The Structure Elucidation and Antimicrobial Activities of Nonsterol Triterpenoids from *Ixeris chinensis*

Qing-Hu Wang, Na-Ren-Chao-Ke-Tu Han, Na-Yin-Tai Dai, Xiao-Lan Wu, Wen-Quan Tai, Jie-Si Wu, and Rong-Jun Wu

College of Traditional Mongolian Medicine, Inner Mongolia University for Nationalities, No. 536 Hulinhe District, Tongliao 028000, P. R. China

Reprint requests to Prof. Qing-Hu Wang. Fax: +86-0475-8314242. E-mail: wqh196812@163.com

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An investigation of the petroleum ether extract from *Ixeris chinensis* Nakai has led to the isolation of a new compound, 3-*O*-acetyl-22,28-cyclobauer-7-en-3β-ol (**1**), together with four known compounds which have been isolated from this plant for the first time, namely taraxast-20-en-3β-ol (**2**), 3-*O*-acetyl-taraxast-20-en-3β-ol (**3**), taraxast-20(30)-en-3β-ol (**4**), and 3-*O*-acetyl-taraxast-20(30)-en-3β-ol (**5**). The structures of the isolated compounds have been elucidated on the basis of spectroscopic methods including UV, IR, ESI-MS, 1D NMR, 2D NMR techniques, and by comparison with data reported in the literature. All compounds have been evaluated for their activity against various bacteria and shown to give good results.

Key words: Ixeris chinensis Nakai, Nonsterol Triterpenoids, Antimicrobial Activity, 3-*O*-Acetyl-22,28-cyclobauer-7-en-3β-ol

Introduction

Ixeris chinensis Nakai is a member of the family Compositae and distributed throughout Inner Mongolia, Shanxi, Xinjiang provinces of China, and is used as a remedy for bronchitis, pneumonia, pharyngitis, dysentery, and poisonous indigestion on the basis of its anti-febrile, antidotal and analgesic effects [1]. Terpenoids [2 – 5] and flavonoids [6, 7] have been reported from *Ixeris chinensis* Nakai. In our phytochemical studies [10], several sesquiterpene lactones and flavonoids were isolated. In continuation of our investigation, we report herein the isolation and characterization of a new compound, 3-*O*-acetyl-22,28-cyclobauer-7-en-3β-ol (**1**), together with four known compounds which were isolated from this plant for the first time, namely taraxast-20-en-3β-ol (**2**), 3-*O*-acetyl-taraxast-20-en-3β-ol (**3**), taraxast-20(30)-en-3β-ol (**4**), and 3-*O*-acetyl-taraxast-20(30)-en-3β-ol (**5**). Terpenoids [8, 9] exhibit significant pharmacological activities including anti-inflammatory, antibacterial, and cytotoxicity effects, against A 549 lung carcinoma, WI-38 lung fibroblast, VA-13 lung malignant tumor, and against HepG2 human liver tumor cells. The antibacterial activities of terpenoids are consistently used in Mongolian medicine. Hence, all compounds have been evaluated against various bacteria.

Results and Discussion

The petroleum ether extract of *Ixeris chinensis* Nakai was separated by chromatography and afforded the new compound 3-*O*-acetyl-22,28-cyclobauer-7-en-3β-ol (**1**), together with four known compounds 2 – 5 which were isolated from this plant for the first time (Fig. 1). The structures of the known compounds were identified by comparing their spectroscopic data with those reported in the literature [11].

Compound **1** was obtained as colorless needle-shaped crystals. The molecular formula was determined to be $C_{32}H_{50}O_2$ by HR-ESI-MS at m/z = 467.3883 $[M+H]^-$. This was in accordauce with the ^{13}C NMR and DEPT spectra, which showed 32 carbon signals (5 quaternary, 7 methine, 9 methylene, 8 methyl, 2 olefinic, and 1 carbonyl carbons). Of these 32 carbon signals (Table 1) 30 were assigned to the triter-

