Tetrahedron. Vol 25, pp 2325 to 2337 Pergamon Press 1969. Printed in Great Britain

LIGNANS OF *ULMUS THOMASII* HEARTWOOD—II

LIGNANS RELATED TO THOMASIC ACID*

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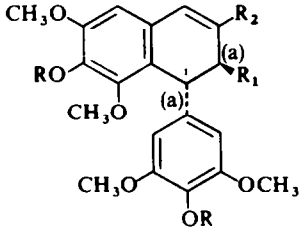
(*Received in the USA* 11 *July* 1968; *Received in the UK for publication* 6 *January* 1969)

Abstract—Three disyringyl lignans closely related to thomasic acid (I) have been isolated from aqueous extracts of the heartwood of *Ulmus thomasii* Sarg. These are thomasidioic acid (II), racemic lyoniresinol (III) and (+)-lyoniresinol-2α-O-rhamnoside (IV). Correlation of I and II with lyoniresinol proves that the series has *trans* stereochemistry in the 1,2-positions. Also isolated were 6-hydroxy-5,7-dimethoxy-2-naphthoic acid and 2,6-dimethoxybenzoquinone.

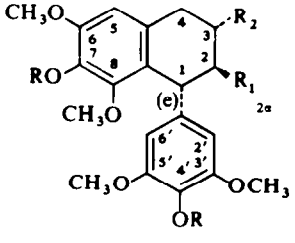
THOMASIC acid, a highly fluorescent disyringyl lignan acid, was previously identified[1] as the major component of aqueous extracts of the heartwood of *Ulmus thomasii*. Wallis[2] proposed that the stereochemistry at C-1 in our original formula for thomasic acid was incorrect. Results reported in this paper prove that the structure of thomasic acid should indeed be change to I.

In addition, three closely related lignans, II, III and IV, have now been isolated from the same source. All are syringyl derivatives as shown by a positive Mäule test, a blue color with *p*-nitrobenzenediazonium salt plus base, and by characteristic disyringyl substitution patterns in their NMR spectra.



	R	R_1	R_2
I *rac*-thomasic acid	H	CH_2OH	COOH
II *rac*-thomasidioic acid	H	COOH	COOH

FORMULA A



	R	R_1	R_2
III *rac*-lyoniresinol	H	CH_2OH	CH_2OH
IV (+)-lyoniresinol-2α-O-rhamnoside‡	H	CH_2ORh	CH_2OH

FORMULA B

* Presented at the annual meeting of the Phytochemical Society of North America, Madison, Wis., August 1967.

† Maintained at Madison, Wis., in cooperation with the University of Wisconsin.

‡ Absolute configuration as shown.

During the isolation of the lignans, other compounds have been obtained in small amounts. A trace of the yellow 2,6-dimethoxybenzoquinone was quickly characterized. A bright white fluorescent compound (V) was identified as 6-hydroxy-5,7-dimethoxy-2-naphthoic acid; its proof of structure will be published later.

Isolation

Crude aqueous extracts of the wood were efficiently fractionated on a single polyamide column; three of the compounds were obtained at once as crystals. First, when the appropriate aqueous eluates were concentrated, 2,6-dimethoxybenzoquinone precipitated on standing. Lignan III precipitated directly from rich 10% methanolic eluates after cooling for several days. I, as previously noted,[1] crystallized in the 40% methanolic eluates soon after its delivery from the column. Of the other compounds, IV, eluting just before III, had to be refractionated on silica but remained amorphous. Finally, II and V were eluted together from polyamide but could be separated on Polyclar. V was crystallized easily, but II, although chromatographically pure, remained amorphous.

Thomasidioic acid (II)

The compound most similar to I is the highly fluorescent lignan II. Spectral characteristics were almost identical. UV maxima (Table 1) were essentially the same, with shifts showing the phenolic and acidic nature of the compound (bathochromic shift with sodium ethylate and hypsochromic shift with sodium acetate). The IR spectrum of II differed significantly from that of I only in a broadening of

TABLE 1. UV DATA FOR LIGNANS AND THEIR DERIVATIVES*

Compound	Solvent and reagents		
	EtOH, 95%	+NaOAc	+NaOEt
II	320†	308	353
	248	240	254
III	281 ($\varepsilon = 3{\cdot}75 \times 10^3$)	No shift	292
	275 ($\varepsilon = 3{\cdot}75 \times 10^3$)		
			251
IV	279	No shift	288
V	301† ($\varepsilon = 7{\cdot}5 \times 10^3$)	293	347
			331
			267 sh
	250 ($\varepsilon = 4{\cdot}95 \times 10^4$)	248	253
VII	278 sh	No shift	No shift
	272 ($\varepsilon = 2{\cdot}11 \times 10^3$)		
VIII	330 sl sh	No shift	No shift
	306 ($\varepsilon = 1{\cdot}50 \times 10^4$)		
	244 ($\varepsilon = 2{\cdot}61 \times 10^4$)		

* λ_{max} in nm; sh = shoulder; sl = slight.

