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LIGNANS OF *ULMUS THOMASII* HEARTWOOD—I

THOMASIC ACID*

M. K. SEIKEL, F. D. HOSTETTLER and D. B. JOHNSON

Forest Products Laboratory,[2]† Forest Service, U.S. Department of Agriculture, Madison, Wisconsin 53705

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Abstract—The compound principally responsible for the vivid yellow-green fluorescence of basified aqueous extracts of *Ulmus thomasii* Sarg. heartwood is an unsaturated lignan in the free acid form with syringyl patterns of substitution. Spectral and degradative studies have shown that it is the 1,2-dihydro-1-phenylnaphthalene I; it has been named thomasic acid.

WOOD anatomists group four elms growing in the United States, *Ulmus thomasii* Sarg., *U. alata* Michx., *U. serotina* Sarg., and *U. crassifolia* Nutt., under the name "rock elm", since their wood is anatomically similar. The four woods are most easily distinguished by differences in the UV fluorescence of the aqueous extracts of the heartwood. Extracts of *U. thomasii* exhibit a bluish fluorescence which brightens to a vivid light yellow-green when base is added. Paper chromatography revealed one principal spot giving this color reaction, plus several minor ones, and work was undertaken to identify the water-soluble compound responsible.

Preliminary paper chromatographic and spectral work showed that the compound was a phenolic acid. Only basic media gave a good assortment on paper of the many spots derived from the extract and, of these, isopropyl alcohol-2N ammonia was outstandingly the best. The acidic character of the compound was also shown by failure of the spot to move in ethyl acetate–ammonia, and movement to the front in ethyl acetate–hydrochloric acid.[1] Because of the fluorescence, it was postulated that the compound was a hydroxycinnamic acid or a derivative.[2,3] Although on paper it gave the typical color reactions of sinapic acid with *p*-nitrobenzenediazonium ion and overspray with carbonate (pink-orange and blue, respectively) and in the Maüle test for syringyl groups, its R_f value was considerably larger (0·60 *vs* 0·30 in isopropyl alcohol-2N ammonia (3:1)). Isolation of small amounts in crude form or in solution by preparative paper chromatography,‡ and spectral studies substantiated the phenolic and acidic character (UV maxima shifted bathochromically by sodium ethylate but far less than observed for hydroxycinnamic esters,§ bathochromically by aluminium chloride,[4] and hypsochromically by sodium acetate[5] (Table 1) and

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

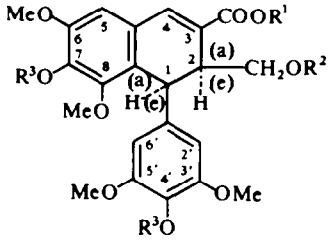
‡ Preparative TLC on silica plates cannot be used, as the compound discolors quickly in daylight on the adsorbent.

§ The shift of 26 mμ approximates that of caffeic acid 3-glucoside (24 mμ), and is far less than the 40–65 mμ shift of the esters.[5]

in the IR a band at 1695 cm^{-1}). Attempted hydrolysis studies* showed that the compound was stable to base (in great part, even to fusion with base), stable to vigorous acidic hydrolytic conditions (refluxing with 3N HCl for 8 hr), and that no sugar or other alcoholic moiety was cleaved from the molecule. Therefore, since the properties of the material checked with none of the well-known hydroxycinnamic acids and it did not degrade to them on hydrolysis, it could not be identified by these simple micro procedures.

Isolation of crystalline material. Isolation of the soluble phenolic acid in 0·2% yield from the heartwood of *U. thomasii* turned out to be relatively simple. It was readily extracted from the ground wood with cold water and readily fractionated from co-occurring related compounds on a nylon (polyamide) column. Large amounts precipitated directly in the 40% methanol eluates from the column even without cooling. The only crucial conditions were the acidity of the applied extract, which had to be reduced to pH 3 or less to keep the acid in unionized form (cf. Table 1, footnote *b*), and a nylon absorbent which had also been acid treated to prevent it from acting as an ion exchanger.

Postulated structure. Analytical and spectral work on the crystalline acid coupled with biogenetic considerations gave a molecular formula of $C_{22}H_{24}O_9$ and led to formula I as by far the most possible structure. Since this 1,2-dihydro-1-phenylnaphthalene was previously unreported, the compound was named thomasic acid. Evidence used for postulating this structure follows.



	R¹	R²	R³
I, thomasic acid	H	H	H
II, dimethyl ether	H	H	Me
III, trimethyl derivative	Me	H	Me
IV, trimethylacetyl	Me	Ac	Me
V, triacetyl derivative	H	Ac	Ac

The acid group was shown by titration in addition to the chromatographic behavior, IR and UV already discussed; that it was monobasic was proven by the mol. wt determination. A chromophoric system similar to that of sinapic acid (4-hydroxy-3,5-dimethoxycinnamic acid) was suggested by the similar color tests and by the similar UV spectral shift with base (sinapic, 322 to 354 mμ). Conjugation of the phenolic group with the unsaturated side chain was indicated by the last, while conjugation of the acid group was indicated by the hypsochromic acetate shift. That the molecule was larger than sinapic acid was shown by the approximate neutralization equivalent and mol. wt determinations. That an extra syringyl group might be present was determined from a careful study of the IR spectra of thomasic and sinapic acids (Fig. 1); sinapic showed fewer aromatic and phenolic hydroxyl bands (e.g. in Fig. 1 the aromatic peak at 1500 cm^{-1} is missing in sinapic acid, that at 1580 cm^{-1} is less prominent, and the region from 1375 to 1190 cm^{-1} where ArO—H and —O—Ar bonds absorb has sharp, distinct peaks instead of broad, overlapped bands).

* The sensitivity of the UV maximum to pH (see Table 1, footnote *b*) contributed to considerable confusion in the early work.

