PERGAMON

Phytochemistry 60 (2002) 295–299

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Inhibition of α-glucosidase by oleanolic acid and its synthetic derivatives

Muhammad Shaiq Ali*, Muhammad Jahangir, Syed Shazad ul Hussan, Muhammad Iqbal Choudhary

H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan

Received 25 September 2001; received in revised form 27 February 2002

Abstract

Oleanolic acid (**1**) and five synthetic derivatives (**2–6**) were tested spectrophotometrically for inhibition of urease, β-lactamase, acetyl cholinesterase and α-glucosidase. All products showed a positive response only against α-glucosidase but not against the other enzymes; IC_{50} calculations showed that the dihydroxy-olide derivative (**4**) was the most potent among all tested samples.
© 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Oleanolic acid; Five synthetic derivatives; α-Glucosidase inhibition; IC_{50} values

1. Introduction

The aim of anti-diabetic therapy is to achieve normoglycaemia in order to avoid diabetes- associated late complications. Hyperglycaemia, which is the result of decreased insulin sensitivity or decreased insulin secretion from pancreatic β-cells, can further inhibit insulin secretion from pancreas and diminish insulin-mediated glucose uptake in peripheral tissues (Wolffenbuttel and Haeften, 1995). Manifestation of late complications like nephropathy, neuropathy, microangiopathy, macroangiopathy, retinopathy and cataract are closely associated with hyperglycaemia, which may lead to severe consequences. Inhibition of α-glucosidase can prevent these late complications by decreasing the postprandial rise in blood glucose. α-Glucosidase is a membrane-bound enzyme at the epithelium of the small intestine, that hydrolyses the cleavage of glucose from disaccharides and oligosaccharides. The inhibitors of this enzyme delay carbohydrate digestion, prolong the overall carbohydrate digestion time and thus cause a reduction in the rate of glucose absorption and lower the postprandial rise in blood glucose. Therefore, inhibition of α-glucosidase is considered important in managing noninsulin-dependent diabetes (Bischoff, 1994).

2. Results and discussion

Oleanolic acid was obtained in good amounts from *Tridax procumbens* and found to be a potential anti-diabetic agent when tested against α-glucosidase. It was then decided to synthesize a number of derivatives to evaluate their behavior against α-glucosidase, in addition to urease, β-lactamase and acetyl cholinesterase. The characterization of synthetic derivatives **2–6** and their activity against α-glucosidase are discussed here.

2.1. Chemistry

Compound **1** was separated as a major constituent in amorphous form from ethyl acetate soluble part of hexane extract of *T. procumbens*. The electron impact mass spectrum of **1** showed the molecular ion peak at *m/z* 456 and HR-EIMS demonstrated the exact mass of this peak at *m/z* 456.3601, in agreement with the molecular formula $C_{30}H_{48}O_3$. The base peak appeared at *m/z* 248, characteristic for pentacyclic triterpene of β-amyrin series with a 12–13 double bond (Waller, 1980). The ^1H NMR spectrum of **1** showed the presence of an olefinic proton resonating at δ 5.51 (1H, *t*, $J = 3.5$ Hz) and a carbinylic proton at δ 3.45 (1H, *dd*, $J = 10.0, 5.0$ Hz), were assigned to H-12 and H-3α, respectively. The same spectrum also showed snglets for seven methyls. The ^{13}C NMR spectrum of **1** showed a total of 30 signals, which were resolved into seven methyls, 10 methylenes, and

---

* Corresponding author. Tel.: +92-21-9243231; fax: +92-21-9243190/91.

E-mail addresses: shaiq303@super.net.pk or shaiq303@hotmail.com (M.S. Ali).

0031-9422/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0031-9422(02)00104-8

five methines through DEPT experiments. From the spectral evidence and comparing with the physical data of **1** with the literature values (Ahmad and Rahman, 1994), the discussed compound was identified as 3β-hydroxyolean-12-en-28-oic acid and commonly known as oleanolic acid (**1**).

Compound **1** was acetylated with acetic anhydride in pyridine. The product **2** was confirmed by the decrement in polarity, FD-MS and further through ^{1}H NMR spectrum. The molecular ion peak of **2** was observed at *m/z* 498 in the FD-MS, which showed the introduction of an acetyl moiety into **1**. The ^{1}H NMR spectrum further supported the introduction of the same moiety. Compound **1** was also treated with diazomethane. The FD-MS of the methylated product (**3**) gave molecular ion peak at *m/z* 470. The ^{1}H NMR spectrum of **3** showed along with seven methyl singlets, an additional singlet at δ 3.59 confirming the synthesis of **3**.

