Phytochemistry, 1979, Vol. 18, pp. 311–313. © Pergamon Press Ltd. Printed in England. 0031-9422/79/0201-0311 $02.00/0

MYCOCHROMONE AND MYCOXANTHONE: TWO NEW METABOLITES FROM *MYCOSPHAERELLA ROSIGENA**

GEMMA ASSANTE†, LORENZO CAMARDA§, LUCIO MERLINI‡ and GIANLUCA NASINI§

†Istituto di Patologia Vegetale and ‡Istituto di Biochimica Generale, Facoltà di Agraria dell'Università, 20133 Milano, Italy and §Politecnico, Istituto di Chimica‖, 20133 Milano, Italy

(*Received* 10 *July* 1978)

Key Word Index—*Mycosphaerella*; Ascomycetes; fungus; xanthone; chromone; structural determination.

Abstract—Two new metabolites, dimethyl 2-(2-ethenyl-5-hydroxy-4H-1-benzopyran-4-onyl-3)-malonate (mycochromone **1a**) and methyl 2-methoxy-8-hydroxy-9-oxo-9H-xanthene-1-carboxylate (mycoxanthone **6a**), have been isolated and identified from the mycelium of *Mycosphaerella rosigena* grown on potato-agar.

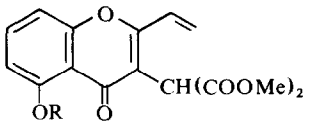
INTRODUCTION

After the screening of the genus *Cercospora* for secondary metabolites [1, 2], which includes several phytotoxic species, in order to find new biologically active substances we have undertaken the screening of the genus *Mycosphaerella*, the perfect form of *Cercospora*. The present work concerns the structural elucidation of mycochromone (**1a**) and mycoxanthone (**6a**), two secondary metabolites produced from *M. rosigena*. This fungus is responsible for the leaf spot of greenhouse roses causing up to 90% defoliation of large plants [3].

RESULTS AND DISCUSSION

The fungus was grown on potato-agar medium and the metabolites extracted with ethyl acetate and purified by chromatography.

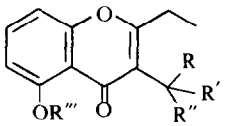
The metabolite (**1a**), that we propose to call mycochromone, is a yellow solid. Analysis and MS give the formula $C_{16}H_{14}O_7$. UV (λ_{max} 267, 290 sh and 342 nm) and IR spectra (3470, OH, 1740, CO, 1650 cm^{-1}, conj. CO), are consistent with the presence of a chromone ring. The PMR spectrum shows two OMe groups, a $-CH{=}CH_2$ group, 3 vicinal aromatic protons (shifted by acetylation), a chelated OH and a proton at δ 5.34 as a singlet which exchanges with D_2O. Combination of all these data gives for (**1a**) a partial structure of a 2,3-disubstituted 5-hydroxychromone. The two substituents must be a vinyl and a diMe malonyl group, as required by the elemental formula, the CO frequency in the IR and the two OMe groups in the PMR spectrum. Hydrogenation of (**1a**) with Pd gives the dihydroderivative (**2**). The presence in the PMR spectrum of a $-CH_2-CH_3$ group instead of a $-CH{=}CH_2$ clearly indicates that the vinyl group only was hydrogenated. Hydrolysis of (**2**) with dil. NaOH gives (**4**), the structure of which is in accordance with the presence in (**1a**) of a malonic group. Methylation of (**2**) with MeI gives a dimethyl derivative (**3**). PMR shows



1a R = H
1b R = COMe

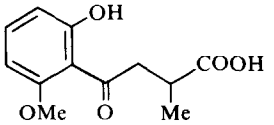
the presence of a new methoxy group, the absence of the acidic proton (at δ 5.21 in (**2**)) and a singlet at δ 1.75 consistent with a Me group on a carbon bearing two carbonyl groups.

