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Research Article

Chemical constituents of the extract Algerian *Reutera lutea* (Desf.) Maire, (Apiaceae)

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ABSTRACT: Phytochemical analysis of the aerial parts of *Reutera lutea* (Apiaceae), resulted in the isolation of two flavonoid C-glucosides and isocoumarine. The structures of the compounds were established as Isoorientin **(1)**, Isoorientin 6″-O-acetate **(2)** and 3,4-dihydro-6, 8-dihydroxy-3-methylisocoumarin **(3)**. The structures of all compounds were elucidated by spectroscopic methods, including (1D and 2D NMR, UV, MS).

KEYWORDS: Apiaceae, *Reutera lutea*, flavonoid, C-glucosides, Isocoumarin

INTRODUCTION

Apiaceae Lindl. (Umbelliferae Juss.) is one of the best known families of flowering plants,[1] many species of this family are widely used in local herbal medicine and food owing to their wide spectrum of pharmacological activities.[2] The aim of the paper in hand is to undertake a phytochemical and biological assessment of one of the Algerian flora's medicinal plants known as *Reutera lutea* (syn. *Pimpinella lutea* Desf., R. Fontanesii Boiss).[3] Diverse separation and purification methods of the methanolic extract of the *Reutera lutea* have led to the isolation of two flavonoid C-glucosides and one isocoumarin. As well as by comparison with literature data, the isocoumarine already described for the genus *Arabidopsis*.[4]

However, such C-glucosides are not of common occurrence within the genus *Reutera*. To our knowledge no reports on the isolation of any secondary metabolites from *R. Lutea* is available to date.

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MATERIALS AND METHODS

Plant material

The aerial parts of *R. lutea* were collected at the flowering stage at Gouraya National Parc (Near Yema Gouraya) (elevation 600 m) in North Algeria and authenticated by Dr Nacira Boulaacheb. A voucher specimen was deposited in the Department of biology and ecology vegetal (University of Setif, Algeria).

Extraction and isolation

Air-dried and powdred aerial parts (1kg) of *Retura lutea* were macerated in methanolic solution (70%) at room temperature. The residue was filtered, concentrated then successively extracted with n-hexane, dichloromethane, ethyl acetate and n-butanol. The butanolic and acetate extracts were concentrated under reduced pressure. The butanolic extract was subsequently subjected to polyamide column chromatography eluting with a system of toluene-methanol, starting the elution with toluene and gradually increasing the polarity of the solvent by addition of the methanol, and finally with 100% methanol to afford 25 fractions. After evaporation, fraction 16 gave a yellow precipate which, after being washed several times with methanol, was identified as a flavonoid (compound 1) (Figure 1). Nevertheless, fraction 15 was chromatographed through a silica gel column using an isocratic system of ethyl acetate:methanol:water (8:1:1) to give another flavonoid (compound 2) (Figure 2).

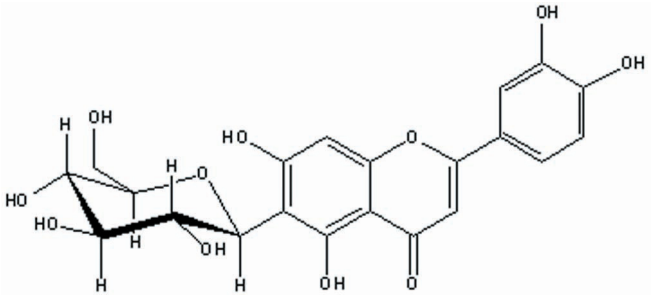

Figure 1. Compound 1 (Isoorientin).

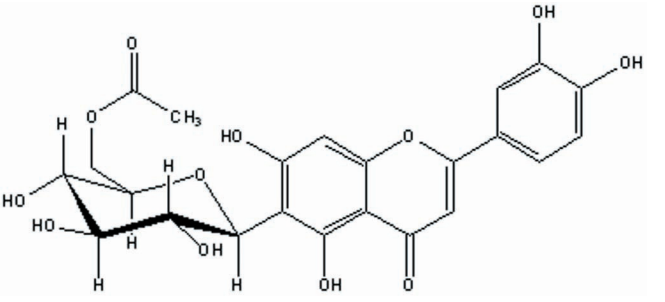

Figure 2. Compound 2 (Isoorientin 6"-O-acetate).

The ethyl-acetate extract was evaporated and chromatographed on silica gel eluting with a system of dichloromethane-ethyl acetate then with a system of ethyl acetate-methanol, starting the elution with dichloromethane and gradually increasing the polarity of the solvent by addition of the ethyl acetate, and finally with 100% ethyl acetate then elution with ethyl acetate and gradually increasing the polarity of the solvent by addition of the methanol, and finally with 100% methanol to provide 30 fractions. An isocoumarin compound (compound 3) was isolated in fraction 24 by using gas chromatography-mass spectroscopy (GC-MS) technique (Figure 3).

