Phytochemistry, 1970, Vol. 9, pp. 1949 to 1952. Pergamon Press. Printed in England.

SHORT COMMUNICATION

# ACCUMULATION OF MANSONONES E AND F IN *ULMUS HOLLANDICA* INFECTED WITH *CERATOCYSTIS ULMI*

J. C. OVEREEM

Institute for Organic Chemistry TNO, Utrecht, The Netherlands

and

D. M. ELGERSMA

Phytopathological Laboratory "Willie Commelin Scholten" Baarn, The Netherlands

(*Received* 12 *January* 1970)

**Abstract**—TLC of alcoholic extracts of the xylem of *Ulmus hollandica* "Belgica" infected with *C. ulmi* revealed three spots which were not detected when the same amount of extract of healthy wood was chromatographed. Two of these spots showed fungitoxic activity. Extracts of *U. hollandica* cl. 390 (resistant against Dutch elm disease) inoculated with *C. ulmi* contained the same compounds. Again the compounds were not detected in healthy wood. The two fungitoxic compounds have been isolated. They proved to be identical with mansonone E (I) and mansonone F (II) respectively.

## INTRODUCTION

RECENTLY several 2-naphthol derivatives have been isolated from the heartwood of different species of the genus *Ulmus* (Ulmaceae).[1-3] In an attempt to discover if phenol oxidation plays a role in the strong discoloration which is observed in the outer xylem vessels of elms infected with *Ceratocystis ulmi* (Buisman) C. Moreau, we have prepared alcoholic extracts of the xylem of healthy young branches of *Ulmus hollandica* Mill. "Belgica" and branches infected with *C. ulmi*. In the same way healthy and inoculated young branches of *U. hollandica* clone 390—which is resistant against Dutch elm disease—were extracted.

## RESULTS

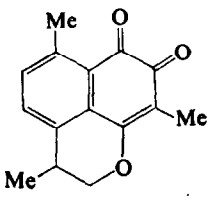
All the elm extracts contained about an equal amount of polyphenols. The latter were tentatively identified as polymeric leucoanthocyanins and (+)-catechin which have earlier been found in leaves and in bark of elms.[4] However, TLC of the extracts of the infected branches revealed three spots which were not observed on chromatograms of extracts of the healthy branches. On silica gel plates eluted with $CHCl_3$–EtOAc (9:1), these three spots appeared at $R_f$ 0·77 (orange), 0·57 (violet) and 0·24 (colourless but fluorescing in u.v. light).

[1] M. FRACHEBOUD, J. W. ROWE, R. W. SCOTT, S. M. FANEGA, A. J. BUHL and J. K. TODA, *Forest Prod. J.* **18**, 37 (1968).
[2] B. O. LINDGREN and C. M. SVAHN, *Phytochem.* **7**, 1407 (1968).
[3] CHEN-LOUNG CHEN and F. D. HOSTETTLER, *Tetrahedron* **25**, 3223 (1969).
[4] D. BEDNARSKA, *Dissertationes Pharm.* **15**, 87 (1963); *Chem. Abs.* **60**, 9599 (1964).

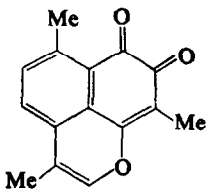
According to a technique described by Dekhuijzen[5] a chromatogram was sprayed with a conidial suspension of *Cladosporium cucumerinum* Ell. et Arth. After a suitable incubation period the dark-coloured mycelium covered the whole plate except for those places where the orange and violet spots were present.

From diseased branches, collected 2 weeks after artificial infection in June 1969, both the orange and violet compounds were isolated. The orange compound proved to be identical with mansonone E (I) and the violet compound with mansonone F (II). These compounds have recently been isolated from the West-African tree *Mansonia altissima* Chev.[6,7]

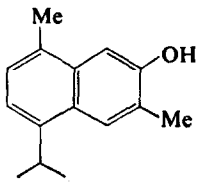
The compounds were identified by means of their melting points, microanalyses, u.v., i.r. and NMR spectra. In the mass spectra of both compounds the M + 2 peak appeared with a greater intensity than the molecular ion peak.* This is a normal phenomenon with 1,2-quinones.[8,9]



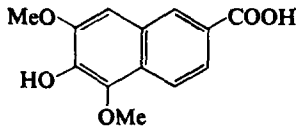

Mansonone E (I)




Mansonone F (II)




7-Hydroxycadalene (III)




(IV)

By means of TLC followed by u.v. spectroscopy of the appropriate fractions, 250 mg of mansonone E and 60 mg of mansonone F could be detected in 10 kg (fresh weight) of diseased branches. At the same time healthy branches were extracted. The $CHCl_3$-soluble part of the extract was purified by column chromatography. TLC revealed the presence of approximately 0·5 mg of mansonone E and less than 0·1 mg of mansonone F in 10 kg of fresh branches. When the experiment was repeated in September no mansonones could be found in healthy wood.

The mansonones show a striking similarity with the naphthols which have been found in the heartwood of *Ulmus rubra*[1] and *U. glabra* and *U. carpinifolia*.[2] Especially the structural relationship with 7-hydroxycadalene (III) is noteworthy. The above-mentioned elms as well as *U. hollandica* all belong to the section *Madocarpus* Dum. of the genus *Ulmus*. In the present investigation we have not been able to detect naphthols in the extracts. This is not too surprising since only young branches were used.

* M.s. were recorded by Dr. J. J. de Ridder, Analytical Laboratory, University of Utrecht, The Netherlands.

