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SHORT COMMUNICATION

Verbascoside, synthetic derivatives and other glycosides from Argentinian native plant species as potential antitumoral agents

Hugo A. Garro[a,b], Ezequiel Bruna-Haupt[a], Valeria Cianchino[a,c], Florencia Malizia[b], Silvina Favier[a,c], Mauricio Menacho-Márquez[b], Diego Cifuente[a], Claudio O. Fernández[b] and Carlos R. Pungitore[a]

[a]Área de Química Orgánica, INTEQUI-CONICET-UNSL, San Luis, Argentina; [b]Max Planck Laboratory for Structural Biology, Chemistry and Molecular Biophysics of Rosario (MPLbioR, UNR-MPIbpC), and Instituto de Investigaciones para el Descubrimiento de Fármacos de Rosario (IIDEFAR, UNR-CONICET), Universidad Nacional de Rosario, Rosario, Argentina; [c]Área de Farmacotecnia, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, San Luis, Argentina

ABSTRACT

A phytochemical study was performed on three native plant species from the central-western zone of Argentina: *Buddleja cordobensis* Grisebach, *Baccharis salicina* Torr. & A. Gray and *Nepeta cataria* L. We could obtain verbascoside (**1**) from *B. cordobensis*. From *N. cataria*, we could obtain 1, 5, 9-*epi*-deoxyloganic acid (**2**) L. Finally, we could isolate 2-β-(*L*-rhamnopyranosyl)-3-angeloyloxy-15-acetyloxy-7,13(14)-*E*-dien-*ent*-labdane (**3**) and 2-β-(*L*-rhamnopyranosyl)-3-α-angeloyloxy-15-hydroxy-7,13(14)-*E*-dien-*ent*-labdane (**4**) from *B. salicina*. Moreover, three derivatives from **1**, and one semi-synthetic derivative from **2**, were prepared. PCR reaction was used to analyse the activity against DNA polymerase and cell culture to determine cytotoxicity and antitumoral activity. Verbascoside (**1**) was strongly active in the nanomolar scale (IC_{50} = 356 nM) against DNA polymerization. Moreover, verbascoside was also strongly active in the nanomolar scale against human melanoma cell line (IC_{50} = 256 nM) and human colorectal cell line (IC_{50} = 320 nM). Furthermore, derivatives **6** and **7** were cytotoxic against both cancer cell lines.

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CONTACT Hugo A. Garro hgarro@unsl.edu.ar; Carlos R. Pungitore carlos.pungitore@conicet.gov.ar

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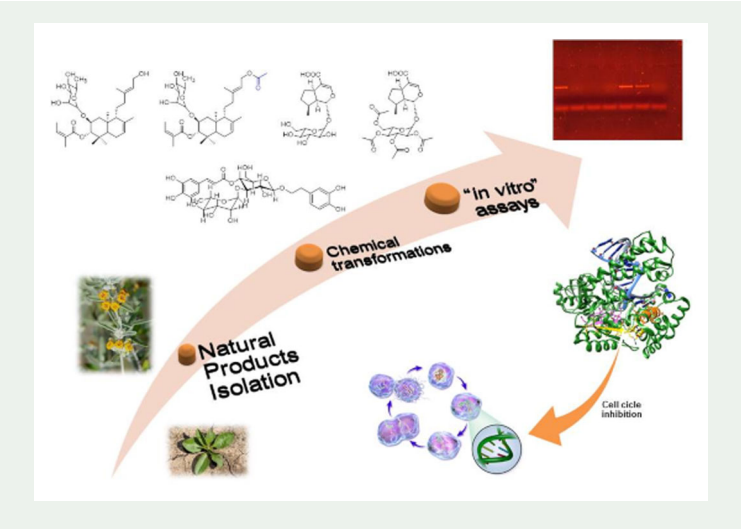

1. Introduction

DNA polymerases are enzymes directly implicated in the genetic material synthesis and replication. The inhibition of these proteins locks DNA synthesis and could affect the unlimited replicative potential of malignant cells, and therefore antitumoral activity (Pungitore 2014; Garro et al. 2015). Natural products may act as precursors or templates for pharmaceutical drugs development (Newman and Cragg 2007; Kabera et al. 2014).

