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BIOGENESIS-LIKE TRANSFORMATION OF SALIDROSIDE TO RENGYOL AND ITS RELATED CYCLOHEXYLETANOIDS OF FORSYTHIA SUSPENSA[1)]

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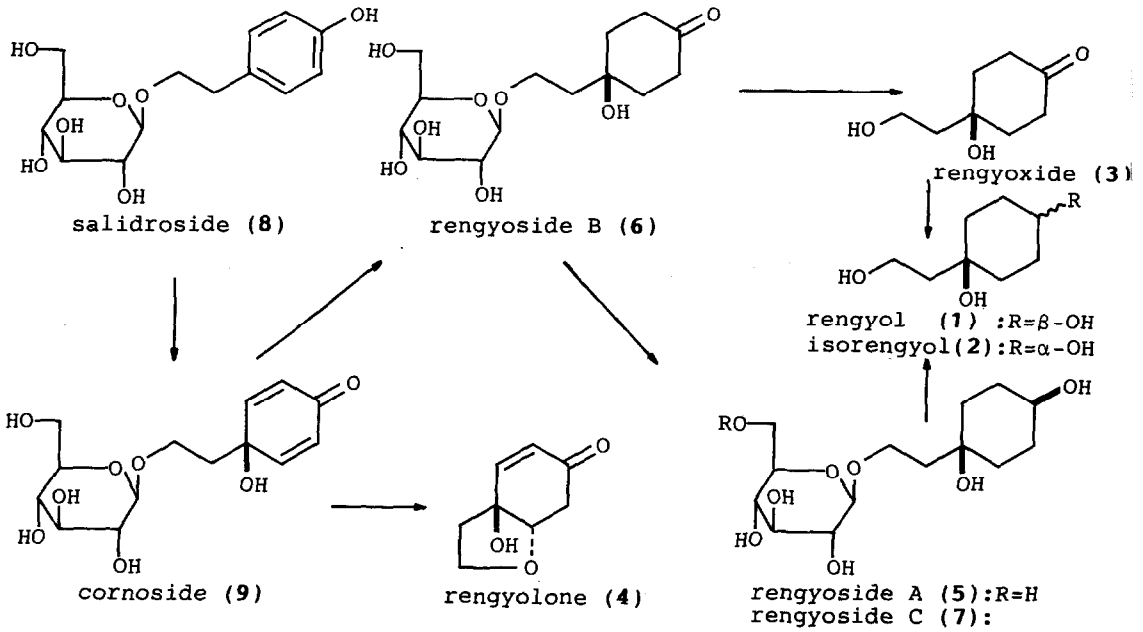
Abstract — Photooxygenation of salidroside (**8**) in methanol in presence of Rose Bengal afforded cornoside (**9**), which, on high pressure hydrogenation with 5 % palladium on activated carbon, yielded rengyoside B (**6**). Reduction of **6** with sodium borohydride gave rengyoside A (**5**) stereoselectively. By enzymatic hydrolysis, **9**, **6** and **5** furnished rengyolone (**4**), rengyoxide (**3**) and rengyol (**1**), respectively.

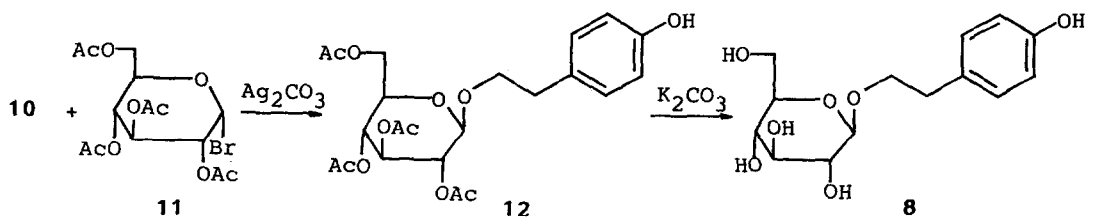
Similarly, p-hydroxyphenylethanol (**10**), the aglycone part of salidroside (**8**), was oxygenated photochemically to a dienone alcohol, which cyclized spontaneously to rengyolone (**4**). Hallerone (**17**) was obtained by the photooxygenation of p-hydroxyphenylethyl acetate (**10b**). Thus the plausible biosynthetic routes from salidroside (**8**) to rengyol (**1**) and the related natural cyclohexylethanoids were simulated chemically.

