Carbohydrate Research 346 (2011) 1302–1306

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Fatty acid synthase inhibitors from the hulls of *Nephelium lappaceum* L.

You-Xing Zhao [a,*], Wen-Juan Liang [c], Hui-Jin Fan [b], Qing-Yun Ma [a], Wei-Xi Tian [b], Hao-Fu Dai [a], He-Zhong Jiang [c], Ning Li [d], Xiao-Feng Ma [b,*]

[a] *Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, China*
[b] *College of Life Sciences, Graduate University of Chinese Academy of Sciences, Beijing 100049, China*
[c] *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China*
[d] *School of Life Sciences, Anhui University, Hefei 230039, China*

ARTICLE INFO

Article history:
Received 29 January 2011
Received in revised form 13 April 2011
Accepted 21 April 2011
Available online 28 April 2011

ABSTRACT

Natural products inhibiting fatty acid synthase (FAS) are appearing as potential therapeutic agents to treat cancer and obesity. The bioassay-guided chemical investigation of the hulls of *Nephelium lappaceum* L. resulted in the isolation of ten compounds (**1**–**10**) mainly including flavonoids and oleane-type triterpene oligoglycosides, in which all of the compounds were isolated from this plant for the first time. Additionally, compounds **8** and **9** were new hederagenin derivatives and were elucidated as hederagenin 3-*O*-(2,3-di-*O*-acetyl-α-L-arabinofuranosyl)-(1→3)-[α-L-rhamnopyranosyl(1→2)]-β-L-arabinopyranoside and hederagenin 3-*O*-(3-*O*-acetyl-α-L-arabinofuranosyl)-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-L-arabinopyranoside, respectively. All these isolates were evaluated for inhibitory activities of FAS, which showed these isolates had inhibitory activity against FAS with IC_{50} values ranging from 6.69 to 204.40 μM, comparable to the known FAS inhibitor EGCG (IC_{50} = 51.97 μM). The study indicates that the hulls of *Nephelium lappaceum* L. could be considered as potential sources of promising FAS inhibitors and the oleane-type triterpene oligoglycosides could be considered as another type of natural FAS inhibitors.

1. Introduction

Fatty acid synthase (EC 2.3.1.85, ab. FAS) present in animals is a key metabolic enzyme catalyzing the de novo synthesis of long chain saturated fatty acids from acetyl-CoA and malonyl-CoA in the presence of the reducing substrate NADPH.[1] Inhibition of FAS suppresses food intake and leads to dramatic weight loss in mice, suggesting that FAS not only functions in providing metabolic substrates, but also plays a role in satiety signaling.[2] It has also been reported that inhibition of FAS by either knockdown of the FAS gene with siRNA or inactivation of FAS leads to caspase-8-mediated tumor cell apoptosis.[3] At the same time, many FAS inhibitors, such as Cerulenin, C75, Orlistat and EGCG, have joint effects of weight-loss and anti-tumor.[4–11] FAS may be a potential target for both anti-obesity and anti-cancer drugs according to several recent reports and may be used as a clinical and experimental target for scientific research and drug discovery.[2,12,13] Therefore, it is important to find novel compounds especially from natural sources that reduce FAS activity or expression levels, which may be useful for the treatment of obesity and cancer. In our preliminary work, we screened dozens of extracts against FAS from fruits including the hulls of *Nephelium lappaceum* L. and *Garcinia mangostana* L. and showed that these fruits had inhibitory FAS properties. Our previous investigation of FAS inhibitors from the hulls of *G. mangostana* L. showed that two types of natural products, xanthones and benzophenones, exhibited strong inhibitory activity of FAS.[14]

Nephelium lappaceum L. commonly known as 'rambutan', is a tropical fruit of the Sapindaceae family.[15] Native to Southeast Asia, rambutan is cultivated for its fruit and sold commercially.[15,16] It is consumed fresh, canned, or processed, and appreciated for its refreshing flavor and exotic appearance.[17] The seeds are bitter and narcotic, and the roots are used for treating fevers, the leaves as poultices, and the bark as an astringent.[16] The fruit is recommended for severe dysentery and as an astringent, an antifebrile, and a warm carminative in dyspepsia.[18] In particular, the rind of rambutan, which is normally discarded, was found to contain extremely high antioxidant and antibacterial activities, and several potential antioxidant activities, including reducing power, β-carotene bleaching, linoleic peroxidation and free radical scavenging activity were also exhibited.[19] As for the chemical constituents of hull of *N. lappaceum*, only a few compounds have been reported, most of them are phenolic compounds that are responsible for these antioxidant and antibacterial activities.[20] During our early screening of activity, the ethanol extract of the hulls of *N. lappaceum* showed a strong capability of inactivating FAS with

* Corresponding authors. Tel./fax: +86 898 66989095 (Y.-X.Z.); tel.: +86 10 88256353 (X.-F.M.).
E-mail addresses: zhaoyx1011@163.com (Y.-X. Zhao), maxiaofeng@gucas.ac.cn (X.-F. Ma).

