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Bioorganic & Medicinal Chemistry Letters 14 (2004) 3841–3845
Bioorganic & Medicinal Chemistry Letters

Yeast and mammalian α-glucosidase inhibitory constituents from Himalayan rhubarb *Rheum emodi* Wall.ex Meisson ☆

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Received 4 March 2004; revised 19 April 2004; accepted 19 April 2004
Available online 25 May 2004

Abstract—The methanolic extract of rhizome of Himalayan rhubarb *Rheum emodi* displayed mild yeast as well as mammalian intestinal α-glucosidase inhibitory activity. However, further fractionation of active extract led to the isolation of several potent molecules in excellent yields, displaying varying degrees of inhibition on two test models of α-glucosidase. Rhapontigenin, desoxyrhapontigenin, chrysophanol-8-*O*–β-D-glucopyranoside, torachrysone-8-*O*-β-D-glucopyranoside displayed potent yeast α-glucosidase inhibition. However chrysophanol-8-*O*–β-D-glucopyranoside, desoxyrhapontigenin and torachrysone-8-*O*–β-D-glucopyranoside displayed potent to moderate mammalian α-glucosidase inhibitory activity. Other compounds displayed mild activity on both the tests. Except desoxyrhapontigenin and rhapontigenin that increased V_{max}, other compounds including crude extract decreased the V_{max} significantly ($p < 0.02$) in yeast α-glucosidase test. Further kinetic analysis on mammalian α-glucosidase inhibition showed that chrysophanol-8-*O*–β-D-glucopyranoside, desoxyrhaponticin and torachrysone-8-*O*-β-D-glucopyranoside may be classified as mixed-noncompetitive inhibitors. However, desoxyrhapontigenin and rhapontigenin may be classified as modulators of enzyme activity. Presence and position of glycoside moiety in compounds appear important for better inhibition of mammalian α-glucosidase. This is the first report assigning particularly, mammalian intestinal α-glucosidase inhibitory activity to these compounds. Chrysophanol-8-*O*–β-D-glucopyranoside, desoxyrhaponticin, desoxyrhapontigenin and rhapontigenin have been isolated in substantial yields from *R. emodi* for the first time. Therefore, these compounds may have value in the treatment and prevention of hyperglycemia associated diabetes mellitus.

1. Introduction

According to the recent estimate human population worldwide appears to be in the midst of diabetic epidemic and despite the great strides that have been made in understanding and management of diabetes, the disease and its related complications are increasing unabated. Hyperglycemia has been the classical risk factor in diabetes and various targets as well as drugs and phytochemicals have been identified to manage this risk factor.[1] The sucrase–isomaltase complexes of mammalian intestinal extract have been classified as a kind of α-glucosidase[2] and have been involved in digestion of carbohydrates and resultantly, increasing the blood glucose level. Thus, inhibitors of this mammalian intestinal α-glucosidase have become exciting candidates to slow down the digestion of carbohydrates and in turn mitigate post-prandial hyperglycemic excursions.[3–5] Furthermore, α-glucosidase inhibitors also offer other benefits like, reducing triglycerides levels[5] and post-prandial insulin levels.[6] Targeting post-prandial hyperglycemia may further provide advantages as it has been linked to cardiovascular mortality.[7]

Rhubarbs, the rhizomes of *Rheum* species are used in remedies of blood stagnation syndrome, also called 'Oketsu syndrome', which includes diabetes, atherosclerosis, ischemia and inflammation in Japanese and Chinese, traditional medicines.[8] Several antioxidants and xanthine oxidase inhibitors have been isolated from methanolic extract of rhubarbs and these properties have been linked to the multiple beneficial effects in

