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Studies on Saudi Compositae: Chemical Constituents of *Echinops spinosissimus* and *Sonchus oleraceus*

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Abstract. From the whole plant extract of *Echinops spinosissimus*, the previously undescribed natural product, pseudo taraxasteryl acetate, together with β-amyrin acetate and ursolic acid have been isolated. The polar fractions of *Sonchus oleraceus* were found to contain 2-methylheptyl benzoate and an acetate ester. The structures of the isolated compounds were determined by spectroscopic and chemical methods.

Introduction

More than twelve percent of the plants mentioned in the flora of Saudi Arabia belong to the family Compositae [1] and various medicinal properties are attributed to these plants in Saudi folk medicine [2]. Although many studies have been made, the family compositae cannot be characterized by any group chemical constituents. As a part of our studies on the chemical constituents of Saudi medicinal plants [3,4], we have now investigated *Echinops spinosissimus* and *Sonchus oleraceus*. β-Sitosterol, β-amyrin acetate (2), apigenin 7-0-glycoside and an alkaloidal compound have been reported from *E. spinoissimus* [5,6] while some flavonoids have been isolated [7] from the polar fractions of *S. oleraceus* extracts. The relatively less polar fractions of the extract of the latter species have received little attention. In this communication, we wish to present our findings on the terpene-fraction of *E. spinosissimus* and on the apolar fractions of *S. oleraceus*.

Experimental

General methods

Plant materials were collected from King Saud University campus in April 1986 and identified by Dr. Hassan M. Hassan, Department of Botany, King Saud Univer-

sity where the voucher specimens were kept for record. Mps were determined on Weignad microscope stage and are uncorrected. MS analyses were performed on Hewlett-Packard 5987 spectrometer at 70 eV with evaporation of the samples in the ion source at ca. 200°C. TLC, unless otherwise stated was carried out on silica gel (Merk 60 PF_{254}). Beckman 4240 (IR) and Perkin-Elmer Lambda 5 UV/Vis instruments were used. Optical rotations were measured in MeOH and $CHCl_3$ on Perkin-Elmer (241 MC) spectrophotometer. The ^1H NMR and ^{13}C NMR spectra were recorded on a Jeol FX 100 (100 MHz) instrument in $CDCl_3$ using TMS as an internal standard.

Echinops spinosissimus

The shade dried and ground plant material (1.2 kg) was first defatted with petroleum ether and then extracted with methanol at room temperature for 10 days. After filtration, the solvent was evaporated under reduced pressure to give a dark green residue (60 g). A portion of this residue (30 g) was dissolved in minimum amount of methanol and subjected to column chromatography on silica gel (400 g) eluting with increasingly polar solvents; mixture of petrol-$CHCl_3$, $CHCl_3$, $CHCl_3$-MeOH were used and various 50 ml fractions were collected. The petrol-$CHCl_3$ (1:1) eluates gave a mixture of β-amyrin acetate(2) and pseudo taraxasteryl acetate(1). This mixture was separated by preparative TLC using petrol-$CHCl_3$ (9:1) as solvent system. Ursolic acid(3) was obtained from $CHCl_3$-MeOH fraction (9:1) of the column. Compounds 2 and 3 were identified by direct comparison with authentic samples and spectroscopic data.

Identification of compound 1. mp, 208-212°C; [α], + 58° ($CHCl_3$); IR(KBr): 2950, 1730 (>C = 0), 1630 (C = C), 1370, 1170 cm^{-1}; ^1H NMR ($CDCl_3$): 0.73, 0.87, 1.04 and 1.25 (each 3H, s,4 × Me), 0.84 (6H, s), 0.91 (3H, d), 1.64 (3H, brs) 2.04 (3H, s, OAc), 4.49 (1H, dd, H-3), 5.66 (1H, m); ^{13}C NMR: see Table 1; EIMS: m/e (relative intensity): 468$[M]_+$ (20%), 453(6), 408(8), 393(7), 249(18), 218(17.5), 204(20), 189 (100), 109(52), 95(68).

