Biosynthesis of the Iridoid Glucosides Cornin, Hastatoside, and Griselinoside † in *Verbena* Species

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^{2}H 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*, thus excluding deoxygeniposide as an intermediate. Deoxygeniposide, loganin, and mussaenoside produced no measurable incorporation into cornin. In *V. hastata* deoxyloganin and dihydrocornin were incorporated into cornin and hastatoside while deoxygeniposide again gave no observable incorporation. Forsythide dimethyl ester was an efficient precursor for griselinoside in *V. hispida*, while no incorporation was observed with deoxygeniposide, geniposide, deoxyloganin, and dihydrocornin. However, the last two compounds were metabolized to cornin, otherwise absent in this species. Incorporation of forsythide dimethyl ester and geniposide into griselinoside in *Griselinia littoralis* was not observed.

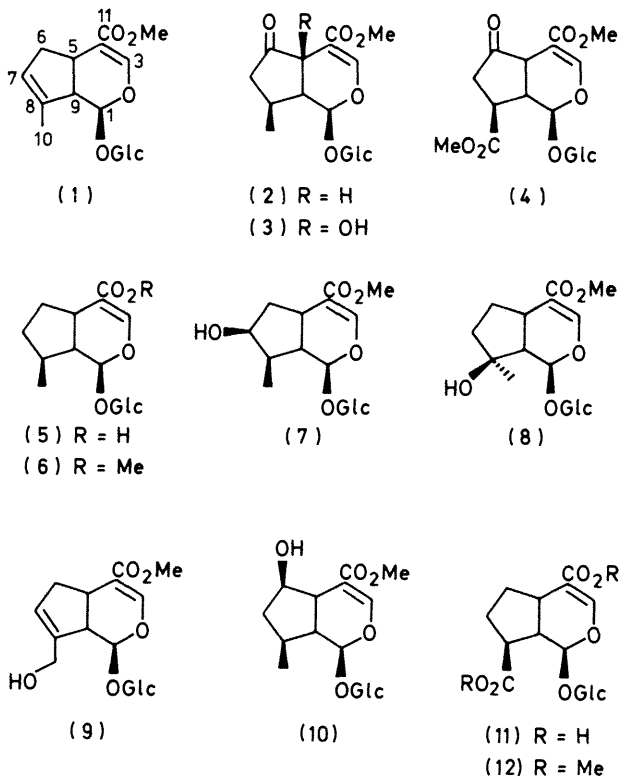
The biosynthesis of iridoid glucosides has been studied in some detail.[1] With the data available in 1974 we proposed [2] deoxygeniposide (1) as a common intermediate for the carboxylic iridoids. However, recent experiments by Inouye *et al.*[3] have shown that compound (1) is not a precursor for certain iridoids with a CH_3 or CH_2OR substituent at C-4. To test our proposal with regard to compounds carrying a methoxycarbonyl group at C-4 we have studied the biosynthesis of cornin (2), hastatoside (3), and griselinoside (4).

The biosynthesis of cornin (2) in *Verbena officinalis* L. has been investigated by Hüni *et al.*[4] who established that mevalonic acid (MVA) was a precursor. Later, Horodysky *et al.*,[5] working with [2-^{14}C]MVA, reported randomization of label at C-3 and C-11 of cornin (2) which varied with the age of the plants; in addition geraniol was shown to be a precursor of compound (2). Inouye *et al.* established that deoxyloganic acid (5) was efficiently incorporated into cornin (2).[6] To account for the formation of the 6-oxo group of cornin (2) Inouye *et al.* proposed a direct oxidation of deoxyloganic acid (5), whereas Hänsel had suggested the aglucone of deoxygeniposidic acid as an intermediate in the biosynthesis of (2).[7]

The fact that deoxyloganic acid (5) was efficiently incorporated (11%) into cornin (2) in *V. officinalis* [6] indicated that the 6-oxo group might be formed by direct oxidation at C-6. However, the discovery of griselinoside (4) in other *Verbena* species [8] suggested that another pathway might exist. Thus, deoxygeniposide (1), with its potentially oxidizable allylic C-6 and C-10 positions, seemed a reasonable precursor for both cornin (2) and griselinoside (4). Oxidation at C-6 and C-10 followed by double-bond reduction could give (4),[2] whereas selective C-6 oxidation would analogously lead to (2).[7]