Keywords: α-Glucosidase; Inhibitors; Modulators; *Rheum emodi*.
☆ IICT communication no.: 040416.
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doi:10.1016/j.bmcl.2004.04.062

these disorders.[8] About 10 species of *Rheum* occur in India and have been used in medicine and culinaries.[9] *Rheum emodi* is the chief source of Himalayan (Indian) rhubarb and is used primarily as purgative and astringent tonic. The calorific value of its rhizome is reported recently[10] to be 3625 kcal g^{-1}. However, like other rhubarbs, its usage in different disease conditions is not reported. In the course of our ongoing programme on identifying antidiabetic principles from traditional medicinal plants,[11,12] we observed yeast α-glucosidase inhibitory property in the methanolic extract of its rhizome, which further displayed its potential in inhibiting mammalian intestinal α-glucosidase activity. These observations for the first time led to the identification of α-glucosidase inhibitory principles from rhubarb and active principles from *R. emodi* in substantial yields.

2. Extraction and isolation

Dried rhizomes of *R. emodi* were procured from Himalayan region of India and were identified by Prof. K. V. B. R. Tilak, former Head, Dept. of Microbiology, Indian Agricultural Research Institute, New Delhi, India. A voucher specimen RE-1, is deposited in Organic Division-1, IICT, Hyderabad.

The dried rhizomes powder (200 g) was first defatted with petrol in a Soxhlet apparatus for 24 h and then extracted with methanol at room temperature for 72 h. The methanol extract (6 g) showed yeast α-glucosidase inhibition activity and was found further active in inhibiting mammalian α-glucosidase. Therefore, the methanolic extract was chromatographed over silica gel (60–120 mesh, 125 g) column and elution was performed taking 100 mL fractions starting with hexane/EtoAc, 98:1, (frs 1–4) to afford chrysophanol (**1**) (0.5 g), hexane/EtoAc, 98:2, frs (5–10) to afford Physcion (**2**) (0.4 g), hexane/EtoAc, 96:4 (frs 16–20) to afford Emodin (**3**) (0.3 g) and then hexane/EtOAc, 95:5 (frs 21–25) to afford Noreugenin (**4**) (0.005 g).

The elution of the column was further continued with $CHCl_3$/MeOH, 96:4 (frs 26–30) to afford Desoxyrhapontigenin (**5**) (1.2 g), $CHCl_3$/MeOH, 95:5 (frs 31–40) to afford Rhapontigenin (**6**) (1.0 g), $CHCl_3$/MeOH, 96:4 (frs 41–50) to afford chrysophanol-8-*O*–β-D-glucopyranoside (**7**) (0.8 g), $CHCl_3$/MeOH, 96:4 (frs 51–60) to afford torachrysone-8-*O*-β-D-glucopyranoside (**8**) (0.2 g), $CHCl_3$/MeOH, 92:8 (frs 80–90) to afford desoxyrhaponticin (**9**) (1.0 g). All the compounds were characterized by spectroscopic methods and compared with the reported literature.[13–16] Compounds **4**–**7** and **9** were isolated for the fist time from *R. emodi*.

3. Biological results and discussion

Yeast α-glucosidase has been frequently used to identify its inhibitors from traditional medicinal plants[11,12] and food items.[17] However, α-glucosidase activity from rat intestinal acetone powder closely mimics the mammalian system[18] and therefore, may be a better model to identify, design and develop antihyperglycemic agents particularly for the management of post perandial hyperglycemia in diabetes. Therefore, we have used both of these test systems in this study in order to verify and compare, if any the differences in enzyme inhibitory activity for phytochemicals of varied nature (Fig. 1).

In both the test systems, since chrysophanol and emodin developed turbidity and noreugenin could not display inhibitory potential at 50 μg/mL concentrations, they were not tested. As we wanted to test and compare the inhibitory activity and potentials of compounds on enzyme α-glucosidase of different origin, we chose a common substrate, *p*-nitrophenyl-α-D-glucopyranoside that is aptly used in yeast α-glucosidase model and kept experimental protocols identical (Fig. 2).

