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ISOLATION OF 4′-DIHYDROABSCISIC ACID FROM IMMATURE SEEDS OF *VICIA FABA**

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Key Word Index—*Vicia faba*; Leguminosae; seed; identification; sesquiterpene; 4′-dihydroabscisic acid.

Abstract—4′-Dihydroabscisic acid [1′, 4′-*cis*-diol of (+)-ABA] was isolated from immature seeds of *Vicia faba*. Its identity was proved by TLC and MS.

INTRODUCTION

In our previous studies of the endogenous hormones of the broad bean during flower and fruit development, we detected ABA in the seed and jasmonic acid in the pericarp as the main plant growth inhibitors in these fruit parts [1, 2]. In the seed, the ABA metabolites PA† and DPA, but no ABA conjugates were found. The pericarp contained only traces of ABA and DPA, indicating the same metabolic pathway of ABA in seed and pericarp. The present investigation was directed to the identification of a compound closely related to ABA, but not identical with the known ABA metabolites PA and DPA.

RESULTS AND DISCUSSION

About 25 kg immature *Vicia faba* seeds were harvested, extracted and worked up according to the procedure described. One half of the Et_2O extract obtained was purified on DEAE-Sephadex A-25 to give a strong inhibitory fraction (wheat seedling bioassay), which was eluted with 0.25 M HOAc in 80% MeOH. TLC of this fraction yielded several fluorescence quenching spots, one of which corresponded to ABA (R_f 0.52) and one (R_f 0.21) which was more polar than PA (R_f 0.32), but less polar than DPA (R_f 0.09). The ABA-like substance was methylated with CH_2N_2 and identified by physical methods as ABA (cf. ref. [1]). The second compound was methylated and purified by TLC (main compound R_f 0.30; ABA-Me, R_f 0.76). The purified methyl derivative could be acetylated within 30 min like DPA (ABA cannot be acetylated under these conditions), indicating the presence of a secondary hydroxyl group [3] (TLC on Si gel GF_{254}, $CHCl_3$–MeOH, 1:1; ABA-Me, R_f 0.76; DPA-Me acetylated, R_f 0.58; acetylated methyl derivative of new compound, R_f 0.90). The MS fragmentation pattern of the methyl derivative was identical to that of the authentic 1′, 4′-*cis*-diol of (+)-ABA-Me, prepared from (+)-ABA. The 1′, 4′-*trans*-diol of ABA differed from the isolated ABA diol both in MS and TLC (Si gel GF_{254}, $CHCl_3$–MeOH, 1:1; 1′, 4′-*cis*-diol of ABA-Me, R_f 0.33; 1′, 4′-*trans*-diol of ABA-Me, R_f 0.61; ABA-Me, R_f 0.79; acetylated 1′, 4′-*cis*-diol of ABA-Me, R_f 0.91; acetylated 1′, 4′-*trans*-diol of ABA-Me, R_f 0.79). Thus, the 1′, 4′-*cis*-diol of ABA (4′-dihydroabscisic acid) has been shown for the first time to be an endogenous compound in plants. In the past both 1′,4′-diols of ABA (*cis* and *trans*) had been synthesized and used for studies in biosynthesis and metabolism. The *cis*-1′,4′-diol of ABA, and to a lesser extent the *trans* isomer, were transformed into ABA in bean axes [4] and wheat leaves [5], but this oxidation was also found to be a non-enzymic reaction proceeding spontaneously in aqueous solution [6]. Therefore, it is difficult to determine exactly the biological activity of these diols and the question of the position and function of the endogenous 4′-dihydroabscisic acid in ABA metabolism remains open. Possibly there may be a parallel to the formation of DPA from PA.

EXPERIMENTAL

Plant material. Plants of *V. faba L.* cv. 'Fribo' were cultivated in a greenhouse in 1979 and seeds (25 kg fr. wt) were harvested about 20 days after flower opening (seeds had reached about 30% of max. fr. wt). The seeds gathered were frozen and stored at −20° until extraction.

