Acid Rearrangement of Secoiridoids Related to Oleuropein and Secologanin

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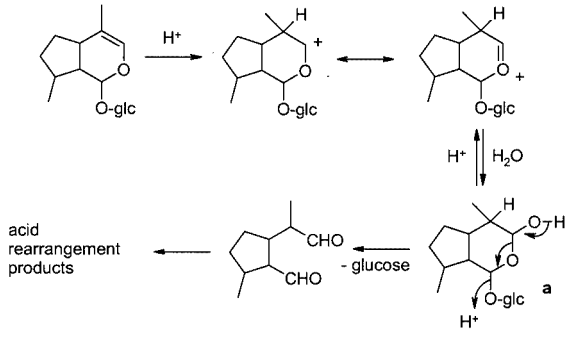
Acid treatment of an iridoid glycoside results in the cleavage of the acetal bond between the sugar unit and the monoterpenoid aglycon. Iridoids possessing non-conjugated enol ether systems, however, undergo the hydration of the iridoid enol ether functionality in acid medium, as well as the hydrolysis of the bond. We examined the acid rearrangement of secoiridoids such as oleuropein (**1**) and secologanin (**2**) and their reduction products oleuropeinol (**3**) and secologaninol (**4**), to examine whether similar behaviour also occurs in this case.

Introduction

The acid hydrolysis of the glucosidic moiety present in monoterpenoids possessing a dihydropyran moiety typical of the iridoid structure does not follow the classical hydrolytic mechanism of glycosides, which consists of the attack of a proton, or of a Lewis acid, on the oxygen of the acetal functionality. On the basis of a series of experimental details, we suggested a different mechanism that depends on the functionalisation of the dihydropyran ring.[1] In fact, it has always been considered that the first event that happens when an iridoid glycoside is treated with acids is the classical hydrolysis of the acetal bond between the sugar unit and the monoterpenoid aglycon. Right from the early research on iridoids, however, there have been numerous experimental indications of the existence of great differences between different iridoids in their ease of hydrolysis. In particular, it can be seen that significant differences exist between iridoids possessing conjugated enol ether systems, and those with non-conjugated systems. As an example, iridoids with conjugated enol ether systems remain stable in 2 M hydrochloric acid at room temperature for several hours, whereas iridoids without conjugated enol ether systems are quantitatively hydrolysed and undergo rearrangement in about an hour under the same conditions. The key to explaining this different behaviour on acid hydrolysis was sought in the reactivity of the enol ether system. It is well known that the unsaturated functionality of dihydropyran is susceptible to electrophilic attack; therefore, in acid medium the hydration of the iridoid enol ether functionality is a parallel reaction with the hydrolysis of the acetal bond. As shown in Scheme 1, the hydration of the C-3/C-4 double bond results in the formation of a hemiacetalic functionality on C-3 (intermediate a). We may therefore suggest the reported mechanism (Scheme 1) for the hydrolysis of sugar, consisting of the hydrolysis of the hemiacetalic intermediate **a**.



Scheme 1. Suggested mechanism for the acid hydrolysis of iridoids with non-conjugated enol ether systems

In this context the greater ease of hydrolysis of iridoids with non-conjugated enol ether systems, relative to those possessing conjugated enol ether systems, can be attributed to the easier hydration of the non-conjugated enol ether system, owing to its greater susceptibility to electrophilic attack. Of course, the presence of a conjugated enol ether system lowers the reactivity of the enol ether system to electrophilic attack. The process resulting in the elimination of the sugar is therefore due only to the acid hydrolysis of the acetal bond and this process, under the same conditions, is much slower.

The aim of this work is to examine the acid rearrangement of secoiridoids, to examine whether analogous behaviour also occurs in the case of this group of monoterpenes.

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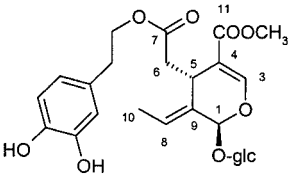
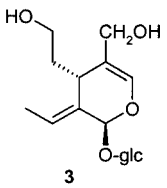


1 Oleuropein



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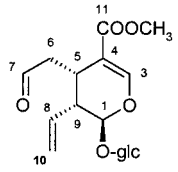
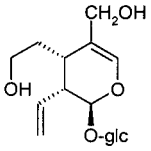


2 Secologanin



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Oleuropein (**1**) and secologanin (**2**), and their reduction derivatives oleuropeinol (**3**) and secologaninol (**4**),[2] were chosen as substrates presenting two typical functionalisations of the dihydropyran ring in iridoids: (i) the methoxycarbonyl group conjugated with the enol ether system and therefore corresponding to the maximum stability in the case of iridoid glucosides, and (ii) the hydroxymethyl group, which corresponds to the maximum ease of glucose cleavage.

