A New Labdane Diterpene from the Flowers of *Solidago canadensis*

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A new labdane diterpene, 9α,16ξ-dihydroxy-6-oxo-7,13-labdadien-15,16-olide (solicanolide, 1) and six known compounds identified as quercetin (2), 3-*O*-caffeoylquinic acid (3, neochlorogenic acid), 5-*O*-caffeoylquinic acid (4, chlorogenic acid), 4,5-di-*O*-caffeoylquinic acid (5), 3,5-di-*O*-caffeoylquinic acid (6) and 3,4-di-*O*-caffeoylquinic acid (7) were isolated from the flowers of *Solidago canadensis*. To our knowledge, compound 7 was isolated for the first time in *S. canadensis*. This work describes the isolation of compounds 1—7 and the structure elucidation of a new compound identified as compound 1. Solicanolide (1) showed cytotoxic activity against A549 (IC_{50}: 13±2 μM), DLD-1 (IC_{50}: 26±2 μM) and WS1 (IC_{50}: 17±1 μM) cell lines.

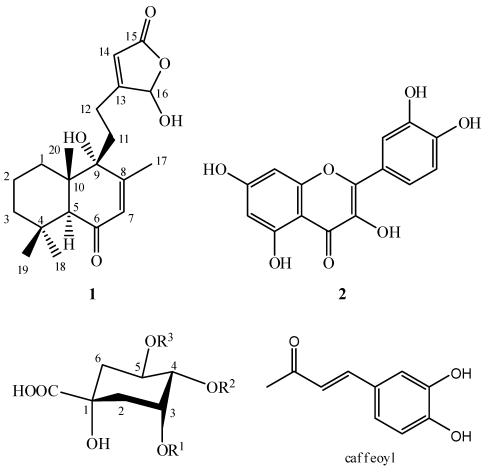
Key words *Solidago canadensis*; isolation; labdane; solicanolide; cytotoxicity; caffeoylquinic acid

Solidago canadensis L. is an Asteraceae widely distributed across North America, occurring in almost every state of the U.S.A. and throughout Canada. Numerous interesting secondary metabolites such as: flavonoids, phenolic acids and glucosides, polysaccharides, diterpenes, triterpenoid saponosides, tannins and essential oils[1] were reported for the genus *Solidago*. Previous phytochemical studies of *S. canadensis* have led to the isolation of flavonoids,[2,3] phenolic acids,[4] sesquiterpenes,[5] diterpenes[6,7] and saponins.[7] The flowers of *S. canadensis* were used in traditional Amerindian medicine as an analgesic,[8] burns and ulcers treatment,[9] febrifuge,[10] gastrointestinal[11,12] and liver[11] aids. In spite of the widespread use of *S. canadensis*, few investigations were carried out on its bioactive secondary metabolites.

Fractionation of the flower extracts of *Solidago canadensis* resulted in the isolation of a new diterpene, 9α,16ξ-dihydroxy-6-oxo-7,13-labdadien-15,16-olide (solicanolide, **1**) and six known compounds identified as quercetin (**2**), 3-*O*-caffeoylquinic acid (neochlorogenic acid) (**3**), 5-*O*-caffeoylquinic acid (chlorogenic acid) (**4**), 4,5-di-*O*-caffeoylquinic acid (**5**), 3,5-di-*O*-caffeoylquinic acid (**6**) and 3,4-di-*O*-caffeoylquinic acid (**7**). To our knowledge, compound **7** was isolated for the first time in *S. canadensis*. This work describes the isolation of compounds **1**—**7** and the structure elucidation of a new compound identified as compound **1**. The cytotoxicity of solicanolide (**1**) was also investigated in this paper.

