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SHORT REPORTS

ACCUMULATION OF *O*-METHYL-INOSITOLS IN WATER-STRESSED *VIGNA* SPECIES

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Key Word Index—*Vigna* species; Leguminosae; water stress; *O*-methyl-inositol accumulation; ononitol; *O*-methyl-scyllo-inositol.

Abstract—Accumulation of *O*-methyl-scyllo-inositol and ononitol in leaves of several water-stressed *Vigna* species is described. It is suggested that the relevant species could be used as convenient sources of these relatively rare compounds.

INTRODUCTION

It has recently been reported that the amounts of D-pinitol (1D-3-*O*-methyl-chiro-inositol) in leaves of the tropical legume siratro (*Macroptilium atropurpureum*) increase markedly under conditions of water stress [1]. Pinitol, although the most commonly occurring *O*-methyl-inositol in the Leguminosae [2], is replaced in some legumes by ononitol (1D-4-*O*-methyl-myo-inositol), e.g. in *Lablab purpureus* and *Vigna unguiculata* (cowpea) [3]. Also, low yields of *O*-methyl-scyllo-inositol have been isolated from mung bean seeds [4]. Cowpea and mung bean are important legume grains in tropical summer rainfall regions, where the risk of encountering water stress during crop growth is high. In continuing investigations relating phytochemical changes to physiological responses in legumes subjected to water stress, it is now shown that ononitol and *O*-methyl-scyllo-inositol, respectively, accumulate in water-stressed leaf tissue of cowpea and mung beans respectively. The results suggest that these species may be convenient sources for extraction of these relatively rare inositols. They can be isolated in quantities and purity not easily obtainable by other means.

RESULTS AND DISCUSSION

Ononitol and *O*-methyl-scyllo-inositol were isolated in yields equivalent to 0.75 and 2.2% dry wt of plant tissue, respectively. Further studies on other *Vigna* species (Table 1) indicated that ononitol is the more commonly accumulated inositol, while *O*-methyl-scyllo-inositol appears confined to the two closely related mung bean species *V. mungo* (black gram) and *V. radiata* (green gram). The yield of *O*-methyl-scyllo-inositol obtained in this work is 11 times that reported from mung bean seeds [4], and represents the first isolation from vegetative plant tissue. It is clear that some species attain much higher concentrations of ononitol during water stress than cowpea (Table 1), and hence would be preferable sources of it if available. It may be of taxonomic interest that the three species which accumulate by far the highest concentrations of ononitol are classified with mung beans in the subgenus *Ceratotropis* (Piper) Verdc. [5]. This group was formely placed in the genus *Phaseolus*.

It is of interest to note that high concentrations of pinitol can also be produced in certain legumes during water stress. For example, water-stressed leaves of the tropical grain legume *Cajanus cajan* (pigeon pea) had pinitol levels of 5.3% dry wt (cf. 1.0% dry wt in well-watered tissue) (Ford, C. W., unpublished results). This compares favourably with 4% in sugar pine heartwood, the usual source of this inositol [6].

This work has thus described a simple technique which increases the availability of some relatively rare inositols. These compounds have value as synthetic starting materials [6], and scyllo-inositol, (easily obtained by demethylation of the *O*-methyl compound), since it is rarely encountered in higher plants, may also provide a useful int. standard in GC analysis of plant carbohydrates [7]. These compounds will also be of interest to plant physiologists and plant biochemists. It is possible that these inositols, which accumulate in leaf tissue during periods of water deficits, may contribute to osmotic adjustment and hence plant survival. The technique could also find application to species outside the Leguminosae to produce increased concentrations of other inositols, e.g. D-bornesitol [3] or other novel products [8].