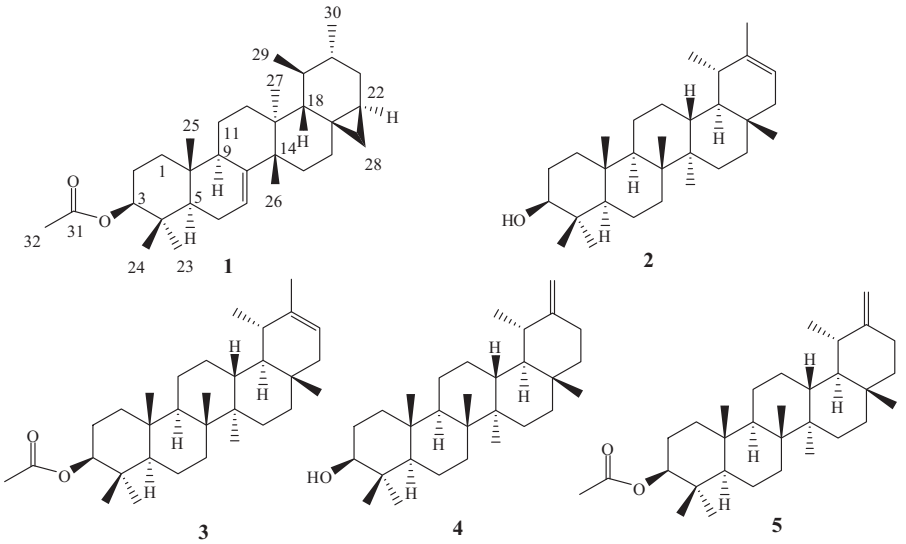

Fig. 1. Structures of compounds **1**–**5**.

pene skeleton and 2 to the acetyl group. The ^{1}H NMR spectrum showed a typical signal at $\delta = 4.51$ ppm (dd, $J = 11.5$, 4.0 Hz) ascribable to an axial H-3, one olefinic proton at $\delta = 5.40$ ppm (dd, 1H, $J = 5.0$, 2.5 Hz) and eight methyl proton signals at $\delta = 0.76$, 0.85, 0.90 (d, J = 6.0 Hz), 0.93, 0.94, 0.99, 1.04 (d, $J = 6.0$ Hz), and 2.06 ppm, which were associated with the relevant carbon resonances at $\delta = 13.0$, 27.5, 22.7, 15.8, 22.5, 23.7, 25.6, and 21.4 ppm, respectively, through the HSQC spectrum. The previously assigned eight methyl and two olefinic carbon signals ($\delta = 116.2$, 145.5 ppm) in the ^{13}C NMR spectrum suggest that compound **1** belonged to a *bauerance-type* saponin [12]. The structure of **1** is similar to that of 3-hydroxy-bauer-7-en-28-oic acid [12], except for C-28. The -COOH (C-28) in 3-hydroxy-bauer-7-en-28-oic acid is substituted by the -CH_2- (C-28) in compound **1**, which was confirmed by HMBC correlations from H-18 ($\delta = 1.29$) to C-28 ($\delta = 37.7$), C-27 ($\delta = 22.5$), C-29 ($\delta = 25.6$), C-16 ($\delta = 31.5$), and C-22 ($\delta = 38.0$), and by ^{1}H-^{1}H COSY correlations of H-28/H-22/H-21. The assignment of an acetyl group was confirmed by correlations from 32-CH_3 ($\delta = 2.06$) to C-31 ($\delta = 171.0$) and from H-3 to C-31 in the HMBC spectrum. The location of the double bond was assigned by HMBC correlations from 26-CH_3 to C-8, and from H-7 to C-5, C-9 and C-14.

The positions of all methyl groups were confirmed by the HMBC spectrum (Fig. 2), in which the correlations of $\delta = 0.76$ (25-CH_3) with C-1 ($\delta = 36.5$), C-5 ($\delta = 50.6$), C-9 ($\delta = 48.1$), C-10 ($\delta = 35.1$), and of $\delta = 0.85$ (23-CH_3) with C-3 ($\delta = 81.1$), C-4 ($\delta = 37.8$), C-5 ($\delta = 50.6$), 24-CH_3 ($\delta = 15.8$), and of $\delta = 0.93$ (24-CH_3) with C-3 ($\delta = 81.1$), C-4 ($\delta = 37.8$), C-5 ($\delta = 50.6$), 23-CH_3 ($\delta = 27.5$), and of $\delta = 0.99$ (26-CH_3) with C-8 ($\delta = 145.5$), C-13 ($\delta = 37.6$), C-14 ($\delta = 41.3$), C-15 ($\delta = 28.9$), and of $\delta = 0.94$ (27-CH_3) with C-12 ($\delta = 32.4$), C-13 ($\delta = 37.6$), C-14 ($\delta = 41.3$), C-18 ($\delta = 54.9$), and of $\delta = 1.04$ (29-CH_3) with C-18 ($\delta = 54.9$), C-19 ($\delta = 35.3$), C-20 ($\delta = 32.0$), and of $\delta = 0.90$ (30-CH_3) with C-19 ($\delta = 35.3$), C-20 ($\delta = 32.0$), C-21 ($\delta = 29.2$) were detected.

