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METABOLITES PRODUCED BY *ALTERNARIA BRASSICAE*, THE BLACK SPOT PATHOGEN OF CANOLA,[1] PART 2, SESQUITERPENOID METABOLITES

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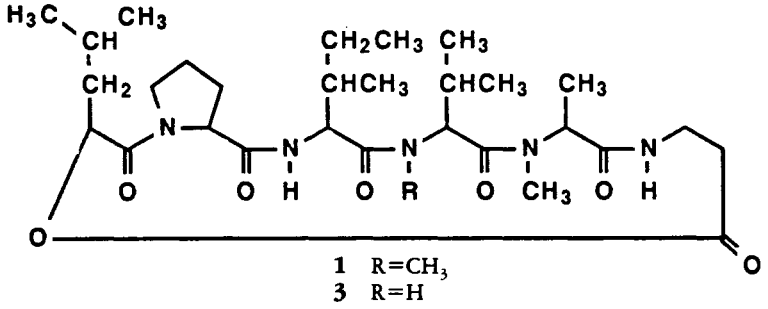
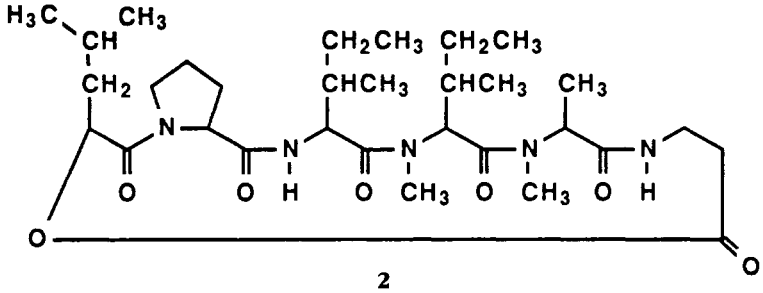
ABSTRACT.—Black spot disease of canola is caused by the fungal pathogen, *Alternaria brassicae*. This fungus, when grown in liquid still culture, produces host specific phytotoxins which have been shown to be cyclodepsipeptides. It also produces several other metabolites including the new drimane sesquiterpenes deoxyuvidin B [**4**], albrassitriol [**8**], and isoalbrassitriol [**9**], as well as brassicadiol [**10**], a C_{15} prenylated pentaketide. These compounds show no phytotoxicity on canola.

Black spot disease of canola is one of the most widespread diseases of rapeseed. The disease, which is caused by the fungal pathogen *Alternaria brassicae* (Berkeley) Saccardo, causes low oilseed yields through reduction in photosynthesis, premature defoliation, and shattering of fruits. The characteristic symptoms of the disease (black lesions surrounded by chlorotic areas on the leaves, fruits, and stems) are caused by a host specific phytotoxin. We have studied the metabolites produced by *A. brassicae* when grown in liquid still culture. In the accompanying paper (1) we describe the isolation and identification of the host specific phytotoxin destruxin B [**1**], as well as the related cyclodepsipeptides, homodestruxin B [**2**] and desmethyldestruxin B [**3**]. In this paper we describe other metabolites including several drimane-type sesquiterpenoids which are produced by this fungus.

Alternaria brassicae was grown in liquid still culture for 20 days and the mycelial and



1 R=CH_3
3 R=H



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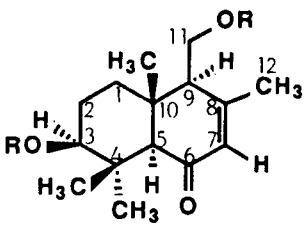
[1]Presented at the 68th Annual Conference, Chemical Institute of Canada, Kingston, Ontario, June 1985.

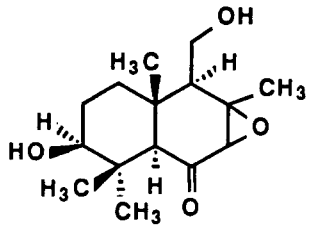
broth extracts worked up as previously described (1). The broth extract was separated into low polarity, medium polarity, and high polarity fractions by solvent partitioning. The medium polarity fraction, which showed the strongest chlorosis-inducing ability when tested for biological activity, was subjected to fractionation by dry flash chromatography over silica gel. Each of the 18 fractions obtained was tested for biological activity and several phytotoxic cyclodepsipeptides, compounds **1-3**, were isolated and identified. Several biologically inactive metabolites including three new drimane sesquiterpenes and a benzofuran derivative were also isolated. The identification of these metabolites is described below.

Compound **A**, the most polar of the sesquiterpenes has a molecular formula of $C_{15}H_{24}O_3$ as determined by hrms. The molecular weight was confirmed by the presence of intense peaks at *m/z* 270 (M+18) and 253 (M+1) in its cims using NH_3 as the reagent gas. The ir spectrum of **A** shows characteristic absorption for an hydroxyl group (3400 cm^{-1}) as well as for the carbonyl group of an α,β-unsaturated system (1652 cm^{-1}). Evidence for the presence of an α,β-unsaturated ketone in **A** is provided by the absorption at 235 nm in the uv spectrum. The ^{1}H-nmr spectrum shows three methyl singlets (δ 1.27, 1.16, and 0.92), a vinyl methyl (δ 2.05, bd, J=0.5 Hz) coupled to an olefinic proton (δ 5.86, bd, J=0.5 Hz), and three carbinyl protons (δ 3.97, 3.86, 3.22).

Treatment of compound **A** with a mixture of Ac_2O/pyridine gave a diacetyl derivative, **A′**. Comparison of the ^{1}H-nmr spectrum of enone **A** with the spectrum of its diacetyl derivative **A′** reveals that three protons *geminal* to oxygen in **A** are shifted downfield in the spectrum of **A′**. Two protons, both doublets of doublets, at δ 3.97 (J= 3.5, 11 Hz) and δ 3.86 (J=6,11 Hz), show downfield shifts of 0.36 ppm suggesting that **A′** contains a primary alcohol (3). The other carbinol proton which appears as a doublet of doublets at δ 3.22 (J=4.5, 10.5 Hz) in the spectrum of **A** is shifted downfield to δ 4.45 in the spectrum of **A**. This downfield acylation shift (2) is characteristic of that observed for a carbinol proton of a secondary alcohol. These data suggest the **A** is a bicyclic sesquiterpene with a β-methyl substituted unsaturated ketone and primary and secondary alcohols. The spectral characteristics of **A** are very similar to those reported for deoxyuvidin B [**4**], a degradation product of uvidin B [**5**] (3).



