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ORIGINAL ARTICLE

New natural compound for the enlargement of the ureter

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Abstract Chemical and phytochemical investigation of the ethanol extract of the roots of *Alhagi maurorum* led to the isolation of a new aliphatic ester, which was named **glyceryl-*n*-tetracosan-17-ol-1-oate** on the basis of spectral data analysis and chemical reactions. This compound, in pure form, was found to enlarge the ureter.

1. Introduction

Alhagi maurorum (Uphof, 1959; Chakravarty, 1960; Brown, 1995) is used in folk medicine as a purgative, laxative, diaphoretic, expectorant and diuretic. Its flowers are used to treat piles, migraine, and warts. Oil from the leaves is used in the treatment of rheumatism. Locally, water extracts of its roots are used to enlarge the ureter and to remove kidney stones. Pharmacological researches on the root extracts of this plant (Marashdah et al., 2006a,b; Mudawi et al., 2007; Marashdah et al., 2008; Marashdah and Farraj, 2010) exhibited the activity of these extracts; therefore, extensive efforts were made to know which compound is the active one. Finally, the aliphatic ester **glyceryl-*n*-tetracosan-17-ol-1-oate** was found to be the active one.

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2. Experimental

2.1. General

Melting points were determined on electrothermal, Model of apparatus Gallenkamp, mpd350-BM 3.5, UK, mpd melting point apparatus and are uncorrected. Infra-red (IR) and FT-IR spectra were recorded as potassium bromide pellets or in chloroform, using Shimadzu FTIR spectrophotometer model FTIR-8400S and Perkin-Elmer IR spectrophotometer model 883.

^{1}H, ^{13}C and 2D nuclear magnetic resonance (NMR) spectra were recorded on Joel JNM-ECP 400 FT NMR system using deuterated solvents in 5 mm NMR tubes.

Mass spectra were obtained using Shimadzu gas chromatograph mass spectrometer model GCMSQP5050A using DP1 30 m column, direct solid probe spectrometer model GCMS-QP5050A.

Column chromatography (CC) was performed using silica gel (s) 60, particle size 0.063–0.200 mm (⩽230 meshes ASTM) from Fluka, in glass columns of different dimensions.

Thin layer chromatography (TLC) was performed using precoated silica gel 60 F254 $20 \times 20 \times 0.25$ from MERCK as preparative plates and 5×20 cm as test plates.

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TLC and the spots were displayed in UV light and by iodine chamber.

2.2. Plant material

The plant was collected from Palestine in December 2008, and identified by the Department of Botany and Microbiology, Faculty of science; King Saud University, Riyadh. The roots were taken out, cleaned and dried in the shade for two weeks and then powdered.

2.3. Separation of the intended compound

The red mass (30.8135 g, 2.7759%) obtained from the ethanolic extract was chromatographed on a wet large glass column (115 cm long, 3.5 cm diameter), packed with silica gel (180 g) and eluted with the following elution systems:

1- Chloroform, (8 fractions).
2- Chloroform:ethyl acetate, 4:1(7 fractions).
3- Chloroform:ethyl acetate, 1:1(3 fractions).
4- Ethyl acetate, (10 fractions).
5- Ethyl acetate:ethanol, 7:3 (10 fractions).
6- Ethyl acetate:ethanol, 1:1 (5 fractions).
7- Ethanol, (3 fractions).

(Total fractions = 46, about 50 ml each)

Fractions 29–46 were spotted on two TLC plates and eluted with chloroform: ethanol, 9:1, but the same situation prevails as with the former fractions although many attempts were made to achieve a notable separation. Therefore, recrystallization was carried out by collecting the fractions which have the same appearance then a less polar solvent was added (pet. ether) drop wise until a turbidity formed and the solution was left overnight. This procedure gave some success with the fractions 5–9 and 10–13 only where a precipitate was formed in each collection.

The precipitate formed from fractions 5–9 was filtered and recrystallized from chloroform:pet. ether, 1:1 and this way was repeated three times. The final precipitate was dried to give (0.1935 g, 0.6279 %), m.p = 92°. The purity of this compound was examined by test TLC to give one spot, and this purity was confirmed by NMR.

