[Chem. Pharm. Bull. 22(3) 676—686 (1974)]

UDC 547.597'457.1.057 : 542.98

# Studies on Monoterpene Glucosides and Related Natural Products. XXIII.[1] Biosynthesis of the Secoiridoid Glucosides, Gentiopicroside, Morroniside, Oleuropein, and Jasminin[2]

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(Received August 1, 1973)

Tracer experiments revealed that (10-$^3$H)-loganin (**2**) was incorporated into gentiopicroside (**3**) in *Gentiana triflora* var. *japonica*. (7-$^3$H)-**2** and (carbo-$^{14}$C-methoxy)-secologanin (**10**) were also found to be incorporated into morroniside (**4**) in *Gentiana thunbergii* and *Cornus officinalis*, respectively. The biosynthetic pathway of oleuropein-type glucosides in *Olea europaea* and *Jasminum primulinum* was further examined by employing (10-$^3$H)-7-deoxyloganic acid (**1**), (10-$^3$H)-loganin (**2**), (carbo-$^{14}$C-methoxy)-secologanin (**10**), (8-$^3$H)- and (carbo-$^3$H-methoxy)-kingiside (**11**), together with (8-$^3$H)- and (carbo-$^{14}$C-methoxy)-8-epikingiside (**18**).

Previously, we have reported on the role of 7-deoxyloganic acid (**1**) and loganin (**2**) for the biosynthesis of several iridoid glucosides[4] and the formation sequences of highly oxidized glucosides of this type.[5]

This paper describes the results obtained afterwards on the biosynthesis of the secoiridoid glucosides, gentiopicroside (**3**), morroniside (**4**), oleuropein (**5**), and jasminin (**6**). From the results of an administration experiment of (2-$^{14}$C)-mevalonic acid, we already proved that gentiopicroside (**3**) is a monoterpenoid. At the same time we suggested that the glucoside could be formed *via* sweroside (**7**) and swertiamarin (**8**).[6] Considering these matters together with the biosynthetic route of indole alkaloids, **3** is supposed to be formed from loganin (**2**) (or loganic acid (**9**)[7]) *via* secologanin (**10**), sweroside (**7**) and swertiamarin (**8**). At the outset of this work, however, a direct proof for the incorporation of **2** or **9** into gentiopicroside (**3**) could not yet be found.

Therefore, we attempted first to establish this biosynthetic pathway. An aqueous solution of (10-$^3$H)-loganin (**2**)[4] was administered to *Gentiana triflora* var. *japonica* by the cotton wick method. After seven days administration, gentiopicroside (**3**) was isolated as its acetate showing 4.5% incorporation. Consequently, it was established that **3** was formed *via* loganin (**2**)[2a] as shown in Chart 1. Almost simultaneously, two other groups, Gröger, *et al.*[8] and Coscia,

1) Part XXII: H. Inouye, T. Yoshida, S. Tobita, K. Tanaka, and T. Nishioka, *Tetrahedron*, **30**, 201 (1974).
2) Preliminary reports of part of this work have been published: *a*) H. Inouye, S. Ueda, Y. Aoki, and Y. Takeda, *Tetrahedron Letters*, **1969**, 2351; *b*) H. Inouye, S. Ueda, and Y. Takeda, *ibid.*, **1971**, 4069; *c*) H. Inouye, S. Ueda, K. Inoue, and Y. Takeda, *ibid.*, **1971**, 4073.
3) Location: *Yoshida-Shimoadachi-cho, Sakyo-ku, Kyoto.*
4) H. Inouye, S. Ueda, Y. Aoki, and Y. Takeda, *Chem. Pharm. Bull.* (Tokyo), **20**, 1287 (1972).
5) H. Inouye, S. Ueda, and Y. Takeda, *Chem. Pharm. Bull.* (Tokyo), **20**, 1305 (1972).
6) H. Inouye, S. Ueda, and Y. Nakamura, *Chem. Pharm. Bull.* (Tokyo), **18**, 2043 (1970); *idem*, *Tetrahedron Letters*, **1967**, 3221.
7) Experimental results obtained in this series of work have been reasonably interpreted by assuming free acids and the corresponding methyl esters to be equivalent although it would be a rather delicate problem. *cf.* K. M. Madyastha, R. Guarnaccia, and C.J. Coscia, *FEBS Letters*, **14**, 175 (1971).
8) D. Gröger and P. Simchen, *Z. für Naturforsch.*, **24b**, 356 (1969).

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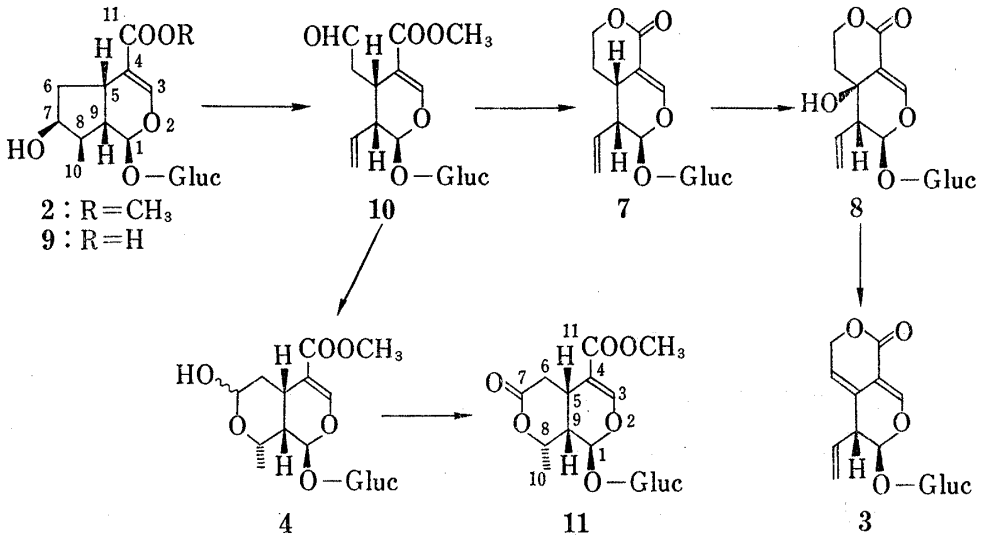


Chart 1

*et al.*,[9] also proved by using different plants that **3** was formed from **2** and **9**, respectively.

Next we investigated the biosynthesis of morroniside (**4**), which had been isolated from *Lonicera morrowii* (Caprifoliaceae) along with secologanin (**10**), sweroside (**7**) and kingiside (**11**) by Souzu and Mitsuhashi.[10] The glucoside (**4**) was later obtained by Endo and Taguchi[11] from *Cornus officinalis* (Cornaceae) besides loganin (**2**) and by our group[12–13] from *Gentiana thunbergii* (Gentianaceae) as its main glucoside. Kingiside (**11**) is the corresponding lactone derived by oxidation of the hemiacetal portion of **4**, both glucosides having related structures to those of **10** or **7**. Considering the fact of the coexistence of these four glucosides in *Lonicera morrowii* together with the occurrence of morroniside (**4**) in *Gentiana thunbergii*, these compounds can be supposed to be biogenetically closely related congeners.

Taking into account the role of secologanin (**10**) in the biosynthesis of secoiridoid glucosides in Gentianaceae and several indole alkaloids, we presumed that the biosynthetic pathway of these glucosides occurred in *Lonicera morrowii* as shown in Chart 1.

