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STEROIDAL SAPONINS GLYCOSIDES FROM FRUITS OF *SOLANUM NODIFLORUM* JACQ.

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ABSTRACT

From the methanolic extract of the fresh berries of *Solanum nodiflorum* Jacq. (Solanaceae), Four known steroidal saponins were reported and identified as: (20S)-3β, 16β-dihydroxy preg-5-ene-22-carboxylic acid-(22,16)-lactone-3-β-chacotrioside (Dumoside **1**) and 3β- dihydroxy pregn-5,16-dien-20-one 3-*O*-β-chacotrioside; (Aethienolone 4), 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl(25*R*,26*R*)-spirost-5-ene-3β, 17α, 26-triol 2 and β-chacotriosyl-(22,26*R*)-furost-5-ene-3β,17α,22,26-tetraol-26-*O*-β-D-glucopyranoside **3**. Their structures were identified by extensive ^{1}H-NMR, ^{13}C-NMR and DEPT-^{13}C-NMR data as well as comparison with reported spectroscopic data.

INTRODUCTION

Solanum, the nightshades, horse nettles and relatives, are a large and diverse genus of annual and perennial plants. They grow as forbs, vines, subshrubs, shrubs, and small trees, and often have attractive fruit and flowers. The genus nowadays contains roughly 1,500-2,000 species. The species usually most commonly called nightshade in North America and England and woody nightshade. Its foliage and egg-shaped berries are poisonous, the active principle being solanine, which can cause convulsions and death if taken in large doses [(1)].

Solanum nodiflorum Jacq, the American nightshade, is a herbaceous flowering plant native to south and west of the United States, south to Paraguay and Peru; it also occurs in Hawaii, where it is considered possibly indigenous or may be a Polynesian introduction[(2)]. The fruit is a shiny black berry 5-10 mm diameter, containing numerous small seeds; when mature they should be considered poisonous as they may contain high levels of the glycoalkaloid solanine, which considered as toxic principle; it breaks down into an alkamine (solanidine), and a sugar (solanose) [(2)]. The Present study deals with identification of the saponin content of *Solanum nodiflorum* fruits as a part of our ongoing studies on Solanaceous plants.

EXPERIMENTAL

NMR spectra (^{1}H, ^{13}C, DEPT) were measured in pyridine-d_5 and recorded at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR, using TMS as internal standard on a JEOL □-500 spectrometer (Japan). Column chromatography was carried out on silica gel 60 (70-230, 230-400 mesh, Merck), HP-20 (75-150 mm, Mitsubishi Chem. Ind.), RP-18 (30-50 □m, Merck). TLC was carried on precoated silica gel plates G60 F_{254} (0.2 mm Aluminium sheets, Merck) and RP-18 F_{254S} (0.2 mm Aluminium sheets, Merck). Plates were visualized by spraying with 20 % *v/v* H_2SO_4 in EtOH, allowed to dry at room temperature and heated at 110-140 °C for 1-2 min. All solvents were double distilled prior using.

TLC solvent system

$CHCl_3$- MeOH (98:2, 95:5, 90:10, 85:15, 80:20)

$CHCl_3$- MeOH: H_2O (9:1:0.1, 9:1:0.2, 8:2:0.1, 8:2:0.2, 7:3:0.1, 7:3:0.2, 7:3:0.5, 65:35:0.1).

MeOH-H_2O (1:1, 4:6, 6:4, 7:3)

Plant Material

Fresh berries of *Solanum nodiflorum* Jacq., were collected from the Botanical garden of Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Kumamoto University, Kumamto, Japan. The plant was authenticated by Dr. Tatemi Yoshida (National Research Institute of Vegetables, Ornamental Plants and Tea, Ministry of Agriculture, Forestry and Fisheries, Ano, Mie, Japan. A voucher specimen was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt.

