

NAPHTHAQUINONE PIGMENTS FROM *TORULA HERBARUM*:
STRUCTURE OF O-METHYLHERBARIN*

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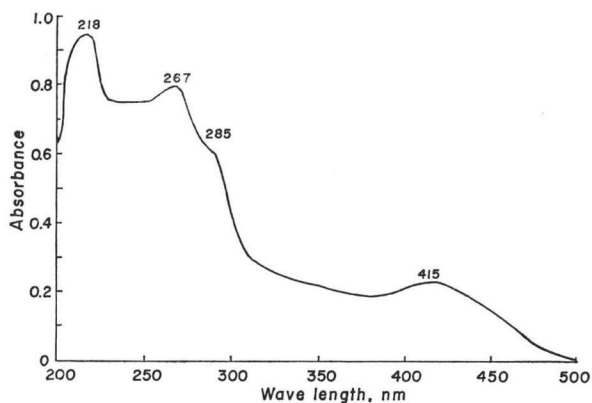
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A third naphthaquinone pigment isolated from *Torula herbarin* is now identified as O-methylherbarin by IR, NMR and mass spectroscopic methods. The structure is confirmed by partial synthesis by methylation of herbarin using methyl iodide and silver oxide. The gas chromatographic-mass spectrometric characteristics of herbarin, dehydroherbarin and O-methylherbarin are described.

In previous papers the isolation of two naphthaquinone pigments, herbarin (**Ia**) and dehydroherbarin (**II**) from *Torula herbarum*^{1,2)} was reported and their structures were described. Both thin-layer (TLC) and gas-liquid chromatography (GLC) of the mixture of pigments revealed the presence of other minor components. One of these minor pigments, described as "fraction 5", was obtained in small quantities by preparative TLC. Fraction 5 was crystallized from ethyl acetate from which it separated as yellow needles and prisms which melt at 188~190°C. The UV and visible spectrum of this product (Fig. 1) was almost identical with that of herbarin (λ_{\max} 218, 267, 285 sh and 415 nm). The infrared spectrum in chloroform solution was also very similar to that of herbarin, but it did not show any hydroxyl absorption. Unlike herbarin, the compound was readily soluble in CDCl_3 and gave a good NMR spectrum (Fig. 2) which afforded information on its structure. The spectrum as in herbarin showed two aromatic protons (meta), two methoxyls, a CH_3 and two CH_2 groups. The spectrum did not show any hydroxyl proton but instead showed a third methoxyl group (6.72 τ) (see Fig. 2 for assignments).

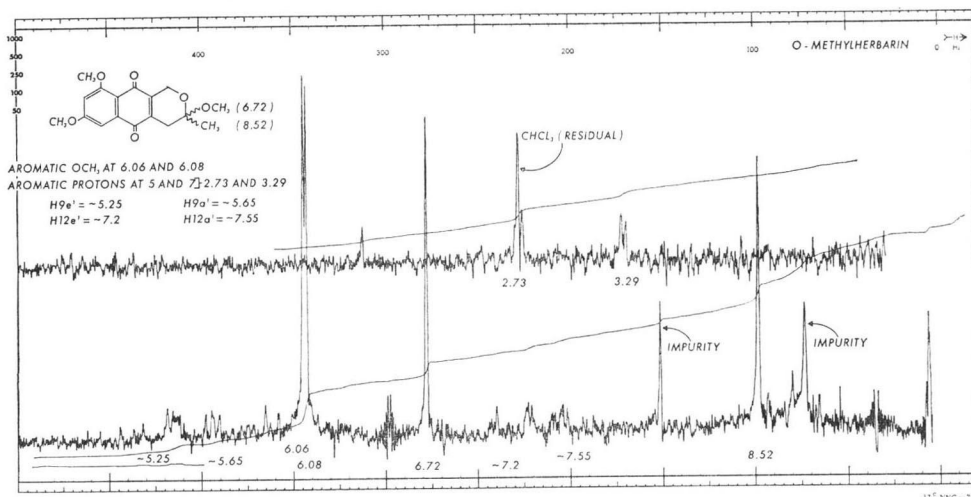
The mass spectrum obtained by direct introduction of sample on a solid probe into the ion source showed the molecular ion (P) at m/e 318 and the fragment ions at m/e 303 ($\text{P}-\text{CH}_3$)⁺, 286 ($\text{P}-\text{CH}_3\text{OH}$)⁺, 271 ($\text{P}-\text{CH}_3\text{OH}-\text{CH}_3$)⁺, 244 ($\text{P}-\text{CH}_3\text{COOCH}_3$)⁺ and 243 ($\text{P}-\text{CH}_3\text{OH}-\text{CH}_3\text{CO}$)⁺. On the basis of these spectroscopic data the compound is assigned the structure of O-methylherbarin (**Ib**). This structure was confirmed by partial synthesis of

Fig. 1. UV and visible spectrum of O-methylherbarin.