In continuation of our drug discovery schedule of antitumoral agents from Argentinean medicinal herbs, our aim here was to elucidate the potential target of action of verbascoside and related semi-synthetic derivatives, and other natural glycosides, as possible polymerase inhibitors. To carry this out, we performed a phytochemical study on three native plant species from Argentina: *Buddleja cordobensis* Grisebach, *Baccharis salicina* Torr. & A. Gray and *Nepeta cataria* L.

2. Results and discussion

The iridoids present in the Genus *Buddleja*, are derivatives of aucubin that include aucubin itself and conjugates with groups derivatives from shikimato as *p*-methoxycinnamoylaucubin. Another large group of iridoids informed contains catalpol as a fundamental structure; for example, catalpol, methylcatalpol, catalposide and *p*-methoxycinnamoylcatalpol have been isolated from *Buddleja globose* Lam. Finally, a third type of iridoids, which contain the ajugol skeleton, have been obtained from the aerial parts from *Buddleja japonica* Hemsl., such as vanillylajugol, feruloylajugol and buddlejoside (Pungitore et al. 2004; Khan et al. 2019). In previous work, we could obtain different iridoids from *Buddleja cordobensis* Grisebach (Pungitore et al. 2004; Garro et al. 2015) and a little account of *per-O*-acetylverbascoside from acetylated fractions, but we could not isolate free verbascoside. In this new phytochemical study, *B.*

cordobensis Grisebach was found to be rich in glycosides, in the TLC analysis, showing the major products in the methanolic extract. Using chromatographic methods from the methanolic fractions from aerial parts of *Buddleja cordobensis* Grisebach we obtained as amorphous brown powders three majority products. We used Si gel column chromatography using mixtures of *n*-chloroform/methanol in increasing polarities to obtain Catalpol (726 mg), 6-*epi*-catalpol (533 mg) and verbascoside (1.050 gr). In our knowledge, verbascoside was isolate for the first time in *Buddleja cordobensis* Grisebach. Compound **1** (verbascoside) was identified (Figure S1) by spectroscopic studies and using bibliographical data (Grisebach 1874; Gomez-Aguirre et al. 2012) as a brown amorphous solid. The ^{1}H NMR spectrum (200 MHz, MeOD) exhibited characteristic signals arising from an ester of (*E*)-caffeic acid and 3,4-dihydroxyphenethyl alcohol moiety together with the signals for two *trans*-olefinic protons as AB system, δ_H 7.58 and 6.26 ($J_{AB} = 16.0$ Hz), a benzylic methylene proton δ_H 2.76 (t, 2 H, $J = 7.0$ Hz). Additionally, typical proton resonances for methyl group of rhamnose were observed at δ_H 1.08 as doublet (d, 3 H, $J = 6.8$ Hz). The ^{13}C NMR data of compound **1** confirmed the diglycosidic nature exhibiting two anomeric carbon resonances at δ_C 104.2 (β-glucose) and 103.1 (α-rhamnose) and HSQC experiments confirm their corresponding anomeric proton resonances at δ_H 5.18 (α-rhamnose) and 4.36 (β-glucose). All proton and carbon resonances were assigned by ^{1}H, ^{1}H-COSY, ^{1}H-^{13}C HSQC and HMBC experiments. The molecular formula of compound **1** was determined as $C_{29}H_{36}O_{15}$ by the spectroscopic data and ESI-MS: *m/z* 625.21 [$M + H^+$].

