Dopaol 2-Keto- and 2,3-Diketoglycosides from *Chelone obliqua*

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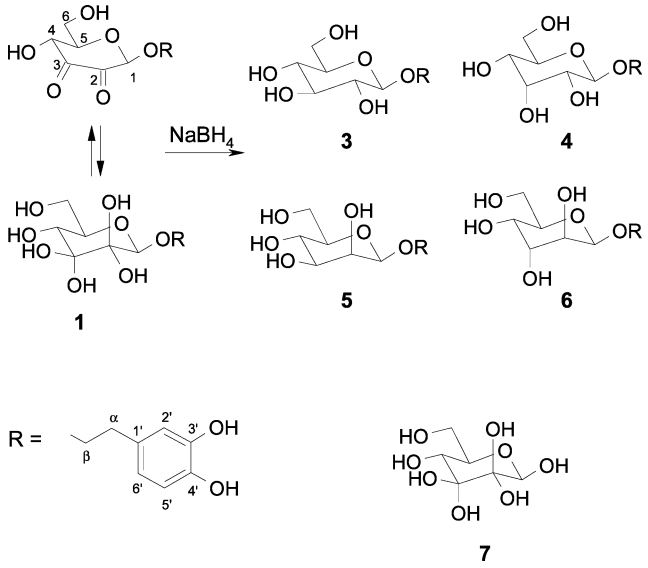
Two unique 2-(3,4-dihydroxyphenyl)ethyl glycosides, namely, dopaol β-D-2-ketoglucopyranoside and dopaol β-D-2,3-diketoglucopyranoside, were isolated from *Chelone obliqua* together with the iridoid glucoside catalpol, dopaol β-D-glucopyranoside, descaffeoylverbascoside, and verbascoside. Glycosides with a diketosugar have not so far been isolated from natural sources.

The genus *Chelone* (Scrophulariaceae) comprises four species with a geographical distribution limited to Northeast America.[1] The two closely related genera *Chionophila* and *Nothochelone*[2] with two and one species, respectively, are both limited to the Western states. There have been only two previous reports of phytochemical work on *Chelone*, namely, the chromatographic detection of the iridoid glucosides catalpol and aucubin in *C. lyonii* Purch., *C. obliqua* L.,[3] and *C. glabra* L.[4] In the course of an investigation of the chemotaxomy of Scrophulariaceae we now report on the water solubles of *C. obliqua*.

The water-soluble part of an ethanolic extract of *C. obliqua* was fractionated by reversed-phase chromatography with H_2O−MeOH mixtures to give the iridoid glucoside catalpol. In addition, two novel glycosides (**1** and **2**) were isolated together with dopaol β-D-glucopyranoside (**3**),[5] decaffeoylverbascoside,[6] and the more common caffeoyl phenylethyl glucoside (CPG) verbascoside.[7]

