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Studies on Monoterpene Glucosides. XII.[1] Biosynthesis of Gentianaceous Secoiridoid Glucosides[2]

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DL-Mevalonolactone-2-^{14}C was administrated to *Gentiana triflora* and *Swertia japonica*. Degradation of labelled gentiopicroside (I) from *Gentiana triflora* and swertiamarin (II) and sweroside (III) from *Swertia japonica* established that these compounds are monoterpenoid glucosides synthesized biologically *via* mevalonic acid.

In the preceding paper,[1] we reported the absolute structures of the gentianaceous bitter glucosides, gentiopicroside (I), swertiamarin (II), and sweroside (III).

As previously mentioned,[1] the carbon skeleton of these glucosides corresponds to that of iridoid glucoside, the cyclopentane ring of which was cleaved between C-7 and C-8. The non-tryptophan derived portion of many indole alkaloids also has the same carbon skeleton as the aglycon of these bitter glucosides. Therefore, the biological pathways for synthesis of these glucosides are always considered together with those of indole alkaloids, and several possible pathways, such as the prephenic acid-,[4] terpenoid-,[5] and acetate–malonate–pathway[6] have been suggested. Since 1965 the terpenoid theory has gradually gained acceptance through experiments on the effects of administration of mevalonic acid[7] and geraniol[8] on indole alkaloids and recently loganin, an iridoid glucoside, was also shown to be incorporated into the alkaloids.[9] However, when this work was started there was no report on the biosynthesis of gentianaceous glucosides.[10]

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1) Part XI: H. Inouye, T. Yoshida, Y. Nakamura, and S. Tobita, *Chem. Pharm. Bull.* (Tokyo), **18**, 1889 (1970).
2) A preliminary report of part of this work has been published. H. Inouye, S. Ueda, and Y. Nakamura, *Tetrahedron Letters*, **1967**, 3221.
3) Location: *Yoshida–Shimoadachi–cho, Sakyo–ku, Kyoto.*
4) E. Wenkert and N.V. Bringi, *J. Am. Chem. Soc.*, **81**, 1474 (1959); E. Wenkert, *Experientia*, **15**, 165(1959).
5) R. Thomas, *Tetrahedron Letters*, **1961**, 544; E, Wenkert, *J. Am. Chem. Soc.*, **84**, 98 (1962).
6) E. Leete and S. Ghosal, *Tetrahedron Letters*, **1962**, 1179.
7) T. Money, I.G. Wright, F. McCapra, and A.I. Scott, *Proc. Natl. Acad. Sci. U.S.*, **53**, 901 (1965); F. McCapra, T. Money, A.I. Scott, and I.G. Wright, *Chem. Commun.*, **1965**, 537; H, Goeggel and D. Arigoni, *ibid.*, **1965**, 538; A.R. Battersby, R.T. Brown, R.S. Kapil, A.O. Plunkett, and J.B. Taylor, *ibid.*, **1965**, 538.
8) E. Leete and S. Ueda, *Tetrahedron Letters*, **1966**, 4915; A.R. Battersby, R.T. Brown, J.A. Knight, J.A. Martin, and A.O. Plunkett, *Chem. Commun.*, **1966**, 346; P. Loew, H. Goeggel, and D. Arigoni, *ibid.*, **1966**, 347; E.S. Hall, F. McCapra, T. Money, K. Fukumoto, J.R. Hanson, B.S. Mootoo, G.T. Philips, and A.I. Scott, *ibid.*, **1966**, 348; T. Money, I.G. Wright, F. McCapra, E.S. Hall, and A.I. Scott, *J. Am. Chem. Soc.*, **90**, 4144 (1968).
9) A.R. Battersby, R.T. Brown, R.S. Kapil, J.A. Martin, and A.O. Plunkett, *Chem. Commun.*, **1966**, 890; A.R. Battersby, R.S. Kapil, J.A. Martin, and L. Mo, *ibid.*, **1968**, 133; P, Loew and D. Arigoni, *ibid.*, **1968**, 137.
10) While we were preparring the manuscript of a short communication of part of this work, the report of Coscia appeared showing that mevalonic acid was incorporated into gentiopicroside (I) in *Swertia caroliniensis* (*cf.* C.J. Coscia and R. Guarnaccia, *J. Am. Chem. Soc.*, **89**, 1280 (1967)). Recently three research groups independently prepared labelled loganin or loganic acid and administrated them to *Swertia caroliniensis*, *Swertia petiolata* and *Gentiana scabra* and obtained specific labelling of gentiopicroside (I) isolated from these plants (*cf.* R. Guarnaccia, L. Botta, and C.J. Coscia, *J. Am. Chem. Soc.*, **91**, 204 (1969); D. Gröger and P. Simchen, *Zeitschrift für Naturforschung*, **24b**, 356 (1969); H. Inouye, S. Ueda, Y. Aoki, and Y. Takeda, *Tetrahedron Letters*, **1969**, 2351).

