Bioorganic & Medicinal Chemistry Letters 25 (2015) 914–918

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

A new iridoid, verbascoside and derivatives with inhibitory activity against *Taq* DNA polymerase

Hugo A. Garro [a,*], Celina García [b], Victor S. Martín [b], Carlos E. Tonn [a], Carlos R. Pungitore [a,*]

[a] *INTEQUI-CONICET, Fac. Qca., Bioqca. y Fcia., Univ. Nac. de San Luis (U.N.S.L), Chacabuco y Pedernera, 5700 San Luis, Argentina*
[b] *Instituto Universitario de Bio-Orgánica 'Antonio González', Departamento de Química Orgánica, Universidad de La Laguna, Avda. Astrofísico Fco. Sánchez, 2 La Laguna 38206, Tenerife, Spain*

ARTICLE INFO

Article history:
Received 17 November 2014
Revised 11 December 2014
Accepted 15 December 2014
Available online 23 December 2014

Keywords:
Natural products
Taq DNA polymerase inhibitors
6-*epi*-Catalpol
Sylil derivatives
Verbascoside

ABSTRACT

DNA polymerases are enzymes that play a crucial role in DNA metabolism such as replication, repair, transcription, recombination, and chromosome segregation during mitosis. Herein we report the isolation of a new iridoid (6-*epi*-catalpol, **2**) and *per*-*O*-acetyl-verbascoside (**11**) from aerial part of *Buddleja cordobensis* Grisebach (Buddlejaceae). From compound **2**, we have obtained eight compounds by chemical transformation. This group of compounds at a concentration of 500 μM was assayed against *Taq* DNA polymerase. Compound **11** (*per*-*O*-acetyl-verbascoside) was the most active with an IC_{50} of 1.21 ± 0.18 μM; compounds **9**, **2** and **8** were strong inhibitors with IC_{50} values of 5.57 ± 0.70, 21.62 ± 0.22 and 78.13 ± 0.93 μM, respectively. Compounds **11** and **9** could be a leader structures to development new anticancer chemotherapy medicines and a useful tool to investigate DNA polymerase activity.

During the last century, advancement in molecular biology contributed to the escalating understanding of the underlying mechanisms related to cancer initiation, promotion, and progression. As a consequence, monoclonal antibodies and small molecules have been developed as anticancer agent. These drugs affect specific molecular targets (typically proteins) implicated in tumor growth and progression, and therefore have become an important quantity of the anticancer armamentarium.[1] The targets include growth factor receptors, signaling molecules, cell-cycle proteins, apoptosis' modulators, and molecules involved in invasion and angiogenesis, which are essential for progress and homeostasis in normal tissues.[2]

DNA polymerases are enzymes that play a crucial role in DNA metabolism such as replication, repair, transcription, recombination, and chromosome segregation during mitosis. For this motive, it has long been accepted that these enzymes are valuable targets for the development of cancer chemotherapeutic agents. Several inhibitors have been introduced into clinical trials including dideoxynucleotides (ddNTPs), phospholipids, fatty acids, flavonoids, iridoids, triterpenoids, camptothecines, anthacyclines, aminoacridines and ellipticines.[3–6]

We have previously presented a series of novel lipophilic catalpol analogs by the regioselective addition of silyl ether groups.[7] These compounds inhibited in a dose-dependent manner the proliferation of a panel of diverse human cancer cell lines through G0/G1 phase arrest. Although we found that they were consistent with the inhibition properties of DNA polymerase exhibited by the parent compound catalpol (**1**).

The synthesis of lipophilic analogs have been successfully applied in the antitumor drug analogs silatecans[8] (silicon-containing camptothecins) and silaplatins (cisplatin analogs),[9] and the HIV-1 reverse transcriptase inhibitor TSAO-T.[10] Taking into account this tactic and previous results where iridoids containing silicon showed an important activity against a panel of human cancer cell,[7] we decide to produce lipophilic analogs containing acetyl, benzoyl and silicon groups in a regioselective manner.

Herein we report the isolation of a new iridoid (6-*epi*-catalpol, **2**) (Fig. 1) from aerial part of *Buddleja cordobensis* Grisebach (Buddlejaceae), *per*-*O*-acetyl-verbascoside (**11**) and the synthesis and inhibitory activity of novel lipophilic analogs of compound **2** against *Taq* DNA polymerase.

