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Chemical Constituents of *Linaria aucheri*

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From the petroleum ether and methanol extracts of the aerial parts of *Linaria aucheri* (Scrophulariaceae) 6 known compounds: β-amyrin (**1**), ergost-7-en-3β-ol (**2**), stigmasta-5,22-*E*-dien-3β-ol (**3**), stigmast-5-en, 24*S*-3β-ol (**4**), antirrinoside (**5**) and linariin (**6**), were isolated and identified.

The structures of these compounds have been elucidated by spectroscopic methods (UV, IR, ^{1}H NMR, ^{13}C NMR, DEPT-135 and GC-MS).

Key Words: *Linaria aucheri*, Scrophulariaceae, lypophylic compounds, β-amyrin, antirrinoside, linariin.

Introduction

The genus *Linaria* (Scrophulariaceae) is represented by 20 species in the flora of Turkey[1]. Several species have been used in traditional medicine as tonics, antiscorbutics, laxatives, antidiabetics and diuretics, as well as for the treatment of wounds, hemorrhoids and vascular disorders[2,3].

Previous studies of *Linaria* species have shown the presence of flavonoids and their glycosides, as well as ionol glucosides, iridoids, alkaloids, diterpenoids and phenylethanoids[3−12]. In the present paper, we report the initial isolation and identification of some known lypophylic compounds, together with an iridoid and flavon glycosides, from the aerial parts of *Linaria aucheri* petroleum ether and methanol extracts.

Experimental

General Experimental Procedures: UV spectra were obtained in MeOH on a Shimadzu spectrophotometer UV 160A. IR spectra were recorded on a Perkin Elmer spectrophotometer FTIR 1720X. The ^{1}H and ^{13}C spectra were obtained on a Bruker WP 200 SY spectrometer at 200 and 50.29 MHz, respectively. Silica gel 60 (0.063-0.200 mm, 7734) for column chromatography was purchased from Merck. Vacuum-liquid

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chromatography (VLC) was performed on a glass column (2.2 x 15 cm) filled with RP-18 (20-45 μm, LiChroprep) material. GC-MS apparatus: Hewlett-Packard 5890 Series II. Ion Sourced: 70 eV electron-impact ion source. Column used: SPB-1, methylsilicone, 12 m x 0.2 mm internal diameter, 0.33 μm width wall. Initial temperature: 100 ^{o}C, 5 ^{o}C increase per min. Final temperature: 300 ^{o}C for 5 min. Carrier gas: He.

Plant Material: *Linaria aucheri* Boiss. (Scrophulariaceae) was collected from Baykuşboğazı, Çankırı (Northeast Anatolia) in July 1997. A voucher specimen has been deposited in the Herbarium of the Faculty of Pharmacy, Hacettepe University (HUEF 97-020).

Extraction and Isolation: The air-dried and powdered aerial parts of *L. aucheri* (950 g) were extracted successively with petroleum ether in a mechanical stirrer (40-60 $^\circ$C) (3 x 2.5 l) and methanol (2 x 1.5 l). The petroleum ether extracts were evaporated to dryness in vacuo (10 g) and then defatted with acetone (6 x 100 mL). The crude extract (4.5 g) was subjected to the Si gel column (3 x 40 cm) with a gradient elution of 100% hexane to 25% ethyl acetate (1 l; 20 mL, each), to give 2 distinctive fractions (AI and AII). Fraction AI (Fr. 27-32, 338 mg) was purified by preparative TLC (5554 Merck) with a mixture of cyclohexane-ethyl acetate (8:2) to yield **1** (17 mg). Fraction AII (Fr. 34-48, 80 mg) is a mixture of compounds **2**–**4**, with a ratio of 1:1:2, respectively.

The methanolic extract (8 g) was subjected to RP-18 [20-45 μm, LiChroprep C-18 (Merck)] VLC with a gradient elution of methanol-water (10% to 90% MeOH) mixture. This yielded 5 main fractions (Frs. BI-BV, each of 100 mL). Fraction BII (1300 mg) yielded an iridoid (**5**), whereas fraction BV (110 mg) yielded a flavonoid (**6**).

Acid Hydrolysis of 5 and 6: Compound **5** was applied to a TLC plate (Silica gel 60 F_{254}, 0.2 mm, Merck) and the plate was treated with concentrated HCl vapor in a closed tank for 1 h. After the evaporation of the concentrated HCl, authentic sugar samples were applied to the TLC plate, and the plate was developed in a EtOAc-MeOH-conc.HOAc-H_2O (60:15:15:10, v/v) solvent system. Spots were visualized by spraying with a Thymol-EtOH-conc.H_2SO_4 (0.5 g:95 mL:5 mL) reagent and heated at 110 $^\circ$C for 5 min. The same procedure was used for compound **6**, though this was treated for 2 h with concentrated HCl vapor. The sugars were identified as glucose for compound **5**, and glucose and rhamnose for compound **6**.