Sonchus oleraceus

The whole plant (1 kg) was dried, powdered and extracted successively with chloroform and methanol. The chloroform extract was concentrated and chromatographed on a column of silica gel (200 g) and eluted with $CHCl_3$ collecting various 100 ml fractions. The fractions obtained were compared by TLC (silica gel using hexane-$CHCl_3$ as a solvent) and those giving similar TLC spots were combined and further purified on silica gel plates to give 2-methylheptyl benzoate(4) and the acetate ester(5) in pure forms.

Compound 4. Colourless oil, [α] + 0.5°; IR (Nujol): 2950, 2920, 2845, 1725, 1600, 1450, 1250, 1120, 1070 cm^{-1}; ^1H NMR ($CDCl_3$]: δ0.92 (3H, t, CH_2CH_3), 0.98

Table 1. ^{13}C **NMR data of 1 in** $CDCl_3$

Carbon No.	Chemical Shift	Carbon No.	Chemical Shift
C-1	38.45	C-16	38.04
C-2	22.54	C-17	36.69
C-3	80.95	C-18	48.72
C-4	37.30	C-19	42.15
C-5	55.42	C-20	139.78
C-6	18.20	C-21	118.83
C-7	34.16	C-22	34.40
C-8	41.09	C-23	16.49
C-9	50.30	C-24	27.94
C-10	37.04	C-25	16.02
C-11	20.95	C-26	16.30
C-12	27.04	C-27	14.70
C-13	39.21	C-28	27.59
C-14	42.33	C-29	21.60
C-15	29.70	C-30	23.72
		OCO *Me*	21.92
		OCO *Me*	170.95

(3H, d, $CHCH_3$), 1.4-1.7 (9H,m), 4.21(2H, d, J = 6Hz, CH_2-O), 7.5-7.7 (5H,m), 13_C NMR: 10.98(C-1), 14.0(C-8), 23.02(C-3), 23.7(C-4), 28.95(C-5), 30.4(C-2), 38.7(C-6), 68.2(C-7), 129.0,(C-3' and C-5'), 130.2(C-1'), 130.3(C-2' and C-6'), 133.8(C-4'), 167.7(>C = O).

Compound 5. Colourless oil, ^{1}HNMR ($CDCl_3$): δ1.4 (3H,d,J = 6.6 Hz), 1.98 (3H,s,$COCH_3$), 3.28 (3H,s,OMe), 3.3 (2H,d, partially overlapped, CH_2-O, 4.98 (1H,m, CH-O). ^{13}C NMR: δ 15.5(q), 20.3(q), 58.02(q), 68.24(t), 74.00(d), 169.5(s).

Results and Discussion

The methanolic extract of the aerial parts of *Echinops spinosissimus* yielded a mixture of triterpenoids. Separation by repeated column chromatography and preparative TLC over silica gel led to the isolation of the new natural pseudo taraxasteryl acetate(1) as well as the isolation of the commonly known triterpenoids, β-amyrin acetate(2) and ursolic acid(3). The identity of the latter was established by comparison (mp, mmp, ^{1}H NMR and ^{13}C NMR) with authentic sample available in our laboratory whereas compound 2 was identified by comparing its melting point and spectral data with those available in the literature [8]. Tests with Lieberman-Bur-