In the case of a hydroxymethyl group at C-4, the hydrolytic process in fact appears even easier than in other non-conjugated iridoid glucosides, owing to the presence of an allylic hydroxy group that allows a course of hydrolysis we hypothesise as being as depicted in Scheme 2. We think that the key step appears to be the protonation of the allylic hydroxy group, which induces addition of water and therefore the hydrolytic process shown in Scheme 2.

Results and Discussion

Oleuropein-type Rearrangement

Oleuropein (**1**) was isolated from *Olea europaea*.[3] Oleuropeinol (**2**) was prepared from a crude sample of oleuropein (**1**) by reduction with $NaBH_4$ in water.[4]

Acid Rearrangement in Methanol: Oleuropein (**1**) in methanol does not show significant modifications on medium timescales (2−6 h at room temperature), at least at the level of the monoterpene unit. The only modification concerns the ester linkage with the hydroxytyrosol unit, which is transesterified with formation of a methoxycarbonyl function at C-7 and the production of oleoside dimethyl ester.[5]

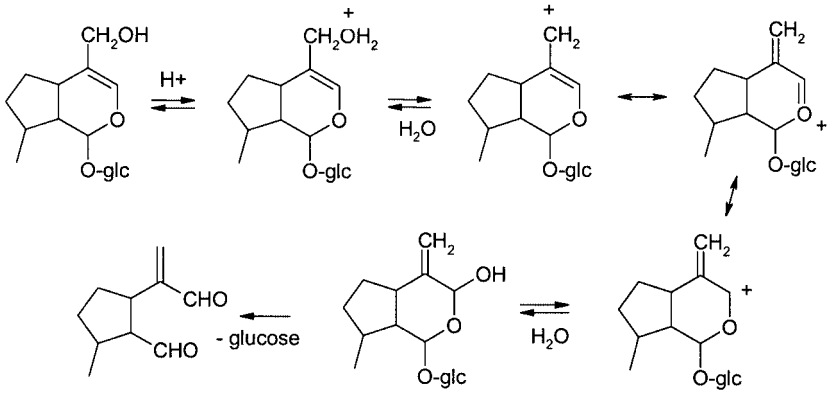
In contrast, the acid rearrangement of oleuropeinol (**3**) in methanol rapidly gave rise to two major products (Scheme 3), of which the first (**5**) is the result of the formation of a cyclic hemiacetal moiety between the primary alcohol function at C-7 and the aldehyde function at C-1, which successively affords the cyclic acetal of **5**. The structure of **5** was ascertained from its ^1H and ^{13}C NMR spectra (see Exp. Sect.). The second product probably arises from the open aldehyde form, which affords, in the reaction medium, the acetal structure **6**. This assumption is based on analysis of the ^1H NMR spectrum of **6**, in which there is no aldehyde signal, but four signals due to the methoxy groups are present. In addition, the base peak in the mass spectrum is 75, corresponding to $[CH(OCH_3)_2]^+$.

TLC monitoring showed that if the acid rearrangement of **3** is left for several hours (18−24 h) only one major product is obtained; it corresponds to compound **5**. This suggests that there is an equilibrium between **5** and **6**, which is slowly converted into the more stable **5**. TLC monitoring also showed two minor products that have not been identified.

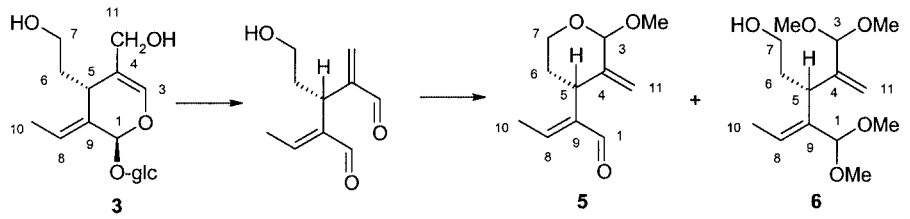
Acid Rearrangement in Water: Oleuropein (**1**), in aqueous acidic medium, does not show significant modification on medium timescales (2−6 h at room temperature), and these data, together with the results of the experiment in alcoholic acidic medium (see before), confirm the stability of methoxycarbonyl-substituted iridoids in acid medium.

The acid rearrangement of **3** in water showed a pattern similar to that seen in methanol. Only one product was observed by TLC, whereas ^1H NMR showed an equilibrium between the open dialdehyde form **7** and the closed acetal form **8** (Scheme 4), corresponding to **6** and **5**, respectively, the same products as observed in methanolic acid.

