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# Corchorusides A and B, new flavonol glycosides as α-glucosidase inhibitors from the leaves of *Corchorus olitorius*

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ABSTRACT

*Corchorus olitorius*, a highly fibrous vegetable commonly known as moroheiya, has long been recognized for its hypoglycemic activity. Bioassay-guided fractionation of the leaf extract led to the isolation of two new flavonol glycosides named corchorusides A and B, in addition to a major component, capsugenin-25,30-*O*-β-diglucopyranoside. Corchoruside A comprises a kaempferol moiety connected with caffeic acid, glucose, and a rare methyl glucuronate (MeGlcA). The occurrence of a caffeoyl moiety in corchoruside A enhanced significantly its inhibitory effect toward α-glucosidase compared to that in corchoruside B.



Type 2 or non-insulin-dependent diabetes mellitus (NIDDM) is a metabolic disorder characterized by insufficient use of insulin. NIDDM is often associated with obesity and develops when chronic overnutrition combines with genetic susceptibility. Recently, several clinical studies have demonstrated that modification of food intake, by consuming dietary fiber, significantly enhanced insulin secretion, inhibited glucosidase activity and delayed glucose absorption into the bloodstream.[1] Therefore, it is of interest to employ vegetables containing high dietary fiber as an alternative antidiabetic food. In our search for α-glucosidase inhibitors from natural sources,[2,3] we have focused on *Corchorus olitorius* due to its historic recognition as an antidiabetes remedy and potent inhibitory effect in our screening. *C. olitorius* (Tiliaceae family) is indigenous to Egypt and the Middle East, and its young leaves have been introduced to East Asia and Japan as a healthy vegetable, typically recognized as Moroheiya. Its health benefits have been reported in terms of antitumor promotion,[4] antioxidant properties[5] and antibacterial activity.[6] Recently, Innami demonstrated that *C. olitorius* leaf extract significantly suppressed postprandial blood glucose level in rats and humans.[7] In addition, both the diffusion rate of glucose and permeation rate of glucose in cultured Caco-2 cells were significantly reduced by the addition of leaf extract.[7] To date, the active principles that are largely associated with the delayed absorption of glucose have not been identified. An attempt to characterize the active components in *C. olitorius* leaves using α-glucosidase inhibition-guided fractionation resulted in the isolation of two new flavonol glycosides.

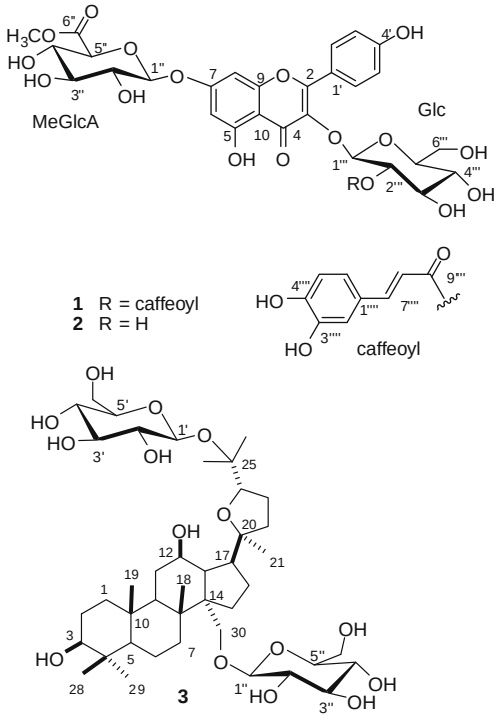


**Figure 1.**

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The dried leaves (1.8 kg) of *C. olitorius* were extracted with MeOH to afford a dark-green residue which was further partitioned with hexane and $CH_2Cl_2$. The resulting aqueous MeOH extract was chromatographed on Diaion HP-20, which was successively eluted with $H_2O$, MeOH, and acetone. The active MeOH fraction was subsequently purified using Sephadex LH-20 (MeOH), silica gel chromatography (10:90→70:30 MeOH–$CH_2Cl_2$), and RP-HPLC (55:45 MeOH–$H_2O$) to yield two new flavonol glycosides named corchorusides A (**1**, 162 mg) and B (**2**, 51 mg), together with the major triterpenoid glycoside capsugenin-25,30-*O*-β-diglucopyranoside[8] (**3**, 209 mg) (Fig. 1).

