignans from Koelreuteria henryi

Yan-Nong Song, Hai-Lan Zhang, Ching-jer Chang, and Daniel M. Bollag

J. Nat. Prod., **1994**, 57 (12), 1670-1674• DOI: 10.1021/np50114a008 • Publication Date (Web): 01 July 2004

Downloaded from http://pubs.acs.org on April 4, 2009

More About This Article

The permalink http://dx.doi.org/10.1021/np50114a008 provides access to:

- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

ACS Publications
High quality. High impact.

Journal of Natural Products is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Journal of Natural Products
Vol. 57, No. 12, pp. 1670-1674, December 1994

CYTOTOXIC CYCLOLIGNANS FROM *KOELREUTERIA HENRYI*

YAN-NONG SONG, HAI-LAN ZHANG, CHING-JER CHANG,*

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907

and DANIEL M. BOLLAG

Merck Research Laboratories, West Point, Pennsylvania 19486

ABSTRACT.—Chemical investigation of the cytotoxic fraction of *Koelreuteria henryi* resulted in the isolation of three cyclolignans. A new cyclolignan, named koelreuterin-1 was elucidated as furo[3′,4′:6,7]naphtho[2,3-d]-1,3-dioxol-6(8H)-one,5-(7-methoxy-1,3-benzodioxol-5-yl) [**1**]. Two known cyclolignans were characterized as austrobailignan-1 [**2**] and austrobailignan-2 [**3**]. The structure elucidation of **1** was based on extensive ^{1}H- and ^{13}C-nmr spectral analyses. Further chemical conversion of **2** to **3** and oxidative transformation of **2** to **1** unambiguously confirmed the structure of **1**. The cytotoxicity and tubulin polymerization inhibitory activity of **1–3** are discussed.

As part of our continuing research on the discovery of novel antitumor agents from plants we have studied the potential antitumor constituents of *Koelreuteria henryi* Dummer (Sapindaceae) (*K. formosana* Hayata), an indigenous tree known as the "Flame Golden Tree" in Taiwan (1,2). Earlier investigations on the chemical constituents of different species of the genus *Koelreuteria* have resulted in the isolation of triterpenoid glycosides (3) and cyanolipids (4–7). Our previous study of the EtOH extract of the leaves and twigs of *K. henryi* resulted in the isolation of several flavonoids with protein-tyrosine kinase (PTK) inhibitory activity (8). In addition, we found that the MeOH fraction showed strong cytotoxicity against human lung carcinoma (A-549), breast adenocarcinoma (MCF-7), and colon adenocarcinoma (HT-29) cells. In this report, we describe the isolation and structural elucidation of a new cyclolignan, koelreuterin-1 [**1**] and the identification of two known cyclolignan constituents, austrobailignan-1 [**2**] and austrobailignan-2 [**3**], which showed strong cytotoxicity against various human tumor cells.

RESULTS AND DISCUSSION

The cytotoxic MeOH residue was chromatographed on a Si gel column and followed by a reversed-phase C_8 Lobar column to yield three cyclolignans [**1–3**]. High-resolution eims (M^+ 378.0749) of **1** suggested a molecular formula of $C_{21}H_{14}O_7$ (calcd 378.0740), which was in agreement with the number of protons and carbons found in the corresponding ^{1}H- and ^{13}C-nmr spectra. The ir spectrum disclosed absorption bands associated with a conjugated γ-lactone (1759 cm^{-1}) and a methylenedioxy ether (940 cm^{-1}). The presence of a lactone in the structure was confirmed by the resonance signals for a carbonyl carbon (δ 169.6) and a methylene carbon attached to oxygen (δ 68.0) in the ^{13}C-nmr spectrum. The uv spectrum of **1** displayed an absorption at 350 nm, indicative of a conjugated aromatic chromophore. The ^{1}H-nmr spectrum showed the signals of five aromatic protons at δ 7.70 (s), δ 7.20 (s), δ 7.13 (s), δ 6.52 (d, J=1.5 Hz), and δ 6.50 (d, J=1.5 Hz). These spectral data suggested that **1** is closely related to 1,2,3,4-dehydrodesoxypodophyllotoxin, which was previously synthesized from desoxypodophyllotoxin (9). However, **1** contained only one methyl proton signal at δ 3.89 and two methylenedioxy signals, an AB quartet at δ 6.10 and δ 6.07 (1H each, J=1.5 Hz) and a singlet at δ 6.09 (2H). These functional groups were confirmed by the presence of ^{13}C-nmr signals at δ 56.6 (CH_3), 101.8 (CH_2), and 101.9 (CH_2). Therefore,

