Langaside, a novel secoiridolactone glycoside derivative from *Tachiadenus longiflorus* Griseb. (Gentianaceae) formed by a [2+2] cycloaddition reaction.

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Abstract

Langaside (**1**), a secoiridoid lactone glucoside possessing a novel skeleton formed by a [2+2] cycloaddition reaction between the secoiridolactone glucoside, 1,9-*trans*-9,5-*cis*-sweroside, and *p*-coumaric acid was isolated from the fruits and flowers of the Malagasy *Tachiadenus longiflorus* Griseb. (Gentianaceae), alongside another seven known compounds. The structure of langaside was established using HRESIMS, IR and NMR spectroscopy and comparison of experimental and calculated electronic circular dichroism (ECD) spectra. Langaside was screened for its neuritogenic activity against SHSY-5Y cells and anticancer activity against the NCI59 human tumour cell panel but not found to be active.

Keywords

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This paper forms part of a special issue of Phytochemistry Letters dedicated to the memory of Andrew Marston (1953-2013), an outstanding phytochemist who is much missed by his friends.

Tachiadenus longiflorus, langaside, secoiridolactone, sweroside, gentiopicrin, SHSY-5Y cells.

1. Introduction

The suffrutescent plant *Tachiadenus longiflorus* Griseb. (Gentianaceae) occurs in the foothills and the highland areas of Madagascar, with all eleven species comprising the genus *Tachiadenus* endemic to Madagascar (Leistner, 2000). It is locally called felampelana or tsifelampelana, meaning beautiful flower, and is easily recognized, even from a distance, by the white flowers that contrast with surrounding plants almost all the year around. The fruit is a one-celled capsule filled with numerous minute seeds (Klackenberg, 1990). In Madagascar, *T. longiflorus* is currently used by the Bezanozano, Sihanaka and Merina tribes as a purgative and to treat fever and fatigue (Randrianarivelojosia, et al., 2006, Yuan et al., 2003). The stem of *T. longiflorus* has been investigated previously and yielded scoparone, scopoletin (Del Rio et al., 2004, Heitz et al., 1979), oleanolic acid (Heitz et al., 1979), oleanolic acid methyl ester (Mulholland et al., 2006), diosmin, diosmetin (Del Rio et al., 2004, Heitz et al., 1979), and the iridoid, angelone (Mulholland et al., 2006). Attempts to isolate more angelone from the fruits and flowers of *T. longiflorus* led to the isolation of langaside (**1**) and seven known compounds, 1,9-*trans*-9,5-*cis*-sweroside (**2**), gentiopicrin (**3**), scopolin, scoparone, scopoletin, oleanolic acid and lupeol.

2. Results and discussion

Langaside (**1**), (Figure 1) was isolated as a white solid and HR-ESIMS showed a quasi $[M-H]^+$ ion peak at *m/z* 503.1557 (cald. 503.1553), indicating a molecular formula of $C_{25}H_{28}O_{11}$ and 12 degrees of unsaturation. The IR spectrum indicated the presence of hydroxyl (3400 cm^{-1}) and lactone carbonyl (1736 cm^{-1}) stretches. The ^1H, ^{13}C and DEPT NMR spectra indicated the presence of two ester carbonyl carbons, an aromatic ring and a monosubstituted alkene group, leaving five further rings in the molecule. The NMR spectra showed similarities to those of the co-isolated 1,9-*trans*-9,5-*cis*-sweroside (**2**), but were more complex and lacked resonances due to the Δ^3-double bond. Resonances due to carbons of a glucopyranosyl group occurred at δ_C 100.9 (C-1'), 80.7 (C-2'), 74.7 (C-3'), 72.1 (C-4'), 79.9 (C-5') and 62.8 (C-6') in the ^{13}C NMR spectrum and the coupling constant of the anomeric proton (δ_H 4.77, d, *J*=8.2 Hz, H-1') and correlations seen in the NOESY

spectrum between H-1'/H-5', H-5'/H-3' and H-2'/H-4', H-4'/H-6' confirmed the presence of β-D-glucose (Bruch, 1996).