A or **4** R=H
A′ or **4a** R=Ac



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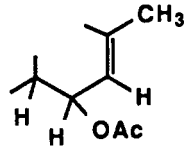
Confirmation of the structure of **A** was obtained by transformation of **A** to uvidin B [**5**], a metabolite obtained from the mushroom *Lactarius uvidus* Fries. Compound **A** was treated with alkaline H_2O_2 (4). The reaction product was purified by preparative tlc. The more polar component of the reaction mixture was shown to be identical with an authentic sample of uvidin B [**5**].[2] Thus, compound **A** is deoxyuvidin B [**4**], a compound which has not previously been reported from natural sources.

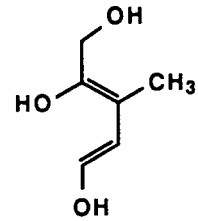
[2]We thank Prof. P. Vita-Finzi, Instituto di Chimica Organica dell'Universita, Pavia, Italy, for kindly providing us with a sample of uvidin B.

A second sesquiterpene, **B**, has the molecular formula $C_{15}H_{26}O_3$ as determined by ms. The ir spectrum of **B** shows hydroxyl (3300 cm^{-1}) and carbon-oxygen (1006 cm^{-1}) stretching bands but lacks absorption in the carbonyl region, suggesting that the three oxygens in **B** are present as alcohols and/or ethers. The fact that these three oxygens are present as hydroxyl groups was first indicated by fragments observed in the hrms. An intense peak at M-31 (*m/z* 223, $C_{14}H_{23}O_2$), resulting from the fragmentation of a primary alcohol, is followed by peaks at *m/z* 205 ($C_{14}H_{21}O$) and *m/z* 187 ($C_{14}H_{19}$) corresponding to loss of one and two molecules of H_2O, respectively. The ^{1}H-nmr spectrum of **B** displays three methyl singlets (δ 1.18, 1.08, and 0.88), a vinyl methyl (δ 1.84) coupled to a vinyl hydrogen (δ 5.52), and three low-field protons (δ 4.17, 3.78, 3.60).

Acetylation of compound **B** afforded a diacetyl derivative, **B′**, which displays two acetyl methyl signals in its ^{1}H-nmr spectrum. The ir spectrum of **B′** confirms the presence of the acetyl groups by the presence of an absorption band at 1733 cm^{-1}. In addition, there is strong hydroxyl absorption (3480 cm^{-1}), which suggests that the third oxygen of **B** is present as part of a tertiary hydroxyl. The nature of the acetylated alcohols was determined to be primary and secondary by comparison of the ^{1}H-nmr spectra of **B** and **B′**. A one proton doublet at δ 3.78 (J=11 Hz) in the spectrum of **B** appears as a doublet of doublets of doublets (J=1.5, 1.5, 10.5 Hz) at δ 5.37 in the spectrum of the diacetyl derivative **B′**. The magnitude of the "acylation shift" (>1 ppm) is characteristic of that shown by carbinol protons of secondary alcohols on acetylation (2). The spectrum of triol **B** displays methylene protons of a primary alcohol (δ 4.17 and 3.60) which show a small downfield acetylation shift (δ 4.26 and 4.21) in the spectrum of **B′**. Thus, **B** contains a primary, a secondary, and a tertiary alcohol, and **B′** is its diacetyl derivative. Decoupling experiments with the diacetate **B′** show that the carbinol proton of the secondary alcohol (δ 5.37) is coupled to a methine proton (δ 2.12) and a vinylic hydrogen (δ 5.44). These data are consistent with partial structure **a** which can be accommodated by a drimane skeleton.



a

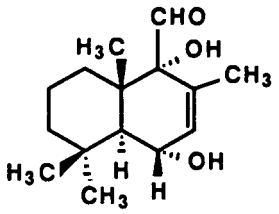


b

The base peak in the hrms of triol **B** occurs at *m/z* 130 ($C_6H_{10}O_3$). This ion, which contains all three oxygens of the molecule, can be explained by a retro-Diels Alder fragmentation of a drimane sesquiterpene giving rise to fragment **b**.

Acid-catalyzed treatment of **B** with 2,2-dimethoxypropane (4) gave rise to an acetonide, consistent with the presence of a 1,2 (or 1,3) diol grouping. The ^{1}H-nmr spectrum of the product, **B″**, shows two methyl singlets at δ 1.49 and 1.39, while in the hrms of **B″** the expected ion arising from a retro-Diels-Alder fragmentation was observed.

On the basis of the preceding spectroscopic data and comparison with similar data reported for related naturally-occurring drimanes, e.g., mukaadial [**6**] (5) and cinnamodial [**7**] (6,7), compound **B** may be assigned structure **8** (without stereochemical implication). Compound **8** is a new natural product for which we propose the name albrassitriol.



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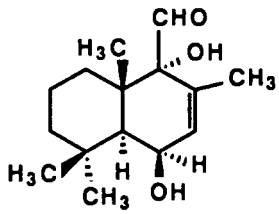

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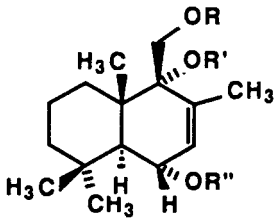


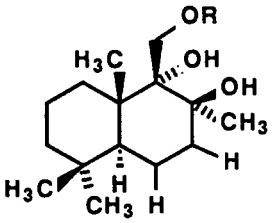
B or **8** R=R′=R″=H
B′ or **8a** R=R″=Ac, R′=H
B″ or **8b** R,R′=$-C(CH_3)_2-$, R″=H

The stereochemistry of albrassitriol [**8**] was established in the following way. The assignment of the *trans*-configuration of the AB ring junction is based on the chemical shift observed for the angular methyl carbon (16.8 ppm) in the ^{13}C nmr of **8**. It is known that the signal for the ring junction methyl in the *trans*-isomer occurs 11-12 ppm further upfield than that of the *cis*-isomer (8,9), the latter usually resonating at approximately 30 ppm. This requires that the proton at C-5 is axial. Since the coupling constant between H-5 and H-6 in the ^1H-nmr spectrum of **8** is large (10 Hz), the two hydrogens must be in a *trans*-diaxial arrangement and, thus, the C-6 hydroxyl group is equatorial (α-orientation). The coupling constant observed for **8** is in agreement with the coupling constant reported for the same protons in the drimane mukaadial [**6**] (5). In cinnamodial [**7**], epimeric at C-6 with **6**, the H-5, H-6 coupling constant is 5 Hz (6,7).

