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# 8-Hydroxy-3-Methyl-3,4-Dihydro-*1h*-Isochromen-1-One from Static Cultures of the Fungus *Xylaria Badia*

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**ABSTRACT**

The fungus *X. badia* was cultured on 3% aqueous malt enriched with 6% glucose in ten Thompson bottles for eight weeks. Solvent extraction with ethyl acetate and subsequent drying on a rotary evaporator, afforded a dark brown gummy solid (5.0 g). TLC studies indicated that the crude extract was a mixture of four components. The dark brown gum was chromatographed over silica gel in a column of size 80 cm x 2.5 cm. The column was eluted with toluene, ethyl acetate and acetic acid (50:49:1) and the eluent collected in volumes of 3.0 ml. Fraction 3 gave a yellowish oil (150 mg). The oil was triturated with n-hexane to give a white powdery solid (12 mg). The solid was recrystallised from the same solvent system, yielding white crystals (8 mg). Spectroscopic analysis of this compound indicated that it is R-mellein. This is the first report of this secondary metabolite from *Xylaria badia*.



## Introduction

*Xylaria badia* is found worldwide. The isolate that was examined for its secondary metabolites was collected from Thailand. Following the isolation and characterisation of a benzoquinone and naphtol glucoside from the first culture of *X. badia* [1],. This earlier study, generated the interest in a second culture of the fungus to isolate the other secondary metabolites produce by *X. badia*. The fungus was cultured under similar conditions. The culture medium was harvested and extracted with neat ethyl acetate after the mycelium had been removed by sieving through a muslin. Drying the extract on a rotary evaporator afforded a dark brown gummy solid (5.0 g). TLC studies indicated that the crude solid was a mixture of four components.

## Materials and Methods

The fungus *X. badia* was cultured on 3% aqueous malt enriched with 6% glucose in four Thompson bottles for eight weeks for the second time. Solvent extraction with neat ethyl acetate and subsequent drying on a rotary evaporator afforded a dark brown gummy solid (5.0 g). TLC studies indicated that this crude extract was a mixture of four components. One of the components gave a dark brown colour on TLC. The dark brown gum was chromatographed over silica gel in a column of size 80 cm x 2.5 cm. The column was eluted with toluene, ethyl acetate and acetic acid (50:49:1) and the eluent collected in volumes of 3.0 ml.

Fraction 3 (tubes 55-74) gave a yellowish oil (150 mg), n-hexane (1.0 ml) was added to the oil and warmed to boiling point. The solution was filtered hot and allowed to cool overnight. A white solid (12 mg) was obtained. The solid was recrystallised in the same solvent system to give R-(-)-mellein white crystals (8 mg), $C_{10}H_{10}O_3$; mp. 48-50 $^oC$, (lit.,[5] 48$^oC$), m/z 166 ($M^+$), $[\alpha]_D^{23}$ -92$^o$ [c=0.93, MeOH], $\nu_{max}$ (KBr) $cm^{-1}$ 3620-3300, 1670, 1615 and 1580. $\delta_H$ ($CDCl_3$) 1.53 (11-$CH_3$, d, *J* 6.4 Hz), 2.91 (4-$CH_2$, d, *J* 7.1 Hz), 4.70 (3-CH, m). 6.69 (Ar-CH, d, *J* 7.4 Hz), 6.88 (Ar-CH, d, *J* 8.4 Hz), 7.40 (Ar-CH, t, *J* 7.3, Hz). $\delta_C$ ($CDCl_3$) 20.85 (11-$CH_3$), 34.68 (4-$CH_2$), 76.79 (3-CH), 108.36 (7-C), 116.31 (5-CH), 117.99 (6-CH), 136.24 (9-CH), 139.47 (10-C), 162.25 (8-C) and 170.06 (1-C=O).

Procedure involved in collecting the physical data include: All chromatography columns, thick layer (PLC) and thin layer (TLC) plates were made up using Merck Kieselgel $GF_{254}$ . Column size used was 80,0 cm x 2.5 cm. Reagents used for detecting spots on plates were prepared as follows. Anisaldehyde spray reagent; addition of 1 $cm^3$ each of anisaldehyde solution and: concentrated sulphuric acid solution to 98 $cm^3$ of glacial acetic acid solution (1:1:98).