The fruits of Forsythia suspensa Vahl (Oleaceae) is named "rengyo" in Oriental medicine and a number of new phenol glucosides, forsythosides A, C, D and E,[2)] have been characterized as the antibacterial principles. Subsequent works further revealed the presence of many novel cyclohexylethanoids from the drug, namely, rengyol (**1**), isorengyol (**2**), rengyoxide (**3**), rengyolone (**4**) and their glucosides, rengyosides A (**5**), B (**6**) and C (**7**), along with two new constituents, salidroside (**8**) and cornoside (**9**), of known constitution.[3-5)] A few of the related compounds have also been characterized from other sources.[6)] These unusual cyclohexylethanoids, non-aromatic C_6-C_2 carbon skeletons, might have been derived from the phenylpropanoids through **8** and **9** (Scheme 1), rather than the polyketide route, since this drug is rich in the dimeric phenylpropanoids, namely the lignan derivatives.[7)]



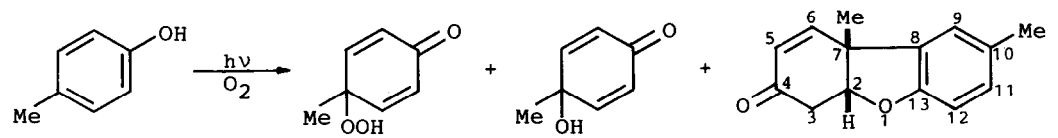
Scheme 1

The biosynthetic transformations from shikimic acid to phenylpropanoids have already been studied and established,[8] and further, the possible pathway to phenylethanoids have also been suggested as proceeding through decarboxylation of phenylpyruvate or its equivalents.[9] It is of great significance, therefore, to characterize p-hydroxyphenylacetic acid in rengyoside C (7),[5] since the acid links phenylpyruvic acid in the biosynthetic pathway with p-hydroxyphenylethanol occurring in salidroside (8). In this paper, we exemplified chemically such biogenetic transformations from salidroside (8) to rengyol (1) and the related natural cyclohexylethanoids by oxidation and successive reduction.



In order to secure sufficient starting material, preparation of salidroside (**8**) by condensation of p-hydroxyphenylethanol (**10**) and glucose was first investigated, and the Koenigs-Knorr method gave a satisfactory result.[10] Thus the condensation of 2, 3, 4, 6-tetra-O-acetyl-α-D-glucopyranosyl bromide (**11**) and **10** with silver carbonate afforded the corresponding β-glucoside (**12**) exhibiting an axial anomeric hydrogen signal at δ 4.45 (J=7 Hz) in its ^{1}H NMR spectrum, stereo and regioselectively, which on hydrolysis with aqueous potassium carbonate furnished salidroside (**8**) in 74 % overall yield.

The first step of the biogenesis-like transformation of salidroside (**8**) to rengyol (**1**) consists of oxygenation of **8** to cornoside (**9**). The phenolic compounds are easily transformed to quinol derivatives in various oxidative conditions,[11] and we have chosen a photosensitized oxygenation reaction because of its experimental simplicity and also referring to the involvement of active oxygen species in the biological processes. Then, the reactivity of p-cresol (**13**), the simplest 4-alkylphenol, was tested as a model. By irradiating a solution of **13** and Rose Bengal with a halogen lamp, under bubbling of oxygen, quinohydroperoxide (**14**), quinol (**15**) and a minor by-product (**16**) were obtained. The ^{1}H NMR spectra of **14** and **15** displayed dienone hydrogen signals at δ 6.23, 6.97 (each 2H d, J=10 Hz) and δ 6.05, 6.89 (each 2H d, J=10 Hz), respectively. The C-1 carbon signal of **15** (δ 66.8 s) was found at a higher field than that of **14** (δ 78.4 s) consistent with the expected structures, and further **14** was easily reduced to **15** with dimethyl sulfide.



13 **14** **15** **16**

The product **16** showed an ion peak at m/z 214.0990 (M^+) in its high resolution mass spectrum, corresponding to the molecular formula $C_{14}H_{14}O_2$. Inspection of ^{13}C NMR spectrum of **16** secured 14 signals to match the molecular formula indicating it to have a dimeric structure of **13**, and analysis of the ^{1}H NMR spectrum led to the structure **16** identical to the Pummerer's ketone.[12]

The solvent effect was examined for methanol, acetone and ethyl acetate and this photosensitized oxygenation proceeded much better in methanol than ethyl acetate, though the yield of **16** was almost independent on the solvent (Table 1). The O-methyl and O-acetyl derivatives of p-cresol (**13**) were not