The stereochemistry at C-9 of albrassitriol was determined by nuclear Overhauser enhancement (nOe) experiments [in related compounds the α-hydroxyl configuration is usually biogenetically favored (7)]. In the case of **8**, irradiation of the angular methyl singlet (C-15, δ 1.08) results in a 4% enhancement of one of the C-11 methylene protons (δ 4.17). This indicates that the $-CH_2-OH$ group is in the β-orientation and, thus, the C-9 hydroxyl is α. In addition, the α-orientation of the C-9 hydroxyl group was confirmed by observing the pyridine-induced solvent shifts in the ^1H-nmr spectrum of the diacetyl derivative **B′**. The signal which showed the largest deshielding effect ($\Delta=-0.42$, $\Delta=\delta CDCl_3-\delta C_5D_5N$) was the doublet ($J=10$ Hz) corresponding to the α-proton at C-5. This is in agreement with the report that protons occupying positions 1,3-diaxial to a hydroxyl function experience deshielding effects of the order of 0.20-0.40 ppm in pyridine relative to $CHCl_3$ (10). Since H-5 is known to be α, the C-9 hydroxyl group must also be α. Thus, the stereochemistry of albrassitriol can be assigned as that depicted in structure **8**.

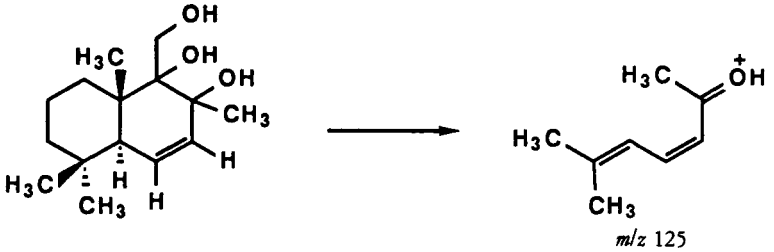
A third drimane sesquiterpene **C** ($C_{15}H_{26}O_3$), which is isomeric with albrassitriol, was isolated from the extracts of *A. brassicae*. The ir spectrum of **C** is very similar to that of albrassitriol, showing hydroxyl but no carbonyl absorption. The ^1H-nmr spectrum of **C** displays three methyl singlets (δ 1.08, 0.96, and 0.89), and the hrms shows an ion at *m/z* 137 ($C_{10}H_{17}$) characteristic of many drimane sesquiterpenes (11,12). Acetylation of compound **C** afforded a monoacetyl derivative **C′**.

Comparison of the nmr spectrum of **C** with the spectrum of its monoacetate derivative **C'**, indicates that two carbinyl protons (δ 4.11, d, J=12 Hz and 3.86, d, J=12 Hz) in the spectrum of **C** are shifted downfield by about 0.45 ppm (δ 4.54 and δ 4.33) in the spectrum of **C'**. This again indicates the presence of a primary alcohol. The ^{1}H-nmr spectrum of compound **C** also displays two vinylic protons, (δ 5.77, dd, 3, 10 Hz and 5.55, dd, 3, 10 Hz), each coupled to a methine proton at δ 2.37 (dd, 3, 3 Hz). The spectrum of **C** lacks a vinylic methyl group, but a three proton singlet at δ 1.37 characteristic of a methyl group *geminal* to oxygen is observed. On the basis of this information structure **9** is proposed for compound **C**. Support for this structure is obtained from its hrms. The base peak at *m/z* 125 ($C_8H_{13}O$) can be accounted for as the fragment resulting from the cleavage of the 3,4, 8,9 and 10,5 carbon-carbon bonds as shown in Scheme 1.



C or **9** R=H
C' or **9a** R=Ac

Attempted formation of an acetonide of the monoacetyl derivative **C'** by treatment with 3,3-dimethoxypropane and *p*-toluenesulfonic acid resulted in the recovery of starting material. This result indicates that the *vicinal* tertiary hydroxyl groups are in a *trans*-diaxial arrangement. If we assume that the C-9 hydroxyl group of **9** has an axial orientation (α) by analogy with albrassitriol, then the C-8 hydroxyl group is also axial and, consequently, β. It appears that compound **9** has not been reported previously. Because it is isomeric with albrassitriol [**8**], we suggest the trivial name isoalbrassitriol for **9**. Both **8** and **9** are stable to treatment with dilute HOAc in the presence of Si gel, but the possibility that **9** is an artifact formed by allylic rearrangement of **8** during the isolation process cannot be excluded.



SCHEME 1

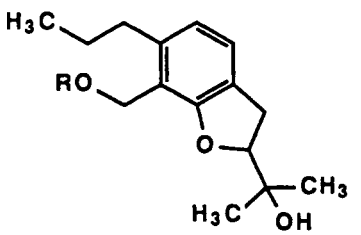
Metabolite **D**, also isolated from the medium polarity fraction of the fungal extract, proved to be of different structural type from those discussed thus far, although it also has fifteen carbons. The molecular formula, deduced by ms, is $C_{15}H_{22}O_3$. The ir spectrum shows hydroxyl and aromatic (1590 cm^{-1}) absorption but no carbonyl absorption. The ^{1}H-nmr spectrum of **D** displays doublets at δ 7.07 (J=8 Hz) and 6.69 (J=8 Hz) indicating the presence of *ortho*-related aromatic protons. Two somewhat deshielded methyl singlets (δ 1.28 and 1.14) are present. Decoupling experiments show the presence of two isolated spin systems: a lowfield methine (δ 4.64, dd, J=9, 9 Hz)

coupled to methylene protons at δ 3.17 and 3.11 (each dd, J=9, 15 Hz); and an *n*-propyl group [a methylene group at δ 1.58 coupled to a methylene triplet (δ 2.63) and to a methyl triplet (δ 0.97)]. Comparison of chemical shifts with those reported for similar systems (2,14,15) suggests that the *n*-propyl group is located on the aromatic ring.

