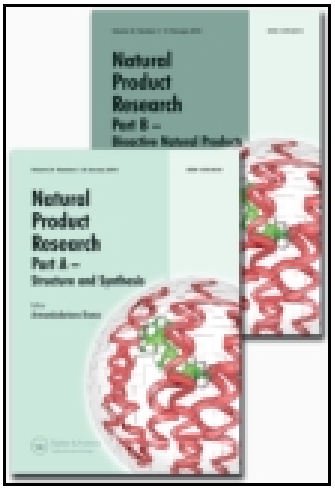

Natural Product Research: Formerly Natural Product Letters

Publication details, including instructions for authors and subscription information:
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A trehalose ester from Lancea tibetica

Shi-Jun Liu[ab], Zhi-Xin Liao[ab], Chao Liu[a] & Yan-Bo Qu[a]

[a] Department of Pharmaceutical Engineering, School of Chemistry and Chemical Engineering, Southeast University, Nanjing 211189, P.R. China

[b] Jiangsu Province Hi-Tech Key Laboratory for Bio-medical Research, Southeast University, Nanjing 211189, P.R. China

Published online: 26 Jun 2014.

To cite this article: Shi-Jun Liu, Zhi-Xin Liao, Chao Liu & Yan-Bo Qu (2014) A trehalose ester from Lancea tibetica, Natural Product Research: Formerly Natural Product Letters, 28:19, 1613-1618, DOI: 10.1080/14786419.2014.930859

To link to this article: http://dx.doi.org/10.1080/14786419.2014.930859

Natural Product Research, 2014
Vol. 28, No. 19, 1613–1618, http://dx.doi.org/10.1080/14786419.2014.930859

A trehalose ester from *Lancea tibetica*

Shi-Jun Liu[a,b], Zhi-Xin Liao[a,b]*, Chao Liu[a] and Yan-Bo Qu[a]

[a]*Department of Pharmaceutical Engineering, School of Chemistry and Chemical Engineering, Southeast University, Nanjing 211189, P.R. China;* [b]*Jiangsu Province Hi-Tech Key Laboratory for Bio-medical Research, Southeast University, Nanjing 211189, P.R. China*

(*Received 30 March 2014; final version received 1 June 2014*)

A phytochemical study of the 95% ethanolic extract of the whole plant of *Lancea tibetica* Hook. f. et Thoms. led to the isolation of a new trehalose ester, 6-*O*-undecanoyl-α,β-trehalose (**1**), along with 23 known compounds (**2**–**24**), of which compounds **2**–**17** were isolated from this plant for the first time. The structures of these compounds were established on the basis of spectroscopic methods. Compound **1** was evaluated for their *in vitro* anti-proliferative activities against MCF-7, NCI-H460 and Hep-G2 tumour cell lines. Compound **1** exhibited potent inhibitory activity against NCI-H460 cell growth, in contrast to moderate cytotoxic activity against MCF-7 and Hep-G2 cells.

Keywords: *Lancea tibetica*; trehalose ester; triterpenoid; flavone; anti-tumour activity

1. Introduction

The genus *Lancea* (Scrophulariaceae) is represented in China by only two species (*Lancea tibetica* and *Lancea hirsuta* Bonati) (Hou 1982). *L. tibetica*, a perennial herb, is used as a traditional Tibetan medicine for the treatment of leukaemia, intestinal angina, heart disease and cough (Chinese Herbs editor 2002). Previous phytochemical studies on *L. tibetica* have revealed that it is a rich source of lignans (Su et al. 1999; Li et al. 2008). To ascertain its chemical composition and medicinal value, the 95% ethanol extract of the whole plant of *L. tibetica* was investigated.

