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METABOLISM OF LIMONOIDS IN CALAMONDIN: CONVERSION OF CALAMIN TO CYCLOCALAMIN

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Key Word Index—*Citrus reticulata* var. *austera x Fortunella* sp, Rutaceae; calamondin, citrus hybrid; calamin, cyclocalamin, limonoids, metabolism

Abstract—[^{14}C] Calamin fed to the detached stem of calamondin (*Citrus reticulata* var. *austera* x *Fortunella* sp) seedlings was metabolized to cyclocalamin.

INTRODUCTION

Limonoids are a group of chemically related triterpene derivatives found in the Rutaceae and Meliaceae. There are 37 limonoids known to be present in *Citrus* and its hybrids Limonin (**3**) is a bitter member of the group and the major cause of limonoid bitterness in a variety of citrus juices. This bitterness has significant negative economic impact on the world-wide citrus industry.

Calamondin (*Citrus reticulata* var. *austera* x *Fortunella* sp), a citrus hybrid, possesses, in addition to citrus limonoids, calamin-type limonoids. This group includes calamin (**1**), cyclocalamin (**2**), retrocalamin, 6-keto-7β-deacetylnomilol, isocylocalamin and methyl isoobacunoate diosphenol [1–4] This group of limonoids differs from the citrus limonoids in that the carboxyl group at C-3 is methylated and the B-ring is oxygenated at C-6. The genes for their formation are most likely inherited from *Fortunella* We have shown that kumquat (*Fortunella* sp) possesses the calamin-type group as the major limonoids [2]

The biosynthetic pathways of calamin-type limonoids in calamondin have been proposed [2, 4] The objective of the present study was to demonstrate how calamin (**1**) is metabolized in calamondin.

RESULTS AND DISCUSSION

Citrus stems are the site of limonoid biosynthesis starting from acetate [5, 6] Detached stems of young citrus seedlings are excellent tools for studies of the biosynthesis and biodegradation of limonoids [6] In radioactive tracer work, the major advantage of using detached stems over attached stem is that radioactive metabolites can be very efficiently recovered. Therefore, in this study, we used the detached stems of young calamondin seedlings

Labelled calamin (**1**) was fed to a 3 cm long detached stem and incubated at 22° for three days The TLC radiochromatogram of the extract is shown in Fig. 1 Peak A was identified as the substrate About 50% of the substrate was metabolized under the conditions. Peak B, which consisted of about 50% of the total metabolites, had an R_f identical to that of cyclocalamin (**2**) when the TLC plate was developed with solvent system *a* (see Experimental). Peak B was scraped off from the plate and the labelled compound was extracted with ethyl acetate to give a radiochromatographically pure compound. The purified compound was further analysed by TLC with solvent systems *b*, *c* and *d* (Table 1). The R_f values of the metabolite were identical to those of cyclocalamin (**2**) with four solvent systems. Therefore, the metabolic product was identified as **2**.

We demonstrated in this study that **1** was converted to **2** in the detached stem of calamondin seedlings, and confirmed the previous suggestion that **1** is a precursor of **2** [2]

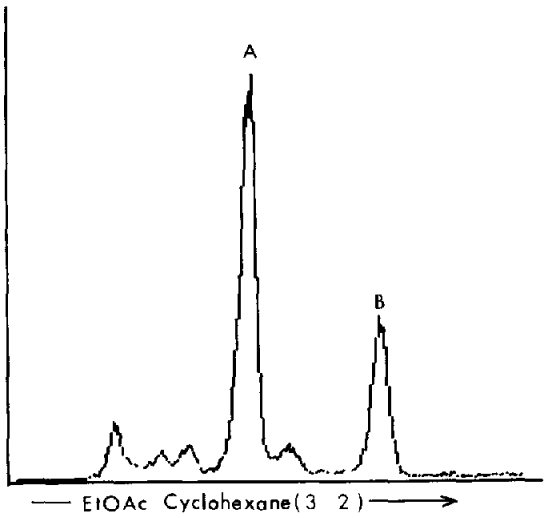
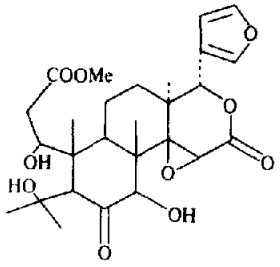
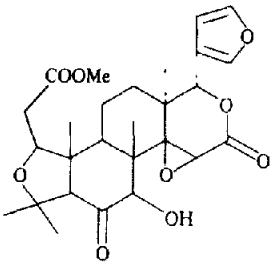


Fig 1 Radiochromatogram of the extract obtained from the detached stem of a calamondin seedling fed with [^{14}C] calamin Peaks A and B were identified as calamin and cyclocalamin, respectively.



1



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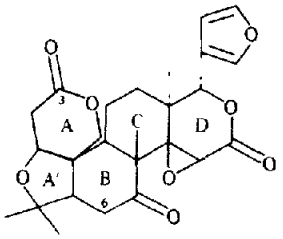

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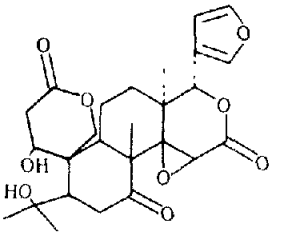

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The conversion of calamin to cyclocalamin is apparently catalysed by calamin A′-ring hydrolase This work shows for the first time the presence of hydrolase activity in citrus or its hybrids which is directly involved in the A′-ring of limonoids. This suggests that citrus also possesses this type of hydrolase activity. Therefore, ichangin (**4**) could be a precursor of limonin (**3**)

EXPERIMENTAL

Materials Calamondin seedlings (about 15 cm in height with 10 leaves) were grown in the greenhouse [1-^{14}C] Sodium acetate (56 mCi/mmol) was purchased from the DuPont New Products, Billerica, Massachusetts

[^{14}C] Calamin (**1**) was prepared biologically with calamondin seedlings and labelled acetate [1-^{14}C] Acetate (25 μCi) was fed to the stem of a calamondin seedling by the procedure described previously [6] After 3 days incubation, radioactive materials were extracted from the stem by the procedure of ref [6] Labelled **1** was isolated from the extracts on a silica gel column (0 7 × 10 cm) The column was eluted, stepwise, by increasing concentrations of EtOAc in hexane The fractions containing labelled calamin were further fractionated with TLC using the solvent *b* to give 130000–150000 cpm of radioactively pure **1**

Feeding experiments An aq soln of [^{14}C] **1** (120000 cpm) was fed to a detached 3 cm long stem which was placed in a small V-shaped vial After 3 days of incubation at 22°, radioactive materials were extracted by the procedure designed to extract mainly lactones and acids [6] The extract was spotted on silica gel TLC plates The plates were developed with the solvent systems (a) EtOAc–cyclohexane (3 2), (b) CH_2Cl_2–MeOH (97 3), (c) EtOAc–CH_2Cl_2 (2 3) and (d) toluene–EtOH–H_2O–HOAc (200 47.15 1, upper layer) Radiochromatograms were scanned with a Berthold Automatic TLC-linear Analyzer LB 2832 Total radioactivity was counted with a Beckman Liquid Scintillation system LS-3133P

Table 1 Identification of the metabolite by TLC

Compound	R_f*			
	Solvent system			
	a	*b*	*c*	*d*
Peak B	0 75	0 53	0 93	0 41
Cyclocalamin	0 75	0 53	0 93	0 41

*Solvent key see Experimental

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