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Nematicidal activity of the derivatives of oleanolic acid

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Key words: Oleanolic acid, Acyl derivatives, Nematicidal activity, *Meloidogyne incognita*

Abstract

In this study, four 3 *O*-acylated derivatives of oleanolic acid (1a-1d) have been prepared. Compounds 3-*β*-[2-Triflouromethylbenzoyloxy]-olean-12-en-28-oic acid (1c) and 3-*β*-[4-Triflouromethylbenzoyloxy]-olean-12-en-28-oic acid (1d) are new and 3-*β*-[Benzoyloxy]-olean-12-en-28-oic acid (1a) and 3-*β*-Dichloroacetoxy-olean-12-en-28-oic acid (1b) have synthesized earlier. Their nematicidal activity against root knot nematode *Meloidogyne incognita* was determined and compared with the standard nematicide furadan. These derivatives were characterized through UV, IR and ^{1}H-NMR. Oleanolic acid (1) was found to be most active with 75% toxicity and among these derivatives, 2-triflouromethyl benzoyl derivative (1c) showed highest toxicity 74% at 1% concentration. This is the first report of the nematicidal activity of these derivatives of oleanolic acid.

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Introduction

The most illustrative group of phytochemicals are triterpenoids, and they includes more than 20,000 documented compounds. Though squalene cyclization they are biosynthesized in plants. Based on their structural skeletons they can be classified into groups like cycloartanes, oleananes, holostanes, dammaranes, freidelene, lupanes, cucurbitances, prtostanes, ursanes, isomalabaricanes, lanostanes, euphanes, triucallanes, hopanes etc. Triterpenes embraces a sustenance chemical group of active principles, which are associated in the pharmacological effect and mechanism of action of many medicinal plants.

They are used against several disease in folk medicine in which the immune system is implicated. In Asian countries, triterpenes traditionally used as analgesics, anti-inflammatory, hepatoprotective, cardiotonic and sedative agents. Further studies have also revealed their antipruritic, antioxidant, antimicrobial, antiallergic and antiangiogenic potential (Almeida *et al.*, 2015; Gill *et al.*, 2016; Nazaruk and Borzym-Kluczyk, 2015).

Oleanolic acid (3β-hydroxy-olean-12-en-28-oic acid, 1) is a natural pentacyclic triterpenoid worldwide. It is evidently indicated that chemical structure of oleanolic acid, consisting three active sites; the hydroxyl group at C-3, double bond between C-12 and C-13 and carboxylic acid group at C-28 are present, which may be chemically modified and eventually fine tune its physical and pharmacological effects. Many derivatives like acyl, alkyl and hydrazide have been reported earlier (Bednarczyk-Cwynar *et al.*, 2012; Li *et al.*, 2006; Nkeh-Chungag *et al.*, 2015; Pollier & Goosens, 2012). Oleanolic acid possess numerous medicinal activities such as antiulcer, anticancer, antipyretic, anti-inflammatory, antidiabetic, hepatoprotective, antioxidant and anti HIV effects. Consequences of studies on oleanolic acid pharmacological activity demonstrated its comprehensive variety of action and its impending advantageous determinations on various diseases. In this study, oleanolic acid presented as multitargetted agent what makes it a good candidate for further use as starting point for a new drug design (Ayeleso *et al.*, 2017; Feng *et al.*, 2020; Sultana and Ata, 2008).

Nematodes are among the numerous and most complex organisms on the planet. Due to root-knot nematodes, the loss of agricultural products has been increasingly annually, worldwide. Root knot nematodes belongs to the genus *Meloidogyne* and are one most important causes of damage to major cultivated crops like sweet potato, potato, cabbage, pepper, tomato, lettuce, okra etc. A major parasitic nematode species, *Meloidogyne incognita*, root knot nematode, is one of the most harmful in affecting the crop production annually. It is not only decrease the plant growth but also accumulates in plant and causes nutrients deficiency. Research has been done on various additives have been extracted in plants and exhibited good activity against nematode parasite (Hassan *et al.*, 2013; Radwan *et al.*, 2012). The plant roots are damaged after being infected and loss their functions to nutrients from sorts and generally absorb water. In general, a diversity of procedures are used to limit *Meloidogyne* damage including the control of cultivation environments through sterilization by sunlight and improvement of soil, the development of resistant crops, the biological control of root-knot nematode using microorganism or plant extracts and the treatment of plant disease using chemical nematicides. Use of chemical nematicides is commonly more effective than other strategies. The interest in the development of new nematicidal agents that do not influence farmers, consumers, crop or the environment is going (Laquale *et al.*, 2020; Echeverrigaray *et al.*, 2010; Park *et al.*, 2020; Nguyen *et al.*, 2013).

