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A NOVEL ABSCISIC ACID METABOLITE FROM CELL SUSPENSION CULTURES OF *NIGELLA DAMASCENA*

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Key Word Index—*Nigella damascena*, Ranunculaceae, cell suspension cultures, abscisic acid, metabolism, (*S*)-3-methyl-5-(1′-hydroxy-2′-hydroxymethyl-6′-dimethyl-4′-oxo-cyclohex-2′-enyl)-penta-2*Z*, 4*E*-dienoic acid

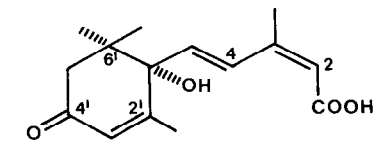
Abstract—The structure of a novel abscisic acid metabolite isolated from cell suspension cultures of *Nigella damascena* fed [2-^{14}C]abscisic acid was identified spectroscopically as (*S*)-3-methyl-5-(1′-hydroxy-2′-hydroxymethyl-6′-dimethyl-4′-oxo-cyclohex-2′-enyl)-penta-2*Z*, 4*E*-dienoic acid

INTRODUCTION

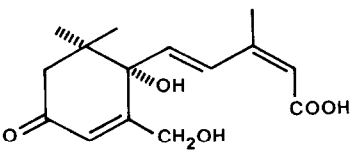
It is known that the plant hormone abscisic acid (ABA) is metabolized to acidic compounds and to conjugates. The pathway by which ABA is metabolized to acidic compounds involves the hydroxylation of its pro-6′(*S*)-methyl group, followed by a rearrangement to phaseic acid and reduction to dihydrophaseic acid and epi-dihydrophaseic acid, respectively [1–4]. In studying the biotransformation of ABA in cell suspension cultures of *Nigella damascena* a novel type of acidic ABA metabolites was detected in which the 2′-methyl group of the cyclohexenyl ring is oxidized to a hydroxymethyl group

RESULTS AND DISCUSSION

The new ABA metabolite (**2**) was found after application of labelled ABA (0.1 mg/50 ml) to cell suspension cultures of *Lycopersicon peruvianum*, *Nigella damascena*, *Papaver alpinum* and *Portulaca grandiflora* [7]. For isolation of **2**, 6 mg [2-^{14}C]ABA was added to 300 ml cell suspension cultures of *Nigella damascena*. After 72 hr the cells were separated by filtration Compound **2** was extracted both from cells and culture filtrate and was purified by TLC followed by CC on Sephadex LH-20.



1



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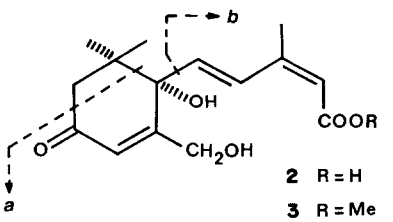

2 R = H
3 R = Me

The IR spectrum of **2** indicates the presence of double bonds (1600 cm^{-1}), a conjugated carbonyl (1668 cm^{-1}) and hydroxy groups (3480 cm^{-1}). The mass spectra of **2** and its methyl ester (**3**), obtained after treatment with diazomethane, show [M]$^+$s of low intensity at *m/z* 280 and 294, respectively. The fragmentation of the abscisic acid derivatives **2** and **3** is mainly characterized by substituent elimination, the RDA-reaction of the C-2′–C-3′ double bond (**a**, [M − C_4H_8]$^+$) and side chain cleavage leading to ion **b** [5]. The subsequent loss of water and methanol, respectively, from ion **a** shows there are two geminal methyl groups at C-6′ The *m/z* values of **b** in **2** and **3** indicate that the C-3 methyl group of the side chain is not hydroxylated.

The ^{1}H NMR spectrum of **2** is very similar to that of ABA (**1**) [6], clearly indicating the 2*Z*, 4*E* configuration of the side chain There are only two marked differences. The signal at δ 1.94 (3H, *s*), assigned to Me-2′ in the spectrum of **1**, is absent and two signals, coupling each other, are present at δ 4.24 and 4.46 (each 1H, *d*, *J* = 18 Hz) indicating hydroxyl substitution of the 2′-methyl group. This is confirmed by a slight downfield shift ($\Delta\delta$ 0.2) of the olefinic proton H-3′. Although long range couplings between these protons are not visible, not even by Gaussian multiplication of the FID, they are detectable by decoupling. The line width of the signal at δ 5.75 (1H, *s*, H-3′) becomes smaller and its intensity increases considerably when the methylene protons (CH$_2$OH-2′), centred at δ 4.35, are irradiated Therefore, it can be concluded from NMR and mass spectral data that the C-2′ methyl group is hydroxylated. Consequently, **2** is (*S*)-3-methyl-5-(1′-hydroxy-2′-hydroxymethyl-6′-dimethyl-4′-oxo-cyclohex-2′-enyl)-penta-2*Z*, 4*E*-dienoic acid

The metabolite **2** has been detected as an endogenous compound in leaves from *Vicia faba* by TLC in different solvent systems as well as by HPLC analysis using **2** from the cell cultures of *N. damascena* as test substance (Lehmann, H., unpublished results).

