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A NEW NOR OLEANANE TRITERPENE FROM *VITIS VINIFERA*

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نبات العنب هو من أحد النباتات الهامة فى الطب الشعبى ، فبلأضافة إلى قيمته الغذائيـة العاليـة فإن أوراق النبات وثماره المجففة لهـا تـأثير ملين كمـا أن أوراق النبـات لهـا تـأثير نـاجع فـى عـلاج الالتهابات الكبدية والدوالى. وفى دراسة لقلف النبات تم فصل مركب جديد من نوع التربينات الثلاثية وتـم تسميته علـى أنـه ٣،٢٨-ثنـائى هيدروكسـى ، ١٩-كيتـو ، ٢٧-نـور أوليانـان بالإضافـة إلـى المركبات المعروفة وهى بيتا-سيتوستيرول ، بتيولين وحمض البتيولينيك.

هذا وقد تم التعرف على هذه المركبات عن طريق دراسة خواصها الطبيعية والطيفية المختلفة ومقارنتها بالمنشور سابقا.

A new nor-oleanane having the structure 3β,28β-dihydroxy, 19-oxo, 27-nor oleanane has been isolated from the stem bark of Vitis vinifera L variety thmpson seedless, beside the known compounds, β-sitostreol, betulin and betulinic acid.

The identification of these compounds was based on different methods of physical and spectral analysis.

INTRODUCTION

Different varieties of *Vitis vinifera* L. (Vitaceae) are cultivated in Egypt. The leaves of the plant is used in folkmedicine as astringent, anti-inflammatory, antihepatotoxic and in treatment of varicose and capillary fragility.[1a,b] Grapes are nourishing and mild laxative while the dried fruits is a mild laxative, stomachic, diuretic and demulcent.[2]

Different classes of compounds including flavonoids, biflavonoids, carotenes, tannins, sugars, organic acids, pectins, anthocyanins, vitamins, stilbene glucosides, megastigmanes, oligostilbenes and hemiterpene glycosides have been reported from the leaves and fruits of the plant and plant wine.[1,3-8]

However nothing could be traced concerning the constituents of the bark of the plant and this work describes a new nor-oleanane type triterpene besides two known lupane type triterpenes of first report in Vitaceae from this plant organ.

EXPERIMENTAL

General

MPS: on Stuart scientific (SMPI) melting point (England), EI-MS on Joel JMS 600 at 70 ev. ^{1}H and ^{13}C-NMR at 400 and 100 MHz respectively, IR on Schimadzu infrared 470 spectrophotometer, TLC with precoated silica gel sheets (Aluminium foil, E. Merck).Column chromatography with SiO_2 (70-230 mesh, E. Merck).

The following solvent systems were used: (A) hexane-EtOAc (95:5), (B) hexane-EtOAc (80:20), (C) $CHCl_3$-MeOH (98:2).

Plant material

Stem bark of *Vitis vinifera* L. variety Thmpson seedless was collected from farms of Assiut province in November 1998. The plant was kindly identified by Prof. Dr. K. I. Ahmed, Faculty of Agriculture, Department of Horticulture, Assiut University, Assiut, Egypt.

Extraction and isolation

500 g of dried and powdered stem bark of

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Vitis vinifera L. variety Thmpson seedless were extracted in a soxhlet apparatus with n-hexane till exhaustion to afford about 10 g of brownish residue. The hexane extract was chromatographed on SiO_2 CC (500 g SiO_2 in 3 x 100 cm column) using n-hexane-ethyl acetate gradient. Elution with hexane-ethyl acetate (97:3) afforded compound **1** (R_f value 0.43, system A) while further elution with 85:15 afforded compound **2** (R_f value 0.73, system B), elution with (80:20) afforded compound **3** (R_f value 0.78, system C) and finally compound **4** (R_f value 0.56, system C) was eluted with (70:30).