Namely, the cyclization to a hemiacetal could take place through the introduction of an oxygen function into the C-8 position of **10** derived from **2** to give morroniside (**4**). Oxidation of the hemiacetal moiety of **4** could afford kingiside (**11**). On the other hand, reduction of the aldehyde group at C-7 of secologanin (**10**) followed by lactonization could give rise to sweroside (**7**).

In an attempt to establish these biosynthetic pathways, (7-$^3$H)-**2**[4] and (carbo-$^{14}$C-methoxy)-**10** were administered respectively to *Gentiana thunbergii* and *Cornus officinalis* and incorporations of the radioactivity into **4** were examined.

An aqueous solution of (7-$^3$H)-**2** was administered hydroponically to *Gentiana thunbergii*. Radioactive **4** isolated from the plant was purified as its pentaacetate (**12**), which was submitted to partial hydrolysis with $KHCO_3$ followed by Jones oxidation and successive methylation with diazomethane giving rise to kingiside tetraacetate (**13**) and keto-dimethyl ester (**14**), both of which were non-radioactive. It was thus clarified that the tritium labelling of (7-$^3$H)-**2** had been incorporated into the C-7 position of **4** without randomization (Chart 2).

(Carbo-$^{14}$C-methoxy)-**10** was then prepared starting from the tetraacetate (**15**) of secologanin (**10**) obtained from *Lonicera morrowii*, which was hydrolyzed with 0.25N aqueous NaOH

9) R. Guarnaccia, L. Botta, and C.J. Coscia, *J. Am. Chem. Soc.*, **91**, 204 (1969).
10) I. Souzu and H. Mitsuhashi, *Tetrahedron Letters*, **1969**, 2725; *idem*, *ibid.*, **1970**, 191.
11) T. Endo and H. Taguchi, *Yakugaku Zasshi*, **93**, 30 (1973).
12) H. Inouye and Y. Nakamura, *Yakugaku Zasshi*, **91**, 755 (1971).
13) H. Inouye, S. Tobita, Y. Akiyama, K. Ito, and T. Shingu, *Chem. Pharm. Bull.* (Tokyo), **21**, 846 (1973).

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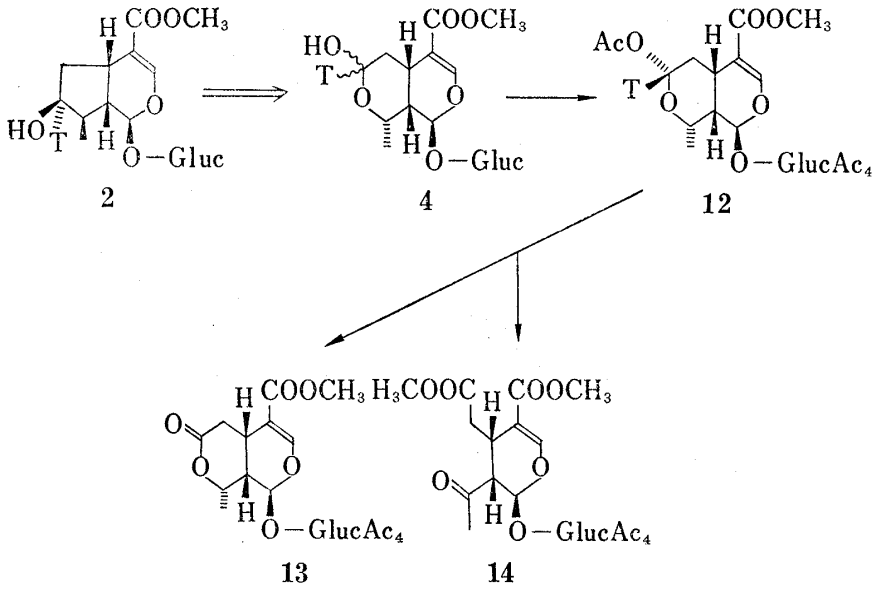
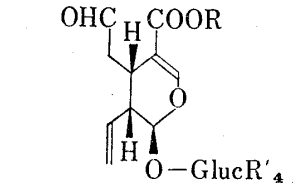



Chart 2




15 : R=$CH_3$, R'=Ac
16 : R=R'=H

Chart 3

Table I. Incorporation of (7-$^3$H)-Loganin (2) and (Carbo-$^{14}$C-methoxy)-secologanin (10) into Morroniside (4) in *Gentiana thunbergii* and *Cornus officinalis*

| Plant | Glucoside fed amt. and spec. activity, dpm/mmole | Morroniside (4) isolated spec. activity, dpm/mmole | Incorporation % |
|---|---|---|---|
| *G. thunbergii* | (7-$^3$H)-loganin (2) 9.9 mg, $3.41\times10^9$ | $1.26\times10^7$ | 0.13 |
| *C. officinalis* | (carbo-$^{14}$C-methoxy)-secologanin (10) 22.0 mg, $7.25\times10^8$ | $1.33\times10^5$ | 0.09 |

and the resulting secologanic acid (**16**) was treated with $^{14}CH_2N_2$ generated from N-nitroso-$\beta$-($^{14}$C)-methylaminoisobutyl methyl ketone.[14] An aqueous solution of thus obtained (carbo-$^{14}$C-methoxy)-**10** was applied hydroponically to a twig of *Cornus officinalis* plant bearing fruits. After five days, the fruits were worked up by the conventional method giving radioactive morroniside (**4**) which was characterized as its pentaacetate (**12**).

The results of these two administration experiments (Table I) revealed that **4** is biosynthesized as expected by the route shown in Chart 1. The fact of retention of the tritium labelling of (7-$^3$H)-loganin (**2**) at the corresponding position of **4** clearly indicates that **4** is not derived *via* substances such as 7-dehydrologanin (**17**) or **11**.

Next we examined the biosynthetic pathway of oleuropein-type secoiridoid glucosides occurring in the plants of Oleaceae. Differing from secoiridoid glucosides of Gentianaceae, oleaceous glucosides such as oleuropein (**5**) and jasminin (**6**) possess a carboxy ester group at C-7 and an ethylidene side chain. These glucosides, after formation of the iridoid skeleton, are also supposed to be formed incipiently according to the same common biosynthetic pathway as gentianaceous secoiridoid glucosides.

(10-$^3$H)-7-Deoxyloganic acid (**1**) and (10-$^3$H)-loganin (**2**)[4] were thus administered to *Jasminum primulinum* to examine their incorporation into jasminin (**6**). The results of the experiments indicated that both glucosides were incorporated as expected into **6** to give radioactive glucosides (Table II). Therefore, it has become clear that the biosynthetic pathway

14) E. Redemann, F.O. Rice, R. Roberts, and H.P. Ward, "Organic Syntheses," Coll. Vol. 3, ed. by E.C. Horning, John Wiley and Sons, Inc. New York, 1955, p. 244.

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to oleuropein (**5**) and jasminin (**6**) could follow the same route as that of gentianaceous secoiridoid glucosides at least to the stage of **2**.

The next problem was the examination of the biosynthetic route including ring cleavage of **2** leading to **5** and **6**. Thereby, two possible biogenetic routes were considered.

In the first route (route 1 in Chart 4), secologanin (**10**) was also thought to be a precursor as it was found to be in the biosynthesis of the glucosides of Gentianaceae, the migration of the double bond and the oxidation of the aldehyde group at C-7 of **10** leading to the secoiridoid moiety of **5** or **6**. The possible occurrence of gentiopicroside-type Glucosides in *Fontanesia phillyreoides* (Oleaceae)[15] and the actual participation of **10** as a usual and important intermediate for the biosynthesis of gentianaceous secoiridoid glucosides and indole alkaloids would support this route.

