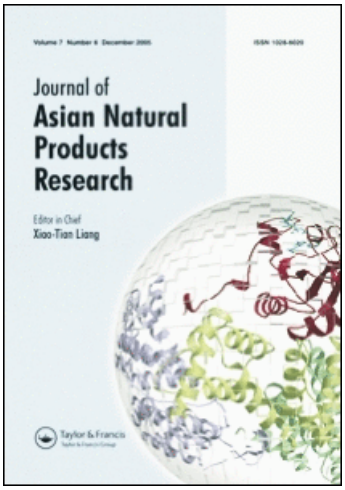

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Trollioside, a new compound from the flowers of *Trollius chinensis*

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TROLLIOSIDE, A NEW COMPOUND FROM THE FLOWERS OF *TROLLIUS CHINENSIS*

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A new compound, named trollioside, together with nine known compounds, proglobeflowery acid, isoswertisin, isoswertiajaponin, cirsimaritin, veratric acid, vitexin, orientin, β-sitosterol and daucosterol, were isolated from the ethanol extract of the dried flowers of *Trollius chinensis* Bunge. The isolation and structural determination of these compounds are discussed.

Keywords: *Trollius chinensis*; Rannuculaceae; Trollioside; NMR spectra

INTRODUCTION

The flower of *Trollius chinensis* Bunge (Rannuculaceae), which is a Traditional Chinese Medicine, has long been used to treat respiratory infections, pharyngitis, tonsillitis and bronchitis. The dried flowers of *T. chinensis* and another species *T. sativus* L. in genus *Trollius* are clinically used with the same name, Jinlianhua [1]. Our experimental material was identified as the former. The original plant is endemic in North China [2]. Previous studies on the chemical constituents of the flowers of *T. chinensis* revealed the existence of vitexin, orientin, veratric acid, dimethoxybenzamide, palmitic acid and volatile oil [3]. The present paper deals with the isolation and structure elucidation of a new compound named trollioside (**1**). In addition, nine known compounds, proglobeflowery acid (**2**), isoswertisin (**3**), isoswertiajaponin (**4**), cirsimaritin (**5**), veratric acid (**6**), vitexin (**7**), orientin (**8**), β-sitosterol (**9**) and daucosterol (**10**), were also obtained from the flowers. Compounds **1**, **3**–**5** were isolated from the title plant for the first time. Cirsimaritin (**5**) exhibited antibacterial activity against both Gram positive and Gram negative bacteria such as *Staphylococcus aureus* and *E. coli* [4], while **2**, **7** and **8** showed antiviral activity against parainfluenza type 3 virus [5].

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RESULTS AND DISCUSSION

Compound **1** exhibited the molecular formula $C_{19}H_{26}O_9$ by its HR-ESI-MS spectrum. The UV spectrum of **1** showed aromatic absorption bands at 292 and 251 nm. Its IR spectrum displayed C–O stretching bands at 1070 and 1040 cm^{-1}, diagnostic of glycoside. The absorption bands at 3346, 1709, and 1590 and 1454 cm^{-1} suggested the presence of hydroxyl, unsaturated carbonyl and aromatic groups, respectively. The ^1H NMR spectrum showed resonances for two aromatic proton signals, two olefinic proton signals and other proton signals for aliphatic and sugar moieties. The ^{13}C NMR, DEPT and HMQC experiments revealed that this compound contains three methyls, two methylenes, eight methines, and six quaternary carbons (Fig. 1).

