Isolation and identification of six mansonones from *Ulmus americana* infected with *Ceratocystis ulmi*

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Summary. Mansonones A and C to G, sesquiterpene quinones possessing antifungal properties, were isolated from the elm *Ulmus americana* infected with *Ceratocystis ulmi*. Scopoletin and β-sitosterol were also present in the extracts. The mansonones were not detected on chromatographic analysis of extracts from healthy elm tissue. The extent to which these antifungal compounds may contribute to resistance to an aggressive strain of *C. ulmi* induced in *U. americana* by a non-aggressive strain of the fungus remains to be determined.

Hubbes and Jeng[2] demonstrated that *Ulmus americana* could be induced to develop resistance to an aggressive strain of *Ceratocystis ulmi* by inoculation with a non-aggressive strain of the pathogen. Mansonones E and F (fig.) were tentatively identified in stems of *U. americana* seedlings artificially infected with *C. ulmi*[3]. Mansonones E and F, originally isolated along with several closely related quinonoid pigments from the West-African tree *Mansonia altissima* Chev.[4–6], were first observed to accumulate in *C. ulmi*-infected *U. americana* by Elgersma and Overeem[7]. Accumulation of mansonones E and F occurred to a greater degree in *U. hollandica*, both in susceptible ('Belgica') and resistant (390) clones, when inoculated with *C. ulmi* or subjected to various other treatments, and Elgersma and Overeem concluded that accumulation of these compounds does not contribute in a major way to the resistance of the latter clone to Dutch elm disease[7,8]. These observations do not exclude the possibility that sufficient levels of mansonones (or other antifungal compounds) induced in elm tissue prior to inoculation with an aggressive strain of *C. ulmi* could hinder establishment of the latter.

The present study was undertaken to determine whether antifungal compounds other than mansonones E and F are produced by *U. americana* as a response to natural or artificial infection by *C. ulmi*. Such information could help to shed light on one aspect of the mechanism(s) of induced resistance.

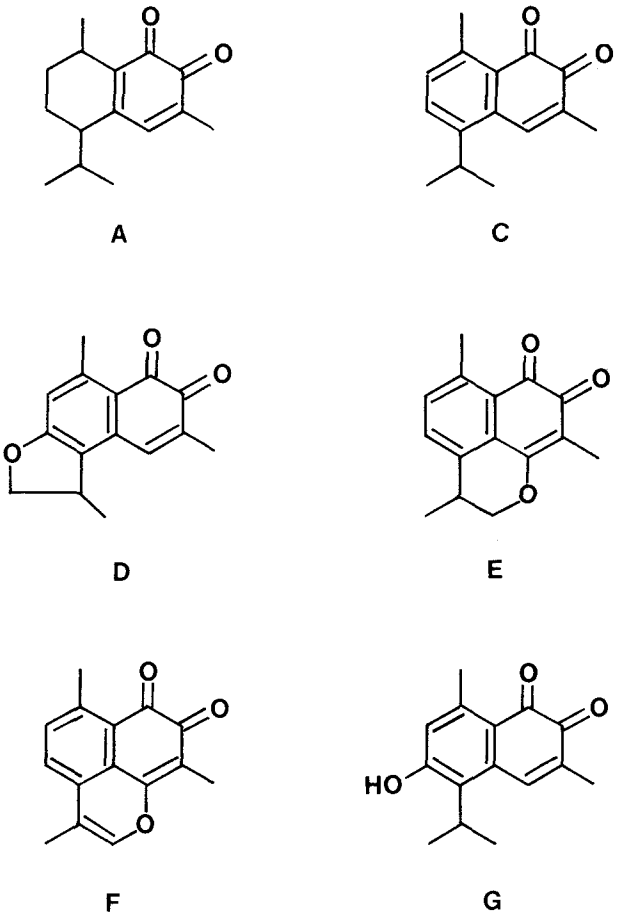
Branches whose leaves displayed wilting were collected during July and August 1982, from several white elms, 8–10 years old, growing in areas around Fredericton, New Brunswick. Infection by *C. ulmi* was confirmed before each sample was extracted. The infected branches, 0.4–1.0 cm diameter, were debarked and cut into sections about 1 cm long, which immediately were extracted repeatedly with 80% ethanol at ambient temperature. The volume of the solution was reduced in vacuo at 30 °C on a Buchi rotovapor, until most of the ethanol was removed. The aqueous residue was extracted exhaustively with chloroform, and the chloroform solution was evaporated to dryness at 30 °C under reduced pressure. The residue was chromatographed on a column of silica gel, using chloroform-ethyl acetate (95:5) as eluent. Fractions displaying similar mobilities on TLC were pooled, and 4 groups of compounds were thus separated. In the order of their emergence from the column, these pooled fractions contained 1. mansonones A and C, 2. mansonone D, 3. mansonone E and β-sitosterol, and 4. mansonones F and G, together with the fluorescent coumarin scopoletin.

The constituent compounds were separated by column chromatography on silica gel using appropriate eluting solvents: chloroform-ethyl acetate (99:1) for A and C; chloroform-hexane (90:10) for E and β-sitosterol; chloroform-ethyl acetate (80:20) for F and G and scopoletin.