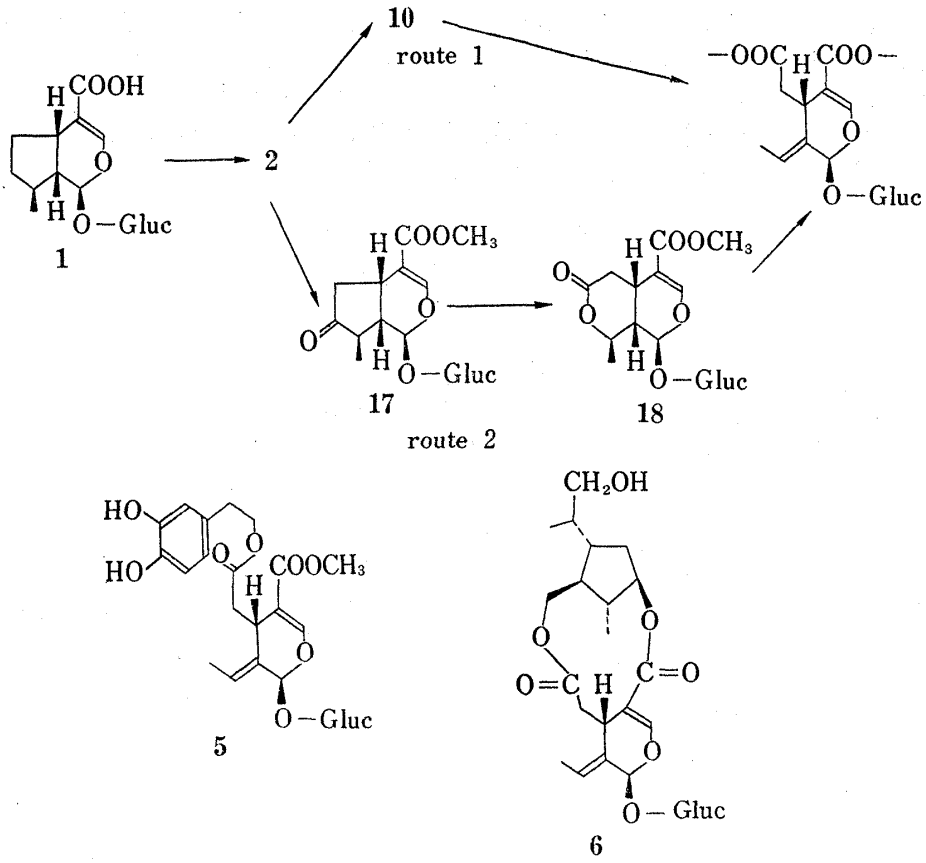


Chart 4

In the second route (route 2 in Chart 4), oxidation at C-7 of **2** leads to 7-dehydrologanin (**17**), the Baeyer-Villiger-type oxidation of which gives 8-epikingiside (**18**), and the following dehydration gives rise to the secoiridoid moiety of **5** or **6**. The stereochemistry of a series of reactions followed in the chemical correlation[1,16] of asperuloside (**19**) with oleuropein (**5**) furnished the parallel model for this route. Namely, the Baeyer-Villiger reaction of 7-dehydrologanin tetraacetate (**20**) gives 8-epikingiside tetraacetate (**21**). Compound **22** having the same configuration at C-8 as **21** is further converted as shown in Chart 5 over several reaction steps to the dimethyl ester (**23**), which is identical with the compound derived from **5**. On the other hand, the same series of reactions applied to compounds possessing the opposite configuration at C-8 gives a geometrical isomer of **23**. Frequent occurrences of natural products

15) H. Budzikiewicz, C. Horstmann, K. Pufahl, and K. Schreiber, *Chem. Ber.*, **100**, 2798 (1967).
16) H. Inouye, T. Yoshida, S. Tobita, K. Tanaka, and T. Nishioka, *Tetrahedron Letters*, **1970**, 2459, a preliminary communication of the paper of footnote 1.

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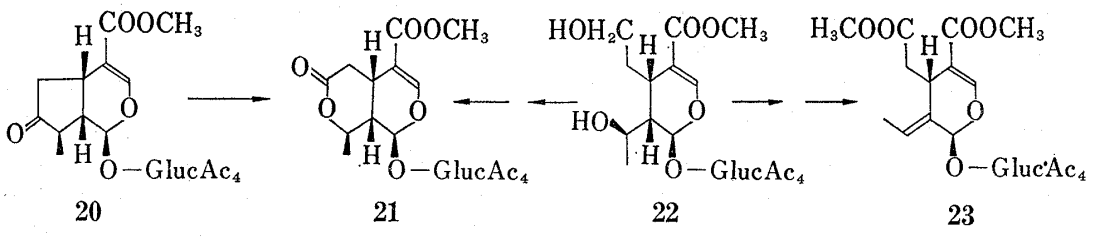


Chart 5

such as obacunone which are supposed to be formed by a Baeyer-Villiger-type oxidation also would give support to this route.[17]

In an attempt to investigate both biosynthetic routes described above, we carried out the following administration experiments. In order to examine the first route, (carbo-$^{14}$C-methoxy)-secologanin (**10**) was administered to *Olea europaea* to find 0.34% incorporation of **10** into **5**. The second route was examined by administration experiments with (8-$^{3}$H)-8-epikingiside (**18**) and (8-$^{3}$H)-kingiside (**11**). Labelled compounds were prepared according to the route established before as shown in Chart 6.[1,16]