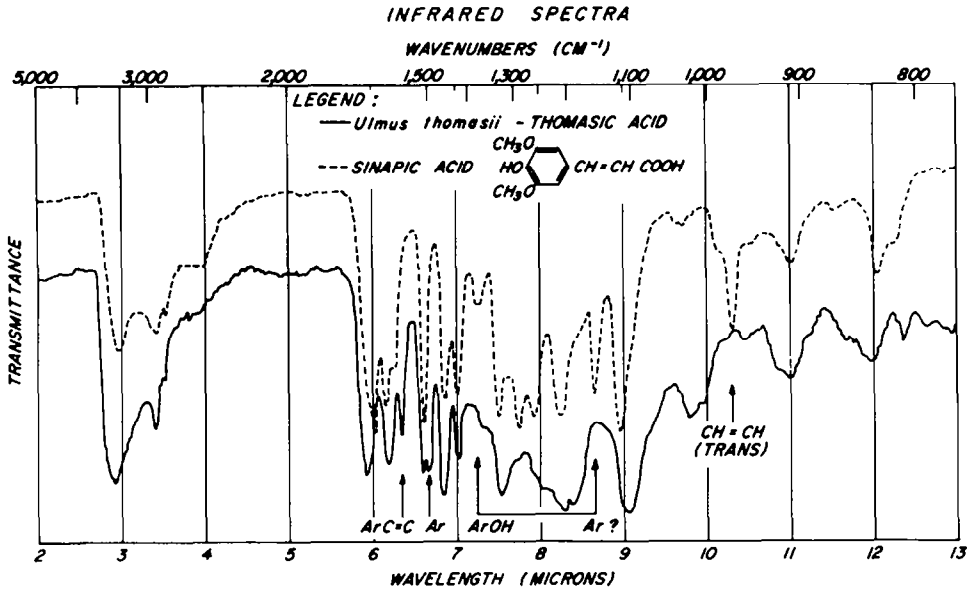


FIG. 1. Infrared spectra of thomasic and sinapic acids. M 130 178

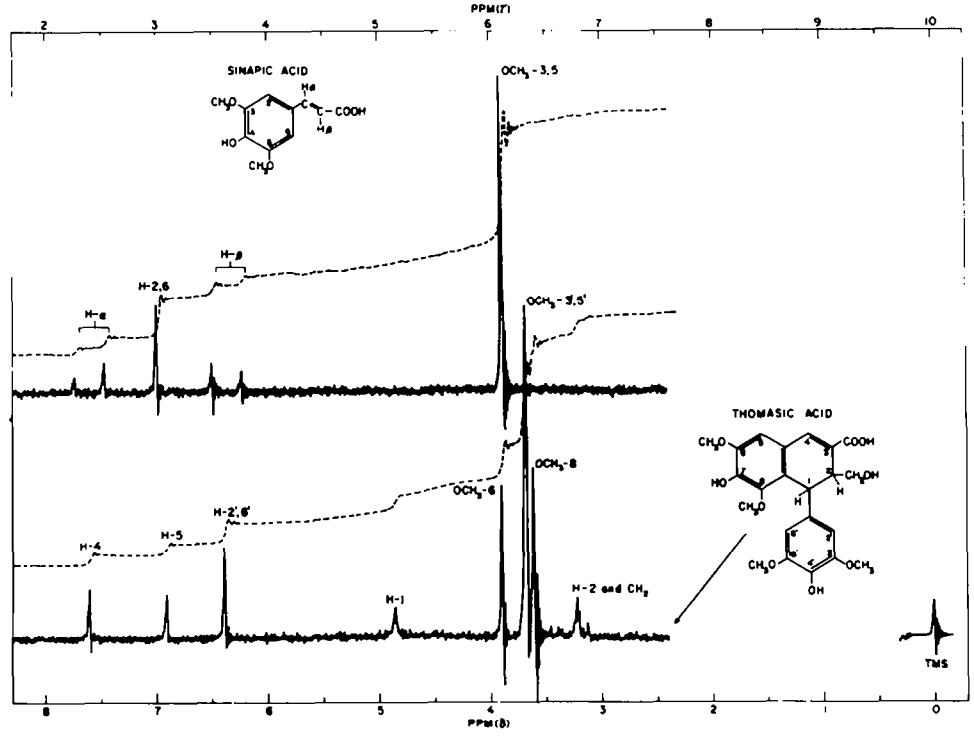


FIG. 2. NMR spectra of thomasic and sinapic acids. M 132 956

Further and corroborative evidence for structure I was obtained from the NMR spectrum, the simplicity of which with seven singlet peaks (Fig. 2) placed severe restrictions on the interpretation. The four OMe groups originally postulated from the analytical data were clearly substantiated by the three peaks representing four OMe groups at $\delta = 3{\cdot}9$–$3{\cdot}6$. Of these, only the downfield signal was the same as sinapic and also ferulic acids, and that was assigned to the OMe in the 6 position of thomasic acid. The two similar OMe groups upfield from this (at 3·70 ppm) were assigned to the unconjugated syringyl group and, in fact, study of the saturated model 2,6-dimethoxy-4-propylphenol showed its OMe groups at 3·80 ppm. The farthest upfield peak was assigned to the 8-OMe, since a model showed that the *peri* situated syringyl group in equatorial conformation would force the OMe group above the plane of this benzene ring and hence into the region shielded by the ring current.

In the aromatic region the lone ethylenic hydrogen, H-4, was considered responsible for the downfield singlet, since it showed the same shift as the H-α doublet of sinapic acid. Another singlet had the same shift as the H-β doublet of sinapic acid but, since it represented two H-atoms and fell in the same region as the singlet of the two aromatic protons of 2,6-dimethoxy-4-propylphenol, it was assigned to the more shielded H-2′,6′ of the pendant syringyl group. The third aromatic singlet at 6·92 ppm fell only slightly upfield from the H-2,6 signal of sinapic acid and was assigned to H-5.

The seventh singlet, at 4·84 ppm, was assigned to a dibenzylic hydrogen, H-1, for which assignment there is considerable support in the lignan literature.[6,7] That this was unspilt, although broad, must mean that the dihedral angle between H-1 and H-2 approaches 90°.[8] The H-2 and —CH_2O— protons must be responsible for the diffuse multiplet equal to two protons at 3·1–3·5 ppm and for the one proton under the OMe peak at 3·7 ppm.

