Cytotoxic Agents from *Bursera morelensis* (Burseraceae): Deoxypodophyllotoxin and a New Lignan, 5′-Desmethoxydeoxypodophyllotoxin

S. D. JOLAD, R. M. WIEDHOPF, and J. R. COLE ˣ

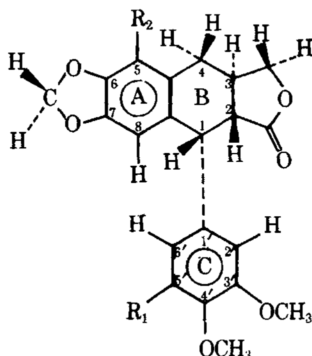
Abstract □ The isolation and identification of deoxypodophyllotoxin and a new lignan, 5′-desmethoxydeoxypodophyllotoxin, from the dried exudate of *Bursera morelensis* (Burseraceae) are reported. Deoxypodophyllotoxin showed high activity in the KB and PS test systems; the new lignan, although highly active against the KB test system, demonstrated only marginal activity against the PS test system. A structure is suggested for the new lignan, which was named morelensin.

Keyphrases □ Deoxypodophyllotoxin—and 5′-desmethoxy derivative, isolated from dried exudate of *Bursera morelensis*, cytotoxic activity evaluated □ *Bursera morelensis*—deoxypodophyllotoxin and 5′-desmethoxy derivative isolated from dried exudate, cytotoxic activity evaluated □ Cytotoxic activity—deoxypodophyllotoxin and 5′-desmethoxy derivative evaluated

During a continuing search for plants having tumor-inhibitory constituents, the ethanol extract of the dried exudate of the Mexican plant *Bursera morelensis* (Burseraceae[1]) demonstrated biological activity against two test systems, the P-388 lymphocytic leukemia (PS) and the human epidermoid carcinoma of the nasopharynx[2] (KB). The activity against these two test systems was due to two constituents, deoxypodophyllotoxin (I) and a new lignan, 5′-desmethoxydeoxypodophyllotoxin (II), which was named morelensin.

RESULTS AND DISCUSSION

Exhaustive extraction of the dried exudate of *B. morelensis* with ethanol, followed by partition of the ethanol extract between chloroform and water, yielded a chloroform-soluble syrup. The syrup was separated into methanol-soluble and methanol-insoluble fractions. The methanol-insoluble fraction was dissolved in ether, and precipitation with isopropyl ether yielded a gum. Extraction of the gum with ether afforded almost a single-component residue. On crystallization, this residue yielded a known lignan, I, mp 167°. Compound I [$C_{22}H_{22}O_7$, *m/e* 398 (M^+)] was identified by its IR, NMR, and mass spectral properties and by direct comparison with an authentic specimen.



I: $R_1 = OCH_3, R_2 = H$ III: $R_1 = H, R_2 = OCH_3$
II: $R_1 = R_2 = H$

The ether-soluble fraction was essentially a mixture of two components. Separation of these two components by silica gel column chromatography and subsequent crystallization yielded, in addition to I, a new lignan, II, mp 181°. This new compound was 1-(3′,4′-dimethoxyphenyl)-3-hydroxymethyl-6,7-methylenedioxy-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid lactone.

The molecular formula of II ($C_{21}H_{20}O_6$) was established by carbon and hydrogen analysis and mass spectrometry. The intense molecular ion peak (*m/e* 368) in the mass spectrum of II immediately suggested that the new lignan was desmethoxy-I. Biogenetically, the 5′-desmethoxy compound (*i.e.*, Structure II) is most favorable. Support of this hypothesis was found in the spectra of this new lignan. The IR spectrum indicated the absence of hydroxyl groups and the presence of methoxy (2840 cm^{-1}), methylenedioxy (2780 cm^{-1}), and γ-lactone (1775 cm^{-1}) groups. The NMR spectrum supported these findings, exhibiting signals for two methoxy groups (δ 3.79 and 3.86, both three-proton singlets) and one methelenedioxy group (δ 5.90, two-proton singlet). A comparison of the NMR signals contributed by the tetrahydronaphthalene-γ-lactone moiety of Compounds I and II indicated that the A, B, and lactone rings of both compounds were identical.