the C-ring of **1** must possess 3′,4′ -methylenedioxy and 5′-methoxy substituents. The assignments for H-2′ and H-6′ were based on the observation of a selective nuclear Overhauser effect (nOe) (26%) at H-6′ upon irradiation of the methoxyl signal. Similarly, irradiation of the H-4 signal yielded a 20% nOe on H-5. These unambiguous assignments permitted us to determine unequivocally the corresponding carbon resonances by a HETCOR experiment and the neighboring carbon signals based on long-range HETCOR nmr data (Table 1). Thus, the ^{13}C-nmr spectral data further confirmed the structure of **1**.

High-resolution ms data of **2** and **3** indicated that they are structural isomers with the molecular formula, $C_{21}H_{18}O_7$. Further 2D ^{1}H- and ^{13}C-nmr analyses suggested that **2** and **3** are identical to austrobailignan-1 and austrobailignan-2, respectively, which were previously isolated from *Austrobaileya scandens* (10). ^{13}C-Nmr spectral interpretations were based on the HETCOR and long-range HETCOR results and further comparison with the spectral data of compounds in the podophyllotoxin series (11).

In order to unambiguously confirm the proposed structures, **2** was chemically transformed into **1** and **3**, respectively (Figure 1). Treatment of **2** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) afforded **1** (9). Reflux of **2** (2,3-*trans*-γ-lactone) with anhydrous NaOAc in absolute EtOH yielded **3** (2,3-*cis*-γ-lactone) (12).

All three compounds were tested against human tumor cells and the results are summarized in Table 2. These compounds exhibited significant cytotoxicity against all human tumor cells in which they were tested. Compound **2** is structurally similar to podophyllotoxin and is the most cytotoxic compound. Badawi *et al.* (13) also observed its cytotoxic activity against human nasopharynx carcinoma (KB) cells. Conversion of the 2,3-*cis*-γ-lactone [**2**] into the *trans*-γ-lactone [**3**] drastically reduced its activity. Compound **1** is less active than **2**. A similar trend was also observed in the podophyllotoxin series using KB cells (14). In addition, **1** demonstrated a somewhat selective cytotoxic profile against human ovarian carcinoma (SK-OV-3) and melanoma (SK-MEL-5) cells, which will be further evaluated in a human tumor cell panel by the National Cancer Institute. These three compounds were also evaluated for their ability to inhibit tubulin polymerization (Table 3), since the mechanism of cytotoxic action of podophyllotoxins has been previously attributed to the prevention of tubulin polymerization and the disruption of the assembly and function of microtubules (15). For these three compounds, the inhibition of tubulin polymerization correlated quite well with their cytotoxicity. Picropodophyllotoxin with a cis-lactone ring is known to be much less cytotoxic than its stereoisomer podophyllotoxin with a trans-lactone ring (16,17), presumably because of the weaker inhibition of tubulin polymerization. This stereospecificity was also shown in the relative inhibitory activity of tubulin polymerization and cytotoxicity of **1–3** against eight different human tumor cells. All of these results confirm that stereochemistry of cyclolignans is critical to their antitumor activity.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Mel-Temp apparatus and are uncorrected. The optical rotations were determined on a Perkin-Elmer 241 polarimeter. Uv spectra were taken in $CHCl_3$ on a Beckman DU-7 spectrometer. Ir spectra were recorded on a Perkin-Elmer 1420 IR spectrometer. ^{1}H-Nmr, COSY, ^{13}C-nmr, and HETCOR and long-range HETCOR nmr spectra were obtained in $CDCl_3$ with TMS as internal standard, employing a Varian VXR-500S spectrometer. Eims data were recorded with a Kratos MS50 spectrometer, and hreims spectra were obtained on a Kratos MS50 spectrometer through peak matching. Reversed-phase chromatography was carried out using a Hitachi 655A-11 hplc system with an ISCO UA-5 detector using a Lobar column (240–10 μm) LiChroprep™ RP8 (40–63 μm). Tlc was performed on Whatman Si gel plates (0.25 mm, flourescent at 254 nm) and visualized with uv light and 5% phosphomolybdic acid in EtOH. Podophyllotoxin was purchased from Aldrich Chemical Company, Inc. and recrystallized from hexane/EtOAc.