Solids were obtained from extracts by trituration or crystallisation using single or mixed solvent system. These had been specified in both the crude and pure state.

Melting points were determined on a Kofler hot-stage apparatus. IR spectra was taken on a Perkin–Elmer 681 instruments. Mass spectra was determined on an AEI MS 902 spectrometer (EI).

$^1H$ and $^{13}C$ NMR spectra were recorded on a JEOL ECA 600 FT NMR spectrometer fitted with an autotune 5 mm X-H probe with field gradient coils. $^1H$ NMR spectra were acquired with 64 K data points over a range of 11.26 kHz and 8-16 scans. *J* values were measured in Hz. Carbon atom types were assigned by employing a combination of $^{13}C$ NMR spectra, broadband proton decoupled (values stated to 2 significant figures, the second significant figure may be ignored) and distortionless enhancement by polarisation transfer (DEPT) experiments.

## Results and Discussion

The dark brown gum (5.0 g) of crude extract from culture medium of *X. badia* was chromatographed over silica gel in a column of size 80 cm x 2.5 cm. The column was eluted with toluene, ethyl acetate and acetic acid (50:49:1) and the eluent collected in volumes of 3.0 ml.

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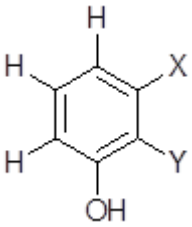
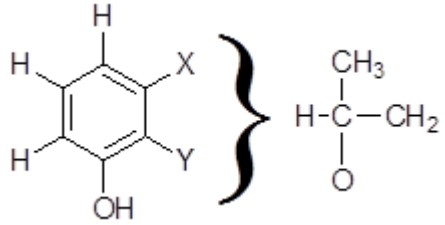
Tubes (**55-74**), fraction 3 gave a yellowish oil (150 mg). The oil was triturated with n-hexane to give a white powdery solid (12 mg). The solid was recrystallised from the same solvent system yielding white crystals (8 mg). mp 48-49 $^{o}$C, m/z 178 ($M^{+}$), ($C_{10}H_{10}O_3$). $[\alpha]_D^{23}$ -92$^{o}$ (c = 0.93, MeOH), IR (KBr/cm) 3620-3300, 1670, 1615 and 1580. $^{1}$H NMR determined in $CDCl_3$ had a strong singlet at $\delta_H$ 11.02 indicating the presence of a chelated OH. Three signals were observed in the aromatic region of this spectrum ($\delta_H$ 6.50-8.5), two doublets at $\delta_H$ 6.69 (*J* 7.39 Hz) and $\delta_H$ 6.89 (*J* 8.4 Hz) coupled to a pseudo triplet at 7.40 (*J* 7.6 Hz). The coupling constants were typical ortho coupling constants. In the $^{13}$C NMR determined in $CDCl_3$, signals for six aromatic carbons occured at $\delta_C$ 108.36, 116.31, 117.99, 136.24, 139.47 and 162.25. Three of these aromatic protons were quaternary protons, suggesting that the aromatic portion of this compound is tri-substituted as shown in fragment (**A**).



**(A)**

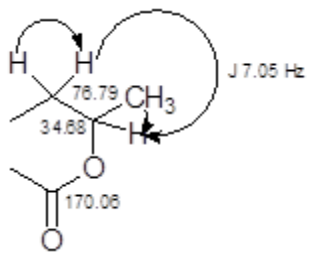
The phenolic nature of the compound was confirmed by treating a solution of the compound with ferric chloride to give violet colouration. The molecular formula of the compound was deduced to be $C_{10}H_{10}O_3$.

Thus, $C_6H_4O$ had been accounted for, leaving $C_4H_6O_2$. In the high field region of the $^{1}$H NMR, there were three signals at $\delta_H$ 4.70 (1H, m), $\delta_H$ 2.91 (2H, d, *J* 7.1 Hz) and $\delta_H$ 1.53 (d, *J* 6.4 Hz). The position of the methine proton at $\delta_H$ 4.75 indicates that it is attached to a C-O group. A $^{1}$H-$^{1}$H COSY spectrum showed the presence of subunit (**B**).



