Cytotoxic Agent from *Penstemon deustus* (Scrophulariaceae): Isolation and Stereochemistry of Liriodendrin, a Symmetrically Substituted Furofuranoid Lignan Diglucoside

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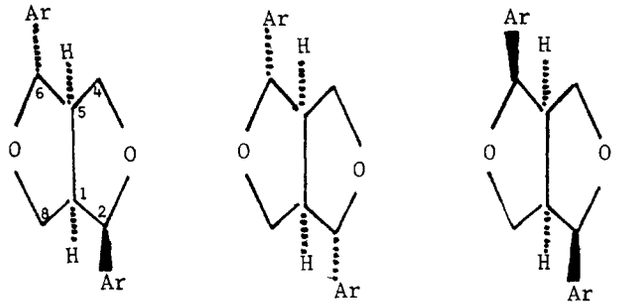
Received October 22, 1979

The isolation and characterization of liriodendrin (mp 269–270 °C), a lignan di-β-D-glucoside from the bark of *Liriodendrin tulipifera*, was first reported by Dickey.[1] The stereochemistry of the lignan portion was not clear as hydrolysis appeared to give three isomeric aglycones: lirioresinol-A [(+)-episyringaresinol] (Ia), lirioresinol-B [(+)-syringaresinol] (IIa) and lirioresinol-C [(+)-diasyringaresinol] (IIIa). Acid hydrolysis gave Ia and IIa whereas



I II III

a: Ar = 4-hydroxy-3,5-dimethoxyphenyl

b: Ar = 3,4,5-trimethoxyphenyl

c: Ar = 3,4-dimethoxyphenyl

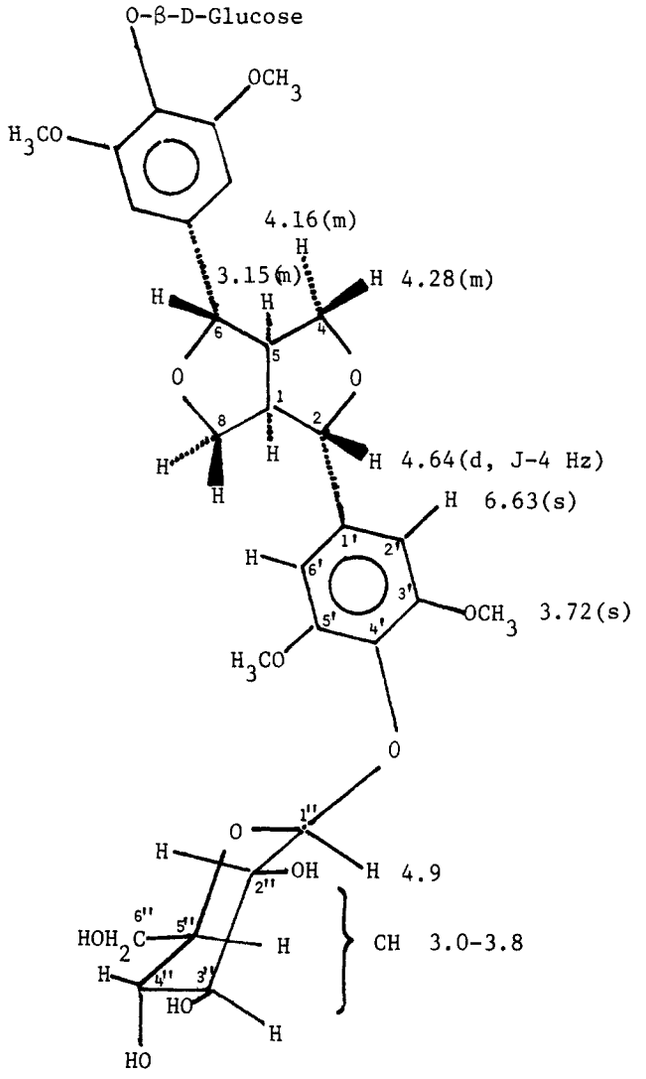
d: Ar = 3,4-methylenedioxyphenyl

enzymatic hydrolysis was claimed to yield IIIa. It was believed that epimerization accompanied the acid hydrolysis, and the stereochemistry of liriodendrin itself was left open.