The two *meta*-coupled doublets at δ 7.49 (1H, d, $J = 2.0$ Hz, H-2) and 7.46 (1H, d, $J = 2.0$ Hz, H-6) in the ^1H NMR spectrum of **1** and their HMBC correlations with C-4 (δ 148.6) showed that this compound has a benzene ring with four substituents (Fig. 2). Both aromatic protons (H-2 and H-6) had HMBC correlations with C-11 (δ 169.7), which indicated the substituent at C-1 (δ 127.8) was a carboxyl group. The substituent at C-3 (δ 153.0) should be a methoxyl group because the HMBC correlations between the methoxyl proton signal at δ 3.88 (3H, s, H-12) and C-3 were observed. The other substituent could be assigned to an isopentenyl group on the basis of the ^1H–^1H COSY, HMQC and HMBC of **1**, and it is attached to the benzene ring at C-5 (δ 138.1) because of the distinct HMBC correlations between a proton signal at δ 3.57 (1H, dd, $J = 15.0$ Hz and 7.5 Hz, H-7a) and two aromatic carbons C-4 or C-6 (δ 124.7). Major HMBC correlations were also observed between a proton signal at δ 3.50 (1H, dd, $J = 15.0$ Hz and 7.5 Hz, H-7b) and C-6 or C-4, between two methyl proton signals at δ 1.74 (3H, s, H-10a) or 1.72 (3H, s, H-10b) and C-8 (δ 123.7), and between H-7a or H-7b and a quaternary carbon C-9 (δ 133.7). These suggest that the aglycone of **1** is proglobeflowery acid [6]. The D-glucose residue could be established from the 1D and 2D NMR of **1**. HMBC correlations between the anomeric proton signal at δ 5.08 (1H, d, $J = 7.5$ Hz, H-1′) and C-4 supported that the D-glucosyl was attached to the 4-hydroxyl group of proglobeflowery acid with a β-configuration (Fig. 2). Therefore, the structure of **1** was determined as 3-methoxy-5-(3-methyl-but-2-enyl)-4-(-*O*-D-β-glucopyranosyl)benzoic acid, which was named trollioside.

The results of the hydrolysis of **1** confirmed the above conclusion. Acid hydrolysis of **1** released D-glucose and **1a** (Fig. 3). Compared with **1**, the ^1H and ^{13}C NMR spectra of **1a** indicated that the olefinic proton and the olefinic carbon signals in **1** disappeared, and an

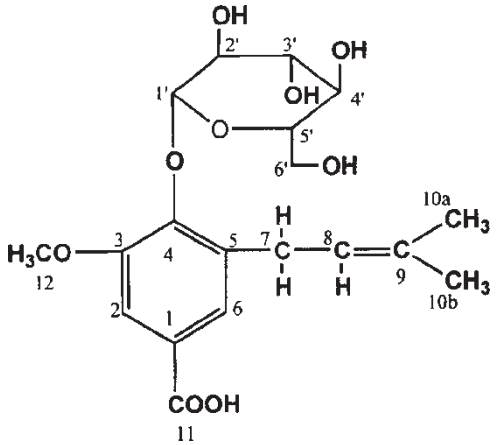

FIGURE 1 Chemical structure of **1**.

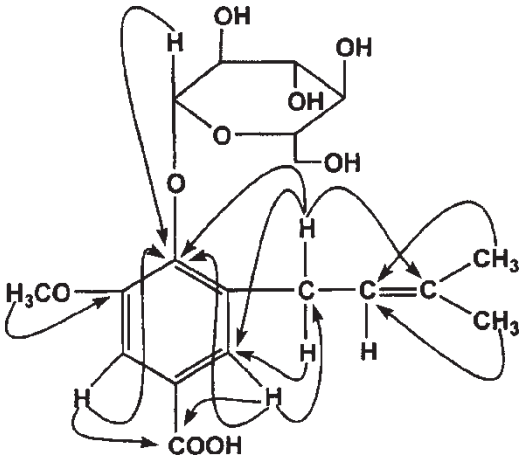

FIGURE 2 Important HMBC correlations of **1**.

additional methylene [δ_H 1.85 (2H, t, $J = 6.6$ Hz, H-8); δ_C 27.0 (C-8)] and an oxygenated quaternary carbon at δ 76.9 (C-9) appeared. These spectral data of **1a** accord with those of a compound named globeflowery acid [3]. Cellulase hydrolysis of **1** yielded D-glucose and a compound whose NMR spectral data were the same as those of compound **2**. These evidences confirmed that the aglycone of **1** was **2**, and **2** was transformed into **1a** under acidic conditions during heating upon hydrolysis.

The known compounds **2**−**10** were characterized by comparing their detailed spectral data (UV, IR, MS and NMR) with those from the literature [3,6–8].

