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OCCURRENCE OF JASMONIC ACID ANALOGUES IN *VICIA FABA*

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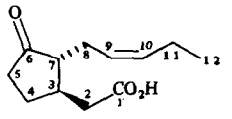
Key Word Index—*Vicia faba*; Fabaceae; fruits; (−)-9,10-dihydrojasmonic acid; 3,7-didehydrojasmonic acid; (+)-6-*epi*-7-iso-cucurbic acid.

Abstract—In addition to the known plant growth regulators (−)-jasmonic acid and (+)-7-*iso*-jasmonic acid, three structurally related cyclopentanoidal C_{12}-acids have been isolated from immature fruits of *Vicia faba* and identified as (−)-9,10-dihydrojasmonic acid, 3,7-didehydrojasmonic acid and (+)-6-*epi*-7-*iso*-cucurbic acid.

INTRODUCTION

Cyclopentanoidal C_{12}-acids and/or their methyl esters have been isolated from different plant species [1–3]. Some of them are important odoriferous substances [4] and some represent a new group of endogenous plant growth regulators [5, 6]. In the broad bean, *Vicia faba* L., the major compounds of this type are (−)-jasmonic acid (**1**) and (+)-7-*iso*-jasmonic acid (**2**) [7, 8], and additionally, some amino acid conjugates of **1** have been isolated recently [9, 10]. The biosynthesis of **1** and **2** has been studied in broad bean fruits [11], and the distribution of jasmonic acid-like compounds within *V. faba* was measured by radioimmunoassay [12]. In continuation of our work in this field, we have isolated and characterized three further cyclopentanoidal acids from young broad bean fruits.



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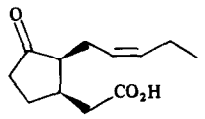

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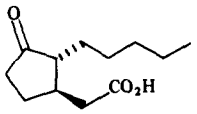

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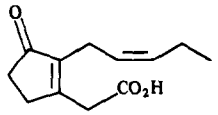

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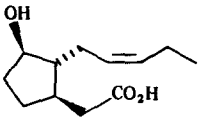

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RESULTS AND DISCUSSION

Column chromatography on silanized silica gel of the acidic fraction of the ethyl acetate extracts of immature fruits of *V. faba* and subsequent preparative HPLC gave in addition to **1** and **2** small amounts of three further C_{12}-acids which were identified as (−)-9,10-dihydrojasmonic acid (**3**), 3,7-didehydrojasmonic acid (**4**), and (+)-6-*epi*-7-*iso*-cucurbic acid (**5**).

The structures of these new compounds were determined from the MS, UV, IR, and ^{1}H NMR (compound **5**) spectra as well as their $[\alpha]_D$ values. The deduced structures of **3** and **5** were confirmed by comparison with synthesized authentic (±)-**3** obtained by hydrogenation of (±)-**1** and **5** obtained by sodium borohydride reduction of (−)-**1** [8], respectively. On the other hand, catalytic hydrogenation of **4**-methyl ester [13] with Adam's catalyst yielded (±)-**3**-methyl ester identified by combined GC/MS.

The 3 (7) position of the ring double bond of **4** was further supported by the MS fragmentation pattern of **4**. Thus, the MS of **4** showed a strong base peak at *m/z* 149 indicating cleavage between C-2 and C-3. Other characteristic fragments were *m/z* 222 $[M]^+$, 193 $[M-C_2H_5]^+$ and 163 $[M-CO_2Me]^+$. On the other hand, the fragment *m/z* 156 $[M-C_5H_8]^+$ observed in the MS spectrum of **1** was absent. All these data are in good agreement with the fragmentation pattern of the analogue methyl (15*Z*)-12-oxo-9(13),15-phytodienoate [14]. As shown by combined GC/MS of **3**, this compound contains *ca* 10% of its 7-isomer, the 9,10-dihydro derivative of (+)-7-*iso*-jasmonic acid (**2**) [3]. Because this acid is easily isomerized to **3**, it cannot be excluded that its content in the plant material is much higher as demonstrated for **2** by recent investigations [8].

The cyclopentanoidal C_{12}-acids **3**–**5** have been found for the first time in nature. However, it should be mentioned that the isoleucine conjugate of **3** is a metabolite of *Gibberella fujikuroi* [15].

EXPERIMENTAL

Plant material. Plants of *Vicia faba* L. var. *minor* cv. 'Fribo' were cultivated in a greenhouse. Young fruits of 2–6 cm length were harvested and immediately extracted.