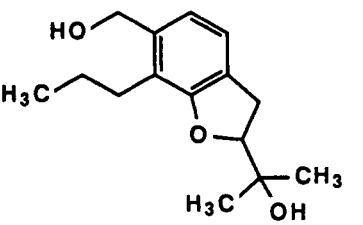
Further evidence for the aromatic nature of **D** is given by the ^{13}C-nmr spectrum which shows six sp^2 carbons (δ 159, 141, 125, 124, 122, 121). The chemical shift of the low field aromatic carbon (δ 159) suggests that it bears an oxygen substituent (13). These data account for four of the five unsaturation equivalents implied by the molecular formula, and suggest that an additional ring is present since all sp^2 carbons have been assigned.

The oxygens in **D** are present as a primary alcohol, a tertiary alcohol, and an ether. The ^{13}C nmr of **D**, which we have named brassicadiol, indicates the presence of 3 oxygenated sp^3 carbons. Compound **D** shows two D_2O-exchangeable protons in its ^{1}H-nmr spectrum. Acetylation yields a monoacetyl derivative **D′** which has hydroxyl absorption in the ir and one D_2O exchangeable proton in the ^{1}H nmr. The acetoxyl is located on a primary benzylic carbon as indicated by chemical shift and the downfield shift of the two proton signal at δ 4.73 in **D** to 5.27 (1H) and 5.08 (1H) in **D′**. Further, monoacetate **D′** is resistant to oxidation with Jones' reagent.

The evidence at this stage indicates that brassicadiol contains an aromatic ring with four contiguous substituents, one of which is a propyl group, another a hydroxymethyl group. By exclusion, the remaining substituents, one of which must be an oxygen, must form a dihydrofuran ring bearing an hydroxyisopropyl group [to account for the



D or **10** R=H
D′ or **10a** R=Ac



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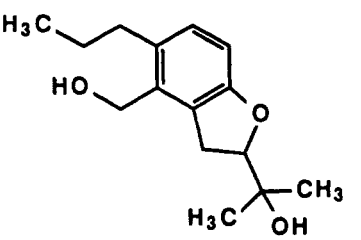

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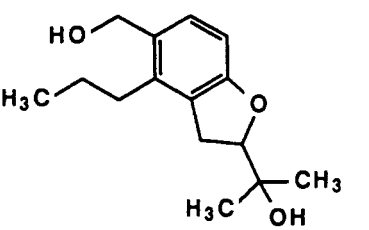

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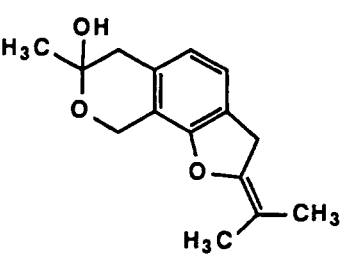

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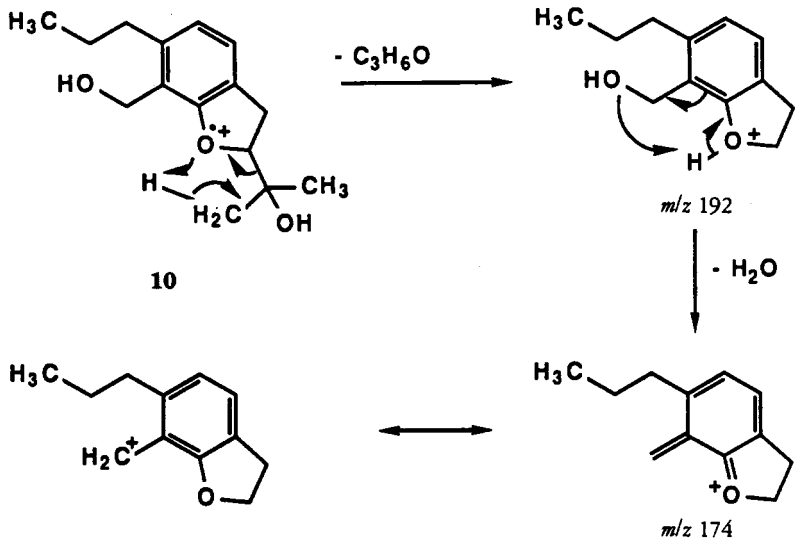
singlet methyls and a peak at *m/z* 59 (C_3H_7O) in the hrms] at the α-position (single ethereal proton as an apparent triplet at δ 4.64).

Thus, it appeared that we were dealing with a prenylated pentaketide, and biogenetic considerations suggested that the propyl group and the hydroxymethyl group are *ortho* to one another (16). Structures **10**, **11**, **12**, and **13** fulfill the requirements outlined to this point, with **10** and **13** preferred on biogenetic grounds (ethereal oxygen *ortho* or *para* to the hydroxymethyl group). The structure of the fungal metabolite pergillin [**14**] (17) illustrates these biogenetic considerations.

Closer analysis of the ^{1}H-nmr spectrum of monoacetate **D'** showed that the aromatic proton at δ 7.06 is, in fact, a doublet of doublets of doublets (J=1, 1, 7.6 Hz). Irradiation of the dihydrofuran methylene protons (δ 3.15) resulted in the disappearance of the small couplings, thus indicating a long range coupling between the methylene protons and the aromatic proton. Of the four possible structures for compound **D** only **10** and **11** would give rise to such long range coupling. Accordingly structures **12** and **13** were eliminated. Irradiation of the benzylic methylene of the propyl group (δ 2.62) resulted in the sharpening of the aromatic doublet at δ 6.69. This is consistent with structure **10** but unlikely for structure **11**. On the basis of these arguments we favor structure **10** for brassicadiol. The base peak in the mass spectrum of brassicadiol appears at *m/z* 174 ($M^+ - C_3H_8O_2$). Structure **10** allows for the rationalization of this peak as illustrated in Scheme 2. Initial loss of acetone, a typical fragmentation observed in this type of system (18), followed by loss of H_2O provides the resonance stabilized ion *m/z* 174 shown in Scheme 2.