2. Results and discussion

The ethanolic extract of *L. tibetica* has afforded a new trehalose ester, 6-*O*-undecanoyl-α,β-trehalose (**1**), along with 23 known compounds, viz. betulinic acid (**2**) (Siddiqui et al. 1988); (3β)-lup-20(29)-en-3-yl stearate (**3**) (Silva de Miranda et al. 2007); 2α,3α-dihydroxyolean-12-en-28-oic acid (**4**) (Lahlou et al. 1999); dillenic acid B (**5**) (Nick et al. 1994); chrysoeriol (**6**) (Jia et al. 1986); luteolin (**7**) (Miyazawa & Hisama 2003); selagin (**8**) (Voirin et al. 1976); tricin (**9**) (Jiao et al. 2007); 5-hydroxy-4′-methoxyflavone (**10**) (Boltenkov et al. 2005); 7-*O*-(2″-*O*-glucuronosyl) glucuronosyltricin (**11**) (Kim et al. 2008); 6‴-sinapoylsaponarin (**12**) (Ohkawa et al. 1998); 1-hydroxy-8-methoxyxanthone (**13**) (Gengan et al. 2003); menthalactone (**14**), (Villasenor & Sanchez 2009); (−)-phillygenin (**15**) (Messiano et al. 2008); myo-inositol (**16**) (Yoo et al. 2002); lycopene (**17**); oleanolic acid (**18**) (Hu et al. 2011); sylvatesmin (**19**) (Banerji & Pal 1982); phillyrin (**20**) (Rahman et al. 1990); simplocosin (**21**) (Su et al.1999); lantibeside B (**22**) (Li et al. 2008); sesamin (**23**) (Zhang et al. 1987) and sucrose (**24**). The structure of known compounds was confirmed by comparison with

*Corresponding author. Email: zxliao@seu.edu.cn

spectral data available with us and in the literature. However, the structure of compound **1** was established as 6-*O*-undecanoyl-α,β-trehalose based on spectroscopic data (IR, NMR and MS spectra) in combination with chemical degradation.

Compound **1** was obtained as a white powder. $[\alpha]_D^{20} = +114$ ($c = 0.20$, MeOH). m.p. 142–143°C. The molecular formula was deduced to be $C_{23}H_{42}O_{12}$ based on the ^{1}H, ^{13}C NMR data and the quasi-molecular ion peak at *m/z* 509.2593 $[M-H]^-$ (calcd for $C_{23}H_{41}O_{12}$: 509.2597) in the HR-ESI-MS. The IR (KBr) absorptions at 3466 (br) and 1731 cm^{-1} suggested the presence of hydroxyl and ester groups. The assignment of ^{1}H and ^{13}C NMR signals was confirmed with the aid of 2D NMR (HSQC, HMBC and ROESY) spectra.

The ^{1}H NMR spectrum displayed 21 signals between 6.65 and 2.90 ppm appropriate for two pyranose residues, signals for the 21 lipid chain protons (δ 2.26, t, 2H; 1.50, m, 2H; 1.23, br s, 14H; 0.83, t, 3H). The ^{13}C NMR spectrum (Table S1) displayed the appropriate signals for the 12 carbons in the two sugar residues, signals for a long lipid chain and one ester carbonyl. A ROESY experiment (Figure 1) assisted by an HSQC experiment clearly identified the two pyranose residues as one α-glucose and one β-glucose residues, as indicated by the coupling constants of the relevant protons. The coupling constants of the two anomeric protons (4.89 and 4.29 ppm) are 3.7 and 6.1 Hz, respectively, indicating that both glucose units have the α configuration and β configuration at the anomeric centres, respectively. The α and β configurations are also consistent with the ^{13}C NMR shifts of the two anomeric carbons (92.2 and 96.8 ppm) (Yang et al. 2007).

The connectivities between the two glucose residues and the undecanoyl chain were established by a HMBC experiment (Figure 1). The long-range H–C correlations from H-1 (4.89 ppm) to C-1′ (96.8 ppm) indicated that the two glucose residues were connected at C-1–C-1′ through a glycosidic linkage to form an α,β-trehalose moiety. The correlations from both H-6α (4.28 ppm), H-6β (3.96 ppm) and H-2″ (2.26 ppm), H-3″ (1.50 ppm) to the ester carbonyl at 172.9 ppm suggested that the lipid chain was attached to the oxygen on C-6. The ^{1}H NMR data (from H-2″ to H-11″), ^{13}C NMR data (from C-1″ to C-11″) and the quasi-molecular ion peak at *m/z* 509.2593 $[M-H]^-$ (calcd for $C_{23}H_{41}O_{12}$: 509.2597) indicated that the two main fragments of compound **1** are trehalose and undecanoyl.