The sugar was joined to the iridoid structure at C-1' as in the known sweroside, confirmed by a correlation seen in the HMBC spectrum between the H-1'and C-1 (δ_C 101.1) resonances. HMBC correlations were seen between the corresponding H-1 (δ_H 5.02 *J*=1.3 Hz) and C-3 (δ_C 67.9), C-5 (δ_C 31.4), C-8 (δ_C 134.0) and C-1' (δ_C 100.9) resonances. The ^{1}H-^{1}H COSY spectrum showed coupling between H-1/ H-9, H-9/ H-8, H-8/H-10$_{cis}$, H-8/H-10$_{trans}$ and H-10$_{cis}$/ H-10$_{trans}$ and between H-9/H-5, H-5/H-6α/H-6β and H-6α/H-6β/H-7α/H-7β resonances (Figure 2). Correlations were seen in the HMBC spectrum between H-7/C-11 (δ_C 174.7), H-3/C-11, H-5/C-11 and between H-3 and H-9 and a fully substituted C-4 (52.8 C) carbon resonance. Langaside has nine extra carbons than sweroside, with the extra carbons assigned to a *p*-coumaric acid unit This was indicated by a pair of two-proton doublets (δ_H 7.13, *J*=8.5 Hz, H-5"/H-9", 6.73, *J*=8.5 Hz, H-6"/H-8"), a pair of *trans*-coupled methine proton resonances (H-3'', δ_H 4.21 d *J* = 4.2 Hz, H-2'', δ_H 3.63 dd *J* = 4.2, 11.0 Hz,) and a carbonyl carbon resonance at δ_C 176.3 (C-1''). The COSY spectrum showed coupling between the H-2'' and H-3 resonances and HMBC correlations between the H-3/C-1'', H-5/C-3'' H-3''/C-3, H-3/C-1'' and H-2'/C-1'' resonances showed the *p*-coumarate unit was joined both to the sweroside unit and β-D-glucose unit and supported the cyclobutane ring structure. Compound **1** was acetylated to give the tetra-acetate derivative (Table 1).

The relative configuration of **1** was assigned using its NOESY spectrum and, for confirmation, the NOESY spectrum of the acetylated derivative, which showed correlations between H-3/H-1, H-3/H-2", H-3/H-9'', H-3/H-8, H-1/H-8, H-1/H-1', H-1'/H-3' and H-1'/H-5' resonances in the NOESY spectrum, indicating that H-1, H-3, H-8, H-1', H-3' and H-5' were on the α-face of the molecule. Further correlations between H-5/H-3'', H-5/H$_β$-6, H-5/H-9, H-3"/H-9", H-3"/H-5", H-3"/H-2', H-2'/H-4' and H-4'/H$_b$-6' resonances suggested that H-2', H-4', H-5, H$_β$-6, H-3" and H-9 were on the β face of **1**. The absolute configuration of **1** was determined by comparing the calculated and experimental ECD curves. Conformational searches were done on two possible isomers of langaside (1*R*, 3*R*,

4*R*, 5*S*, 9*R*, 1'*S*, 2'*S*, 3'*R*, 4'*R*, 5'*R*, 2"*R* and 3"), **1,** and its enantiomer *ent*-langaside (1*S*, 3*S*, 4*S*, 5*R*, 9*S*, 1'*R*, 2'*R*, 3'*S*, 4'*S*, 5'*S*, 2"*S* and 3"*S*), (**1a**), that were consistent with correlations seen in the NOESY spectrum. An initial conformational search of **1** using Spartan10 at ground state with a molecular mechanics force field (MMFF) basis set gave ten conformers, three of which were under 2 kcal/mol. The three conformers were subjected to TDDFT calculations using a B3LYP method at 6-31G (d, f) level in Gaussian09 software (Frisch et al., 2010). The resulting ECD curves of the three conformers were Boltzmann weighted and compared to the experimental ECD curve for **1** (Figure 4). This was also done for *ent*-langaside and and yielded 10 conformers, 4 of which were under 2 kcal/mol and were subjected to TDDFT calculations using the same basis set used for **1** to give ECD curves that were Boltzmann weighted. By comparing the experimental ECD curve and the calculated ECD curves for **1** and **1a,** the absolute configuration could be unambiguously assigned as 1*R*, 3*R*, 4*R*, 5*S*, 9*R*, 1'*S*, 2'*S*, 3'*R*, 4'*R*, 5'*R*, 2"*R* and 3"*R*.

