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Xanthones from *Cudrania Tricuspidata* displaying potent α-glucosidase inhibition

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Abstract—We have proven that xanthones **1**–**8** isolated from the root of *C. tricuspidata* possess highly potent αα-glucosidase inhibition properties. Compound **1** was identified as a new isoprenylated tetrahydroxy xanthone, *1,3,6,7-tetrahydroxy-2-(3-methylbut-2-enyl)-8-(2-methylbut-3-en-2-yl)-9H-xanthen-9-one* (**1**). These are the first natural xanthones documented to exhibit such inhibition. The IC_{50} values of compounds **1**–**8** inhibiting α-glucosidase activity were determined to be up to 16.2 μM. Mechanistic analysis showed the xanthones **1**–**8** exhibited full mixed inhibition.

α-Glucosidase inhibitors have come to the fore of biomedical research into the treatment of numerous diseases including diabetes mellitus type II,[1] cancer,[2] and HIV.[3] The diverse therapeutic roles of these inhibitors stem from the paramount role played by carbohydrates in biochemistry.[4] For instance: by retarding the cleavage of complex carbohydrates, postprandial glucose absorption in vivo can be attenuated, thus regulating blood sugar levels in diabetics[5]; the spread of cancer as well as the structural changes of cell surface glycoconjugates within neoplasmic cells is proliferated by glycosidases in the sera and interstitial fluid around the tumor, thus by effecting glycosidase inhibition, cancer growth may be retarded[6]; finally, cellular signaling/recognition is principally orchestrated by glycoproteins. In HIV glycoprotein 120 (gp120) is responsible for choreographing the union of the virus and its $CD4^+$ T-cell targets, via a specific interaction with CD4.

Furthermore, the ensuing syncytium formation (aggregation of healthy T cells with the infected cell), which grossly accelerates the rate of initial infection, is even more dependent on the gp120-CD4 interaction. α-Glucosidase inhibitors have thus reduced the rate of viral proliferation in HIV infection.[7] Currently the most widely used glucosidase inhibitors, such as acarbose and voglibose, are iminosugars. Although numerous glucosidase inhibitors have been developed, including chalcones,[8] azasugars,[9] isoxazoles,[10] and aminosugars,[11] these can be tedious to synthesize and are thus not optimal. Recently, certain synthetic xanthones[12] have also been identified as α-glucosidase inhibitors, but to date no such inhibitors derived from natural sources have been evaluated.

Cundrania tricuspidata is one of the most ubiquitous traditional herbal remedies in East Asia. This plant's beneficial effects have been traditionally associated with anti-inflammatory, anti-tumor, and anti-gastritis activity. In our recent publications, we have elucidated the vast biological potential of these species through various biological studies.[13,14] For instance, xanthones possessing a vicinal dihydroxy group on one phenyl ring were shown to exhibit excellent radical scavenging activities.[15] Interestingly, xanthone-derived species were later demonstrated to possess potent suppressing properties toward LDL oxidation, which we linked to the anti-atherosclerotic and anti-inflammatory nature of *C. tricuspidata*.[13]

Keywords: α-Glucosidase inhibition; Xanthone; *Cudrania tricuspidata*.
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In this manuscript, we present our most recent results surveying the α-glucosidase inhibitory ability of a range of xanthones from *C. tricuspidata*, as defined by their IC_{50} values. We hope that this study can elucidate further the multitude of roles ascribed to this important plant (Fig. 1).

The methanol extract of *C. tricuspidata* showed glycosidase inhibitory activity. In our ensuing investigations, we isolated eight xanthones **1**–**8** from it. The identification of isolated xanthones **1**–**8** was performed using 2D-NMR together with other spetroscopic data, all of which was compared with previous work. Compounds **2**–**8** were identified as Macluraxanthone B (**2**), Cudraxanthone L (**3**), 1,3,7-trihydroxy-4-(1,1-dimethyl-2-propenyl)-5,6-(2-2-dimethylchromeno)xanthone (**4**), Cudraxanthone M (**5**), Cudraxanthone D (**6**), Cudratricusxanthone F (**7**), and Cudratricusxanthone A (**8**).[16,17]