The stereochemistry of lirioresinols I–IIIa was established by Briggs et al.,[2] who isolated the dimethyl ethers IIb and IIIb from *Macropiper excelsum*. They found that the substance believed by Dickey to be lirioresinol-C (IIIa) was in fact impure lirioresinol-B (IIa) but had no liriodendrin and did not comment on its stereochemistry. We now report the isolation (from a plant of another family) of liriodendrin, its cytotoxic activity, and its complete stereochemistry.

Results and Discussion

The isolation and characterization of penstemide, a cytotoxic isovaleroyl-type iridoid glucoside from the ethanol extract of the roots, stems, leaves, flowers, and fruits of *Penstemon deustus* Dougl. ex Lindl. (Scrophulariaceae) has been reported.[3] This ethanol extract, when subjected to partition between chloroform and water, yielded a water-soluble fraction which on further partition between butanol and water afforded a water-soluble syrup. Column chromatography of this syrup yielded, in addition to mannitol, sucrose, and unidentified sugars, a very small amount of a lignan we have shown to be liriodendrin (IV).



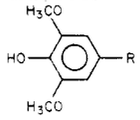
IV

The isolated lignan IV did not dissolve in most solvents but crystallized from pyridine as colorless needles, mp 265–266 °C, and was optically active, $[\alpha]^{25}_{D}$ −12.1°.[4] The molecular formula $C_{34}H_{46}O_{18}$ was established from elemental analysis, and the molecular weight was inferred from the mass spectrum which was very informative (Table I).[4] The lower part of the spectrum (up to *m/e* 418) was very close to that of lirioresinol-B dimethyl ether (IIb) with appropriate shifts to lower mass numbers. The lignan did not display a recognizable molecular ion peak but did exhibit an intense peak at *m/e* 418 (base peak), corresponding to IIa derived presumably from the molecular

(1) E. E. Dickey, *J. Org. Chem.*, 23, 179 (1958).
(2) Lindsay H. Briggs, R. C. Cambie, and R. A. F. Couch, *J. Chem. Soc.*, 3042 (1968).
(3) S. Jolad, J. J. Hoffmann, R. M. Wiedhopf, J. R. Cole, R. B. Bates, and G. R. Kriek, *Tetrahedron Lett.*, 46, 4119 (1976); S. R. Jensen, B. J. Nielsen, C. B. Mikkelsen, J. J. Hoffmann, S. D. Jolad, and J. R. Cole, *ibid.*, 3261 (1979).
(4) The optical rotation and spectral properties of liriodendrin have not been reported previously.

0022-3263/80/1945-1327$01.00/0

Table I. Major Fragment Ions in the Mass Spectrum of Lignan IV

fragment ions	R	m/e[a]
IIa		418 (446)
IIa-CH_2O		388 (416)
IIa-OMe		387 (415)
	$C_5H_6O_2^+$	251
	$C_7H_7O^+$	236 (250)
	$C_6H_6O^+$	235 (249)
	$COCH_2CH_2OH^+$	226
	$CH{=}CHCH_2OH^+$	210 (224)
	$CH_2CH{=}CH_2^+$	193 (207)
	$C{\equiv}O^+$	181 (195)
	CH_2^+	167 (181)
	H	154 (168)

[a] Figures in parentheses represent analogous ions in the mass spectrum of lirioresinol-B dimethyl ether (IIb).