The proposed substitution pattern in ring C of II is identical to the pattern of 5′-desmethoxy-β-peltatin-A methyl ether (III), previously isolated from *B. fagaroides* (Burseraceae) (1). Indeed, a comparison of the aromatic region of the NMR spectra of II and III (subtracting A-ring signals) showed nearly identical signals for C-ring protons. This proposed C-ring substitution in II was supported by its mass spectrum. The significant peak at *m/e* 77 can be visualized as having arisen from the dimethoxybenzene ion (*m/e* 138) *via* the protonated oxepin ion (*m/e* 95) followed by the expulsion of a molecule of water. Among the three isomeric dimethoxybenzenes, only the *o*-isomer displays a mass spectrum with this fragmentation pattern (2). Morelensin (II) was optically active, $[\alpha]_D^{24}$ −125°, indicating one A-type isomer (*cis*, 1/2; *trans*, 2/3). Since the absolute configuration of III was established previously by X-ray crystallography (3), the stereochemistry of II was assumed to be identical to that of III.

Compound I demonstrated activities of 194 (one cure) and 161% test/control (T/C) at 12.50 and 6.25 mg/kg, respectively. Compound II demonstrated activity of 127% test/control (T/C) at 50 mg/kg. Activity in the PS test system is defined as an increase in the survival of treated animals over that of control animals resulting in a $T/C \geq 125\%$ (4).

In the KB test system, I and II demonstrated activities of 2.48×10^{-4} and 3.99×10^{-4} μg/ml, respectively. Activity in the KB test system is defined as $ED_{50} \leq 20$ μg/ml (5).

EXPERIMENTAL[3]

The dried exudate of *B. morelensis*, collected in Mexico during October 1974, was stored at −10° prior to extraction.

The exudate (0.68 kg) was extracted exhaustively with 95% ethanol. The air-dried chloroform-soluble fraction (syrup) was dried further as much as possible under vacuum to a total of 80 g and then dissolved in a minimum amount of methanol. The solution was left in a freezer for 2 days, and the resulting residue was separated by decanting the supernate. The residue was then dissolved in a minimum amount of ether, and isopropyl ether was added until the solution was slightly turbid. The mixture was left in a freezer overnight, and the gummy residue that separated was removed by decantation. Trituration of the residue with ether followed by stirring with a magnetic stirrer gave a colorless residue, which was filtered. The residue (2.13 g), which on TLC showed essentially

[1] Identification was confirmed by Dr. Robert E. Purdue, Medicinal Plant Resources Laboratory, Plant Genetics and Germ Plasma Institute, Beltsville, Md. A reference specimen was deposited in that herbarium.

[2] Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Md.

[3] Carbon and hydrogen analyses were carried out by Chemalytics, Inc., Tempe, Ariz. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra were run on a Beckman IR-33 instrument. NMR and mass spectra were run using a Varian T-60 spectrometer and Hewlett-Packard model 5930 spectrometer, respectively.

a single spot corresponding to I, was further purified by crystallization.

Solvent was removed under vacuum from the ether-soluble fraction (13 g), which on TLC showed two major spots corresponding to I and II. It was then subjected to silica gel 60 (500 g) column chromatography. Elution with dichloromethane with increasing concentration of ethyl acetate yielded fractions containing pure I and pure II, which were further purified by crystallization.

Deoxypodophyllotoxin (I)—This compound was obtained as colorless prisms, mp 167° (ether–dichloromethane). It was identical in all respects with the authentic specimen.

Anal.—Calc. for $C_{22}H_{22}O_7$: C, 66.32; H, 5.57; mol. wt. 398. Found: C, 66.49; H, 5.68; *m/e* 398 (M^+).

5′-Desmethoxydeoxypodophyllotoxin (II)—This compound was obtained as colorless needles, mp 181° (ether–dichloromethane). The IR ($CHCl_3$: 3000, 2900, 2840, 2780, 1775, and 930 cm^{-1}), NMR [$CDCl_3$: δ 2.9 (3H, m), 3.79 (3H, s), 3.86 (3H, s), 3.9 (2H, m), 4.5 (2H, m), 5.90 (2H, s), 6.37 (1H, dd, J = 8 and 2 Hz), 6.49 (1H, s), 6.66 (1H, s), 6.67 (1H, d, J = 8 Hz), and 6.90 (1H, d, J = 2 Hz)], and mass [*m/e* 368 (M^+, base), 353, 340, 338, 337, 323, 309, 308, 253, 230, 212, 185, 151, 138, 95, and 77] spectra were in accord with Structure II. This compound was optically active, $[\alpha]_D^{24}$ − 125° (c 0.014 in chloroform).