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doi:10.1016/j.carres.2011.04.028

an IC_{50} value at 6.77 μg/mL. To investigate the FAS-inhibiting constituents of the hull of rambutan, a detailed chemical study on this plant was carried out and two new oleane-type triterpene oligoglycosides, together with eight known compounds, were isolated from the methanol extract of the hulls of rambutan. This paper describes, for the first time, the isolation, structural elucidation and anti-FAS activity of all the isolates from the hulls of *N. lappaceum*.

2. Results and discussion

2.1. Identification of compounds from the hulls of *Nephelium lappaceum*

A phytochemical study on the hulls of *N. lappaceum* resulted in the isolation of two new oleane-type triterpene oligoglycosides (**8** and **9**) together with eight known compounds (**1**–**7**, and **10**) (Fig. 1). The structures of eight known compounds were identified as kaempferol (**1**),[21] kaempferol-3-β-D-(6-*O*-*trans*-*p*-coumaroyl)-glucopyranoside (**2**),[22] kaempferol-3-α-L-rhamnopyranoside (**3**),[23] kaempferol 3-*O*-(6-*O*-caffeoyl)-β-D-glucopyranoside (**4**),[24] isocopoletin (**5**),[25] ethyl gallate (**6**),[26] hederagenin (**7**),[27] hederagenin 3-*O*-(2,3-di-*O*-acetyl-α-L-arabinofuranosyl)-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-L-arabinopyranoside (**8**), hederagenin 3-*O*-(3-*O*-acetyl-α-L-arabinofuranosyl)-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-L-arabinopyran-oside (**9**), and hederagenin 3-*O*-α-L-arabinofuranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-L-arabinopyranoside (**10**)[28] by comparison of their spectroscopic data with those reported in the literature. All ten compounds were obtained from the hull of this plant for the first time. Compounds **9** and **10** may be the natural products, or may be derived from compound **8** due to deacetylation during the extraction process.

Compound **8** was obtained as an amorphous powder with a $[\alpha]_D^{22}$ −15.24 (*c* 0.38, MeOH). The IR spectrum of **8** showed absorption bands at 1733 and 1630 cm^{-1} ascribable to an ester carbonyl, carboxyl, olefin functions and broad bands at 3441 cm^{-1} suggestive of hydroxyl groups (including carboxyl hydroxyl). In the negative-ion ESI-mass spectrum of **8**, a quasimolecular ion peak was observed at *m/z* = 989 $[M+Na]^+$ in accordance with the molecular formula $C_{50}H_{78}O_{18}Na$, which was further confirmed by a signal at *m/z* = 989.5076 $[M+Na]^+$ in the HR-ESI-mass spectrum (calcd for $C_{50}H_{78}O_{18}Na$, 989.5085). The ^1H NMR spectrum (Table 1) of **8** exhibited six methyl groups [δ: 0.68, 0.78, 0.88, 0.91, 0.94, 1.14 (3H each, all s, H_3-24, 26, 29, 30, 25, 27)], an olefinic proton [δ: 5.24 (1H, br s, H-12)], and three anomeric protons [δ: 4.57 (1H, s, inner-Ara-H-1), 5.13 (1H, s, Rha-H-1) and 5.24 [1H, s, Ara*f*-H-1] suggesting three sugar units, together with two methyl of acetyl groups [δ: 2.08 (6H, s, 2 × $COCH_3$)].

The assignments of the ^1H NMR signals in Table 1 were determined by HMQC spectra combined with the literature.[28,29] Analysis of the ^{13}C NMR and DEPT spectra (Table 2) showed 50 carbon resonances, similar to those of hederagenin 3-*O*-α-L-arabinofuranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-L-arabinopyranoside (**10**),[28] except for the additional shifts at δ_C 172.4, 171.9 and 20.9, 20.8 for two acetyl groups and the different chemical shifts of Ara*f* in **8** and **10**, suggesting compound **8** was a hederagenin glucoside.

