Natural Product Research: Formerly Natural Product Letters

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gnpl20

Synthesis and biological evaluation of (-)-13,14-dihydroxy-8,11,13-podocarpatrien-7-one and derivatives from (+)-manool

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Published online: 30 Jul 2014.

To cite this article: María L. Novoa, Yelisbeth Escalante, Liliana Maldonado, Iván Galindo-Castro, Annamil Álvarez, Katherine Figarella, Sabrina Marsiccobetre, Irina Arocha, Jais Nieves, Franklin Salazar, Carlos Gámez, Nieves Canudas, Eleonora Tropper, Teresa González & José E. Villamizar (2015) Synthesis and biological evaluation of (-)-13,14-dihydroxy-8,11,13-podocarpatrien-7-one and derivatives from (+)-manool, Natural Product Research: Formerly Natural Product Letters, 29:3, 207-212, DOI: 10.1080/14786419.2014.942299

To link to this article: http://dx.doi.org/10.1080/14786419.2014.942299

Natural Product Research, 2015
Vol. 29, No. 3, 207–212, http://dx.doi.org/10.1080/14786419.2014.942299

Synthesis and biological evaluation of (−)-13,14-dihydroxy-8,11,13-podocarpatrien-7-one and derivatives from (+)-manool

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(*Received 10 March 2014; final version received 3 July 2014*)

13,14-Dihydroxy-8,11,13-podocarpatrien-7-one (**1**) and a series of ring C aromatic diterpene derivatives were synthesised from (+)-manool (**4**) and evaluated for their cytotoxic, leishmanicidal and trypanocidal activities. Our results indicated that compound **1** and other podocarpane-type intermediates are cytotoxic. Cleavage of C6–C7 bond of compound **7** improved cytotoxic activity, indicating that, in particular, the 6,7-*seco*-podocarpane-type compound **20** might serve as a lead compound for further development.

Keywords: podocarpane; abietane; cytotoxicity; leishmanicidal; trypanocidal

1. Introduction

Plants have an outstanding ability to synthesise aromatic substances, most of which are either phenols or their oxygen-substituted derivatives. In many cases, these substances serve as plant defence mechanisms against predation by microorganisms, insects and herbivores (Cowan 1999). Abietane-type and biosynthetically related polycyclic diterpenes are a major group of ring C aromatic diterpenes (Nakano 1989). They exhibit interesting biological properties such as antimalarial (Achenbach et al. 1992), antibiotic (Batista et al. 1995; Dellar et al. 1996) and cytotoxic (Gao & Han 1997) activities. Moreover, podocarpane-type diterpenes are present only in several genera, such as *Azadirachta* (Ara et al. 1988a, 1988b, 1990; Siddiqui et al. 1988), *Humirianther* (Das et al. 1981), *Micrandropsis* (de Alvarenga et al. 1981) and *Podocarpus* (Cambie & Mander 1962). In Kuo et al. (2000) isolated the podocarpane-type diterpene (−)-13,14-dihydroxy-8,11,13-podocarpatrien-7-one (**1**) from the bark of *Taiwaniacryptomerioides* and Lu et al. (2006) isolated this from the Chinese medicinal plant *Celastruspaniculatus*. Other bioactive podocarpane-type catechols isolated were (+)-deoxynimbidiol (**2**) (Xiong et al. 2006) and (+)-nimbidiol (**3**) (Majumder et al. 1987) (Figure 1).

To date, a number of synthetic approaches to these biologically active podocarpane-type diterpenes have been reported via polyene cyclisation (Burnell & Caron 1992; Majetich et al. 1997; Tada et al. 2000; Ishihara et al. 2001, 2002; Ishibashi et al. 2004; Harring & Livinghouse

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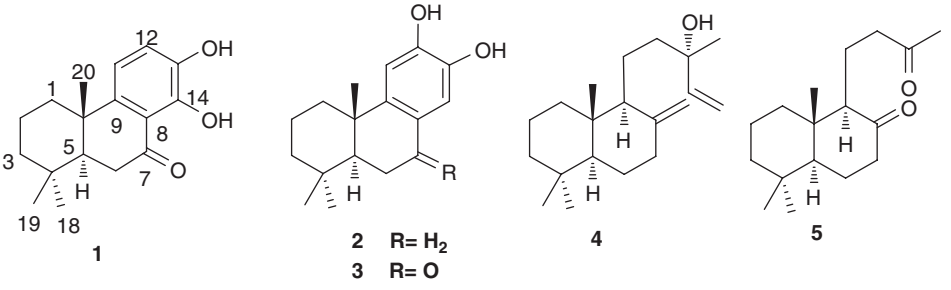


Figure 1. Structures of compounds **1**–**5**.