TABLE 1. ^{1}H- and ^{13}C-Nmr Data of **1**, **2**, and **3** in $CDCl_3$.[a]

Position	Compound					
	1		**2**		**3**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1		140.0	4.57 (d, J=4.0)	43.5	4.34 (d, J=3.0)	45.0
2		139.7	2.76 (m)	47.4	3.29 (dd, J=9.5, 3.0)	46.3
3		118.8	2.78 (m)	32.6	3.02 (ddddd, J=9.5,7.5,6.5, 5.5,3.0)	32.9
4	7.70 (s)	119.1	α: 3.06 (dd, J=20, 9.0) β: 2.76 (m)	33.1	α: 2.84 (dd, J=15.5, 6.5) β: 2.48 (dd, J=15.5, 5.5)	32.0
5	7.20 (s)	103.6	6.65 (s)	108.5	6.65 (s)	108.8
6		149.9		147.0		146.8[b]
7		148.6		146.7		146.7[b]
8	7.17 (s)	103.7	6.50 (s)	110.3	6.58 (s)	109.8
9		130.3		130.7		130.4
10		134.5		128.2		128.1
11	5.38 (s)	68.0	α: 4.38 (dd, J=9.0, 8.0) β: 3.92 (dd, J=9.0, 8.0)	72.1	α: 4.44 (dd, J=9.0, 7.5) β: 3.97 (dd, J=9.0, 3.0)	72.8
12		169.6		174.8		178.2
1′.....		128.8		135.1		137.1
2′.....	6.52 (d, J=1.5)	104.3	6.06 (d, J=1.5)	104.6	6.25 (d, J=1.5)	101.8
3′.....		148.6		148.1		149.1
4′.....		135.2		134.0		133.8
5′.....		143.3		142.9		143.5
6′.....	6.50 (d, J=1.5)	109.6	6.59 (d, J=1.5)	110.7	6.35 (d, J=1.5)	107.6
13	6.09 (s)	101.8	5.92 (s)	101.3	5.94 (s)	101.4
14	3.89 (s)	56.6	3.88 (s)	56.6	3.86 (s)	56.8
15	6.10, 6.07 (ABq, J=1.5)	101.9	5.89, 5.87 (ABq, J=1.5)	101.1	5.94, 5.92 (ABq, J=1.5)	101.0

[a] ^{13}C-Nmr assignments are based on HETCOR and long-range HETCOR spectral results, and direct comparison with the previous data of related lignans (11).

[b] Assignments interchangeable.

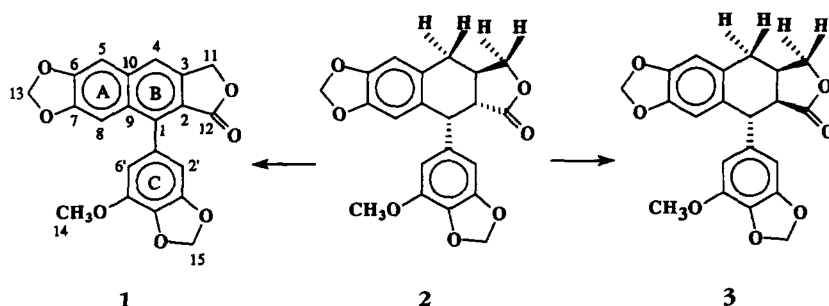

FIGURE 1. Structures and chemical transformations of cyclolignans isolated from *K. henryi*.

