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A LIMONOID FROM YOUNG CALAMONDIN SEEDLINGS

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Key Word Index—*Citrus* hybrid; calamondin (*Citrus reticulata* cv. 'Austera' × *Fortunella* sp.); seedlings; 6-keto-7β-deacetylnomilol; limonoids.

Abstract—Radioactive tracer work resulted in the finding of a new limonoid in young seedlings of calamondin (*Citrus reticulata* cv. 'Austera' × *Fortunella* sp.). The compound was identified by NMR and TLC analyses as 6-keto-7β-deacetylnomilol. The results show that this compound was biosynthesized and accumulated during early stages of growth.

INTRODUCTION

Bitterness due to limonoids in a variety of citrus juices is a major problem of the worldwide citrus industry and has significant negative economic impact. In the past few years we have made substantial progress in the area of limonoid biochemistry in citrus [1–4]. At present there are 29 limonoids shown to be present in *Citrus* and *Citrus* hybrids, and their possible biosynthetic pathways have been proposed [4]. Recently, we found a new limonoid in young seedlings of calamondin (*Citrus reticulata* cv. 'Austera' × *Fortunella* sp.). We report here the isolation and identification of this compound.

RESULTS AND DISCUSSION

When [^{14}C]acetate was fed to the stem of a young calamondin seedling, three major peaks were observed by TLC analysis. Two of them were identified by the procedures previously described [1, 5, 6] as nomilin and retrocalamin. The most polar compound did not correspond to any of the limonoids known to be present in *Citrus*. Therefore, this compound was isolated from calamondin seedlings and identified.

Limonoids were extracted from 15 seedlings by the procedure, which was designed to extract mainly lactones [1]. The total extract (480 mg) was separated on a silica gel column. Combined fractions containing the compound of interest (52 mg) were still contaminated with nomilin. Therefore, the compound was further purified by prep. TLC using solvent system b (see Experimental).

TLC analysis showed that the isolate had R_f values identical to those of synthetic 6-keto-7β-deacetylnomilol (**1**) with three solvent systems. The ^{1}H NMR and ^{13}C NMR spectra were also identical to those of 6-keto-7β-deacetylnomilol. Therefore, the isolate was identified as 6-keto-7β-deacetylnomilol (**1**). This compound had previously been synthesized by treatment of calamin (methyl 6-keto-7β-deacetylnomilolate) with HCl in dichloromethane [5], but it was not recognized as a natural product.

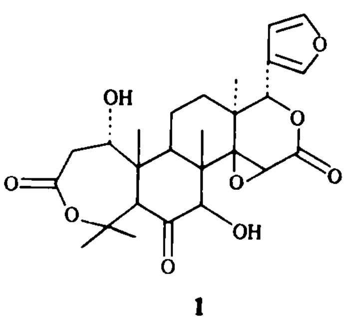
This compound appears to be biosynthesized and accumulated during the early stages of calamondin growth. In young seedlings of lemon, grapefruit, Valencia orange and tangerine, nomilin is the major, if not only, limonoid biosynthesized [1].

Analyses of calamondin seeds showed only a trace amount of 6-keto-7β-deacetylnomilol. The low levels precluded the previous identification of this compound [5, 6]. The major limonoids in the seeds consist of two groups. One is the calamin group consisting of calamin, cyclocalamin, retrocalamin and methyl isoobacunoate diosphenol; in all of these compounds the 3-carboxyl group is in the form of a methyl ester and the B-ring contains 6-keto and 7β-hydroxyl substituents. The other is the common citrus limonoid group such as limonin, nomilin, obacunone and deacetylnomilin [5, 6], in which the 3-carboxyl group is part of a lactone ring and the B-ring contains a 7-keto substituent. The new limonoid contains structural features of both groups and thus may represent a biosynthetic link between them. In fact, preliminary radioactive tracer work suggests that it is the precursor of the calamin group of limonoids.

EXPERIMENTAL

Materials. Calamondin seedlings (about 15 cm height with 10 leaves) were grown in our greenhouse. [^{14}C]Sodium acetate (56 μCi/μmol) was purchased from new England Nuclear, Boston, MA.