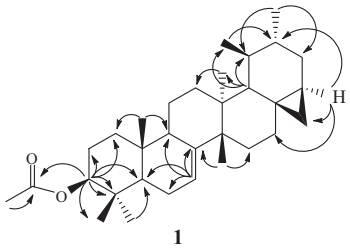

Fig. 2. Selected HMBC correlations for **1**.

The relative configuration at the stereogenic centers of **1** were determined by NOESY experiments (Fig. 3). The NOESY interactions H-C(25)/H-C(26), and H-C(26)/H-C(24)/H-18/H-C(29) indicated that

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data of compound **1** in $CDCl_3$ with coupling constants *J* in Hz in parentheses.

Position	δ_C	DEPT	δ_H	HMBC (H→C)
1	36.5	CH_2	1.67 m, 1.20 m	C-5
2	24.2	CH_2	1.68 m, 1.63 m	
3	81.1	CH	4.51 dd (11.5, 4.0)	C-1, C-2, C-4, C-5, C-23, C-24, C-31
4	37.8	C		
5	50.6	CH	1.40 m	
6	24.0	CH_2	2.14 m, 1.98 m	
7	116.2	CH	5.40 dd (5.0, 2.5)	C-5, C-6, C-9, C-14
8	145.5	C		
9	48.1	CH	2.23 m	
10	35.1	C		
11	16.8	CH_2	1.54 m, 1.48 m	
12	32.4	CH_2	1.56 m, 1.46 m	
13	37.6	C		
14	41.3	C		
15	28.9	CH_2	1.50 m, 1.40 m	
16	31.5	CH_2	1.62 m, 1.11 m	
17	32.0	C		
18	54.9	CH	1.29 m	C-12, C-16, C-27, C-28, C-29
19	35.3	CH	1.16 m	C-13, C-18
20	32.0	CH	1.54 m	
21	29.2	CH_2	1.25 m, 1.18 m	
22	38.0	CH	1.04 m	C-16, C-18, C-20
23	27.5	CH_3	0.85 s	C-3, C-4, C-5, C-24
24	15.8	CH_3	0.93 s	C-3, C-4, C-5, C-23
25	13.0	CH_3	0.76 s	C-1, C-5, C-9, C-10
26	23.7	CH_3	0.99 s	C-8, C-13, C-14, C-15
27	22.5	CH_3	0.94 (s, 3H)	C-12, C-13, C-14, C-18
28	37.7	CH_2	1.50 m 1.18 m	
29	25.6	CH_3	1.04 d (6.0)	C-18, C-19, C-20
30	22.7	CH_3	0.90 d (6.0)	C-19, C-20, C-21
31	171.0	C		
32	21.4	CH_3	2.06 s	C-31

24-CH_3, 25-CH_3, 26-CH_3, 29-CH_3 and H-C(18) were all β-oriented. In addition, the NOESY interactions H-3/H-C(23)/H-5/H-9, and H-9 /H-C(27)/H-C(30)/H-22 suggested the α-orientation of H-3, H-5, H-9, 23-CH_3, 27-CH_3, and 30-CH_3. These data indicate that **1** is 3-*O*-acetyl-22,28-cyclobauer-7-en-3β-ol.

The antimicrobial activity of the above compounds was evaluated against various bacteria and fungi. The results reported in Table 2 indicate that compounds **1**–**3** show good activity against *Bacillus coagulans* while being less active against *Proteous vulgaris* and *Eschecheria coli* as compared to Streptomycin. Compounds **4** and **5** show similar activity as Streptomycin against *Eschecheria coli* and are less active against *Staphylococcus aureus*, *Bacillus coagulans*, and *Proteous vulgaris*. With regard to fungicidal activity, compounds **1**–**3** are more active than Griseofulvin against *Aspergillus niger* and are less active against *Fusarium oxysporum*. Compounds **4** and **5** are found to be highly active against *Fusarium oxysporum* and less active against *Aspergillus niger*.