The identities of the two main fragments of compound **1**, trehalose and undecanoyl, are also supported by the results of alkaline hydrolysis. The major non-volatile products of alkaline hydrolysis of compound **1** are identified as α,β-D-trehalose and undecanoic acid, identical with authentic samples (TLC, IR, $[\alpha]_D$ and NMR). On the basis of the above analysis, compound **1** was identified as 6-*O*-undecanoyl-α,β-trehalose.

The cytotoxic activity of compound **1** was evaluated *in vitro*. Compared with cisplatin, compound **1** was found to show potent cytotoxicity in NCI-H460 cell lines with IC_{50} value of 16.2 μM (cisplatin: 29.6 μM) and weak cytotoxic activity in MCF-7 (32.8 μM) (cisplatin:

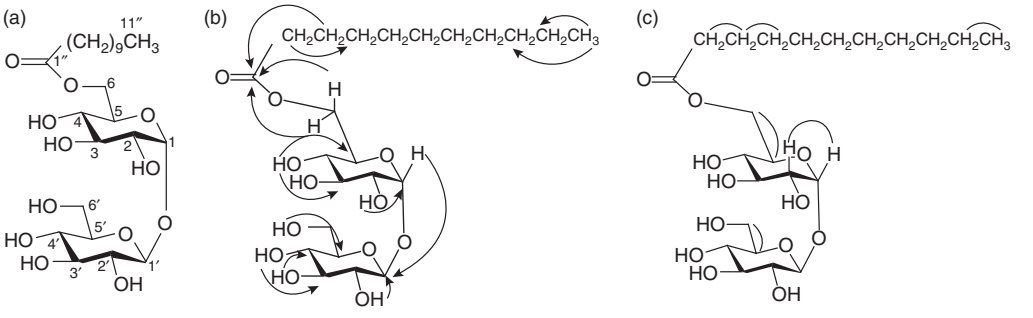

Figure 1. (a) Structure of **1**. (b) Key HMBC (H → C) correlations of **1**. (c) Key ROESY correlations of **1**.

26.2 μM) and Hep-G2 (20.6 μM) (cisplatin: 15.8 μM). Cisplatin was used as a positive control (Table 1).

3. Experimental

3.1. *General experimental procedures*

Optical rotations were measured on a Perkin Elmer-343 spectropolarimeter (Perkin-Elmer, Waltham, MA, USA). IR spectra were recorded on a NICOLET IR200 FT-IR spectrophotometer (Thermo Electron, Waltham, MA, USA). ^{1}H NMR spectra were scanned on a Bruker Avance DRX-500 spectrometer (Bruker Co., Fällanden, Switzerland) at 500 MHz (^{1}H). ^{13}C NMR spectra were obtained on a Bruker Avance DRX-300 spectrometer (Bruker Co., Fällanden, Switzerland) at 75 MHz (^{13}C). HR-ESI-MS was carried on an Agilent Technologies 6224 TOF LC-MS apparatus (Agilent Technologies, Santa Clara, CA, USA). Column chromatography (CC) was performed using silica gel (200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, Shandong Province, China). MCI GEL CHP20p (75–150 μm, Mitsubishi Kasei Corporation, Chiyoda-ku, Tokyo, Japan) was also used for CC. TLC was conducted on silica gel GF_{254} plates (10–40 μm; Qingdao Marine Chemical, Inc., Qingdao, Shandong Province, China). Petroleum ether, ethyl acetate, *n*-BuOH and other reagents were purchased from Nanjing Wanqing reagent, Inc., Nanjing, Jiangsu Province, China. Spots were observed by UV light as well as by spraying with 10% H_2SO_4–EtOH followed by heating. Tumour cells were incubated in an HF-212UV CO_2 incubator and observed under an OLYMPUS CKX41 (Olympus, Japan) inverted microscope. Optical density values were read under a BIO-RAD Model 680 (Bio-Rad Company, Hercules, CA, USA) microplate reader.

3.2. *Plant material*

The dried whole plant of *L. tibetica* Hook. f. et Thoms. was purchased in April 2013 from 'Jiukang' Tibetan medicine market in Xining of Qinghai Province, China. It was identified by Prof. Xue-Feng Lu of the Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, China, and a voucher specimen (No. 13-01-03) has been deposited at the laboratory of Zhi-Xin Liao, Southeast University, Nanjing, China.

