Acta Pharm. 54 (2004) 277–285 Original research paper

2D NMR spectroscopic analyses of archangelicin from the seeds of *Angelica archangelica*

MELANIE MULLER[1]
MAUREEN BYRES[1]
MARCEL JASPARS[2]
YASHODHARAN KUMARASAMY[1]
MOIRA MIDDLETON[1]
LUTFUN NAHAR[3]
MOHAMMAD SHOEB[1]
SATYAJIT D. SARKER[1,*]

[1] *Phytopharmaceutical Research Laboratory, School of Pharmacy The Robert Gordon University, Schoolhill Aberdeen AB10 1FR, Scotland, UK*

[2] *Marine Natural Products Laboratory Department of Chemistry, University of Aberdeen, Meston Walk Aberdeen AB24 3UE, Scotland, UK*

[3] *School of Life Sciences, The Robert Gordon University, St Andrew Street Aberdeen AB25 1HG, Scotland, UK*

Received April 28, 2004
Accepted September 17, 2004

A total of six coumarins, bergapten (**1**), xanthotoxin (**2**), imperatorin (**3**), isoimperatorin (**4**), phellopterin (**5**) and archangelicin (**6**), have been isolated from an *n*-hexane extract of the seeds of *Angelica archangelica*. The results of comprehensive 2D NMR analyses of archangelicin are discussed.

Keywords: Angelica archangelica (*Apiaceae*), furanocoumarins, archangelicin, 2D NMR

Angelica archangelica L. (*Apiaceae*), commonly known as European angelica or wild parsnip, is native to Austria, Belgium, Denmark, Germany, Greenland, Hungary, Iceland, Poland and central Russia, and has been naturalised in the UK and other parts of Europe (1, 2). *Angelica archangelica* is believed to possess angelic healing power. This plant has been used in traditional and folk medicine as a remedy for nervous headaches, fever, skin rashes, wounds, rheumatism, and toothaches (2). The roots of this plant have been used internally for digestive problems, including gastric ulcers, anorexia, and migraine, bronchitis, chronic fatigue, and menstrual and obstetric complaints. It has been shown to stimulate gastric and pancreatic secretions. *A. archangelica* can be used as an

* Correspondence, e-mail: s.sarker@rgu.ac.uk

antiseptic, expectorant, emmenagogue and a diuretic. Previous phytochemical investigations on *A. archangelica* revealed the presence of various types of secondary metabolites, predominantly furanocoumarins (2–4). As part of our recently initiated phytochemical studies on the genus *Angelica* (5, 6), we now report on the convenient isolation protocol of six furano/dihydrofuranocoumarins (**1**–**6**) (Fig. 1) and, for the first time, present comprehensive 2D NMR spectroscopic analyses of archangelicin (**6**) leading to the unequivocal assignment of ^{1}H and ^{13}C NMR chemical shifts for this compound.