The intended compound did not respond to the tetranitromethane test indicating the saturated nature of the molecule. Its IR ($CHCl_3$) spectrum illustrated an intense broad absorption band for hydroxyl group at 3372 cm^{-1}. It also showed a carbonyl absorption at 1709 cm^{-1} attributable to an ester carbonyl stretching.while the C—(=O)—O stretching band (intense and broad) appeared at 1090 cm^{-1}. The C–H stretching bands in long saturated chain appeared at 2892 and 2923 cm^{-1}

The mass spectrum of this compound showed the presence of a large number of fragment ions with a uniform difference of 14 mass units, thereby indicating the presence of a long aliphatic chain. It showed a weak molecular ion peak at *m/z* 458 (0.8%) corresponding to the molecular formula $C_{27}H_{54}O_5$ (DBE = 1). The familiar fission of ester led to the formation of a peak at *m/z* 91(1.5%) which is attributed to the glyceryl group; normally loses two water molecules to give a base peak at *m/z* 55 (100%). The other part of the ester gave a peak at *m/z* 367 (7.5%) which in turn loses one water molecule to give a peak at *m/z* 349 (3.5%) or it may subject to C_{16}–C_{17} fission which leads to the formation of [$^{\bullet}CH_2(CH_2)_{14}C{\equiv}O^+$] at *m/z* 238 (1.6%) and [$CH_3(CH_2)_6{}^{\bullet}CHOH$] at *m/z* 129 (12.5%). The other possibility of fragmentation in this part is the fission of C_{17}–C_{18} which leads to the formation of [$CH_3(CH_2)_6]^{\bullet}$ at *m/z* 99 (9.8%) and [$CHOH(CH_2)_{15}C{\equiv}O^+$] at *m/z* 269 (1.5%). These patterns of fragmentation suggest the attachment of the hydroxyl group to C-17.

The ^{1}H NMR spectrum of the compound exhibited resonance of oxygenated methylene protons (H-1′) as doublet of doublet (*dd*) at δ 4.14 and 4.05 (J = 7.3 Hz), one methine proton (H-2′) attached to hydroxyl carbon pentet at δ 3.3, methylene protons (H-3′) attached to hydroxyl carbon doublet of doublets at δ 3.66 and 3.60 (J = 5.9 Hz), methylene protons adjacent to carbonyl (H-2) triplet at δ 2.34 (J = 7.3 Hz). The spectrum also showed terminal methyl protons (H-24) triplet at δ 0.9 (J = 6.6 Hz), two methylene protons (H-16 and H-18) quartets at δ 1.5, 1.6 (J = 6.6 Hz) in addition to methylene protons (H-3) pentet at the same δ and methine proton (H-17) pentet at δ 3.8 (J = 5.12 Hz).

The ^{13}C NMR of compound showed an important signals for ester carbonyl group at δ174.3 and four signals due to oxygenated carbons at δ 70.3, 65.2, 63.3 and 63 attributed to C-17, 2′, 3′ and 1′, respectively. The spectrum also displayed three signals at δ 34.2, 32.8, and 31.9 which were attributed to C-18, C-16 and C-2 respectively. The terminal methyl carbon resonates at δ14. The signals of the remaining methylene carbons gave signals between δ 25 and 29.7.

These spectral data led to formulate this compound as **glyceryl-n-tetracosan-17-ol-1-oate**, a **new phytoconstituent**.

CH_2 (OH) CH (OH) CH_2OCO $(CH_2)_{15}CHOH$ $(CH_2)_6CH_3$

2.4. Standard procedures for biological activity tests

- The effects of the extracts on mice rectal temperature were studied following the method described by (Gray et al., 1987).
- The effects of the different extracts on the rectus abdominis muscle were studied following the method described by (Fleisher et al., 1960).
- The effects of the different extracts on the heart using the ECG (Electro-cardio gram) were studied following the method described by (Bakheet et al., 1999).
- The effects of the different extracts on the guinea – pig ureter were studied using the method described by (Yoshiaki, 1968).