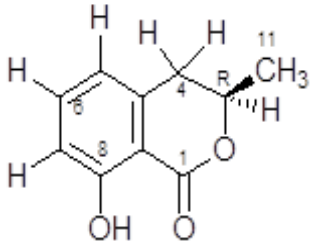
(**B**)

A methyl carbon at $\delta_C$ 20.85, a methylene carbon at $\delta_C$ 34.68 and a methine at $\delta_C$ 76.79 in the $^{13}$C NMR spectra supports the above proposal. One carbon and an oxygen atom are left unaccounted for. On TLC, the compound gave a yellow colour with 2,4-DNP spray reagent, indicating the presence of a C=O group. In the $^{13}$C NMR spectrum, there was a quaternary carbon at $\delta_C$ 170.06, typical of a carbonyl of a lactone. The phenolic fragment (**A**) might be joined to fragment (**C**).



(**C**)

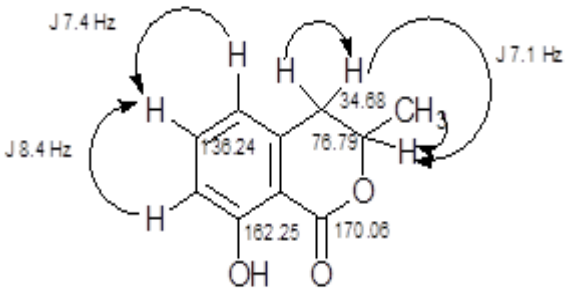
The orientation of fragment (**A**) and (**C**) was revealed by the chelation of the phenolic OH with the carbonyl group. The chelated OH occurred at $\delta_H$ 11.02, indicating that the OH is in the neighbourhood of the carbonyl group. The optical rotation is negative. This gave the compound as **R-(-)-mellein (1)**.



A summary of the $^{1}$H and $^{13}$C NMR assignments is shown in Table 1 and structure (2)

**Table 1. $^{1}$H and $^{13}$C NMR designation of R-(-)-mellein.**

| ($\delta_H$) | ($\delta_C$) | $C_{\#}$ |
|---|---|---|
| 1.53 (3H,d, *J* 6.3 Hz) | 20.85 | 11 |
| 2.91 (2H, d, 7.1 Hz) | 34.68 | 4 |
| 4.70 (1H, m) | 76.79 | 3 |
| 6.69 (1H, d, *J* 7.4 Hz) | 116.31 | 7 |
| 6.88 (1H, d, *J* 8.4 Hz) | 117.99 | 5 |
| 7.40 (1H, d, *J* 7.6,) | 136.24 | 6 |
| 11.03 (1H, s(br)) | - | - |
| - | 108.36 | 9 |
| - | 139.4710 | 10 |
| - | 162.25 | 8 |
| | 170.06 | 1 |



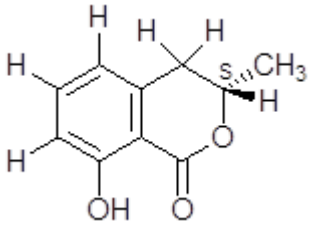
**(2)**

ACD/Labs, was used to assist in the identification of the compound. The software was used to predict the $^{13}$C NMR data which was compared with the experimental values obtained (Table 2).

**Table2.Comparison of the experimental and calculated values of $^{13}$C NMR of mellein**

| (Expt.) ($\delta_C$) | (Cal.) ($\delta_C$) | $C_{\#}$ |
|---|---|---|
| 20.85 | 20.73 | c-11 |
| 34.68 | 34.62 | c-4 |
| 76.79 | 76.07 | c-3 |
| 108.36 | 108.30 | c-7 |
| 116.31 | 116.46 | c-5 |
| 117.99 | 117.88 | c-6 |
| 136.24 | 136.11 | c-9 |
| 139.47 | 139.39 | c-10 |
| 162.25 | 162.21 | c-8 |
| 170.06 | 169.91 | c-1 |

Mellein is a secondary metabolite found in both higher and lower plants. It was first isolated in 1933 from *Aspergillus melleus*. Later researchers worked out the stereochemistry of mullein [2]. It was found to exist naturally in two main forms; the more common form R-(-)-mellein (**1**) and the less common isomer S-(+)-mellein (3).



