Isolation and Characterization of Cytotoxic Saponin Chloromaloside A from *Chlorophytum malayense*

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Received: September 9, 1999; Accepted: October 9, 1999

Abstract: **A cytotoxic steroidal glycoside was isolated from *Chlorophytum malayense* Ridley and its structure was characterized as a known compound, neohecogenin 3-*O*-*β*-D-glucopyranosyl(1→2)-[*β*-D-xylopyranosyl(1→3)]-*β*-D-glucopyranosyl(1→4)-*β*-D-galactopyranoside (chloromaloside A). The structural identification was performed using 2D-NMR and LC/MS/MS analysis. The previous, erroneously assigned ^{1}H-NMR spectral data were revised whereas the published ^{13}C-NMR spectral assignments were confirmed. This compound showed *in vitro* cytotoxicity against several human cancer cell lines.**

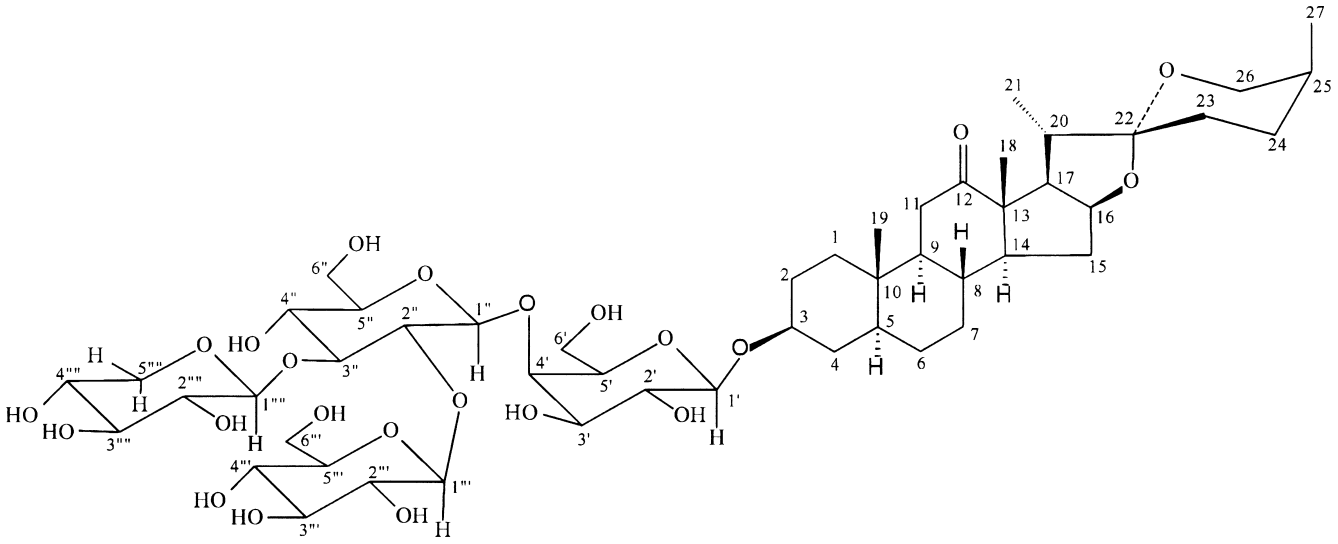
Steroidal glycosides constitute an important class of secondary metabolites from seaweed, fungi and higher terrestrial plants. Some steroidal glycosides were shown to exhibit cytotoxic (1), (2) and antineoplastic activities (3). As part of our search for novel anticancer agents from higher plants, an *n*-BuOH extract of the rhizomes of *Chlorophytum malayense* Ridley (Anthericaceae), a species indigenous to Southeast Asia and the southwest of Yunnan Province, China, was found to exhibit potential cytotoxicity against several human cancer cell lines. Bioassay-guided fractionation of this extract led to the isolation of a cytotoxic steroidal saponin (**1**) as at least one of the active constituents responsible for the cytotoxicity exhibited by the MeOH extract of *C. malayense*.