† Exact wavelength is pH dependent, ranging from 321 to 318 nm for II, and 307 to 299 nm for V in neutral or acid media.

the carbonyl band to two peaks at 1690 and 1700 cm^{-1}, indicative of both conjugated and nonconjugated acid carbonyl groups, and the lack of a peak at 1022 cm^{-1} that in the spectrum of I was assigned to the primary alcohol group. The NMR spectrum of II (Table 2) showed identical patterns in the methoxy, aromatic, and vinyl hydrogen regions. On the basis of the spectral differences, the diacid structure II was proposed in which the hydroxymethyl group of I is replaced by the nonconjugated carboxylic acid group.

The structure of II was proved by correlation to a derivative of I as shown in Fig. 1. In Part 1,[1] the oxidation of Ia with Jones reagent to 6,7,8-trimethoxy-3-methoxycarbonyl-1-(3,4,5-trimethoxyphenyl)-1,2-dihydro-2-naphthoic acid is described. A simple methylation of this compound with diazomethane gave a compound

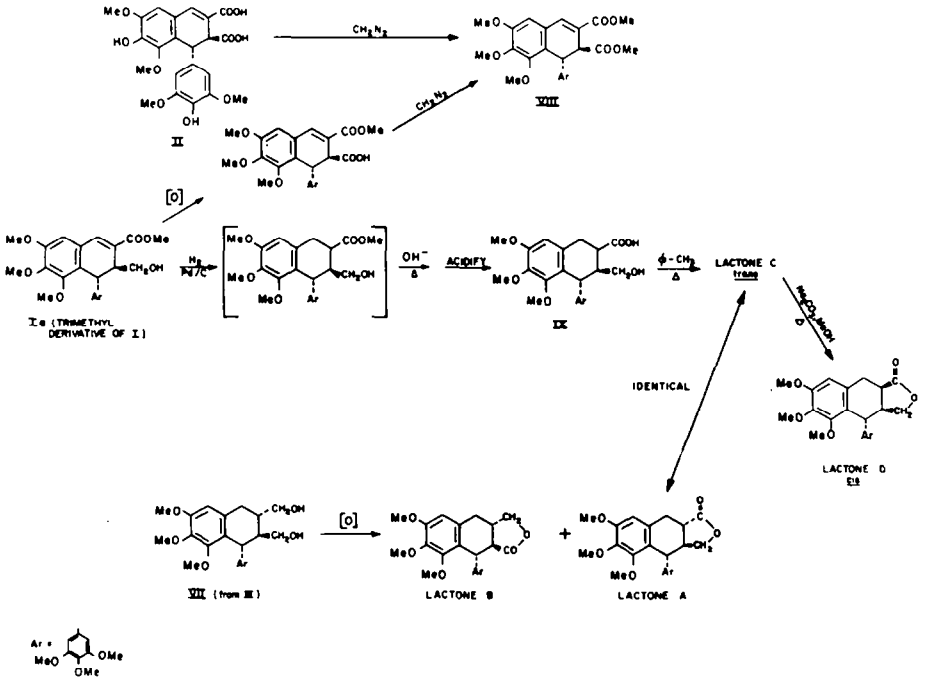


FIG. 1. Relationship of lignans I, II and III, from *Ulmus thomasii*.

identical to the dimethyl ether-dimethyl ester of II (VIII). Therefore thomasidioic acid is *rac-trans*-1,2-dihydro-7-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-6,8-dimethoxy-2,3-naphthalenedicarboxylic acid.

(±)-*Lyoniresinol* (III)

Lignan III is nonfluorescent. Its UV spectrum (Table 1) showed only a typical phenolic peak λ_{max} 275–281 nm that gave a bathochromic shift with sodium ethoxide. There was no carbonyl band in the IR spectrum, and the aromatic region was similar to that of I except that it lacked the peaks at 1621 and 1572 cm^{-1} assigned to the conjugated double bond. In the NMR spectrum (Table 2), the four methoxyl peaks of a disyringyl backbone were present. The vinyl hydrogen singlet of I was missing.

TABLE 2.

Compound	Solvent	H-1	H-2	—CH_2O— (2α or 3α)	H-3
II§	d_6-acetone	5·05	?	¦¦	‖
III (hydrate)	d_6-acetone	4·32 (d, J = 5·5)	¶	3·62 (m)	¶
IV	d_6-acetone	4·34 (d, J = 6)	¶	3·5 (m)	¶
VI	$CDCl_3$	4·33 (m)¶	?2·1 (m)	4·17 (m)	?2·1 (m)
VII	$CDCl_3$ (100 mc)	3·95 (d, J = 7·5)	1·80 (m)	3·35–3·75 (m)	1·80 (m)
	d_6-DMSO	4·30 (d, J = 5·5)	?1·5–2 (m)	¶	?1·5–2 (m)
	pyridine	4·87 (d, J = 5·5)	¶	4·03 (m)	¶
VIII	$CDCl_3$	5·01 (d, J = 1)	4·08 (d, J = 1)	‖	‖
X	$CDCl_3$ (100 mc)	3·96 (d, J = 7·5)	1·82 (m)	3·36–3·75 (m)	1·82 (m)
	d_6-DMSO	4·28 (d, J = 5·5)	?1·5–2 (m)	¶	?1·5–2 (m)
XI	$CDCl_3$ (100 mc)	4·3 (m)	¶	3·5 (m)	¶

