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SOME METABOLITES OF *BOTRYTIS CINEREA* RELATED TO BOTCINOLIDE

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Key Word Index—*Botrytis cinerea*; Moniliaceae; phytotoxic metabolite; botcinolide.

Abstract—Four new botcinolide derivatives 4-*O*-methylbotcinolide, 3-*O*-acetyl-5-*O*-methylbotcinolide, 3-*O*-acetyl-2-epibotcinolide and 2-epibotcinolide have been isolated from the plant pathogen, *Botrytis cinerea*. Their structures have been established by extensive spectroscopic methods.

INTRODUCTION

The plant pathogen [1], *Botrytis cinerea*, produces several groups of phytotoxic metabolites [2–10]. Botcinolide (**1**) is a phytotoxic metabolite isolated from a strain of the fungus [11,12] growing on fruit. The structure of this highly substituted lactone may be biogenetically related to botrylactone (**2**), a metabolite isolated previously from a different strain of *B. cinerea* [13].

In the course of our investigations on the fungus *B. cinerea* (strain UCA 992) [14], examination of the fermentation broth led to the isolation and characterization of four new metabolites with a botcinolide skeleton. These are 4-*O*-methylbotcinolide (**3**), 3-*O*-acetyl-5-*O*-methylbotcinolide (**4**), 3-*O*-acetyl-2-epibotcinolide (**5**) and 2-epibotcinolide (**6**).

RESULTS AND DISCUSSION

B. cinerea (UCA 992), isolated from grapes, was cultured on a Czapek–Dox medium for 19 days. The fermentation broth was extracted with ethyl acetate and separated into neutral and acidic fractions. Chromatography of the acidic fraction on silica gel followed by final purification using HPLC (normal phase, petrol–ethyl acetate) led to the isolation of the compounds **3–6**.

The EI-mass spectrum of compound **3** contained a molecular ion peak at *m/z* 417 $[M+1]^+$ and high-resolution measurement established the molecular composition as $C_{21}H_{36}O_8$. A DEPT experiment established that the ^{13}C NMR spectrum contained signals arising from six methyls, three methylenes, nine methines and three nonprotonated carbon atoms, indicating that compound **3** contained 21 carbons and 33 hydrogen atoms bonded to carbon. Because **3** had a $[M-CH_3]^+$ peak (*m/z* 385) in its mass spectrum, a ^1H NMR signal at δ 3.29 (3H, singlet) and a methyl signal at δ_c 50.9 in its ^{13}C NMR spectrum, there was a methoxyl group present in the molecule. The remaining ^1H and ^{13}C NMR signals were very close to those of botcinolide (**1**) indicating that compound **3** must be a methyl derivative. Because the chemical shifts of H-3 and H-5 (δ 3.5 and 3.9 respectively) were very similar to those of H-3 and H-5 of botcinolide (**1**) (δ 3.6 and 3.8) the methoxyl group must be at C-4.

The relative stereochemistry of compound **3** was apparent from a qualitative analysis of NOE experiments. NOE interactions were observed between H-2 and H-3, H-17 and H-18; between H-5 and H-7 and H-19 and between the methoxyl group and H-20. These correlations, together with the magnitude of the coupling constants, imply that the nine-membered ring has the same relative configuration as that proposed for botcinolide (**1**) [2].

Compound **4** was obtained as a colourless oil from the acid fraction. The ^{13}C NMR spectrum showed 23 signals and the mass spectrum possessed an $[M+1]^+$ (*m/z* 459) peak consistent with the molecular formula, $C_{23}H_{38}O_9$. The ^1H NMR spectrum of compound **4** was very similar to that of botcinolide (**1**) except for the presence of two new signals at δ_H 3.64 (3H, *s*) and 2.22 (3H, *s*) which indicated that compound **4** possessed a methoxyl and an acetoxyl group. The presence of these groups was confirmed by the ^{13}C NMR spectrum which contained a signal at δ_C 51.49 (OCH_3) and two signals at δ 21.92 ($\underline{C}H_3CO$) and 172.89 ($CH_3\underline{C}O$). The location of the acetoxyl group at C-3 followed from the downfield shift of H-3 (δ_H 3.57 in **1** to δ_H 4.97 in **4**) and C-3 (δ_C 74.25 in **1** to δ_C 78.29 in 4) in the ^1H and ^{13}C NMR spectra. On the other hand, conversely the signals corresponding to H-5 and C-5 were shielded (δ_H 3.78 in **1**, δ_H 3.09 in **4**; δ_C 78.53 in **1**, δ_C 72.07 in 4). Hence, in contrast to compound **3** the methoxyl group is located