The crude methanolic extract, physcion, desoxyrhapontigenin, rhapontigenin, desoxyrhaponticin, chrysophanol-8-*O*–β-D-glucopyranoside, torachrysone-8-*O*-β-D-glucopyranoside displayed varying degrees of α-glucosidase inhibitory potentials in both the test models (Fig. 2A and B). However, they displayed superior inhibitory potential on yeast α-glucosidase than mammalian α-glucosidase. Furthermore, their inhibitory pattern and potentials also varied in these two test models. The pattern and potentials in decreasing order of yeast α-glucosidase inhibition was observed as follows: rhapontigenin > desoxyrhapontigenin > chrysophanol-8-*O*–β-D-glucopyranoside > torachrysone-8-*O*-*β*-D-glucopyranoside > desoxyrhapontigenin > physcion > crude extract (Fig. 2A). However, rhapontigenin was observed at fourth place in inhibiting mammalian α-glucosidase and the order of inhibitory potential was observed as follows: chrysophanol-8-*O*–β-D-glucopyranoside > desoxyrhaponticin > torachrysone-8-*O*-β-D–glucopyranoside > rhapontigenin > physcion > desoxyrhapontigenin >

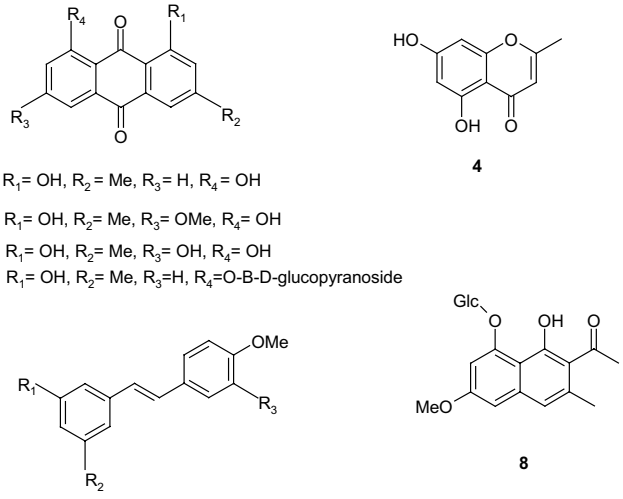

Figure 1.