Keeping in veiw the interesting chemistry and biological properties of oleanolic acid. The aim of this study was to examine the nematicidal effect of oleanolic acid and its 3 O-acyl derivatives. In current research, we are reporting the acylation of hydroxyl group at C-3 position of oleanolic acid and characterization through different spectral studies like UV, IR and ^{1}H-NMR and determination of their nematicidal activity.

Materials and methods

General

Melting points were recorded in glass capillary tubes on BUCHI 535 melting point apparatus and are uncorrected. Infrared spectra were obtained on a JASCO A-302 spectrophotometer. Ultraviolet spectra were recorded on a Hitachi-U-3200 spectrop hotometer. The[1]H-NMR spectra were recorded on Bruker Avance spectrometer operating at 400MHz. The Chemical shifts are expressed in δ (ppm) referenced to the residual solvent signal and the coupling constants (J) are in Hz. TLC was performed on Kieselgel 60 F254 precoated aluminium cards (0.2mm thickness, Merck) and spots were visualized under UV light at 254/365 nm and by spraying with 5% H_2SO_4.

Derivatization of Oleanolic acid

50mg of oleanolic acid (1) in 10mL of chloroform with few pellets of dimethyl amino pyridine was mixed for ten minutes and kept at room temperature overnight (Fig 1). The obtained residue was separated through separating funnel with chloroform and water. The organic phase was washed with 2% aq. NaOH, then with water and dried over mg SO_4. A colorless crystalline solid was obtained which was purified through column chromatography (petroleum ether; petroleum ether-EtOAc) in order of increasing polarity which furnished 14 fractions. Fraction 9 (petroleum ether-EtOAc 7.5:2.5 eluate) afforded corresponding acyl derivatives.

3-*β*-[Benzoyloxy]-olean-12-en-28-oic acid (1a). Colorless crystals, mp. 265-267ºC, yield: 66%. UV λ_{max} ($CHCl_3$-MeOH 1:1, nm): 207 and 236. IR spectrum (KBr , ν_{max},cm^{-1}): 3436, 2665 (Br, COOH), 2925, 2860 (CH), 1725 (ester C=O), 1702 (acid C=O), 1628 (C=C), 1530 (Ar C=C), 1125 (C-O). ^{1}H-NMR: (300MHz, $CDCl_3$, δ, ppm, J/Hz): δ 7.74 (2H, m, H-2' and H-6'), 7.02 (2H, m, H-3' and H-5'), 7.44 (1H, m, H-4'), 5.28 (1H, t, *J* = 3.4 Hz, H-12), 4.62 (1H, t, *J*=7.8 Hz, H-3*α*), 2.82 (1H, dd, *J*=14.1, 3.9 Hz, H-18), 1.1 (3H, s, Me), 1.01 (3H, s, Me), 0.98 (3H, s, Me), 0.94 (3H, s, Me), 0.91 (3H, s, Me), 0.88 (3H, s, Me) and 0.76 (3H, s, Me).

3-*β*-Dichloroacetoxy-olean-12-en-28-oic acid (1b). Colorless crystals yield 69%, mp. 283-284 ºC. UV λ_{max} ($CHCl_3$-MeOH 1:1, nm): 206. IR spectrum (KBr , ν_{max},cm^{-1}): 3440-2656 (Br, COOH), 2946, 2855 (CH), 1728 (ester C=O), 169 (acid C=O), 1628 (C=C) and 1124 (C-O). ^{1}H-NMR: (300MHz, $CDCl_3$, δ, ppm, J/Hz) δ 5.26 (1H, t, *J*=3. Hz, H-12), 4.89 (1H, s, H-1'), 4.54 (1H, t, *J*=7.5 Hz, H-3*α*), 2.88 (1H, dd, *J*=14.0 , 3.8 Hz, H-18), 1.11 (3H, s, Me), 0.94 (6H, s, 2xMe), 0.90 (6H, s, 2xMe), 0.88 (3H, s, Me) and 0.81 (3H, s, Me).

