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Studies on Monoterpene Glucosides and Related Natural Products. XIII.[1] Incorporation of [10-^{14}C]-Sweroside into Gentiopicroside and the Alkaloids in *Vinca* and *Cincona* Plants[2]

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Feeding of [10^{14}-C]-sweroside (I), prepared by the route shown in Chart 1, to plants established that this glucoside (I) was incorporated into gentiopicroside (III) and vindoline (VI). Substance I was also incorporated into reserpinine (VII) and quinine (V). These data confirmed the biosynthetic sequence of the gentianaceous secoiridoid glucosides and the biological conversion of sweroside (I) to *Vinca* and *Cinchona* alkaloids.

In the preceding paper,[1] we reported experiments on the feeding of [2-^{14}C]-mevalonic acid (MVA) to *Gentiana triflora* Pall. var *japonica* (Kusenz.) Hara (Japanese name "Ezorindo") and *Swertia japonica* Makino (Japanese name "Semburi") establishing that the gentianaceous bitter glucosides, sweroside (I), swertiamarin (II) and gentiopicroside (III) are monoterpene glucosides synthesized in the plants *via* MVA. By comparisons of the incorporation of radioactivities into these glucosides with their structures, we concluded that the biosynthetic pathway of these glucosides was as follows: MVA→→→ sweroside (I)→swertiamarin (II)→ gentiopicroside (III).

At the beginning of this work, many research groups substantiated that the non-tryptophan portion of many indole alkaloids originates from MVA[4] *via* geraniol[5] and loganin (IV)[6,7] and that quinine (V), a *Cinchona* alkaloid, is also derived from geraniol.[8] Sweroside (I) has the same skeleton as that of secologanin, the then supposed precursor of I, formed in the next step after loganin (IV) in the biosynthetic pathway of indole alkaloid. Thus it seemed likely that when this glucoside (I) was administered to plants, it must be incorporated into the alkaloids after some transformation such as oxidation of the alcohol at position C-7 to aldehyde.

Accordingly, we prepared [10-^{14}C]-sweroside (I) and administered it first to *Gentiana scabra* Bunge *var.* Buergeri (Miq.) Maxim. (Japanese name "Rindo") and then to *Vinca*

1) "Studies on Monoterpene Glucosides," I–XII by H. Inouye constitute part of this series of work. Part XII: H. Inouye, S. Ueda, and Y. Nakamura, *Chem. Pharm. Bull.* (Tokyo), **18**, 2043 (1970).
2) A preliminary report of part of this work has been published. H. Inouye, S. Ueda, and Y. Takeda, *Tetrahedron Letters*, **1968**, 3453; *idem*, *ibid.*, **1969**, 407.
3) Location: *Yoshida-Shimoadachi-cho, Sakyo-ku, Kyoto.*
4) 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.
5) 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).
6) 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.
7) P. Loew and D. Arigoni, *Chem. Commun.*, **1968**, 137.
8) E. Leete and J.N. Wemple, *J. Am. Chem. Soc.*, **88**, 4743 (1966); *idem*, *ibid.*, **91**, 2698 (1969); A.R. Battersby, R.T. Brown, R.S. Kapil, J.A. Knight, J.A. Martin, and A.O. Plunkett, *Chem. Commun.*, **1966**, 810.

rosea L., *Vinca major* L., and *Cinchona succirubra* PAVON ex. KLOTZSCH. As expected, results showed that it was a precursor of gentiopicroside (III) and that it was also incorporated into vindoline (VI), reserpinine (VII), and quinine (V).

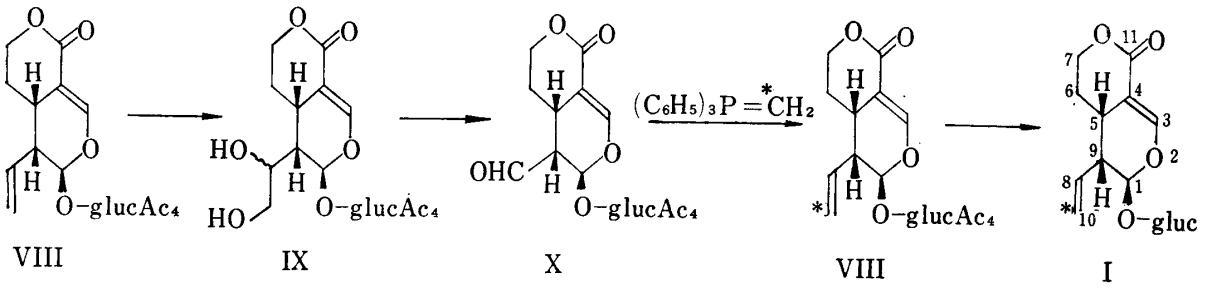

Chart 1

[10-^{14}C]-Sweroside (I) was prepared by the route shown in Chart 1. Osmium tetroxide oxidation of sweroside tetraacetate (VIII) gave the glycol (IX), $C_{24}H_{32}O_{15}3/2H_2O$, mp 148—150°. The glycol (IX) was treated with sodium periodate to afford the aldehyde (X), $C_{23}H_{28}O_{14}1/2H_2O$, as a white powder. The nuclear magnetic resonance (NMR) spectrum of X showed a doublet at 9.68 ppm (J=1.5 Hz) due to an aldehyde proton. This compound (X) was then allowed to react with methylene triphenylphosphorane to give a product, $C_{24}H_{30}O_{13}$, which was confirmed to be sweroside tetraacetate (VIII) by admixture and by comparisons of its infrared (IR) and NMR spectra with those of an authentic sample. The acetate (VIII) underwent the Zemplén reaction to regenerate sweroside (I).