Compound **1** was isolated as colorless needles. It gave a quasi-molecular ion observed at *m/z* = 1047 $[M - H]^-$ in the negative mode electrospray mass spectrum, consistent with the molecular formula of $C_{50}H_{80}O_{23}$. The molecular formula was further supported by the ^{13}C-NMR (DEPT) spectrum (4 CH_3, 14 CH_2, 28 CH and 4 C). Inspection of the ^{1}H- and ^{13}C-NMR spectra of **1** revealed they are almost superimposable with those of neohecogenin 3-*O*-*β*-D-glucopyranosyl(1→2)-[*β*-D-xylopyranosyl(1→3)]-*β*-D-glucopyranosyl(1→4)-*β*-D-galactopyranoside, namely chloromaloside A, which is a known compound previously isolated from the title plant (4).

The structure of chloromaloside A (**1**) was previously determined by interpreting the NMR spectral data of the prosapogenins from partial acidic hydrolysis, with the released free

Planta Med 66 (2000) 587 – 590

ISSN: 0032-0943



sugar being identified by TLC comparison with the corresponding authentic samples (4), although the absolute configurations are left to be assigned. In the present work, the identity of the **1** as chloromaloside A was confirmed by use of electrospray LC/MS/MS and 2D NMR spectral analysis.

Negative ion mode electrospray mass spectrum of **1** gave the quasimolecular ion $[M - H]^-$ at m/z = 1047 as the only peak. From the collision-induced MS/MS significant daughter ions of the parent ion at m/z = 1047 were detected at m/z = 915 $[M - H - 132]^-$, 885 $[M - H - 162]^-$, 753 $[M - H - 132 - 162]^-$, 591 $[M - H - 132 - 162 - 162]^-$, respectively. This fragmentation pattern was in good agreement with the sugar linkage of **1**, implying the presence of a branched glycone chain with both a pentose and a hexose as terminal sugar units. Furthermore, the presence of an inner hexose directly bound to the aglycone was evident from the observation of a daughter ion at m/z = 161, corresponding to a hexose unit, from the parent fragment ion at m/z = 591. This is one of the few examples for the use of electrospray MS/MS in the structure elucidation of natural saponins (5).

The interpretation of the through-bond connectivity and through-space relaxation information from DQF-COSY, HOHAHA, HMQC, HMBC and ROESY experiments resulted in the unambiguous assignment of all ^{13}C and the well-resolved ^1H signals. It was found that the ^{13}C resonance assignments are in good agreement with those reported in the literature (4), which were deduced from chemical shift arguments based on the literature data following a buildup scheme incorporating α- and β-glycosidation effects in an empirical manner. With regard to the ^1H-NMR data, other than the confirmation of the methyl group signals, the previously erroneously assigned resonances of H-20, H-11, H-17, H-26, H_2-5‴, H-2′, H-6′ were unequivocally assigned correctly for the first time. Further, it is noteworthy that the previously assigned anomeric proton signals of xylose (H-1‴) at δ 5.59 and of glucose (H-1‴) at δ 5.25 must be revised, since the signal resonating at δ 5.25 showed a significant HMBC cross peak to the C-3″ signal of the inner glucose at δ 86.6, while the δ 5.59 proton showed a ^1H-^{13}C long-range heteronuclear correlation to C-2″ at δ 81.2 of the inner glucose (Fig. **1**). The absolute configurations of all the sugars (glucose, galactose and xylose) were assigned to D based on the follow assumptions and evidence: i) The D-isomer predominates in nature; ii) the calculated $[\alpha]_D$ value of