Extraction and fractionation

The fresh fruits (4.7 Kg) of *Solanum nodiflorum* were cut into small pieces and exhaustly extracted twice with hot methanol (5 L, each time). The methanolic extract was concentrated under reduced pressure to a syrupy consistency. The solvent free residue (103.5 g), which labeled SNF, was dissolved in distilled H_2O and fractionated over to Diaion HP-20P column using distilled water followed by MeOH; fractionation was monitored by using TLC with system $CHCl_3$:MeOH:H_2O (7:3:0.5); similar fractions were collected together to give one fraction (22.17 g) which was subjected to further successive fractionation . The dried methanol fraction after HP-20 column chromatography (17 g) was dissolved in the least amount of $CHCl_3$:MeOH:H_2O (9:1:0.1) and chromatographed over silica gel C.C. Elution was carried out using $CHCl_3$-MeOH:H_2O:NH_4OH mixtures in a gradient elution technique, from (9:1:0.1:0.1) to (6:4:1:0.1). Elutes were collected in 10 ml fractions. The fractions were concentrated under reduced pressure and monitored on precoated silica gel plates using solvent systems $CHCl_3$:MeOH:H_2O (9:1:0.1, 8:2:0.1, 8:2:0.2, 7:3:0.2 and 7:3:0.5). The chromatograms were sprayed with 20% sulphuric acid. Fractions of similar R_f values were collected together and concentrated. Seven groups of subfractions were obtained labeled SNF-1 to SNF-5. The group labeled SNF-3 (1.9 g), was chromatographed on RP-18 C.C. using MeOH-H_2O mixtures 30% to MeOH. Nine subfractions were

obtained labeled SNF-3-1 to SNF-3-9. The subfraction SNF-3-5 (200 mg) was subjected to further RP-18 C.C. using MeOH-H_2O mixtures 60% to MeOH to give to give compound **1**(9 mg), which was crystallized from methanol. The Fraction labeled SNF-3-8 (100 mg) was subjected to silica gel C.C. using $CHCl_3$:MeOH:H_2O (9:1:0.1, 8:2:0.1, 8:2:0.2, 7:3:0.2 and 7:3:0.5) as eluent methanol to give compound **2** (10 mg), which was then subjected for crystallization from methanol. The group SNF-4 (167 mg) was dissolved in the least amount MeOH-H_2O (45%) and chromatographed over RP-18 C.C. using MeOH-H_2O (45%) to MeOH. The respective elutes were separately collected, evaporated to dryness under reduced pressure and monitored by TLC. 10 fractions were obtained labeled SNF-4-1 to SNF-4-10. Fraction SNF-4-7 (30 mg) was chromatographed on silica gel C.C. using $CHCl_3$-MeOH-H_2O (9:1:0.1 to 7:3:0.5) mixtures in a manner of increasing polarities. The subfractions eluted with $CHCl_3$:MeOH:H_2O (8:2:0.1) gave one spot on TLC. The solvent was distilled off under reduced pressure and the residue was dissolved in methanol and left for crystallization to afford compound **3** (9 mg). Fraction SNF-4-10 (100 mg) was chromatographed on silica gel C.C. using $CHCl_3$-MeOH-H_2O-NH_4OH (8:2:0.1:0.1 to 7:3:0.5:0.1) mixture. Subfractions showed single spot on TLC were collected together. The solvent was distilled off under reduced pressure and the residue was dissolved in methanol and left for crystallization to afford compound **4** (7 mg).

Compound 1:white amorphous powder, ^{1}H-NMR (Pyridine-d_5, 500 MHz), δ_H 0.72 (3H, s, H-18), 1.00 (3H, s, H-19), 1.26 (3H, d, *J*= 7.7 Hz, H-21), 1.6 (3H, d, *J*= 5.4 Hz, H-6‴), 1.73 (3H, d, *J*= 4.88 Hz, H-6″), 2.66 (1H, m, H-20), 3.48 (1H, m, H-3), 4.48 (1H, br. s, H-1′), 4.89 (1H, m, H-16), 5.42 (1H, m, H-6), 5.76 (1H, s, H-1‴), 6.30 (1H, s, H-1″).

