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ADDENDUM

Incorporation of [2-¹⁴C]Mevalonic Acid into 28-Isocuposterol by Leaves of *Pisum sativum*

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During a previous examination of the sterols of pea (*Pisum sativum*) leaves we noted a minor component that was tentatively identified as fucosterol on the basis of g.l.c. retention results (Goad & Goodwin, 1966). However, the isomer of fucosterol, 28-isocuposterol, has since been identified as a minor constituent of oat seeds and a few other plants (Knights, 1965; B. H. Knights, personal communication). It therefore became of interest for us to reinvestigate the pea leaf sterol, and the availability of a sample of 28-isocuposterol (Gibbons, Goad & Goodwin, 1968) made this possible. Comparison of the g.l.c. retention times of fucosterol and 28-isocuposterol on the stationary phase cyclohexane dimethanol succinate (CHDMS, also known as Hi-EFF-8B) showed that they can be distinguished when they constitute the major component, as described by Knights (1965). However, when these sterols comprised only a small percentage of the total sterol mixture, as was the case in the pea leaf sterols (Goad & Goodwin, 1966), it was difficult on our column to differentiate between the two components with certainty. We therefore resorted to radiochemical methods to confirm the presence of 28-isocuposterol in pea leaves.

EXPERIMENTAL AND RESULTS

Methods were generally as described previously (Goad & Goodwin, 1966; Williams, Goad &

Goodwin, 1967; Goad, Gibbons, Bolger, Rees & Goodwin, 1969). Peas (variety Onward) were germinated for 7 days and 3.0g. of the freshly developed leaves chopped into strips and moistened with 1.0ml. of a solution of [2-¹⁴C]mevalonic acid (5.0 μ C). After 6 hr. incubation at 25° with illumination, the non-saponifiable lipids (10.0mg.; 8.0 $\times$ 10⁵ c.p.m.) were extracted in the usual manner. Preparative t.l.c. on silica gel developed with chloroform gave the 4-demethyl sterol fraction (2.0mg.; 1.9 $\times$ 10⁵ c.p.m.). To this material 28-isocuposterol (1.0mg.) was added, the mixture was subjected to t.l.c. on 10% silver nitrate-impregnated silica gel developed with chloroform, and the

Table 1. *Crystallization of the labelled 24-ethylidene sterol of pea leaves with 28-isocuposterol or fucosterol*

Crystallization no.	Sp. radioactivity (c.p.m./mg.)	
	28-Isocuposterol	Fucosterol
1	245	256
2	199	188
3	226	137
4	214	90
5	204	90
6	177	77
7	174	67
8	207	28

β -sitosterol (3.27×10^4 c.p.m.) and 28-isofucosterol (3.14×10^4 c.p.m.) bands were eluted. In addition, marker spots of α -spinasterol and ergosterol were run on the plate and the bands corresponding to the Δ^7 sterol (1.43×10^4 c.p.m.) and the $\Delta^{5,7}$ sterol (1.36×10^4 c.p.m.) were also eluted but were not further characterized. Equal portions (5.23×10^3 c.p.m.) of the material co-chromatographing with 28-isofucosterol were added to carrier 28-isofucosterol (20.1 mg.) or fucosterol (19.7 mg.) and the mixtures crystallized several times (Table 1). Crystallization with 28-isofucosterol resulted in essentially constant specific radioactivity after the first crystallization; however, with fucosterol as carrier there was a continual decrease in specific radioactivity until a very low value was reached, at which point insufficient material prevented further crystallization.

DISCUSSION

The above results establish that the 24-ethylidene sterol of pea leaves, into which [2- 14 C]mevalonic acid is readily incorporated, is 28-isofucosterol and not fucosterol as earlier suggested. This is in accord with the results obtained with leaves of *Larix decidua* (Goad *et al.* 1969).

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