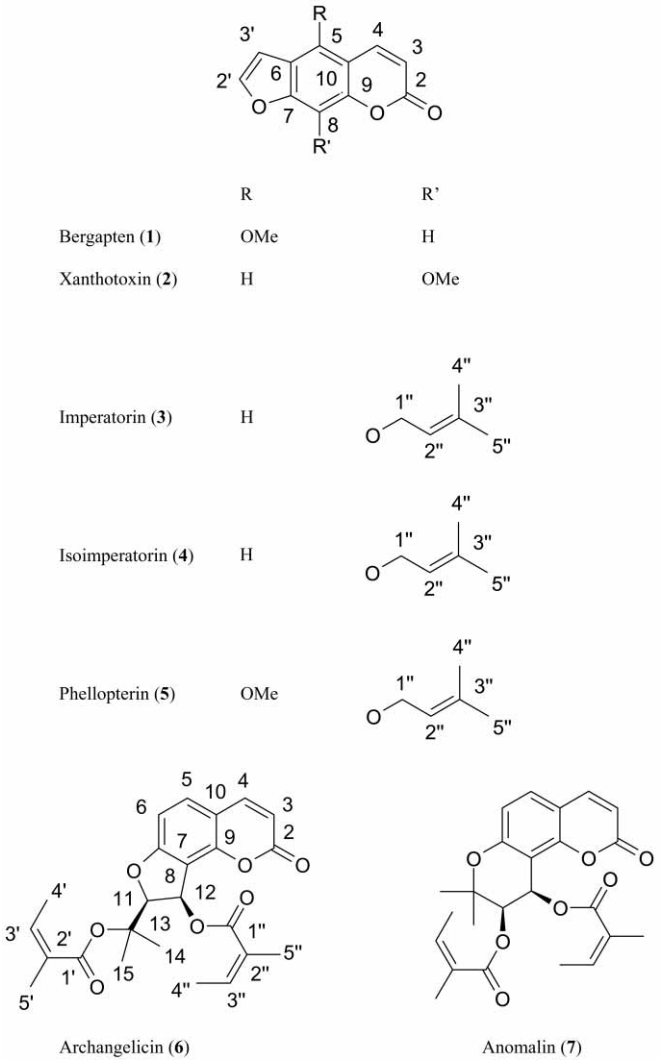

Fig. 1. Structures of coumarins **1**–**6** isolated from *A. archangelica* (*Apiaceae*).

EXPERIMENTAL

General procedures

UV spectra were obtained in MeOH using a Hewlett-Packard 8453 UV-Vis spectrometer (Agilent, Germany). FT-IR spectra were obtained using the KBr disc method on AVATAR 360 FT-IR (Thermo Nicolet, UK). Chemical Ionisation Mass Spectrometry (CIMS) analyses were performed in the EPSRC Central Mass Spectroscopy Facility in Swansea, UK, on a Micromass Quattro II triple quadrupole instrument (Waters, UK) in the chemical desorption mode (ammonia as CI gas, mass accuracy within 0.4 Da, CI source temperature 170 °C and electron energy 59eV, internal standard polyethylimine). The optical rotation was measured on an ADP 220 Polarimeter, Bellingham-Stanley (UK). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were recorded on a Varian Unity INOVA 400 MHz NMR spectrometer (Varian, UK). Heteronuclear Multiple Bond Coherence (HMBC) spectra were optimized for a long range J_{H-C} of 9 Hz and the Nuclear Overhauser Effect Spectroscopy (NOESY) experiment was carried out with a mixing time of 0.8 s. Silica gel 60H and silica gel PF_{254} (Merck, Germany) were used for vacuum liquid chromatography (VLC) and thin layer chromatography (TLC), respectively.

Plant material

The seeds of *Angelica archangelica (Apiaceae)* were purchased from B & T World Seeds Sarl, Paguignan, France. A voucher specimen (PH00131103 SDS) has been deposited in the herbarium of the Plant and Soil Science Department, University of Aberdeen, Scotland, UK.

Extraction and isolation

The seeds (113 g) were ground using a coffee grinder, and extracted sequentially with *n*-hexane, dichloromethane and methanol (1.1 L in each case) using Soxhlet apparatus. The dichloromethane and methanol extracts did not contain any significant amounts of non-polar coumarins and were not processed further. On standing at room temperature, the *n*-hexane extract produced a yellow precipitate (8.7 g). The precipitate was separated from the liquid by filtration and dried off on filter paper. Recrystallisation of a portion (1.7 g) of the precipitate using $CHCl_3$ yielded mixed crystals (609 mg) of imperatorin (**3**) and isoimperatorin (**4**). The mixed crystals were dissolved in $CHCl_3$ and subjected to preparative thin-layer chromatography (PTLC) (mobile phase: 10% EtOAc in *n*-hexane) to purify **3** and **4**. The rest of the *n*-hexane extract was concentrated using a rotary evaporator operating at 45 °C to obtain 24.8 g of oily substance. A portion of this oily material (2.5 g) was subjected to VLC and eluted with a mobile phase of increasing polarity: *n*-hexane → *n*-hexane/EtOAc → EtOAc → EtOAc/MeOH → MeOH. The preparative TLC (mobile phase: 20% EtOAc in *n*-hexane) of the VLC fraction eluted with 40% EtOAc in *n*-hexane yielded bergapten (**1**, 12 mg) and xanthotoxin (**2,** 22 mg). Similar purification of another VLC fraction (25% EtOAc in *n*-hexane) provided phellopterin (**5**, 23 mg) and archangelicin (**6**, 17 mg).

