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BIOACTIVE LIGNANS FROM THE SEEDS OF *CENTAUREA MACROCEPHALA*

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ABSTRACT

Four dibenzylbutyrolactone-type lignans, arctiin, lappaol A, matairesinol and matairesinoside were isolated from a methanol extract of the seeds of *Centaurea macrocephala*. The structures of these isolates were elucidated by spectroscopic means. The general toxicity and antioxidant activity of the isolated lignans were evaluated, respectively, by the brine shrimp lethality assay and the DPPH assay. The distribution of these lignans within the genus *Centaurea* has also been discussed.

Keywords: *Centaurea macrocephala*, Asteraceae, matairesinoside, arctiin, matairesinol, lappaol A

INTRODUCTION

Centaurea macrocephala Muss-Puschk. Ex Willd. (Family: Compositae *alt.* Asteraceae), commonly known as 'big-head knapweed', is a splendid border plant with large, yellow, thistle-like flower heads, endemic to Armenia, Iran and Turkey, and also naturalised in many other countries of the world (1). Many species of the genus *Centaurea* have been used in traditional medicine to cure various ailments, e.g. diabetes, diarrhoea, rheumatism, malaria, hypertension etc., and a variety of secondary metabolites have been reported from different species of this genus (2). A lignan, arctigenin, and a number of flavonoids, 6-*C*-glucosyl-luteolin, isoquercitrin, 3-*O*-glucosyl-isorhamnetin, trifolin, rutin, isoscoparin and isovitexin were previously isolated from the aerial parts of *C. macrocephala* (3,4). The isolation, identification, biological activities and distribution of four dibenzylbutyrolactone lignans, arctiin [**I**], matairesinoside [**II**], matairesinol [**III**] and lappaol A [**IV**] from the seeds of *C. macrocephala* are reported here.

MATERIALS AND METHODS

General procedures

UV spectra were obtained in MeOH using a Hewlett-Packard 8453 UV-Vis spectrometer. FT-IR spectra were obtained on AVATAR 360 FT-IR. MS analyses were performed on a Quattro II triple quadrupole instrument. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were recorded on a Varian Unity INOVA 400 MHz NMR spectrometer. The optical rotation was measured on ADP 220 Polarimeter, Bellingham, Stanley, Ltd. HPLC separation was performed using a Dionex prep-HPLC system coupled with Gynkotek GINA50 autosampler and Dionex UVD340S Photo-Diode-Array detector. A Luna C_{18} preparative HPLC column (10m, 250 mm × 21.2 mm) was used. Sep-Pak Vac 35 cc (10 g) C_{18} cartridge (Waters) was used for pre-HPLC fractionations.

Plant material

Seeds of *C. macrocephala* were purchased from an authentic seed supplier B & T World Seeds sarl, Pauguignan, 34210 Olonzac, France, and a voucher specimen (PHSH0001) has been retained in the herbarium of the Plant and Soil Science Department, University of Aberdeen, UK.

Extraction, isolation and structure elucidation

Ground seeds (100 g) of *C. macrocephala* were Soxhlet-extracted, successively, with *n*-hexane, dichloromethane and methanol (1.1 L each). The

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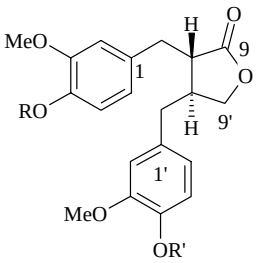
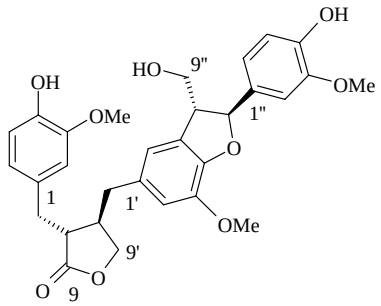
methanolic extract was fractionated using solid phase extraction on a Sep-Pak C_{18} (10 g) cartridge eluting with a step gradient: 30, 60, 80 and 100% MeOH in water (200 ml each). Preparative-HPLC (Luna C_{18} column 10 μm, 250 mm × 21.2 mm, eluted with a liner of gradient-water: MeOH= 70:30 to 20:80 over 50 minute followed by 80% MeOH for 10 minute, 20 ml/min, monitored by photo-diode-array detector) of the Sep-Pak fraction, which was eluted with 30% MeOH, yielded fraction A (70.5 mg; t_R = 14.1) and [**I**] (314.1 mg; t_R = 16.5 min). Fraction A was further purified by prep-HPLC (isocratic elution with 40% methanol in water, 20 ml/min) to obtain [**II**] (5.8 mg, t_R = 6.8 min). Preparative HPLC, using the same condition as for the Sep-Pak 30% methanolic fraction, of 60% Sep-Pak fraction afforded more of [**I**] (49.5 mg), [**III**] (33.2 mg, t_R = 21.6 min) and fraction B. Compound [**IV**] (5.2 mg. t_R = 19.8 min) was purified from fraction B by HPLC, eluted with a liner gradient water : acetonitrile = 75:25 to 40:60 over 50 min, 20 ml/min. The structures of all four compounds were elucidated by comprehensive spectroscopic analysis and also by comparison with published data.



