Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 66 (2005) 811–815
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Cytotoxic lignans from *Larrea tridentata*

Joshua D. Lambert [a,*], Shengmin Sang [a], Ann Dougherty [b], Colby G. Caldwell [b], Ross O. Meyers [c], Robert T. Dorr [c], Barbara N. Timmermann [b]

[a] *Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, 164 Frelinghuysen Road, Piscataway, NJ 08854, USA*
[b] *Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ 85721, USA*
[c] *Arizona Cancer Center, University of Arizona, Tucson, AZ 85724, USA*

Received 9 March 2004; received in revised form 6 December 2004

Abstract

Six lignans, including the cyclolignan 3,4′-dihydroxy-3′,4′-dimethoxy-6,7′-cyclolignan, were isolated from the flowering tops of *Larrea tridentata*. Additionally the flavanone, (S)-4′,5-dihydroxy-7-methoxyflavanone, was isolated for the first time from *L. tridentata* or any member of the family Zygophyllaceae. All of the compounds were assessed for their growth inhibitory activity against human breast cancer, human colon cancer and human melanoma cell lines. The lignans had IC_{50} values of 5–60 μM with the linear butane-type lignans being the most potent, and it was found that colon cancer cells were the least sensitive cell type tested. The relative potency of linear butane type lignans against human breast cancer appears to correlate positively with the number of *O*-methyl groups present on the molecule.

Keywords: *Larrea tridentata*; Zygophyllaceae; Lignans; Flavanone; Cytotoxicity; Cancer; Creosote bush

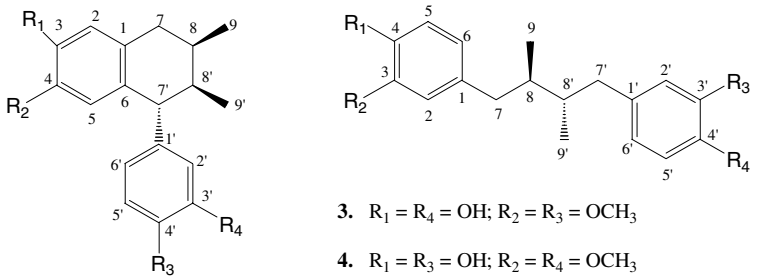
1. Introduction

Larrea tridentata (Moc. & Sess.) Cov. (Zygophyllaceae) has a long history of ethnobotanical use among native peoples in northern Mexico and the southwestern USA. Cancer, tuberculosis, menstrual pains, and diabetes treatment are among the indications listed for chaparral, as the plant is commonly known (Tyler, 1992). Others have previously shown the efficacy of nordihydroguaiaretic acid (NDGA), a lignan found at high levels in this plant, as an anticancer compound (Khan et al., 1993; Moody et al., 1998; Snyder et al., 1989). This compound inhibits a number of enzymes including lipoxygenase, phospholipase A_2, and NADH oxidase (Avis et al., 1996; Burk and Woods, 1963; Lanni and Becker, 1985). Additionally, NDGA has been shown to block the formation of actinic keratoses, a use for which it was previously approved by the Food and Drug Administration (Kulp-Shorten et al., 1993; Olsen et al., 1991). We and others have shown that a tetra-*O*-methyl derivative of NDGA (M4N) retains the anticancer activity of NDGA and inhibits tumor growth in vivo (Heller et al., 2001; Lambert et al., 2001). The present study was undertaken to isolate further lignans from *L. tridentata* and determine the anticancer activity, if any, of these compounds.

Here, we describe the isolation of the new cyclolignan, 3,4′-dihydroxy-3′,4-dimethoxy-6,7′-cyclolignan (**1**), as well as five known lignans and (S)-4′,5-dihydroxy-7-methoxyflavanone (**2**) which had not been previously isolated from any member of the Zygophyllaceae family (Fig. 1). All isolated compounds were assessed for cytotoxicity in several human tumor cell lines.