NMR DATA OF LIGNANS AND THEIR DERIVATIVES*†

H-4 or H-4a, 4b‡	H-5	H-2′, 6′	OMe		Acetyl and sugar protons
			8	Other (position if known or relative intensity)	
7·67	6·95	6·40	3·63	3·90 (6) 3·70 (3′, 5′)	
2·60, 2·72	6·63	6·50	3·42	3·87 (6) 3·77 (3′, 5′)	
2·63 (d, J = 3) 2·75	6·57	6·40	3·40	3·83 (6) 3·72 (3′, 5′)	*rhamnose protons* 4·78 (d, J = 2) C-1 1·19 (d, J = 6) C-Me
2·83, 2·71	6·55	6·35	3·22	3·82 (6) 3·73 (3′, 5′)	2·28 (R_1, acetyl (2)) 2·08, 2·04 (ali. acetyl—C_{2a}, C_{3a})
2·68, 2·65, 2·59 (m)	6·44	6·32	3·22	3·83 (1·1·3) 3·77 3·74	
2·7 (m)	6·66	6·40	3·36	3·80 (1:3:1) 3·68 3·63	
3·03 (m)	6·70	6·78	3·53	3·80 (2:1:2) 3·73 3·61	
7·65	6·72	6·29	3·66	3·89 (6, 7) 3·78 (4′) 3·73 (3′, 5′) 3·78 (aro. ester) 3·66 (ali. ester)	
2·69, 2·65, 2·60 (m)	6·42	6·30	3·22	3·83 (1:1:3) 3·77 3·74	
2·7 (m)	6·62	6·37	3·33	3·78 (1:3:1) 3·66 3·61	
¶	6·50	6·30	3·31 (d, J = 8)**	3·80 (6) 3·70 (3′, 5′)	2·26 (R_1, acetyl (2)) 2·12, 2·02, 2·00, 1·95 (ali. acetyl) 4·8 (C-1 proton of sugar)

TABLE 2—*continued*

Compound	Solvent	H-1	H-2	—CH_2O— (2α or 3α)	H-3
XII	$CDCl_3$	4·17 (m)¶	?2·1 (m)¶	4·07–4·23 (m)	?2·1 (m)¶
	pyridine	4·20–4·54 (m)¶	?	4·20–4·54 (m)¶	?
XIII	$CDCl_3$	4·17 (m)¶	?2·1 (m)¶	4·06–4·25 (m)	?2·1 (m)¶
	pyridine	4·21–4·56 (m)¶	?	4·21–4·56 (m)	?

* Formulas of compounds not previously shown:

		R	R_1	R_2
Formula A	VIII	Me	COOMe	COOMe
Formula B	VI	Ac	CH_2OAc	CH_2OAc
	VII	Me	CH_2OH	CH_2OH
	X[a]	Me	CH_2OH	CH_2OH
	XI	Ac	$CH_2ORh(Ac)_3$	CH_2OAc
	XII	Me	CH_2OAc	CH_2OAc
	XIII[a]	Me	CH_2OAc	CH_2OAc

[a] Absolute configuration as shown.

The signals of the three aromatic hydrogens were still present although closer together; apparently the hydrogens are more nearly similar than in I or II. One methoxyl peak appeared upfield from the other three, and in fact farther upfield than the corresponding peaks for I and II; this indicated that the 8-OMe of the lignan structure was even more strongly shielded by the *peri*-situated benzene ring at C-1. The NMR spectrum of the tetraacetyl derivative (VI) indicated two aliphatic and two aromatic hydroxyl groups. This data suggested a disyringyl lignan with a saturated B ring and two hydroxymethyl groups.

The proposed structure III possesses three centers of asymmetry. Of the possible isomers, two optically active ones and one racemate have already been reported. The first, lyoniresinol[3] (also called dimethoxy-isolariciresinol),[4] was isolated by Yasue and Kato[3] from *Lyonia ovalifolia* var. *elliptica* and by Freudenberg and Weinges[4] from *Alnus glutinosa* (as the xyloside). Lyoniresinol was reported to have m.p. 171°, $[\alpha]_D^{22} = 68{\cdot}4°$, and the stereochemistry was shown to be 1,2-*trans*-2,3-*trans* (our numbering). Its dimethyl ether had m.p. 158–160°, $[\alpha]_D^{28} = 30{\cdot}1°$. Kato[5]

H-4 H-4a, 4b‡	H-5	H-2′, 6′	OMe		Acetyl and sugar protons
			8	Other (position if known or relative intensity)	
2·80, 2·67	6·52	6·33	3·33	3·88 (1:1:3) 3·80 3·78	2·09 } (ali. acetyl— 2·03 } $C_{2\alpha}$, $C_{3\alpha}$
2·88, 2·76	¶	¶	3·50	3·83 (1:1:1:2) 3·81 3·78 3·70	2·03 } (ali. acetyl— 2·00 } $C_{2\alpha}$, $C_{3\alpha}$)
2·79, 2·67	6·51	6·33	3·33	3·88 (1:1:3) 3·80 3·78	2·09 } (ali. acetyl— 2·03 } $C_{2\alpha}$, $C_{3\alpha}$
2·90, 2·78	¶	¶	3·50	3·84 (1:1:1:2) 3·82 3·78 3·70	2·03 } (ali. acetyl— 2·00 } $C_{2\alpha}$, $C_{3\alpha}$

† All spectra on 60 mc instrument unless noted. δ values in ppm relative to tetramethylsilane. Singlets except as noted; d = doublet, m = ill-defined multiplet. J in Hz.