2010). However, they generally require long reaction sequences, and furthermore, almost all of them produce the racemic mixture of the natural substance.

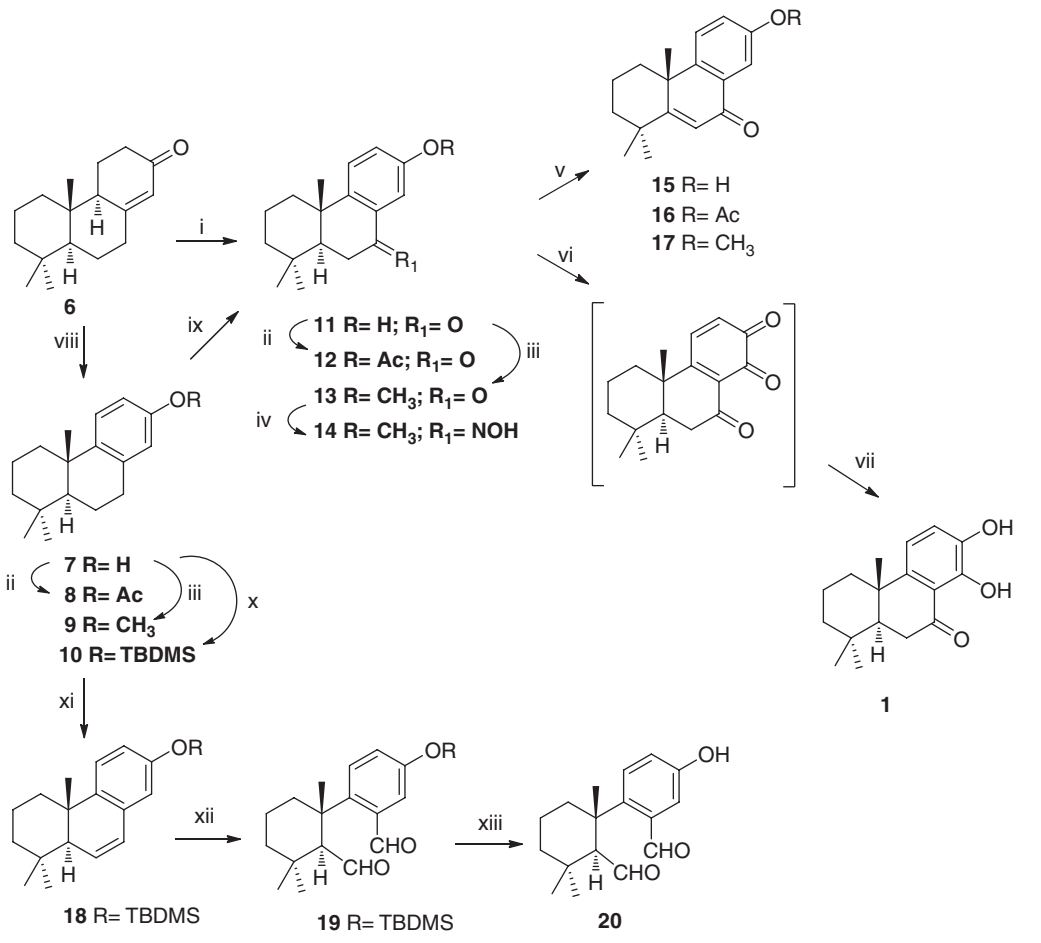
(+)-Manool (**4**) is a commercially available natural labdane-type diterpene with established absolute stereochemistry that has been used as a starting material for the syntheses of drimane-type sesquiterpenes (Salazar & Villamizar 2013a) and abietane-type and labdane-type diterpenes (Salazar & Villamizar 2013b). Recently, we have reported a new synthetic route to 13-hydroxy-8,11,13-podocarpatriene compounds from compound **4**, but this strategy involves the use of potassium permanganate (Villamizar et al. 2010, 2011). Continuing our research programme on the synthesis of bioactive diterpenes, the natural compound **1** and derivatives were synthesised from compound **4** and screened for their *in vitro* cytotoxic activity against two human cancer cell lines, and for antitrypanosomal and antileishmanial activities against *Trypanosoma cruzi* and *Leishmania mexicana*.