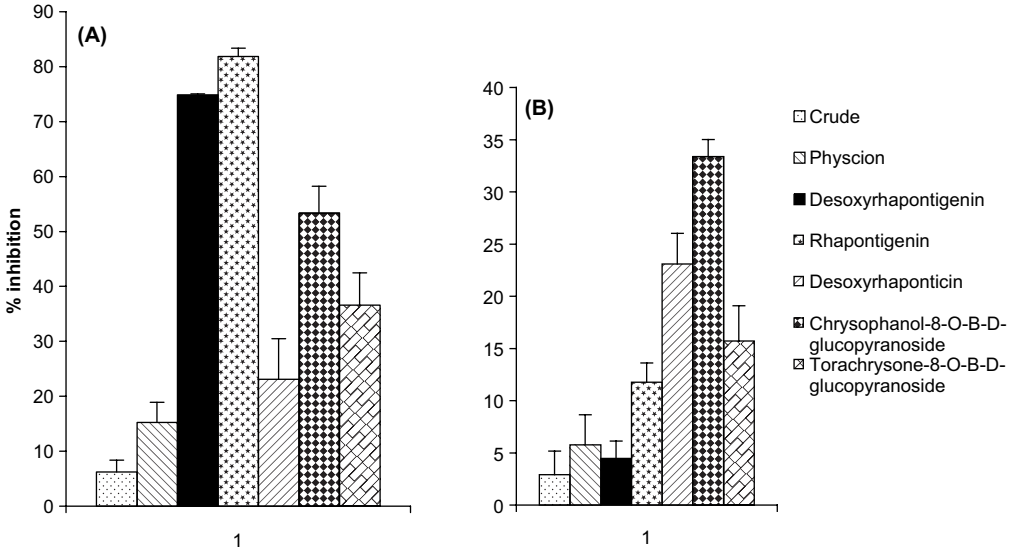

Figure 2. Yeast (A) and mammalian intestinal (B) α-glucosidase inhibitory activity by compounds isolated from *R. emodi*. Yeast α-glucosidase was prepared as reported earlier[11] in 100 mM phosphate buffer (pH 6.8). Rat, intestinal acetone powder (Sigma Chemicals, USA) in normal saline (100:1, w/v) was sonicated properly and supernatant was treated as mammalian α-glucosidase[18] after centrifugation at 3000 rpm × 30 min. 10 μL compound (5 mg/mL DMSO) or DMSO in control and blank was incubated in 100 μL buffer with 50 μL enzyme for 5 min and thereafter absorbance 405 nm was read spectrophotometrically (SPECTRAmaxPLUS[384], Molecular Devices, USA) by further incubating (25–26 °C) samples for another 5 min with 50 μL substrate (5 mM, *p*-nitrophenyl-α-D-glucopyranoside prepared in same buffer). Blanks and control were substituted with same amount of buffer for the substrate. Percent inhibition of enzyme activity by compounds was calculated accordingly.[11] Each data represent mean, SD of triplicate readings.

crude extract (Fig. 2B). These observations reveal the fact that the presence and position of glycoside moiety may play an important role in inhibiting mammalian α-glucosidase, as all the three superior active compounds possess glucoside linkage. This feature became further important as desoxyrhaponticin displayed better inhibitory potential than its aglycon, desoxyrhapontigenin, which was reverse in yeast α-glucosidase inhibition.

The V_{max} of an enzyme is a measure of how fast the reaction it catalyzes can proceed once the enzyme–substrate complex is formed and therefore, may provide the information on inhibitors nature and type of enzyme inhibition. Figure 3 presents V_{max} data of fifth minute for yeast α-glucosidase at fixed substrate concentration. It was observed that desoxyrhapontigenin and rhapontigenin increased the V_{max} substantially, however, other compounds including methanolic extract decreased V_{max} significantly ($p < 0.02$) than the control. Therefore, further analysis of the compounds in order to reveal the type of enzyme inhibition on mammalian α-glucosidase, was done with different substrate concentrations. Based on Lineweaver–Burk plots (Fig. 4) compounds chrysophanol-8-*O*–β-D-glucopyranoside, desoxyrhaponticin and torachrysone-8-*O*-β-D-glucopyranoside may be classified as mixed-noncompetitive inhibitors.[19]

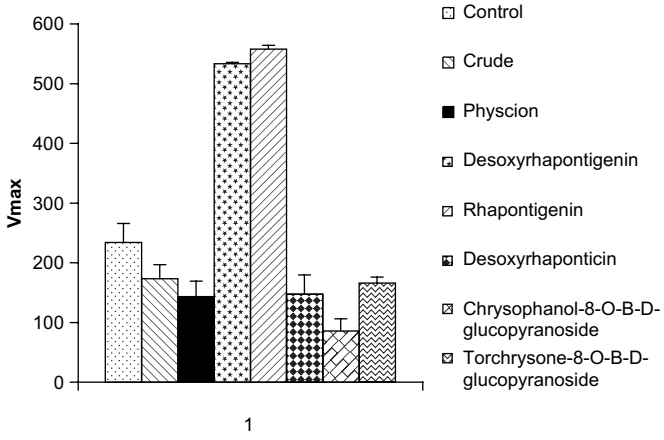

Figure 3. Changes in V_{max} recorded at fifth minute for yeast α-glucosidase. It was observed that rhapontigenin and desoxyrhapontigenin increased the V_{max} where as other compounds decreased it significantly ($P < 0.02$) than the control. Each data represents mean, SD of triplicate readings.