TABLE 2. Cytotoxicity (ED_{50}, μg/ml) Against Human Tumor Cells.

Cell line	Podophyllotoxin	**1**	**2**	**3**	Adriamycin
A-431	1.1×10^{-6}	1.8×10^{-1}	3.6×10^{-6}	9.6×10^{-1}	1.2×10^{-2}
A-498	3.4×10^{-7}	7.8×10^{-2}	3.2×10^{-6}	7.6×10^{-1}	3.2×10^{-3}
A-549	7.7×10^{-8}	4.9×10^{-2}	3.9×10^{-6}	4.6×10^{-1}	3.1×10^{-4}
HT-29	4.7×10^{-8}	2.9×10^{-1}	3.7×10^{-6}	1.0×10^{-1}	5.6×10^{-4}
MCF-7	1.1×10^{-6}	9.7	5.2×10^{-5}	4.1	2.0×10^{-2}
PC-3	1.2×10^{-7}	1.5×10^{-1}	3.3×10^{-6}	3.1	1.0×10^{-2}
SK-MEL-5	4.1×10^{-8}	3.5×10^{-3}	3.0×10^{-7}	7.7×10^{-1}	2.8×10^{-4}
SK-OV-3	4.2×10^{-5}	6.7×10^{-3}	2.7×10^{-6}	8.32×10^{-1}	2.5×10^{-3}

PLANT MATERIAL.—The 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 specimen was deposited.

EXTRACTION AND ISOLATION.—The dried, pulverized leaves and twigs (5.8 kg) of *K. henryi* were extracted by maceration with 95% EtOH three times at room temperature. The EtOH extract (522 g) was partitioned between H_2O-CH_2Cl_2 (1:1) to give a H_2O fraction (337 g) and a CH_2Cl_2 fraction (213 g). The CH_2Cl_2 fraction was found to be cytotoxic (A-549, $ED_{50}=5.78\times10^{-2}$; MCF-7, $ED_{50}=1.08\times10^{-1}$, HT-29, $ED_{50}=2.48\times10^{-1}$ μg/ml), and was dissolved in 90% MeOH and then extracted with hexane to give a MeOH fraction (89 g), resin-like precipitates (12 g), and a hexane fraction (110 g). Screening of these fractions for cytotoxicity indicated that the main active constituents were in the MeOH fraction (A-549, $ED_{50}=3.95\times10^{-2}$; MCF-7, $ED_{50}=3.65\times10^{-2}$; HT-29, $ED_{50}=3.13\times10^{-2}$ μg/ml). This fraction (89 g) was chromatographed on a Si gel column, using hexane, hexane/$CHCl_3$, and $CHCl_3$/MeOH as the solvents, resulting in the collection of 49 fractions. These fractions were pooled as fractions A–O according to their tlc patterns. A cytotoxic fraction D (3.0 g, A-549, $ED_{50}=<10^{-2}$; MCF-7, $ED_{50}=<10^{-2}$; HT-29, $ED_{50}=<10^{-2}$ μg/ml) was further resolved on a Si gel column, eluted with a gradient of hexane/EtOAc, to yield **2** (641 mg), **3** (32.7 mg), and mixtures of **3** and **1**. The mixture was further chromatographed on a RP-8 Lobar column, using MeOH-H_2O (60:40) for elution, to yield pure **3** (5.5 mg) and **1** (3.0 mg).

Koelreuterin-1 [**1**].—White needles from hexane/EtOAc; mp 270–272°, uv λ max ($CHCl_3$) 261 (ε 23850), 309 (10219), 350 (7375) nm; ir ν max (film) 1759 (γ-lactone), 1628 (aromatic), 940 (methylenedioxy) cm^{-1}; hreims m/z $[M]^+$ 378.0749 (calcd for $C_{21}H_{14}O_7$, 378.0740); eims m/z $[M]^+$ 378 (100), 319 (5), 291 (8), 163 (8), 149 (10); ^{1}H- and ^{13}C-nmr data, see Table 1.