In a first step, we obtained as an amorphous brown powder *per*-*O*-acetyl-6-*epi*-catalpol (**3**) (Fig. 2) by Si gel column chromatography using mixtures of *n*-hexane/ethyl acetate in increasing polarities from the acetylated fractions. This compound showed a similar polarity than *per*-*O*-acetyl-catalpol and the same molecular formula ($C_{27}H_{34}O_{16}$), deduced from the elemental analysis (anal. C 52.16%, H 5.54%, calcd for C 56.20%, H 4.53%) and supported by ^{13}C NMR spectral data, showing similar pattern of signals in ^{13}C and ^{1}H NMR spectra, like an olefinic hydrogen at 6.33 ppm. Moreover, two

* Corresponding authors. Tel.: +54 2644 423789; fax: +54 2664 426711.
E-mail addresses: hugocanaya@yahoo.com.ar (H.A. Garro), carlos.pungitore@conicet.gov.ar (C.R. Pungitore).

http://dx.doi.org/10.1016/j.bmcl.2014.12.052
0960-894X/

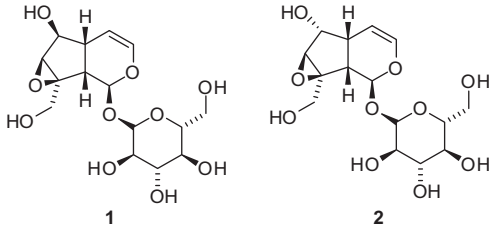


Figure 1. Structures of catalpol (**1**) and a new epimer 6-*epi*-catalpol (**2**).

protons resonances at higher field at δ_H 2.66 (dd, J = 9.5; 8.0 Hz) and 2.56 multiplete assigned as aglycone ring fused hydrogens. Finally the ^{1}H NMR spectrum displayed signals for a β-glucose unit, like an anomeric proton at 4.98 ppm and a triplet δ_H 5.16 (J = 10.0 Hz) attributed to H-4′. Surprisingly, signal attributed to H-6 appeared at lower field than *per*-*O*-acetyl-catalpol at 4.90 ppm. The ^{13}C NMR spectrum also showed 27 signals like as *per*-*O*-acetyl-catalpol, 12 of which were attributed to six acetyl groups, 13 methines, and 2 methylenes assigned to C-10 of aglycone and C-6′ of glucose. Anomeric signal appeared at 97 ppm and C-4 in 141 ppm region. It was in this kind of experiment when the highfield shift for C-6 was significantly higher compared to *per*-*O*-acetyl-catalpol isomer, appearing now at 79.52 ppm as opposed to *per*-*O*-acetyl-catalpol where this signal appears at 86.90 ppm. HSQC experiments was shown correlation between this signal at 79.52 ppm whit H-6 in 4.90 ppm region, and the fact that a change in the ^{13}C NMR chemical shift was significant (because this technique is highly sensitive to stereochemical changes) we proposed a new epimer at position six of catalpol *per*-*O*-acetylated named *per*-*O*-acetyl-6-*epi*-catalpol.

Posteriorly, we used Na/MeOH to get 6-*epi*-catalpol (**2**) and two acetyl derivatives, 10-*O*-acetyl-6-*epi*-catalpol (**4**) and 6,6′,10-tri-*O*-acetyl-6-*epi*-catalpol (**5**) (Scheme 1). The compound **2** showed a value of $[\alpha]_D^{25}$ −72.48° (*c* 0.98; ethanol) different to the observed value for catalpol (**1**), $[\alpha]_D^{25}$ −102.00° (*c* 0.98; ethanol).

In a second step, we produced silylations of hydroxyl groups with TBDPS and TBDMS in compound **2**. In both reactions, we used similar conditions, imidazole and CH_2Cl_2 as base and solvent, respectively. In this way, we obtained the compounds: 6,10,6′-tri-*O*-*tert*-butyldiphenylsilyl-6-*epi*-catalpol (**6**); 6,10,3′,6′-tetra-*O*-*tert*-butyldiphenylsilyl-6-*epi*-catalpol (**7**); 10,6′-di-*O*-*tert*-butyldimethylsilyl-6-*epi*-catalpol (**8**) and 6,10,6′-tri-*O*-*tert*-butyldimethylsilyl-6-*epi*-catalpol, respectively, (**9**) (Scheme 2). Then, we generated the compound 6,10,3′,4′,6′-penta-*O*-benzoyl-6-*epi*-catalpol (**10**) (Scheme 3) by standard method using Et_3N and CH_2Cl_2 as base and solvent, respectively.

This group of nine iridoids compounds and at a concentration of 500 μM was assayed and three compounds showed activity (33.33% of active compounds) against *Taq* DNA polymerase. To determinate IC_{50} values serial dilutions (1:2) were performed. Compound **9** was the most active one iridoid with an IC_{50} of 5.57 ± 0.70 μM and compounds **2** and **8** were strong inhibitors with