Corchoruside A (**1**)[9] has the molecular formula $C_{37}H_{36}O_{20}$ as established by HRESIMS and NMR data. The UV spectrum of **1** exhibited absorptions [267 (3.68), 333 (3.77)] that were ascribable to the flavonol skeleton.[10] The $^1$H NMR spectrum of **1** (Fig. 2a), recorded in $CD_3OD$, contained proton resonances in aromatic and glycosidic regions only, in addition to a methoxy signal [$\delta_H$ 3.64 (3H, s)]. Despite signal broadening around 6.6–7.1 ppm (Fig. 1a), two separate aromatic spin systems were established by COSY data and coupling constant analysis. The *meta*-coupled resonances [$\delta_H$ 6.38 (1H, br s, H-8) and 6.65 (1H, br s, H-6)] as well as the aromatic protons [$\delta_H$ 8.02 (2H, br d, $J$ = 8.6 Hz, H-2′ and H-6′) and 6.91 (2H, overlapped, H-3′ and H-5′)] were assigned to rings A and B of the kaempferol unit, respectively. The *trans*-olefinic protons [$\delta_H$ 7.56 (1H, d, $J$ = 16.0 Hz, H-7‴″) and 6.29 (1H, d, $J$ = 16.0 Hz, H-8‴″)] in conjunction with an aromatic ABX spin system [$\delta_H$ 6.78 (1H, m, H-5‴″), 6.90 (1H, m, H-6‴″) and 7.04 (1H, br s, H-2‴″)] were assigned to a caffeoyl moiety, as was evident from diagnostic HMBC cross peaks (H-2‴″/C-7‴″, H-6‴″/C-7‴″, H-7‴″/C-9‴″, and H-8‴″/C-9‴″). In the glycosidic region, the anomeric resonances at $\delta_H$ 5.13/$\delta_C$ 99.6 and $\delta_H$ 5.68/$\delta_C$ 99.2 indicated the presence of two sugar residues. However, the identity of these units and glycosidic

