

(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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1. Sumner, J. B. *The Enzymes*, 1st Ed., Vol. 1, Part 2 (1951) p. 886.
2. Blakeley, R. L., Webb, E. C. and Zerner, B. *Biochemistry* 8 (1969) 1984.
3. Sehgal, P. P. and Naylor, A. W. *Plant Physiol.* 41 (1966) 567.
4. Carlsson, J., Axén, R., Brocklehurst, K. and Crook, E. M. *Eur. J. Biochem.* 44 (1974) 189.
5. Brocklehurst, K., Carlsson, J., Kierstan, M. P. J. and Crook, E. M. *Biochem. J.* 133 (1973) 573.
6. Carlsson, J. and Svenson, A. *FEBS Lett.* 42 (1974) 183.
7. Axén, R., Drevin, H. and Carlsson, J. *Acta Chem. Scand. B* 29 (1975) 471.
8. Carlsson, J., Drevin, H. and Axén, R. *Unpublished results.*
9. Carlsson, J., Axén, R. and Unge, T. *Eur. J. Biochem.* (1975). *In press.*
10. Reithel, F. J. *The Enzymes*, 3rd Ed., Vol. IV (1971) p. 1.

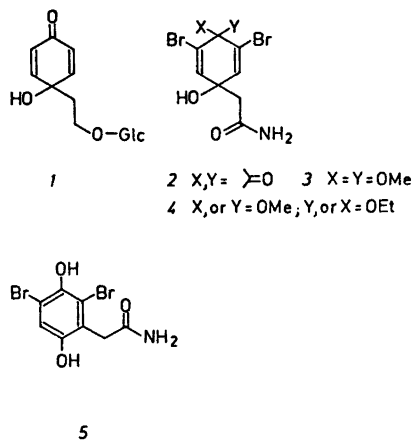
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## Biosynthesis of a Quinol Glucoside in *Cornus*

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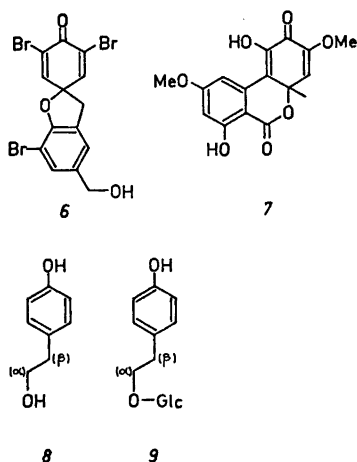
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The glucoside **1**, first isolated from *Cornus foemina* Mill.,<sup>1</sup> but subsequently recognized as a component of probably all species of the *Cornus* subgenera *Kraniopsis* and *Mesomora*,<sup>2</sup> has no known obvious structural relative 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**,<sup>3</sup> **3**,<sup>4</sup> **4**,<sup>5</sup> and **5**,<sup>6</sup> all from marine sponges of the genus *Verongia*; **6** (thelepin) from a marine worm;<sup>7</sup> and botrallin **7** of fungal origin.<sup>8</sup> An oxidative *in vivo* derivation of **1** from tyrosol **8**, possibly *via* salidoside **9**, a phenolic glucoside encountered within the families *Crassulaceae*, *Ericaceae*, *Oleaceae*, and *Salicaceae*, but also accompanying **1** in *C. foemina*<sup>1</sup> and other *Cornus* species,<sup>2</sup> appeared likely and has now been confirmed. We report the results.



The potential precursors, L-tyrosine, tyrosol **8**, and salidoside **9**, labelled with <sup>14</sup>C or <sup>3</sup>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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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.<sup>9</sup> [ $\alpha, \beta$ -<sup>3</sup>H][glucose-*U*-<sup>14</sup>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 incorporation was accompanied by only a minor change in the <sup>3</sup>H:<sup>14</sup>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 (for-

mula 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<sup>10</sup> and *Digitalis purpurea* L.<sup>10,11\*</sup> are now to be counted as established sources of 1. The facile conversions of the tetraacetate of 1 into homogentisyl or resorcinol derivatives,<sup>1</sup> may conceivably have their enzymic counterparts in Nature.

The detailed character of the enzymic oxidation of 9 to 1 is unknown. Singlet oxygen participation<sup>12</sup> and the intermediacy of arene oxides<sup>5</sup> 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<sup>12</sup> suggesting its identity with (ii)<sup>11,13</sup> that arises from intramolecular cyclization of (iii), liberated on enzymic hydrolysis of 1 in *Cornus* extracts.<sup>1,11</sup>

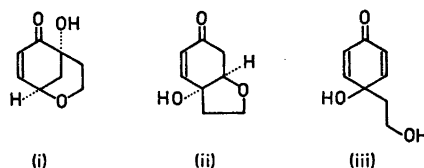


Table 1. Absolute incorporations of <sup>14</sup>C- and <sup>3</sup>H-labelled precursors into salidroside 9 and the quinol glucoside 1 in *Cornus stolonifera* Michx.

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

<sup>a</sup> The <sup>3</sup>H:<sup>14</sup>C-ratio was 6.0 after correction for a radioactive impurity in the salidroside administered.

<sup>b</sup> <sup>3</sup>H:<sup>14</sup>C = 6.7. <sup>c</sup> <sup>3</sup>H:<sup>14</sup>C = 6.7.

