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PENTACYCLIC TRITERPENES FROM *FICUS PANDURATA* HANCE. FRUIT

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من ثمار نبات الفيكس باندیوراتا تم فصل ستة مركبات تربينية وثلاثة استيرويدية وقد تم التعرف عليها من خلال دراسة خواصها الطبيعية والطيفية مثل طيف الأشعة تحت الحمراء والرنيين النووي المغناطيسي بنوعيه البروتوني والكربوني ومطياف الكتلة.

From Ficus pandurata Hance fruits, six triterpenes and three sterols were isolated. The structure of these compounds were elucidated using physical and spectral characters including IR, ^{1}H, ^{13}C-NMR including DEPT experiment and MS.

INTRODUCTION

Ficus is a large genus of trees or shrubs cultivated for their ornamental leaves and their edible fruits (*F. sycomorus* L.), while others for providing shade and as ornamental plants[1-6]. Ficus species has several biological activities[7-11]. Many phytoconstituents including triterpenes belonging to different skeleton and sterols were isolated and identified from different species of Ficus[12-25]. Several Ficus species are indigenous to Egypt, others are recently introduced as *Ficus pandurata. Ficus pandurata* (the "Fiddle Leaf Fig") is one of the large leaved *Ficus.* In a previous study a variety of chemical constituents were isolated from the leaves and stem bark of *Ficus pandurata*[26]. In the present study triterpenes and sterols of the fruit were isolated and identified using different methods of analysis.

EXPERIMENTAL

Instruments and apparatus

1- Melting points (uncorrected) were determin-ed by electrothermal model 550.
2- Schimadzu infra red-470 spectrophotometer (Japan) was used for measuring IR spectra as KBr discs.
3- ^{1}H-NMR and ^{13}C-NMR were recorded on JEOL-TNM-LA 500 spectrometers using TMS as an internal standard.
4- MS spectra were measured on SX102A.
5- Column chromatography was performed with silica gel 60 (E-Merck).
6- Precoated sheets of silica gel 60 G_{254} (E-Merck) and RP-C_{18} F_{254} were used for TLC purposes.
7- The spots were visualized by UV lamp (254, 366 nm, VL, LC, Marinc Lavalec-Codex, France) and sprayed with 10% H_2SO_4.
8- Authentic samples were obtained from Department of Pharmacognosy, Faculty of Pharmacy, Assiut University.
9- Solvent systems used are:
I-*n*-Hexane-EtOAc (9:1).
II-$CHCl_3$-MeOH (9.5:0.5)
III- $CHCl_3$-MeOH (9:1)

Plant material

Fruits of *Ficus pandurata* was collected in April-June 2008 from the front of Faculty of Pharmacy, Assiut University, Assiut.

The plant was kindly identified and authenticated by Prof. Dr. Salah EL-Naggar (Professor of Botany, Faculty of Science, Assiut University).

Extraction and isolation

The air dried powdered fruits (3 kg) of *Ficus pandurata* were exhaustively extracted with MeOH to yield 70 g of concentrated methanolic extract. Part of the concentrated combined methanolic extract (60 g) was successively fractionated with *n*-hexane, chloroform and ethyl acetate.

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The *n*-hexane soluble fraction (25 g) was chromatographed over silica gel C C using *n*-hexane-EtOAc gradiently (fractions 50 ml, each, were collected). Fractions eluted with *n*-hexane-EtOAc (95: 5) afforded compound **1**, compound **2** and compound **3**. Fractions eluted with *n*-hexane-EtOAc (9: 1) were purified by rechromatography over silica gel CC using $CHCl_3$-MeOH gradiently. Subfractions eluted with $CHCl_3$-MeOH (95: 5) gave compounds **4**, **5** and **6**. Fractions eluted with *n*-hexane-EtOAc (85: 15) were purified by rechromatography over silica gel column using $CHCl_3$-MeOH gradients where sub-fractions eluted with $CHCl_3$-MeOH (9: 1) gave compounds **7** and **8** respectively. The $CHCl_3$ soluble fraction (15 g) was chromatographed over a silica gel column using gradient $CHCl_3$-MeOH (fractions 50 ml, each, were collected). Fractions eluted with $CHCl_3$-MeOH (9: 1) afforded compound **9**, which was identified as β-sitosterol glucoside.