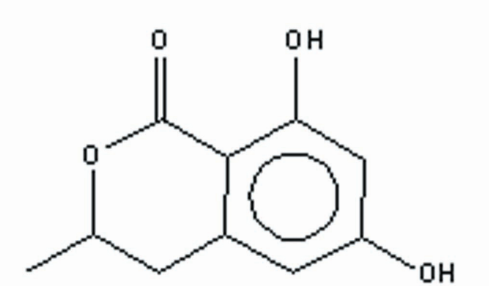

Figure 3. Compound 3 (3,4-dihydro-6,8-dihydroxy-3-methyl-isocoumarin).

The structure elucidation of the isolated compounds was performed on the basis of the spectroscopic data (1D and 2D NMR, UV, MS).

RESULTS AND DISCUSSION

Compound 1: Isoorientin (Luteolin 6-C-glucoside), (Figure 1, Table 1)

The molecular formula of compound **1** was determined as $C_{21}H_{20}O_{11}$ by eilectrospray mass spectrum, peak at *m/z* 449 $[M+H]^+$. The ^{1}H-NMR and ^{13}C-NMR spectral data of compound **1** indicated a Isoorientin.[5–8]

^{1}H NMR: (300 MHz, DMSO-d6, **δ** in *ppm*) data: 13.57 (1H, brs, 5-OH), 7.44 (1H, dd, *J* = 8.2 Hz, 2.2 Hz, 6'-H), 7.40 (1H, d, *J* = 2.2 Hz, 2'-H), 6.89 (1H, d, *J* = 8.2 Hz, 5'-H), 6.68 (1H, s, 3-H), 6.48 (1H, s, 8-H), 4.58 (1H, d, *J* = 9.8 Hz, 1"-H).

^{13}C NMR: (300 MHz, DMSO-d6, **δ** in *ppm*) data: 163.6 (C-2), 102.7 (C-3), 181.8 (C-4), 160.6 (C-5), 108.8 (C-6), 163.3 (C-7), 93.4 (C-8), 156.1 (C-9), 103.3 (C-10), 121.3 (C-1'), 113.2 (C-2'), 145.7 (C-3'), 149.7 (C-4'), 116.0

Table 1: ^{1}H (300 MHz) and ^{13}C (100 MHz) NMR data of compound 1 (DMSO-d6, TMS, δ ppm)

No	δ_H (J Hz)	δ_C	DEPT	No.	δ_H (J Hz)	δ_C	DEPT
2		163.6	C	**3′**		145.7	C
3	6.68 s	102.7	CH	**4′**		149.7	C
4		181.8	C	**5′**	6.89 d (8.2)	116.0	CH
5	13.57 (5-OH)	160.6	C	**6′**	7.44 dd (8.2, 2.2)	118.9	CH
6		108.8	C	**1″**	4.58 d (9.8)	73.0	CH
7		163.3	C	**2″**		70.6	CH
8	6.48 s	93.4	CH	**3″**		78.9	CH
9		156.1	C	**4″**		70.1	CH
10		103.3	C	**5″**		81.6	CH
1′		121.3	C	**6″**		61.4	CH_2
2′	7.40 d (2.2)	113.2	CH				

Table 2: ^{1}H (300 MHz) and ^{13}C (100 MHz) NMR data of compound 2 (DMSO-d6 , TMS, δ ppm)

No	δ_H (J Hz)	δ_C	DEPT	No.	δ_H (J Hz)	δ_C	DEPT
2		163.6	C	**4′**		149.7	C
3	6.69 s	102.7	CH	**5′**	6.89 d (8.2)	116.0	CH
4		181.8	C	**6′**	7.44 dd (8.2, 2.2)	118.9	CH
5	13.65 (5-OH)	160.9	C	**1″**	4.66 d (9.8)	72.9	CH
6		108.1	C	**2″**		68.4	CH
7		163.3	C	**3″**		78.6	CH
8	6.49 s	93.3	CH	**4″**		67.7	CH
9		156.3	C	**5″**		81.4	CH
10		103.3	C	**6″**		61.1	CH_2
1′		121.3	C	**COO**		169.9	C
2′	7.40 d (2.2)	113.2	CH	**CH_3**	2.03 s	21.25	CH_3
3′		145.7	C				

(C-5'), 118.9 (C-6'), 73.0 (C-1"), 70.6 (C-2"), 78.9 (C-3"), 70.1 (C-4"), 81.6 (C-5"), 61.4 (C-6").

Comparing with the reported data, the ^{1}H-NMR and ^{13}C-NMR data are in agreement with those of Isoorientin in the literature.[5–8]

Compound 2: Isoorientin 6"-O-acetate (5,7,3',4'-tetrahydroflavone 6-C- (6"-O-acetylglucoside) (Figure 2, Table 2).

The molecular formula of compound **2** was determined as $C_{23}H_{22}O_{12}$ by electrospray mass spectrum, peak at m/z 491 $[M+H]^+$. The ^{1}H-NMR and ^{13}C-NMR spectral data of compound 2 indicated a Isoorientin 6"-O-acetate.[10]

^{1}H NMR: (300 MHz, DMSO-d6, δ in *ppm*) data: 13.65 (1H, brs, 5-OH), 7.44 (1H, dd, *J* = 8.2 Hz, 2.2 Hz, 6'-H), 7.40 (1H, d, *J* = 2.2 Hz, 2'-H), 6.89 (1H, d, *J* = 8.2 Hz, 5'-H), 6.69 (1H, s, 3-H), 6.49 (1H, s, 8-H), 4.66 (1H, d, *J* = 9.8 Hz, 1"-H).

