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Chemical Investigation of *Euphorbia schimperi* C. Presl

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Abstract: Three triterpenoids; cycloart-25-en-3β,24-diol, cycloart-23-en-3β,25-diol, and α-amyrin, in addition to β-sitosterol-β-D-O-glucoside, scopoletin, luteolin and kampferol were isolated for the first time from the chloroform fraction of the alcoholic extract of *Euphorbia schimperi* C. Presl (F. Euphorbiaceae). The isolated compounds were identified using different spectroscopic methods (EIMS, ^{1}HNMR, ^{13}CNMR, HMQC, HMBC and COSY). The cytotoxic activity of the chloroform fraction was also studied using brain and breast carcinoma cell lines.

Keywords: *Euphorbia schimperi*, triterpenoids; cycloartane, β-sitosterol-β-D-O-glucoside, scopoletin, luteolin; kampferol, cytotoxic activity.

1. Introduction

Euphorbiaceae is a large family of about 300 genera and 6000 or more species. Most members are trees or shrubs and few are herbs. Some genera (e.g. *Euphorbia*) are xerophytic [1]. Certain *Euphorbia* species have been reported to possess antitumor activity and was recommended to be used as anticancer remedies [2, 3]; on the other hand some *Euphorbia* species have been reported as carcinogens [4, 5]. Several other species have been reported to exhibit antimicrobial [6, 7], antimalarial [8], insecticidal [9], mollusicidal [10, 11], anti-inflammatory and antipyretic activities [12]. Different di- and triterpenes were isolated from different *Euphorbia* species [13-17].

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In the present study, seven compounds were isolated for the first time from the chloroform fraction of the alcoholic extract of *E. schimperi* C. Presl growing in Kingdom of Saudi Arabia. The cytotoxic activity against brain and breast carcinoma cell lines of the chloroform fraction was reported.

2. Materials and Methods

2.1. Plant Material

The aerial parts of *Euphorbia schimperi* C. Presl were collected from Jeddah-Al-Taif road, Kingdom of Saudi Arabia, in March 2005 and was identified by Dr. Farag A. Al-Ghamdi, Plant Taxonomy Department, College of Science, King Abdulaziz University, Kingdom of Saudi Arabia. A specimen was deposited in the herbarium of College of Pharmacy, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia (#. ES1084).

2.2. General

Electro thermal 9100 was used for determination of melting point. Beckman UV spectra were recorded on Du-7 spectrophotometer. Mass spectra were measured using Finningan Mat SSQ 7000, 70 ev. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) and 2D NMR spectra were measured on Varian Mercury-400BB spectrophotometer or on Varian Mercury-VX-300 NMR spectrophotometer. TLC was performed on precoated silica gel plates using solvent systems S_1: n-hexane-EtOAc (4:1); S_2: $CHCl_3$-MeOH (95:5) and S_3: $CHCl_3$-MeOH (4:1), the chromatograms were visualized under UV light (at 254 and 366 nm) before and after exposure to ammonia vapour, as well as spraying with anisaldehyde-sulphuric acid spray reagent.

2.3 Extraction and fractionation

Dried aerial parts of *E. schimperi* C. Presl (1.5 kg) were macerated in alcohol (95%) on cold till exhaustion (3 L each time). The combined extracts were evaporated under reduced pressure ($\leq$ 60^{o}C) to give 285 g of green residue. The alcoholic extract (110 g) was fractionated into n-hexane (15 g), chloroform (30 g), ethyl acetate (5.6 g) and n-butanol (10.2 g).

The chloroform fraction (10 g) was chromatographed on a VLC column of silica gel G 60 (7 x 20 cm) using C_6H_{12}-$CHCl_3$ and $CHCl_3$-MeOH mixtures. Fractions 200 ml each were collected and monitored by TLC (see experimental) to give four main fractions, fr-1 (1.76 g), fr-2 (0.5 g), fr-3 (1 g) and fr-4 (2.25 g). Fr-1 eluted between 1600-2600 ml using C_6H_{12}-$CHCl_3$ (3:7) and $CHCl_3$-MeOH (96:4) mixtures was subjected to chromatography on a Sephadex LH-20 column (MeOH) followed by series of silica gel columns using hexane-ethyl acetate mixtures to afford compounds **1** (10 mg), **2** (40 mg), **3** (15 mg) and **4** (20 mg). Fr-2 eluted between 3200 and 3400 ($CHCl_3$-MeOH, 88:12) afforded compound **5** (21 mg) after purification on sephadex LH-20 (MeOH). Fr-3 eluted between 3400 and 3600 ml using $CHCl_3$-MeOH (86:14) was purified by successive columns of sephadex LH-20 using MeOH and MeOH-H_2O mixtures to afford compound **6** (42 mg). Fr-4 eluted between 3600 and 3800 mL ($CHCl_3$-MeOH, 84:16) afforded compound **7** (29 mg) by chromatography on a silica gel column ($CHCl_3$-MeOH, 98:2).