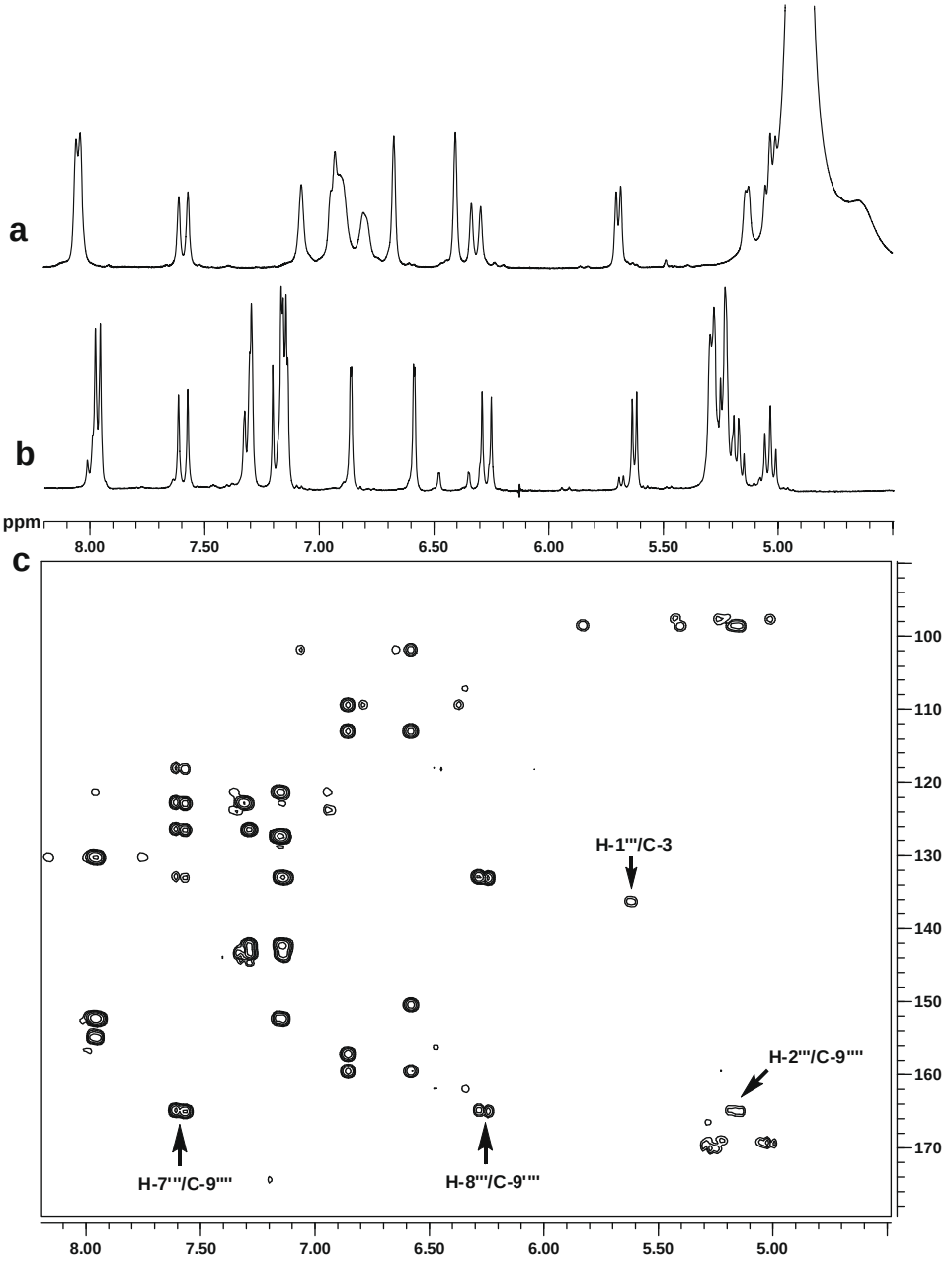


**Figure 2.** NMR spectra of corchoruside A (**1**) and its nonaacetate (**1a**). (a) $^1$H NMR spectrum of **1** in $CD_3OD$, (b) $^1$H NMR spectrum of **1a** in $CDCl_3$, and (c) HMBC spectrum of **1a**. Particular diagnostic HMBC cross peaks are indicated by solid arrows.

**Table 1**
NMR data (400 MHz) of corchoruside A (**1**, $CD_3OD$) and its nonaacetate derivative (**1a**, $CDCl_3$)

| Position | **1** | | **1a**[a] | |
|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$, mult, $J$ in Hz | $\delta_C$ | $\delta_H$, mult, $J$ in Hz |
| 2 | 157.7 | | 152.2 | |
| 3 | 133.8 | | 136.0 | |
| 4 | 174.8 | | 172.0 | |
| 5 | 156.4 | | 157.3 | |
| 5-OH | | | | 12.39, s |
| 6 | 94.2 | 6.65, br s | 102.1 | 6.86, d, 2.0 |
| 7 | 162.6 | | 159.6 | |
| 8 | 99.1 | 6.38, br s | 109.6 | 6.59, d, 2.0 |
| 9 | 160.4 | | 150.4 | |
| 10 | 106.4 | | 113.5 | |
| 1′ | 120.7 | | 121.4 | |
| 2′, 6′ | 131.1 | 8.02, br d, 8.6 | 130.0 | 7.97, d, 8.4 |
| 3′, 5′ | 115.0 | 6.91, m | 121.5 | 7.17, d, 8.8 |
| 4′ | 160.4 | | 155.0 | |
| MeGlcA | | | | |
| 1″ | 99.6 | 5.13[b] | 98.1 | 5.29, d, 8.0 |
| 2″ | 70.2 | 3.40, m | 68.2 | 5.06, m |
| 3″ | 77.4 | 3.34, m | 72.0 | 5.24, m |
| 4″ | 71.6 | 3.62, m | 68.8 | 5.30, m |
| 5″ | 75.3 | 4.16, d, 9.2 | 72.8 | 4.23, d, 9.2 |
| 6″ | 169.4 | | 166.0 | |
| 7″ | 49.5 | 3.64, s | 53.0 | 3.68, s |
| Glc | | | | |
| 1‴ | 99.2 | 5.68, d, 8.0 | 99.2 | 5.98, d, 7.6 |
| 2‴ | 74.3 | 5.03, m | 74.3 | 5.21, m |
| 3‴ | 74.8 | 3.64, m | 74.8 | 5.28, m |
| 4‴ | 73.1 | 3.52, m | 73.1 | 5.27, m |
| 5‴ | 75.6 | 3.55, m | 75.6 | 5.17, m |
| 6‴ | 61.2 | 3.57, m<br>3.76, m | 61.2 | 3.95, m |
| Caffeoyl | | | | 4.02, m |
| 1⁗ | 126.5 | | 132.9 | |
| 2⁗ | 114.1 | 7.04, br s | 122.8 | 7.30, s |
| 3⁗ | 145.4 | | 142.5 | |
| 4⁗ | 148.4 | | 143.9 | |
| 5⁗ | 115.5 | 6.78, m | 123.8 | 7.15, br s |
| 6⁗ | 122.0 | 6.90, m | 126.3 | 7.32, br s |
| 7⁗ | 146.1 | 7.56, d, 16.0 | 144.0 | 7.59, d, 16.0 |
| 8⁗ | 114.0 | 6.29, d, 16.0 | 118.1 | 6.27, d, 16.0 |
| 9⁗ | 166.4 | | 165.0 | |