Results and Discussion

β-Amyrin (1): UV λ_{max} (MeOH): 240.2 nm; IR γ_{max} (KBr) cm^{-1}: 3397, 2932, 1645, 1465, 1379, 1029, 900; ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.12 (m, H-12), 3.23 (m, H-3), 1.13, 0.99, 0.97, 0.94, 0.87 (x 2), 0.83, 0.79 (CH_3). ^{13}C NMR (50.29 MHz, $CDCl_3$): δ 38.14 (C-1), 27.51 (C-2), 79.12 (C-3), 38.88 (C-4), 55.27 (C-5), 18.44 (C-6), 33.03 (C-7), 38.82 (C-8), 47.81 (C-9), 37.00 (C-10), 23.48 (C-11), 121.82 (C-12), 145.28 (C-13), 42.18 (C-14), 26.12 (C-15), 27.37 (C-16), 32.04 (C-17), 47.22 (C-18), 46.93 (C-19), 31.34 (C-20), 34.83 (C-21), 37.26 (C-22), 28.22 (C-23), 15.47 (C-24), 15.72 (C-25), 16.97 (C-26), 25.56 (C-27), 28.44 (C-28), 33.44 (C-29), 23.48 (C-30); EI-MS: m/z 426 (M^+, 0.6%), 411 (0.1%), 218 (100%), 272 (0.1%), 189 (30%), 135 (34%), 95 (48%).

Ergost-7-en-3β-ol (2): EI-MS: m/z 400 (M^+, 100%), 382 (43%), 367 (30%), 273 (32%), 255 (36%), 161 (48%), 147 (40%), 107 (77%), 43 (84%).

Stigmasta-5,22*E*-dien-3β-ol (3): EI-MS: m/z 412 (M^+, 66%), 369 (11%), 300 (36%), 271 (55%),

255 (56%), 159 (60%), 147 (43%), 133 (58%), 97 (54%), 83 (100%), 55 (98%).

Stigmast-5-en, 24*S*-3β-ol (4): EI-MS: m/z 414 (M^+, 98%), 381 (26%), 329 (52%), 303 (50%), 273 (33%), 213 (54%), 145 (76%), 119 (61%), 107 (88%), 95 (81%), 43 (100%).

Antirrinoside (5): UV λ_{max} (MeOH) nm: 212, 232 sh; IR γ_{max} (KBr) cm^{-1}: 3392 (OH), 2922 (=C-H), 1657 (C=C), 1402, 1231, 1014, 960, 894, 860; ^{1}H and ^{13}C NMR (see Table 1).

Table 1. ^{1}H and ^{13}C NMR spectroscopic data of **5** (δ ppm, CD_3OD).

C/H atom	δ_H	δ_C
Aglycone		
1	5.31 (d, J= 7.3 Hz)	95.0
3	6.30 (d, J = 6.4 Hz)	142.9
4	4.80 (d, J = 6.4 Hz)	107.6
5	-	74.5
6	3.88 (d, J = 1.6 Hz)	78.1
7	3.31 (d, J = 1.6 Hz)	66.2
8	-	64.1
9	2.30 (br.d, J = 7.3 Hz)	53.0
10	1.38 (3H,s)	17.6
Glucose		
1′	4.59 (d, J = 7.3 Hz)	99.5
2′	3.09-3.26*	74.5
3′	3.09-3.26*	78.3
4′	3.09-3.26*	71.6
5′	3.09-3.26*	77.5
6′a	3.83 (dd, J = 1.8/11.7 Hz)	62.8
6′b	3.52 (dd, J = 6.2/11.7 Hz)	

*Signal pattern unclear due to overlapping

Linariin (6): UV λ_{max} (MeOH) nm: , 230sh, 277, 328; (NaOMe) nm: 294.5; ($AlCl_3$) nm: 230.5sh, 288.5, 300, 355; ($AlCl_3$+HCl) nm: 230.5sh, 289.5, 299, 353; (NaOAc) nm: 276, 328; (NaOAc+H_3BO_3) nm: 276.5, 328.5; IR γ_{max} (KBr) cm^{-1}: 3402, 2916, 1723, 1688, 1610, 1583, 1514, 1490, 1429, 1301, 1189, 835. ^{1}H and ^{13}C NMR (see Table 2).