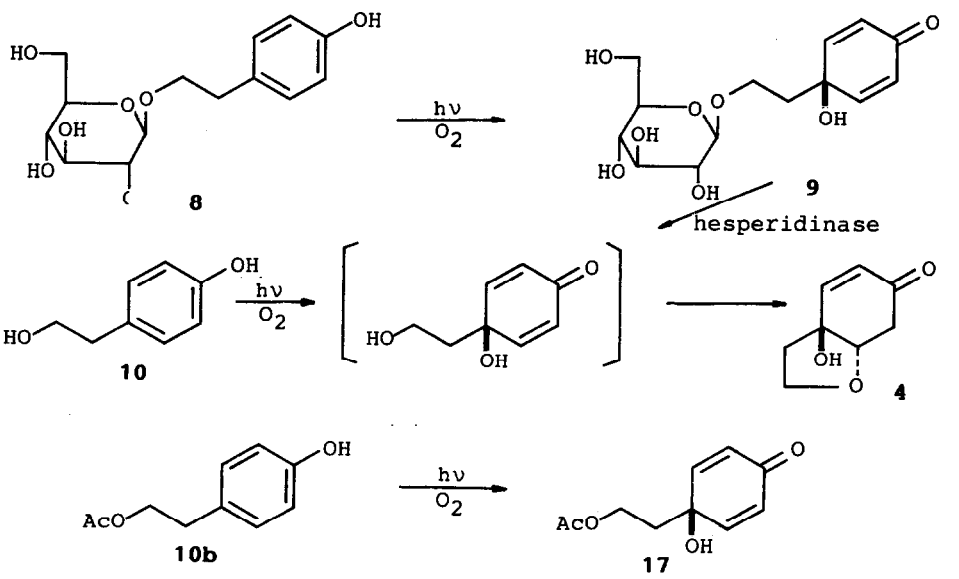
oxygenated under these conditions. And hence, the oxygenation of **13** seemed to proceed through the ionic transition state, while condensation of **13** and the quinol, which is related to the biosynthesis of usnic acid,[13] may occur either by ionic or radical mechanisms.

Table 1.
Solvent Effect on the Photosensitized Oxygenation of p-Cresol.

Product (mg)	Solvent		
	MeOH	acetone	AcOEt
Hydroperoxide (**14**)	795	400	72
Dimer (**16**)	27	27	16

Reaction condition: 1.08 g (10 mmole) of p-cresol; 100 mg of Rose Bengal; 150 ml of solvent; O_2 (bubbling); 100 W Halogen lamp; ice-cooling; 10h.

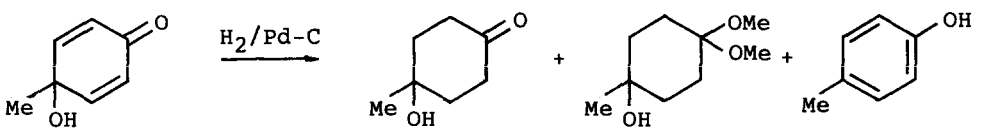
Based on the informations described above, photosensitized oxygenation of salidroside (**8**) was conducted in methanol in presence of Rose Bengal, and subsequent reduction with dimethyl sulfide yielded cornoside (**9**) in the corrected yield of 95 %. The similar treatment of **10**, followed by silica gel column chromatography resulted, via a spontaneous intramolecular ring



Scheme 2

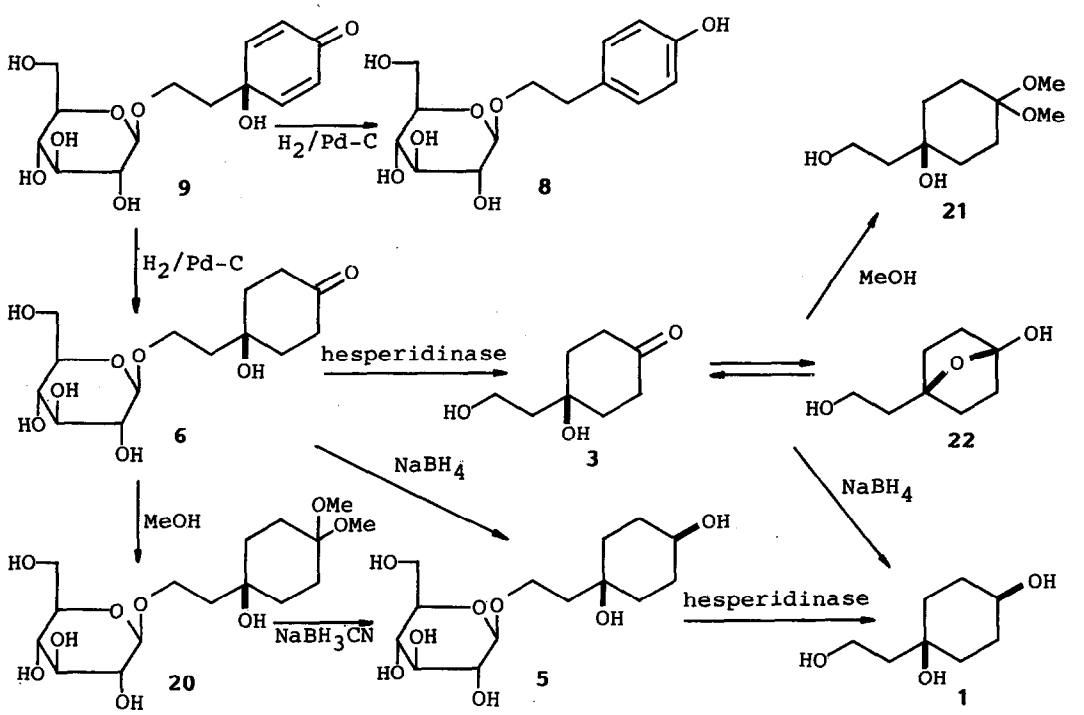
closure, to yield rengyolone (**4**) in 26 % yield, which was also obtained by enzymatic hydrolysis of **9**. The p-hydroxyphenylethyl acetate (**10b**) analogously afforded hallerone (**17**),[15] isolated from Halleria lucida,[6] though in a poor yield (13 %). Cornoside (**9**) was found fairly stable under the oxygenation condition, but the reaction products of **10** and **10b** were decomposed slowly, and hence, it was not possible to increase their yields by elongation of reaction time even though a large amount of the starting substance was still present in the reaction mixture. The greater stability of **9** may due mainly to the steric protective effect of the large glucosyl group.