* Corresponding author. Tel.: +1 732 445 3400; fax: +1 732 445 0687.
E-mail address: joshua_lambert@hotmail.com (J.D. Lambert).

0031-9422/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2005.02.007



1. R_1=R_3= OH; R_2=R_4= OCH_3

7. R_1=R_3= OH; R_2= OCH_3; R_4= H

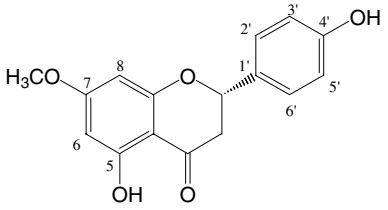
8. R_1=R_4= OCH_3; R_2=R_3= OH

3. R_1 = R_4 = OH; R_2 = R_3 = OCH_3

4. R_1 = R_3 = OH; R_2 = R_4 = OCH_3

5. R_1 = R_4 = OCH_3; R_2 = R_3 = OH

6. R_1 = R_2 = R_4 = OCH_3; R_3 = OH



2.

Fig. 1. Lignans and a flavanone isolated from *Larrea tridentata*.

2. Results and discussion

Compound **1** was isolated as a brown gum, $[\alpha]_D^{25} = -19.2^\circ$ ($CHCl_3$). Its molecular formula was determined as $C_{20}H_{24}O_4$ by HRESIMS. Its IR spectrum featured strong absorptions at 3442 and 3539 cm^{-1} due to hydroxyl groups. The ^1H and ^{13}C NMR spectroscopic data showed that **1** had two methyl groups (δ_H 0.86 *d*, J = 6.6 Hz and 0.88 *d*, J = 7.8 Hz; δ_C 15.4 and 16.4), two methoxyl groups (δ_H 3.66 *s* and 3.78 *s*; δ_C 55.9 and 55.9), one methylene group (δ_H 2.40 *dd*, J = 8.4, 16.8 Hz and 2.82 *dd*, J = 5.4, 16.8 Hz; δ_C 34.5), three methine signals (δ_H 1.89 *m*, 1.99 *m*, and 3.63 *d*, J = 6.0 Hz; δ_C 28.5, 40.9, and 51.1), and two sets of aromatic resonances (δ_H 6.30 *s*, 6.49 *dd*, J = 1.8, 7.8 Hz, 6.50 *brs*, 6.64 *s*, and 6.77 *d*, J = 7.8 Hz; δ_C, see Table 1). These signals corresponded to a molecule similar in structure to the di-*O*-methyl cyclolignan, isoguaiacin (**8**). HMBC and NOESY experiments were used to assign the *O*-methyl substitutions. One of the *O*-methyl substituent (δ_H 3.78) showed a three bond coupling with a carbon at δ_H 146.4. This carbon showed a two-bond coupling with H-2′ (δ_H 6.49) and a three bond coupling with H-5′ (δ_H 6.78). Thus, δ_C 146.4 was assigned to C-3′: this methoxyl group was located to position 3′. This assignment was further confirmed by the cross-peak between δ_H 3.78 and H-2′ δ_H 6.49) in the NOESY spectrum (Fig. 2). In the HMBC spectrum, the cross-peaks between δ_H 6.64 and C-7 (δ_C 34.5) and δ_H 6.30 and C-7′ (δ_C 51.1) indicated that δ_H 6.64 was assigned to H-2 and δ_H 6.30 to H-5. Therefore, the other *O*-methyl group was assigned to position 4 according to the cross-peak between δ_H 3.66 and H-5 in the NOESY spectrum (Fig. 2). Thus, the skeleton of compound **1** was confirmed.