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On feeding mevalonic acid to *Gentiana triflora* Pall. var. *japonica* (Kusnez.) Hara (Japanese name "Ezorindo") and *Swertia japonica* Makino (Japanese name "Semburi") we found that these gentianaceous bitter glucosides are monoterpenoid glucosides formed from mevalonic acid.

An aqueous solution of DL-mevalonolactone-2-^{14}C was administrated by the cotton wick method to *Gentiana triflora* plants during their flowering period. The plants were harvested after seven days and radioactive gentiopicroside (I) was isolated from the shoots and roots, respectively. I was then converted to its tetraacetate (IV) and recrystallized to give material of constant activity. The specific activities of IV from the shoots and roots were 1.90×10^5 and 4.89×10^3 dpm/m mole, respectively. The total incorporation of radioactivity was about 0.06%.

The labelled gentiopicroside tetraacetate (IV) was treated with ammonia and then with dil. hydrochloric acid to give gentianine (VII)[11] which had the same specific activity as gentio-

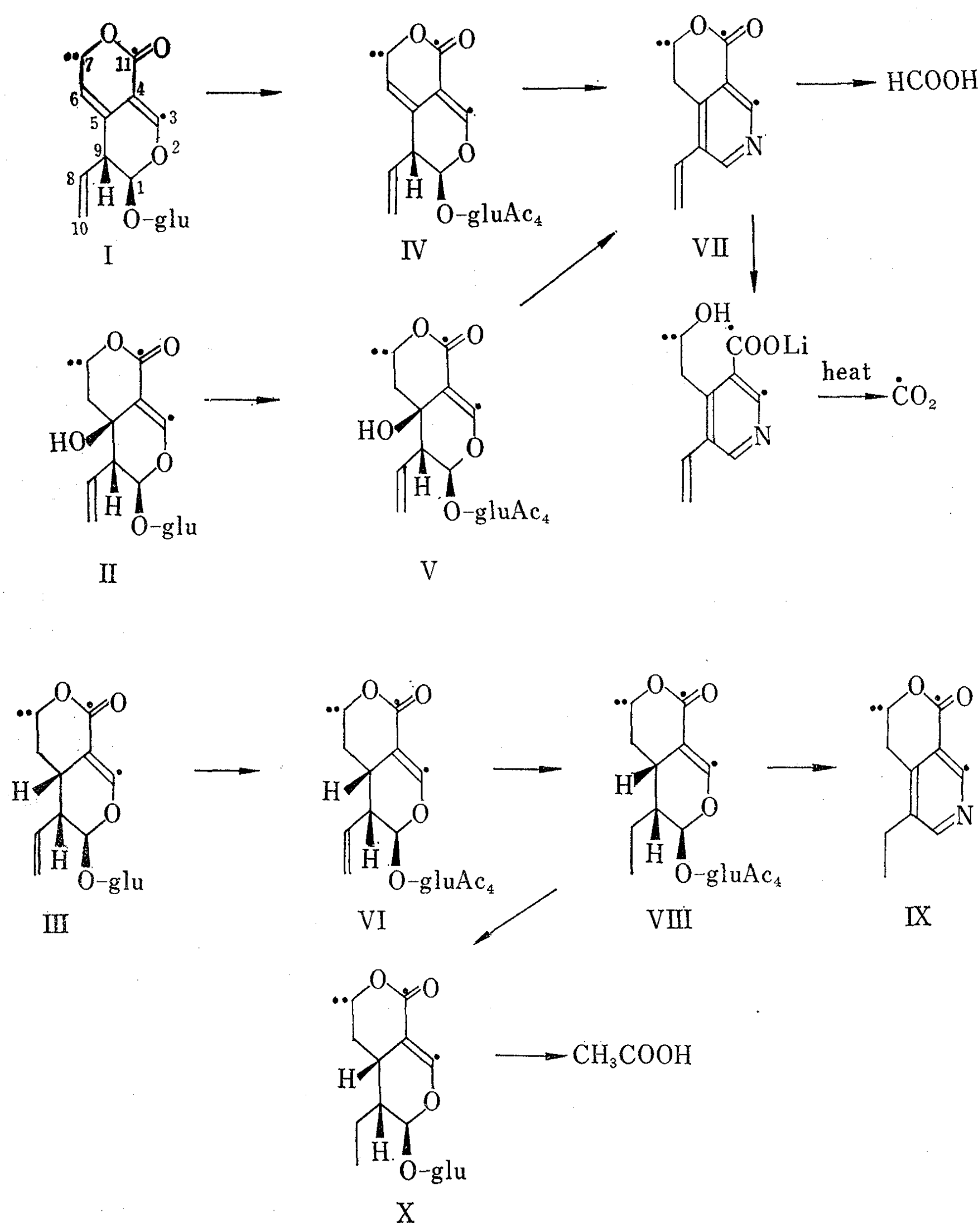

Chart 1. Degradation of Radioactive Glucosides and Their Derivatives

11) *a*) T. Kubota and Y. Tomita, *Bull, Chem. Soc. Japan*, **34**, 1345 (1961); *b*) T. Kubota and T. Kamikawa, *ibid.*, **35**, 1046 (1962); *c*) H. Inouye, S. Ueda, and N. Shimokawa, *Yakugaku Zasshi*, **86**, 1202 (1966).

