CHEMISTRY LETTERS, pp. 339 - 340, 1984.

ISOLATION AND STRUCTURE OF A NEW NATURAL CYCLOHEXADIENONE DERIVATIVE, WASABIDIENONE-A, FROM POTATO CULTURE SOLUTION OF *PHOMA WASABIAE* YOKOGI

Osamu SOGA,* Hidetoshi IWAMOTO, Satoru DATE, Takeshi WATANABE,
Kazuhiko TANAKA, Kuniharu HATA, Akio TAKUWA, and Mitsuru NAKAYAMA*†
Department of Chemistry, Faculty of Science, Shimane University,
Nishikawatsu-cho, Matsue 690
†Department of Chemistry, Faculty of Science, Hiroshima University,
Higashisenda-machi, Naka-ku, Hiroshima 730

A yellow pigment isolated from the title solution is proposed to be a tautomeric mixture of 3-hydroxy-5-methoxy-2,6-dimethyl-6-(2-methylbutyryloxy)-2,4-cyclohexadien-1-one and 3-hydroxy-5-methoxy-2,4-dimethyl-4-(2-methylbutyryloxy)-2,5-cyclohexadien-1-one on the basis of methylation experiment which give two products corresponding to both forms.

In previous papers, we reported the isolation of flaviolin(1) and 2-hydroxy-6-methoxy-3,5-dimethyl-1,4-benzoquinone(2) from the potato culture solution of *Phoma wasabiae* YOKOGI. The latter benzoquinone seemed to be produced by the oxidative degradation of an unknown yellow pigment during isolation. In order to confirm the structure of this yellow pigment, this work was carried out.

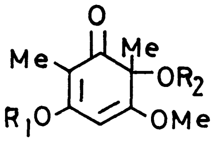
Phoma wasabiae was cultivated on the potato culture solution containing 2% glucose at 25 °C. After three weeks of the cultivation, the blackish culture solution (6.0 l) was concentrated in vacuo. The concentrated solution was extracted with $CHCl_3$ and the solvent was removed; the resulting oily substance (0.24 g/l) showed five yellow bands on silica-gel plate using C_6H_6-CH_3OH (9:1) as the solvent (Rf values: 0.45, 0.33, 0.26, 0.19, and 0.12). A broad yellow band (Rf 0.45) was collected and then extracted with Et_2O to give the oily substance, wasabidienone-A (1), yield 0.03 g/l, $C_{14}H_{20}O_5$ [m/z M^+ 268 (GC/MS)], IR(CCl_4) ν 3450-3200, 1735, 1675, 1620 cm^{-1}. The ^1H NMR spectral feature of 1 was rather complex. It was predicted that 1 was a mixture of unseparable compounds. Moreover, 1 gradually changed from yellow to brown in color by the air oxidation when it was left on the silica-gel plate. The resulting brown color substance was identical with 2-hydroxy-6-methoxy-3,5-dimethyl-1,4-benzoquinone (2) previously reported.(2)

The methylation of 1 with diazomethane gave two methyl ethers with ratio of 1:1, 3 (mp 75-76 °C, pale yellow needles from hexane) and 4 (colorless oil), which were easily separated on preparative TLC with C_6H_6-CH_3OH (9:1).

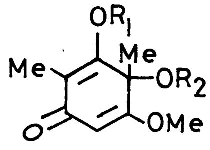
Compound 3, $[\alpha]_D$ +142.0° (c 2.0, $CHCl_3$), had a molecular formula $C_{15}H_{22}O_5$ (Found: m/z 282.1479. Calcd: M, 282.1467 and Anal. C, H). From the spectral data of 3, IR ν 1730 cm^{-1}, MS *m/z* 198(M-C_5H_8O), ^1H NMR($CDCl_3$) δ 0.92(3H, t, J=7.0 Hz), 1.15(3H, d, J=7.0 Hz), 2.48(1H, m) with decoupling experiments, and ^{13}C NMR,3) **3** was presumed to be an ester of 2-methylbutyric acid. The compound **3** was hydrolysed in aq 1M NaOH solution to give (R)-2-methylbutyric acid ($[\alpha]_D$ -16.0° (c 0.64, $CHCl_3$)),4) which was identical with authentic sample. Further structural characteristics of **3** deduced from ^{1}H and ^{13}C NMR spectra were the presence of two methyl groups [δ 1.49, 1.78(each 3H, s)], two methoxyl groups [δ 3.74, 3.90(each 3H, s)], one olefinic proton [δ 5.47(1H, s)], one tetrasubstituted and one trisubstituted double bond, one carbonyl carbon, and one quarternary carbon. On the basis of IR(ν 1650 cm^{-1}) and UV[λ_{max}^{EtOH}nm(log ε) 226(4.41), 369(3.84)] spectra, the presence of a pentasubstituted cyclohexadienone moiety is strongly suggested. The flanking of the olefinic proton by two methoxyl protons was proved by the NOE experiments (30% and 35% enhancement respectively). Moreover, the reduction of **3** with Zn in AcOH gave 3,5-dimethoxy-2,6-dimethylphenol (**5**), which was identical with synthetic one.5) Therefore, the structure of **3** was determined as 3,5-dimethoxy-2,6-dimethyl-6-(2-methylbutyryloxy)-2,4-cyclohexadien-1-one.