Compound **1** was obtained as yellowish solid having the molecular formula $C_{23}H_{24}O_6$ and 12 degrees of unsaturation from its HREIMS data. The UV and IR spectra display the properties of 1,3,6,7-tetrahydroxyxanthone derivatives. The presence of the 1,1-dimethylallyl group was deduced from the connectivity between H-18 and the vinylic proton H-19$_{\alpha/\beta}$ (δ_H 5.34) and the correlation between C-16, 17 (δ_C 27.6) and H-18 in the HMBC experiment. This group resided at C-2 because C-15 correlated with the H-bonded OH at C-1 and H-4 in the HMBC experiment. The presence of a 3,3-dimethylallyl group was determined on the basis of successive connectivities from C-10 to C-14 in the ^{1}H-^{1}H COSY spectrum. HMBC correlation of C-8 with H-10 proved that the 3,3-dimethylallyl group was located at C-8. Furthermore, HMBC results of **1** were compared with those of **3** and **7** to resolve any ambiguity in the position of the 3,3-dimethylallyl group: a strong correlation between C-9 and H-10 was observed in compound **3** but was absent in compound **1**; furthermore, the pattern of correlation for H-5 was similar in both **1** and **7**. These results are consistent with **1** displaying similar regiochemistry to **7**. The structure of compound **3** was fully confirmed with X-ray crystallographic analysis (CCDC-222304) and HMBC correlation in our previous work.[16] Thus, compound **1** was identified as *1,2,6,7-tetrahydroxy-2-(3-methylbut-2-enyl)-8-(2-methylbut-3-en-2-yl)-9H-xanthen-9-one*.[18]

The isolated compounds were tested for their enzymatic inhibitory activities against α-glucosidase from baker's yeast. The enzyme was assayed according to standard procedures by following the hydrolysis of nitrophenyl glycoside spectrophotometrically.[19,20] All compounds showed dose-dependent inhibitory effect on α-glucosidase activity (Fig. 2). The inhibitory potencies and capacities of these polyphenol toward α-glucosidase activity were investigated.

As shown in Table 1, all xanthones investigated apart from xanthone **5** exhibited a significant degree of α-glucosidase inhibition (IC_{50} 16.2–52.9 μM). However the activity was significantly affected by subtle changes in

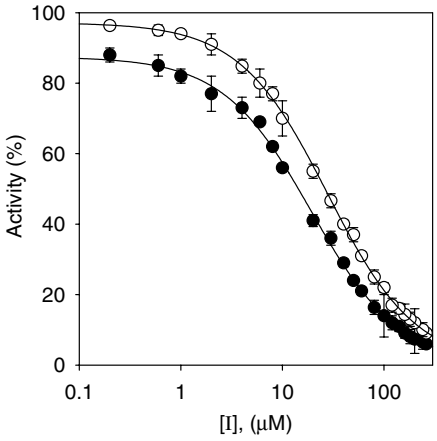

Figure 2. Effect of compounds **4** (○) and **7** (●) on the activity of α-glucosidase for the hydrolysis of *p*-nitrophenyl α-D-glucopyranoside.