chardt reagent and tetranitromethane indicated the new isolate(1) to be an unsaturated triterpenoid. The molecular weight of 1 was determined by mass spectrometry as M^+, m/e 468, $C_{32}H_{52}O_2$. The mass spectrum of 1 was closely related to that of pentacyclic triterpenes [9]. Its IR spectrum showed absorptions at 1730 (> C = O) and 1630 cm^{-1} (unsaturation). The ^1H NMR spectrum of 1 exhibited singlets for six methyl groups, a doublet for one methyl and a broad singlet for olefinic methyl (see experimental). The singlet (3H) at δ 2.04 was attributed to an acetoxyl methyl group. The double doublet at δ 4.49 (1H) was assigned to the proton at the acetoxy-bearing carbon whereas the signal (1H,m) at δ 5.66 was attributed to an olefinic proton. The ^1H NMR spectrum of 1 was found to be closely related to that one of pseudo taraxasterol 6 [10] excepting the downfield positions of the protons adjacent to the oxygen functionalities (found δ 4.49 in 1 and δ3.24 in 6). The trisubstituted nature of the double bond present in the structure of 1 was also confirmed from the ^{13}C NMR spectrum of the latter (Table 1) which displayed signals at δ 118.3(d) and δ 139.78(s). Assuming that compound 1 was of the taraxastane type, the only possible location of the trisubstituted double bond is between C-20 and C-21.

The mass spectrum of 1 showed typical fragment ions of pentacyclic triterpenoids [9] with the formation of prominent peaks at m/e 249 and 189 from ring C cleavage. The formation of the latter peaks located the acetoxyl group at ring A and B. Based on ^1H NMR data and biogenetic grounds, the acetoxyl group in 1 was considered to be at C-3. Furthermore, the acetoxyl group at C-3 must have a β-equatorial configuration according to the position of the signal at δ 80.95 (d, ^{13}C NMR) a characteristic feature of C-3 carrying a β-acetoxyl group in pentacyclic triterpenoids [11]. Further support in favour of structure 1 came from its ^{13}C NMR spectrum (Table 1) in which 32 carbon atoms were assigned by the aid of the single frequency off-resonance decoupling (SFORD) techniques and by comparison with reported ^{13}C NMR data of structurally related triterpenses [11]. Moreover, ^{13}C NMR chemical shifts of carbons in 1 were in good agreement with those in 7 except as expected, for the chemical shifts of the double bond carbons [12]. Thus, the above data confirmed structure 1 for this new compound isolated from *E. spinosissimus*.

The methanolic extracts of *S. oleraceus* afforded on chromatographic separation (silica gel), mainly sucrose which was identified by direct comparison with an authentic sample. Flavonoids were also detected in these extracts. Efforts was then concentrated on the chloroform extracts. The different fractions obtained as described in the experimental yielded upon further preparative TLC compounds 4 and 5. Compound 4 was an ester of benzoic acid as deduced from its IR and NMR spectra. Alkaline hydrolysis of 4, gave a compound which was found to be identical in all respects (mmp, IR and NMR) with an authentic specimen of benzoic acid. The presence of a carbonyl group in 4 was inferred from its IR spectrum (> C = O, at 1715 cm^1). The ^1H NMR spectrum of compound 4 showed signals for five aromatic protons in the region δ 7.5-7.7 as well as a doublet (two protons) at δ 4.21 = 6 Hz) which

was attributed to the methylene protons in -O-CH_2-CH. The latter spectrum showed also signals assigned to two methyls and methylenes (see experimental). The above data are consistent with the proposed structure for 4. Evidence in favour of structure 4 was also provided by its ^{13}C NMR spectrum which showed in addition to the signals of aromatic carbons. eight signals for aliphatic moieties. All the ^{13}C NMR signals were assigned unambiguously [13].

The structure of 5 was followed from its IR and NMR spectra. The IR spectrum of the latter revealed the presence of a carbonyl ester (1730 cm^{-1}). The ^1H NMR spectrum of 5 displayed signals for three methyl groups, a doublet at δ 1.4 (CH_3CH) and two singlets at δ 1.98 (acetoxyl methyl) and at δ 3.28 (methoxy groups). In addition, the spectrum showed a doublet at δ 3.3 assigned to a methylene group and a multiplet (δ 4.98) which integrated for one proton. Irradiation of the latter signal turned the signal at δ 3.3 to a singlet proving their proximity. The ^{13}C NMR spectrum of 5 corroborates the structure since it exhibited in total six signals, and the assignment of carbons was made in a straightforward manner based on multiplicities and chemical shifts.