EXPERIMENTAL

General Experimental Procedures

Melting points were measured on an XT-4A apparatus without correction. Optical rotations were measured with an AA-10R polarimeter manufactured by the Optical Activity Co. Ltd. UV spectra were recorded on a Varian Cary Eclipse 300 spectrometer using MeOH as the solvent. IR spectra (film) were determined on a Thermo Nicolet Nexus 470 FT-IR

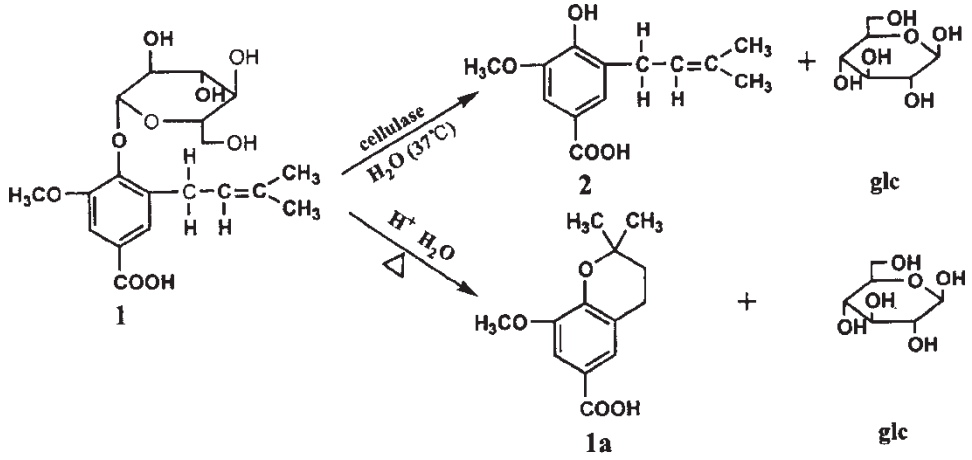

FIGURE 3 Hydrolysis of **1**.

spectrometer. NMR spectra were obtained on a Bruker DRX 500 and a JEOL JNM-AL 300 NMR spectrometer with the solvents as internal standards. The HR-ESIMS spectrum was measured on a Bruker APEX II mass spectrometer and TOF-MS spectra were detected with a PE Q-STAR ESI-TOF-MS/MS spectrometer. Cellulase was supplied by Shanghai Boao Biotech. Co. Ltd. Silica gel (200–300 mesh) for column chromatography was provided by Tsingtao Marine Chemistry Co. Ltd. Sephadex LH-20 (18–110 μm) was manufactured by Pharmacia Co. Ltd. and polyamide (30–60 and 200 mesh) was produced by Wuxi Electronic Teaching Apparatus Co. Ltd. in Jiangsu Province, China.

Plant Material

The experimental material (Jinlianhua) was purchased in October 2000 from the Chinese crude drug market in Anguo, Hebei Province of China, and authenticated by Professor Shao-qing Cai as the flowers of *T. chinensis*. A voucher specimen (No. 2594) has been deposited in the herbarium of Pharmacognosy, School of Pharmaceutical Sciences, Peking University.

Extraction and Isolation

Some 8 kg of Jinlianhua (dried flowers of *T. chinensis*) was extracted with 95% ethanol under reflux (2 h × 3). After concentration *in vacuo*, the ethanol crude extract (1.6 kg) was dissolved in water, and partitioned successively with light petroleum, ethyl acetate and n-butanol.

Initial separation of the EtOAc-soluble part (205 g) was carried out by polyamide (30–60 mesh) column chromatography, eluted with H_2O, 20% EtOH, 50% EtOH, 70% EtOH and 95% EtOH, which afforded 8 major fractions (Fr.A-H). Compound **6** (1050 mg) was crystallized in a light petroleum–acetone mixture from Fr.B (3.5 g). Fr.C (8.6 g) was subjected to silica gel chromatography, employing gradient $CHCl_3$-MeOH from 30:1 to 3:1 as the eluent, to give six fractions (Fr.C1-C6). Fr.C2 (820 mg) was then isolated using silica gel column chromatography, eluted with $CHCl_3$–MeOH from 30:1 to 10:1, to afford **2** (14 mg). Fr.C3 (1.3 g) was isolated with silica gel column chromatography, employing $CHCl_3$–MeOH 30:1–3:1 as eluent, to obtain **1** (212 mg). Further purification of Fr.C4 (2.6 g) with silica gel column chromatography eluted with $CHCl_3$–MeOH (30:1–5:1) mixtures yielded **10** (320 mg). Fr.E (5.2 g) was further separated by polyamide (200 mesh) column chromatography, eluted with $CHCl_3$–MeOH (30:1–3:1) mixtures, to obtain five further fractions (Fr.E1-E5). Fr.E2 (2.1 g) was purified by Sephadex LH-20 with MeOH as eluent to yield **3** (304 mg) and **4** (73 mg). Fr.E4 (1.8 g) was separated by polyamide (200 mesh) column chromatography with $CHCl_3$–MeOH (30:1–2:1) mixtures as eluent to afford **7** (210 mg) and **8** (208 mg). Compound **5** (51 mg) was isolated from Fr.G (1.8 g) by silica gel chromatography eluted with $CHCl_3$–MeOH (20:1–3:1) mixtures.