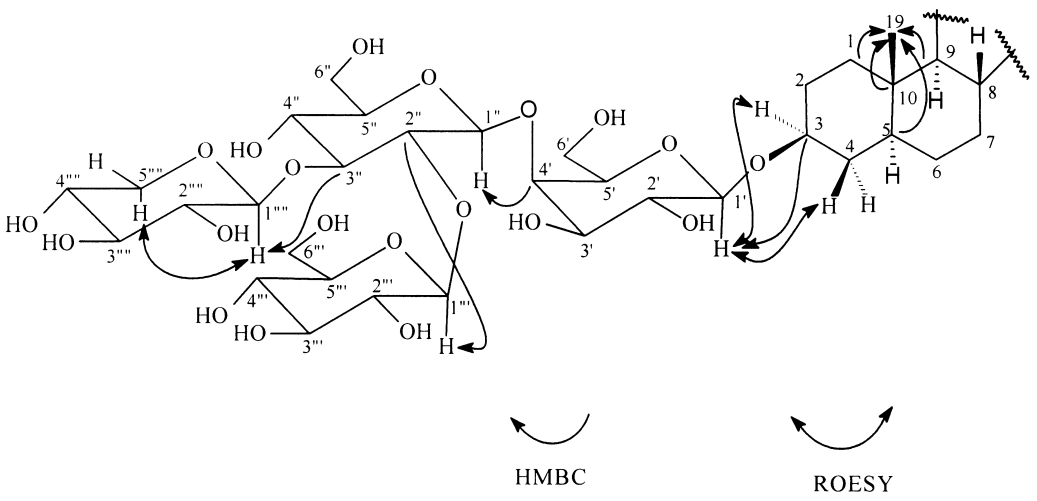


Fig. 1 Key long-range ^{13}C-^1H NMR (HMBC) and ROE (ROESY) correlations for chloromaloside A (**1**).

Table 1 Cytotoxic activity of chloromaloside A (**1**)[a].

Compound	Cell Line[b] Lu-1	KB	KB-V (+VLB)	KB-V (–VLB)	LNCaP	BC1	Col2	ASK[c]
chloromaloside A (1)	1.9	2.3	5.0	5.0	1.4	4.8	3.8	–
colchicine[d]	0.2	0.2	0.6	3.5	0.06	0.008	0.001	+
ellipticine[d]	0.2	0.04	0.2	0.3	0.8	0.2	0.3	–

[a] Results are expressed as ED_{50} values (μg/ml).

[b] Key to cell lines used: Lu-1 = human lung cancer; KB = human oral epidermoid carcinoma; KB-V = vinblastine-resistant KB test in the presence (+VLB) or absence (–VLB) of 1 μg/ml vinblastine; LNCaP = hormone-dependent human prostate cancer; ZR-75-1 = hormone-dependent human breast cancer; BC1 = human breast cancer; Col2 = Human colon cancer; ASK = rat glioma.

[c] Test compound that reverse astrocye formation is scored as (+) and non-reverse astrocyte formation is scored as (–) in the ASK test at the concentration of 100 μg/ml.

[d] Positive control compounds.

compound **1** (–97.5°) according to the Klyne rule (6) is very close to the experimental data (–67.1°).

As shown in Table **1**, compound **1** showed broad cytotoxicity against several human cancer cell lines using a panel of human cell lines and established protocols (7). Despite the observation that the cytotoxic activity of pennogenin glycosides from *Majanthemum dilatatum* increased with more monosaccharides attached to the aglycone core pennogenin, parallel with increasing soap activity (8), it was widely accepted that the cytotoxicity of the steroidal glycosides is not just due to their detergent activity but some unique subtle structural features are required for optimal activity (1). It was envisioned that, based on the data available, the cytotoxic activity of steroidal glycosides is very sensitive to their precise functionalization, therefore, more extensive studies are needed before a clear structure-activity relationship can be reached.

Materials and Methods

General experimental procedures: IR spectra were recorded in a KBr pellet on a MIDAC FT-IR interferometer. The optical rotations were measured with a Perkin-Elmer 241 polarimeter. The ^{1}H-, ^{13}C-NMR, APT, DQF-COSY, HOHAHA, HMQC, HMBC and ROESY spectra were recorded using a Bruker Advance DPX-300 instrument in C_5D_5N solution with TMS as internal standard. Electrospray MS/MS was performed on a Macro mass Quattro II electrospray triple quadrupole mass spectrometer. TLC was performed on precoated Kieselgel 60 F_{254} plates (Merck) and detection was achieved by spraying with 10% H_2SO_4 followed by heating.

Plant material: Rhizomes of *Chlorophytum malayense* Ridley were collected in Xishuangbanna, Yunnan province, China, and identified by Prof. H. Li of Kunming Institute of Botany, Chinese Academy of Science, China. A voucher specimen (KIB-93-878) has been deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation: The milled air-dried rhizomes (150 g) were extracted with MeOH (0.5 liter × 3). The combined MeOH extract was concentrated, in vacuum, and the residue (40 g) was suspended in H_2O, extracted with petroleum ether, and then with *n*-BuOH (saturated with H_2O). The *n*-BuOH layer was shown to be cytotoxic and were concentrated to dryness, and subjected to chromatography over silica gel (450 g), using a gradient mixture of $CHCl_3$-MeOH-H_2O (50 : 10 : 1 to 10 : 10 : 1, v/v) as eluent. Compound **1** (22 mg) was obtained as colorless needles from the fraction eluted $CHCl_3$-MeOH-H_2O (40 : 10 : 1). The homogeneity of **1** was shown by normal-phase and reverse-phase TLC in the following solvent system: silica gel, $CHCl_3$-MeOH-H_2O (6 : 4 : 1), R_f = 0.6; reverse-phase silica gel, MeOH-H_2O (7 : 3), R_f = 0.5.