The coupling constants of the two methylene protons (δ_H 2.40 *dd*, J = 8.4, 16.8 Hz and 2.82 *dd*, J = 5.4, 16.8 Hz) suggested the H-8 to be at one axial position. In the NOESY spectrum, H-6′ showed cross-peaks with H-8 and H-8′ (Fig. 2). According to the three-dimensional structure model, this indicated that H-8′ and the phenyl group were on the same side as H-8, indicating that both H-7″ and H-8′ were in the equatorial position. This stereochemistry was further confirmed by the chemical shifts of the two methyl groups (δ_H 0.86 and 0.88) which are comparable to that of stereochemically similar isoguaiacin (δ_H 0.86 and 0.84) but not to guaiacin (δ_H 0.84 and 1.85) which has a different stereochemistry (Wang et al., 2000). All of the signals in the ^1H and ^{13}C NMR spectra were unquestionably assigned on the basis of extensive two-dimensional NMR spectral studies (Table 1).

Six other known lignans and one known flavanone were also isolated. Three butane-type di-*O*-methylated lignans (**3**–**5**), one butane-type tri-*O*-methyl lignan (**6**) and the cyclolignan, 3′-demethoxyisoguaiacin (**7**) were identified. To our knowledge, the flavanone, (*S*)-4′,5-dihydroxy-7-methoxyflavanone (**2**), has not previously been isolated from any member of the Zygophyllaceae family.

Growth inhibition was assessed in human melanoma (ACC375), human breast cancer (MCF7) and human

Table 1
NMR spectroscopic data for 3,4-dihydroxy-3′,4-dimethoxy-6,7′-cyclolignan (**1**)

Position	δ_H	δ_C	HMBC	NOESY
1	–	129.3	–	
2	6.64 (*s*)	114.1	C1, C3, C4, C6	
3	–	143.8	–	
4	–	144.9	–	
5	6.30 (*s*)	112.6	C4	
6	–	129.2	–	
7α	2.82 (*dd*, *J* = 5.4, 16.8 Hz)	34.5	C8, C9	H8
7β	2.40 (*dd*, *J* = 8.4, 16.8 Hz)	34.5	C8, C9	H8
8	1.99 (*m*)	28.5	C7, C9	H8
9	0.88 (*d*, *J* = 7.8 Hz)	15.4	C7, C8, C8′	
1′	–	139.5	–	
2′	6.50 (*s*)	122.1	C3′, C4′, C6′	
3′	–	146.4	–	
4′	–	144.0	–	
5′	6.77 (*d*, *J* = 7.8 Hz)	113.7	C4, C6, C7′, C3	
6′	6.49 (*dd*, *J* = 1.8, 8.4 Hz)	111.4	C1′	
7′	3.63 (*d*, *J* = 6 Hz)	51.1	C1, C1′, C3′, C4, C4′, C6′	H8′
8′	1.89 (*m*)	40.9	C7, C7′, C9′	H7′
9′	0.86 (*d*, *J* = 6.6 Hz)	16.4	C7′, C8′, C8′	
OH	5.41 (*s*)	–	–	
OH	5.43 (*s*)	–	–	
OCH_3	3.66 (*s*)	55.90	C4	
OCH_3	3.78 (*s*)	55.92	C3′	

colon cancer (SW480) cell lines after 120 h using the sulforhodamine B assay (Skehan et al., 1990). Table 2 shows the IC_{50} values of compounds **1**–**7** in each of the cell lines tested. SW480 cells appeared to be approximately twofold less sensitive to all of the assayed compounds when compared to ACC375 or MCF7 cells. While all of the lignans showed IC_{50} values in the micromolar range, (S)-4′,5-dihydroxy-7-methoxyflavanone (**2**) was relatively inactive. Similarly, the cyclolignans (**1** and **7**) were significantly less potent against all three cell lines suggesting that free movement of the butyl chain, which is prevented in the cyclolignans, may be required to allow the molecules to assume the optimal conformation for cytotoxicity.

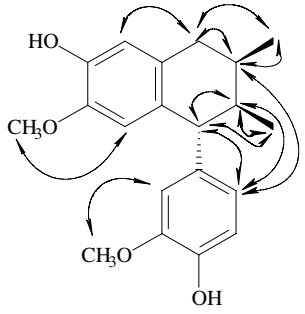


Fig. 2. Significant NOESY correlations and proposed stereochemistry of compound **1**.