We have tested this hypothesis by feeding deoxyloganin (6), deuterium-labelled at the 8-position, to *V. officinalis* plants to produce cornin (2). If deoxygeniposide (1) were an intermediate in the biosynthesis of (2) from (6), the label at C-8 would be lost. The hydrogenation of geniposide pentaacetate (9a) ‡ to yield (6a) has previously been reported,[9] and deuteriated deoxyloganin (6d) labelled at the 7, 8, 10, and CO_2CH_3 positions was thus made by catalytic deuteriation of (9a) over Pd–C followed by transesterification with $C^2H_3O^2H$–C^2H_3ONa.[10] The labels at C-10 and in the methoxycarbonyl group served as internal standards for measuring the degree of incorporation and for estimating whether the label at C-8 was retained or not. Furthermore, a constant $CO_2C^2H_3$: 10-^{2}H ratio during biosynthesis would ensure that no transesterification had taken place. A combination of m.s. and ^{2}H n.m.r. spectroscopy showed the following distribution of label in compound (6d): 0.9 ^{2}H in the methoxycarbonyl group, 1.8 ^{2}H at C-10, and 2.0 ^{2}H at positions 7 and 8 (probably including small fractions at positions 5, 6, and 9 due to scrambling during the synthesis).

In addition to the experiment with (6d) as precursor we tested deuterium-labelled analogues of deoxygeniposide (1), loganin (7), and mussaenoside (8). As the experiment with the doubly labelled compound (6d) had shown that no methyl ester exchange takes place during the biosynthesis of (2) from (6) we decided to label the compounds only in the methoxy-



† Part of this work has been presented as a preliminary report: S. Damtoft, S. R. Jensen, and Bent Juhl Nielsen, *J. Chem. Soc., Chem. Commun.*, 1980, 42.

‡ Unless otherwise indicated the letter a in the numbering of the compounds denotes a fully acetylated compound, and the letter d denotes a compound deuteriated as specified in the text.

carbonyl group. Deoxygeniposide (1) was prepared by partial hydrogenation of geniposide penta-acetate (9a) over Pd–C, followed by deacetylation. Transesterification using $C^2H_3O^2H$–C^2H_3ONa yielded compound (1d) containing 1.9 ^2H in the methoxycarbonyl group. Loganin (7) and mussaenoside (8) were isolated from *Menyanthes trifoliata* and *Melampyrum arvense*, respectively, and were then saponified and the products treated with an ethereal solution of diazodideuteriomethane in EtO^2H. The reagent was conveniently prepared by exchange of an ethereal solution of diazomethane with deuterium oxide containing NaO^2H and a phase-transfer catalyst.[11] The two products, compounds (7d) and (8d), contained 2.8 ^2H and 2.7 ^2H, respectively, in the methoxycarbonyl group.

The labelled compounds were administered as aqueous solutions by the cotton-wick method to *V. officinalis* plants during the flowering period in July. After a metabolic period of three days, work-up of the plant material yielded cornin (2) which was converted into its tetra-acetate (2a) and purified.

During work-up of cornin (2) from the plant fed with compound (6d), possible deuterium at C-5 and C-7 was removed by exchange in aqueous alkali. The ^2H n.m.r. spectrum of compound (2a) from this experiment showed signals at δ 7.27 (C^2HCl_3 internal standard), 3.74 ($CO_2C^2H_3$), 2.3 (8-^2H), 2.0 (OAc of natural abundance), and 1.2 p.p.m. (10-^2H), in agreement with the ^1H n.m.r. spectrum. The relative intensities of the signals, after correction for natural-abundance deuterium, were $CO_2C^2H_3$: 8-^2H : 10-^2H 0.9 : 1.0 : 1.7. From the relative intensities of the C^2HCl_3 and $CO_2C^2H_3$ peaks the incorporation was estimated to be 5%, corresponding to 1% enrichment. A comparison of the above relative intensities with the corresponding relative intensities from the precursor (6d) (CO_2H_3 : 7-^2H + 8-^2H : 10-^2H 0.9 : 2.0 : 1.8) showed that the label at C-8 was retained during the biosynthesis of cornin (2) from deoxyloganin (6) (as the deuterium at C-7 has been removed). This was confirmed by deacetylation of the sample and by subsequently recording the ^2H n.m.r. spectrum in H_2O. This spectrum showed signals at δ 4.8 (H^2HO of natural abundance), 3.7 ($CO_2C^2H_3$), 2.5 (8-^2H), and 1.2 p.p.m. (10-^2H), in agreement with the ^1H n.m.r. spectrum. These results show that deoxygeniposide (1) cannot be an intermediate in the biosynthesis of (2) from (6) in *V. officinalis*, and also that no significant methyl ester exchange had taken place during the biosynthesis. Furthermore, when feeding compound (1d) to the plant, no observable incorporation was seen in the isolated cornin. The results are summarized in Table 1. From these results it is evident that deoxygeniposide (1), loganin (7), and mussaenoside (8) are not on the main biosynthetic pathway to cornin (2) in *V. officinalis*.