13C NMR: (100 MHz, DMSO-d6, δ = *ppm*) data: 163.6 (C-2), 102.7 (C-3), 181.8 (C-4), 160.9 (C-5), 108.1 (C-6), 163.3 (C-7), 93.3 (C-8), 156.3 (C-9), 103.3 (C-10), 121.3 (C-1'), 113.2 (C-2'), 145.7 (C-3'), 149.7 (C-4'), 116.0 (C-5'), 118.9 (C-6'), 72.9 (C-1"), 68.4 (C-2"), 78.6 (C-3"), 67.7 (C-4"), 81.4 (C-5"), 61.1 (C-6").

Comparing with the reported data, the ^{1}H NMR and ^{13}C NMR data are in agreement with those of Isoorientin and Isoorientin 6"-O-acetate in the literature.[8–11]

Compound 3: 3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin (6-hydroxymellein) Figure 3[4]

Analytical CPG/SM of the ethyl acetate extract of *Retura lutea* indicated the presence of three major volatile compounds. The major constituent which eluted at a lower temperature was identified as 3,4-dihydro-6, 8-dihydroxy-3-methylisocoumarin) with a retention time of 18.270 min (Figure 4). By comparison with the CPG retention times and mass spectra in the library, the compound had strikingly similar mass spectra (Figure 5). The molecular

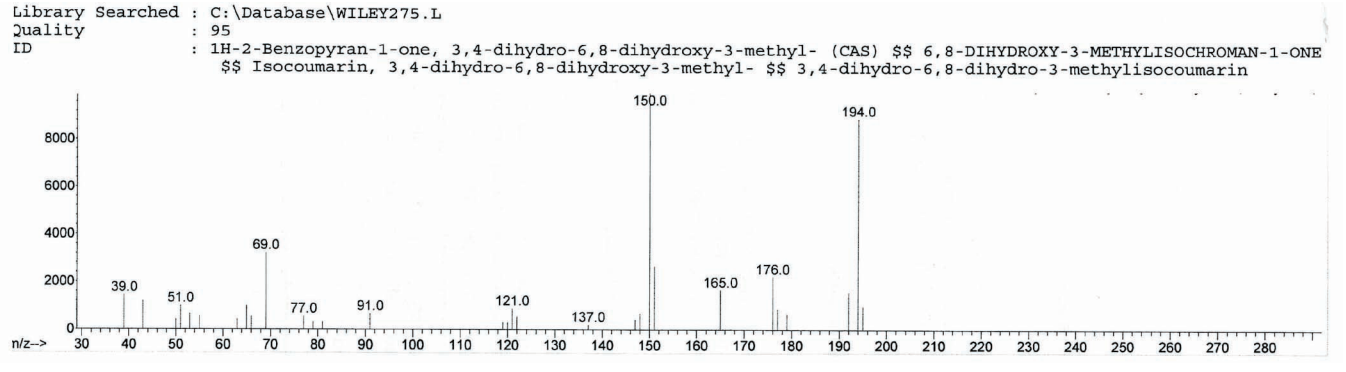

Figure 4. Mass spectrum of isocoumarine, 3,4-dihydro-6, 8-dihydroxy-3-methylisocoumarin.

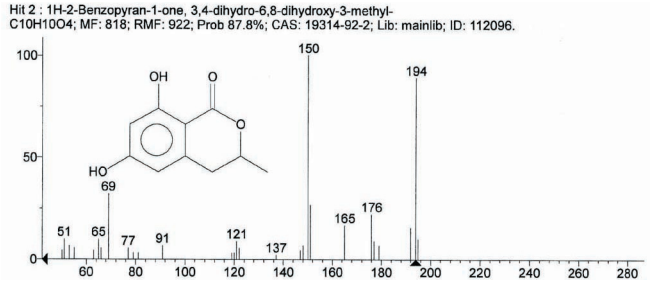

Figure 5. Mass spectrum of 3,4-dihydro-6, 8-dihydroxy-3-methylisocoumarin in the library.

formula of compound **3** was determined as $C_{10}H_{10}O_4$ by CPG/SM spectrum showing a molecular ion peak at m/z 194. The Compound (M+ m/e 194, 90.3%) displayed an abundant ion at m/e 150 (base peak) (M-44. 99.9%) (loss of CO_2) and fragment ions at m/e 121 (M-44-29) (Loss of CO2 and HCO) respectively. Another fragment at m/e 176 (M-18) (loss H_2O), and at m/e 165 (M-29) (loss of HCO) and at m/e 137 (M-29-28) (loss HCO and CO) respectively. These peaks were compared to those reported in the literature.[4]

CONCLUSIONS

The phytochemical study of *Reutera lutea* revealed the presence of two c-glucoside flavones and isocoumarine identified for the first time in this species.

ACKNOWLEDGEMENTS

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