[a] The acetate signals resonated at: $\delta_H$ 2.34, 2.27, 2.24, 2.23, 1.99, 1.97, 1.94, 1.91, and 1.89; $\delta_C$ 21.2, 21.1, 21.0, 20.9, 20.6, 20.4 (4 × $CH_3$), 170.6, 170.3, 170.2, 169.7 (3 × C=O), 169.4, 169.2, and 169.0.
[b] Overlapped with the signal for water.

linkages could not be confirmed due to the lack of diagnostic 2D NMR correlations. In fact, this problem has been encountered frequently in the case of samples dissolved in certain polar solvents such as $CD_3OD$ and acetone-$d_6$, leading to signal broadening. We have demonstrated that acetylated glycosides produce sharper and better-separated signals than their parent congeners.[11] Thus **1** was acetylated using $Ac_2O$/pyridine, affording corchoruside A nonaacetate (**1a**)[12] in which all the hydroxy groups, except for 5-OH, were acetylated. Therefore, the complete structure of **1** was established as its acetylated derivative (Fig. 3).

Corchoruside A nonaacetate (**1a**) produced sharp and well-separated signals that allowed clear HMBCs (Fig. 2b and c). Two sugar residues were identified by interpretation of the 2D NMR data along with analysis of $^1H$–$^1H$ coupling constants. Starting with the anomeric proton at $\delta_H$ 5.29, five contiguous methines were apparent. In the HMBC spectrum, H-5″ ($\delta_H$ 4.23) correlated with a carbon at $\delta_C$ 166.0 (C-6″), which was in turn coupled with a methoxy proton ($\delta_H$ 3.68), indicating that this sugar residue was glucose-derived and possessed a methyl carboxylate (Fig. 3). Therefore, this unit was assigned as methyl glucuronate (MeGlcA). In the same manner, the anomeric proton at $\delta_H$ 5.98 and its contiguous oxygenated protons were assigned to a glucose moiety (Glc).

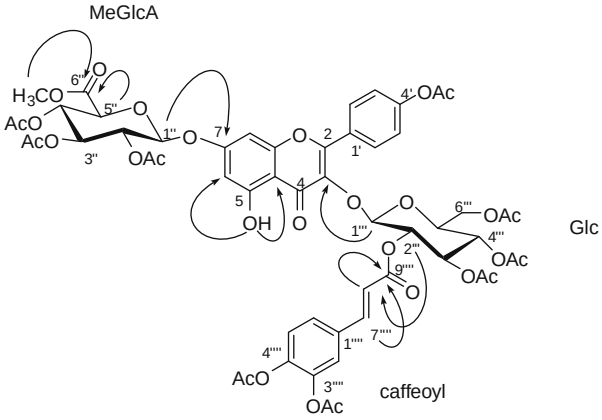


**Figure 3.** Selected HMBCs of **1a**.

The glycosidic linkages were established by HMBC data analysis. MeGlcA was connected to C-7 of kaempferol through β-orientation as indicated by the HMBC between H-1″ and C-7 as well as the large coupling constant (8.0 Hz) for the anomeric proton. Similarly, the HMBC cross peak between H-1‴ and C-3 and the large $J$ value of 7.6 Hz suggested the connectivity of Glc to the aglycone via β-orientation. The caffeoyl moiety was attached at C-2‴ of Glc as demonstrated by the HMBCs between H-2‴/C-9⁗, H-7⁗/C-9⁗ and H-8⁗/C-9⁗, thus completing the structure of corchoruside A.

