Phytochemistry, 1972, Vol. 11, pp. 2513 to 2517. Pergamon Press. Printed in England.

CHEMOTAXONOMY OF *ULMUS**

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(*Received* 8 *February* 1972)

Key Word Index—*Ulmus*; Ulmaceae; sesquiterpenes; lignans; sterols; chemotaxonomy.

Abstract—The heartwoods of 10 elm species have been examined for the sesquiterpenes, 7-hydroxycadalenal (I), 7-hydroxy-3-methoxycadalenal (II), 7-hydroxycadalene (III), 7-hydroxycalamenenal (IV), 7-hydroxycalamenene (V), and mansonone C (VI), and for the lignans, thomasic acid (VII), thomasidioic acid (VIII), lyoniresinol (IX), and lyoniresinol 2a-*O*-rhamnoside (X). Cholesterol, campesterol, stigmasterol, sitosterol, 24-methylenelophenol, and citrostadienol were also found. The Section Madocarpus was distinctly different in its chemistry from the other four sections of this genus.

INTRODUCTION

THE MOST characteristic symptom of Dutch elm disease caused by *Ceratocystis ulmi* (Buis. M.) C. Moreau is the formation of a brown discoloration in the last one or two annual growth rings. Phenolic substances identical to those obtained by extraction of heartwood but not present in sapwood were found among the extractives from this 'brown ring' area. This made it desirable to identify phenolic substances and to explore the possible relationship between their occurrence in different elm species with known disease resistance.

Research on the extractives of elm heartwood is also desirable to distinguish between anatomically closely related species. The lumber of four elms of the United States, *Ulmus thomasii*, *U. alata*, *U. serotina*, and *U. crassifolia*, are commercially grouped under 'rock elm' because their wood is anatomically very similar. Previous research on the extractives of elm heartwood attempted to develop a chemotaxonomic test by which the wood anatomist could differentiate these four species by the characteristic fluorescent aqueous extractives. Although this objective was only partially met, our examination of the extractives of *U. thomasii* yielded four lignans, racemic thomasic acid (VII), racemic thomasidioic acid (VIII), racemic lyoniresinol (IX), and (+)-lyoniresinol-2a-*O*-rhamnoside (X),[1,2] as well as

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* A preliminary report was presented at the 5th International Symposium on the Chemistry of Natural Products, London, July 1968 (p. F20 of Abstracts).

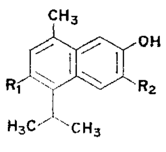
✠ Deceased, see *Phytochem.* **9**, 679 (1970).

† Maintained in cooperation with the University of Wisconsin.

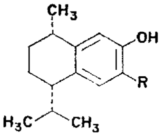
[1] M. K. Seikel, F. D. Hostettler and D. B. Johnson, *Tetrahedron* **24**, 1475 (1968).

[2] F. D. Hostettler and M. K. Seikel, *Tetrahedron* **25**, 2325 (1969).

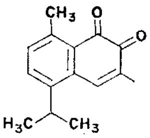
two new naphthoic acids[3] and 2,6-dimethoxy-*p*-benzoquinone.[2] It is interesting that the naphthoic acids and 2,6-dimethoxy-*p*-benzoquinone can be regarded as *in vivo* cleavage products of thomasic acid.



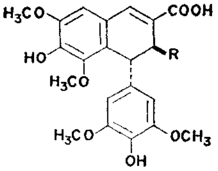
(I) R_1=H, R_2=CHO
(II) R_1=CH_3O, R_2=CHO
(III) R_1=H, R_2=CH_3



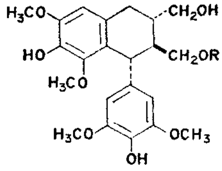
(IV) R = CHO
(V) R = CH_3



(VI)



(VII) R = CH_2OH
(VIII) R = COOH



(IX) R = H
(X) R = rhamnoside

Other research on staining problems from *U. rubra* heartwood led to the isolation of the new sesquiterpenes, 7-hydroxycadalenal (I), 7-hydroxy-3-methoxycadalenal (II), 7-hydroxycadalene (III), and (—)-7-hydroxycalamenenal (IV).[4] When we compared the rock elms with *U. rubra* as well as *U. americana* and several English elms, another new sesquiterpene, (—)-7-hydroxycalamenene (V) was discovered in 0·5% yield of the air-dry heartwood of *U. thomasii*. This was correlated with IV as well as with (—)-calamenene from other sources, and the absolute stereochemistry of the 5-isopropyl group in IV, V, and (—)-calamenene proved to be *S* as illustrated by correlation with (—)-copaene.[5] The 1-methyl group in these compounds undoubtedly is *S* also (*cis* to the isopropyl).