To increase the number of compounds for biological assays we prepare chemical derivatives using verbascoside as substrate. The first transformation of verbascoside (**1**) was started using acetone in acidic medium (Figure S1) to obtain an acetonide derivative (**5**) with 92% yield. The NMR spectra exhibited characteristic signals arising from a typical 2,2-dimethyl-dioxolane moiety. The ^{1}H NMR spectrum exhibited six new hydrogens at higher field between 1.3–1.5 ppm, and the ^{13}C NMR spectrum showed a new ketal carbon at 110.25 ppm (quaternary carbon) and signals at 26.79 and 28.55 ppm attributed to two geminal methyl groups. It was assumed that only one cyclic ketal protected *regio*- selectively positions 2′and 3′ of *L*-rhamnose, since only these positions have *cis* configuration in the rhamnosyl moiety. Moreover, the adjacent anomeric signal appeared now at 99.48 ppm instead of 103.11 ppm, and HMBC spectrum showed correlations between sugar alcohol base hydrogen at 3.76 ppm with ketal Carbon at 110.25 ppm. Furthermore, with derivative **5** as substrate, a dimethyl derivative (**6**) was obtained using Me_2SO_4 as alkylating agent, without affecting the 2,2-dimethyl-dioxolane moiety previously incorporated with 79% yield (Figure S1). The ^{1}H NMR spectrum exhibited signals arising from two methyl groups, showing six new Hydrogens at $\delta_{\Η}$ 3.84 and prominent signals at 57.0 and 59.0 ppm in ^{13}C spectra. Besides, methylation of hydroxyl groups was carried out in a *regio*-selective manner. It seems that the phenolic hydroxyl groups were preferentially alkylated compared to sugar alcohols. One methyl group on caffeoyl moiety was assumed to be positioned at the C-3′ on the basis of the deshielding of the H-7′and H-8′ of α,β-unsaturated carbonyl, and the HMBC cross-peak observed between O-CH_3 Hydrogens and the aromatic Carbons (δ ^{13}C in 146.0–158.0). Instead, the methyl group on phenylethanoid moiety was assumed to be positioned at the C-4. Furthermore, the subtle deshielding of aglycone Hydrogens at

position seven (β) and the HMBC correlation from methyl to aromatic Carbons suggest another methyl attached at position three of phenylethanoid moiety.

In the last step, we produced compound **7** using verbascoside (**1**) as substrate and ethyl bromide. We generated a *regio*-alkylation of the primary hydroxyl from glucose (Figure S2). The selection of this position against phenolic and secondary hydroxyls may be steric (ethyl is larger than methyl). The ethyl group was assumed to be attached to the hydroxymethylene of the glucose unit based on the migration of the signal attributed to C-5′. Furthermore, in the ^{1}H NMR spectrum a new signal appeared for two Hydrogens (dd, 2 H, $J = 8.0$; 6.4 Hz) at 4.07 ppm and another signal at higher field (t, 3 H, $J = 3.6$ Hz) for three Hydrogens at 1.28 ppm.

The *Nepeta* genus phytochemistry has two main kinds of compounds. The first is the nepetolactone chemotype, and the second is the 1,8-cineole and/or linalool chemotype (Salehi et al. 2018). From *Nepeta cataria* L. we could isolate the iridoid 1, 5, 9-*epi*-deoxyloganic acid, also called β–nepetanudoside (**2**). Compound **2** was identified by spectroscopic studies and using bibliographical data (Murai et al. 1984). The ^{1}H NMR spectrum of compound **2** exhibited typical absorptions of iridoid glycosides. Signals at δ_H 7.41 and 5.31 should be assigned to H-3 and H-1, respectively. H-1 appeared as a doublet (d, $J = 3.9$ Hz) and H-3 as a singlet. Also, a doublet at δ_H 1.06 (d, $J = 6.9$ Hz) corresponds to H-10. The ^{13}C NMR spectra of **2** showed two absorptions in the region 100-105 ppm. The signal at δ_C 100.9 and 104.1 correspond to C-1 and C-1′, respectively. Then, using DMAP as a catalyst, the *per-O*-acetylated derivative (**8**) was obtained as the sole product, 64% yield (Figure S3).

