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STEROLS AND TRITERPENES FROM *ALESTONIA SCHOLARIS* R.BR.

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سبق للباحثين إجراء دراسة كيميائية على المكونات الفعالة لنبات الألستونيا شولاريز أر بى أر التابع للعائلة الدفلية والمنزرع فى جزيرة النباتات بمدينة أسوان. وقد تم فصل عدد من الفلافونيدات وتم التعرف على تركيبها الكيميائى. ونظرا للإستخدامات الشعبية العديدة لهذا النبات وأهميته القصوى فى علاج العديد من الأمراض مثل الروماتزم وبعض حالات الحمى وبعض الأمراض المعدية فقد قام الباحثون بإجراء المزيد من الدراسة لأوراق هذا النبات وقد أمكن فصل خمسة مركبات من خلاصة الهكسان وتم التعرف عليها بواسطة الطرق الكيميائية والكروماتوجرافية والطيفية الحديثة والتى تشمل الأشعة فوق الحمراء والرنين النووى المغناطيسى بنوعيه البروتونى والكربونى وتم التعرف عليها وهى: ٤-ألفا ، ١٤-ألفا ، ٢٤-ثلاثى ميثيل-٩-بيتا ، ١٩-سيكلو-٥-ألفا-كوليست-٢٤(٢٨)-أين-٣-بيتا أول (سيكلوايوكالينول) والستجماستيرول وهى من الاستيزولات التى تفصل لأول مرة من هذا النبات بالإضافة إلى ثلاثة مركبات ترايتربينية تفصل أيضا لأول مرة من هذا النبات وهى البتيولين وحمض البيتيولينيك وخلات الألفا أميرين.

From the hexane fraction of the alcoholic extract of the leaves of Alestonia scholaris R.Br. (Apocynaceae), five compounds have been isolated and identified as 4α,14α,24-trimethyl-9β,19-cyclo-5α-cholest-24(29)-en-3β ol; stigmasterol, betulin, betulinic acid, and α-amyrin acetate. The structures of the isolated compounds were principally deduced by physical, chromatographic characters as well as by spectroscopic analyses. The isolated compounds are reported here for the first time.

INTRODUCTION

In a previous publication,[1] the presence of a number of flavonoidal aglycones and glycosides for the first time from the leaves of *Alestonia scholaris* R.Br. was reported. Consequently a systemic chemical examination of the plant with the objective of isolating the steroidal and triterpenoidal contents of *A. scholaris* was undertaken.

In this paper, the isolation and characterization of 4α,14α,24-trimethyl-9ß,19-cyclo-5α-cholest-24(29)-en-3ß ol (**2**), stigmasterol (**4**), betulinic acid (**1**), betulin (**5**), and α-amyrin acetate (**3**) are reported. These isolated compounds are reported here for the first time in *Alestonia scholaris* R.Br.

EXPERIMENTAL

Plant material

The leaves of *Alestonia scholaris* R.Br. were collected in May 1995 from the trees growing in the Botanic Island in Aswan (Upper Egypt). The plant was identified and authenticated by Prof. Dr. A. Fayed, Professor of Taxonomy, Faculty of Science, Assiut University to whom we are indebted. A voucher sample is kept in Dept. of Pharmacognosy, Faculty of Pharmacy, Assiut University.

General experimental procedures

Melting points were uncorrected. IR as KBr pellets. ^{1}HNMR and ^{13}CNMR spectra were recorded in $CDCl_3$ (except compound (**5**) where $CDCl_3$-CD_3OD is used) using JEOL JNMA-500 spectrometer (500 MHz for ^{1}HNMR and 125

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MHz for ^{13}CNMR) using TMS as internal standard.

Thin layer chromatography was carried out on silica gel G plates (Kieselgel 60 F_{254}, E.Merck) and for column chromatography, silica gel (E.Merck, 230-400 mesh) was used. The spray reagent used was 50% H_2SO_4 in MeOH.