As the second step, the transformations of cornoside (**9**) to rengyosides A and B (**5**, **6**) were examined (Scheme 3). Direct reduction of such type of dienone to the corresponding ketone has not been described extensively, and so, a few test reactions were run. Direct hydrogenation of **15**, catalyzed by 5 % palladium on activated carbon at room temperature, afforded **18** and p-cresol (**13**) in 38 % and 40 % yields respectively, indicating that the second hydrogenation was competitive with aromatization. Interestingly, the dimethylketal (**19**), which displayed the methoxyl signals at δ 3.13 and 3.17 (each 3H s) in the ^{1}H NMR spectrum, was produced in 14 % yield during this process. The ketal **19** was easily transformed into **18** in aqueous media. On the other hand, the reduction of **15** with zinc in acetic acid under refluxing condition proceeded smoothly but only to give aromatization product, while the reduction of **15** by the Wilkinson's catalyst was not effective at all.[14]



15 **18** **19** **13**

Since there seemed few alternatives, direct hydrogenation of **9** catalyzed by 5 % palladium on activated carbon in methanol was examined. In the normal atmospheric pressure, however, no hydrogenation occured even at the refluxing temperature. The glucosyl group, which protected the dienone system from the excessive photooxygenation, blocked the reaction in this time. But the reaction proceeded slowly when **9** was hydrogenated at a high pressure (10 atm) at 80° affording the tetrahydro derivative, rengyoside B (**6**), in 23 % yield, and its dimethylketal (**20**) in 21 % yield, in addition to the aromatization product, **8**, in 34 % yield.



Scheme 3

Such a strong tendency to ketal formation was also encountered during the isolation of rengyoxide (**3**) using methanol as a solvent. Thus silica gel chromatography of crude **3** occasionally afforded forerunning fractions exhibiting sharp singlets at δ 3.13 and 3.18 in addition to a triplet at δ 3.73 (J=7 Hz) in its ^{1}H NMR spectrum and an ion peak at m/z 205 (M^++1) in the mass spectrum. These data were compatible with the structure **21**.[3] Therefore, this characteristic seems typical to the 4-hydroxycyclohexanone system. The unusual hemiketal structure **22**, which is in equiblium with the structure **3**, described for rengyoxide may also be the closely related behavior, although the detailed understanding of these phenomena was not feasible yet by these experimantal results.

Subsequently, either the sodium borohydride reduction of **6** or the sodium cyanoborohydride reduction of **20** at pH 4 afforded rengyoside A (**5**) stereoselectively. And finally, enzymatic hydrolysis of **6** and **5** with crude hesperidinase converted the glucosides to rengyol (**1**) and rengyoxide (**3**) in the quantitative yield, respectively.

Rengyol (**1**) is isolated from the crude drug by about 0.1 % of its weight,[3] which is a fairly large content, and hence, may be considered as a

sort of accumulation product. Further, as has been shown by this work, the glucosides **5**-**8** may stand as the metabolic intermediates of **1**,[5] while other minor congeners **2**-**4** are the products branched out from the main metabolic path by hydrolysis of the corresponding glucosides. The fact that rengyolone (**4**) occures as the racemic mixture probably because of non-enzymatic cyclization is consistent with this view.

The present work has fully substantiated these possible biosynthetic courses by modifying the aromatic ring by oxidation followed by the extensive reduction and enzymatic hydrolysis. Physiological significance of the metabolite in the plant still remains to be clarified.

Experimental

Melting points were taken on a hot-stage microscope (Mitamura Riken) and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL JNM FX-100 spectrometer. TMS was used throughout as an internal standard. Mass spectra (MS) were taken with a Hitachi-M52 or JEOL JMS-01SG-2 (high-resolution MS) spectrometer.

Photosensitized oxygenation was conducted by irradiating a sample solution in a Pyrex reactor, cooled by ice-water, with a 100 watt halogen lamp (USHIO, ICV 100-200GS). O_2 gas was bubbled into the reaction mixture.