V. hastata has been shown [8] to produce cornin (2) and hastatoside (3). The biosynthesis of the latter compound had not previously been investigated but should be closely related to that of cornin (2). We decided to feed with [7,8,10,CO_2CH_3-^2H]deoxyloganin (6d) and [CO_2CH_3-^2H]deoxygeniposide (1d) as well as [CO_2CH_3-^2H]dihydrocornin (10d). Dihydrocornin (10) co-occurs with cornin (2) in some *Cornus* species [12] and might be an intermediate in the biosynthesis of (2) from (6). [CO_2CH_3-^2H]Dihydrocornin (10d) was made by transesterification using a saturated solution of $Ba(OH)_2$ in perdeuteriomethanol.

The results of the feeding experiments are presented in Tables 2 and 3.

The spectrum of compound (2a) obtained from the feeding experiment with (6d) is in accord with that discussed above. However, the lower enrichment (0.14%) complicates an interpretation of the fate of 8-H in this experiment. The uncertainty is mainly caused by the difficulty in separating the 8-H signal from that of the natural-abundance deuterium in the acetate groups. The feeding experiment with (1d), however, establishes that (1) is not on the biosynthetic pathway to (2) and (3) in *V. hastata*.

Table 1. Administration of tracers to *Verbena officinalis*

Administered precursor (amount of precursor; enrichment; [a] amount of plant material)	Acetate isolated (2a) (mg)	Incorporation (%)
[7,8,10,CO_2CH_3–^2H]Deoxyloganin (6d) (16.6 mg; 30%; 39 g)	105	5
[CO_2CH_3–^2H]Deoxygeniposide (1d) (10.3 mg; 64.4%; 43 g)	125	<0.3 [b]
[CO_2CH_3–^2H]Loganin (7d) (6.5 mg; 92.7%; 35 g)	117	<0.1 [b]
[CO_2CH_3–^2H]Mussaenoside (8d) (33.0 mg; 91.3%; 48 g)	181	<0.1 [b]

[a] Enrichment of the methoxycarbonyl group. [b] Small amounts of the administered precursor were isolated. No decrease in its enrichment could be detected.

In Table 2 it is seen that compound (10d) is incorporated into compounds (2) and (3) to a smaller degree than is (6d). However, this does not exclude the possibility that dihydrocornin (10) is an intermediate between (6) and (3). In a recent study Inoue *et al.*[13] have shown that deoxyloganic acid (5) is more efficiently incorporated into secologanin in *Lonicera morrowii* than is loganin (7) (30 and 20%, respectively) despite the fact that (7) is an intermediate between (5) and the secoiridoids. A rather appealing biosynthetic sequence in accord with the present results * would be (6) ⟶ (10) ⟶ (2) ⟶ (3).

Owing to the structural resemblance of cornin (2) and griselinoside (4) and their occurrence in various species of *Verbena* it seemed likely that their biosyntheses were similar. We had earlier proposed that (2) might be a precursor of (4). Other pathways, however, seemed possible. We decided to test these possibilities by feeding labelled analogues of compounds (1), (6), (9), and (10), and forsythide dimethyl ester (12) to *V. hispida* [which produces griselinoside (4)].[8] The feeding experiments with *V. hispida* were performed in two series. In the first, compounds (1d), (6d), and (10d) were tested as precursors.

Griselinoside (4) and its acetate were purified by chromatographic methods designed to ensure separation of (4) from any non-metabolized precursor.

The ^2H n.m.r. spectrum of (4a) obtained from the feeding with (6d) furnished five absorptions: δ 7.27 (C^2HCl_3 of natural abundance) 3.7, 2.3, 2.0, and 1.2 p.p.m. As compound (4a), however, does not contain a C-8 methyl group which could give rise to the absorption at 1.2 p.p.m. it was evident that an impurity, highly enriched with deuterium, was present. The ^2H n.m.r. spectrum was virtually identical with that of (2a). Therefore the sample was recrystallized, but the impurity remained. To prove the nature of the impurity, unlabelled (2a) was added and the mixture was rechromatographed in another solvent system; the tetra-acetate (4a) obtained in this way only contained deuterium at natural abundance, whereas the isolated compound (2a) revealed the characteristic enriched spectrum. Based on the first spectrum the conversion of (6d) into cornin was estimated to be 5%.

The ^2H n.m.r. spectrum of compound (4a) obtained from the feeding experiment with (10d) indicated that the incorpor-

* In recent work we have demonstrated that (10) is an intermediate in the biosynthesis of (2), see S. Damtoft, M. U. Jars, S. R. Jensen, O. Kirk, and B. J. Nielsen, *Phytochemistry*, 1983, **22**, 695.