Compound **1** was obtained as an amorphous glass, prone to decompose partially upon high-vacuum drying, and it was thus only characterized by NMR spectroscopy and by HRFABMS. From the ^{1}H and ^{13}C NMR spectra of **1** in D_2O, it was obvious that it was a glycoside closely related to **3**. The observed $[M - H]^-$ ion of *m/z* 311.0767 showed the molecular formula to be $C_{14}H_{16}O_8$ and demonstrated the presence of two additional unsaturations when compared to **3**. Besides the signals from the dopaol aglycon, the ^{13}C NMR spectrum revealed two low-intensity resonances (δ 94.3 and 95.8), signals from an anomeric carbon at δ 101.0, two oxygenated methines at δ 70.2 and 76.0, and finally a hydroxymethyl signal at δ 62.4. The ^{1}H NMR signals were consistent with this and displayed the coupling system $HOCH_2$−CHOR−CHOH− together with a signal from an anomeric proton (δ 4.66, linked to the carbon at δ 101.0 according to HSQC) appearing as a sharp singlet. Thus we concluded that the carbohydrate part of **1** in solution was the dihydrate of a 2,3-diketo-β-D-glucopyranoside (i.e., a β-D-erythrohexos-2,3-diulopyranoside), which would explain the two low-intensity signals. Full assignments were performed by using the HSQC and HMBC spectra; in the latter correlations were seen between C-2 (δ 94.3) and H-1 (δ 4.66) as well as between C-3 (δ 95.8) and H-4 (δ 3.63). Further proof was obtained by subjecting **1** to sodium borohydride reduction, which gave rise to all four possible fully reduced dopaol glycosides. The approximate distribution between the β-D-glucoside (**3**), β-D-alloside (**4**),[8] β-D-mannoside (**5**), and β-D-altroside (**6**) of dopaol was ca. 2:4:8:1; the last two compounds proved to be inseparable even by repeated HPLC. This showed that hydride addition to the α-face of the 2-keto group was only slightly prevalent, while hydride addition to the α-face of the 3-keto group was more dominant. The structures of **5** and **6** were based upon 2D NMR assignment of the ^{13}C NMR spectra and comparison of these with reported data for methyl β-D-mannopyranoside and methyl β-D-altropyranoside.[9] The present isolation of a naturally occurring 2,3-diketoglycoside is to our knowledge unprecedented. However, the dihydrate of the corresponding free sugar, β-D-*erythro*-hexos-2,3-diulopyranose (**7**), has been prepared enzymatically and its X-ray structure determined.[10,11] The free diketoaldose was shown to exist as a single dihydrate form even in DMSO solution; it was stable for several hours, and the 2-monoketo form could only be detected by NMR spectroscopy after equilibration for 1 day.[11] Similar treatment of **1**, however, led to partial decomposition of the compound. Comparison of the NMR data of the free 2,3-diketosugar (δ 94.3, 94.7, 93.5, 69.4, 75.7, 61.8) in DMSO[11] with those of **1** showed a high degree of similarity, even allowing for the difference in solvent, except for the expected downfield ^{13}C chemical shift for C-1 in the glycoside.

Scheme 1



Compound **2** could not be obtained in an entirely pure state, partly because **1** and **2** eluted in overlapping fractions, and even by repeated HPLC only a 10:1 mixture of

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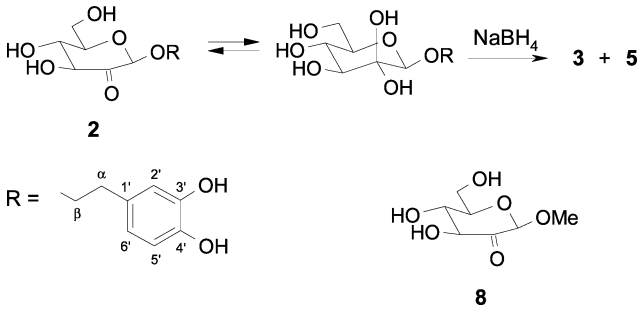
Scheme 2



2 and **1** was obtained. Hence **2** was also only characterized by NMR spectroscopy and by HRFABMS. Again, the overall appearances of the ^{1}H and ^{13}C NMR spectra of compound **2** were similar to those of **1** and **3**. The observed $[M - H]^-$ ion of *m*/*z* 313.0924 gave the molecular formula $C_{14}H_{18}O_8$, corresponding to one less unsaturation than in **1**. As above, the ^{13}C NMR spectrum of **2** in D_2O showed an unusual carbohydrate moiety to be present in addition to the signals arising from a dopaol aglycon. The glycon comprised six carbon atoms, of which one appeared as a low-intensity signal at unusually low field (δ 93.9), while the other signals seemed to constitute three oxygenated methines, a hydroxymethyl group, and an anomeric carbon (δ 102.3). As was the case with **1**, the anomeric proton (δ 4.47) appeared as a sharp singlet, and consequently the quaternary carbon at δ 93.9 could be assigned as a hydrated 2-keto group. Using the COSY correlations and starting from the typical hydroxymethyl signals (δ 3.90 and 3.71) with the usual large geminal coupling constant typical of aldohexoses, it was possible to assign the remaining three one-proton signals in the region δ 3.9−3.4 to a single coupling system, namely, $HOCH_2-CHOR-CHOH-CHOH-$. Thus, **2** was the hydrated form of a 2-ketoaldopyranoside. This also allowed assignment of the ^{13}C NMR signals using the HSQC and HMBC spectra. Additional proof for the structure of **2** was obtained by sodium borohydride reduction, which gave a 1:3 mixture of dopaol-β-D-glucoside (**3**) and dopaol-β-D-mannoside (**5**). In this case, hydride addition on the 2-keto group had proceeded with the expected preference for the less hindered α-face.