After these preliminary experiments, the aldehyde (X) was subjected to the Wittig reaction with ^{14}C-methylene triphenylphosphorane to obtain [10-^{14}C]-sweroside tetraacetate (VIII), which was then deacetylated to give [10-^{14}C]-sweroside (I).

An aqueous solution of the [10-^{14}C]-sweroside (I) was administered by the cotton wick method to *Gentiana scabra* plants during the flowering period. The plants were harvested after four days and gentiopicroside (III) was isolated by the conventional method. It was then converted to the tetraacetate (XI) and recrystallized from ethanol to a constant specific activity of 2.96×10^3 dpm/mmole (40% incorporation). The radioactive compound (XI) was then subjected to ozonolysis to obtain formaldehyde, which was isolated as the crystalline

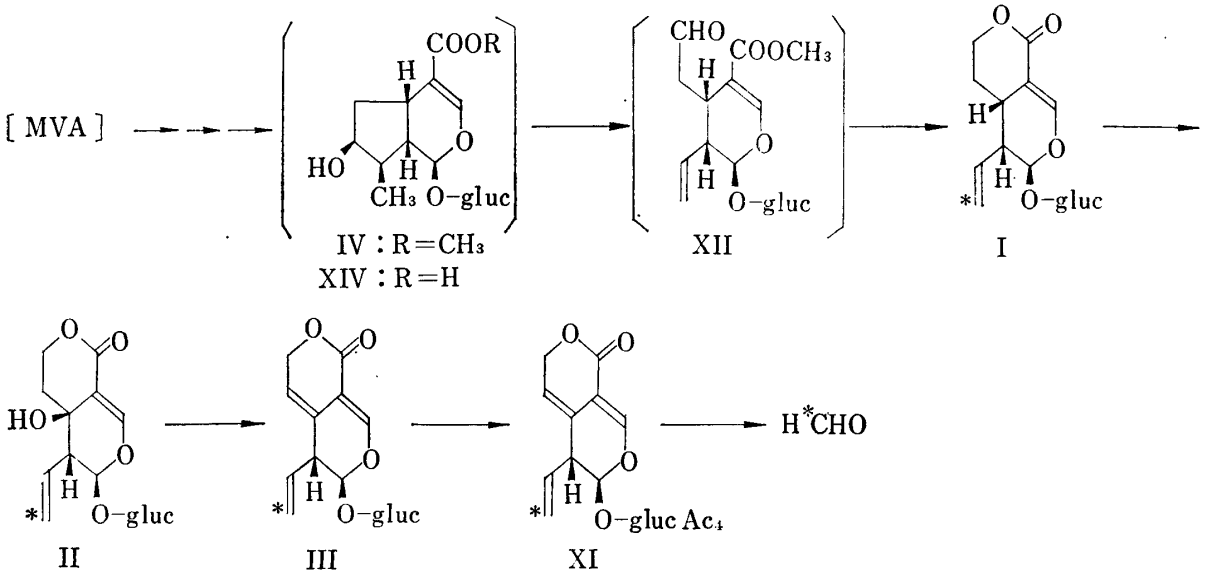

Chart 2

dimedone derivative and purified by distillation. The specific activity amounted to 2.88×10^3 dpm/mmole, corresponding to 98% of the radioactivity of the intact gentiopicroside tetraacetate (XI).

Table I. Results of the Feeding Experiments of [10-^{14}C]-Sweroside (I) to *Gentiana*, *Vinca*, and *Cinchona*-Plants

	Amt. & sp. Activity (dpm/mmole) of I fed	sp. Activity (dpm/mmole) of isolated substances & their degradation Prod.	Incorporation %
(*Gentiana scabra*)	8.7 mg 6.90×10^5		
Gentiopicroside tetraacetate (XI)		2.96×10^3	40
HCHO		2.88×10^3	
(*Vinca rosea*)	26.4 mg 1.54×10^6		
Vindoline (VI)		1.75×10^5	11
Deacetylvindoline (XIII)		1.70×10^5	
CH_3COOH from C-4 Ac		0	
CH_3COOH from C-5 Et		1.63×10^5	
(*Vinca major*)	9.4 mg 1.75×10^7		
Reserpinine (VII)		7.94×10^3	0.03
(*Cinchona succirubra*)	26.4 mg 2.09×10^6		
Quinine (V)		6.75×10^4	0.6

Thus in this plant there was a high rate of conversion of sweroside (I) to gentiopicroside (III) without randomization. Therefore, from these results and those reported previously[1,9] and from the finding of secologanin (XII)[10] in *Vinca rosea*, it is evident that these glucosides are synthesized by the route shown in Chart 2. The high incorporation of radioactivity into gentiopicroside (III) is compatible with the facts that this glucoside (III) is the end product on this biosynthetic pathway and that of the three secoiridoid glucosides described above this plant contains only gentiopicroside (III).