3-*β*-[2-Triflouromethylbenzoyloxy]-olean-12-en-28-oic acid (1c): Colorless crystals, yield 62%, mp. 291-292ºC. UV λ_{max} ($CHCl_3$-MeOH 1:1, nm): 208 and 256. IR ν_{max} (KBr)cm^{-1}: 3430-2635 (Br, COOH), 2928, 2858 (CH), 1726 (ester C=O), 1700 (acid C=O), 1625 (C=C), 1548 (Ar C=C) and 1127 (C-O). ^{1}H-NMR: ($CDCl_3$, 300MHz): 8.13 (1H, m, H-3'), 7.82 (1H, m, H-6'), 7.67 (2H, m, H-4', 5'), 5.28 (1H, t, *J*=3.5 Hz, H-12), 4.64 (1H, t, *J*=7.7 Hz, H-3*α*), 2.80 (1H, dd, *J*=14.1, 3.9 Hz, H-18), 1.13 (3H, s, Me), 1.01 (3H, s, Me), 0.99 (3H, s, Me), 0.95 (3H, s, Me), 0.93 (3H, s, Me), 0.88 (3H, s, Me) and 0.76 (3H, s, Me).

3-*β*-[4-Triflouromethylbenzoyloxy]-olean-12-en-28-oic acid (1d): Colorless crystals, yield 64%, mp 287-288ºC. UV λ_{max} ($CHCl_3$-MeOH 1:1, nm): 207 and 254. IR ν_{max} (KBr)cm^{-1}: 3432-2640 br. (COOH), 2928, 2858 (CH), 1725 (ester C=O), 1702 (acid C=O), 1630 (C=C), 1550 (Ar C=C) and 1120 (C-O). ^{1}H-NMR: ($CDCl_3$, 300MHz): 8.23 (1H, d, J = 7.8Hz, H-3', 5'), 7.81 (1H, d, J = 7.8Hz, H-2', 6'), 5.27 (1H, t, *J*=3.4 Hz, H-12), 4.62 (1H, t, *J*=7.7 Hz, H-3*α*), 2.81 (1H, dd, *J*=14.2, 3.8 Hz, H-18), 1.14 (3H, s, Me), 1.00 (3H, s, Me), 0.98 (3H, s, Me), 0.94 (3H, s, Me), 0.91 (3H, s, Me), 0.87 (3H, s, Me) and 0.78 (3H, s, Me).

Determination of Nematicidal Activity

For nematicidal activity 100 larvae were count in a counting chamber for each dose and replicate to introduce in 3 × 3 glass cavity block. Population of J2 infective stage juveniles of *M. incognita* was collected from pure culture maintained on tomato plants *Lycopersicon esculentum* in microplot of a screen house in National Nematological Research Center, University of Karachi, Karachi, Pakistan. Egg masses was extracted from the roots of infected tomato plant and transferred to a small cavity block contained water. The cavity block was incubated for egg hatching at 28 ˚C for 3 days. Three concentrations 1%, 0.5% and 0.125% were applied at a rate of 1mL at each cavity block. The stock solutions (10mg/mL) from plant extracts were prepared in 5% dimethyl sulfoxide (DMSO). After expected time durations, the movement of the nematodes was checked by needle whereas the larvae were counted under a stereoscopic microscope for mortality. The nematodes, which exhibited mortality after 72h of exposure, were transferred in another cavity block containing distilled H_2O, and their mortality was confirmed after 24h of observation. For positive control, standard nematicide furadan was used whereas for negative control, 5% aq. DMSO was used. The mortality percentage was confirmed by an average of three replicates analysis.

Results and discussions

Chemistry

In this study, four (1a-1d) 3 *O*-acyl derivatives of oleanolic acid (1) was prepared in good yield. Among them, two compounds 1c and 1d are new while other two 1a and 1b have been reported earlier (Chouaib *et al.*, 2015; Silva *et al.*, 2012). IR shows the presence of ester carbonyl which confirms the structure. In the proton NMR of the acylated compounds the signals of H-3*α* shifted downfield and appeared at around 4.5 instead of δ 3.20 in oleanolic acid. In dichloroacetyl derivatives (1b) the proton attached with dichloro group was observed in proton NMR with δ_H 4.89. While in benzyl derivatives (1a), the aromatic protons were observed at δ_H 7.02-7.74 and in case of triflouromethyl benzoyl group (1c & 1d) the aromatic protons were observed at δ_H 7.67-8.23.

