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Isoflavonoids and Alkaloids from *Spartidium saharae*

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Abstract – A new isoflavone, (+)-4'-*O*-methyl-8-*C*-β-D-glucopyranosylgenistein, was isolated from the aerial parts of *Spartidium saharae* together with the known isoflavone, (+)-8-*C*-β-D-glucopyranosylgenistein as well as dipiperidine alkaloids, (+)-ammodendrine and (+)-*N*-acetyl hystrine. Details of their structure elucidation are based on chemical and spectroscopic methods. N-formyl ammodendrine was detected by GC-MS. The potential chemotaxonomic value of the alkaloid content is explored. Cytotoxic activity has been determined for both alcoholic extract and isolated compounds.
Key words – Fabaceae, aerial parts, isoflavone-*C*-glycosides, (+)-4'-*O*-methyl-8-*C*-β-D-glucopyranosylgenistein, dipiperidine alkaloids, chemotaxonomy, cytotoxic activity

Introduction

Spartidium saharae Coss. et Dur. Pomel (=*Genista saharae* Coss. et Dur.) belonging to Fabaceae is a small leafless wild desert shrub growing in Libya (Jafri and El-Gaadi, 1980). It is a monotypic genus, confined to the saharan desert areas, especially Libya and Algeria. The status of *Spartidium* as a distinct genus was based on morphological difference from *Genista*, since the calyx of *Spartidium* is campanulate, 5-dentate, with short triangular teeth, while *Genista* has two- lobed calyx (Jafri and El-Gaadi, 1980; Bourquin *et al.*, 1987). However, Bourquin *et al.* (Bourquin *et al.*, 1987) stated that, no chemotaxonomic differences between *Genista* and *Spartidium* based on their chemical study on *Spartidium saharae* grown in Algeria where they reported the isolation and identification of (+)-ammodendrine, while *N*-methyl ammodendrine, *N*-acetyl hystrine, anabasine, sparteine, lupanine and cytisine were tentatively chracterized by GC-MS. In the course of our studies on leguminous plants (Abdel-Halim *et al.*, $1992_{a,b}$, 1995, 1997; Abdel-Halim, 1995), we have investigated the constituents of the aerial parts of *Spartidium saharae* growing in Libya. In the present study, the isolation and structural elucidation of isoflavone glycosides as well as some dipiperidine alkaloids was reported. Identification of isolated compounds was established through chemical and spectroscopic methods. The chemotaxonomic value of the alkaloid content is discussed. Cytotoxic activity has been assessed for both alcoholic extract and isolated compounds.

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Experimental

Plant material – The aerial parts of *Spartidium saharae* were collected from Tripoli (Libya) during flowering season. A voucher specimen was identified by Professor Dr. A. El-Gady, Faculty of Science, El-Faateh University, Tripoli and deposited in Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.

Extraction – The air dried powdered aerial parts of *Spartidium saharae* (1 kg) were extracted by percolation with 75% EtOH until the last fraction gave a negative response to Dragendorff's reagent. The extract was concentrated under reduced pressure, diluted with equal volume of water and divided into two equal portions. The first portion was defatted with pet. ether then extracted with EtOAc (4×400 ml). The EtOAc extract was concentrated to afford 11.0 gm extract (flavonoid fraction). The second portion was acidified with 0.5 N HCl and left standing at room temperature for 30 minutes. After centrifugation, the supernatant was alkalinized with NH_4OH to pH 9, extracted with CH_2Cl_2 (4×300 ml). The CH_2Cl_2 extract was dried over anhydrous Na_2SO_4 and the solvent was evaporated in vacuum. The alkaloid mixture was obtained as pale yellow oil in yields of 1.5% of the dry weight.

Isolation of flavonoids – 7 gm of EtOAc extract was chromatographed on Diaion HP-20 (Mitsubishi

Chemicals San., Tokyo, Japan), eluted with water with increasing proportions of MeOH to give 6 main fractions. Fraction 4 (50% MeOH) afforded compound $\mathbf{F_1}$ (300 mg), while fraction 5 (55% MeOH) afforded fraction enriched in $\mathbf{F_1}$ together with $\mathbf{F_2}$. Pure $\mathbf{F_2}$ (200 mg) was obtained from these fractions by further purification on silica gel C.C. using solvent system c.

