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BIOSYNTHESIS OF THE IRIDOIDS AUCUBIN AND ANTIRRINOSIDE FROM 8-*EPI*-DEOXYLOGANIC ACID

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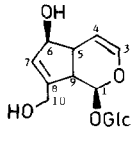
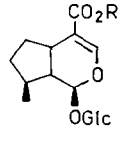
Key Word Index—*Scrophularia racemosa*; *Antirrhinum majus*; Scrophulariaceae; *Plantago major*; Plantaginaceae; biosynthesis; ^{2}H NMR; 8-*epi*-deoxyloganic acid; deoxyloganic acid; aucubin; antirrinoside.

Abstract—^{2}H NMR spectroscopy shows that 8-*epi*-deoxy[6,7,8,10-$^2H_4$]loganic acid is efficiently incorporated into aucubin in *Plantago major* and *Scrophularia racemosa*, and into antirrinoside in *Antirrhinum majus*. Deoxyloganic acid produced no observable incorporation in these species.

In studies on the biosynthesis of aucubin (**1**), one of the most widespread iridoid glucosides, by *Aucuba japonica* (Cornaceae), ^{3}H-labelled deoxyloganic acid (**2a**) and scandoside (**3**) were reported to give incorporations of 0.5 and 0.04%, respectively [1, 2]. The biosynthesis of antirrinoside (**4**) has not been investigated previously. The incorporation of deoxyloganic acid (**2a**) into aucubin (**1**) was in accordance with the general acceptance of **2a** as a key intermediate in iridoid biosynthesis. Recently, however, it has been demonstrated that 8-*epi*-deoxyloganin (**5b**) rather than deoxyloganin (**2b**) is incorporated into lamiide (**6a**) and ipolamiide (**6b**) in *Hebenstreitia dentata* (Scrophulariaceae) [3]. When ^{2}H-labelled analogues of **2b** and **5b** were fed to *Melampyrum cristatum* (Scrophulariaceae), containing aucubin (**1**) and 8-*epi*-loganin (**7**) as well as other iridoid glucosides, neither **2b** nor **5b** were incorporated into **1**, whereas **5b** gave a significant incorporation (5%) into **7** [4]. It was assumed that the biosynthesis of **1** from either **2b** or **5b** was blocked by the methyl ester group, so it was decided to synthesize ^{2}H-labelled analogues of the free acids **2a** and **5a** as potential precursors for aucubin (**1**).



1



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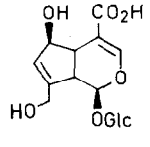
2b R = Me



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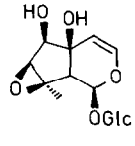

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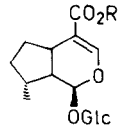
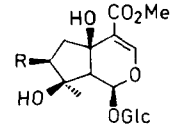
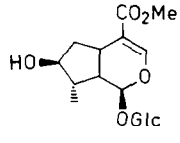


5a R = H
5b R = Me



6a R = OH
6b R = H



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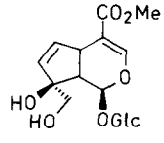

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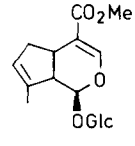

9

Deoxy[6,7,8,10-$^2H_4$]loganic acid (**2a**), was prepared by acid catalysed allylic rearrangement of gardenoside (**8**), followed by acetylation and catalytic deuteration over palladium–charcoal, and saponification. Similarly, labelled 8-*epi*-deoxyloganic acid (**5a**) resulted from treatment of gardenoside hexa-acetate with palladium–charcoal in dioxane, containing two equivalents of $^2HCO_2{}^2H$, to yield **9** labelled in positions 6 and 10, followed by catalytic deuteration over rhodium–charcoal and saponification. The total amount of deuterium in (**5a**) was 4.1 ^{2}H (determined by mass spectrometry) of which 1.8 ^{2}H were located in the 10 position (as measured from a 270 MHz NMR spectrum).*

*270 MHz ^{1}H NMR spectroscopy showed that the **2a** employed contained 13% of its 8-epimer, while the 8-epimer content of **5a** was less than 5%.

Table 1. Incorporation of [6,7,8,10-$^2H_4$]**2a** and **5a** into iridoids

Plant	Amount of plant material (g)	Precursor		Iridoid isolated		Incorporation* (%)	Enrichment (%)
		Compound	Amount (mg)	Compound	Amount (mg)		
P. major	32.5	**2a**	23.5	**1**	72	< 0.5	—
	40.5	**5a**	25.5	**1**	89	10	6.8
S. racemosa	41.0	**2a**	25.1	**1**	112	< 0.5	—
	41.0	**5a**	27.1	**1**	70	7	1.8
A. major	26.0	**2a**	23.8	**4**	117	< 0.5	
	24.5	**5a**	25.0	**4**	241	13	1.9

*The figures for degree of incorporation have been corrected to allow for the fact that **2a** contained 13% of **5a**.

