Tetrahedron 66 (2010) 9785–9789

Contents lists available at ScienceDirect

Tetrahedron

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

Enantioselective synthesis of 3(*S*)-hydroxy polygodial derivatives and evaluation of their vanilloid activity

Mariantonietta D'Acunto [a], Carmela Della Monica [a], Irene Izzo [a], Luciano De Petrocellis [b], Vincenzo di Marzo [c], Aldo Spinella [a,*]

[a] *Dipartimento di Chimica, Università di Salerno, Via Ponte Don Melillo, 84084 Fisciano, Salerno, Italy*
[b] *Endocannabinoid Research Group, Istituto di Cibernetica, C.N.R., Via Campi Flegrei 34, 80078 Pozzuoli NA, Italy*
[c] *Endocannabinoid Research Group, Istituto di Chimica Biomolecolare del CNR, Via Campi Flegrei 34, 80078 Pozzuoli NA, Italy*

ARTICLE INFO

Article history:
Received 30 June 2010
Received in revised form 12 October 2010
Accepted 1 November 2010
Available online 5 November 2010

This work is dedicated to the memory of Professor G. Sodano

ABSTRACT

The enantioselective synthesis of 3(*S*)-hydroxy polygodial and its acetyl derivative is described. The construction of the 3-hydroxy drimane skeleton was based on the titanium-catalyzed radical cyclization of (10*S*)-10, 11-epoxy-farnesyl acetate. Only underivatized 3(*S*)-hydroxy polygodial showed activity in assays on VR1 vanilloid receptor in HEK cells transfected with the human VR1.

1. Introduction

Hot tasting natural compounds, such as capsaicin, are considered pleasant spices in the culinary traditions of many countries. Furthermore, some of them are used in traditional medicine as antinociceptives.[1] However, these irritant compounds play a defensive role in many organisms because they are avoided by predators. For many years, pungency of capsaicin has been correlated to the activation of a membrane receptor in sensory neurons. The presence of a vanillyl-like moiety in capsaicin was the reason why this receptor was named vanilloid receptor and all interacting compounds were termed vanilloids. In 1997 TRPV1, a temperature sensitive ion channel belonging to the transient receptor potential family, was identified and cloned as molecular target of capsaicin.[2] Many natural dialdehydes, such as polygodial (**1**), used as deterrent against predators by many terrestrial and marine organisms,[3] are hot tasting and have been considered as potential agonists of the 'capsaicin' receptor.[4,5] Some studies have suggested that biological activities of these compounds are linked to the dialdehyde moiety and to reactivity toward nucleophiles.[6] The observation that some bulkier molecules, e.g., scalaradial (**2**), were not hot tasting, suggested that the size of the molecule is also important.[7] However, the hot taste reported for compound **4**, a deacetoxy derivative of the tasteless sesterterpenoid scalaradial,[8] pointed to the influence of an oxygenated function, such as the acetoxy group, on the bioactivity of these compounds (Fig. 1). This stimulated our investigation on the influence of a polar substituent, such as the hydroxy group, on the bioactivity of these 1,4-dialdehydes. Initially we investigated the influence of the hydroxyl group at C-1 through the synthesis of 1-(*R*)-hydroxypolygodial (**3**). Vanilloid activity bioassays showed that **3** was completely inactive.[9]

This result pointed out that the introduction of a polar substituent in active compounds, such as **1** and **4**, caused the loss of vanilloid activity. At this point we wondered if it was just the presence of hydroxy group by itself the reason of the inactivity and

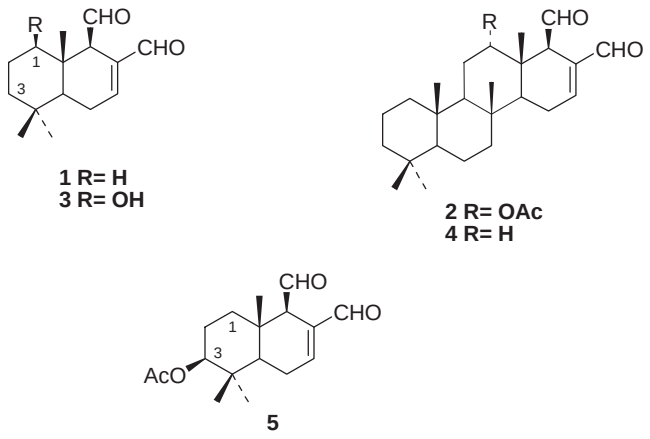


Fig. 1. Some terpenoid 1,4-dialdehydes.