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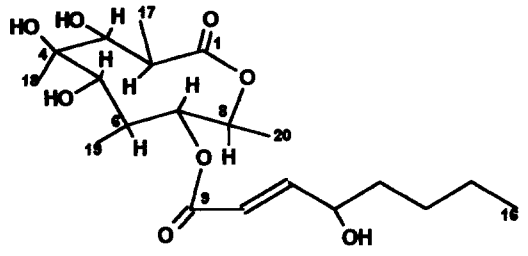

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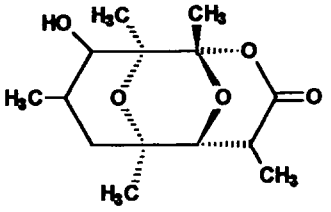

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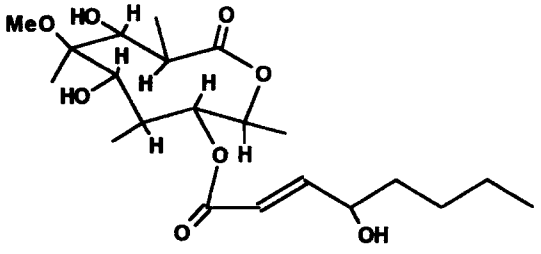

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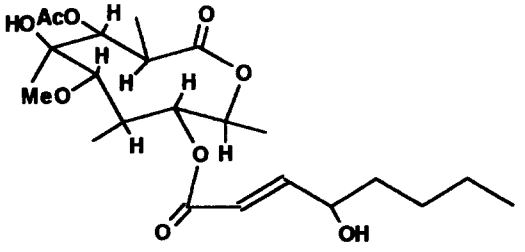

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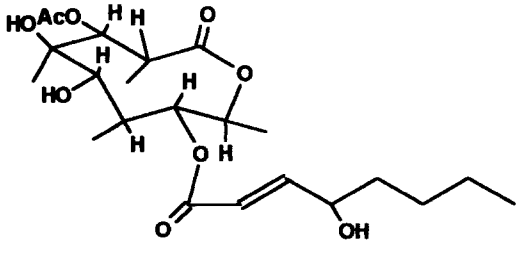

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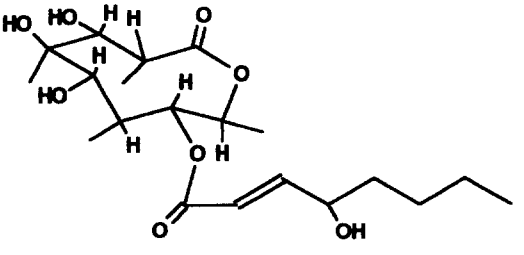

6

at C-5. The coupling constants and NOE interactions indicated that **4** has the same relative configuration as botcinolide (**1**).

Compound **6** was also a colourless oil and possessed a molecular formula, $C_{20}H_{34}O_8$ and hence it is isomeric with botcinolide (1). Although the ^1H and ^{13}C NMR spectra were similar to those of botcinolide the chemical shifts of H-2, H-17, H-3 and H-6 were different while the magnitude of the coupling constant, $J_{2\text{-}3}$ had changed from 2.3 Hz in botcinolide (**1**) to 9.5 Hz in **6**. Hence, this compound was the C-2 epimer of botcinolide (**1**). A qualitative analysis of the NOE experiments confirmed this. In particular there were NOE interactions between H-2 and H-3 and H-5 and between H-5 and H-2 and H-7.