It is proposed that langaside is formed by an intramolecular [2+2]-cycloaddition reaction between the Δ^2-double bond of *p*-coumaric acid and the Δ^3-double bond of sweroside (**2**), which was co-isolated from the plant along with the related gentiopicrin (**3**). Although many secoiridoid compounds are known, langaside (**1**) is the first derivative of this type to be reported. Littoralisone, a compound formed by [2+2] cycloaddition between the iridolactone (-) brasoside and *p*-coumaric acid has been shown to elicit a 30% enhancement of NGF-mediated neurite outgrowth from PC12D cells (Li et al., 2001). Thus, the ability of **1** to potentiate the activity of nerve growth factor (NGF) in the neuroblastoma cell line SHSY-5Y cells was assessed. However, no significant potentiation of NGF-mediated neurite outgrowth was found at the two concentrations tested, 0.78µg/ml and 50µg/ml with NGF (100ng/ml, 50ng/ml, 25ng/ml, 1ng/ml and 0ng/ml). Compounds **1**, **2** and **3** were submitted to the U.S. National Cancer Institute for screening against the NCI 59 human tumour cell lines panel for potential anti-cancer efficacy (Shoemaker, 2006). All three compounds were found to be inactive at the single concentration of 10 µM against 59 human tumour cell lines (data not shown).

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a JASCO P-1020 polarimeter. The ECD spectrum for compound **1** was determined using an Applied Photophysics Chirascan CD spectrometer using a 1 mm cell in acetonitrile. FTIR spectra were recorded using a Perkin-Elmer (2000) spectrometer. 1D and 2D NMR spectra were recorded in MeOD on a 500 MHz Bruker AVANCE NMR instrument at room temperature. Chemical shifts (δ) are expressed in ppm and were referenced against the solvent resonances at 4.87 and 49.15 ppm for ^{1}H and ^{13}C NMR spectra respectively. HR-ESI mass spectra were recorded on a Bruker MicroToF mass spectrometer using an Agilent 1100 HPLC to introduce samples (University of Oxford). Column chromatography was undertaken using 1 or 4 cm diameter columns suitable to be used as flash systems packed with silica gel (Merck 9385 grade). TLC analysis was performed on aluminium-backed precoated silica gel plates (Alugram$^{(R)}$ SIL G/UV_{254}) visualised using anisaldehyde spray reagent.

3.2 Plant material

Tachiadenus longiflorus (Pall.) Regel (Gentianaceae) was collected at Mangoro, in the eastern foothill area of Madagascar and a voucher specimen (002MJ/MDUL 2005-TAN) was retained at the Botany Department of the University of Antananarivo and also at the National Botanical Garden Tsimbazaza.

3.3 Extraction and isolation

The dried, powdered fruits and flowers (232 g) of *T. longiflorus* were extracted using hexane, CH_2Cl_2, EtOAc and MeOH successively in a Soxhlet apparatus to yield 5.5 g, 9.8 g, 6.5 g and 46.3 g of extract respectively. Thin layer chromatographic (TLC) analysis and column chromatography (CC) over silica gel (Merck 9385) with a hexane/ CH_2Cl_2 step gradient starting with 100% hexane and gradually increasing the CH_2Cl_2 concentration to 100% followed by 5% MeOH in CH_2Cl_2 was used to separate the non-polar/semi polar (CH_2Cl_2) crude extracts to yield fractions (75 ml each) which were combined depending on TLC analysis. The polar (EtOAc and MeOH) combined extracts were eluted using flash CC and a step gradient of CH_2Cl_2 and MeOH to yield fractions (75 ml each) which were combined based on TLC analysis. Fractions from the combined EtOAc and MeOH extracts were purified to yield the following: Fractions 1-41 gave a mixture of triglycerides and

fatty acids that were not further investigated. Fractions 41 – 82 eluted using 5% MeOH/CH_2Cl_2 gave langaside (**1**, 5% MeOH/CH_2Cl_2, 210 mg), sweroside (**2**, 100% EtOAc, 120 mg) (Takeda et al., 1999), gentiopicrin (**3**, 100% EtOAc, 150 mg) (Takeda et al., 1999) and scopolin (5% MeOH/CH_2Cl_2, 15 mg) (Kisiel and Michalska, 2002). From the CH_2Cl_2extract: Fraction 15-18 gave scoparone (100% CH_2Cl_2, 25 mg) (Razdan et al., 1987), fraction 19-20 eluted using 100% CH_2Cl_2 gave scopoletin (**6**, 100% CH_2Cl_2, 15 mg) (Imai et al., 1989), fraction 5-13 eluted using 1:1 hexane:methylene chloride gave oleanolic acid (**7**, 10% EtOAc/hexane, 70 mg) (Ahmad and Rahman, 1994) and lupeol (**8**, 10% EtOAc/hexane, 20 mg) (Mulholland et al., 2006).