Extraction and isolation

The air-dried leaves (1 kg) of *Alestonia scholaris* R.Br. were extracted with ethanol (70%) till exhausion. The ethanolic extract was concentrated under reduced pressure till nearly dryness. The syrupy extract (220 g) was successively exhausted with n-hexane, the hexane fraction was concentrated under reduced pressure to a syrupy consistency giving (65 g).

Chromatographic study of the hexane residue was done using silica gel G plates, the solvent system was pet. ether-EtOAc (9:1) and 50% H_2SO_4 in MeOH as a spray reagent. The chromatoplates show five spots having h R_f: 81, 68, 53, 47 and 38 respectively. About 20 g of the hexane residue were chromatographed over a silica gel column (400 g, 5 x 150 cm) using pet. ether-EtOAc mixture in a manner of increasing polarities. Fractions (250 ml, each) were collected and monitored by using TLC as mentioned before. Similar fractions were collected and concentrated under reduced pressure. The fractions eluted with pet. ether-EtOAc (98:2) afforded (**5**), fractions eluted with pet. ether-EtOAc (95:5) afforded (**2**); fractions eluted with pet. ether-EtOAc (9:1) afforded the isolation of (**3**), (**4**) and (**1**).

Compound (1): betulinic acid, white needles from MeOH (135 mg); m.p 276-277°. IR: 346(OH-stretch.), 3010, 2920, 2890 (C-H stretch.), 1685 (C=O stretch.), 1640 (C=C stretch.), 1445, 1330 (C-H bend.), 1040 and 880 cm^{-1}. ^{1}HNMR ($CDCl_3$-CD_3OD): δ 4.69 (1H, d, J= 1.2 Hz), 4.56 (1H, d, J= 1.2 Hz), 3.78 (1H, d, J= 10 Hz), 3.27 (1H, d, J= 10 Hz), 3.15 (1H, m), 1.65 (3H, s), 0.93 (3H, s), 0.85 (6H, s), 0.78 (3H, s), 0.66 (3H, s). ^{13}CNMR: see Table 2.

Compound (2): Cycloeucalenol, white needles from acetone (47 mg); m.p 140°. ^{1}HNMR ($CDCl_3$): δ 4.79 (1H, bd, J= 2.3 Hz), 4.65 (1H, bd, J= 2.3 Hz), 3.32 (1H, m, J= 5.2 Hz), 1.15 (6H, d, J= 6.7 Hz), 0.97 (3H, s), 0.96 (3H, d, J= 6.7 Hz), 0.92 (3H, s), 0.85 (3H, d, J= 6.7 Hz), 0.22 (1H, d, J= 4.2 Hz) and 0.38 (1H, d, J= 4.2 Hz). ^{13}CNMR: see Table 1.

Compound (3): α-amyrin acetate, white needles from acetone (145 mg), m.p 231-233°. ^{1}HNMR ($CDCl_3$): δ 5.15 (1H, dd, J= 7.5, 2.5 Hz), 4.5 (1H, m), 2.12 (3H, s), 1.12 (3H, s), 1.00 (3H, s), 0.98 (3H, s), 0.93 (3H, s), 0.87 (6H, d, J= 6.2 Hz) and 0.80 (3H, s). ^{13}CNMR: see Table 2.

Compound (4): Stigmasterol, white needles from MeOH (75 mg), m.p 170°. ^{1}HNMR ($CDCl_3$): δ 5.40 (1H, dd, J= 7.5, 2.5 Hz), 5.12 (1H, dd, J= 15.0, 8.5 Hz), 5.00 (1H, dd, J= 15.0, 8.5 Hz), 3.6 (1H, m, J= 10, 10, 4.5, 4.5 Hz), 1.16 (3H, d, J= 6.5 Hz), 1.02 (3H, s), 0.94 (3H, d, J= 6.5 Hz), 0.90 (3H, t, J= 7.5 Hz), 0.80 (3H, d, J= 6.5 Hz) and 0.70 (3H, s). ^{13}CNMR: see Table 1.