Anal.—Calc. for $C_{21}H_{20}O_6$: C, 68.47; H, 5.77; mol. wt. 368. Found: C, 68.47; H, 5.47; *m/e* 368 (M^+).

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[x] To whom inquiries should be directed.

Modified Electron-Capture GLC Assay for Salsolinol in Brain Tissue

PATRICK J. O'NEILL [*] and RALF G. RAHWAN [x]

Abstract □ A modified assay for a neuroamine-derived tetrahydroisoquinoline, salsolinol, is presented. It combines the ease and rapidity of solvent extraction from brain tissue with the sensitivity of electron-capture GLC. Detection of salsolinol (1,2,3,4-tetrahydro-1-methyl-6,7-isoquinolinediol), the alkaloid derived from condensation of dopamine and acetaldehyde, at levels of 5–10 ng/g of brain tissue is possible. The advantages afforded by the modifications are discussed in relation to existing procedures.

Keyphrases □ Salsolinol—electron-capture GLC analysis, mouse brain tissue □ GLC, electron capture—analysis, salsolinol in mouse brain tissue □ Tetrahydroisoquinolines, substituted—salsolinol, electron-capture GLC analysis in mouse brain tissue □ Alkaloids—salsolinol, electron-capture GLC analysis in mouse brain tissue

The *in vivo* formation of tetrahydroisoquinoline alkaloids from aldehydes and endogenous biogenic amines has been suggested to mediate some effects of alcohol (1, 2). These alkaloids possess a variety of pharmacological actions *in vitro* and *in vivo* (3). Tetrahydropapaveroline (the condensation product of dopamine with dopaldehyde) and salsolinol (1,2,3,4-tetrahydro-1-methyl-6,7-isoquinolinediol) (the condensation product of acetaldehyde with dopamine) were detected in the urine of humans (4) and in rat brain (5, 6). Likewise, tetrahydroprotoberberines (the cyclization derivatives of tetrahydropapaveroline) were recovered from human urine (7).

The analyses used in some of these studies (4, 5, 7) involved costly mass fragmentography. Recently, a sensitive electron-capture GLC assay was reported for salsolinol in rat brain (8). However, the tissue extraction method was time consuming (adsorption onto and elution from alumina, followed by overnight lyophilization) and presented additional problems such as inadequate buffering of the extract prior to alumina adsorption and incomplete removal of the derivatizing reagent prior to injection into the chromatograph. Even with appropriate modifications to eliminate the latter problems, the long extraction procedure still proved undesirable.

The present report describes a more rapid and convenient method for the detection of salsolinol in mouse brain using a simplified solvent extraction and electron-capture GLC system. Detection of salsolinol at levels of 5–10 ng/g of brain tissue [approximately 1% of the endogenous dopamine level (9)] is possible with this method.

EXPERIMENTAL

Extraction—The extraction method from tissue is essentially that of Maruyama and Takemori (9) with some modifications. A single mouse brain was added to an all-glass grinding vessel[1] containing 1 ml of 0.05 *N* oxalic acid, saturated with sodium chloride, and 3.5 ml of 25% 1-butanol in 2-propanol and was homogenized. Standards were added at this point and mixed by passing the pestle through the homogenate.

The homogenate was transferred to a conical test tube and centrifuged at 3000×*g* for 5 min in a clinical centrifuge. The lower phase was discarded, and a 3-ml aliquot of the upper solvent phase was removed. Hexane, 3 ml, and 0.5 ml of 0.5 *M* sodium phosphate buffer (pH 6.5) were added to this aliquot, and the mixture was shaken for 5 min, followed by a 5-min centrifugation. The upper phase was discarded, and a 0.5-ml

[1] Size 22, Kontes Glass Co., Vineland, N.J.