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picroside (I). The compound (VII) was converted to its lithium salt and submitted to pyrolysis in a stream of nitrogen. The resulting carbon dioxide contained 20.1% of the radioactivity of the original gentianine (VII). VII was further submitted to Kuhn–Roth oxidation and the resulting formic acid was converted to N-formyl-α-naphthylamine which was no longer radioactive. These results show that mevalonic acid was specifically incorporated into gentiopicroside (I).

The fact that carbon dioxide derived from the C-11 carbon contained 20.1% of the total activity in gentianine (VII) or gentiopicroside (I) explains the occurrence of randomization between the C-11 and C-3 carbons in the biosynthesis of the glucoside.

This isotope distribution is in accord with that found in plumieride by Schmid[12] and in indole alkaloids by Scott, Battersby, Arigoni and their respective coworkers.[7]

Schmid suggested that randomization of radioactivity between the C-11 and C-3 carbons occurred after formation of the iridoid skeleton. Observation of this same labelling pattern in iridoid, secoiridoid, and indole alkaloid would provide strong support for the biosynthetic route from iridoid to indole alkaloid *via* secoiridoid.

Table I. Specific Activities of Gentianaceous Secoiridoid Glucosides and Their Derivatives

Gentiana triflora var. *japonica*

	Specific activity dpm/m mole	Incorporation ratio %
(shoots)		
Gentiopicroside (I)		0.06
Gentiopicroside tetraacetate (IV)	1.90×10^5	
Gentianine (VII)	1.98×10^5	
$BaCO_3$	4.00×10^4	
HCOOH	0	
(roots)		
Gentiopicroside (I)		0.004
Gentiopicroside tetraacetate (IV)	4.89×10^3	

