ARBUTIN AND SUCROSE IN THE LEAVES OF THE RESURRECTION PLANT MYROTHAMNUS FLABELLIFOLIA

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Abstract—Forty-two per cent of the desiccated leaves of *Myrothamnus flabellifolia* was extracted in methanol, and contained two major components, sucrose (30%) and arbutin (65%). Its arbutin content can probably explain the purported medicinal properties of this plant. By analogy with other desiccation-tolerant organisms, sucrose and arbutin may be partially responsible for the extreme resistance of *Myrothamnus* to environmental stress.

INTRODUCTION

'Resurrection plants' are those that, by various means, are able to recover from complete desiccation [1]. A remarkable example of this habit is *Myrothamnus flabellifolia* Welw. [2], a small shrub native to southern Africa where it grows on shallow granitic soils subjected to drought during the dry winter months. The desiccated branches of *Myrothamnus* are collected from the wild for use as an herbal medicine. Infusions of the leaf are purported, among other things, to be effective against colds, breast diseases, backache, kidney troubles and haemorrhoids. Chewing the leaves was thought to be a remedy for scurvy, halitosis and Vincent gingivitis [3]. Major components in the essential oil obtained from *Myrothamnus* are the terpenes (+)-carvone and (-)-perillic alcohol [4], but the medicinal principle, if any, has not been identified.

In winter, Myrothamnus plants desiccate entirely, without shedding their leaves, which dry and fold close to the stems. The leaves, with the rest of the plant, rehydrate at the onset of the summer rains. Leaves on cut branches rehydrate within 24 hr of the branches being placed in water. The ability to rehydrate is little affected by prolonged desiccation, or by exposure to high (80°) or low (-195°) temperatures [5]. The mechanism by which leaves of *Myrothamnus*, or of other resurrection plants, are able to withstand prolonged and extreme desiccation is not known. In drought-tolerant animals there is a large accumulation of the disaccharide trehalose during desiccation [6]. Studies with isolated enzymes and model membrane systems indicate that disaccharides may be directly involved in desiccation tolerance [7]. In the present study we examined the soluble metabolite composition of dried leaves of Myrothamnus.

RESULTS AND DISCUSSION

Dry branches of *Myrothamnus flabellifolia* which were still living were used. Entire leaves of the plant were refluxed with methanol yielding an extract containing ca 42% of the leaf dry weight. The woody stems contained only 9% methanol-soluble material. The leaf extract

was fractionated between water and chloroform, the aqueous phase was lyophilized to yield a reddish solid (73%, extract A), and the organic phase dried to a greenish solid (23%, extract B).

Elemental analysis of extract A indicated the absence of nitrogen, and the ¹H and ¹³C NMR spectra suggested a relatively simple mixture of components of carbohydrate and aromatic nature. Attempts to analyse components of extract A using HPLC (reversed-phase C18 column and methanol-water as mobile phase) were unsatisfactory, and the mixture was therefore acetylated and the major constituents separated by prep. TLC. Two major components were detected and isolated. The slower moving component was identified as sucrose octa-acetate. For the faster moving component a M_r of 482 was observed in the CIMS (m/z 483, [M+1]), and the base peak at m/z 331 as well as peaks at 271, 211 and 169, suggested a glycosidic structure with a tetra-acetylated hexose sugar unit, subsequently identified as β -glucose [8]. The non-sugar part of the molecule appeared to be hydroquinone, since ¹H NMR showed one phenolic acetate and four accidentally equivalent aromatic protons. This identification was consistent with the presence of only four signals in the aromatic region of the ¹³CNMR spectrum, two for the unsubstituted carbon atoms (δ 122.5 and 118.1), and two for the quaternary carbon atoms bonded to oxygen (δ 154.5 and 146.4). The tentative identification of the glycoside as hydroquinone- β -D-glucopyranoside pentaacetate [(-)-arbutin pentaacetate], was confirmed by comparison with a sample prepared from commercially available (-)-arbutin. From ¹³CNMR measurements it was inferred that arbutin and sucrose (2:1 ratio) constituted more than 90% of extract A.

