

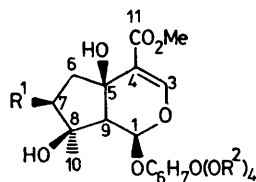
Biosynthesis of Lamiide and Ipolamiide from 8-*epi*-Deoxyloganin Studied by ^2H N.M.R. Spectroscopy

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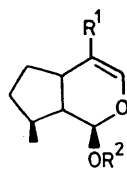
Summary ^2H N.m.r. spectroscopy shows that selectively labelled 8-*epi*-deoxyloganin is efficiently incorporated into lamiide and ipolamiide in *Hebenstreitia dentata*, whereas selectively labelled deoxyloganin provides no observable incorporation.

In biosynthetic studies of the iridoid glucoside lamiide (**1a**) in *Lamium amplexicaule*, mevalonic acid, iridodial (**2a**), iridodial glucoside (**2b**), 11-hydroxyiridodial glucoside (**2c**), and deoxyloganin acid (**2d**) were incorporated with efficiencies ranging from 0.003% [(**2c**)] to 0.025% [(**2d**)]; mevalonic acid was incorporated into ipolamiide (**1b**) to the extent of 0.024% in the same plant. On this basis it was concluded that (**1a**) and (**1b**) were formed *via* (**2a**) and (**2d**).¹



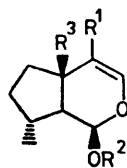
(1)

- a; $\text{R}^1 = \text{OH}$,
 $\text{R}^2 = \text{H}$
b; $\text{R}^1 = \text{R}^2 = \text{H}$
c; $\text{R}^1 = \text{OAc}$,
 $\text{R}^2 = \text{Ac}$
d; $\text{R}^1 = \text{H}$,
 $\text{R}^2 = \text{Ac}$



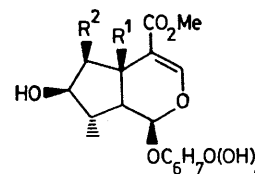
(2)

- a; $\text{R}^1 = \text{Me}$,
 $\text{R}^2 = \text{H}$
b; $\text{R}^1 = \text{Me}$,
 $\text{R}^2 = \text{C}_6\text{H}_7\text{O}(\text{OH})_4$
c; $\text{R}^1 = \text{CH}_2\text{OH}$,
 $\text{R}^2 = \text{C}_6\text{H}_7\text{O}(\text{OH})_4$
d; $\text{R}^1 = \text{CO}_2\text{H}$,
 $\text{R}^2 = \text{C}_6\text{H}_7\text{O}(\text{OH})_4$
e; $\text{R}^1 = \text{CO}_2\text{Me}$,
 $\text{R}^2 = \text{C}_6\text{H}_7\text{O}(\text{OH})_4$



(3)

- a; $\text{R}^1 = \text{Me}$,
 $\text{R}^2 = \text{R}^3 = \text{H}$
b; $\text{R}^1 = \text{CHO}$,
 $\text{R}^2 = \text{R}^3 = \text{H}$
c; $\text{R}^1 = \text{CHO}$,
 $\text{R}^2 = \text{C}_6\text{H}_7\text{O}(\text{OH})_4$, $\text{R}^3 = \text{H}$
d; $\text{R}^1 = \text{CHO}$,
 $\text{R}^2 = \text{C}_6\text{H}_7\text{O}(\text{OH})_4$, $\text{R}^3 = \text{OH}$
e; $\text{R}^1 = \text{CO}_2\text{Me}$,
 $\text{R}^2 = \text{C}_6\text{H}_7\text{O}(\text{OH})_4$, $\text{R}^3 = \text{H}$

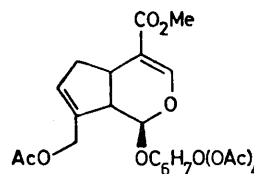


(4)

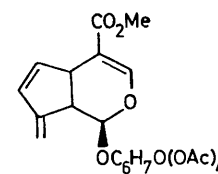
- a; $\text{R}^1 = \text{R}^2 = \text{OH}$
b; $\text{R}^1 = \text{R}^2 = \text{H}$

To test the validity of this possibility, labelled analogues of (**2e**) and (**3e**) were fed in parallel experiments to *Hebenstreitia dentata* L. (Scrophulariaceae-Selaginaceae) normally producing (**1b**)⁷ and (**1a**).

Labelled deoxyloganin (**5a**) was prepared as described.⁸ The product contained a mean of 4.1 deuterium atoms per molecule with 64% of the label ($2.6 \text{ } ^2\text{H}$) in the methoxy-carbonyl group. The labels in the other positions (mainly

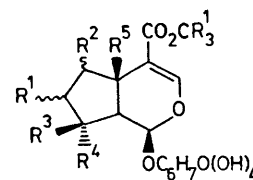


(6)



(7)

Other pathways should be considered, however, for the following reason: the aldehyde boschnalioside (**3c**) was for many years the only simple compound of this type with the 8-methyl group in the α -configuration.² Recently, however, additional compounds (**3d**),³ (**4a**),⁴ and (**4b**)⁵ of this series were isolated from natural sources suggesting their biogenetic derivation from 8-*epi*-iridodial (**3a**).[†] Furthermore, the formation of the compounds (**1a**) and (**1b**), so far rationalized as being *via* iridodial (**2a**) through a net inversion at C-8, could as well be rationalized by assuming 8-*epi*-iridodial (**3a**) as a precursor, taking the small incorporations from the previous experiment into account.