Corchoruside B (**2**)[13] gave an $[M+Na]^+$ ion at $m/z$ 661.1375 in the HRESIMS spectrum, which corresponded to a molecular formula of $C_{28}H_{30}O_{17}Na$. The $^1H$ NMR spectrum of **2** (pyridine-$d_5$) was similar to that of **1** ($CD_3OD$) even though they were recorded in different solvents. Careful examination of the $^1H$ and $^{13}C$ NMR spectra of **2** (Table 2) revealed the absence of olefinic protons and certain aromatic resonances. In the aromatic region, $m$-coupled protons [$\delta_H$ 7.13 (1H, d, $J$ = 2.0 Hz) and 6.89 (1H, d,

**Table 2**
NMR data (400 MHz) of corchoruside B (**2**) in pyridine-$d_5$

| Position | **2** | |
|---|---|---|
| | $\delta_C$ | $\delta_H$, mult, $J$ in Hz |
| 2 | 156.4 | |
| 3 | 133.4 | |
| 4 | 173.8 | |
| 5 | 155.4 | |
| 6 | 93.5 | 7.13, d, 2.0 |
| 7 | 161.9 | |
| 8 | 98.7 | 6.89, d, 2.0 |
| 9 | 161.3 | |
| 10 | 105.6 | |
| 1′ | 120.5 | |
| 2′, 6′ | 130.5 | 8.24, d, 8.8 |
| 3′, 5′ | 114.8 | 7.28, d, 8.8 |
| 4′ | 160.4 | |
| MeGlcA | | |
| 1″ | 100.2 | 4.82, d, 7.2 |
| 2″ | 73.1 | 4.52, m |
| 3″ | 76.5 | 4.56, m |
| 4″ | 71.5 | 4.71, m |
| 5″ | 75.8 | 5.03, d, 9.6 |
| 6″ | 168.8 | |
| 7″ | 50.8 | 3.77, s |
| Glc | | |
| 1‴ | 102.2 | 5.20, d, 7.2 |
| 2‴ | 74.7 | 4.50, m |
| 3‴ | 77.0 | 4.47, m |
| 4‴ | 70.5 | 4.36, m |
| 5‴ | 77.8 | 4.18, m |
| 6‴ | 61.2 | 4.38, m<br>4.52, m |

**Table 3**
α-Glucosidase inhibitory effects

| Compound | α-Glucosidase inhibitory effect ($IC_{50}$, mM) |
|---|---|
| **1** | 0.18 ± 0.01 |
| **2** | 0.72 ± 0.03 |
| **3** | 1.42 ± 0.03 |
| Acarbose | 0.62 ± 0.03 |
| 1-Deoxynojirimycin (DNJ) | 0.17 ± 0.02 |

$J$ = 2.0 Hz)] and *o*-coupled resonances [$\delta_H$ 8.24 (2H, d, $J$ = 8.8 Hz) and 7.28 (2H, d, $J$ = 8.8 Hz)] suggested that **2** possessed a kaempferol core structure. Two anomeric protons ($\delta_H$ 5.20 and 4.82) revealed an identical 2D NMR correlation pattern as observed in **1**, suggesting that **2** possibly comprised MeGlcA and Glc residues, which was further supported by acid hydrolysis. The difference in molecular mass of 162 amu ($C_9H_6O_3$) in connection with the absence of the aforementioned resonances indicated that **2** was a decaffeoyl congener of **1**.

Corchoruside A (**1**) inhibited α-glucosidase activity with an $IC_{50}$ value of 0.18 mM, which is threefold more active than that of the standard diabetes drug acarbose and comparable to DNJ (Table 3). On the other hand, the congener **2** demonstrated significantly reduced inhibition ($IC_{50}$ 0.72 mM), suggesting that the caffeoyl moiety is critical in blocking enzyme function.