Subsequently, an aqueous solution of [10-^{14}C]-sweroside (I) was administered by the cotton wick method to *Vinca rosea* plants during their flowering period. After administration for four days, the plants were harvested and vindoline (VI) was isolated in the usual manner. It was recrystallized from *n*-hexane as colorless needles with a specific activity of 1.75×10^5 dpm/mmole (11% incorporation). This compound was subjected to acid hydrolysis affording deacetylvindoline (XIII) which had the same specific activity as that of the intact vindoline (VI). Kuhn–Roth oxidation of XIII yielded acetic acid containing 96% of the radioactivity of the intact base.

To study the incorporation of sweroside (I) into reserpinine (VII), a *Corynanthe* alkaloid, and quinine (V), a *Cinchona* alkaloid, the radioactive glucoside (I) was then administered to *Vinca major* and *Cinchona succirubra*, respectively.

Vinca major plants were harvested after administration of [10-^{14}C]-sweroside (I) for eight days and radioactive reserpinine (VII) was isolated by the conventional method. It was recrystallized from acetone to a constant specific activity of 7.9×10^3 dpm/mmole (0.03% incorporation).

9) H. Inouye, S. Ueda, and Y. Nakamura, *Tetrahedron Letters*, **1967**, 3221.
10) A.R. Battersby, A.R. Burnett, and P.G. Parsons, *J. Chem. Soc.* (C), **1969**, 1187.

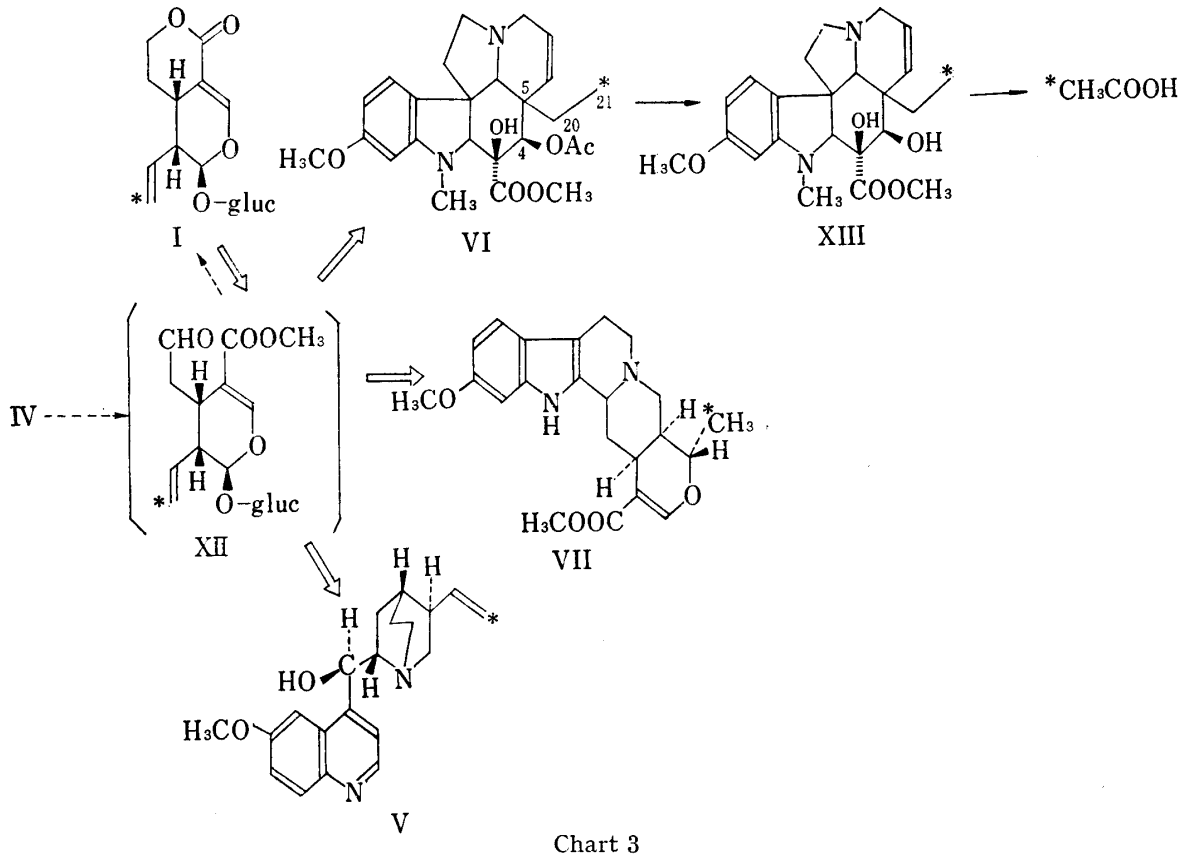

Chart 3

An aqueous solution of [10-^{14}C]-sweroside (I) was also fed to a *Cinchona succirubra* plant by the cotton wick method for fourteen days. Then quinine (V)[11] was isolated by a combination of silica gel column chromatography and preparative paper chromatography. The alkaloid was further purified by conversion to its sulfate. The specific activity of the purified free base V was 6.75×10^4 dpm/mmole (0.6% incorporation).

