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STEROLS OF ROOTS AND NITROGEN-FIXING ROOT NODULES OF SOME NON-LEGUMINOUS SPECIES

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Key Word Index—*Alnus glutinosa*; *A. rubra*; *A. cordata*; Betulaceae; *Casuarina cunninghamiana*; Casuarinaceae; roots; nodules; sterols.

Abstract—Sterols of both roots and nodules of three species of *Alnus* were found to consist only of sitosterol, whereas *Casuarina cunninghamiana* contained substantial amounts of campesterol, stigmasterol and sitosterol. In all four cases more sterol was extracted from nodules than from roots.

INTRODUCTION

In a previous report, we described the sterols of roots and their associated nitrogen-fixing nodules from *Vicia faba* [1]. Qualitatively, the same sterols were present in both tissues but a greater amount of sterol per unit fresh weight was recorded for nodules. The three sterols characterized were 24-methylcholesterol, 24-ethylcholesterol and 24-ethyl-5,*E*22-cholestadien-3β-ol.

In the present work we report on the sterols of three species of *Alnus* (Betulaceae) and one of *Casuarina* (Casuarinaceae), which are all species of plants bearing nitrogen-fixing nodules formed in association with a class of micro-organism (Actinomycetes) not closely related to *Rhizobium*, the endophyte of the Leguminosae.

RESULTS

On the basis of relative GLC and of MS data for TMSi derivatives, it was found that all three species of *Alnus* examined (*A. glutinosa*, *A. rubra*, *A. cordata*) afforded only one sterol, identical in each case with 24-ethylcholesterol. A sample isolated from *A. glutinosa* afforded ^{1}H NMR data at 90 MHz identical with data found for authentic sitosterol (24*R*) 24-ethylcholesterol and distinguishable from that for the (24*S*)-isomer clionasterol [2–4]. (Found for *A. glutinosa* sterol: δ 0.682 *s*: C-18; 0.837 *d*, J = 7.5 Hz: C-26/27; 0.85 *t*, J = 7.0 Hz: C-29; 0.923 *d*, J = 6.5 Hz: C-21; 1.009 *s*: C-19). Three peaks were observed in GLC of sterol extracts from *Casuarina cunninghamiana* roots and nodules. In each case the data for TMSi derivatives were identical to those recorded previously by us [1, 5] for respectively 24-methylcholesterol, 24-ethyl-5,*E*22-cholestadien-3β-ol and 24-ethycholesterol. Quantitative data are listed in Table 1 for the four species examined.

DISCUSSION

It is generally assumed that where 24-ethylcholesterol occurs in nature, it is the (24*R*)-isomer (sitosterol) in higher plants and the (24*S*)-isomer (clionasterol) in lower plants and micro-organisms [6]. If this correlation is generally true, then the presence of the (24*R*)-isomer sitosterol in both roots and nodules of *A. glutinosa* can be taken as a strong indication that the probable source of sterols in both roots and nodules is the host plant. This is in agreement with our earlier work for *V. faba* where no sterol could be detected in pure cultures of the endophyte *Rhizobium leguminosarum*. Currently, our attempts to

Table 1. Sterol analyses of root and nitrogen-fixing root nodules of three *Alnus* species and *Casuarina cunninghamiana*

Experiment*	Species	Harvest time	Material	Sample No.†	Weight (g)	Replicate No.‡	Total sterol (mg/g dry wt)
1	*Alnus glutinosa*	July 1980	roots	1	1.0	1	1.18
	Alnus glutinosa	July 1980	roots	2	1.0	1	1.18
	Alnus glutinosa	July 1980	roots	2	1.0	2	1.13
	Alnus glutinosa	July 1980	nodules	1	1.0	1	1.60
	Alnus glutinosa	July 1980	nodules	2	1.0	1	1.77
	Alnus glutinosa	July 1980	nodules	2	1.0	2	1.81
	Alnus glutinosa	July 1980	nodules	2	1.0	3	1.56
	Alnus glutinosa	July 1980	nodules	3	2.0	—	1.72
	Alnus glutinosa	July 1980	nodules	4	3.0	—	1.41
2	*Alnus glutinosa*	July 1980	roots	1	1.0	2	1.24
	Alnus glutinosa	July 1980	nodules	1	1.0	2	1.50
	A. rubra	Sept. 1980	roots	—	3.1	—	1.78
	A. rubra	Sept. 1980	nodules	—	1.11	—	2.26
	A. cordata	Sept. 1980	roots	—	1.11	—	1.79
	A. cordata	Sept. 1980	nodules	—	0.31	—	1.98
3	*A. glutinosa*	Jan. 1980	roots	—	1.0	—	0.74
	A. glutinosa	Jan. 1980	nodules	—	1.0	—	1.05
4	*Casuarina cunninghamiana*	Jan. 1980	roots	—	—	—	3.11§
	C. cunninghamiana	Jan. 1980	nodules	—	—	—	3.47‖

*All analyses in any one experiment were conducted as a single batch.
†These numbers represent replicate samplings of plant material.
‡Replicate isolations of sterols from the extracts of plant material indicated by '†'.
§0.34 + 1.13 + 1.66
‖0.36 + 1.16 + 1.95
respectively of 24-methyl-, 24-ethyl-Δ^{22}-, and 24-ethylcholesterol.

isolate the endophyte of *Alnus glutinosa* under conditions which will allow us to analyse for sterols have been unsuccessful, although isolation of the endophyte from this and other species of *Alnus* has been reported [7–9].