Extraction and purification. The extraction procedure was performed as described in ref. [2]. The seeds were homogenized in 80% aq. MeOH at low temp. and then extracted with the same solvent. After filtration, the MeOH was removed from the aq. MeOH extract *in vacuo* and the aq. remainder frozen. After thawing at 4° the lipid material was separated by filtration through a layer of cellulose powder. The aq. filtrate was adjusted to pH 2.5 and extracted with Et_2O (4 × 1/3 vol.). The Et_2O extracts were combined, partitioned

*Part 5 in the series "Endogenous Plant Hormones of the Broad Bean, *Vicia faba*". For Part 4 see ref. [2].

†Abbreviations: DPA, 4′-dihydrophaseic acid; PA, phaseic acid.

with 0.1 M $KHCO_3$–0.1 M K_2CO_3 (80:3; pH 9; 1 × 1/3 vol) and the aq. phase acidified again to pH 2.5 and repartitioned with Et_2O. The Et_2O extract was dried (dry Na_2SO_4) and evaporated to a gum (3.4 g), 1.7 g of which were purified on a column of DEAE-Sephadex A-25 (2 × 50 cm). The elution was performed with a discontinuous gradient of HOAc in 80% MeOH according to ref. [7]. The diol was eluted with 0.25 M HOAc (ABA-containing fractions). TLC of this fraction (0.3 mm Si gel GF_{254}: C_6H_6–Me_2CO–EtOAc–HOAc, 40:10:5; 1) gave ABA (R_f 0.52) and the diol (R_f 0.21, eluted with EtOAc–MeOH, 1:1) which was then methylated with ethereal CH_2N_2 and purified by TLC (0.3 mm Si gel CF_{254}: $CHCl_3$–EtOAc, 1:1, R_f 0.3).

Preparation of 1',4' - *cis* - *and* 1',4'; - trans - *diol of ABA.* (+) - ABA - Me prepared from (+)-ABA (ethereal CH_2N_2 followed by TLC in the system (R_f 0.76) used to purify the Me ester of the diol), isolated from immature *V. faba* seeds during these experiments was reduced in 5 ml MeOH–H_2O (2:1) containing some crystals of $NaBH_4$ for 2 hr at room temp. according to ref. [8]. The MeOH was evaporated and the aq. remainder partitioned at pH 7.0 with Et_2O. The Et_2O extract was separated on TLC (Si gel GF_{254}; $CHCl_3$–MeOH, 1:1) to give the 1',4'-*cis*-diol of (+)-ABA-Me (R_f 0.33) and *trans*-diol (R_f 0.61). 1',4' - *Cis* - diol of ABA-Me MS (15 eV) *m/z* (rel. int.): 280 $(M)^+$ (27), 262 (42), 244 (23), 248 (20), 230 (28), 224 (39), 206 (65), 192 (44), 174 (84), 146 (98), 125 (100), 111 (84). 1',4' - *Trans* - diol of ABA-Me. MS (15 eV) *m/z* (rel. int.): 280 $[M]^+$ (8), 262 (42), 244 (38), 248 (10), 230 (33), 224 (16), 206 (48), 192 (31), 174 (65), 146 (80), 125 (100), 111 (75).

Bioassay. Wheat seedling bioassay was performed as described previously [9].

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8β-HYDROXY DEHYDROZALUZANIN C FROM *ANDRYALA PINNATIFIDA**

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Key Word Index—*Andryala pinnatifida*; Compositae; sesquiterpene lactone; guaianolide.

Abstract—The roots of *Andryala pinnatifida* afforded 8β-hydroxy dehydrozaluzanin C.

From the genus *Andryala* (tribe Lactuceae), so far only the isolation of taraxasterol from *A. pinnatifida* Ait (= *A. canariensis*) has been reported [1]. A re-investigation of the roots of this species afforded, in addition to taraxasteryl acetate and cinnamic acid, small amounts of a sesquiterpene lactone, molecular formula $C_{15}H_{16}O_4$. The ^1H NMR spectrum (Table 1) indicated the presence of a methylene lactone with two further exomethylene groups. As the signals for one of these groups were downfield shifted doublets as in dehydrozaluzanin C [2], a guaianolide with a 3-keto group was indicated. The presence of an additional hydroxyl group followed from the IR spectrum and a broad signal at δ 4.46, which was coupled

*Part 419 in the series "Naturally Occurring Terpene Derivatives". For Part 418, see Bohlmann, F., Adler, A., King, R. M. and Robinson, H. (1982) *Phytochemistry* **21**, 1169.