Compound 2: white amorphous powder, ^{1}H-NMR (Pyridine-d_5, 500 MHz), δ_H 0.94 (3H, s, H-18), 1.08 (3H, s, H-19), 1.13 (3H, d, *J*= 6.72 Hz, H-21), 1.26 (3H, d, *J*= 6.1 Hz, H-27), 1.6 (3H, d, *J*= 6.1 Hz, H-6‴), 1.74 (3H, d, *J*= 5.49 Hz, H-6″), 3.43 (3H, s, OCH_3), 3.86 (1H, m, H-3), 4.52 (1H, H-16), 4.54 (1H, d, *J*=7.94 Hz, H-26), 4.81 (1H, d, *J*=8.16 Hz, H-1′), 5.33 (1H, br.s, H-6), 5.76 (1H, s, H-1‴), 6.30 (1H, s, H-1″).

Compound 3: white amorphous powder, ^{1}H-NMR (Pyridine-d_5, 500 MHz), δ_H 0.99, assigned to H-18 (3H, s, H-18), 1.02 (3H, d, *J*= 6.1 Hz, H-27), 1.08 (3H, s, H-19), 1.35 (3H, d, *J*= 6.7 Hz, H-21), 1.6 (3H, d, *J*= 6.1 Hz, H-6‴), 1.73 (3H, d, *J*= 6.1 Hz, H-6″), 4.77 (1H, d, *J*=7.93 Hz, H-1′), 4.90 (1H, d, *J*=7.91 Hz, H-1⁗), 5.31 (1H, H-6), 5.54 (1H, s, H-1‴), 6.30 (1H, s, H-1″).

Compound 4: white amorphous powder, ^{1}H-NMR (Pyridine-d_5, 500 MHz), δ_H 0.94 (3H, s, H-18), 1.05 (3H, s, H-19), 1.6 (3H, d, *J*= 6.1 Hz, H-6‴), 1.74 (3H, d, *J*= 5.49 Hz, H-6″), 2.23 (3H, s, H-21), 3.42 (1H, m, H-3), 4.96 (1H, d, *J*=7.93 Hz, H-1′), 5.39 (1H, H-6), 5.77 (1H, s, H-1‴).

RESULTS AND DISCUSSION

Compound **1**: The ^{13}C-NMR spectrum of compound **1** exhibited two carbon signals at δ_C 79.03 and 82.66 related to the oxygenated aglycone moiety [3, 4]. The carbon signals at δ_C (121.50 and 140.97) and 181.07 indicating the olefinin and carbonylic functionalities, respectively in the molecule [3-5]. ^{13}C chemical shifts values of compound **1** showed three characteristic methyl signals at δ_C 13.61, 19.4 and 17.95; suggesting that the aglycone pertains to a pregnane type steroid [3-5]. ^{1}H-NMR spectrum displayed ^{1}H signals at δ_H 3.48 (1H, m) and 4.89 (1H, m) corresponding to δ_C 79.03 (C-3) and 82.66 (C-16), indicating the position of the glycosidic linkage and the presence of the lactone ring, respectively [4-6]. ^{1}H-NMR spectrum of compound **1** exhibited three anomeric proton signals at δ_H 4.48 (1H, br. s), 5.76 and 6.30 each (1H, s). This was in accordance with ^{13}C-NMR spectrum, which showed three anomeric carbon resonances at δ_C 100.38, 101.98 and 102.95. Other sugar signals at δ_C (100.38, 78.21, 76.87, 77.94, 77.94 and 61.46), (101.98, 72.72, 72.50, 73.90, 69.46 and 18.45) and (102.95, 72.83, 72.72, 74.16, 70.47 and 18.61), were indicative for the presence of two terminal β-L-rhamnosyl moieties attached to inner glucosyl moiety. Comparison of the ^{13}C-NMR data of the sugar moieties with non substituted β-D-glucopyranoside indicated that the C-2 and C-4 of the glucosyl moiety were downfield shifted to δ_C 78.21 and 77.94 from δ_C 74.8 and 70.30 respectively [3]. The significant glycosylation shifts clearly showed that two terminal rhamnopyranoses linked to C-2 and C-4 positions of the inner glucopyranose moiety [4-7]. With the help of published data and the above discussion compound **1** was established as (20S)-3β,16β-dihydroxy preg-5-ene-22-carboxylic acid-(22,16)-lactone-3-β-chacotrioside known as Dumoside [4,5] which is a known compound isolated before time from *Asparagus dumosus* [6,7]. This is the first reported occurrence in *Solanum nodiflorum*.

