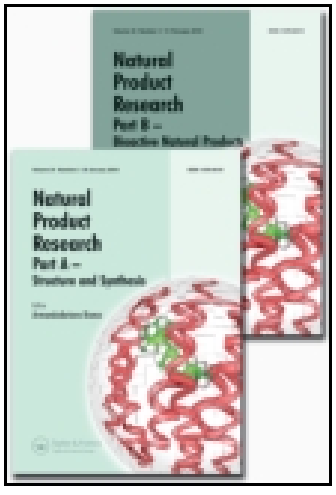

Natural Product Research: Formerly Natural Product Letters
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gnpl20

(-)-Arctigenin as a lead compound for anticancer agent

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Published online: 20 Aug 2013.

To cite this article: Gui-Rong Chen, Hong-Fu Li, De-Qiang Dou, Yu-Bin Xu, Hong-Shuai Jiang, Fu-Rui Li & Ting-Guo Kang (2013) (-)-Arctigenin as a lead compound for anticancer agent, Natural Product Research: Formerly Natural Product Letters, 27:23, 2251-2255, DOI: 10.1080/14786419.2013.821120

To link to this article: http://dx.doi.org/10.1080/14786419.2013.821120

Natural Product Research, 2013
Vol. 27, No. 23, 2251–2255, http://dx.doi.org/10.1080/14786419.2013.821120

SHORT COMMUNICATION

(−)-Arctigenin as a lead compound for anticancer agent

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(*Received 5 March 2013; final version received 2 June 2013*)

(−)-Arctigenin, an important active constituent of the traditional Chinese herb Fructus Arctii, was found to exhibit various bioactivities, so it can be used as a good lead compound for further structure modification in order to find a safer and more potent medicine. (−)-Arctigenin derivatives **1**–**5** of (−)-arctingen were obtained by modifying with ammonolysis at the lactone ring and sulphonylation at C (6′) and C (6″) and *O*-demethylation at CH_3O-C (3′), CH_3O-C (3″) and CH_3O-C (4″), and their anticancer bioactivities were examined.

Keywords: (−)-arctigenin; structure modification; synthetic derivatives; anticancer bioactivity

1. Introduction

Fructus Arctii is the dried ripe fruit of *Arctium lappa* L. which contains mainly dibenzylbutyrolactone lignans of arctiin and (−)-arctigenin. The pharmacological research indicated that (−)-arctigenin exhibited stronger activity than arctiin. The research has also confirmed (−)-arctigenin to possess anticancer activity (Hirano et al. 1994; Takasi et al. 2000) and antiviral actions (Yang et al. 1996) and also that it functions as a platelet-activating factor antagonist (Han et al. 1992; Iwakami et al. 1992). However, (−)-arctigenin has showed lower bioavailability and has been limited to clinical application. Owing to a wide range of biological activities, (−)-arctigenin can be used as a useful precursor for structure modification to improve the bioavailability and enhance the bioactivity and expand the clinical application. Based on the aforementioned facts, (−)-arctigenin derivatives **1**–**5** were designed and synthesised, and their anticancer bioactivities were examined. The structures of (−)-arctigenin derivatives were elucidated on the basis of MS and NMR spectra.

2. Results and discussion

2.1. *Synthesis of compounds 1–5*

Two new ammonoylsis derivatives of **1** and **2** (Figure 1) were prepared following reaction of (−)-arctigenin and two *N*-substituted primary amines. The new synthetic sulphonate derivative (**5**) (Figure 1) is water soluble and was obtained with the treatment of (−)-arctigenin and sulphuric acid stirring at room temperature for 12 h (Yao et al. 2009). The demethylated derivatives (**3, 4**) (Figure 1) were synthesised by treating (−)-arctigenin and demethylating