Experimental

General Experimental Procedures NMR spectra were recorded in methanol-d_4 on a Bruker Avance 400 spectrometer (5 mm QNP with Z-gradient probe) operating at 400.13 MHz (^{1}H) or 100.61 MHz (^{13}C). Chemical shifts were referenced relative to the corresponding residual solvent signals ($\delta_{H/C}$ 3.31/49.0 ppm, respectively). The accurate mass determination was carried out with an Applied Biosystems QSTAR XL Hybrid LC/MS/MS system. Optical rotation was obtained on a Jasco DIP-360 digital polarimeter. Analytical HPLC-DAD-MS analysis were performed on an Agilent 1100 series HPLC-DAD-MS system. A Zorbax ODS C18 column (5 μm, 150×4.6 mm) maintained at 25 °C was utilised. The flow rate was 1 ml/min. Agilent G1315B diode array detector was used for UV detection. The UV spectra were recorded from 190 to 400 nm. An Agilent mass selective detector (VL model) equipped with an atmospheric chemical ionisation source (APCI) was employed for MS detection. All mass spectra were acquired in the negative ion mode. The full scan mass spectrum was recorded over the range of *m/z* 100—1000. Temperature of the drying gas (N_2) was 350 °C with a gas flow rate of 10 l/min and a nebulizing pressure of 40 psi. The ionisation voltage was 4000 V and the corona current was 15 mA. All HPLC separations were performed on a preparative Agilent 1100 series (Agilent Technologies Canada Inc.) with a ZORBAX ODS column C18 (2.1×25 cm; 7 μm) at a flow rate of 16 ml/min. Compounds were detected by UV absorption at 254 nm. For all HPLC procedures, solvent A was H_2O+0.1% HCOOH, solvent B was MeOH+0.1% HCOOH and solvent C was ACN+0.1% HCOOH. Silica gel 60 (230—400 mesh, Silicycle), Diaion HP-20 (Supelco) and C18 silica gel (230—400 mesh, EMD) were used for column chromatography. The solvents were purchased from EMD. Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} aluminium sheets (Silicycle) using EtOAc–MeOH–H_2O (100 : 16.5 : 13.5) or $CHCl_3$–MeOH (10 : 1) as solvent systems. Detection of the phenolic compounds was carried out by spraying natural products with NP/PEG reagent (1%



3 R^1 = caffeoyl, R^2 = R^3 = H
4 R^1 = R^2 = H, R^3 = caffeoyl
5 R^1 = R^2 = caffeoyl, R^3 = H
6 R^1 = R^3 = caffeoyl, R^2 = H
7 R^1 = H, R^2 = R^3 = caffeoyl

Fig. 1. Structures of Compounds **1**—**7**

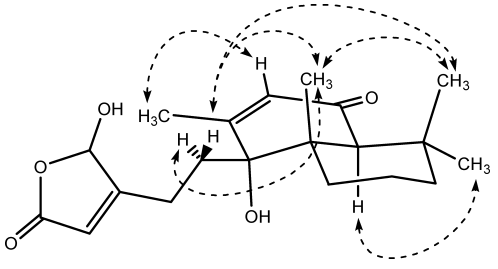


Fig. 2. ^{1}H–^{1}H NOESY Correlations of Compound **1**

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diphenylboric acid 2-aminoethylester in methanol and 5% polyethylene glycol in ethanol) and observing under UV light before and after revelation (254, 365 nm). Quercetin and chlorogenic acid standards are from Sigma-Aldrich.

Plant Material The flowers of *Solidago canadensis* L. were collected in September 2004 in St-Félicien (48°40′N, 72°27′W), Québec, Canada. The specimens were identified by Mr. Patrick Nadeau (Département des Sciences Fondamentales, Université du Québec à Chicoutimi, Québec, Canada). A voucher specimen was deposited in the Louis-Marie Herbarium of Université Laval, Québec, Canada (QFA-492115).

Extraction and Isolation The air-dried flowers of *Solidago canadensis* (285 g) were extracted with hot methanol (3×2.5 l) and then with hot methanol 80% (3×2.5 l) for 2 h. The combined extracts were evaporated under a reduced pressure, yielding a residue (103.81 g), which was suspended in water (400 ml) and successively extracted with dichloromethane (3×200 ml) and ethyl acetate (6×400 ml), yielding dichloromethane, ethyl acetate and aqueous fractions.

The ethyl acetate fraction (15.6 g) was separated into two fractions (A, B) by chromatography over Diaion (3.2×40.5 cm) eluting with MeOH–H_2O (from 50 to 100%). Fraction B (3.0 g) was chromatographed on a silica gel column (3.6×120.0 cm) with $CHCl_3$–MeOH (10 : 1) to obtain six fractions (B1—B6). From B1 (91.4 mg), compound **1** (5.4 mg) was purified by preparative HPLC (40% solvent A, 60% solvent B). Fractions B5 and B6 were combined and then fractionated on a silica gel column (3.0×45.0 cm) with $CHCl_3$–MeOH (10 : 1) to give compound **2** (53.6 mg).