The carboxylic, phenolic, and alcoholic protons gave no clear signals in the NMR spectrum, although the addition of deuterium oxide indicated that 3–4 protons were concealed in a broad band from 4–5·5 ppm.

Structures closely related to structure I have been reported, although no lignans with a dihydronaphthalene nucleus are known. Unsaturation has been found in noncyclized lignans such as guaiaretic acid[9] and savinin,[10] and in naphthalene derivatives such as justicidin A and B, diphyllin,[11] dehydropodophyllotoxin,[12] and dehydroguaiaretic acid.[13] Most acidic lignans exist as lactones such as conidendrin, the podophyllotoxins and peltatins,[9] but recently free plicatic acid has been isolated.[6] Other disyringyl lignans have been found in hardwoods; namely, dimethoxyisolariciresinol (lyoniresinol) as its xyloside, lyoniside in *Alnus*[14] and in *Lyonia*,[7] and lirioresinols and syringaresinols in *Populus*[15] and as a glycoside in *Liriodendrin*.[16]

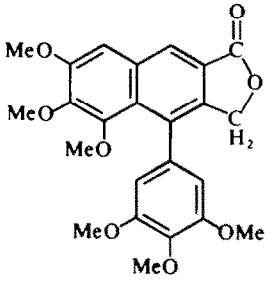
Proof of structure. Preparation of derivatives of thomasic acid and a study of their UV, IR, and NMR spectra has corroborated structure I; see Tables 1, 2, and 3.

Two phenolic groups and the acid group were indicated by conversion to the dimethyl and trimethyl derivatives II and III with diazomethane. That III was an ester was shown by the lack of base shift in the UV and a carbonyl band at 1700 cm^{-1} in the IR. Also, simple basic hydrolysis of III gave II which then showed a hypsochromic UV shift with base resulting from the free, conjugated carboxyl group. The

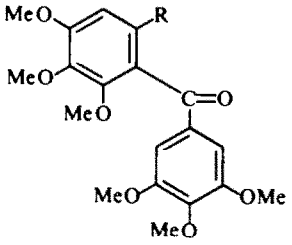
NMR spectra and OMe analyses substantiated the number of new OMe groups in these derivatives.

The presence of a third and alcoholic OH group was shown by the preparation of a triacetyl derivative V and the monoacetyl derivative of III; the number of Ac groups was clearly shown by NMR (Table 2).

Attempts to prove the 1,2-position of the carboxyl and the hydroxymethyl groups by aromatizing II to VI failed. With N-bromosuccinimide, palladium-carbon, dichlorodicyanobenzoquinone, and lead tetraacetate, no naphthalenic lactone could be isolated. We conclude that this failure to aromatize must be caused by steric difficulties because of the *cis* and axial position of the hydroxymethyl group.

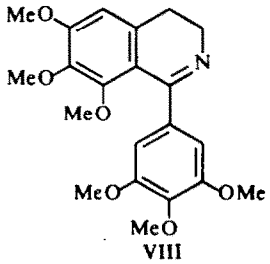


VI

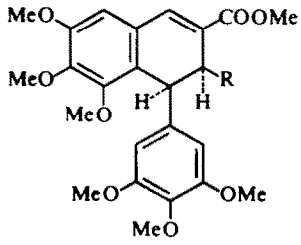


VII, R = COOH
XI, R = CH=CH$_2$

The hexa-oxygenated diphenylmethane backbone and the relative position of the second side chain was proven by a permanganate oxidation of II or III to 3,4,5-trimethoxy-2-(3,4,5-trimethoxybenzoyl)benzoic acid, VII, and 3,4,5-trimethoxybenzoic acid. The latter was identified by chromatography and UV spectra. The sample of VII obtained melted 5° below the recorded value;[17] it gave, however, the expected NMR spectrum (Table 2), a UV spectrum with a double maximum, one of which shifted with base as expected for a conjugated carboxyl group, and an IR spectrum indicating two different CO groups in the molecule, one of which showed a base shift with diethylamine characteristic of a carboxylic carbonyl.[18] Because of the discrepancy in m.ps, VII was synthesized by two methods. A Friedel-Crafts reaction between ethyl 3,4,5-trimethoxybenzoate and 3,4,5-trimethoxybenzoyl chloride gave an infinitesimal yield of material with the same m.p., R_f, UV, and IR spectra as the degradation product. A more successful synthesis paralleled a synthesis of a triethoxymethoxy-2-benzoylbenzoic acid[19] by starting from the trimethoxybenzoyl derivative of mescaline and proceeding through the new compounds VIII-XI.



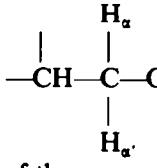
VIII



XII, R = CHO
XIII, R = CH=NNHC$_6$H$_3$(NO$_2$)$_2$
XIV, R = COOH

Oxidation of the vinyl compound XI produced a benzoylbenzoic acid, the m.p. and IR spectrum of which checked exactly the properties of the acid obtained by degradation.

Proof of the primary $-CH_2OH$ group and its position was more difficult to obtain. Since the NMR spectra of I and II both showed an uninterpretable multiplet for these protons plus H-2, the two acetyl derivatives IV and V were prepared in order to study the downfield shift of the methylene protons. Although the expected shift of about 1 ppm for primary alcohols was obtained and the integration suggested two protons, no clearcut multiplet was obtained, and it was partially hidden by the OMe signals. Even with the solvents acetonitrile and pyridine, the multiplets obtained, although shifted slightly more downfield, were not improved. A reaction of I *in situ* with trichloroacetyl isocyanate[20] gave similar results, and again the shift of 0·8 ppm was of the magnitude expected for a primary alcohol. It was concluded that the



—CH—C—OR was acting as an ABC group, H-α and H-α′ being different because of the asymmetry of C-2.[21] In a different approach, the signal of the OH group and its splitting by H-α and H-α′ as observed in DMSO[22] was determined for III. The result was an unsymmetrical multiplet,* which did not change in character as the temperature was raised to 100°, although it shifted upfield. Study of this band on a 100 mc NMR instrument† showed this group as an unclear quartet, indicative of splitting by two different protons and hence in a line with a $-CH_2OH$ group attached to an asymmetric carbon as outlined above.