Glucosylation of **10** with Ag_2CO_3——A mixture of **10** (0.83 g, 6 mmole) and Ag_2CO_3 (2.07 g, 7.5 mmole) in dry benzene-ether (9:1, 10 ml) was stirred at room temperature under N_2 atmosphere for 1.5 h. A solution of **11** (2.06 g, 5 mmole) and Ag_2CO_3 (1.66 g, 6 mmole) in dry benzene (5 ml) was added rapidly to the mixture and stirring continued for 12.5 h. The reaction mixture was treated with satd. aq. $NaHCO_3$ and was extracted with CH_2Cl_2. The extract was washed with brine and then dried over $MgSO_4$. Removal of the solvent afforded a residue, which was subjected to column chromatography on silica gel (80 g). Elution with hexane-ether (2:1) gave a product **12** (1.72 g, 74 %) as a colorless oil; $[\alpha]_D$ -16.7° (*c* 0.24, MeOH); ^{1}H NMR ($CDCl_3$) δ: 1.91, 1.98, 2.01, 2.08 (each 3H s, OAc), 2.79 (2H t, *J*=7 Hz, C7'-H_2), 3.64 (2H t, *J*=7 Hz, C8'-H_2), 3.68 (1H m, C5-H), 4.08 (1H d, *J*=12, 2 Hz, C6-H), 4.22 (1H d, *J*=12, 4 Hz, C6-H), 4.45 (1H d, *J*=7 Hz, C1-H), 4.8-5.2 (3H m, C2,3,4-H), 6.70 (2H d, *J*=9 Hz, C3',5'-H), 7.01 (2H d, *J*=9 Hz, C2',6'-H).

Alkaline hydrolysis of **12**——To a solution of **12** (0.77 g, 1.64 mmole) in MeOH (10 ml), K_2CO_3 (2.27 g, 16.4 mmole) was added. After stirring overnight, the reaction mixture was passed through celite. The filtrate was acidified with Amberlite IRA-120 to pH 6, concentrated under a reduced pressure, and the residue was subjected to column chromatography over silica gel (30 g). Elution with MeOH-$CHCl_3$ (1:4) gave a product **8** (0.49 g, 100 %) as amorphous powder; $[\alpha]_D$ -30.0° (*c* 1.78, H_2O); ^{13}C NMR (pyridine-d_5) δ: 35.8 t (C-7'), 62.6 t (C-6'), 71.1 t (C-8), 71.5 d (C-4), 74.9 d (C-2), 78.2 d (C-3), 78.2 d (C-5), 104.4 d (C-1), 116.0 d (C-3',5'), 129.3 s (C-1'), 130.3 d (C-2',6'), 157.0 s (C-4'); FD-MS *m/z*:300 (M^+). The ^{1}H NMR spectrum (CD_3OD) was identical to that of natural salidroside (**8**), $[\alpha]_D$ -27.7° (*c* 0.13, H_2O).[5]

Photooxygenation of p-cresol (**13**)——1) A solution of **13** (1.08 g, 10 mmole) and Rose Bengal (100 mg) in MeOH (150 ml) was irradiated for 10 h. The solution was then concentrated under a reduced pressure to give a

residue, which was chromatographed over a silica gel column (50 g). Elution with hexane-ether (1:1) gave the products **14** (0.66 g, 47 %), **15** (0.12 g, 10 %) and **16** (27 mg, 2.6 %).

14: Colorless needles from $CHCl_3$-hexane; mp 102-104°; ^{1}H NMR ($CDCl_3$) δ: 1.39 (3H s, Me), 4.22 (1H s, OOH), 6.23 (2H d, J=10 Hz, C3,5-H), 6.97 (2H d, J=10 Hz, C2,6-H); ^{13}C NMR ($CDCl_3$) δ: 22.7 q (Me), 78.4 s (C-1), 129.7 d (C-3,5), 150.9 d (C-2,6), 186.2 s (C-4); MS m/z: 124, 108 (base peak), 107, 96.

15: Colorless needles from $CHCl_3$-hexane; mp 74-77°; ^{1}H NMR ($CDCl_3$) δ: 1.44 (3H s, Me), 3.48 (1H brs, OH), 6.05 (2H d, J=10 Hz, C3,5-H), 6.89 (2H d, J=10 Hz, C2,6-H); ^{13}C NMR ($CDCl_3$) δ: 26.7 q (Me), 66.8 s (C-1), 126.3 d (C-3,5), 153.8 d (C-2,6), 186.4 s (C-4); MS m/z: 124 (M^+), 109 (base peak), 97, 82; High-resolution MS: Calcd for $C_7H_8O_2$, 124.0524. Found, 124.0499.