3.3. *Extraction and isolation*

The dried and powdered plant material (5.0 kg) of *L. tibetica* was percolated four times with 95% ethanol at room temperature. The filtrates were consolidated and evaporated in vacuum to give a concentrate. The crude extract was subsequently suspended in H_2O (5000 mL) and extracted with petroleum ether (4 × 2000 mL), ethyl acetate (4 × 2000 mL) and *n*-BuOH (4 × 2000 mL), successively. After the removal of the solvents, three separate residues were obtained. The petroleum ether fraction was first purified over MCI GEL eluted with 80% ethanol to remove the

Table 1. Cytotoxicity of compound **1** (mean ± SD, $n = 5$).

	Median inhibitory rate (IC_{50}) (μM)		
Sample	MCF-7[a]	Hep-G2[a]	NCI-H460[a]
1	32.8 ± 0.5	20.6 ± 0.4	16.2 ± 0.3
Cisplatin[b]	26.2 ± 0.4	15.8 ± 0.3	29.6 ± 0.5

[a] Clinical strain.
[b] Positive control.

pigments. The remaining material was evaporated for further isolation, which was separated by repeated silica gel CC eluted with petroleum ether–ethyl acetate to yield **23** (20.0 mg), **2** (18.0 mg), **3** (15.0 mg), **19** (105 mg), **4** (26.0 mg), **5** (32.0 mg), **18** (5.60 g), **17** (2.00 mg), **14** (15.0 mg) and **1** (20.0 mg). The ethyl acetate fraction was subjected to silica gel CC eluted with a gradient system of petroleum ether–ethyl acetate to give **15** (16.5 g), **10** (15.0 mg), **13** (20.0 mg), **6** (30.0 mg), **9** (5.00 mg), **7** (18.0 mg) and **8** (308 mg). The *n*-BuOH extract fraction was fractionated by CC over silica gel by repeating with the gradient system of increasing polarity (chloroform–methanol) to give **20** (10.8 g), **12** (30.0 mg), **11** (16.0 mg), **22** (18.0 mg), **21** (15.3 g), **16** (20.0 mg) and **24** (10.0 mg).

3.4. *6-O-Undecanoyl-α,β-trehalose (1)*

White powder; m.p. 142–143°C, $R_f = 0.48$; petroleum ether–ethyl acetate, 1:1; $[\alpha]_D^{20} + 114$ (methanol, $c = 0.20$); IR (KBr) ν_{max} (cm^{-1}): 3466, 2920, 2850, 1731; HR-ESI-MS: *m/z* 509.2593 $[M - H]^-$ (calcd. for $C_{23}H_{41}O_{12}$: 509.2597); ^{1}H NMR (DMSO-d_6, 500 MHz): 0.83 (3H, t, $J = 6.9$ Hz, H-11″), 1.23 (14H, br s, H-4″-10″), 1.50 (2H, m, H-3″), 2.26 (2H, t, $J = 6.9$ Hz, H-2″), 2.90 (1H, m, H-2′), 3.03 (1H, m, H-4′), 3.04 (1H, m, H-3), 3.12 (1H, m, H-2), 3.12 (1H, m, H-3′), 3.31 (1H, m, H-5), 3.42 (1H, m, H-5′), 3.76 (1H, m, H-4), 3.94 (1H, m, H-6′b), 3.96 (1H, m, H-6b), 4.24 (1H, m, H-6′a), 4.28 (1H, m, H-6a), 4.29 (1H, d, $J = 6.1$ Hz, H-1′), 4.89 (1H, d, $J = 3.7$ Hz, H-1); ^{13}C NMR (DMSO-d_6, 75 MHz): 13.9 (C-11″), 22.0 (C-10″), 24.4 (C-3″), 28.6 (C-5″), 28.7 (C-6″), 28.9 (C-7″), 29.0 (C-8″), 29.0 (C-9″), 31.2 (C-4″), 33.4 (C-2″), 63.8 (C-6), 63.8 (C-6′), 69.1 (C-4), 70.1 (C-3), 70.5 (C-4′), 72.1 (C-2), 72.8 (C-5′), 73.5 (C-5), 74.6 (C-2′), 76.4 (C-3′), 92.2 (C-1), 96.8 (C-1′), 172.9 (C-1″); for ^{1}H and ^{13}C NMR data (DMSO-d_6), see Table S1. For ROESY and HMBC correlations, see Figure 1.

