

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

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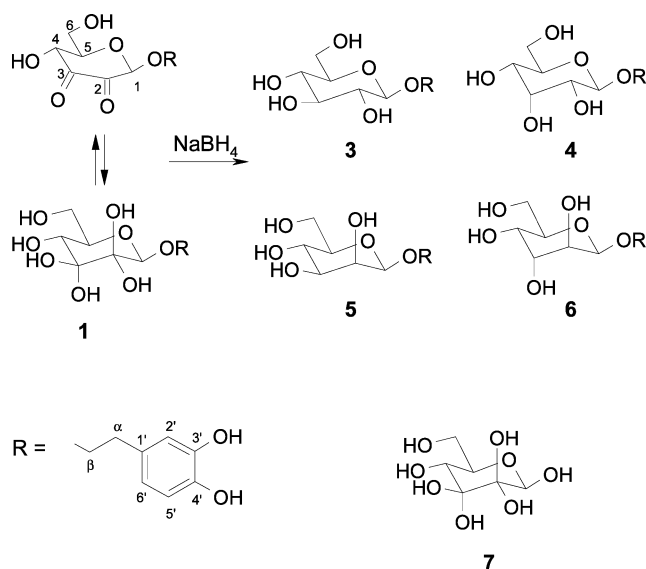
Two unique 2-(3,4-dihydroxyphenyl)ethyl glycosides, namely, dopaol  $\beta$ -D-2-ketoglucofuranoside and dopaol  $\beta$ -D-2,3-diketoglucofuranoside, were isolated from *Chelone obliqua* together with the iridoid glucoside catalpol, dopaol  $\beta$ -D-glucofuranoside, descaffeoylverbascoside, and verbascoside. 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 North-east America.<sup>1</sup> The two closely related genera *Chionophila* and *Nothochelone*<sup>2</sup> 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.,<sup>3</sup> and *C. glabra* L.<sup>4</sup> In the course of an investigation of the chemotaxonomy 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<sub>2</sub>O–MeOH mixtures to give the iridoid glucoside catalpol. In addition, two novel glycosides (**1** and **2**) were isolated together with dopaol  $\beta$ -D-glucofuranoside (**3**),<sup>5</sup> decaffeoylverbascoside,<sup>6</sup> and the more common caffeoyl phenylethyl glucoside (CPG) verbascoside.<sup>7</sup>

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 <sup>1</sup>H and <sup>13</sup>C NMR spectra of **1** in D<sub>2</sub>O, it was obvious that it was a glycoside closely related to **3**. The observed [M – H]<sup>–</sup> ion of *m/z* 311.0767 showed the molecular formula to be C<sub>14</sub>H<sub>16</sub>O<sub>8</sub> and demonstrated the presence of two additional unsaturations when compared to **3**. Besides the signals from the dopaol aglycon, the <sup>13</sup>C NMR spectrum revealed two low-intensity resonances ( $\delta$  94.3 and 95.8), signals from an anomeric carbon at  $\delta$  101.0, two oxygenated methines at  $\delta$  70.2 and 76.0, and finally a hydroxymethyl signal at  $\delta$  62.4. The <sup>1</sup>H NMR signals were consistent with this and displayed the coupling system HOCH<sub>2</sub>–CHOR–CHOH– together with a signal from an anomeric proton ( $\delta$  4.66, linked to the carbon at  $\delta$  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- $\beta$ -D-glucofuranoside (i.e., a  $\beta$ -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 ( $\delta$  94.3) and H-1 ( $\delta$  4.66) as well as between C-3 ( $\delta$  95.8) and H-4 ( $\delta$  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 distribu-