The presence of the primary OH group was evidenced by a study of the position of the OH band in the fundamental region of the near IR. II gave the band in exactly the same position (3634 cm^{-1}) as a series of model primary alcohols; it was well above those of secondary and tertiary alcohols.

The primary alcohol group has been substantiated chemically. Oxidation of II with a chromic-sulfuric acid mixture produced an aldehyde, XII, purified as its dinitrophenylhydrazone XIII, and an acid XIV. That XII was an aldehyde and not a ketone was shown by the singlet NMR signal at δ 9·60, and by the UV spectrum of XIII and its change with base according to the method of Jones *et al.*[23] The bright yellow color of XIII also showed that the CO was nonconjugated. The NMR spectrum of XIII checked exactly that given by Curtin *et al*[24] for the dinitrophenylhydrazone of an aliphatic aldehyde with the system —CHCH=NNH—; it showed the expected doublet at δ 7·44 (J = 4·5 c/s) for the originally aldehydic proton. The same splitting was observed in a quartet at 4·08 ppm which was therefore assigned to H-2. This coupling of vicinal protons in XIII contrasted with the lack of splitting of the signal of the aldehydic proton in the spectrum of the free aldehyde XII. However, J_{AB} is always small for aldehydic protons; for example, for the closely related cyclohexanal J = 1 c/s.[25]

* Several model compounds, including tetrahydrofurfuryl alcohol and cinnamyl alcohol, gave sharp triplets.

† Kindness of H. Kagan, Collège de France, Paris.

Evidence for the asymmetric C-2 and its attached proton was available from the NMR spectra of XII, XIII, and XIV, and of III in DMSO. The asymmetry was supported by the dissimilarity of H-α and H-α′ as revealed in the multiplicity of various signals. H-2 was visualized clearly as a distinct peak in the spectrum of the acid XIV. It showed very weak coupling with H-1, enough to split the peak slightly, although the H-1 signal was merely broad. H-2 also showed as a quartet in the spectrum of XIII, where it was coupled with H-α and weakly with H-1, and as a doublet in the spectrum of XII in acetonitrile, where it was coupled only weakly with H-1. In these two cases, the H-1 signal was just discernibly split. This evidence corroborated earlier ideas on the approximate 90° dihedral angle between H-1 and H-2.

A few points on the structure have not been definitely clarified by the degradation studies, but for the most part depend on spectral results and bear repetition. They

TABLE 1. UV DATA FOR THOMASIC ACID AND DERIVATIVES[a]

Compound	Solvents and reagents			
	EtOH, 95%	+NaOAc	+NaOEt	+$AlCl_3$
I[b]	330·5 (ε = 13,500)[c]	311	356	334
			294 sh	
	246 (ε = 22,600)	242	253[d]	247
II	305		298	308
	242		239	
			234	
III	307 (ε = 14,700)	No shift	No shift	
	243 (ε = 25,600)			
V	330 sh	320 sh	292–288[e]	
	295	292		
	238	286 sh		
		238 sh		
VII	282 (ε = 14,300)	281	281	286
	264 (ε = 13,600)	248 tr sh	248 tr sh[f]	263
XIII	350 (in $CHCl_3$)		510 sh[g]	
			433	
XIV	305			
	243		No shift	

[a] λ_{max} in mμ; sh = shoulder, tr = trace.

[b] An electrometric titration with aqueous base coupled with a determination of the absorption maximum at every pH unit or less showed that the acid existed in the unionized form, λ_{max} in water 322 mμ, only at less than pH 3·5; the carboxyl group is almost completely ionized by pH 4, λ_{max} 307–304 mμ from pH 4 to 8·5; and the phenolic hydroxyl ionizes from pH 8·5 to 9, the λ_{max} rising rapidly to 356 mμ, and the solution assuming a pale yellow color.

[c] Exact wavelength very dependent on pH.

[d] Reverted to 323, 247 mμ on addition of HCl; see *c*.

[e] After 25 min, gave 358 mμ due to hydrolysis.

[f] Reverted on addition of HCl to 281, 264 mμ.

[g] With 0·25N NaOH in EtOH according to procedure of Jones *et al.*[23]

TABLE 2. NMR DATA FOR THOMASIC ACID AND ITS DERIVATIVES[a]

Compound	Solvent	H-1[b]	H-2	—C(H)—OR²(H)	H-4	H-5	H-2',6'	No. of OCH_3 signals	OCH_3 and other
I	acetone-d_6	4·84	[3·1–3·7 m]		7·60	6·92	6·40	3	OCH_3-3',5'; 3·70 -8; 3·61 -6; 3·92
	pyridine	5·67	4·42 (q, J = 8,3) *c*					3	OCH_3-3',5'; 3·61 -8; 3·83 -6; 3·91
II	$CDCl_3$	4·67	[3·2–3·5 m]		7·68	6·73	6·27	4	OCH_3[d] COOH; 6·66
III[e]	DMSO-d_6	4·69	?3·47[f]	2·91 (d, J = 5) 3·10 (q, J = 5,2)	7·59	7·06	6·22	6	CH_2O^{II}; 4·90 (q)[g]
IV	$CDCl_3$	4·50	3·37 (d, J = 6)[f]	4·07 (d, J = 6)[f]	7·59	6·69	6·24	4	OCH_3[d] ali. acetyl; 2·00
V	$CDCl_3$	4·54	4·12 (m)[f]		7·71	6·77	6·29	3	OCH_3[d] ali. acetyl; 1·95 aro. acetyl; 2·28 2·33