3.5. *Alkaline hydrolysis of compound 1*

A solution of compound **1** (10 mg) in 95% EtOH (2 mL) and 5% NaOH (2 mL) was refluxed for 3 h. After cooling, the mixture was diluted to 10 mL, acidified to pH 5 with dilute HCl and extracted with $CHCl_3$. Concentration of the $CHCl_3$ extract yielded undecanoic acid (2.60 mg, 95%), which was identical with the authentic sample. The aqueous solution was concentrated to dryness and acetylated with Ac_2O/pyridine/DMAP to produce α,β-D-trehalose octaacetate, which was also identical with the authentic sample (TLC, IR, $[\alpha]_D$ and NMR).

3.5.1. *Undecanoic acid*

White solid; $R_f = 0.52$; petroleum ether–ethyl acetate, 10:1. IR (KBr) ν_{max} (cm^{-1}): 2922, 2853, 2673, 1710, 1464, 1411, 1377, 1364, 1286, 1240, 1221, 936, 720, 624, 480; ^{13}C NMR ($CDCl_3$, 75 MHz): 14.0, 22.7, 24.2, 29.1, 29.2, 29.3, 29.4, 29.7, 31.0, 34.0, 179.2.

3.5.2. *α,β-D-Trehalose octaacetate*

White powder; $R_f = 0.42$; petroleum ether–ethyl acetate, 8:1; $[\alpha]_D^{20} + 64$ ($CHCl_3$, $c = 0.20$); IR (KBr) ν_{max} (cm^{-1}): 2924, 2858, 1245, 1053, 969, 898, 845; ^{13}C NMR ($CDCl_3$, 75 MHz): 20.3, 20.3, 20.4, 20.5, 20.5, 20.5, 20.6, 20.6, 60.5, 61.9, 66.9, 68.3, 68.4, 69.8, 71.1, 71.8, 72.4, 72.9, 89.8, 95.2, 169.4, 169.5, 169.6, 170.2, 170.2, 170.4, 170.8, 171.2.

3.6. In vitro *cytotoxic assay*

IC_{50} values for compound **1** were determined as described previously (Lu et al. 2013). The anti-tumour activities of compound **1** against NCI-H460, MCF-7 and Hep-G2 cells were tested using

the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) MTT assay, and cisplatin was used as a positive control. IC_{50} values of compound **1** and cisplatin were determined using average data over five replicates. The cell suspensions were distributed into 96-well cell culture plates and cultured at 36–37°C in a 5% CO_2 incubator for 24 h. Cell viability was measured by the MTT method after 48 h of incubation. Experiments were conducted in quintuplicate. Cytotoxicity was expressed as the concentration of compound inhibiting cell growth by 50% (IC_{50}). Origin 7.5 computer program (Data Analysis and Graphics Software, Origin 7.5, Northampton, MA, USA) was used to determine the median inhibitory rate (IC_{50}), and the results are shown in Table 1.

4. Conclusion

6-*O*-Undecanoyl-α,β-trehalose (**1**), a new trehalose ester, has been isolated from the genus *Lancea* for the first time. Its structure was determined using spectroscopic methods. α,β-Trehalose skeleton of **1** was rarely reported in the literature. Cisplatin was used as a positive control. Compared with cisplatin, compound **1** was found to show potent cytotoxicity in NCI-H460 cell lines with IC_{50} value of 16.2 μM (cisplatin: 29.6 μM) and weak cytotoxic activity in MCF-7 (32.8 μM) (cisplatin: 26.2 μM) and Hep-G2 (20.6 μM) (cisplatin: 15.8 μM) (Table 1). Compounds **2**–**17** were isolated from this plant for the first time.

Supplementary material

Supplementary material relating to this article is available online, alongside Table S1 and Figures S1–S6.

Acknowledgement

We thank Prof. Xue-Feng Lu of the Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, China, for the identification of the plant material.

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