Antitumor Activity of *Bursera schlechtendalii* (Burseraceae): Isolation and Structure Determination of Two New Lignans

P. B. McDONIEL and J. R. COLE▲

Abstract □ *Bursera schlechtendalii* (Burseraceae) has shown antitumor activity against the 9KB (adenocarcinoma of nasal pharynx) test system. Two new lignans isolated from the biologically active plant fraction were identified as (−)-*trans*-2-(3″,4″,5″-trimethoxybenzyl)-3-(3′,4′-methylenedioxybenzyl)butyrolactone and (−)-*trans* - 2 - (3″,4″ - dimethoxybenzyl) - 3 - (3′,4′ - methylenedioxybenzyl)butyrolactone. In addition, the triterpene α-amyrin was isolated from the active fraction.

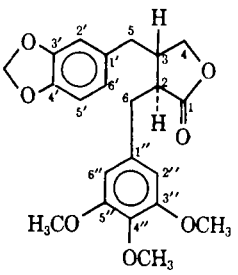
Keyphrases □ Lignans—isolated from *Bursera schlechtendalii*, structure determination □ *Bursera schlechtendalii* (Burseraceae)—isolation and structure determination of two new lignans □ Antitumor activity—isolation and structure determination of two lignans from *Bursera schlechtendalii* □ α-Amyrin—isolated from *Bursera schlechtendalii*

As a result of continuous screening of Southwestern plants for potential antitumor activity, it was found that the chloroform extract of the Mexican plant, *Bursera schlechtendalii* (Burseraceae)[1] demonstrated antitumor activity in the 9KB (adenocarcinoma of nasal pharynx) test system of the Cancer Chemotherapy National Service Center at 1 mg./ml. Activity is defined as $ED_{50} \leq 10$ mg./ml. for plant extracts (1).

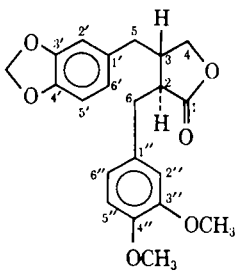
By utilizing an elution chromatography system with an alumina column, it was possible to separate the plant extract into essentially two portions. The first contained α-amyrin, and the second contained a mixture of lignans. The lignans were subsequently isolated by utilizing a silica gel G dry column (2). They were identified as (−) - *trans* - 2 - (3″,4″,5″ - trimethoxybenzyl)-3-(3′,4′-methylenedioxybenzyl)butyrolactone (I) and (−)-*trans*-2-(3″,4″-dimethoxybenzyl)-3-(3′,4′-methylenedioxybenzyl)butyrolactone (II).

DISCUSSION

Compound I, $[\alpha]^{23}_{546\,nm.}$ −29.83° ($CHCl_3$, c 3.0%), has $\lambda_{max}^{CHCl_3}$ 286 and 246 nm. (log ε 3.52 and 3.76) and $\nu_{max}^{CHCl_3}$ 1770 (γ-lactone) and 1582 (aromatic) cm.$^{-1}$. Elemental analyses (C, H, and —OCH_3) along with mass spectrometry, which suggested a molecular ion at *m/e* 400, support the empirical formula $C_{22}H_{24}O_7$. The NMR spectrum was determined in $CDCl_3$ solution (15%) at 100 mc.; it indicated the presence of three methoxyl groups (τ 6.23), a methylenedioxy (τ 4.18), four benzylic protons (τ 7.5), a methylene attached to the lactone oxygen (τ 5.8–6.0), five aromatic protons (τ 3.3–3.8), and two *trans*-protons at the C-2 and C-3 positions (τ 7.12 and 7.20, doublet).