* Corresponding author. Fax: +39 089969603; e-mail address: spinella@unisa.it (A. Spinella).

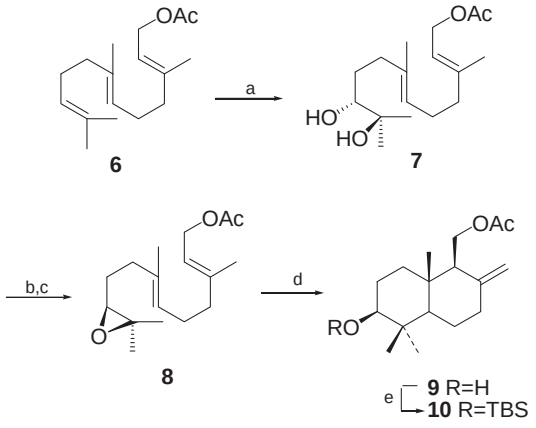
0040-4020/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2010.11.002

planned the synthesis of an analog with the polar function in a different position. We decided to insert the hydroxy group at C-3, because some bioactive hydroxyl polygodial derivatives have been isolated from natural source, such as 3-β-acetoxypolygodial (**5**) from *Canella winterana*.[10]

In this communication we report an enantioselective synthesis of 3-(*S*)-hydroxypolygodial and its acetyl derivative, in order to evaluate their vanilloid activity.

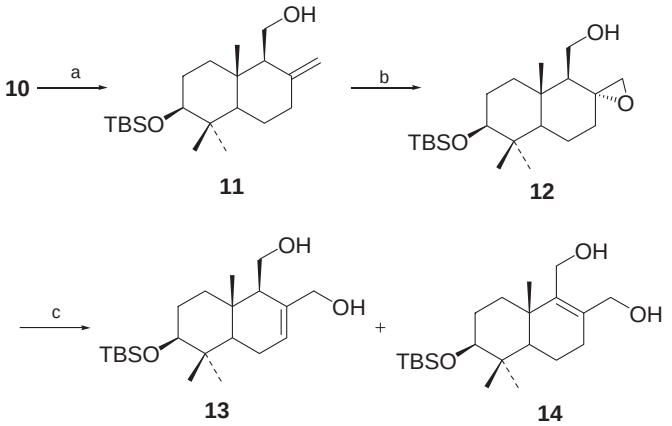
2. Results and discussion

The construction of the 3-hydroxy drimane skeleton (Scheme 1) was based on the titanium-catalyzed radical cyclization of epoxypolyprenes recently developed by Barrero et al.[11] In order to obtain an enantioselective process, we applied this titanocene-catalyzed cyclization to an epoxide with high optical purity. To this purpose we prepared (10*S*)-10,11-epoxy-farnesyl acetate (**8**) starting from (2*E*,6*E*)-farnesyl acetate (**6**) using a procedure described by Vidari et al.[12] Highly enantioselective dihydroxylation of (2*E*,6*E*)-farnesyl acetate (**6**) employing AD-mix-β afforded diol **7**. Conversion of diol **7** to the corresponding 10-*O*-mesylate followed by reaction with K_2CO_3 in CH_3OH gave (10*S*)-10,11-oxidofarnesol, which was subjected to acetylation affording **8**. Titanocene-catalyzed cyclization of (10*S*)-10,11-epoxy-farnesyl acetate (**8**) afforded a mixture of products in which the major product was **9** (34%).



Scheme 1. Reagents and conditions: (a) AD-mix-β, *t*-BuOH/H_2O (1:1), 35%, 95% ee; (b) (i) MsCl, pyridine, CH_2Cl_2, 0 °C to rt. (ii) K_2CO_3, MeOH, rt, 65% two steps; (c) Ac_2O, pyridine, DMAP, CH_2Cl_2, 20 h, 85%; (d) Cp_2TiCl_2, Mn, Me_3SiCl, collidine, THF, rt, 34% (e) TBSCl, imidazole, CH_3CN, 90 °C, 3 h, 75%.

Silylation of **9** with *tert*-butyldimethylsilylchloride (TBSCl) provided protected 3-hydroxy drimane derivative **10** in 75% yield. Acetyl group was removed by treatment with K_2CO_3 in MeOH. The introduction of allylic alcohol was obtained through acid catalyzed opening of the epoxide **12** easily prepared from **11** (Scheme 2). In fact, treatment of **11** with *m*-CPBA afforded epoxide **12** as a single isomer. The opening of epoxide in **12** into the corresponding allyl alcohol was subject to several trials. Camphorsulfonic acid (CSA) treatment in CH_3CN/DMSO (10:1) as solvent at room temperature[13] successfully yielded the desired diol **13** in 58% yield together with diol **14** (20%). Reactions performed at lower temperatures (10−15 °C) resulted in lower yield of diol **13**.