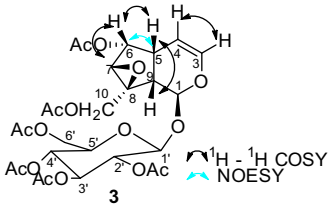
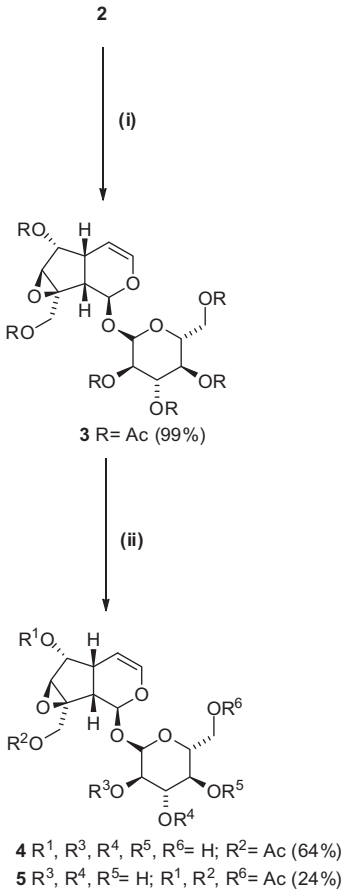


Figure 2. Structure and correlations ^{1}H–^{1}H COSY and ^{1}H–^{1}H NOESY for *per*-*O*-acetyl-6-*epi*-catalpol.

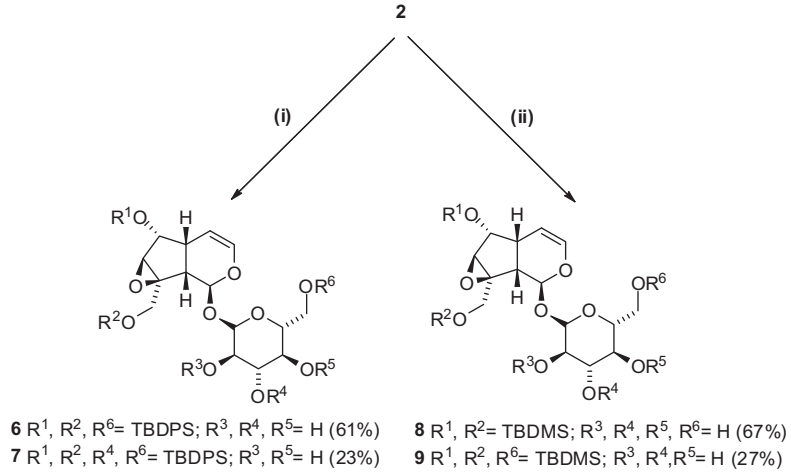


Scheme 1. Reagents and conditions: (i) 2.3 equiv Ac_2O, Py, DMAP, rt. (ii) Na, CH_3OH, rt.

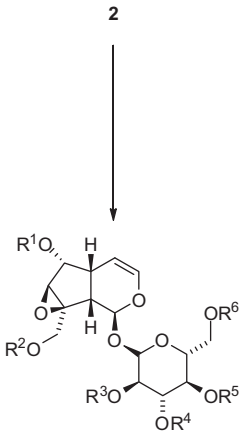
IC_{50} values of 21.62 ± 0.22 and 78.13 ± 0.93 μM, respectively, (Table 1).

In the last step, we obtained as an amorphous dark brown powder *per*-*O*-acetyl-verbascoside (**11**) from the acetylated fractions with a molecular formula of $C_{47}H_{54}O_{24}$ deduced from the elemental analysis (anal. C 55.54%, H 5.43%, calcd for C 56.20%, H 4.53%) and supported by ^{13}C NMR spectral data.

The ^{1}H NMR spectrum exhibited characteristic signals arising from an ester of (*E*)-caffeic acid and 3,4-dihydroxyphenethyl alcohol acetylated moiety together with the signals for two *trans*-olefinic protons (AB system, J_{AB} = 16.0 Hz), a benzylic methylene proton δ_H 2.87 (t, 2H, J = 7.5 Hz) and two non-equivalent protons δ_H 4.10 (br m, 1H) and 3.64 (br m, 1H). Additionally, two anomeric proton resonances were observed at δ_H 4.40 (d, J = 8.0 Hz) and 4.84 (br m, 1H) indicating a disaccharidic structure. The anomeric proton signals were consistent with the β configuration of one glucose and α configuration for one rhamnose. The ^{13}C NMR data of compound **11** confirmed the diglycosidic nature exhibiting two anomeric carbon resonances at δ_C 100.7 (β-glucose) and 99.0 (α-rhamnose). All proton and carbon resonances were assigned by ^{1}H, ^{1}H-COSY, ^{1}H–^{13}C HSQC and HMBC experiments. The caffeoyl group was supposed to be positioned at the C-4″ of the glucose unit on the basis of the significant deshielding of the H-4″, signal of glucose δ_H 5.21 (t, 1H, J = 9.6 Hz) and the HMBC cross-peak observed between H-4″ and the carbonyl carbon (δ_C 165.0). A prominent HMBC correlation from the anomeric proton of glucose (δ_H 4.40) to the C-α atom of the 3,4-dihydroxyphenethyl acetylated moiety (δ_C 70.0) revealed that the glucose moiety was attached at the C-α position. On the other hand, the downfield shifted H-3″ signal δ_H 3.89 (t, 1H, J = 9.6 Hz) of glucose indicated that a glycosidation



Scheme 2. Reagents and conditions: imidazole 3.3 equiv, CH_2Cl_2, rt; (i) 3.3 equiv TBDPSCl; (ii) 3.3 equiv TBDMSCl.