These results prove that sweroside (I) was incorporated into reserpinine (VII), a *Corynanthe* alkaloid, and quinine (V), a *Cinchona* alkaloid, as well as vindoline (VI), an *Aspidosperma* alkaloid. The biosynthetic pathways of the indole alkaloid and the *Cinchona* alkaloid from the secoiridoid glucoside were thus established for the first time.

Accordingly, these experiments together with those on the incorporation of loganin (IV) into indole alkaloids by Battersby[6] and Arigoni[7] establish the following biosynthetic sequence: iridoid glucosides→secoiridoid glucosides→indole alkaloids, *Cinchona* alkaloids.

Shortly after the publication of our preliminary report[2] on the incorporation of [10-^{14}C]-sweroside (I) into gentiopicroside (III) and vindoline (VI), Battersby, *et al.* reported that secologanin (XII), a C-7 aldehyde and the C-11 carbomethoxy equivalent of sweroside (I) is a precursor of the indole alkaloid.[10,12]

In the meantime, Coscia, *et al.*,[13] Gröger, *et al.*[14] and we[15] independently found that loganin (IV) (or loganic acid (XIV)) was incorporated into gentiopicroside (III). These findings also support the biosynthetic pathway shown in Chart 2. A more detailed report on the work with loganin (IV) will be presented later.

11) The quinine (V) was considered to be contaminated by quinidine, its C-9 stereoisomer, but further purification was impossible because of the small amount of sample available.
12) A.R. Battersby, A.R. Burnett, and P.G. Parsons, *Chem. Commun.*, **1968**, 1280.
13) R. Guarnaccia, L. Botta, and C.J. Coscia, *J. Am. Chem. Soc.*, **91**, 204 (1969); C.J. Coscia, L. Botta, and R. Guarnaccia, *Arch. Biochem. Biophys.*, **136**, 498 (1970).
14) D. Gröger and P. Simchen, *Z. für Naturforsch.*, **24b**, 356 (1969).
15) H. Inouye, S. Ueda, Y. Aoki, and Y. Takeda, *Tetrahedron Letters*, **1969**, 2351.

Experimental[16]

Osmium Tetroxide Oxidation of Sweroside Tetraacetate (VIII)——To a solution of sweroside tetraacetate (VIII) (215 mg) in anhyd. pyridine (0.5 ml) was added anhyd. ether (4 ml) and then an ethereal solution of OsO_4 (125 mg) with stirring and the reaction mixture was allowed to stand in the dark. After 42 hr, the resulting dark brown precipitate was collected and dissolved in EtOH. H_2S was passed into this solution and the resulting black precipitate of osmium sulfide was removed by filtration through a layer of celite. The filtrate was treated with charcoal and evaporated to dryness *in vacuo* to give a yellowish residue. This was chromatographed on silica gel (50 g). The column was eluted successively with $CHCl_3$ (400 ml), $CHCl_3$:MeOH (99:1 v/v, 150 ml), (98:2 v/v, 150 ml) and finally $CHCl_3$:MeOH (97:3 v/v) yielded a colorless oil which was crystallized from EtOH yielding 70 mg of colorless needles (IX), mp 148—150°, $[\alpha]_D^{21}$ −259° (c=2.14, $CHCl_3$). UV λ_{max}^{EtOH} mμ (log ε):246 (3.94): IR ν_{max}^{KBr} cm^{-1}: 3600—3300 (OH), 1750 (O–$COCH_3$), 1705 (lactone), 1620 (enolic double bond). NMR (pyridine-d_5) ppm: 1.95—2.12 (4×CH_3CO), 7.21 (1H, enolic H). *Anal.* Calcd. for $C_{24}H_{32}O_{15}$. 3/2 H_2O: C, 49.10; H, 5.67. Found: C, 49.24; H, 5.94.