3.3.1 Langaside (**1**) white solid, m.p. 150-152, $[\alpha]^{26}_D$ - 45.8 (*c* 0.5, MeOH), IR (neat) ν_{max} 3400, 2921, 2850, broad 1736, 1633, 1460, 1377, 1270, 1072cm^{-1}, for ^{1}H and ^{13}C NMR see Table 1, CD (CH_3CN, *c* 0.009M, 0.1 cm path length): 198 ($\Delta\varepsilon$ + 3.0), 216 ($\Delta\varepsilon$ -10.0), 227 ($\Delta\varepsilon$ -7.1) and 280 nm ($\Delta\varepsilon$ - 1.5), HR-ESIMS *m/z* 503.1557 $[M-H]^+$ (calcd. for $C_{25}H_{27}O_{11}$, 503.1553)

3.4 Computational methods

The conformational search was carried out for langaside (**1**) and *ent*-langaside (**1a**) at the molecular mechanics level of theory employing MMFF force field basis set incorporated in Spartan10 (Wavefunction, Irvine, CA) software package. Conformers under 2 kcal/mol were selected for TDDFT calculations. The selected conformers were subjected to TDDFT calculations using a B3LYP method at 6-31G (d,f) level built into Gaussian09 software (Frisch et al., 2010). The ECD curves were Boltzmann weighted and compared.

3.5 Biological assays

3.5.1 Neuritogenic activity against human derived neuroblastoma cell line (SHSY-5Y)

The SHSY-5Y cells were maintained in Dulbecco's modified eagle medium (DMEM) supplemented with 10% (*v/v*) foetal calf serum, 1% (*v/v*) penicillin/streptomycin and non-essential amino acids at 37°C in a humidified 5% CO_2/95% air environment. For the neuritogenic assay, cells were plated in 24 well plates at a density of 1×10^4 cells/well, and incubated at 37^0C in a humidified 5% CO_2/95% air environment for 24 hours to allow the

cells to attach. Next, DMEM was aspirated and medium containing NGF (100ng/ml, 50ng/ml, 25ng/ml, 1ng/ml, 0 ng/ml) and langaside (**1**) (0.78µg/ml, 50µg/ml) was added and incubated for 24 hours under the same conditions. Following compound exposure, neurite growth was determined: For each of treatment condition, three wells were used and light micrographs (x100 magnification) from three random fields captures. Ten differentiated cells with neurites were identified in each field, and the neurite length in micrometres determined using ImageJ (available from http://rsbweb.nih.gof/ij/inex.html). All values were expressed as the mean ± SEM.. One way ANOVA followed by the Dunnett test and two way ANOVA was performed and *p*-values of < 0.05 were considered significant.

3.5.1 Neuritogenic activity against human derived neuroblastoma cell line (SHSY-5Y)

The SHSY-5Y cells were maintained in T75 tissue flasks in Dulbecco's modified eagle medium (DMEM) and supplemented with 10% foetal calf serum, a mixture of 1% penicillin/streptomycin and non-essential amino acids at 37°C in a humid 5% CO_2/95% air environment. The neuritogenic assay was done by detaching SHSY-5Y cells from the tissue T75 flasks using trypsin and resuspending them in fresh DMEM. The cells were placed in a 24 well plate in 100µl of medium at a density of 1×10^4 cells/well, 900µl of fresh DMEM was added to all the wells and the plate was incubated at 37^0C in a humid 5% CO_2/95% air environment for 24 hours to allow the cells to attach to the wells. The DMEM was aspirated and medium containing NGF (100ng/ml, 50ng/ml, 25ng/ml, 1ng/ml, 0 ng/ml) and langaside (**1**) (0.78µg/ml, 50µg/ml) was added and incubated under the same conditions. Images used for determining neurite growths were photographed after 24 hours using an inverted microscope (×100 magnification) and manual measurements of the length of the neurites were carried out using ImageJ software (available from http://rsbweb.nih.gof/ij/inex.html). Treatments were performed in triplicate and 3 fields were obtained per well. Ten differentiated cells with neurites were identified, labelled and the neurites were measured in micrometres. All values were expressed as the mean ± SEM. Data were analysed using Graph pad prism software. One way ANOVA followed by the Dunnett test and two way ANOVA was performed and *p*-values of < 0.05 were considered significant.