Keto forms of monosaccharides in aqueous solution exist mainly as the hydrates, as seen from data for both anomers of 2-ketoglucose (glucosone)[12] and also for methyl 3-ketoglucosides[13] obtained from the free sugars by enzymatic processes.[12−14] Likewise, chemical oxidation[15,16] of unprotected methyl glycosides has produced several 2-keto-, 3-keto-, and 4-ketoglycosides, and NMR data (in D_2O) for the dihydrate form of methyl β-D-2-ketoglucopyranoside (**8**) were also reported. We have found only two reports of naturally occurring 2-ketoglycosides in the literature, namely, on two saponins (gymnemic acids) from *Gymnema sylvestre*[18] (Asclepediaceae) as well as on antibiotics from a *Streptomyces* sp.[19] However, several examples of iridoid 3-ketoglucosides are known. In the genus *Penstemon* (closely related to *Chelone*), serruloside[19] has been isolated from *P. serrulatus*, and dihydroserruloside[20] has been reported from *P. confertus*. Also, suspensolide[21] from *Viburnum suspensum* (Sambucaceae) and clandonoside[22] together with 8-*O*-acetylclandonoside from *Caryopteris* × *Clandonensis* (Lamiaceae) are naturally occurring iridoid 3-ketoglucosides. All of these iridoid compounds were shown to exist as the keto forms when their NMR spectra were recorded in methanol; however, the last two compounds were found to be hydrated when dissolved in water.[22]

Neither *Chelone lyonii* nor *Nothochelone nemorosa* (Douglas ex Lindl.) Straw appeared to contain any dopaol glycosides as seen by an initial screening by NMR and HPLC.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DRX600 instrument at 600 and 150 MHz, respectively, in D_2O using acetone (δ 31.4 and 2.23) as the standard. HRFABMS (JEOL JMS-AX505W) were recorded in negative mode, using a bis-(hydroxyethyl)disulfide matrix.

Plant Material. Flowering stems of *Chelone obliqua* were collected from plants cultivated in The Botanical Garden of The University of Copenhagen, Denmark. A voucher (IOK-4/2000) has been deposited at the Botanical Museum, The University of Copenhagen, Denmark (C).

Extraction and Isolation. Frozen flowering stems (50 g) of *C. obliqua* were homogenized with EtOH (0.3 L), and the extract was partitioned in H_2O-Et_2O (1:10, 150 mL). The aqueous layer was concentrated to yield a water-soluble extract (2.44 g), which was separated on a Merck Lobar (RP-18) reversed-phase column (size C). Elution with $H_2O-MeOH$ mixtures (25:1 to 1.5:1) gave, after the polar front, first catalpol (105 mg), then a fraction of slightly impure dopaol β-D-2,3-diketoglucoside (**1**; 60 mg), a ca. 1:1 mixture (20 mg) of **1** and dopaol β-D-2-ketoglucoside (**2**), followed by dopaol β-D-glucoside (**3**; 120 mg), descaffeoylverbascoside (30 mg), a fraction (200 mg) containing di- or oligomeric hemiketals of **1**, and finally verbascoside (330 mg) was obtained. The above fraction (200 mg) containing hemiacetalic ketoglucosides was dissolved in H_2O and allowed to stand for 24 h, upon which analytical HPLC showed the sample to be an approximately 1:1 mixture of **1** and the original hemiketalic mixture. From this fraction a further amount of **1** could be obtained by chromatography.