I

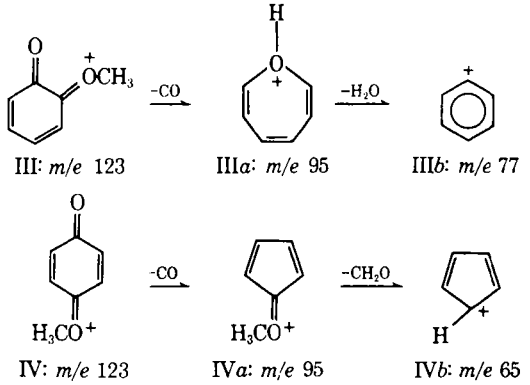


II

By opening the methylenedioxy ring in I and methylating the resulting *ortho*-hydroxy compound with diazomethane, (±)-*trans*-2 - (3″,4″,5″ - trimethoxybenzyl) - 3 - (3′,4′ - dimethoxybenzyl)-butyrolactone[2] was formed. The methylenedioxy ring was opened in a sealed tube at 175° after 7 hr. in the presence of potassium hydroxide and methanol, using a method established by Keimatsu and Ishiguro (4) with a related lignan. Comparative TLC using silica gel G (three systems), mass spectra, NMR spectra, and IR spectra of the authentic sample[2] and the sample synthesized from Compound I were identical. The mass spectra of the samples showed molecular ions at *m/e* 416 and strong tropylium ions at *m/e* 151 and 181 derived from the 3′,4′-dimethoxy- and 3″,4″,5″-trimethoxybenzyl units, respectively (5). The *m/e* 181 ion was the base peak in both spectra and was only slightly more intense than the *m/e* 151 ion, whereas the parent ion was very weak. The NMR spectra of both samples differed from Compound I by the absence of the methylenedioxy group (τ 4.1) and addition of two methoxy groups (τ 6.18). The mass spectrum of Compound I revealed a strong tropylium ion, *m/e* 135, derived from the 3′,4′-methylenedioxybenzyl unit; the *m/e* 181 ion was also the base peak in the mass spectrum of Compound I (5).

Compound II, $[\alpha]^{23}_{546\,nm.}$ −45.26° ($CHCl_3$, c 3.8%), has $\lambda_{max}^{CHCl_3}$ 286 and 246 nm. (log ε 3.78 and 3.76) and $\nu_{max}^{CHCl_3}$ 1770 (γ-lactone), 1600, and 1582 (aromatic) cm.$^{-1}$. Mass spectrometry, which suggested a molecular ion at *m/e* 370, and elemental analyses support the molecular formula $C_{21}H_{22}O_6$. The NMR spectrum, determined in $CDCl_3$ solution (15%) at 100 mc., indicated the presence of two methoxy groups (τ 6.18), a methylenedioxy (τ 4.12), four benzylic protons (τ 7.5), a methylene adjacent to the lactone oxygen (τ 5.8–6.0), six aromatic protons (τ 3.2–3.6), and two *trans*-protons at the C-2 and C-3 positions (τ 7.06 and 7.12, doublet).

The structure of Compound II was determined by showing that it differed from Compound I in that II lacked the 5″-methoxy. The mass spectrum of II revealed a molecular weight 30 mass units less



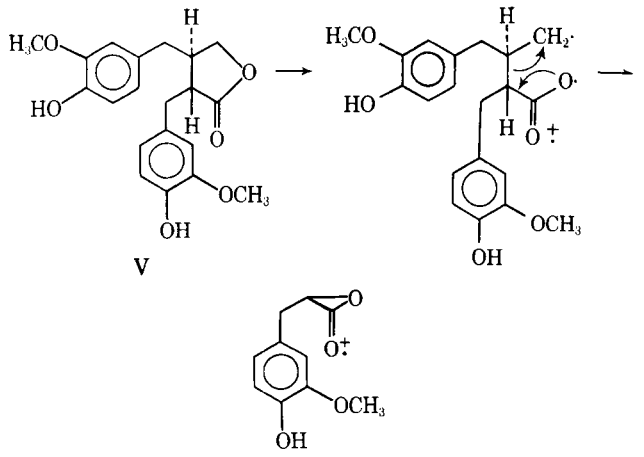
III: *m/e* 123 — IIIa: *m/e* 95 — IIIb: *m/e* 77

IV: *m/e* 123 — IVa: *m/e* 95 — IVb: *m/e* 65

Scheme I

[1] Identification confirmed by Robert J. Barr, College of Pharmacy, and Dr. Charles Mason, Botany Department, University of Arizona, Tucson. A reference specimen was also deposited in the University of Arizona Herbarium. The sample utilized for this study, consisting of only the stems and leaves, was collected near Puebla, Mexico.