The presence of the two acetyl was further identified by the treatment of **8** with 0.5% sodium methoxide in methanol yielding a deacetylated derivative, exhibiting quasimolecular ion peaks at *m/z* = 905 $[(M+Na)-42-42]^+$ in the positive-ion ESI-mass spectrum. The linkages of two acetyl groups to Ara*f*-C-3 and Ara*f*-C-2 in **8** were characterized from analysis of the HMBC spectrum (Fig. 2) exhibiting key long-range correlations from the Ara*f*-H-2 [δ_H: 5.18 (1H, m)] and Ara*f*-H-3 [δ_H: 4.30 (1H, m)] to the two acetyl carbonyl carbons, and comparison of ^{13}C NMR spectra at C-2 and C-3 in Ara*f* from **8** with **10**. The correlations of the Ara*f*-H-1 [δ_H: 5.24 (1H, br s)] with the Ara*f*-C-3 (δ_C: 80.9), Rha-H-1 [δ_H: 5.13 (1H, br s)] with the Ara*f*-C-2 (δ_C: 76.5) and the Ara*f*-H-1 [δ_H: 4.57 (1H, br s)] with the C-3 (δ_C: 82.4) of the aglycone indicated one terminal Ara*f* moiety was attached to the inner-Ara*f*-C-3, another terminal Rha*p* moiety was attached to the inner-Ara*f*-C-2 and this sugar chain was linked to C-3 in **8**. On the basis of the above mentioned evidence, compound **8** was determined as hederagenin

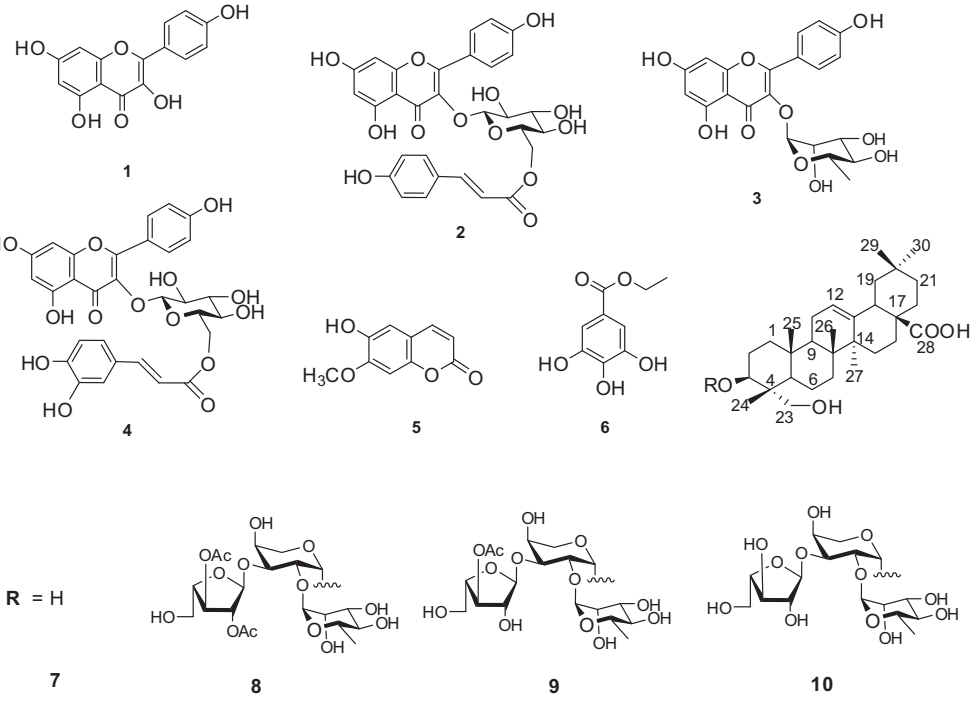

Figure 1. Structures of compounds **1**–**10** isolated from the hulls of *Nephelium lappaceum*.