Compound **5** possessed an ion at *m/z* 427 ($C_{22}H_{36}O_9$-OH) in its mass spectrum. The ^1H and ^{13}C NMR spectra were also consistent with the formula, $C_{22}H_{36}O_9$. The presence of an acetoxyl group was indicated by signals at δ_H 2.11 (3H, *s*) and δ_C 20.58 ($\underline{C}H_3CO$) and δ_C 170.07 ($CH_3\underline{C}O$). The location of this group at C-3 was established by the change in the chemical shift of H-3 (δ_H 3.95 in compound **6** to δ_H 5.4 in compound **5**). The coupling constant $J_{2\text{-}3}$ 9.6 Hz indicated that this compound belonged to the 2-epi-botcinolide series. In accordance with this there were NOE interactions between H-2 and H-3 and H-5. Hence, compound **5** was the C-3 acetyl derivative of compound **6**.

The isolation of these compounds from the acid fraction suggested that the lactone ring is readily cleaved and reformed. However, the compounds were

unstable and we were unable to isolate any hydrolysis product. An interesting aspect of the structure of the nine-membered ring, which may however be fortuitous, is that it can be dissected into four propionate units.

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR: 400 MHz, solvents as given below, TMS as int. standard; MS: VG12.250, 70 eV; TLC: MN Alugran SIL G/UV 254 plates, 0.25 mm thick; CC: -Merck silica gel.

Isolation culture and Botrytis cinerea. Accession No. UCA 992, was isolated from grape growing in Jerez de la Frontera, Cádiz, Spain. Culture conditions for this organism, and details of the bioassay-directed isolation of botcinolide derivatives are reported elsewhere [4].

4-O-*Methylbotcinolide* (**3**). Oil; IR (film) υ_{max} cm^{-1}: 3434, 2931, 2875, 1726, 1460, 1170 cm^{-1}; ^{1}H NMR (400 MHz, C_6D_6): δ 7.04 (*dd*, 1H, J_{11-10} = 15.6 Hz and J_{11-12} = 4.4 Hz, H-11), 6.18 (*d*, J_{10-11} = 15.6 Hz, H-10), 4.72 (*dd*, 1H, J_{7-6} = 10.2 Hz and J_{7-8} = 9.6 Hz, H-7), 3.87 (*d*, 1H, J = 11.0 Hz, H-5), 3.77 (*m*, 1H, H-12), 3.55 (*dq*, 1H, J_{8-7} = 9.6 Hz, J_{8-20} = 6.2 Hz, H-8), 3.49 (*d*, 1H, J = 1.9 Hz, H-3), 3.29 (*s*, 3H, OC$\underline{H}_3$), 2.68 (*dq*, 1H, J_{2-17} = 7.1 Hz and J_{2-3} = 1.9 Hz, H-2), 1.89 (*m*, 1H, J_{6-7} = 10.2 Hz, J_{5-6} = 11.0 Hz, J_{6-19} = 6.3 Hz, H-6), 1.82 (*m*, 2H, H-13), 1.30 (*d*, 3H, J_{17-2} = 7.1 Hz, H-17), 1.09 (*s*, 3H, H-18), 1.09 (*d*, 3H, J_{20-8} = 6.2 Hz, H-20), 1.05 (*d*, 3H, J_{19-6} = 6.3 Hz, H-19), 0.77 (*t*, 3H, J = 7.0 Hz, H-16); ^{13}C NMR (400 MHz, C_6D_6): δ 14.09 (*q*, C-16), 14.2 (*q*, C-19), 14.5 (*q*, C-18), 17.2 (*q*, C-17), 18.4 (*q*, C-20), 22.8 (*t*, C*-15), 27.6 (*t*, C*-14), 36.5 (*t*, C-13), 38.4 (*d*, C-6), 38.5 (*d*, C-2), 50.9 (*q*, O$\underline{C}$H$_3$), 68.5 (*d*, C-8), 70.9 (*d*, C-12), 71.8 (*d*, C-5), 77.8 (*d*, C-7), 77.5 (*s*, C-3), 79.1 (*s*, C-4), 119.7 (*d*, C-10), 151.7 (*d*, C-11), 165.8 (*s*, C-9), 176.5 (*s*, C-1) *interchangeable signals; HRMS: Obsd. m/z = 417.2490 $[M + 1]^+$, $C_{21}H_{37}O_8$ requires m/z = 417.2488.