EXPERIMENTAL

N damascena cells (10 g) were inoculated into 250 ml of Murashige and Skoog medium and grown for 7 days before incubation with 6 mg [2-^{14}C]ABA (35 kBq). Cells (65 g) were harvested after 72 hr by filtration and homogenized in EtOAc–MeOH (5 2). After filtration of the homogenate the extract was evaporated to dryness and dissolved in 500 μl EtOAc–MeOH for chromatography. The culture filtrate, obtained after filtration of cells, was extracted with EtOAc–*n*-PrOH–*n*-BuOH (10 1 2) (×4). The combined extracts were evaporated to dryness. The extracts were subjected to TLC on Si gel HF_{254} (Merck) developed with toluene–EtOAc–MeOH–HOAc (50 30 7 4). The developed plates were scanned for radioactivity and the zones containing the new metabolite (**2**) (R_f 0.43, ABA R_f 0.68) eluted with MeOH. Further purification by CC on Sephadex LH-20 using 1,2-dichloroethane–MeOH (2 1) gave 1.8 mg **2** as a colourless intractable gum. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH, *br*), 1668 (C=C–C=O), 1600 (>C=C<), UV λ_{max}^{MeOH} nm (log ε): 256 (3, 24), EIMS, 80 eV, *m/z* (rel. int.): 280 $[M]^+$ (2), 262 $[M-H_2O]^+$ (17), 244 $[M-2H_2O]^+$ (20), 229 $[M-2H_2O-Me]^+$ (14), 224 **a** (13), 218 $[M-H_2O-CO_2]^+$ (41), 206 (**a** – H_2O) (75), 188 (**a** – $2H_2O$) (92), 161 (83), 122 (48), 111 **b** (78), 97 (100). ^{1}H NMR (200.13 MHz, $CDCl_3$, TMS): δ 1.01 (3H, *s*, Me-6'), 1.10 (3H, *s*, Me-6'), 2.03 (3H, *s*, Me-3), 2.28 and 2.44 (each 1H, *d*, J = 18 Hz, H-5'), 4.24 and 4.46 (each 1H, *d*, J = 18 Hz, CH_2OH-2'), 5.75 (1H, *s*, H-2), 6.18 (1H, *s*, H-3'), 6.14 (1H, *d*, J = 16 Hz, H-5), 7.65 (1H, *d*, J = 16 Hz, H-4).

3: EIMS, 80 eV, *m/z* (rel. int.): 294 $[M]^+$ (1), 276 $[M-H_2O]^+$ (2), 263 $[M-OMe]^+$ (2), 258 $[M-2H_2O]^+$ (2), 243 $[M-2H_2O-Me]^+$ (2), 238 **a** (4), 220 (**a** – H_2O) (6), 206 (**a** – MeOH) (25), 188 (**a** – MeOH – H_2O) (34), 161 (22), 125 **b** (51), 83 (100).

The MS were recorded on a Varian MAT 111 instrument (direct inlet system, electron energy 80 eV, source temp. *ca* 300°, inlet temp. *ca* 120°).

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ISOLATION AND CHARACTERIZATION OF DIHYDROMALEIMIDE AND ITS GLUCOSIDE AS GROWTH INHIBITORS FROM DWARF PEA

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Key Word Index—*Pisum sativum*, Leguminosae, dwarf pea, growth inhibitor, *R*-dihydromaleimide, *R*-dihydromaleimide-β-D-glucoside.

Abstract—Two new growth inhibitors, *R*-dihydromaleimide and *R*-dihydromaleimide β-D-glucoside, were isolated from 2-week-old pea shoots.

In spite of many studies [1–7], plant dwarfism is not fully understood in terms of growth inhibitors. We report now on the isolation of two new growth inhibitors (**1** and **2**) from 12 kg fr. wt of dwarf pea (cv Progress No. 9) shoots grown under *ca* 1 klx of white fluorescent light at 18–23° for 2 weeks. The bioassay procedures used were the lettuce germination and hypocotyl elongation tests.

Compound **1** (0.8 g), colourless prisms, mp 103–105°, $C_4H_5NO_2$, $[\alpha]_D^{23}$ −126°, was characterized as (−)-dihydromaleimide (**1**) by its spectroscopic properties, and by direct comparison with (±)-dihydromaleimide derived from maleimide by a partial reduction by sodium boro-

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