RESULTS AND DISCUSSION

Five linear furanocoumarins, bergapten (**1**), xanthotoxin (**2**), imperatorin (**3**), isoimperatorin (**4**) and phellopterin (**5**) and an angular dihydrofuranocoumarin archangelicin (**6**) (Fig. 1), have been isolated from the *n*-hexane extract of the seeds of *Angelica archangelica* by crystallisation and PTLC. The structures of these compounds have been elucidated by UV, IR, MS and comprehensive NMR analyses.

All compounds **1–6** displayed UV and IR absorption peaks, and ^{1}H and ^{13}C NMR signals (Tables I–IV) characteristic of a coumarin nucleus (12). Particularly, the spectroscopic data of compounds **1–5** were typical of linear furanocoumarins (8, 12, 13). Coumarins **1–5** could be readily identified by comparison of their UV, IR, MS and NMR data with those published for the linear furanocoumarins, bergapten (**1**), xanthotoxin (**2**), imperatorin (**3**), isoimperatorin and phellopterin (**5**), respectively (7–9), and also by co-TLC with authentic samples previously isolated and identified in our labs. However, the unambiguous assignment of the ^{1}H and ^{13}C NMR data, based on comprehensive 2D NMR analyses, for these compounds is presented here.

The CIMS revealed the $[M+NH_4]^+$ ion at *m/z* 444 daltons corresponding to the molecular formula $C_{24}H_{26}O_7$ for **6**. In addition to the signals due to the coumarin nucleus, the ^{1}H and ^{13}C NMR spectra (Table III) of **6** displayed signals that could be attributed to

*Table I. Physical and spectroscopic data for coumarins **1–6***

Coumarins	Physical state	CIMS data (*m/z*) $[M+NH_4]^+$	UV, λ_{max} (nm)	IR, ν_{max} (cm^{-1})	Optical rotation $[\alpha]_D^{25}$	Reference
Bergapten (**1**)	Amorphous solid	234	223, 243, 249, 259, 268, 311	3100, 3070, 3050, 1726, 1620, 1602, 1575, 1540, 885;	NA	7, 8
Xanthotoxin (**2**)	Amorphous solid	234	223, 242, 248, 269, 273, 313	3110, 3080, 3040, 1705, 1626, 1580, 1540, 875	NA	7, 8
Imperatorin (**3**)	Crystalline solid	288	219, 248, 302	1721, 1586, 1147, 835	NA	7, 9
Isoimperatorin (**4**)	Crystalline solid	288	218, 248, 303	1721, 1587, 1147, 836	NA	7, 9
Phellopterin (**5**)	Amorphous solid	318	205, 220, 249, 264, 302	3055, 2925, 1705, 1584, 1465, 1377, 1146, 1081, 1027	NA	9
Archangelicin (**6**)	Colourless needles	444	216, 246, 256, 258, 266, 302, 322	3055, 2925, 1740, 1738, 1716, 1623, 1584, 1490, 1460	+ 112.8° (*c* 0.002 g L^{-1} MeOH)	10, 11

NA – Not applicable

*Table II. ^1H NMR data of furanocoumarins **1–5**[a]*

Carbon number	Chemical shift (δ ppm)				
	1	**2**	**3**	**4**	**5**
3	6.26 d (9.8)	6.35 d (9.5)	6.36 d (9.5)	6.26 d (9.6)	6.25 d (9.6)
4	8.16 d (9.8)	7.75 d (9.5)	7.75 d (9.5)	8.08 d (9.6)	8.09 d (9.6)
5	–	7.34 s	7.35 s	–	–
5–OMe	4.26 s	–	–	–	4.25 s
8	7.16 s	–	–	7.22 s	–
8–OMe	–	4.30 s	–	–	–
2′	7.60 d (2.5)	7.67 d (2.4)	7.68 d (2.4)	7.58 d (2.3)	7.60 d (2.3)
3′	7.01 d (2.5)	6.82 d (2.4)	6.82 d (2.4)	6.97 d (2.3)	7.02 d (2.3)
1″	–	–	4.95 d (7.2)	4.84 d (7.0)	4.82 d (7.0)
2″	–	–	5.61 t (7.2)	5.53 t (7.0)	5.34 t (7.0)
4″	–	–	1.73 s	1.71 s	1.72 s
5″	–	–	1.69 s	1.69 s	1.69 s