Dopaol β-D-2,3-diketoglucoside (1): ^{1}H NMR δ 4.66 (1H, s, H-1), 3.63 (1H, d, J = 10.1 Hz, H-4), 3.55 (1H, ddd, J = 10.1, 6.1 and 2.1 Hz, H-5), 3.89 (1H, dd, J = 12.2 and 2.1 Hz, H-6a), 3.71 (1H, dd, J = 12.2 and 6.1 Hz, H-6b), 2.86 (2H, t, J = 7.0 Hz, H-α), 4.10 (1H, dt, J = 10.1 and 7.0 Hz, H-β), 3.90 (1H, dt, J = 10.1 and 7.0 Hz, H-β), 6.89 (1H, d, J = 2.1 Hz, H-2′), 6.88 (1H, d, 7.9 Hz, H-5′), 6.78 (1H, dd, 7.9 and 2.1 Hz, H-6′); ^{13}C NMR δ 101.0 (C-1), 94.3 (C-2), 95.8 (C-3), 70.2 (C-4), 76.0 (C-5), 62.4 (C-6), 35.7 (C-α), 72.2 (C-β), 132.7 (C-1′), 118.0 (C-2′), 145.0 (C-3′), 143.5 (C-4′), 117.5 (C-5′), 122.4 (C-6′); HRFABMS *m*/*z* 311.0767 $[M - H]^-$ (calcd for $C_{14}H_{15}O_8$, 311.0757).

Dopaol β-D-2-ketoglucoside (2). Fractions from several workups containing mixtures of **1** and **2** were rechromatographed three times. Finally, a fraction containing ca. 90% of **2** was obtained: ^{1}H NMR δ 4.47 (1H, s, H-1), 3.48 (1H, d, J = 9.5 Hz, H-3), 3.42 (1H, t, J = 9.5 Hz, H-4), 3.42 (1H, obsc, H-5), 3.90 (1H, dd, J = 12.1, 1.7 Hz, H-6a), 3.71 (1H, dd, J = 12.1, 5.7 Hz, H-6b), 2.85 (2H, t, J = 7.0 Hz, H-α), 4.07 (1H, dt, J = 10.1, 7.0 Hz, H-β), 3.86 (1H, dt, J = 10.1, 7.0 Hz, H-β), 6.87 (1H, d, J = 1.8 Hz, H-2′), 6.86 (1H, d, 8.1, H-5′), 6.77 (1H, dd, 8.1, 1.8 Hz, H-6′); ^{13}C NMR δ 102.3 (C-1), 93.9 (C-2), 77.5 (C-3), 69.9 (C-4), 77.1 (C-5), 62.1 (C-6), 35.6 (C-α), 72.2 (C-β), 132.7 (C-1′), 118.0 (C-2′), 145.1 (C-3′), 143.5 (C-4′), 117.5 (C-5′), 122.4 (C-6′); HRFABMS *m*/*z* 313.0924 $[M - H]^-$ (calcd for $C_{14}H_{15}O_8$, 313.0923).

Dopaol β-D-glucoside (3): ^{13}C NMR δ 103.4 (C-1), 74.3 (C-2), 77.0 (C-3), 70.9 (C-4), 77.1 (C-5), 62.0 (C-6), 35.7 (C-α), 72.1 (C-β), 132.7 (C-1′), 118.0 (C-2′), 145.1 (C-3′), 143.5 (C-4′), 117.5 (C-5′), 122.4 (C-6′), essentially as reported,[5] except for the different solvent used (MeOH-d_4).