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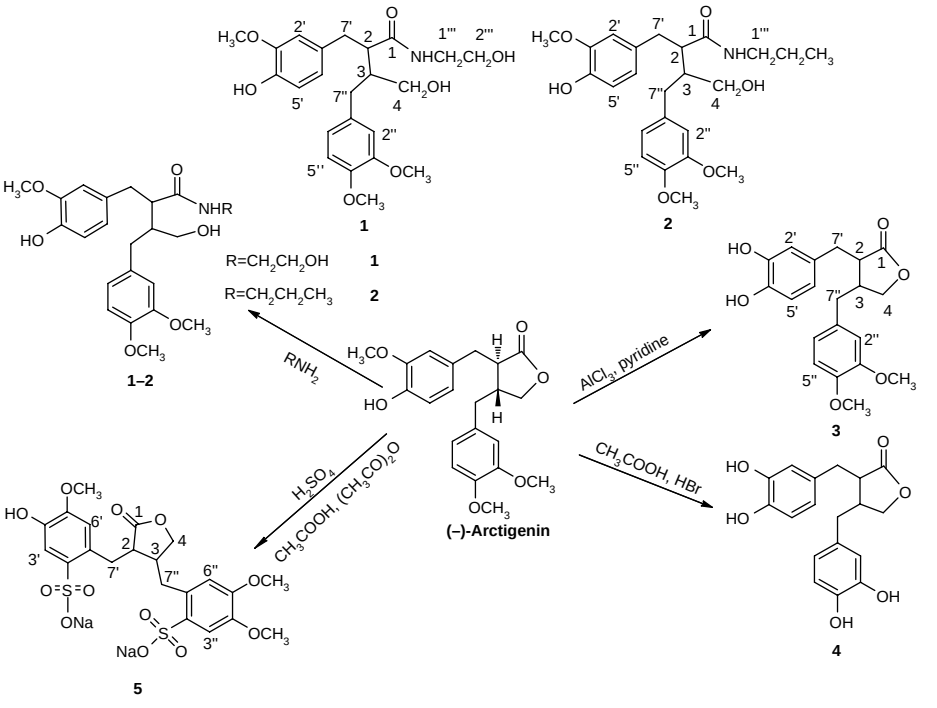

Figure 1. The synthetic derivatives of (−)-arctigenin.

agents, respectively (Ding & Zhang 2007). On the basis of the spectral data of ^{1}H and ^{13}C NMR, HSQC and HMBC, the structures of compounds **1**–**5** were elucidated.

2.2. *Structure elucidation of the five synthetic derivatives*

Compound **1**, $[\alpha]_D^{20} = +24.6$, was afforded as white powder. HR-ESI-MS gave its quasi molecular ion peak at *m/z* 433.2106 $[M]^+$ (calcd 433.2101), exhibiting that the molecular formula of **1** was confirmed as $C_{23}H_{31}O_7N$. The structure of **1** contains an additional —$NHCH_2CH_2OH$ fragment compared with the molecular weight of (−)-arctigenin. In the NMR spectra of **1**, three methoxyls were observed by carbon signals at δ 56.4, 56.5 and 56.6, respectively, in the ^{13}C NMR spectrum and their corresponding signal at δ 3.80 (9H, overlapped) in the ^{1}H NMR spectrum according to the HSQC spectrum of **1**. Six aromatic protons appeared at δ 6.69 (1H, d, $J = 1.9$ Hz), 6.86 (1H, d, $J = 8.2$ Hz), 6.59 (1H, dd, $J = 1.9$ and 8.2 Hz), 6.81 (1H, d, $J = 1.9$ Hz), 6.67 (1H, d, $J = 8.1$ Hz) and 6.77 (1H, dd, $J = 1.9$ and 8.1 Hz) in the ^{1}H NMR spectrum, indicating two 1,2,4-trisubstituted phenyl groups. Six aromatic protons coupled with the ^{13}C NMR signals of **1** at low field, and the structure of **1** was inferred to contain two 1,2,4-trisubstituted phenyl groups. The carbon signal at δ 177.6 belonged to an amide carboxyl group. The proton signals at δ 2.77 (1H, dd, $J = 5.9$ and 13.2 Hz), 2.70 (1H, dd, $J = 8.3$ and 13.7 Hz), 2.88 (1H, dd, $J = 5.7$ and 13.5 Hz), 2.82 (1H, dd, $J = 9.5$ and 13.2 Hz), 2.60 (1H, m) and 1.98 (1H, m) in the ^{1}H NMR spectrum and the carbon signals at δ 35.5, 37.1, 51.9 and 45.7 in the ^{13}C NMR spectrum belonged to two —CH_2— and two —CH— groups. With the help of HSQC, the ^{13}C NMR signal at δ 61.9 correlated with the proton signals at δ 3.54 (1H, dd, $J = 4.6$ and 11.3 Hz) and 3.58 (1H, dd, $J = 4.9$ and 11.4 Hz), which showed the presence of one —CH_2OH fragment. In addition, the proton signals at δ 3.15 (1H, m), 3.25 (1H, m) in the ^{1}H NMR spectrum due to methylene in —$NHCH_2CH_2OH$ showed long-range correlation with δ

177.6 due to the characteristic carbonyl carbon signal of C-1, which shows that—$NHCH_2CH_2$-OH is linked to C-1. With the aid of HMBC and HSQC spectra, the carbon and proton signals were attributable. Thus, the structure was established to be 2-(4′-hydroxyl-3′-methoxybenzyl)-3-(3″,4″-dimethoxy benzyl)-4-hydroxy-*N*-hydroxyethylbutanamide, and the NMR spectral data are shown in supplementary material (Tables S1, S2).