Table 1
^{1}H NMR spectroscopic data (CD_3OD) of **8** and **9**

Compound	8	9	Compound	8	9
1	1.60 (m), 0.94 (m)	1.62 (m), 1.00 (m)	**Ara**		
2	1.87 (m), 1.78 (m)	1.86 (m), 1.73 (m)	**1′**	4.57 (s)	4.53 (s)
3	3.62 (dd, 12.0, 3.2)	3.62 (d, 10.0)	**2′**	3.72 (s)	3.70 (s)
5	1.25 (s)	1.24 (d, 6.0)	**3′**	3.82 (m)	3.85 (m)
6	1.75 (m), 1.35 (m)	1.76 (m), 1.38 (m)	**4′**	3.74 (m)	3.75 (m)
7	1.61 (m), 1.25 (s)	1.62 (m), 1.24 (d, 6.0)	**5′**	3.50 (d, 9.6), 3.35 (d, 11.2)	3.51 (d, 10.4), 3.36 (d, 10.8)
9	1.63 (m)	1.65 (m)	**Rha**		
11	1.93 (m), 1.87 (m)	2.00 (m), 1.89 (m)	**1″**	5.13 (s)	5.18 (s)
12	5.24 (s)	5.24 (m)	**2″**	4.02 (m)	4.09 (s)
15	1.79 (m), 1.04 (m)	1.75 (m), 1.08 (m)	**3″**	3.50 (m)	3.50 (br s)
16	2.08 (m), 1.95 (m)	2.07 (m), 1.98 (m)	**4″**	3.69 (s)	3.69 (s)
18	2.82 (d, 12.0)	2.86 (d, 12.0)	**5″**	3.85 (m)	3.85 (m)
19	1.66 (m), 1.10 (m)	1.69 (m), 1.10 (m)	**6″**	1.25 (s)	1.25 (s)
21	1.26 (m), 1.18 (m)	1.39 (m), 1.18 (m)	**Ara(*f*)**		
22	1.78 (m), 1.64 (m)	1.73 (m), 1.52 (m)	**1‴**	5.24 (s)	5.26 (s)
23	3.82 (m), 3.50 (d, 9.6)	3.86 (m), 3.51 (d, 10.4)	**2‴**	5.18 (m)	4.23 (br s)
24	0.68 (s)	0.71 (s)	**3‴**	4.30 (m)	4.83 (m)
25	0.94 (s)	0.97 (s)	**4‴**	4.26 (m)	4.19 (m)
26	0.78 (s)	0.81 (s)	**5‴**	4.30 (m), 4.26 (m)	3.76 (m), 3.70 (m)
27	1.14 (s)	1.18 (s)	**Acetyl**		
29	0.91 (s)	0.94 (s)	**2‴**	2.08 (s)	2.08 (s)
30	0.88 (s)	0.91 (s)	**3‴**	2.08 (s)	

Table 2
^{13}C NMR spectroscopic data (CD_3OD) of **7**–**10**

Compound	7	8	9	10	Compound	7	8	9	10
1	34.3	39.8	39.8	39.8	**28**	182.4	182.1	181.7	181.9
2	27.9	26.2	26.3	26.6	**29**	24.5	23.9	24.0	24.0
3	74.3	82.4	82.4	82.4	**30**	33.9	33.5	33.6	33.6
4	45.3	43.5	44.0	44.0			**Ara**	**Ara**	**Ara**
5	50.1	47.9	47.6	47.3	**1′**		103.5	104.7	104.7
6	18.2	18.2	18.3	18.4	**2′**		76.5	76.5	76.5
7	34.0	33.0	33.4	33.4	**3′**		80.3	79.7	79.4
8	40.5	40.0	40.5	40.5	**4′**		67.8	69.5	69.5
9	48.8	48.4	48.4	48.4	**5′**		64.6	65.4	65.5
10	38.3	37.3	37.6	37.6			**Rha**	**Rha**	**Rha**
11	24.9	24.8	24.8	24.5	**1″**		100.8	101.6	101.4
12	124.0	123.1	123.1	123.6	**2″**		71.2	71.8	71.8
13	145.7	144.8	144.8	145.2	**3″**		72.3	72.8	73.0
14	44.3	42.3	42.6	43.0	**4″**		73.3	73.6	74.0
15	29.3	28.8	28.6	28.6	**5″**		69.3	70.3	70.2
16	24.4	23.8	24.2	24.3	**6″**		17.8	18.0	18.0
17	48.2	46.8	46.7	47.3			**Ara(*f*)**	**Ara(*f*)**	**Ara(*f*)**
18	41.1	42.5	43.0	42.7	**1‴**		110.4	110.9	110.2
19	47.7	47.2	47.2	47.6	**2‴**		82.5	80.9	82.7
20	32.1	31.3	31.6	31.6	**3‴**		80.9	80.8	78.9
21	35.3	34.8	34.9	35.0	**4‴**		83.0	84.4	86.6
22	33.9	33.2	33.8	33.8	**5‴**		64.2	63.2	63.4
23	67.7	64.5	64.6	64.5	Acetyl				
24	13.2	13.5	13.8	13.8	**2‴**		172.4		
25	16.7	16.3	16.4	16.4			20.9		
26	20.3	17.5	17.8	17.8	**3‴**		171.9	172.4	
27	26.9	26.4	26.5	26.5			20.8	20.8	