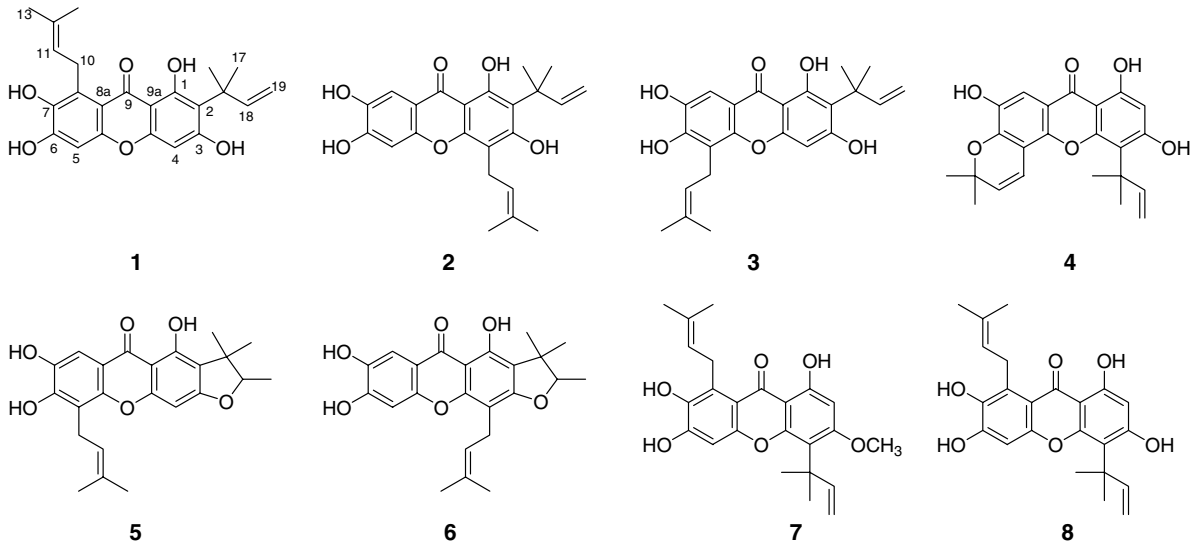

Figure 1. Isolated xanthones **1**–**8** from the roots of *C. tricuspidata*.

Table 1. α-Glucosidase assay results for compounds **1–8**

Compound	IC_{50}[a] (μM)	K_i value
1	35.8 ± 1.7	31.7
2	38.2 ± 0.8	8.9
3	52.9 ± 2.1	7.4
4	24.9 ± 1.1	5.8
5	>100	NT
6	32.0 ± 2.7	15.7
7	16.2 ± 0.4	7.0
8	37.7 ± 1.8	12.4

[a] Values are means of three experiments, standard deviation is given in parentheses (NT, not tested).

structure. Perhaps most poignantly, inactive xanthone **5** differed only by the relative position of the 3,3-dimethylallyl appendage (C-4 vs C-5) from one of the most effective inhibitors screened, xanthone **6**. However, C-4 and C-8 regioisomeric xanthones **1** and **2** were of almost equal efficacy, as were regioisomers **1** and **8**. Perhaps most importantly, compound **3**, a regioisomer of **1**, **2**, and **8** possessing substitution at C-4, exhibited the second lowest activity. It thus seems that alkyl substitution in the 4-position diminishes the potency of the inhibitors greatly, whilst other positions show significant tolerance.

This was however not the only effect at play in this system. C3*O*-Methylated species **7** was by far the most effective inhibitor, but its demethylated analog **8** was much less efficient. This implicates H-bond donor ability or polarity as an important facet in defining the activity. Finally, it has been shown previously in synthetic xanthones that large conjugated systems serve as optimal inhibitors.[12] The potency of the most effective inhibitor **7** (IC_{50} = 16.2 μM) compares with sugar-derived glucosidase inhibitors currently used for therapeutic purposes, such as voglibose (IC_{50} = 23.4 μM)[21] and deoxynojirimycin (IC_{50} = 3.5 μM).[22]

All inhibitors manifested the same relationship between enzyme activity and enzyme concentration. The inhibition of α-glucosidase by compound **6** is illustrated in Figure 3, representatively. Plots of the initial velocity versus enzyme concentrations in the presence of different concentrations of compound **6** gave a family of straight lines, all of which passed through the origin. Increasing the inhibitor concentration resulted in the lowering of the slope of the line, indicating that these compounds were reversible inhibitors. We progressed to analyze the mode of inhibition using Lineweaver–Burk plots (Fig. 4), which showed that all xanthones displayed mixed inhibition.