$NaIO_4$ Oxidation of Diol (IX)——To a solution of diol (IX) (50.6 mg) in MeOH (2 ml) was added $NaIO_4$ (40 mg) in 1 ml each of H_2O and MeOH at room temperature with stirring and stirring was continued for 35 min. Then H_2O (20 ml) was added and the reaction mixture was extracted with three 30 ml portions of $CHCl_3$. The $CHCl_3$ layer was dried over anhyd. $MgSO_4$ and the solution was evaporated *in vacuo* to give a colorless syrup. This residue was chromatographed on silica gel (20 g) with $CHCl_3$ as eluent and 35.5 mg of a white powder (X) was obtained from the eluate. $[\alpha]_D^{21}$+71.5° (c=1.23, $CHCl_3$). UV λ_{max}^{EtOH} mμ (log ε): 244.5 (3.90). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750 (O–$COCH_3$), 1715 (CHO), 1700 (lactone), 1620 (enolic double bond). NMR ($CDCl_3$) ppm: 1.95—2.12 (4×CH_3CO), 5.85 (1H, d, J=2 Hz, C-1 H), 7.53 (1H, d, J=2 Hz, enolic H), 9.68 (1H, d, J=1.5 Hz, CHO). *Anal.* Calcd. for $C_{23}H_{28}O_{14}$·1/2 H_2O: C, 51.44; H, 5.44. Found: C, 51.53; H, 5.41.

Wittig Reaction of Aldehyde (X) with Methylene-triphenylphosphorane (Formation of Sweroside Tetraacetate (VIII)——i) Cold Run: To a suspension of triphenylmethylphosphonium bromide[17] (150 mg) in ether (15 ml) was added 0.8N *n*-BuLi–ether (1 ml) under a nitrogen atmosphere with stirring for 2.5 hr. The reaction mixture became yellow. Aldehyde (X) (46.7 mg) in anhyd. tetrahydrofuran (20 ml) was added to this reaction mixture under a nitrogen atmosphere and the mixture was stirred for 2 hr. The solvent was then removed *in vacuo*. The residue was chromatographed on silica gel (20 g) with $CHCl_3$ as eluent and fractions of *ca.* 20 ml were collected. Fractions No. 2—7 were combined and rechromatographed on silica gel (20 g) with ether as eluent. Fractions of 5 ml of eluate were collected. Fractions No. 20—26 were combined and recrystallized from EtOH to give colorless needles, mp 165—166°, yield 6.2 mg, which was identified with an authentic sample of sweroside tetraacetate (VIII) by the mixed melting point and by comparisons of the UV and IR spectra.

ii) Hot Run: ^{14}C-Triphenylmethylphosphonium bromide (59 mg, specific activity 1.37×10^8 dpm/mmole), prepared from [^{14}C]-methyl bromide and triphenylphosphine, was diluted with 60 mg of carrier. This was suspended in ether (7 ml) and 0.8N *n*-BuLi–ether (0.35 ml) was added under a nitrogen atmosphere and the mixture was stirred for 2.5 hr. Then a solution of the aldehyde (X) in anhyd. tetrahydrofuran (5 ml) was added and stirring was continued for 2 hr. The solvent was removed *in vacuo*. The residue was chromatographed on silica gel (10 g, 2×12 cm) with $CHCl_3$ as eluent and fractions of 15 ml were collected. Fraction No. 2 was diluted with non-radioactive sweroside tetraacetate (VIII) (11.0 mg) and chromatographed on silica gel (10 g, 2×12 cm) with ether as eluent and fractions of 10 ml were collected. Fractions No. 4—7 were combined and recrystallized from EtOH to give 21.5 mg of [10-^{14}C]-sweroside tetraacetate (VIII) as colorless needles, mp 165—166°, specific activity 1.87×10^7 dpm/mmole. This substance was identified with an authentic sample of sweroside tetraacetate (VIII) by its mixed melting point and TLC (silica gel, ether).

Zemplén Reaction of Sweroside Tetraacetate (VIII)——i) Cold Run: To a solution of sweroside tetraacetate (VIII) (30 mg) in anhyd. MeOH (1.8 ml) was added anhyd. 0.1N CH_3ONa–MeOH (0.1 ml)

16) Melting points were determined in a Yanagimoto Micro-Melting Point Apparatus and were not corrected. Unless specified otherwise, paper chromatography (PPC) 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 acc. to Stahl (E. Merck) or Alumina G (E. Merck) was used for thin-layer chromatography (TLC). Unless otherwise noted, spots were detected by exposing the plates to iodine vapour. Silica gel (Mallinckrodt) or alumina (Woelm, neutral) was used for column chromatography. Radioactivity was measured in a Beckman liquid scintillation counter, Model LS-100, with samples dissolved in scintillation mixture consisting of toluene (10 ml), 2,5-diphenyloxazole (PPO) (40 mg), and 2,2'-*p*-phenylenebis(5-phenyloxazole) (POPOP) (0.5 mg) or dioxane (10 ml), naphthalene (1 g), PPO (70 mg), POPOP (5 mg). Specific activities are expressed as values before dilution.