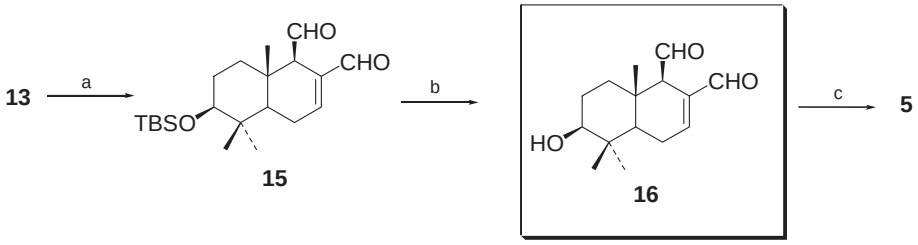


Scheme 2. Reagents and conditions: (a) K_2CO_3, MeOH, reflux, 1 h, 92%; (b) *m*-CPBA, CH_2Cl_2, 0 °C to rt, 1 h, 78%; (c) CSA, CH_3CN/DMSO (10:1), rt, overnight, 58%.

The final stages of the synthesis are shown in Scheme 3. Oxidation of both primary alcohol functionalities of **13**, conducted under Swern conditions, gave **15** in excellent yield (76%). Finally, removal of the TBS protective group (HF, CH_3CH aq) afforded 3-β-hydroxypolygodial (**16**). 3-β-acetoxypolygodial (**5**) was prepared by reaction of **16** with acetic anhydride.

3. Assays on TRPV1 receptors

To assess vanilloid activity of the synthetized compounds we performed assays on TRPV1 vanilloid receptor in HEK cells transfected with the human TRPV1. Vanilloid activity was evaluated by measuring the entry of Ca^{2+} (the concentration of internal calcium [Ca^{2+}] before and after the addition of test compounds). Vanilloid activity assays showed that only compound **16** was active (maximal response at 100 μM=49.0±1.5% of the effect of 4 μM ionomycin). In fact, although both compounds **5** and **16** caused Ca^{2+} influx activity into human embryonic kidney HEK-293 cells transfected with the human TRPV1, this activity was completely inhibited with the selective TRPV1 antagonist 5-iodo-resiniferatoxin (IRTX) only for compound **16** while activity resulted unaffected by the presence of the specific inhibitor in the assays with compound **5**. Furthermore when the compound **16** at a concentration of 10 μM was administered at HEK cells transfected with the human TRPV1 for 5 min, it caused a significant inhibition of the effect capsaicin 0.1 μM, a desensitization effect typical of TRP receptor channels.[14]



Scheme 3. Reagents and conditions:(a) DMSO, $(COCl)_2$, NEt_3, −78 °C, 2 h, 76%; (b) 48% HF (aq), CH_3CN, rt, 4 h, 70%; (c) Ac_2O, NEt_3, DMAP, 1 h, 62%.

4. Conclusion

In conclusion, a total synthesis of 3-(*S*)-hydroxypolygodial (**16**) and its acetyl derivative **5** has been accomplished in a highly stereoselective way through a synthetic strategy based on the titanocene(III)-catalyzed radical cyclization of (10*S*)-10,11-epoxy-farnesyl acetate (**8**). Only underivatized 3(*S*)-hydroxypolygodial (**16**) showed vanilloid activity while 3-β-acetoxypolygodial (**5**) did not show specific activity toward TRPV1 vanilloid receptor. This intriguing result furnished an interesting insight in the study of the structure–activity relationship. The vanilloid activity measured for 3(*S*)-hydroxypolygodial (**16**) and the inactivity evidenced for 1-(*R*)-hydroxypolygodial[9,10] show how the introduction of a hydroxy group on polygodial can have different effects on vanilloid activity depending on the position of insertion.