VII[k]	$CDCl_3$					7·46	7·03	4	OCH_3[l]
XII	$CDCl_3$	5·02	3·93[f]	9·60[j]	7·76	6·71	6·27	5	OCH_3[d]
	CH_3CN	4·97 (d, J = 1)	3·93 (d, J = 1)	9·60[j]	7·72	6·92	6·30	4	OCH_3[k]
XIII	$CDCl_3$	4·97[b]	4·12 (q, J = 4,1)	7·44 (d, J = 4)[j]	7·68	6·73	6·29	4	OCH_3[d]
XIV	$CDCl_3$	5·06[b]	4·09 (d, J = 1,5)		7·72	6·78	6·33	4	OCH_3[d]

[a] δ values in ppm relative to tetramethylsilane. Singlets except as noted; d = doublet, m = ill-defined multiplet, q = quartet.
[b] Broad, but generally unsplit; occasionally J = 1.
[c] Hidden by OMe signal.
[d] See Table 3 for average values for OMe protons.
[e] Figures given for 100 mc instrument with deuterated dimethylsulfoxide (footnote 30).
[f] Unclear and perhaps multiplet hidden by OMe signal.
[g] Diffuse; disappears with D_2O.
[h] Shifts catalogued under original position of proton in thomasic acid.
[i] 3·97, 3·92, 3·83, and 3·73 for 2,1,2, and 1 OMe groups respectively.
[j] Aldehydic H.
[k] 3·86, 3·80, 3·72, and 3·67 for 1,1,1, and 4 OCH_3 groups respectively.

TABLE 3. AVERAGE PROTON SHIFTS IN NMR SPECTRA OF THOMASIC ACID DERIVATIVES[a, b]

Proton	H-1	H-4	H-5	H-2',6'	OCH_3 protons in positions				
					6	8	3',5'	7	4' and R[1]
Shift, δ	variable	7·69 ±0·04	6·74 ±0·03	6·28 ±0·02	3·90 ±0·01	3·63 ±0·03	3·73 ±0·02	3·90 ±0·005	3·80 ±0·02

[a] For II, IV, V, XII, XIII, and XIV, where applicable, in $CDCl_3$.

[b] With exception of hydroxyl, $—CH_2O—$, and H-2 protons, the signals of which were missing or not clearly defined.

include the following. That the carboxyl group is definitely attached to C-3 is attested for by the spectral shift on the addition of sodium acetate which shows that it is part of the conjugated system (acid XIV shows no such shift). That the unsaturation is Δ^3 and not Δ^1 is proven by the presence of only one, not two, benzylic protons in the NMR spectra. The syringyl pattern of substitution is supported by the Maüle test and in ring A by the spectral shift with base and the similarity of the NMR spectrum with that of sinapic acid. Two hydrogens and two OMe groups are equivalent in all the NMR spectra, so the substitution in the 3',4',5'-positions of ring C (proven by degradation) must be the symmetrical syringyl pattern.

The stereochemistry of thomasic acid can be deduced in part from the evidence at hand. Since it does not exist as a lactone as do the members of the podophyllin and picropodophyllin series, as well as conidendrin,[9] and since the carboxyl group must be planar, the hydroxymethylene group on C-2 must be axial. Since the equatorial H-2 and H-1 couple only very slightly, the dihedral angle of their bonds must approach 90°. This could occur if (a) they were *cis* and the syringyl group equatorial or (b) they were *trans* and the syringyl axial. Condition (a) seems more probable from steric considerations and is supported by the marked upfield shift of the 8-OMe group. When the pendant Ph group is placed in an equatorial position in a model, the OMe forces this Ph group to twist into a position out of the plane of the remainder of the molecule. This places the OMe directly above the π system of the ring where it would be shielded and its signal shifted upfield. If condition (b) existed, the 8-OMe and the pendant syringyl group would be widely separated and would show no interaction.

Thomasic acid seems to occur in an optically inactive form. Both it and its derivative III show zero rotation at the D line, and attempts* to obtain an ORD curve of III failed to produce clear evidence for activity.

Thomasic acid, therefore, is 6-hydroxy-4(4-hydroxy-3,5-dimethoxyphenyl)-3-hydroxymethyl-5,7-dimethoxy-*cis*-3,4-dihydro-2-naphthoic acid, I, an unsaturated syringa-cyclolignan[26] (a 1,2-dihydro-1-phenylnaphthalenic lignan).

Related compounds. Three other phenolic compounds in the aqueous extract of *Ulmus thomasii* heartwood have been investigated, and two of these are closely related to thomasic acid. This work will be reported later.

* Kindness of Prof. W. Klyne, University of London, and of Prof. J. P. Kutney, University of British Columbia.

EXPERIMENTAL

All m.ps are uncorrected.

UV spectra. Spectra were determined on a Beckman DK recording spectrophotometer. Except for spectra of H_2O extracts of the wood and H_2O—MeOH eluates of the polyamide column, all spectra were determined in 95% EtOH except as noted. Diagnostic shifts were obtained by adding 3 drops of the following solns to 3 ml of the soln in the quartz cell: (1) 1N NaOEt in EtOH (or 10% NaOH to H_2O solns); (2) excess solid NaOAc; and (3) 5% $AlCl_3$ in EtOH.

IR spectra. All spectra were determined on a Baird infrared recording spectrophotometer in KBr pellets except as noted.

NMR spectra. Spectra were determined in the solvents noted in Table 2 on a Varian A-60 NMR spectrometer. To some solutions, D_2O was added to check on exchangeable hydrogen.

*Paper chromatography (PC)** One- and two-dimensional paper chromatograms were run on Whatman No. 1 paper in isopropyl alcohol-2N ammonia (3:1) and (2:1), respectively. Sheets were routinely viewed in UV in the presence of NH_3; a few unfumed sheets were sprayed with 0·5% p-$NO_2C_6H_4N_2^+BF_4^-$ and oversprayed with 20% Na_2CO_3.