SCHEME 2

Compounds **4**, **8**, and **9** did not show phytotoxicity towards canola. Compound **10**, obtained in very small amount, was not subjected to bioassay.

EXPERIMENTAL

General experimental details are given in part 1 of this series (1).

ISOLATION OF METABOLITES.—Extracts of *A. brassicae* (UAMH 4936)[3] were obtained as described in the previous paper of this series (1). The medium polarity fraction was subjected to chromatographic

[3]Cultures of *A. brassicae* used in this study have been deposited with the University of Alberta Microfungus Collection, Devonian Botanic Garden, under the assession number UAMH4936.

separation over Si gel. Like fractions (as determined by tlc) were combined, and a total of 18 fractions were collected (1). Further purification as described below led to the isolation of the following compounds: fraction 7, isoalbrassitriol [**9**]; fraction 9, brassicadiol [**10**]; fraction 10, albrassitriol [**8**], destruxin B [**1**]; fraction 11, homodestruxin B [**2**]; fraction 12, deoxyuvidin B [**4**]; and fraction 13, desmethyldestruxin B [**3**].

ISOLATION OF DEOXYUVIDIN B [**4**].—Deoxyuvidin B [**4**] was isolated (1.6 mg) as a white powder by the preparative tlc (20×20 cm, $CHCl_3$-MeOH, 95:5, 2×) purification of fraction 12 (39.8 mg), tlc: Rf 0.24 ($CHCl_3$-MeOH, 95:5), 0.29 (C_6H_6-Me_2CO, 70:30); ir ($CHCl_3$ cast) 3440, 2925, 1652, 1440, 1383, 1237, 1047 cm^{-1}; uv (MeOH) λ max 235 nm (ε 1763); ^1H nmr (400 MHz, $CDCl_3$) δ 5.86 (1 H, bd, 0.5 Hz), 3.97 (1 H, dd, 3.5, 11 Hz), 3.86 (1 H, dd, 6, 11 Hz), 3.22 (1 H, dd, 4.5, 10.5 Hz), 2.29 (1 H, bs), 2.13 (1 H, bs), 2.05 (3 H, bd, 0.5 Hz), 1.27 (3 H, s), 1.16 (3 H, s), 0.92 (3 H, s); hrms *m/z* calcd. for $C_{15}H_{24}O_3$ (M^+): 252.1725; found: 252.1725 (18), 234 (17), 221 (3), 216 (17), 203 (4), 149 (10), 123 (100), 81 (17); cims (NH_3) 270 (20), 254 (21), 253 (100), 252 (5).

0,0-DIACETYLDEOXYUVIDIN B (**A'** [**4a**], 3β,11-DIACETOXY-6-OXODRIM-7-ENE).—Deoxyuvidin B (**4**, 1.1 mg) was treated with Ac_2O (1 ml) and pyridine (0.5 ml) overnight at room temperature. The reaction mixture was poured over H_2O-ice (20 ml) and extracted with EtOAc (3×10 ml). Workup of the organic layer in the usual fashion afforded the crude acetylated product (1.8 mg). Purification by preparative tlc (10×10 cm, C_6H_6-Me_2CO, 95:5, 2×) yielded pure *0,0*-diacetyldeoxyuvidin B (0.4 mg), tlc: Rf 0.51 (C_6H_6-Me_2CO, 90:10), 0.34 (Skellysolve B-Me_2CO, 70:30), 0.3 (Skellysolve B-EtOAc, 75:25); ir ($CHCl_3$ cast) 2920, 1738, 1671, 1366, 1241 cm^{-1}; ^1H nmr (400 MHz, $CDCl_3$) δ 5.87 (1 H, bd, 1 Hz), 4.45 (1 H, dd, 4.5, 10 Hz), 4.3 (1 H, dd, 3, 12 Hz), 4.22 (1 H, dd, 6, 12 Hz), 2.49 (1 H, bs), 2.20 (1 H, s), 2.07 (6 Hz, s), 1.95 (1 H, bs), 1.23 (3 H, s), 1.15 (3 H, s), 0.96 (3 H, s); hrms *m/z* calcd. for $C_{17}H_{24}O_3$ (M^+-$C_4H_4O_2$, parent ion not observed: 276.1725; found: 276.1727 (20), 234 (2), 216 (28), 175 (22), 135 (100), 122 (38), 81 (8); cims (NH_3) 673 ($2M^+$ +1, 2), 354 (M^+ +18, 34), 338 (24), 337 (M^+ +1, 100), 335 (30), 278 (15), 277 (76), 217 (21), 216 (27).

EPOXIDATION OF DEOXYUVIDIN B [**4**].—Deoxyuvidin B (**4**, 0.3 mg) was dissolved in MeOH (0.5 ml) containing 30% H_2O_2 (1 drop). The solution was cooled to 20° and 6 N NaOH (21 drops) was added. The reaction mixture was stirred at room temperature. After 18 h the tlc showed that all of the starting material had disappeared. The mixture was diluted with H_2O (10 ml) and the resulting aqueous suspension extracted with Et_2O (3×5 ml). The organic extract was washed with saturated NaCl (10 ml), dried (Na_2SO_4), and concentrated to give crude product (0.4 mg). Ir (CH_2Cl_2 cast) 3400, 1700, 1677 cm^{-1}; hrms *m/z* 234 (48), 220 (2), 219 (18), 216 (7), 167 (5), 149 (8), 123 (5).

The crude reaction product showed two main components by tlc. The least polar component was uv active while the other component showed a similar Rf value to that of the starting material but was not visible under uv light. The two components were separated by preparative tlc (5×7.5 cm, $CHCl_3$-MeOH, 95:5, 2×). The spectral data for the less polar component (0.1 mg) indicates that it is actually a mixture of two compounds. This mixture was not further resolved. The more polar compound (0.2 mg) was identified as uvidin B [**5**] by comparison (tlc, ir) with an authentic sample.