Table 2
IC_{50} values of *L. tridentata* compounds **1**–**6** for growth inhibition of human melanoma (ACC375), breast (MCF7), and colon cancer (SW480) cell lines

Compounds	ACC375 (μM)	MCF7 (μM)	SW480 (μM)
1	50	25	50
2	>80	60	>80
3	7	10	30.2
4	13.7	13.7	28
5	10	12.1	31
6	7	5.3	55
7	22	9.6	55.1
NDGA	7.9	24	27

NDGA is included as a positive control for growth inhibition.

Finally, the number of *O*-methyl groups on butane-type lignans appears to be positively correlated with the potency of those compounds, at least against MCF7 human breast cancer cells. Previously, we have reported that M_4N, which has four *O*-methyl groups, inhibits MCF7 cells with an IC_{50} of 6 μM while NDGA, which has no *O*-methyl groups, has an IC_{50} of 24 μM against the same cells (Lambert, 2001; Lambert et al., 2001). Here, it is seen that the three di-*O*-methyl compounds (**3**–**5**) are approximately twofold more potent than NDGA but half as potent as compound **6**. Compound **6** has three *O*-methyl groups and a similar potency to M_4N. The reason for this increased potency with *O*-methylation may be due simply to increased lipophilicity which allows more compound to cross the plasma membrane of the cell. Alternatively, it may relate to the ability of the molecule to bind to a lipophilic target, such as the estrogen receptor (ER) which are expressed in MCF7 cells. This hypothesis is supported by the fact that the effect seems confined to the ER-positive MCF7 breast cancer cells. Previously, others have shown that

NDGA binds to the ER to elicit an estrogenic response (Sathyamoorthy et al., 1994). Perhaps methylation increases the affinity of the molecule for the ER, and may also change the compound's effect to that of an antiestrogen.

3. Experimental

3.1. General

Optical rotations were measured on a JASCO P1020 polarimeter. IR spectra were determined using a Thermo Nicolet Avatar 360 FT-IR spectrometer (Waltham, MA). UV spectra were determined using CH_3CN as a solvent and a Varian Cary 50 Conc UV spectrophotometer (Palo Alto, CA). ^1H and ^{13}C NMR spectra were recorded on a Bruker AMX-500 (Billerica, MA) or Bruker DRX-600 (500 or 600 MHz for ^1H and 125 or 150 MHz for ^{13}C) in $CDCl_3$. The chemical shifts are given in δ (ppm) values relative to that of the solvent ($CHCl_3$). HMBC spectra were acquired with $1/2J = 0.05$ *s*. Medium pressure liquid chromatography (MPLC) was performed using a Büchi 688 pump (Zurich, Switzerland) with a C_{18} column (40 mm × 490 mm) and a CH_3CN–H_2O mobile phase grandient (30–100% CH_3CN, flow rate = 20–30 mL/min). Semi-preparative high performance liquid chromatography (HPLC) was performed on a Varian 9012 chromatograph (Palo Alto, CA, USA) equipped with a Varian 9065 Polychrome diode array detector with a C_{18} reversed phase column (Reliasil C_{18}, 10 mm × 250 mm, pore size = 5 μm, Column Engineering, Ontario, CA, USA, flow = 5 mL/min). Compounds **1**, **3**–**5**, and **7** were eluted in CH_3CN–H_2O (1:1) containing 0.15% HCO_2H with t_R = 10.9, 15, 16.5, 17.5, and 11.9 min, respectively. Compound **2** was eluted in CH_3CN–H_2O (4:6) containing 0.15% HCO_2H (t_R = 17.3 min), and compound **6** was eluted in CH_3CN–H_2O (7:3) containing 0.15% HCO_2H (t_R = 17.8 min). Elution was monitored at λ_{max} = 280 nm.

