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ISOFLAVONOIDS FROM *SALSOLA SOMALENSIS*

YORDANOS WOLDU and BERHANU ABEGAZ*

Department of Chemistry, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia

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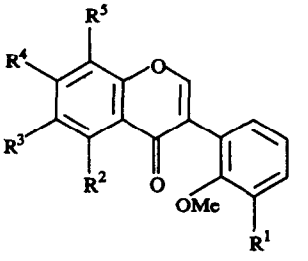
Abstract—Three new isoflavones, 5,3′-dihydroxy-7,8,2′-trimethoxy isoflavone, 5,3′-dihydroxy-2′-methoxy-6,7-methylenedioxyisoflavone and 5,3′-dihydroxy-6,7,8,2′-tetramethoxyisoflavone, have been isolated and identified from the roots of *Salsola somalensis* These are found to possess an unusual 2′,3′-oxygenation pattern. ^{1}H and ^{13}C NMR data are given for the compounds and their methyl ethers.

INTRODUCTION

The genus *Salsola* consists of over 100 species found in the drier parts of Asia, Europe and Africa. Two species are reported from Ethiopia, namely *S somalensis* N.E. Br and *S. spinescens Salsola somalensis* (synonym *Halotamnus somalensis* and *S. bottae*) is used in local medicine for the treatment of tapeworm infestations Root parts (*ca* 3 g) are employed as tooth sticks, the fibrous roots slowly disintegrating in the mouth, and the juice is swallowed. This is claimed to result in the expulsion of the parasite

The chemical literature contains some information on Asian *Salsola* species Triterpenes and their glycosides [1], common flavonoids such as rutin, quercetin and isorhamnetin glycoside [2], the antihypertensive isoquinoline alkaloids salsoline and salsolidine [3] as well as coumarins and glycosides [4] have been reported There is, however, no report in 'Chemical Abstracts' on *S. somalensis*.

As part of our continuing interest on the phytochemical investigation of marketed medicinal plants, we have studied the composition of the chloroform extract of the roots of *S. somalensis* We report here the isolation and structural elucidation of three new isoflavones **1**–**3** besides some known triterpenes.



1 $R^1 = R^2 = OH$, $R^3 = H$, $R^4 = R^5 = OMe$

2 $R^1 = R^2 = OH$, $R^3, R^4 = OCH_2O$, $R^5 = H$

3 $R^1 = R^2 = OH$, $R^3 = R^4 = R^5 = OMe$

4 $R^1 = R^2 = R^4 = R^5 = OMe$, $R^3 = H$

5 $R^1 = R^2 = OMe$, $R^3, R^4 = OCH_2O$, $R^5 = H$

RESULTS AND DISCUSSION

The residue from the chloroform extract of the roots were defatted with petrol The defatted sample was examined by TLC and found to be rich in terpenoids and phenolic compounds. The initial trials to determine the nature of the components demonstrated a rather difficult-to-separate mixture of compounds. Attempts to isolate pure compounds by column and preparative layer chromatography (silica gel) were unsuccessful. In fact fractions obtained from column, and which seemed homogeneous on normal TLC plates (Kieselgel 60) using a variety of solvents, were found to be mixtures when examined by NMR and with HPTLC plates. The most suitable procedure involved successive fractionations by VLC and column followed by preparative TLC using thin layer plates (Sil G-25 UV_{254}, 0.25 mm) as described in the Experimental. Accordingly, the chloroform extract yielded three pure isoflavones These were characterized as 5,3′-dihydroxy-7,8,2′-trimethoxyisoflavone (**1**), 5,3′-dihydroxy-2′-methoxy-6,7-methylenedioxyisoflavone (**2**) and 5,3′-dihydroxy-6,7,8,2′-tetramethoxyisoflavone (**3**).