EXPERIMENTAL

Plant material. Plants were grown in large cylinders (1 × 0.25 m i.d.) in a controlled environment (500 μE/m^2/g photosynthetic quatum flux for a 17 hr day; 30°/25° day/night air temp.) and were watered daily to a H_2O content equivalent to *pF*2. After 22 days watering of plants ceased. Samples of last fully expanded leaves were harvested just before com-

Table 1. The effect of water-stress on *O*-methyl-inositol concentrations in leaflets and petioles of *Vigna* species

Plant		Water potential Ψ_l (−MPa)	*O*-Methyl-inositol content (% dry wt) Leaflet	Petiole
*V. unguiculata** CPI 60452	W	0.40	0.2	0.6
	D	1.00	1.1	1.7
*V. pubescens** CPI 60435	W	0.45	0.1	0.8
	D	0.90	1.1	1.7
*V. schimperi** CPI 28705	W	0.50	0.8	2.2
	D	1.30	1.5	2.0
*V. parkeri** CPI 72529	W	0.65	0.1	1.9
	D	1.20	0.4	1.1
*V. luteola** cv Dalrymple	W	0.55	0.3	1.2
	D	1.15	2.6	2.7
V. aconitifolia† CPI 50750	W	0.25	0.7	2.4
	D	2.05	6.4	2.9
V. trilobata† CPI 13671	W	0.50	0.4	2.2
	D	1.00	4.0	3.7
V. umbellata† CPI 2779	W	0.45	1.4	3.1
	D	1.05	4.2	3.0
V. mungo† cv Regur	W	0.40	0.8‡	nd
	D	2.30	2.8	nd
V. radiata† cv Berken	W	0.40	0.9‡	nd
	D	1.65	1.9	nd

*Subgenus *Vigna*.
†Subgenus *Ceratotropis*.
‡*O*-Methyl-scyllo-inositol. All other values are amounts of ononitol.
nd, Not determined; W, wet; D, dry.

mencement of the H_2O-stress period, and then again after the plants had been without H_2O for 34 days. The remaining H_2O-stressed leaves were bulked for each species. All plant material was immediately frozen in dry ice followed by lyophilization, drying in vacuum over P_2O_5 and grinding through a 1 mm sieve.

General. Mps were recorded on a Kofler hot stage apparatus and are uncorr. Evaporations were performed under red. pres. at less than 40°. Thin-layer electrophoresis (TLE) was carried out on glass plates (20 × 20 cm) coated with Whatman CC41 cellulose (0.25 mm) in sodium borate (0.05 M) at 1500 V for 15 min. Spots were visualized with alkaline $AgNO_3$ [9]. GC separations were effected in stainless steel columns (1800 × 3 mm) packed with (a) 3% GE-SE 30 or (b) 10% Carbowax 20 M. Injector and detector (FID) temps. were 220 and 285° respectively. Column temps. were initially 140°, programmed to rise at 2°/min. Carrier gas was N_2 at 25 ml/min. TMSi derivatives were prepared using TMSi–imidazole [10]. Leaf H_2O potentials (Ψ_l) were measured in a pressure chamber.

Extraction. For quantitative *in situ* analyses, single trifoliate leaves were used (*ca* 200 mg). For isolation purposes, bulked H_2O-stressed leaf from *V. unguiculata* (CPI 28215) and *V. mungo* (cv Regur) (8.7 g and 9.3 g respectively) was used. Dry, ground tissue was sequentially extracted in a Soxhlet apparatus with Et_2O and 95% aq. EtOH. Inositols in the single leaf EtOH extracts were determined by GC.