Treatment of **1** with ozone produced derivative **4** which showed a molecular ion peak at *m/z* 472 in its FD-MS suggesting a formula $C_{30}H_{48}O_4$. The ^{1}H NMR of **4** showed a new methane signal at *δ* 3.87 ($J=2.85$) in addition to the one at *δ* 3.20 (*dd*, $J=11.4$, 4.8 Hz, H-3). The new signal was assigned to H-12 (Poehland et al., 1987). The β stereochemistry of H-12 was suggested by the small coupling constant ($J=2.85$ Hz) observed. The ^{1}H NMR showed resonances for seven methyls groups (*δ* 0.76, 0.86, 0.89, 0.97, 0.98, 1.13, 1.29) and the formation of **4** could be explained by the formation of an olide-type bridge between C-13 and C-28. The proposed mechanism for **4** is shown in Scheme 1. Derivative **4** was identified as 3β, 12α, 13β-trihydroxy 28-oic acid-28→13-olide oleanane. The ^{13}C assignments are given in the Experimental section.

Compound **4** containing two hydroxyl functions was acetylated with acetic anhydride in pyridine. The proton NMR spectrum of product **5** showed two double doublets at *δ* 4.45 (1H, $J=9.8$, 6.0 Hz) and 5.05 (1H, $J=3.3$, 2.1 Hz) assigned to H-3 and H-12, respectively. The downfield shift of these protons in agreement, both having acetoxyl moieties. The same spectrum also showed additional methyl signals at *δ* 2.08 and 2.03 due to the acetoxyl methyls. The two hydroxyls in **4** were converted into ketonic functions by treating with chromium trioxide. The ^{1}H NMR spectrum of **6** was confirmed the conversion of two hydroxyls into ketonic functions by the disappearance of the double-doublets due to the H-3 and H-12 methines. The assigned structure was thus 13β-hydroxy 3,12-dioxo-28-oic acid-28→13-olide oleanane. The derivatization of oleanolic acid (**1**) into derivatives **2–6** is summarized in Scheme 2.

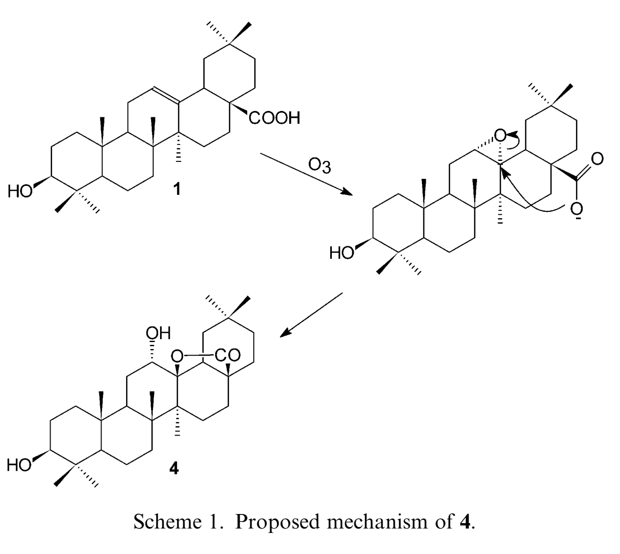
2.2. Bioactivity

Oleanolic acid and its synthetic derivatives shown in Scheme 2, were evaluated against various enzymes (urease, β-lactamase, acetyl cholinesterase and α-glucosidase). All tested samples gave positive response only against α-glucosidase. The IC$_{50}$ of compounds **1–6** were also determined and compound **4** was found to be the most active for the inhibition of α-glucosidase (IC$_{50}$ 7.97 μM). All the tests were performed using deoxynojirimycin as a positive control. See Table 1 and Fig. 1a–f.

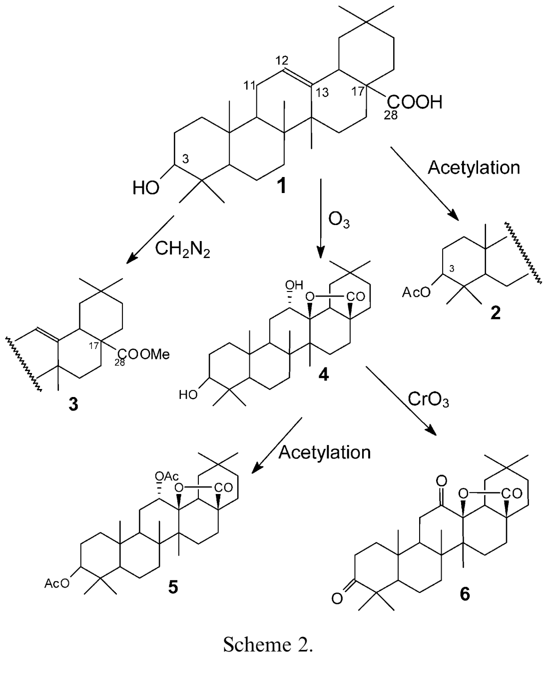
3. Experimental

3.1. General

The ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, recorded on a Bruker AM-300 spectrophotometer.