Compound 2 :^{1}H-NMR spectrum displayed signals due to pair of tertiary methyl signals at δ_H 0.94 and 1.08 each (3H, s) and four secondary methyls at δ_H 1.13 (3H, d, *J*= 6.72 Hz), 1.26 (3H, d, *J*= 6.1 Hz), 1.60 (3H, d, *J*= 6.1 Hz) and 1.74 (3H, d, *J*= 5.49 Hz), in addition to three anomeric protons at δ_H 4.81 (1H, d, *J*= 8.16 Hz), 5.76 (1H, s) and 6.30 (1H, s). The ^{13}C-NMR signals of compound **2** exhibited 46 carbon signals among which, 18 signals were assigned to the sugar part. Signals at δ_C (102.9, 72.47, 72.73, 73.91, 70.49 and 18.61) and (101.9, 72.5, 72.84, 74.18, 69346 and 18.45) were assigned to two terminal rahmnosyl moieties [7-10]. The residual signals observed at δ_C (100.3, 77.96, 79.07, 77.96, 76.87 and 61.45) were attributed to an inner glucose unit, which together could be ascribed to β-

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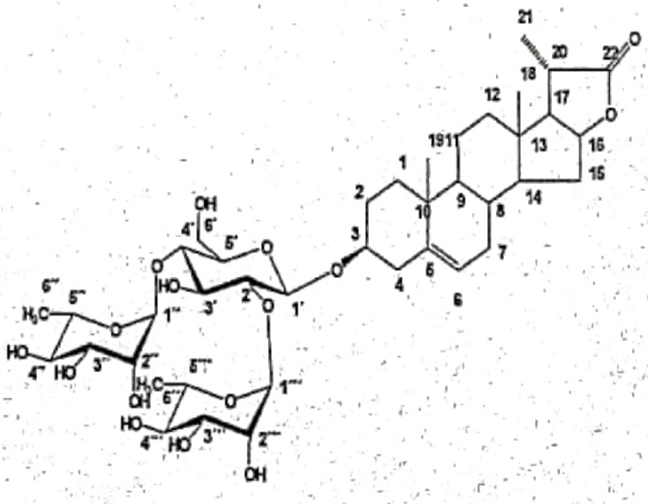
chacotriosyl moiety [8-11]. The remaining 28 signals could be assigned to a spirostanol derivative in which, an oxygen bearing methylene carbon signal allocated to C-26 disappeared and a new ketal signal at 103.30 appeared suggesting the substitution of the hydroxyl group at C-26 [6-9]. Moreover, a signal due to C-17 usually observed at □C 63.0 was vanished and another signal appeared at □C 90.2 indicating the existence of hydroxyl group at this position [7,8]. The substitution at C-17 caused good □, □ and □ substitution shifts at C-12 to C-16 and C-20 to C-22 and 1, 3 diaxial interactions with C-22 [9-12]. The proton coupling constant of a doublet signal of H-26, which appeared at □H 4.54 (1H, d, *J*= 7.94 Hz) indicated that a trans diaxial configuration between H-25 and H-26 that suggesting 25*R* and 26*R* configuration [10,11]. The presence of methyl group resonating at □H 3.43 (3H, s) was confirmed from ^{13}C-NMR signal at □C 55.85 indicated the presence of a methoxy group attached to C-26 [8]. The aglycone part could be identified as pennogenin [7,8]. Consequently, the structure of compound 2 was deduced to be: 3-*O*-□-L-rhamnopyranosyl-(1→2)-[□-L-rhamnopyranosyl-(1→4)]-□-D glucopyranosyl (25*R*, 26*R*)-spirost-5-ene-3□, 17□, 26-triol [8], which is a known compound isolated previously from the *Solanum nodiflorum* [8].