Scheme 1



tion between the  $\beta$ -D-glucofuranoside (**3**),  $\beta$ -D-alloside (**4**),<sup>8</sup>  $\beta$ -D-mannoside (**5**), and  $\beta$ -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  $\alpha$ -face of the 2-keto group was only slightly prevalent, while hydride addition to the  $\alpha$ -face of the 3-keto group was more dominant. The structures of **5** and **6** were based upon 2D NMR assignment of the <sup>13</sup>C NMR spectra and comparison of these with reported data for methyl  $\beta$ -D-mannopyranoside and methyl  $\beta$ -D-altropyranoside.<sup>9</sup> The present isolation of a naturally occurring 2,3-diketoglycoside is to our knowledge unprecedented. However, the dihydrate of the corresponding free sugar,  $\beta$ -D-erythrohexos-2,3-diulopyranose (**7**), has been prepared enzymatically and its X-ray structure determined.<sup>10,11</sup> The free diketosugar 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.<sup>11</sup> Similar treatment of **1**, however, led to partial decomposition of the compound. Comparison of the NMR data of the free 2,3-diketosugar ( $\delta$  94.3, 94.7, 93.5, 69.4, 75.7, 61.8) in DMSO<sup>11</sup> with those of **1** showed a high degree of similarity, even allowing for the difference in solvent, except for the expected downfield <sup>13</sup>C chemical shift for C-1 in the glycoside.

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

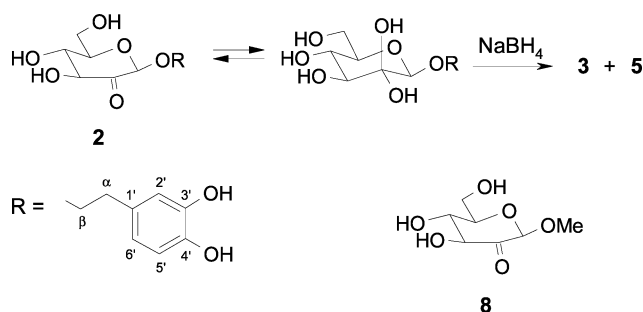
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## Scheme 2



**2** and **1** was obtained. Hence **2** was also only characterized by NMR spectroscopy and by HRFABMS. Again, the overall appearances of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of compound **2** were similar to those of **1** and **3**. The observed  $[\text{M} - \text{H}]^-$  ion of  $m/z$  313.0924 gave the molecular formula  $\text{C}_{14}\text{H}_{18}\text{O}_8$ , corresponding to one less unsaturation than in **1**. As above, the  $^{13}\text{C}$  NMR spectrum of **2** in  $\text{D}_2\text{O}$  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 ( $\delta$  93.9), while the other signals seemed to constitute three oxygenated methines, a hydroxymethyl group, and an anomeric carbon ( $\delta$  102.3). As was the case with **1**, the anomeric proton ( $\delta$  4.47) appeared as a sharp singlet, and consequently the quaternary carbon at  $\delta$  93.9 could be assigned as a hydrated 2-keto group. Using the COSY correlations and starting from the typical hydroxymethyl signals ( $\delta$  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  $\delta$  3.9–3.4 to a single coupling system, namely,  $\text{HOCH}_2\text{-CHOR-CHOH-CH-OH-}$ . Thus, **2** was the hydrated form of a 2-ketoglucopyranoside. This also allowed assignment of the  $^{13}\text{C}$  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- $\beta$ -D-glucoside (**3**) and dopaol- $\beta$ -D-mannoside (**5**). In this case, hydride addition on the 2-keto group had proceeded with the expected preference for the less hindered  $\alpha$ -face.

Keto forms of monosaccharides in aqueous solution exist mainly as the hydrates, as seen from data for both anomers of 2-ketoglucose (glucosone)<sup>12</sup> and also for methyl 3-ketoglucosides<sup>13</sup> obtained from the free sugars by enzymatic processes.<sup>12–14</sup> Likewise, chemical oxidation<sup>15,16</sup> of unprotected methyl glycosides has produced several 2-keto-, 3-keto-, and 4-ketoglycosides, and NMR data (in  $\text{D}_2\text{O}$ ) for the dihydrate form of methyl  $\beta$ -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*<sup>18</sup> (Asclepiadaceae) as well as on antibiotics from a *Streptomyces* sp.<sup>19</sup> However, several examples of iridoid 3-ketoglucosides are known. In the genus *Penstemon* (closely related to *Chelone*), serruloside<sup>19</sup> has been isolated from *P. serrulatus*, and dihydroseruloside<sup>20</sup> has been reported from *P. confertus*. Also, suspensolide<sup>21</sup> from *Viburnum suspensum* (Sambucaceae) and clandonoside<sup>22</sup> together with 8-O-acetylclandonoside from *Caryopteris*  $\times$  *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.<sup>22</sup>