‡ One vinyl (Δ^3) proton as singlet in II and VIII; two protons in all others (saturated bond). This appears as two broad peaks in III, VI, XII and XIII, but as three broad peaks in VII and X.

§ Crude.

‖ Does not apply.

¶ Splitting too complex to make definite assignments. Signals may be hidden beneath other peaks or within solvent peaks.

** Collapses to singlet at 60°.

was able to convert the dimethyl ether to the 1,2-*trans*-2,3-*cis* isomer, *epi*-lyoniresinol dimethyl ether, m.p. 177°, $[\alpha]_D = +16{\cdot}4°$. Freudenberg and Weinges[4] synthesized *rac*-lyoniresinol, m.p. 115–118°, tetraacetate, m.p. 141–143° from syringaresinol. Because III and its dimethyl ether (VII) were optically inactive, m.p. 193–194° and 164–164·5° respectively, it was obvious that we did not have either of these two active isomers. The inactive isomer was also eliminated at first because of its reported melting point although the acetate of III, (VI), m.p. 145–146° was not distinctly different.

To prove the structure of III, VII was first oxidized to 3,4,5-trimethoxy-2-(3,4,5-trimethoxybenzoyl)benzoic acid, identical to that obtained from I; this verified the linkage of the rings in the molecule.

III was then correlated with I. By a mild oxidation of VII (the dimethyl ether of III) two lactones, A and B, were obtained as shown in Fig. 1; these differed only in relative positions of the acid and hydroxymethyl group that formed the lactone ring. Lactone C obtained from Ia (the dimethyl ether of I) (see Fig. 1), was shown to be identical with A. Thus the diol III must have the same stereochemistry as thomasic acid in the 1,2-positions.

That the 2,3-relationship of the lactones is *trans* was shown by the fact that the

lactone ring of lactone C was difficult to close because 2 hr of refluxing in toluene was required and by the fact that it could be epimerized to a new lactone, D, by treatment with base (see Fig. 1). That C and D were respectively *trans* and *cis* lactones was supported by (1) comparison with reactions of somewhat similar lactones in the podophyllin series[6] and the lyoniresinol series[5] that showed that a *cis* lacton in this type of ring system is considerably less strained and therefore more stable than a *trans*; epimerization can occur at the carbon α to the carbonyl if it is thermodynamically favored;[5,6] (2) the position of the carbonyl band in their IR spectra, namely at 1784 and 1774 cm^{-1}; these figures check reported values for *trans* and *cis* lactones respectively in related lignans.[6]

When new NMR evidence reported by Wallis[2] suggested that I had a *trans* 1,2-relationship, the possibility arose that III was the racemic form of lyoniresinol.[3] An authentic sample of (+)-lyoniresinol dimethyl ether* was found to give the same NMR spectrum in pyridine (Table 2), the same IR spectrum in chloroform, and the same R_f values on TLC as VII, and authentic (+)-lyoniresinol† had the same chromatographic properties on paper and TLC as III. Also the NMR spectrum of lactone C was found to be indistinguishable from that reported for resinolide.[6] Thus III is *rac*-lyoniresinol,‡ *rac*-9,9′-dihydroxy-*trans*-7,8-*trans*-8,8′-syringacyclolignan.[7]

This identification of III confirms the proposal of Wallis[2] for 1,2-*trans* stereochemistry for the related thomasic acid (I). Note, however, that although I and II have axial syringyl groups, the NMR shift of the 8-methoxyl hydrogens for III and IV at 3·40 ppm and for the more sterically crowded 7-O-methyl and 7-O-acetyl derivatives at 3·22–3·33 ppm suggests, in line with the work of Wallis, that the aryl group is now equitorial so that the methoxy groups are more directly above the plane of the phenyl ring.

(+)-*Lyoniresinol*-2α-O-*rhamnoside* (IV)

Lignan IV is also nonfluorescent; its UV spectrum and the aromatic region of its IR and NMR spectra were almost identical with those of III. In a preliminary analysis of IV, it appeared to be a simple glycoside of III. Acid hydrolysis of the compound gave rhamnose and an aglycone with chromatographic properties identical to those of III. The NMR also showed IV to be a monorhamnosyl compound from its integration, with the rhamnose Me at δ 1·19 (J = 6 Hz) and the C-1 proton at 4·78 (J = 2 Hz).