The IR spectrum of **1** showed absorption bands at 3421 cm^{-1} (–OH), 1651 cm^{-1} (C=O) and several bands assignable to aromatics and C–O functionalities In the HRMS the molecular ion was observed at *m/z* 344.0905 suggesting the molecular formula $C_{18}H_{16}O_7$. The UV spectrum showed an absorption band at 260 nm which underwent a bathochromic shift of 13 nm in the presence of aluminium trichloride, indicating the presence of an hydroxy group at C-5 [5]. In addition the ^{1}H NMR showed a signal at δ12.55 confirming the presence of a chelated hydroxy group. The ^{1}H NMR spectrum also showed a signal at δ8.02 (1H, *s*) which is due to the proton at C-2 of isoflavonoids The 400 MHz ^{1}H NMR showed a 3H ABX system at δ7.07 (1H, *t*, *J*=7.8 Hz) at δ7.00 (1H, *dd*, *J*=8.15 and 1.69 Hz) at 6.89 (1H, *dd*, *J*=7.57

Table 1 ^{13}C NMR spectral data of compounds **1**–**5** (22 5 MHz, DMSO for **1** and **2** $CDCl_3$ for **3**–**5**)

C	1	2	3	4	5
2	155 273	159 649	154 792	151.885	151 842
3	120 875	125 178	120 004	123 038	122 954
4	180 578	185 174	180 950	174 990	174 903
5	158 613[a]	158 552[b]	153 256	156 923[c]	154 962
6	96 326	134 222	136 849	94 869	135 948
7	157 175[a]	157 601[b]	145 052	156 354[c]	148 136
8	129 625	94 120	128 292	131 489	93 336
9	149 540	154.603	149 258	148 120	142 115
10	104.663	111.843	107 647	110 795	114 374
1′	125 019	129.298	123.563	126 424	126 489
2′	150 202	150 702	150 006	153 077	153 158
3′	146 375	145 778	145 455	152 373	152 841
4′	117 633	121 984	116 492	113 450	113 545
5′	123 800	128 152	124 855	124 284	124 198
6′	121 923	126 299	123 246	123 418	126 515
–OMe	56 711	64 305	61 106	60 687	56 184
	59 929		61 179	61 472	60 646
	61 221		61 667	56 162	61 208
			62 178	56 570	
				57 138	
–OCH_2O–		107 406			

[a–c]Assignments may be interchanged

and 1.69 Hz) suggesting a trisubstituted ring. The proton at C-6 gave a signal at 6.46 (1H, *s*). Signals attributed to three methoxy groups were observed at δ3 95, 3.91 and 3.65 The above data, in conjunction with the ^{13}C NMR spectra, (editing DEPT sequence) were found to be consistent with an isoflavone structure consisting of 10 quaternary, five tertiary and three primary carbons The presence of $[M-31]^+$ in the mass spectrum indicated a methoxy group at position C-2′ [6]. An ion at *m/z* 196 was assigned to the retro-Diels–Alder fragmentation product Ring A should thus be substituted with two methoxy and one hydroxy groups The two methoxy groups should be at C-7 and C-8 to explain the one proton singlet signal at δ6.46 observed in the ^{1}H NMR spectrum The alternative structure which would have this proton at C-8 and the two methoxy groups at C-6 and C-7 was ruled out by the finding that the chemical shifts of the five methoxy groups in the ^{13}C NMR spectrum of the pentamethyl ether (**4**) showed the presence of only two *ortho* disubstituted methoxy groups [7] (Table 1)

The HR mass spectrum of **2** 328.060 suggested the molecular formula $C_{17}H_{12}O_7$ The spectroscopic data for this compound were similar to those of compound **1** and it was assumed to be an isoflavone ^{1}H NMR showed the same ABX pattern for ring B, at δ6 50 and 8 00 (1H, *s*, each), at δ6 10 (2H, *s*), one methoxy at 3 60 and chelated hydroxy at δ12 60 of C-5 which was also confirmed by a bathochromic shift of 8 nm in the presence of aluminium trichloride [5]. The presence of $[M-31]^+$ in the mass spectrum and the chemical shift in the ^{13}C NMR spectrum of the methoxy at 64 305 indicated the location of the methoxy at C-2′ Ring A has the –OCH_2O– group at C-6, C-7 and an hydroxy group at C-5. The alternative structure, –OCH_2O– at C-7 and C-8, was ruled out by the finding that the chemical shifts of the three methoxy groups in the ^{13}C NMR spectrum of the tetramethyl ether (**5**) showed the presence of two *ortho* disubstituted methoxy groups [7].