In summary, we have succeeded in identifying two new flavonol glycosides **1** and **2**, which display antidiabetic activity, from the leaf extract of *C. olitorius*. Corchoruside A comprises a kaempferol moiety connected to glucose and caffeoyl residues, in addition to a rare sugar, methyl glucuronate (MeGlcA).[14] We also demonstrated that despite signal broadening and the lack of diagnostic 2D NMR correlations in the original spectra of **1**, the overall structure could be eventually established by transformation of **1** into its peracetylated derivative **1a**. Flavonol glycosides possessing 7-glucuronic acid are encountered only in particular species such as *Allium cepa*,[15] *Tanacetum parthenium*,[16] and *Tulipa gesneriana*.[17] The markedly improved α-glucosidase inhibitory effect of **1** over that of **2** clearly pointed to the crucial role of the caffeoyl residue in blocking the enzyme function. Although a variety of compounds containing the caffeoyl moiety have been synthesized and validated for glucosidase inhibition,[18,19] the nature of the interaction between the caffeoyl moiety and binding sites of the enzyme remains unknown.

## Acknowledgments

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9. *Corchoruside A* (**1**): pale yellow powder; $[\alpha]_D^{26}$ −88.7 (*c* 0.05, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 245 (3.64), 267 (3.68), 333 (3.77); positive HRESIMS *m*/*z* $[M+Na]^+$ 823.1715 (calcd for $C_{37}H_{36}O_{20}Na$, 823.1692); $^1H$ and $^{13}C$ NMR (see Table 1).
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12. *Corchoruside A nonaacetate* (**1a**): white powder; $[\alpha]_D^{26}$ −94.5 (*c* 0.05, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 255 (3.82), 284 (3.87), 357 (3.97); negative HRESIMS *m*/*z* $[M-H]^-$ 1177.2755 (calcd for $C_{55}H_{53}O_{29}$, 1177.2751); $^1H$ and $^{13}C$ NMR (see Table 1).
13. *Corchoruside B* (**2**): pale yellow powder; $[\alpha]_D^{26}$ −10.2 (*c* 0.05, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 265 (3.53), 346 (3.64); positive HRESIMS *m*/*z* $[M+Na]^+$ 661.1370 (calcd for $C_{28}H_{30}O_{17}Na$, 661.1375); $^1H$ and $^{13}C$ NMR (see Table 2).
14. To confirm that the MeGlcA moiety in **1** and **2** is of natural origin, extraction of *C. olitorius* leaves using three different solvents (MeOH, EtOH, PrOH) was performed. The dried leaves (each *ca.* 5 g) were soaked in the solvents (2 × 300 mL) at room temperature for 2 d. The extracts were concentrated and partitioned with $CH_2Cl_2$ to remove chlorophyll and lipophillic residues. The resulting extract was analyzed by HPLC (5C18-AR-II∅ 4.6 × 250 mm, 55:45 MeOH–$H_2O$, flow rate 1.0 mL/min, UV 254 nm). The extracts obtained using MeOH, EtOH, and PrOH showed the presence of **1** ($t_R$ 31.2 min) and **2** ($t_R$ 28.4 min). For reports of flavonoids containing MeGlcA, see: (a) Yang, H.; Protiva, P.; Cui, B.; Ma, C.; Baggett, S.; Hequet, V.; Mori, S.; Weinstein, I. B.; Kennelly, E. J. *J. Nat. Prod.* **2003**, *66*, 1501–1504; (b) Tezuka, Y.; Terazono, M.; Kusumoto, T. I.; Kawashima, Y.; Hatanaka, Y.; Kadota, S.; Hattori, M.; Namba, T.; Kikuchi, T.; Tanaka, K.; Supriyatna, S. *Helv. Chim. Acta* **1999**, *82*, 408–417; (c) Allais, D. P.; Simon, A.; Bennini, B.; Chulia, A. J.; Kaouadji, M.; Christiane, D. *Phytochemistry* **1991**, *30*, 3099–3101; (d) Nawwar, M. A. M.; Souleman, A. M. A.; Buddrus, J.; Linscheid, M. *Phytochemistry* **1984**, *23*, 2347–2349.
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