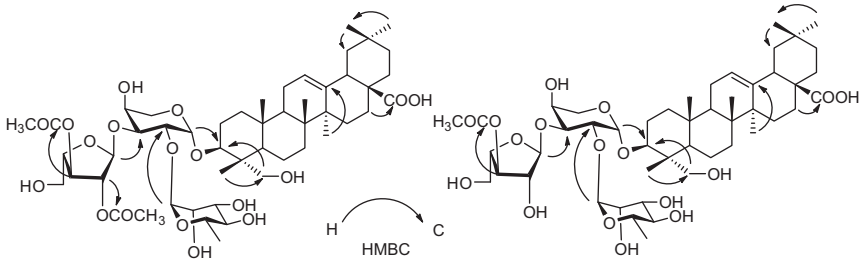

Figure 2. Selected HMBC correlations of compounds **8** and **9**.

3-*O*-(2,3-di-*O*-acetyl-α-L-arabinofuranosyl)-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-L-arabinopyranoside.

Compound **9**, isolated as an amorphous powder with a $[\alpha]_D^{22}$ −19.05 (*c* 0.32, MeOH), possessed the molecular formula $C_{48}H_{76}O_{17}$ as established by a quasimolecular ion peak at *m*/*z* = 925 $[M+H]^+$ in ESI-MS and HR-ESI-MS at *m*/*z* = 925.5176 $[M+H]^+$ (calcd for $C_{48}H_{77}O_{17}$, 925.5160). The IR spectrum of **9** showed the presence of ester carbonyl (1729 cm^{-1}), carboxyl (1696 cm^{-1}), olefin functions (1643 cm^{-1}), and hydroxyls (3427 cm^{-1}). The ^1H NMR spectra (Table 1) and ^{13}C NMR and DEPT spectra (Table 2) of compound **9** were also similar to those of hederagenin 3-*O*-α-L-arabinofuranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-L-arabinopyranoside (**10**),[28] except for the additional acetyl group signals at δ_H 2.08 and δ_C 172.4, 20.8 and the different chemical shifts of Ara*f* in **9** and **10**. The presence of the acetyl was further identified by the deacetylatic product exhibiting quasimolecular ion peaks at *m*/*z* = 883 $([M+H]-42)^+$ in the positive-ion ESI-MS spectrum. The linkage of the acetyl group to Ara*f*-C-3 was assigned from the downfield shift of C-3 (δ_C 80.8, Δ = +1.9 ppm) in Ara*f* in **9** compared to **10** (Table 2) and analysis of the HMBC spectrum (Fig. 2), in which key long-range correlation from the Ara*f*-H-3 [δ_H: 4.83 (1H, s)] to the acetyl carbonyl carbon. On the basis of the above evidence, compound **9** was determined as hederagenin 3-*O*-(3-*O*-acetyl-α-L-arabinofuranosyl)-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-L-arabinopyranoside.

2.2. Anti-FAS activities of the isolates

All the isolates were assayed for their inhibitory activity against FAS, and the results are presented in Table 3. The known FAS inhibitor EGCG (IC_{50} = 51.97 μM) was used as a positive control in the assay. All of the isolates inhibited FAS in a dose-dependent manner and some compounds were more potent than EGCG. Of the compounds tested, compound **1** exhibited the strongest inhibitory activity with a lower IC_{50} value of 6.69 μM. The new compound **8** showed a higher activity of anti-FAS, while other compounds, such as compounds **3**, **4**, and **5** demonstrated equivalent inhibitory activities of FAS to EGCG. The order of inhibitory activity against FAS assay was found to be: **1** > **2** > **8** > **3** > EGCG > **5** > **4** > **7** > **9** > **6** > **10**. In general, among compounds **1**–**10**, the flavonoid structures (compounds **1**–**4**) showed excellent inhibitory activity of FAS. Additionally, the oleane-type triterpenes also exhibited effective inhibition of FAS. Moreover, the inhibitory activities of the oligoglycosides against FAS showed a trend that with increasing number of acetyl groups in the compound, the higher the activity. This was based on an analysis of the inhibitory order, which was found to be **8** > **9** > **10**, indicating that the acetyl groups may be a positive factor and are required for the activity of oleane-type triterpene oligoglycosides. In summary, the oleane-type triterpenes can be considered as another promising class of FAS inhibitors following our previous discovery of FAS inhibition by xanthones and benzophenones.[14] Although the oleane-type triterpenes have a broad range of biological activities, including anti-tumor and inhibitory activities to pancreatic lipase,[29,30] to our best knowledge, this is the first time FAS inhibitory activity of this type of natural products has been shown.