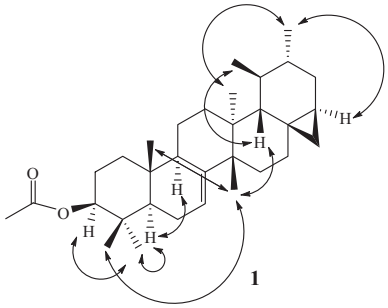

Fig. 3. Selected NOESY correlations for **1**.

Experimental Section

General experimental procedures

Melting points were determined using an X-4 micro melting point apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ at 25 °C on a Perkin-Elmer 241 polarimeter. The UV spectra were recorded on a Shimadzu UV-2201 spectrometer. The IR spectra were recorded in KBr discs on a Thermo Nicolet 200 double beam spectrophotometer. The HR-ESI-MS spectra were measured on a Bruker Daltonics MicroTOFQ instrument. NMR spectra were measured on a Bruker AVANCE III-500 NMR spectrometer with tetramethylsilane (TMS) as the internal reference, chemical shifts being expressed in $\delta(ppm)$.*Column* chromatography was performed by using silica gel (200 – 300 mesh, Marine Chemical Factory, Qingdao, China). Fractions were monitored by TLC (silica gel GF_{254} 10 – 40 μm, Marine Chemical Factory, Qingdao, China), and spots were visualized by heating silica gel plates sprayed with 10 % H_2SO_4 in EtOH.

Plant material

The whole plants of *Ixeris chinensis* Nakai were collected in Tongliao, Inner Mongolia of China, in June 2012, and identified by Prof. Buhebateer (Inner Mongolia University for Nationalities). A voucher specimen (no. 20120602) has been deposited in the School of Traditional Mongolian Medicine of Inner Mongolia University for Nationalities.

Table 2. Antibacterial and antifungal activity of compounds **1**–**5**.

Compound[a]	Antibacterial activity (zone of inhibition in mm)					Antifungal activity (zone of inhibition in mm)		
	E. coli	*S. aureus*	*B. coagulans*	*P. vulgaris*		*P. digitatum*	*F. oxysporum*	*A. niger*
1 (1.02)	15.81	17.97	20.24	14.78		16.82	17.21	18.65
2 (0.98)	13.25	18.24	19.75	12.47		15.78	17.40	18.24
3 (0.96)	13.65	18.74	20.13	12.87		15.34	16.87	17.89
4 (1.04)	19.02	15.24	16.84	15.84		14.65	19.74	15.47
5 (1.02)	18.76	16.23	17.02	15.47		14.68	18.67	15.87
Streptomycin	19.87	18.64	19.91	18.54	Griseofulvin	17.61	20.91	16.02

[a] In parentheses: tested concentration in mg mL^{-1}.

Extraction and isolation

The air-dried whole plant of *Ixeris chinensis* Nakai (8.0 kg) was powdered and extracted twice with refluxing petroleum ether (P. E.) (30 L). Evaporation of the solvent under reduced pressure delivered the P. E. extract. The P. E. extract (50.0 g) was subjected to column chromatography on silica gel and eluted with a P. E.-EtOAc (100 : 1 to 20 : 1) gradient to give 10 fractions (Fractions 1 – 10). Fraction 5 (1.0 g) was subjected to silica gel column chromatography using P. E.-EtOAc with increasing polarity (80 : 1 to 50 : 1) to give **1** (35 mg) and **3** (22 mg). Fraction 6 (600 mg) was subjected to silica gel column chromatography using P. E.-EtOAc with increasing polarity (70 : 1 to 50 : 1) to give **5** (20 mg) and **2** (11 mg). Fraction 7 (300 mg) was subjected to silica gel column chromatography using P. E.-EtOAc with increasing polarity (60 : 1 to 30 : 1) to give **4** (17 mg).

Antimicrobial activity

The antimicrobial activity of compounds **1** and **2** has been determined by the filter paper disc diffusion method [13]. The various bacterial species were first incubated at 45 °C for 48 h. The sterile filter paper discs (6 mm) were soaked with standard antibacterial agent and various test samples and were dried at 50 °C. The discs were then placed on soft nutrient agar (2 %) petri plates previously seeded with a suspension of each bacterial species. The diameter of the zone of inhibition was measured at 37 ± 1 °C after 24 h. For antifungal activity, Saborauds broth media [14] with 4 % agar was used for the preparation of plates and incubated with spores and mycelium suspensions of fungi obtained from one week old cultures. The diameter of the zone of inhibition was measured at 28 ± 1 °C after 48 h. The results are listed in Table 2.

