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SAPONINS FROM ZYGOPHYLLUM ALBUM L.

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ABSTRACT

Four saponins were isolated from the overground parts of Z.album L. along with B-sitosterol-B-D-glucopyranoside. Their structures were identified as 14-decarboxyquinovic acid-O-(3→1)-∝-L- rhamnopyranoside (1), quinovic acid-(28→1)-∝-L-rhamnopyranosyl ester (2), which is new; quinovic acid-O-(3→1)-∝-L-rhmnopyranoside (3) and quinovic acid-O(3→1)-B-D-glucopyranoside (4). This identification was based on chemical studies. Glc, Gc/Ms and Mass spectral analysis.

INTRODUCTION

Zygophyllum album L. is a wild salty desert herb belonging to the family Zygophyllaceae[1]. The plant is characterized by containing triterpene saponins and producing toxic symptoms to domestic animals[2]. Z. album is known by Egyptian farmers as "Biz El-Kalba[1].

Hylands.P.J. et al[3] isolated quinovic acid from Z.album L. and the ^{13}C-NMR of its acetate and dimethylacetate were assigned.

The present work represents the isolation and identification of triterpenoid saponins with particular interest to the study of their sugar moieties.

EXPERIMENTAL

Materials

Z. album L. was collected from the Red Sea Coastal region near Marsa Alam City in May 1985. Its identification was confiermed by Prof. Dr. M.N.El-Hadidy, Professor of Taxonomy, Dept. of Botany, Faculty of Science, Cairo University.

Melting points were determined by an electrothermal appratus and were uncorrected. GLC analysis was performed on Varian 3300. WCOT column (0.25 mm/25 m). Carrier gas is nitrogen (0.8 ml/min. Split : 1:20). Temperature programme of 175-205°C (1°C/min) and 15 min isothermal. Detector FID. GC/MS analysis was carried out on Hewlett-packard GC 5890 A coupled with selective mass detector 5970 B and HP work station 300; carrier gas, helium (0.8 ml/min. split = 1 : 50); Column, Durabond fused silica (0.25 mm/30 m) DB 1701-30W, 25 um thickness; Temperature, programme 170-210°C(1°C/min) and 210°C(10 min, isothermal). EIMS was carried out at 70 eV with direct inlet technique.

TLC : Silica gel precoated plates (E Merck).

system : a) $CHCl_3$ - MeOH (90 : 10).

b) $CHCl_3$ - MeOH (85 : 15).

spots visualized with 40 % H_2SO_4 in MeOH, then heated at 130°C for 10 min.

cc : silica gel (60 - 120 mesh; E Merck).

Reference materials :

Authentic B-sitosterol-B-D-glucoside and quinovic acid were kindly supplied by Prof. Dr. D. Bishay, Dept. of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

Isolation of saponins :

The air-dried powdered plant (1 Kg) was macerated on cold with alcohol 70 %. The extract (6L) was concentrated under vacuum to syrupy consistancy. Then diluted with water (1 L) and successively extracted with chloroform and n-butanol. The concentrated n-butanol extract (15 g) was

chromatographed over silica gel column. Elution was started with $CHCl_3$-MeOH-H_2O(95:1:0.1)and the polarity was increased gradually.Fraction were collected (250 ml each) and tested by TLC. Fractions containing similar single spots were pooled and purified. This resulted in the isolation and identification of five glycosides labelled ZS_1-ZS_5.

Acid hydrolysis :

Each saponin (5 mg) was separately dissolved in 2M TFA (trifluoroacetic acid) in a sealed tube and autoclaved at 120°C/1 bar for one hour. TFA was removed by distillation under vacuum and the residue obtained was extracted with 1 ml distilled demineralized water. The obtained aqueous extract was directed to the study of sugars. The water insoluble residue was dissolved in MeOH and left for crystallization to give the aglycone.

Alkaline hydrolysis[4] :

Each saponin (5 mg) was separately hydrolyzed with 5 N NH_4OH in a sealed tube by autoclaving at 120°C/1 bar for 3 hours. The sample was neutralized with dil.acetic acid and then lyophilized and worked up as mentioned under acid hydrolysis.

Alditol acetates :

All alditol acetates were separately prepared following the method of Blackeny, et al[5]. The aqueous hydrolysate of each compound was reduced by 1 ml of 2 % $NaBH_4$ in 0.1 ml IN NH_3 at 60°C for 90 min. Acetylation was performed with 2 ml acetic anhydride and 0.2 ml 1-methyl imidazole as catalyst.

