Asian Journal of Chemistry Vol. 18, No. 3 (2006), 2348–2352

Isolation of Coumarins and Coumarin Glucoside from *Launaea resedifolia*

NOUREDDINE GHERRAF*, ASHRAF A. EL-BASSUONY†, AMMAR ZELLAGUI SALAH RHOUATI†, AHMED A. AHMED‡ and MOHAMED RIDHA OUAHRANI**

Department of Chemistry, Faculty of Science
Laboratory of Natural Products and Organic Synthesis
University Mentouri-Constantine, Algeria
Fax: (208)6342601; Tel: (201)24470241; E-mail: ngherraf@yahoo.com

From the aerial parts of *Launaea resedifolia*, four coumarin compounds were isolated: cichoriin I, esculetin II, scopoletin III and isoscopoletin IV.

Key Words: *Launaea resedifolia*, Asteraceae, Coumarins, Coumarin glucoside.

INTRODUCTION

The genus *Launaea* (tribe *Lactucaea*, family Asteracea) comprises about 40 species. Its importance in folk medicine is illustrated in its use in bitter stomachic and skin diseases and for its antitumour and insecticide activities. The genus *Launaea* presents interesting phytochemical features such as the occurrence of terpenoids[1-5], phenolics[6, 7], flavones[8] and coumarins[9, 10].

Therefore, the chemical constituents of *Launaea resedifolia* (O.K.) from Algeria is investigated. The methylene chloride-methanol (1 : 1) extract of the aerial parts gave four compounds: cichoriin (I), esculetin (II), scopoletin (III) and isoscopoletin (IV). The structures of the compounds were elucidated by ^{1}D, ^{2}D NMR and HR-CIMS analyses.

EXPERIMENTAL

IR spectra were obtained in a Perkin-Elmer 1000 FTIR instrument with KBr pellets. The NMR spectra were recorded on Bruker AC 500 [500 MHz (^{1}H) and 125 MHz (^{13}C)] spectrometer. Chemical shifts were recorded in δ (ppm) using TMS as internal standard. EIMS were obtained at 70 eV using a VG-ZAB-E instrument. Column chromatography (CC) was performed using silica gel 60 (Merck, 0.063–0.2 mm). TLC analyses were performed with silica gel (Merck, Kieselgel). Spots were visualized by UV (λ_{max} 259 and 360 mm). HPLC was

†Basic Science Department, Industrial Education College, Beni Suef, Egypt. E-mail: ahnnbass@yahoo.com
‡Department of Chemistry, Faculty of Science, El-minbisa University, El-minia 61519, Egypt.
**Laboratoire De Valorisation Des Ressources Sahariennes, Universite De Ouargla, Algeria.

carried out in the reverse phase on Knauer pump 64 and different refractometer (column: RP-8, 250 × 25 mm, flow: 17 mL/min, elution with MeOH-H_2O, mixtures, refractive index).

Aerial parts of *L. resedifolia* were collected in March 2002 from 25 km north of Ouargla, Algeria, during flowering period. A voucher specimen was deposited at the herbarium of Chemistry Department, Faculty of Sciences, Constantine University, under the code number SR 101, Algeria.

Extraction and Isolation of the Constituents

The aerial parts of *L. resedifolia* (1 Kg) were dried, powdered and extracted with methylene chloride-methanol (1 : 1) at room temperature. The solvent was distilled under reduced pressure furnishing a residue (10 g). The residue was submitted to flash column chromatography, being eluted with *n*-hexane, methylene chloride and methanol, in increasing polarity. The extracts were pre-fractionated by CC (6 × 120 cm) on silica gel eluting with *n*-hexane followed by a gradient of *n*-hexane-CH_2Cl_2 up to 100% CH_2Cl_2 and CH_2Cl_2-MeOH up to 15% MeOH. The fractions were further purified by CC (2 × 40 cm); a Sephadex LH-20 eluted with *n*-hexane-CH_2Cl_2-MeOH (6 : 4 : 1) giving a complex mixture. The mixture was purified by HPLC (MeOH-H_2O, 65 : 35, R_t = 5.6 and 6.0 min).