3-*β*-[Benzoyloxy]-olean-12-en-28-oic acid (1a) was found to be as colorless crystals with yield 66% having mp. 265-267ºC. It showed λ_{max} in UV spectrum at 207 and 236 nm. IR spectrum shows presence of COOH, CH, ester C=O, acid C=O, C=C and C-O. The ^{1}H-NMR spectra showed singlet at δ_H 0.76, 0.88, 0.91, 0.94, 0.98, 1.01, 1.14 attributed to seven methyl groups. A one proton double doublet appeared at δ_H 2.82 (*J* = 14.1, 3.9 Hz) characteristic of H-18 of oleanane triterpenoids. A one proton triplet appeared at δ_H 4.62 (*J* = 7.8 Hz) which indicated *β* – oriented ester function at C-3. Compound further showed three multiplets at δ_H 7.74 for H-2' and H-6', δ_H 7.02 for H-3' and H-5' and δ_H 7.44 for H-4' confirms the presence of benzoyl ring. One proton triplet also occurs at δ_H 5.28 (*J* = 3.4 Hz) confirm the presence of double bond at C-12. These data confirm the structure of 3-*β*-[Benzoyloxy]-olean-12-en-28-oic acid.

3-*β*-Dichloroacetoxy-olean-12-en-28-oic acid (Ib). It physical appearance is also colorless crystals having mp. 283-284ºC having 69% yields. It showed λ_{max} in UV spectrum at 206 nm and IR spectrum at COOH, acid C=O, ester C=Oand C=C. The analysis from ^{1}H-NMR data seven methyl singlets at δ_H 0.81, 0.88, 0.90, 0.94 and 1.11 and one proton double doublet for H-18 at δ_H 2.88 (*J* = 14.0, 3.8 Hz) confirmed that it is an oleanane triterpenoids. A peak at δ_H 4.54 (*J* = 7.5 Hz) was also approved that this compound has a *β* oriented ester moiety at C-3. The presence of one proton triplet at δ_H 5.26 (*J* = 3.4 Hz) supported that a double bond present at C-12. A one proton singlet appears at δ_H 4.89 for H-1' confirm the presence of dichloroacetyl group. These data confirm the structure of 3-*β*-Dichloroacetoxy-olean-12-en-28-oic acid.

3-*β*-[2-Triflouromethylbenzoyloxy]-olean-12-en-28-oic acid (1c): It was found to be as colourless crystals having yield 62% with mp. 291-292ºC. It showed λ_{max} in UV spectrum at 208 and 256 nm and IR peaks for the presence of COOH, acid C=O, ester C=O and C=C, C-O. The analysis from ^{1}H-NMR data seven methyl singlets at δ_H 0.76, 0.88, 0.93, 0.95, 0.99, 1.03 and 1.13 and one proton double doublet for H-18 at δ_H 2.80 (*J* = 14.1, 3.9 Hz) confirmed that it is an oleanane triterpenoids. A peak at δ_H 4.64 (*J* = 7.7 Hz) was also approved that this compound has a *β* oriented ester moiety at C-3. The presence of one proton triplet at δ_H 5.28 (*J* = 3.5 Hz) supported that a double bond present at C-12. Three multiplets appeared at 8.13 for H-3', 7.82 for H-6' and 7.67 for H-4', 5' respectively. Singlet appears at δ_H 4.89 for H-

1' confirm the presence of dichloroacetyl group. These data confirm the structure of 3-β-[2-Triflourome thylbenzoyloxy]-olean-12-en-28-oic acid.