Compound (5): Betulin, white needles from MeOH (120 mg), m.p 246-248°. IR: 3420 (OH-stretch.), 3020, 3000, 2890 (CH stretch.), 1640 (C=C stretch.), 1375, 1360 (C-H bending) and 880 cm^{-1}. ^{1}HNMR ($CDCl_3$): δ 4.68 (1H, d, J= 2 Hz), 4.58 (1H, d, J= 2 Hz), 3.2 (1H, m), 1.68 (3H, s), 1.12 (3H, s), 0.98 (3H, s), 0.96 (3H, s), 0.83 (3H, s), and 0.76 (3H, s). ^{13}CNMR: see Table 2.

The structural formulae, from the obtained data, for the compounds [**1-5**] are illustrated in Figure 1.

RESULTS AND DISCUSSION

The tetracyclic structure of (**2**) is deduced from the study of the methyl signals in both ^{1}HNMR (see experimental) and ^{13}CNMR spectra of the isolated compounds (Table 1). 9ß,19-cyclostructure is based on the appearance of an AB quartet at δ 0.22 and 0.38 (J= 4.2 Hz) in

Table 1: The ^{13}CNMR spectra of the steroidal compounds (**2**) and (**4**).

Carbon No.	(2)	(4)
1	30.80	37.50
2	34.78	29.30
3	76.63	71.72
4	44.57	39.36
5	45.36	140.93
6	24.67	121.93
7	28.00	32.07
8	46.87	32.18
9	23.56	50.36
10	29.53	36.90
11	25.36	21.30
12	35.35	42.49
13	45.36	42.49
14	48.91	56.08
15	32.80	24.50
16	26.99	25.70
17	52.22	56.93
18	17.79	12.40
19	26.99	19.00
20	36.13	36.96
21	18.35	21.48
22	35.01	138.83
23	31.33	129.48
24	156.92	51.43
25	33.56	32.18
26	22.00	19.45
27	21.88	21.30
28	14.40	23.15
29	105.95	12.55
30	19.14	-

Table 2: The ^{13}CNMR spectral data of the triterpenoids compounds (**3**), (**1**) and (**5**).

Carbon No.	(3)	(1)	(5)
1	38.4	39.0	38.7
2	23.6	27.6	27.4
3	80.7	78.2	79.1
4	37.6	39.0	38.8
5	55.3	55.5	55.3
6	18.3	18.4	18.3
7	32.8	31.5	31.3
8	40.1	40.8	41.0
9	47.6	50.7	50.5
10	36.8	37.3	37.3
11	23.2	21.0	20.9
12	124.1	25.6	25.2
13	139.4	38.2	37.2
14	42.1	42.2	42.8
15	28.7	30.8	27.1
16	26.7	32.6	29.2
17	33.8	56.3	47.9
18	59.0	47.1	47.9
19	39.7	49.4	48.8
20	39.7	150.5	150.5
21	31.3	29.9	29.8
22	41.5	37.3	34.0
23	28.1	28.2	28.1
24	16.8	15.6	15.4
25	15.7	16.1	16.1
26	16.8	16.1	16.1
27	23.2	14.7	14.8
28	28.1	178.9	60.6
29	17.5	19.4	19.1
30	21.4	109.4	109.7
OCOMe	21.2	-	-
OCOMe	170.4	-	-