[**I**] R = β-D-glucopyranosyl R' = Me
[**II**] R = β-D-glucopyranosyl R' = H
[**III**] R = H R' = H



[**IV**]

Figure 1: Structures of dibenzylbutyrolactone lignans (**1**-**4**) isolated from the seeds of *C. macrocephala*

Arctiin [**I**]. Gum, $[\alpha]^{23}_D$ -55.3° (*c* 0.0033, MeOH); UV λ_{max} (MeOH): 279, 225; IR ν_{max} (neat): 3459, 1765, 1591, 1514, 1460 and 1266 cm^{-1}; CIMS *m/z* 552 $[M+NH_4]^+$; ^1H NMR (400 MHz, CD_3OD): Table 1; ^{13}C NMR (100 MHz, CD_3OD): Table 2.

Matairesinoside [**II**]. Gum, $[\alpha]^{23}_D$ -48.8° (*c* 0.002, MeOH); UV λ_{max} (MeOH): 279, 222; IR ν_{max} (neat): 3373, 1760, 1600, 1514, 1452 and 1270 cm^{-1}; ESIMS *m/z* 543 $[M+Na]^+$; ^1H NMR (400 MHz, CD_3OD): Table 1; ^{13}C NMR (100 MHz, CD_3OD): Table 2.

Matairesinol [**III**]. Gum, $[\alpha]^{23}_D$ -47.2° (*c* 0.0022, MeOH); UV λ_{max} (MeOH): 282, 228; IR ν_{max} (neat): 3430, 1760, 1610, and 1525 cm^{-1}; ESIMS *m/z* 381 $[M+Na]^+$; ^1H NMR (400 MHz, CD_3OD): Table 1; ^{13}C NMR (100 MHz, CD_3OD): Table 2.

Lappaol A [**IV**] Amorphous, $[\alpha]^{23}_D$ -17.6° (*c* 0.0021, MeOH); UV λ_{max} (MeOH): 282, 225; IR ν_{max} (neat): 3410, 1765, 1591, 1514 and 850 cm^{-1}. ESIMS *m/z* 559 $[M+Na]^+$; ^1H NMR (400 MHz, CD_3OD): Table 3; ^{13}C NMR (100 MHz, CD_3OD): Table 3.

Free radical scavenging activity (the DPPH assay)

2,2-Diphenyl-1-picrylhydrazyl (DPPH), molecular formula $C_{18}H_{12}N_5O_6$, was obtained from Fluka Chemie AG, Bucks, UK. Quercetin was obtained from Avocado Research Chemicals Ltd, Shore Road, Heysham, Lancs, UK. The method used by Takao et. al. (5) was adopted with suitable modifications (6). DPPH (4 mg) was dissolved in MeOH (50 ml) to obtain a concentration of 80 μg/ml. Compound [**I-IV**] were dissolved in MeOH to obtain a concentration of 0.5 mg/mL. Dilutions were made to obtain concentrations of $5x10^{-2}$, $5x10^{-3}$, $5x10^{-4}$, $5x10^{-5}$, $5x10^{-6}$ $5x10^{-7}$, $5x10^{-8}$, $5x10^{-9}$, $5x10^{-10}$ mg/mL. Diluted solutions (1.00 mL) were mixed with DPPH (1.00 mL) and allowed to stand for 30 min for any reaction to occur. The absorbance was recorded at 517 nm. The experiment was performed in triplicate and the average absorption was noted for each concentration. The same procedure was followed for the positive control (quercetin).