The labelled compounds were administered to the plants as aqueous solutions during their period of growth in May. Work-up of the plant material gave a water-soluble fraction from which the iridoid glucosides were isolated by reversed phase chromatography.

The compounds were acetylated, purified by prep. TLC, recrystallized and the ^2H NMR spectra recorded. Aucubin hexa-acetate, obtained from *Plantago major* and *Scrophularia racemosa* which had been fed with labelled 8-*epi*-deoxyloganic acid (**5a**), gave two distinct signals: at δ 7.27 (C^2HCl_3; internal standard) and 4.7 (^2H-10), whereas the 7-position showed no enrichment. The incorporation could be estimated from the relative intensities of the signals at δ 7.27 and 4.7, after correction of the latter for natural abundance ^2H. The hexa-acetate of antirrinoside (**4**), obtained from *Antirrhinum majus*, to which labelled 8-*epi*-deoxyloganin acid (**5a**) was fed also displayed two distinct signals in the ^2H NMR spectrum, namely at δ 7.27 (C^2HCl_3; internal standard) and 1.4 (^2H-10). The results are presented in Table 1.

In this laboratory, neither **2a** nor **5a** produced observable incorporation into aucubin (**1**) in *A. japonica*. The non-incorporation of **2a**, which is at variance with previous results [1, 2], seems attributable to the toxic effect of the precursor on the plant.

These results show that 8-*epi*-deoxyloganic acid (**5a**) rather than deoxyloganic acid (**2a**) is a precursor for aucubin (**1**) and antirrinoside (**4**) in members of the order Scrophulariales (see Dahlgren [5]). At present studies on the biosynthesis of aucubin (**1**) in the other orders of the angiosperms are being carried out.

EXPERIMENTAL

^2H NMR spectra were recorded at 41.43 MHz on a Bruker HX-270 instrument in $CHCl_3$, without proton noise-decoupling.

A forthcoming paper will report the synthesis of the ^2H-labelled precursors. Their physical data were as follows.

Deoxy [6,7,8,10-$^2H_4$]loganic acid mp 152–159°, $[\alpha]_D$-93° (MeOH; *c* 1.22). ^1H and ^{13}C NMR data were in accordance with those of ^2H-labelled deoxyloganin previously used [6, 7]. MS showed a distribution (%) of label of $^2H_1$, 3; $^2H_2$, 8; $^2H_3$, 16; $^2H_4$, 21; $^2H_5$, 24; $^2H_6$, 19; $^2H_7$, 8; and $^2H_8$, 1 (calculated from *m/z* 198–206) with a mean of 4.5 ^2H atoms/mol. ^2H NMR showed that 1.8 ^2H were located at the 10-position.

epi-Deoxy[6,7,8,10-$^2H_4$]loganic acid mp 213–214°, $[\alpha]_D$-122° (MeOH; *c* 0.29). ^1H and ^{13}C NMR data were in accordance with those of *epi*-deoxyloganin [7]. MS showed a distribution (%) of label of $^2H_1$, 4; $^2H_2$, 11; $^2H_3$, 19; $^2H_4$, 23; $^2H_5$, 23; $^2H_6$, 16; and $^2H_7$, 4 (calculated from *m/z* 198–206) with a mean of 4.1 ^2H atoms/mol. ^1H NMR showed that 1.8 ^2H were located in the 10-position.

General procedure for the administration of labelled precursors and for the isolation of iridoids. The precursor was administered as an aq. soln during the period of growth in May. In the expts with *Scrophularia* and *Antirrhinum*, the wick method was used, and in the expts with *Plantago*, the cut leaves were dipped into the soln of the precursor. After harvesting, the plants were stored in polyethylene bags at −23° until work-up. The frozen plant was extracted twice with EtOH, evaporated, dissolved in H_2O and extracted with Et_2O to remove fats, etc. The aq. soln was filtered through a column of neutral Al_2O_3 followed by washing with H_2O. The eluate was concd and treated with activated C. The isolation of the iridoids was accomplished by reversed phase chromatography (RP-8) with H_2O–MeOH (4:1 and 3:1) as the eluent.

The iridoids were acetylated and recrystallized for ^2H NMR.

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