Table II. ^{13}C NMR[a] Chemical Shifts of Lignan IV Compared with Certain Shifts in the Eudesmin Series

atom	lignan IV[b]	eudesmins[c] EQ-AX (Ic)	DIEQ (IIc)	DIAX (IIIc)
		Fused Furan		
C-1 } C-5 }	53.6 (d)	{ 54.44 } { 50.11 }	54.31	49.49
C-4 } C-8 }	71.3 (t)	{ 70.94 } { 69.64 }	71.72	68.78
C-2 } C-6 }	85.1 (d)	{ 87.53 } { 81.96 }	85.77	83.96
		Aromatic		
C-1′	133.9 (s)	{ 133.51, } { 130.81 }	134.04	131.38
C-2′	104.3 (d)			
C-3′	152.7 (s)			
C-4′	137.2 (s)			
OCH_3	56.4 (q)			
		Glucose		
C-1″	102.7 (d)		104.0 (d)[d]	
C-2″	74.2 (d)		74.1 (d)[d]	
C-3″	76.5 (d)		76.8 (d)[d]	
C-4″	70.0 (d)		70.6 (d)[d]	
C-5″	77.2 (d)		76.8 (d)[d]	
C-6″	60.9 (t)		61.8 (t)[d]	

[a] All values given in parts per million downfield from Me_4Si (δ). [b] In CD_3SOCD_3. [c] In $CDCl_3$. [b] Methyl-β-D-glucose.[10]

ion by fission at the two glucoside linkages with a transfer of two protons. This aglycone (IIa) then gives rise to all the principal fragment ions at m/e 388, 387, 251, 236, 235, 226, 210, 193, 181, 167, and 154 by a breakdown pattern characteristic of aryl-substituted furofuranoid lignans.[5,6]

The IR spectrum of the lignan suggested the presence of a β-glucose moiety (890 cm^{-1}, β sugar[7]) with a broad hydroxyl band centered at 3400 cm^{-1}, a phenyl ring with an isolated H (1595, 1500, 1460, and 805 cm^{-1}), a CH_2–O linkage (1416 cm^{-1}), and methyl (1365 cm^{-1}) groups. This was supported by the ^1H and ^{13}C NMR spectra (Table II) of the lignan which clearly indicated twofold symmetry. The ^{13}C NMR spectrum showed only 14 peaks for the 34 carbons, as expected for structure IV. This finding precluded the possibility of an unsymmetrical (equatorial–axial) configuration (I). The diaxial configuration (III) was ruled out in three ways for the lignan. The first way was a comparison of the proton chemical shifts (see numbering on formula IV) with those of isomeric aglycones Ia, IIa, IIIa reported by Briggs et al.[2] which follow the general criteria established by Pelter et al.[9] with IIc and IIId. According to Pelter, the signals for the benzylic protons in the diequatorial series appear between δ 3.75 and 4.7 whereas in the diaxial series they appear between δ 3.25 and 4.0. Thus, finding the signals due to protons at C-2 and C-6 at δ 4.64 in the ^1H NMR spectrum of the lignan strongly suggests that it is of the diequatorial type (II); the other ^1H NMR shifts also fit well for this stereochemistry. Second, comparison of the ^{13}C NMR shifts (Table II) of 1′ carbon atoms, which are characteristic of the stereochemistry of attachment to the central bicyclic skeleton, clearly established the diequatorial configuration for the lignan, in agreement with the deductions derived from the ^1H NMR spectrum discussed before. A similar comparison of other ^{13}C NMR shifts (Table II) provides strong support for this view. For all four types of carbons (C-1, C-2, C-4, and C-1′) which can be used, the values for lignan IV are much closer to those for the diequatorial model (IIc). Third, the method of molecular rotation differences (which should work quite well here with the two sugars well separated from the central chiral portion of the molecule) gives further support to the diequatorial arrangement for lignan IV and, in addition, shows its absolute configuration to be as shown; pertinent values are given in Table III.