The light petroleum-soluble part (385 g) yielded **9** (1250 mg) and **6** (214 mg) after repeated silica gel column chromatography eluted with light petroleum–acetone (20:1–1:1) mixtures. Both **7** (152 mg) and **8** (141 mg) were also obtained from the n-butanol-soluble part (285 g) separated with repeated column chromatography such as silica gel, polyamide and Sephadex LH-20.

Trollioside (1)

White powder, mp 172–174°C; $[\alpha]_D^{20}$ −2.3 (MeOH; *c* 0.50); UV (MeOH) λ_{max} (log ε) (nm): 213 (4.06), 251 (2.76), 292 (2.02); IR (KBr) ν_{max} (cm^{-1}): 3346 (OH), 2975 (CH), 1709 (C═O), 1590, 1465 (-CH═CH- aromatic), 1070 and 1007 (C–O); ^1H NMR (CD_3OD, 500 MHz): δ 7.49 (1H, d, J = 2.0 Hz, H-2), 7.46 (1H, d, J = 2.0 Hz, H-6), 3.57 (1H, dd, J = 15.0 Hz, 7.5 Hz, H-7a), 3.50 (1H, dd, J = 15.0 Hz, 7.5 Hz, H-7b), 5.32 (1H, m, H-8), 1.74 (3H, s, H-10a), 1.72 (3H, s, H-10b), 3.88 (3H, s, H-12), 5.08 (1H, d, J = 7.5 Hz, H-1′),

3.45 (1H, H-2′, overlapped with H-3′), 3.44 (1H, H-3′, overlapped with H-2′), 3.38 (1H, t, $J = 9.5$ Hz, H-4′), 3.22 (1H, m, H-5′), 3.79 (1H, dd, $J = 12.0$ Hz, 5.0 Hz, H-6′a), 3.66 (1H, dd, $J = 12.0$ Hz, 5.0 Hz, H-6′b); ^{13}C NMR (CD_3OD, 125 MHz): δ 127.8 (C-1), 112.6 (C-2), 153.0 (C-3), 148.6 (C-4), 138.1 (C-5), 124.7 (C-6), 29.8 (C-7), 123.7 (C-8), 133.7 (C-9), 18.0 (C-10a), 25.9 (C-10b), 169.7 (C-11), 56.5 (C-12), 104.3 (C-1′), 75.9 (C-2′), 78.0 (C-3′), 71.6 (C-4′), 78.3 (C-5′), 62.7 (C-6′); HR-ESIMS (positive) *m/z*: 399.1656 $[M + 1]^+$ (calcd for $C_{19}H_{27}O_9$, 399.1649); TOF-MS (negative and positive) *m/z*: 397 $[M - 1]^-$ and 399 $[M + 1]^+$.

*Acid hydrolysis of **1***. Compound **1** (16 mg) was refluxed with 2 M HCl in H_2O–EtOH (3:1, 4 mL) for 2 h. After evaporating EtOH *in vacuo*, the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was then concentrated to dryness to afford a white powder (10 mg) of **1a**. D-Glucose was identified from the aqueous layer by silica gel TLC (R_f 0.4) with a $CHCl_3$–MeOH–H_2O (5:5:1) mixture and 10% H_2SO_4 spray reagent.