3.5.2 Anticancer activity against NCI 59 human tumour cell lines

Compounds **1**, **2** and **3** were submitted to the NCI and evaluated at the single concentration of 10 µM against 59 human tumour cell lines according to the NCI protocol (Shoemaker, 2006).

Acknowledgements

This research was funded by the University of Surrey through a PhD studentship for ML and the South African National Research Foundation (NRF). Cytotoxic assays were performed by the NCI.

References

Ahmad, V. U., Rahman, A. U. 1994. Handbook of Natural Products Data, Pentacyclic Triterpenoids, 2, Elsevier, Amsterdam, Netherlands.

Bruch, M. D. 1996. *NMR Spectroscopy Techniques*. 2nd Ed. Mercel Dekker, Inc: New York.

Chiba, K., Yamazaki, M., Kikuchi, M., Kakuda, R., Kikuchi, M. 2010. New physiological function of secoiridoids: neuritogenic activity in PC12h cells. J. Nat. Med. 65, 186-190

Del Rio, J.A., Fuster, M.D., Gomez, P., Porras, I., Garcia-Lidon, A., Ortuno, A. 2004. Citrus limon: a source of flavonoids of pharmaceutical interest. Food Chem. 84, 457-461

Dewick, P.M. 2002. Medicinal Natural Products, A Biosynthetic Approach, 2nd edition. John Wiley and Sons, Chichester, England. P. 189-190

Frisch, M.J., Trucks, G.W., Schlegel, H.B., Scuseria, G.E., Robb, M.A., Cheeseman, J.R., Scalmani, G., Barone, V., Mennucci, B., Petersson, G.A., Nakatsuji, H., Caricato, M., Li, X., Hratchian, H.P., Izmaylov, A.F., Bloino, J., Zheng, G., Sonnenberg, J.L., Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai, H., Vreven, T., Montgomery, Jr. J. A., Peralta, J. E., Ogliaro, F., Bearpark, M., Heyd, J.J., Brothers, E., Kudin, K.N., Staroverov, V.N., Keith, T., Kobayashi, R., Normand, J., Raghavachari, K., Rendell, A., Burant, J.C., Iyengar, S.S., Tomasi, J., Cossi, M., Rega, N., Millam, J.M., Klene, M., Knox, J.E., Cross, J.B., Bakken, V., Adamo, C., Jaramillo, J., Gomperts, R., Stratmann, R.E., Yazyev, O., Austin, A.J., Cammi, R., Pomelli, C., Ochterski, J.W., Martin, R.L., Morokuma, K., Zakrzewski, V.G., Voth, G.A., Salvador, P., Dannenberg, J.J., Dapprich, S., Daniels, A.D., Farkas, O., Foresman, J.B., Ortiz, J.V., Cioslowski, J., Fox, D. J., 2010. Gaussian, Inc., Wallingford CT, Gaussian 09, Revision B.01.

Ganesan, A. 2008. The impact of natural products upon modern drug discovery. Cur. Opin. Chem. Biol. 12, 306 – 317

Heitz, S., Durgeat, M., Billet, D. 1979. Constituents of *Tachiadenus longiflorus*. Bull. Mus. Natl. Hist. Nat. Sect. B. 1, 65-66.

Imai, F., Itoh, K., Kishibuchi, N., Kinoshita, T., Sankawa, U. 1989. Constituents of the root bark of *Murraya paniculata* collected in Indonesia. Chem. Pharm. Bull. 37, 119-123

Klackenberg J. 1990. *Flore de Madagascar et des Comores. Famille 168 – Gentianacées*. Musée National d'Histoire Naturelle, Paris.

Kisiel, W., Michalska, K. 2002. A new coumarin glucoside ester from *Cichorium intybus*. Fitoterapia. 73, 544-546

Leistner, O.A. 2000. *Seed plants of Southern Africa: Families and Genera*, Strelitzia 10, National Botanical Institute, Pretoria.