3.2. Plant materials

Flowering tops of *L. tridentata* were collected in May, 2000 in Tucson, AZ, USA. This material was shade-dried and ground to sub-millimeter particles. A voucher specimen was deposited at the University of Arizona Herbarium (Lambert s.n., 08 April 2002, Tucson, AZ).

3.3. Extraction and isolation

Ground plant material (500 g) was extracted for 24 h at room temperature with Et_2O:toluene (1 L, 1:9). The solvent was evaporated in vacuo to yield a crude extract, (55 g) which was fractionated using silica-gel flash chromatography with acetone (0%, 2.5%, 5%, 7.5%, 10%, 12.5%, 15%, 20%, 30%, 40%, 50%, 60%, 75%, 100% (1 L each)) in hexane. This yielded 29 fractions (250–1000 mL) that were recombined based on thin layer chromatographic analysis. Fractions 14–18 were recombined and subjected to silica gel flash chromatography (Silica gel 60, 40–63 mesh, Scientific Adsorbents, Inc., Atlanta, GA, USA) with EtOAc:hexane (8:2, 7.5:2.5, 7:3, 6:4, 1:1) to yield 23 fractions. Fractions 11–13 were recombined and separated by MPLC to yield 35 fractions. These were further separated using HPLC with CH_3CN–H_2O mobile phase containing 0.15% HCO_2H to yield compounds **1**–**7** (4–20 mg). Compounds **1**, **3**–**5**, and **7** were eluted in CH_3CN–H_2O (1:1) containing 0.15% formic acid with t_R = 10.9, 15, 16.5, 17.5, and 11.9 min, respectively. Compound **2** was eluted in CH_3CN–H_2O (4:6) containing 0.15% HCO_2H (t_R = 17.3 min), and compound **6** was eluted in CH_3CN–H_2O (7:3) containing 0.15% HCO_2H (t_R = 17.8 min).

3.4. 3,4′-Dihydroxy-3′,4-dimethoxy-6,7′-cyclolignan (*1*)

Brown gum; $[\alpha]_D^{25} = -19.2°$ (c = 0.5 in $CHCl_3$); HRESIMS (negative) *m/z* 327.1911 (Calc. 327.1596 for $C_{20}H_{24}O_4$); UV (CH_3CN) λ_{max} = 213 and 280 nm; IR (neat) ν_{max} 3539, 3442 cm^{-1}; for ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$), HMBC, and NOESY analyses, see Table 1.

3.5. Cell lines and growth inhibition assay

Human colon cancer (SW480) and human breast cancer (MCF7) cell lines were maintained in log-phase growth in either PDRG (ACC375 or MCF7) or RPMI 1640 (SW480) medium supplemented with 5% calf serum, 100 U/mL penicillin/streptomycin, and 2 mM L-glutamine. For growth inhibitory assays, cells were plated in 96-well microtitre plates ($5–10 \times 10^3$ cells per well) and treated with test compounds (in DMSO) at a final concentration of 0–100 μM for 120 h at 37 °C (Leibovitz et al., 1976; Levenson and Jordan, 1997). Inhibition of growth was assessed using the sulfarhodamine B (SRB) assay (Skehan et al., 1990). Cells treated with 0.1% DMSO or NDGA served as a negative or positive control, respectively. Dose–response curves were prepared and the IC_{50} value for each compound was determined. Each compound was tested three times with six replicates per determination.

Acknowledgements

Supported in part by the ICBG "Bioactive Agents from Drylands Biodiversity of Latin America" grant 5UO1TW00316 from the National Institutes of Health

(NIH) and National Science Foundation (NSF) (to BNT); and the Arizona Disease Control Research Commission (ADCRC) grant 20009.

References

Avis, I.M., Jett, M., Boyle, T., Vos, M.D., Moody, T., Treston, A.M., Martinez, A., Mulshine, J.L., 1996. Growth control of lung cancer by interruption of 5-lipoxygenase-mediated growth factor signaling. J. Clin. Invest. 97, 806–813.