That the sugar is attached at the 2α position was shown by the crude hexaacetyl derivative XI. The NMR spectrum of XI showed all the expected characteristics (Table 2), except that the easily recognized 8-OMe signal was no longer a singlet, but a doublet. If the sugar were on the nearest aliphatic hydroxyl (the 2α-OH), models show that extreme crowding in the hexaacetylated derivative would prevent free rotation of the 8-OMe. It could be caught on either side of the ring to which it is attached, thereby giving rise to the two peaks. In fact, at elevated temperatures the doublet collapsed to a singlet as expected. From this steric crowding we infer that the rhamnose must be in the 2α position.

* Kindness of Y. Kato.

† Kindness of K. Weinges.

‡ The discrepancy between the reported melting point of III and of *rac*-lyoniresinol can be explained by hydrate formation. (See Experimental.)

On further examination, methylation of lignan IV followed by hydrolysis was expected to give VII, the dimethyl ether of III. The product, X, was similar to VII in many respects, but its IR spectrum (in KBr) was slightly different, mostly in aliphatic C—C and C—H regions; its melting point was lower; and it had an optical rotation of +21° compared to 0° for VII. This was the first derivative of the lignans from *Ulmus thomasii* isolated thus far to show any optical activity. A careful comparison of compounds VII and X by NMR as well as of their diacetyl derivatives, XII and XIII, by NMR and by IR in solution showed that the two were indistinguishable; thus it was concluded that X was an optically pure isomer of VII. Finally comparison of X (m.p. 156–156·5°) with the authentic sample of (+)-lyoniresinol dimethyl ether[3] by IR (in KBr), by TLC, and by mixed melting point showed that the principal aglycone derived from the rhamnoside was (+)-lyoniresinol.

ORD data are given in Table 3. Because VII gave no ORD curve, its racemic nature and that of III is verified. Although IV, from which optically active X was

TABLE 3.
ORD DATA ON LIGNANS AND LIGNAN DERIVATIVES

Compound	$\lambda_{(nm)}$	ϕ
IV*	283	+5270 pk
	265	−681 tr
	255	+5180 (cutoff)
VII†	0	0
X†	281	+4200 pk
	267	+730 tr
	248	+14200 pk
	232	−42500 tr

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† By Prof. W. Klyne, University of London.

derived, gave no rotation at the D-line, it gave a good ORD curve and a positive Cotton effect at 274 nm. X also gave a good ORD curve with two Cotton effects; the first positive one centered at about 275 nm and the second at about 240 nm. The first positive Cotton effect, according to the work of Swan and Klyne[8] on a wide sampling of cyclolignans, indicates that the configuration at C-1 (C-4 by their numbering) in IV and X is most likely α-aryl. The absolute configuration of (+)-lyoniresinol and its rhamnoside would then be 1R:2S:3S. This assignment is opposite to that given for (+)-lyoniresinol by Kato on the basis of other data.[5]

DISCUSSION

Several points are significant about the lignans isolated from *Ulmus thomasii*. All are closely related syringacyclolignans. Thomasic acid and thomasidioic acid fall, with plicatic acid,[9] into the very small class of free lignan acids, that is, lignans containing an acid group that does not exist in the much more common lactone form. Thomasidioic acid is the first lignan diacid reported; this acid and thomasic acid are the only naturally occurring lignans thus far shown to possess the unusual 1,2-dihydro-1-phenylnaphthalene skeleton.

A most unusual property of the first three lignans in this series, I, II and III, is their lack of optical activity. Naturally occurring lignans have until now been found to be optically active.[7] Biosynthesis of these three by a free radical mechanism such as postulated by Weinges and Spänig[7] seems highly probable. The last lignan in this series, the rhamnosyl lignan IV, does show optical activity; therefore no conclusion can be made on the mechanism of its biosynthesis.

EXPERIMENTAL

All m.ps are uncorrected.

Spectra. All spectra were determined as described in Part I of this series.[1]

Paper and TLC. Chromatographic methods have been outlined in Part I of this series.[1] For PC, solvents used were predominantly isopropyl alcohol–2N ammonia (ipram) (2:1) and (3:1) and butanol–27% acetic acid (1:1) (BAW). Preparatory TLC was carried out on fluorescent SiO_2 plates, 1 mm thick, and developed with varying percentages of methanol in chloroform as noted. The developed plates were viewed under a shortwave UV lamp, the bands scraped off, and the silica eluted with 95% ethanol.

Column chromatography. Nylon powder and silica gel (Merck), described in Part I,[1] were used for isolation, as well as Polyclar-AT powder (General Aniline and Film Corporation, New York, N.Y.). Polyclar and polyamide were purified as previously outlined. For the Polyclar column, 53 g were slurried in 40% methanol to give a column 23·5 × 3·5 cm, and the column was developed with the same solvent.

