Short Communications

[Agr. Biol. Chem., Vol. 32, No. 1, p. 117~118, 1968]

Isolation of a Novel Auxin, Methyl 4-Chloroindoleacetate from Immature Seeds of *Pisum sativum*

Sir:

The neutral extract of immature seeds of *Pisum sativum* exhibited fairly strong growth regulating activity, when it was assayed by hypocotyl swelling of *Phaseolus mungo*.¹⁾ The active substance contained in it was assumed, by our preliminary experiment, not to be a simple derivative of indoleacetic acid, because of its faint coloration against the Ehrlich reagent. This report describes the isolation and identification of methyl 4-chloroindoleacetate from the immature seeds.

The materials (276 kg) were extracted according to the usual procedure to give 6.5 g of the neutral fraction which is soluble in acetonitrile and insoluble in hexane. It was chromatographed on an alumina column, and the biologically active fraction was eluted with ethyl acetate-hexane (15:85). After repeated recrystallization, the active substance (25 mg) was obtained as slightly brownish prisms, mp 122°C. This substance was positive for the Beilstein test, and its mass spectrum showed a parent peak at m/e 223 along with 30% intensity of $P+2$, strongly suggesting that the most probable molecular formula should be $C_{11}H_{10}NO_2Cl$. The presence of indole nucleus was indicated by UV spectrum, λ_{max}^{EtOH} 223, 275, 280 and 292 m μ ($\log \epsilon$ 4.59, 3.80, 3.84 and 3.75); methoxycarbonyl by ν_{KBr} 1708 cm^{-1} (C=O), δ (CDCl₃) 3.72 (CH₃, s) and m/e 164 (M-59, COOCH₃); methylene group by δ

FIG. 1.

—: Synthetic methyl 4-chloroindoleacetate
---: An auxin isolated from immature seeds

(CDCl₃) 4.01 (CH₂, s); NH by ν_{KBr} 3340 cm^{-1} . Therefore, the active substance should be a derivative of methyl indoleacetate containing a chlorine atom on the indole nucleus. The possibility to be 2-chloro substitution was excluded by the comparison with the Porter and Thimann's synthetic compound.²⁾ The active substance was hydrolyzed to the free acid, mp 185~188°C, which was in accord with the reported value of either 4- or 6-chloro derivative.³⁾ 4-Chloroindoleacetic acid was prepared by the method described by Fox and Bullock³⁾ and methylated with diazomethane. The substance isolated from immature seeds and its hydrolyzed acid were completely identical with the synthesized samples of methyl ester and acid, respectively, with respect to the infrared (shown in Fig. 1) and ultraviolet spectra, mixture melting point and biological activity. 4-Chloro derivative exhibited auxin activity comparable to methyl indoleacetate against the *Avena* first internode and section tests. It is not clear that methyl

1) The bioassay procedure has not been published. A related reference: J. L. Key, J. B. Hanson and R. F. Bills, *Plant Physiol.*, **35**, 177 (1966).

2) W. L. Porter and K. V. Thimann, *Phytochem.*, **4**, 229 (1965).

3) S. W. Fox and M. W. Bullock, *J. Am. Chem. Soc.*, **73**, 2756 (1951).

ester itself is present in immature seeds, because methanol was used for extraction of the seeds. It is noteworthy that chlorine containing organic compound was found in higher plant as a phytohormone.

We wish to express our thanks to Mr. K. Aizawa, the University of Tokyo, for NMR measurements.

Shingo MARUMO
Hiroshi ABE
Hiroyuki HATTORI
Katsura MUNAKATA

*Department of Agricultural
Chemistry
Nagoya University,
Nagoya*

Received November 10, 1967