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SHORT REPORTS

IDENTIFICATION BY GC–MS OF 4-CHLOROINDOLYLACETIC ACID AND ITS METHYL ESTER IN IMMATURE *VICIA FABA* SEEDS

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Key Word Index—*Vicia faba*; Fabaceae; 4-chloroindolylacetic acid; 4-chloroindolylacetic acid methyl ester; auxins.

Abstract—4-Chloroindolylacetic acid and its methyl ester have been converted to the *N'*-heptafluorobutyryl methyl ester derivative. An extract of immature seeds of *Vicia faba* has been similarly derivatized. It gave in its mass spectrum the same fragmentation pattern as the synthetic heptafluorobutyryl derivative. The chlorine atom was assigned to the 4-position on the indole ring after comparison by GLC of the extract and of four monochlorinated IAA isomers.

INTRODUCTION

4-Chloroindolylacetic acid methyl ester (4-Cl-IAA) has been detected independently by two groups of workers [1, 2] in young, immature seeds of *Pisum sativum*. The free acid was then isolated from 240 kg of peas [3]. This compound has a high biological activity; 4-Cl-IAA is 10 times more active than IAA itself in the *Avena* straight growth assay [4]. This may be due to a reduced destruction due to the low reactivity of 4-Cl-IAA to peroxidase [5].

Until now, natural chlorinated indolic compounds have been identified by rigorous methods only in immature seeds of *Pisum sativum*. Nevertheless other species might contain this substance. Radioactive Cl is indeed incorporated into compounds migrating close to 4-Cl-IAA methyl ester on TLC [6]. We report here the occurrence of 4-Cl-IAA and its methyl ester in *Vicia faba* immature seeds, as shown by GC–MS.

RESULTS AND DISCUSSION

The immature seeds of *Vicia faba* have been shown to contain two compounds (one acidic and one neutral) which, after derivatization to the N-HFB methyl ester, appear with the same retention time on GLC and present the same fragmentation pattern in GC–MS as the synthetic 4-Cl-IAA derivative in the range of *m/e* 300–460. The lower *m/e* values are disturbed by many fragments of impurities. However with this method of extraction and derivatization, further purification is not necessary. This is important because of the sensitivity of indole compounds to oxygen, acid and light.

In spite of the limited purification, the occurrence of 4-Cl-IAA and its methyl ester in *Vicia faba* immature seeds is based on the following evidence: (1) a molecular ion at *m/e* 419/421 and a base fragment at *m/e* 360/362 corresponding to the stable quinolinium ion characteristic of substituted indoles [7]; (2) the MS shows for these peaks the typical 3:1 ratio of $^{35}Cl/^{37}Cl$ at *m/e* values two units apart; and (3) the position of the Cl atom is confirmed by GLC. The methyl esters of the various isomers have retention times which differ by at least 1 min. There was only one peak in the *Vicia* extract corresponding to the 4-Cl-IAA derivative and no peaks corresponding to the other isomers.

We could further confirm by the same methods the occurrence of 4-Cl-IAA and its methyl ester in immature *Pisum* seeds [1, 2, 8]. Whereas there is six times more ester than the free acid [3] in *Pisum*, the determination of both compounds in *Vicia* indicates about equal amounts, i.e. GLC determination shows 125 ng/g 4-Cl-AA and 142 ng/g of the methyl ester.

The above procedure was applied to 30 g of *Phaseolus* seeds and 1000 g *Zea mays* kernels, but neither compound could be detected unequivocally. It seems that the occurrence of chlorinated IAA may be restricted to the tribe Vicieae, although Engvild [6] demonstrated that the incorporation of ^{36}Cl into indoles does occur to a limited extent in *Phaseolus* and *Zea*. The lack of MS evidence for the chloroindoles may be due to the low level of the substances and the relative low sensitivity of the MS system.

EXPERIMENTAL

Chlorinated IAA's were a generous gift from K. C. Engvild, Denmark. All plant materials were obtained from the market.

Extraction. 30–1000 g of plant material were frozen under liquid N_2 and thawed with addition of 1 ml/g 100 mM Na_2CO_3 and 5 mM ascorbic acid adjusted to pH 9.5 in order to avoid oxidation and acidic destruction of the indole compounds. This mixture was stirred under N_2 for 5 min at $-1°$ and centrifuged.

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Purification. The supernatant was Et_2O extracted and the Et_2O fraction brought to dryness (alkaline fraction). The H_2O fraction was adjusted to pH 3, Et_2O extracted and discarded. The Et_2O was extracted by pH 9 buffer and the Et_2O discarded. This procedure was repeated 5 × under N_2. The same procedure was performed with the alkaline fraction after hydrolysis with 1 ml 1N NaOH for 2 hr at 25°.

Derivatization. Both fractions were methylated by ethereal CH_2N_2 and dried in N_2. After addition of 10 μl dry acetonitrile and drying, the extracts were dissolved in N_2 in 10 μl of heptafluorobutyryl anhydride, NEt_3 (9:1) and allowed to stand at room temp. for 1 hr before analysis.

Gas chromatography. For determination of the ring position of the Cl atom, a Packard 427 equipped with a ^{63}Ni ECD was used. Carrier gas: N_2(25 ml/min, make-up 15 ml/min). A silylated glass column filled with 10% SE-30 on chromosorb W/AW 80/100 mesh was used.

GC–MS. A Varian 1400 equipped with a 1.25 m × 3 mm stainless steel column containing 3% SE 30 on Varaport 30, 100–120 Mesh was interfaced to a Varian MAT. 2 μl were injected. Program T° 50–250°, 20°/min. The 4-Cl-IAA derivative was detected at 210°. The mass spectrometer was used as a detector writing out the total ion current and the single ion current at *m/e* 419. When this peak appeared, the scans (*m/e* 300–460) were run. The electron impact mass spectra were obtained at 70 ev. The MS of the synthetic *N*-heptafluorobutyryl 4-chloroindolylacetate methyl ester gives the following characteristic ions, *m/e* (%Σ 40): 419 (7.6), 421 (2.5) [$M^{+\cdot}$], 360 (24.0), 362 (8.1) [M − $COOCH_3$], 169 (8.4) [C_3F_7], 163 (15.9), 165 (5.3) [360, 362 − COC_3F_7], 128 (6.5) [163 − Cl], 69 (5.2) [CF_3].

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