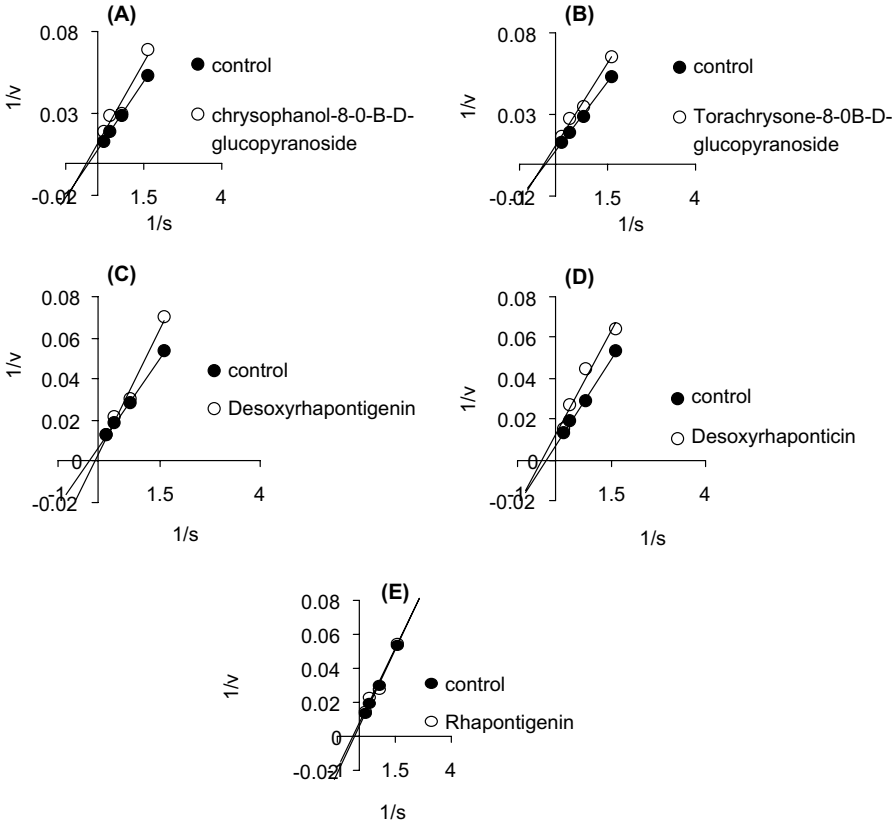

Figure 4. Lineweaver–Burk plots for the hydrolysis of substrate by mammalian intestinal α-glucosidase. Experimental procedures remain same as in Figure 2 except immediately after addition of the substrate, V_{max} kinetics was recorded with or without addition of 50 μL test compounds with different substrate concentrations. Plots A, B and D show that the slope for the inhibitors crosses slope of the control left to the intercept above or below the *X*-axis. Therefore, compounds may be designated as mixed noncompetitive inhibitors. However, in plots C and E compounds slope crossed the control slope right to the intercept. Therefore, these two compounds appear to reduce the enzyme's catalytic property by rather modulating enzyme activity. Desoxyrhapontigenin (C) may be classified as positive, homotropic, K-modulator whereas rhapontigenin (E) a weak, negative, homotropic, M-modulator.

However, plot (C and E) in Figure 4 shows that slopes for desoxyrhapontigenin and rhapontigenin, respectively, crosses control's slope right to the intercept axis. Therefore, these compounds might be reducing the enzyme's catalytic activity by modulating the regulatory site distinct from the catalytic or active site of the enzyme. In the present observations, increase or decrease in the substrate concentration is responsible for changing the nature of substrate/velocity plots. At lower concentration of the substrate, desoxyrhapontigenin reduces the V_{max}, however, as substrate concentration increase, it increases the V_{max}. Therefore, desoxyrhapontigenin may be classified as positive, homotropic, K-modulator. Though very narrow, the reverse was observed in case of rhapontigenin, therefore, it might be classified as weak, negative, homotropic, M-modulator.[19,20] However, further detail analyses are required to delineate their mechanism of action.