Compound 1: White amorphous powder, (15 mg), IR ν cm^{-1} (KBr): 1670 and 1720. EI-MS (m/z): 482. ^{1}H-NMR ($CDCl_3$, 500 MHz), δ: 0.78 (3H, s, CH_3-25), 0.85 (3H, s, CH_3-24), 0.89 (3H, s, CH_3-28), 1.14 (3H, d, J= 7.0 Hz, CH_3-29), 1.22 (3H, s, CH_3-23), 1.39 (6H, s, CH_3-26, CH_3-27), 1.58 (3H, s, CH_3-30), 2.03 (3H, s, CH_3-32), 4.5 (1H, dd, J= 4.5, 12.0 Hz, H-3), 5.01 (1H, s, H-21). ^{13}C-NMR ($CDCl_3$, 125 MHz) spectral data are listed in Table (1).

Compound 2 (α-amyrin): White needles (ethanol) (10 mg), m.p. 202-204°C, IR ν cm^{-1} (KBr): 3455, 2945, 1375 and 1040.

Compound 3 (β-amyrin): White needles (acetone), (25 mg), m.p. 185-187°C, IR ν cm^{-1} (KBr): 3455, 2945, 1380 and 1037.

Compound 4 (β-sitosterol): White needles (methanol), (15 mg), m.p. 134-136°C.

Compound 5 (stigmasterol): Obtained as white crystalline needles (acetone), m.p. 168-170°C. IR ν_{max} (KBr) cm^{-1}: 3420 (OH), 2965 (C-H) and 1636 (C=C).

Compound 6: White amorphous powder (10 mg), IR ν cm^{-1} (KBr): 3430, 1670. EI-MS (m/z): 438, 428, 230, 208. ^{1}H-NMR ($CDCl_3$, 500 MHz), δ: 0.79 (3H, s, CH_3-25), 0.81 (3H, s, CH_3-24), 0.89 (3H, s, CH_3-28), 0.97 (3H, d, J= 7.0 Hz, CH_3-29), 1.22 (3H, s, CH_3-23), 1.39 (6H, s, CH_3-26, CH_3-27), 1.49 (3H, s, CH_3-30), 3.5 (1H, dd, 4.5, 12.0, H-3), 5.01 (1H, s, H-21), 5.11 (1H, m, H-12). ^{13}C-NMR ($CDCl_3$, 125 MHz) spectral data are listed in Table (1).

Compound 7: White amorphous powder, (15 mg), IR ν cm^{-1} (KBr): 3435 and 1720. EI-MS (m/z): 484, 440 (2.2), 425 (3.6), 266 (4), 218 (7.9), 206 (4.2), 203 (9) and 133 (13.6). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ: 0.77 (3H, s, CH_3-25), 0.86 (3H, s, CH_3-24), 0.94 (3H, s, CH_3-28), 0.97 (3H, s, CH_3-29), 1.00 (3H, s, CH_3-23), 1.03 (6H, s, CH_3-26, CH_3-27), 1.23 (3H, s, CH_3-30), 2.07 (3H, s, CH_3-32), 4.33, (1H, d, 14.0, H-3), 5.01 (1H, m, H-2), 5.14 (1H, m, H-12). ^{13}C-NMR ($CDCl_3$, 125 MHz) spectral data are listed in Table (1).

Compound 8: White amorphous powder, (10 mg), IR ν cm^{-1} (KBr): 3430. EI-MS (m/z): 442, 427, 424 and 207 ^{1}H-NMR ($CDCl_3$, 500 MHz), δ: 0.79 (3H, s, CH_3-30), 0.85 (3H, s, CH_3-24), 0.90 (3H, s, CH_3-28), 0.97 (3H, CH_3-29), 1.00 (3H, s, CH_3-26), 1.03 (6H, s, CH_3-23), 1.16 (3H, s, CH_3-25), 1.23 (3H, s, CH_3-27), 3.28 (1H, dd, 4.0, 14.0, H-3), 3.55 (1H, m, H-22).

Table 1: ^{13}C-NMR data of compounds **1,6-8** ($CDCl_3$, 125 MHz).