3-O-*Acetyl*-5-O-*methylbotcinolide* (**4**). Oil; IR (film) υ_{max} cm^{-1}: 3471, 2933, 2875, 1723, 1657, 1261, 1169 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.70 (*dd*, 1H, J_{11-10} = 15.6 Hz and J_{11-12} = 4.7 Hz, H-11), 6.03 (*bd*, J_{10-11} = 15.6 Hz and J_{10-12} = 1.7 Hz, H-10) 4.97 (*d*, 1H, J = 3.8 Hz, H-3), 4.40 (*dd*, 1H, J_{7-6} = 10.7 Hz and J_{7-8} = 9.6 Hz, H-7), 4.32 (*m*, 1H, H-12), 3.64 (*s*, 3H, -OC$\underline{H}_3$), 3.56 (*dq*, 1H, J_{8-7} = 9.6 Hz, J_{8-20} = 6.2 Hz, H-8), 3.1 (*dd*, 1H, J_{5-6} = 10.5 Hz and J = 4.5 Hz, H-5), 3.09 (*m*, 1H, H-2), 2.2 (*s*, 3H, -COC$\underline{H}_3$), 1.90 (*m*, 1H, J_{6-7} = 10.7 Hz, J_{5-6} = 10.5 Hz, J_{6-19} = 6.2 Hz, H-6), 1.6 (*m*, 2H, H-13), 1.33 (*s*, 3H, H-18), 1.20 (*d*, 3H, J_{17-2} = 7.3 Hz, H-17), 1.01 (*d*, 3H, J_{20-8} = 6.2 Hz, H-20), 0.97 (*d*, 3H, J_{19-6} = 6.2 Hz, H-19), 0.89 (*t*, 3H, J = 7.1 Hz, H-16); ^{13}C NMR (400 MHz, $CDCl_3$): δ 13.9 (*q*, C-16), 14.2 (*q*, C-18), 14.9 (*q*, C-17), 16.1 (*q*, C-19), 17.9 (*q*, C-20), 20.9 (*q*, C$\underline{H}_3$CO-), 22.5 (*t*, C*-15), 27.3 (*t*, C*-14), 36.3 (*t*, C-13), 37.0 (*d*, C-6), 38.8 (*d*, C-2), 51.5 (*q*, O$\underline{C}$-H$_3$), 68.1 (*d*, C-8), 71.13 (*d*, C-12), 72.1 (*d*, C-5), 76.6 (*d*, C-7), 77.9 (*s*, C-4), 78.3 (*d*, C-3), 119.5 (*d*, C-10), 151.2 (*d*, C-11), 165.9 (*s*, C-9), 172.9 (*s*, $\underline{C}$†OCH$_3$), 173.9 (*s*, C†-1), *†interchangeable signals; HRMS: Obsd. m/z = 459.2603 $[M + 1]^+$, $C_{23}H_{39}O_9$ requires m/z = 459.2594.