Compound 1: 200 mg, fine needles, m.p 134-136° (ether) not depressed by authentic sample of ß-sitosterol. Co-chromatography and IR comparison with authentic sample of ß-sitosterol showed their identity.

Compound 2: 400 mg, white powder, m.p 215-217° (MeOH), IR (ν_{max}, KBr, cm^{-1}): 3445, 2936, 1706, 1473, 1388, 1363, 1255, 1035, EI-MS, m/z (Rel. int.%.): 444 (M^+, 98), 426 (M-H_2O, 72.1), 413 (M-CH_2OH, 26.1), 395 (M-H_2O-CH_2OH, 56.3), 237 (ion y, Scheme 1, 4.2), 235 (ion y-2H, 8.3), 207 (ion x, Scheme 1, 69.4), 206 (ion y-CH_2OH, 17.1), 189 (ion x-H_2O, 87.6). ^{1}H-NMR ($CDCl_3$, 400 MHz); δ ppm: 0.76 (3H, s), 0.82 (3H, s), 0.96 (3H, s), 1.00 (9H, s), 2.16 (1H, d, J= 2.1 Hz, H-18), 3.18 (1H, dd, J= 14.8 and 6.4 Hz), 3.23 (1H, d, J= 11.0 Hz), 3.78 (1H, d, J= 11.0 Hz). ^{13}C-NMR (see Table).

Compound 3: (Betulin), 625 mg, fine needles, m.p 245-248° (MeOH) EI-MS m/z (Rel. int.%) 442 (M^+, 70.4), 427 (M^+-Me, 17.3), 424 (M^+-H_2O, 22.1), 411 (M-CH_2OH, and/or M-isopropenyl, 91), 393 (M^+-H_2O-CH_2OH, 17.3), 234 (45.5, Scheme 2), 220 (29.6, Scheme 2), 207 (84, Scheme 2), 203 (75.1, Scheme 2), 189 (100% Scheme 2). IR (ν_{max}, KBr, cm^{-1}) 3420 (O-H Stretching), 3020, 3000, 2890 (C-H Stretching), 1640 (C=C Stretching).

^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm: 0.76 (3H, s), 0.82 (3H, s), 0.96 (3H, s), 0.98 (3H, s), 1.12 (3H, s), 1.68 (3H, s), 3.19 (1H, d, J= 10.2, 5.7 Hz, H3), 3.33 (1H, d, J= 10.5 Hz, H28a) 3.80 (1H, d, J= 10.5 Hz, H28b), 4.58 (1H, d, J= 2 Hz, H30a), 4.68 (1H, d, J= 2 Hz, H30b). ^{13}C-NMR see Table.

Compound 4: Betulinic acid, 10 mg, fine needles, m.p 276-277° (MeOH). IR ν_{max}^{KBr} cm^{-1}: 3460 (O-H stretching) 3010, 2920, 2890 (C-H stretching), 1685 (C=O stretching), 1640 (C=C stretching). EI-MS m/z (Rel. int.%) 456 (M^+, 15.2), 438 (M^+-H_2O, 7), 425 (M^+-isopropenyl), 411 (M-COOH, 3), 248 (23.4, Scheme 2), 220 (18.0, Scheme 2), 207 (52.7, Scheme 2), 189 (81.8, Scheme 2).

RESULTS AND DESCUSSION

The molecular formula of **2** was deduced as $C_{29}H_{52}O_3$ (MS and ^{13}C-NMR with DEPT mode measurments).

The IR spectrum of **2** revealed hydroxyl function(s) centered at 3445 cm^{-1} and a ketonic carbonyl at 1706 cm^{-1}.