Partially acetylated methylated sugar :

Following Harris et al[6] each saponin (10 mg) was dissolved in DMSO and 0.4 ml of dimethylsulphenyl carbanion was added in an argon atmosphere and left for 2 hours at room temperature with stirring. 0.3 ml methyl iodide was added on cooling then left for one hour. The obtained methylated saponin was diluted with distilled water and extracted with chloroform. The chloroform extract was evaporated under nitrogen atmosphere. Complete methylation was checked by IR and the obtained residue was then hydrolysed and the alditol acetates were prepared as mentioned above.

RESULTS AND DISCUSSION

Compound ZS_1 was obtained from CC-fractions (2-4) eluted with $CHCl_3$- MeOH-H_2O (95:1:0.1). It occurs as needle crystals (500 mg) (MeOH), mp 265-67°C, R_f 0.65 (system a). It was identified as B-sitosterol-B-D-glucoside by direct comparison with authentic material (mp,mmp,R_f) and reported mass spectrum[2].

Compound ZS_2(1) : subsequent elution with the same solvent mixture (fractions[5-8] and crystallization from methanol afforded compound ZS_2 as needle crystals (25 mg). R_f0.60 (system a) Ms,M^+ 442, m/z427 (M^+- Me), 409 (M^+- H_2O), 234 for retro-Diels Alder fragment $[C_{15}H_{26}O]^+$ of norreiterpene having a COOH-group at ring D or E[7], a fragment peak at m/z 207 $[C_{14}H_{23}O]^+$ suggesting the presence of OH-group at the usual C_{-3} position[8] and a peak at m/z 189 for subsequent loss of H_2O. These results are in good agreement with those reported for 14-decarboxyquinovic acid (27-nor-methyl-ursolic acid)[9].

In order to determine the linkage and number of sugar units in ZS_2, it was subjected to permethylation by Harris et al method[6]. The permethylated glycoside was hydrolysed with acid and the resulting partially methylated sugar was identified by GLC and GC/MS analysis of its alditol acetate as 1,5-di-O-acetyl-2,3,4-O-trimethyl-rhamnose (R_t 7.99 min) having the following main fragments m/z 175(8%), 161(27%), 131(77%), 117(64%), 115(50%), 101(100%),89 (60%) and 72(25%)[10].

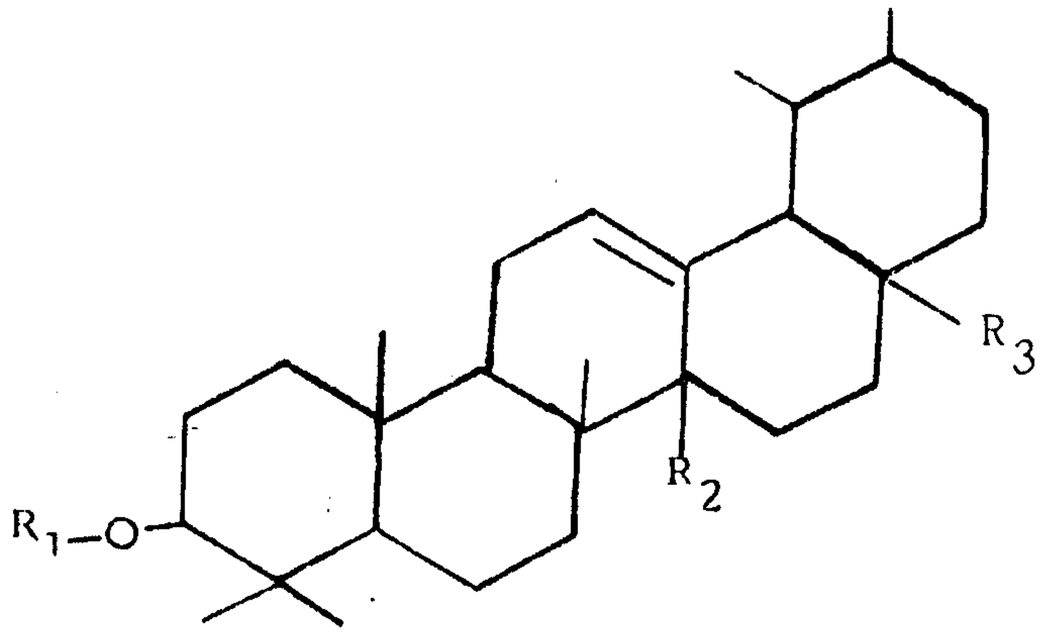
The alkaline hydrolysis did not affect the glycoside indicating ether linked rhamnose. Therefore, saponin ZS_2 proved to be 14-decarboxyquinovic acid-D-(3⟶1)-∝-L-rhamnopyranoside (1). This compound was previously identified in Z-coccenium based on quantitative acid hydrolysis[9].