**(3)**

Table 3 is a summary of the source and physical properties of naturally occurring R-(-)-mellein reported in literature.

**Table 3. Source and physical properties of R-(-)-mellein reported in literature.**

| Melting point ($^{o}$C) | Optical rotation | Source |
|---|---|---|
| 56 | -102.5$^{o}$ (c 1.0, $CHCl_3$ at 25 $^{o}$C) | *Aspergillus onika* [3] |
| 58 | -100$^{o}$ (c 1.0, $CHCl_3$ at 22 $^{o}$C) | *Hypoxylon howieanum* [4] |
| 56-58 | -80$^{o}$ (c 0.02, $CHCl_3$ at 22 $^{o}$C) | *Aspagillus melleus* |
| 47-48 | -94$^{o}$ (c 0.48, $CHCl_3$ at 22 $^{o}$C) | *Lasiodiplodia spp.*[5] |
| 55-56 | -100.8$^{o}$ (c 1.01, $CHCl_3$ at 22 $^{o}$C) | *Synthesis* [6] |
| 55.5-56 | -101.3$^{o}$ (c 0.07, $CHCl_3$ at 26 $^{o}$C) | *Synthesis* [7] |

The R-mellein isolated from *X. badia* is very similar in physical properties to that reported from *Lasiodiplodia spp* [5].

**Conclusion**

Mellein is a secondary metabolite found in both higher and lower plants. It was first isolated in 1933 from *Aspergillus melleus*[7]. Later researchers worked out the stereochemistry of mellein. It was found to exist naturally in two main forms; the more common form R-(-)-mellein and the less common isomer S-(+)-mellein. Melleins are 3,4-dihydroisocoumarins mainly produced by fungi, but also by plants, insects and bacteria. These specialized metabolites play important role in the life cycles of the producers and they are involved in many biochemical reactions. This is the first report of the isolation and characterization of R-(-)mellein from the fungus *Xylaria badia*.

**References**

1. Oppong, E. K., Edwards, R. L., Maitland, D. J., & Hanson, R. (2010). Metabolites from static cultures of the fungus Xylaria badia. *International Journal of Applied Chemistry*, *6*(3), 387-394.

2. Garson, M. J., Staunton, J., & Jones, P. G. (1984). New polyketide metabolites from Aspergillus melleus: structural and stereochemical studies. Journal of the Chemical Society, Perkin Transactions 1, 1021-1026.

3. Sasaki, M., Kaneko, Y., Oshita, K., Takamatsu, H., Asao, Y., & Yokotsuka, T. (1970). Studies on the compounds produced by molds: Part VII. Isolation of isocoumarin compounds. *Agricultural and Biological Chemistry*, *34*(9), 1296-1300..

4. Anderson, J. R., Edwards, R. L., & Whalley, A. J. (1983). Metabolites of the higher fungi. Part 21. 3-Methyl-3, 4-dihydroisocoumarins and related compounds from the ascomycete family Xylariaceae. Journal of the Chemical Society, PerkinTransactions 1, 2185-2192.

5. Cimmino, A., Andolfi, A., Abouzeid, M., & Evidente, A. (2013). Polyphenols as fungal phytotoxins, seed germination stimulants and phytoalexins. Phytochemistry reviews, 12(4), 653-672.

6. Mori, K., & Gupta, A. K. (1985). Chiral synthesis of (R)-(-)-mellein and (3R, 4aS)-(+)-ramulosin. Tetrahedron, 41(22), 5295-5299.

7. Dimitriadis, C., Gill, M., & Harte, M. F. (1997). The first stereospecific approach to both enantiomers of mellein. *Tetrahedron: Asymmetry*, *8*(13), 2153-2158.