As was found for *V. faba*, more sterol per unit weight of tissue was isolated from nodules than from roots. Although the differences were not always very large, they appeared to be consistent and reproducible and may reflect the greater membrane development associated with nodules when compared with roots. Further knowledge of the lipoidal constituents of actinomycetous root nodules is of importance in view of the reported stimulation of growth of endophyte isolates by ethanolic extracts of nodules [7] and by commercially available soybean lecithin [10].

EXPERIMENTAL

Roots and nodules from *Alnus glutinosa* (L.) Gaertn. were collected in the wild from a plantation on the east side of Loch Lomond at Balmaha, Scotland, National Grid Ref.: NS415915. In addition, *A. glutinosa*, *A. rubra* Bong. and *A. cordata* (Louis.) Desf. were all inoculated with *A. glutinosa* crushed nodule inoculum and grown in combined N_2-free culture in Peralite in a glasshouse. *Casuarina cunninghamiana* Mig. was grown similarly, but was inoculated with crushed nodules from plants from Melbourne, Australia, originally inoculated with nodules.

Sterols were isolated by extractions ($CHCl_3$–MeOH) of freeze-dried and crushed material, followed by TLC. GLC and GC/MS analyses of TMSi ethers were by standard procedures [1], and the ^{1}H NMR analyses were performed using a Perkin–Elmer R.32 spectrometer (Mr. J. Gall, Department of Chemistry). Quantitative analyses were achieved by the addition of cholesterol (1 mg) to the original crude extract. Sterols were then isolated by TLC of an aliquot and analysed by GLC of the derived TMSi ethers. Peak areas were measured by triangulation and sterol amounts were calculated by comparison with the area for the known amount of cholesterol added to the extract. The relative response for the main sterol, sitosterol, was found to be the same as that for cholesterol and, where appropriate, other sterols are assumed to respond similarly.

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ANTHRAQUINONES FROM *VISMIA* SPECIES*

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Key Word Index—*Vismia cayennensis*; *V. japurensis*; Guttiferae; anthraquinones; physcion; vismiaquinone; vismiaketone.

Abstract—1,8-Dihydroxy-3-methyl-6-methoxyanthraquinone (physcion) and two derivatives, 7-(*trans*-3-methyl-1-butenyl)-physcion (vismiaquinone) and 7-(3-methyl-2-oxobutyl)-physcion (vismiaquinone B), were isolated respectively from *Vismia cayennensis* and *V. japurensis*.

Vismia cayennensis (Jacq.) Pers. [1] and *V. japurensis* Reich. (Guttiferae) [2] are large shrubs which occur in north-eastern South America. The latter species has recently been located also in central Brazil (Prof. José Badini, private information). Wood samples were found to contain respectively physcion (**1a**), besides sitosterol, lupeol and betulinic acid, and the two physcion derivatives **1b** and **1c**, besides sitosterol, friedelin and friedelan-3β-ol.

The structures of **1b** and **1c** resulted from a comparison of their spectral data with those of physcion (**1a**) [3]. The ^{1}H NMR spectra were very similar with respect to the signals of H-5 (δ 7.38 $\pm$ 0.04), H-4 (δ 7.59 $\pm$ 0.04), H-2 (δ 7.05 $\pm$ 0.03), Me-3 (δ 2.43 $\pm$ 0.02), OMe-6 (δ 3.98 $\pm$ 0.02) and two chelated hydroxyls. The only significant differences were that the signal of H-7 (δ 6.68) in **1a**, was replaced by signals assigned to a *trans*-3-methyl-1-butenyl group in **1b** and a 3-methyl-2-oxobutyl group in **1c**. All assignments and, most importantly, the fact that the C—Me group is flanked by two aromatic protons, were confirmed by double irradiation experiments. The IR spectra were consistent with the proposed structures indicating the existence in all three compounds of chelated (ν_{max}^{KBr} 1623 $\pm$ 2 cm^{-1}) and unchelated (ν_{max}^{KBr} 1666 $\pm$ 1 cm^{-1}) anthraquinone carbonyls and in **1c** additionally of an unconjugated ketone function (ν_{max}^{KBr} 1706 cm^{-1}).

Physcion (**1a**) is a well known natural compound and has been isolated for instance from *Harungana madagascariensis* Poir. which also belongs to the Guttiferae family [4]. 7-(3-Methyl-1-butenyl)-physcion (**1b**) has been reported previously as one of the alkaline decomposition products of vismione A, a constituent of the berries of *Vismia baccifera* (L.) Tr. et Pl. subsp. *dealbata* (H.B.K.) Ewan [5]. Gonçalves and Mors [6], who isolated **1b** while our work on *V. japurensis* was in progress and who named the compound vismiaquinone, demonstrated, however, that the product is a genuine constituent of the leaves of *V. reichardtiana* (O. Ktze.) Ewan.

In addition to vismione A and vismiaquinone, only the flavones artocarpin, norartocarpin [7] and chaplashine [8] from *Artocarpus* (Moraceae) species have been reported to contain 3-methyl-1-butenyl side chains. Delle Monache *et al.* [5] postulated that these groups may be formed from the more usual γ,γ-dimethylallyl chains by shift of the double bond into conjugation with the aromatic nucleus. The isolation of the novel vismiaquinone B (**1c**) reveals a possible mechanism for this shift. Oxygenation of the β-carbon of γ,γ-dimethylallyl groups does occur in Guttiferae, as demonstrated by psorospermin (**2**) [9], and an analogous intermediate substituted by $-CH_2.CHOR.CHMe_2$ may be a common

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