In order to establish the exact position of the two substituents in (**1a**) we reacted (**3**) by refluxing with aq. NaOH and obtained product (**5**) together with resorcinol



2 R = R′ = COOMe; R″ = R‴ = H
3 R = R′ = COOMe; R″ = R‴ = Me
4 R = COOH; R′ = R″ = R‴ = H

mono Me ether and 2-hydroxy-6-methoxybenzoic acid. The MS of (**5**) has M^+ at *m/e* 238 and the IR spectrum gives two characteristic bands at 1705 cm^{-1}, acidic CO, 1630 cm^{-1}, conj. CO. The PMR shows 3 vicinal aromatic protons, a OMe group, a CH_3-CH as a doublet, 3 aliphatic protons of a $-CO-CH_2-CH-CO-$ system, a chelated OH and another OH group at δ 11.18. The ^{13}C NMR spectrum shows 12 carbons: 6 aromatic carbons (3 singlets and 3 doublets), a OMe (quartet), two aliphatic carbon (a triplet and a doublet), and two carbonyl (singlets). These data can be explained by hydrolysis of the malonate, decarboxylation and degradation of the chromone ring with alkali. Thus (**5**) must have the formula shown. Therefore, the structure of mycochromone is (**1a**).



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*Part VI in the series "Secondary Mould Metabolites". For Part V see ref. [1].
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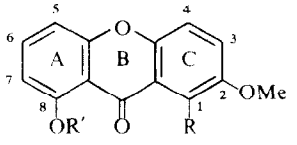
The metabolite, (**6a**), that we propose to call mycoxanthone, is a yellow solid. Analysis and MS (M^+ at *m/e* 300, *m/e* 268 ($M^+ - 32$) and *m/e* 240 ($M^+ - 60$) give the formula $C_{16}H_{12}O_6$. UV (λ_{max} 236.5, 262, 290, 315 sh, 324 sh and 386 nm) and IR spectra (3430 cm^{-1}, OH), 1742 cm^{-1}, ester CO, 1652 cm^{-1}, conj. CO) suggest the presence of a xanthone ring. The PMR shows 3 vicinal aromatic protons (shifted by acetylation), two aromatic protons in the *ortho* position, two OMe groups and a chelated OH. All these data give for (**6a**) a partial structure of a 8-hydroxyxanthone disubstituted in ring C. In order to establish the exact substitution of the ring C, (**6a**) was hydrolysed with aq. alkali and the acid (**6c**) was decarboxylated with copper chromite/quinoline to give the known [4] 2-methoxy-8-hydroxyxanthone (**7**).



6a R = COOMe; R′ = H
6b R = COOMe; R′ = COMe
6c R = COOH; R′ = H
7 R = R′ = H

Therefore mycoxanthone must have a OMe substituent in position 2, and since (**7**) shows the presence of a new aromatic proton at low field (δ 7.65) near the carbonyl group [5] and two *ortho* protons in the PMR spectrum, the only possible location for the carbomethoxy group is in position 1. It follows that its structure is (**6a**).

The carbon skeleton of both (**1a**) and (**6a**) could be biosynthetically derived from an anthraquinone via the degradation scheme already established for other fungal xanthones [6]. Biosynthetic studies to confirm this suggestion are in progress.

EXPERIMENTAL

Mps are uncorr. UV spectra were measured in 95% EtOH. NMR spectra were recorded at 90 and 100 MHz, chemical shifts are in ppm (δ), from TMS as internal standard. Column chromatography and TLC were performed with Si gel. Where not otherwise indicated the purity of the products was checked by TLC, NMR and MS and deemed sufficient for the purposes of structural elucidation.

Isolation and purification of the metabolites. A strain of *M. rosigena* 330.51 obtained from C entraalbureau voor Schimmelcultures, Baarn grown on potato-agar in Roux flasks was extracted twice with EtOAc after 3 weeks growth at room temp. The extract was dried over Na_2SO_4 and evapd to give a yellow-brown mixture of crude pigments (1 g from each 2 l. flask). This mixture was adsorbed on the top of a chromatographic column and eluted with a mixture of hexane and EtOAc. Two main metabolites (**1a**) (250 mg) and (**6a**) (50 mg) were isolated with hexane–EtOAc mixtures of 7:3 and 6:4, respectively.