Li, Y, S., Matsunaga, K., Ishibashi, M., Ohizumi, Y. 2001. Littoralisone, a novel neuritogenic iridolactone having an unprecedented heptacyclic skeleton including four- and nine-membered rings consisting of glucose from *Verbena littoralis.* J. Org. Chem. 66, 2165 – 2167.

Mulholland, D. A., Langlois, A., Randrianarivelojosia, M., Derat, E., Nuzillard, J.M. 2006. The structural elucidation of a novel iridoid derivative from *Tachiadenus longiflorus* (Gentianaceae) using the LSD programme and quantum chemical computations. Phytochem. Anal. 17, 87-90.

Qi, J., Ojika, M., Sakagami, Y. 1999. Differentiation in a rat PC12 cell line induced by ostruthin and (-)-bornyl ferulate, constituents of a Chinese herbal medicine. Biosci. Biotechnol. Biochem. 63, 1501-1502

Randrianarivelojosia, M., Langlois, A., Mulholland, D.A. 2006. Investigations of the Malagasy species *Tachiadenus longiflorus* Grisebach (Gentianaceae): linking chemical finding and traditional usage. J. Ethnopharmacol. 105, 456-458.

Shoemaker, R.H. 2006. The NCI60 human tumour cell line anticancer drug screen. Nat. Rev. Cancer. 6, 813-823

Takeda, Y., Masuda, T., Honda, G., Takaishi, Y., Ito, M., Ashurmetov, O. A., Khodzhimatov, O. K., Otsuka, H. 1999. Secoiridoid glycosides from *Gentiana olivieri*. Chem. Pharm. Bull. 47, 1338-1340

Waight, ES., Razdan, T.K., Qadri, B., Harkar, S. 1987. Chromones and coumarins from *Skimmia laureola*. Phytochem. 26, 2063-2069

Wang, Z.J., Nie, B. M., Chen, H.Z., LU, Y. 2006. Panaxynol induces neurite outgrowth in PC12D cells via cAMP- and MAP kinase-dependent mechanisms. Chem. Biol. Inter. 159, 58-64

Yuan, Y., Wohlhauser, S., Moller, M., Chassot, P., Mansion, G., Grant, J., Kupfer, P., Klackenberg, J. 2003. Monophyly and relationships of the tribe Exaceae (Gentianaceae) inferred from nuclear ribosomal and chloroplast DNA sequences. Mol. Phylogenet. Evol. *28*, 500–517.

Table 1. ^{1}H and ^{13}C NMR data of 1 (in MeOD) and its tetra-acetate derivative (in $CDCl_3$)[a]

	1		**1Ac**	
No.	δ_C, Carbon type	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)
1	101.1 CH	5.02 (d, 1.3)	101.1 CH	5.05 (br s $W_{1/2}$3.7)
3	67.9 CH	5.67 (d, 11.0)	66.7 CH	5.73 (d, 11.2)
4	52.8 C	-	51.3 C	-
5	31.4 CH	3.31 m	30.1 CH	3.30 m
6α	25.7 CH_2	2.21 m	24.6 CH_2	1.89 m
6β		1.85 m		2.14 m
7α	66.6 CH_2	4.10 m	65.0 CH_2	4.09 m
7β		4.23 m		4.09 m
8	134.0 CH	5.54 m	131.8 CH	5.56 m
9	47.9 CH	2.43 (ddd, 1.3, 4.0, 10.4)	46.3 CH	2.50 (dd, 3.5, 10.3)
10_{cis}	122.6 CH_2	5.25 (dd, 10.4, 1.8)	123.0 CH_2	5.30 (dd, 10.7, 1.0)
10_{trans}		5.21 (dd, 17.0, 1.8)		5.20 (dd, 17.0, 1.0)
11	174.7 C	-	171.3 C	-
1’	100.9 CH	4.77 (d, 8.2)	99.3 CH	4.82 (d, 8.2)
2’	80.7 CH	4.82 m	76.1 CH	5.15 m
3’	74.7 CH	3.70 m	71.1 CH	5.41 (t, 9.6)
4’	72.1 CH	3.39 m	68.5 CH	5.15 m
5’	79.9 CH	3.38 m	73.6 CH	3.77 (ddd, 2.1, 10.5, 4.3)
6’a	62.8 CH_2	3.89 (dd, 2.0, 11.0)	61.9 CH_2	4.26 (dd, 4.3, 12.4)
6’b		3.69 m		4.15 m
1’’	176.3 C	-	174.1 C	-
2’’	52.3 CH	3.63 (dd, 4.2, 11.0)	51.3 CH	3.57 (dd, 4.0, 11.2)
3’’	49.8 CH	4.21 (d, 4.2)	48.5 CH	4.21 (d, 4.0)
4’’	128.9 C	-	133.8 C	-
5’’/9’’	130.9 CH	7.13 (d, 8.5)	129.5 CH	7.21 (d, 8.5)
6’’/8’’	117.2 CH	6.73 (d, 8.5)	122.4 CH	7.05 (d, 8.5)
7’’	158.5	-	150.6 C	-
Ac-3’	-	-	170.2 C, 20.7 CH_3	2.02 s
Ac-4’	-	-	169.7 C, 20.8 CH_3	2.04 s
Ac-6’	-	-	170.8 C, 21.3 CH_3	2.09 s
Ac-7”	-	-	169.3 C, 24.6 CH_3	2.26 s