The HR mass spectrum of **3** 374.0996 indicated the molecular formula $C_{19}H_{18}O_8$ ^{1}H NMR showed similar ABX pattern for ring B, at 8 05 ppm (1H, *s*), four methoxy groups at δ4.11–3 65 and a chelated hydroxy at δ12.50 The latter was also confirmed by UV data which showed a bathochromic shift of 12 nm in the presence of aluminium trichloride [5] The 1H, *s* at δ6 46 and 6.50 in **1** and **2** respectively was not observed in the ^{1}H NMR spectrum of **3** The presence of $[M-31]^+$ and the chemical shifts of the methoxyls in the ^{13}C NMR spectrum supported the structure [7].

The structures of these isoflavones are unusual because they lack oxygenation at 4′-position. There are only a few such isoflavonoids reported so far Two of these (7,2′-dihydroxy-6-methoxyisoflavone and 2′-hydroxy-6,7-methylenedioxyisoflavone) have been isolated from *Salicornia europaea* [8] and one (2′-hydroxy-5-methoxy-6,7-methylenedioxyisoflavone), from *Beta vulgaris* [9] It may be chemotaxonomically significant that both these plants belong to the Chenopodiaceae as does *S somalensis* All three isoflavonoids presented in this report have identical substitution in ring B and also possess an hydroxy group at C-5 The absence of oxygenation at C-4′ is biosynthetically significant and makes the spirodienone intermediate often proposed during the biosynthesis of the usual isoflavonoids improbable [10] for these isoflavonoids However an isomeric spirodienone intermediate resulting from 2′-hydroxy precursor cannot be ruled out

EXPERIMENTAL

Plant material Root parts of *Salsola somalensis* were collected 200 km along the Bale Goba-Imi road, alt 710 m during September 1987 A voucher sample is deposited at the Herbarium of

Addis Ababa University (BA 020). The plant material collected from Bale province was compared (TLC) with the root part of the plant sold in the market and found to be identical For extraction purposes *S somalensis* was bought from a street vendor at the main entrance to the Anwar Mosque in Merkato, Addis Ababa.

Extraction. Air-dried powdered roots were first extracted with petrol and then exhaustively with $CHCl_3$. The residue, after evapn of $CHCl_3$ was extracted again with petrol to remove fatty substances The crude extract was soaked with $CHCl_3$ and the soluble part was applied on VLC using $CHCl_3$ as eluent and the following, 100 ml each, fractions were collected 1–5 contains β-amyrin, β-sitosterol and lupeol, 6–10 contained mixture of the compounds **1–3** and some terpenes, 11–17 contained mixture of **1–3** and more polar compounds. Fractions 6–10 and 11–17 were combined and soaked in $CHCl_3$. The $CHCl_3$-soluble part was applied on silica gel column and eluted with 5% EtOAc in CH_2Cl_2. Fractions were monitored using HPTLC Finally the mixtures were purified on prep thin layer plates, Sil G-25 UV_{254}, 0 25 mm, using the same solvent system to give pure **1–3**

5,3′-*Dihydroxy*-7,8,2′-*trimethoxyisoflavone* (**1**). Light yellow crystals, mp (MeOH–CH_2Cl_2 mixture) 222–224°. ^{1}H NMR (400 MHz, $CDCl_3$) 12 55 (1H, *s*, OH-5), 8 02 (1H, *s*, H-2), 7 07 (1H, *t*, *J*=7 8 Hz, H-5′), 7 00 (1H, *dd*, *J*=8.15, 1.69 Hz, H-6′), 6.89 (1H, *dd*, *J*=7 57, 1 69 Hz, H-4′), 6.46 (1H, *s*, H-6), 3.95, 3 91, 3.65 (3H each, *s*, –OMe at 2′, 7, 8,) UV λ_{max}^{MeOH} nm (log ε) 260 (4 3190) and showed a bathochromic shift of 13 nm in $AlCl_3$. IR ν_{max}^{KBr} cm^{-1} 3421, 3106, 2940, 2860, 1651, 1581, 1501, 1456, 1421, 1361, 1306, 1251, 1211, 803 MS *m/z* (rel int.) 344.0905 (80 67), 329 (100), 313 (9 2), 197 (0 7), 181 (6 7).