Compound **2**, $[\alpha]_D^{20} = -39.3$, was obtained as white crystalline columnar. HR-ESI-MS gave its quasi molecular ion peak at *m/z* 431.2298 $[M]^+$ (calcd 431.2308), exhibiting that the molecular formula of **2** was confirmed as $C_{24}H_{33}NO_6$. The structure of **2** contains an additional —$NHCH_2CH_2CH_3$ group by comparing with the molecular weight of (−)-arctigenin. The proton signals at δ 3.00 (2H, m) in the ^{1}H NMR spectrum due to methylene in —$NHCH_2CH_2CH_3$ showed long-range correlation with δ 177.1 due to the characteristic carbonyl carbon signal of C-1, indicating that —$NHCH_2CH_2CH_3$ is linked to C-1. By comparing the spectral data with those of **1**, the complete molecule of **2** was established by ^{1}H and ^{13}C NMR, HSQC and HMBC experiments, whose structure was almost identical to that of **1**, besides those of the side chain. Therefore, the structure of **2** was determined to be 2-(4′-hydroxyl-3′-methoxybenzyl)-3-(3″,4″-dimethoxy benzyl)-4-hydroxy-*N*-propylbutanamide, and the NMR spectral data are shown in the supplementary material (Tables S1, S2).

Compound **3** was obtained as colourless oil. Two methoxyls were observed by the signal at δ 3.78 (6H, s) in the ^{1}H NMR spectrum and their corresponding carbon signals were attributed at δ 56.5 and 56.6, respectively, in the ^{13}C NMR spectrum according to the HSQC spectrum of **3**. Six aromatic protons emerged in the ^{1}H NMR spectrum at δ 6.64 (1H, d, $J = 1.8$ Hz), 6.68 (1H, d, $J = 8.0$ Hz), 6.48 (1H, dd, $J = 8.0$ and 1.8 Hz), 6.60 (1H, d, $J = 1.9$ Hz), 6.82 (1H, d, $J = 8.2$ Hz) and 6.61 (1H, dd, $J = 8.2$ and 1.9 Hz), suggesting two ABX coupling systems in two phenyl groups. They coupled with the ^{13}C NMR signals of **3** at the low field, and the structure of **3** was deduced to contain two 1,2,4-trisubstituted phenyl fragments. The carbon signal at δ 181.5 belonged to the carboxyl group. The carbon signals at δ 35.3, 47.6, 38.8 and 42.7 in the ^{13}C NMR spectrum and the proton signals at δ 2.77 (1H, dd, $J = 5.0$ and 13.3 Hz), 2.87 (1H, dd, $J = 5.5$ and 13.4 Hz), 2.49 (1H, dd, $J = 8.5$ and 13.8 Hz), 2.59 (1H, dd, $J = 5.8$ and 13.1 Hz), 2.62 (1H, m) and 2.47 (1H, m) in the ^{1}H NMR spectrum belonged to two —CH_2— and two —CH— fragments. The complete molecule of **3** was confirmed by ^{1}H and ^{13}C NMR, HSQC and HMBC experiments, whose structure was almost identical to that of (−)-arctigenin, expect for the lost of methyl at 3′-OCH_3. Therefore, the structure of **3** was determined to be 3-(3′,4′-dihydroxylbenzyl)-4-(3″,4″-dimethoxy benzyl)dihydrofuran-2(3*H*)-one, and the NMR spectral data are shown in the supplementary material (Tables S1, S2).

Compound **4** was afforded as white powder. In the NMR spectra of **4**, methoxyl signals were not observed in the ^{1}H NMR spectrum and their corresponding carbon signals were absent in the ^{13}C NMR spectrum. Comparing the spectral data with that of **3**, the complete molecule of **4** was determined by ^{1}H and ^{13}C NMR, HSQC and HMBC experiments, and the structure of **4** was determined to be 3-(3′,4′-dihydroxylbenzyl)-4-(3″,4″-dihydroxylbenzyl)dihydrofuran-2(3*H*)-one, whose NMR spectral data are shown in the supplementary material (Tables S1, S2).