Compound 2: White amorphous powder; mp 148-150 ^{o}C; R_f 0.8 (TLC, S_1); EIMS *m/z* (rel. int.%): 442 $[M]^+$ $C_{30}H_{50}O_2$ (14), 424 $[M- H_2O]^+$ (43), 409 (40), 391(8), 381(23), 355 (7), 315 (18), 303 (16), 173 (36); ^{1}H NMR (400 MHz): δ_H 4.81 (1H, *br s*, H-26$_a$), 4.91 (1H, *br s*, H-26$_b$), 3.99 (1H, *t, J*= 6.8 Hz, H-24), 3.24 (1H, *dd, J*=4.8, 11.6 Hz, H-3), 1.70 (3H, *s*, H-27), 0.94 (2X 3H, *s*, H-18, H-30), , 0.85 (3H, *d, J*= 5.2 Hz, H-21), 0.78 (3H, *s*, H-29), 0.33, 0.55 (2H, *d, J*= 4 Hz, H-19$_{a, b}$) and ^{13}C NMR (100 MHz) see table 1.

Compound 3: White needle crystals ($CHCl_3$); mp 160-162 ^oC; R_f 0.74 (TLC, S_1); EIMS *m/z* (rel. int.%): 442 $[M]^+$ $C_{30}H_{50}O_2$ (8), 424 $[M- H_2O]^+$ (29), 409 (20), 381 (14), 363 (2), 355 (2), 313 (6), 302 (44), 269 (13), 175 (43); ^1H NMR (400 MHz): δ_H 5.57 (2H, *m,* , H-23, H-24), 3.24 (1H, *dd, J*= 4.4, 11.2 Hz, H-3), 1.29 (2 X 3H, *s*, H-26, H-27), 0.94 (2X 3H, *s*, H-18, H-30), 0.86 (3H, *s*, H-28), 0.83 (3H, *d, J*= 6.8 Hz, H-21), 0.78 (3H, *s*, H-29), 0.31, 0.53 (2H, *d, J*= 4 Hz, H-$19_{a, b}$) and ^{13}C NMR (100 MHz) see tables 1.

Compound 4: Colourless needle crystals ($CHCl_3$); mp 223-225^oC; R_f 0.5 (TLC, S_1); UV, λ_{max} nm (MeOH) 229, 250_{sh}, 260_{sh}, 295_{sh} and 342 nm. ^1H-NMR (300 MHz, $CDCl_3$) δ_H 6.26 and 7.58 (2H, *d*, *J*= 9.6 Hz, H-3 and H-4), 6.85 and 6.92 (2H, *s*, H-8 and H-5) and 3.92 (3H, s, Me-6).

Compound 5: Yellow powder; mp 278-280^oC; R_f 0.48 (TLC, S_2); UV λ_{max} nm, MeOH: 268 and 363, MeOH/NaOMe: 279 and 423, MeOH/$AlCl_3$: 269 and 423, MeOH/$AlCl_3$/HCl: 269 and 423, MeOH/ NaOAc: 215 and 378, MeOH/NaOAc/H_3BO_3: 268 and 363; ^1H-NMR (300 MHz, DMSO) δ_H 6.17 and 6.42 (2H, d, J = 2.1 Hz, H-6 and H-8), 6.91 and 8.02 (each 2H, d, J = 8.7, H-3`-5` and H-2`, - 6`).

Compound 6: Yellow powder; mp 328-330 ^oC; R_f 0.45 (TLC, S_2); UV, λ_{max} nm, MeOH: 263 and 350, MeOH/NaOMe: 271 and 404, MeOH/$AlCl_3$: 273 and 421, MeOH/$AlCl_3$/HCl: 265 and 371, MeOH/NaOAc: 273 and 370, MeOH/NaOAc/H_3BO_3: 263 and 371.

Compound 7: White powder; mp 270-272 ^oC; R_f 0.4 (TLC, s_2); ^1H-NMR (300 MHz, DMSO), δ_H 0.64 and 1.02 (each 3H, *s*, H-18 and H-19), 0.78-0.84 (9 H, *m*, H-26, 27 and H-29), 0.89 (3H, *d*, *J*=6.6 Hz, H-21), 0.95 (3H, *m*, H-29), 4.39 (1H, *m*, H-3), 5.38 (1H, broad *s*, H-6) and 4.20 (1H, *d*, *J* = 7.8 Hz, H-1′).