17) N.A. Milas and C.P. Priesing, *J. Am. Chem. Soc.*, **79**, 6295 (1957).

and the mixture was refluxed for 2 min. Then it was cooled in an ice bath and neutralized with Amberlite IRC-50 (–COOH form). The ion exchange resin was filtered off and the solvent was removed *in vacuo*. The residue was dissolved in H_2O (10 ml) and washed with $CHCl_3$ (10 ml). The aqueous layer was concentrated to dryness *in vacuo* giving 18.8 mg of a white powder, which was identified with an authentic sample of sweroside (I) by IR and NMR spectral comparisons and TLC (silica gel, $CHCl_3$:MeOH 7:3).

ii) Hot Run: To a solution of sweroside tetraacetate (VIII) (73.9 mg), specific activity 2.34×10^6 dpm/mmole) in anhyd. MeOH (4 ml) was added anhyd. methanolic 0.1N CH_3ONa (0.15 ml) and the mixture was refluxed for 2 min. It was cooled in an ice bath and neutralized with Amberlite IRC-50 (–COOH form) and the solvent was removed *in vacuo*. The residue was dissolved in H_2O (50 ml) and washed with $CHCl_3$ (30 ml). The aqueous layer was concentrated *in vacuo* and the residue was dried under reduced pressure over silica gel to afford a colorless syrup (52.8 mg). This substance was identified with an authentic sample of sweroside (I) by TLC (silica gel, $CHCl_3$:MeOH 8:2). The specific activity was 2.09×10^6 dpm/mmole.

Administration of [10-^{14}C]-Sweroside (I) to *Gentiana scabra* Bunge var. *Buergeri* Maxim.——[10-^{14}C]-Sweroside (I) (8.7 mg, specific activity 6.90×10^5 dpm/mmole) was dissolved in H_2O (1.5 ml) and the aqueous solution was placed in glass tubes and administered by the cotton wick method to nine *Gentiana scabra* var. *Buergeri* plants (*ca.* 8 cm in height) during their flowering period in December. After the solution had been absorbed by the plants, more H_2O (total 5 ml) was put into the tubes so that all the remaining radioactive material could be absorbed by the plants. Four days from the beginning of the administration, the plants were harvested (wet weight 34.5 g).

Extraction and Isolation of Glucoside from *Gentiana scabra* var. *Buergeri* ——The whole plants were cut into pieces and extracted with four 150 ml portions of hot MeOH. The extract was concentrated *in vacuo* and the residue was dissolved in H_2O (50 ml). Water insoluble material was removed by filtration through a celite layer. The filtrate was then washed with four 10 ml portions of AcOEt. The aqueous layer was concentrated to *ca.* 15 ml *in vacuo* and extracted with four 20 ml portions of *n*-BuOH. After washing with a very small amount of H_2O, the *n*-BuOH layer was concentrated *in vacuo* and dried under reduced pressure over silica gel to obtain crude gentiopicroside (III) as a yellowish powder, yield 800 mg. The crude glucoside (III) (377 mg) was acetylated by the usual method with Ac_2O (4 ml) and pyridine (2 ml). The resulting crude acetate (XI) was dissolved in EtOH and decolorized by treatment with activated charcoal and 173.6 mg of gentiopicroside tetraacetate (XI) were obtained as colorless needles, mp 142—143°. This substance was identified with an authentic sample of gentiopicroside tetraacetate (XI) by its mixed melting point and by TLC (silica gel, ether). The radioactive gentiopicroside tetraacetate (XI) was recrystallized from EtOH to the constant activity shown in Table I.

Ozonolysis of Gentiopicroside Tetraacetate (XI)——^{14}C-Labelled gentiopicroside tetraacetate (XI) (78.9 mg, specific activity 2.96×10^3 dpm/mmole) was diluted with carrier (XI) (70.2 mg) and dissolved in anhyd. $CHCl_3$ (20 ml). The solution was cooled in a dry ice–MeOH bath and a stream of ozone was passed through the solution for 6 hr. After the reaction had stopped, the solvent was removed *in vacuo* at room temperature. H_2O (5 ml) was added to the residue and the mixture was heated to distil the resulting formaldehyde with H_2O. During the distillation H_2O (25 ml) was added dropwise to maintain the volume of the reaction mixture at about 5—10 ml. Heating was continued for 1 hr and the distillate was collected in a flask containing dimedone (72.9 mg) in acetate buffer (pH 4.5, 20 ml). The resulting white precipitate was collected by centrifugation and dried under reduced pressure at room temperature over P_2O_5. Yield 42.2 mg, mp 191—193°. The formaldehyde-dimethone was further purified by distillation, $bp_1$150°. The specific activity was 2.88×10^3 dpm/mmole, corresponding to 98% of that of the intact ^{14}C-labelled gentiopicroside tetraacetate (XI).

Administration of [10-^{14}C]-Sweroside (I) to *Vinca rosea* L.——A solution of [10-^{14}C]-sweroside (I) (26.4 mg, specific activity 1.54×10^6 dpm/mmole) in H_2O (6 ml) was placed in glass tubes and administered by the cotton wick method to twenty *Vinca rosea* plants (*ca.* 25—30 cm in height) in a green house, during their flowering period. After the solution had been absorbed into the plants more H_2O (total 15 ml) was put into the glass tubes so that the remaining radioactive material could be absorbed by the plants. Five days from the beginning of administration, the shoots were harvested. Wet weight 203 g.