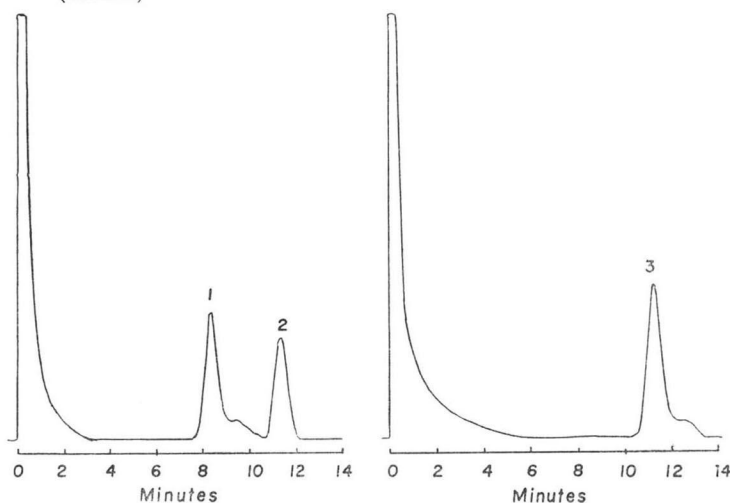


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Fig. 2. NMR spectrum of O-methylherbarin in CDCl_3 .Fig. 3. Gas-liquid chromatograms of natural herbarin, O-methylherbarin and synthetic O-methylherbarin. (3% OV-101 on gaschrom Q 6-ft column 210° iso N_2 40 ml/min).

Herbarin and O-methylherbarin (natural) O-Methylherbarin from herbarin



this compound from herbarin (**Ia**) by refluxing it with methyl iodide and silver oxide in a solution of methylene chloride. The UV, IR, NMR and mass spectra of the synthetic and natural products were identical. The identity was further confirmed by TLC and GLC. Gas-liquid chromatograms of natural herbarin, O-methylherbarin and synthetic O-methylherbarin are shown in Fig. 3.

Gas-liquid Chromatography—Mass Spectrometry (GC-MS)

Gas-liquid chromatography was done on a 6-ft, 3% OV-101 on GCQ column at 210°C isothermal with N_2 as the carrier gas at a flow-rate of 40 ml/min on a Barber Colman 5700 instrument. Both herbarin and dehydroherbarin had the same retention time (8 minutes 18 seconds) suggesting the possible dehydration of herbarin (**Ia**) to dehydro compound (**II**). This was confirmed by GC-MS where both herbarin and dehydroherbarin gave identical mass spectra corresponding to structure **II**. O-Methylherbarin had a retention time of 11 minutes 15 seconds and the mass spectrum was identical with that obtained on direct solid sample probe. The GC-MS data were obtained on a Varian CH-7 mass spectrometer interfaced with a Varian 2740 gas chromatograph,

ionization potential 70 eV, source temperature 280°C, separator temperature 300°C and helium flow-rate 30 ml/min.

Mass-spectral Characteristics of Herbarins

The mass spectra of dehydroherbarin (II), herbarin (Ia) and O-methylherbarin (Ib) are shown in Figs. 4, 5 and 6. Of these, high resolution data were also obtained for herbarin. While the fragmentations are those generally expected of the naphthaquinone structure, some special features are worthy of mention. The base peak in dehydroherbarin is $(M-CH_3)^+$ at m/e 271, while in herbarin the base peak is at m/e 286, the $(M-18)^+$ ion, and in O-methylherbarin the base peak is at m/e 244 by loss of CH_3COOCH_3 from the molecular ion. The fragment ions in the spectra at m/e 244 in herbarin and O-methylherbarin and at m/e 243 in dehydroherbarin arise by a similar mechanism as shown in the scheme.

The fragmentation mechanism is confirmed by the distinct metastable ions (m^* 195.8 and 187.2) seen in the mass spectra of Ia and Ib. Similarly the other fragmentations for the three structures are supported by appropriate metastable transitions noted in the spectra. These are given in Table 1.

Another feature in the spectra of these compounds, which could be used for their identification by GC-MS, is the appearance in all three spectra of doubly charged ions of high intensity at m/e 121.5 and 129.5, and of ions at m/e 135.5 and 151.5 in the spectrum of O-methylherbarin.

Fig. 4. Mass spectrum of dehydroherbarin.