The aqueous fraction (66.13 g) was separated into three fractions (C, D, E) by chromatography on a silica gel column (7.0×60.0 cm) using an isocratic solvent system of EtOAc–MeOH–H_2O (100 : 16.5 : 13.5). Fraction D (6.70 g), which contained the major constituents, was divided into six fractions (D1—D6) using Diaion (3.2×40.5 cm) by elution with MeOH 20%. Fraction D2 (1.03 g) was separated into nine fractions (D2A—D2I) by chromatography on a C18 silica gel column (1.6×46.0 cm) and eluted with MeOH 10%. From D2A (214.2 mg), compounds **3** (3.8 mg) and **4** (9.3 mg) were purified by preparative HPLC (94% solvent A, 6% solvent C). Fractions D2E (135.1 mg) and D2F (64.9 mg) were combined and then, fractionated by preparative HPLC (84% solvent A, 16% solvent C) to yield compounds **5** (9.1 mg), **6** (7.0 mg) and **7** (11.4 mg).

Solicanolide (**1**): Yellow oil; $[\alpha]_D^{25}$ −30.1° (c=0.1, MeOH); UV λ_{max} (MeOH) 216 nm; HR-ESI-MS m/z 371.1834 $[M+Na]^+$ (Calcd for $C_{20}H_{28}O_5Na$, 371.1842); ^{1}H- and ^{13}C-NMR: see Table 1.

Cell Culture The human lung carcinoma A549 (#CCL-185), colorectal adenocarcinoma DLD-1 (#CCl-221) and skin fibroblast WS1 (#CRL-1502) cell lines were all obtained from the American Type Culture Collection (ATCC, Manassas, U.S.A.). Cells lines were grown in minimum essential medium containing Earle's salts (Mediatech Cellgro®, Herndon, U.S.A.), supplemented with 10% fetal calf serum (Hyclone, Logan, U.S.A.), 1×solution of vitamins, 1×sodium pyruvate, 1×non-essential amino acids, 100 IU of penicillin and 100 μg/ml of streptomycin (Mediatech Cellgro®). Cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2.

Cytotoxic Assay Exponentially growing cells were plated at a density of 5×10^3 cells per well in 96-well microplates (BD Falcon) in 100 μl of culture medium and were allowed to adhere for 16 h before treatment. The cells were then incubated for 48 h in the presence or absence of 100 μl of increasing concentrations of compounds **1**—**7** dissolved in culture medium and an appropriate solvent. The final concentration of solvent in the culture medium was maintained at 0.25% (v/v) to avoid toxicity. Cytotoxicity was assessed using the resazurin reduction test.[13] Fluorescence was measured on an automated 96-well Fluoroskan Ascent FI™ plate reader (Labsystems) using an excitation wavelength of 530 nm and an emission wavelength of 590 nm. Cytotoxicity was expressed as the concentration of drug inhibiting cell growth by 50% (IC_{50}).

Results and Discussion

Compound **1** was obtained as an optical active yellow oil ($[\alpha]_D^{25}$ −30.1°). The molecular formula of compound **1** was established as $C_{20}H_{28}O_5$ on the basis of HR-ESI-MS (Found at m/z 371.1834 $[M+Na]^+$, Calcd for $C_{20}H_{28}O_5Na$, 371.1842). ^{1}H-, ^{13}C-NMR and DEPT spectra revealed four methyls, five methylenes, four methines including two olefinics and seven quaternary carbons including a carbonyl and a quaternary alcohol (Table 1). The NMR spectra suggests that compound **1** is a labdane diterpene.[14,15]

Table 1. NMR Assignments of Compound **1**

Position	^{13}C[a]	Connected ^{1}H[b]	H–H COSY[c]	HMBC[d]	NOESY[e]
1	32.6 (t)	1.92 (m), 1.57 (m)		H5, 20	
2	19.0 (t)	1.66 (m), 1.53 (m)	H3		
3	43.9 (t)	1.35 (m), 1.19 (m)	H2	H18, 19	
4	33.3 (s)	—		H5, 18, 19	
5	56.8 (d)	2.88 (s)	H20	H18, 19, 20	H18
6	202.9 (s)	—		H5	
7	129.6 (d)	5.66 (s)	H17	H17	H17
8	159.1 (s)	—		H17	
9	76.7 (s)	—		H5, 7, 17, 20	
10	48.1 (s)	—		H5, 20	
11	31.0 (t)	2.11 (m), 1.94 (m)			H14, 19, 20
12	25.4 (t)	2.65 (m)			
13	172.8 (s)	—			H14, 16
14	117.6 (d)	5.95 (s)		H16	H12, 14
15	173.7 (s)	—		H14, 16	
16	101.1 (d)	6.06 (s)		H14	H12
17	20.5 (q)	2.02 (s)	H7	H7	H7
18	34.3 (q)	1.14 (s)		H19	H5
19	22.2 (q)	1.18 (s)		H3, 5, 18	H11, 20
20	18.8 (q)	1.02 (s)	H5	H5	H11, 19