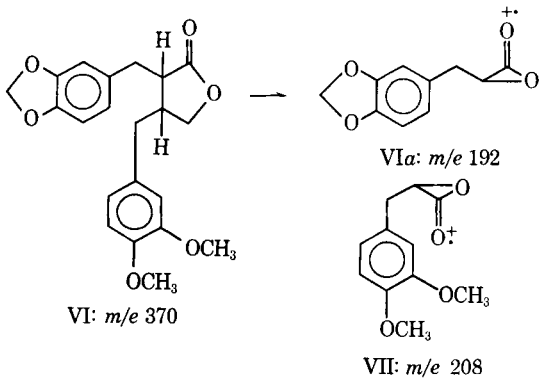
[2] A sample of this compound was provided by Dr. Harold MacLean, Department of Forestry and Rural Development, Vancouver, Canada (3).



V

Va

Scheme II



VI: *m/e* 370

VIa: *m/e* 192

VII: *m/e* 208

Scheme III

than I. A methoxy determination supported this difference, as did the NMR spectrum, which indicated three less methoxy protons and an additional aromatic proton. The base peak in the mass spectrum of Compound II was *m/e* 151, indicative of a dimethoxybenzyl unit, whereas the base peak in Compound I was *m/e* 181, indicative of a trimethoxybenzyl unit (5). The *m/e* 151 ion in II was only slightly more intense than the *m/e* 135 ion derived from the methylenedioxybenzyl unit.

Mass spectral evidence was utilized to establish that the methoxy groups in II were *ortho* by comparing the relative intensities of fragments *m/e* 123, 95, and 77 in II with the same fragments in *o*-, *m*-, and *p*-dimethoxybenzenes (Scheme I). Both the *o*- and *p*-dimethoxybenzenes lose a methyl radical to give stable and, hence, prominent quinoidal fragments, *m/e* 123. The *m*-isomer gives an ion of negligible abundance at *m/e* 123. The *o*- and *p*-quinoidal fragments, *m/e* 123 (III and IV, respectively), in turn lose carbon monoxide to give different *m/e* 95 fragments. The *m/e* 95 fragment derived from *o*-dimethoxybenzene (III*a*) subsequently loses water to give an *m/e* 77 fragment (III*b*). The *m/e* 95 fragment from *p*-dimethoxybenzene (IV*a*) loses a CH_2O group to give an *m/e* 65 fragment (IV*b*) (6). In the mass spectrum of II, the abundance of the ions at *m/e* 123, 95, and 77 is in the same proportion as those recorded for *o*-dimethoxybenzene.

The assignment of the methoxy groups in the 3″,4″-*ortho*-position is supported by NMR evidence. Identical coupling constants of the 5″- and 6″-protons and the presence of an isolated 2″-proton leave no doubt that the methoxy groups are *ortho* and occupy the 3″- and 4″-positions.

The orientation of the benzylic units relative to the lactone was established by considering the substituted 3-phenylpropene ion (V*a*), which was shown to have arisen in the mass spectrum of (−)-matairesinol (V) (Scheme II) (5).

The benzylic units could have been oriented as shown in II or as shown in VI (Scheme III). If the benzylic units were oriented as in VI, the resulting 3-phenylpropene moiety would be *m/e* 192 (VI*a*); however, for the benzylic units to be oriented as in II, the analogous 3-phenylpropene moiety would reside with the 3′,4′-dimethoxy unit, *m/e* 208 (VII). The mass spectrum of II suggested an ion at *m/e* 208 (7%) and only a relatively insignificant ion at *m/e* 192, which indicate that the benzylic units are oriented as in the structure proposed for Compound II. The mass spectral data for Compound VI were reported, and it is significant to note that an *m/e* 192 ion was reported and an *m/e* 208 ion was not (7).

To test the validity of using the 3-phenylpropene moiety to determine the orientation of the benzylic units, Compound II was deuterated alpha to the carbonyl carbon. The mass spectrum of the deuterated compound showed that the intensity of the *m/e* 208 fragment was decreased to 1–2%, the *m/e* 209 fragment was increased from 0 to 6%, and the *m/e* 192 fragment was unaffected. The molecular ion shifted from *m/e* 370 to 371, indicating that only one proton was replaced by deuterium. This was supported by the NMR spectrum of the deuterated compound where the integration of the doublet in the 7.06–7.12 region of II decreased from two protons to slightly more than one proton. The authors are, therefore, confident that the 3-phenylpropene moiety can be used in determining the orientation of the benzylic units in the butyrolactone series of lignans.