Compound **5**, $[\alpha] = +15.5$, was obtained as white needle crystal. The molecular formula of compound **5** was confirmed as $C_{21}H_{22}O_{12}S_2Na_2$ by HR-ESI-MS spectroscopic data. Compared with the molecular weight of (−)-arctigenin, the structure of **5** contains two additional —SO_3Na groups. In the NMR spectra of **5**, four aromatic protons emerged in the ^{1}H NMR spectrum at δ 7.27 (1H, s), 6.35 (1H, s), 7.35 (1H, s) and 6.33 (1H, s). They coupled with the ^{13}C NMR signals of **5** at low field and the structure of **5** was deduced to posses two 1,2,4,5-four-substituted phenyl fragments by comparing with that of (−)-arctigenin. Three methoxyls were observed by the signals at δ 3.89 (3H, s) and 3.62 (6H, overlapped) in the ^{1}H NMR spectrum and their corresponding carbon signals at δ 55.9, 56.1 and 56.3, respectively, in the ^{13}C NMR spectrum according to the HSQC spectrum of **5**. The carbon signal at δ 184.2 belonged to carboxyl

group. With the aid of HSQC, the carbon signals at δ 32.3, 35.5, 47.4 and 40.7 in the ^{13}C NMR spectrum and the proton signals at δ 3.40 (1H, t), 2.87 (1H, dd, $J = 13.7$ and 3.3 Hz), 3.31 (1H, t), 2.77 (1H, dd, $J = 13.7$ and 4.0 Hz), 3.00 (1H, m), 3.00 (1H, m) in the ^{1}H NMR were attributed to two —CH_2— and two —CH— fragments. Thus, the structure was determined to be 2-((4-(4″,5″-dimethoxy-2″-sulphonatobenzyl)-2-oxotetrahydrofuran-3-methyl)-5′-hydroxy-4′-methoxybenzen-sulphonate sodium, and the NMR spectral data are shown in the supplementary material (Tables S1, S2).

2.3. *Bioactivity essay of (−)-arctigenin and its structural derivatives 1–5*

All the synthetic derivatives were screened for anticancer activity by different human cancer cells, such as HCT116 (human colon), MGC-803 (human gaster), NCI-H460 (human lung), PANC-1 (human pancreas), Bel7402 (human hepatocellular carcinoma), SGC7901 (human gastric carcinoma), NCI-H460 (human lung cancer), Du145 (human prostate cancer) and HEPG-2 (human hepatoma) (Qiu et al. 2009).

The anticancer activities of (−)-arctigenin and the synthetic derivatives were investigated and the results (Tables S3, S4) indicated that the cleavage derivatives **1**–**2** showed hardly activity on the five different human cancer cells compared with (−)-arctigenin. The results showed that cleavage in the lactone was considered to be unfavoured method for structure modification. Although demethylation derivatives **3**–**4** and sulphonate derivative **5** exhibited low activity on SGC7901 compared with (−)-arctigenin, the derivative **5** showed stronger water solubility than (−)-arctigenin, which was suitable to be prepared for parenteral solution.

3. Conclusion

The synthetic derivatives of **1**–**5** were obtained and the structures were elucidated with the aid of spectral data. The anticancer bioactivity of **1**–**5** and (−)-arctigenin was tested by different human cancer cells. The results indicated that the anticancer bioactivity of **1** and **2** was lower than that of (−)-arctigenin. Anticancer bioactivity of **3**–**5** was close to that of (−)-arctigenin. But compound **5** was water soluble and it can be prepared for parenteral solution of (−)-arctigenin. As concluded from this study, the lactone ring and the phenolic hydroxyl of (−)-arctigenin played an important role in anticancer bioactivity.

Supplementary material

Supplementary material relating to this article is available online, alongside Tables S1–S4.

Acknowledgements

Thanks for the funding of the National Eleventh Five-year Plan in R&D of Important New Medicine (2009ZX09103-423), Shenyang City Science and Technology Project (F11-264-1-20) and Program for Liaoning Innovative Research Team in University (LT2013020). De-Qiang Dou was sponsored by the funding.

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