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NIGELLIC ACID—AN ENDOGENOUS ABSCISIC ACID METABOLITE FROM *VICIA FABA* LEAVES

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Key Word Index—*Vicia faba*; Fabeae; abscisic acid metabolite; nigellic acid; gas chromatography/mass spectrometry.

Abstract—Nigellic acid [(+)(*S*)-7′-hydroxy-ABA], previously isolated from cell suspension cultures of *Nigella damascena* fed (±) abscisic acid was detected as an endogenous compound in leaves of *Vicia faba* using gas chromatography/mass spectrometry.

INTRODUCTION

Nigellic acid [(+)(*S*)-7′-hydroxy-ABA] (**1**) and/or its 1′-enantiomer (**2**) were detected in cell cultures of *Chelidonium majus*, *Papaver alpinum*, *Portulaca grandiflora*, and *Nigella damascena* [1, 2], in intact wheat seedlings [3], in leaves of *Hordeum vulgare* [4] and *Xanthium strumarium* [5] as a metabolite of applied (±)ABA. Here, we present the determination of nigellic acid as an endogenous compound in leaves from *Vicia faba*.

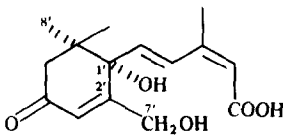
RESULTS AND DISCUSSION

Nigellic acid was isolated from leaves of *Vicia faba*. The plant material was extracted by ethyl acetate and acidic compounds were eluted from the organic phase by NaOH. After acidification, the acidic compounds were extracted by EtOAc–Et_2O and the extract purified by TLC. After elution of the nigellic acid, methyl esters were formed. Further purification was by reverse phase HPLC on Lichrosorb Si 100. Fractions containing nigellic acid methyl ester were collected and analysed by GC/MS. The spectrum obtained was identical to that of authentic nigellic acid methyl ester.

Nigellic acid and its 1′-enantiomer are known to be metabolites of applied (±)ABA in other plant material. Whereas in *Xanthium* leaves only the unnatural (−)ABA was converted to (−)-nigellic acid [5] in cell suspension cultures of *Nigella damascena*, both nigellic acid and (−)-nigellic acid were observed after application of (±)ABA [1]. ORD measurement showed that the (−)ABA was converted preferentially [3]. Static cultures of *Portulaca grandiflora* cells contained after (±) [2′-^{14}C]ABA application radioactive labelled ABA (8%), phaseic acid (13%), conjugated forms (23%) and 52% of their radioactivity as 7′-hydroxy-ABA. Therefore also in this plant both isomers of ABA were transformed to nigellic acid and (−)-nigellic acid. Now it was found that nigellic acid is a natural compound in leaves of *Vicia faba*. Our result do not support the speculation on the biochemical mechanism for the hydroxylation of ABA at the 7′-position given by Boyer and Zeevaart [5]. The authors concluded that the oxygenase catalysing the conversion of (+)ABA to its 8′-OH-derivative also catalyses the hydroxylation of the 7′-methyl group of the (−)ABA during the formation of (−)-nigellic acid by fixing the 1′-hydroxyl and 4′-keto groups at the enzyme surface and inversion of the cyclohexene ring. As natural ABA and its natural metabolites have *S*-configuration at C-1′, we conclude that the endogenous nigellic acid also has the *S*-configuration.

EXPERIMENTAL

100 g lyophilized leaves of *Vicia faba* were pulverized and homogenized in EtOAc using Ultra-Turrax (3 min). After filtration the organic phase was extracted × 3 with 0.1 M NaOH. After acidification the acids were removed from the aq. phase by extraction with EtOAc–Et_2O (2:1). The residue obtained after washing of the organic phase with H_2O and evaporation was purified by TLC (silica gel HF_{254}; solvent system toluene:



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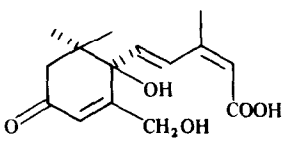

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Dedicated to Prof. Klaus Schreiber on the occasion of his 60th birthday.

EtOAc–MeOH–HOAc (50:30:7:4; nigellic acid R_f: 0.43). The zone corresponding to authentic nigellic acid was eluted with MeOH. Addition of ethereal CH_2N_2 yielded the Me ester of nigellic acid (**3**) which was purified by HPLC (Lichrosorb Si 100, RP-18, 5 μm, 250 × 4.6 mm) using 50% MeOH–H_2O. Fractions corresponding to **3** were collected and evapd before GC/MS analysis.

3 EIMS: 70 eV, *m/z* (rel. int.): 294 $[M]^+$ (1), 276 $[M-H_2O]^+$ (5), 263 $[M-OMe]^+$ (3), 258 $[M-H_2O]^+$, 238 $[M-C_4H_8]^+$, a (7), 221 (11), 220 $[a-H_2O]^+$ (20), 206 $[a-MeOH]^+$ c (83), 188 $[c-H_2O]^+$ (100), 178 $[c-CO]^+$ (6), 161 (50), 160 (32), 125 $[C_7H_9O_2]^+$ b (39). GC/MS was carried out using a Finnigan 4015 GC-MS-data system. Derivatized samples were injected (260°) in 1 μl aliquots into a fused silica capillary column (WCOT OV-101, 25 m × 0.32 mm) using the Grob splitless injection method. The column temp. was maintained at 50° for 1 min, then programmed at 15°/min to 120° and at 4°/min to 260°. The He flow rate was 2 ml/min. The split (50:1) was opened 1 min after injection. The column effluent was led direct into the ion source at 290°. Electron energy was 70 eV, emission current 0.26 mA.

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