[a] $CDCl_3$, 400 MHz, coupling constant *J* in Hz in parentheses

*Table III. ^{13}C NMR data of furanocoumarins **1–5**[a]*

Carbon number	Chemical shift (δ ppm)				
	1	**2**	**3**	**4**	**5**
2	160.3	160.7	160.6	161.3	161.3
3	112.6	114.8	113.0	112.4	112.5
4	139.6	144.4	144.4	139.6	140.0
5	149.7	112.9	114.8	152.6	152.8
5–MeO	60.4	–	–	–	60.8
6	114.0	126.3	126.0	114.0	114.0
7	158.3	147.7	148.6	158.1	158.9
8	94.0	132.6	132.0	94.1	132.9
8–OMe	–	61.2	–	–	–
9	152.7	143.9	143.8	148.9	144.1
10	106.4	116.5	116.5	106.7	106.5
2′	144.9	146.7	146.6	145.0	146.2
3′	105.2	106.8	106.7	105.4	105.8
1″			69.9	69.8	69.1
2″			119.6	119.1	118.9
3″			139.7	139.8	139.7
4″			18.2	18.4	18.2
5″			25.9	25.7	25.8

[a] ($CDCl_3$, 100 MHz)

two angeloyl moieties (δ_H 6.00, 5.95, 1.93, 1.92, 1.81, 1.78; δ_C 167.3, 165.6, 139.3, 137.5, 128.8, 127.0, 20.6, 20.3, 15.7, 15.6), two oxymethines (δ_H 7.09, 5.30, δ_C 68.0, 88.7), two geminal dimethyl groups (δ_H 1.70, 1.64; δ_C 24.8, 22.8) and an oxygenated quaternary carbon (δ_C 81.0). All these NMR signals could be assigned to the structure of either archangelicin (**6**) or anomalin (**7**). To date, the available published NMR data for archangelicin (**6**) are not conclusive enough to differentiate between these two structures. Only a combination of 2D NMR experiments on this compound are deemed appropriate to solve this structure. The ^{1}H–^{1}H COSY (Homonuclear Correleted Spectroscopy) (Table IV) displayed all ^{1}H–^{1}H scalar couplings, the most significant being H–11 ↔ H–12 and H–3′ ↔ Me–4′. The ^{1}H–^{13}C Heteronuclear Single Quantum Coherence (HSQC) spectrum (Table

*Table IV. $^1H^a$ and ^{13}C NMR^b data, and ^1H–^1H COSY, ^1H–^{13}C HSQC and ^1H–^{13}C HMBC correlations of archangelicin (**6**)^c*

Carbon number	Chemical shift (δ ppm)		^{1}H–^{1}H COSY	^{1}H–^{13}C HSQC	^{1}H–^{13}C HMBC	
	δ_H	δ_C		(^1J)	^2J	^3J
2	–	159.7	–	–	–	–
3	6.19 d (9.6)	113.2	H–4	113.2	C–2	C–10
4	7.57 d (9.6)	143.4	H–3	143.4	C–3, C–10	C–2, C–5, C–9
5	7.37 d (8.2)	131.2	H–6	131.2	C–6, C–10	C–7, C–9, C–4
6	6.80 d (8.2)	107.5	H–5	107.5	C–7	C–8, C–10
7	–	163.6	–	–	–	–
8	–	113.3	–	–	–	–
9	–	151.6	–	–	–	–
10	–	113.0	–	–	–	–
11	5.30 d (7.2)	88.7	H–12	88.7	C–13, C–12	C–14, C–15, C–7
12	7.09 d (7.2)	68.0	H–11	68.0	C–8	C–1′′, C–7, C–9
13	–	81.0	–	–	–	–
14	1.64 s	22.8	–	22.8	C–13	C–15
15	1.70 s	24.8	–	24.8	C–13	C–14
1′	–	167.3	–	–	–	–
2′	–	127.0	–	–	–	–
3′	5.95 m	137.5	Me–4′	137.5		C–5′
4′	1.93 d (7.2)	15.7	H_3–3′	15.6	C–3′	C–2′
5′	1.81 s	20.6	–	20.3	C–2′	C–1′, C–3′
1′′	–	165.6	–	–	–	–
2′′	–	128.8	–	–	–	–
3′′	6.00 m	139.3	Me–4′′	139.3	–	C–5′′
4′′	1.92 d (7.2)	15.6	H_3–3′′	15.7	C–3′′	C–2′′
5′′	1.78 s	20.3	–	20.6	C–2′′	C–1′′, C–3′′