Scheme 3. Reagents and conditions: (i) 3.3 equiv BzOCl, TEA, Cl_2CH_2, rt.

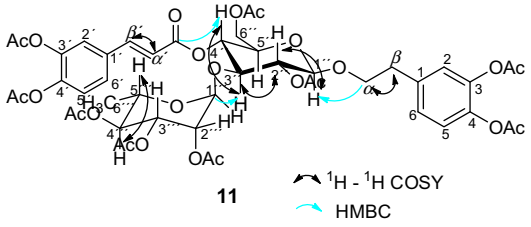


Figure 3. Structure and correlations ^1H–^1H COSY and HMBC for *per-O*-acetyl-verbascoside.

Table 1
Inhibitory activity at a concentration of 500 μM and IC_{50} values for the compounds panel

Compounds	IC_{50}[a] values	Compounds	IC_{50}[a] values
2	21.62 ± 0.22	**7**	NI
3	NI	**8**	78.13 ± 0.93
4	NI	**9**	5.57 ± 0.70
5	NI	**10**	NI
6	NI	**11**	1.21 ± 0.18

[a] IC_{50} values were determined by interpolation from plots and enzyme activity versus inhibitor concentration. The IC_{50} values are means from at least three independent experiments and standard deviation never exceeded 20%. NI: No Inhibition. The results are expressed in μM.

took place at the C-3″ carbon atom. A heteronuclear long-range correlation observed between the H-1‴ (δ_H 4.84) of rhamnose and C-3″ (δ_C 80.4) of glucose showed that the rhamnose unit was attached to the C-3″ carbon atom of glucose. On the other hand, the upfield shift of the C-6″ (δ_C 62.3) resonance of glucose revealed the presence of an acetyl group in this position. Furthermore, the deshielded carbon resonance of the C-4‴ at 72.0 ppm of the rhamnose unit was suggestive of acetylation at this position (Fig. 3).[11]

This acetylated phenylethanoid at a concentration of 500 μM was assayed against *Taq* DNA polymerase. To determinate IC_{50} values serial dilutions (1:2) were performed. Compound **11** was the most active essayed with an IC_{40} of 1.21 ± 0.18 μM (Table 1).[12]

In previous studies, the iridoid catalpol has shown significant inhibition of *Taq* DNA polymerase.[13] In vitro experiments and theoretical calculations suggest that the mechanism of *Taq* DNA polymerase inhibition may occur in a competitive way with deoxynucleoside triphosphates (dNTPs) at the binding site of the enzyme.[14–16] The idea behind molecular targeting is to design proceedings that specifically attack the molecular pathways that cause illness, without disturbing the normal functions in our cells. Drugs developed using this advance can be less toxic and more active than current medicines. From the results can be inferred two important concepts. First, the introductions of three silyl ether groups improve in an important way the activity of iridoids. Second, the silyl ether groups have to be bulky but without planes functional group. Also, it should not be introduced more of three silyl groups because the activity disappear. On the other hand, the incorporation of acetyl and aromatic moieties seem to be irrelevant for protein recognition and inhibition. Finally, phenylethanoids like verbascoside shows interesting pharmacology activities, such as antioxidant capacity and their ability to reduce ROS levels in human prostate cancer cells.[17] The inhibitory action for *per-O*-acetyl-verbascoside against a polymerase should be a new type of activity for this kind of compounds.

In summary, we have reported a new iridoid and a series of novel lipophilic 6-*epi*-catalpol analogs by the regioselective addition of silyl ether groups and acetyl groups. The compound **9** showed an excellent inhibitory activity against *Taq* DNA polymerase with an IC_{50} value equal to 5.57 ± 0.70 μM. *per-O*-Acetyl-verbascoside (**11**) also showed an excellent inhibitory activity against *Taq* DNA polymerase with the appearance of polymerization products recently at nM concentrations, and an IC_{50} value equal to 1.21 ± 0.18 μM. Compounds **9** and **11** could be a leader structures to develop new

anticancer chemotherapy medicines and a useful tool to investigate DNA polymerase activity.

Acknowledgments

Financial supports from CONICET (PIP 00360), UNSL (PROICO 2/1214), ANPCyT (PICT-2011-1416) and PCI-A/025750/09 are gratefully acknowledged. H.A. Garro thanks to CONICET for a post-doctoral grant. C.R. Pungitore and C.E. Tonn are part of CONICET researcher's staff. This research was also supported by the Spanish MINECO (CTQ2011-28417-C02-01). We wish to thank to M.L. Mascotti, C.S. Lucero, M. Purino and O. Donadel for their help. This work is a part of the doctoral thesis of Hugo A. Garro.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.12.052.