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.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker Avance DRX600 instrument at 600 and 150 MHz, respectively, in  $\text{D}_2\text{O}$  using acetone ( $\delta$  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-A/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  $\text{H}_2\text{O}$ –Et $_2\text{O}$  (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  $\text{H}_2\text{O}$ –MeOH mixtures (25:1 to 1.5:1) gave, after the polar front, first catalpol (105 mg), then a fraction of slightly impure dopaol  $\beta$ -D-2,3-diketoglucoside (**1**; 60 mg), a ca. 1:1 mixture (20 mg) of **1** and dopaol  $\beta$ -D-2-ketoglucoside (**2**), followed by dopaol  $\beta$ -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  $\text{H}_2\text{O}$  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 hemiacetalic mixture. From this fraction a further amount of **1** could be obtained by chromatography.

**Dopaol  $\beta$ -D-2,3-diketoglucoside (1):**  $^1\text{H}$  NMR  $\delta$  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- $\alpha$ ), 4.10 (1H, dt,  $J$  = 10.1 and 7.0 Hz, H- $\beta$ ), 3.90 (1H, dt,  $J$  = 10.1 and 7.0 Hz, H- $\beta$ ), 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');  $^{13}\text{C}$  NMR  $\delta$  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- $\alpha$ ), 72.2 (C- $\beta$ ), 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  $[\text{M} - \text{H}]^-$  (calcd for  $\text{C}_{14}\text{H}_{15}\text{O}_8$ , 311.0757).

**Dopaol  $\beta$ -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:  $^1\text{H}$  NMR  $\delta$  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- $\alpha$ ), 4.07 (1H, dt,  $J$  = 10.1, 7.0 Hz, H- $\beta$ ), 3.86 (1H, dt,  $J$  = 10.1, 7.0 Hz, H- $\beta$ ), 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');  $^{13}\text{C}$  NMR  $\delta$  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- $\alpha$ ), 72.2 (C- $\beta$ ), 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  $[\text{M} - \text{H}]^-$  (calcd for  $\text{C}_{14}\text{H}_{15}\text{O}_8$ , 313.0923).

**Dopaol  $\beta$ -D-glucoside (3):**  $^{13}\text{C}$  NMR  $\delta$  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- $\alpha$ ), 72.1 (C- $\beta$ ), 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,<sup>5</sup> 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  $\text{NaBH}_4$  (5 mg) in  $\text{H}_2\text{O}$  (2 mL) at 0  $^\circ\text{C}$  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  $\text{H}_2\text{O}$  and then  $\text{H}_2\text{O}$ –MeOH (15:1 to 6:1). This gave an approximately 1:2 mixture (9 mg) of dopaol  $\beta$ -D-glucoside (**3**) and dopaol  $\beta$ -D-alloside (**4**), an intermediary fraction containing an approxi-

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

**Experiment A.** **1** (11 mg) was reduced with NaBH<sub>4</sub> (2 mg) at 0 °C for 0.5 h, then more NaBH<sub>4</sub> (2 mg) was added and the temperature was allowed to rise to 20 °C. Analysis of the reaction mixture by <sup>1</sup>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<sub>4</sub> (2 × 4 mg). <sup>1</sup>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<sub>4</sub> (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<sub>2</sub>O and then with H<sub>2</sub>O–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<sub>2</sub>O–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  $\beta$ -D-alloside (**4**):** <sup>13</sup>C NMR  $\delta$  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- $\alpha$ ), 72.1 (C- $\beta$ ), 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,<sup>8</sup> except for the different solvent used (MeOH-*d*<sub>4</sub>).