Cichoriin **I:** colourless oil, HR-CIMS $[M + 1]^+$, m/z 341 (80), $C_{15}H_{16}O_9$, 178 $[M + H\text{-Glu.}]^+$ (75). IR λ_{max} (KBr, cm^{-1}): 3295.9, 2934.5, 1595.9, 1452.5, 1125.8. ^1H NMR (500 MHz, CD_3OD) (Table-1). ^{13}C NMR (500 MHz, CD_3OD) δ: 160.57 (C-2), 112.97 (C-3), 143.59 (C-4), 144.17 ($C\text{-}4_a$), 112.65 (C-5), 113.46 (C-6), 148.81 (C-7), 103.37 (C-8), 147.79 ($C\text{-}8_a$), 101.00 (C-1′), 75.86 (C-2′), 72.28 (C-3′), 73.17 (C-4′), 69.81 (C-5′), 60.74 (C-6′).

Cichoriin acetate **(Ia):** Compound I (5 g) was refluxed in 1 mL of AC_2O-C_5H_5N (1 : 1) for 2 h. The mixture was cooled to room temperature and extracted with CH_2Cl_2 to give the acetate **Ia** (3.5 g). Colourless oil, IR λ_{max} (KBr, cm^{-1}): 2960.5, 1588.6, 1445.8; HR-CIMS m/z (rel. int.) 551 $[M + H]^+$ (80); $C_{25}H_{26}O_{14}$; ^1H NMR spectral data (500 MHz, CD_3OD) (Table-1).

Esculetin **II:** yellowish brown oil, HRCIMS $[M + 1]^+$, m/z 179 (95), $C_9H_6O_4$. IR λ_{max} (KBr, cm^{-1}): 3286.5, 2922.5, 1590.9, 1450.5. ^1H NMR (500 MHz, CD_3OD) (Table-1).

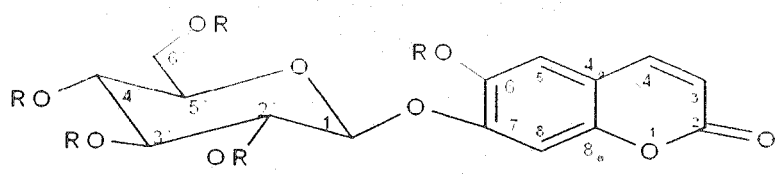
Scopoletin **III:** grayish oil, HR-CIMS $[M + 1]^+$, m/z 193 (90), $C_{10}H_8O_4$. IR λ_{max} (KBr, cm^{-1}): 3230.5, 2900.6, 1700.5, 1452.5. ^1H NMR (500 MHz, CD_3OD) (Table-1).

Isoscopoletin **IV:** grayish oil, HR-CIMS $[M + 1]^+$, m/z 193 (93), $C_{10}H_8O_4$. IR λ_{max} (KBr, cm^{-1}): 3235.5, 2905.6, 1700.6, 1450.5. ^1H NMR (500 MHz, CD_3OD) (Table-1).

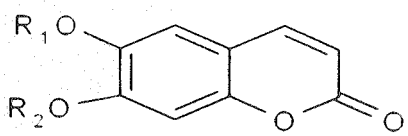
RESULTS AND DISCUSSION

Investigation of the CH_2Cl_2-MeOH (1 : 1) extract of the aerial parts of *Launaea resedifolia* afforded four compounds. Compound **I**, colourless oil, CIMS, showed a molecular ion peak $[M + 1]^+$ at m/z 341 in accordance with the molecular formula $C_{15}H_{16}O_9$. ^{13}C NMR spectrum of compound **I** displayed fifteen carbon