5. Experimental

5.1. General

All reactions were carried out under a dry N_2 atmosphere using freshly distilled solvents, unless otherwise noted. Tetrahydrofuran (THF) was distilled from sodium–benzophenone complex. Dichloromethane was distilled from calcium hydride. Glassware was flame-dried (0.05 Torr) prior to use. Starting materials and reagents purchased from commercial suppliers were generally used without purification. Reaction temperatures were measured externally; reactions were monitored by thin layer chromatography on Merck silica gel plates (0.25 mm) and visualized by UV light and spraying with phosphomolybdic acid, *p*-anisaldehyde or $Ce(SO_4)_2$ solutions and drying. Flash chromatography was performed on Merck silica gel 60 (particle size: 0.040–0.063 mm). Yields refer to chromatographically and spectroscopically (^1H and ^{13}C NMR) pure materials. The NMR spectra were recorded at room temperature on a Bruker DRX 400, a Bruker DRX 300 or a Bruker AV 250 spectrometers. Chemical shifts are reported relative to the residual solvent peak ($CHCl_3$: δ=7.26, $^{13}CDCl_3$: δ=77.0). Assignments in the ^{13}C NMR spectra were confirmed by DEPT spectroscopy experiments. ESIMS spectra were performed on a Micromass Quattro micro API™ mass spectrometer equipped with an electrospray ionization source operating in positive mode. IR spectra were obtained at a resolution of 2.0 cm^{-1} with a Vector 22 Bruker Spectrometer. Optical rotations were measured with a JASCO DIP-1000 polarimeter. Elemental analyses were performed on Flash EA 1112 (Thermo Electron Corporation) analyzer.

5.2. Synthesis

The epoxide **8** ($[\alpha]_D^{22}$ −2.9 (*c* 2.4, $CHCl_3$)) was prepared according to a known procedure starting from (2*E*,5*E*)-farnesyl acetate (**6**).[12] The enantiomeric excess was determined as 95% by ^1H NMR analysis of the corresponding mono-(*S*)-MTPA ester of diol **7**.[15]

*5.2.1. Preparation of ((2S, 4aS, 5S, 8aR)-decahydro-2-hydroxyl-1,1,4a-trimethyl-6- methylenenaphthalen-5-yl) methyl acetate (**9**).* Strictly deoxygenated THF (16.7 mL) was added to a mixture of Cp_2TiCl_2 (106.6 mg, 0.42 mmol) and Mn dust (940.6 mg, 17.1 mmol) under an Ar atmosphere and the suspension was stirred at room temperature until it turned green (after about 15 min). A solution of epoxide **8** (600 mg, 2.14 mmol) in THF dry (1.67 mL) was added via cannula to this suspension and then collidine (2.26 mL) and Me_3SiCl (1.10 mL) were added sequentially. The solution was stirred overnight. The reaction was then quenched with aq HCl 2 N (50 mL) and was stirred until no effervescence and a change in color from green to brown were observed. THF was removed under vacuum and the residue aqueous layer was extracted with EtOAc. The combined organic layers were dried (anhydrous $MgSO_4$) and concentrated under reduced pressure. TBAF dry (1 M in THF, 10.6 mmol, 10.6 mL) was added to the residue and the solution was stirred under N_2 atmosphere for 3 h. THF was removed under vacuum and the residue was diluted with EtOAc and H_2O. The aqueous layer was extracted with AcOEt, the combined organic phases were washed with brine, dried (anhydrous Na_2SO_4), and solvent removed. The residue was flash chromatographed on silica gel (10→50% AcOEt in petroleum ether) giving 207.2 mg of **9** (34%) as a yellow oil. [Found: C, 73.13; H, 9.87. $C_{17}H_{28}O_3$ requires C, 72.82; H, 10.06]; R_f (30% EtOAc/hexane) 0.40; $[\alpha]_D^{27}$ +18.0 (*c* 1.7, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ 4.86 (1H, br s), 4.53 (1H, br s), 4.31 (1H, dd, *J*=11.0, 3.9 Hz), 4.18 (1H, dd, *J*=11.0, 8.6 Hz), 3.27 (1H, dd, *J*=10.7, 3.9 Hz), 2.45–2.38 (1H, m), 2.09–1.97 (2H, m), 2.01 (3H, s), 1.80–1.30 (6H, m), 1.12 (1H, dd, *J*=12.5, 2.4 Hz), 1.00 (3H, s), 0.78 (3H, s), 0.75 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.6 (s), 146.4 (s), 107.8 (t), 78.8 (d), 61.7 (t), 54.7 (d), 54.5 (d), 39.4 (s), 38.9 (s), 37.6 (t), 37.2 (t), 28.5 (q), 27.9 (t), 23.7 (t), 21.3 (q), 15.7 (q), 15.3 (q). ES-MS (*m*/*z*)=281 ($M+H^+$), 221 ($M-AcOH+H^+$).