Dimethyl 2-(2-ethenyl-5-hydroxy-4H-1-benzopyran-4-onyl-3)-malonate (**1a**). Yellow needles mp 148–149° (EtOAc). Mass 318, 286, 254. λ_{max} 267, 290 sh and 342 nm (ε 34000, 9500, 5500). ν_{max}^{KBr} cm^{-1}: 3470 (OH), 1740 (COOMe), 1650 (conj. CO). (Found: C, 60.38; H, 4.53. $C_{16}H_{14}O_7$ requires: C, 60.38; H, 4.43%). PMR ($CDCl_3$, 100 MHz): δ 3.77 (6H, *s*, —COO<u>Me</u>), 5.34 (1H, *s*, —<u>CH</u>(COOMe)$_2$), 5.84 (1H, *dd*, J_{cis} = 10 Hz, J_{gem} = 2 Hz, *cis*—CH=C<u>H</u>—H), 6.42 (1H, *dd*, J_{trans} = 18 Hz, J_{gem} = 2 Hz, *trans*—CH=C<u>H</u>—H), 6.78 (1H, *dd*, J_{cis} = 10 Hz, J_{trans} = 18 Hz, —C<u>H</u>=CH$_2$), 6.78 and 6.9 (2H, *d*, *J* = 8 Hz, C—6 and C—8), 7.52 (1H, *t*, *J* = 8 Hz, C—7), 12.2 (chel. OH).

Methyl 2-methoxy-8-hydroxy-9-oxo-9H-xanthene-1-carboxylate (**6a**). Yellow solid, mp 222–223°. Mass 300, 268, 254, 240, 225. λ_{max} 236.5, 262, 290, 315 sh, 324 sh and 386 nm (ε 19600, 21800, 18700, 16200, 16000, 17800). ν_{max}^{nujol} cm^{-1}: 3430 (OH), 1742 (COOMe), 1652 (conj. CO). (Found: C, 63.88; H, 4.04. $C_{16}H_{12}O_6$ requires: C, 64.00; H, 4.03%). PMR ($CDCl_3$, 100 MHz) δ 3.92 and 4.05 (6H, *s*, 2 OMe), 6.78 and 6.9 (2H, *dd*, *J* = 8 and 2 Hz, C-5 and C-7), 7.4 (1 H, *d*, *J* = 8 Hz, C-4), 7.54 (1 H, *d*, *J* = 8 Hz, C-3), 7.59 (1H, *t*, *J* = 8 Hz, C-6) 12.24 (chel. OH).

Dimethyl 2-(2-ethenyl-5-acetoxy-4H-1-benzopyran-4-onyl-3)-malonate (**1b**). **1a** (100 mg) in 1 ml dry C_5H_5N and 1 ml Ac_2O were left for 12 hr at 4°. The mixture was dissolved in $CHCl_3$ and treated with a satd soln of $NaHCO_3$, H_2O, satd $KHSO_4$, H_2O and finally dried with Na_2SO_4. PLC (hexane–EtOAc, 2:1) gave a monoacetate (**1b**), mp 151–153° (dec) λ_{max} 251, 258, 286 and 313 nm (ε 30500, 30000, 10900, 9900). $\nu_{max}^{CHCl_3}$ cm^{-1}: 1757 (CO acetate), 1740 (COOMe), 1640 (conj. CO). PMR ($CDCl_3$, 100 MHz): δ 2.42 (3H, *s*, CO<u>CH</u>$_3$), 3.74 (6H, *s*, COOMe), 5.49 (1H, *s*, —<u>CH</u>(COOMe)$_2$), 5.78 (1H, *dd*, J_{cis} = 10 Hz, J_{gem} = 2 Hz, *cis*-CH=C<u>H</u>—H), 6.37 (1H, *dd*, J_{trans} = 18 Hz, J_{gem} = 2 Hz, *trans*-CH=C<u>H</u>—H), 6.75 (1H, *dd*, J_{trans} = 18 Hz, J_{cis} = 10 Hz, —C<u>H</u>=CH$_2$), 7.01 and 7.38 (2H, *dd*, *J* = 8 and 2 Hz, C-6 and C-8), 7.64 (1H, *t*, *J* = 8 Hz, C-7).

