Three New Sesquiterpenoid Metabolites of *Botrytis cinerea*

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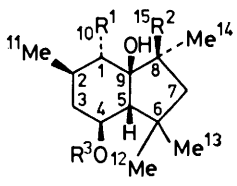
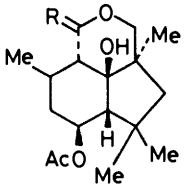
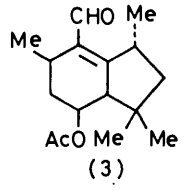
The C-15 carboxylic acid corresponding to botrydial and its C-4 alcohol, together with the C-10 carboxylic acid related to dihydrobotrydial, have been isolated from *Botrytis cinerea*. They have been inter-related with dihydrobotrydial.

EXAMINATION of the neutral sesquiterpenoid metabolites of the fungal plant pathogen, *Botrytis cinerea*, has led to the isolation of botrydial (1),[1] dihydrobotrydial (2),[1] and norbotryal acetate (3).[2] The structure of dihydrobotrydial (2) has been confirmed by *X*-ray analysis.[3] In continuation of our biosynthetic studies [4] on these metabolites, we have examined the constituents of the acid fraction from the fermentation broth.





(1) $R^1 = R^2 = CHO$; $R^3 = Ac$
(2) R = H,OH
(4) $R^1 = CHO$, $R^2 = CO_2H$, $R^3 = H$
(10) R = O
(5) $R^1 = CHO$, $R^2 = CO_2H$, $R^3 = Ac$
(6) $R^1 = CO_2H$, $R^2 = CH_2OH$, $R^3 = Ac$
(7) $R^1 = CHO$, $R^2 = CO_2Me$, $R^3 = H$
(8) $R^1 = CHO$, $R^2 = CO_2Me$, $R^3 = Ac$
(9) $R^1 = R^2 = CO_2Me$, $R^3 = Ac$
(11) $R^1 = CO_2Me$, $R^2 = CH_2OH$, $R^3 = Ac$
(12) $R^1 = CO_2Me$, $R^2 = CHO$, $R^3 = Ac$



(3)

RESULTS AND DISCUSSION

This has afforded three new acids, botryaloic acid (4) and botryaloic acid acetate (5) together with botryoloic acid (6). The acids were purified as their methyl esters. Methyl botryaloate (7), $C_{16}H_{26}O_5$, had i.r. absorption indicative of hydroxy, aldehyde, and ester groupings, whilst the ^1H n.m.r. spectrum contained signals assigned to a secondary methyl group, three tertiary methyl groups, and a secondary alcohol. The aldehyde-CHO resonance was a doublet. Acetylation of methyl botryaloate afforded the second metabolite as its methyl ester (8). The i.r. spectrum of this still contained a hydroxy absorption, assigned to a tertiary alcohol. In the ^1H n.m.r. spectrum, the C*H*OAc resonance appeared as a sextet (δ 5.06, *J* 4.5, 11, and 11 Hz) indicative of a $CH_2CH(OAc)CH$ grouping. Spin-decoupling experiments showed that the aldehyde signal (δ 9.89, *J* 3 Hz) was coupled to a methine proton which in turn had a *trans*-diaxial coupling (11 Hz) to a further methine proton. The ^{13}C n.m.r. spectra (see Table) contained signals attributable to four *C*-methyl groups, three methylenes, three methine carbons, two quaternary carbons, a secondary and a tertiary alcohol, an ester and an aldehyde carbonyl, together with the methoxy and acetoxy signals. This data suggested the structures (7) and (8) for the methyl ester. These were confirmed by an inter-relationship with dihydrobotrydial (2).[1]

Oxidation of methyl acetylbotryaloate (8) with 8N-chromium trioxide and subsequent methylation with diazomethane, gave the acetoxydimethyl ester (9). Dihydrobotrydial (2) was oxidized with chromium trioxide in pyridine to afford the δ-lactone (10) [1] which was then hydrolysed with potassium carbonate to the parent hydroxy-acid (6). The acid was methylated with diazomethane. Examination of the n.m.r. spectrum of this ester showed that only the δ-lactone, and not the acetate, had been hydrolysed under these conditions. The primary alcohol of this ester (11) was oxidized with chromium trioxide to the aldehyde (12). This was autoxidized to the corresponding acid which was isolated, after methylation with diazomethane, as the acetoxydimethyl ester (9) which was identical to the material obtained from botryaloic acid acetate. The hydroxy-acid (6), botryoloic acid acetate, was also isolated from the fungus and characterized as its methyl ester. It was identical to material obtained by hydrolysis of the δ-lactone (10). In this case the carboxy group was at C-10 rather than at C-15 as in the other two metabolites.