Extraction and Isolation of Vindoline (VI) from *Vinca rosea*——The excised *Vinca rosea* shoots were cut into pieces and extracted with four 500 ml portions of hot MeOH. The MeOH extracts were combined and the solvent was removed *in vacuo*. The residue was dissolved in 1% HCl (total 140 ml) and insoluble material was removed by filtration through a celite layer. The resulting brownish red filtrate was made alkaline with conc. NH_4OH and extracted with three 100 ml portions of benzene. The benzene layer was washed with H_2O containing a small amount of ammonia and dried over anhyd. $MgSO_4$. The solvent was removed *in vacuo* and the residue was dissolved in $CHCl_3$ (20 ml). The $CHCl_3$ solution was extracted successively with McIlvaine buffer solutions (double strength, pH 4.0, 2.95 and 2.0). The $CHCl_3$ layer was dried over anhyd. $MgSO_4$ and the solvent was removed *in vacuo*. The residue was dissolved in $CHCl_3$ and mixed with Al_2O_3 (activity grade III, 5 g) and the solvent was removed *in vacuo*. The residue was placed on the top of a column of Al_2O_3 (activity grade III, 40 g) and eluted successively with petroleum ether (90 ml), benzene

(300 ml), $CHCl_3$:benzene (1:4 v/v) (120 ml), $CHCl_3$:benzene (1:3 v/v) (200 ml) and then with $CHCl_3$:benzene (1:2 v/v). The fractions eluted with $CHCl_3$:benzene (1:3 v/v) were collected and the solvent was removed *in vacuo* to obtain 44.1 mg of vindoline (VI) as colorless needles, mp 177°. This substance was identified with non-radioactive vindoline (VI) by TLC (silica gel, $CHCl_3$:MeOH 95:5 v/v). The spots were detected by spraying with a solution of ceric ammonium sulfate in phosphoric acid. The specific activity was 1.75×10^5 dpm/mmole (11% incorporation).

Acid Hydrolysis of Vindoline (VI)——^{14}C-Labelled vindoline (VI) (39.7 mg, specific activity 1.75×10^5 dpm/mmole) was diluted with non-radioactive vindoline (VI) (74.2 mg) and dissolved in 1/2N H_2SO_4 (8.6 ml). After refluxing the solution for 4 hr the reaction mixture was distilled and during the distillation H_2O (30 ml) was added dropwise to maintain its volume. The distillate was neutralized with 1/10N NaOH and then the solvent was removed *in vacuo*. PPC of the residue gave a single spot corresponding to that of sodium acetate (*Rf* 0.27). To a solution of the resulting sodium acetate in H_2O (0.5 ml) were added α-naphthylamine–HCl (5 mg), N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide–HCl (50 mg) and a drop of conc. HCl with stirring. The resulting-brown precipitate was extracted with three 2 ml portions of benzene and dried over anhyd. $MgSO_4$, and the solvent was removed *in vacuo*. The residue was distilled under reduced pressure giving N-acetyl-α-naphthylamine as colorless needles, mp 155—156°. This material was non-radioactive. Then, conc. HCl (6 ml) was added and the mixture was heated on a water bath for 7 min, when it became dark blue. The mixture was poured into ice water, made alkaline with conc. NH_4OH, and extracted with three 5 ml portions of $CHCl_3$. The $CHCl_3$ layer was dried over anhyd. $MgSO_4$ and the solvent was removed *in vacuo*. The residue was dried under reduced pressure over silica gel yielding deacetylvindoline (XIII) (57.2 mg). This substance was identified with non-radioactive deacetylvindoline (XIII) by TLC (silica gel, $CHCl_3$:MeOH 95:5 v/v). Spots were detected by spraying with a solution of ceric ammonium sulfate in phosphoric acid. The specific activity of this substance (XIII) was 1.70×10^5 dpm/mmole.

Kuhn–Roth Oxidation of Deacetylvindoline (XIII)——The radioactive deacetylvindoline (XIII) (57.2 mg) was dissolved in 2N H_2SO_4 10 ml) containing CrO_3 (3 g) and heated to distil the resulting acid. During the distillation H_2O (40 ml) was added to maintain the volume of the reaction mixture. The distillate was neutralized with 1/10N NaOH and the solvent was removed *in vacuo*. PPC of an aliquot of the residue gave a single spot corresponding to that of sodium acetate but no spot of sodium propionate was detectable. The sodium acetate was converted to N-acetyl-α-naphthylamine in the usual manner and purified by distillation *in vacuo*. The specific activity was 1.63×10^5 dpm/mmole corresponding to 96% of the radioactivity of the intact deacetylvindoline (XIII).

Administration of [10-^{14}C]-Sweroside (I) to *Vinca major* L.——[10-^{14}C]-Sweroside (I) (specific activity 1.75×10^7 dpm/mmole, 9.4 mg) was dissolved in H_2O (7 ml), put in glass tubes and administered by the cotton wick method to sixteen *Vinca major* plants (*ca.* 20 cm in height) in November. After the solution had been absorbed into the plants H_2O (total 17 ml) was added to the tubes to permit absorption of the remaining radioactive material by the plants. Seven days from the beginning of administration the plants were harvested (wet weight 66 g).