Our results are summarized in Table 1, which also includes findings by Lindgren and Svahn[6] on *U. laevis*, *U. carpinifolia*, and *U. procera* (= *U. glabra* Huds.). We have confirmed and extended their findings on *U. procera*. I, II, and mansonone C (VI) were recently found in *U. procera* heartwood.[7] Therefore this survey was extended to include mansonone C.[*,8]

RESULTS AND DISCUSSION

Our results for the compounds are summarized in Table 1. Due to the death of M.K.S. we were unable either to expand our survey to additional elms as planned or to check variability in the depth desired. We have, however, noted that the relative amounts of the extractives vary considerably. The percentage of I in *U. rubra*, for example, varied fifteenfold. Some of the data resulted from examining either a few samples or just one sample; thus variation in the relative amounts of the different extractives may well be found in other samples. Considerable variation in the phenolics with the season the tree was cut was also observed. Since compounds I–VI and VII–X were analyzed by different procedures, the relative amounts indicated should be applied only within each group.

[*] After this work was completed, III, VI, mansonone E,[8] and two new cadalenic sesquiterpenes, lacinilene A and B, were reported in *U. laciniata* Mayr. (Madocarpus) heartwood by H. Suzuki, S. Yasuda and M. Hanzawa, *J. Japan Wood Res. Soc.* **17**, 221 (1971). A third sesquiterpene, lacinilene C, has also been isolated, and lacinilene A has been proved to be 8-methyl-5-isopropyl-2-naphthol (demethyl-III) by synthesis (Prof. Hanzawa, private communication). Compound I has also been found in *U. wallichiana* heartwood (Dr. K. Venkataraman, private communication).

[3] C.-L. Chen and F. D. Hostettler, *Tetrahedron* **25**, 3223 (1969).

[4] M. Fracheboud, J. W. Rowe, R. W. Scott, S. M. Fanega, A. J. Buhl and J. K. Toda, *Forest Prod. J.* **18** (2), 37 (1968).

[5] J. W. Rowe and J. K. Toda, *Chem. & Ind.* 922 (1969).

[6] B. O. Lindgren and C. M. Svahn, *Phytochem.* **7**, 1407 (1968).

[7] V. Krishnamoorthy and R. H. Thomson, *Phytochem.* **10**, 1669 (1970).

[8] N. Tanaka, M. Yasue and H. Imamura, *Nippon Mokuzai Gakkaishi* **13**, 12 (1967).

The number of phenolics present appears to increase with increasing resistance to Dutch elm disease as is observed in most areas in North America. This, however, may result from the predominant use of *U. americana* that could determine the pathogenicity of the existing strain or strains of *C. ulmi*.

TABLE 1. COMPOUNDS IN *Ulmus* HEARTWOODS

Section*	*Ulmus* species	I	II	III	IV	V	VI	VII	VIII	IX	X
Blepharocarpus	*laevis* Pall.†	–	–	–	–						
	americana L.	–	–	+	–	+++	++	–	–	–	–
Madocarpus	*carpinifolia* Gleditsch†	+	+	+	+						
	minor Mill.	+	–	–	+	++	+	–	–	–	–
	procera Salisb.	+++	+	+	++	+++	+	–	–	–	–
	rubra Mühl.	+++	+	+	++	+	–	–	–	–	–
	diversifolia Melville‡	+++	–	+	++	++	±	–	–	–	–
	pumila L.	+	+	+	+	++	–				
Trichoptelea	*serotina* Sarg.	–	–	–	–	++	+	+	±	+	++
Microptelea	*crassifolia* Nutt.	±	–	–	±	++	±	±	–	±	+
	parvifolia Jacq.§	+	+				+				
Chaetoptelea	*thomasii* Sarg.	–	–	±	–	+++	±	+++‖	+	+++	++
	alata Michx.	–	–	–	±	++	–	+	+	±	+

Key: – Absent; ± Possibly present in minute amounts; + Present in small amounts; ++ Present in moderate amounts; +++ Present in major amounts.

* A. REHDER, *Manual of Cultivated Trees and Shrubs*, 2nd Edition, pp. 174–182, Macmillan, New York (1956).

† Data from Ref. 6.

‡ R. MELVILLE, *J. Bot. London* **77**, 140 (1939).

§ F.-C. CHEN, Y.-M. LIN and A.-H. CHEN, *Phytochem.* **11**, 1190 (1972). They also found mansonone G, sitosterol, and three unidentified compounds.

‖ In freshly cut wood, especially strong; in older wood, not as strong.

Several conclusions are immediately apparent from Table 1. V appears to be ubiquitous in elm heartwood. Care must be used in analyzing for III, since it was observed that this was readily formed from V by autoxidation. Presumably, I could also be formed by autoxidation of IV. The major sesquiterpenes, I–IV, are obviously characteristic of the Section Madocarpus, which is also characterized by patterns of phenolics in the water extract quite similar to one another, but remarkably free of syringyl compounds such as VII–X. *U. americana* gave a pattern of water-soluble extractives dramatically different from all of the other elms.