[a] 400 MHz, coupling constant *J* in Hz in parentheses
[b] 100 MHz
[c] Spectra taken in $CDCl_3$

IV) revealed all ^1H–^{13}C direct couplings and helped in the unambiguous assignment of ^1H and ^{13}C NMR signals for all methine and methyl groups. Among these 2D NMR techniques, the ^1H–^{13}C HMBC was found to be the most informative regarding the structure elucidation of **6**. The ^1H–^{13}C long-range key correlations observed in the HMBC (Table IV) that could differentiate archangelicin (**6**) from anomalin (**7**) were: from H–11 (δ_H 5.30), ^3J correlations to C–7 (δ_C 163.6), C–8 (δ_C 113.3), C–14 (δ_C 22.8) and C–15 (δ_C 24.8), and ^2J correlations to C–12 (δ_C 68.0) and C–13 (δ_C 81.0); from H–12 (δ_H 7.09), ^3J correlations to C–1′′ (δ_C 165.6), C–9 (δ_C 151.6), C–7 (δ_C 163.6) and C–13 (δ_C 81.0), and ^2J correlations to C–11 (δ_C 88.7) and C–8 (δ_C 113.3). These correlations were only possible for the structure of **6**, not **7**. If the structure had been **7**, both oxymethine protons (δ_H 5.30 and 7.09) would have shown ^3J correlation to the angeloyl carbonyls (δ_C 167.3 and 165.6), and the oxymethine signal δ_H 5.30 would not have shown any ^3J correlation to δ_C 163.6. The *cis*-configuration of H–11 and H–12 was evident from the vicinal coupling constant J = 7.2 Hz. The relative stereochemistry at C–11 and C–12 was further established from a strong NOE interaction between H–11 and H–12 observed in the ^1H–^1H NOESY spectrum (Fig. 2). Thus coumarin **6** was identified unequivocally as archangelicin. To our knowledge, unambiguous and comprehensive ^1H and ^{13}C NMR data, based on extensive 2D NMR analyses, are not available in the published literature, and are thus presented here for the first time.

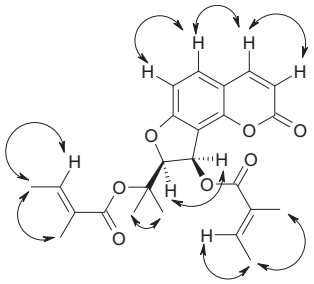

Fig. 2. Key NOE interactions observed in the ^1H–^1H NOESY spectrum of **6**.

While coumarins **1**–**5** have previously been reported from various parts of *A. archangelica*, archangelicin (**6**) has not been reported from the seeds before. Compounds **1**–**5** are of common occurrence within the genus *Angelica* and the family *Apiaceae* (5), but the distribution of **6** is apparently restricted to various subspecies of *A. archangelica* (4, 11), *A. keiskei* (10) and *Athamanta longeradiata* (4).

CONCLUSIONS

It is almost impossible to distinguish between anomalin (**7**) and archangelicin (**6**) simply on the basis of ^1H and ^{13}C NMR data interpretation. It has been demonstrated in this paper that comprehensive 2D NMR experiments including COSY, HMBC, HMQC and NOESY are essential to unequivocally deduce the structure of the compound archangelicin (**6**).

Acknowledgement. – We thank the EPSRC National Mass Spectrometry Service Centre (Department of Chemistry, University of Wales Swansea, Swansea, Wales, UK) for CIMS analyses.