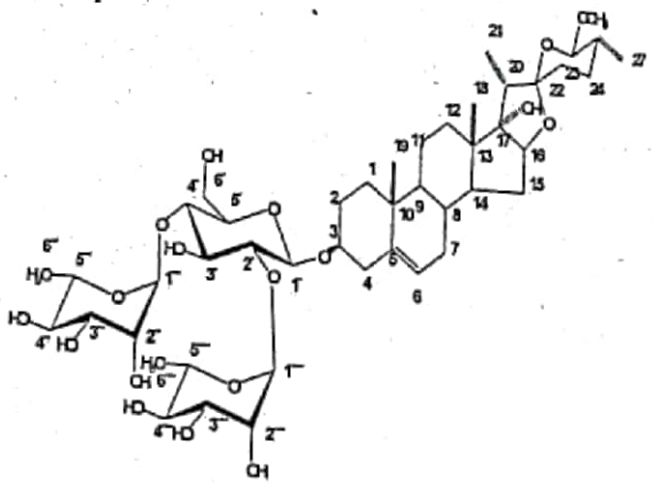
Compound 3: ^{1}H-NMR spectrum displayed signals due to two tertiary methyl signals at □H 0.99 and 1.08 each (3H, s) and four secondary methyls at □D 1.01 (3H, d, *J*= 6.1 Hz), 1.35 (3H, d, *J*= 6.7 Hz), 1.59 (3H, d, *J*= 6.1 Hz) and 1.0 (3H, d, *J*= 6.1 Hz), in addition to four anomeric protons at □D 4.77 (1H, d, *J*= 7.91 Hz), 4.90 (1H, d, *J*= 7.93 Hz), 5.54 (1H, s) and 6.29 (1H, s). The ^{13}C-NMR signals of compound 3 exhibited 51 carbons; among which, 27 signals could be assigned to a furostanol derivative in which oxygen bearing methylene carbon signal ascribable to C-26 disappeared and a new signal at □C 75.25 appeared suggesting the substitution of the hydroxyl group at C-26 [6-8]. Signals, which arise from the sugar residue, indicated the presence of three anomeric carbons at □C 100.32, 102.92 and 101.98. This assumption was further declared from ^{1}H-NMR signals at □H 4.9 (1H, d, *J*= 7.93 Hz), 5.54 (1H, s) and 6.29 (1H, s) in addition to two secondary methyls at □H 1.59 (3H, d, *J*= 6.1 Hz) and 1.7 (3H, d, *J*= 6.1 Hz). The ^{13}C-NMR showed signals at □C (102.92, 72.80, 72.69, 73.87, 70.47, 18.44) and (101.98, 72.69, 72.81, 74.13, 69.46 and 18.59), which were assigned to two terminal rhamnosyl moieties, also it showed signals at □C (100.32, 78.11, 78.56, 77.91, 76.81 and 61.42) assigned to an inner glucosyl unit substituted at C-2 and C-4 and this was indicated from their down field shift upon comparison with non substituted glucose [6-8, 12,13]. The sugar part was identified as □-chacotriosyl. These data in conjunction with ^{1}H-NMR information revealed the attachment of □- chacotriosyl moiety at C-3 of the aglycone moiety [4-8, 9-13]. Moreover the ^{1}H-NMR spectrum showed the presence of an additional anomeric proton at □H 4.9 (1H, d, *J*=7.93 Hz), indicated the presence of an extra sugar moiety. This was cleared from ^{13}C-NMR signals at □C (104.86, 75.18, 78.11, 71.80, 78.56 and 62.89), which represents a set of terminal glucose signals, located at C-26 [14]. Moreover, a signal due to C-17 usually observed at □C 63.0 was vanished and another new signal appeared at □C 90.81 indicating the presence of hydroxyl group at this position [8, 9-13]. The substitution at C-17 caused good □, □ and □ substitution shifts at C-12 to C-16 and C20 to C-22. The appearance of signals at □C 75.3, 111.4 and 90.8 were assigned to C-26 and C-22 of the furostanol moiety in addition to C-17 with a hydroxyl group respectively [9-13]. Consequently, the structure of compound 3 could be identified as: □-chacotriosyl-(22,26*R*)-furost-5-ene-3□,17□,22,26-tetraol-26-*O*-□-D-glucopyranoside [8, 11-13], which is a known compound isolated previously from the *Solanum nodiflorum* [8].