^{13}C N.m.r. spectra of methyl botryaloate and its acetate (in $CDCl_3$, p.p.m. from $SiMe_4$)

Carbon atom	(7)	(8)
1	68.5	68.3
2	28.5	28.2
3	43.5	38.6
4	70.0	72.9
5	66.3	61.7
6	38.6	38.6
7	54.9	55.0
8	55.1	55.0
9	88.2	88.0
10	204.1	203.5
11	20.7	20.9 *
12	27.8	27.5
13	36.2	35.7
14	20.7	20.5 *
15	179.0	178.9
OMe	52.5	52.6
OAc		21.4
		170.3

* These assignments may be interchanged.

The metabolites which have hitherto been isolated from *Botrytis cinerea* possess either an aldehyde or a carboxy function at C-10, whilst the oxidation level of

C-15 may be either an alcohol, aldehyde, or acid. In all cases oxygen functions are present at C-4 and C-9, which suggests that cleavage of the C-10–C-15 bond is a late event in the biosynthesis.

EXPERIMENTAL

I.r. spectra were determined as Nujol mulls; ^1H and ^{13}C n.m.r. spectra were determined in deuteriochloroform at 90 and 25.15 MHz respectively; optical rotations were determined in chloroform.

Isolation of the Metabolites from Botrytis cinerea.—The original culture of *Botrytis cinerea* was obtained from the Glasshouse Crops Research Institute. The fungus was grown in Thomson bottles for 8 d on surface culture on a Czapek Dox medium containing 0.1% yeast extract and 5% glucose. The broth (5.25 l) was saturated with sodium chloride and acidified to pH 2 with hydrochloric acid. The broth was extracted with ethyl acetate. The extracts were then shaken with aqueous sodium hydrogencarbonate. The sodium hydrogencarbonate extracts were acidified to pH 2 with 6N-hydrochloric acid and extracted with ethyl acetate. These extracts were dried and the solvent evaporated to give an oil which was taken up in ether and treated with an excess of diazomethane. The solvent and excess of reagent were evaporated to give an oil which was chromatographed on a dry column of silica (Merck). Elution with 20% ethyl acetate–light petroleum gave *methyl acetylbotryaloate* (8) (700 mg) which crystallized from ether as needles, m.p. 106—108 °C; $[\alpha]_D$ +81° (*c* 0.46) (Found: C, 63.5; H, 8.3. $C_{18}H_{28}O_6$ requires C, 63.5; H, 8.3%); $\nu_{max.}$ 3 445, 2 760, 2 720, 1 740, and 1 710 cm^{-1}; δ 0.86 (3 H, d, *J* 5 Hz), 1.06, 1.30, 1.36, 2.03, and 3.70 (each 3 H, s), 5.06 (1 H, sextet, *J* 4.5, 11, and 11 Hz), and 9.86 (1 H, d, *J* 3 Hz). Further elution with 25—30% ethyl acetate–light petroleum gave *methyl botryaloate* (100 mg) (7) which crystallized from ether as needles, m.p. 115—117 °C; $[\alpha]_D$ +67° (*c* 0.43) (Found: C, 64.35; H, 8.8. $C_{16}H_{26}O_5$ requires C, 64.4; H, 8.8%); $\nu_{max.}$ 3 460, 3 360, 2 750, 1 730, and 1 710 cm^{-1}; δ 0.90 (3 H, d, *J* 5 Hz), 1.33 (9 H, s), 3.68 (3 H, s, OMe), 3.95 (1 H, sextet, *J* 4.5, 11, and 11 Hz), and 9.86 (1 H, d, *J* 3 Hz). The fractions eluted with 40% ethyl acetate–light petroleum were rechromatographed on preparative layer plates in chloroform–ethyl acetate–acetic acid (40 : 10 : 1) to afford methyl botryoloate (8 mg) identical (i.r. and n.m.r.) to the material described below.

Oxidation of Dihydrobotrydial.—Dihydrobotrydial (600 mg) in pyridine (10 ml) was treated with a solution of chromium trioxide (2.25 ml) [prepared from chromium trioxide (5 g) in water (3 ml) and pyridine (10 ml)] for 3 d. The reaction mixture was diluted with water and extracted with ethyl acetate. The extract was washed with dilute hydrochloric acid, aqueous sodium hydrogencarbonate, and water, and dried. The solvent was evaporated to afford a gum which was purified by preparative layer chromatography on silica in chloroform–ethyl acetate–acetic acid (40 : 10 : 1) to afford the δ-lactone (10) which crystallized from ethyl acetate–light petroleum as needles (335 mg), m.p. 214—215 °C (lit.,[1] 217—219 °C).