Thin-layer chromatography (TLC). Fluorescent SiO_2 plates (silica HF_{254}, Brinkman Instruments, Inc., New York) were developed with 0·7–10% MeOH in $CHCl_3$, depending on the polarity of the compounds. For acids, 3% acetic acid in $CHCl_3$ was preferred. Carbonyl compounds were visualized with a 0·05% 2,4-dinitrophenylhydrazine in 2·4N HCl spray, the benzoyl-benzoic acid VII with a conc H_2SO_4 in ether spray plus heating.

Column chromatography. Commercial nylon powder (type 66-D, the Polymer Corporation, Reading, Pa.) was purified and acidified by stirring successively with the following solvents: acetone (2×), hot MeOH (2×), boiling MeOH—H_2O (7:3) (2×), 1N HCl (1×), and cold H_2O (about 8×) until the pH of the filtrate was that of distilled H_2O. It was air-dried for storage. To prepare the column, about 90 g was suspended in 500 ml H_2O, stirred ½ hr, and poured into a large column (63 × 4·5 cm). The column was allowed to settle overnight before press was applied.

High-grade silica gel (Merck, 0·05–0·2 mm) was used in ratio of 300:1 of applied material, and columns were packed by a deaerated slurry and developed by the solvents noted.

Isolation of thomasic acid

Extraction of heartwood. Heartwood of *U. thomasii* from the Nicolet National Forest, Wisconsin, fresh-cut or up to 18 months old, was ground to pass a 40-mesh screen. A 575 g sample was packed fairly tightly into a 79 × 7·5 cm column and chromatographically extracted in two portions with cold H_2O. The first 600 ml of dark brown eluate was used unconcentrated, the second 1500 ml was freeze-dried (to avoid the excessive foaming of rotary evaporation *in vacuo*) to reduce the volume to about ⅓. The content of thomasic acid and related compounds in the extracts was determined by obtaining the absorbance of the base-shifted maximum of the UV spectrum and calculating it in terms of thomasic acid; approximately 4·6 g was obtained from the above sample, or 0·8%.

Fractionation on polyamide column. The aqueous extracts were acidified to pH 1-3 with HCl and filtered. The ppts were redissolved in carbonate, and the soln reacidified and refiltered. The combined filtrates (approx 600 ml) were applied to a 22 × 4·5 cm polyamide column and light press applied. After elution with 200 ml water and 300 ml 20% MeOH, I was eluted with 40% MeOH, the course of the fractionation being followed by PC. Almost pure material crystallized from the three or four consecutive 20 ml fractions containing the greatest amount of I, either on standing or after cooling. Further lots could be isolated by evaporation of the filtrates and from later fractions shown by UV spectral analysis to possess only the 318 and 239 mμ peaks. The yield of crystalline material from the above sample (two columns) was 1·0 g, 0·18% of the wood. It could be recrystallized from water (4 mg/1 ml), or if very impure it was purified by reapplication to another column.

Properties. I crystallizes in colorless flat needles, m.p. 232–234°, after drying over P_2O_5 *in vacuo*, $[\alpha]_D^{25}$ 0°, approx neut eq 450 (calc 432) approx M.W. 480, positive Mañle test, pale yellow with NaOH, vivid fluorescent green-yellow in dilute NaOH in UV, yellow changing to orange with conc H_2SO_4, R_f in isopropyl alcohol-ammonia (3:1) 0·62, (2:1) 0·74, IR bands (Fig. 1) at 3440 (OH), 1695 (carboxyl C=O), 1615 (C=C, non-aromatic), 1578 (ArC=C), 1515 and 1500 (both Ar), 1460 and 1425 (COOH),

* Preliminary developmental paper chromatographic work was done by Linda Feldman.

1327 (CH_2OH), 1205 and 1190 (ArOH), 1103 (OMe), 1022 (CH_2OH), and 910 cm^{-1} (COOH dimer). (Found: C, 61·25; H, 5·72; OMe, 28·86. $C_{22}H_{24}O_9$ requires: C, 61·10; H, 5·59; OMe, 28·71%.)

Derivatives of thomasic acid

Dimethyl ether (II). I (about 0·2 g) was methylated in MeOH with an ethereal soln of diazomethane by the procedure of Hartwell *et al.*[27] Generally two treatments with the reagent were required before TLC showed that the reaction was complete. After filtration from polymeric products of the diazomethane and evaporation of the solvents, 5 ml each of MeOH and 2N KOH were added, and the mixture refluxed 1 hr. After evaporation of the MeOH, acidification, and cooling overnight, the crude gummy ppt was heated with water until it grew crystalline; then AcOH was added for recrystallization. After two recrystallizations, the tiny colorless needles, m.p. 174–175° (50–60% yield) showed a single spot on TLC (R_f 0·18 in $CHCl_3$—MeOH (9:1)),* $[\alpha]_D^{25}$ 0°, new IR bands at 1410 and 1345 (both C—H), and 1118 cm^{-1} (OMe); IR bands also at 3634 (CH_2OH) and 3543 cm^{-1} (COOH). (Found: C, 62·70; H, 6·55; OMe, 40·8. $C_{24}H_{28}O_9$ requires: C, 62·60; H, 6·13; OMe, 40·4%.)

Trimethyl derivative (III). The preceding preparation was interrupted before the hydrolysis step and the ester crystallized from the residue by triturating with aq MeOH or heating with hot water. Samples which did not crystallize readily were separated from polymeric material by chromatography on a silica column developed first with $CHCl_3$ and then with 1·5% MeOH in $CHCl_3$. III was obtained after two or three recrystallizations from MeOH—H_2O (1:2) in 50–70% yield as compact white rosettes, m.p. 121·5–122·5°, R_f 0·95 in $CHCl_3$—MeOH (9:1),* new IR bands at 1700 (ester C=O), 1250 cm^{-1} (ester C—OMe). (Found: C, 63·26; H, 6·63; OMe, 45·72. $C_{25}H_{30}O_9$ requires: C, 63·28; H, 6·37; OMe, 45·78%.)