signals. DEPT experiments indicated these signals as: one carbonyl carbon at δ_C 160.57 (s, C-2), one methylene carbon at δ_C 60.74 (t, C-6′); nine methine carbons at δ_C: 143.59 (d, C-4), 112.97 (d, C-3), 112.65 (d, C-5), 103.37 (d, C-8), 100.99 (d, C-F), 77.28 (d, C-3′), 75.86 (d, C-2′), 73.17 (d, C-4′) and 69.81 (d, C-5′) and four quaternary carbons at δ_C: 148.81 (s, C-7), 147.79 (s, C-8_a), 144.17 (s, C-4_a) and 113.45 (s, C-6). ^{1}H NMR spectrum showed characteristic signals of glucose moiety, whereas the methylene protons H-6a′ and H-6b′ appeared as two double doublets at δ_H 3.93 (J = 12.0, 3.0 Hz) and 3.72 (J = 12.0, 3.0 Hz). The anomeric proton H-F appeared downfield as doublet signal at δ_H 4.96 (J = 7.5 Hz), the other methine protons H-2′, H-3′, H-4′ and H-5′ appeared at δ_H 3.54 (dd, J = 7.5, 8.5 Hz), 3.50 (dd, J = 8.5, 9.0 Hz), 3.41 (dd, J = 9.0, 9.0 Hz) and 3.51 (ddd, J = 3.0, 5.0, 9.0 Hz), respectively. The coumarin moiety exhibited characteristic signals as a doublet at δ_H 7.81 (H-4, J = 9.5 Hz), which correlated in ^{1}H-^{1}H COSY with doublet at δ_H 6.27 (H-3, J = 9.5 Hz). The two singlet signals appearing at δ_H 7.20 and 7.03 were assigned for H-8 and H-5, respectively. All proton and carbon signals were assigned by ^{1}H-^{1}H and ^{1}H-^{13}C COSY. In ^{1}H-^{13}C COSY, the signal at δ_H 4.96 (H-1′) showed correlation with the carbon signal at δ_C 101.0 (C-1′). The two double doublet signals at δ_H 3.93 and 3.72 correlated with carbon signal at δ_C 60.74 (C-6′). The presence of sugar moiety in position 7 was proved by NOE spectrum (Fig. 1), which showed correlation between doublet at δ_H 7.81 (H-4) and the two signals at δ_H 7.03 (H-5) and doublet at δ_H 6.27 (H-3) and correlation between singlet at δ_H 7.20 (H-8) and the doublet at δ_H 4.96 (H-1′). Therefore, compound I was identified as cichoriin[11].



I R = H
I_a R = AC



II R_1 = H R_2 = H
III R_1 = Me R_2 = H
IV R_1 = H R_2 = Me

Acetylation of a portion of compound I gives the acetylated derivative Ia. HR-CIMS provides a molecular ion peak $[M + 1]^+$ at m/z 551 corresponding to $C_{25}H_{26}O_{14}$. ^{1}H NMR spectrum revealed the five acetyl signals at δ_H 2.04, 2.07, 2.08, 2.14 and 2.29. The protons of the sugar and coumarin moieties were determined by ^{1}H-^{1}H COSY and given in Table-1.

TABLE-1
^{1}H NMR SPECTRAL DATA OF I–IV
(500 MHz, CD_3OD, TMS as int., standard, δ-values)*

Position of C	I δ_H	Ia† δ_H	II δ_H	III δ_H	IV δ_H
3	6.27 (d, 9.5)	6.37	6.15	6.25	6.30
4	7.81 (d, 9.5)	7.65	7.75	7.90	7.65
5	7.03 (s)	7.05	6.74	6.80	6.86
8	7.20 (s)	7.20	6.93	7.25	6.93
1′	4.96 (d, 7.5)	5.15			
2′	3.54 (dd, 7.5, 8.5)	5.35			
3′	3.50 (dd, 8.5,9.0)	5.37			
4′	3.41 (dd, 9.0, 9.0)	5.36			
5′	3.51 (ddd, 3.0, 5.0, 9.0)	3.95			
6′	3.93 (dd, 12.0, 3.0)	4.35			
	3.72 (dd, 12.0, 3.0)	4.22			
OMe				3.85 (s)	4.00 (s)

*Homonuclear ^{1}H-^{1}H COSY spectra were also used for these assignments.
†OAc, 2.04, 2.07, 2.08, 2.14 and 2.29.