Compound ZS_3(2) : obtained from fractions (10,11) eluted with solvent mixture $CHCl_3$-MeOH-H_2O (90:3.5:0.2) as needle crystals (MeOH)(30 mg), R_f 0.50 (system b).

The sugar moiety : GLC of the alditol acetate of the aqueous extract of the hydrolysate revealed the presence of D-glucose (R_t 22.32 min). GC/MS showed that the suger linked through its $C-_1$ with the aglycone and gave the fragments m/z 205 (23%), 161(62%), 145(57%), 129(66%), 117(62%), 113(14%), 101 (100%), 88(22%), 87(37%), 71(25%) and 45(73%).

The alkaline hydrolysis did not affect the glucoside indicating the presence of ether linkage. Consequently the glucoside ZS_5 was elucidated as quinovic acid-O-(2⟶1)-B-D-glucopyranoside.

This compound is reported for the first time in Zygophyllaceae and reported before in family Rubiaceae[12,13].



Compound		R_1	R_2	R_3
ZS_2	(1)	L-rhamnopyranoside	H	COOH
ZS_3	(2)	H	COOH (rhamnose ester with either R_2 or R_3)	COOH
ZS_4	(3)	L-rhamnopyranoside	COOH	COOH
ZS_5	(4)	B-D-glucoside	COOH	COOH

The aglycone of compound ZS_3 was isolated after acid hydrolysis and proved to be quinovic acid by direct comparison with authentic sample (R_f, mp and mmp) and reported data[9].

The sugar moiety on GLC and GC/MS analysis of the alditol acetate of the aqueous hydrolysate of compound ZS_3 revealed the presence of one mole of L-rhammose.

The alkaline hydrolysis showed that rhamnose was cleaved indicating that it is linked to the aglycone through ester linkage.

The above mentioned date proved that glycoside ZS_3 is a quinovic acid [28 or 27$\longrightarrow$1]-α-L-rhamnopyranosyl ester. To the best of our knowledge, ,this compound was not previously isolated or reported.

Compound ZS_4(3) : obtained from fractions (6-10) eluted with $CHCl_3$-MeOH-H_2O(90:3.5:0.2) as needle crystals (MeOH)(40 mg), R_f 0.45(system b).

The glycoside ZS_4 was similar to glycoside ZS_3. but differs in alkaline hydrolysis i.e. ZS_4 was not affected indicating that the sugar moiety(L-rhamnose) linked with the aglycone (quinovic acid) through ether linkage. Therefore glycoside ZS_4 is quinovic acid-O- (3$\longrightarrow$1)-α-L-rhamnopyranoside.

This represents the first report for isolation of this glycoside from the family Zygophyllaceae but was reported in Rubiaceae[11].

Compound ZS_5(4) : fractions (11-16) eluted from CC with the same solvent on crystallization from methanol afforded needle crystals (40 mg), R_f 0.41 (system b).

Acid hydrolysis of compound ZS_5 furnished an aglycone and a sugar. The aglycone was found to be the same as that obtained from glycoside ZS_4 (quinovic acid) on the bases of R_f.mp. mmp and MS.

Saponins From *Zygophyllum Album* L.

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صابونينات من نبات زيجوفيللوم ابيـــــض ل

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يتناول هذا البحث فصل والتعرف على الصابونينات الموجودة فى عشب نبات ريجوفيللوم أبيض الذى ينمو فى المناطق الصحراوية الموجودة على ساحل البحـــــر الأحمر بالغرب من مدينة مرسى علم • وتم فى هذا البحث التعرف على خمس مركبات موجودة على هيئة جلوكوزيدات وهـــــى :ـ

١ ـ بيتاسيتوستيرول ـ بيتا ـ د ـ جلوكوز •

٢ ـ ١٤ ـ ديكربوكسى حمض كينوفيك ـ أ ـ (٣ ـــ ١) ـ رامنوزيد •

٣ ـ حمض كينوفيك ـ (٢٨ أو ٢٧ ـــ أ) ـ الفا ـ ل رامنوزيد (جديد) •

٤ ـ حمض كينوفيك ـ أ ـ (٣ ـــ أ) ـ الفا ـ ل ـ رامنوزيد •

٥ ـ حمض كينوفيك ـ أ ـ (٣ ـــ أ) ـ بيتا ـ د ـ جلوكوزيد •

وأستخدمت الطرق المختلفة لفصل والتعرف على هذه المواد مثل كروماتوجرافيا الطبقـــة الرقيقة سابقة التجهيز وكروماتوجرافيا العمود وكذلك كروماتوجرافيـــا الغاز وكروماتوجرافيـــا الغاز مع مطياف الكتلة بالأضافة الى تحضير مشتقـــــات السكاكر كيميائيـــــا •

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