(5)

- a; $\text{R}^1 = \text{R}^4 = ^2\text{H}$, $\text{R}^2 = \text{H}$, $\text{R}^3 = \text{CH}_2^2\text{H}$, $\text{R}^5 = \text{H}$
b; $\text{R}^1 = \text{R}^2 = \text{R}^3 = ^2\text{H}$, $\text{R}^4 = \text{CH}_2^2\text{H}$, $\text{R}^5 = \text{H}$
c; $\text{R}^1 = \text{R}^4 = ^2\text{H}$, $\text{R}^2 = \text{H}$, $\text{R}^3 = \text{CH}_2^2\text{H}$, $\text{R}^5 = \text{OH}$

TABLE 1. Tracer experiments in *Hebenstreitia dentata*.

Precursor	Amount (in g) of plant material	Amount (in mg) of glucosides		Amount (in mg) of acetates	
		(1a)	(1b)	(1c)	(1d)
(5a) (20.2 mg)	36	48	96	67	131
(5b) (18.8 mg)	29	41	77	53	117

[†] The biosynthesis might equally well proceed through (**3b**), in analogy with the secoiridoid biosynthesis proposed by Arigoni *et al.*⁹

at C-10) served as internal standards to ascertain that the iridoid skeleton was preserved during the biosynthetic transformations. The stereochemical analogue (**5b**) was prepared from geniposide penta-acetate (**6**) by palladium-catalysed elimination of acetic acid [$\text{Pd}(\text{OAc})_2$, PPh_3 in toluene]⁹ to give (**7**). Catalytic deuteration ($\text{Rh}-\text{C}$, $^2\text{H}_2$ in ethanol), followed by saponification and treatment with $\text{C}^2\text{H}_5\text{N}_2$, gave (**5b**).¹⁰ The product contained a mean of 2.5 deuterium atoms per molecule. In this case the methoxy-carbonyl group contained 85% (2.1 ^2H) of the label present. ‡

The labelled compounds were administered in aqueous solutions by the cotton wick method to *Hebenstreitia dentata* plants during the flowering period in August. After 11 days

mined by comparing the relative intensities of the signals at 7.27 (CHCl_3 internal standard) and 3.7 p.p.m. (CO_2CH_3) after correcting the latter for natural-abundance deuterium. The results are presented in Table 2.

These results demonstrate that 8-*epi*-deoxyloganin (**3e**), rather than deoxyloganin (**2e**), is a precursor for ipolamiide (**1b**) and for lamiide (**1a**) in *Hebenstreitia dentata*. This is the first time that a compound of the 8-*epi*-iridodial series (**3a—e**) has been shown to be a precursor for an 8 β -hydroxy-substituted iridoid. Iridoid glucosides with this feature are very common in the orders *Scrophulariales-Lamiales*.

The experiments illustrate further the utility of ^2H n.m.r. spectroscopy for biosynthetic investigations in higher plants.

TABLE 2. Enrichments and incorporations into lamiide and ipolamiide in *Hebenstreitia dentata*.

Precursor	Lamiide		Ipolamiide	
	Enrichment	Incorporation	Enrichment	Incorporation
Deoxyloganin (5a)	—	$\leq 0.3\%$	—	$\leq 0.1\%$
8- <i>epi</i> -Deoxyloganin (5b)	0.4%	1.0%	2.6%	16%

the plants were harvested. Work-up of the plant material gave a water-soluble fraction from which (**1a**) and (**1b**) were isolated by reversed-phase h.p.l.c. The compounds were acetylated and subjected to preparative t.l.c. The results are given in Table 1.

From the feeding experiment with (**5a**) a small amount (<3 mg) of a highly deuteriated compound was isolated. It was tentatively identified (^1H , ^{13}C , and ^2H n.m.r., mass spectrometry) as (**5c**). Its mass spectrum showed that no dilution with unlabelled material had occurred.

The ^2H n.m.r. spectra of the purified acetates (**1c**) and (**1d**) in CHCl_3 were recorded. The enrichments were deter-

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‡ 270 MHz ^1H n.m.r. spectroscopy showed that (**5a**) contained ca. 1.5% of its 8-epimer, while the 8-epimer content of (**5b**) was 4.2%. In the labelled deoxyloganin previously used,⁸ the 8-epimer content was 5—10% as judged by ^{13}C n.m.r. spectroscopy.

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⁹ J. Tsuji, T. Yamakawa, M. Kaito, and T. Mandai, *Tetrahedron Lett.*, 1978, 2075.

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