Brine shrimp Lethality assay

Brine shrimp eggs were purchased from The Pet Shop, Kittybrewster Shopping Complex, Aberdeen, UK. The bioassay was conducted following the procedure described by Meyer et al. (7). The eggs were hatched in a conical flask containing 300 mL of artificial seawater. The flasks were well aerated with the aid of an air pump, and kept in a water bath at 29-30 °C. A bright light was left on and the nauplii hatched within 48 h. The compounds [**I-IV**] were

dissolved in 20% aq. DMSO to obtain a concentration of 1 mg/mL. These were serially diluted two-times, and seven different concentrations were obtained. Solution of each concentration (1 mL) was transferred into clean sterile universal vials with pipette and aerated sea water (9 mL) was added. About 10 nauplii were transferred into each vial with pipette. A check count was performed and the number alive after 24 h was noted. LD_{50}s were determined using the Probit analysis method (8).

RESULTS AND DISCUSSION

Reversed-phase preparative-HPLC analysis of the methanol extract of the seeds of *C. macrocephala* afforded four dibenzylbutyrolactone lignans which on the basis of comprehensive spectroscopic analyses (e.g. UV, ESIMS or CIMS, and 1D and 2D NMR), were characterised as arctiin [**I**], matairesinoside [**II**], matairesinol [**III**] and lappaol A [**IV**] (Figure 1).

All four compounds [**I-IV**] displayed characteristic UV absorption maxima of dibenzylbutyrolactone-type lignans. A strong absorption band at around 1765 cm^{-1} in the IR spectrum of each of these compounds could be attributed to the carbonyl functionality of the lactone ring. A CIMS spectrum of [**I**] revealed $[M+NH_4]^+$ ion peak at *m/z* 552, suggesting *M*r=534, and the molecular formula $C_{27}H_{34}O_{11}$. The ^1H and ^{13}C NMR spectral data of [**I**] (Tables 1 and 2) were similar to those published for arctiin [**I**] (9, 10). However, a combination of HMQC, HMBC, COSY and NOESY 2D NMR spectral analyses led to the unambiguous assignment of all ^1H and ^{13}C NMR signals of [**I**] and confirmed unequivocally its identity as arctiin [**I**].

The ^1H and ^{13}C NMR spectra of [**II**]and [**III**] (Tables 1 and 2) displayed similar signals with the exceptions that the signals for two methoxy groups (instead of three in [**I**]**)** were present in the spectra of [**II**] and [**III**], and the signals due to the β-D-glucopyranosyl moiety were absent in the spectra of [**III**]. The ESIMS spectrum of [**II**] revealed $[M+Na]^+$ ion at *m/z* 543, suggesting Mr=520, and the molecular formula $C_{26}H_{32}O_{11}$ which confirmed the findings from the NMR data of [**II**] that it contained 14 mass units less than arctiin [**I**], i.e. instead of a methoxy group, it had a hydroxyl group present. The ^1H and ^{13}C NMR data of [**II**] were in good agreement with the published data of matairesinoside (9, 10). The ESIMS spectrum of [**III**] revealed $[M+Na]^+$ ion at *m/z* 381, suggesting Mr=358, and the molecular formula $C_{20}H_{22}O_6$ which confirmed the findings from the NMR data of [**III**] that, unlike [**II**], it did not have a glucosyl moiety. The spectroscopic data of [**III**] were in good agreement with the published data of matairesinol (9, 10). However, detailed 2D NMR spectral analyses helped to assign unambiguously all the ^1H and ^{13}C NMR signals of [**II**] and [**III**], and confirmed unequivocally their identities, respectively, as matairesinoside [**II**] and matairesinol [**III**].

The ESIMS spectrum of [**IV**] revealed $[M+Na]^+$ ion peak at *m/z* 559 indicating the molecular formula $C_{30}H_{32}O_9$. In the ^1H NMR spectrum (Table 3), the signals at δ 6.95, 6.82, 6.76, 6.70, 6.68 and 6.57 could be assigned to two trisubstituted benzene ring protons, while the signals at δ 6.59 and 6.55 were due to the protons of a tetra-substituted benzene ring. In addition to a dibenzylbutyro-lactone system, presence of a phenyl propanol moiety was evident from its ^1H and ^{13}C NMR spectra (Table 3). The HMBC correlations (Table 3) between H-7 to C-9 and C-8', H-7' to C-9', C-2' and C-6', H-8' to C-9' and C-1', and H-9' to C-9, C-7' and C-8 confirmed the presence of the dibenzylbutyrolactone structure, similar to that in [**I-III**]. The ^1H NMR signals at δ 5.54, 3.46 and 3.82, and the ^{13}C NMR chemical shifts at δ 89.2, 55.0 and 65.2 could be assigned to the protons and carbons of a dihydrofuran ring system, and a primary alcohol group. The ^1H-^1H COSY spectrum displayed correlation between H-7'' to H-8'' and H-8'' to H-9''. The HMBC correla-tions between H-7'' to C-2'', C-6'', C-4' and C-5', H-8'' to C-7'', C-9'', C-1'', C-4' and C-5' and H-2'' to C-7'' confirmed that the dihydrofuran system was fused with one of the two phenyl rings of the dibenzylbutyrolactone system to form the structure of lappaol A [**IV**]. The spectroscopic data of [**IV**] were in good agreement with the published data of sesquilignan lappaol A (11, 12). The relative stereochemistry at the chiral centres in [**IV**] was assigned by direct comparison of its ^1H and ^{13}C NMR data with published data. The unambiguous assignment of all ^1H and ^{13}C NMR chemical shift, based on extensive 2D NMR analysis of [**IV**], is present here for the first time.