Table III. Molecular Rotation of Model Lignans Compared with That of Lignan IV

	lignan	$[\alpha]^t_D$, deg	$[M]^t_D$, deg	av value, deg	value for IV, deg: predicted[a]	value for IV, deg: obsd
DIEQ	IIa[2]	+62	+259	+242	−118	−90
	IIb[2]	+47	+210			
	IIc[9]	+64	+247			
	IId[9]	+71	+251			
DIAX	IIIb[2]	+284	+1267	+1283	+923	−90
	IIIc[9]	+316	+1220			
	IIId[9]	+385	+1363			

[a] Estimated by adding the average value to twice −63° ($[M]^t_D$ − 180°) which is the rotation observed for *p*-methoxyphenyl β-D-glucoside.[11]

Experimental Section

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Carbon and hydrogen analyses were carried out by Chemalytics Inc. and the University Analytical Center, Tucson, Arizona. Optical rotations were measured in pyridine by using a Perkin-Elmer 241MC polarimeter. Ultraviolet (UV) and infrared (IR) spectra were run on Cary 15 and Beckman IR-33 spectrometers, respectively. ^1H NMR spectra were run at 60 MHz on a Varian T-60 spectrometer and at 250 MHz on a Bruker Spectrospin spectrometer, and values are given as parts per million downfield from Me_4Si (δ). ^{13}C NMR spectra were measured in Me_2SO-d_6 at 90 MHz by using a Bruker WH-90 spectrometer. Mass spectra were recorded on a Hewlett-Packard 5930 A spectrometer.

Liriodendrin (IV). The dried plant (roots, stems, leaves, flowers, and fruits) of *Penstemon deustus*, collected in California in May 1973, was milled in a Wiley mill and stored at −10 °C prior to extraction. The ground material was extracted exhaustively

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(6) A. M. Duffield, *J. Heterocycl. Chem.*, **4**, 16 (1967).
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(9) A. J. Birch, P. L. Macdonald, and A. Pelter, *J. Chem. Soc.*, 1968 (1967); C. K. Atal, K. L. Dhar, and A. Pelter, *ibid.*, 2229 (1967).
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in a Lloyd-type extractor in turn with petroleum ether and 95% ethanol, and the air-dried ethanol extract was subjected to partitioning between chloroform and water. The water-soluble extract was lyophilized and stored at −10 °C prior to further fractionation.

A 600-g portion of the lyophilized water extract was dissolved in water (2500 mL) and repeatedly extracted in portions with *n*-butanol (3500 mL). The butanol-insoluble phase was air-dried, and the resulting syrup was dissolved in the minimum amount of methanol (800 mL) and left in the refrigerator overnight. The supernatant liquid, after filtration by decantation, was passed through a small column of silica gel 60 and eluted exhaustively with methanol. The methanol eluent on standing overnight at room temperature gave a dark brown residue which was removed by decantation. The clear methanol eluent was concentrated and subjected to silica gel 60 (2 kg) column chromatography. The column was eluted with hexane/dichloromethane/methanol (20:20:10) as the initial solvent system followed by a gradual change in the concentration of dichloromethane and methanol. Forty 1-L fractions were collected, and the solvent from each fraction was evaporated off in vacuo. The resulting residue was dissolved in the minimum amount of ethyl acetate/methanol (1:3) and left at room temperature for a few days. Liriodendrin (IV), which was separated from fractions 6–10 as a colorless residue (mp 256–257 °C), crystallized from pyridine as colorless needles: mp 265–266 °C; $[\alpha]^{25}_{D}$ −12.1° (*c* 0.596, pyridine); UV (H_2O) λ_{max} 268 (ϵ 1966). The mixture melting point with an authentic sample showed no depression. The IR, mass, and ^1H and ^{13}C NMR spectra (described under Results and Discussion) were in accord with structure IV.

Anal. Calcd for $C_{34}H_{46}O_{18}$: C, 54.98; H, 6.24. Found: C, 55.26; H, 6.15.

Liriodendrin demonstrated an activity of 147 [test/control (T/C)] at 12.5 mg/kg in the PS test system. Activity in the PS test systems is defined as an increase in the survival of treated animals over that of controls resulting in a T/C value of ≥126%.[12]

Liriodendrin Octaacetate. Acetylation of liriodendrin with acetic anhydride–pyridine at room temperature for 48 h afforded an octaacetate, mp 121–124 °C (MeOH) (lit.[1] mp 124–125 °C).