[a]Recorded at 500 MHz (^{1}H NMR) or 125 MHz (^{13}C NMR). *J* in Hz.

Figure 1. Chemical structures of langaside (**1**), sweroside (**2**) and gentiopicrin (**3**)

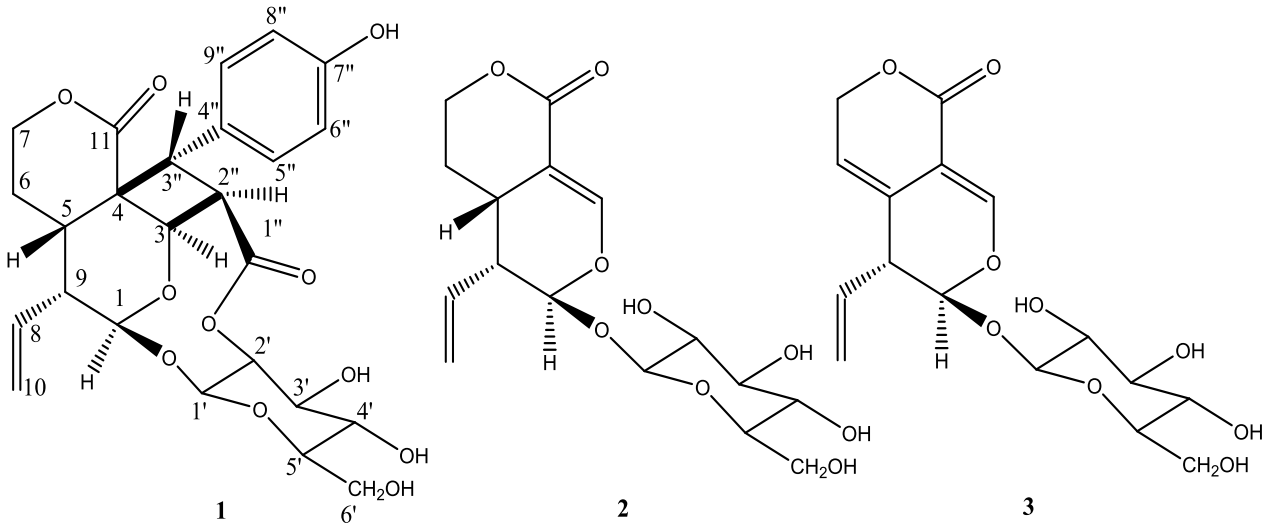


Figure 2. Selected ^{1}H-^{1}H COSY and key HMBC correlations for 1

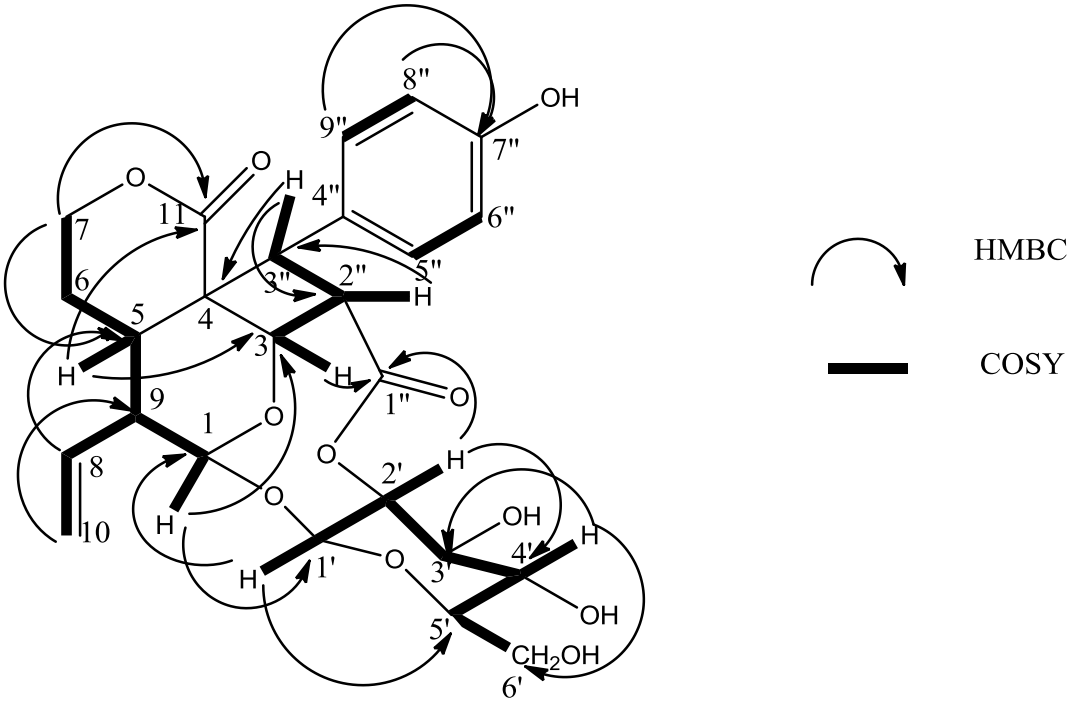