(+)-4'-*O*-methyl-8-*C*-β-D-glucopyranosylgenistein ($\mathbf{F_1}$); white amorphous solid, mp. 214-220°, $[\alpha]_D^{26}$ +12°(MeOH, c. 0.15); UV λ_{max}^{MeOH} nm: 258, 320_{sh}; IRν_{max}^{KBr} cm^{-1}: 3400 (OH), 1640 (C=O), 1615, 1580 (C=C); HR-FABMS, *m/z*: (447.1290[M+H]$^+$, $C_{22}H_{23}O_{10}$ calcd. 447.1291); EIMS *m/z* (rel. int.): 429 ([M-OH]$^+$ 22), 415(100), 397 (62), 314(13), 297 (32), 283 (40), 281(17), 254 (16), 165 (18), 153(16), 132(18); ^{1}H-NMR (CD_3OD, 400 MHz) and ^{13}C-NMR (CD_3OD, 100 MHz): Table 1.

(+)-8-*C*-β-D-glucopyranosyl genistein ($\mathbf{F_2}$); yellow crystals, mp. 215-217° (EtOAc), $[\alpha]_D^{26}$ +16.6° (MeOH, c. 0.12); UV λ_{max}^{MeOH} nm: 255, 326_{sh}; IRν_{max}^{KBr} cm^{-1} : 3420 (OH), 1638 (C=O), 1620, 1585 (C=C); EIMS *m/z* (rel. int.): 414 ([M-H_2O]$^+$, 82), 310(86), 284 (30), 283 (100), 270(72), 165(15), 153(16), 148(16), 118 (24); ^{1}H-NMR (CD_3OD, 400 MHz) and ^{13}C-NMR (CD_3OD, 100 MHz): Table 1.

$FeCl_3$ oxidation of compounds $\mathbf{F_1}$ and $\mathbf{F_2}$ – as previously reported (Markham, 1982).

Bioassay of cytotoxic activity against mouse P388 – as previously reported (Twentyman and Luscombe, 1987).

Analysis of alkaloids by GC/MS – The total alkaloid was analyzed by combined Gas chromatography-mass spectrometry. Fragmentation patterns were compared with published data (6,13,14,19, 20). Three bases could be identified as follows: ammodendrine, mass spectrum *m/z* (rel. int.) 208 ([M]$^+$ 48), 179(41). 165(100), 137 (40), 136(50), 110 (78), 109(65), 43(45), t_R 11.7, relative % composition 83.6.

***N*-acetyl hystrine**: mass spectrum *m/z* (rel. int.) 206([M]$^+$62), 163(100), 135(15), 108(32), 43(41), t_R 12.45, relative % composition 12.18.

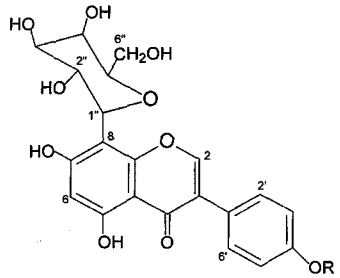
***N*-formyl ammodendrine**: mass spectrum *m/z* (rel. int.) 236([M]$^+$10), 218(100), 208(22), 175(66), 150(32), 122(29), 43(21), t_R 14.26, relative % composition 3.02.

Isolation of alkaloids – The mixture of bases (5 gm) was chromatographed on a silica gel-packed column (Merck, type 60, 230-400 mesh, 200 g) and gradient elution using MeOH in CH_2Cl_2-28% NH_4OH (500:1) as reported in previous papers (4-5). Compound $\mathbf{A_1}$ (1.1 gm) was eluted with 7% MeOH in CH_2Cl_2-28% NH_4OH (500:1). Fractions enriched in compound $\mathbf{A_2}$ (82 mg) were eluted with 8% MeOH in CH_2Cl_2-28% NH_4OH (500:1) together with $\mathbf{A_1}$. Pure $\mathbf{A_2}$ (8 mg) was obtained from these fractions by further purification on preparative TLC (Silica gel, Kiselgel 60, F254, 0.5 mm layer thickness) in Et_2O-MeOH-28% NH_4OH (17:2:1).