16: Colorless prisms from ether-hexane; mp 110-113°; ^{1}H NMR ($CDCl_3$) δ: 1.55 (3H s, Me), 2.30 (3H s, Me), 2.90 (1H dd, J=17, 4 Hz, C3-H), 2.99 (1H ddd, J=17, 4, 1 Hz, C3-H), 4.66 (1H m, C4-H), 5.89 (1H dd, J=10, 1 Hz, C5-H), 6.41 (1H dd, J=10, 2 Hz, C6-H), 6.67 (1H d, J=8 Hz, C12-H), 6.95 (1H brd, J=8 Hz, C11-H), 6.98 (1H s, C9-H); ^{13}C NMR ($CDCl_3$) δ: 20.8 q (Me), 21.4 q (Me), 37.5 t (C-3), 45.0 s (C-7), 86.5 d (C-2), 110.0 d (C-12), 123.1 d (C-9), 125.6 d (C-11), 129.6 d (C-5), 131.0 s (C-8), 132.2 s (C-10), 149.5 d (C-6), 156.6 s (C-13), 194.9 s (C-4); MS m/z: 214 (M^+), 199 (M^+-Me), 171 (M^+-Me-CO); High-resolution MS: Calcd for $C_{14}H_{14}O_2$, 214.0992. Found, 214.0990.

2) A mixture of **13** (1.08 g, 10 mmole) and Rose Bengal (100 mg) in acetone (150 ml) was irradiated for 10 h. The reaction mixture was treated with Me_2S (1.87 ml, 25 mmole) with stirring overnight, and was then worked up as above to give **15** (354 mg, 29 %) and **16** (28 mg, 2.6 %).

3) A mixture of **13** (1.08 g, 10 mmole) and Rose Bengal (100 mg) in AcOEt (150 ml) was iradiated for 10 h to give **14** (44 mg, 3 %), **15** (25 mg, 2 %) and **16** (16 mg, 1.5 %).

Photooxygenation of salidroside (**8**)——A mixture of **8** (0.53 g, 1.76 mmole) and Rose Bengal (50 mg) in MeOH (150 ml) was irradiated for 19 h. The reaction mixture was treated with Me_2S (0.33 ml, 4.4 mmole) with stirring overnight. The suspension was concentrated under a reduce pressure to give a residue, which was chromatographed on Sephadex LH-20 (300 g, MeOH) and on silica gel (20 g, $CHCl_3$-MeOH (6:1)) to give a product **9** (0.15 g, 27 %) and recovered **8** (0.38 g).

9: Amorphous powder; $[\alpha]_D$ -10.5° (c 0.42, EtOH); FD-MS m/z: 316 (M^+). The ^{1}H NMR spectrum (CD_3OD) and TLC were identical to those of natural cornoside (**9**), $[\alpha]_D$ -10.5° (c 0.26, MeOH).[4]

Photooxygenation of **10**——A solution of **10** (0.69 g, 5 mmole) and Rose Bengal (100 mg) in MeOH (150 ml) was irradiated for 10 h. The reaction mixture was treated with Me_2S (0.93 ml, 12.5 mmole) overnight and worked up followed by chromatography on silica gel (50 g) which was eluted with ether to give a product **4** (0.17 g, 22 %) and recovered **10** (0.10 g).

4: A colorless oil; MS m/z: 154 (M^+), 110, 83 (base peak). The ^{1}H NMR spectrum ($CDCl_3$) and TLC were identical to those of natural rengyolone (**4**).[3]

Photooxygenation of **10b**——A solution of **10b** (180 mg, 1 mmole) and Rose Bengal (10 mg) in MeOH (150 ml) was irradiated for 24 h followed by work up and chromatography to give a product **17** (19 mg, 10 %) and recovered **10b** (50 mg).

17: A colorless oil; ^{1}H NMR ($CDCl_3$) δ: 2.02 (3H s, OAc), 2.10 (2H t, J=6 Hz, C7-H_2), 4.16 (2H t, J=6 Hz, C8-H_2), 6.16 (2H d, J=10 Hz, C3,5-H), 6.85 (2H d, J=10 Hz, C2,6-H); ^{13}C NMR ($CDCl_3$) δ: 20.9 q (Me), 38.7 t (C-7), 59.6 t (C-8), 68.3 s (C-1), 128.2 d (C-3,5), 150.2 d (C-2,6), 170.7 s (C=O), 185.1 s (C-4); MS m/z: 154 (M^+-Ac), 136 (M^+-AcOH), 109 ($C_6H_5O_2$), 43 (base peak). These data were identical to those of natural hallerone (**17**).[6]