References and notes

1. Miranda, P.; Padrón, J. M.; Padrón, J. I.; Villar, J.; Martín, V. *Chem. Med. Chem.* **2006**, *1*, 323.
2. Sung, S.; Hsieh, C.; Wu, D.; Chung, L.; Johnstone, P. *Curr. Probl. Cancer* **2007**, *31*, 36.
3. Hamdi, H.; Castellon, R. *Biochem. Biophys. Res. Commun.* **2005**, *334*, 769.
4. Konoshima, T.; Takasaki, M.; Tokuda, H.; Nishino, H. *Cancer Lett.* **2000**, *157*, 87.
5. Mizushina, Y.; Iida, A.; Ohta, K.; Sugawara, F.; Sakaguchi, K. *Biochem. J.* **2000**, *350*, 757.
6. Ortiz de Urbina, A.; Martín, M.; Fernández, B.; San Román, L.; Cubillo, L. *Planta Med.* **1994**, *60*, 512.
7. Pungitore, C.; Leon, L.; García, C.; Martín, V.; Tonn, C.; Padrón, J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1332.
8. (a) van Hattum, A.; Pinedo, H.; Schlüper, H.; Hausheer, F.; Boven, E. *Int. J. Cancer* **2000**, *88*, 260; (b) Bence, A.; Mattingly, C.; Burke, T.; Adams, V. *Cancer Chemother. Pharmacol.* **2004**, *54*, 354.
9. Anderson, W.; Kasliwal, R.; Houston, D.; Wang, Y.; Narayanan, V.; Haugwitz, R.; Plowman, J. *J. Med. Chem.* **1995**, *38*, 789.
10. Bonache, M.; Chamorro, C.; Velázquez, S.; De Clercq, E.; Balzarini, J.; Rodríguez-Barrios, F.; Gago, F.; Camarasa, M.; San Félix, A. *J. Med. Chem.* **2005**, *48*, 653.
11. Spectroscopy data for compounds **2**–**11**.
6-epi-Catalpol (**2**). ^{1}H NMR (400 MHz, MeOD) 2.30 (br m, 1H), 2.56 (dd, 1H, *J* = 8.0; 2.0 Hz), 3.35–3.20 (br m, 1H), 3.35–3.25 (br m, 1H), 3.35–3.30 (br m, 1H), 3.45–3.40 (br m, 1H), 3.47 (br m, 1H), 3,66 (dd, 1H, *J* = 6.5; 5.5 Hz), 3.81 (d, 1H, *J* = 13.0 Hz), 3.91 (br m, 1H), 3.93 (br m, 1H), 4.15 (d, 1H, *J* = 13.0 Hz), 4.79 (d, 1H, *J* = 8.0 Hz), 5.05 (d, 1H, *J* = 10.0 Hz), 5.09 (dd, 1H, *J* = 6.0; 5.0 Hz), 6.37 (dd, 1H, *J* = 6.0; 5.0 Hz); ^{13}C NMR (100 MHz, MeOD) 37.67, 42.16, 60.17, 61.14, 61.47, 64.81, 70.32, 73.40, 76.26, 77.16, 78.13, 93.88, 98.29, 102.61, 140.36.
Per-O-acetyl-6-epi-catalpol (**3**). ^{1}H NMR (400 MHz, MeOD) 2.15–2.05 (br m, 18H), 2.56 (br m, 1H), 2.66 (dd, 1H, *J* = 9.5; 8.0 Hz), 3.67 (br m, 1H), 3.71 (br m, 1H), 3.97 (d, 1H, *J* = 13.0 Hz), 4.20 (dd, 1H, *J* = 12.5; 4.0 Hz) 4,31 (dd, 1H, *J* = 12.5; 2.0 Hz), 4.82 (br m, 1H), 4,85 (d, 1H, *J* = 9.1 Hz) 4.90–4.85 (br m, 1H), 4.95–4.90 (br m, 1H), 4.95 (br m, 1H), 5.00–4.95 (br m, 1H), 5.16 (t, 1H, *J* = 10 Hz), 5.25 (t, 1H, *J* = 8.0 Hz), 6.33 (d, 1H, *J* = 6.0 Hz); ^{13}C NMR (100 MHz, MeOD) 20.94–20.60 (6 C), 34.74, 41.39, 58.59, 61.14, 62.29, 62.57, 68.13, 70.51, 72.24, 72.50, 79.52, 94.16, 96.54, 101.92, 141.10, 171.1-169.1 (6 C). Elemental anal. C 52.16%, H 5.34%, calcd for ($C_{27}H_{34}O_{16}$).
10-O-Acetyl-6-epi-catalpol (**4**). ^{1}H NMR (400 MHz, MeOD) 2.08 (br m, 3H) 2.29 (m, 1H), 2.61 (dd, 1H, *J* = 7.5; 2.0 Hz), 3.20–3.10 (t, 1H, *J* = 8.0 Hz), 3.25–3.20 (br m, 1H), 3.40–3.35 (br m, 1H), 3.45–3.40 (br m, 1H), 3.47 (br m, 1H), 3.65 (dd, 1H, *J* = 6.5; 5.5 Hz), 3,92 (br m, 1H), 3.95 (br m, 1H), 4.07 (d, 1H, *J* = 12.5 Hz), 4.76 (d, 1H, *J* = 8.0 Hz), 4.95 (d, 1H, *J* = 12.5 Hz), 5.06 (d, 1H, *J* = 10 Hz), 5.07 (dd, 1H, *J* = 6.0; 5.0 Hz), 6.37 (dd, 1H, *J* = 6.0; 5.0 Hz); ^{13}C NMR (100 MHz, MeOD) 19.29, 37.54, 42.10, 60.17, 61.26, 61.60, 61.94, 63.11, 70.17, 73.39, 76.44, 77.07, 78.03, 94.04, 98.65, 102.33, 140.40, 171.35.
6,′6,10-Tri-O-acetyl-6-epi-catalpol (**5**). ^{1}H NMR (400 MHz, MeOD) 2.11–2.03 (br m, 9H), 2.28 (br m,1H), 2.60 (dd, 1H, *J* = 9.5; 8.0 Hz), 3.20 (br m, 1H), 3.38 (br m, 1H), 3.40–3.35 (br m, 2H), 3.49 (br m, 1H), 3.90 (d, 1H, *J* = 8.0 Hz), 3.94 (d, 1H, *J* = 12.5 Hz), 4.24 (dd, 1H, *J* = 7.0; 5.0 Hz), 4.53 (dd, 1H, *J* = 10.0; 2.0 Hz), 4.74 (d, 1H, *J* = 8.0 Hz), 4.89 (d, 1H, *J* = 10.0 Hz), 4.95 (d, 1H, *J* = 12.5 Hz), 5.08 (dd, 1H, *J* = 6.0; 1.0 Hz), 6.37 (dd, 1H, *J* = 6.0; 2.0 Hz); ^{13}C NMR (100 MHz, MeOD) 19.34–13.03 (3 C), 37.52, 41.94, 60.96, 61.80, 62.19, 63.25, 69.63, 73.25, 74.33, 76.23, 78.25, 94.20, 98.78, 102.39, 140.52, 171.30 (3 C).