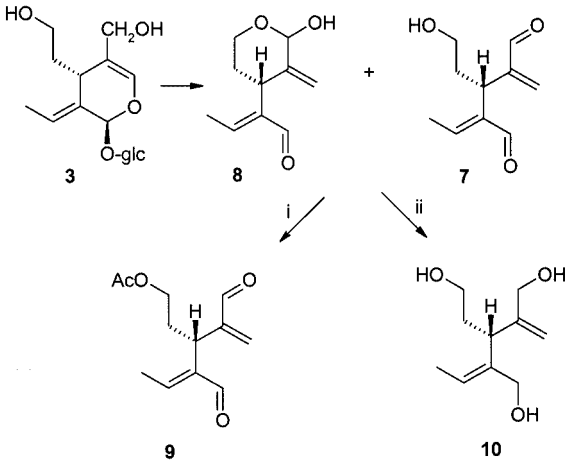
We considered it also to be of interest in this case to examine whether the rearrangement procedure had an in-



Scheme 2. Suggested mechanisms for the acid hydrolysis of iridoids with a hydroxymethyl at C-4



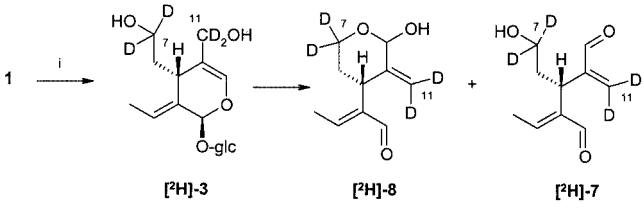
Scheme 3. Suggested mechanism for the acid hydrolysis of oleuropeinol (**3**)



Scheme 4. (i) Ac_2O/pyridine; ii) $NaBH_4$/MeOH

fluence on the products obtained. A prolonged rearrangement of **3** was performed for 24 h, but the rearrangement products were slowly degraded to black tar. This is in contrast to the prolonged rearrangement in methanol, in which only one major product was obtained.

The equilibrium mixture of **7** and **8** was acetylated, and gave only one main product **9**, according to TLC. By ^1H NMR analysis, compound **9** was found to be the monoacetyl derivative of the open form **7**. This could be explained in terms of an equilibrium shift towards the open form **7**, which is therefore acetylated. The equilibrium mixture of **7** and **8** was also reduced with $NaBH_4$ in methanol for a few minutes, with only one product being obtained, corresponding to the expected structure **10**. The structural determination was achieved from the ^1H and ^{13}C NMR spectra (see Exp. Sect.). The pathway in the rearrangement of oleuropeinol (**3**) and the structures of obtained compounds were also confirmed by the preparation of labelled oleuropeinol (**[^{2}H]-3**) by reduction of oleuropein (**1**) with $NaBD_4$



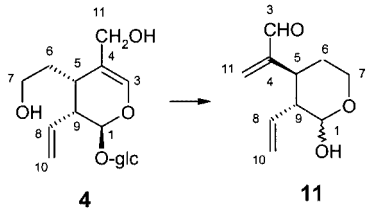
Scheme 5. Synthesis of deuterium-labelled **3**, **7** and **8**; (i) $NaBD_4$/D_2O

(Scheme 5). The ^{13}C NMR of **[^{2}H]-3** shows the typical pattern of deuterated compounds. The two singlets at δ = 61.92 and 62.97 ppm, which correspond to the two primary alcoholic functions at C-7 and C-11 in the spectrum of **3**, were reduced in size due to the multiple ^2H-C coupling, owing to the presence of two deuterium atoms at C-7 and C-11.

The production of **[^{2}H]-7** and **[^{2}H]-8** is in agreement with the acid rearrangement of **3** seen before. The ^{13}C NMR of **[^{2}H]-7** shows the "disappearance" of the peak at δ = 136.6 (C-11) and the peak at δ = 60.5 (C-7), while the carbon spectrum of **[^{2}H]-8** shows the "disappearance" of the peak at δ = 110.7 (C-11) and the peak at δ = 59.5 (C-7).