2. Results and discussion

2.1. *Chemistry*

To synthesise podocarpane-type diterpenes from labdane-type diterpene, the construction of the C-ring on the chiral podocarpane skeleton is necessary. This could be achieved by an intramolecular aldol condensation of 14,15,17-trinorlabdan-8,13-dione (**5**) (Álvarez-Manzaneda et al. 2007; Villamizar et al. 2010, 2011). Recently, Rogachev et al. (2012) reported that (+)-manool (**4**) was converted into compound **5** by ozonolysis in toluene in the presence of 10% sodium hydroxide in low yield. In an attempt to increase this yield, the reaction was studied in different solvents, resulting that the most favourable solvent to obtain compound **5** was toluene. In addition, when toluene was used as solvent and catalytic amounts of TBAB were added, compounds **5** and **6** were obtained in 64% and 10% yield, respectively. The approaches for the synthesis of all derivatives are outlined in Scheme 1. In our ongoing effort to synthesise novel bioactive podocarpane-type derivatives, we needed to prepare two key intermediates, natural phenols **7** (Kuo et al. 2000) and **11** (Minh et al. (2012). We previously reported the synthesis of compound **7** (Villamizar et al. 2010, 2011). Oxidation of compound **6** with sodium acetate and air afforded compound **11** (91% yield) (Scheme 1). The oxime derivative **14** was obtained by treatment of compound **13** with NH_2OH/AcOK (93% yield). The α,β-unsaturated ketones **15** and **17** were obtained from compounds **11** and **13**, respectively, by use of SeO_2 (71% and 88% yields, respectively). Compounds **12** and **13** can also be obtained from derivatives **8** and **9**, respectively, using different oxidation systems. Only oxidation with Na_2CrO_4 yielded compound **16** in low yield (Scheme 1).

To prepare 6,7-*seco*-podocarpane-type molecules, compound **10** was directly converted to the olefin **18** with *N*-bromosuccinimide (84% yield). Ozonolysis of compound **18** was carried out in CH_2Cl_2 and the reaction was quenched with PPh_3 to afford dialdehyde **19** (89% yield).



Scheme 1. Reagents and conditions: (i) AcONa, air, DMF, 120°C, 18 h; (ii) Ac_2O, TEA, DMAP, acetone, reflux, 12 h; (iii) K_2CO_3, MeI, acetone, reflux, 30 h; (iv) NH_2OH, AcOK, MeOH, reflux, 1 h; (v) **13**, SeO_2, AcOH, H_2O, reflux, 2.5 h → **17**. For **11**, same conditions, 17 h → **15**; (vi) IBX, $CHCl_3$–MeOH (4:1), rt, 1 h; (vii) then $NaBH_4$, rt, 3 h; (viii) Villamizar et al. (2010, 2011); (ix) *Method A*: **8**, CrO_3, AcOH, rt, 120 h → **12**; *Method B*: **8**, Na_2CrO_4, AcOH, Ac_2O, benzene, reflux, 24 h → **12** and **16**. For **9**, same conditions → **13**; *Method C*: **8**, PDC, TBHP, Celite, benzene, 10°C, 15 min, then rt, 4 h → **12**; (x) Im, TBDMSCl, DMAP, DMF, rt, 30 h; (xi) **10**, NBS, BPO, CCl_4, reflux, 2 h; (xii) (a) O_3, CH_2Cl_2, −78°C, 10 min; (b) PPh_3, CH_2Cl_2, −78°C, 1 h, then rt, 2 h; (c) H_2O_2, 0°C, 1 h; (xiii) TBAF, THF, 0°C, 5 min, then rt, 40 min.