[5] H. M. DEKHUIJZEN, *Nature* **191**, 198 (1961).
[6] G. B. MARINI BETTÒLO, C. G. CASINOVI and C. GALEFFI, *Tetrahedron Letters* **52**, 4857 (1965).
[7] N. TANAKA, M. YASUE and H. IMAMURA, *Tetrahedron Letters* **24**, 2767 (1966).
[8] S. UKAI, K. HIROSE, A. TATEMATSU and T. GOTO, *Tetrahedron Letters* **49**, 4999 (1967).
[9] R. W. A. OLIVER and R. M. RASHMAN, *J. Chem. Soc.* B, 1141 (1968).

Naphthalene derivatives of the cadalene type have not been found in the heartwood of *U. laevis* (section *Blepharocarpus* Dum.) and *U. thomassii* [section *Chaetoptelea* (Liebm.) C. Schn.].[2] *U. thomassii* contains naphthalene derivatives of a different type, for instance IV.[3] It seems reasonable to expect that after infection with *Ceratocystis ulmi* these elms will not respond with the synthesis of mansonones.

Fungitoxicity of the mansonones was tested according to the roll-culture method described by Pluijgers and Kaars Sijpesteijn.[10] The results are listed in Table 1.* It appears that *C. ulmi* is relatively insensitive.

TABLE 1. ANTIFUNGAL ACTIVITY OF MANSONONES E AND F

| | *Botrytis allii* | *Penicillium italicum* | *Aspergillus niger* | *Cladosporium cucumerinum* | *Ceratocystis ulmi** |
|---|---|---|---|---|---|
| Mansonone E | 10 | 5 | 50 | 20 | >500† |
| Mansonone F | 2 | 2 | >50 | 5 | >100† |

Medium: glucose-mineral salts agar; pH 6·3. Minimum inhibitory concentration in ppm after 8 days' incubation at 24°.

* Pyridoxal and biotin were added to the growth medium.

† Growth retarded in comparison with control. Due to lack of material the compounds were not tested at higher concentrations.

We have not found a significant difference in the amounts of mansonones which accumulate in *U. hollandica* "Belgica" and in *U. hollandica* clone 390 after infection with *C. ulmi*. Therefore, formation of mansonones cannot be one of the major factors which govern susceptibility or resistance against Dutch elm disease. The possibility remains that the mansonones are at least partly responsible for the sharp decrease in fungal material which is observed in resistant as well as in susceptible elms in the second week after the inoculation.[11]

## EXPERIMENTAL

### *Extraction*

*Ulmus hollandica* "Belgica" and *U. hollandica* cl. 390 were inoculated with a conidial suspension of *C. ulmi* in June 1969. 2 weeks later branches were cut from both trees. At the same time healthy branches were cut. The bark was stripped off and the branches were broken into small pieces. The wood was then immediately plunged into boiling 80% ethanol. After boiling for 2 hr the mixture was cooled and filtered. The alcohol was evaporated *in vacuo* and the aqueous residue was extracted twice with EtOAc. The extract was dried over $Na_2SO_4$ and evaporated *in vacuo* yielding a dark-coloured residue (about 10 g from 4 kg of branches).

### *Polyphenols*

In all cases the residues obtained after evaporation of the EtOAc showed absorption maxima at 277 nm (in ethanol). In different experiments the extinction value at 277 nm varied between 0·25 and 0·35 calculated on the basis of 10 g of wood/1 l. of solvent. TLC (silica gel, $CHCl_3$–MeOH, 4:1) revealed two spots which gave a strong colour after spraying with "Echtblausalz B" (Merck). One of these spots cochromatographed with (+)-catechin. The strongest spot remained at $R_f$ 0·0. It was probably caused by polymeric leucoanthocyanins since heating the extractives with 5% HCl in *n*-butanol (cf. Swain and Hillis)[12] yielded a solution which gave an anthocyanidin spectrum.

* The test was carried out at the Biochemical Department of the Institute for Organic Chemistry TNO, under the guidance of Dr. A. Kaars Sijpesteijn.

[10] C. W. PLUIJGERS and A. KAARS SIJPESTEIJN, *Ann. Appl. Biol.* **57**, 465 (1966).
[11] D. M. ELGERSMA, Diss. Amsterdam, Meded. Phytopathol. Lab. Willie Commelin Scholten, No. 77 (1969).
[12] T. SWAIN and W. E. HILLIS, *J. Sci. Food. Agri.* **10**, 63 (1959).

*Isolation of Mansonones*

TLC (silica gel, $CHCl_3$–EtOAc, 9:1) showed the presence of an orange compound at $R_f$ 0·77, a violet compound at 0·57 and a compound which fluoresced in u.v. light at 0·24 only in the extracts of the infected branches.

The EtOAc-soluble extractives of 7·5 kg of diseased *U. hollandica* "Belgica" branches were boiled with $CHCl_3$. The solution was filtered and evaporated to a small volume. The solution was chromatographed on a silica gel column (length 70 cm, diameter 3·5 cm). Elution of the orange and violet compounds was performed with $CHCl_3$–EtOAc, 9:1. To obtain pure compounds the appropriate fractions were carefully rechromatographed. The orange compound (mansonone E) crystallized from light petroleum in needles. Yield 80 mg, m.p. 146–147° (m.p. microscope). The violet compound (mansonone F) crystallized in glistening needles from a small amount of ethanol. Yield 22 mg, m.p. 220–222° (m.p. microscope). Spectral data were completely in accordance with those recorded for mansonone E and mansonone F respectively.[6,7]