It was not possible to conclusively differentiate between the four rock elms on the basis of the fluorescent aqueous extractives. The considerable individual variation within a species in the content and the concentration of the phenolics could not be correlated with age or season in which the wood was cut (samples varied in age as much as 40 yr and were taken from trees cut from early spring to late autumn). In general, the aqueous extractives were most distinctive in freshly cut samples of *U. thomasii*. This was due to the high concentration of the UV fluorescent lignans VII and VIII and other syringyl derivatives (including IX and X) that give a blue color with the chromogenic, paper chromatographic spray. By contrast, *U. crassifolia* contained primarily unidentified vanillyl compounds that give a purple color with the same spray. *U. alata* and *U. serotina* contained some of each type of phenolic.

Samples of *U. serotina* contained the smallest amounts of these aqueous extractives; *U. thomasii*, the largest.

Preliminary TLC of extracts from the wood of other genera of the Ulmaceae, namely *Celtis occidentalis* L. and *Zelkova serrata* (Thumb.) Mark., indicated that only the *Zelkova* species was similar to the elms in containing III and V, but both species contained some polar unidentified spots observed also in elms.

We also compared the composition of the free sterols in *U. thomasii* and *U. rubra*; these sterols are probably not taxonomically significant. *U. thomasii* contained 0·13% free sterols, consisting of 2% cholesterol, 6% campesterol, 2% stigmasterol, 85% sitosterol, 4% 24-methylenelophenol, and 1% citrostadienol[9] by GLC of the TMS ethers on SE-30. The corresponding percentages for *U. rubra*, which contained 0·02% free sterols, are 1, 3, 7, 88, 0, and 2. However, the esterified sterols of this species (0·005%) contained these sterols in the percentages 1, 2, 0, 70, 3, and 24. It is interesting that stigmasterol was absent in the esters, whereas citrostadienol and 24-methylenelophenol were found almost entirely esterified. This is, apparently, a general phenomenon.[10] Sitosterol and campesterol were found also in *U. americana*.

Table 2. TLC analysis of sesquiterpenes

Compound	r_l	Color With longwave UV	 On warming with H_2SO_4	 With diazotized benzidine followed by 1 N NaOH
I	1·00	Dark spot	Grey	Violet
II	0·85	Dark spot	Maroon	Bluish grey
III	0·53	Not visible	Tan	Pink
IV	1·06	Yellow fluorescence	Dark green	Yellow
V	0·54	Not visible	Pink	Yellow
VI	0·18	Dark spot	Brown	Orange

EXPERIMENTAL

GLC procedure for identifying sesquiterpenes. GLC of a light petroleum extract of the heartwood was on an SE-30 column (3% on 70/80 Anakrom ABS, 1·8 m × 0·317 cm, 185°). Retention times relative to I (elution time = 5·6 min) are: I = 1·00, II = 1·93, III = 0·92, IV = 0·67, V = 0·48, and VI = 1·24. Under these conditions, none of the elm samples showed any other major detectable components in a 30-min elution.

TLC procedure for identifying sesquiterpenes. The light petroleum extract was also spotted on a glass plate coated with silica gel HF and developed with benzene–light petroleum (1:4) with 4% EtOAc* (Table 2). All compounds produced a dark spot under shortwave UV. A series of fatty acid esters was visible at r_l 1·36–1·74. Phytosterols (r_l 0·15) and other more polar spots were also visible.

PC procedure for identifying lignans. Water was slowly percolated through 2 g of finely ground heartwood packed into a thin column. The first ml of eluate was spotted onto paper and developed with *i*PrOH–2 N NH_3 (3:1) for 50–55 hr. After drying, the papers were examined under shortwave and longwave UV both before

* Hexane–acetone (8:2) also gave good resolution.

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[9] R. B. Bates, A. D. Brewer, B. A. Knights and J. W. Rowe, *Tetrahedron Letters* 6163 (1968).

[10] B. A. Nagasampagi, J. W. Rowe, R. Simpson and L. J. Goad, *Phytochem.* **10**, 1101 (1971).

and after fuming with NH_3. The papers were then aired, sprayed with 0·05% aqueous *p*-nitrobenzenediazonium tetrafluoroborate–2 N HCl (9:1), dried, and oversprayed with 20% Na_2CO_3.[11] Syringyl compounds give a blue color; vanillyl compounds, a purple color.

Acknowledgements—The authors thank Professors R. H. Thomson, for a sample of mansonone C; W. A. G. Morsink, for identifying and collecting elm samples; and Peter Yates, for his encouragement. They acknowledge the aid of the Forest Products Research Laboratory of England. They are grateful to Jan Toda Kuperman, Frances Hostettler, and D. Charles for technical assistance. The authors acknowledge a research grant from the National Research Council of Canada (E.J.).

[11] M. J. Strohl and M. K. Seikel, *Phytochem.* **4**, 383 (1965).