Tetrahedron 64 (2008) 6324–6328

Contents lists available at ScienceDirect

Tetrahedron

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

Total synthesis of a dienynone from *Echinacea pallida*

Stefania Morandi [a], Federica Pellati [a], Stefania Benvenuti [a,*], Fabio Prati [b,*]

[a] Department of Pharmaceutical Sciences, University of Modena and Reggio Emilia, via Campi 183, 41100 Modena, Italy
[b] Department of Chemistry, University of Modena and Reggio Emilia, via Campi 183, 41100 Modena, Italy

ARTICLE INFO

Article history:
Received 4 February 2008
Received in revised form 10 April 2008
Accepted 24 April 2008
Available online 29 April 2008

Keywords:
Echinacea pallida
Total synthesis
Acetylenes
Polyenes
Cytotoxicity

ABSTRACT

The first total synthesis of (8*Z*,13*Z*)-pentadeca-8,13-dien-11-yn-2-one is described. This dienynone was recently isolated from the *n*-hexane extract of *Echinacea pallida* roots and displayed a selective cytotoxic activity toward cancer cells, thus featuring as a potential anticancer lead. The product was obtained in 11 steps in 25% overall yield.

1. Introduction

The in vitro cytotoxic and pro-apoptotic activities of the *n*-hexane root extracts from the three medicinally important *Echinacea* species (*Echinacea purpurea*, *Echinacea angustifolia* var. *angustifolia*, and *Echinacea pallida*; Asteraceae family) have been recently demonstrated.[1] A more pronounced cytotoxic effect for *E. pallida* root extracts in comparison with the other two species was observed.[1] This is in agreement with the different chemical composition of this species with respect to the others belonging to the genus *Echinacea*: in fact, the typical hydrophobic constituents of *E. pallida* extracts were found to be polyacetylenes and polyenes, whereas the lipophilic extracts of *E. purpurea* and *E. angustifolia* mainly consist of alkamides.[2–7] More recently, the bioassay-guided isolation and characterization of most of the lipophilic constituents of *E. pallida* roots have been performed.[6,7] Among them, (8*Z*,13*Z*)-pentadeca-8,13-dien-11-yn-2-one (**1**) was found to be one of the major constituents (0.98 mg/g in the plant material and 0.19–1.90 mg/g in the herbal products)[6] as well as the most active compound.[7,8] Compound **1** displayed a very low IC_{50} value toward the colonic COLO320 cancer cell line (IC_{50}=2.34 μM) and a noteworthy activity toward pancreatic Mia PaCa-2 cancer cell line (IC_{50}=32.17 μM), the latter being a type with generally low sensitivity to therapeutic agents.[9]Apoptotic cell death was found to be involved in the cytotoxic activity of this molecule.[8] Dienynone **1** displayed a selective effect on cancer cells versus non-cancer cells, with an IC_{50} value higher than 100 μM against human embryonic kidney HEK-293 cell.[8] Furthermore, this compound was found to be able to cross the Caco-2 monolayer,[8] which is an accepted model of intestinal absorption,[10] indicating a potential good absorption in humans after oral administration.

Due to the difficulty in purifying this compound from *E. pallida* roots, whose extracts contain many other constituents of similar polarity, and owing to the need of higher amounts of **1** for biological assays, the total synthesis of this secondary metabolite was undertaken.

2. Discussion

2.1. Retrosynthesis

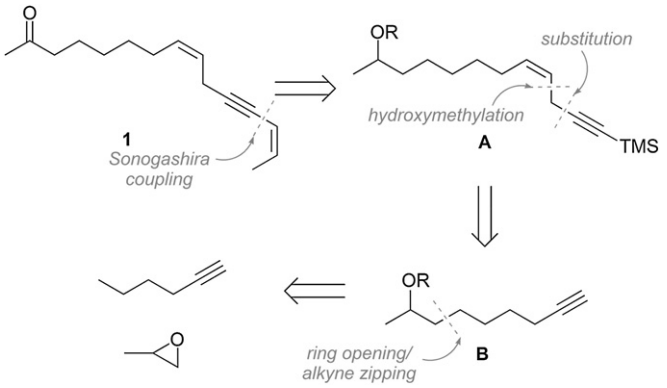
By analogy to the total synthesis of related structures,[11,12] our synthetic strategy hinged on a Sonogashira coupling reaction for the insertion of the terminal alkene moiety. Enyne precursor **A** can be disconnected by chain extension and selective reduction to alkyne **B**, which can be traced back to commercially available 1-hexyne and propylenoxide (Scheme 1).