*3-O-Acetyl-22,28-cyclobauer-7-en-3β-ol (**1**)*

Colorless needles. M. p. 307 – 309 °C; $[\alpha]_D^{25} = -11.6$ (c = 0.1, $CHCl_3$). – IR (KBr): ν = 1705 (C=O), 1640 (C=C) cm^{-1}. – HRMS ((–)-ESI): m/z = 467.3883 (calcd. 467.3889 for $C_{32}H_{51}O_2$, $[M+H]^-$). – ^{1}H and ^{13}C NMR spectra: see Table 1.

*Taraxast-20-en-3β-ol (**2**)*

Colorless needles. M. p. 301 – 302 °C; $[\alpha]_D^{25} = -19.8$ (c = 0.1, $CHCl_3$). – IR (KBr): ν = 3302 (OH), 1632 (C=C) cm^{-1}. – HRMS ((–)-ESI): m/z = 425.3772 (calcd. 425.3778 for $C_{30}H_{49}O$, $[M–H]^-$). – ^{1}H NMR (500 MHz, in $CDCl_3$): δ = 5.27 (1H, m, H-21), 3.20 (1H, dd, J = 11.5, 4.0 Hz, H-3), 0.95 (3H, s, 23-CH_3), 1.01 (3H, d, J = 6.0 Hz, 29-CH_3), 1.63 (3H, s, 30-CH_3), 0.85 (3H, s, 25-CH_3), 1.04 (3H, s, 26-CH_3), 0.77 (3H, s, 24-CH_3), 0.73 (3H, s, 28-CH_3), 0.94 (3H, s, 27-CH_3). – ^{13}C NMR (125 MHz, in $CDCl_3$): δ = 139.9 (C-20), 118.9 (C-21), 79.0 (C-3), 55.4 (C-5), 50.5 (C-9), 48.6 (C-18), 42.3 (C-14), 42.2 (C-22), 41.1 (C-8), 39.2 (C-13), 38.9 (C-4), 38.4 (C-1), 37.1 (C-10), 36.7 (C-16), 36.3 (C-19), 34.4 (C-17), 34.2 (C-7), 28.0 (C-23), 27.6 (C-11), 27.0 (C-15), 27.4 (C-2), 22.6 (C-29), 21.7 (C-30), 21.6 (C-12), 18.3 (C-6), 17.7 (C-28), 16.3 (C-25), 16.1 (C-26), 15.4 (C-24), 14.8 (C-27).

*3-O-Acetyl-taraxast-20-en-3β-ol (**3**)*

Colorless needles. M. p. 309 – 310 °C; $[\alpha]_D^{25} = -12.4$ (c = 0.1, $CHCl_3$). – IR (KBr): ν = 1708 (C=O), 1642 (C=C) cm^{-1}. – HRMS ((–)-ESI): m/z = 467.3879 (calcd. 467.3884 for $C_{32}H_{51}O_2$, $[M–H]^-$). – ^{1}H NMR (500 MHz, in $CDCl_3$): δ = 5.28 (1H, m, H-21), 4.51 (1H, dd, J = 11.5, 4.0 Hz, H-3), 0.88 (3H, s, 23-CH_3), 1.01 (3H, d, J = 6.0 Hz, 29-CH_3), 1.61 (3H, s, 30-CH_3), 2.07 (3H, s, 32-CH_3), 0.76 (3H, s, 28-CH_3), 0.87 (3H, s, 24-CH_3), 0.90 (3H, s, 25-CH_3), 1.07 (3H, s, 26-CH_3), 0.97 (3H, s, 27-CH_3). – ^{13}C NMR (125 MHz, in $CDCl_3$): δ = 171.0 (C-31), 139.8 (C-20), 118.9 (C-21), 80.9 (C-3), 55.4 (C-5), 50.3 (C-9), 48.7 (C-18), 42.3 (C-14), 42.2 (C-22), 41.1 (C-8), 39.2 (C-13), 38.4 (C-1), 37.8 (C-4), 37.0 (C-10), 36.7 (C-16), 36.3 (C-19), 34.4 (C-17), 34.2 (C-7), 28.0 (C-23), 27.6 (C-11), 27.0 (C-15), 23.7 (C-2), 22.6 (C-29), 21.7 (C-30), 21.6 (C-12), 21.4(C-32), 18.2 (C-6), 17.7 (C-28), 16.5 (C-24), 16.4 (C-25), 16.1 (C-26),14.7 (C-27).