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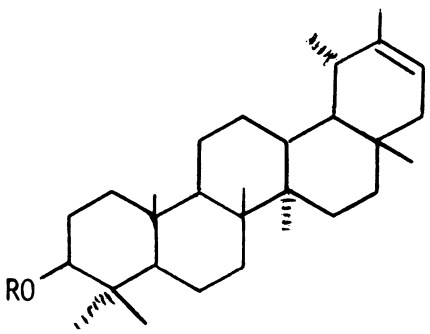
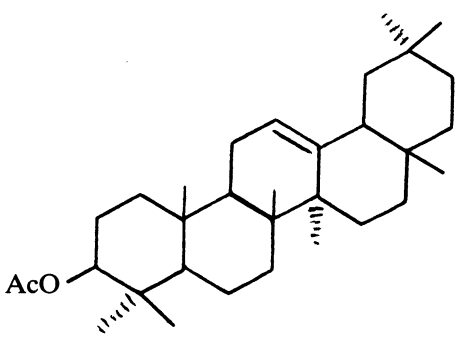


1. R = Ac
6. R = H



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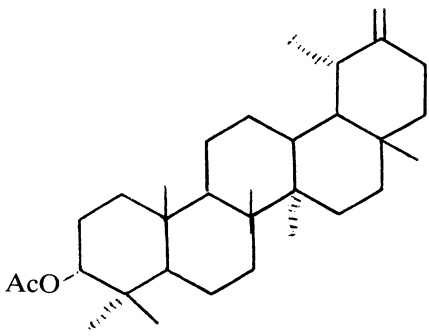

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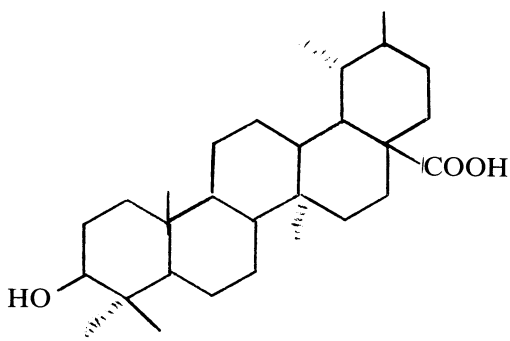

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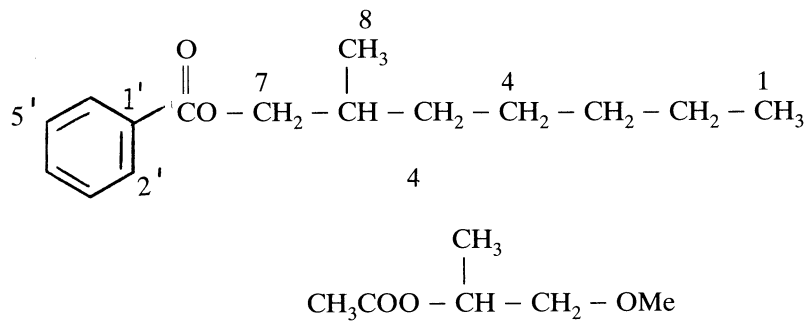

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دراسات على العائلة المركبة: المكونات الكيميائية لكل من نبات أيكونبس سبينوسيسمس (شوك الجمل) ونبات سونكس أولوريسيس (خويش)

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ملخص البحث. لقد تم استخلاص كل من سودو ـ تراكاستريل اسيتات الذي تم عزله من الطبيعة لأول مرة، وبيتا ـ أميرين أسيتات وحمض أرسول من نبات شوك الجمل. وبالمثل فإنه قد تمت الدراسة على خلاصة نبات خويش وبالذات الخلاصات غير القطبية والتي وجد أنها تحوي بصورة رئيسة كلا من ٢ ـ مثيل هبتيل بنزوات ومشتق الأسيتات. وقد تم التعرف على المركبات المعزولة من النباتين المذكورين أعلاه بالطرق الطيفية الحديثة وكذلك بمقارنتها بمركبات قياسية معروفة.