Table 2. Administration of tracers to *Verbena hastata*

Administered precursor (amount of precursor; enrichment; [a] amount of plant material)	Compounds isolated after acetylation: (2a) Amount (mg)	(2a) Incorporation (%)	(3a) * Amount (mg)	(3a) * Incorporation (%)
[7,8,10,CO_2H_3-^{2}H]Deoxyloganin (6d) (24.6 mg; 30%; 75 g)	227	3.0	88	1.3
[CO_2CH_3-^{2}H]Deoxygeniposide (1d) (19.7 mg; 64.4%; 100 g)	404	<0.3 [b]	115	<0.1 [b]
[CO_2CH_3-^{2}H]Dihydrocornin (10d) (28.5 mg; 72.4%; 82 g)	319	1.9	129	0.5

[a] and [b] as in Table 1.
* (3a) Denotes hastatoside tetra-acetate.

Table 3. Enrichments [a]

Administered precursor	Compounds isolated after acetylation: (2a) Enrichment (%)	(3a) Enrichment (%)
V. officinalis		
[7,8,10,CO_2CH_3-^{2}H]Deoxyloganin (6d)	0.34	
V. hastata		
[7,8,10,CO_2CH_3-^{2}H]Deoxyloganin (6d)	0.14	0.19
[CO_2CH_3-^{2}H]Dihydrocornin (10d)	0.19	0.14

[a] Enrichment of the methoxycarbonyl group.

Table 4. Administration of tracers to *Verbena hispida*. Series I

Administered precursor (amount of precursor; enrichment; [a] amount of plant)	Acetate isolated (4a) (mg)	Incorporation (%) into (4)	into (2)
[7,8,10,CO_2CH_3-^{2}H]Deoxyloganin (6d) (24.6 mg; 30%; 101 g)	46	<0.3	5
[CO_2CH_3-^{2}H]Deoxygeniposide (1d) (23.0 mg; 64.4%; 75 g)	97	<0.3 [b]	
[CO_2CH_3-^{2}H]Dihydrocornin (10d) (24.0 mg; 72.4%; 63 g)	85	<0.1	3

[a] and [b] as in Table 1.

ation was 3%. In this case, however, it was impossible to see from the spectrum whether the labelled compound was (4a) or (2a), as the precursor had been labelled only in the methoxycarbonyl group. By repeating the aforementioned procedure it was shown that (4a) was unlabelled, indicating that the plant was able to convert (10) into (2) but not into (4). Feeding with (1d) gave no observable incorporation. The results are summarized in Table 4.

In the second series of experiments, the selectively deuteriated derivatives of dihydrocornin, (10d), forsythide dimethyl ester, (12d), and geniposide, (9d), were used as precursors. The last two compounds were made by reaction of the corresponding iridoid acids with diazodideuteriomethane.

The deuteriated forsythide dimethyl ester (12d) was efficiently incorporated into compound (4a) (5.5%), whereas no incorporation was observed with (9d). The enrichment of griselinoside (4), biosynthesized from (12d), was 1.5%. Again, dihydrocornin (10) was converted into cornin (2) which otherwise is absent in the plant. The results are presented in Table 5.

The only precursor for griselinoside (4) found in this series of experiments was forsythide dimethyl ester (12). The results

Table 5. Administration of tracers to *Verbena hispida*. Series II

Administered precursor (amount of precursor; enrichment; [a] amount of plant)	Acetate isolated (4a) (mg)	Incorporation (%) into (1)	into (2)
[CO_2CH_3-^{2}H]Dihydrocornin (10d) (28.0 mg; 72.4%; 178 g)	189	<0.2	6.7
Forsythide [CO_2CH_3-^{2}H]dimethyl ester (12d) (57.0 mg; 68%; 163 g)	273	5.5	
[CO_2CH_3-^{2}H]Geniposide (9d) (50.5 mg; 78.8%; 201 g)	225	<0.1 [b]	

[a] and [b] as in Table 1.

indicate that the methoxycarbonyl group at C-8 is formed before that at C-4. The conversion of deoxyloganin (6) and dihydrocornin (10) (the latter is otherwise absent in the plant) suggests that the formation of (4) from (12) in *V. hispida* proceeds analogously to the formation of (2) from (6) in *V. officinalis* and *V. hastata*.

Griselinoside (4) is also a constituent of *Griselinia littoralis*.[14] Since the deuterium-labelled compounds were available we decided to test (9d) and (12d) as precursors in this plant. Neither of them provided, however, observable incorporations when fed to shoots (detection limit 0.1%).

Experimental

General.—M.p.s are uncorrected. ^2H_2O and $C^2H_3O^2H$ contained 99.8 and 99.5% ^{2}H, respectively. Preparative t.l.c. (p.l.c.) was performed on plates with 1-mm silica gel and the compounds were detected by u.v. light. Mass spectra were recorded at 70 eV.