*Cellulase hydrolysis of **1***. Compound **1** (12 mg) was incubated with cellulase (60 U) in H_2O (2 mL) at 37°C for 2 h. The reaction mixture was then extracted with $CHCl_3$. The $CHCl_3$ layer was concentrated to dryness to afford a white powder of the aglycone (**2**) (7 mg). D-Glucose was identified using the same method as that of acid hydrolysis of **1**.

Globeflowery Acid (1a)

White powder, mp 212–213°C; ^{1}H NMR (CD_3OD, 500 MHz): δ 7.44 (1H, d, $J = 2.0$ Hz, H-2), 7.37 (1H, d, $J = 2.0$ Hz, H-6), 2.83 (2H, t, $J = 6.6$ Hz, H-7), 1.85 (2H, t, $J = 6.6$ Hz, H-8), 1.35 (3H, s, H-10a), 1.34 (3H, s, H-10b), 3.88 (3H, s, H-12); ^{13}C NMR (CD_3OD, 125 MHz): δ 125.5 (C-1), 111.6 (C-2), 149.8 (C-3), 149.7 (C-4), 125.5 (C-5), 122.5 (C-6), 33.7 (C-7), 27.0 (C-8), 76.9 (C-9), 14.7 (C-10a), 23.3 (C-10b), 170.1 (C-11), 56.4 (C-12); TOF-MS (negative) *m/z*: 235 $[M - 1]^-$.

Proglobeflowery Acid (2)

White powder, mp 141–142°C; ^{1}H NMR (CD_3OD, 500 MHz): δ 7.40 (1H, d, $J = 2.0$ Hz, H-2), 7.43 (1H, d, $J = 2.0$ Hz, H-6), 3.32 (2H, d, $J = 7.2$ Hz, H-7), 5.32 (1H, t, $J = 7.2$ Hz, H-8), 1.71 (3H, s, H-10a), 1.73 (3H, s, H-10b), 3.88 (3H, s, H-12); ^{13}C NMR (CD_3OD, 125 MHz): δ 121.9 (C-1), 111.1 (C-2), 150.2 (C-3), 148.2 (C-4), 128.9 (C-5), 125.4 (C-6), 28.9 (C-7), 123.3 (C-8), 133.6 (C-9), 17.8 (C-10a), 25.9 (C-10b), 170.4 (C-11), 56.5 (C-12); TOF-MS (negative) *m/z*: 235 $[M - 1]^-$.

Isoswertisin (3)

Yellow powder, mp 285–286°C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.87 (3H, s, CH_3O), 4.73 (1H, d, $J = 10.2$ Hz, H-1″), 6.52 (1H, s, H-6), 6.82 (1H, s, H-3), 6.90 (2H, d, $J = 8.7$ Hz, H-3′, H-5′), 8.05 (2H, d, $J = 8.4$ Hz, H-2′, H-6′); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 182.3 (C-4), 164.4 (C-2), 163.3 (C-7), 161.3 (C-5), 155.1 (C-9), 129.1 (C-2′, C-6′), 121.5 (C-1′), 115.8 (C-3′, C-5′), 105.7 (C-8), 104.4 (C-10), 102.4 (C-3), 95.0 (C-6), 81.9 (C-5″), 78.6 (C-3″), 73.1 (C-1″), 70.8 (C-2″), 70.5 (C-4″), 61.2 (C-6″); FAB-MS *m/z*: 447 $[M - 1]^-$.

Isoswertiajaponin (4)

Yellow powder, mp 237 − 238°C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.88 (3H, s, CH_3O), 4.73 (1H, d, $J = 9.9$ Hz, H-1″), 6.52 (1H, s, H-6), 6.70 (1H, s, H-3), 6.88 (1H, d, $J = 8.4$ Hz,

H-5′), 7.50 (1H, s, H-2′), 7.57 (1H, d, $J = 8.4$ Hz, H-6′); ^{13}C NMR (DMSO-d$_6$, 125 MHz): δ 182.3 (C-4), 164.5 (C-2), 163.3 (C-7), 161.3 (C-5), 155.1 (C-9), 150.0 (C-4′), 145.9 (C-3′), 121.8 (C-1′), 119.6 (C-6′), 115.7 (C-5′), 114.1 (C-2′), 105.6 (C-8), 104.4 (C-10), 102.4 (C-3), 95.0 (C-6), 82.1 (C-5″), 78.7 (C-3″), 73.1 (C-1″), 70.7 (C-2″), 70.6 (C-4″), 61.6 (C-6″); FAB-MS *m*/*z*: 461 $[M - 1]^-$.