Chromatographic methods. CC: silanized silica gel, RP 2 (Merck), (600 × 20 mm), elution with a stepwise gradient of

EtOAc in $CHCl_3$; HPLC: Si 100 Polyol RP 18 (25 × 0.46 cm) mobile phase MeOH–0.1% H_3PO_4 (11:9), flow rate 1 ml/min, UV-detection at 228 nm; prep. TLC (silica gel, 1 mm): (a) *n*-hexane–EtOAc–HOAc (60:40:1), (b) $CHCl_3$–MeOH–H_2O (140:20:1); anal. TLC: detection with anisaldehyde reagent and heating for 5–10 min at 120° [16] or UV_{254} (compound **4**).

Isolation procedure. Immature fruits (5 kg) were homogenized in 1 l EtOAc at 4° and filtered through celite. The organic phase was partitioned with satd $NaHCO_3$ soln (3 × 100 ml) and the aq. phase re-extracted with $CHCl_3$ (2 × 50 ml) after acidification to pH 3.5 with 4 M HCl. The extract was dried with Na_2SO_4 and the solvent evapd. The crude acids were purified by CC and fractions eluted with $CHCl_3$–EtOAc (9:1) combined and the solvent evapd. Prep. HPLC gave a mixture (12 mg) of the known compounds **1** and **2** (R_t 9.5 min) [8] and another fraction which on further purification by prep. TLC gave:

(−)-9,10-*Dihydrojasmonic acid* [(1R, 2R)-(−)-3-*oxo*-2-*pentylcyclopent*-1-*yl-acetic acid*] (**3**). 0.9 mg; R_t 13.5 min; R_f 0.45 (solvent system a); $[\alpha]_D^{22}$ −28.3° (MeOH; *c* 0.1). MS and IR identical with [15] and authentic (±)-**3**. Fractions eluted with $CHCl_3$–EtOAc (4:1) were purified by prep. TLC (solvent system a) and HPLC (× 2) to give **4**.

3,7-*Didehydrojasmonic acid* [3-*oxo*-2-(2*Z*-*pentenyl*)-1-*cyclopenten*-1-*yl-acetic acid*] (**4**). 0.2 mg; R_t 7.2 min; R_f 0.42 (solvent system a); IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 3000, 1705, 1740, 1644, 1435; UV λ_{max}^{MeOH} nm 236 (log ε 3.81); MS (80 eV) *m/z* (rel. int.) of **4**-methyl ester: 222 $[M]^+$ (24), 193 (78), 167 (27), 163 (22), 149 (100), 135 (38), 133 (44), 121 (33), 109 (53), 105 (49), 95 (33), 91 (64), 83 (18), 79 (62). Catalytic hydrogenation of **4**-methyl ester [13] with Adams catalyst yielded (±)-**3**-methyl ester identified by combined GC/MS. Fractions eluted with $CHCl_3$–EtOAc (2:3) were further purified by prep. TLC (solvent systems a and b) to give **5**.

(+)-6-epi-7-*iso-cucurbic acid* [(1R,2R,3R)-(+)-3-*hydroxy*-2-(2*Z*-*pentenyl*) *cyclopent*-1-*yl-acetic acid*] (**5**). 1 mg, R_f 0.32 (solvent system a) and 0.17 (b) $[\alpha]_D^{22}$ +6.1 (EtOH; *c* 0.1); IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 3600, 1710, 1650; MS (80 eV) *m/z* (rel. int.) of **5**-methyl ester: 226 $[M]^+$ (2), 208 (11), 195 (6), 153 (15), 152 (24), 139 (31), 134 (100), 119 (38), 107 (39), 105 (38), 93 (39), 83 (46), 79 (50); ^1H NMR (200.13 MHz, $CDCl_3$, TMS as int. standard) δ 5.33–5.54 (2H, *m*, H-9, H-10), 3.91 (1H, *m*, H-6), 2.58 (1H, *m*), 1.44–2.45 (12H, *m*), 0.97 (3H, *t*, $J = 7$ Hz, H-14). Identical with authentic **5** prepared from (−)-**1** by $NaBH_4$ reduction [8, 17].

Analytical methods. Methyl esters of all carboxylic acids were prepared by treatment with ethereal CH_2N_2. Combined GC/MS was performed with an 80 eV mass spectrometer and a glass column (1.80 m × 2 mm) containing 10% EG SS-X on Gas Chrom P (100–120 μm), column temp. 175°, He 15 ml/min, R_t (min) of the methyl esters: **1** 10.3, **2** 12.6, **3** 8.5, **4** 18.8, **5** 11.9.

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