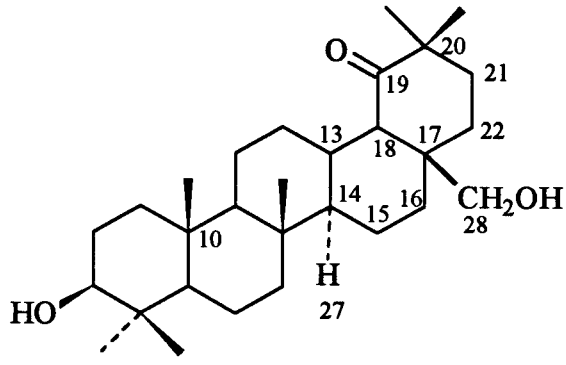
The MS spectrum showed a molecular ion peak at m/z 444 [M^+] and further significant fragments at m/z 207 (Scheme 1) for the lower part of the nor-triterpene skeleton having one hydroxyl group and at m/z 237 for the upper part.[9] The ^{1}H-NMR displayed six sharp methyl singlets at δ 0.76-1.0, 2.16 (1H, d, J= 2.1 Hz, H18), an AB quartet at δ 3.23 and 3.78 (each 1H, d, J= 11.6 Hz) assigned for a CH_2OH function and an axial methine proton geminal to a hydroxyl group at δ 3.18 (1H, dd, J= 14.8, 6.4 Hz, H-3α).

The ^{13}C-NMR with DEPT mode measurments showed 29 carbon signals including six CH_3, eleven CH_2 comprising an oxygenated one at δ_c 60.7, six CH signals, one of them is oxygenated (δ_c at 78.9) and six quaternary carbon signals, one of which is a carbonyl function at δ_c 212. All of these data are suggestive of a nor-oleanane skeleton of **2**.

The ^{13}C-NMR showed absence of the most downfield shifted methelene group of C-19 of oleanane skeleton at δ_c 46 suggesting the presence of the carbonyl function at this

Table: ^{13}C-NMR data of Compound **2** and **3** ($CDCl_3$).

Carbon No.	2	3
1	38.6	38.7
2	27.3	27.4
3	78.9	79.1
4	38.0	38.8
5	55.2	55.3
6	18.2	18.3
7	33.9	34.3
8	38.8	41.0
9	49.6	50.5
10	40.8	37.3
11	20.8	20.9
12	27.6	25.2
13	36.1	37.2
14	50.2	42.8
15	26.9	27.1
16	27.2	29.2
17	47.8	47.9
18	52.0	48.8
19	212.2	47.9
20	42.5	150.5
21	28.8	29.8
22	34.1	34.0
23	28	28.1
24	14.7	15.4
25	15.4	16.1
26	15.9	16.1
27	---	14.8
28	60.7	60.6
29	29.4	109.7
30	16.0	19.1



m/z 444 (Rel. Int.% 98)

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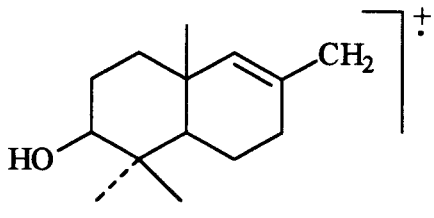
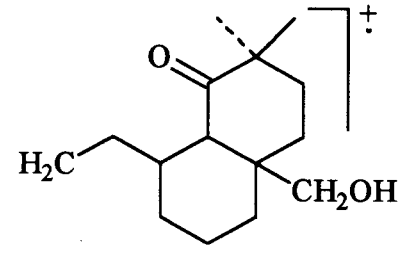


Ion x
m/z 207 (Rel. Int.% 69.4)

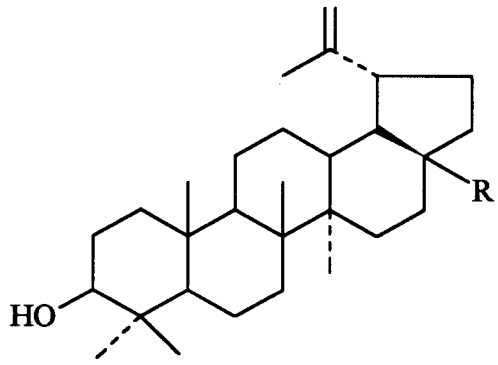


Ion y
m/z 237 (Rel. Int.% 4.2)

