(same as above) and the pH was adjusted to 7.2 by addition of 0.5 M NaOH. The final extract thus contained 25 % ethanol.

Covalent chromatography. A column with a total volume of 6.3 ml (1 × 8 cm) was prepared from the agarose-2-pyridyl disulfide gel. The column was equilibrated with 0.05 M Tris-HCl buffer pH 7.2 containing 0.1 M KCl and 1 mM EDTA. Jack bean meal extract (250 ml) was passed through the column at a flow rate of 20 ml/h. Ultraviolet absorption at 280 nm and ureolytic activity were determined in fractions of the effluent. Most of the UV-absorbing material passed through the column unretained. In the first 150 ml of eluate no urease activity was detected. A small activity that gradually increased to that of the sample applied was then observed within the next 100 ml of eluate. The column was then washed with Tris-HCl buffer (same as above) until A_{280} in the eluate was less than 0.04. Covalently bonded material was detached from the chromatographic material with 20 mM dithiothreitol (20 ml) dissolved in 0.05 M Tris-HCl buffer pH 8.0 containing 0.1 M KCl and 1 mM EDTA.

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Biosynthesis of a Quinol Glucoside in *Cornus*

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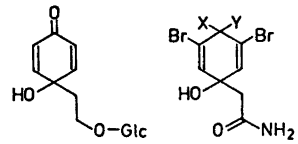
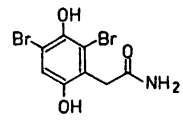
The glucoside *1*, first isolated from *Cornus foemina* Mill.,[1] but subsequently recognized as a component of probably all species of the *Cornus* subgenera Kraniopsis and Mesomora,[2] has no known obvious structural relative among constituents of higher plants, though the parent 4-alkyl-4-hydroxy-cyclohexa-2,5-dienone (quinol) system, or its formal addition or rearrangement derivatives, has been repeatedly encountered in natural products, such as *2*,[3] *3*,[4] *4*,[5] and *5*,[6] all from marine sponges of the genus *Verongia*; *6* (thelepin) from a marine worm;[7] and botrallin *7* of fungal origin.[8] An oxidative *in vivo* derivation of *1* from tyrosol *8*, possibly *via* salidroside *9*, a phenolic glucoside encountered within the families Crassulaceae, Ericaceae, Oleaceae, and Salicaceae, but also accompanying *1* in *C. foemina*[1] and other *Cornus* species,[2] appeared likely and has now been confirmed. We report the results.



1 *2* X,Y = =O *3* X = Y = OMe *4* X, or Y = OMe; Y, or X = OEt



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The potential precursors, L-tyrosine, tyrosol *8*, and salidroside *9*, labelled with ^{14}C or ^{3}H, were administered to young shoots of *C. stolonifera* Michx. After a metabolic period of 44 h, the glucosides *9* and *1* were isolated and purified by chromatography before their total radioactivities were determined. The results are presented in Table 1.

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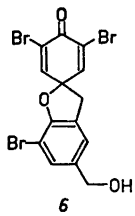

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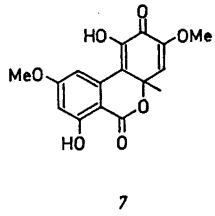

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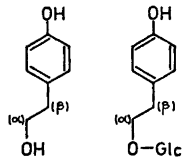

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9

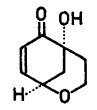
It appears that tyrosol *8* and salidroside *9* are both efficiently converted into *1*, whereas L-tyrosine and glucose, not surprisingly, act as far inferior precursors. Though unproven, the observed incorporation of L-tyrosine may conceivably proceed through *p*-hydroxyphenylacetaldoxime and tyrosol, an established pathway in certain dicotyledonous angiosperms.[9] [α,β-^{3}H][*glucose-U*-^{14}C]Salidroside *9* was administered to the plant in order to learn to what extent incorporation proceeded in a direct step, *i.e.* without rupture of the glucosidic linkage. Even when its corporation was accompanied by only a minor change in the ^{3}H:^{14}C ratio (Table 1), a moderate *in vivo* exchange of glucose in *9* was noticed in recovery experiments. The simultaneous operation of alternative, minor pathways, such as oxidation of tyrosol to the aglucone of *1* (formula iii in the footnote), followed by glucosylation of the latter, cannot be dismissed on the basis of the present results.

The natural distribution of the glucoside *1* is unknown. Apart from the two formerly specified subgenera within *Cornus*, *Forsythia* species [10] and *Digitalis purpurea* L.[10,11]* are now to be counted as established sources of *1*. The facile conversions of the tetraacetate of *1* into homogentisyl or resorcinol derivatives,[1] may conceivably have their enzymic counterparts in Nature.

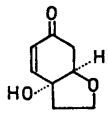
The detailed character of the enzymic oxidation of *9* to *1* is unknown. Singlet oxygen participation [13] and the intermediacy of arene oxides [5] are both conceivable reactions among which the present results, however, cannot distinguish.

Experimental. Plant material and feeding technique. The experiments were performed in the last week of April on small spring shoots of *C. stolonifera* Michx. The radioactive com-

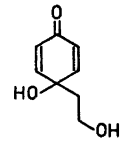
* A cyclohexanone derivative, isolated from the growing tips of *D. purpurea* and formulated as (i) (or its mirror image) possesses properties and spectroscopical data [12] suggesting its identity with (ii) [11,13] that arises from intramolecular cyclization of (iii), liberated on enzymic hydrolysis of *1* in *Cornus* extracts.[1,11]



(i)



(ii)



(iii)

Table 1. Absolute incorporations of ^{14}C- and ^{3}H-labelled precursors into salidroside *9* and the quinol glucoside *1* in *Cornus stolonifera* Michx.