Hydrogenation of (**1a**) *with* $Pd/BaSO_4$. **1a** (30 mg) in 10 ml EtOAc were hydrogenated in the presence of 10% Pd on $BaSO_4$ at room temp. Evapn of the solvent and PLC (hexane–EtOAc, 8:2) gave 25 mg of (**2**), mp 136–137°. Mass 320, 288, 256, λ_{max} 328 nm (ε 4900). ν_{max}^{nujol} cm^{-1}: 1760 (COOMe), 1650 (conj. CO). (Found: C, 59.66; H, 4.90. $C_{16}H_{16}O_7$ requires: C, 60.00; H, 5.04%). PMR ($CDCl_3$, 100 MHz) δ 1.3 (3H, *t*, *J* = 6 Hz, <u>CH</u>$_3$—CH$_2$—), 2.73 (2H, *q*, *J* = 6 Hz, CH$_3$—<u>CH</u>$_2$—), 3.79 (6H, *s*, COOMe), 5.21 (1H, *s*, —<u>CH</u>(COOMe)$_2$), 6.78 and 6.85 (2H, *d*, *J* = 8 Hz, C-6 and C-8), 7.52 (1H, *t*, *J* = 8 Hz, C-7), 12.28 (chel. OH). The same product was obtained by reaction of (**1a**) with Zn and 5% H_2SO_4 in MeOH.

Hydrolysis of (**2**). Hydrolysis of 100 mg of (**2**) by refluxing with 10% NaOH in MeOH for 4 hr gave (**4**) as white needles, mp 180–181°. Mass 248, 204. λ_{max} 237, 258 sh and 326 nm (ε 28900, 15500, 7200). ν_{max}^{KBr} cm^{-1}: 1700 (COOH), 1650 (conj. CO). PMR (Me_2CO-d_6, 100 MHz) δ 1.32 (3H, *t*, *J* = 6 Hz, <u>CH</u>$_3$—CH$_2$—), 2.84 (2H, *q*, *J* = 6 Hz, CH$_3$—<u>CH</u>$_2$—), 3.61 (2H, *s*, R—<u>CH</u>$_2$—COOH), 5 (1H, *br* OH), 6.76 and 6.99 (2H, *d*, C-6 and C-8), 7.61 (1H, *t*, *J* = 8 Hz, C-7), 12.6 (*br* chel. OH).

Methylation of (**2**) *with* MeI. MeI (1 ml) was added dropwise to 100 mg of (**2**) in 10 ml of dry Me_2CO and 300 mg of anhyd. K_2CO_3 and the mixture refluxed for 4 hr. Filtration and evapn gave a quantitative yield of (**3**), mp 113–114° (EtOAc–ligroin–Et_2O). Mass 348, 316, 289, 273, 257. λ_{max} 230, 257, 268 sh and 316 nm (ε 27000, 15500, 8500, 6500). ν_{max}^{nujol} cm^{-1}: 1733 and 1760 COOH), 5 (1 H, *br* OH), 6.76 and 6.99 (2H, *d*, C-6 and C-8), 7.61 *t*, *J* = 6 Hz, <u>CH</u>$_3$—CH$_2$—), 1.75 (3H, *s*, <u>CH</u>$_3$—C), 2.4 (2H, *q*, *J* = 6 Hz, <u>CH</u>$_3$—CH$_2$—), 3.78 (6H, *s*, COOMe), 3.9 (3H, *s*, OMe), 6.72 and 6.92 (2H, *d*, *J* = 8 Hz, C-6 and C-8), 7.5 (1H, *t*, *J* = 8 Hz, C-7),