2.2. Synthesis

Alkynol **3**[13] (Scheme 2) was synthesized in 72% overall yield by coupling of propyleneoxide and 1-hexyne (**2**) with *n*-butyllithium and boron trifluoride[14] followed by migration of the triple bond to

* Corresponding authors. Tel.: +39 059 205 5056; fax: +39 059 373543 (F.P.); tel.: +39 059 205 5144; fax: +39 059 205 5131 (S.B.).
E-mail addresses: stefania.benvenuti@unimore.it (S. Benvenuti), fabio.prati@unimore.it (F. Prati).

0040-4020/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2008.04.094



Scheme 1. Retrosynthetic analysis of dienynone **1**.

the chain terminus by means of the strong base KAPA (potassium hydride/1,3-diaminopropane).[15] The formation of the desired product **3** was confirmed by the presence of a diagnostic triplet in the ^{1}H NMR spectrum at 1.92 ppm ($^4J_{H\text{-}7\text{–}H\text{-}9}$=2.7 Hz, δ_C 68.2 ppm), referable to the terminal alkyne proton.

Finally, the resulting alcohol was protected as a THP ether (85% yield) following the classical protocol,[16] affording a mixture of stereoisomers clearly discernable by NMR analysis.

Protected alkynol **4** was converted into enyne **5** following the protocol described by Kraus,[11] which, in our hands, resulted in an improved 64% overall yield over four steps. In particular, hydroxymethylation of compound **4** was accomplished by reaction with ethylmagnesium bromide and formaldehyde. Subsequently, nucleophilic displacement of the hydroxy group with iodine followed by copper-catalyzed coupling with trimethylsilylacetylene allowed the insertion of the second acetylene moiety. Finally, compound **5** was obtained by regioselective reduction of the internal alkyne using nickel acetate and sodium borohydride.[17]

Enyne **5** was then desilylated using silver nitrate and potassium cyanide in methanol/water,[18] thus affording terminal alkyne **6** in 90% yield. Removal of the TMS group was easily confirmed by ^{1}H NMR analysis, which showed a highly diagnostic triplet at δ 1.94 ppm, corresponding to the terminal alkynyl proton (δ_C 67.5–67.8 ppm). Compound **6** was subjected to Sonogashira coupling[19] with commercial *cis*-bromopropene in the presence of $Pd(PPh_3)_4$ and copper iodide in piperidine[20] to afford the highly stable **7**[21] in 93% yield. The structure of **7** was confirmed by mass spectrometry and NMR spectroscopy. In particular, the ^{1}H NMR spectrum was characterized by the presence of a diagnostic doublet of doublet at δ 1.85 ppm, corresponding to the terminal allylic methyl group (δ_C 15.7 ppm). Due to the overlapping of olefinic proton signals in the ^{1}H spectrum, the configuration of the double bonds could not be assigned by direct measurement of coupling constants ($^3J_{H\text{–}H}$). For this purpose, a non-decoupled gHMBC experiment was run and accurate analysis of ^{13}C NMR satellites[22] allowed to assign the *Z* stereochemistry of both double bonds, ($^3J_{H\text{-}2\text{–}H\text{-}3}$=10.9 Hz, $^3J_{H\text{-}7\text{–}H\text{-}8}$= 5.7 Hz), thus confirming that the coupling reaction proceeded with retention of the cis-configuration.