Isolation of lignans

Fractionation by polyamide column. Aqueous extracts of the heartwood of *Ulmus thomasii* were acidified, then fractionated on polyamide as described in Part I.[1] The major components of the extracts were eluted from the column in the following order: 2,6-dimethoxybenzoquinone (H_2O), IV (H_2O or 10% MeOH), III (10% MeOH), I (40% MeOH), II and V (40–60% MeOH, wide overlapping bands). *rac*-Lyoniresinol (III) precipitated after the fractions containing it had stood in the refrigerator several days. The amount of III varied significantly in different trees and did not diminish in concentration in the aqueous extract as rapidly as did I.[1] Highest yields from 575 g of heartwood were approximately 0·5 g or 0·09%. Fractions containing II, IV and V were fractionated further on other types of columns as will be described.

Separation of II *and* V *by Polyclar* All extracts containing compounds II and V were combined, evaporated *in vacuo* to a few ml, and rediluted to 100 ml with 40% MeOH. A few ml of conc HCl were added. The entire soln was applied to a Polyclar column; it was eluted with 40% MeOH, the course of the fractionation being followed by PC. II was eluted first, appearing as a dark band because of the acidity of the applied soln (although it is strongly green fluorescent in basic soln), followed by V, a bright white fluorescent band. On evaporation *in vacuo* of fractions containing II, crude amorphous material (380 mg) was obtained, yield perhaps 0·03%; this compound was relatively pure by PC. A considerably smaller amount of crystalline V was similarly obtained.

Purification of IV *on silica.* Combined residues containing lignan IV from polyamide columns were applied in 10% MeOH in $CHCl_3$ to a silica column, then the column was developed with 10–20% MeOH in $CHCl_3$. Total yield of amorphous residue was approximately 0·09% of the ground wood.

Thomasic acid (I)

Conversion to VIII. 6,7,8-Trimethoxy-3-methoxycarbonyl-1-(3,4,5-trimethoxyphenyl)-1,2-dihydro-2-naphthoic acid, synthesized in Part I from lignan I,[1] was methylated in MeOH with ethereal diazomethane by addition of diazomethane until the yellow color of the soln was just retained. After evaporation, the residue was crystallized from MeOH–H_2O to give VIII, m.p. 141·5–142·5°, identical by IR to that synthesized from II.

Conversion to lactone C. (a) Reduction. A suspension of 300 mg of the trimethyl derivative of lignan I (Part I), namely Ia, and 10% palladium on charcoal (60 mg) in 35 ml of glacial acetic acid was hydrogenated at room temperature under a slight positive pressure for 2 hr or until hydrogen uptake (approximately 18 ml) had ceased. The soln was filtered from the catalyst and evaporated to dryness.

(b) Hydrolysis and ring closure to lactone C. To the oily residue from the reduction, MeOH (10 ml) and 1N KOH (10 ml) were added, and the soln was refluxed 1·5 hr. The MeOH was boiled off, and the soln cooled and acidified. The aqueous soln was then extracted with $CHCl_3$, and the $CHCl_3$ layer evaporated. Toluene (50 ml) was added, and the solution was refluxed for 2 hr. In one run, the soln was allowed to

cool, and a precipitate was filtered from it. This was the reduced free acid (IX), m.p. 173–174° [IR, 1705 cm^{-1} (C=O)]. In most runs, the toluene was evaporated *in vacuo* without cooling, and no acid remained. The residue from evaporation was then taken up in hot MeOH–H_2O and left to cool and crystallize. After a second recrystallization, the yield of lactone C was 83 mg; m.p. 176·5–177·5°. (Found: C, 64·86; H, 6·42; OCH_3, 41·97. $C_{24}H_{28}O_8$ requires: C, 64·85; H, 6·35; OCH_3, 41·89%); IR bands at 3430 (OH), 1785 (lactone C=O), 1595, 1510, 1496 and 1460 (all Ar), 1424, 1409, 1338, 1272 and 1242 (ArO), 1187 (lactone C=O), 1128 (OMe), 1062, 1034, 1001, 990 (several ring deformation bands), 922, 830 and 717 cm^{-1}. When 7·5 mg were refluxed for 1 hr in 0·5 ml of methanol saturated with Na_2CO_3, lactone D was obtained as the water-insoluble product, IR bands essentially unchanged except: C=O at 1774, much weaker 990, stronger 781, new 1354, lost 1272, 1062 and 1001 cm^{-1}.

Thomasidioic acid (II)

Properties. Lignan II could not be crystallized, so analytical data are given only for its dimethyl ester-dimethyl ether VIII. Crude II gave the same syringyl reactions as I and III. It had R_f in ipram (3:1) 0·21, a fluorescent yellow-green spot in the UV in presence of NH_3; IR bands at 3400 (OH), 1700 (aliph acid C=O), 1690 (conj acid C=O), 1621 and 1572 (C=C, phenyl conj), 1613, 1515, 1500, 1458 (all Ar), 1425 (COOH), 1321 and 1205 (ArOH), 1205 (OAr ether), 1103 (OMe), 920–910 (COOH), 885.