Acknowledgment. This investigation was supported by Grant No. 5-R01-CA-22336-02, awarded by the National Cancer Institute, Department of Health, Education, and Welfare, Bethesda, MD 20014. The authors are grateful to Dr. E. E. Dickey, Appleton, WI, for providing a small sample of liriodendrin.

Registry No. IIa, 21453-69-0; IV, 573-44-4; IV octaacetate, 66007-45-2.

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Formation of Dimethylmercury in the Oxidation of Methylhydrazine by Mercuric Oxide

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Received November 27, 1979

With the objective of finding a method for the synthesis of 1,4-dimethyl-2-tetrazene, we have recently examined the oxidation of monomethylhydrazine by mercuric oxide. Renouf[1] reported in 1880 that 1,1,4,4-tetramethyl-2-tetrazene is formed by the corresponding reaction of *unsym*-dimethylhydrazine with mercuric oxide (eq 1). It was

$$2(CH_3)_2NNH_2 + 2HgO \rightarrow (CH_3)_2NN{=}NN(CH_3)_2 + 2Hg + 2H_2O \quad (1)$$

(1) E. Renouf, *Ber. Dtsch. Chem. Ges.*, 13, 2169 (1880).

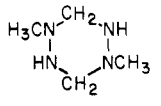
Table I

measd mass	calcd mass for $(CH_3)_2Hg$	measd mass	calcd mass for $(CH_3)_2Hg$
234.0209	234.0204	230.0139	230.0153
232.0162	232.0176	229.0133	229.0152
231.0158	231.0172		

hoped that methylhydrazine would react similarly. The reaction was carried out in anhydrous ethyl ether and, in addition to a variety of products, not including 1,4-dimethyl-2-tetrazene, a considerable amount of dimethylmercury was obtained. To our knowledge this highly hazardous substance has not previously been detected in the products of mercuric oxide oxidations of the methylhydrazines.

Results and Discussion

In addition to those resulting from the solvent and air, three peaks were observed on the gas chromatograph of the liquid reaction mixture from the methylhydrazine–mercuric oxide reaction. Two of these were at retention times 5.81 and 4.83 min and can be attributed, respectively, to water and to $CH_3NHN{=}CH_2$ or its dimer.[2]



The third major component was isolated by drying the solution over $CaSO_4$ for 14 h and evaporating it under reduced pressure. Approximately 1 mL of a colorless liquid whose peak on the gas chromatograph had a retention time identical with the third peak of the mixture, viz., 2.42 min, remained. On the basis of the gas chromatographic analysis, this liquid contains about 97.9% dimethylmercury. The proton NMR spectrum of this material gave a single resonance at τ 9.71 in agreement with that reported for dimethylmercury.[3] Anal. Calcd for $(CH_3)_2Hg$: C, 10.40; H, 2.61. Found: C, 11.41; H, 2.92. The identification of this compound was further confirmed by comparing its infrared and mass spectra with those reported[4,5] for dimethylmercury.

The infrared spectrum of the product contained the following peaks: 2970–1900 (s), 1640 (w), 1400 (br), 1240 (w), 1110–1000 (m), 750 (s), 520 (s) cm^{-1}. The 1240-cm^{-1} peak may result from an impurity.

The mass spectrum of this product is in excellent agreement with that expected for dimethylmercury. The precisely measured masses of the parent peaks agreed well with those calculated for dimethylmercury as shown in Table I. We are continuing to explore this and similar reactions. We recommend care in such processes to minimize the danger introduced by the possible formation of dimethylmercury.

On the basis of the gas chromatographic data, the yield of dimethylmercury in this reaction approximated 5–10%.

Experimental Section

Materials. Methylhydrazine obtained from the Aldrich Chemical Co. was refluxed and distilled over solid KOH no more than 1 day before use and was stored at −4 °C. The fraction boiling at 87.5 °C was collected and used in the study. The mercuric oxide (yellow) used was CP grade.

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0022-3263/80/1945-1329$01.00/0