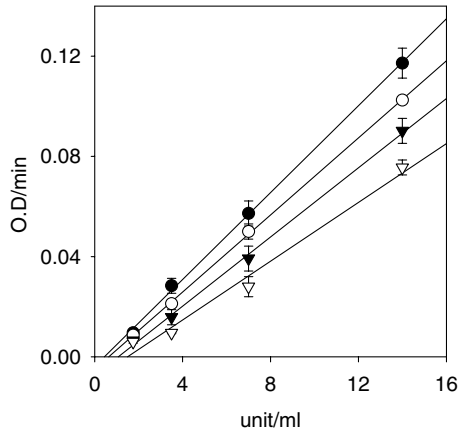

Figure 3. Relationship of the hydrolytic activity of α-glucosidase with enzyme concentrations at different concentrations of compound **6**. Concentrations of compound **6** for curve from top to bottom: 0, 5, 10, and 20 μM.

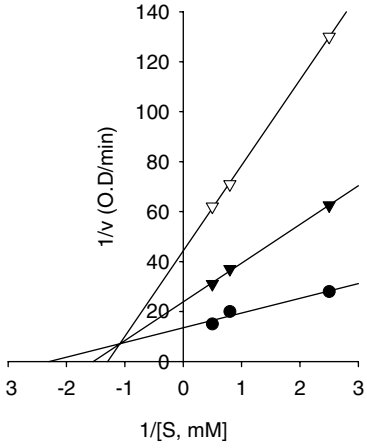

Figure 4. Lineweaver–Burk plots of compound **6** (●, control; ▼, 10 μM; ▽, 20 μM).

In conclusion, we have proven that numerous xanthones isolated from the root bark of *C. tricuspidata* possess highly potent α-glucosidase inhibition properties. These represent the first natural xanthone-derived α-glucosidase inhibitors documented.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.10.007.

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18. Selected compounds, spectroscopic data; compound **1**: mp 144–145 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.56 (3H, s, H-14), 1.59 (3H, s, H-17), 1.59 (3H, s, H-18), 1.72 (3H, s, H-17), 3.09 (2H, d, J = 7.0 Hz, H-11), 5.01 (1H, d, J = 7.0 Hz, H-12), 5.34 (1H, d, J = 10.6 Hz, H-15a), 5.44 (1H, d, J = 17.8 Hz, H-15b), 6.29 (1H, dd, J = 10.6, 17.8 Hz, H-19), 6.29 (1H, s, H-5), 7.43 (1H, s, H-4); compound **2**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.68 (3H, s, H-16), 1.68 (3H, s, H-17), 1.70 (3H, s, H-13), 1.83 (3H, s, H-14), 3.44 (2H, d, J = 7.0 Hz, H-10), 5.20 (1H, m), 5.40 (2H, dd, J = 10.5, 17.8 Hz, H-19), 6.41 (1H, s, H-5), 6.53 (1H, dd, J = 10.5, 17.8 Hz, H-18), 7.51 (1H, s, H-8); compound **4**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.43 (3H, s, H-16), 1.43 (3H, s, H-17), 1.62 (3H, s, H-13), 1.63 (3H, s, H-14), 5.30 (1H, d, J = 10.5 Hz, H-19a), 5.39 (1H, d, J = 17.8 Hz, H-19b), 5.75 (1H, d, J = 10.2 Hz, H-11), 6.39 (1H, dd, J = 10.5, 17.8 Hz, H-18), 6.77 (1H, s, H-8), 7.93 (1H, d, J = 10.2 Hz, H-10); compound **5**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.28 (3H, s, H-16), 1.42 (3H, d, J = 6.6 Hz, H-19), 1.51 (3H, s, H-17), 1.65 (3H, s, H-13), 1.81 (3H, s, H-14), 3.25 (2H, m, H-10), 4.53 (1H, q, J = 6.6 Hz, H-18), 5.14 (1H, m, H-11), 6.33 (1H, s, H-4), 7.50 (1H, s, H-8); compound **7**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.62 (3H, s, H-16), 1.62 (3H, s, H-17), 1.77 (3H, s, H-13), 1.88 (3H, s, H-14), 3.82 (3H, s, OCH_3), 4.30 (2H, br d, J = 6.8 Hz, H-10), 4.84 (1H, d, J = 10.6 Hz, H-19a), 4.90 (1H, d, J = 17.4, H-19b), 5.30 (1H, m, H-11), 6.25 (1H, dd, J = 10.6, 17.4 Hz, H-18), 7.26 (1H, s, H-5).
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