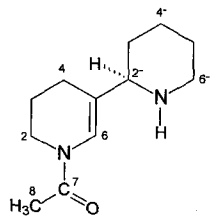
(+)-Ammodendrine ($\mathbf{A_1}$); pale yellow oil, $[\alpha]_D^{26}$ +7.5°(EtOH, c. 0.30) UV λ_{max}^{MeOH} nm: 318, 241; IRν_{max}^{CHCl3} cm^{-1}: 1642(carbamido group), 1425 (C = C); ^{1}H NMR ($CDCl_3$, 400 MHz), 7.14, 6.54(1H, s, H-6), 3.55(1H, m, H-2), 3.01(2H, dm, H-2',$6'_{eq}$), 2.62(1H, m, H-$6'_{ax}$), 2.1, 2.06(3H, s, CH_3), ^{13}C-NMR ($CDCl_3$, 100 MHz): as reported in (Saito *et al.*, 1989).

(+)-*N*-acetyl hystrine ($\mathbf{A_2}$); colorless oil, $[\alpha]_D^{26}$ + 11° (EtOH, c. 0.08); IRν_{max}^{CHCl3} cm^{-1}: 1640(carbamido group), 1430 (C=C); ^{1}H-NMR ($CDCl_3$, 400 MHz), 7.18, 7.78(1H, s, H-6), 3.63(2H, m, 2H-6'), 2.02(3H, s, CH_3); EIMS for $\mathbf{A_1}$, $\mathbf{A_2}$: identical to those mentioned under GC-MS.

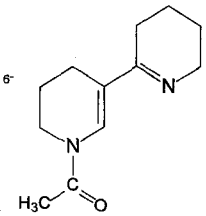
Some of ^{1}H NMR spectral peaks of $\mathbf{A_1}$, $\mathbf{A_2}$ are doubled because of the presence of two conformer



$\mathbf{F_1}$ R=CH_3
$\mathbf{F_2}$ R=H



$\mathbf{A_1}$



$\mathbf{A_2}$

isomers from restricted rotation about *N*-Ac bond.

Identification procedures – Mps. Uncorr. IR; in $CHCl_3$ or KBr; optical rotations: 10 cm path length in the solvent stated; ^{1}H-NMR and ^{13}C-NMR: 400 and 100 MHz, respectively. TMS was used as internal standard in $CDCl_3$ and CD_3OD; EIMS: 70 ev.; FAB-MS: Kratos MS-25 using xenon as a bombardment gas and glycerol as a viscus matrix. TLC: Silica gel (kiselgel 60, F254) of 0.25 mm layer thickness in CH_2Cl_2-MeOH-28% NH_4OH (43:6:1)[a], Et_2O-MeOH-28% NH_4OH (17:2:1)[b] and EtOAc-MeOH-H_2O (10:1:0.8)[c]. GC/MS was performed with Hewlett-Packard 598011/5971A, EI-MS (probe, 70eV), equipped with DB-1 (0.25 mm×30 m) (J & W Scientific Co.) column using He as a carrier gas (0.6 ml/min.). The oven was kept for 2 min. at 100°, then programmed at 15°/min.to 300°, hold for 5 min.

Results and Discussion

Fractionation of the ethyl acetate fraction of the ethanolic extract of the aerial parts of *Spartidium saharae* on Diaion HP-20 followed by silica gel column chromatography, afforded two major compounds **F$_1$** and **F$_2$**. The isolated compounds gave positive tests for flavonoid glycosides (Markham, 1982). **F$_2$** was identified as (+)-8-C-β-D-glucopyranosyl genistein on basis of mp, $[\alpha]^D$, IR, MS, ^{1}H-NMR and ^{13}C-NMR as reported previously (Heerden *et al.*, 1980). It is first reported in the title plant.

F$_1$ was isolated as amorphous solid, mp 218-220°. The UV spectrum of **F$_1$** in methanol displayed λ_{max} at 258 nm and a shoulder at 320 nm, typical of an isoflavone type (Markham, 1982). The IR spectrum suggested the presence of hydroxyl (3400 cm^{-1}), carbonyl (1640 cm^{-1}) and aromatic (1615, 1580 cm^{-1}) groups. The HR-FABMS afforded an $[M+H]^+$ *m/z* 447.1290, corresponding to $C_{22}H_{23}O_{10}$ (calcd. 447.1291). The ElMS spectrum of **F$_1$** afforded fragment ions at *m/z* 429&415, corresponding to $[M-OH]^+$ and $[M-OCH_3]^+$, respectively. Also fragment ion at *m/z* 297 is consistent with aglycone fragment containing CH_2-reminant of the C-linked sugar, indicating the presence of C-glucoside (Oelrichs *et al.*, 1968). The retro-Diels-Alder cleavage of the latter fragment was observed at *m/z* 165 indicating the presence of two hydroxyl groups in ring-A and also at *m/z* 132 supported the presence of one methoxyl group in ring-B. The ^{1}H-NMR and ^{13}C-NMR spectra of **F$_1$** and **F$_2$** are almost the same (Table 1), except for the presence of OMe group in **F$_1$** at δ 3.78 and 56.4 ppm.