Burk, D., Woods, A., 1963. Hydrogen peroxide, catalase, glutathione peroxidase, quinones, nordihydroguaiaretic acid, and phosphopyridine nucleotides in relation to X-ray action on cancer cells. Radiat. Res. 3 (Suppl.), 212–246.

Heller, J.D., Kuo, J., Wu, T.C., Kast, W.M., Huang, R.C., 2001. Tetra-*O*-methyl nordihydroguaiaretic acid induces G2 arrest in mammalian cells and exhibits tumoricidal activity in vivo. Cancer Res. 61, 5499–5504.

Khan, M.A., Hoffbrand, A.V., Mehta, A., Wright, F., Tahami, F., Wickremasinghe, R.G., 1993. MK 886, an antagonist of leukotriene generation, inhibits DNA synthesis in a subset of acute myeloid leukaemia cells. Leuk. Res. 17, 759–762.

Kulp-Shorten, C., Konnikov, N., Callen, J.P., 1993. Comparative evaluation of the efficacy and safety of masoprocol and 5-fluorouracil cream for the treatment of actinic keratoses of the head and neck. J. Geriatr. Dermatol. 1, 161–168.

Lambert, J.D., 2001. Anticancer pharmacology of natural and semisynthetic lignans from *Larrea tridentata* (Moc. and Sess.) Cov. (Zygophyllaceae). Ph.D. Dissertation, Graduate Program in Pharmacology and Toxicology, University of Arizona, Tucson.

Lambert, J.D., Meyers, R.O., Timmermann, B.N., Dorr, R.T., 2001. Tetra-*O*-methylnordihydroguaiaretic acid inhibits melanoma in vivo. Cancer Lett. 171, 47–56.

Lanni, C., Becker, E.L., 1985. Inhibition of neutrophil phospholipase A2 by *p*-bromophenylacyl bromide, nordihydroguaiaretic acid, 5,8,11,14-eicosatetraynoic acid and quercetin. Int. Arch. Aller. Appl. Immunol. 76, 214–217.

Leibovitz, A., Stinson, J.C., McCombs III, W.B., McCoy, C.E., Mazur, K.C., Mabry, N.D., 1976. Classification of human colorectal adenocarcinoma cell lines. Cancer Res. 36, 4562–4569.

Levenson, A.S., Jordan, V.C., 1997. MCF-7: the first hormone-responsive breast cancer cell line. Cancer Res. 57, 3071–3078.

Moody, T.W., Leyton, J., Martinez, A., Hong, S., Malkinson, A., Mulshine, J.L., 1998. Lipoxygenase inhibitors prevent lung carcinogenesis and inhibit non-small cell lung cancer growth. Exp. Lung Res. 24, 617–628.

Olsen, E.A., Abernethy, M.L., Kulp-Shorten, C., Callen, J.P., Glazer, S.D., Huntley, A., McCray, M., Monroe, A.B., Tschen, E., Wolf Jr., J.E., 1991. A double-blind, vehicle-controlled study evaluating masoprocol cream in the treatment of actinic keratoses on the head and neck. J. Am. Acad. Dermatol. 24, 738–743.

Sathyamoorthy, N., Wang, T.T., Phang, J.M., 1994. Stimulation of pS2 expression by diet-derived compounds. Cancer Res. 54, 957–961.

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S., Boyd, M.R., 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Inst. 82, 1107–1112.

Snyder, D.S., Castro, R., Desforges, J.F., 1989. Antiproliferative effects of lipoxygenase inhibitors on malignant human hematopoietic cell lines. Exp. Hematol. 17, 6–9.

Tyler, V.E., 1992. The Honest Herbal, a Sensible Guide to the Use of Herbs and Related Remedies. Pharmaceutical Press, New York, pp. 87–88.

Wang, B.G., Hong, X., Li, L., Zhou, J., Hao, X.J., 2000. Chemical constituents of two chinese magnoliaceae plants, *Tsoongiodendron odorum* and *Manglietiastrum sinicum*, and their inhibition of platelet aggregation. Planta Medica 66, 511–515.