Swertia japonica

	Specific activity dpm/m mole	Incorporation ratio %
Swertiamarin (II)		0.006
Swertiamarin tetraacetate (V)	3.18×10^4	
Gentianine (VII)	3.15×10^4	
Sweroside (III)		0.06
Sweroside tetraacetate (VI)	4.37×10^5	
Dihydrosweroside tetraacetate (VIII)	4.30×10^5	
Dihydrogentianine (IX)	4.12×10^5	
CH_3COOH	0	

DL-Mevalonolactone-2-^{14}C was administrated by the cotton wick method to *Swertia japonica* plants during their flowering period. After seven days the plants were harvested and a mixture of swertiamarin (II) and sweroside (III) was obtained. This mixture was acetylated and chromatographed over silica gel to give swertiamarin tetraacetate (V) and sweroside tetraacetate (VI). The specific activity of sweroside tetraacetate (VI) was 4.37×10^5 dpm/m mole (incorporation, 0.06%) and that of swertiamarin tetraacetate (V) was 3.18×10^4 dpm/m mole (incorporation, 0.006%), which was about one fourteenth of that of VI. This result suggested that sweroside (III) might be a precursor of swertiamarin (II).

12) D.A. Yeowell and H. Schmid, *Experientia*, **20**, 250 (1964).

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Swertiamarin tetraacetate (V) isolated as mentioned above was treated with aqueous ammonia and then with hydrochloric acid to give gentianine (VII) with the same specific activity as that in V. Catalytic hydrogenation of sweroside tetraacetate (VI) over palladized charcoal afforded dihydrosweroside tetraacetate (VIII), which also had the same specific activity as sweroside tetraacetate (VI). On treatment with aqueous ammonia and then with hydrochloric acid VIII was converted to dihydrogentianine (IX) retaining all its radioactivity. Substance VIII was subjected to the Zemplén reaction followed by Kuhn–Roth oxidation to yield acetic acid which was counted as its α-naphthylamine derivative and found not to be radioactive. These results demonstrate the specific incorporation of mevalonic acid into sweroside (III) and swertiamarin (II) as well as gentiopicroside (I). The fact that the acetic acid obtained by Kuhn–Roth oxidation of dihydrosweroside (X) was not radioactive indicates that the C-8 and C-10 carbons are not radioactive. This is compatible with the prediction that 50% of the radioactivity is present in the C-7 carbon.

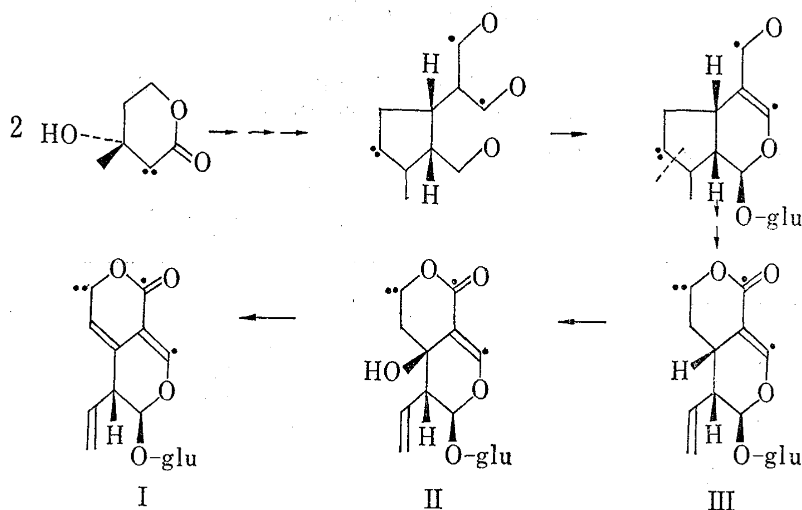

Chart 2. Biosynthetic Pathway of Gentianaceous Secoiridoid Glucosides

These data of experiments using *Gentiana triflora* and *Swertia japonica* plants show that I, II, and III are monoterpenoids originating from mevalonic acid and suggest that gentiopicroside (I) is derived from sweroside (III) *via* swertiamarin (II).[13] The conversion of II to I in plants is highly probable since structurally I is the dehydrated form of II, and II is actually transformed into I[11a)] *in vitro*.