The identity of the precursors was verified by ^{1}H and ^{13}C n.m.r. spectroscopy. The ^{13}C n.m.r. data of the unlabelled compounds are given in Table 6. The purity of the precursors was checked by t.l.c. and ^{1}H n.m.r. spectroscopy. Only in the case of (6d) could an impurity be detected.

When not otherwise indicated the ^{1}H n.m.r. spectra were recorded in C^2HCl_3 or ^2H_2O with tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulphonate (DDS) as internal standard. The ^{2}H n.m.r. spectra were recorded in $CHCl_3$ or H_2O in 10-mm tubes at 41.43 MHz with a repetition time of 0.82 s. The spectra were recorded without proton noise-decoupling as decoupling did not significantly simplify them. C^2HCl_3 of natural abundance served as an internal standard (signal at δ 7.27 p.p.m.) for the measurement of both chemical shifts and the absolute amount of deuterium in the sample. We estimated the amount of deuterium in a

Table 6. ^{13}C N.m.r. data (δ_C/p.p.m.) of unlabelled precursors [a]

Compound	C-1	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	OMe	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
(1)	96.9	153.2		33.6	38.7	127.9	139.2	49.9	15.5			99.5	73.6	76.6	70.4	77.2	61.5
(6)	98.0	152.2	113.2	33.6	32.2	33.2	35.7	48.4	20.1	170.1	52.6	99.6	73.5	76.6	70.3	77.1	61.5
(7)	97.5	151.7	113.8	30.6	41.2	74.8	40.9	45.7	12.8	170.5	52.5	99.4	73.6	76.5	70.4	77.1	61.5
(8)	95.2	151.9	113.3	30.3	29.6	40.4	80.4	51.4	23.7	170.6	52.6	99.1	73.4	76.4	70.4	77.1	61.5
(9)	98.1	153.4	112.4	35.1	38.9	129.8	142.3	46.6	60.6	170.8	52.7	99.8	73.6	76.5	70.3	77.1	61.5
(10)	96.5	153.0	110.1	41.8	77.7	41.8	34.0	47.1	19.9	170.5	52.7	99.4	73.5	76.5	70.4	77.2	61.5
(12)	97.3	153.0	111.6	34.8	32.3	28.6	46.0	45.3	178.3	170.2	52.5 53.4	99.7	73.4	76.4	70.3	77.0	61.5

[a] The spectra have been aligned (δ C-6′ 61.5 p.p.m. for all compounds).[15]

certain peak by comparing the peak area to the peak area of the C^2HCl_3-peak (of natural abundance). The natural-abundance deuterium content of the chloroform was checked against [$^2H_6$]DMSO.* It varied between 0.016 and 0.032% and thus had to be controlled for each batch. By recording a series of standard spectra we established the uncertainty of a single deuterium determination to be *ca.* 10%.

Preparation of Deoxyloganin (6).—Geniposide penta-acetate (9a) (200 mg), dissolved in a mixture of EtOH (10 ml) and EtOAc (2 ml), was hydrogenated over Pd–C (10%; 40 mg). After 3 h the catalyst was removed by filtration, $CHCl_3$ was added, and the organic phase was washed with saturated aqueous $NaHCO_3$, dried, and evaporated. T.l.c. (Et_2O) of the residue (194 mg) showed one u.v.-active spot, but ^1H n.m.r. indicated that the product was a 9 : 1 mixture of deoxyloganin tetra-acetate (6a) and its 8-epimer. Repeated crystallizations from EtOH–pentane gave a product, m.p. 103—106 °C, with unchanged composition. An aliquot of the above mixture was deacetylated and crystallized (EtOAc), m.p. 149.5—151.5 °C; the ^1H n.m.r. spectrum of the main constituent, deoxyloganin (6) (270 MHz; D_2O; DSS), showed δ_H 7.45 (br s, 3-H), 5.33 (d, $J_{1,9}$ 4.5 Hz, 1-H), 3.73 (s, OCH_3), 3.3—4.0 (glucoside H_5), 2.92 (br q, $J_{5,9}$ 7.5 Hz, 5-H), 2.15 (m, 6β-H), 1.78—1.91 (m, 7α-, 8-, and 9-H), 1.38 (m, 6α-H), 1.24 (m, 7β-H), and 1.06 (d, $J_{8,10}$ 6.2 Hz, 10-H_3). Owing to the impurity it was impossible by decoupling experiments to distinguish between the α- and β-proton at C-6 and C-7. The assignments were therefore based on the shift values of cyclohexane and of 3α-H and 3β-H in cholestane, H_{ax} absorbing at higher field than H_{eq}. From a molecular model of compound (6), based on the known coupling constants, it appeared that 7α-H and 6β-H were pseudo-equatorial and thus supposedly were the low-field protons. Signals from the impurity were observed at 7.48 (br s, 3-H), 5.52 (d, $J_{1,9}$ 3.2 Hz, 1-H), and 0.99 (d, $J_{8,10}$ 6.5 Hz, 10-H_3). In the ^{13}C n.m.r. spectrum of the mixture two sets of signals could be discerned corresponding to those of deoxyloganin (6) and its 8-epimer. The spectra of the individual compounds have been published elsewhere.[15] We have tried numerous methods to find a more selective way of converting geniposide (9) into deoxyloganin (6) but none of them were successful.

