

## AGE-DEPENDENT VARIATIONS OF THE EFFICIENCY OF IRIDOID BIOSYNTHESIS IN *VERBENA OFFICINALIS*

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**Key Word Index**—*Verbena officinalis*; Verbenaceae; biosynthesis;  $^2\text{H}$  NMR; experimental parameters; deoxyloganin; dihydrocornin; cornin.

**Abstract**— $^2\text{H}$  Labeled deoxyloganin gave ca a 30% incorporation into each of dihydrocornin and cornin in *Verbena officinalis* plants before their flowering. The incorporation, as measured by mass spectrometry and  $^2\text{H}$  NMR spectroscopy, decreased during the flowering period, stopping when the plants had ceased flowering.

### INTRODUCTION

In an earlier paper [1], we reported the effects of metabolic period, dose and application method on the incorporation of deoxyloganin (**1**) into dihydrocornin (**2**) and cornin (**3**) in flowering plants of *Verbena officinalis* L. We found that the incorporation of **1** into **3** was efficient in the dose range 0.5–5  $\mu\text{mol/g}$  plant. Furthermore, a metabolic period of 7 days seemed optimal, whereas the method of application (wick- or cut stem-feeding) had little effect on the degree of incorporation. As a supplement to these results, we were interested in the effect of the plants' age on the incorporation of **1**. Previously, Horodysky *et al.* showed [2] that the incorporation of [ $2\text{-}^{14}\text{C}$ ]mevalonate into **3** in *V. officinalis* increased until the plants were 4 months old. Past flowering, the incorporation was very low. We were, however, interested in knowing whether the same conclusion was valid for an advanced precursor such as **1**. Hence, we report the results

of a series of feeding experiments with  $^2\text{H}$ -labelled **1** to *V. officinalis* at different stages of development.

### RESULTS AND DISCUSSION

[Methoxyl- $^2\text{H}$ ] deoxyloganin (**1**) was prepared by treatment of deoxyloganic acid with  $\text{C}^2\text{H}_5\text{N}_2$  [1]. We had shown earlier [3] that the methoxycarbonyl group of **1** is preserved during the conversion of **1** into cornin (**3**). The precursor was fed as an aqueous solution to *V. officinalis* plants by letting the cut stems suck up the solution. After the experiment, the glucosides were isolated and the iridoids were separated by reversed-phase chromatography. The  $^2\text{H}$  content in dihydrocornin (**2**) was measured by mass spectrometry whereas cornin was acetylated to give cornin tetra-acetate (**3**) which was crystallized from ethanol. The  $^2\text{H}$  content of **3** was estimated by  $^2\text{H}$  NMR spectroscopy [3].

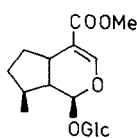
Table 1. Incorporation of **1** into **2** and **3** in *V. officinalis*\*

Stage of development	Young shoots (5–10 cm)	Just before flowering (15–20 cm)	In the middle of flowering period†	Late in the flowering period
Amount of plant (g)	18	14	16	18
Amount of [Methoxyl- $^2\text{H}$ ]- <b>1</b> fed (mg)	22.0	21.9	24.0	11.6
Mol/g plant	3.3	4.2	4.8	1.7
<b>Isolated 2</b>				
Amount (mg)‡	11.5	9.4	7.5	2.3
Enrichment (%)	62	67	76	78
Incorporation (%)	33	29	23	15
<b>Isolated 3</b>				
Amount (mg)	240	143	149	101
Enrichment (%)	2.8	4.0	2.4	0.5
Incorporation (%)	31	27	14	4.5

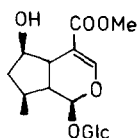
\*The precursor was administered as an aqueous solution to shoots cut from second year plants.

†These data have been reported earlier [1].

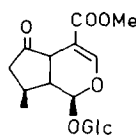
‡Measured by HPLC [1].



1



2



3

The results presented in Table 1 show that the incorporation of **1** into **2** and **3** in *V. officinalis* was extremely efficient in young shoots. During the flowering period, however, the incorporation decreased significantly. Previously, we had shown [3] that the incorporation of **1** into **3** in *V.*

*officinalis* plants, past the flowering stage, was too small to be measured by  $^2\text{H}$  NMR ( $< 0.5\%$ ).

These results suggest that immature plants should be used in biosynthetic experiments with advanced precursors, unless other information is at hand.

#### EXPERIMENTAL

The experimental techniques, as well as the synthesis and physical properties of the precursor, have been described previously [1].

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#### REFERENCES

1. Damtoft, S., Jars, M. U., Kirk, O., Jensen, S. R. and Nielsen, B. J., (1983) *Phytochemistry* **22**, 695.
2. Horodysky, A. G., Waller, G. R. and Eisenbraun, E. J. (1969) *J. Biol. Chem.* **244**, 3110.
3. Damtoft, S. (1980) Thesis, The Technical University of Denmark, Lyngby.

## GUAIANOLIDES FROM *CENTAUREA KOTSCHYI*

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**Key Word Index**—*Centaurea kotschyi*; Compositae; sesquiterpene lactones; guaianolides.

**Abstract**—The aerial parts of *Centaurea kotschyi* (var. *kotschyi*) afforded, besides three known sesquiterpene lactones, a new derivative of linichlorin B. The structure was determined by spectral analysis.

#### INTRODUCTION

In continuation of our phytochemical investigation of the genus *Centaurea* [1], we report here the isolation and structure elucidation of a new guaianolide.

*Centaurea kotschyi* Boiss., endemic to Turkey, had not been investigated chemically. The aerial parts of the plant gave, in addition to the three known guaianolides desacylcynaropicrin (**1**) [2], cynaropicrin (**2**) [3] and linichlorin B (**3**) [4], a new lactone (**4**) which is closely related to linichlorin B.

#### RESULTS AND DISCUSSION

Compound **4**,  $\text{C}_{19}\text{H}_{24}\text{O}_7$ ,  $[\text{M}]^+$  364, exhibited the typical IR absorption bands of an  $\alpha,\beta$ -unsaturated- $\gamma$ -lactone group ( $1750\text{ cm}^{-1}$ ), hydroxyl group(s) ( $3400\text{ cm}^{-1}$ )

and an acyl group ( $1730$  and  $1270\text{ cm}^{-1}$ ). The  $^1\text{H}$  NMR spectrum (Table 1) showed that compound **4** was very similar to linichlorin B, and that they both had the same ring structure, therefore the only difference was in the side chain. A pair of doublets at  $\delta$  3.87 and 3.64 ( $J = 11\text{ Hz}$ ) indicated the presence of a  $-\text{CH}_2-\text{O}-$  group [5, 6] and a methyl singlet  $\delta$  1.40, in addition to the signals that were assigned for the ring protons, suggested that the acyl group was an  $\alpha,\beta$ -dihydroxyisobutyryl moiety. In the mass spectrum peaks at  $m/z$  244  $[\text{M} - \text{C}_4\text{H}_8\text{O}_4]^+$  and 119 corroborated the presence of this group.

#### EXPERIMENTAL

**Extraction and isolation.** The dried, aerial parts of *C. kotschyi* (var. *kotschyi*) (1.5 kg), collected near Ankara in July 1980 (voucher No. 45503 deposited at the herbarium of the Faculty of