Hydrolysis of the δ-Lactone (10).—The lactone (100 mg) was suspended in 1N-methanolic potassium carbonate (1 ml) overnight. The solution was acidified and extracted with ethyl acetate. The solvent was evaporated to give a white solid which was insoluble in ether but soluble in methanol. The methanolic solution was methylated with diazomethane and the product purified by preparative layer chromatography to afford *methyl botryoloate* (11) (74 mg) which crystallized from aqueous methanol as plates, m.p. 123—125 °C; $[\alpha]_D$ +34° (*c* 0.24) (Found: C, 61.0; H, 8.9. $C_{18}H_{30}O_6 \cdot 0.5H_2O$ requires C, 61.5; H, 8.9%); $\nu_{max.}$ 3 560, 3 480 (sh), 3 230 (br), 1 725, and 1 705 cm^{-1}; δ 0.92 (3 H, d, *J* 6 Hz), 1.04, 1.10, 1.31, and 2.08 (each 3 H, s), 3.28 and 3.60 (each 1 H, d, *J* 13 Hz), 3.80 (3 H, s), and 5.10 (1 H, sextet, *J* 4, 11, and 11 Hz).

Oxidation of Methyl Botryoloate.—The above ester (11) (60 mg) in pyridine (5 ml) was treated with the above chromium trioxide reagent (1 ml) overnight. The solution was poured into water and the product recovered in ethyl acetate. The extract was washed with dilute hydrochloric acid and water, dried, and the solvent evaporated to afford the *aldehyde* (12) which crystallized from ether–light petroleum, m.p. 130—132 °C (Found: C, 63.6; H, 8.3. $C_{18}H_{28}O_6$ requires C, 63.5; H, 8.3%); $\nu_{max.}$ 3 450, 2 730, and 1 725 (br) cm^{-1}; δ 0.92 (3 H, d, *J* 6 Hz), 1.12, 1.32, 1.48, and 2.06 (each 3 H, s), 3.68 (3 H, s, OMe), and 5.10 (1 H, sextet, *J* 4, 11, and 11 Hz), and 9.50 (1 H, s).

Preparation of the Dimethyl Ester (9).—(*a*) The aldehyde (12) (25 mg) in ethyl acetate (2 ml) was allowed to stand in contact with the air over several weeks. When t.l.c. showed almost complete conversion to a slower running spot, the solvent was evaporated and the product methylated with diazomethane and chromatographed on silica. Elution with 20% ethyl acetate–light petroleum gave the dimethyl ester (9) which crystallized from light petroleum as needles, m.p. 125—126 °C, identical (i.r. and n.m.r.) to the material described below.

(*b*) Methyl acetylbotryaloate (8) (40 mg) in acetone (5 ml) was treated with the 8N-chromium trioxide reagent until the orange colour persisted. Excess of reagent was then destroyed with sodium sulphite. The reaction mixture was diluted with water, the acetone was removed *in vacuo*, and the product recovered in ethyl acetate. The ethyl acetate extract was then extracted with aqueous sodium hydrogencarbonate. This extract was then acidified and the organic product recovered in ethyl acetate. The solvent was evaporated to give a gum which was methylated with diazomethane. The solvent and excess of diazomethane were evaporated and the *dimethyl ester* (9) (15 mg) recrystallized from light petroleum as needles, m.p. 125—126 °C (Found: C, 61.5; H, 8.1. $C_{19}H_{30}O_7$ requires C, 61.6; H, 8.2%); $\nu_{max.}$ 3 435, 1 735 (br), and 1 700 (sh) cm^{-1}; δ 0.88 (3 H, d, *J* 6 Hz), 1.10, 1.29, 1.52 and 2.05 (each 3 H, s), 3.68 and 3.72 (6 H), and 5.10 (1 H, m).

Acetylation of Botryaloic Acid Ester.—The ester (7) (50 mg) in pyridine (1 ml) was treated with acetic anhydride (0.5 ml) overnight. The solution was poured onto ice, acidified with dilute hydrochloric acid and the product recovered in ethyl acetate. The extract was washed with dilute hydrochloric acid, aqueous sodium hydrogencarbonate, and water, dried, and evaporated to afford an oil. This was identical (t.l.c. and n.m.r.) with methyl acetylbotryaloate but it would not crystallize. It was oxidized in acetone (5 ml) with the 8N-chromium trioxide reagent as above to afford the dimethyl ester (9) (6 mg) which crystallized from light petroleum as needles, m.p. 123—125 °C (identified by its i.r. and n.m.r. spectrum).

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