Preparation of [7,8,10,OCH_3-^2H]*Deoxyloganin* (6d).—Geniposide penta-acetate (9a) (400 mg) and Pd–C (10%; 160 mg) were suspended in EtO^2H [16] (10 ml) and treated with deuterium, generated from ^2H_2O and sodium under nitrogen and collected in a polyethylene bag. After 4 h the product (330 mg) was isolated and deacetylated, and the glucoside was purified by p.l.c. [$CHCl_3$–MeOH (3 : 1)]. The main fraction (158 mg), together with a similar fraction (125 mg) from another experiment, was dissolved in $C^2H_3O^2H$ and sodium was added. After 4 h the solution was neutralized with IR 120 resin, the resin was removed, and the solvent was evaporated off. With longer reaction times addition of methanol to the 3,4-double bond occurred. The crude product (6d) (290 mg) was chromatographed on a Sephadex G15 column (2.5 × 110 cm) with MeOH–water as eluant. Fractions containing compound (6d) were combined (230 mg) and recrystallized from wet EtOAc to give crystals (140 mg), m.p. 148—151 °C. The deuterium content was determined by mass spectroscopy: $^2H_0$, 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; and $^2H_{10}$, 1.4% (calculated from *m*/*z* 212—222 peaks) with a mean of 4.7 deuterium molecule^{-1}. The product (6d) contained 10—15% of its 8-epimer as judged by the ^1H n.m.r. spectrum (absorptions of the 1-Hs).

Preparation of Deoxygeniposide (1).—Geniposide penta-acetate (9a) (700 mg) was suspended in EtOH (20 ml). After the addition of acetyl chloride (0.8 ml) and Pd–C (5%; 100 mg) the suspension was hydrogenated for 5 min, CH_2Cl_2 was added, and the catalyst was removed. The organic phase was washed with saturated aqueous $NaHCO_3$, dried, and evaporated. The residue (620 mg) was chromatographed on a Sephadex LH-20 column. The fractions containing the tetra-acetate (1a) were combined and subjected to p.l.c. (Et_2O). Pure compound (1a) was deacetylated in a mixture of $CHCl_3$ (3 parts) and MeOH saturated with $Ba(OH)_2$ (1 part). Crystallization from EtOAc and recrystallization from EtOAc–EtOH gave deoxygeniposide (1) (150 mg), m.p. 159—161 °C. An analytical sample obtained by recrystallization from acetone had m.p. 167—168 °C, $[\alpha]_D^{20}$ −4.1° (*c* 0.7 in MeOH) (Found: C, 54.65; H, 6.5. $C_{17}H_{24}O_9$ requires C, 54.85; H, 6.5%); δ_H 7.51 (s, 3-H), 5.54 (br s, 7-H), 5.46 (d, $J_{1,9}$ 4.5 Hz, 1-H), 3.75 (s, CO_2CH_3), and 1.81 (br s, 10-H_3).

Preparation of [CO_2CH_3-^2H]*Deoxygeniposide* (1d).—Deoxygeniposide (1) (225 mg) was dissolved in $C^2H_3O^2H$ (1 ml) and sodium (*ca.* 5 mm^3) was added. After 20 h the solution was neutralized with IR 120 resin. Filtration, followed by evaporation of the solvent, gave compound (1d) which was crystallized from EtOAc (85 mg), m.p. 150—153 °C. The deuterium distribution in compound (1d) was 35.6% $^2H_0$, 64.4% $^2H_3$ (calculated from *m*/*z* 210—213 peaks).

Preparation of [CO_2CH_3-^2H]*Dihydrocornin* (10d).—Dihydrocornin (10) (250 mg), isolated from *Viburnum dentatum*,[17] was dissolved in $C^2H_3O^2H$ saturated with $Ba(OH)_2$, and the exchange was followed by ^1H n.m.r. spectroscopy. After 2.5 h the reaction mixture was neutralized with IR 120 resin and evaporated. The residue was treated with activated carbon and crystallized from water, m.p. 90—100 °C (lit.,[12] 90—100

* DMSO is dimethyl sulphoxide.