The ^{1}H NMR spectrum of compound **1** indicated the presence of 1 vinylic proton (δ 5.12, t) and 8 methyl groups (δ 0.99, H-23; 0.79, H-24; 0.94, H-25; 0.97, H-26; 1.13, H-27; 0.83, H-28; 0.87, H-29, H-30). The structure of **1** is supported by the appearance of 8 methyl, 10 methylene, 5 methine and 7 quarternary carbons of 30 signals in the ^{13}C NMR and DEPT-135 spectra.

The EIMS spectrum of compound **1** showed the molecular ion at m/z 426, consistent with the formula $C_{30}H_{50}O$. The structure of **1** was identified by GC-MS. The IR and NMR (^{1}H, ^{13}C and DEPT-135) spectra, as well as the GC-MS data, permitted the structure of **1** to be assigned as β-amyrin[13−15].

The remaining compounds were identified as ergost-7-en-3β-ol (**2**), stigmasta-5,22-*E*-dien-3β-ol (**3**) and stigmast-5-en, 24*S*-3β-ol (**4**) by direct comparison of their GC-MS data with those given in the literature[16−21].

Table 2. ^{1}H and ^{13}C NMR spectroscopic data of **6** (δ ppm, CD_3OD).

H/C atom	δ_H	δ_C
Aglycone		
2	-	103.8
3	6.80 (1H, s)	182.8
4	-	
5-OH	12.90 (1H, br.s)	152.8
6	-	133.1
7	-	152.8
8	6.91 (1H, s)	94.9
9	-	156.9
10	-	106.4
1′	-	123.3
2′/6′	7.97 (d, J = 9.0 Hz)	128.8
3′/5′	7.08 (d, J = 9.0 Hz)	115.1
4′	-	163.0
6-OMe	3.88 (3H, s)	60.6
4′-OMe	3.82 (3H, s)	55.7
Glucose		
1′	5.12 (d, J = 7.3 Hz)	100.6
2″	3.2-3.9 (6 H, m)*	73.7
3″	3.2-3.9 (6 H, m)*	76.9
4″	3.2-3.9 (6 H, m)*	69.7
5″	3.2-3.9 (6 H, m)*	75.9
6″	3.2-3.9 (6 H, m)*	65.9
Rhamnose		
1‴	4.59 (1H, br.s)	100.5
2‴	3.2-3.9 (3 H, m)*	70.8
3‴	3.2-3.9 (3 H, m)*	68.8
4‴	4.69 (t, J = 9.8 Hz)	74.3
5‴	3.2-3.9 (3 H, m)*	66.3
6‴	0.82 (d, J = 6.2 Hz)	17.4
-OCO$\underline{C}H_3$	1.93 (3H, s)	20.9
-O$\underline{C}OCH_3$		170.6

*Signal pattern unclear due to overlapping

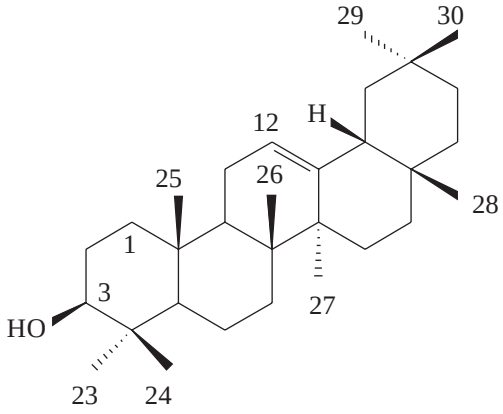

Figure 1. β-Amyrin (**1**).

Substance **5**, which is 1 of the 2 major compounds, is an iridoid glucoside. The UV spectrum of **5**

showed the characteristic absorption bands for a non-conjugated iridoid enol-ether system at 212 and 232(sh) nm (MeOH). In the IR spectrum, it exhibited a distinctive absorption band of an enol-ether function at 1657 cm^{-1}, as well as a broad hydroxyl absorption band at 3392 cm^{-1}. The ^{1}H, ^{13}C NMR and DEPT-135 (Table 1) spectra showed that compound **5** was an hydroxylated epoxy iridoid glucoside. The anomeric proton signal at δ 4.59 (*d*, J = 7.3 Hz), as well as the doublets at δ 3.83 (J = 1.8/11.7 Hz) and 3.52 (J = 6.2/11.7 Hz), were assigned to a β-oriented glucopyranose moiety in **5**. On the other hand, the ^{13}C NMR and DEPT-135 data of the related carbon atoms also supported the presence of a β-glucopyranose in compound **5**. Hydroxyl groups of the aglycon appeared to be located at C-5 and C-6, owing to the broad doublet at δ 4.80, which was assigned to the H-4 proton that coupled with H-3 ($J_{3.4}$= 6.4 Hz) and H-9 protons ($J_{4,9} < 1$ Hz). This assumption was also proved by the chemical shift values of C-5 (δ 74.5) and C-6 (δ 78.1). The ^{1}H and ^{13}C NMR resonances at C-7 and C-8 positions were indicative of the presence of an epoxy-system between C-7 and C-8, as a result of comparing these values with those analogues containing a similar epoxy-system at C-7 and C-8 [22,23]. A singlet at δ 1.33 (3H) was assigned to the methyl group in the α position, which was assumed to be placed at C-8 (δ 64.1, s). Concerning the stereochemistry of the hydroxyl group at C-6, the chemical shift value of $J_{6,7}$(J = 1.6 Hz) is indicative of a *cis*- configuration between H-6 and H-7. The H-9 proton was observed as a broad doublet (J = 7.3 Hz) due to the coupling between H-1 and H-9. Based on its spectroscopic data as well as a comparison with those published in the literature, the structure of **5** was established as anthirrinoside[7–9,23,24].