Carbon no.	Chemical shift			
	Compound (**1**)	Compound (**6**)	Compound (**7**)	Compound (**8**)
1	39.85	38.7	40.6	38.6
2	24.14	27.2	64.39	27.4
3	82.0	78.3	80.59	77.7
4	36.77	38.7	38.70	38.9
5	59.0	55.2	58.99	54.9
6	18.55	18.3	18.24	18.3
7	33.67	32.9	32.60	33.2
8	41.0	40.0	40.91	42.7
9	51.3	47.7	47.65	48.4
10	36.76	36.9	36.60	36.9
11	22.34	23.3	23.92	23.8
12	27.55	121.7	121.22	28.7
13	37.81	140.6	144.00	132.8
14	43.4	42.0	42.07	43.8
15	27.2	28.7	29.29	25.1
16	28.34	26.6	26.27	33.0
17	54.1	55.9	32.26	40.2
18	45.2	45.1	47.65	133.5
19	36.82	35.6	45.0	37.9
20	167.7	165.3	31.25	32.2
21	125.0	125.9	34.55	43.5
22	199.70	202.5	37.75	78.8
23	28.2	28.5	28.09	28.1
24	16.50	16.4	15.72	15.6
25	17.66	15.1	14.80	16.3
26	16.76	17.0	16.86	18.8
27	15.52	15.5	26.60	20.4
28	17.55	17.55	28.66	16.7
29	21.43	21.30	34.42	25.1
30	20.60	20.50	23.7	32.2
31	171.0	-	174.00	-
32	21.42	-	22.69	-

RESULTS AND DISCUSSION

Phytochemical study of *Ficus pandurata* fruits resulted in the isolation and identification of nine compounds.

Compounds **2**, **3**, **4,** **5** and **9** were identified as α-amyrin, β-amyrin, β-sitosterol, stigmasterol and β-sitosterol-3-*O*-β-glucoside respectively by comparing their physical and chromatographic characters with authentic samples.

Compound **1** was obtained as amorphous powder, which gave a positive Liebermann-Burchard's test for triterpenes. Its IR displayed absorption bands attributed to conjugated ketone group (1670 cm^{-1}) and acetoxyl group (1720 cm^{-1}). The EI-MS spectrum of compound **1** showed [M^+] at m/z 482. The molecular formula was deduced to be $C_{32}H_{50}O_3$ from the MS, ^{1}H-, ^{13}C-NMR including DEPT experiments.

The ^{1}H-NMR gave signals due to eight methyl groups, one is doublet and seven are singlets at (δ_H 0.78, 0.85, 0.89, 1.14, 1.22, 1.39 (2 Me) and 1.58), in addition to an acetyl group. In the low filed region, an olefinic proton (δ_H 5.01) vicinal to carbonyl group and a proton adjacent to acetyl group (δ_H 4.5) were observed. These data are similar to a taraxstane triterpene previously isolated from the same plant[26]. The ^{13}C-NMR spectrum of compound **1** had thirty two carbon signals including two olefinic signals (δ_C 167.7 and 125.0) as singlet and doublet respectively, one oxygenated carbon at δ_C 82.0 and one conjugated carbonyl carbon at δ_C 199.7 (C-22). Comparing the ^{13}C-NMR data of compound **1** with those reported for 3β-acetoxy-20-taraxasten-22-one**,** they seemed to be identical. So compound **1** was identified as 3β-acetoxy-20-taraxasten-22-one and it was isolated previously from *Ficus pandurata* Hance leaves and stem bark [26].

Compound **6** gave a positive Liebermann-Burchard's test for triterpenes, it showed hydroxyl group (3430 cm^{-1}) and conjugated ketone group (1670 cm^{-1}) in the IR spectrum. The molecular formula was deduced to be $C_{30}H_{46}O_2$ from the MS, ^{1}H-, ^{13}C-NMR including DEPT experiments.