6,10,6′-Tri-O-t-butyl-diphenylsilan-6-epi-catalpol (**6**). ^{1}H NMR (400 MHz, MeOD) 1.11–0.94 (br m, 27H), 2.59 (br m, 1H), 2.64 (br m, 1H), 3.09 (br m, 1H), 3.19 (br m, 1H), 3.35–3.30 (br m, 1H), 3.40–3.35 (br m, 1H), 3.52 (br m, 1H), 3.73 (br m, 2H), 3,81 (d, 1H, *J* = 12.5 Hz), 3.90 (d, 1H, *J* = 7.5 Hz), 4.11 (d, 1H, *J* = 12.5 Hz), 4.59 (d, 1H, *J* = 12.5 Hz), 4.64 (d, 1H, *J* = 8.0 Hz), 4.97 (br m, 1H), 6.22 (d, 1H, *J* = 2.0 Hz), 7.80–7.26 (br m, 30H); ^{13}C NMR (100 MHz, MeOD) 26.85–19.00 (9 C), 38.58, 42.03, 60.41, 61.31, 65.09, 65.23, 72.70, 73.05, 74.57, 76.17, 79.79, 94.32, 97.98, 103.38, 135.80-127.57 (36 C), 140.10.
6,10,3′,6′-Tetra-O-t-butyl-diphenylsilan-6-epi-catalpol (**7**). ^{1}H NMR (400 MHz, MeOD) 1.12–0.94 (br m, 36H), 2.15 (br m, 1H), 2.59 (br m, 1H), 3.00 (t, 1H, *J* = 9.0 Hz), 3.12–3.07 (br m, 1H), 3.15–3.10 (br m, 1H), 3.28 (br m, 1H), 3.36 (dd, 1H, *J* = 9.5; 6.0 Hz), 3.54 (br m, 2H), 3.67 (d, 1H, *J* = 12.5 Hz), 3.85 (d, 1H, *J* = 8.0 Hz), 3.93 (d, 1H, *J* = 12.5 Hz), 4.60 (d, 1H, *J* = 12.5 Hz), 4.83 (d, *J* = 9.5 Hz), 4.94 (dd, 1H, *J* = 7.0; 4.5 Hz), 6.25 (d, 1H, *J* = 12.5 Hz), 7.79–7.05 (br m, 36H); ^{13}C NMR (100 MHz, MeOD) 26.96–18.97 (12 C), 38.78, 42.41, 59.29, 60.20, 65.43, 65.58, 72.54, 74.19, 75.46, 78.02, 79.76, 92.53, 96.91, 102.57, 135.80–127.45 (48 C), 139.91.
10,6′-Di-O-t-butyl-dimethylsilan-6-epi-catalpol (**8**). ^{1}H NMR (400 MHz, MeOD) 0.12–0.04 (br m, 12H), 0.97–0.87 (br m, 18H), 2.32 (br m, 1H), 2.50 (br m, 1H), 3.23 (br m, 1H), 3.30–3.25 (br m, 3H), 3.44 (br m, 1H), 3.82 (d, 1H, *J* = 12.8 Hz), 3.83 (dd, 1H, *J* = 8.0 Hz), 4.05 (d, 1H, *J* = 12.8 Hz), 4.14 (dd, 1H, *J* = 12.8; 3.2 Hz), 4.76 (d, 1H, *J* = 8.0 Hz), 4.96 (d, 1H, *J* = 10.0 Hz), 5.09 (dd, 1H, *J* = 6.0; 1.6 Hz), 6.36 (dd, 1H, *J* = 6.0, 1.2 Hz).
6,10,6′-Tri-O-t-butyl-dimethylsilan-6-epi-catalpol (**9**). ^{1}H NMR (400 MHz, MeOD) 0.16–0.07 (18H), 0.97–0.90 (br m, 27H), 2.45 (br m, 1H), 2.47 (br m, 1H), 3.25–3.20 (br m, 1H), 3.30–3.25 (br m, 2H), 3.40–3.35 (br m, 1H), 3.40 (br m, 1H), 3.71 (dd, 1H, *J* = 7.2; 4.4 Hz), 3.95 (dd, 1H, *J* = 8.0; 1.2 Hz), 4.04 (d, 1H, *J* = 2.0 Hz), 4.08 (d, 1H, *J* = 12.8 Hz), 4.19 (d, 1H, *J* = 12.8 Hz), 4.76 (d, 1H, *J* = 8.0 Hz), 4.97 (d, 1H, *J* = 10.0 Hz), 5.00 (br m, 1H), 6.37 (br m, 1H); ^{13}C NMR (100 MHz, MeOD) 1.00–0.00 (6 C), 25.07–24.85 (9 C), 38.58, 42.23, 60.41, 62.36, 63.13, 64.72, 70.53, 73.70, 76.64, 77.51, 79.66, 93.46, 97.94, 102.37, 140.56.
6,10,3′,4′,6′-Penta-O-benzoyl-6-epi-catalpol (**10**). ^{1}H NMR (400 MHz, MeOD) 2.71 (br m, 1H), 2.82 (dd, 1H, *J* = 9.5; 8.0 Hz 1H), 3.53 (t, 1H, *J* = 8.0 Hz), 3.78 (br m, 1H), 3.87–3.82 (br m, 2H), 4.38 (d, 1H, *J* = 12.5 Hz), 4.68 (dd, 1H, *J* = 10.0; 4.5 Hz), 4.73 (dd, 1H, *J* = 10.0; 4.5 Hz), 4.95 (d, 1H, *J* = 7.5 Hz), 5.03 (d, 1H, *J* = 6.0 Hz), 5.05 (d, 1H, *J* = 8.0 Hz), 5.10 (d, 1H, *J* = 9.5 Hz), 5.21 (d, 1H, *J* = 12.5 Hz), 5.26 (t, 1H, *J* = 9.5 Hz), 6.39 (dd, 1H, *J* = 6.0; 1.5 Hz) 8.12–7.46 (br m, 25H); ^{13}C NMR (100 MHz, MeOD) 35.43, 42.05, 58.71, 62.36, 62.70, 62.90, 68.49, 71.85, 74.16, 77.75, 80.45, 94.14, 99.12, 101.22, 133.17-128.02 (30 C), 141.00, 166 (5 C).