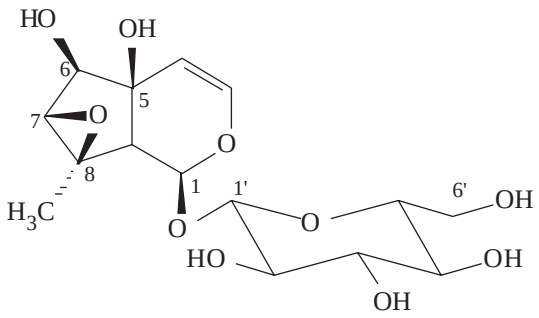

Figure 2. Antirrinoside (**5**).

The absorption maxima at 328 and 277 nm in the UV spectrum of compound **6** (MeOH) were attributed to a flavone skeleton. The absence of a bathochromic shift in the UV spectrum with the presence of sodium acetate showed the glycosylation of the 7-OH group of the aglycon. In the IR spectrum of **6**, the absorption band at 1730 cm^{-1} was characteristic of an ester group.

The signals in the ^{1}H, ^{13}C NMR and DEPT-135 spectroscopic data of **6** (Table 2) supported the presence of a flavone skeleton. The ^{1}H NMR signals at δ 5.12 (d, J = 7.3 Hz) and 4.59 (br s) were attributed to the anomeric protons of a β-glucopyranose and α-rhamnopyranose units, respectively. In addition, the methyl signal at δ 0.82 (d, J = 6.2 Hz) also confirmed the presence of the rhamnose moiety in **6**. The downfield shift of C-6″ (δ 65.9) carbon resonance of the glucose unit was suggestive of the linkage of the rhamnose unit at the C-6″ position. In the ^{1}H NMR spectrum of **6**, the resonances at δ 7.08 (2H, d, J = 9 Hz) and 7.97 (2H, d, J = 9 Hz), which appeared as an AA′BB′ system, were assigned to the H-3′/H-5′ and H-2′/H-6′ protons of the B ring, respectively. Moreover, 2 aromatic protons at δ 6.80 (1H, s) and 6.81 (1H, s) were readily attributed to H-3 and H-8, respectively. On the other hand, 2 methoxyl resonances at δ 3.82

(3H, s) and 3.88 (3H, s) were assigned to the C-4′ and C-6 positions of the aglycon, respectively. Moreover, a ^{1}H NMR singlet resonance at δ 1.93 ppm suggested the presence of an acetyl group, attached to the C-4‴ hydroxyl group of the rhamnose moiety, due to the downfield shifts of the H-4‴ (δ 4.69, t, J = 9.8 Hz) and C-4‴ (δ 74.3) resonances of rhamnose.

Based on the above NMR data, the structure of **6** was established as 4‴-*O*-acetyl pectolinarin (linariin) [4,25,26].

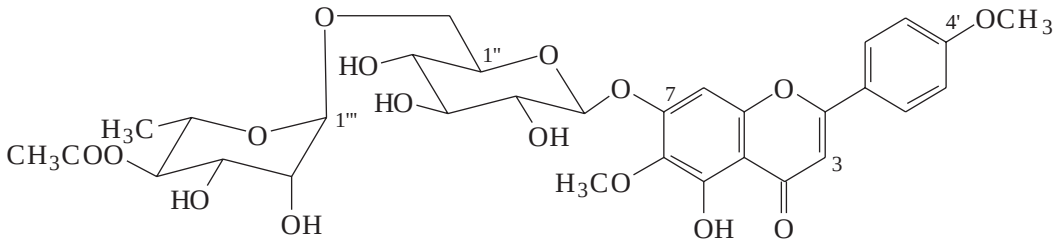

Figure 3. Linariin (**6**).

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