This is the first report on the isolation of arctiin, matairesinoside, matairesinol and lappaol A from *C. macrocephala*. While lignans [**I-III**] have previously been isolated from several other *Centaurea* species (13-15), lappaol A [**IV**] has only been found in *C. napifolia* (16), *C. nicaensis* (17) and *C. sphaerocephala* (11). The distribution of these lignans within the genus *Centaurea* is summarised in Table 4. The co-occurrence of these lignans in the species of *Centaurea* might have some chemotaxonomic significance. All lignans (**I-IV**) showed prominent free radical scavenging activity (antioxidant activity) in the DPPH assay (Table 5). While the anti-oxidant

Table 1. ^{1}H (400 MHz, coupling constant *J* in Hz in parentheses) of compound [**I-III]**

Carbon no.	Chemical shift δ in ppm		
	[I]	**[II]**	**[III]**
2	6.53, d (2)	6.54, d (1.6)	6.63, d (2)
5	6.76, d (8.4)	6.66, d (8.4)	6.67, d (8.4)
6	6.53, dd (8.4, 2)	6.64, dd(8.4, 2)	6.54, dd (8.4, 2)
7	2.86, dd (14.1, 7.3) 2.76, dd (14.1, 6.2)	2.84, dd (14.0, 5.3) 2.76, dd (14.0, 6.5)	2.81, dd (14.0, 5.6) 2.78, dd (14.0, 6.8)
8	2.62 , m	2.64, m	2.61, m
2’	6.69, d (2)	6.72, d (2)	2.51, d (1.6)
5’	7.00, d (8.4)	7.2, d (8.0)	6.64, d (8)
6’	6.60, dd (8.4, 2)	6.48, dd (8.0, 2)	6.46, dd (8, 1.6)
7’	2.50, m	2.58, m	2.50, m
8’	2.41, m	2.46, m	2.48, m
9’	4.18, dd (8.9, 7.6) 3.84, dd (7.8, 7.6)	4.15, dd (8.7, 7.5) 3.90, dd (8,4, 7.5)	4.11, dd (9.2, 7.6) 3.82, dd (8.8, 7.6)
1’’	4.60, d (7.6)	4.80, d (7.6)	-
2’’	3.46, m	3.44, m	-
3’’	3.46 dd (6.8, 4.8)	3.44, m	-
4’’	3.36, dd (4.8, 3.2)	3.36, m	-
5’’	3.36, m	3.35, m	-
6’’	3.82, m	3.76, m	-
	3.62, m	3.66, m	-
OCH_3(3)	3.72, s	3.77, s	3.70, s
OCH_3(3’)	3.70, s	3.74, s	3.70, s
OCH_3(4)	3.70, s	-	-

Table 2. ^{13}C NMR (100 MHz) data of compound [**I-III**]

Carbon no.	Chemical shift δ in ppm		
	[I]	**[II]**	**[III]**
1	131.5	130.1	130.2
2	112.4	112.1	112.7
3	149.2	147.9	147.8
4	147.9	145.6	145.0
5	111.8	115.0	114.9
6	120.9	121.8	121.8
7	34.2	34.1	34.1
8	46.4	46.4	46.5
9	180.1	180.3	180.4
1’	133.1	133.1	129.5
2’	113.7	113.7	112.1
3’	149.2	149.5	147.8
4’	145.6	145.1	145.2
5’	116.6	116.7	115.0
6’	121.8	121.1	121.0
7’	37.7	37.2	37.7
8’	41.3	41.4	41.3
9’	71.7	71.7	71.7
1’’	101.7	101.7	-
2’’	73.7	73.7	-
3’’	76.6	76.6	-
4’’	70.1	70.1	-
5’’	76.9	76.9	-
6’’	61.3	61.3	-
O-CH_3 (3)	55.4	55.5	55.1
O-CH_3 (3’)	55.6	55.2	55.1
O-CH_3 (4)	55.4		