Acid hydrolysis of both **F$_1$** and **F$_2$** with dil HCl failed to give any sugar product or aglycone while $FeCl_3$ oxidation (Markham, 1982) afforded glucose confirming the presence of C-glucopyranoside unit.

From all the foregoing chemical and spectroscopic evidences, it can therefore be established that, the new compound **F$_1$** is (+)-4'-*O*-methyl-8-*C*-β-D-glucopyranosylgenistein.

The alkaloid profile of 75% ethanol extract of the dried aerial parts of *Spartidium saharae* is clarified using combined gas chromatography-mass spectrometry. Three bases could be identified on the basis of their mass spectra and fragmentation pattern *viz.* ammodendrine, *N*-acetyl hystrine and *N*-formyl ammodendrine. Only two bases could be isolated by repeated silica gel column chromatography and completely identified as (+)-ammodendrine **A$_1$** and (+)-*N*-acetyl hystrine **A$_2$** on the basis of physicochemical properties and spectral data including $[\alpha]^D$, IR, MS, ^{1}H-NMR and ^{13}C-NMR as reported previously (Bourquin *et al.*, 1987; Abdel-Halim *et al.*, 1997; Saito *et al.*, 1989; Fitch *et al.*, 1974). The presence of *N*-formyl ammodendrine in trace amount precludes its separation and characterization by other spectral techniques. N-formyl ammodendrine is first reported

Table 1. ^{1}H-NMR and ^{13}C-NMR spectra (ppm from TMS) of **F$_1$** & **F$_2$** in CD_3OD

Carbon No.	F$_1$		F$_2$	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
2	8.0*s*	153.3	8.08*s*	154.9
3		124.7		124.7
4		180.9		182.1
5		162.5		162.7
6	6.4*s*	97.5	6.3*s*	98.1
7		163.9		164.9
8		105.7		106.8
9		158.6		159.1
10		104.0		104.6
1'		124.4		123.5
2',6'	7.3 *d* (8.5)	131.5	7.32 *d*(8.5)	131.6
3',5'	6.8 *d*(8.5)	116.1	6.8 *d*(8.5)	116.6
4'		158.6		159.1
Glc-1"	3.4-3.7 *m*	75.7	3.5-3.8 *m*	75.7
2"		73.0		73.1
3"		80.1		80.4
4"		71.7		72.1
5"		82.7		82.9
6"		62.8		63.2
OMe	3.78*s*	56.4		

J in Hz between parentheses

in the title plant.

On the bases of our present study, *Spartidium saharae* yields apperciable quantities of isoflvones which are quite common in *Genista* species. However, the alkaloid content in *Spartidium saharae,* ammodendrine, *N*-acetyl hystrine, *N*-formyl ammodendrine, was found to be confined principally to dipiperidine alkaloids. TLC, GC and GC-MS make sure that the Lybian *Spartidium saharae* is free from sparteine or α-pyridone-type alkaloids, in contrary to the Algerian one reported before (Bourquin *et al.*, 1987). Lupin alkaloids might be considered as systematic marker for chracterization of closely related genera (Kinghorn and Balendrin, 1984). Most *Genista* species accumulate mainly, sparteine- and α-pyridone -types alkaloids, while few species accumulate in addition, small amount of simple dipiperdine alkaloids (Kinghorn and Balendrin, 1984; Harborne *et al.*, 1971; Faugeras, 1971). Thus, the presence of high yield of the dipiperdine alkaloids in the title plant is likely to receive attention as potential taxonomic marker for *Spartidium.*

The cytotoxic activity of the alcoholic extract of *Spartidium saharae* showed a moderate activity against mouse P388 leukemia cells with IC_{50} 11 μgml^{-1}. However, none of the isolated compounds has displayed a significant cytotoxic activity.

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