The most widespread secondary metabolites present in the genus *Buddleja* are clerodane and labdane diterpenoids, as well as triterpenoids of the oleanane series. Moreover, kaurane diterpenoids, cinnamic acid esters, coumarin derivatives and flavonoids are frequent and bioactive (Abad et al. 2005). For example, hexane extracts from *Baccharis salicina* Torr. & A. Gray were active against mice edema (Cifuente et al. 2001a). We could isolate from *Baccharis salicina* Torr. & A. Gray two *ent*-labdane diterpene glycosides: 2-β-(*L*-rhamnopyranosyl)-3-α-angeloyloxy-15-acetyloxy-7,13(14)-*E*-dien-*ent*-labdane (**3**) and 2-β-(*L*-rhamnopyranosyl)-3-α-angeloyloxy-15-hydroxy-7,13(14)-*E*-dien-*ent*-labdane (**4**) (Figure S4). Interestingly, these products showed a *L*-rhamnosyl moiety like verbascoside (Cifuente et al. 2001b).

Finally, we used all products to evaluate their potential antitumoral therapeutic properties against *Taq* DNA polymerase, as possible DNA replication inhibitors. Compound **1** showed a remarkable IC_{50} value of 356.20 ± 1.15 nM, while compounds **4**-**7** had IC_{50} values equal to 104.83 ± 0.94, 4.22 ± 0.08, 3.08 ± 0.29 and 3.96 ± 0.77 μM, respectively (Table S1 and Figure S7).

In order to determine the putative antitumoral activity of the compounds, their effect on cell proliferation was evaluated in two different cancer cell lines. Two semi-synthetic compounds (**6** and **7**), showed a significant reduction of cell proliferation in the two tumor cell lines tested (Figure S9). Moreover, by the use of MTT assays, we observed that these compounds effectively decreased cancer cell proliferation *in vitro* in a dose dependent manner (Figure S6 and S5). Indeed, the IC_{50} values obtained for the cell lines tested fit into the micromolar range (Table S2). Verbascoside (**1**), seemed to be more effective than the others, affecting cell proliferation at lower doses in the

Table 1. Selectivity index.

Compound / *Cell Line*	**7**	**6**	**1**
A375 (melanoma)	0.8772	0.568	1.28159

It was made comparing the activities of compounds **1**, **6** and **7** in A375 melanoma cell line with normal MDCK cells.

nanomolar range (Figure S9) (human melanoma cancer cell line $IC_{50} = 256\,nM$ and human colorectal cancer cell line $IC_{50} = 320\,nM$). Interestingly, the three compounds (**1**, **6** and **7**), showed polymerase inhibition and cytotoxic activity (Table S2 and S1).

The results obtained revealed that, except for compounds **2**, **3** and **8**, all the assayed products were active below IC_{50} values of 125.0 μM in the polymerase inhibition. Furthermore, the activity of compound **1** is very powerful, throwing inhibition values to the nanomolar scale (Table S1 and Figure S7a). The activity of compound **1** cannot be improved with chemical transformations; however, it was possible to obtain three derivatives with excellent activity. It is clear that the lack of free hydroxyl groups in verbascoside derivatives and the increase of the hydrophobicity is an important reason for inactivity or activity decrease. For glycosides **3** and **4,** the lack of acetyl moiety is clearly a determinant for activity. Compound **4** has a free hydroxyl group, showing activity at 104.83 μM against DNA replication.

The analysis of the effect on tumor cells proliferation showed that some glycosides have an important impact on cell growth (Figure S10), showing IC_{50} values at the micromolar range (Table 1). For compound **4** there is some cytotoxicity with A375 cell lines. Even more, verbascoside (**1**) exhibits a powerful activity in the nanomolar range as DNA polymerase inhibitor ($IC_{50} = 356\,nM$) and cytotoxic agent ($IC_{50} = 256\,nM$). Moreover, verbascoside has more activity against melanoma cancer cell lines than normal MDCK cells (Table 1), showing some selectivity. This finding makes verbascoside to a possible candidate for further antitumoral development.

3. Conclusions

In this study, we have highlighted the pharmacological potential on the DNA replication by verbascoside, chemical derivatives from it, and 2-β-(*L*-rhamnopyranosyl)-3-α-angeloyloxy-15-hydroxy-7,13(14)-*E*-dien-*ent*-labdane against DNA polymerase. Moreover, the antitumor effects at nanomolar level observed for the verbascoside obtained from *B. cordobensis* suggest the development of new pharmacological studies in the future.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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