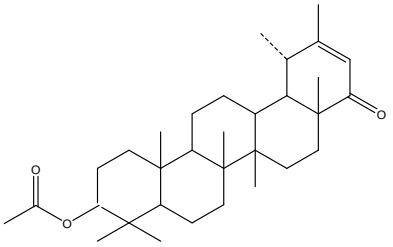
The ^{1}H-NMR showed seven singlet methyl groups at δ_H 0.79, 0.81, 0.89, 1.22, 1.39, 1.49 and 1.60 and one doublet methyl group at δ_H 0.97 (d), one oxygenated methine proton at δ_H 3.5 (1H, dd, J= 4.5, 12 Hz), in addition to two olefinic protons, one vicinal to a carbonyl group δ_H 5.01 (1H, s, H-21) and the second at δ_H 5.11 (1H, m, H-12). When compared with compound **1**, it was suggested to be a taraxstane triterpene. The two signals appeared in the spectrum of compound **1** at δ_C 171.0 and 21.42 for the acetyl group were disappeared in the spectrum of compound **6**, also the signals for C-2 and C-4 in compound **6** were downfield shifted by 3.06 and 1.93 ppm respectively, while the signal for C-3 (in compound **6**) was upfield shifted by 3.7 ppm confirming the absence of the acetate group from C-3 position of compound **6**. The IR spectrum also showed the disappearance of the acetoxyl group (at 1720 cm^{-1}). In the other hand another olefinic proton was appeared in compound **6** at δ_H 5.11 (1H, m, H-12) by comparing with compound **1** and other similar triterpenes, indicating the presence of a second double bond in this compound. The location of the second double bond must behave one doublet carbon (δ_C121.7) and another singlet one (δ_C140.0). The EI-MS of compound **6** exhibited peaks due to the characteristic fragmentation at m/z 230 (D/E) and 208 (A/B ring) by retro-Diels-Alder fission supported this structure clearly, the fragment at m/z 230 indicating a C-12/C-13 double bond (Fig. 2). The peak at m/z 420 is corresponding to the loss of water from the molecule, indicating the presence of OH group in the structure. The β configuration of the OH group was assigned according to the fact that the proton appeared as *dd*, where the large coupling constant (J=12.0) established an axial configuration of this proton and β OH group[27]. Therefore compound 6 was identified as 3β-hydroxy-12, 20-taraxastdiene-22-one, and this is the first report for its isolation from genus Ficus.

Compound **7** was obtained as amorphous powder. The IR spectrum showed the presence of hydroxyl and acetoxyl groups absorption bands at 3435 and1720 cm^{-1}. The molecular formula of compound **7** was deduced to be $C_{32}H_{52}O_3$ from the MS, ^{1}H-, ^{13}C-NMR and DEPT experiment. The ^{1}H-NMR spectrum showed the presence of eight singlet methyls, one olefinic proton 5.14 (1H, m, H-12), two oxymethine protons δ_H 4.33 (1 H, d, J= 14.0, H-3) and 5.01 (1H, m, H-2) and one acetyl group 2.07 (3H, s, CH_3-32). The ^{13}C-NMR spectrum revealed the existence of 32 carbons ascribable to 9 methylenes, 9 methyls, 6 methines and 8 quaternary carbons by DEPT spectrum. Both the ^{1}H- and ^{13}C-NMR depicted data typical of a triterpene of oleanane or ursane skeleton with a double bond between C-12/C-13 and one acetyl group[28]. This was further supported by the characteristic retro Diels-Alder cleavage of C-12 double bond pentacyclic triterpene skeleton leading to m/z 218 ($C_{16}H_{26}$, 7.9) and 266 (C_{16} H_{26} O_3, 4), thus indicating that the hydroxyl group and the acetyl group were present in the rings A/B of the molecule[29] (Fig. 2). The olean-12-ene was inferred from the chemical shifts of C-12 (δ_C 121.2) and C-13 (δ_C 144.0)[30]. The signal at δ_H 4.33was assigned for the H-3 proton where the large coupling constant (J= 14.0) established an axial configuration of this proton and β acetyl group. Comparing the chemical shifts of each

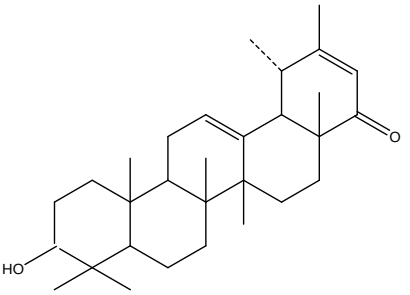
carbon with those reported for β-amyrin, it was suggested that the acetyl group was located at C-3 and the hydroxyl group at C-2. So, compound **7** was identified as 2 β– hydroxyl-3 β–acetoxy β-amyrin, and this is the first report for its isolation from genus Ficus.