Extraction and Isolation of Reserpinine (VII) from *Vinca major*——Excised *Vinca major* plants were cut into pieces and extracted with four 200 ml portions of hot MeOH. The MeOH extracts were combined and the solvent was removed *in vacuo*. The residue was extracted with 5% HCl (60 ml) and insoluble material was removed by filtration through a layer of celite. The filtrate was washed with four 20 ml portions of ether. The 5% HCl extract was made alkaline with conc. NH_4OH and extracted with four 15 ml portions of $CHCl_3$ containing 10% MeOH. The $CHCl_3$ layer was washed with dilute aq. ammonia, dried over anhyd. $MgSO_4$ and evaporated *in vacuo*. The residue was dissolved in $CHCl_3$ and mixed with Al_2O_3 (activity grade III, 5 g) and the solvent was removed *in vacuo*. The residue was placed on the top of a column of Al_2O_3 (activity grade III, 1.5 × 20 cm, 20 g) and eluted in order with the following solvents: petroleum ether (150 ml), petroleum ether:benzene (1:1 v/v, 150 ml) and benzene. Fractions of 25 ml were collected. Fractions No. 12—13 gave reserpinine (VII) as colorless needles, mp 247—248°, yield 6.4 mg. This substance was identified with non-radioactive reserpinine (VII) by its mixed melting point and TLC (silica gel, $CHCl_3$: MeOH 98:2 v/v). Radioactive reserpinine (VII) (4.39 mg) was diluted with carrier reserpinine (VII) (4.36 mg) and recrystallized repeatedly from acetone to give material of constant activity. The specific activity was 7.94×10^3 dpm/mmole (0.03% incorporation).

Administration of [10-^{14}C]-Sweroside (I) to *Cinchona succirubra* Pavon ex. Klotzsch.——[10-^{14}C]-Sweroside (I) (26.4 mg, specific activity 2.09×10^6 dpm/mmole) was dissolved in H_2O (5 ml) and fed to a 3-year-old *Cinchona succirubra* plant with four leaves (*ca.* 25 cm in height) in May using cotton wicks inserted into the stem. After the solution had been taken up by the plant more H_2O (total 10 ml) was added to the tubes to permit absorption of the remaining radioactive material by the plant. The *Cinchona* plant was harvested two weeks from the beginning of the administration. Wet weight 34.5 g.

Extraction and Isolation of Quinine (V) from *Cinchona succirubra*——The excised plant was cut into pieces and extracted with four 250 ml portions of hot MeOH. The MeOH extracts were combined and evaporated to dryness *in vacuo*. The residue was extracted repeatedly with 5% HCl (total 85 ml) and insoluble material was removed by filtration through a layer of celite. The filtrate was washed with ether,

made alkaline with conc. NH_4OH and extracted with four 25 ml portions of $CHCl_3$. The $CHCl_3$ extracts were combined, washed with dil. NH_4OH, dried over anhyd. $MgSO_4$, and evaporated to dryness *in vacuo*. The residue was chromatographed on a silica gel column (1×20 cm, 15 g) with $CHCl_3$ (170 ml) and then with $CHCl_3$:MeOH (99:1 v/v) as eluent collecting fractions of ml.10 Fractions No. 24—34 contained only quinine (V)[11] while later fractions contained quinine (V) contaminated with other alkaloids. Th latter fractions were combined and subjected to preparative paper chromatography on Toyo Roshi No. 526 filter paper with cyclohexanol saturated with 3.5N HCl as solvent. The area of filter paper containing quinine (V) was cut out and extracted with three 20 ml portions of hot MeOH and the solvent was removed *in vacuo*. The residue was dissolved in 5% HCl (2 ml) and filtered. The filtrate was made alkaline with conc. NH_4OH and extracted with three 5 ml portions of $CHCl_3$. The $CHCl_3$ extract was dried over anhyd. $MgSO_4$ and evaporated *in vacuo*. The residue was dried under reduced pressure over silica gel giving quinine (V).[11] This was combined with the purified V, mentioned above, obtained by silica gel column chromatography. The total yield of quinine (V) was 4.4 mg. The ^{14}C-labelled quinine (V) (4.4 mg) was diluted with nonradioactive quinine (V) (3.5 mg) in EtOH (0.1 ml) and 10% (v/v) ethanolic H_2SO_4 was added. The resulting precipitate was collected by centrifugation and suspended in $CHCl_3$ and conc. NH_4OH (15 ml) was added to the suspension. After shaking the suspension, the $CHCl_3$ layer was washed with H_2O, dried over anhyd. K_2CO_3, and evaporated. The residue was dried under reduced pressure over P_2O_5 giving 0.8 mg of the alkaloid (V). The specific activity was 6.75×10^4 dpm/mmole (0.6% incorporation).

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