Austrobailignan-1 [**2**].—White needles from hexane/EtOAc; mp 182–184° [lit. (10) 183–185°]; $[\alpha]^{25}D$ $-119°$ (c=0.2, $CHCl_3$) [lit. (10) $-110°$, c=1.2, $CHCl_3$]; uv λ max ($CHCl_3$) 244 (ε 7418), 288 (4223) nm; ir ν max (film) 1770 γ-lactone), 1634 (aromatic), 940 (methylenedioxy) cm^{-1}; hreims m/z $[M]^+$ 382.1041 (calcd for $C_{21}H_{18}O_7$, 382.1053); eims m/z $[M]^+$ 382 (100), 337 (7), 297 (9), 230 (7), 185 (16), 165 (22); ^{1}H- and ^{13}C-nmr data, see Table 1.

Austrobailignan-2 [**3**].—White needles from hexane/EtOAc; mp 219–221° [lit. (10) 217–219°]; $[\alpha]^{25}D$ $+43°$ (c=0.2, $CHCl_3$) [lit. (10) $+88°$, c=2.5, $CHCl_3$]; uv λ max ($CHCl_3$) 244 (ε 7387), 289 (5096) nm; ir ν max (film) 1765 (γ-lactone), 1633 (aromatic), 930 (methylenedioxy) cm^{-1}; hreims m/z $[M]^+$ 382.1041 (calcd for $C_{21}H_{18}O_7$, 382.1053); eims m/z $[M]^+$ 382 (100), 337 (7), 323 (8), 310 (16), 297 (13), 213 (8), 185 (6), 165 (12); ^{1}H- and ^{13}C-nmr data, see Table 1.

SYNTHESIS OF **1** FROM **2** (9).—A mixture of **2** (38.2 mg) and DDQ (72.8 mg) in 4 ml of dry C_6H_6 was refluxed for 18 h. After cooling, the reaction mixture was evaporated *in vacuo* and chromatographed on a Si

TABLE 3. Inhibition of Tubulin Polymerization.

	IC_{50} (μM)	
	Turbidity assay	Sedimentation assay
Podophyllotoxin	0.6	1.0
1	5.0	20.0
2	0.8	1.0
3	40.0	45.0

gel column using hexane/EtOAc for elution, to give 14 mg of **1** (37%). White needles were obtained from hexane/EtOAc. Its mp, ^{1}H-nmr, eims, uv, and ir spectra were identical to those of **1** isolated from *K. henryi*.

CONVERSION OF **2** TO **3** (11).—A mixture of **2** (10 mg) and anhydrous NaOAc (40 mg) in 1 ml of absolute EtOH was refluxed for 18 h. After cooling, the reaction mixture was evaporated and partitioned between $CHCl_3/H_2O$. The $CHCl_3$ fraction was used for ^{1}H-nmr analysis (100% conversion). White needles were obtained from hexane/EtOAc. Its mp, ^{1}H-nmr, eims, uv, and ir spectra were identical to those of **3** isolated from *K. henryi*.

CYTOTOXICITY ASSAYS.—Cytotoxicity against human solid tumor cells was measured at the Purdue Cell Culture Laboratory, Purdue Cancer Center for the A-549 lung carcinoma, MCF-7 breast adenocarcinoma, HT-29 colon adenocarcinoma, A-431 epidermoid carcinoma, SK-VO-3 ovarian adenocarcinoma, A-498 renal carcinoma, PC-3 prostate adenocarcinoma, and SK-MELS melanoma, using a standard MTT method with adriamycin as a control substance (18,19).

TUBULIN POLYMERIZATION.—Microtubule protein (MTP) was prepared from bovine brains (20). Samples contained 1 mg/ml MTP plus 1 mM GTP and varying concentrations of the inhibitory compounds. The positive-control sample contained MTP and GTP whereas the negative control sample contained only MTP. Turbidity increases (21) were measured at 340 nm and the experiment was carried out for 30 min at 37° in a Molecular Devices THERMOmax Microplate Reader. Microtubules were sedimented by centrifugation at 100,000 g for 5 min following a 30 min incubation at 37°. The protein content of the supernatant (150 μl) was determined by the BCA method and the amount of sedimented protein was calculated relative to the positive and negative controls.