5,3′-*Dihydroxy*-2′-*methoxy*-6,7-*methylendioxyisoflavone* (**2**) Crystals, mp (MeOH–$CHCl_3$ mixture) 235–237°. ^{1}H NMR (90 MHz, $CDCl_3$): 12 60 (1H, *s*, OH-5), 8 00 (1H, *s*, H-2), 7 10–6 70 (3H, *m*, H-4′,5′,6′), 6 50 (1H, *s*, H-8), 6 10 (2H, *s*, –OCH_2O–6, 7), 3 60 (3H, *s*, –OMe at 2′). UV λ_{max}^{MeOH} nm (log ε): 268 nm (4.2277) showed a bathochromic shift of 8 nm in the presence of $AlCl_3$ IR ν_{max}^{KBr} cm^{-1} 3366, 3106, 2941, 2861, 1671, 1621, 1556, 1486, 1461, 1321, 1283, 1201, 1031, 731 MS *m/z* (rel int.): 328.060 (100), 297 (88 70), 181 (22 58), 148 (12.90).

5,3′-*Dihydroxy*-6,7,8,2′-*tetramethoxyisoflavone* (**3**) Yellow amorphous solid, mp 127–129° ^{1}H NMR (90 MHz, $CDCl_3$) 12 50 (1H, s, OH-5), 8 05 (1H, *s*, H-2), 7.10–6 80 (3H, *m*, H-4′, 5′,6′), 4 11, 3 94, 3 65 (3H, 6H, 3H resp., *s*, –OMe at 2′,6,7,8). UV λ_{max}^{MeOH} nm (log ε). 264 (4 379) and showed a bathochromic shift of 12 nm in the presence of $AlCl_3$ IR ν_{max}^{KBr} cm^{-1} 3356, 3107, 2951, 2861, 1651, 1576, 1456, 1416, 1361, 1326, 1264, 1206, 1100, 1019 MS *m/z* (rel int.) 374 0996 (100), 359 (77 83), 343 (4), 227 (1 02), 147 (2.03)

5,7,8,2′,3′-*Pentamethoxyisoflavone* (**4**). Compound **1** (100 mg) was refluxed for 24 hr with 0.6 ml Me_2SO_4 and 3 g dry K_2CO_3 in 25 ml dry Me_2CO The soln was cooled, filtered and evapd to dryness The residue was purified on silica gel column and gave 80 mg crystals of **4** mp (MeOH–CH_2Cl_2 mixture) 142–143°. ^{1}H NMR (90 MHz, $CDCl_3$) 7 86 (1H, *s*, H-2), 7.10–6 80 (3H, *m*, H-6′,5′,4′), 6 43 (1H, *s*, H-6) and 3 97, 3 92, 3.89, 3.85, 3 69 (3H each, *s*, –OMe at 2′, 3′, 5, 7, 8) UV λ_{max}^{MeOH} nm 247 IR ν_{max}^{KBr} cm^{-1} 2962, 2856, 1641, 1600, 1570, 1456, 1346, 1261, 1064

5,2′,3′-*trimethoxy*-6,7-*methylendioxyisoflavone* (**5**). Compound **2** (200 mg) was refluxed for 24 hr with 1 3 ml Me_2SO_4 and 6 g dry K_2CO_3 in 25 ml dry Me_2CO. The solution was cooled, filtered, evapd and purified in silica gel column to give 160 mg of crystals (MeOH–$CHCl_3$ mixture) mp 138–139° ^{1}H NMR (90 MHz, $CDCl_3$): 7.77 (1H, *s*, H-2), 7.15–6 80 (3H, *m*, H-6′, 5′, 4′), 6 60 (1H, *s*, H-8), 6.01 (2H, *s*, –OCH_2O-6, 7), 4 03, 3 85, 3.70 (3H each, *s*, –OMe at 2′, 3′ ,5) UV λ_{max}^{MeOH} 247 nm IR ν_{max}^{KBr} cm^{-1} 2962, 2848, 1617, 1462, 1422, 1252, 1042

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