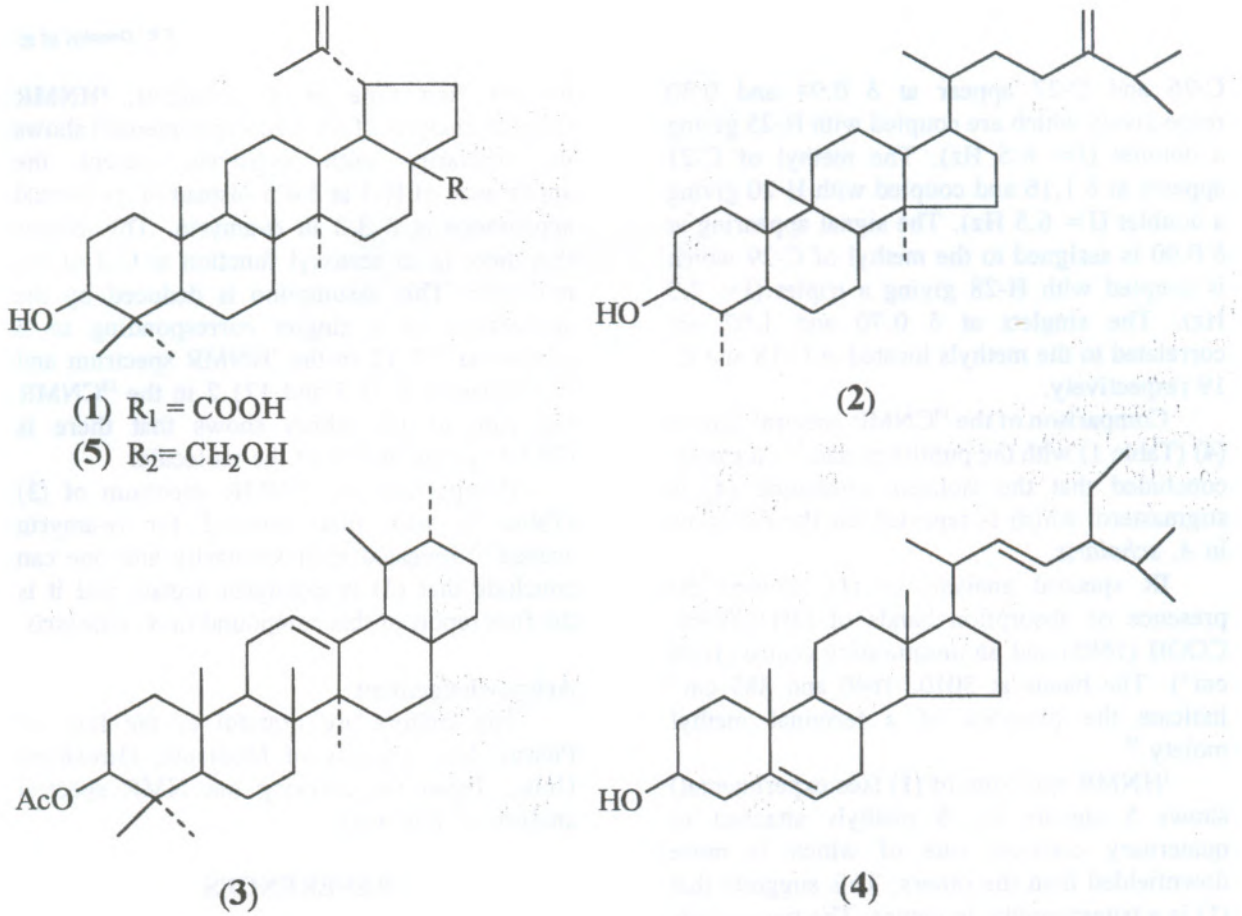

Fig. 1: The structural formulae of the isolated compounds **1-5.**

the ^{1}HNMR spectrum of (**2**). The α-orientation of H-3 was assigned on the basis of ^{1}HNMR signal at δ 3.32 (t, J= 5.2 Hz)[2,3]. The presence of C-25, C-26 and C-27 as a terminal isopropyl unit is based on the ^{1}HNMR signal at δ 1.15 (6H, d, J= 6.7 Hz) and ^{13}CNMR signals of these three carbons.[4,5] The signals appearing at δ 156.3 and 105.6 confirmed the position of the exomethylenic double bond of the side chain at C-24.[4,5]

Comparison of ^{13}CNMR spectral data of (**2**) with the published data[4] shows that (**2**) is assignedas4α,14α,24-trimethyl-9ß,19-cyclo-5α-cholest-24(29)-en-3ß-ol. Reviewing the current literature, it was found that (**2**) is similar in all aspects with the previously isolated cycloeucalenol which was reported eariler in some plants belonging to Solanaceae.[6] Consequently, cycloeucalenol is reported for the first time in *Alestonia scholaris* R.Br.