Dialdehyde **20** was then obtained in quantitative yield after treatment of compound **19** with TBAF (Scheme 1). Even though the spectral data (^{1}H NMR, ^{13}C NMR and EI-MS) for dialdehyde **20** were completely consistent with its structure, further confirmation was sought by means of single crystal X-ray analyses. X-ray quality crystals of compound **20** were grown by slow evaporation of a hexane–ethyl ether solution. An ORTEP depiction of compound **20** is shown in Figure S25 (see Supplementary material). It is interesting to notice the cis position of methyl C20 with respect to the aldehyde C7.

In order to obtain the desired catechol **1**, compound **11** was oxidised according to the method of Magdziak et al. (2002) and Pezzella et al. (2005) to afford compound **1** with 50% yield (Scheme 1), whose physical and spectroscopic properties were identical with those reported (Kuo et al. 2000).

2.2. *Biological evaluation*

Compounds **1**, **7**, **9**, **12**–**16**, **19** and **20** were tested for cytotoxicity on human tumour cells, as well as for leishmanicidal and trypanocidal activities. First, these compounds were tested *in vitro* for potential cytotoxic activity to determine the concentration of the compound that induces 50% growth inhibition (IC_{50}) of human breast cancer-derived cells (MCF-7) or human prostate cancer-derived cells (PC3). Primary cultures of human dermal fibroblast were used as reference for non-malignant cells. Selectivity index (SI) for each compound was expressed as the ratio of IC_{50} on fibroblasts to the IC_{50} on the corresponding cell type. Cell viability and specific cytotoxicity were assessed by the MTT method (Denizot & Lang 1986). As can be seen in Table S1 (see Supplementary material), the highest cytotoxic activity on both MCF-7 and PC3 cells was shown by the oxime **14** with almost similar IC_{50} values, 19.9 ± 2.7 and 20.5 ± 4.3 μM, respectively. However, the IC_{50} on fibroblasts showed similar values (18.0 ± 3.2 μM). It is noteworthy that the phenolic dialdehyde **20** exhibited the highest SI, with values greater than 3.5 and 2.8 for MCF-7 and PC3 cell lines, respectively. This compound also showed the lowest cytotoxicity on non-cancerous cells (fibroblasts) with an IC_{50} value above 180 μM. By contrast, phenolic compounds **1**, **7** and **15**, all of them of podocarpane skeleton, were more cytotoxic to fibroblasts than to tumour cells. This trend seems to be reversed when the skeleton is broken at C6–C7 bond, as seen in compound **20**. Compound **9** exhibited the highest SI with values greater than 2.8 and 3.1 for *L. mexicana* and *T. cruzi*, respectively. According to these SI values, this molecule is the most effective compound tested against the parasites studied. It is interesting that compound **7**, although very similar to compound **9**, showed a lower SI value. This behaviour could be attributed to the hydrophobicity of the methyl group in compound **9**, which may facilitate the entry of the molecule through the cytoplasmic membrane. Compound **20** showed the highest IC_{50} value on parasite cells and phenolic compounds **1**, **7** and **15** were more cytotoxic against fibroblasts than to any parasite or tumour cell tested (see Table S1 Supplementary material).

3. Conclusions

We reported the first enantiospecific synthesis of (−)-13,14-dihydroxy-8,11,13-podocarpatrien-7-one (**1**), prepared several podocarpane-type derivatives and tested them for their *in vitro* tumour-derived cell cytotoxicity, as well as antileishmanial and antitrypanosomal activities. We found that compound **20** was the most effective on the tumour-derived cells tested (higher SI values), implicating that cleavage of C6–C7 double bond in the phenol **7** is likely to enhance the SI. We also demonstrated that compound **9** exhibited the highest SI value on both parasites assayed, showing that some functional groups play a key role in compound bioactivity.

Supplementary material

Supplementary material relating to this article is available online, alongside Table S1 and Figures S1–S25.

Acknowledgements

The authors thank the laboratories of Mass Spectrometry (Ana Angarita and Matilde Gómez), NMR (Sara Pekerar, Alberto Fuentes, Liz Cubillan and Ligia Llovera) and Elemental Analyses (EleinneSeverino) of IVIC for the research support.

Funding

This research was supported by FONACIT [grand number 2007000960], [grand number 2007001522], Foundation IDEA (POA-2012), and FONDEN.

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