2.4.1. Preparation of test samples

The compounds were used in the form of suspension in 0.25% aqueous sodium carboxy methyl cellulose. After suspension, the mixture was emulsified by vortexing (shaking). A glass rod is usually inserted inside a tube containing the suspension to facilitate emulsification during vortexing in the vortex mixer.

2.4.2. Biological activity tests

1- Effect on rectal temperature: Rectal temperature of mice was measured using an Aplex rectal thermometer (Haly) fitted with thermistor probes. The probe was inserted to a depth of 2.5 cm into the rectum of each mouse and

temperature reading was allowed to stabilize, recorded and the probe then removed. The temperature was taken at an interval of 30 min.

2- Effect on the rectus abdominis muscle of the frog: Initially, each frog was decapitated and then pithed by inserting a needle into the spinal cord. The frog was then fixed to a wooden board with its abdomen facing upward. The outer skin of the frog was cut longitudinally and horizontally to expose the abdominal wall. The two rectus abdomini muscles were located and they were cut and removed completely from the animal and placed in Krebs' solution. Then each muscle was suspended vertically in the chamber of an organ bath containing Krebs' solution and aerated using (95% O_2 + 5% CO_2). The temperature of the bathing fluid was adjusted to 37 °C. Each tissue was allowed to equilibrate with the bathing fluid for 30 min. Each dose of the drug was allowed to contact the tissue for a time to obtain the maximum response. Each compound was allowed to contact the tissue for 5 min before 3 additions of the nicotine agonist. The percentage inhibition induced by the compounds on the agonist submaxional dose was then calculated.

3- Effect on the Electro-Cardio Gram (ECG) of rats: Male Wister rates (250 g) were anesthetized with urethane and prepared for measurement of the ECG waves. The limbs of the animal were fastened to a dissection board with the animal lying on its back. The ECG lead II was recorded by the aid of subcutaneous needle electrodes and the record was displayed in chart of the instrument. The recording speed was adjusted to 25 mm/sec. The compounds were injected intraperitoneally and the effect was then followed for twenty minutes to observe any disturbances in the cardiac rhythm. The heart rate before and after the compound injection was recorded and the percentage changes were then calculated.

4- Effect on the ureter: Guinea-pigs were killed by blows on the neck, and the abdominal cavity of each animal was opened. The kidney and the ureters were located and the whole length of the ureter (from the kidney to the urinary bladder) was cut and placed in Krebs' solution. The ureter was then prepared for the study of the effect of the compounds. 2 cm length of each ureter was suspended in an organ bath containing oxygenated (95% O_2 + 5% CO_2) Krebs' solution. Each tissue was connected to an isometric transducer connected to a physiograph (Narco Biosystems, USA). Each tissue was allowed to equilibrate with the bathing fluid for 30 min. Initially the effects of different doses of the compounds were determined. The effect of the compounds on the standard agonists (Histamine, Acetyl Colin (ACh)) were then determined. Each compound was allowed to contact the tissue for 5 min before the addition of the agonist. The percentage inhibition induced by the compound on the selected dose of the agonist was then calculated.

2.4.3. Pharmacological activity of the compound

1- Intraperitoneal administration of the compound in mice a dose of 200 mg / kg of body weight resulted to reduce body temperature by 4.1 and 5.2 after one and two hours respectively of the injected compound Listen Read phonetically.

2- Treatment the ureter isolated from animal hair material histamine to cause contractions of multiple and reached to 32 contraction during 100 s, but when treated the ureter with two doses of 20 micrograms and 40 micrograms/ml of solution, physiological surrounding the ureter for 5 min, reduced the ability of histamine to the events contractions multi to 8 and zero contractions through 100 s a percentage equal to 75% and 100%, respectively.

3- The compound possessed a heart rate stimulant action and a myocardial depressant action.

4- The compound induced relaxations to the guinea – pig ureter and suppressed histamine – induced spasms. It seemed to possess an anticolic action and a ureter relaxing action that can enhance getting rid of renal stones and relieve of the accompanying pain (contraction of the ureter).

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