REFERENCES

1. A. R. Clapham, T. G. Tutin and E. F. Warburg, *Flora of the British Isles*, The University Press Cambridge, London 1952.
2. S. D. Sarker and L. Nahar, Natural medicine: The genus *Angelica*, *Current Med. Chem.* **11** (2004) 1479–1500.
3. Phytochemical and Ethnobotanical Databases, USDA – ARS – NGRL, Beltsville Agricultural Research Center, Beltsville, Maryland, USA (2004) (http://www.ars-grin.gov/duke/).
4. Dictionary of Natural Products on CD ROM, version 9.1, Chapman & Hall/CRC Press, Boca Raton, 2000.
5. E. M. Murphy, L. Nahar, L. M. Byres, M. Shoeb, M. Siakalima, M. M. Rahman, A. I. Gray and S. D. Sarker, Coumarins from the seeds of *Angelica sylvestris* (*Apiaceae*) and their distribution within the genus *Angelica*, *Biochem. Syst. Ecol.* **32** (2004) 203–207.
6. S. D. Sarker, E. Eynon, K. Fok, Y. Kumarasamy, E. M. Murphy, L. Nahar, E. M. Shaheen, N. M. Shaw and M. Siakalima, Screening the extracts of the seeds of *Achillea millefolium*, *Angelica sylvestris* and *Phleum pratense* for antibacterial, antioxidant activities and general toxicity, *Oriental Pharm. Exp. Med.* **3** (2003) 157–162.
7. T. Masuda, M. Takasugi and M. Anetai, Psoralen and other linear furanocoumarins as phytoalexins in *Gleihnia littoralis*, *Phytochemistry* **47** (1998) 13–16.
8. S. D. Sarker, *Phytochemical and Chemotaxonomic Studies in the Tribe Boronieae (Rutaceae)*, Ph. D. Thesis, University of Strathclyde, Glasgow 1995.
9. O. Bergendorff, K. Dekermendjian, M. Nielsen, R. Shan, R. Witt, J. Ai and O. Sterner, Furanocoumarins with affinity to brain benzodiazepine receptors *in vitro*, *Phytochemistry* **44** (1997) 1121–1124.
10. T. Okuyama, M. Takata, J. Takayasu, T. Hasegawa, H. Tokuda, A. Nishino and H. Nishino, Antitumour promotion by principles obtained from *Angelica keiskei*, *Planta Med.* **57** (1991) 243–246.
11. B. E. Nielsen and J. Lemmich, Constituents of umbelliferous plants III. The structure of archangelicin, a coumarin from *Angelica archangelica* L. subsp. *litoralis* Thell., *Acta Chem. Scand.* **18** (1964) 932–936.
12. R. D. H. Murray, J. Mendez and S. A. Brown, *The Natural Coumarins: Occurrence, Chemistry and Biochemistry*, Wiley, Bristol 1982.
13. B. E. Nielsen, *Coumarins of Umbelliferous Plants*, The Royal Danish School of Pharmacy, Copenhagen 1970.

S A Ž E T A K

2D NMR spektroskopske analize arhangelicina iz sjemenki biljke *Angelica archangelica*

MELANIE MULLER, MAUREEN BYRES, MARCEL JASPARS, YASHODHARAN KUMARASAMY, MOIRA MIDDLETON, LUTFUN NAHAR, MOHAMMAD SHOEB i SATYAJIT D SARKER

Iz *n*-heksanskog ekstrakta sjemenki biljke *Angelica archangelica* izolirano je šest kumarina, bergapten (**1**), ksantotoksin (**2**), imperatorin (**3**), izoimperatorin (**4**), felopterin (**5**) i arhangelicin (**6**). U radu su detaljno analizirani 2D NMR spektri arhangelicina.

Ključne riječi: Angelica archangelica (Apiaceae), furanokumarini, arhangelicin, 2D NMR spektroskopija

School of Pharmacy, The Robert Gordon University, Schoolhill, Aberdeen AB10 1FR, Scotland, UK

Department of Chemistry, University of Aberdeen, Meston Walk, Aberdeen AB24 3UE, Scotland, UK

School of Life Sciences, The Robert Gordon University, St Andrew Street, Aberdeen AB25 1HG, Scotland, UK