Table 3. ^{1}H (coupling constant *J*=Hz in parentheses) and ^{13}C NMR data, ^{1}H-^{13}C long-range (^{2}J and ^{3}J) HMBC key correlations of **[IV]**

Carbon number	Chemical shift δ in ppm		HMBC correlations	
	δ_H	δ_C	^{2}J	^{3}J
1		130.9		
2	6.57, brs	113.6	C-3’	C-7, C-4’, C-6’
3		149.1		
O-CH_3(3)	3.73, s	56.9		C-3
4		146.5		
5	6.68, d (8.0)	115.1	C-4’, C-6’	C-1’, C-3’
6	6.70, m	119.2	C-1’	C-7, C-4’
7	2.92, dd (14.0, 5.4) 2.85, dd (14.0, 6.6)	35.7	C-8, C-1	C-6, C-9, C-8’
8	2.58, m	48.0	C-9	
9		181.8		
1’		131.2		
2’	6.59, d (2)	113.2	C-7’	
3’		146.6		
O-CH_3(3’)	3.90, s	56.5	C-3’	
4’		147.5		
5’		132.0		
6’	6.55, d (2)	123.0	C-2’, C-7’	
7’	2.67, dd (13.5, 6.5) 2.56, m	39.1	C-8’	C-2’, C-6’, C-9’
8’	2.52, m	42.8	C-9’	C-1’
9’	4.20, dd (9.0, 7.5) 3.92, dd (9.0, 7.0)	73.1	C-8’	C-8, C-9, C-7’
1’’		134.9		
2’’	6.95, d (2)	110.7	C-1’’, C-3’’	C-6’’, C-4’’, C-7’’
3’’		149.2		
O-CH_3(3’’)	3.75	56.5	C-3’’	
4’’		147.7		
5’’	6.76, d (8)	116.4	C-4’’, C-6’’	C-1’’, C-3’’
6’’	6.82, dd (8, 2)	119.9	C-2’’, C-4’’,	C-7’’
7’’	5.54, dd (6)	89.2	C-1’’, C-8’’	C-4’, C5’, C-2’’, C-6’’, C-9’’
8’’	3.46, br. qr, (6)	55.0	C5’, C-7’’, C-9’’	C-4’, C-1’’
9’’	3.82, m	65.2		C-5’

Table 4. Distribution of lignans **[I-IV]** within the genus *Centaurea*

Species	Lignans				Reference.
	[I]	**[II]**	**[III]**	**[IV]**	
C. americana Nutt	+	+	-	-	14
C. nicaensis L.	-	-	-	+	17
C. napifolia L	-	-	-	+	16
C. nigra L	+	+	+	-	13
C. scabiosa L	-	+	+	-	15
C. scoparia L	-	-	+	-	19
C. solstitialis L	-	-	+	-	20
C. sphaerocephala L	+	-	+	+	11
C. tweediei Hook. Et Arn	-	-	+	-	21

(+) denotes presence of lignan

Table 5. Antiodixant and brine shrimp activity of compound [**I-IV**]

Compounds	IC_{50} (mg/mL)	LD_{50} (mg/mL)
I	16.0×10^{-2}	9.8×10^{-2}
II	2.19×10^{-3}	1.65×10^{-2}
III	2.02×10^{-3}	5.5×10^{-3}
IV	3.6×10^{-2}	9.25×10^{-3}
Quercetin	2.88×10^{-5}	-
Podophyllotoxin	-	2.79×10^{-3}

activity of **[II]** and **[III]** was identical to that of recently published data (18), **[I]** and **[IV]** have never been tested in the DPPH assay. The anti-oxidant activity of **[I]** and **[IV]** (IC_{50} = 16 x 10^{-2} and 3.6 x 10^{-2} mg/ml, respectively) was found to less than that of **[II]** and **[III]**. The antioxidant activity of **[I-IV]**, like other natural phenolic antioxidants, e.g. flavonoids, is a consequence of the presence of the phenolic moieties in the structures. The antioxidant activity of phenolic natural products is predominantly due to their redox properties, i.e. the ability to act as reducing agents, hydrogen donors and singlet oxygen quenchers, and to some extent, could also be due to their metal chelation potential.

The shrimp lethality assay, which has been proven to be an effective and rapid assay method to screen compounds for potential cytotoxic activity was used to determine the general toxicity of these lignans (Table 5). The general toxicity of **[II]** and **[III]** has recently been reported (18). The general toxicity of lappaol A (**IV**) was found to be ~50% less than that of **[III]**. The level of toxicity was very low with arctiin (**IV**).

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