2.4. Cytotoxic activity

The chloroform fraction of the alcoholic extract of *E. schimperi* C. Presl was tested for its cytotoxic activity, *in vitro* using sulphorhodamine B assay [18] against brain (U251) and breast human carcinoma (MCF7) cell lines, National Cancer Institute, Cairo University, Egypt.

3. Results and Discussion

Seven compounds (**1**-**7**) were isolated by chromatographic fractionation of the chloroform fraction of the alcoholic extract of *E. schimperi* C. Presl using series of columns chromatography on silica gel and sephadex LH-20.

Compound **1** was isolated as white powder, and identified as α-amyrin by co-chromatography with a reference substance and by mixed mp [mp. 195-197 ^oC (uncorrected)].

The inspection of ^1H and ^{13}C-NMR spectra of compounds **2** and **3** revealed a tetracyclic cycloartanol triterpenoid skeleton [19-21].

Compound **2** (Figure 1) showed a side chain terminating in an isopropenyl group, which was indicated from the signals of two olefenic protons at δ_H 4.81 and 4.91 (br s, H_2-26) both correlated to carbon signal at δ_C 111.1 (C-26), and from the methyl signal at δ_H 1.70 (s, H_3-27) and δ_C 17.4. A secondary OH group at C-24 was indicated from a downfield allylic proton signal at δ_H 3.99 (*t, J*=6.8 Hz). In HMBC spectrum of **2**, an allylic carbinol carbon at δ_C 76.6 showed long range connectivity with the methyl protons at δ_H 1.70 (H_3-27) and with the exo methylene protons at δ_H 4.81 and 4.91

(H_2-26), confirming the position of OH group at C-24. The identification of compound **2** as cycloart-25-en-3*β*,24-diol was further confirmed from the analysis of ^{1}H-^{1}H COSY and HMQC and HMBC spectra and by comparison to the reported spectral data in literature [19-21].

Compound **3** (Figure 1) showed undistinguished ^{1}H and ^{13}C NMR spectral data to those of **2**, except for the proton and carbon signals of the side chain. The ^{1}H NMR of **3** showed the presence of two olefinic protons appeared as broad multiplet at δ_H 5.57 (2H, H-23 and -24) which is directly correlated to carbon signals at δ_C 125.8 and 139.5, respectively (HMQC). The position of the double bond between C-23 and C24 was confirmed from the long range correlation (HMBC) between proton signal at δ_H 1.29 (H-26 and H-27) and carbon signal at δ_C 139.5 (C-24). In addition, the long range correlation between proton signal at δ_H 0.83 (*d*, *J*=6.8Hz, H-21) and the methylene carbon signal at δ_C 39.2 (C-22) further confirmed the position of double bond. The position of OH group at C-25 was evident from the relation between carbon signal at δ_C 70.9 and the proton signal at δ_H 1.29 (H-26 and -27) in HMBC spectrum. From the aforementioned data and by comparison to the reported data in literature, compound **3** could be identified as cycloart-23-en-3*β*,25-diol [20, 21].

The assignment of C-12 and C-15 for compounds **2** and **3** was found reversed in the literature and was confirmed from the long range correlation between H_3-18 (δ_H 0.94) and C-12 (δ_C 33.0) and between H_3-28 (δ_H 0.86) and C-15 (δ_C 35.8) in the HMBC spectrum of compound **3**. Also, the assignment of the chemical shits of carbon signals of the double bond in compound **3** was reversed by Teresa *et al.*, (1987) [21], and was confirmed from HMBC spectrum (see table 1).

Compound **4** showed strong blue fluorescence under UV light (366 nm) and on exposure to ammonia vapour turned into greenish yellow fluorescence. The UV spectrum of **4** in MeOH showed absorption bands at 229, 250_{sh}, 260_{sh}, 295_{sh} and 342 nm which suggested a 6,7-dioxgenated coumarin skeleton [22]. From the results of ^{1}H-NMR analysis [22] and by co-chromatography with reference substance, compound **4** was identified as scopletin.

Compound **5** was identified as kaempferol from the analysis of its UV spectra in MeOH before and after addition of the different shift reagents [23], and from the analysis of its ^{1}H-NMR spectral data [23] and further confirmed by co-chromatography with reference substance.

Compound **6** was identified as luteolin from the analysis of its UV spectra in MeOH before and after addition of the different shift reagents [23] and further confirmed by co-chromatography with reference substance.

Compound **7** gave a positive Liebermann and Molish tests indicating its steroidal and glycosidal nature. From the analysis of ^{1}H-NMR spectrum and by comparison to reported spectral data in literature [24-26], compound **7** was identified as *β*-sitosterol-*β*-D-O-glucoside, and further confirmed by co-chromatography with reference substance.