Trimethyl-acetyl derivative (IV). III (100 mg) was acetylated by the method described below for the preparation of V. After recrystallization from benzene–ligroin, a 59% yield of white crystals was obtained, m.p. 113·5–114·5°. This was analyzed only by NMR (Table 2).

Triacetyl derivative (V). I (117 mg) was acetylated according to the procedure of Hartwell and Detty[28] by simple refluxing for 1 hr with Ac_2O. The gum obtained on working up the product with water was crystallized most effectively by warming for 1·5 hr with water, although the product still showed minor impurities on TLC. After one recrystallization from C_6H_6-ligroin (57% yield) and two from MeOH–H_2O, the needles melted at 197·5–198°, new IR bands at 1765 and 1735 (aliphatic and aromatic acetyl C=O), 1710 (acid C=O), 1192 (phenolic acetate), 1130 (in addition to 1112 cm^{-1}). (Found: C, 60·19; H, 5·33; Ac, 23·03. $C_{28}H_{30}O_{12}$ requires: C, 60·21; H, 5·41; Ac, 23·12%.)

Oxidative degradation to keto acid (VII)

According to the procedure of King *et al*,[18] 500 mg of powdered $KMnO_4$ was added during the course of 1 hr to a refluxing soln of 100 mg either II or III in 15 ml acetone. After concentration to 3 ml, the addition of 3 ml H_2O to decompose excess MnO_4^-, and 10 min of further refluxing, the mixture was diluted with water and MnO_2 was removed by filtration. The filtrate, after the acetone was distilled off on the steam bath, was purified by extraction with ether and then acidified. Long cooling finally yielded successive small lots of gummy ppt; the final clear filtrate was evaporated to ½, and 15·6 mg crystals (18% yield) then separated. After two recrystallizations from MeOH–H_2O (1:2), the keto acid melted at 181–182° [recorded for 3,4,5-trimethoxy-2-(3,4,5-trimethoxybenzoyl)benzoic acid · 2½ H_2O, 186·7–187°[23]], negative test with 2,4-dinitrophenylhydrazine, bright purple with conc H_2SO_4,[29] a single spot on TLC, IR bands at 2620 (carboxyl OH), 1720 (aryl acid), 1675 (diaryl ketone), 1590 and 1505 (both Ar), 1455, 1415, 1333,† and 1228 (aromatic ether), 1130 and 1112 (both OMe), and 990 cm^{-1} (aryl ether); IR in $CHCl_3$, 1710 and 1680 (aryl acid), and 1660 (diaryl ketone), plus Et_2NH[24] 1660 (diaryl ketone) and 1625 (COO^-). (Found: C, 59·12; H, 5·39. Calc for $C_{20}H_{22}O_9$: C, 59·11; H, 5·46%.)

This material did not lower the m.p. of the synthetic sample of VII, showed the same color reactions, gave the same R_f values on TLC (0·66 in 5% AcOH in $CHCl_3$),‡ and gave identical UV, IR, and NMR spectra.

A second soluble acid was isolated by evaporating the filtrate of VII and by separating the components by preparative TLC with 5% MeOH in $CHCl_3$. Elution of the principal band with EtOH gave a soln,

* I, R_f 0 in same solvent.

† Most intense band, occurring in three diaryl ketones (VII, X, and XI) and in aldehyde derivative XIII.

‡ Distinguished from trimethoxybenzoic acid (see following paragraph) by H_2SO_4 spray, giving a purple color.

the spectral and chromatographic properties of which checked those of 3,4,5-trimethoxybenzoic acid: UV, λ_{max} 261 mμ shifted to 264 mμ (increased intensity) with $AlCl_3$ and with NaOAc to 250 mμ with a shoulder at 283 mμ; PC, an absorbing spot, R_f in isoPrOH–2N NH_3 (3:1) 0·70, 5% AcOH 0·80, C_6H_6—AcOH–H_2O (125:72:sat) 0·85; TLC, 5% AcOH in $CHCl_3$ 0·85, 5% MeOH in $CHCl_3$ 0·35.

Synthesis of 3,4,5-*trimethoxy*-2-(3,4,5-*trimethoxy*-2-*benzoyl*) *benzoic acid* (VII)[19]

6,7,8-*Trimethoxy*-1-(3,4,5-*trimethoxyphenyl*)-3,4-*dihydroisoquinoline* (VIII). 3,4,5-Trimethoxy-N-[2-(3,4,5-trimethoxyphenyl)ethyl]benzamide* (8·2 g, m.p. 176–177°) in 30 ml toluene was refluxed with 8·5 ml $POCl_3$ for 2 hr. After removal of the solvents, the residue was dissolved in boiling 10% HCl and the mixture again evaporated to dryness *in vacuo*. The residue was dissolved in 350 ml $CHCl_3$, the soln shaken twice with 2N Na_2CO_3, dried over $MgSO_4$, and the solvent evaporated. The oily residue was crystallized from a minimum of hot benzene plus pet. ether and, after recrystallization from benzene–ligroin, yielded 6·5 g (87%) of VIII, m.p. 119–120°, IR bands at 1600, 1585, 1562, 1505, 1490 (all Ar), 1455, 1410, 1358, 1318, 1232 (aryl ether), 1192, 1158, 1110 (OCH_3), 1095, 1053, 1011, 1000 (aryl ether), and 833 cm^{-1}. (Found: C, 65·13; H, 6·63. $C_{21}H_{25}NO_6$ requires: C, 65·10; H, 6·50%.)

Methiodide of VIII (IX). A soln of VIII (4·5 g) in 20 ml MeI was refluxed for 20 min, the solvent removed *in vacuo*, and the crystalline residue extracted with isopropyl alcohol and recrystallized from H_2O, 5·8 g (95%), m.p. 196–197°, new IR bands at 1620, 1375, and 1202 cm^{-1}, and loss of bands at 1505 and 1192 cm^{-1}. (Found: C, 50·06; H, 5·60. $C_{22}H_{28}NIO_6$ requires: C, 49·91; H, 5·33%.)