Compound **8** was obtained as amorphous powder. The IR spectrum showed the presence of hydroxyl group (3430 cm^{-1}). The molecular formula of compound **8** was deduced to be $C_{30}H_{50}O_2$ from the MS, ^{1}H-, ^{13}C-NMR and DEPT experiment. The MS spectrum showed [M^+] at m/z 442. The ^{1}H –NMR spectrum showed the presence of 8 singlet tertiary methyl signals δ_H 0.79, 0.85, 0.90, 0.97, 1.00, 1.03, 1.16, 1.23, the signal at δ_H 3.28 was assigned for the H-3 proton where the large coupling constant (J=14.0 Hz) clearly established an axial (α configuration of this proton and β-hydroxyl group. The ^{13}C-NMR spectrum showed 30 carbon signals i.e. 8 singlets, 4 doublets, 10 triplets and 8 quartets. The signals at δ_C 77.7 and δ_C 78.8 were assigned for two oxygen bearing carbons, C-3 and C-22 respectively, this was confirmed by the presence of fragment at m/z 207 in the EIMS spectrum, which indicates that one hydroxyl group was present at rings A/B of the molecule and the other at rings D/E. The peak at m/z 424 is corresponding to the loss of water from the molecule, indicating the presence of OH group (s) in the structure. The two olefinic signals at δ_C 132.8 and δ_C 133.3 as singlets supported the location of the double bond at C-13 and C-18 respectively[31]. The assignments of each carbon signal are cited in Table (1). Comparing the chemical shifts of each carbon with those reported for abrisopagenol G[31], compound **8** was identified as 3, 22 β-dihydroxy-13 (18) oleanene.

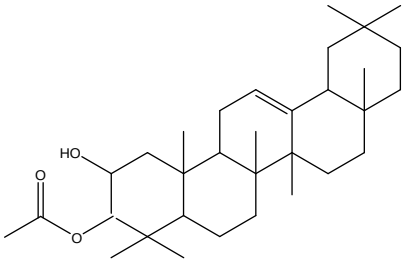
According to the available current literatures compounds **6** and **7** are new compounds.



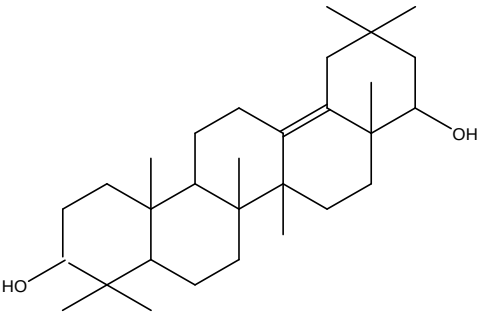
Compound 1



Compound 6

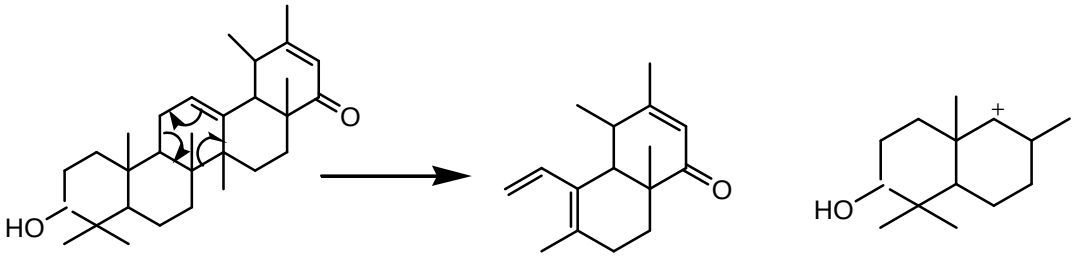


Compound 7

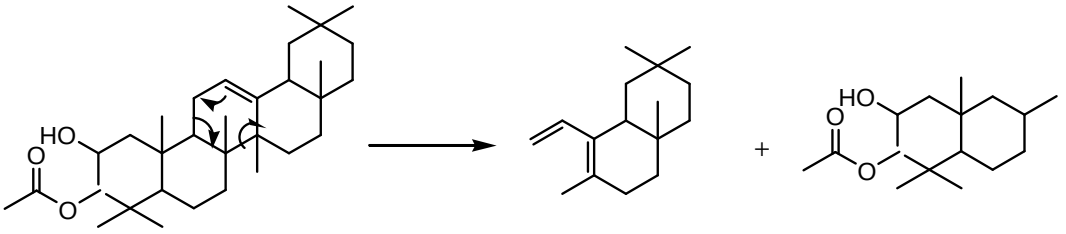


Compound 8

Fig. 1: Compounds isolated from *Ficus pandurata* Hance.



Compound 6



Compound 7

Fig. 2: Some important MS fragments of compound **6** and compound **7**.

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