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STEROLS OF ROOTS AND NITROGEN FIXING ROOT NODULES OF *VICIA FABA*

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Abstract.—Sterols of both roots and nodules of *Vicia faba* were found to be mainly 24-methylcholesterol, 24-ethyl-5,22-cholestadien-3β-ol and 24-ethylcholesterol. Nodules contained nearly twice the quantity of sterols present in roots, mainly due to an increase in 24-ethylcholesterol from about 40–60% of the total sterol content of root extract to over 80% of nodule extracts. Insignificant amounts of sterol were detected in nodule bacteroid fractions and in free-living *Rhizobium leguminosarum.*

INTRODUCTION

The main structural features which distinguish plant sterols from those of animals have been well-documented [1, 2] and the similarities and divergences of their respective pathways of biosynthesis discussed in terms both of the mechanisms involved [2] and of the possible biological significance of these differences [3]. Studies on animal and microbial physiology suggest that sterols can act as hormones or hormone precursors and that they are of importance in membrane structure and function. Little is known of their function in higher plants, although it has been suggested that they may adopt the same roles as in other organisms [4–6].

Nitrogen fixing root nodules possess certain characteristics which may favour their use in investigations into the role of sterols in higher plants. Firstly, such nodules contain large amounts of all three classes of plant hormones—auxins, gibberellins and cytokinins [7–9] so that the question of interactions between these compounds and steroids in the growth and development of plant tissues might be examined profitably [10, 11]. Secondly, nodule membrane structure almost certainly possesses special features which facilitate the massive bidirectional exchange of solutes between the host plant cell and endophyte and between the nodule and root which takes place during nitrogen fixation [12].

Considerable synthesis of membrane material, particularly of the peribacterial membrane, occurs during nodule development in legumes and differences in the proteins and lipids present have been demonstrated [13]. An analysis of the sterols of roots and nodules of *Vicia faba* is reported here as a preliminary to investigations of their involvement in nodule development and function.

RESULTS AND DISCUSSION

Results of GLC and GC–MS analyses of sterol TMS derivatives from roots and nodules of *Vicia faba* var. 'The Midget' are summarised in Table 1. The three principal compounds were 24-methylcholesterol (peak 1), 24-ethyl-5,22-cholestadien-3β-ol (peak 2) and 24-ethylcholesterol (peak 3). No data were obtained for the stereochemistry at C-24 in these compounds so it is only a presumption that they are respectively campesterol, stigmasterol and sitosterol. Traces of cholesterol and Δ^5-avenasterol could be detected in chromatograms and MS. In addition, substantial quantities of pentacyclic triterpenes (M^+ at *m/e* 498 and 496) could be detected in all extracts.

There were no qualitative differences between sterols present in roots or nodules of varieties 'The Midget' and 'Aquadulce'. However, there was a notable quantitative difference between the sterols of roots

Table 1. GC–MS data for TMS derivatives of sterols from roots and nodules of *V. faba* var. 'The Midget'

Identity	GLC Peak	*RR*$_t$*	M^+(*m/e*)	Other important ions	mg/g fr. wt		
					Nodules	Roots	Roots of non-inoculated plants
24-Methylcholesterol	1	1.24	472	457, 389, 367, 343, 129	0.07	0.09	0.04
24-Ethyl-5,22-cholestadien-3β-ol	2	1.32	484†	394, 379, 351, 255, 129	0.17	0.40	0.22
24-Ethylcholesterol	3	1.49	489	471, 396, 381, 357, 329, 303, 275, 255, 129.	1.03	0.30	0.38

*Cholesterol TMS = 1.0. †Also contains ions of material overlapping from Peak 1.

(roots from both nodulated and non-nodulated plants were essentially the same) and of nodules. Thus, nodules contained both a greater total quantity (1.5–2 ×) of sterol and a greatly increased proportion of the mixture was sitosterol (81% in nodules and 38–59% in roots). Insignificant amounts of sterol were detected on analysis of the nodule bacteroid fraction (isolated by differential centrifugation of nodule homogenates) and no sterols were detected in extracts from 0.5 g dry weight of cultured *Rhizobium leguminosarum*. Therefore, it is probable that virtually all the sterols of nodules are of host cell origin.

It seems likely that the increased synthesis of sitosterol may be associated with changes in the properties of host cell membranes during nodule development. Young growing tissues of the hypocotyl and root of mung bean are characterised by a high sitosterol and a low stigmasterol content and the ratio of stigmasterol/sitosterol increases as the tissues age [14]. Sitosterol is less effective than both campesterol and cholesterol in reducing the rate of methanol induced leakage of β-cyanin from *Beta vulgaris* [15] and of electrolytes from barley roots [16]. In addition, sterols with side chain double bonds (e.g. ergosterol and stigmasterol) impose greater elasticity to yeast protoplast membranes, as measured by resistance to osmotic shock [17]. The location and possible function of sitosterol in nodule membranes remains to be demonstrated.

EXPERIMENTAL

Seeds of *Vicia faba* var. 'Aqualdulce' were sown in garden soil and plants harvested after at least 14 weeks. Nodules formed as a result of infection by the natural soil population of Rhizobium. Nodulated plants of *Vicia faba* var. 'The Midget', inoculated with *Rhizobium leguminosarum*, strain PN27 (originally obtained from Landbouwhogeschool, Wageningen, The Netherlands) were grown in controlled environment rooms as described previously [9]. Nodule-free plants (non-inoculated) were grown similarly, but supplied with NH_4NO_3 (20 mg N l.$^{-1}$ culture solution) and harvested when approximately 2 months old. Plant material was lyophilised on harvest. The ground material was extracted ($CHCl_3$–MeOH), sterols isolated (TLC–$CHCl_3$) and subjected to GLC (of TMS ethers on OV-17 and OV-101) by standard methods. GC–MS was performed using an OV-17 column.

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