Isolation. Both EtOH solns from the bulked tissues were evaporated to a syrup, hydrolysed with 10 ml H_2SO_4 (0.5 M, 1 hr, 100°), neutralized ($BaCO_3$) and the sugars oxidized with 0.05 M $Ba(OH)_2$ (60 ml, 1 hr, 100°). After centrifugation, the solns were treated with ion-exchange resins (Amberlite IR 120 (H^+) and Bio Rad AGI × 8 (acetate)). Both solns deposited crystals on evaporation and cooling in EtOH–H_2O (9:1). Further purification by CC [Cellulose, EtOAc–HOAc–HCO_2H–H_2O (18:3:1:4)] to remove small amounts of co-pptd free inositols myo-inositol and scyllo-inositol (only from mung bean) resulted in the isolation from aq. EtOH of crystalline ononitol (65 mg, mp 168–172°, lit. 172°) [3] and *O*-methyl-scyllo-inositol (206 mg, mp 243–244°, lit. 243°) [4], homogeneous by GC and TLE. Identities were confirmed by comparison with authentic compounds [mmp, GC, RR_i, columns (a) and (b), TLE] Demethylation [11] produced myo-inositol and scyllo-inositol respectively.

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CARDIOLIPIN OF *CHLAMYDOMONAS REINHARDTII* 137$^+$

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Key Word Index—*Chlamydomonas*; phospholipid; cardiolipin; fatty acid.

Abstract—The fatty acids of cardiolipin from the phototrophic green alga *Chlamydomonas reinhardtii* 137$^+$ have been quantitatively analysed. Comparison is made at the molecular level between the cardiolipin of *Chlamydomonas* and that of higher plant tissue.

INTRODUCTION

Cardiolipin (diphosphatidylglycerol) has been analysed at the molecular level in only a few higher plant tissues [1–2]. Recently, the phospholipid was localized exclusively in the inner membrane of the plant mitochondrion and the fatty acids associated with the mitochondrial cardiolipin were quantitated [3]. The technical difficulty of obtaining purified mitochondria from green algae [4] and the relatively minor contribution that cardiolipin makes to plant tissue lipid [1–3] has hindered comparable direct study of cardiolipin from lower green plants. We have obviated these limitations by isolating cardiolipin from cellular lipid extracts of the green alga *Chlamydomonas reinhardtii* 137$^+$ grown phototrophically. Reported here is a quantitative analysis of the acyl groups associated with the cardiolipin of this typical chlorophyte.

RESULTS AND DISCUSSION

Purified *Chlamydomonas* cardiolipin was transesterified with sodium methoxide [5] and the fatty acid methyl ester derivatives recovered. The methyl ester fraction was analysed by GC either as recovered or after separation into classes of unsaturation by silver nitrate-TLC [6] with comparable results. In phototrophic *Chlamydomonas*, cardiolipin has an ester group unsaturated–saturated ratio of 2.41 ± 0.12 (s.d.; $n = 4$). Monoenes constitute 36.1% of the unsaturates; dienes 32.3%; trienes 19.9% and tetraenes 11.7%. Cardiolipin is one of the most highly unsaturated phospholipids of the alga; only phosphatidylglycerol is more unsaturated [7].

The fatty acid profile of *Chlamydomonas* cardiolipin is detailed in Table 1. Together, 16-carbon and 18-carbon acyl chains constitute over 70% of the major fatty acids, with lesser contributions from 14-, 20- and 22-carbon acyl groups. Prominent fatty acids are 16:0, 18:0 and 18:3. The prevalence of 16- and 18-carbon fatty acids is qualitatively reminiscent of the cell as a whole [7, 8] and of its major membrane, the chloroplast thylakoid [7].

Cauliflower (*Brassica oleracea* [1]) buds, mung bean (*Vigna radiata* [2, 3]) hypocotyls and sycamore (*Acer pseudoplatanus* [3]) cells are as yet the only plants from which cardiolipin has been analysed at the molecular level. In these higher plants at least 93% of the total cardiolipin fatty acids are C_{18}. The unsaturated–saturated ratio for higher plant cardiolipin ranges from *ca* 13 in mung bean [2, 3] to *ca* 32 in sycamore cells [3]. Comparison of these characteristics with the properties of *Chlamydomonas* cardiolipin demonstrates that the algal cardiolipin has both a wider variety of acyl groups and greater fatty acid saturation than do the higher plant cardiolipins.

Since the number of detail analyses on cardiolipin

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