[3,4,5-*Trimethoxy*-2-(3,4,5-*trimethoxybenzoyl*)*phenethyl*]*trimethylammonium iodide* (X). The methiodide IX (2 g) in 12 ml MeI was stirred vigorously with 80 ml 1N NaOH for 1·5 hr, the organic layer drawn off and the aqueous layer extracted with $CHCl_3$. The oil obtained after drying the combined organic layers with $MgSO_4$ and evaporating the solvents was crystallized from a minimum of hot C_6H_6 and pet. ether; after recrystallization from EtOH, ether (trace) and C_6H_6, the 2·2 g (91%) melted at 219–220°, new IR bands at 1655 (C=O), 1335,[41] 1125 cm^{-1} (OMe) and loss of bands at 1620, 1600, 1350–1360, 1050–1060 cm^{-1}. (Found: C, 49·95; H, 6·23. $C_{24}H_{34}NIO_7$ requires: C, 50·09; H, 5·96%.)

2,3,3',4,4',5'-*Hexamethoxy*-6-*vinylbenzophenone* (XI). The iodide X (1·8 g) was suspended in 50 ml 2N NaOH and heated 15 min on a steam bath, during which time a strong amine odor was given off. The oil that separated crystallized slowly on standing after the aqueous layer was decanted, 0·9 g (74%). After recrystallization from pet. ether, it melted at 73·5–74·5°; had IR bands much sharper than the preceding compounds, with bands at 1660 (C=O), 1590, 1560, 1505, 1490, (all Ar), 1450, 1435, 1415, 1400, 1330,[41] 1252, 1237 and 1228 (aryl ether), 1198, 1150, 1125 (OCH_3), 1074, 1032, 992 (vinyl), 930, and 920 and 850 cm^{-1} (both vinyl); and showed a typical vinyl pattern in the NMR spectrum, δ 6·53 (1H, *q*, J = 17 and 11), 5·62 (1H, *q*, J = 17·5 and 1·5), and 5·18 (1H, q, J = 10·5 and 1·5) as well as 7·13 (2H), 6·98 (1H), and the five OMe shifts, 3·99, 3·94, 3·92, 3·86 (two OMe), and 3·78. (Found: C, 64·94; H, 6·30. $C_{21}H_{24}O_7$ requires: C, 64·93; H, 6·23%.)

3,4,5-*Trimethoxy*-2-(3,4,5-*trimethoxybenzoyl*)*benzoic acid* (VII). Crude XI (400 mg) was dissolved in 60 ml boiling acetone, 850 mg $KMnO_4$ added slowly, and the mixture boiled 45 min longer. After addition of 100 ml water, removal of acetone on the steam bath, filtration, concentration to ½, acidification, and cooling, VII precipitated. It was recrystallized from MeOH—H_2O (1:2) giving 117 mg, m.p. 181·5–182·5°, IR bands the same as the keto acid from the oxidative degradation, and the same R_f on TLC (0·46 in 3% AcOH in $CHCl_3$). (Found: C, 59·04; H, 5·55. Calc for $C_{20}H_{22}O_9$: C, 59·11; H, 5·46%.)

Selective oxidation of hydroxymethyl group by the Jones reagent[30]

To aldehyde XII. A soln of 230 mg of the trimethyl derivative III in 100 ml of acetone was cooled and then treated dropwise, with stirring and under N_2, with 12–15 drops of the Jones reagent (CrO_3—dil H_2SO_4). After 5 min of stirring, a little MeOH was added, to decompose excess reagent, and then 5 volumes of benzene. The mixture was extracted with an equal volume of sat NaCl aq, and the organic layer washed with dil HCl, $NaHCO_3$, and H_2O. The residue from evaporation of the organic layer was checked by TLC and showed one principal spot, which gave a positive test with 2,4-dinitrophenylhydrazone, plus many minor spots.

This major component was purified first by preparative TLC, and then the 47 mg (20% yield) of crude aldehyde thus obtained was freed from chromium salts by an ion exchange column (Dowex 50W-X8).

* Prepared through a series of known compounds from 3,4,5-trimethoxybenzoic acid by the method in reference 25.

The crystals obtained were recrystallized from cyclohexane containing a small amount of $CHCl_3$; XII, m.p. 128·5–130°, IR bands at 1710 (C=O, aldehyde and ester), 1630, 1592, 1495, 1450, 1410, 1250, 1210, 1200, and 1112 cm^{-1}. The crude aldehyde, after spectral study, was converted into its yellow derivative, XIII, *6,7,8-trimethoxy-3-methoxycarbonyl-1-(3,4,5-trimethoxyphenyl)-1,2-dihydro-2-naphthaldehyde 2,4-dinitrophenylhydrazone* after three recrystallizations from EtOH—EtOAC, m.p. 218–219°, IR bands at 1710 (shoulder, ester), 1685 (C=N), 1620, 1595, 1510, 1333,[41] 1255, 1125, and 1110 cm^{-1}. (Found: C, 57·17; H, 4·93; N 8·49. $C_{31}H_{32}N_4O_{12}$ requires: C, 57·04; H, 4·94; N, 8·59%.)

To acid XIV. From the bicarbonate layer obtained in the above oxidation, 59 mg of a single crude acid was isolated. To improve its crystallizability, it was run through an ion exchange column (Dowex 50W-X8) and then recrystallized from 10% $CHCl_3$ in cyclohexane; XIV, *6,7,8-trimethoxy-3-methoxycarbonyl-1-(3,4,5-trimethoxyphenyl)-1,2-dihydro-2-naphthoic acid*, m.p. 143·5–144·5°. IR bands at 2560 (carboxyl OH), 1710 (shoulder, ester C=O), 1695 (acid C=O), 1630, 1590, 1485, 1450, 1410, 1342, 1255, 1239, and 1125, and 1105 cm^{-1}. (Found: C, 61·45; H, 5·85; OMe, 44·36. $C_{25}H_{28}O_{10}$ requires: C, 61·47; H, 5·78; OMe, 44·47%.)

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