Table 3
The inhibitory activity of **1**–**10** isolated from *Nephelium lappaceum* against FAS

Compound	FAS inhibitory activity IC_{50} (μM) [a]
1	6.69 ± 0.42
2	29.88 ± 0.34
3	51.43 ± 0.53
4	65.61 ± 0.31
5	52.18 ± 0.89
6	173.18 ± 0.71
7	128.51 ± 0.72
8	34.46 ± 0.38
9	166.20 ± 0.51
10	204.40 ± 0.60
EGCG[b]	51.97 ± 1.65

[a] IC_{50} values were determined by regression analyses and expressed as means ± SD for three distinct experiments.
[b] Positive controls.

3. Experimental

3.1. General methods

Optical rotations were measured with a Horiba SEPA-300 polarimeter/UV-2401A spectrophotometer. CD spectra were measured on a JASCO J-810 spectrophotometer. A tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on DRX-500 spectrophotometers with TMS as the internal standard. Unless otherwise indicated, chemical shifts (δ) are expressed in ppm with reference to the solvent signals. HR-ESI-MS were performed on a VG Autospec-3000 spectrophotometer. Column chromatography was performed with Si gel (200–300 mesh; Qingdao Marine Chemical Inc., Qingdao, China), Lichroprep RP-18 gel (40–63 μm; Merck, Darmastadt, Germany). The fractions were monitored by TLC, and spots were visualized by heating the silica gel plates sprayed with 5% H_2SO_4 in EtOH.

3.2. Plant material

The fruit of *Nephelium lappaceum* was purchased from a market in Kunming, China, and identified by Doctor Rong Li, Kunming Institute of Botany, Chinese Academy of Sciences. The specimen (LWJ 2008-12-01) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3. Extraction and isolation

The air-dried fruit hulls of *N. lappaceum* (10 kg) were powdered and extracted three times with 95% EtOH under reflux. The combined extracted EtOH solution (18 L) was evaporated under reduced pressure, then suspended in water (1.5 L) and partitioned with petroleum ether, EtOAc, and *n*-BuOH sequentially to yield petroleum ether soluble (7.5 g), EtOAc-soluble (159.5 g) and *n*-BuOH-soluble (102.5 g) fractions. A portion (154.5 g) of the EtOAc-soluble fraction was separated by silica gel column chromatography (CC, 200–300 mesh, 1.4 kg) and eluted with $CHCl_3$–MeOH (100: 1, 98: 2, 95: 5, 90: 10, 80: 20, 70: 30, 0: 100, v:v) gradient solvent system to give ten fractions (1–10).

Fraction 2 (11 g) was subjected to column chromatography over silica gel, eluting with petroleum ether–EtOAc (2:1) to give five subfractions 2a–2e. Subfraction 2b was separated by chromatography using elution with petroleum ether–EtOAc (4:1) to yield compounds **1** (6 mg), **2** (10 mg), **3** (18 mg), and **4** (33 mg). Subfraction 2c was separated by chromatography using elution with petroleum ether–EtOAc (5:1) to give compound **6** (233 mg). Fraction 3 (8 g) was subjected to column chromatography over silica gel eluting with petroleum ether–EtOAc (1:1) to give four subfractions 3a–3d. Subfraction 3b was separated by chromatography using elution with petroleum ether–EtOAc (2:1) to yield compounds **5** (7 mg) and **7** (22 mg). Fraction 10 (20 g) was chromatographed over a silica gel column eluting with a petroleum ether–EtOAc solvent system (20:1 to pure EtOAc) to give six subfractions (10a–10f). Compounds **8** (32 mg), **9** (473 mg) and **10** (500 mg) were obtained

from Subfraction 10f (2 g) by repeated silica gel column chromatography with petroleum $CHCl_3$–MeOH (95:15), RP-18 column eluted with MeOH–H_2O gradient system (60–100%) and then chromatography over a Sephadex LH-20 column, using $CHCl_3$–MeOH (1:1) as the solvent.