3-O-*Acetyl*-2-*epibotcinolide* (**5**). Oil; IR (film) υ_{max} cm^{-1}: 3476, 2939, 1757, 1744, 1239, 1168 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.00 (*dd*, 1H, J_{11-10} = 15.6 Hz and J_{11-12} = 4.7 Hz, H-11), 6.05 (*bd*, J_{10-11} = 15.6 and J_{10-12} = 1.7 Hz, H-10) 5.40 (*d*, 1H, J_{3-2} = 9.6 Hz, H-3), 4.52 (*dd*, 1H, J_{7-6} = 10.7 Hz and J_{7-8} = 9.6 Hz, H-7), 4.33 (*m*, 1H, H-12), 3.80 (*d*, 1H, J_{5-6} = 10.9 Hz, H-5) 3.67 (*m*, 1H, H-8), 3.15 (*dq*, 1H, J_{2-3} = 9.6 Hz and J_{2-17} = 7.1 Hz, H-2), 2.18 (*ddq*, 1H, J_{6-7} = 10.7 Hz, J_{5-6} = 10.9 Hz, J_{6-19} = 6.2 Hz, H-6), 2.11 (*s*, 3H, COC$\underline{H}_3$), 1.58 (*m*, 2H, H-13), 1.34 (*m*, 4H, H-14 and H-15), 1.25 (*s*, 3H, H-18), 1.10 (*d*, 3H, J_{17-2} = 7.0 Hz, H-17), 1.08 (*d*, 3H, J_{20-8} = 6.2 Hz, H-20), 1.05 (*d*, 3H, J_{19-6} = 6.2 Hz, H-19), 0.93 (*t*, 3H, J = 7.1 Hz, H-16); ^{13}C NMR (400 MHz, $CDCl_3$): δ 10.2 (*q*, C-17), 11.9 (*q*, C-18), 13.7 (*q*, C-19), 13.9 (*q*, C-16), 18.2 (*q*, C-20), 20.6 (*q*, C$\underline{H}_3$CO-), 22.5 (*t*, C*-15), 27.3 (*t*, C*-14), 35.4 (*d*, C-6), 36.3 (*t*, C-13), 37.3 (*d*, C-2), 68.4 (*d*, C-8), 71.1 (*d*, C-12), 74.2 (*d*, C-3), 75.2 (*s*, C-4), 76.0 (*s*, C-7), 78.5 (*d*, C-5), 119.0 (*d*, C-10), 151.8 (*d*, C-11), 165.9 (*s*, C-9), 170.1 (*s*, $\underline{C}^{\#}$OCH$_3$), 173.8 (*s*, C$^{\#}$-1); HRMS: Obsd. m/z 427.2350 $[M + 1\text{-}H_2O]^+$, $C_{22}H_{35}O_8$ requires m/z = 427.2332.

2-*epibotcinolide* (**6**). Oil; IR (film) υ_{max} cm^{-1}; 3471, 2933, 2875, 1723, 1459, 1168 cm^{-1}; ^{1}H NMR (400 MHz, MeOD): δ 7.91 (*dd*, 1H, J_{11-10} = 15.6 Hz and J_{11-12} = 4.6 Hz, H-11), 5.94 (*dd*, J_{10-11} = 15.6 Hz and J_{10-12} = 1.9 Hz, H-10) 4.40 (*dd*, 1H, J_{7-6} = 10.5 Hz and J_{7-8} = 9.6 Hz, H-7), 4.13 (*m*, 1H, H-12), 3.97 (*d*, 1H, J_{2-3} = 9.5 Hz, H-3), 3.83 (*d*, 1H, J = 11.0 Hz, H-3) 3.66 (*dq*, 1H, J_{8-7} = 9.5 Hz, J_{8-20} = 6.1 Hz, H-8), 3.10 (*m*, 1H, J_{2-3} = 9.5 Hz and J_{2-17} = 7.1 Hz, H-2), 2.10 (*m*, 1H, J_{6-7} = 10.5 Hz, J_{6-19} = 6.3 Hz, H-6), 1.45 (*m*, 2H, H-13), 1.25 (*m*, 4H, H-14 and H-15), 1.06 (*s*, 3H, H-18), 1.02 (*d*, 3H, J_{17-2} = 7.1 Hz, H-17), 0.96 (*d*, 3H, J_{20-8} = 6.1 Hz, H-20), 0.90 (*d*, 3H, J_{19-6} = 6.3 Hz, H-19), 0.81 (*t*, 3H, J = 5.0 Hz, H-16); ^{13}C NMR (400 MHz, $CDCl_3$): δ 10.3 (*q*, C-17), 10.9 (*q*, C-18), 13.7 (*q*, C-19), 13.9 (*q*, C-16), 18.2 (*q*, C-20), 22.5 (*t*, C*-15), 27.3 (*t*, C*-14), 35.6 (*d*, C-6), 36.4 (*t*, C-13), 38.4 (*d*, C-2), 68.5 (*d*, C-8), 71.1 (*d*, C-12), 74.1 (*d*, C-3), 76.3 (*s*, C-4), 77.2 (*d*, C-7), 78.5 (*d*, C-5), 119.2 (*d*, C-10), 151.7 (*d*, C-11), 170.1 (*s*, $\underline{C}$†OCH$_3$), 173.2 (*s*, C†-1); HRMS: Obsd. m/z = 385.2232 $[M + 1 - H_2O]^+$, $C_{20}H_{33}O_7$ requires m/z = 385.2226.

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