Experimental[14)]

Administration of DL-Mevalonolactone-2-^{14}C to *Gentiana triflora* PALL. var. *japonica* (KUSNEZ.) HARA—DL-mevalonolactone-2-^{14}C (0.15 mCi, specific activity 5.03 mCi/m mole) was dissolved in H_2O (3 ml) and the

13) The biosynthetic route from III to I was demonstrated by a feeding experiment using 10-^{14}C-sweroside. *cf.* H. Inouye, S. Ueda, and Y. Takeda, *Tetrahedron Letters*, **1968**, 3453. Further details will be reported in the forthcoming paper.

14) Melting points were determined in a Yanagimoto Micro Melting Point Apparatus and were not corrected. Paper chromatography was carried out on Toyo Roshi No. 50 filter paper with 10% NaOH–95% EtOH (1: 10 v/v) as solvent. Spots were detected by spraying the paper with a solution of bromocresol blue (50 mg) and citric acid (200 mg) in 100 ml of H_2O. Silica gel G–, or Alumina G–plates (acc. to Stahl) were used for thin-layer chromatography (TLC). Spots were detected by exposing the plates to iodine vapour. All ^{14}C-labelled compounds were counted in a Nuclear Chicago Model 6807 scintillation counter, the samples being dissolved in scintillation mixture consisting of toluene (10 ml), 2,5-diphenyloxazole (PPO) (40 mg), and 2-*p*-phenylene-bis(5-phenyloxazole) (POPOP) (0.5 mg) (*cf.* E. Rapkin, *Anal. Biochem.*, **1960**, 1279). DL-Mevalonolactone-2-^{14}C was supplied by Daiichi Pure Chemicals Co. Ltd.

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aqueous solution was placed in glass tubes and administered by the cotton wick method to seven *Gentiana triflora* var. *japonica* plants (*ca.* 50 cm in height) during their flowering period in August. After three days when the solution had been absorbed into the plants more H_2O (3 ml) was put into the glass tubes so that all the remaining radioactive material could be absorbed by the plants. Seven days from the beginning of the administration period, the plants were harvested and their shoots (60 g) and roots (80 g) respectively were combined.

Extraction and Isolation of Glucosides from *Gentiana triflora* var. *japonica*——The roots were cut into pieces and extracted with five 200 ml portions of hot MeOH. The extract was concentrated *in vacuo* and the residue was dissolved in 100 ml of H_2O. The aqueous solution was then extracted with four 50 ml portions of *n*-BuOH. The *n*-BuOH extract was washed with H_2O and concentrated to dryness *in vacuo* and then dissolved in 100 ml of H_2O. Insoluble material was removed by filtration through a layer of celite. The filtrate was extracted with four 40 ml portions of AcOEt and then with four 50 ml portions of *n*-BuOH. The *n*-BuOH extract was concentrated to dryness *in vacuo* and the residue was chromatographed on a charcoal column (3×4 cm, consisting of 4 g each of charcoal and celite) with MeOH as eluent. The eluate was concentrated to dryness *in vacuo* and the residue was recrystallized from EtOH to give colorless needles, mp 116—117°, which were shown to be identical with gentiopicroside (I) by measuring the mixed melting point and TLC (SiO_2 eluted with $CHCl_3$: MeOH (7: 3 v/v)). This material was acetylated by the usual method with Ac_2O and pyridine and the resulting acetate was recrystallized from EtOH to give 712 mg of gentiopicroside tetraacetate (IV) as colorless needles, mp 138—139°. This was also identified with an authentic sample by the mixed melting point and TLC (SiO_2 eluted with ether). The specific activity was 4.89×10^3 dpm/m mole.

The shoots of this plants were treated in the same way to give crude gentiopicroside (I), which was acetylated and the resulting acetate was chromatographed on a charcoal column (2.7×2.2 cm, consisting of 2 g each of charcoal and celite) with EtOH as eluent. The eluate was concentrated to dryness *in vacuo* and the residue was recrystallized from EtOH to give 275 mg of fine colorless needles, mp 137—138°. The specific activity was 1.90×10^5 dpm/m mole.

