Application of ^2H N.M.R. Spectroscopy to a Study of the Biosynthesis of the Iridoid Glucoside Cornin in *Verbena officinalis*

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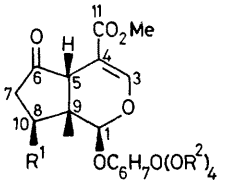
Summary ^2H N.m.r. spectroscopy has been used to demonstrate that in specifically labelled deoxyloganin, the label at C-8 is retained during its conversion into cornin in *Verbena officinalis*, suggesting that the 6-oxo group is produced by oxidation in an unactivated position.

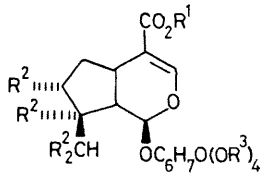
THE biosynthesis of the iridoid glucoside cornin† (**1a**) has been investigated previously,[1] and it has been shown that deoxyloganic acid (**2a**) is efficiently incorporated (11%) into (**1a**) in *Verbena officinalis*, perhaps, as proposed by Inouye *et al.*,[1] by direct oxidation at C-6. However, the finding of griselinoside (**1b**) in other *Verbena* species[2] suggests that a more general pathway may exist. Thus, deoxygeniposide (**3a**), with the potentially oxidizable allylic C-6 and C-10, constitutes a conceivable precursor for both (**1a**) and (**1b**); oxidation at C-6 and C-10 followed by double-bond reduction could give (**1b**),[3] whereas selective C-6 oxidation would lead to (**1a**).[4] We have tested this hypothesis by feeding deoxyloganin (**2b**), labelled with deuterium at C-8, to *Verbena officinalis* plants to produce (**1a**). If (**3a**) were an intermediate the C-8 label would be lost.

An appropriate precursor, (**2c**), was prepared from geniposide tetra-acetate (**3b**) by catalytic deuteriation (Pd/C, $^2H_2$) followed by deacetylation and exchange of the methyl ester group ($C^2H_3O^2H$–C^2H_3ONa). Significant



(1)

(**a**) $R^1 = Me$; $R^2 = H$
(**b**) $R^1 = CO_2Me$; $R^2 = H$
(**c**) $R^1 = Me$; $R^2 = MeCO$

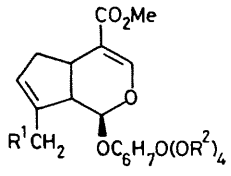


(2)

(**a**) $R^1 = R^2 = R^3 = H$
(**b**) $R^1 = Me$; $R = R^3 = H$
(**c**) $R^1 = C^2H_3$; $R^2 = {}^2H$; $R^3 = H$
(**d**) $R^1 = C^2H_3$; $R^2 = {}^2H$; $R^3 = MeCO$

scrambling occurred during the synthesis as revealed by the mass spectrum of (**2c**) [$^2H_0$: 2·0; $^2H_1$: 5·3; $^2H_2$: 10·0; $^2H_3$: 13·5; $^2H_4$: 16·4; $^2H_5$: 17·3; $^2H_6$: 13·4; $^2H_7$: 9·8; $^2H_8$: 7·0; $^2H_9$: 3·8; $^2H_{10}$: 1·4% (calculated from *m/e* 212—222) with a mean of 4·7 deuterium atoms per molecule]. The ^2H n.m.r. spectrum ($CHCl_3$, 41·4 MHz) of the tetra-acetate (**2d**) showed a distribution of label of *ca.* 19% (0·9 ^2H) in the methoxycarbonyl group, *ca.* 38% (1·8 ^2H) at C-10 and *ca.* 43% (2·0 ^2H) in positions 7 and 8 (probably including small fractions in positions 5, 6, and 9). The labels at C-10 and in the methoxycarbonyl group served as internal

† The name 'cornin' has priority over 'verbenalin', see S. R. Jensen, B. J. Nielsen, and A. Kjaer, *Acta Chem. Scand.*, 1973, **27**, 2581.



(3)

(**a**) $R^1 = R^2 = H$
(**b**) $R^1 = MeCO_2$; $R^2 = MeCO$

standards for measuring the degree of incorporation and for estimating whether the label at C-8 was retained or not.

The labelled compound (16·6 mg) was administered as an aqueous solution by the cotton wick method to 15 *Verbena officinalis* plants during the flowering period late in July. After a metabolic period of 3 days, work-up of the plant material (39 g) yielded (**1a**) (99 mg), which was converted into the tetra-acetate (**1c**) and purified (105 mg). During the work-up possible deuterium at C-5 and C-7 was removed by exchange in aqueous alkali. The ^2H n.m.r. spectrum of the isolated (**1c**) (2700 transients) showed signals at 7·27 ($CHCl_3$ internal standard), 3·74 (CO_2CH_3), 2·3 (H-8), 2·0 (OAc of natural abundance), and 1·2 p.p.m. (10-CH_3), in agreement with the ^1H n.m.r. spectrum. The relative intensities of the signals, after correction for natural abundance calculated from the intensity of the $CHCl_3$‡ peak, were: $CHCl_3$: CO_2CH_3 : H-8 : 10-CH_3 = 1·4 : 0·9 : 1·0 : 1·7. From the relative intensities of the $CHCl_3$ and CO_2CH_3 peaks the incorporation was estimated to be 5%, corresponding to 1% enrichment at C-8. A comparison of the integrals with those of (**2c**) recorded above showed that the label at C-8 had been retained in the biosynthetic conversion of (**2b**) into (**1a**), thereby supporting the originally proposed direct oxidation at C-6.[1]

To our knowledge, only two papers reporting the use of deuterium n.m.r. spectroscopy in biosynthesis studies in higher plants have been published.[5] In these, enrichments of 56[5a] and 0·24%,[5b] respectively, were obtained, and the authors of the latter paper estimate that the lower limit with the present technique would be 0·1—0·2% of deuterium enrichment. Although we largely agree with this, we have, in another experiment, obtained an enrichment in the methoxycarbonyl group of 0·08% and a ^2H n.m.r. spectrum with an acceptable S : N ratio of 7 : 1 (72,000 transients; rep. time 0·82 s).

We thank Professor H. Inouye, The University of Kyoto, for a generous gift of geniposide acetate; Mr. P. Rasmussen, The Botanical Garden of the University of Copenhagen, for growing the plant material; The Danish Natural Science Research Council for use of its Bruker HX-270 n.m.r. instrument, and Dr. K. Schaumburg, The University of Copenhagen, for recording the n.m.r. spectra.

(*Received*, 1*st October* 1979; *Com.* 1045.)

‡ The ^2H content in commercial chloroform is variable and was checked for each batch.

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