°C). The m.s. showed 27.6% $^2H_0$ and 72.4% $^2H_3$ (from *m/z* 182—185 peaks).

Preparation of $C^2H_2N_2$ *and Esterification of Iridoid Acids.*—A solution of CH_2N_2 (*ca.* 0.5 g) was prepared in Et_2O. Deuterium exchange was effected with a solution of NaO^2H (115 mg) and trimethylmyristylammonium bromide (50 mg) in ^2H_2O (5 ml).[10] Half of this solution was stirred for 15 min with the ethereal solution of CH_2N_2, after which the aqueous phase was removed with a syringe. The exchange was repeated with the other half of the alkaline solution. The resulting $C^2H_2N_2$ solution was used without purification.

The iridoid acid, from which EtO^2H and/or ^2H_2O had been evaporated several times, was dissolved in EtO^2H and treated with the above solution of $C^2H_2N_2$ at room temperature until the solution remained yellow, excess of $C^2H_2N_2$ being destroyed with acetic acid.

Preparation of [CO_2CH_3-^{2}H]*Loganin* (7d).—Loganin (7) (161 mg), isolated from *Menyanthes trifoliata*, was dissolved in water (10 ml) and $Ba(OH)_2$ (600 mg) was added. After 20 h the solution was neutralized with IR 120 resin and, after removal of the resin, concentrated under reduced pressure. Treatment with $C^2H_2N_2$ then gave crude compound (7d) which was purified by p.l.c. [$CHCl_3$–MeOH (3 : 1)]. The main fraction (53 mg) was crystallized from EtOH to give crystals (40 mg), m.p. 210—212 °C. The distribution of label was as follows: 3.8% $^2H_1$, 14.4% $^2H_2$, and 81.8% $^2H_3$, calculated from *m/z* 228—232 peaks with a mean of 2.78 ^{2}H molecule^{-1}.

Preparation of [CO_2CH_3-^{2}H]*Mussaenoside* (8d).—By the above procedure mussaenoside (8), isolated from *Melampyrum arvense*, and with ^{1}H n.m.r. data as published [18] (310 mg) yielded the deuteriated product (8d) as a syrup (150 mg) containing 3.8% $^2H_1$, 18.4% $^2H_2$, and 77.8% $^2H_3$ (calculated from *m/z* 228—232 peaks) with a mean of 2.75 ^{2}H molecule^{-1}.

Preparation of [CO_2CH_3-^{2}H]*Geniposide* (9d).—Geniposidic acid (1.1 g), obtained from the saponification of geniposide (9) (1.3 g), was acetylated and the product (1.6 g) was treated with $C^2H_2N_2$. The crude [CO_2CH_3-^{2}H]geniposide penta-acetate was crystallized from EtOH and recrystallized from Et_2O to give the deuteriated penta-acetate (9ad) (387 mg), m.p. 131—134 °C (lit.,[19] 137—138 °C). This sample was kept for other purposes. The mother-liquor (1.1 g) was deacetylated. P.l.c. [$CHCl_3$–MeOH (3 : 1)] of the residue (734 mg) gave the deuteriated geniposide (9d) (388 mg). Crystallization from acetone gave crystals of compound (9d) (98 mg), m.p. 155—157 °C (lit.,[19] 163—164 °C). The m.s. showed 5.0% $^2H_0$, 11% $^2H_1$, 26.3% $^2H_2$, and 57.6% $^2H_3$ (calculated from *m/z* 225—228 peaks) with a mean of 2.37 ^{2}H molecule^{-1}.

Preparation of Forsythide [CO_2CH_3-^{2}H]*Dimethyl Ester* (12d).—Crude forsythide (11), isolated from *Forsythia viridissima*,[9] was treated with $C^2H_2N_2$ and the product (323 mg) was purified by p.l.c. [$CHCl_3$–MeOH (3 : 1)]. Forsythide [CO_2CH_3-^{2}H]dimethyl ester (12d) was crystallized from EtOH (151 mg), m.p. 140—142 °C (lit.,[9] 141.5—142.5 °C). It contained 13% $^2H_0$, 0% $^2H_1$, 7% $^2H_2$, 18% $^2H_3$, 6% $^2H_4$, 20% $^2H_5$, and 36% $^2H_6$ (calculated from *m/z* 256—262 peaks) with a mean of 4.1 ^{2}H molecule^{-1}.