Hydrogenation of 15——To a solution of **15** (124 mg, 1 mmole) in MeOH (10 ml), 5% Pd-C (50 mg) was added and the resulting suspension was kept stirring under H_2 atmosphere at room temperature for 9 h. The catalyst was filtered off and the filtrate was concentrated to afford a residue, which was subjected to column chromatography on silica gel (20 g). Elution with hexane-ether (2:3) gave the products **18** (49 mg, 38 %), **19** (24 mg, 14 %) and p-cresol (43 mg, 40 %).
18: A colorless oil; ^{1}H NMR ($CDCl_3$) δ: 1.36 (3H s, Me), 1.7-2.1 (4H m, C2,6-H_2), 2.1-2.9 (4H m, C3,5-H_2), 1.96 (1H brs, OH); ^{13}C NMR ($CDCl_3$) δ: 28.9 q (Me), 36.3 t (C-2,6), 39.7 t (C-3,5), 67.8 s (C-1), 211.1 s (C-4); MS m/z: 128 (M^+), 110 (M^+-H_2O), 113 (M^+-Me); High-resolution MS: Calcd for $C_7H_{12}O_2$, 128.0712. Found, 128.0756.
19: A colorless oil; ^{1}H NMR ($CDCl_3$) δ: 1.22 (3H s, Me), 1.3-1.8 (8H m, C2,3,5,6-H_2), 3.13, 3.17 (each 3H s, OMe); ^{13}C NMR (pyridine-d_5) δ: 29.9 q (Me), 36.3 t (C-2,6), 39.3 t (C-3,5), 47.3 q (OMe), 47.4 q (OMe), 67.8 s (C-1), 100.2 s (C-4); MS m/z: 143 (M^++1), 142 (M^+), 125 (M^+-H_2O+1), 101 (M^+-H_2O-Me).

Hydrogenation of cornoside (9)——A mixture of **9** (142 mg, 0.45 mmole) and 5 % Pd-C (142 mg) in MeOH (30 ml) was stirred at 80° under hydrogen atmosphere (10 atm). After 6 h of reaction, the catalyst was filtered off and the suspension was concentrated under a reduce pressure to give a residue, which was chromatographed on a silica gel column (20 g). Elution with $CHCl_3$-MeOH (6:1) gave products **6** (32 mg, 23 %), **20** (34 mg, 21 %) and **8** (46 mg, 34 %).
6: Amorphous solid; $[\alpha]_D$ -10.4° (c 0.26, MeOH); ^{13}C NMR (pyridine-d_5) δ: 37.5 t (C-2',6'), 37.7 t (C-7'), 41.9 t (C-3',5'), 49.6 d (C-5), 62.6 t (C-8'), 66.3 t (C-6), 68.8 s (C-1'), 71.6 d (C-2), 78.4 d (C-3), 78.4 d (C-4), 104.6 d (C-1), 211.2 s (C-4'); MS m/z: 284 (M^+-$2H_2O$). The ^{1}H NMR spectrum (pyridine-d_5) and TLC were identical with those of natural rengyoside B (**6**), $[\alpha]_D$ -10.4° (c 0.28, MeOH).[5]
20: Amorphous solid; ^{1}H NMR (CD_3OD) δ: 2.81 (2H t, J=7 Hz, C7'-H_2), 3.13, 3.17 (each 3H s, OMe), 4.24 (1H d, J=7 Hz, C1-H); ^{13}C NMR (pyridine-d_5) δ: 34.6 t (C-2',6'), 34.7 t (C-3',5'), 35.7 t (C-7'), 47.3 q (OMe), 47.4 q (OMe), 55.1 d (C-5), 62.6 t (C-8'), 66.5 t (C-6), 69.3 s (C-1'), 71.6 d (C-2), 78.4 d (C-3), 78.4 d (C-4), 100.2 s (C-4'), 104.6 d (C-1).

Borohydride Reduction of rengyoside B (6) and 20——1) To a solution of **6** (7.0 mg, 0.022 mmole) in MeOH (0.3 ml), $NaBH_4$ (0.8 mg, 0.022 mmole) was added, and left standing for 30 min with stirring at room temperature. The reaction mixture was concentrated under a reduce pressure to give a residue, which was chromatographed on a silica gel column (10 g). Elution with $CHCl_3$-MeOH (2:1) gave **5** (7.1 mg, 100 %) as amorphous powder, $[\alpha]_D$ - 13.3° (c 0.35, MeOH). The ^{1}H NMR spectrum (CD_3OD) and TLC were identical to those of rengyoside A (**5**).[5]
2) To a cooled solution of **20** (14 mg, 0.040 mmole) in AcOH-H_2O (1 ml, pH 4), $NaBH_3CN$ (5 mg, 0.079 mmole) was added. After 30 min of stirring at room temperature, the reaction mixture was worked up to give **5** (9 mg, 70 %).