ISOLATION OF ALBRASSITRIOL (**8**, 6α,9α,11-TRIHYDROXYDRIM-7-ENE).—Chromatography fraction 10 (3.5 mg) from the medium polarity crude extract gave a positive bioassay and displayed two components by tlc, one of which was identified as destruxin B [**1**]. The second component was purified by dry flash column chromatography (1) (C_6H_6-Me_2CO 80:20, 10 cm long, 0.7 cm diameter). Albrassitriol (**8**, 2.6 mg) was obtained as a white amorphous powder, mp 100-104°; tlc, Rf 0.37 (C_6H_6-Me_2CO, 70:30), 0.15 (Et_2O-Skellysolve B, 75:25), 0.19 ($CHCl_3$-MeOH, 97.5:2.5); ir ($CHCl_3$ cast) 3299, 2922, 1450, 1006 cm^{-1}; ^1H nmr (400 MHz, $CDCl_3$) δ 5.52 (1 H, bd, 1 Hz), 4.17 (1 H, dd, 1, 10 Hz), 3.78 (1 H, d, 11 Hz), 3.60 (1 H, dd, 1, 10 Hz), 2.52 (1 H, bs), 2.09 (1 H, bs), 1.84 (3 H, bdd, 1.5 Hz), 1.72 (1 H, dt, 3.5, 11.5 Hz), 1.63 (1 H, d, 10 Hz), 1.18 (3 H, s), 1.08 (3 H, s), 0.88 (3 H, s); ^{13}C nmr ($CDCl_3$) δ 131.5 (s), 123.6 (d), 76.7 (s), 68.7 (d), 62.0 (t), 50.4 (d), 42.9 (t), 42.7 (t), 38.0 (s), 33.3 (s), 32.6 (q), 22.8 (q), 19.6 (q), 18.6 (t), 16.8 (q); hrms *m/z* calcd. for $C_{15}H_{26}O_3$ (M^+): 254.1882; found: 254.1876 (3), 236 (16), 223 (75), 205 (27), 187 (14), 154 (10), 137 (11), 130 (100), 112 (63); cims (NH_3) 272 (51), 254 (100), 237 (54), 219 (94), 201 (48).

0,0-DIACETYLALBRASSITRIOL (**B'** [**8a**], 6α,11-DIACETOXY-9α-HYDROXYDRIM-7-ENE).—A mixture of albrassitriol (**8**, 2.6 mg), Ac_2O (1 ml), and pyridine (0.5 ml) was allowed to stir overnight at room temperature. The reaction mixture was poured over H_2O-ice (20 ml), extracted with EtOAc (3×10 ml), and worked up in the usual manner to afford 3.6 mg of crude product. Purification of the acetylated product by dry flash column chromatography (C_6H_6-Me_2CO, 97:3, 10 cm long, 0.7 cm diameter) yielded *0,0*-diacetylalbrassitriol (**8a**, 1.2 mg). Tlc, Rf 0.32 (C_6H_6-Me_2CO, 95:5), 0.50 (C_6H_6-Me_2CO, 90:10), 0.43 ($CHCl_3$-MeOH, 98:2); ir ($CHCl_3$ cast) 3480, 2930, 1733, 1372 cm^{-1}; ^1H nmr (400 MHz, $CDCl_3$) δ 5.44 (1 H, dd, 1.5, 1.5 Hz), 5.37 (1 H, ddd, 2, 2, 10 Hz), 4.26 (1 H, d, 12 Hz), 4.21 (1 H, d, 12 Hz),

2.36 (1 H, s), 2.12 (1 H, d, 10 Hz), 2.08 (3 H, s), 2.05 (3 H, s), 1.77 (3 H, dd, 1.5, 1.5 Hz), 1.01 (6 H, s), 0.98 (3 H, s); ^{13}C nmr ($CDCl_3$) δ 170.7 (2×, s), 137.6 (s), 127.5 (d), 75.0 (s), 71.8 (d), 64.8 (t), 46.0 (t), 43.5 (d), 42.7 (t), 35.6 (s), 33.2 (s), 32.9 (q), 23.1 (q), 21.8 (q), 21.0 (q), 19.3 (q), 18.6 (q), 17.6 (t); hrms *m/z* calcd. for $C_{17}H_{26}O_3$ ($M+-C_2H_4O_2$, parent ion peak not seen): 278.1882; found: 278.1886 (87), 236 (13), 218 (85), 214 (27), 205 (82), 200 (3), 154 (19), 137 (10), 112 (100); cims (NH_3) 356 (M^+ +18, 1), 296 (26), 280 (20), 279 (100), 219 (16), 201 (54).

ALBRASSITRIOL ACETONIDE (**B″ [8b]**, 9α,11-*O*-ISOPROPYLIDINE-6α-HYDROXYDRIM-7-ENE).—Albrassitriol (**8**, 0.8 mg), 2,2-dimethoxypropane (1.5 ml), and *p*-toluenesulfonic acid (catalytic amount) were allowed to stir at room temperature. The course of the reaction was monitored by tlc, and after 2 h no starting material was observed. The reaction mixture was diluted with Et_2O (30 ml) and washed with saturated $NaHCO_3$ (3×10 ml). The organic extract was dried (Na_2SO_4) and concentrated to give a yellowish oil (1 mg). Purification by means of dry flash column chromatography (C_6H_6-Me_2CO, 96:4, 10 cm long, 0.7 cm diameter) afforded albrassitriol acetonide (0.4 mg) as a colorless oil. Tlc Rf 0.45 (C_6H_6-Me_2CO, 90:10), 0.23 ($CHCl_3$-MeOH, 98:2); ir ($CHCl_3$ cast) 3440, 2921, 1450, 1380, 1260, 1061 cm^{-1}; ^{1}H nmr (400 MHz, $CDCl_3$) δ 5.56 (1 H, bs), 4.10 (1 H, bs), 3.89 (2 H, q, 10 Hz), 2.17 (1 H, d, 2 Hz), 1.86 (3 H, dd, 2, 2 Hz), 1.59 (1 H, d, 10 Hz), 1.49 (3 H, s), 1.39 (3 H, s), 1.17 (3 H, s), 1.05 (3 H, s), 0.83 (3 H, s); hrms *m/z* calcd. for $C_{18}H_{30}O_3$ (M^+): 294.2195; found: 294.2190 (29), 279 (16), 236 (7), 219 (4), 201 (4), 170 (30), 137 (5), 112 (100); cims (NH_3) 312 (7), 294 (14), 278 (21), 277 (100), 219 (12).

