

**Isolation of a New Natural Quinone,
2-Hydroxy-6-methoxy-3,5-dimethyl-
1,4-benzoquinone,
from the Potato Culture Solution
of *Phoma wasabiae* Yokogi**

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Benzoquinone,
2-Hydroxy-6-methoxy-3,5-dimethyl-1,4-
benzoquinone, *Phoma wasabiae* Yokogi

2-Hydroxy-6-methoxy-3,5-dimethyl-1,4-
benzoquinone was found in the blackish potato
culture solution of *Phoma wasabiae*.

Previously, flaviolin was isolated from the blackish culture solution of *Phoma wasabiae* [1]. In continuation of the study on the metabolites of this mold, we have now isolated 2-hydroxy-6-methoxy-3,5-dimethyl-1,4-benzoquinone. This benzoquinone was already synthesized [2], but has not been found as a natural product. In this paper we wish to report on the isolation of the above quinone from the blackish culture solution.

Phoma wasabiae was cultivated on the potato culture solution containing 20% glucose at 25 °C for 3 or 4 weeks. After 3 or 4 weeks of the cultivation, the blackish culture solution was filtered and a 20% solution of lead acetate added at the ratio of 3 ml to 100 ml of the blackish culture solution to precipitate some dark brown substances. After centrifuging, the supernatant solution was concentrated and centrifuged again. The aqueous layer was acidified with sulfuric acid to remove lead sulfate and filtered. The filtrate was extracted 3 times with ether of the same volume as the aqueous solution. The residue showed five yellow bands (R_f values 0.2, 0.4, 0.5, 0.7 and 0.9) and one purple band (R_f value 0.6) on a silica-gel plate (E. Merck,

Kiesel gel nach Stahl, Type 60) with a mixture of benzene-methanol (9:1). The purple band changed the color into red with ammonia, yellow with acids, and red with ferric chloride solution.

The purple band was collected, extracted with ether, and the extract evaporated; the resulting precipitates were crystallized from petroleum ether (30 ~ 50 °C), to give orange needles, m. p. 116 ~ 117 °C [3]. The isolated yield of the compound was ca. 1.5 ~ 2.0 mg per 4000 ml of the culture solution. The mass spectral pattern was found to be similar to that [4] of methyl or methoxy derivative of *p*-benzoquinone, m/e 182 (M^+), 167 ($M^+ - CH_3$), 154 ($M^+ - CO$), 139 ($M^+ - CH_3 - CO$), 111 ($M^+ - CH_3 - 2CO$), 83 ($M^+ - CH_3 - 3CO$) (base). IR (KBr) ν_{max} 3360, 1660, 1625, 1615 cm^{-1} ; UV (EtOH) λ_{max} (log ϵ) 217(4.00), 287(4.14), 4.10(2.61) nm; 1H -NMR ($CDCl_3$) δ 1.91, 1.96 (each s, 3H, CH_3), 3.93 (s, 3H, OCH_3), 6.80 (s, 1H, OH).

The methylation of the *p*-quinone with diazomethane gave a methyl derivative, which was purified by use of thin layer chromatography with benzene; recrystallized from petroleum ether m. p. 102.5 ~ 103.7 °C. IR (KBr) ν_{max} 2930, 1665, 1655, 1630, 1612 cm^{-1} ; 1H NMR ($CDCl_3$) δ 1.93 (s, 6H, 2 CH_3), 3.94 (s, 6H, 2 OCH_3). These results suggested that the orange compound was one of three isomers of hydroxy-methoxy-dimethyl-*p*-benzoquinone derivatives: 2-hydroxy-6-methoxy-3,5-dimethyl-1,4-benzoquinone (1) (m. p. 116.6 °C [1]), 2-hydroxy-3-methoxy-5,6-dimethyl-1,4-benzoquinone (2) (m. p. 168 ~ 180 °C [5]), and 2-hydroxy-5-methoxy-3,6-dimethyl-1,4-benzoquinone (3) (m. p. 70 °C [6]). The melting point of the orange compound showed no depression on mixed with the authentic sample of the quinone (1), which was synthesized from 2,6-dimethylphenol [7, 8]. Furthermore, IR and 1H NMR spectra of the orange compound and its methyl derivative were good agreement with those of synthetic compounds; therefore, it was confirmed that the orange compound was 2-hydroxy-6-methoxy-3,5-dimethyl-1,4-benzoquinone (1). Although the band of R_f value 0.7 was quickly decolorized, a yellow compound was isolated from the band and, in polar solvent as methanol or ethanol, changed to a compound identified with the above quinone (1) on TLC (purple, R_f value 0.6).

The structure of the yellow compound of R_f value 0.7 is under investigation.

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[1] O. Soga, Z. Naturforsch. **31b**, 124 (1976).

[2] C. Bosse, Monatsh. **21**, 1021 (1900).

[3] All melting points were determined on a Yanagimoto Micro-melting point apparatus and are uncorrected. IR spectra were taken with a Hitachi 215 spectrometer as a KBr disc. NMR spectra were observed with a JEOL MH-100 spectrometer in $CDCl_3$ solution using TMS as the internal standard. Mass spectra were measured with a Hitachi RMU-6L mass spectrometer. Ultra-

violet spectra were recorded by means of a Hitachi 124 spectrophotometer.

[4] R. H. Thomson, Naturally Occurring Quinones, 2nd ed, p. 78, Academic Press, London 1971.

[5] G. Schill and R. Henschel, Liebigs Ann. Chem. **731**, 113 (1970).

[6] G. Petterson, Acta Chem. Scand. **18**, 2303 (1964).

[7] E. B. Vliet, Org. Synth., Coll. Vol. 1, 317 (1941).

[8] R. E. Moore, H. Singh, C. W. J. Chang, and P. J. Scheuer, Tetrahedron **23**, 3271 (1967).