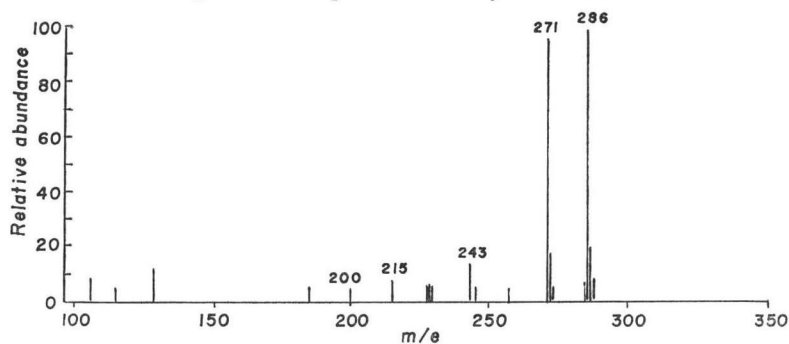


Fig. 5. Mass spectrum of herbarin.

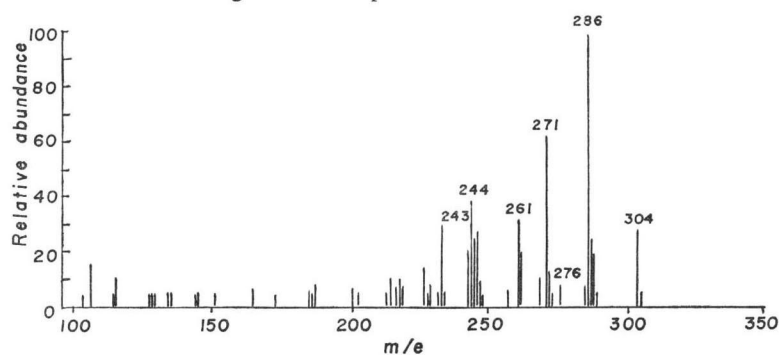
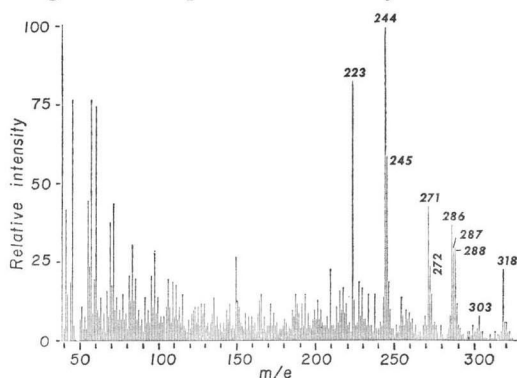
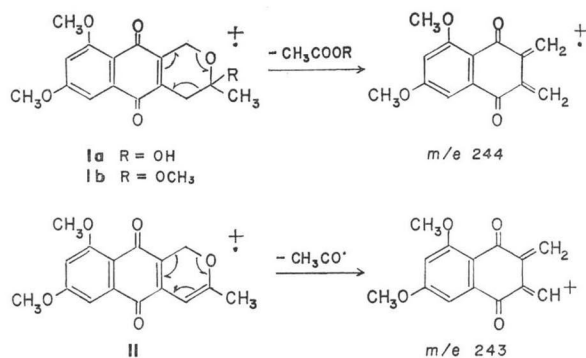


Fig. 6. Mass spectrum of O-methylherbarin.





Experimental

A solution of herbarin (20 mg) in methylene chloride (100 ml) was refluxed with methyl iodide (1 ml) and silver oxide (1 g) for 12 hours. The mixture was filtered, the solids were washed with 10 ml of methylene chloride and the filtrate plus washings was evaporated under vacuum. TLC on silica gel G showed that the major portion of the product was O-methylherbarin with traces of unreacted

Table 1. Metastable transitions in the mass spectra of herbarin derivatives and the parent and daughter ions

Compound	Parent <i>m/e</i>	Daughter <i>m/e</i>	Metastable <i>m</i> *
Herbarin	304 ^a	286	269.3
	286	271	256.6
	304	276	250.8
	304	244	195.8
	286	258	232.4
	243	215	190.2
	271	243	217.9
Dehydroherbarin	286 ^a	271	256.4
	271	243	217.9
	243	215	190.2
	243	228	213.9
O-Methylherbarin	318 ^a	303	288.7
	318	286	257.2
	318	244	187.2
	286	271	256.6
	271	243	217.9
	243	215	190.2
	215	187	162.5

^a Molecular ion.

herbarin. After purification by preparative TCL on silica gel G, the fast moving band corresponding to O-methylherbarin was eluted with chloroform-methanol (1 : 1). The residue obtained after evaporation was used directly for TLC, GLC and spectroscopic analysis. The TLC, GLC, UV, IR and mass spectral characteristics of the methylated product were identical to those of natural O-methylherbarin.

Acknowledgement

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References

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