The isolated compound (**2**) belongs to a class of compounds known as cycloartenols which are of relatively rare existence in plants kingdom. Their occurrence is restricted in some plant families.[4,5,13] By reviewing the current literature; this represents the first isolation of cycloaudenol from family *Apocynaceae*.

^{1}HNMR spectral analysis of (**4**) (see experimental) shows a doublet of doublets at δ 5.4 (J= 7.5, 2.5 Hz) which is assigned for H-6 (which is coupled with H-7). H-22 and H-23 appear at δ 5.12 and 5.00 with an olefinic trans coupling (J= 15 Hz) as well as a vicinal coupling (J= 8.5 Hz). The signal at δ 3.6 is assigned for H-3 which is coupled with H-4 and H-2 giving a tt (J= 10, 4.5 Hz). The methyls at

C-26 and C-27 appear at δ 0.94 and 0.80 respectively which are coupled with H-25 giving a doublet (J= 6.5 Hz). The methyl of C-21 appears at δ 1.16 and coupled with H-20 giving a doublet (J= 6.5 Hz). The signal appearing at δ 0.90 is assigned to the methyl of C-29 which is coupled with H-28 giving a triplet (J= 7.5 Hz). The singlets at δ 0.70 and 1.02 are correlated to the methyls located at C-18 and C-19 respectively.

Comparison of the ^{13}CNMR spectral data of (**4**) (Table 1) with the published data[7-9], it can be concluded that the isolated compound (**4**) is stigmasterol which is reported for the first time in *A. scholaris*.

IR spectral analysis of (**1**) showed the presence of absorption bands of OH (3450), COOH (1685) and an unsaturation centre (1640 cm^{-1}). The bands at 3010, 1640 and 885 cm^{-1} indicate the presence of a terminal methyl moiety.[10]

^{1}HNMR spectrum of (**1**) (see experimental) shows 5 signals for 6 methyls attached to quaternary carbons, one of which is more downfielded than the others. This suggests that (**1**) is a triterpenoidal in nature. The two signals at δ 4.56 and 4.70 (1H, each) and the singlet at 1.65 (3H) show the presence of one olefinic methyl group.[10] The full assignment of (**1**) is achieved by comparing its ^{13}CNMR data (Table 2) with the published data for lupane-type triterpenes.[11] It is concluded that (**1**) is betulinic acid which is reported for the first time in *A. scholaris*.

On comparing IR spectrum of (**5**) with that of (**1**), it is concluded that (**5**) has absorption bands corresponding to OH, unsaturation centre and a terminal methylene moiety. Lack of the absorption band at 1685 cm^{-1} means the absence of CO function in (**5**). Close inspection of the ^{1}HNMR spectrum of (**5**) (see experimental) shows the triterpenoidal nature of (**5**).[10] The full assignment of (**5**) is deduced by comparing its ^{13}CNMR (Table 2) with the published data of lupane-type triterpenes.[11] It was found that ^{13}CNMR spectrum is coincided with that reported for betulin. Consequently, it is concluded that (**5**) is betulin which is reported for the first time in *A. scholaris*. ^{1}HNMR spectral analysis of (**3**) (see experimental) shows its similarity with α-amyrin except the appearance of H-3 at δ 4.5 instead of its normal appearance at δ 3.3 in α-amyrin. This means that there is an acetoxyl function at C-3 of the molecule. This assumption is deduced by the appearance of a singlet corresponding to 3 protons at δ 2.12 in the ^{1}HNMR spectrum and the signals at δ 21.5 and 171.2 in the ^{13}CNMR spectrum of (**3**) which shows that there is CH_3CO group at C-3 of the molecule.

Comparison of ^{13}CNMR spectrum of (**3**) (Table 2) with that reported for α-amyrin acetate[12] revealed their similarity and one can conclude that (**3**) is α-amyrin acetate and it is the first report of this compound in *A. scholaris*.

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