Treatment of Gentiopicroside Tetraacetate (IV) with Ammonia and HCl (Formation of Gentianine (VII))——To a solution of gentiopicroside tetraacetate (IV) obtained from the shoots of *Gentiana triflora* var. *japonica* (130 mg, specific activity 1.90×10^5 dpm/m mole) in 6 ml of MeOH was added conc. NH_4OH (7 ml) at room temperature. After standing the mixture for six days the solvent was removed *in vacuo*. Then 17.5 ml of 5% HCl were added to the residue and the mixture was heated on a water bath for 1 hr. It was made alkaline with conc. NH_4OH and the resulting base was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O and dried over anhydrous K_2CO_3. Removal of the solvent yielded 9 mg of crude base, which was chromatographed on a silica gel column (Mallinckrodt 3 g, 1.5×4 cm) with $CHCl_3$ as eluent and then was recrystallized from a mixture of benzene and hexane to give 7.1 mg of gentianine (VII) as colorless needles, mp 82—83°. This was identified with an authentic sample by measuring the mixed melting point and by TLC (SiO_2 eluted with $CHCl_3$: MeOH (98:2 v/v)). The specific activity was 1.89×10^5 dpm/m mole.

Decarboxylation of Gentianine (VII)——To a solution of LiOH (8 mg) in MeOH (2 ml) and H_2O (2 drops), was added gentianine (VII) (50 mg, specific activity 1.98×10^5 dpm/m mole) and the mixture was heated on a water bath until all of VII had dissolved. The solvent was removed *in vacuo* and the resulting Li salt of gentianine (VII) was dried over P_2O_5 under reduced pressure. It was heated at 200—320° for 7 hr and a stream of N_2 was passed slowly over it. The CO_2 evolved was collected in an aq. solution of 0.2N $Ba(OH)_2$. The precipitate of $BaCO_3$ formed was collected by centrifugation, washed successively with H_2O, MeOH, and ether, and dried over P_2O_5 under reduced pressure. A 3.377 mg aliquot of $BaCO_3$ was mixed with 1 ml of conc. H_2SO_4 and the CO_2 evolved was trapped in a solution of "NCS" solubilizer (0.1 ml) in toluene (0.5 ml) and its radioactivity was determined by the usual method. The specific activity was 4.00×10^4 dpm/m mole, corresponding to 20.1% of the total activity in gentianine (VII).

Kuhn–Roth Oxidation of Gentianine (VII)——VII (50 mg, specific activity 1.98×10^5 dpm/m mol) was dissolved in 2N H_2SO_4 (10 ml) containing CrO_3 (3 g) and heated to distill the resulting formic acid with H_2O. During the distillation H_2O (60 ml) was added to maintain the volume of the reaction mixture at about 10—20 ml. Heating was continued for 70 min and the distillate was neutralized with aq. N/10 NaOH using phenolphthalein as an indicator. The solvent was removed *in vacuo* and an aliquot of the resulting residue was monitored by PPC. The *Rf* value (0.24) was identical with that of HCOONO. This residue (5 mg) and α-naphthylamine hydrochloride (5 mg) were dissolved in H_2O (0.5 ml) and N-ethyl-N'(3-dimethylamino-propyl)carbodiimide hydrochloride (50 mg) was then added and the mixture was stirred for 10 min. The resulting oily precipitate was extracted with ether, washed with H_2O, and dried over anhydrous $MgSO_4$, and the solvent was removed *in vacuo*. The residue was sublimed under reduced pressure to give colorless needles of N-formyl-α-naphthylamine, mp 135—138°. This compound was found to be non-radioactive.

Administration of DL-Mevalonolactone-2-^{14}C to *Swertia japonica* Makino——DL-Mevalonolactone-2-^{14}C (0.2 mCi, specific activity 5.03 mCi/m mole) was dissolved in H_2O (12.5 ml) and the solution was placed in glass tubes and administrated by the cotton wick method to twenty five *Swertia japonica* plants (*ca.* 10—20 cm in height) during their flowering period in October. Next day when the solution had been absorbed

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into the plants more H_2O (10 ml) was put into the glass tubes to allow all the remaining radioactive material to be absorbed by the plants. Seven days from the beginning of the administration, the plants were harvested.