Secologanin-type Rearrangement

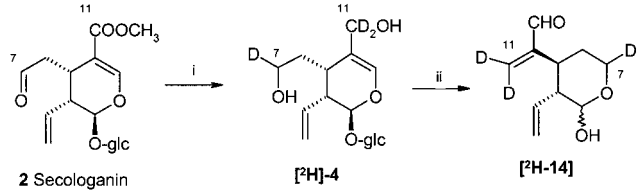
Secologanin (**2**) was isolated from *Rauwolfia grandiflora*.[6] Secologaninol (**4**) was prepared from a crude sample of **2** by reduction with $NaBH_4$ in water.[4] Secologanin (**2**), in aqueous acidic medium at room temperature, does not show any modification, confirming the stability of methoxycarbonyl-substituted secoiridoids under mildly acidic conditions. Secologaninol (**4**) in aqueous acidic medium is rapidly hydrolysed, affording, in practically quantitative yields, only compound **11** (Scheme 6), as the anomeric mixture at C-1.



Scheme 6

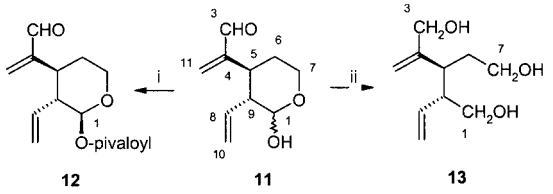
Compound **11** is the result of the opening of the dihydropyran ring of **2** in a process similar to that described in Scheme 2, with the dehydration of the vinylic hydroxymethyl group at C-4 and hemiacetalization between the hydroxy group at C-7 and the formyl group at C-1. Compound **11** is a mixture of the two anomeric forms, as clearly confirmed by NMR spectra showing the presence of two compounds differing in the configuration at the anomeric carbon. To have unambiguous assignments of the resonances in both the ^1H and the ^{13}C NMR spectra, we prepared the deuterio derivative **[^{2}H]-4** by reduction of secologanin **2** with sodium deuterioborohydride (Scheme 7), by

the same procedure as reported for the preparation of deuterioleuropeinol (**[^{2}H]-3**). Deuteriosecologaninol (**[^{2}H]-4**), treated in acidic medium similarly to its unlabelled parent **4**, afforded the deuterium-labelled rearrangement product **[^{2}H]-11**, confirming the proposed structure for **[^{2}H]-4**.



Scheme 7. (i) $NaBD_4$; ii) H^+

In addition, to obtain chemical support for the proposed structure, the anomeric mixture of **11** was esterified with pivaloyl chloride, providing only the more stable @ anomer ester **12**, and the same anomeric mixture of **11** was reduced with sodium borohydride, furnishing only one polyol **13**, as depicted in Scheme 8.



Scheme 8. (i) piv-Cl; ii) $NaBH_4$

The experimental data from the acid hydrolysis of oleuropein (**1**), secologanin (**2**) and their derivatives **3** and **4** indicate, as expected, that the behaviour of secoiridoids in acidic medium is similar to that of iridoids, being linked to the functionality of the dihydropyran ring.

Experimental Section

General: ^{1}H and ^{13}C NMR spectra were measured with a Mercury 300 spectrometer, and chemical shifts are expressed in ppm from TMS. MS spectra were recorded with a Kratos MS80 instrument. Microanalyses: CE Instruments. Optical rotations were registered with a Jasco DIP 370 polarimeter. Product purification was achieved by column chromatography on Merck 0.0623−0.20 mm silica gel. Merck TLC plates precoated with Kieselgel 60 F_{254} were employed to monitor the reactions, with use of 2 N H_2SO_4 as spraying reagent and heating at 120 °C for 2 min.

Oleuropein (1): Isolation of oleuropein (**1**) from *Olea europaea* was performed by the procedure described in refs.[7,8].

Oleuropeinol (3): Oleuropein (**1**, 1.0 g) was dissolved in water (70 mL), and $NaBH_4$ (1.5 g) was added in small portions. The reduction was followed by TLC ($CHCl_3$/MeOH, 8:2). After 40 h the reduction was complete, and the solution was neutralised with CO_2. Charcoal (10 g) was added, whilst stirring for 15 min. The suspension was stratified on a Gooch funnel and washed with water until no more salt was observed (400 mL). The charcoal was then washed with MeOH (100 mL), the organic phase was evaporated, and a crude sample of oleuropeinol (**3**, 803 mg) was obtained. This was chromatographed on a silica gel column (10 g) with $CHCl_3$/MeOH (85:15). This gave pure oleuropeinol (**3**, 794 mg, 85%). $[\alpha]_D = -24$ (c = 0.2, MeOH). ^{1}H NMR (D_2O): δ = 1.55 and 1.95 (m, 6-H_2), 1.64 (d, 10-H), 4.05 (d, 11-H_2), 5.60 (s, 1-H), 5.95 (q, 8-H), 6.37 (s, 3-H) ppm. ^{13}C NMR (D_2O): δ = 15.5 (C-10), 33.6 (C-6), 38.5 (C-5), 61.9 (C-7), 63.0 (C-11), 63.5 (C-6′), 72.4 (C-5′), 75.6 (C-4′), 78.6 (C-3′), 79.1 (C-2′), 97.2 (C-1′), 102.2 (C-1), 120.2 (C-8), 125.8 (C-4), 134.8 (C-9), 142.6 (C-3) ppm. $C_{16}H_{26}O_9$ (362): calcd. C 53.04, H 7.18; found C 52.89, H 7.29.