Feeding experiment. [^{14}C] Acetate (20 μCi) was fed to a seedling



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through the stem by the procedure described previously [1]. After 2 days of incubation in the greenhouse, the young shoot was harvested and analysed.

Extraction and isolation of a new limonoid. Limonoids were extracted from 15 seedlings (82 g) by procedures previously described [1]. The total extract was placed on the top of a silica gel column (1.6 × 18 cm). The column was eluted, stepwise, by increasing concns of EtOAc in hexane; 500 ml each of 35, 40, 45, 50 and 55% EtOAc. Fractions containing the compound of interest were still contaminated with nomilin. Therefore, the combined fractions were further purified by a preparative TLC plate, which was developed with solvent system b (see below).

Analysis of limonoids. The isolate obtained above was analysed by TLC, and ^1H NMR and ^{13}C NMR spectra. NMR spectra were obtained with a JEOLFT spectrometer, JNM-GX 270 WB. Labelled metabolites obtained from [^{14}C]acetate feeding were analysed with a Berthold Automatic TLC-linear Analyzer LB 2832. TLC plates were developed with solvent systems: (a) EtOAc–cyclohexane (3:2); (b) CH_2Cl_2–MeOH (97:3) and (c) EtOAc–CH_2Cl_2 (2:3). Limonoids were revealed by spraying plates with Ehrlich's reagent followed by HCl gas exposure [7].

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A BIANTHRAQUINONE AND A TRITERPENOID FROM THE SEEDS OF *CASSIA HIRSUTA*

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Key Word Index—*Cassia hirsuta*; Leguminosae; seeds; 4,4′-bis(1,3,8-trihydroxy-2-methyl-6-methoxy anthraquinone); pentacyclic triterpernoid sapogenol; isohopane series; 3β,16β,22-trihydroxyisohopane.

Abstract—From the seeds of *Cassia hirsuta* a new bianthraquinone, 4,4′-bis(1,3,8-trihydroxy-2-methyl-6-methoxy anthraquinone) and a triterpenoid 3β,16β,22-trihydroxyisohopane have been isolated.

INTRODUCTION

Species of *Cassia* are rich sources of flavonoids [1], anthraquinones [2] and polysaccharides [3] and the plant possesses important medicinal properties [4].

RESULTS AND DISCUSSION

A yellow crystalline pigment (**1**), $C_{32}H_{22}O_{12}$, ($[M]^+$ at m/z 598), mp > 300° (dec.) and a triterpenoid (**2**), $C_{30}H_{52}O_3$, ($[M-H_2O]^+$ at m/z 442), mp 211° were isolated from the ethyl acetate extract of the defatted seeds of *Cassia hirsuta*.

Compound **1** contained two methyl groups, two methoxyl groups and gave a pink colour with methanolic magnesium acetate [5] for the presence of a chelated (α-hydroxy) hydroxyl group. The solubility of the compound in 5% aq. sodium bicarbonate and a peak at 3440 cm⁻¹ in its IR spectrum showed the presence of at least one β-hydroxyl group. A medium band at 2910 and 2890 cm⁻¹ indicated the presence of a methoxyl and a methyl group, respectively. Sharp and strong bands at 1630 and 1668 cm⁻¹ suggested the presence of chelated and non-chelated carbonyl groups. The λ_{max}^{EtOH} at 260, 390 and 440 nm indicated that **1** was a polyhydroxy anthraquinone [6, 7].

On acetylation, a hexaacetyl derivative, $C_{44}H_{34}O_{18}$ (**1a**), mp 213°, was obtained. The IR spectrum of **1a** showed a phenolic acetate band at 1770 cm⁻¹ and a non-chelated carbonyl at 1668 cm⁻¹. Thus, out of twelve oxygen atoms in compound **1**, four occur as quinone carbonyl groups, which are both chelated (1630 cm⁻¹) and non-chelated (1668 cm⁻¹) as revealed from a com-