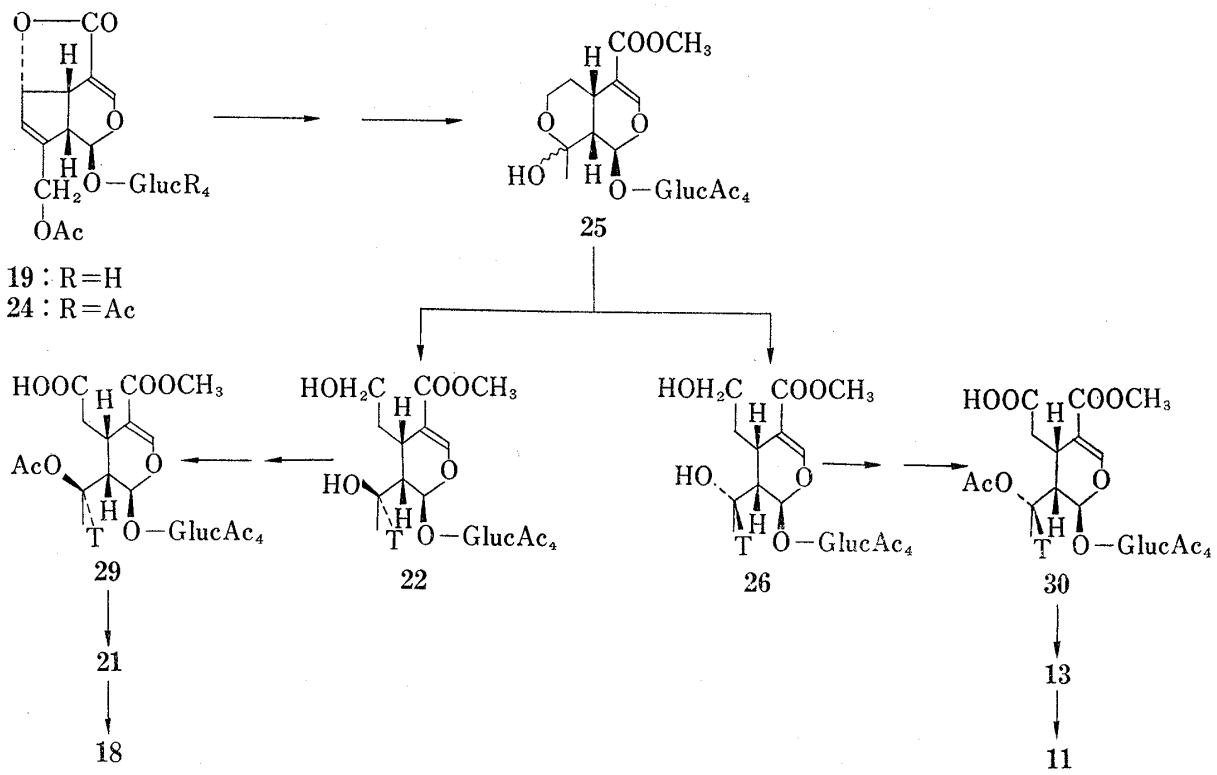


Chart 6

Hemiketal (**25**) prepared from asperuloside tetraacetate (**24**) was reduced with NaB$^{3}$H$_4$ yielding (8-$^{3}$H)-diol I (**22**) and (8-$^{3}$H)-diol II (**26**). These diols were isolated and successively submitted to tritylation, acetylation and Jones oxidation giving rise to the corresponding carboxylic acids (**29**) and (**30**), respectively. After deacetylation of these acids with methanolic Ba(OH)$_2$, the reaction products were reacetylated to give (8-$^{3}$H)-8-epikingiside tetraacetate (**21**) and (8-$^{3}$H)-kingiside tetraacetate (**13**), both of which were finally deacetylated to (8-$^{3}$H)-8-epikingiside (**18**) and (8-$^{3}$H)-kingiside (**11**). Both radioactive compounds were

17) D. Lavie and E.C. Levy, *Tetrahedron Letters*, **1970**, 1315; H.E. Conrad, R. Dubus, M.-J. Namtvedt, and I.C. Gunsalus, *J. Biol. Chem.*, **240**, 495 (1965).

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administered respectively to *Jasminum primulinum*. As is shown in Table II, incorporation of these tracers into jasminin (**6**) has been found to be about the same.

In an attempt to determine the intrinsic precursor between **11** and **18**, (carbo-$^{14}$C-methoxy)-8-epikingiside (**18**) and (carbo-$^{3}$H-methoxy)-kingiside (**11**) have been prepared and both compounds were administered simultaneously to the plant. Both labelled compounds were prepared in the following way (Chart 7).

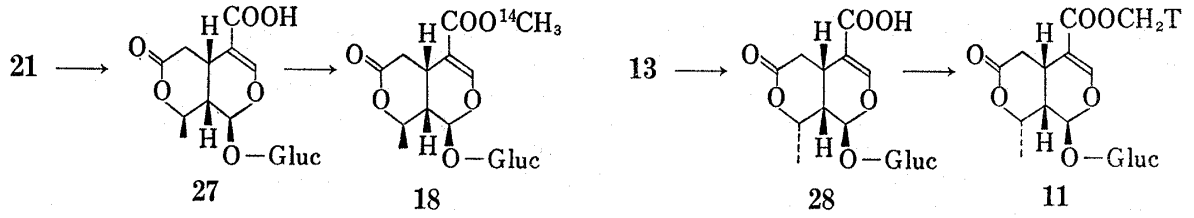


Chart 7

Table II. Administration of Iridoid and Secoiridoid Glucosides into *Jasminum primulinum* and *Olea europaea*

| Plant | Amt. and spec. activity of glucoside fed dpm/mmole | Feeding time day | Spec. activity of glucoside isolated dpm/mmole | Incorporation % |
|---|---|---|---|---|
| *J. primulinum* | (10-$^{3}$H)-7-deoxyloganic acid (**1**) 9.67 mg, $1.99\times10^{11}$ | 7 | jasminin (**6**) $1.35\times10^{6}$ | 0.01 |
| *J. primulinum* | (10-$^{3}$H)-loganin (**2**) 44.1 mg, $1.72\times10^{7}$ | 4 | jasminin (**6**) $7.50\times10^{5}$ | 0.03 |
| *O. europaea* | (carbo-$^{14}$C-methoxy)-secologanin (**10**) 22.0 mg, $7.25\times10^{8}$ | 5 | oleuropein (**5**) $3.92\times10^{5}$ | 0.34 |
| *J. primulinum* | (8-$^{3}$H)-8-epikingiside (**18**) 21.5 mg, $9.45\times10^{7}$ | 8 | jasminin (**6**) $1.86\times10^{4}$ | 0.02 |
| *J. primulinum* | (8-$^{3}$H)-kingiside (**11**) 42.7 mg, $2.08\times10^{8}$ | 8 | jasminin (**6**) $7.42\times10^{4}$ | 0.02 |
| *O. europaea* | (carbo-$^{14}$C-methoxy)-8-epikingiside (**18**) 25.1 mg, $2.92\times10^{8}$ | 5 | oleuropein (**5**) $4.34\times10^{5}$ | 0.13 |
| *O. europaea* | (carbo-$^{3}$H-methoxy)kingiside (**11**) 28.3 mg, $1.47\times10^{8}$ | 5 | oleuropein (**5**) $2.38\times10^{5}$ | 0.13 |

After hydrolysis of 8-epikingiside tetraacetate (**21**) with 0.5N NaOH, the hydrolysate was fractionated by the counter current distribution giving 8-epikingisidic acid (**27**) which was treated with $^{14}CH_2N_2$ to yield (carbo-$^{14}$C-methoxy)-8-epikingiside (**18**). On the other hand, the hydrolysate of kingiside tetraacetate (**13**) obtained in the same way was purified by the counter current distribution to give kingiside (**11**) and kingisidic acid (**28**). Compound **28** in $D_2O$-dioxane was methylated with a dried ethereal solution of diazomethane to afford (carbo-$^{2}$H-methoxy)-kingiside (**11**). Comparison of the nuclear magnetic resonance (NMR) spectrum of this compound with that of kingiside (**11**) revealed that one deuterium atom had been introduced into the carbomethoxyl group because the intensity of the signal at 3.45 ppm assignable to the carbomethoxyl group of deuterated **11** decreased by one proton. Working up of kingisidic acid (**28**) in a similar manner in a mixture of $^{3}H_2O$ and dioxane gave (carbo-$^{3}$H-methoxy)-kingiside (**11**).

A mixture of (carbo-$^{14}$C-methoxy)-8-epikingiside (**18**) and (carbo-$^{3}$H-methoxy)-kingiside (**11**) ($^{3}H/^{14}C=1.97$) was then applied to *O. europaea* plant, and radioactive oleuropein (**5**) was isolated, whose $^{3}H/^{14}C$ ratio was 1.84. Therefore, it can be assumed that there was no significant change in $^{3}H/^{14}C$ ratios between the administered material and the isolated oleuropein (**5**).

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Judging from these experimental results, we cannot accept route 2, where only 8-epikingiside (**18**) could be the precursor. Although the results of the foregoing experiments suggest that route 1, where secologanin (**10**) intervenes, seems to be the more probable route for the biosynthesis of **5** and **6**, further examination is still necessary in order to draw definitive conclusions.