Rearrangement of 3 in Methanol/Acid. Compounds 5 and 6: Compound **3** (89 mg) was dissolved in HCl/MeOH (2 N, 3 mL). After 15 min, TLC ($CHCl_3$/MeOH, 8:2) showed 100% conversion. TLC tests show that 1 min in MeOH is enough for 100% rearrangement. The reaction was neutralised with $NaHCO_3$, and water (50 mL) was added to dissolve the salts. The solution was extracted with Et_2O (2 × 25 mL), which was washed with brine. The Et_2O extract was dried with $NaSO_4$, and the solvents were evaporated to give a mixture of **5** and **6** (48 mg). This was chromatographed on a silica gel column (4 g) with hexane/diethyl ether (7:3), which gave **5** (19 mg, 40%) and **6** (21 mg, 39%).

Compound 5: ^{1}H NMR (D_2O): δ = 2.02 (d, 10-H), 2.35 (m, 6-H_2), 3.72 (dd, 5-H), 4.00 (q, 7-H), 4.53 and 4.95 (s, 11-H_2), 4.93 (s, 3-H), 6.78 (d, 8-H), 9.41 (s, 1-H) ppm. ^{13}C NMR (D_2O): δ = 16.5 (C-10), 30.3 (C-6), 33.8 (C-5), 54.7 (OCH_3), 59.9 (C-7), 102.4 (C-3), 111.2 (C-11), 142.8 (C-9), 143.5 (C-4), 153.8 (C-8), 194.8 (C-1) ppm. $C_{11}H_{16}O_3$ (196): calcd. C 67.35, H 8.16; found C 67.16, H 8.25.

Compound 6: ^{1}H NMR ($CDCl_3$): δ = 1.12 (d, 10-H), 2.91−3.22 (m, 5-H, 1-H, 3-H), 3.25, 3.27, 3.31, 3.33 (4s, 4 × OCH_3), 3.98 (m, 27-H), 4.61−5.10 (m, 11-H_2), 6.59 (d, 8-H) ppm. MS: m/z = 75 $[CH(OCH_3)_2]^+$. $C_{14}H_{26}O_5$ (274): calcd. C 61.31, H 9.49; found C 61.19, H 9.59.

Rearrangement of 3 in Water. Compounds 7 and 8: Compound **3** (73 mg) was dissolved in HC1 (2 M, 4 mL), and after 5 min the solution was neutralised with $NaHCO_3$. TLC showed 100% rearrangement. The solution was extracted with EtOAc (3 × 25 mL), which was washed with brine. The EtOAc extract was dried with $NaSO_4$ and concentrated. This afforded an equilibrium mixture (35 mg) consisting of about 80% of **7** and 20% of **8**. The unseparated mixture was analysed by NMR spectroscopy.

Compound 7: ^{1}H NMR (D_2O): δ = 2.05 (d, 10-H), 2.10 (m, 6-H_2), 6.18 and 6.58 (s, 11-H_2), 6.75 (q, 8-H), 9.26 (s, 3-H), 9.43 (s, 1-H) ppm. ^{13}C NMR (D_2O): δ = 15.0 (C-10), 30.8 (C-5), 32.6 (C-6), 60.5 (C-7), 136.6 (C-11), 143.0 (C-9), 150.2 (C-4), 154.5 (C-8), 194.4 (C-3), 195.5 (C-1) ppm.

Compound 8: ^{1}H NMR (D_2O): δ = 4.50 and 4.96 (s, 11-H_2), 5.47 (s, 3-H), 6.78 (q, 8-H), 9.39 (s, 1-H) ppm. ^{13}C NMR (D_2O): δ = 16.2 (C-10), 30.9 (C-5), 32.7 (C-6), 59.5 (C-7), 95.4 (C-3), 110.7 (C-11), 143.2 (C-9), 150.4 (C-4), 153.8 (C-8), 194.7 (C-1) ppm. Elemental analysis of the mixture: $C_{10}H_{14}O_3$ (182.2): calcd. C 65.93, H 7.69; found C 65.87, H 7.88.