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

**Radioactive compounds.** D-[U-<sup>14</sup>C] Glucose and L-[ $\alpha,\beta$ -<sup>3</sup>H]tyrosine were obtained from Amer-sham Radiochemical Centre, England. [ $\alpha,\beta$ -<sup>3</sup>H]Tyrosol 8 was synthesized by a modification of the method of Ehrlich:<sup>14</sup> to a solution of L-tyrosine (6 mg) and sucrose (0.5 g) in water (5 ml) were added 220  $\mu$ Ci (1  $\mu$ Ci/ $\mu$ l) of L-[ $\alpha,\beta$ -<sup>3</sup>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<sub>3</sub>, and continuously extracted with ether. After drying and evaporation, the ether solution afforded a crystalline residue which was purified by chromatography on SiO<sub>2</sub>-plates (CHCl<sub>3</sub>:MeOH, 5:1) to give homogeneous [ $\alpha,\beta$ -<sup>3</sup>H]tyrosol (18.9 mg). [ $\alpha,\beta$ -<sup>3</sup>H]Salidroside 9 was prepared, essentially as reported,<sup>15</sup> by reaction of the total amount of <sup>3</sup>H-tyrosol described above with tetra-*O*-acetyl- $\alpha$ -D-glucopyranosyl bromide to give a crude product, separated on SiO<sub>2</sub>-plates (CHCl<sub>3</sub>:MeOH, 95:5) into [ $\alpha,\beta$ -<sup>3</sup>H]-2-[4-hydroxyphenyl]ethyl tetra-*O*-acetyl- $\beta$ -D-glucopyranoside (49 mg) and [ $\alpha,\beta$ -<sup>3</sup>H]tyrosol, the latter isolated after addition of carrier (37 mg) and possessing a total activity of 30.1  $\mu$ Ci. The tetraacetate was converted into salidroside 9 on standing in MeOH (8 ml), saturated with NH<sub>3</sub> at 0 °C, for 17 h at 20 °C. The product was purified by chromatography on SiO<sub>2</sub>-plates (CHCl<sub>3</sub>:MeOH, 4:1) to give a homogeneous product (20 mg) with a total activity of 15.3  $\mu$ Ci. [glucose-U-<sup>14</sup>C]Salidroside (55 mg, total activity 23  $\mu$ Ci) was prepared similarly, by deacetylation of the tetraacetate (121 mg), resulting from the reaction between tyrosol (44 mg) and tetra-*O*-acetyl- $\alpha$ -D-[U-<sup>14</sup>C]glucopyranosyl bromide, the latter produced by dissolving D-glucose (57 mg), together with the residue from 225  $\mu$ l (55.1  $\mu$ Ci) of a D-[U-<sup>14</sup>C]glucose solution, in pyridine (2 ml), to which Ac<sub>2</sub>O (0.5 ml) was added. After 1 h at 100 °C, H<sub>2</sub>O (2 ml) was added. After 15 min, the solution was extracted with CHCl<sub>3</sub> (4  $\times$  5 ml) and the organic phase was washed with H<sub>2</sub>SO<sub>4</sub>, NaHCO<sub>3</sub>, and H<sub>2</sub>O. The remaining syrup was dissolved in CHCl<sub>3</sub> (5 ml), containing HBr/AcOH (30 % v/v) (1.3 ml), and kept for 2 h at 20 °C. Additional CHCl<sub>3</sub> 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<sub>2</sub>O; the aqueous phase was extracted twice with ether, and concentrated *in vacuo*. After passage through a column of neutral Al<sub>2</sub>O<sub>3</sub>, 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<sub>2</sub>-plates, first with CHCl<sub>3</sub>:MeOH (4:1) and then with BuOH:MeOH:H<sub>2</sub>O (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<sub>2</sub>O (the precursors) or MeOH (the glucosides). The solutions were mixed with 10 ml scintillation solvent.<sup>16</sup> Counting efficiency and <sup>14</sup>C-overlap were determined by counting <sup>14</sup>C- and <sup>3</sup>H-labelled hexadecane standards under the same circumstances.

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- Jensen, S. R., Kjær, A. and Nielsen, B. J. *Acta Chem. Scand.* 27 (1973) 367.
- Jensen, S. R., Kjær, A. and Nielsen, B. J. *Biochem. System. Ecol.* 3 (1975) 75.
- Sharma, G. M. and Burkholder, P. R. *Tetrahedron Lett.* (1967) 4147; Fattorusso, E., Minale, L. and Sodano, G. *J. Chem. Soc. Perkin Trans. 1* (1972) 16.
- Sharma, G. M., Vig, B. and Burkholder, P. R. *J. Org. Chem.* 35 (1970) 2823.
- Andersen, R. J. and Faulkner, D. J. *Tetrahedron Lett.* (1973) 1175.
- Krejcárek, G. E., White, R. H., Hager, L. P., McClure, W. O., Johnson, R. D., Rinehart, Jr., K. L., McMillan, J. A., Paul, I. C., Shaw, P. D. and Brusca, R. C. *Tetrahedron Lett.* (1975) 507.
- Higa, T. and Scheuer, P. J. *J. Amer. Chem. Soc.* 96 (1974) 2246.
- Kameda, K., Aoki, H., Namiki, M. and Overeem, J. C. *Tetrahedron Lett.* (1974) 103.
- Kindl, H. and Schiefer, S. *Phytochemistry* 10 (1971) 1795.
- Jensen, S. R., Nielsen, B. J. and Dahlgren, R. *Bot. Not.* 128 (1975) 148, p. 174.
- Jensen, O. S. *Unpublished results.*
- Raymakers, A. and Compennolle, F. *Phytochemistry* 12 (1973) 2287.
- Saito, I., Chujo, Y., Shimazu, H., Yamane, M., Matsuura, T. and Cahnmann, H. J. *J. Amer. Chem. Soc.* 97 (1975) 5272.
- Ehrlich, F. *Ber. Deut. Chem. Ges.* 44 (1911) 139.
- Troshchenko, A. T. and Yuodvirshis, A. M. *Khim. Prir. Soedin.* 5 (1969) 256.
- Turner, J. C. *Int. J. Appl. Radiat. Isotopes* 19 (1968) 557.

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