Enzymatic hydrolysis of 9——Crude hesperidinase (5 mg) was added to a solution of **9** (6.3 mg, 0.020 mmole) in H_2O (1 ml) and the mixture was incubated at 40° for 20 h. The reaction mixture was concentrated under a reduce pressure, and the residue was subjected to column chromatography on silica gel (10 g). Elution with CH_2Cl_2-MeOH (9:1) gave a product **4** (2.9 mg, 97 %), as a colorless oil; MS m/z: 154 (M^+), 110, 83 (base peak). The ^{1}H NMR spectrum ($CDCl_3$) and TLC were identical to those of natural rengyolone (**4**).[3]

Enzymatic hydrolysis of 5——A mixture of **5** (7.1 mg, 0.022 mmole) and crude hesperidinase (5 mg) in a citrate-phosphate buffer (pH 4.1, 1 ml) was treated and work up as above to give **1** (3.5 mg, 99 %) as colorless prisms

from MeOH, mp 121-123°; ^{1}H NMR (CD_3OD) δ: 1.68 (2H t, J=7 Hz, C7-H_2), 3.54 (1H m, W_H=20 Hz, C4-H), 3.72 (2H t, J=7 Hz, C8-H_2); MS m/z 142 (M^+-H_2O), 115 (M^+-C_2H_4OH). These data and TLC were identical with those of natural rengyol (**1**).[3]

Enzymatic hydrolysis of **6**——A mixture of **6** (7.6 mg, 0.024 mmole) and crude hesperidinase (5 mg) in a citrate-phosphate buffer (pH 4.1, 1 ml) was treated and work up as above to give **3** (3.8 mg, 100 %) as a colorless oil. The ^{1}H NMR spectrum (CD_3OD) and TLC were identical to those of natural rengyoxide (**3**).[3]

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References

+) Deceased on December 8 th, 1988.
1. Outline of this content has been presented at the 27 th Symposium on the Chemistry of Natural Products, Hiroshima, Japan, Oct. 1985; Chem. Abstracts **1986**, 104, 164877h. Recently similar transformations of p-hydroxyphenylethanol (**10**) and its acetate (**10b**) to hallerone (**17**) and rengyolone (**4**), respectively, have also been reported.[15]
2. (a) Endo, K.; Takahashi, K.; Abe, T.; Hikino, H. Heterocycles **1981**, 16, 1311-1315. (b) Endo, K.; Hikino, H. ibid. **1982**, 19, 2033-2036.
3. Endo, K.; Hikino, H. Can. J. Chem. **1984**, 62, 2011-2014.
4. Endo, K.; Seya, K.; Hikino, H. Tetrahedron **1987**, 43, 2681-2688.
5. Seya, K.; Endo, K.; Hikino, H. Phytochemistry **1989**, 28, in press.
6. (a) Messana, I.; Sperandei, M.; Multari, G.; Galeffi, C.; Marini Bettolo, G. B. Phytochemistry **1984**, 23, 2617-2619. (b) Navarro, E.; Trujillo, J.; Breton, J. L.; Boada, J. ibid. **1986**, 25, 1990-1991. (c) Abdullahi, H.; Nyandat, E.; Galeffi, C.; Messana, I.; Nicoletti, M.; Marini Bettolo, G. B. ibid. **1986**, 25, 2821-2823.
7. Nishibe, S.; Chiba, M.; Hisada, S. Yakugaku Zasshi **1977**, 97, 1134-1137.
8. (a) Levin, J. G.; Sprinson, D. B. J. Biol. Chem. **1964**, 239, 1142-1150. (b) Morell, H.; Clark, M. J.; Knowles, P. F.; Sprinson, D. B. ibid. **1967**, 242, 82-90.
9. Neish, A. C. Can. J. Botany **1959**, 37, 1085-1100.
10. (a) Koenigs, W.; Knorr, E. Ber. **1901**, 34, 957-981. (b) Troshchenko, A. T.; Juodvirshis, A. M. Khim. Prir. Soedin. **1969**, 5, 256-260.
11. (a) Saito, I.; Chujo, Y.; Shimazu, H.; Yamane, M.; Matsuura, T.; Cahnmann, H. J. J. Am. Chem. Soc. **1975**, 97, 5272-5277. (b) Ohmori, H.; Ueda, C.; Tokuno, Y.; Maeda, H.; Masui, M. Chem. Pharm. Bull. **1985**, 33, 4007-4011.
12. (a) Barton, D. H. R.; Deflorin, A. M.; Edwards, O. E. J. Chem. Soc. **1956**, 530-534. (b) Haynes, C. G.; Turner, A. H.; Waters, W. A. ibid. **1956**, 2823-2831. (c) Kametani, T.; Ogasawara, K. Chem. Pharm. Bull. **1968**, 16, 1138-1139.
13. (a) Taguchi, H.; Sankawa, U.; Shibata, S. Chem. Pharm. Bull. **1969**, 17, 2054-2060. (b) Penttila, A.; Fales, H. M. J. Chem. Soc., Chem. Comm. **1966**, 656-657.
14. (a) Young, J. F.; Osborn, J. A.; Jardine, F. H.; Wilkinson, G. J. Chem. Soc., Chem. Comm. **1965**, 131-132. (b) Birch A. J.; Walker, K. A. M. Tetrahedron Lett. **1967**, 3457-3458. (c) Piers, E.; Cheng, K. F. Can. J. Chem. **1968**, 46, 377-383.
15. Breton, J. L.; Llera, L. D.; Navarro, E.; Trujillo, J. Tetrahedron **1987**, 43, 4447-4451.