3.4. Identification

Hederagenin 3-*O*-(2,3-di-*O*-acetyl-α-L-arabinofuranosyl)-(1→3)-[α-L-rhamnopyranosyl (1→2)]-β-L-arabinopyranoside (**8**): Amorphous powder; $[\alpha]_D^{22}$ −15.24 (*c* 0.38, MeOH); IR (KBr) ν_{max} 3441, 2942, 1733, 1630, 1460, 1375, 1240, 1051 cm^{-1}; ^1H NMR and ^{13}C NMR see Tables 1 and 2; HR-ESI-MS *m*/*z*: (positive-ion) 989.5076 $[M+Na]^+$ (calcd for $C_{50}H_{78}O_{18}Na$, 989.5085). ESI-MS *m*/*z*: (positive-ion) 989 $[M+Na]^+$.

Hederagenin 3-*O*-(3-*O*-acetyl-α-L-arabinofuranosyl)-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-L-arabinopyranoside (**9**): Amorphous powder; $[\alpha]_D^{22}$ −19.05 (*c* 0.38, MeOH); IR (KBr) ν_{max} 3427, 2945, 1729, 1696, 1643, 1461, 1387, 1255, 1051, 644 cm^{-1}; ^1H NMR and ^{13}C NMR see Tables 1 and 2; HR-ESI-MS *m*/*z*: (positive-ion) 925.5176 $[M+H]^+$ (calcd for $C_{48}H_{77}O_{17}$, 925.5160). ESI-MS *m*/*z*: (positive-ion) 925 [M+H]+.

3.5. Deacetylation of 8 and 9

A solution of compound **8** or **9** in 0.5% NaOMe–MeOH was stirred at room temperature for 3 h. The reaction mixture was neutralized with Dowex HCR-W2 (H^+ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure gave a residue.

3.6. Inhibitory activities of FAS assays

The preparation, storage, and usage of FAS from chicken liver, which shares 63% identity with the amino acid sequence of human FAS, were performed as described previously.[31] FAS activity was measured at the constant temperature of 37 °C by the changes of NADPH absorption at 340 nm with a UV–vis spectrophotometer (Amersham Pharmacia Ultrospec 4300, England, UK). The reaction mixture contained 100 mM, pH 7.0, KH_2PO_4–K_2HPO_4 buffer; 1 mM EDTA; 1 mM dithiothreitol; 3 μM acetyl-CoA; 10 μM malonyl-CoA; 35 μM NADPH and 10 μg FAS in a total volume of 2 mL. The preparative concentrations of FAS and its substrate were determined by the spectrophotometry with the following extinction coefficients: FAS, $4.83 \times 10^5\ M^{-1}\ cm^{-1}$ at 279 nm; acetyl-CoA, $1.54 \times 10^4\ M^{-1}\ cm^{-1}$ at 259 nm, pH 7.0; malonyl-CoA, $1.46 \times 10^4\ M^{-1}\ cm^{-1}$ at 260 nm, pH 6.0; acetoacetyl-CoA, $1.59 \times 10^4\ M^{-1}\ cm^{-1}$ at 259 nm, pH 7.0; NADPH, $6.02 \times 10^3\ M^{-1}\ cm^{-1}$ at 340 nm, and $1.59 \times 10^4\ M^{-1}\ cm^{-1}$ at 259 nm, pH 9.0.

Acknowledgments

This work was supported by National Nonprofit Institute Research Grant of CATAS-ITBB (110301), the Natural Science foundation of Yunnan (2008CD159). The authors are thankful to the members of the analytical group of the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, for the spectral measurements.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2011.04.028.