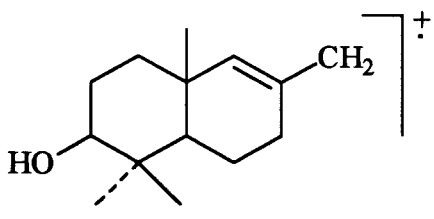
-2H

m/z 235 (Rel. Int.% 8.2)

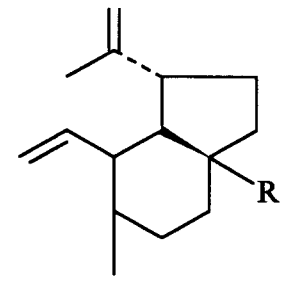
Scheme 1: MS fragmentation of **2**



m/z 456 (R= COOH, Compound **4**) betulinic acid (Rel. Int.% 15.2)
442 (R= CH_2OH, Compound **3**) betulin (Rel. Int.% 70.4)

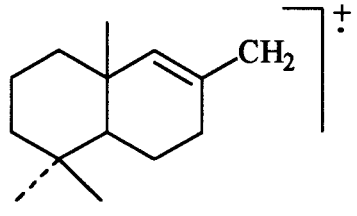


m/z 207 **4** (Rel. Int.% 52.7)
3 (Rel. Int.% 84)



m/z 248 (R= COOH) (Rel. Int.% 23.4)
m/z 234 (R= CH_2OH) (Rel. Int.% 45.5)

$-H_2O$



m/z 189 **4** (Rel. Int.% 100)
3 (Rel. Int.% 81.8)

Scheme 2: MS fragmentation of **3** and **4**

position. This was supported by the downfield shift of C-18 and C-20 (ca 10 ppm) in comparison with similar skeletons.[10]

The CH_2OH function was located on C17 and C-28) based on comparison of C-17 resonance with compounds having the same function[10] and also to absence of C-28 methyl resonance from ^{13}C-NMR spectra of **2**. Also comparison of the ^{13}C-NMR spectra of **2** with a series of oleanane skeletons[10] showed absence of C-27 methyl resonance and instead, a CH signal at δ 50.2 has appeared. These data are clearly showing that **2** has the structure 3ß,28ß-dihydroxy 19-oxo, 27-demethyl oleanane.

The above conclusion was confirmed by 2D-NMR measurment (CH-COSY and COLOC) where the COLOC spectrum displayed significant correlation peaks between H-18 and both C-19 and C-13.

Also one of the protons of the CH_2OH function at δ 3.78 displayed significant correlation peaks with C-21, C-22 and C-16.

Compound **1** was identified as the common ß-sitosterol (m.p, IR and co-chromatography).

Compound **3** was identified as a lupene derivative from its significant MS data (M-isopropenyl), ^{1}H-NMR (two olefenic protons of the exomethylene group of exomethelene function at δ 4.58 and δ 4.68; see exp.) and from the ^{13}C-NMR significant signals of exomethelene function at δ 109.8 and δ 150.5. Finally the compound was identified as betulin from its spectral data (IR, MS, ^{1}H and ^{13}C-NMR) and by comparison with literature.[11,12]

Compound **4** was identified as betulinic acid (m.p, IR, MS, and co-chromatography).

It is worthy to mention that betulin and betulinic acid are first reported in *Vitaceae*. Betulin which is the major compound in the bark of the plant is the precursor for semisynthesis of betulinic acid which is a specific, selective, cheap and safe anticancer agent. The specificity of betulinic acid for melanoma cells is unique in comparison to other chemotherapeutic agents[13] and this studreflects the importance of the bark of the plant as a rich source of betulin.

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