a) Multiplicities were determined by DEPT. *b*) Connections were determined by gHSQC. *c*) Determined by gCOSY. *d*) Correlation from C to the indicated protons. *e*) unambiguous NOESY cross peaks.

HMBC correlations between H-17 (δ_H 2.02) and C-7, C-8, C-9 (δ_C 129.6, 159.1, 76.7 respectively) showed that the methyl group was branched β on an α,β-insaturated ketone and that the quaternary alcohol group was on C-9. The position of the ketone was further confirmed by the HMBC correlation between H-5 (δ_H 2.88) and C-6 (δ_C 202.9). The side chain was identified as a γ-hydroxy-α,β-unsaturated-γ-lactone by the presence of typical[16] ^{1}H (δ_H 5.95, 6.06) and ^{13}C-NMR signals (δ_C 101.1, 117.6, 172.8, 173.7).

The relative stereochemistry of compound **1** was partially determined from the 2D NOESY spectrum. Cross-peaks between H-11 (δ_H 1.94, 2.11), H-19 (δ_H 1.18) and H-20 (δ_H 1.02) showed that all these groups are positioned on the same side. Unfortunately, NOESY correlations between H-16 (δ_H 6.06) and both H-11 (δ_H 1.94, 2.11) were observed with the same intensity which prevents the determination of the stereochemistry of the hydroxyl function at C-16. However, while some authors observed both epimers at H-16 of related compounds,[17,18] its single signal in the ^{1}H- and ^{13}C-NMR spectra suggests that only one epimer was isolated.

The determination of the absolute stereochemistry by the Mosher ester method was precluded by the limited amount of this natural product. However, the chemotaxonomic information indicated that all labdanes from *Solidago* genus belong to the normal series[5,19,20] and by analogy, compound **1** could be assigned to this series. Therefore, the structure of compound **1** was identified as 9α,16ξ-dihydroxy-6-oxo-7,13-labdadien-15,16-olide.

The other compounds isolated were identified as quercetin (**2**), 3-*O*-caffeoylquinic acid (**3**, neochlorogenic acid), 5-*O*-caffeoylquinic acid (**4**, chlorogenic acid), 4,5-di-*O*-caffeoylquinic acid (**5**), 3,5-di-*O*-caffeoylquinic acid (**6**) and 3,4-di-*O*-caffeoylquinic acid (**7**). Identification of these compounds was established by NMR (^{1}H, ^{13}C, DEPT, COSY, HSQC and HMBC) and HR-ESI-MS analyses. The data ob-

Table 2. Cytotoxic Activity of Isolated Compounds against A549 Lung Carcinoma Cells, DLD-1 Colon Carcinoma Cells and Normal Fibroblasts, WS1

Compound	IC_{50} (μM)		
	A549	DLD-1	WS1
Etoposide	1.1±0.1	4.8±0.8	n.d.
5-Fluorouracil	4.8±0.6	11±2	n.d.
1	13±2	26±2	17±1
2	>199	>199	>199
3	>57.5	>57.5	>57.5
4	>56	>56	>56
5	>109	>109	>109
6	>107	>107	>107
7	>36.8	>36.8	>36.8

Data are expressed as means±standard deviations of three determinations; n.d.=not determined.

tained were compared with those reported in the literature[21,22] and standards. To our knowledge, this is the first report of the isolation of compound **7** from *Solidago canadensis*.

All isolated compounds were evaluated for their *in vitro* cytotoxic activity against human lung cancer A549, colon cancer DLD-1 and normal fibroblasts, WS1 cell lines. Etoposide and 5-fluorouracil was used as positive controls. As presented in Table 2, only compound **1** was found to be active against A549 (IC_{50}: 13±2 μM), DLD-1 (IC_{50}: 26±2 μM) and WS1 (IC_{50}: 17±1 μM) cell lines.

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