References

1. Wakil, S. J. *Biochemistry* **1989**, *28*, 4523–4530.
2. Loftus, T. M.; Jaworsky, D. E.; Frehywot, G. L.; Townsend, C. A.; Ronnett, G. V.; Lane, M. D.; Kuhajda, F. P. *Science* **2000**, *288*, 2379–2381.
3. Knowles, L. M.; Yang, C.; Osterman, A.; Smith, J. W. *J. Biol. Chem.* **2008**, *283*, 31378–31384.
4. Drent, M. L.; Larsson, I.; William-Olsson, T.; Quaade, F.; Czubayko, F.; Von Bergmann, K.; Strobel, W.; Sjostrom, L.; Van der Veen, E. A. *Int. J. Obes. Relat. Metabol. Disord.* **1995**, *19*, 221–226.
5. Drent, M. L.; van der Veen, E. A. *Obes. Res.* **1995**, *3*, 623s–625s.
6. Kao, Y. H.; Hiipakka, R. A.; Liao, S. *Endocrinology* **2000**, *141*, 980–987.
7. Kridel, S. J.; Axelrod, F.; Rozenkrantz, N.; Smith, J. W. *Cancer Res.* **2004**, *64*, 2070–2075.
8. Kuhajda, F. P.; Pizer, E. S.; Li, J. N.; Mani, N. S.; Frehywot, G. L.; Townsend, C. A. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 3450–3454.
9. Pizer, E. S.; Jackisch, C.; Wood, F. D.; Pasternack, G. R.; Davidson, N. E.; Kuhajda, F. P. *Cancer Res.* **1996**, *56*, 2745–2747.
10. Wang, X.; Tian, W. X. *Biochem. Biophys. Res. Commun.* **2001**, *288*, 1200–1206.
11. Yang, G. Y.; Liao, J.; Kim, K.; Yurkow, E. J.; Yang, C. S. *Carcinogenesis* **1998**, *19*, 611–616.
12. Kuhajda, F. P. *Cancer Res.* **2006**, *66*, 5977–5980.
13. Menendez, J. A.; Lupu, R.; Colomer, R. *Mol. Gen. Metabol.* **2005**, *84*, 293–295.
14. Jiang, H. Z.; Quan, X. F.; Tian, W. X.; Hu, J. M.; Wang, P. C.; Huang, S. Z.; Cheng, Z. Q.; Liang, W. J.; Zhou, J.; Ma, X. F.; Zhao, Y. X. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6045–6047.
15. Wall, M. M. *J. Food Comp. Anal.* **2006**, *19*, 655–663.
16. Ragasa, C. Y.; de Luna, R. D.; Cruz, W. C.; Rideout, J. A. *J. Nat. Prod.* **2005**, *68*, 1394–1396.
17. Ong, P. K. C.; Acree, T. E.; Lavin, E. H. *J. Agric. Food Chem.* **1998**, *46*, 611–615.
18. Palanisamy, U.; Ming, C. H.; Masilamani, T.; Subramaniam, T.; Teng, L. L.; Radhakrishnan, A. K. *Food Chem.* **2008**, *109*, 54–63.
19. Thitilertdecha, N.; Teerawutgulrag, A.; Rakariyatham, N. *LWT-Food Sci. Techol.* **2008**, *41*, 2029–2035.
20. Thitilertdecha, N.; Teerawutgulrag, A.; Kilburn, J. D.; Rakariyatham, N. *Molecules* **2010**, *15*, 1453–1465.
21. Wang, L. L.; Liu, B.; Shi, R. B.; Tu, G. Z. *J. Beijing Univ. Trad. Chin. Med.* **2008**, *31*, 116–118.
22. Tsukamoto, S.; Tomise, K.; Aburatani, M.; Onuki, H.; Hirorta, H.; Ishiharajima, E.; Ohta, T. *J. Nat. Prod.* **2004**, *67*, 1839–1841.
23. Matthes, H. W. D.; Luu, B.; Ourisson, G. *Phytochemistry* **1980**, *19*, 2643–2650.
24. Yoshida, K.; Hishida, A.; Iida, O.; Hosokawa, K.; Kawabata, J. *J. Agric. Food Chem.* **2008**, *56*, 4367–4371.
25. Li, H. B.; Zhou, C. X.; Pan, Y. X. *Planta Med.* **2005**, *71*, 1128–1133.
26. Yu, Z. B.; Yang, G. Y.; Wu, X.; Zhou, Y. W. *Nat. Prod. Res. Dev.* **2007**, *19*, 67–69.
27. Morota, T.; Yang, C. X.; Sasaki, H. *Phytochemistry* **1995**, *39*, 1153–1157.
28. Jayasinghe, L.; Shimada, H.; Hara, H.; Fujimoto, Y. *Phytochemistry* **1995**, *40*, 891–897.
29. Ovesna, Z.; Vachalkova, A.; Horvathova, K.; Tothova, D. *Mini-Rev. Neoplasma* **2004**, *51*, 327–333.
30. Morikawa, T.; Xie, Y. Y.; Asao, Y.; Okamoto, M.; Yamashita, C.; Muraoka, O.; Matsuda, H.; Pongpiriyadacha, Y.; Yuan, D.; Yoshikawa, M. *Phytochemistry* **2009**, *70*, 1166–1172.
31. Tian, W. X.; Hsu, R. Y.; Wang, Y. S. *J. Biol. Chem.* **1985**, *260*, 11375–11387.