Scheme 1. Proposed mechanism of **4**.



Scheme 2.

3.2. Collection and identification

The plant material (*T. procumbens*) was collected from Gulshan-e-Maymar (Karachi) and identified by Prof. Khan Usmanghani, Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, where the voucher specimen (No. UG 187) is deposited.

Table 1

Oleanolic acid and its derivatives	IC_{50} (μM)	Fig.
1	11.16±0.493	1a
2	55.097±2.635	1b
3	19.012±0.835	1c
4	7.97±0.214	1d
5	89.71±2.105	1e
6	21.63±2.3352	1f
(Standard) deoxynojirimycin	330	

pH: 6.8; temperature: 37 °C.

3.3. Extraction and isolation

The plant material was dried under the shade for two weeks and the air-dried material (9 kg) was then soaked in hexane (15 l) after in vacuo removal of the hexane the resulting extract (107 g) was partitioned between ethyl acetate and water. The ethyl acetate soluble fraction was concentrated and then subjected to silica gel column chromatography using hexane, hexane:chloroform, chloroform and chloroform: methanol as eluting solvents. The fraction eluted with 80% chloroform in hexane afforded an impure solid material, which on washing with hexane and then with methanol, gave **1** as the major constituent (6.5 g) (Ahmad and Rahman, 1994).