## Experimental[18]

**Administration of (10-$^3$H)-Loganin (2) to *Gentiana triflora* and Isolation of Gentiopicroside (3)**——(10-$^3$H)-Loganin (2) (30.3 mg, spec. activity $2.27 \times 10^6$ dpm/mmole) was dissolved in water (5 ml), placed in glass tubes and administered by the cotton wick method to two *G. triflora* plants (20 cm in height) in August. During the administration, water (total 8 ml) was added into the glass tubes in order to let the remaining radioactive compound be absorbed into the plants.[19] Seven days after the beginning of the administration the shoots were harvested (wet weight 12.5 g), cut into pieces and extracted with four 150-ml portions of MeOH under reflux. The MeOH extracts were combined and the solvent was removed *in vacuo*. The residue was dissolved in water (30 ml) and the insoluble material was filtered off through a celite layer. The celite layer was washed four times with water (total 40 ml). The filtrate and the washings were combined, washed with AcOEt ($3 \times 30$ ml) and concentrated *in vacuo* to about 20 ml. This solution was then extracted with *n*-BuOH ($3 \times 30$ ml). The *n*-BuOH extracts were combined and concentrated *in vacuo*. The residue was chromatographed on silica gel (30 g, $2 \times 27$ cm) eluted successively with $CHCl_3$ (fractions (fr.) No. 1—4), $CHCl_3$: MeOH 95: 5 (fr. No. 5—7), $CHCl_3$: MeOH 93: 7 (fr. No. 8—10), and $CHCl_3$: MeOH 90: 10 (fr. No. 11—34), collecting 30 ml fractions. Fr. No. 9—33 were combined and the solvent was removed *in vacuo* to give gentiopicroside (3) as a colorless syrup (103.8 mg). 94.3 mg of this substance was acetylated by the usual method with $Ac_2O$ and pyridine (0.94 ml each). The reaction product was chromatographed on silica gel (20 g, $2.5 \times 10$ cm) with ether as eluent and 15 ml fractions were collected. Fr. No. 14—21 were combined and the solvent was removed *in vacuo* to give a colorless syrup (64.1 mg), which was identified with an authentic sample of gentiopicroside tetraacetate by thin-layer chromatography (TLC) (ether). This compound (61.5 mg) was diluted with carrier (48.6 mg) and repeatedly recrystallized from EtOH to constant activity, mp 142—143°.

**Administration of (7-$^3$H)-Loganin (2) to *Gentiana thunbergii* and Isolation of Morroniside (4)**——(7-$^3$H)-Loganin (2) (9.9 mg, spec. activity $3.41 \times 10^9$ dpm/mmole) was dissolved in water (3 ml) and administered hydroponically to five clumps of *G. thunbergii* plants (about 1 cm in height) in April to May. Five days after the beginning of the administration, all the plants (wet weight 6.5 g) were extracted with four 50-ml portions of MeOH under reflux for 30 min. The combined MeOH extracts were concentrated *in vacuo* and the residue was chromatographed on silica gel (40 g, $2.8 \times 14$ cm), eluted successively with $CHCl_3$ (fr. No. 1—8), $CHCl_3$: MeOH 97: 3 (fr. No. 9—12), $CHCl_3$: MeOH 95: 5 (fr. No. 13—15), $CHCl_3$: MeOH 93: 7 (fr. No. 16—20), $CHCl_3$: MeOH 90: 10 (fr. No. 21—33) and $CHCl_3$: MeOH 85: 15 (fr. No. 34—38) collecting 25 ml each of the fractions. Fr. No. 25—36 were combined and concentrated *in vacuo*. The residue (12.7 mg) was acetylated with $Ac_2O$ and pyridine (0.3 ml each) by the usual method. The reaction product was chromatographed on silica gel (10 g, $1.2 \times 16$ cm) with ether as eluent collecting 15 ml fractions. The combined fractions (No. 4—5) were concentrated *in vacuo* and the residue was recrystallized from ether-petr. ether affording colorless needles (5.6 mg), which were identified with an authentic sample of morroniside pentaacetate (12) by TLC (ether). This substance was diluted with carrier (101 mg) and repeatedly recrystallized as mentioned above to constant activity, mp 149°.

**Partial Hydrolysis of (7-$^3$H)-Morroniside Pentaacetate (12) and Oxidation of the Hydrolysate with Jones Reagent**——To a solution of (7-$^3$H)-morroniside pentaacetate (12) (32.1 mg) and carrier (68.4 mg) in EtOH (16 ml) was added a solution of $KHCO_3$ (19.0 mg) in water (8 ml) and the mixture was left standing at 35° for 50 hr. After removal of the solvent *in vacuo*, the resulting residue was chromatographed on silica gel (10 g, $1.3 \times 15$ cm) eluted with $CHCl_3$ (fr. No. 1—9) and then with $CHCl_3$: MeOH 9: 1 (fr. No. 10—24) collecting 25 ml fractions. Fr. No. 13—19 were combined and the solvent was removed *in vacuo* giving a colorless

18) Unless otherwise noted, the experimental procedure were the same as described in the footnote 18 of the 17th paper of this series.[4] Paper chromatography (PPC) was carried out on Toyo Roshi No. 50 filter paper with *n*-BuOH: EtOH: $H_2O$ (4: 1: 5) as solvent. The paper strips were cut into 1 cm pieces and the radioactivity was determined by scintillation counting. The counter current distribution was performed by using a Mitamura Model 4-53 fully automatic distributor with the same solvent system as in the case of PPC. Ultraviolet (UV) and infrared (IR) spectra were recorded on a Hitachi EPS-2U and a Hitachi EPI-S2 spectrophotometer, respectively. NMR spectra were measured by a Varian A-60 instrument with tetramethylsilane (TMS) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as internal standards. All chemical shifts are given in ppm downfield from TMS or DSS.

19) The same procedure was employed in all the administration experiments described in this paper.

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syrup (35.1 mg). Jones reagent (0.5 ml) was added to a solution of this syrup in acetone (4 ml) and stirred at room temperature for 1 hr. After an addition of water (20 ml), the reaction mixture was extracted with three 10 ml portions of $CHCl_3$. The $CHCl_3$ extracts were combined and dried over anhyd. $MgSO_4$ and the solvent was removed *in vacuo*. The residue was methylated with $CH_2N_2$–ether and the reaction product was chromatographed on silica gel (15 g, 1.5×15 cm) with ether as eluent and 10 ml each of the fraction was collected. Fr. No. 7—11 were combined and the solvent was removed. Recrystallization of the residue from EtOH gave colorless needles, mp 103—105°, yield 26.6 mg, which were identified with an authentic sample of ketodimethyl ester (**14**) by mixed melting point and TLC (ether). Fr. No. 14—15 gave a substance (1.0 mg) whose *Rf* value on TLC (ether) was the same as that of kingiside tetraacetate (**13**). Further purification of the former by recrystallization and the latter by preparative TLC (silica gel, 0.25 mm in thickness, ether) revealed that both compounds were non-radioactive.

**Hydrolysis of Secologanin Tetraacetate (15)**——Tetraacetate (**15**) (2.2 g) of secologanin (**10**) obtaind from *Lonicera morrowii* was mixed with aq. 0.25 N NaOH (60 ml) and stirred at room temperature for 2 hr. After adjustment of the pH of the solution to 3 with Amberlite IR-120 (H-form), the ion exchange resin was filtered off and the filtrate was concentrated *in vacuo* to give 503 mg of the residue. The residue was subjected to the counter current distribution. After 383 transfers the fractions of tube No. 130—175 were combined and the solvent was removed *in vacuo* giving secologanic acid (**16**) as a white powder (163.5 mg). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 239 (4.00). IR $\nu_{max}^{Nujol}$ ($cm^{-1}$): 3500—3050, 1700, 1690, 1680. *Anal.* Calcd. for $C_{16}H_{22}O_{10}$: C, 51.38; H, 5.93. Found: C, 50.97; H, 6.20. This substance was treated with $CH_2N_2$–ether for a short time giving secologanin (**10**), which was identical with an authentic sample by TLC ($CHCl_3$: MeOH: 8: 2). The fractions of tube No. 30—50 were combined and concentrated to give 29.8 mg of the residue, the acetate of which was presumed to be a mixture consisting of both epimers of lactol acetate. NMR ($CDCl_3$) $\delta$: 7.60 (1H, d, $J$=2 Hz, H-3), 6.42—6.60 (1H, m, H-7), 1.95—2.17 (3H each, s, 5×$OCOCH_3$). The fractions of tube No. 10—20 gave 13.0 mg of the residue, which was left unexamined.