Per-O-acetyl-verbascoside (**11**). ^{1}H NMR (400 MHz, MeOD) 1.04 (d, 3H, *J* = 6.4 Hz), 2.31–1.88 (s, 27H), 2.87 (t, 2H, *J* = 7.0; 3.4 Hz), 3.64 (br m, 2H), 3.79 (br m, 1H), 3.89 (t, 1H, *J* = 9.6 Hz), 4.10 (br m, 1H), 4.20–4.05 (br m, 2H), 4.40 (d, 1H, *J* = 8.0 Hz), 4.84 (br m, 1H), 4.95 (t, 1H, *J* = 9.6 Hz), 5.15–5.04 (br m, 3H), 5.21 (t, 1H, *J* = 9.6 Hz), 6.35 (d, 1H, *J* = 16.0 Hz), 7.04 (s, 1H) 7.09 (br m, 2H), 7.23 (d, 1H, *J* = 8.0 Hz), 7.37 (br m, 1H), 7.40 (dd, 1H, *J* = 2.0; 8.0 Hz), 7.66 (d, 1H, *J* = 16.0 Hz); ^{13}C NMR (100 MHz, MeOD) 17.4, 20.9-20.6 (9 C), 35.3, 62.3, 67.3, 68.6, 69.6, 69.8, 70.0, 70.5, 72.0, 72.1, 80.4, 99.0, 100.7, 117.9, 122.8, 123.8 (2 C), 124.1, 126.4, 127.3, 132.7, 137.5, 140.5, 141.7, 142.5, 143.8, 144.4, 165.0, 170.7-167.9 (9 C).
12. Experimental: *Plant material:* aerial parts of *Buddleja cordobensis* Griseb. (Buddlejaceae) were collected during March 2008, in Cerro de La Cruz, Departamento La Capital, San Luis, Argentina, and a voucher specimen was deposited at the Herbarium of Universidad Nacional de San Luis: L.A. Del Vitto & E.M. Petenatti-4868 (UNSL).
Extraction and isolation: the dry aerial parts (4.025 kg) were chopped and macerated three times for 7 day periods with MeOH. The solvent was evaporated under reduced pressure at low temperature, and the residue (1.165 kg) taken up in $CHCl_3$ and partitioned against H_2O. The aqueous layer was subjected to lyophilization, and the brown amorphous residue was purified by Si gel column chromatography using mixtures of $CHCl_3$–MeOH in increasing polarities. After successive purifications yielded, among others, a white amorphous solid (1.880 g) as a pure compound $[\alpha]_D^{25}$ −102.00 (*c* 0.98, ethanol), which subjected to NMR studies proved the iridoid catalpol (**1**). The fractions obtained after isolation of catalpol (**1**) showed the presence of two compounds of higher polarity, one capable of being revealed by UV light (due to the presence of aromatic chromophores). Unfortunately it was impossible to isolate using a mixture of $CHCl_3$/MeOH, to which we resorted to an acetylation reaction. The purpose of this transformation is to form ester linkages to the hydroxyl groups present and thus significantly reduce its polarity. In this way it was possible to chromatographic separation using a solvent mixture comprised of *n*-hexane/ethyl acetate (This mix has a lower density than a mixture of $CHCl_3$/MeOH, and thus produces a lower diffusion of the analytes). After of this reaction of *per*-acetylation we could obtain a derivative of the natural iridoid (3.723 g) that compared with the spectroscopic data of catalpol and *per*-*O*-acetyl-catalpol proved to be an iridoid not described in literature which presented an investment in the configuration of C-6, which we named as *per*-*O*-acetyl-6-*epi*-catalpol (**3**). Additionally it was possible to isolate a compound of higher polarity and UV active character on the same acetylation reaction. This *per*-acetylated product was introduced as a brown amorphous solid (9.236 g) with similar macroscopic characteristics of a glycoside. Based on the NMR and quantitative elemental analysis, we proposed the structure of *per*-*O*-acetylverbascoside (**11**) for the isolated phenylethanoid disaccharide.
PCR assays: the assayed compounds were all dissolved in DMSO. The PCR master mixture consisted of 40 mM Trisacetate pH 8.3, 15 mM $MgCl_2$, 2.5 U of *Taq* DNA polymerase (Sigma–Aldrich), 20 mM each oligonucleotide primer, and 2.5 mM each desoxynucleotide triphosphate (dNTP). Inhibition studies were carried out with varying compound concentrations. All PCRs were done in 20 μL reaction volumes. The sequence of the sense primer was 5′-TAG AGC