General Procedure for the Administration of Labelled Precursors and for the Isolation of Iridoids.—The precursor was administered as an aqueous solution by the cotton-wick method during the flowering period of the plants (July–September). After 3—4 d the plants were harvested and stored in polyethylene bags at −23 °C until work-up. The sample of frozen plant was extracted twice with EtOH, the extracts were evaporated, and the residue was dissolved in water and extracted with Et_2O to remove fats, *etc.* The aqueous solution was filtered through a column of neutral Al_2O_3 which was then washed with water. The combined eluates were concentrated and fixed on a column of silica gel, and the iridoids and other glucosides were eluted with acetone. The acetone fraction was concentrated to give 'crude glucosides.'

Further purification will be described under separate headings. However, if the enrichment of an acetate was below the detection limit, the experiment is not further described.

Isolation of cornin (2) *from* Verbena officinalis *fed with* [7,8,10,CO_2CH_3-^{2}H]*deoxyloganin* (6d). *Table* 1. The crude glucosides (280 mg) were dissolved in water (10 ml) and K_2CO_3 (133 mg) was added to catalyse the exchange of possible deuterium at C-5 and C-7. After 1 h the solution was neutralized with IR 120 resin. Removal of the resin and concentration of the filtrate under reduced pressure gave a residue (218 mg) which was subjected to p.l.c. [$CHCl_3$–MeOH (4 : 1)]. No precursor could be isolated, and the main fraction was cornin (2) (99 mg). Acetylation gave an acetate (140 mg) which, after p.l.c. (Et_2O), furnished compound (2a) (105 mg). Based on the ^{2}H n.m.r. spectrum the enrichment of the methoxycarbonyl group was estimated to be 0.34%.

Isolation of cornin (2) *and hastatoside* (3) *from* Verbena hastata *fed with compound* (6d). *Table* 2. P.l.c. of the crude glucosides (953 mg) [$CHCl_3$–MeOH (6 : 1) (twice) and then (5 : 1)] gave cornin (2) (379 mg) as the faster moving zone, followed by hastatoside (3) (198 mg).

The cornin (2) fraction was dissolved in water (10 ml) and K_2CO_3 (150 mg) was added. After 1 h the solution was neutralized with 2M H_2SO_4 and concentrated under reduced pressure. Acetylation yielded the tetra-acetate (2a) (515 mg) which was purified by p.l.c. (Et_2O) (recovery 277 mg) and recrystallized from EtOH to constant activity.

Hastatoside (3) was dissolved in water (10 ml) and K_2CO_3 (150 mg) was added. After 1 h the solution was neutralized with 2M H_2SO_4 and concentrated under reduced pressure. Acetylation yielded the crude tetra-acetate (3a) (252 mg), purified by p.l.c. [Et_2O–pentane (10 : 1) (twice)]. This gave compound (3a) (88 mg) which was recrystallized to constant activity.

Isolation of cornin (2) *and hastatoside* (3) *from* V. hastata *fed with* [CO_2CH_3-^{2}H]*dihydrocornin* (10d). *Table* 2. Work-up as in the experiment above, except for the K_2CO_3 treatment, gave the acetates (2a) (319 mg) and (3a) (129 mg). The acetates were recrystallized from EtOH to constant activity.

Isolation of griselinoside (4) *from* V. hispida *fed with compound* (6d). *Table* 4. P.l.c. of the crude glucosides (398 mg) [$CHCl_3$–MeOH (4 : 1) (twice)] gave griselinoside (4) which was acetylated (product yield 142 mg). P.l.c. (Et_2O) gave the tetra-acetate (4a) (46 mg). The ^{2}H n.m.r. spectrum indicated the presence of labelled (2a). An aliquot (30 mg) was diluted with unlabelled (4a) (28 mg) and recrystallized from EtOH to give crystals (48 mg), m.p. 185—186 °C (lit.,[14] 189—189.5 °C). The ^{2}H n.m.r. spectrum of the product indicated that labelled (2a) was still present. Unlabelled (2a) was added and the mixture was subjected to p.l.c. [$CHCl_3$–EtOAc (2 : 1)], whereby labelled (2a) and unlabelled (4a) were obtained.

Isolation of griselinoside (4) *from* V. hispida *fed with* [CO_2CH_3-^{2}H]*dihydrocornin* (10d). *Table* 4. Crude glucosides (207 mg) were purified as above giving labelled (2a) and unlabelled (4a).

Isolation of griselinoside (4) *from* V. hispida *fed with forsythide* [CO_2CH_3-^{2}H]*dimethyl ester* (12d). *Table* 5. Crude glucosides (544 mg) were treated essentially as above, giving

labelled (4a) which was recrystallized from EtOH to constant activity.

Isolation of griselinoside (4) *from* V. hispida *fed with* [CO_2CH_3-^{2}H]*dihydrocornin* (10d). *Table* 5. Labelled (2a) and unlabelled (4a) were obtained from crude glucosides (440 mg) as in the experiment recorded in Table 4.

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