Compound 4: ^{1}H-NMR spectrum displayed signals due to two of tertiary methyl signals at □H 0.94 and 1.05 each (3H, s). The aglycone was identified as pregnenolone type of steroidal glycosides [4-9, 16,17]. Concerning the sugar moiety, the ^{1}H-NMR spectrum showed the presence of three anomeric protons as □H 4.96 (1H, d, *J*=7.93 Hz), 5.77 (1H, s) and 6.31 (1H, s), this was apparent from ^{13}C-NMR signals at □C 100.38, 102.01 and 102.98, indicated the occurrence of three anomeric carbons. The ^{1}H-NMR also showed the existence of two secondary methyls at □H 1.6 (3H, d, *J*=6.1 Hz) and 1.78 (3H, d, *J*=5.49 Hz), assigned to the terminal methyl group of two rhamnosyl moieties [15,16]. Inspection of other ^{1}H and ^{13}C-NMR signals indicated the incidence of two units of rhamnosyl moiety attached to the C-2 and C-4 of an inner glucopyranosyl moiety confirmed from their downfield shift upon comparison with non-substituted glucose moiety [6-8, 15,16]. From the aforementioned data and by comparison with literature [4,6,15,16], it could be concluded that the structure of compound 4 is Aethienolone, which is a known compound isolated previously from *Solanum lyratum* [6]. This is the first reported occurrence in *Solanum nodiflorum*.