Dimethyl ether-dimethyl ester of II (VIII). Crude II (100 mg) was dissolved in 10 ml of MeOH and methylated with diazomethane. The product was purified on a 20- × 2·5-cm silica column, developed, and eluted with $CHCl_3$. The residue yielded crystalline VIII after cooling overnight in MeOH–H_2O. Two recrystallizations from MeOH–H_2O gave 27 mg of white needles, m.p. 142·5–143°. IR bands at 1730 (aliph ester C=O), 1708 (conj ester C=O), 1630 and 1565 (C=C, phenyl conj), 1590, 1505, 1488, 1455 (all Ar), 1250 (ArO ether, C—O ester), 1125 and 1107 (OMe). (Found: C, 62·36; H, 5·92; OCH_3, 49·59. $C_{26}H_{30}O_{10}$ requires: C, 62·14; H, 6·02; OCH_3, 49·41%).

rac-*Lyoniresinol* (III)

Properties. Lignan III crystallized in heavy pinkish prisms, as a hydrate, m.p. 130–140° (loss of water), followed by slow resolidification and remelting at 193–194°. It was recrystallizable only with difficulty from water or $CHCl_3$. From $CHCl_3$ it precipitated in the anhydrous form, m.p. 193–194°. For analysis it was recrystallized as the hydrate and dried at 125° and 1 mm for 2 days. III was difficultly soluble in most solvents except alcohols, had $[\alpha]_D = 0°$, with $FeCl_3$ gave a yellow-brown to yellow-green color changing slowly to a stable dark blue color, a positive Mäule test, and a typical syringyl reaction, orange to blue color, with p-$NO_2C_6H_4N_2^+BF_4^-$ spray and 20% Na_2CO_3 overspray. It had R_f in ipram (2:1) 0·87 and (3:1) 0·89, BAW 0·87, an absorbing spot in the UV; IR bands at 3410 (OH), 1612, 1515, 1502, 1455 (all Ar), 1320 (ArOH), 1218 (OAr ether, ArOH), 1110 (OMe), 1060 (prim OH). (Found: C, 63·17; H, 6·88; OMe, 29·20. $C_{22}H_{28}O_8$ requires: C, 62·84; H, 6·71; OMe, 29·52%).

Tetraacetate (VI). Acetylation by refluxing III in acetic anhydride gave a tetraacetyl derivative VI with m.p. 145–146° after recrystallization from MeOH–H_2O and then benzene–ligroine (Found: C, 61·16; H, 6·23; AcO, 29·33; OMe, 21·08. $C_{30}H_{36}O_{12}$ requires: C, 61·21; H, 6·17; AcO, 29·22; OMe, 21·08%).

Dimethyl ether (VII). By the procedure described in Part I,[1] III was methylated with diazomethane to give VII, white needles in 78% yield, m.p. 164–164·5° from MeOH–H_2O, $[\alpha]_D^{20} = 0°$. It had IR bands at 3390 (OH), 2930 and 2820 (CH), 1590, 1490 and 1450 (all Ar), 1420, 1405, 1325, 1230 (OAr ether), 1115 (OMe), 1050 (prim OH), 1028, 1013, 925, 893, 835. (Found: C, 64·28; H, 7·27; OMe, 41·61. $C_{24}H_{32}O_8$ requires: C, 64·27; H, 7·19; OMe, 41·52%).

Lignan III was also methylated with dimethyl sulfate by the procedure described for IV, and subjected to the same hydrolysis conditions to assure that no isomerization under the acid conditions was occurring. After TLC purification, VII was obtained as the only product.

Dimethyl ether-diacetate (XII). The acetylation procedure described for III gave a diacetyl derivative of VII after purification by TLC and crystallization from MeOH–H_2O, m.p. 103–104°. It had IR bands (CS_2) 2925 (C—H), 1736 (ester C=O), 1355, 1325, 1228 (ArO ether, C—O ester), 1112 (OMe), 1031, 1010, 942, 920, 826. (Found: C, 63·21; H, 6·93. $C_{28}H_{36}O_{10}$ requires: C, 63·14; H, 6·81%).

Oxidation to a benzoylbenzoic acid. Mild permanganate oxidation was carried out on 60 mg of VII as outlined in Part I[1] to give 3,4,5-trimethoxy-2-(3,4,5-trimethoxybenzoyl)benzoic acid. Refluxing time was lengthened to 1·5 hr to assure complete reaction. The product was purified by TLC; the silica plates were developed in 25% MeOH in $CHCl_3$. After elution of the major band with ethanol and evaporation of the solvent, dilute acid had to be added to obtain a crystalline product. It was purified further from traces of silica by dissolving in 1% Na_2CO_3, filtering, and reprecipitating with HCl, to give 3·5 mg, m.p. 180·5–

181·5°. Its IR spectrum checked exactly with that of the keto acid synthesized as partial proof of the structure of I.[1]

Oxidation to lactone A. To VII (200 mg) in 70 ml of acetone stirred under nitrogen 15 drops of Kiliani soln were added as in Part I,[1] and the soln was stirred and worked up as reported. The residue from the benzene was purified by preparatory TLC by development in 1% MeOH in $CHCl_3$ (redeveloped four times). The two major bands, which had R_f values of 0·46 (A) and 0·40 (B), gave 14 mg of lactone A, m.p. 163–164° (softened), 178–179° (melted) after recrystallization from MeOH–H_2O; IR the same as lactone C; and a much smaller amount of lactone B, m.p. 157·5–159°.