**Dopaol  $\beta$ -D-mannoside (**5**):** <sup>13</sup>C NMR  $\delta$  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- $\alpha$ ), 71.6 (C- $\beta$ ), 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  $\beta$ -D-altroside (**6**):** <sup>13</sup>C NMR  $\delta$  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- $\alpha$ ), 71.6 (C- $\beta$ ), 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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## References and Notes

- (1) Nelson, A. D.; Elisens, W. J. *Am. J. Bot.* **1999**, *86*, 1487–1501.
- (2) Wolfe, A. D.; Elisens, W. J.; Watson, L. E.; Depamphilis, C. W. *Am. J. Bot.* **1997**, *84*, 555–564.
- (3) Kooiman, P. *Acta Bot. Neerl.* **1970**, *19*, 329–340.
- (4) Bowers, M. D.; Boockvar, K.; Collinge, S. K. *J. Chem. Ecol.* **1993**, *19*, 815–823.
- (5) Shimomura, H.; Sashida, Y.; Adachi, T. *Phytochemistry* **1987**, *26*, 249–251.
- (6) Kikuchi, M.; Yamauchi, Y. *Yakugaku Zasshi* **1985**, *105*, 542–546.
- (7) (a) Scarpati, M. L.; Delle Monache, F. *Ann. Chim. (Rome)* **1963**, *53*, 356–367.
- (8) Birkofer, L.; Kaiser, C.; Thomas, U. *Z. Naturforsch.* **1968**, *23b*, 1051–1058.
- (9) Toyota, M.; Saito, T.; Asakawa, Y. *Phytochemistry* **1996**, *43*, 1087–1088.
- (10) Bock, C.; Pedersen, C. *Adv. Carbohydr. Chem. Biochem.* **1983**, *41*, 27–66.
- (11) Volc, J.; Sedmera, P.; Havlicek, V.; Prikrylová, V.; Daniel, G. *Carbohydr. Res.* **1995**, *278*, 59–70.
- (12) Sedmera, P.; Volc, J.; Havlicek, V.; Pakhomova, S.; Jegorov, A. *Carbohydr. Res.* **1997**, *297*, 375–378.
- (13) Freimund, S.; Baldes, L.; Huwig, A.; Giffhorn, F. *Carbohydr. Res.* **2002**, *337*, 1585–1587.
- (14) Freimund, S.; Huwig, A.; Giffhorn, F.; Köpper, S. *Chem. Eur. J.* **1998**, *4*, 2442–2455.
- (15) Volc, J.; Sedmera, P.; Halada, P.; Prikrylová, V.; Daniel, G. *Carbohydr. Res.* **1998**, *310*, 151–156.
- (16) Liu, H.-M.; Sato, Y.; Tsuda, Y. *Chem. Pharm. Bull.* **1993**, *41*, 491–501.
- (17) Tsuda, Y.; Hanajima, M.; Matsuhira, N.; Okuno, Y.; Kanemitsu, K. *Chem. Pharm. Bull.* **1989**, *37*, 2344–2350.
- (18) Vértessy, L.; Aretz, W.; Fehlhaber, H.-W.; Kogler, H. *Helv. Chim. Acta* **1995**, *78*, 46–60.
- (19) Kiuchi, F.; Liu, H. M.; Tsuda, Y. *Chem. Pharm. Bull.* **1990**, *38*, 2326–2328.
- (20) Junior, P. *Planta Med.* **1984**, *50*, 417–420.
- (21) Gering, B.; Junior, P.; Wichtl, M. *Phytochemistry* **1987**, *26*, 3011–3013.
- (22) Iwagawa, T.; Hase, T. *Phytochemistry* **1989**, *28*, 2393–2396.
- (23) Hannedouche, S.; Jacquemond-Collet, I.; Fabre, N.; Stanislas, E.; Moulis, C. *Phytochemistry* **1999**, *51*, 767–769.

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