Matsuda et al.[8] have reported recently potent antioxidant, free radical scavenging and xanthine oxidase inhibitory activities for rhapontigenin and desoxyrhapontigenin along with the other compounds isolated from methanolic extract of rhubarbs and have described structural requirements for these activities. However, in the present study compounds have given varied results and also belong to different classes, therefore, structure–activity relationship could not be proposed. All the compounds isolated from the rhizomes of *R. emodi* in this study are in superior yields than other rhubarbs.[8]

Indian rhubarb gained importance during the World War II when the supplies of Chinese rhubarb became scare.[9] To our knowledge, this is the first report assigning α-glucosidase particularly, mammalian intestinal α-glucosidase inhibitory activity as well as modulatory activity to the compounds isolated from *R. emodi*. These compounds therefore, may present promising therapeutic potential controlling hyperglycemia. Furthermore, the most potent mammalian intestinal α-glucosidase inhibitory principles chrysophanol-8-*O*-β-D-glucopyranoside, desoxyrhaponticin and rhapontigenin have been isolated for the first time from *R. emodi* in substantial yields. Though the plant has been consumed as food and culinary for long time, observations of the varied nature and behaviour of compounds in this report for α-glucosidase inhibition needs further detailed studies for their use in prevention and treatment of hyperglycemia and consequently, diabetes mellitus.

Acknowledgements

Authors thank to D'IICT for constant encouragement and K.S.B. thanks CSIR, New Delhi for financial support.

References and notes

1. Tiwari, A. K.; Rao, J. M. *Curr. Sci.* **2002**, *83*, 30.
2. Conklin, K. A.; Yamashiro, K. M.; Gray, G. M. *J. Biol. Chem.* **1975**, *250*, 5735.
3. Moordian, A. D.; Thurman, J. E. *Drugs* **1999**, *57*, 19.
4. Goke, B.; Herrmann-Rinke, C. *Diabetis Metab. Res. Rev.* **1998**, *14*(suppl. 1), 531.
5. Lebowitz, H. E. *Diabetes Rev.* **1998**, *6*, 132.
6. Johnston, P. S.; Coniff, R. F.; Hoogwerf, B. J.; Santiago, J. V.; Pi-Sunyer, F. X.; Kral, A. *Diabetes Care* **1994**, *17*, 20.
7. Donahue, R. P.; Abbott, R. D.; Reed, D. M.; Yano, K. *Diabetes* **1987**, *36*, 689.
8. Matsuda, H.; Morikawa, T.; Toguchida, I.; Park, J.-Y.; Harima, S.; Yoshikawa, M. *Bioorg. Med. Chem.* **2001**, *9*, 41.
9. *The Wealth of India*, Raw materials, New Delhi (CSIR), 1972, *9*, p 3.
10. Prasad, P.; Purohit, M. C. *Curr. Sci.* **2001**, *80*, 734.
11. Rao, R. J.; Tiwari, A. K.; Kumar, U. S.; Reddy, S. V.; Ali, A. Z.; Rao, J. M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2777.
12. Reddy, S. V.; Tiwari, A. K.; Kumar, U. S.; Rao, R. J.; Rao, J. M. *Phytother. Res*. **2004**, in press.
13. Tane, P.; Ayafor, J. F.; Sondengam, B. L.; Connolly, J. D. *Phytochemistry* **1990**, *29*, 1004.
14. Chaudhari, M.; Jain, G. K.; Sarin, J. P.; Khanna, N. M. *Ind. J. Chem.* **1983**, *22*(B), 1163.
15. Kashiwada, Y.; Nonaka, G. I.; Nishioka, I. *Chem. Pharm. Bull.* **1984**, *32*, 3501.
16. Kubo, I.; Murari, Y.; Soediro, I.; Soetarno, S.; Sastrodihardjo, S. *Phytochemistry* **1992**, *31*, 1063.
17. Matsui, T.; Yashimoto, C.; Osajima, K.; Oki, T.; Osajima, Y. *Biosci. Biotechnol. Biochem.* **1996**, *60*, 2019.
18. Ohta, T.; Sasaki, S.; Oohori, T.; Yoshikawa, S. *Biosci. Biotechnol. Biochem.* **2002**, *66*, 1552.
19. Wong, J. T.-F. *Kinetics of Enzyme Mechanisms*; Academic: London, 1975.
20. Neet, K. E. In *Contemporary Enzyme Kinetics and Mechanisms*; Purich, D. L., Ed.; Academic: London, 1983.