ISOLATION OF ISOALBRASSITRIOL ([**9**], 8β,9α,11-TRIHYDROXYDRIM-6-ENE).—Chromatography fraction 7 (2.8 mg) of the medium polarity crude extract was further purified by dry flash column chromatography (C_6H_6-Me_2CO, 90:10, 10 cm long, 0.7 cm diameter). This afforded isoalbrassitriol (**9**, 0.7 mg) as a clear oil. Tlc, Rf 0.55 (C_6H_6-Me_2CO, 70:30), 0.59 (CH_2Cl_2-Me_2CO, 70:30); ir ($CHCl_3$ cast) 3400, 2926, 1458, 1383, 1260, 1074 cm^{-1}; ^{1}H nmr (400 MHz, $CDCl_3$) δ 5.77 (1 H, dd, 3, 10 Hz), 5.55 (1 H, dd, 3, 10 Hz), 4.11 (1 H, d, 12 Hz), 3.86 (1 H, d, 12 Hz), 3.87 (1 H, bs), 2.37 (1 H, t, 3 Hz), 2.00 (1 H, d, 2 Hz), 1.37 (3 H, s), 1.17 (1 H, d, 10 Hz), 1.08 (3 H, s), 0.96 (3 H, s), 0.89 (3 H, s); hrms *m/z* calcd. for $C_{15}H_{26}O_3$ (M^+): 254.1882; found: 254.1877 (6), 236 (9), 221 (10), 218 (6), 205 (35), 193 (48), 175 (34), 137 (10), 125 (100), 107 (28); cims (NH_3) 272 (4), 255 (6), 254 (26), 253 (25), 237 (72), 219 (100), 201 (47).

O-ACETYLISOALBRASSITRIOL (**C′ [9a]**, 11-ACETOXY-8β-9α-DIHYDROXYDRIM-6-ENE).—A mixture of isoalbrassitriol (**9**, 0.9 mg), Ac_2O (1 ml), and pyridine (0.5 ml) was allowed to stir overnight at room temperature. The reaction mixture was poured over H_2O-ice (10 ml), extracted with EtOAc (3×10 ml), and worked up in the usual manner. Dry flash column chromatography (C_6H_6-Me_2CO, 96:4, 10 cm long, 0.7 cm diameter) purification of the crude acetylated product (1.1 mg) afforded *O*-acetylisoalbrassitriol (**9a**, 0.5 mg). Tlc, Rf 0.36 (C_6H_6-Me_2CO, 90:10), 0.33 ($CHCl_3$-MeOH, 98:2); ir ($CHCl_3$ cast) 3520, 2927, 1730, 1460, 1383, 1238, 1039 cm^{-1}; ^{1}H nmr (400 MHz, $CDCl_3$) δ 5.76 (1 H, dd, 2, 10 Hz), 5.53 (1 H, dd, 3, 10 Hz), 4.54 (1 H, d, 12 Hz), 4.33 (1 H, d, 12 Hz), 2.49 (1 H,s), 2.40 (1 H, bt, 2 Hz), 2.13 (3 H, s), 1.37 (3 H, s), 1.08 (3 H, s), 0.96 (3 H, s), 0.88 (3 H, s); hrms *m/z* calcd. for $C_{17}H_{28}O_4$ (M^+): 296.1988; found: 296.1964 (3), 278 (3), 236 (37), 218 (13), 205 (41), 200 (3), 193 (40), 175 (53), 137 (15), 125 (100), 107 (28); cims (NH_3) 314 (20), 296 (38), 279 (100), 219 (14), 201 (29).

TREATMENT OF *O*-ACETYLISOALBRASSITRIOL [**9a**] WITH 2,2-DIMETHOXYPROPANE.—*O*-Acetylisoalbrassitriol (**9a**, 0.3 mg) was dissolved in 2,2-dimethoxypropane (1 ml) and a catalytic amount of *p*-toluenesulfonic acid added. The solution was allowed to stir at room temperature, and the course of the reaction was monitored to tlc. After 48 h of reaction, no significant change was observed. The reaction mixture was diluted with Et_2O (30 ml), and the organic layer washed with saturated $NaHCO_3$ (3×10 ml). Concentration of the extract yielded a crude product which showed *O*-acetylisoalbrassitriol [**C′**] as the major component on tlc.

ISOLATION OF BRASSICADIOL (**D [10]**, 2,3-DIHYDRO-6-PROPYL-2-[2-HYDROXYPROPYL]-7-HYDROXYMETHYLBENZOFURAN).—Purification of chromatography fraction 9 (10.7 mg) of the medium polarity crude extract by dry flash column chromatography (C_6H_6-Me_2CO, 90:10, 10 cm long, 0.7 cm diameter) gave brassicadiol (**10**, 2.7 mg) as a milky-white oil. Tlc, Rf 0.43 (C_6H_6-Me_2CO, 70:30), 0.37 ($CHCl_3$-MeOH, 96:4), 0.33 (Et_2O-Skellysolve B, 75:25); ir (CH_2Cl_2 cast) 3335, 2957, 2928, 1590, 1442, 1380 cm^{-1}; ^{1}H nmr (400 MHz, $CDCl_3$) δ 7.02 (1 H, d, 7.5 Hz), 6.69 (1 H, d, 7.5 Hz), 4.73 (2 H, s), 4.64 (1 H, dd, 9, 9 Hz), 3.17 (1 H, dd, 9, 15.5 Hz), 3.11 (1 H, dd, 9, 15.5 Hz), 2.62 (1 H, bt, 7.5 Hz), 2.16 (1 H, m), 2.04 (1 H, bs), 1.58 (2 H, ddd, 8, 8, 9.5 Hz), 1.28 (3 H, s), 1.14 (3 H, s), 0.97 (3 H, t, 7.5 Hz); ^{13}C nmr ($CDCl_3$) 158.5 (s), 141.0 (s), 124.7 (s), 124.1 (d), 122.0 (d), 120.6 (s), 89.9 (d), 72.0 (s), 57.5 (t), 34.7 (t), 30.6 (t), 26.3 (q), 25.1 (t), 24.3 (q), 14.1 (q); hrms *m/z* calcd. for $C_{15}H_{22}O_3$ (M^+): 250.1569; found: 250.1565 (24), 192 (2), 174 (100), 131 (13), 59 (25); cims (NH_3) 501 (2 M^+ 1, 5), 500 (16), 269 (14), 268 (75), 251 (18), 250 (100), 233 (16).