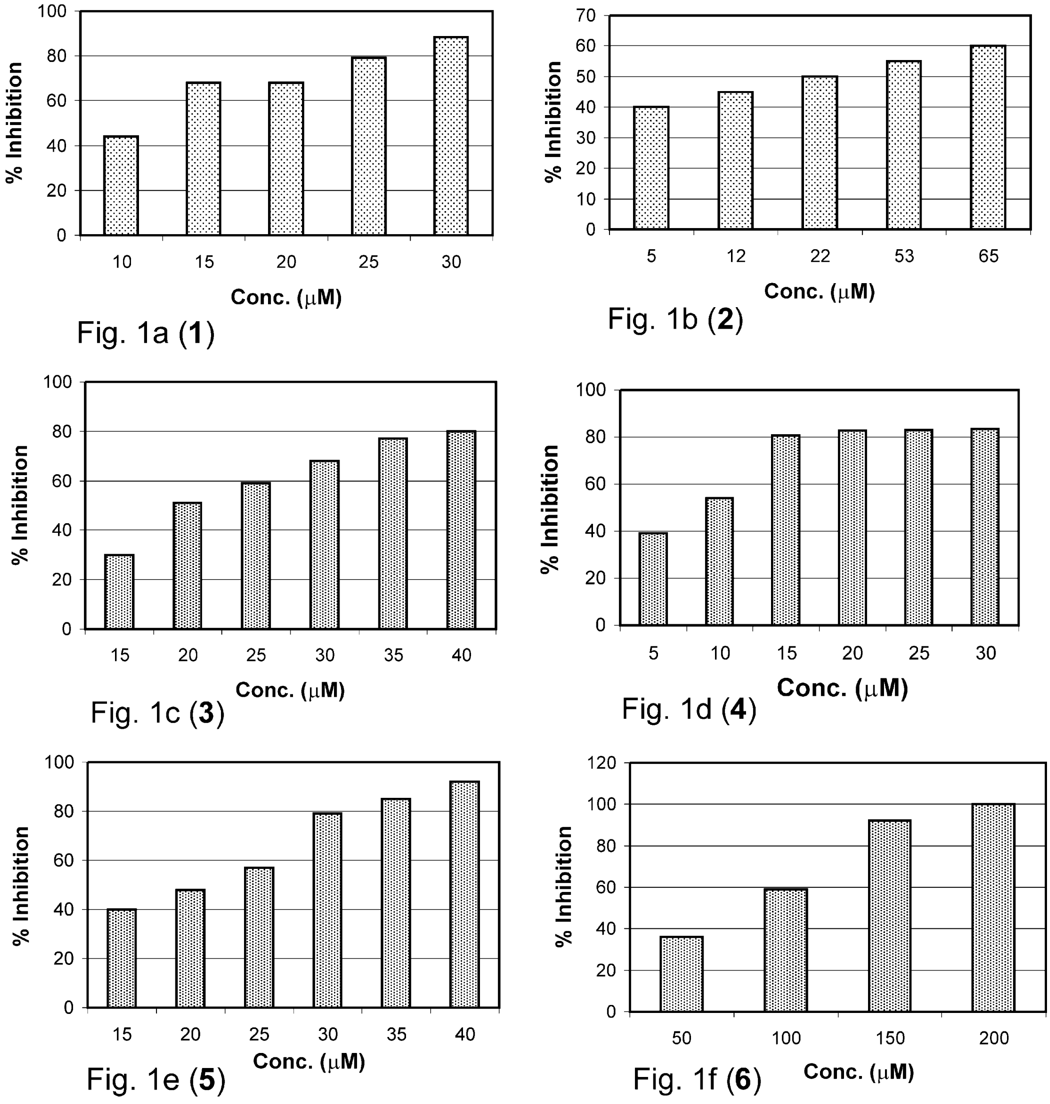


Fig. 1a (**1**)

Fig. 1b (**2**)

Fig. 1c (**3**)

Fig. 1d (**4**)

Fig. 1e (**5**)

Fig. 1f (**6**)

Fig. 1. (a) **1**; (b) **2**; (c) **3**; (d) **4**; (e) **5**; (f) **6**.

3.3.1. Acetylation of **1**

Oleanolic acid (**1**, 50 mg) was dissolved in pyridine (4 ml) and then treated with acetic anhydride (2 ml) overnight. The reaction mixture was diluted with water and the product was recovered in ethyl acetate. The organic layer was washed with a saturated copper sulfate solution and then with water. After concentration and column chromatography, **2** was obtained as a white solid (31 mg) (Alves et al., 1966).

3.3.2. Treatment of **1** *with diazomethane*

Oleanolic acid (**1**, 100 mg) in methanol (10 ml) was treated with ethereal diazomethane until the solution remained yellow. The excess reagent was neutralized with a few drops of acetic acid and the solvent was removed by evaporation to give **3** as white solid (98 mg) (Ikuta and Itokawa, 1986).

3.3.3. Ozonolysis of **1**

Oleanolic acid (**1**, 4 g) was dissolved in ethyl acetate (100 ml) and ozone was passed through the solution at −70 °C, until the light blue color persisted. Excess O_3 was reduced by treating with zinc-dust (2 g) and acetic acid (4 ml) overnight under continuous stirring at room temperature. Zinc-dust was removed by filtration and the solvent by evaporation. On chromatography, **4** (1.75 g) was obtained in pure form.

Mp: > 300 °C (dec.); IR (KBr): $\upsilon_{\max}=3525$, 2940, 1775, 1735, 1470, 1255 cm^{-1}; FD-MS: $m/z=472$; HR-EIMS: $m/z=472.3488$ (calcd. 472.3552 for $C_{30}H_{48}O_4$); ^1H NMR ($CDCl_3$, 300 MHz): $\delta=3.87$ (1H, *t*, $J=2.85$ Hz, H-12), 3.20 (1H, *dd*, $J=11.4$, 4.8 Hz, H-3), 1.29, 1.13, 0.98, 0.89, 0.97, 0.86, 0.76 ($7\times CH_3$); ^{13}C NMR: ($CDCl_3$, 75 MHz): δ 38.9 (C-1), 28.1 (C-2), 78.8 (C-3), 39.2 (C-4), 55.2 (C-5), 17.7 (C-6), 34.0 (C-7), 42.1 (C-8), 44.6 (C-9), 36.5 (C-10), 21.2 (C-11), 77.0 (C-12), 90.5 (C-13), 42.3 (C-14), 28.8 (C-15), 27.3 (C-16), 45.3 (C-17), 44.6 (C-18), 38.9 (C-19), 31.6 (C-20), 34.0 (C-21), 27.9 (C-22), 27.5 (C-23), 20.3 (C-24), 15.3 (C-25), 18.6 (C-26), 18.5 (C-27), 179.8 (C-28), 33.2 (C-29) and 23.9 (C-30).

3.3.4. Acetylation of **4**

Compound **4** (50 mg) was subjected to acetylation. The product (**5**, 36 mg) was obtained as a white solid.