GTG AGG TCG ACA C-3′, and the antisense primer, 5′ TCA AGT TAG ACG TGG CCG TC 3′. Thermocycling conditions consisted of 35 cycles of denaturation at 95 °C for 1 min followed by primer annealing at 56 °C and primer extension at 72 °C for 90 seg.

Analysis of PCR products: relative intensities of ethidium bromide stained PCR products were analyzed by using the optical scanner and the image program. The image of stained agarose gel was captured using a photography camera Kodak 2320 and then was scanned (Hewlett–Packard 3200 C). The digitized band images were processed using the Image processing program (Scion Image, public domain program); IC_{50} values were determined by the GraphPad Prism program.

13. Pungitore, C.; Juri Ayub, M.; Borkowski, E.; Tonn, C.; Ciuffo, G. *Cell. Mol. Biol.* **2004**, *50*, 767.
14. Pungitore, C.; Juri Ayub, M.; García, M.; Borkowski, E. J.; Sosa, M.; Ciuffo, G.; Giordano, O.; Tonn, C. *J. Nat. Prod.* **2004**, *67*, 357.
15. Martin, O.; Garro, H.; Kurina Sanz, M.; Pungitore, C.; Tonn, C. *J. Mol. Mod.* **2011**, *17*, 717.
16. Garro, H.; Manzur, J.; Ciuffo, G.; Tonn, C.; Pungitore, C. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 760.
17. Kirmizibekmez, H.; Ariburnu, E.; Masullo, M.; Festa, M.; Capasso, A.; Yesilada, E.; Piacente, S. *Fitoterapia* **2012**, *83*, 130.