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RICININE IN PHLOEM SAP OF *RICINUS COMMUNIS*

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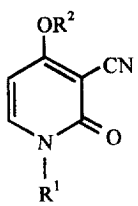
Key Word Index—*Ricinus communis*; Euphorbiaceae; phloem transport; alkaloids; ricinine.

Abstract—Ricinine was detected in the phloem sap of *Ricinus communis* seedlings providing strong evidence for transport in the phloem as expected previously. Ricinine was also isolated from the yellow cotyledons of *R. communis*.

INTRODUCTION

Ricinus communis is known due to its seed oil used as a purgative in medicine and its very toxic lectin, ricin [1]. The oil and the plant have been investigated by several authors and many natural products were identified (for a comprehensive survey see Refs [2] and [3]). Ricinine (**1**, 4-methoxy-1-methyl-2(1H)-pyridone-3-carbonitrile) attracted the attention of plant physiologists mainly due to the investigations of Waller and co-workers, who postulated that it is involved in the senescence of the leaves [4–6]. In the early stages of senescence, **1** is degraded to *N*-demethylricinine (**2**) and, to a much lesser extent, to *O*-demethylricinine (**3**). At the end of the senescence (i.e., when old leaves are completely yellow), **1** and **2** cannot be detected because both are translocated to young parts of the plants, especially to young growing tissue (small amounts of **1** and **2** in yellow leaves were interpreted to be due to the incomplete process of senescence, since naturally detached leaves were found to be devoid of both compounds). Feeding labelled **1** to senescing leaves for one week, led to labelling of all the ricinine pools in the plant, especially in the growing part again. It was suspected to be translocated via the phloem, since movement through the xylem would have led to accumulation in the mature transpiring leaves [7]. Direct analysis of phloem sap for ricinine had not been performed. We have analysed the phloem sap of *R. communis* seedlings, which can be obtained in sufficient quantities for unequivocal chemical identification of secondary metabolites.



1 $R^1 = R^2 = Me$

2 $R^1 = H$, $R^2 = Me$

3 $R^1 = Me$, $R^2 = H$

RESULTS AND DISCUSSION

We could identify **1** in the phloem sap by GC-MS and GC-coinjection with an authentic synthetic sample providing strong evidence for transport in the phloem as expected previously. One remaining question to be answered is, if **1** is actively transported or passively accompanies other substances moving along by mass flow. The concentration was determined by GC and has a mean value of 2.05 ± 0.44 mg g^{-1} fr. wt (12.5 ± 2.7 mM). The reason for the high standard error is probably due to the large tailing of **1**, which makes an exact determination of the peak area to be integrated very difficult. This is a lower concentration than we found in excised yellow cotyledons (ca 5 mg g^{-1} fr. wt (50.8 mM)) taken from 6- to 8-day-old etiolated seedlings and is in agreement with other studies [8]. The structure of synthesized ricinine (**1**) was proven unequivocally by means of EI mass spectrometry, ^{1}H NMR, ^{13}C NMR, APT and HMBC. The ^{13}C NMR signals could be exactly assigned by HMBC. The assignment of the ^{1}H NMR signals was the same as published previously [9].

In general, the ricinine content in cotyledons and in phloem sap is among the highest reported, like in shoot apices and developing flowers [5]. In contrast to the latter, the cotyledons are an exporting organ, although they themselves may have taken up **1** from the endosperm. From the concentration of **1** in coty-

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ledons (50 mM), a downhill gradient to the phloem exists, so that active ricinine loading into the sieve tubes seems to occur. The accumulation of **1** in growing parts of the adult plant may be driven by the sucrose mass flow in the phloem, so that minor compounds like **1** are continuously delivered to the sink tissues, even if these contain already a higher ricinine level than the sieve tube sap itself.

The biological implication of the ricinine transport, if it happens, is however unclear. The physiological situation in our study is different from senescence, the cotyledons contain non-green proplastids in the former in contrast to gerontoplasts in the latter case. So two major suggestions are most likely: **1** is transported as a wound response and therefore its appearance is a consequence of the phloem sap collection procedure, or it is normally transported through the whole plant from the leaves.