**Preparation of (Carbo-$^{14}$C-methoxy)-secologanin (10)**——To a solution of secologanic acid (**16**) (40.2 mg) in a mixture of water (1.5 ml) and dioxane (5 ml) was added $^{14}CH_2N_2$–ether generated from N-nitroso-$\beta$-($^{14}$C)-methylaminoisobutyl methyl ketone (27.5 mg), which was derived from $^{14}CH_3NH_2$–HCl (0.5 mCi), and its carrier (16.7 mg) and the mixture was left standing for 20 sec. After an addition of AcOH (3 drops), the solvent was removed *in vacuo* giving a colorless syrup (44.0 mg), which was identified with an authentic sample of secologanin (**10**) by TLC ($CHCl_3$: MeOH 8: 2). PPC of the substance gave a single radioactive spot (*Rf* 0.81). The specific activity was $7.25\times10^8$ dpm/mmole.

**Administration of (Carbo-$^{14}$C-methoxy)-secologanin (10) to *Cornus officinalis* and Isolation of Morroniside (4)**——(Carbo-$^{14}$C-methoxy)-secologanin (**10**) (22.0 mg, spec. activity $7.25\times10^8$ dpm/mmole) was dissolved in water (2 ml) and administered hydroponically to a twig of *C. officinalis* plant (about 15 cm in length with five leaves and fourteen fruits) in July. Five days after the beginning of the administration, the fruits (wet weight 14 g) were treated in a conventional way giving syrupy morroniside (**4**) (111.7 mg), which was acetylated with 1.2 ml each of $Ac_2O$ and pyridine. The reaction product was recrystallized from ether–petr. ether yielding morroniside pentaacetate (**12**) (101 mg) as colorless needles. This substance was recrystallized repeatedly from the same solvent mixture to constant activity, mp 149°.

**Administration of (10-$^3$H)-7-Deoxyloganic Acid (1) to *Jasminum primulinum* and Isolation of Jasminin (6)**——(10-$^3$H)-7-Deoxyloganic acid (**1**) (9.67 mg, spec. activity $1.99\times10^{11}$ dpm/mmole) was dissolved in water (1 ml) and administered hydroponically to three twigs of *J. primulinum* plants (about 20 cm in length) in May. Seven days after the beginning of the administration, the plants (wet weight 4.1 g) were extracted with three 100 ml portions of MeOH under reflux. The MeOH extracts were combined and the solvent was removed *in vacuo*. The residue was dissolved in water (30 ml) and the insoluble material was removed by filtration through a celite layer. Being concentrated to about 10 ml *in vacuo*, the filtrate was extracted with four 20-ml portions of AcOEt. The AcOEt extracts were combined, dried over anhyd. $MgSO_4$ and the solvent was removed *in vacuo*. The residue was chromatographed on silica gel (10 g, 1.2×10 cm) eluted successively with $CHCl_3$ (fr. No. 1—3), $CHCl_3$: MeOH (98: 2. fr, No. 4—5), $CHCl_3$: MeOH (96: 4, fr. No. 6—7), $CHCl_3$: MeOH (95: 5, fr. No. 8—12) and $CHCl_3$: MeOH (93: 7, fr. No. 13—16) collecting 50 ml fractions. Fr. No. 13—16 were combined and concentrated *in vacuo* to give 34.1 mg of the residue, which was acetylated with $Ac_2O$ (0.7 ml) and pyridine (0.5 ml). The reaction product was chromatographed on silica gel (7 g, 1.2×7 cm) eluted with $CHCl_3$ (fr. No. 1—8) and then with $CHCl_3$: MeOH (99: 1, fr. No. 9—14) collecting 30 ml fractions. The combined fr. No. 10—13 were concentrated *in vacuo* to give crystalline compound (41.9 mg), which was identified with an authentic sample of jasminin tetraacetate by TLC (ether). It was further recrystallized repeatedly from EtOH–water to constant activity.

**Administration of (10-$^3$H)-Loganin (2) to *Jasminium primulinum* and Isolation of Jasminin (6)**——(10-$^3$H)-Loganin (**2**) (44.1 mg, spec. activity $1.72\times10^7$ dpm/mmole) was dissolved in water (2 ml) and administered hydroponically to three twigs of *J. primulinum* plants (about 20 cm in length) in May. Four days after the beginning of the administration, the plants (wet weight 2.6 g) were treated as described above and radioactive jasminin tetraacetate (21.0 mg) was obtained as colorless needles, mp 124—125°.

**Administration of (Carbo-$^{14}$C-methoxy)secologanin (10) to *Olea europaea* and Isolation of Oleuropein (5)**——(Carbo-$^{14}$C-methoxy)-secologanin (**10**) (22.0 mg, spec. activity $7.25\times10^8$ dpm/mmole) was dissolved

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in water (2 ml) and administered hydroponically to a twig (about 20 cm in length) of *O. europaea* plant. Five days after the beginning of the administration, the plant (wet weight 8.5 g) was cut into pieces and extracted with four 70-ml portions of MeOH under reflux for 15 min. The MeOH extracts were combined and the solvent was removed *in vacuo* to give an oily residue, which was extracted four times with water (total 60 ml). The aq. extract was washed with $CHCl_3$ ($3\times30$ ml) and then concentrated *in vacuo*. The residue was chromatographed on carbon (carbon for chromatography, Wako, 10 g, $3\times8$ cm) eluted successively with water (fr. No. 1), water: EtOH (4: 1, fr. No. 2), water: EtOH (1: 1, fr. No. 3), water: EtOH (3: 7, fr. No. 4), EtOH (fr. No. 5—9) and $CH_2Cl_2$ (fr. No. 10) collecting 200 ml fractions. Fr. No. 5—10 were combined and the solvent was removed *in vacuo*. The residue was chromatographed on silica gel (15 g, $2\times12$ cm) eluted successively with $CHCl_3$ (fr. No. 1—3), $CHCl_3$: MeOH (97: 3, fr. No. 4—7), $CHCl_3$: MeOH (96: 4, fr. No. 8—10), $CHCl_3$: MeOH (95: 5, fr. No. 11—17), $CHCl_3$: MeOH (93: 7, fr. No. 18—23) and $CHCl_3$: MeOH (90: 10, fr. No. 24—28) collecting 25 ml fractions. Fr. No. 18—26 were combined and the solvent was removed *in vacuo* to give a colorless syrup (195 mg), which was identified with an authentic sample of oleuropein (**5**) by TLC ($CHCl_3$: MeOH 8: 2). This substance was acetylated with $Ac_2O$ and pyridine (2 ml each) and then chromatographed on silica gel with ether as eluent giving a colorless syrup (284 mg), which was identified with an authentic sample of oleuropein hexaacetate (TLC, ether). This substance was further purified by column chromatography (silica gel, ether) to constant activity.