Extraction of Glucosides from *Swertia japonica*——Whole plants were cut into pieces and extracted with three 170 ml portions of hot MeOH. The extract was concentrated *in vacuo* and the residue was dissolved in H_2O (100 ml). The aqueous solution was extracted with four 75 ml portions of AcOEt, and then with four 50 ml portions of *n*-BuOH. The *n*-BuOH layer was washed with H_2O and then concentrated *in vacuo*. The residue was chromatographed over Al_2O_3 (Woelm neutral, activity grade III, 70 g (3×12 cm)) with acetone: H_2O (1: 1 v/v) as eluent and fractions of 50 ml of eluate were collected. Fractions No. 2—5 were combined and the solvent was removed *in vacuo*. The residue was dissolved in H_2O and lyophilized to give 450 mg of crude glucoside mixture. This was acetylated by the usual method with 4 ml each of Ac_2O and pyridine. The resulting acetate was recrystallized from EtOH to give 430 mg of colorless needles, which were found to be a mixture of swertiamarin tetraacetate (V) and sweroside tetraacetate (VI) by silica gel or alumina TLC using ether as developing solvent.

Isolation of Swertiamarin Tetraacetate (V) and Sweroside Tetraacetate (VI)——A sample of 25 mg of the mixture of V and VI was chromatographed on a silica gel column (Mallinckrodt; 15 g, 1.8×13 cm) using $CHCl_3$ as eluent. Fractions No. 7—19 were combined and the solvent was removed. The residue was recrystallized from EtOH to give colorless needles, mp 167—168°, yield 9.3 mg. This material was identified with an authentic sample of sweroside tetraacetate (VI) by the mixed melting point and by silica gel or alumina TLC using ether as developing solvent. The specific activity was 4.37×10^5 dpm/m mole. Fractions No. 22—35 were combined and the solvent was removed. The residue was recrystallized from EtOH to give colorless needles, mp 192—193°, yield 13.2 mg. This compound was identified with swertiamarin tetraacetate (V) by the mixed melting point and by silica gel or alumina TLC using ether as developing solvent. The specific activity was 3.18×10^4 dpm/m mole. The remainder of the mixture of V and VI was treated in the same way. The total yields of swertiamarin tetraacetate (V) and sweroside tetraacetate (VI) were 227 mg and 160 mg, respectively.

Treatment of Swertiamarin Tetraacetate (V) with Ammonia and HCl (Formation of Gentianine (VII))——To a solution of swertiamarin tetraacetate (V) (55 mg, specific activity 3.18×10^4 dpm/m mol) in MeOH (3 ml) was added conc. NH_4OH (3.5 ml) at room temperature and the solution was allowed to stand for seven days. The solvent was removed *in vacuo* and 5% HCl (8 ml) was added to the residue. After heating on a water bath for 1 hr, the mixture was cooled and made alkaline with conc. NH_4OH. The resulting base was extracted with $CHCl_3$, washed with H_2O, and dried over anhydrous K_2CO_3 and the solvent was removed *in vacuo*. The residue was chromatographed on a silica gel column (Mallinckrodt; 3 g, 1.54×4 cm) with $CHCl_3$ as eluent. Fractions of 50 ml of eluate were collected. Fractions No. 3—6 were combined and the solvent was removed. The residue was recrystallized from a mixture of benzene and hexane to give 11 mg of gentianine (VII) as colorless needles, mp 82°. This material was identified with an authentic sample by the mixed melting point and by TLC (SiO_2 eluted with $CHCl_3$: MeOH (98: 2 v/v)). The specific activity was 3.15×10^4 dpm/m mole.