3-β-[4-Triflouromethylbenzoyloxy]-olean-12-en-28-oic acid (1d): It was shown as colourless crystals having yield 64% and showed mp at 287-288°C. It exhibited λ_{max} in UV spectrum at 207 and 254 nm and COOH, CH, ester C=O, acid C=O, C=C, C-O group presence confirmed through the IR spectrum. The analysis from ^{1}H-NMR data seven methyl singlets at δ_H 0.78, 0.87, 0.91, 0.94, 0.98, 1.00 and 1.14 and one proton double doublet for H-18 at δ_H 2.81 (J = 14.2, 3.8 Hz) confirmed that it is an oleanane triterpenoids. A peak at δ_H 4.62 (J = 7.7 Hz) was also approved that this compound has a β oriented ester moiety at C-3. The presence of one proton triplet at δ_H 5.27 (J = 3.4 Hz) supported that a double bond present at C-12. Three multiplets appeared at 8.23 for H-3', 5' and 7.81 for H-2' 6' respectively. Singlet appears at δ_H 4.89 for H-1' confirm the presence of dichloroacetyl group. These data confirm the structure of 3-β-[4-Triflouromethylbenzoyloxy]-olean-12-en-28-oic acid.

Nematicidal Activity

In the present investigations, nematicidal activity of oleanolic acid and its 3 O-acyl derivatives (1a-1d) against root knot nematode *Meloidogyne incognita* at three different concentrations (0.1, 0.5 and 0.125%) after 24, 48 and 72h of exposure were determined (Table 1). The tested acyl derivatives showed good toxicity (68-80%) against *M. incognita* at 1% concentration after 72h. It was observed that mortality increased with increasing concentration and time duration. After 48 h all these derivatives displayed moderate to good inhibition 58-70% at 1% concentration. It is to be noted that the parent compound and its derivatives showed more activity than the standard furadan at 0.125% concentration after 24h. The parent compound 1 showed 75% mortality after 72h at 1% concentration. Compound 1b was found to be most active with 80% mortality after 72h at 1% concentration. Among all aromatic derivatives compound 1c (2-triflouromethyl benzoyl derivative) showed highest activity (74%) after 72h at the same concentration.

This is the first report of the nematicidal activity of the acyl derivatives of oleanolic acid.

Table 1. Mortality of oleanolic acid and its derivatives against *Meloidogyne incognita*

Compound	Concentration [%] in 5% DMSO	Mortality[%] Hours		
		24	48	72
Oleanolic Acid (1)	1	60	70	75
	0.5	30	52	67
	0.125	10	30	52
3-β-[Benzoyloxy]-olean-12-en-28-oic acid (1a)	1	47	58	68
	0.5	30	40	45
	0.125	30	40	44
3-β-Dichloroacetoxy-olean-12-en-28-oic acid (1b)	1	45	65	80
	0.5	40	54	64
	0.125	37	49	54
3-β-[2-Triflouromethylbenzoyloxy]-olean-12-en-28-oic acid (1c)	1	42	67	74
	0.5	35	53	68
	0.125	32	50	66
3-β-[4-Triflouromethylbenzoyloxy]-olean-12-en-28-oic acid (1d)	1	40	65	72
	0.5	30	60	68
	0.125	26	56	60
Furadan	1	100	100	100
	0.5	50	100	100
	0.125	20	100	100

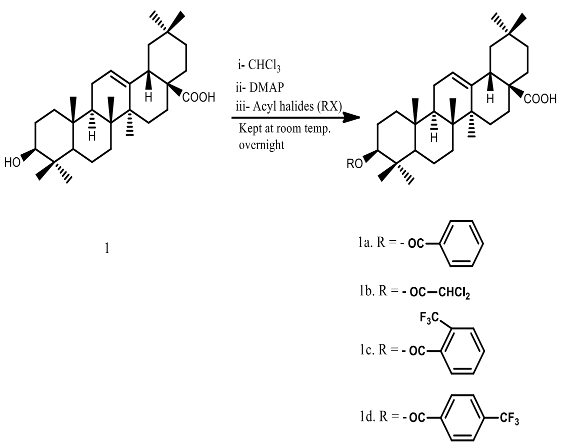

Fig. 1. Acylation of oleanolic acid (1).

Conclusion

In the present study oleanolic acid and its four acyl derivatives were prepared and characterized through ^{1}H-NMR, IR and UV spectroscopy. Oleanolic acid and its derivatives showed activity against *M. incognita*. In which oleanolic and 1b (3-β-dichloroacetoxy-olean-12-en-28-oic acid) was found to be more active. The description of the activity of oleanolic acid and its derivatives opens a new window for investigation.

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