Acetylation of 7 and 8. Compound 9: The equilibrium mixture containing **7** and **8** (15 mg) was acetylated with Ac_2O in pyridine (the sample was dissolved in pyridine (0.2 mL) and Ac_2O (0.4 mL) was added; the reaction mixture was allowed to stand at room temperature for 2 h). MeOH (5 mL) was then added and the solution was evaporated six times with MeOH (5 mL) until no more pyridine was present. The residue was chromatographed on a silica gel col-

umn (1 g) with benzene/Et_2O (9:1), to afford **9** (11 mg, 60%). ^1H NMR ($CDCl_3$): δ = 2.05 and 2.35 (m, 6-H_2), 2.10 (d, 10-H), 6.20 and 6.63 (s, 11-H_2), 6.69 (q, 8-H), 9.24 (s, 3-H), 9.42 (s, 1-H) ppm. $C_{12}H_{16}O_4$ (224): calcd. C 64.29, H 7.14; found C 64.15, H 7.27.

Reduction of 7 and 8 . Compound 10: A mixture of **7** and **8** (31 mg) was dissolved in MeOH, and reduced with $NaBH_4$. After 3 min. the solution was neutralised with CO_2, and a little water was added to dissolve the salts. The MeOH/water phase was extracted with EtOAc (50 mL), according to the amount of starting material. TLC in $CHCl_3$/MeOH (9:1) showed one spot. The crude product (34 mg) was chromatographed on silica gel, with elution with $CHCl_3$/MeOH (98:2 and finally 9:1). This afforded **10** (29 mg, 93%). ^1H NMR (D_2O): δ = 1.71 (d, 10-H), 2.0 (m, 6-H_2), 3.5 (m, 3-H), 3.65 (m, 7-H), 4.00 and 4.03 (s, 1-H), 5.03 and 5.21 (s, 11-H_2), 5.76 (q, 8-H) ppm. $C_{10}H_{18}O_3$ (186): calcd. C 64.51, H 9.68; found C 64.36, H 9.77.

Preparation of Deuterio-oleuropeinol [^{2}H]-3 and Rearrangement of [^{2}H]-3 to [^{2}H]-7 and [^{2}H]-8: The crude fraction of **1** (253 mg) was dissolved in water (15 mL), and $NaBD_4$ (100−150 mg) was added. This was left for 2 h. TLC ($CHC1_3$/MeOH, 7:3) did not show full reduction, so further $NaBD_4$ (105 mg) was added and the system was left for another 2 h. The solution was neutralised with CO_2, and charcoal (2.5 g) was added whilst stirring for 15 min. The resulting suspension was stratified on a Gooch funnel, and washed with water (250 mL) until no more salts were present. The charcoal was eluted with MeOH (70 mL). The MeOH phase was evaporated, affording a crude product (235 mg), which was chromatographed on a silica gel column (5 g) with $CHCl_3$/MeOH (9:1 to 8:2). This gave pure **[^{2}H]-3** (212 mg, 90%). ^1H NMR (D_2O): δ = 1.53 and 1.94 (m and df, 6-H_2), ***((←Author: what is the meaning of df?))*** 1.64 (d, 10-H), 5.64 (s, 1-H), 5.96 (q, 8-H), 6.37 (s, 3-H) ppm. ^{13}C NMR (D_2O): δ = 15.3 (C-10), 33.6 (C-6), 38.2 (C-5), 63.4 (C-6′), 72.3 (C-5′), 75.5 (C-4′), 78.5 (C-3′), 79.1 (C-2′), 97.3 (C-1′), 102.2 (C-1), 120.2 (C-8), 125.8 (C-4), 134.8 (C-9), 142.6 (C-3) ppm. Acid rearrangement of **[^{2}H]-3** (70 mg) was performed as described previously for **3** and an equilibrium mixture (34 mg) consisting of about 80% of **[^{2}H]-7** and 20% of **[^{2}H]-8** was obtained. The inseparable mixture was analysed by NMR spectroscopy.

Compound [^{2}H]-7: ^1H NMR (D_2O): δ = 2.08 (d, 10-H), 2.10 (m, 6-H_2), 4.02 (t, 5-H), 6.66 (q, 8-H), 9.30 (s, 3-H), 9.46 (s, 1-H) ppm.

Compound [^{2}H]-8: ^1H NMR (D_2O): δ = 2.05 (nm, 10-H), 2.2 (m, 6-H_2), 4.0 (m, 5-H), 5.50 (s, 3-H), 6.79 (q, 8-H), 9.41 (s, 1-H) ppm.