Exp. No.	Compound administered	Total activity administered (mμCi)	Total activity isolated in *9* (mμCi)	% Incorporation into salidroside *9*	Total activity isolated in *1* (mμCi)	% Incorporation into the quinol glucoside *1*
1	D-[*U*-^{14}C] Glucose	4860	12	0.25	6.2	0.13
2a	L-[α, β-^{3}H] Tyrosine	4850	2.3	0.05	8.1	0.17
2b	L-[α, β-^{3}H] Tyrosine	4830	2.6	0.05	7.1	0.15
3a	[α, β-^{3}H] Tyrosol, *8*	2760	730	26.4	430	15.7
3b	[α, β-^{3}H] Tyrosol, *8*	2730	560	20.5	480	17.6
4a	[α, β-^{3}H] Salidroside, *9* + [*glucose-U*-^{14}C] Salidroside, *9*, (^{3}H:^{14}C = 5.6)[a]	3629 653			784[b] 116[b]	21.6 17.8
4b	[α, β-^{3}H] Salidroside, *9* + [*glucose-U*-^{14}C] Salidroside, *9* (^{3}H: ^{14}C = 5.6)[a]	3799 683			691[c] 102[c]	18.2 15.0

[a] The ^{3}H: ^{14}C-ratio was 6.0 after correction for a radioactive impurity in the salidroside administered. [b] ^{3}H: ^{14}C = 6.7. [c] ^{3}H: ^{14}C = 6.7.

pounds were administered to the leaves through the petioles. The metabolic period was 44 h.

Radioactive compounds. D-[*U*-^{14}C] *Glucose* and L-[α,β-^{3}*H*]*tyrosine* were obtained from Amersham Radiochemical Centre, England. [α,β-^{3}*H*]*Tyrosol 8* was synthesized by a modification of the method of Ehrlich:[14] to a solution of L-tyrosine (6 mg) and sucrose (0.5 g) in water (5 ml) were added 220 μCi (1 μCi/μl) of L-[α,β-^{3}H]tyrosine and baker's yeast (250 mg). At the end of 5 days at 22 °C, carrier tyrosol (14.4 mg) was added. After centrifugation, the supernatant, combined with the washings, was adjusted to pH 7 with solid $NaHCO_3$, and continuously extracted with ether. After drying and evaporation, the ether solution afforded a crystalline residue which was purified by chromatography on SiO_2-plates ($CHCl_3$:MeOH, 5:1) to give homogeneous [α,β-^{3}H]tyrosol (18.9 mg). [α,β-^{3}*H*]*Salidroside 9* was prepared, essentially as reported,[15] by reaction of the total amount of ^{3}H-tyrosol described above with tetra-*O*-acetyl-α-D-glucopyranosyl bromide to give a crude product, separated on SiO_2-plates ($CHCl_3$:MeOH, 95:5) into [α,β-^{3}H]-2-[4-hydroxyphenyl]ethyl tetra-*O*-acetyl-β-D-glucopyranoside (49 mg) and [α,β-^{3}H]tyrosol, the latter isolated after addition of carrier (37 mg) and possessing a total activity of 30.1 μCi. The tetraacetate was converted into salidroside *9* on standing in MeOH (8 ml), saturated with NH_3 at 0 °C, for 17 h at 20 °C. The product was purified by chromatography on SiO_2-plates ($CHCl_3$:MeOH, 4:1) to give a homogeneous product (20 mg) with a total activity of 15.3 μCi. [glucose-U-^{14}*C*]*Salidroside* (55 mg, total activity 23 μCi) was prepared similarly, by deacetylation of the tetraacetate (121 mg), resulting from the reaction between tyrosol (44 mg) and tetra-*O*-acetyl-α-D-[*U*-^{14}C]glucopyranosyl bromide, the latter produced by dissolving D-glucose (57 mg), together with the residue from 225 μl (55.1 μCi) of a D-[*U*-^{14}C]glucose solution, in pyridine (2 ml), to which Ac_2O (0.5 ml) was added. After 1 h at 100 °C, H_2O (2 ml) was added. After 15 min, the solution was extracted with $CHCl_3$ (4 × 5 ml) and the organic phase was washed with H_2SO_4, $NaHCO_3$, and H_2O. The remaining syrup was dissolved in $CHCl_3$ (5 ml), containing HBr/AcOH (30 % v/v) (1.3 ml), and kept for 2 h at 20 °C. Additional $CHCl_3$ was added; the organic phase, after washing and drying, gave a syrup (159 mg) used in the reaction described above.

Isolation of the quinol glucoside 1. The plant material (5 – 10 g) was homogenized in EtOH and the suspension filtered. The combined filtrate and washings were taken to dryness and the residue was dissolved in H_2O; the aqueous phase was extracted twice with ether, and concentrated *in vacuo*. After passage through a column of neutral Al_2O_3, and thorough washing of the column, the solution was taken to dryness. The residue was taken up in MeOH and the solution evaporated after the addition of silica gel (3 g). The powder was placed on top of a silica gel column, packed in acetone, and the column was eluted with acetone; the fractions containing *1* and *9* were combined and evaporated to dryness. The residue was purified by preparative chromatography on SiO_2-plates, first with $CHCl_3$:MeOH (4:1) and then with BuOH:MeOH:H_2O (7:1:3) as the mobile phase.

Determination of radioactivity. All measurements were made by liquid scintillation counting on a Packard Tricarb scintillation spectrometer model 3320. Samples were dissolved in 1 ml H_2O (the precursors) or MeOH (the glucosides). The solutions were mixed with 10 ml scintillation solvent.[16] Counting efficiency and ^{14}C-overlap were determined by counting ^{14}C- and ^{3}H-labelled hexadecane standards under the same circumstances.

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