Mansonones C and G were crystallized from hexane, E from cyclohexane, and F from chloroform-hexane. Mansonones A and D were not crystallized, but were further purified on a column prepacked with Li Chroprep Si 60 (Merck), eluting under a pressure of 0.5 kg cm^{-2} with chloroform-ethyl acetate (99:1) and (97:3), respectively.

Mansonones isolated from *Ulmus americana* infected by *C. ulmi*

Mansonones	% Yield based on dry weight of elm extracted	Melting point (°C)
A	0.013	
C	0.0067	128–129 (Marini-Bettolo et al.[4]; 134–138)
D	0.0087	
E	0.030	143–145 (Overeem and Elgersma[8]; 146–147)
F	0.013	222–224 (Overeem and Elgersma[8]; 220–222)
G	0.0071	179–182 (Galeffi et al.[6]; 201–203)



The identities of the compounds were established by comparison of their spectroscopic characteristics with published data[4-6], and with spectra obtained from other workers[9]. Scopoletin and β-sitosterol were identified by comparison with authentic samples. The structures and concentrations of the mansonones are shown in the figure and the table, respectively.

The same compounds were detected chromatographically in *U. americana* seedlings 4 weeks after inoculation with a non-aggressive strain (strain 311[2]) of *C. ulmi*. None of the mansonones was detected by TLC in extracts from healthy *U. americana*, but a fluorescent compound showing chromatographic behavior similar to that of scopoletin was present in these extracts. (An unidentified fluorescent substance reported in xylem of infected *U. hollandica* by Overeem and Elgersma was not observed by these authors in healthy wood[8].) To our knowledge, accumulation of mansonones A, C, D, and G in infected *U. americana* has not been observed previously.

In preliminary assays using the mehod of Homans and Fuchs[9] with 3% potato dextrose agar as medium, all the mansonones isolated displayed antifungal properties when tested against *Cladosporium cucumerinum* and *C. ulmi*. Scopoletin and β-sitosterol did not show activity against these organisms.

Assessment of the extent to which these antifungal compounds may contribute to induced resistance to Dutch elm disease must await the outcome of further research.

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Difference in the absorption coefficient of enantiomers for arbitrarily polarized light in a magnetic field: A possible source of chirality in molecular evolution

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Summary. It is predicted that a static magnetic field parallel to the direction of propagation of an incident light beam causes a small shift in the value of the absorption coefficient of a chiral molecule. This shift is not a circular differential effect. It should occur with arbitrarily polarized light. However, for enantiomers, the sign of the shift is opposite. This effect, though relatively small, may lie at the origin of a mechanism by which, in early stages of molecular evolution and starting from a particular racemic mixture, a change in the relative concentration of the two enantiomers was induced, without the prerequisite of an asymmetric material environment or of an external source of circularly polarized light.

It may be shown by quantum mechanics[1] that a static magnetic field parallel to the direction of propagation of an incident light beam causes a small shift in the value of the absorption coefficient of a chiral molecule. This shift is not a circular differential effect. In a molecule of given handedness it has the same sign for left and right circularly polarized light. It should therefore also occur with light which is arbitrarily polarized or 'unpolarized'. However, for enantiomers, the sign of the shift is opposite. This phenomenon, which for the sake of brevity we shall call MIAD (for magnetic field-induced absorption difference) is not to be confounded with natural CD (circular dichroism) or MCD (magnetic field-induced circular dichroism). Both CD and MCD correspond to differences in the absorption coefficient for circularly polarized light, whereas in MIAD the direction of polarization is irrelevant. MIAD like CD, but unlike MCD, vanishes in racemic mixtures. For a given antipode, the shift in the absorption coefficient corresponding to MIAD changes sign if the relative direction of the light beam with respect to the magnetic field is reversed.

The effect is indeed a small one. It is meaningful to discuss the order of magnitude of the MIAD signals to be expected in comparison with MCD[2]. For a strong MCD B-Term in a field of, say, 5T, the ratio $\Delta\varepsilon/\varepsilon \equiv (\varepsilon_L - \varepsilon_R)/\frac{1}{2}(\varepsilon_L + \varepsilon_R)$ is at best of the order of 10^{-3}, $\varepsilon_L - \varepsilon_R$ designate the absorption coefficient of a given molecular species for left and right circularly polarized light, respectively. Whereas in the tensor governing MCD we find products of 2 electric dipole transition moments and 1 magnetic dipole transition moment, in MIAD the corresponding products contain 1 electric dipole transition moment and 2 magnetic dipole transition moments (or an electric dipole transition moment, a magnetic dipole transition and an electric quadrupole transition moment)[1]. The ratio between the magnitude of the 2 effects should therefore be of the order of $(e\hbar/2\,mc)/ea_0$, or 10^{-2} to 10^{-3}. Consequently for MIAD at the above-mentioned magnetic field strength, $\Delta\varepsilon/\varepsilon \equiv (\varepsilon_A - \varepsilon_B)/$