**Reduction of Hemiketal (25) with $NaB^3H_4$**——To a solution of hemiketal (**25**) (350 mg) in dioxane (11 ml) was added a suspension of $NaB^3H_4$ (25 mCi) and carrier $NaBH_4$ (175 mg) in dioxane containing 12.5% water (5 ml) and stirred at room temperature for 1 hr. After decomposition of the excess reagent with AcOH, water (50 ml) was added to the reaction mixture and extracted with three 50 ml portions of $CHCl_3$. The $CHCl_3$ extracts were combined and dried over anhyd. $MgSO_4$. After evaporation of the solvent *in vacuo*, the resulting residue was chromatographed on silica gel (15 g, $1.5\times17$ cm) with ether as eluent, collecting 20 ml fractions. Fr. No. 16—26 were combined and the solvent was removed *in vacuo*. The residue was recrystallized from EtOH to give colorless needles (24.0 mg), mp 142—144°, which were identified with an authentic sample of diol-I (**22**) by mixed melting point and TLC (ether). Evaporation of the solvent *in vacuo* from the combined fr. No. 32—44 gave a colorless syrup (39.6 mg), which was identified with an authentic sample of diol-II (**26**) by TLC (ether).

**Preparation of (8-$^3$H)-8-Epikingiside (18) from (8-$^3$H)-Diol-I (22)**——To a solution of (8-$^3$H)-diol-I (**22**) (24.0 mg) and carrier (71.4 mg) in anhyd. pyridine (2 ml) was added trityl chloride (136 mg) and the mixture was stirred at room temperature overnight. Ice water (20 ml) was added to the reaction mixture and the resulting white precipitate was extracted with three 20 ml portions of $CHCl_3$. The $CHCl_3$ extracts were combined, dried over anhyd. $MgSO_4$ and concentrated *in vacuo* to dryness. After acetylation of the residue with $Ac_2O$ and pyridine (2 ml each), the resulting product was dissolved in acetone (10 ml) and worked up with Jones reagent (1 ml) at room temperature for 2 hr. The reaction mixture was diluted with water (50 ml), extracted with $CHCl_3$ ($3\times30$ ml) and the $CHCl_3$ extract was dried over anhyd. $MgSO_4$ and the solvent was removed *in vacuo*. The residue was chromatographed on silica gel (20 g, $2.5\times11$ cm) with ether as eluent collecting 20 ml fractions. The combined fr. No. 5—9 were concentrated *in vacuo* to give a colorless syrup (69.4 mg), which was identified with an authentic sample of carboxylic acid-I (**29**) by TLC (ether). To a solution of this compound (**29**) in MeOH (5 ml) were added a saturated methanolic $Ba(OH)_2$ and a few drops of water to adjust the pH of the solution to 10. After standing at room temperature for 1.5 hr, the reaction mixture was treated with Amberlite IR-120 to adjust the pH to 3. For the sake of completing lactonization, the mixture was left standing for 30 min and then the ion exchange resin was filtered off. After concentration of the filtrate under reduced pressure, the residue (48.4 mg) was acetylated with $Ac_2O$ and pyridine (0.5 ml each) and the resulting acetate was chromatographed on silica gel (10 g, $1.5\times11$ cm) with ether as eluent collecting 15 ml fractions. Fr. No. 7—15 were combined and the solvent was removed *in vacuo*. Recrystallization of the residue from EtOH gave colorless needles (11.8 mg), mp 104—105°, which were identified with an authentic sample of 8-epikingiside tetraacetate (**21**) by mixed melting point and TLC (ether). Three recrystallizations of the radioactive compounds (**21**) diluted with carrier (48.0 mg) from EtOH showed a single radioactive spot on TLC (ether). To a solution of (8-$^3$H)-8-epikingiside tetraacetate (**21**) (27.1 mg) in MeOH (3 ml) were added a saturated methanolic $Ba(OH)_2$ and a few drops of water to adjust the pH of the solution to 10. After standing at room temperature for 1 hr, the solution was treated with Amberlite IR-120 (H-form) to adjust the pH to 3. The ion exchange resin was filtered off and the filtrate was concentrated *in vacuo* to give a colorless syrup (21.5 mg), which was identified with an authentic sample of 8-epikingiside (**18**) by TLC ($CHCl_3$: MeOH 8: 2). The specific activity was $9.45\times10^7$ dpm/mmole.

**Preparation of (8-$^3$H)-Kingiside (11) from (8-$^3$H)-Diol-II (26)**——(8-$^3$H)-Diol-II (**26**) (39.6 mg) was diluted with carrier (170 mg) and worked up in a similar manner as in the case of (8-$^3$H)-diol-I (**22**) to afford kingiside tetraacetate (**13**) (19.0 mg) as colorless needles, mp 162—163.5°, which were identical with an authentic sample (mixed melting point and TLC (ether)). This substance showed a single radioactive spot on TLC (ether). Work up of this substance (**13**) (19.0 mg) diluted with carrier (31.6 mg) in a similar way as in the case of 8-epi-compound (**21**) gave a colorless syrup (42.7 mg), which was identified with an authentic sample of kingiside (**11**) by TLC ($CHCl_3$: MeOH 8: 2). The specific activity was $2.06\times10^8$ dpm/mmole.

**Administration of (8-$^3$H)-8-Epikingiside (18) to *Jasminum primulinum* and Isolation of Jasminin (6)**——

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(8-$^3$H)-8-Epikingiside (**18**) (21.5 mg, spec. activity $9.45 \times 10^7$ dpm/mmole) was dissolved in water (2 ml) and administered hydroponically to a twig of *J. primulinium* plant (about 20 cm in length) in May. Eight days after the beginning of the administration, the plant (wet weight 3.2 g) was worked up as above to give jasminin (**6**) (33.5 mg), which was diluted with carrier (30.9 mg). This substance (56.7 mg) was acetylated with $Ac_2O$ and pyridine and the resulting acetate was recrystallized from EtOH to constant activity.

**Administration of (8-$^3$H)-Kingiside (11) to *Jasminum primulinum* and Isolation of Jasminin (6)**——(8-$^3$H)-Kingiside (**11**) (42.7 mg, spec. activity $2.06 \times 10^8$ dpm/mmole) was dissolved in water (2 ml) and administered to a twig of *J. primulinum* plant (about 15 cm in length) in the same way as described above. Conventional work up of the plant (wet weight 2.5 g) gave jasminin (**6**) (29.4 mg), which was diluted with carrier (44.2 mg) and acetylated. The resulting acetate was recrystallized from EtOH to constant activity.

**Preparation of 8-Epikingisidic Acid (27) and Its Methylation**——8-Epikingiside tetraacetate (**21**) (568 mg) was mixed with aq. 0.5 N NaOH (50 ml) and stirred at room temperature for 2 hr. The reaction solution was acidified with Amberlite IR-120 (H-form) to adjust the pH to 3. The ion exchange resin was filtered off and the filtrate was concentrated *in vacuo*. The residue was subjected to the counter current distribution (charge 335 mg, 376 transfers). The fractions of tube No. 100—140 were combined and concentrated *in vacuo* to give 8-epikingisidic acid (**27**) as a white powder (78.0 mg). Treatment of this substance (23.7 mg) dissolved in a mixture of MeOH (0.5 ml) and dioxane (1 ml) with $CH_2N_2$–ether gave 8-epikingiside (**18**). NMR ($D_2O$) $\delta$: 7.60 (1H, s, H-3), 5.58 (1H, d, $J$=6 Hz, H-1), 3.75 (3H, s, $COOCH_3$), 1.58 (3H, d, $J$=6.5 Hz, $CH_3$-10). This compound (**18**) was acetylated with $Ac_2O$ and pyridine (0.2 ml each) and recrystallized from EtOH to give colorless needles, mp 104—105°, which were identified with an authentic sample of 8-epikingiside tetraacetate (**21**) by mixed melting point, IR (Nujol) and NMR ($CDCl_3$). *Anal.* Calcd. for $C_{25}H_{32}O_{15}$: C, 52.44; H, 5.63. Found: C, 52.25; H, 5.62.