O-ACETYLBRASSICADIOL (**D [10a]**, 2,3-DIHYDRO-6-PROPYL-2-[2-HYDROXYPROPYL]-7-ACETOXYMETHYLBENZOFURAN).—Brassicadiol (**10**, 0.9 mg) was allowed to stir overnight at room temperature with Ac_2O (1 ml) and pyridine (0.5 ml). The reaction mixture was poured over H_2O-ice (10 ml), extracted with EtOAc (3×10 ml), and worked up in the usual way to give crude acetylated product (1.9 mg). The major, most polar component, was purified by dry flash column chromatography (C_6H_6-Me_2CO, 96:4, 10 cm long, 0.7 cm diameter) and identified as *O*-acetylbrassicadiol (0.6 mg). Tlc, Rf 0.37 (C_6H_6-Me_2CO, 90:10), 0.36 (Et_2O-Skellysolve B, 3:2), 0.17 (C_6H_6-Me_2CO, 96:4); ir ($CHCl_3$ cast) 3480, 2960, 1736, 1590, 1445, 1380, 1237, 1020 cm^{-1}; ^1H nmr (400 MHz, $CDCl_3$) δ 7.06 (1 H, ddd, 1, 1, 7.6 Hz), 6.69 (1 H, bd, 7.6 Hz), 5.27 (1 H, d, 11.6 Hz), 5.08 (1 H, d, 11.6 Hz), 4.62 (1 H, dd, 9, 9 Hz), 3.15 (2 H, bd, 9 Hz), 2.62 (1 H, bdd, 8, 14 Hz), 2.54 (1 H, bdd, 8, 14 Hz), 2.16 (1 H, bs), 2.08 (3 H, s), 1.60 (1 H, m), 1.38 (3 H, s), 1.22 (3 H, s), 1.00 (3 H, t, 7 Hz); hrms *m/z* calcd. for $C_{17}H_{24}O_4$ (M^+): 292.1674; found: 292.1673 (12), 217 (7), 174 (100), 131 (9), 59 (34); cims (NH_3) 542 (19), 311 (20), 310 (100), 292 (2), 250 (49), 231 (26).

OXIDATION OF *O*-ACETYLBRASSICADIOL [**10a**].—*O*-Acetylbrassicadiol (0.4 mg) was dissolved in Me_2CO (2 ml), and Jones reagent (0.3 ml) was added. The mixture was allowed to stir at room temperature. After 90 min the reaction was diluted with Et_2O (3×5 ml). The Et_2O extract was dried and concentrated. The reaction product showed starting material as the only component.

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LITERATURE CITED

1. W.A. Ayer and L.M. Pena-Rodriguez, *J. Nat. Prod.*, in press.
2. L.M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Second Edition, Pergamon Press, 1969, p. 176.
3. M.D. Bernardi, G. Mellerio, G. Vidari, G. Fronaz, and P. Vita-Finzi, *J. Chem. Soc. Perkin I*, 221 (1980).
4. W.A. Ayer, T. Yoshida, and D.M.J. van Schie, *Can. J. Chem.*, **56**, 2133 (1978).
5. I. Kubo, T. Matsumoto, A.B. Kakooko, and N.K. Mubiru, *Chem. Lett.*, 979 (1983).
6. C.J.W. Brooks and G.H. Draffan, *Tetrahedron*, **25**, 2887 (1969).
7. L. Canonica, A. Corbella, P. Gariboldi, G. Jommi, J. Krepinsky, G. Ferrari, and C. Casagrande, *Tetrahedron*, **25**, 3895 (1969).
8. J.L. Gough, J.P. Guthrie, and J.B. Stothers, *J. Chem. Soc. Chem. Comm.*, 979b (1972).
9. F.W. Wehrli and T. Wirthlin, "Interpretation of C-13 NMR Spectra," Heyden, London, 1978.
10. P.V. Demarco, E. Farkas, D. Dodrell, B.L. Mylari, and E. Wenkert, *J. Am. Chem. Soc.*, **90**, 5480 (1968).
11. W.A. Ayer and S. Fung, *Tetrahedron*, **33**, 2771 (1977).
12. J.R. Hlubucek, A.J. Aasen, S.O. Almqvist, and C.R. Enzell, *Acta Chem. Scand.*, **B28**, 289 (1974).
13. F.W. Wehrli and T. Nishida, in: "Progress in the Chemistry of Organic Natural Products," Ed. by W. Herz, H. Grizebach, and G.W. Kirby, Vol. 36, 1979, p. 55.
14. W.S. Allen and S. Bernstein, *J. Am. Chem. Soc.*, **78**, 5693 (1956).
15. W.S. Allen and S. Bernstein, *J. Am. Chem. Soc.*, **77**, 1028 (1955).
16. E. Haslam, "Metabolites and Metabolism," The International Series of Monographs on Chemistry, No. 12, Clarendon Press, Oxford, 1985, p. 19.
17. H.G. Cutler, F.G. Crumley, J.P. Springer, R.H. Cox, R.J. Cole, J.W. Dorner and J.E. Thean, *J. Agric. Food Chem.*, **28**, 989 (1980).
18. R.D.H. Murray, M. Sutcliffe, and P.H. McCabe, *Tetrahedron*, **27**, 4901 (1971).

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