In the desiccated state, *Myrothamnus flabellifolia* leaves therefore contain *ca* 20% arbutin and 9% sucrose. Extract B comprised mostly waxes and hydroquinone. The purported medicinal properties of infusions of myrothamnus are probably due to their contents of arbutin [9]. This compound is presently extracted commercially from plants containing from 3 to 7% arbutin; *Myrothamnus* might be an alternative commercial source of this compound.

It seems likely that sucrose and/or arbutin may play important roles in the amazing resistance of Mvrothamnus leaves to extended periods of drought, and other environmental stresses. Arbutin is found in diverse taxa in the plant kingdom, and many of the plants in which it occurs in high levels are adapted to stress conditions, for example arctic low temperatures (Vaccinium spp., Arctostaphylos spp. [9]), or drought stress (Leucodendron [10], Myrothamnus). One feature of the arbutin molecule which might confer protection during these stresses is its tworing hydroxylated structure, which is somewhat analogous to the structure of sucrose and other disaccharides. Additionally, arbutin, as a source of hydroquinone, might reduce the rate of peroxidation of unsaturated plant membrane lipids, one of the events commonly considered to play a role in the deterioration of plant cells during aging or exposure to stress [11]. The effects of sucrose/ arbutin mixtures on the chemical and physical stability of model plant membranes are presently under investigation

EXPERIMENTAL

General. Mps: uncorr. ¹H and ¹³C NMR spectra were obtained at 200 and 50.3 MHz, respectively. MS were obtained with CH_4 CI and by electron ionization at 70 eV. TLC was performed on silica gel 60 F 254 plates.

Plant material. Dry branches of Myrothamnus flabellifolia were obtained from a street market in Harare, Zimbabwe. As evidenced by their ability to revive within 24 hr of being placed in water, the harvested branches were still 'living'. A voucher specimen is deposited at the herbarium of the Departamento de Biologia Vegetal at the University of Málaga (MGC28837).

Extraction and isolation. Entire leaves of the plant (7 g) were extracted twice with MeOH (120 ml) under reflux for 20 hr. The methanolic extract was concd under red. pres. to dryness to give 3 g of solid extract (42%). In the same way, woody stems (2 g) were sliced and extracted to afford 180 mg of methanol-soluble material (9%). A portion of the leaf extract (800 mg) was fractionated between H₂O and CHCl₃, and the aq. phase was lyophilized to yield 580 mg (73%) of a reddish solid (extract A). The chloroformic extract was concd under red. pres. to dryness to give 180 mg (23%) of a greenish solid (extract B).

Acetylation of extract A. A soln of extract A (100 mg) in pyridine (1 ml) was treated with Ac_2O (2 ml) at room temp. for

16 hr. After the usual work-up, the crude product was purified on prep. TLC ($\text{Et}_2\text{O}-\text{CCl}_4$, 5:1). Two major components were detected and isolated. The slower moving component was identified as sucrose octa-acetate by direct comparison with a sample prepared from commercial sucrose (TLC, ¹H, ¹³C NMR, $[\alpha]$).

Arbutin pentaacetate. The faster moving component was crystallized from ethanol. Mp 137–138°; $[\alpha]_D - 27.5°$ (CH₂Cl₂; c 0.10). ¹H NMR (CDCl₃): $\delta 6.98$ (4H, s), 5.28–4.99 (4H, m), 4.27 (1H, dd, J = 12.4 and 5.4 Hz), 4.13 (1H, dd, J = 12.4 and 2.7 Hz), 2.27, 2.06, 2.04, 2.03, 2.01 (each, 3H, s, 5 × OAc). EIMS m/z (rel. int.): 331 (17), 271 (4), 211 (2), 169 (100), 127 (30), 109 (88); CIMS m/z: 483 [M + 1]⁺.

Separation of products. Me₂CO (15 ml) was added to 100 mg of extract A, refluxed for 8 hr, then filtered. The Me₂CO soln was concd to dryness giving 65 mg (65%) of pure arbutin, mp 165°. ¹³C NMR of the acetone-insoluble residue demonstrated the presence mainly of sucrose and trace amount of arbutin.

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