Finally, the THP protecting group was quantitatively removed using catalytic *p*-toluenesulfonic acid in methanol at rt[23] and the resulting alcohol was converted to the corresponding ketone by Swern oxidation,[24] leading to compound **1** in 71% yield from enyne **5**. Spectroscopic data of compound **1** were in full agreement with those reported for the natural product isolated from *E. pallida* roots.[7] Dienynone **1** was found to be particularly prone to allylic oxidation, and required storage under inert atmosphere at −20 °C. In fact, NMR spectra of samples stored at higher temperatures (at 4 °C and at rt) for a few days showed a gradual increase of the oxidation products.[2,7] This is in agreement with what was observed in the course of the extraction and isolation of this compound from the natural matrix, whereby oxidized artifacts were detected upon exposure to atmospheric oxygen. At present, the problem can be circumvented by stocking the precursor **7** and proceeding with deprotection–oxidation steps on demand.

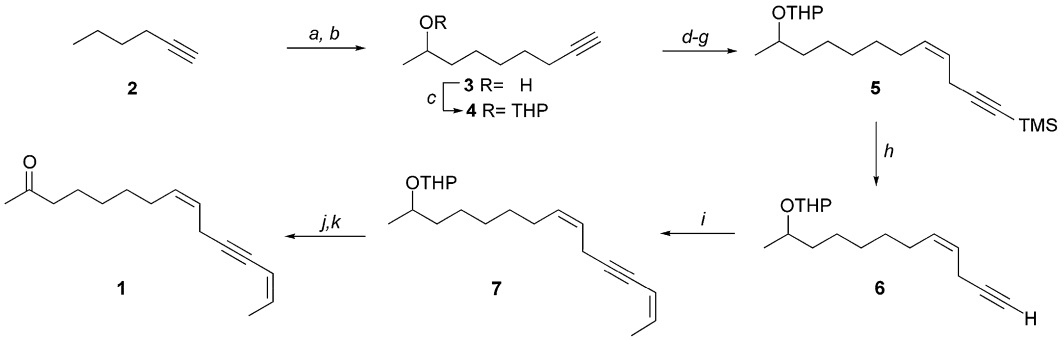
3. Conclusions

In conclusion, we have described the first total synthesis of dienynone **1**, originally isolated from *E. pallida* roots, which displays cytotoxic activity toward pancreatic and colonic cancer cell lines. Compound **1** was synthesized in 11 steps from 1-hexyne and propyleneoxide in 25% overall yield. Further studies on the biological activity of compound **1** are currently ongoing with the aim to shed light on the mechanism of action of this potential anticancer lead.

4. Experimental

4.1. General

All solvents used were anhydrous, unless stated otherwise, and all reactions requiring anhydrous conditions were performed using oven-dried and argon-flushed glassware. Anhydrous THF and diethyl ether were prepared by standard methods and freshly distilled from sodium benzophenone before use. Dichloromethane was dried according to standard procedures and stored upon 3 Å molecular sieves. All reagents were purchased from Aldrich. Chromatographic purification of compounds was carried out on silica gel (60–200 μm). Compounds were visualized by exposure to UV light and by dipping the plates in $KMnO_4$ stain followed by



Scheme 2. Synthesis of compound **1**. (a) *n*-BuLi, $BF_3{\cdot}Et_2O$, propylenoxide, THF, −78 °C, 3 h, 85%. (b) KAPA, rt overnight, 85%. (c) DHP, CH_2Cl_2, rt, 2 h, 85%. (d) EtMgBr, HCHO, THF, reflux 1 h, then rt overnight, 87%. (e) I_2, PPh_3, Et_2O/MeCN, 0 °C→rt, 4 h, 92%. (f) CuI, K_2CO_3, trimethylsilylacetylene, DMF, 0 °C→rt, then 35 °C, 6 h, 86%. (g) $NiAc_2{\cdot}4H_2O$, $NaBH_4$, ethylendiamine, H_2, EtOH, rt, 2 h, 93%. (h) $AgNO_3$, KCN, H_2O/MeOH, rt, 15 min, 86%. (i) *cis*-1-bromopropene, CuI, $Pd(PPh_3)_4$, piperidine, rt, overnight, 96%. (j) PTSA, MeOH, 40 °C, 1 h, 100%. (k) $(COCl)_2$, DMSO, TEA, CH_2Cl_2, −78 °C, 1.5 h, then rt, 1 h, 74%.