Compound **4**, $[\alpha]_D$ +63.6° (c 0.64, $CHCl_3$), $C_{15}H_{22}O_5$, is a structural isomer of **3**. In fact, the ^{1}H and ^{13}C NMR spectra6) of this compound were very similar with those of **3**. The significant differences between **3** and **4** appeared in the absorption bands of IR(ν 1668 cm^{-1}) and UV[λ 315(3.73)] spectra. From these spectral properties and other spectral data,6) the structure of **4** was determined as 3,5-dimethoxy-2,4-dimethyl-4-(2-methylbutyryloxy)-2,5-cyclohexadien-1-one.

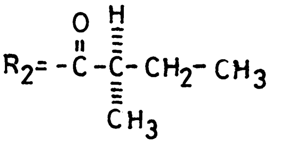
From the above results, it is deduced that wasabidienone-A is the tautomeric mixture of 3-hydroxy-5-methoxy-2,6-dimethyl-6-(2-methylbutyryloxy)-2,4-cyclohexadien-1-one (**1a**) and 3-hydroxy-5-methoxy-2,4-dimethyl-4-(2-methylbutyryloxy)-2,5-cyclohexadien-1-one (**1b**). This is the first example of natural occurring cyclohexadiene pigment.



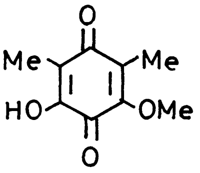
1a R_1=H
3 R_1=CH_3



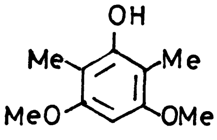
1b R_1=H
4 R_1=CH_3



$R_2 = -\overset{O}{\overset{\|}{C}}-\overset{H}{\underset{CH_3}{C}}-CH_2-CH_3$



2



5

References

1) O. Soga, *Z. Naturforsch.*, B, 31, 124 (1976).

2) O. Soga and H. Iwamoto, *Z. Naturforsch.*, B, 36, 277 (1981).

3) ^{13}C NMR δ s: 194.5, 175.0, 169.0, 167.6, 106.5, 78.0; d: 86.2, 40.1; t: 26.7; q: 55.9, 55.8, 25.0, 16.4, 11.3, 7.3.

4) F. L. Weisenborn, J. W. Bolger, D. B. Rosen, L. T. Mann, L. Johnson, and H. L. Holmes, *J. Am. Chem. Soc.*, 76, 1792 (1954): $[\alpha]_D$ -18.0° (pure liq.).

5) W. Gruber and F. Traub, *Monatsh. Chem.*, 77, 414 (1947).

6) **4**: $C_{15}H_{22}O_5$ (Found: *m/z* 282.1461. Calcd: M, 282.1467); IR ν 1730, 1668 cm^{-1}; UV λ 213(4.41), 315(3.73); ^{1}H NMR δ 0.97(3H, t, J=7.0 Hz), 1.15(3H, d, J=7.0 Hz), 1.50, 1.86(each 3H, s), 2.48(1H, m), 3.76, 3.79(each 3H, s), 5.44(1H, s); ^{13}C NMR δ s: 195.4, 175.6, 172.7, 163.3, 112.8, 79.3; d: 96.1, 40.2; t: 26.7; q: 61.3, 56.3, 24.1, 16.4, 11.4, 9.6.

(Received November 16, 1983)