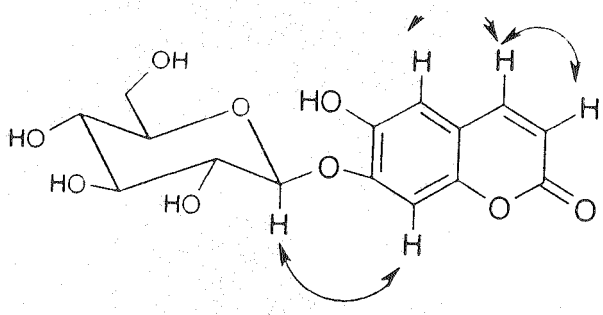


Fig. 1. NOE correlations of compound I

HR-CIMS of compound II showed the molecular ion peak $[M + 1]^+$ at m/z 179 in accordance with the molecular formula $C_9H_6O_4$. ^{1}H NMR spectrum of II showed presence of two singlet signals at δ_H 6.93 (H-8) and 6.74 (H-5) and the two doublets at δ_H 7.75 (H-4, $J = 9.5$ Hz) and 6.15 (H-3, $J = 9.5$ Hz). Therefore, compound II was identified as esculetin.

IR spectrum of III displayed absorption bands characteristic of carbonyl group (1700 cm^{-1}, C=O). The HR-CIMS showed the molecular ion peak $[M + 1]^+$ at m/z 193 in accordance with the molecular formula $C_{10}H_8O_4$. ^{1}H NMR spectrum of compound III revealed the presence of two doublets at δ_H 7.90 (H-4, $J = 6.0$ Hz) and 6.25 (H-3, $J = 6.0$ Hz). The two singlet signals appeared at δ_H 6.80 and 7.25 were assigned for the two protons H-5 and H-8, respectively. The difference

between compounds **II** and **III** was the presence of singlet signal at δ_H 3.85, which was assigned for a methoxy group. Therefore, compound **III** was identified as scopoletin.

^{1}H NMR spectrum of compound **IV** was close to compound **III**. The difference in the chemical shifts of the signals suggested that compound **IV** was an isomer of compound **III** (isoscopoletin), H-8 of compound **III** appeared as singlet at δ_H 7.25, whereas H-8 of compound **IV** appeared as singlet at δ_H 6.93. Also, few differences in the chemical shifts for H-5, H-3 and H-4 were observed (Table-1). HR-CIMS revealed a molecular ion peak $[M + 1]^+$ at m/z 193 which corresponds to the molecular formula $C_{10}H_8O_4$.

ACKNOWLEDGEMENTS

One of the authors (AAE) is grateful to Industrial Education College, Beni Suif, for all facilities present to finish this work. The Algerian authors wish to thank the National Agency of Research Development in Health (ANDRS) in part and the Ministry of Higher Education and Scientific Research for financial support. Thanks are also due to Prof. Dr. Ahmed A. Ahmed for their good reception in his laboratory.

REFERENCES

1. N.M. Sokkar, E.A. Aboutable, W. Kubelka, Z.H. Mohamad and S.H. Hilal, *Plant. Med. Phytother.*, **26**, 368 (1993).
2. H.A. El-Fattah, A.F. Zaghloul, A.F. Halim and E.S. Waight, *Egypt J. Pharm. Sci.*, **31**, 81 (1990).
3. N.A.A. El-Salam, Z.F. Mahmoud and K. Fahimak, *Egypt J. Pharm. Sci.*, **27**, 275 (1986).
4. F. Hook, M. Behari, R. Gupta and T. Matsumoto, *Indian Drugs*, **21**, 366 (1984).
5. T.M. Sarg, A.A. Omar, S.M. Khafagy, M. Grenz and F. Bohlmann, *Phytochem.*, **21**, 1163 (1982).
6. R.M. Giner, J. Diaz, S. Manez, M.C. Recio, C. Sonano and J.L. Rios, *Biochem. Syst. Ecol.*, **20**, 187 (1992).
7. T.M. Sarg, A.A. Omar, A.M. Ateya and S.S. Hafiz, *Egypt J. Pharm. Sci.*, **25**, 35 (1986).
8. R. Gupta and A. Bahar, *Phytochem.*, **24**, 873 (1985).
9. M.R.I. Saleh, A.A.M. Habib, M.G. El-Ghazooly, O.M.K. Gabr and F.K. El-Fiky, *Egypt J. Pharm. Sci.*, **29**, 507 (1988).
10. T.M. Sarg, A.M. Ateya and G.A. Dora, *Fitoterapia*, **58**, 133 (1987).
11. W. Kisiel and K. Michalska, *Fitoterapia*, **73**, 544 (2002).

(*Received*: 12 December 2005; *Accepted*: 6 March 2006) AJC-4722