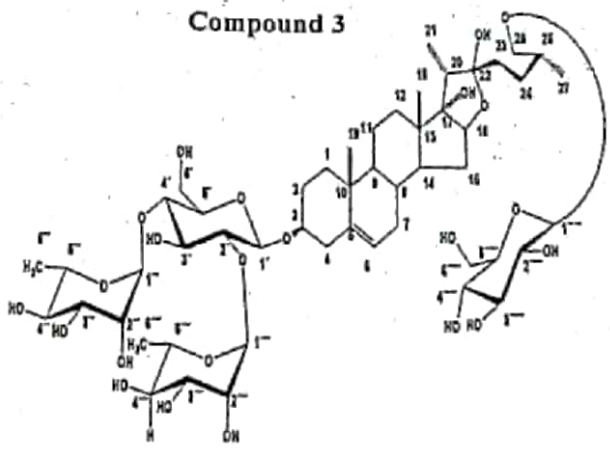
Compound 1



Compound 2



Compound 3



Compound 4

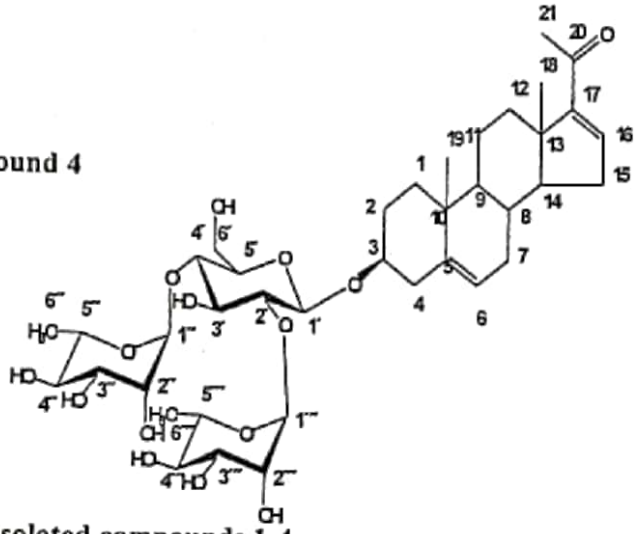


Fig.1: Structures of the isolated compounds 1-4

Table (1): ^{13}C-NMR (125 MHz) of the isolated compounds (1-4) in C_5D_5N

	□C			
Carbon atom	1	2	3	4
C-1	38.14	37.58	37.57	37.43
C-2	30.17	30.2	30.19	30.19
C-3	79.03	77.91	78.11	79.03
C-4	39.01	39.04	39.03	39.09
C-5	140.97	140.9	140.89	141.41
C-6	121.50	121.7	121.82	121.53
C-7	32.16	31.69	31.94	32.34
C-8	31.43	32.30	34.25	30.45
C-9	49.66	50.33	50.36	50.89
C-10	37.18	37.20	37.18	37.23
C-11	20.62	20.99	21.04	20.99
C-12	38.14	31.77	36.79	35.21
C-13	41.55	45.18	45.12	46.38
C-14	54.73	53.18	53.07	56.59
C-15	33.37	32.39	32.50	31.88
C-16	82.66	90.40	90.53	144.49
C-17	58.97	90.20	90.81	155.41
C-18	13.61	16.70	17.26	19.29
C-19	19.40	19.40	19.44	15.97
C-20	36.28	44.87	43.55	196.20

Continue Table (1): ^{13}C-NMR (125 MHz) of the isolated compounds (1-4) in C_5D_5N

	δ_C			
Carbon atom	1	2	3	4
C-21	17.95	9.40	10.30	27.1
C-22	181.07	112.50	111.40	-
C-23	-	32.17	32.31	-
C-24	-	27.90	28.03	-
C-25	-	35.30	32.16	-
C-26	-	103.3	75.25	-
C-27	-	17.1	17.44	-
OCH_3	-	55.85	-	-
Glc				
C-1′	100.38	100.30	100.32	100.38
C-2′	78.21	77.96	78.11	78.25
C-3′	76.87	79.07	78.56	76.91
C-4′	77.94	77.96	77.91	77.98
C-5′	77.94	76.87	76.81	77.93
C-6′	61.46	61.45	61.42	61.45
Rha				
C-1″	102.95	102.9	102.92	102.98
C-2″	72.83	72.47	72.69	72.49
C-3″	72.72	72.73	72.81	72.86
C-4″	74.16	73.91	73.87	73.93
C-5″	70.47	70.49	70.47	70.49
C-6″	18.61	18.61	18.44	18.62
Rha				
C-1‴	101.98	101.9	101.98	102.01
C-2‴	72.72	72.40	72.69	72.53
C-3‴	72.50	72.84	72.81	72.75
C-4‴	73.90	74.18	74.13	74.19
C-5‴	69.46	69.46	69.46	69.48
C-6‴	18.45	18.45	18.59	18.47
Glc′				
C-1⁗	-	-	104.86	-
C-2⁗	-	-	75.18	-
C-3⁗	-	-	78.11	-
C-4⁗	-	-	71.80	-
C-5⁗	-	-	78.56	-
C-6⁗	-	-	62.89	-

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جليكوسيدات الصابونين الستيرويدية من ثمار نبات سولانم نوديفلورم جاك

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من خلاصة الميثانول للثمار الطازجة لنبات السولانوم نوديفلورم و الذى ينتمى للعائلة الباذنجانية تم فصل مركبين لاول مرة من النبات ينتميان لمشتقات الصابونينات الاستيرويدية و قد تم التعرف عليهما كالتالى: دوموزيد (1) و ايثينولون (4) بالاضافة الى اثنان من المركبات المعروفة و قد تم التعرف عليهما كالتالى: بيتا-كاكوترايوزيل (25ر ، 26ر)-سبيروست-5-اين-3بيتا ، 17ألفا ، 26-ترايول (2) و بيتا-كاكوترايوزيل (22 ، 26ر)-فيوروست-5-اين-3بيتا ، 17ألفا ، 22 ، 26 تتراول- 26-أ-بيتا-د-جلوكوبيرانوزيد (3) و قد تم التعرف على التركيب الكميائى للمركبات المفصولة باستخدام الرنين النووى المغناطيسى البروتونى و الكربونى و كذلك بالمقارنة بالقيم الطيفية المنشورة.