Sodium Borohydride Reduction of Ketoglucosides. A sample of the above mixture of **1** and **2** (27 mg) was treated with $NaBH_4$ (5 mg) in H_2O (2 mL) at 0 °C for 0.5 h followed by an additional 4 h at room temperature. Upon addition of 10% HOAc (2 mL), the reaction mixture was loaded on an RP-18 Lobar column (size B), which was eluted with H_2O and then $H_2O-MeOH$ (15:1 to 6:1). This gave an approximately 1:2 mixture (9 mg) of dopaol β-D-glucoside (**3**) and dopaol β-D-alloside (**4**), an intermediary fraction containing an approxi-

mately 1:1 mixture (3 mg) of **3** and dopaol β-D-mannoside (**5**), and finally a fraction of **5** (13 mg) containing a small amount of dopaol β-D-altroside (**6**), as seen by NMR analysis of the fractions.

Experiment A. 1 (11 mg) was reduced with $NaBH_4$ (2 mg) at 0 °C for 0.5 h, then more $NaBH_4$ (2 mg) was added and the temperature was allowed to rise to 20 °C. Analysis of the reaction mixture by ^{1}H NMR and analytical HPLC showed an approximately 4:2:8:1 mixture of **4**, **3**, **5**, and **6** (the last two coeluting).

Experiment B. An approximately 1:1 mixture of **1** and **2** (18 mg) was reduced as above with $NaBH_4$ (2 × 4 mg). ^{1}H NMR and analytical HPLC showed the reaction mixture to be an approximately 1:1:3 mixture of **3**, **4**, and **5**.

Experiment C. An approximately 1:1 mixture of **2** and **3** (9 mg) was reduced as above with $NaBH_4$ (2 × 2 mg). Analysis demonstrated the reaction mixture to be an approximately 5:3 mixture of **3** and **4**.

Purification of Dopaol Glycosides. The above reaction mixtures from experiments A−C were combined and purified first by chromatography on an RP-18 Lobar column (size B), which was eluted with H_2O and then with H_2O−MeOH (4:1) to give a dopaol glycoside mixture, which together with the previously obtained impure fractions (25 mg) from the initial reduction experiment were subjected to further HPLC purification using a Merck Hibar RP-18 column (eluent: H_2O−MeOH, 10:1), which afforded successive fractions of **4** (3 mg), a mixture of **4** and **3** (3 mg), pure **3** (3 mg), and finally a ca. 10:1 mixture (13 mg) of **5** and **6**.

Dopaol β-D-alloside (4): ^{13}C NMR δ 101.2 (C-1), 72.3 (C-2), 71.5 (C-3), 68.1 (C-4), 74.8 (C-5), 62.4 (C-6), 35.7 (C-α), 72.1 (C-β), 132.8 (C-1′), 118.0 (C-2′), 145.1 (C-3′), 143.6 (C-4′), 117.5 (C-5′), 122.5 (C-6′), essentially as reported,[8] except for the different solvent used (MeOH-d_4).

Dopaol β-D-mannoside (5): ^{13}C NMR δ 101.0 (C-1), 71.8 (C-2), 74.2 (C-3), 68.1 (C-4), 77.5 (C-5), 62.3 (C-6), 35.7 (C-α), 71.6 (C-β), 133.1 (C-1′), 118.0 (C-2′), 145.3 (C-3′), 143.7 (C-4′), 117.5 (C-5′), 122.5 (C-6′).

Dopaol β-D-altroside (6): ^{13}C NMR δ 99.5 (C-1), 71.3 (C-2), 71.2 (C-3), 66.2 (C-4), 76.0 (C-5), 62.8 (C-6), 35.7 (C-α), 71.6 (C-β), 133.1 (C-1′), 118.0 (C-2′), 145.3 (C-3′), 143.7 (C-4′), 117.5 (C-5′), 122.5 (C-6′).

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