

SHORT REPORTS

ABSCISIC ACID FROM *ALTERNARIA BRASSICAE*

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Key Word Index—*Alternaria brassicae*; black spot fungus; abscisic acid, identification.

Abstract—Abscisic acid has been characterized in the mycelium of the black spot fungus, *Alternaria brassicae*.

INTRODUCTION

Abscisic acid (ABA) is a naturally occurring plant growth regulator which may be involved in the control of various plant processes [1]. Since its discovery in 1965 extensive research has been carried out on its chemical, biochemical and physiological properties [2]. Seven species of phytopathogenic fungi have been found that produce abscisic acid since Assante *et al.* first reported ABA production by *Cercospora rosicola* [3–5]. In the present studies, we report its isolation from *Alternaria brassicae*, a black spot fungal pathogen of canola/rapeseed.

RESULTS AND DISCUSSION

The active fraction with HPLC retention time of 16.5 min was obtained as colourless oil. Mass spectral analysis gave m/z (% R.A.), 264 $[M]^+$ (8), 246 (12), 203 (17), 208 (20), 190 (100), 162 (38), 234 (38) and 91 (22). Methyl ester of the active fraction gave a molecular ion m/z (% R.A.), 278 $[M]^+$ (1) and other fragments 260 $[M - H_2O]^+$ (18), 190 (100), 162 (41), 234 (39), 125 (34), 112 (H) and 91 (20). Ethanol solution of the methyl ester gave an UV max $\lambda_{\text{max}}^{\text{EtOH}}$, nm (ϵ), 262 (21, 800).

^1H NMR (400 MHz, CDCl_3) of the active substance gave a broad singlet at δ 10.56 confirming the $-\text{COOH}$ proton. Presence of methyl protons at positions 2', 3, 6' (axial) and 6' (equatorial) was readily confirmed by doublets at δ 1.96 ($J = 1.4$ Hz), 2.07 ($J = 1.4$ Hz), 1.10 ($J = 1.2$ Hz) and a singlet at δ 1.05. Hydroxyl protons at C-1' position was indicated by a singlet at δ 4.46, whereas positions of protons at C-3' and 5' were given by quartets at δ 5.86 and δ 2.56, respectively. Doublets at δ 5.76 ($J = 16.0$ Hz) and δ 6.28 ($J = 16.0$ Hz) indicated the protons at C-2 and C-4 position. A double doublet at δ 2.17 ($J = 13.4$ and 6.7 Hz) was given by a proton at 5' position (Fig. 1). Presence of proton at C-5 position was confirmed by a doublet at δ 7.86 ($J = 16.0$ Hz).

^1H NMR spectral analysis of the methyl ester of the active fraction in deuterated chloroform gave doublets at δ 2.32 ($J = 17.2$ Hz) and 2.46 ($J = 17.2$ Hz) characteristic of the methylene protons. Singlets at δ 1.02, 1.14, 3.70 and doublets at δ 1.92, 2.06 and 1.45 were given by the methyl protons. Broad singlets at δ 5.75 and 5.93 and doublets at δ 6.15 ($J = 16.0$ Hz) and δ 7.86 ($J = 16.1$ Hz) confirm the presence of vinyl protons. All the foregoing spectral data are well in accord with that of an authentic ABA and its methyl ester purchased from Sigma.

EXPERIMENTAL

Fungal culture. The fungus *Alternaria brassicae* used in the present investigation was isolated from infected pods collected from the rapeseed field at Agriculture Canada, Research Station, Beaverlodge, Alberta, Canada.

Isolation of abscisic acid. The fungus was cultured on a potato dextrose agar medium at 25° for 20 days under continuous light (light intensity of 80 $\mu\text{W}/\text{cm}^2$). The cultured mycelium and agar were macerated with acetone and the acetone extract was evaporated to an aqueous solution which was shaken at pH 10.0 with EtOAc to remove neutral and basic substances. The residual aqueous solution was then acidified (pH 3.0) and extracted with EtOAc.

The activity of the EtOAc fraction was tested on canola seedlings. Having established the growth inhibitory action of the extract on canola seedlings, the active acidic substance was converted by treatment with ethereal diazomethane to the methyl ester which still showed the inhibitory activity. It was then purified by prep. HPLC (Cosmosil 5 SL silica gel column 4.6 \times 150 mm, CHCl_3 -MeCN-HOAc 99:1:1; flow rate 2.0 ml/min). The UV absorbing peak at retention 16.5 min was collected and dried. The fraction was retested for growth inhibitory actions before being subjected to UV, NMR and mass spectral analysis.

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AN ISOBUTYLAMIDE AND BEYERENE DERIVATIVES FROM *BRACHYCOME* SPECIES

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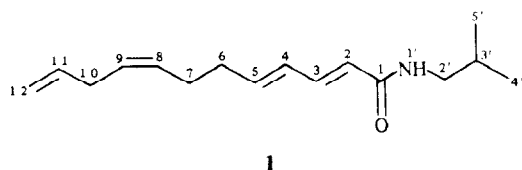
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Key Word Index—*Brachycome* species; Compositae; unsaturated amide; diterpenes; beyerene derivatives.

Abstract—The investigation of some *Brachycome* species gave, in addition to widespread compounds, beyerene derivatives and a new highly unsaturated isobutylamide.

The genus *Brachycome*, with 66 species, shows a large variety of chromosome numbers reaching from two to 45. Most of the species are from Australia, but three are from New Zealand and one from New Guinea [1]. Preliminary chemical studies have shown that the roots contain acetylenes typical for the Astereae [2]. We have now studied the aerial parts of a few species. Those of *Brachycome ciliocarpa* W. Fitzg. gave, in addition to germacrene D, bicyclgermacrene, geranyl- and borneyl acetate the amide 1.

The presence of an isobutylamide followed from the typical ^1H NMR signals [5.48 *br s*, 3.16 *t* (2H), 1.80 *qq* and 0.92 *d* (6H)]. Furthermore nine low field signals were visible. Accordingly, in addition to a vinyl end group (5.82 *ddt*, 5.03 *ddt* and 4.98 *ddt*) three further double bonds were present. Spin decoupling allowed the assignment of all signals and the observed couplings indicated the configurations. Therefore the structure was assigned in agreement with the fragmentation pattern in the mass spectrum.



The aerial parts of *B. iberidifolia* Benth. gave the beyerene derivatives 2–4, the latter so far only being isolated from a *Myriocephalus* species [3]. However, beyerene derivatives are also reported from *Nidorella* [4] and *Baccharis* species [5–7] in the tribe Astereae.

Some further species, *B. ciliaris* (Labill.) Less., *B. ciliaris* (Labill.) Less var. *lanuginosa* and *B. trachycarpa* F. Muell. gave no characteristic compounds.

The isolation of the amide 1 is interesting, as so far these highly unsaturated compounds have only been reported in the Compositae from the tribes Anthemideae