Cirsimaritin (5)

Yellow powder, mp 260–265°C; ^{1}H NMR (DMSO-d$_6$, 500 MHz): δ 3.72 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 6.83 (2H, s, H-3, H-8), 6.93 (2H, d, $J = 7.5$ Hz, H-3′, H-5′), 7.96 (2H, d, $J = 7.5$ Hz, H-2′, H-6′); ^{13}C NMR (DMSO-d$_6$, 125 MHz): δ 182.2 (C-4), 164.0 (C-2), 161.3 (C-4′), 158.6 (C-7), 152.6 (C-9), 152.1 (C-5), 131.8 (C-6), 128.5 (C-2′, C-6′), 121.1 (C-1′), 115.9 (C-3′, C-5′), 105.0 (C-10), 102.6 (C-3), 91.5 (C-8), 60.0 (OCH_3), 56.4 (OCH_3); EI-MS *m*/*z*: 314 $[M]^+$.

Vitexin (6)

Yellow powder, mp 239–240°C; ^{1}H NMR (DMSO-d$_6$, 500 MHz): δ 4.98 (1H, s, H-1″), 6.26 (1H, s, H-6), 6.77 (1H, s, H-3), 6.89 (2H, d, $J = 7.0$ Hz, H-3′, H-5′), 8.02 (2H, d, $J = 7.0$ Hz, H-2′, H-6′); ^{13}C NMR (DMSO-d$_6$, 125 MHz): δ 182.1 (C-4), 163.9 (C-2), 162.6 (C-7), 161.2 (C-4′), 160.4 (C-5), 156.0 (C-9), 129.0 (C-2′, C-6′), 121.6 (C-1′), 115.8 (C-3′, C-5′), 104.6 (C-8), 104.0 (C-10), 102.5 (C-3), 98.2 (C-6), 81.9 (C-5″), 78.7 (C-3″), 73.4 (C-1″), 70.8 (C-2″), 70.5 (C-4″), 61.3 (C-6″); FAB-MS *m*/*z*: 431 $[M - 1]^-$.

Orientin (7)

Yellow powder, mp 256–258°C; ^{1}H NMR (DMSO-d$_6$, 500 MHz): δ 4.69 (1H, d, $J = 9.9$ Hz, H-1″), 6.26 (1H, s, H-6), 6.64 (1H, s, H-3), 6.87 (1H, d, $J = 8.4$ Hz, H-5′), 7.54 (2H, m, H-2′, H-6′); ^{13}C NMR (DMSO-d$_6$, 125 MHz): δ 182.0 (C-4), 164.1 (C-2), 162.8 (C-7), 160.4 (C-5), 156.0 (C-9), 149.7 (C-4′), 145.9 (C-3′), 122.0 (C-1′), 119.4 (C-5′), 115.7 (C-6′), 114.1 (C-2′), 104.6 (C-8), 104.0 (C-10), 102.4 (C-3), 98.2 (C-6), 82.0 (C-5″), 78.8 (C-3″), 73.4 (C-1″), 70.8 (C-2″), 70.7 (C-4″), 61.7 (C-6″); FAB-MS *m*/*z*: 447 $[M - 1]^-$.

Veratric Acid (8)

Colorless needle crystals (MeOH), mp 117–118°C; ^{1}H NMR ($DCCl_3$, 500 MHz): δ 3.93 (6H, s, $OCH_3 \times 2$), 6.91 (1H, d, $J = 8.4$ Hz, H-5), 7.58 (1H, d, $J = 2.1$ Hz, H-2), 7.78 (1H, dd, $J = 8.4$ Hz, 2.1 Hz, H-6); ^{13}C NMR ($DCCl_3$, 125 MHz): δ 172.1 (C = O), 153.8 (C-4), 148.7 (C-3), 124.7 (C-6), 121.8 (C-1), 112.3 (C-2), 110.4 (C-5); TOF-MS (negative) *m*/*z*: 181 $[M - 1]^-$.

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