2-Methyl-4-oxo-4(2-hydroxy-6-methoxyphenyl)-butanoic acid (**5**). **3** (50 mg) was refluxed for 20 min with 5% NaOH in MeOH/H_2O (1:1). Acidification, extraction, evapn and PLC ($CHCl_3$–MeOH, 30:1) gave 2-hydroxy-6-methoxybenzoic acid and the compound (**5**), mp 124–125°. Mass 238, 220, 193, 177, 151. λ_{max} 271 and 338 nm (ε 6500, 1900). ν_{max}^{nujol} cm^{-1}: 1705 (COOH), 1630 (conj. CO). PMR (90 MHz, $CDCl_3$) δ 1.3 (3H, *d*, *J* = 6 Hz <u>CH</u>$_3$—CH), 2.8–3.8 (3H), 3.9 (3H, *s*, OMe), 6.38 and 6.53 (2H, *d*, *J* = 8 Hz, C-3 and C-5), 7.33 (1H, *t*, *J* = 8 Hz, C-4), 11.18 (*br* CO<u>OH</u>), 12.98 (*br* cgel. OH). ^{13}C-NMR ($CDCl_3$) 204.5 (*s*, Ar-CO), 182.2 (*s*, —COOH), 164.6, 161.4 (*s*, C-2 and C-6), 136.3 (*d*, C-4), 110.9 (*s*, C-1), 110.9 (*d*, C-5), 101.1 (*d*, C-3), 55.6 (*q*, OMe), 48.1 (*t*, —CH$_2$—), 34.9 (*d*, —CH—), 17.0 (*q*, CH$_3$—).

Methyl 2-methoxy-8-acetoxy-9-oxo-9H-xanthene-1-carboxylate (**6b**). **6a** (50 mg) in 0.5 ml C_5H_5N and 0.5 Ac_2O was left 18 hr at room temp. The mixture was dissolved in $CHCl_3$ and worked up as described above. The monoacetate (**6b**) has mp 220–221°. Mass 342, 300, 268, 254, 240, 225. λ_{max} 240, 250 sh, 270 sh, 302 sh.

360 and 373 nm (ε 22000, 18500, 5300, 800, 3800, 3400). ν_{max}^{nujol} cm^{-1}: 1762 (CO acetate), 1732 (COOMe), 1660 (conj. CO). PMR (100 MHz, $CDCl_3$) δ 2.38 (3H, *s*, $COCH_3$), 3.84 and 3.96 (6H, *s*, OMe), 6.92 (1H, *dd*, $J = 8$ Hz, $J = 2$ Hz, C-5), 7.06–7.8 (4 aromatic protons).

8-Hydroxy-2-methoxy-9-oxo-9H-xanthene-1-carboxylic acid (**6c**). **6a** (5 mg) was treated with 5% aq. KOH for 4 days at reflux temp. After acidification, extraction and PLC (benzene–Et_2O–HCO_2H, 25:75:0.5 **6c** was obtained. Mass 286, 268.

Decarboxylation of **6c** *with copper chromite/quinoline.* **6c** (10 mg) was dissolved in 1 ml of quinoline, treated with Cu chromite and the mixture stirred and heated at 120° for 20 min. PLC (benzene–Et_2O–HCO_2H, 25:75:0.5 and hexane–EtOAc, 2:1) gave 2-methoxy-8-hydroxyxanthone(**7**)asyellow needles, mp 126–127° (lit. [4] 129.5°). ν_{max}^{nujol} cm^{-1}: 1650 (conj. CO). Mass 242, 227, 212, 199, 171. PMR (100 MHz, $CDCl_3$) δ 3.92 (3H, *s*, OMe), 6.8 and 6.93 (2H, *dd*, $J = 8$ and 2 Hz, C-5 and C-7), 7.34 (1H, *dd*, $J = 8$ and 2 Hz, C-3), 7.44 (1H, *d*, $J = 8$ Hz, C-4), 7.58 (1H, *t*, $J = 8$ Hz, C-6), 7.65 (1H, *d*, $J = 2$ Hz, C-1), 12.68 (chel. OH).

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