(+)-*Lyoniresinol-2α-O-rhamnoside* (IV)

Properties. Lignan IV was obtained as an amorphous solid after evaporation of its fractions. It had R_f in ipram (2:1) 0·83 and (3:1) 0·85, an absorbing spot in the UV; IR bands at 3450 (OH), 2800 (CH), 1612, 1515, 1500, 1460 (all Ar), 1320, 1208, 1105 (OH), 1048, 980, 752. Its optical rotation at the D-line was too small to be measured accurately, but Table 3 gives ORD data.

Hexaacetate (XI). Crude IV (approximately 100 mg) was refluxed with acetic anhydride for 1·5 hr. After workup, a brown solid (76 mg) was recovered that was purified by TLC with 5% acetone in $CHCl_3$. The major product could not be obtained in crystalline form. Because it seemed pure by TLC, an NMR spectrum of the compound was taken (Table 2).

Qualitative acid hydrolysis. On hydrolysis with 1N methanolic HCl, two components were formed: an aglycone that had R_f (on TLC), color (blue with H_2SO_4 spray and heating), and UV characteristics identical to III; and a sugar positively identified as rhamnose by R_f data on PC and TLC and by its characteristic vivid yellow color with H_2SO_4 spray. The R_f value of rhamnose and the hydrolyzed sugar was 0·68 in 30% MeOH in $CHCl_3$, (TLC); R_{glu} in EtOAc–HOAc–H_2O (9:2:2) visualized with aniline phthalate spray, 2·5

(+)-*Lyoniresinol dimethyl ether* (X). A mixture of 300 mg of IV, 1·25 g of anhydrous K_2CO_3, 0·5 ml of freshly distilled dimethyl sulfate, and 5 ml of acetone was refluxed for 4 hr. The soln was then cooled and filtered, and the acetone was air-evaporated. The residue was refluxed for 19 hr in 2% sulfuric acid with a small amount of MeOH to aid solution. The MeOH was then boiled off, and the aqueous soln was extracted with $CHCl_3$. The $CHCl_3$ layer was evaporated, and the residue purified by preparative TLC with 15% MeOH in $CHCl_3$ to give 89 mg of the dimethyl ether, X (35% yield). Two recrystallizations from MeOH–H_2O gave pure X, m.p. 156–156·5° (authentic lyoniresinol, 156–156·5°), $[\alpha]_D = +21·5°$ (recorded, +30°).[3] It gave IR bands at 3360 (OH), 2900 and 2820 (CH), 1590, 1490 and 1450 (all Ar), 1405, 1330, 1230 (OAr ether), 1124 (OMe), 1053 (prim OH), 1031, 1005, 940. (Found: C, 64·23; H, 7·15. $C_{24}H_{32}O_8$ requires: C, 64·27; H, 7·19%).

Diacetate of X (XIII). By the same procedure described for compound XII, a diacetyl derivative of X was obtained, m.p. 93–94° (recorded, 88–89°).[4] IR bands (CS_2) were identical to those of XII. (Found: C, 63·16; H, 6·88. $C_{28}H_{36}O_{10}$ requires: C, 63·14; H, 6·81%).

2,6-*Dimethoxybenzoquinone*

A small amount of yellow compound was obtained from earliest fractions from the polyamide column that had been evaporated, and the residue was taken up in a small amount of ethanol. It had m.p., 255°; two singlet peaks in the NMR ($CDCl_3$) at δ 5·85 (olefinic) and 3·82 (OMe) in a 1:3 ratio; and was identical by IR[10] to an authentic sample.

6-*Hydroxy*-5,7-*Dimethoxy*-2-*Naphthoic Acid* (V)

This is the only nonlignan of the group. It gave pale tan needles from MeOH–H_2O, m.p. 215–217° (226–228° before recrystallization). (Found: C, 62·49; H, 4·70; OMe, 25·35; $C_{13}H_{12}O_5$ requires: C, 62·90; H, 4·87; OMe, 25·01). It had R_f in ipram (3:1) 0·42, a fluorescent white spot in UV, changing to fluorescent lavender-white with NH_3; IR bands at 3450 (OH), 2940 (CH), 2560 (broad, COOH), 1675 (acid C=O), 1625, 1615, 1480 (all Ar), 1410 (ArOH), 1336, 1280 (ArO ether), 1088 (OMe), 1020, 935 (COOH), 893. The proof of structure of this compound will be published later.

Acknowledgements—The authors thank Prof. W. Klyne, of the University of London, for ORD spectra and for his comments on their results; Prof. Y. Kato of the Nagoya City University, Japan, for samples of the dimethyl ethers of (+)-lyoniresinol and *epi*-lyoniresinol; Prof. K. Weinges of the University of Heidelberg for samples of (+)-dimethoxyisolariciresinol and related compounds; Forest Products

Laboratory scientists D. B. Johnson and M. F. Wesolowski for NMR spectra, Dr. D. F. Zinkel for additional ORD determinations, Dr. C.-L. Chen for helpful discussions on the problem; and the North Central Forest Experiment Station, Rhinelander, Wis., for supplying the wood.

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