Mp: < 300 °C (dec.); IR ($CHCl_3$): $\upsilon_{\max}=1770$, 1745, 1450, 1360, 1275 cm^{-1}; EIMS: $m/z=496$ $[M-(Ac+H_2O)]^+$, 436, 300, 249, 234, 205 (100%), 189; ^1H NMR ($CDCl_3$, 300 MHz): $\delta=5.05$ (1H, *dd*, $J=3.3$, 2.1 Hz, H-12), 4.45 (1H, *dd*, $J=9.8$, 6.0 Hz, H-3), 2.08, 2.03 ($2\times CH_3COO$), 1.24, 1.13, 0.94, 0.85, 0.84, 0.82, 0.80 ($7\times CH_3$).

3.3.5. Oxidation of **4**

Compound **4** (60 mg) was dissolved in acetone (8 ml) and treated with 8N chromium trioxide in sulfuric acid at 0 °C. Once, a yellow coloration persisted, the reaction mixture was left for a further 300 min. The excess reagent was destroyed by adding methanol and the solvent was removed by evaporation. The resulting gum was diluted with water and extracted with ethyl acetate. The organic layer was washed with water and dried over sodium sulfate. After concentration and repeated column chromatography, **6** was obtained as white solid (48 mg).

Mp: 300 °C (dec.); IR ($CHCl_3$): $\upsilon_{\max}=1765$ (γ-lactone), 1710 (C=O), 1122 cm^{-1}; FD-MS: $m/z=468$; EIMS: $m/z=468$, 426, 250, 221, 222, 206 (100%), 176, 107, 105, 95, 69.00; ^1H NMR ($CDCl_3$, 300 MHz): $\delta=1.35$, 1.08, 1.04, 1.03, 0.96, 0.95 ($7\times CH_3$).

3.4. α-Glucosidase inhibition

The inhibitory activity of all samples against α-glucosidase type VI (Sigma G6136) was measured spectrophotometrically at pH 6.8 and at 37 °C using 0.7 mM *p*-nitrophenyl α-D-glucopyranoside (PNP-G) as a substrate and 0.017 units/ml enzyme, in 50 mM sodium phosphate buffer containing 100 mM NaCl. 1-Deoxynojirimycin (0.3 mM) was used as a positive control. IC_{50} of 1-deooxynojirimycin was reproduced the same as described by Asanom et al. (1994). The increment in absorption at 400 nm due to the hydrolysis of PNP-G by α-glucosidase was monitored continuously with the spectrophotometer (Molecular Devices USA) (Marsui et al., 1966). The results are shown in Table 1 and Fig. 1a–f.

4. Conclusion

Among the all tested samples for the inhibition of α-glucosidase, dihydroxy-olide (**4**) was found the most active inhibitor (IC_{50} 7.97μM) of α-glucosidase.

Acknowledgement

We are very much thankful to our guest Prof. Y. Tsuda of Kanazawa University, Japan for discussions and finalizing the structure of **4**.

References

Ahmad, V.U., Rahman, Atta-ur, 1994. Handbook of Natural Product Data, Vol. 2. Elsevier, Netherlands, pp. 111–112.

Alves, H.M., Arndt, V.H., Ollis, W.D., Eyton, W.B., Gottlieb, O.R., Magalhaes, M.T., 1966. Triterpenoids isolated from *Machaerium incorruptibile*. Phytochemistry 5, 1327–1330.

Asanom, N., Oseki, E., Kaneko, K.M., 1994. Enzymic synthesis of α- and β-D-glucosidase of 1-deoxynojirimychi and their glycosidase inhibitory activities. Carbohydr. Res. 258, 255–256.

Bischoff, H., 1994. Pharmacology of α-glucosidase inhibition. Eur. J. Clin. Invest. 24, 3–10.

Ikuta, A., Itokawa, H., 1986. Triterpenoids of *Akebia quinata* callus tissue. Phytochemistry 25, 1625–1628.

Marsui, T., Yoshimoto, C., Osajima, K., Oki, T., Osajima, Y., 1966. Inhibition of intestinal glucose absorption by food components. Part 1. *In vitro* survey of α-glucosidase inhibitory food components. Biosci. Biotech. Biochem. 60, 2019–2022.

Poehland, B.L., Carte, B.K., Francis, T.A., Hyland, L.J., Allaudeen, H.S., Troupe, N., 1987. *In vitro* antiviral activity of dammar resin triterpenoids. J. Nat. Prod. 50, 706.

Waller, G.R., 1980. Biochemical Applications of Mass Spectroscopy. John Wiley and Sons, p. 396.

Wolffenbuttel, H.R., Hoeften, W.V., 1995. Prevention of complications in non-insulin-dependent diabetes mellitus (NIDDM). Drugs 50, 263–288.