**Preparation of (Carbo-$^{14}$C-methoxy)-8-epikingiside (18)**——To a solution of 8-epikingisidic acid (**27**) (30.0 mg) in water (0.5 ml) and dioxane (2 ml) was added $^{14}CH_2N_2$–ether generated from N-nitroso-$\beta$-($^{14}$C)-methyl aminoisobutylmethyl ketone (33.5 mg, about 0.5 mCi) and the mixture was left standing for 10 min. After concentration of the reaction mixture, the residue was purified by preparative TLC (silica gel, 2 mm in thickness, $CHCl_3$: MeOH 8: 2) to give a colorless syrup (28.3 mg), which was identified with an authentic sample of 8-epikingiside (**18**) by TLC ($CHCl_3$: MeOH 8: 2). This substance showed a single radioactive spot on PPC, *Rf* 0.59. The specific activity was $1.47 \times 10^8$ dpm/mmole.

**Preparation of Kingisidic Acid (28)**——Kingiside tetraacetate (**13**) (1.5 g) was mixed with aq. 0.5 N NaOH (150 ml) and stirred at room temperature for 1.5 hr. The reaction mixture was then worked up according to the procedure employed at the preparation of 8-epikingisidic acid (**27**). The reaction product was subjected to the counter current distribution. After 873 transfers, the fractions of tube No. 170—204 were combined and concentrated *in vacuo* to give kingisidic acid (**28**) as a white powder (320 mg). *Anal.* Calcd. for $C_{16}H_{22}O_{11} \cdot 1/2H_2O$: C, 48.16; H, 5.81. Found: C, 48.33; H, 6.06. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 235 (3.92). IR $\nu_{max}^{Nujol}$ cm$^{-1}$: 3500—3050, 1710, 1690, 1640. NMR ($D_2O$) $\delta$: 7.58 (1H, s, H-3), 5.70 (1H, d, $J$=4 Hz, H-1), 1.50 (3H, d, $J$=7 Hz, $CH_3$-10). Fr. No. 221—255 were combined and concentrated *in vacuo*. The residue was recrystallized from EtOH giving colorless needles of kingiside (**11**), mp 122—123°, yield 63.0 mg. *Anal.* Calcd. for $C_{17}H_{24}O_{11} \cdot H_2O$: C, 48.34; H, 6.21. Found: C, 48.04; H, 6.52. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 236 (4.04). IR $\nu_{max}^{Nujol}$ cm$^{-1}$: 3500—3200, 1725, 1700, 1635. NMR ($D_2O$) $\delta$: 7.55 (1H, s, H-3), 5.70 (1H, d, $J$=4.5 Hz, H-1), 3.75 (3H, s, $COOCH_3$), 1.50 (3H, d, $J$=7 Hz, $CH_3$-10).

**Preparation of (Carbo-$^2$H-methoxy)-kingiside (11)**——To a solution of kingisidic acid (**28**) (49.7 mg) in $D_2O$ (99.75%, 1 ml) and anhyd. dioxane (1 ml) was added anhyd. $CH_2N_2$–ether[20] under ice cooling and left standing at room temperature for 10 min. After an addition of AcOH (a few drops), the reaction mixture was concentrated *in vacuo*. The residue was chromatographed on silica gel (14 g, 1.5 × 14 cm) eluted successively with $CHCl_3$ (fr. No. 1—6), $CHCl_3$: MeOH (97: 3, fr. No. 7—10), $CHCl_3$: MeOH (95: 5, fr. No. 11—20), $CHCl_3$: MeOH (93: 7, fr. No. 21—30) and $CHCl_3$: MeOH (90: 10, fr. No. 31—35). Fr. No. 27—33 were combined and the solvent was removed *in vacuo* to give a colorless syrup, yield 28.7 mg, which was identified with an authentic sample of kingiside (**11**) by TLC ($CHCl_3$: MeOH 8: 2). NMR ($D_2O$) $\delta$: 7.58 (1H, s, H-3), 5.70 (1H, d, $J$=4.5 Hz, H-1), 3.75 (2H, s, $COOCH_2D$), 1.50 (3H, d, $J$=7 Hz, $CH_3$-10).

**Preparation of (Carbo-$^3$H-methoxy)-kingiside (11)**——To a solution of kingisidic acid (**28**) (42.4 mg) in $^3H_2O$ (454 mCi/ml, 0.05 ml) and anhyd. dioxane (1 ml) was added anhyd. $CH_2N_2$–ether and worked up in a similar way as mentioned above. After decomposition of the excess $CH_2N_2$ on an addition of AcOH the solvent was removed *in vacuo*. Water (2 ml) was then added to the residue and the solvent was removed *in vacuo*. After five repetitions of this procedure, the resulting residue was chromatographed on silica gel (15 g, 1.5 × 15 cm) eluted successively with $CHCl_3$ (fr. No. 1—5), $CHCl_3$: MeOH (97: 3, fr. No. 6—9), $CHCl_3$: MeOH (95: 5, fr No. 10—13), $CHCl_3$: MeOH (93: 7, fr. No. 14—19) and $CHCl_3$: MeOH (90: 10, fr. No. 20—27). Fr. No. 17—26 were combined and the solvent was removed *in vacuo*. The residue was purified twice by preparative TLC (silica gel, 2 mm in thickness, $CHCl_3$: MeOH 8: 2) giving a colorless syrup (25.1 mg),

20) An ethereal solution of $CH_2N_2$ was dried over KOH tablets in a refrigerator overnight.

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which was identified with kingiside (**11**) by TLC ($CHCl_3$: MeOH 8: 2). The specific activity was $2.92 \times 10^8$ dpm/mmole. This substance showed a single radioactive spot on PPC, *Rf* 0.54.

**Administration of (Carbo-$^{14}$C-methoxy)-8-epikingiside (18) and (Carbo-$^{3}$H-methoxy)-kingiside (11) to *Olea europaea* and Isolation of Oleuropein (5)**——A mixture of (carbo-$^{14}$C-methoxy)-8-epikingiside (**18**) (28.3 mg, spec. activity $1.47 \times 10^8$ dpm/mmole) and (carbo-$^{3}$H-methoxy)-kingiside (**11**) (25.1 mg, spec. activity $2.92 \times 10^8$ dpm/mmole) ($^{3}H/^{14}C = 1.97$) was dissolved in water (2 ml) and administered hydroponically to a twig (about 20 cm in length) of *O. europaea* plant. The plant (wet weight 8 g) was worked up in a similar manner as above to give oleuropein (**5**) (41.6 mg) as a colorless syrup. Conventional acetylation of this substance gave oleuropein hexaacetate as a colorless syrup (44.7 mg), which was purified by column chromatography (silica gel, ether) to constant activity. The $^{3}H/^{14}C$ value of the acetate was 1.82.

**Acknowledgement** This work was partly supported by a grant from the Takeda Science Foundation. The authors are grateful to Dr. Hozumi and his co-workers, the Microanalytical Center of this University for elemental analyses, to Dr. T. Shingu and Miss M. Ohkawa of this Faculty for NMR measurements, and Professor I. Nishioka, Kyushu University, Drs. M. Goto and T. Matsuoka, Kyoto Herbal Garden, Takeda Chemical Industries, Ltd., and Mr. K. Kobata, the Kyoto Botanical Garden, for their kind supply of the plant materials.

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