*Taraxast-20(30)-en-3β-ol (**4**)*

Colorless needles. M. p. 304 – 306 °C; $[\alpha]_D^{25} = -21.1$ (c = 0.1, $CHCl_3$). – IR (KBr): ν = 3334 (OH), 1640 (C=C)

cm^{-1}. – HRMS ((–)-ESI): $m/z = 425.3771$ (calcd. 425.3778 for $C_{30}H_{49}O$, $[M–H]^-$). – ^1H NMR (500 MHz, in $CDCl_3$): $\delta = 4.61$ (2H, m, H-30), 3.20 (1H, dd, $J = 11.5$, 4.0 Hz, H-3), 0.95 (3H, s, 23-CH_3), 1.02 (3H, d, $J = 6.0$ Hz, 29-CH_3), 0.87 (3H, s, 25-CH_3), 0.85 (3H, s, 28-CH_3), 1.04 (3H, s, 26-CH_3), 0.77 (3H, s, 24-CH_3), 0.93 (3H, s, 27-CH_3). – ^{13}C NMR (125 MHz, in $CDCl_3$): δ = 154.7 (C-20), 107.2 (C-30), 79.0 (C-3), 55.3 (C-5), 50.4 (C-9), 48.7 (C-18), 42.0 (C-14), 40.9 (C-8), 39.4 (C-13), 39.1 (C-19), 38.9 (C-4), 38.8 (C-1), 38.7 (C-22), 38.3 (C-16), 37.1 (C-10), 34.5 (C-17), 34.1 (C-7), 28.0 (C-23), 27.4 (C-2), 26.7 (C-15), 26.2 (C-12), 25.6 (C-21), 25.5 (C-29), 21.5 (C-11), 19.5 (C-28), 18.3 (C-6), 16.3 (C-25), 15.9 (C-26), 15.4 (C-24), 14.7 (C-27).

*3-O-Acetyl-taraxast-20(30)-en-3β-ol (**5**)*

Colorless needles. M. p. 311 – 312 °C; $[\alpha]_D^{25} = -13.1$ (c = 0.1, $CHCl_3$). – IR (KBr): ν = 1718 (C=O),1639 (C=C) cm^{-1}. – HRMS ((–)-ESI): $m/z = 467.3878$ (calcd. 467.3884 for $C_{32}H_{51}O_2$, $[M–H]^-$). – ^1H NMR (500 MHz, in $CDCl_3$): $\delta = 4.56$ (2H, m, H-30), 4.48 (1H, dd, $J = 11.5$, 4.0 Hz, H-3), 0.89 (3H, s, 23-CH_3), 1.02 (3H, d, $J = 6.0$ Hz, 29-CH_3), 2.08 (3H, s, 32-CH_3), 0.78 (3H, s, 25-CH_3), 0.87 (3H, s, 28-CH_3), 0.91 (3H, s, 26-CH_3), 1.04 (3H, s, 24-CH_3), 0.95 (3H, s, 27-CH_3). – ^{13}C NMR (125 MHz, in $CDCl_3$): δ = 171.1 (C-31), 154.7 (C-20), 107.1 (C-30), 81.0 (C-3), 55.3 (C-5), 50.4 (C-9), 48.6 (C-18), 42.0 (C-14), 40.9 (C-8), 39.4 (C-13), 39.1 (C-19), 38.8 (C-4), 38.4 (C-1), 38.3 (C-22), 37.8 (C-16), 37.1 (C-10), 34.5 (C-17), 34.0 (C-7), 28.0 (C-23), 26.7 (C-15), 26.2 (C-12), 25.6 (C-21), 25.5 (C-29), 23.7 (C-2), 21.5 (C-11), 21.4(C-32), 19.5 (C-28), 18.2 (C-6), 16.5 (C-24), 16.4 (C-25), 15.9 (C-26), 14.7 (C-27).

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