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Phytochemistry 66 (2005) 811–815
PHYTOCHEMISTRY
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Cytotoxic lignans from *Larrea tridentata*

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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.

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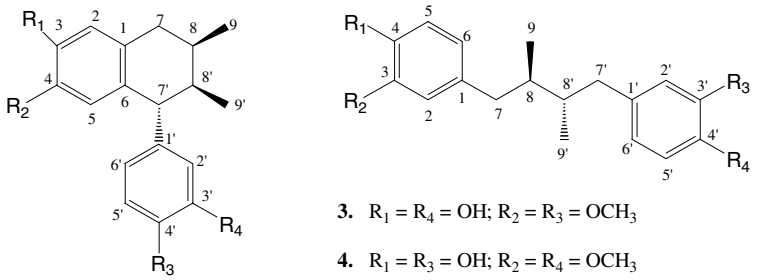
doi:10.1016/j.phytochem.2005.02.007



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

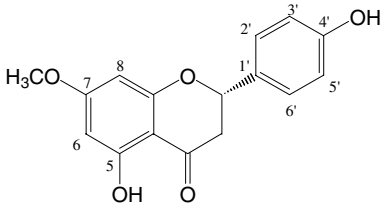
8. R_1=R_4= OCH_3; R_2=R_3= OH

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