ACKNOWLEDGMENTS

We are grateful to the National Cancer Institute (R01 CA49632) for financial support of this research. The cytotoxicity tests were provided by the Cell Culture Laboratory, Purdue University Cancer Center, which was partially supported by the National Cancer Institute (P30 CA23168). We also express our appreciation to Mr. W.-l. Chu, Taiwan Forestry Research Institute, for collecting the plant material used in this project.

LITERATURE CITED

1. J.C. Willis, "A Dictionary of the Flowering Plants and Ferns," Eighth Edition, Cambridge University Press, Cambridge, UK, 1973, p. 619.
2. Y.-c. Liu, "Colored Illustrations of Important Trees in Taiwan," Taiwan Provincial Chung-Hsing University, Taiwan, 1970, p. 450.
3. V.Y. Vhirva, P.K. Kintya, and V.A. Sosnovskii, *Khim. Soed.*, **6**, 328 (1970).
4. R. Ahmad, I. Ahmad, and S.M. Osman, *Chem. Ind.*, 734 (1985).
5. D.S. Seigler and C.S. Butterfield, *Phytochemistry*, **15**, 842 (1976).
6. D.S. Seigler, *Phytochemistry*, **13**, 841 (1974).
7. K.L. Mikolajczak, C.R. Smith, Jr., and L.W. Tjarks, *Lipids*, **5**, 627 (1970).
8. M. Abou-Shoer, G.-E. Ma, X.-H. Li, N.M. Koonchanok, R.L. Geahlen, and C.-j. Chang, *J. Nat. Prod.*, **5**, 967 (1993).
9. H. Yamaguchi, M. Arimoto, M. Tanoguchi, and A. Numata, *Yakugaku Zasshi*, **101**, 485 (1981).
10. S.T. Murphy, E. Ritchie, and W.C. Taylor, *Aust. J. Chem.*, **28**, 81 (1975).
11. S.F. Fonseca, E.A. Ruveda, and J.D. McChesney, *Phytochemistry*, **19**, 1527 (1980).
12. M.M. Badawi, A.A. Seida, A.D. Kinghorn, G.A. Cordell, and N.R. Farnsworth, *J. Nat. Prod.*, **44**, 331 (1981).
13. J.L. Hartwell, A.W. Schrecker, and J.M. Johnson, *J. Am. Chem. Soc.*, **75**, 2138 (1953).
14. S.A. Beers, Y. Imakura, H.-J. Dai, D.-H. Li, Y.-C. Cheng, and K.-H. Lee, *J. Nat. Prod.*, **51**, 901 (1988).
15. I. Jardine, in: "Anticancer Agents Based on Natural Product Models." Ed. by J. Cassady and J. Douros, Academic Press, New York, 1980, p. 319.
16. J. Leiter, V. Downing, J.L. Hartwell, and M.J. Shear, *J. Natl. Cancer Inst.*, **10**, 1273 (1950).
17. J.D. Loike, C.F. Brewer, H. Sternlicht, W.J. Gensler, and S.B. Horwitz, *Cancer Res.*, **38**, 2688 (1978).
18. T. Mosmann, *J. Immunol. Methods*, **65**, 55 (1983).
19. M.C. Alley, D.A. Scudiero, A. Monks, M.L. Heersey, M.Z. Czerwinski, D.L. Fine, B.J. Abbot, Z.G. Mayo, R.H. Shoemaker, and M.R. Boyd, *Cancer Res.*, **48**, 589 (1988).
20. C.F. Asnes and L. Wilson, *Anal. Biochem.*, **98**, 64 (1979).
21. F. Gaskin, C.R. Cantor, and M.L. Shelansky, *J. Mol. Biol.*, **89**, 737 (1974).

Received 7 June 1994