Secologanin (2): The isolation of secologanin (**2**) from *Rauwolfia grandiflora* was performed by the procedure described in ref.[5].

Secologaninol (4): Secologanin (**2**, 500 mg) was dissolved in water (60 mL), and $NaBH_4$ (1.5 g) was added in small portions. The reaction mixture was allowed to stand at room temperature overnight. When the reaction was complete, as evidenced by TLC, the solution was neutralised with CO_2 and filtered, charcoal (10 g) was added, and the resulting suspension was stratified on a Gooch funnel and washed with water until no more salt was observed (400 mL). The charcoal was then washed with MeOH (200 mL), and the organic phase was concentrated in vacuo to give crude **4** (470 mg, 80%). Further purification for analytical purposes was performed by silica gel chromatography in $CHCl_3$/MeOH (8:2). ^{13}C NMR (D_2O): δ = 26.3 (C-5), 29.8 (C-6), 42.8 (C-9), 59.8 (C-7), 61.4 (C-11), 61.4 (C-6′), 70.4 (C-4′), 73.5 (C-2′), 76.4 (C-3′), 76.9 (C-5′), 97.4 (C-1), 98.8 (C-1′), 115.7 (C-4), 120.4 (C-10), 134.1 (C-8), 139.0 (C-3) ppm. $C_{16}H_{26}O_9$ (362): calcd. C 53.04, H 7.18; found C 52.87, H 7.29.

Rearrangement of 4 in Water. Compound 11: Compound **4** (100 mg) was dissolved in HCl (2 N, 5 mL), and the reaction mixture was allowed to stand at room temperature for 30 min. After this time the reaction was complete as evidenced by TLC ($CHCl_3$/CH_3OH, 95:5). The solution was neutralised with saturated $NaHCO_3$ and extracted with Et_2O (3 × 25 mL), which was then washed with brine and dried on anhydrous Na_2SO_4. The volatile material was removed in vacuo, affording crude **11** (51 mg). Compound **11** was purified on silica gel ($CHCl_3$/CH_3OH, 95:5) to afford pure **11** (47 mg, 98%). M.p. 54−54.5 °C; needles from CCl_4. ^1H NMR (D_2O): δ = 1.50−1.80 (m, 6-H_2), 2.18 (dddd, *J* = 8.5, 13.0 Hz, 9-Ha,), 2.50 (dd, *J* = 3.5, 13.0 Hz, 9-Hb), 2.89 (d, *J* = 3.5 Hz, 5-H), 3.24 (d, *J* = 13.0 Hz, 5-H), 3.48−3.78 (m, 7-Hα), 3.95−4.27 (m, 7-Hβ), 4.54 (d, *J* = 8.5 Hz, 1-Hα), 4.96 (d, *J* = 14.0 Hz, 10-H_{trans}), 5.00 (1-Hβ), 5.04 (m, 10-H), 5.52 (m, 8-H), 6.06 (d, *J* = 4.5 Hz, 11-Hb), 6.25 (d, *J* = 4.5 Hz, 11-Ha), 9.41 (s, 3-H) ppm. ^{13}C NMR (D_2O): α-anomer: δ = 31.2 (C-6), 32.2 (C-5), 49.7 (C-9), 59.1 (C-7), 94.1 (C-1), 117.4 (C-10), 135.2 (C-11), 137.5 (C-8), 151.3 (C-4), 194.1 (C-3) ppm. β anomer: δ = 31.5 (C-6), 36.8 (C-5), 52.1 (C-9), 65.4 (C-7), 98.5 (C-1), 118.6 (C-10), 135.3 (C-11), 135.6 (C-8), 152.0 (C-4), 194.3 (C-3) ppm. $C_{10}H_{14}O_3$ (182): calcd. C 65.93, H 7.69; found C 65.88, H 7.77.