It was not surprising to find α-amyrin in this species of Bursera, since it had been reported (8) in other Bursera species. The identification was made on the basis of mixed melting point; superimposable IR, NMR, and mass spectra; preparation of the acetate derivative; and comparison of R_f values with an authentic specimen utilizing TLC, silica gel G, 0.3-mm. plates. The solvent systems employed were dichloromethane–benzene–ethyl acetate (3:6:1), dichloromethane–ether (30:1), and chloroform–benzene–acetone (5:6:1).

EXPERIMENTAL[3]

Materials—Silica gel G (Merck) was utilized in the dry column and TLC.

Extraction—The dry stems and leaves of *B. schlechtendalii* (Burseraceae) were ground to a coarse powder in a Wiley mill. Fifteen kilograms of the dried powder was extracted with chloroform [22.8 l. (6 gal.)] in a Lloyd extractor, yielding 1.24 kg. of crude extract.

Isolation—One hundred grams of this crude extract was dissolved in chloroform, absorbed over 450 g. alumina (grade III) in hexane, and eluted with hexane exhaustively. The eluate was discarded. The column was then eluted with hexane containing increasing concentrations of benzene (9:1, 8:2, ... until 3:7). The elution was then continued with dichloromethane as eluant until the eluate gave no more solid material.

α-Amyrin—The hexane–benzene eluate (3:7), after removal of the solvent, yielded 3.1 g. of solid material. This material was treated with chloroform; the chloroform-soluble fraction, on evaporation of the solvent, yielded crude α-amyrin. The α-amyrin was crystallized from ethanol and had a melting point of 183°. Identity with an authentic sample was established by mixed melting point; TLC, IR, and mass spectra; and preparation of the acetate derivative, m.p. 226° [lit. (9) m.p. 225–226°].

Lignans I and II—The dichloromethane eluate, after evaporation of the solvent, yielded 5.5 g. of lignan mixture (I and II). The two lignans were separated in a pure form, employing the silica gel G dry column technique (2) and dichloromethane–benzene–ethyl acetate (3:6:1) as eluant.

Lignan I—This was identified as (−)-*trans*-2-(3″,4″,5″-trimethoxybenzyl) - 3 - (3′,4′ - methylenedioxybenzyl)butyrolactone; $[\alpha]_{546\ nm.}^{23}$ −29.83° ($CHCl_3$, c 3.0%); $\lambda_{max}^{CHCl_3}$: 286 and 246 nm. (log ε 3.52 and 3.76); $\nu_{max}^{CHCl_3}$: 1770 (γ-lactone), 1600, 1582, 1450 (aromatic), 3010, 1460, 1340, 1250, 1140, and 1020 (CH_3O and CH_2O) cm.$^{-1}$; NMR: 6.23 (9H, CH_3O), 4.18 (2H, methylenedioxy), 7.5 (4H, benzylic), 5.8–6.0 (2H, CH_2 adjacent to the lactone oxygen), a doublet at 7.12 and 7.20 (*trans*-2H, C-2 and C-3), and 3.3–3.8 (5H,

[3] Specific rotations were measured on a Zeiss OLD 4 polarimeter using a 1-dm. cell. UV spectra were obtained using a Beckman DB-G spectrophotometer. IR spectra were obtained on a Perkin-Elmer Infracord model No. 137. Elemental analyses (C, H, and —OCH_3) were performed by Huffman Laboratories, Wheatridge, Colo. Mass spectrometry was done on the Hitachi Perkin-Elmer RMU-6E mass spectrophotometer. NMR spectra were run on the HA 100 manufactured by Varian Associates; tetramethylsilane was used as the internal standard.

aromatic); mass spectroscopy: fragments at *m/e* 385, 265, 238, 219, 181 (base peak), and 135.

Anal.—Calc. for $C_{22}H_{24}O_7$: C, 66.00; H, 6.00; mol. wt. 400. Found: C, 66.85; H, 6.25; CH_3O, 22.02; *m/e* 400.

Lignan II—This was identified as (−)-*trans*-2-(3″,4″-dimethoxybenzyl)-3-(3′,4′-methylenedioxybenzyl)butyrolactone; $[\alpha]_{546\ nm.}^{23}$ −45.26° ($CHCl_3$, c 3.8%); $\lambda_{max}^{CHCl_3}$: 286 and 246 nm. (log ε 3.78 and 3.76); $\nu_{max}^{CHCl_3}$: 1770 (γ-lactone), 1600, 1582, 1450 (aromatic), 3010, 1460, 1340, 1250, 1140, and 1020 (CH_3O and CH_2O) cm.$^{-1}$; NMR: 6.18 (6H, CH_3O), 4.12 (2H, methylenedioxy), 7.5 (4H, benzylic), 5.8–6.0 (2H, CH_2 adjacent to the lactone oxygen), a doublet at 7.06–7.12 (*trans*-2H, C-2 and C-3), and 3.2–3.6 (6H, aromatic); mass spectroscopy: fragments at *m/e* 235, 219, 208, 151 (base peak), 135, 123, 95, and 77.