Chloromaloside A (**1**): Colorless needles, m.p. 224–225 °C, $[\alpha]_D^{25}$: –67.1° (pyridine, *c* 0.03). IR (KBr): ν_{max} = 3440, 3300, 1710, 980, 920, 900, 855 cm^{-1} [intensity 920 > 900, diagnostic of 25(S) spirostane]. Electrospray MS/MS: *m/z* = 1047 [M – H]$^-$, 915 [M – H – 132]$^-$, 885 [M – H – 162], 753 [M – H – 132 – 162]$^-$, 591 [M – H – 132 – 162 – 162]$^-$ and 161 [591 – aglycone]$^-$. ^{1}H-HMR: δ = 0.65 (3H, s, 19-CH_3), 1.06 (3H, d, *J* = 6.9 Hz, 27-CH_3), 1.07 (3H, s, 18-CH_3), 1.37 (3H, d, *J* = 6.9 Hz, 21-CH_3), 1.89 (1H, m, H-20), 2.22 (1H, dd, *J* = 5.1, 13.5 Hz, H-11α), 2.36 (1H, t, *J* = 13.5 Hz, H-11β), 2.74 (1H, dd, *J* = 8.5, 6.2, H-17), 3.37 (1H, d, *J* = 10.6, H-26β), 3.68 (1H, t, *J* = 10.5, H-5‴‴α), 4.02 (1H, obsc., H-26α), 4.21 (1H, obsc, H-6′), 4.23 (1H, obsc, H-5‴‴β), 4.42 (1H, obsc, H-2′), 4.87 (1H, d, *J* = 7.5, H-1′), 5.20 (1H, d, *J* = 7.9 Hz, H-1″), 5.25 (1H, d, *J* = 7.7 Hz, H-1‴), 5.59 (1H, d, *J* = 7.2 Hz, H-1‴). ^{13}C-NMR: δ = 36.6 (t, C-1), 29.6 (t, C-2), 77.0 (d, C-3), 34.6 (t, C-4), 44.4 (d, C-5), 28.6 (t, C-6), 31.4 (t, C-7), 34.3 (d, C-8), 55.5 (d, C-9), 36.2 (s, C-10), 38.0 (t, C-11), 212.8 (s, C-12), 55.3 (s, C-13), 55.9 (d, C-14), 31.7 (t, C-15), 79.7 (d, C-16), 54.1 (d, C-17), 16.3 (q, C-18), 11.7 (q, C-19), 43.1 (d, C-20), 13.8 (q, C-21), 109.8 (s, C-22), 26.3 (t, C-23), 26.1 (t, C-24), 27.5 (d, C-25), 65.2 (t, C-26), 16.1 (q, C-27), 102.4 (d, C-1′), 73.2 (d, C-2′), 75.6 (d, C-3′), 79.9 (d, C-4′), 76.3 (d, C-5′), 60.6 (t, C-6′), 105.2 (d, C-1″), 81.2 (d, C-2″), 86.6 (d, C-3″), 70.5 (d, C-4″), 77.6 (d, C-5″), 62.5 (t, C-6″), 104.9 (d, C-1‴), 75.1 (d, C-2‴), 78.7 (d, C-3‴), 71.1 (d, C-4‴), 77.7 (d, C-5‴), 62.9 (t, C-6‴), 105.0 (d, C-1‴‴), 75.4 (d, C-2‴‴), 78.8 (d, C-3‴‴), 70.8 (d, C-4‴‴), 67.4 (t, C-5‴‴).

Cytotoxicity and antimitotic assays: Cytotoxicity data for compound **1** were obtained from a panel of human cancer lines using established protocols (7) and the antimitotic assay was carried out according to a published method (9).

Acknowledgements

This project was supported, in part, by a grant from Division of Cancer Treatment, national Cancer Institute, Bethesda, MD, USA.

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