Preparation of Deuterio-secologaninol [^{2}H]-4 and Rearrangement of [^{2}H]-4 to [^{2}H]-11: Secologanin (**2**, 250 mg) was dissolved in water (40 mL), and $NaBD_4$ (150 mg) was added. The reaction mixture was allowed to stand at room temperature overnight. When the reaction was complete, as evidenced by TLC ($CHC1_3$/MeOH, 7:3), the solution was neutralised with CO_2, charcoal (5 g) was added, and the resulting suspension was stratified on a Gooch funnel and washed with water until no more salt was observed (400 mL). The charcoal was then washed with MeOH (200 mL), and the organic phase was concentrated in vacuo to give crude **[^{2}H]-4** (230 mg, 91%). ^1H NMR (D_2O): δ = 1.32−1.51 (m, 6-Ha), 1.90−2.05 (m, 6-Hb), 2.50−2.62 (m, 5-H), 2.79−2.92 (m, 9-H), 4.60 (d, 1-H′), 5.12- 5.75 (m, 1-H, 10-H, 8-H), 6.30 (d, 3-H) ppm. ^{13}C NMR (D_2O): δ = 27.2 (C-5), 30.9 (C-6), 44.3 (C-9), 60.3 (C-7), 97.6 (C-1), 116.3 (C-4), 19.8 (C-10), 136.0 (C-8), 139.9 (C-3) ppm. Acid rearrangement of **[^{2}H]-4** (100 mg) was performed as described previously for **4**, and compound **[^{2}H]-11** was obtained (49 mg, 90%). ^{13}C NMR (D_2O): α anomer: δ = 31.1 (C-6), 32.2 (C-5), 49.7 (C-9), 58.9 (C-7), 94.3 (C-1), 117.5 (C-10), 137.4 (C-8), 151.9 (C-4), 194.0 (C-3) ppm. β anomer: δ = 31.4 (C-6), 36.8 (C-5), 52.3 (C-9), 65.1 (C-7), 98.5 (C-1), 118.7 (C-10), 135.4 (C-8), 151.2 (C-4), 194.2 (C-3) ppm.

Compound 12: Compound **11** (50 mg) was dissolved in pyridine (1 mL), pivaloyl chloride (0.5 mL) was added, and the reaction mixture was allowed to stand at room temperature for 2 h. MeOH (5 mL) was then added and the solution was concentrated in vacuo. The residue was dissolved in Et_2O, which was washed with water, cold NaOH (2 N), water, cold aqueous HCl (2 N) and then brine. The organic phase was concentrated in vacuo to give crude **12** (90 mg). Compound **12** was purified on silica gel with elution with hexane/Et_2O (9:1) to afford pure **12** (70 mg, 98%). ^1H NMR (D_2O): δ = 1.50−1.80 (m, 6-H_2), 2.54 (dd, *J* = 3.5, 13.0 Hz, 9-H), 3.28 (d, *J* = 13.0 Hz, 5-H), 3.9−4.2 (m, 7-H), 4.9−5.0 (10-H_2), 6.09 (1-H), 5.55 (m, 8-H), 6.06 (d, *J* = 4.5 Hz, 11-H), 9.41 (s, 3-H), 1.13 (s, 3 CH_3) ppm. ^{13}C NMR (D_2O): δ = 27.0 [(CH_3)$_3$C], 31.0 (C-6), 37.3 (C-5), 38.5 (*C*-CO), 49.7 (C-9), 66.1 (C-7), 95.5 (C-1), 119.5 (C-10), 134.5 (C-11), 135.4 (C-8), 150.9 (C-4), 176.6 (CO), 195.1 (C-3) ppm. $C_{15}H_{22}O_4$ (266): calcd. C 67.67, H 8.27; found C 67.58, H 8.33.

Compound 13: Compound **11** (50 mg) was dissolved in THF/H_2O (3:1, 10 mL), and $NaBH_4$ (100 mg) was added, the reaction being followed by TLC ($CHCl_3$/MeOH, 9:1). After 20 min the reaction was complete and the solution was neutralised with CO_2. After evaporation of THF, charcoal (500 mg) was added. The suspension was stratified on a Gooch funnel and washed with water until no more salt was observed (100 mL), after which the charcoal was washed with MeOH (100 mL). The methanol was concentrated in vacuo and the residue was chromatographed on silica gel ($CHCl_3$/MeOH, 9:1) to afford pure **13** (46 mg, 95%). $[\alpha]_D = -13$ (c = 0.2, MeOH). ^{1}H NMR (D_2O): δ = 1.50−1.80 (m, 6-H_2), 2.11 (m, 9-H), 2.77 (d, J = 3.5 Hz, 5-H), 3.48−3.78 (7-H, 3-H, 1-H), 4.9−5.1 (10-H), 5.52 (m, 8-H), 6.25 (d, J = 4.5 Hz, 11-H) ppm. ^{13}C NMR (D_2O): δ = 35.9 (C-6), 40.6 (C-5), 51.7 (C-9), 61.2 (C-7), 64.5 (C-1), 65.8 (C-3), 111.8 (C-11), 117.6 (C-10), 139.8 (C-8), 150.8 (C-4) ppm. $C_{10}H_{18}O_3$ (186): calcd. C 64.52, H 9.68; found C 64.45, H 9.78.

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