Anal.—Calc. for $C_{21}H_{22}O_6$: C, 68.10, H, 5.94; mol. wt. 370. Found: C, 68.15; H, 6.07; CH_3O, 16.36; *m/e* 370.

Deuterium Exchange of Compound II—A small piece of sodium, about the size of a pinhead, was placed cautiously into 1 ml. of D_2O; 70 mg. of Compound II was dissolved in a minimal amount of tetrahydrofuran and this was added to the sodium–D_2O reaction mixture. Additional tetrahydrofuran was added until the reaction mixture was homogeneous. The mixture was allowed to stand overnight at room temperature; excess D_2O was added to the reaction mixture, followed by extraction with chloroform. Drying of the chloroform extract over anhydrous magnesium sulfate and evaporation yielded 50 mg. of II deuterated alpha to the carbonyl carbon.

Preparation of (±)-*trans*-2-(3″,4″,5″-Trimethoxybenzyl)-3-(3′,4′-dimethoxybenzyl)butyrolactone from I—Compound I (100 mg.) was placed in a Pyrex tube, sealed at one end (8 mm. in diameter), along with 0.8 ml. of methanol and 200 mg. of potassium hydroxide. The tube was sealed and placed in an oil bath maintained at 175° for 7 hr. Upon completion of heating, the tube was allowed to cool and was opened. After adding excess water, the reaction mixture was extracted with three 5-ml. portions of chloroform, and the chloroform extract was discarded. The aqueous layer was acidified with 5% aqueous hydrochloric acid and again extracted repeatedly with 5-ml. portions of chloroform. The combined chloroform extracts were dried over anhydrous magnesium sulfate, and the solvent was removed under vacuum (4). The residue was taken up in methanol and methylated with diazomethane prepared from *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (1 g.) and 40% aqueous potassium hydroxide (5 ml.) covered with ether (19.7 ml.)[4]. The product of this reaction was applied to TLC along with the authentic sample; the portion of the reaction product that corresponded to the authentic sample was purified by preparative silica gel G (0.5 mm.) TLC, yielding 30 mg. of product. The solvent system used was dichloromethane–benzene–ethyl acetate (3:6:1).

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▲ To whom inquiries should be directed.

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Photochemical Studies of Marijuana (Cannabis) Constituents

WILLIAM H. ALLWARDT*, PHILIP A. BABCOCK†, ALVIN B. SEGELMAN†, and JOHN M. CROSS*▲

Abstract □ The marijuana (Cannabis) constituents, cannabidiol, (−)-Δ^9-*trans*-tetrahydrocannabinol, and (−)-Δ^8-*trans*-tetrahydrocannabinol were found to be photoreactive. The only interconversion of these cannabinoids detected by GLC, however, was the conversion of cannabidiol to (−)-Δ^9-*trans*-tetrahydrocannabinol. From a photoreaction mixture obtained by the irradiation of cannabidiol, a sample of (−)-Δ^9-*trans*-tetrahydrocannabinol was isolated and identified by GLC, optical rotation, NMR, and mass spectrometry. A yield of 16% was obtained. The activating energy for the conversion appears to be in the 235–285-nm. wavelength area.

Keyphrases □ Marijuana constituents—photochemical study □ Cannabis constituents—photochemical study □ Cannabidiol—photoreactions □ Tetrahydrocannabinols—photoreactions

Although a number of reports have dealt with the photoreactivity of various cannabinoid substances, the first definitive work in this area was done by Shani and Mechoulam (1, 2). Those authors showed clearly that cannabidiolic acid undergoes an intramolecular photooxidative cyclization when irradiated with UV light in the presence of oxygen. These authors also observed the photoreactivity of cannabidiol in the absence of oxygen (1). In the latter study, various transformation products, including (−)-Δ^9-*trans*-tetrahydrocannabinol, were shown to form when solutions of cannabidiol in different solvents were exposed to UV radiation for rather