Catalytic Hydrogenation of Sweroside Tetraacetate (VI)——A solution of radioactive sweroside tetraacetate (VI) (50 mg, specific activity 4.37×10^5 dpm/m mole) in MeOH (15 ml) was shaken with H_2 in the presence of catalyst prepared by the usual method from 50 mg of activated charcoal (Norit) and 0.3 ml of 5% $PdCl_2$ solution. After the uptake of 3 ml of H_2, the reaction was stopped and the catalyst was removed by filtration. The filtrate was concentrated *in vacuo* and the residue was recrystallized from EtOH to give dihydrosweroside tetraacetate (VIII) as colorless needles, mp 177°, yield 47.8 mg. This was identified with an authentic sample by the mixed melting point and by TLC (Al_2O_3, ether). The specific activity was 4.30×10^5 dpm/m mole.

Treatment of Dihydrosweroside Tetraacetate (VIII) with Ammonia and HCl (Formation of Dihydrogentianine (IX))——To a solution of radioactive dihydrosweroside tertaacetate (VIII) (24 mg, specific activity 4.30×10^5 dpm/m mole) in MeOH (3 ml) were added conc. NH_4OH (2 ml) and $CHCl_3$ (0.5 ml) at room temperature and the solution was stood for three days. The solvent was removed *in vacuo* and 5% HCl (5 ml) was added to the residue and the mixture was heated for 30 min on a water bath. It was made alkaline with conc. NH_4OH and the resulting base was extracted into $CHCl_3$, washed with H_2O and dried over anhydrous K_2CO_3 and the solvent was removed *in vacuo*. The residue was chromatographed on a silica gel column (Mallinckrode, 3 g, 1.54×4 cm) with $CHCl_3$ (60 ml) and then with $CHCl_3$: MeOH (8: 2 v/v) as eluent. Fractions No. 4 and 5 afforded colorless needles, mp 74—76°, yield 1.2 mg, This material was identified with authentic sample of dihydrogentianine (IX) by the mixed melting point and by TLC (SiO_2), $CHCl_3$: MeOH (95: 5 v/v). The specific activity was 4.12×10^5 dpm/m mole.

Zemplén Reaction of Dihydrosweroside Tetraacetate (VIII)——To a solution of radioactive dihydrosweroside tetraacetate (VIII) (76.4 mg, specific activity 4.30×10^5 dpm/m mole) in abs. MeOH (3 ml) was added a methanolic solution of 0.1N NaOMe (0.23 ml). The reaction mixture was refluxed for 25 min and then cooled in ice water. The solution was neutralized with a small amount of Amberlite IRC 50 (H form) and filtered. The filtrate was evaporated to a colorless oily residue *in vacuo*. This was dissolved in H_2O and

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washed with $CHCl_3$ and the aqueous layer was evaporated *in vacuo* to give a white powder, yield 45.1 mg. TLC (SiO_2, $CHCl_3$: MeOH (7: 3 v/v)) gave a single spot corresponding to that of an authentic sample of dihydrosweroside (X).

Kuhn–Roth Oxidation of Dihydrosweroside (X)——Radioactive X (45.1 mg) was dissolved in 2N H_2SO_4 (10 ml) containing CrO_3 (3 g) and heated for 1 hr to distill the resulting acetic acid with H_2O. During the distillation H_2O (50 ml) was added to maintain the volume of the reaction mixture. The distillate was neutralized with N/10 NaOH and the solvent was removed *in vacuo*. PPC in the residue gave a single spot corresponding to that of sodium acetate (*Rf*: 0.22) but no spot corresponding to sodium propionate was detectable. The resulting sodium acetate and α-naphthylamine HCl (5 mg) were dissolved in H_2O (0.5 ml) and N-ethyl-N′-(3-dimethylaminopropyl)carbodiimide HCl (50 mg) was then added to the solution with stirring for 10 min. The resulting precipitate was extracted with ether, washed with H_2O and dried over anhydrous $MgSO_4$ and the solvent was removed *in vacuo*. The residue was sublimed under reduced pressure to give colorless needles of N-acetyl-α-naphthylamine, mp 155—156°. Admixture with an authentic sample gave no melting point depression. This material was non-radioactive.

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