The transport of alkaloids other then ricinine via phloem has been described. In the host–parasite system, *Lupinus albus–Cuscuta reflexa*, the parasite receives the quinolizidine alkaloids from the phloem [10]. Quinolizidine alkaloids formed in the leaves of *Lupinus albus* are translocated via the phloem to the other plant organs [11]. Broom aphids suck the phloem sap of the broom plant (*Cytisus scoparius*) and accumulate quinolizidine alkaloids. The alkaloid pattern of the aphids is different from that of its host plants [12]. The long-distance transport of the pyrrolizidine alkaloid *N*-oxides in *Senecio vulgaris* is mainly accomplished by the phloem [13]. Phloem transport in *Astragalus lentiginosus* has been demonstrated for the indolizidine alkaloid, swainsonine, with the help of phloem-feeding aphids [14]. Tropane and pyridine alkaloids were detected in the phloem sap of *Duboisia myoporoides* [15]. The diterpenoid alkaloid aconitine was isolated from aphids feeding on *Aconitum napellus*. The presence of the alkaloid in aphids suggests that it is transported in the phloem [16].

It is possible that the distribution of **1** from seeds to seedlings or the mobilization from senescing leaves to growing parts serves as a feeding deterrent for herbivores and especially aphids. It may be the presence of this toxic compound in the phloem sap, which "allows" the *R. communis* plant to have a less efficient and less instantaneous phloem sealing response (pathogens or herbivores could not settle easily in the nutrient-rich phloem sap). Besides this deterrent action, **1**, by its quantity is just one of the major nitrogen-containing compounds in phloem sap.

EXPERIMENTAL

The phloem sap of 6-day-old seedlings of *R. communis* L. var. sanguineus (seeds purchased from Jelitto GmbH, Hamburg, Germany) was collected as described in Ref. [17]. Seedlings of *R. communis* L. var. gibsonii Impala were cultivated from seeds (Julius Wagner GmbH, Samenzucht-Samengroßhandel, Heidelberg, Germany).

Quantification of **1** *in phloem sap and cotyledons*

Insoluble proteins of phloem exudate were separated by centrifugation for 5 min. The supernatant (28.3 g) was lyophilized (3.6 g). A sample (545 mg) was partitioned between $CHCl_3$–H_2O. The organic phase was evapd and the content of **1** was determined by GC with synthesized **1** [9] as Cotyledons (31.0 g) of 6- to 8-day-old seedlings (*R. communis* L. var. sanguineus) were frozen in liquid N_2, stored at −20 and homogenized in EtOH (400 ml) using an Ultra-Turrax. After filtration, the extract was concd under red. pres. (4.2 g). Prep. TLC of the crude extract (82 mg) with EtOAc–EtOH–H_2O (7 : 2 : 1) yielded **1** (3 mg, R_f 0.54). ^{13}C NMR of **1** ($CDCl_3$): 37.6 (NMe), 57.1 (OMe), 88.6 (C-3), 93.5 (C-5), 113.7 (CN), 143.5 (C-6), 161.3 (C-2), 172.4 (C-4).

Instrumentation

Capillary GC measurements were carried out under the following conditions: fused silica-glass column DB-1 (J. & W. Scientific, 30 m × 0.32 mm, 0.1 μm film thickness): inj. temp. 270°; column temp. 3 min 80°, heat rate 3° min^{-1} to 280°, 15 min 280°; FID temp. 290°; carrier gas He, flow rate 2 ml min^{-1}, split ratio 1 : 20. Retention indices were calculated in the isothermal part of the temp. programme according to Ref. [18] and in the linear part according to Ref. [19]. GC-MS measurements were performed on an instrument coupled to a GC fitted with a fused silica-glass column (DB-1, 30 m × 0.32 mm, 0.1 μm film thickness): inj. temp. 270°; carrier gas H_2, flow rate 2 ml min^{-1}, splitless injection.

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