Figure 3. Key NOESY correlations of **1**

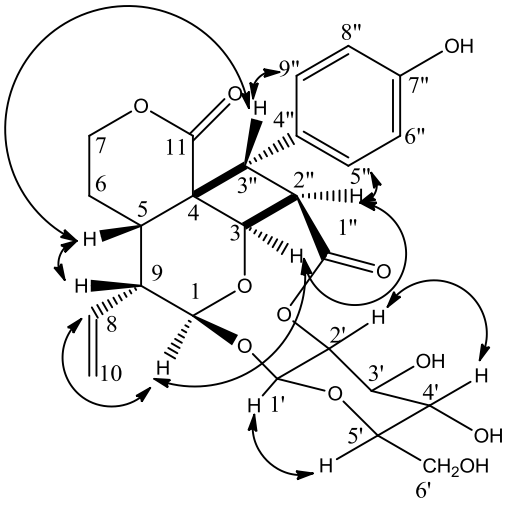
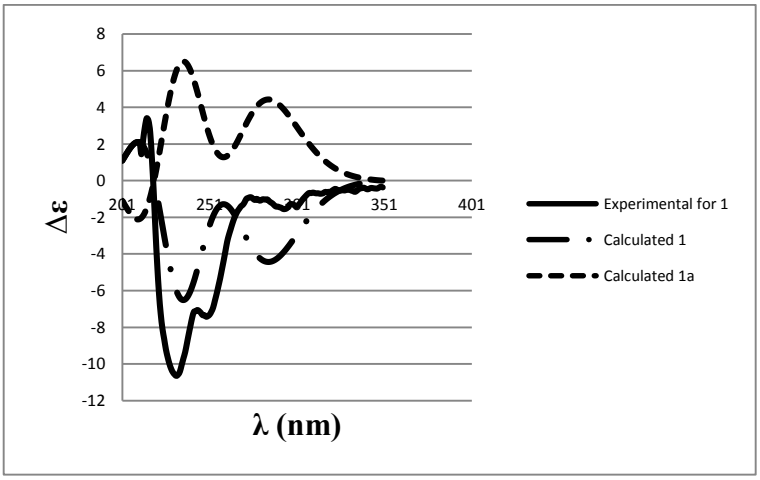


Figure 4. Experimental ECD spectrum (solid line), conformationally averaged calculated ECD spectrum of **1** and of **1a** (dashed lines).



Scheme 1. Proposed formation of **1** from sweroside