The chloroform fraction of the alcoholic extract of *E. schimperi* C. Presl exhibited moderate cytotoxic activity against brain and breast carcinoma cell lines, the surviving cells were decreased by 40% and 50% at concentration 10 µg/mL for brain and breast carcinoma, respectively.

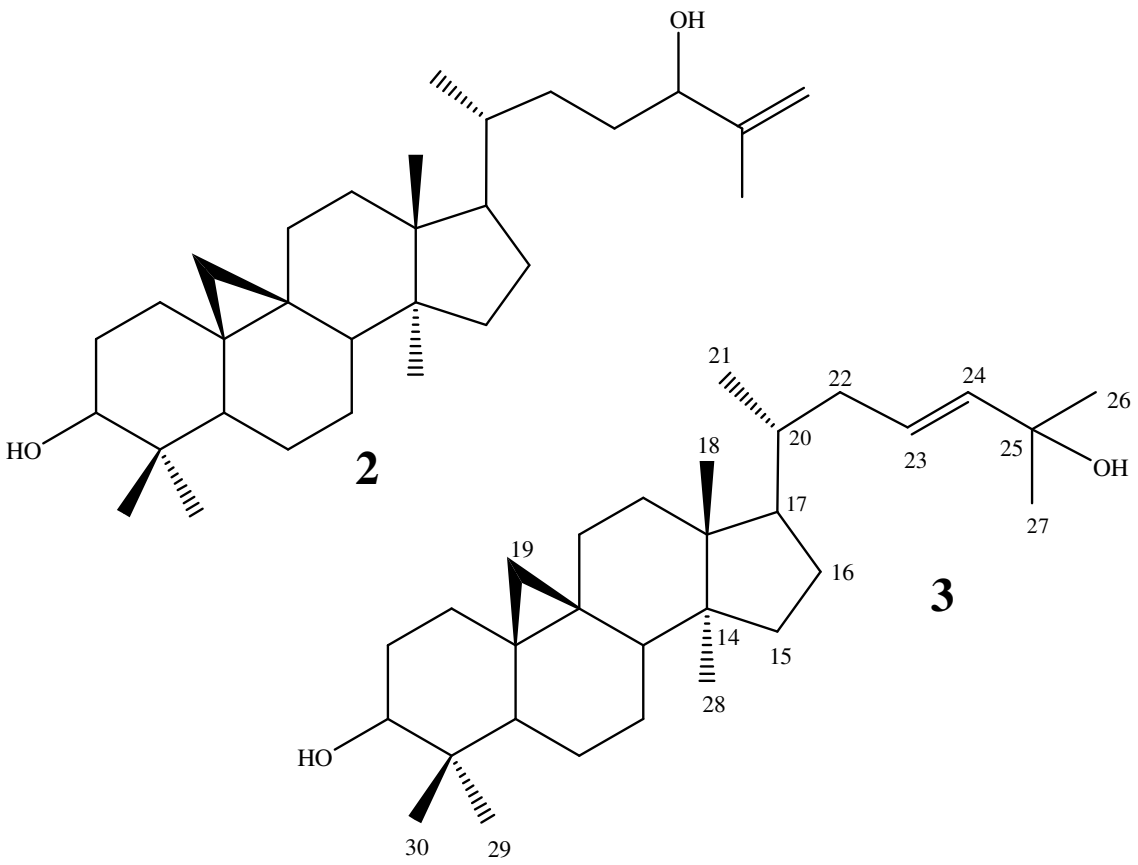

Figure 1. Structure of compounds **2** and **3**

Table 1. ^{13}CNMR spectral data of compounds 2 and 3 in $CDCl_3$ (δ in ppm).

C	2	3	C	2	3
1	32.1	32.1	**16**	26.7	26.3
2	30.5	30.5	**17**	52.4	52.2
3	79.1	79.0	**18**	17.8	18.3
4	40.7	40.7	**19**	29.9	30.3
5	47.3	47.3	**20**	36.1	36.6
6	21.3	21.3	**21**	18.5	18.5
7	28.3	28.2	**22**	31.9	39.2
8	48.2	48.1	**23**	29.1	125.8
9	20.2	20.2	**24**	76.6	139.5
10	26.3	26.3	**25**	147.7	70.9
11	26.2	26.2	**26**	111.1	29.9
12	33.0	33.0	**27**	17.4	30.1
13	45.5	45.5	**28**	19.5	19.5
14	49.1	49.0	**29**	14.2	14.2
15	35.8	35.8	**30**	25.6	25.6

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