*5.2.2. Synthesis of compound **10**.* To a solution containing **9** (175 mg, 0.62 mmol), imidazole (493 mg, 7.24 mmol), CH_3CN (3 mL) under N_2 atmosphere, TBSCl (467 mg, 3.1 mmol) was added. The solution was heated to 90 °C and stirred for 3 h. CH_3CN was removed under N_2 flux, the residue was diluted with H_2O, and the aqueous layer was extracted with AcOEt. The combined organic phases were dried (Na_2SO_4), filtered, and evaporated in vacuo. The crude product was flash chromatographed (5→40% Et_2O in petroleum ether) to afford 185 mg (75%) of **10** as colorless oil. [Found C, 70.45; H, 11.02. $C_{23}H_{42}O_3Si$ requires C, 70.00; H, 10.73]; R_f (15% EtOAc/hexane) 0.91; $[\alpha]_D^{25}$ +26.0 (*c* 0.4, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ 4.85 (1H, br s), 4.52 (1H, br s), 4.32 (1H, dd, *J*=11.2, 3.7 Hz), 4.16 (1H, dd, *J*=11.2, 9.0 Hz), 3.22 (1H, dd, *J*=10.2, 5.6 Hz), 2.43–2.36 (1H, m), 2.05–2.01 (1H, m), 2.00 (3H, s), 1.99–1.97 (1H, m), 1.75–1.71 (1H, m), 1.69–1.67 (1H, m), 1.60–1.54 (2H, m), 1.44–1.28 (2H, m), 1.09 (1H, dd, *J*=12.5, 2.5 Hz), 0.91 (3H, s), 0.88 (9H, s), 0.74 (6H, br s), 0.04 (3H, s), 0.03 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.3 (s), 146.4 (s), 107.3 (t), 79.0 (d), 61.5 (t), 54.5 (d), 54.2 (d), 39.7 (s), 38.6 (s), 37.5 (t), 36.9 (t), 28.7 (q), 28.1 (t), 25.9 (q, 3C), 23.7 (t), 21.1 (q), 18.1 (s), 15.9 (q), 15.1 (q), −3.8 (q), −4.9 (q). ES-MS (*m*/*z*)=395 ($M+H^+$).

*5.2.3. Synthesis of compound **11**.* To a solution of **10** (185 mg, 0.47 mmol) in MeOH (2.5 mL), at room temperature, K_2CO_3 (78 mg, 0.56 mmol) was added. The reaction mixture was heated to reflux and stirred for 1 h. MeOH was removed in vacuo, the residue was diluted with H_2O, and the aqueous layer was extracted with AcOEt. The combined organic phases were washed with aq HCl 1 N, dried (anhydrous Na_2SO_4), filtered, and evaporated in vacuo. The crude product was flash chromatographed (5→20% AcOEt in petroleum ether) to afford 152 mg (92%) of **11** as colorless oil. [Found: C, 71.35; H, 11.24. $C_{21}H_{40}O_2Si$ requires C, 71.53; H, 11.43]; R_f (15% EtOAc/hexane) 0.62; $[\alpha]_D^{25}$ +10.1 (*c* 0.6, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ 4.93 (1H, br s), 4.63 (1H, br s), 3.85–3.70 (2H, m), 3.24–3.20 (1H, m), 2.42 (1H, ddd, *J*=13.0, 4.2, 2.4 Hz), 2.00 (1H, ddd, *J*=13.0, 12.8, 4.9 Hz), 1.93–1.89 (1H, m), 1.77–1.71 (1H, m), 1.67–1.63 (1H, m), 1.58–1.54 (1H, m), 1.45–1.36 (1H, m), 1.32–1.28 (1H, m), 1.09 (1H, dd, *J*=12.5, 2.8 Hz), 0.91 (3H, s), 0.88 (9H, s), 0.73 (6H, s),0.04 (3H, s), 0.03 (3H, s) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 147.5 (s), 106.5 (t), 79.1 (d), 58.9 (d), 58.8 (t), 54.4 (d), 39.7 (s), 38.6 (s), 37.8 (t), 36.9 (t), 28.7 (q), 28.1 (t), 25.9 (q), 23.9 (t), 18.1 (s), 15.9 (q), 15.3 (q), −3.8 (q), −4.9 (q) ppm. ES-MS (*m*/*z*)=35 ($M+H^+$).

*5.2.4. Synthesis of epoxide **12**.* A solution of **11** (152 mg, 0.43 mmol) in CH_2Cl_2 dry (3.2 mL), under N_2 atmosphere, was cooled to 0 °C and *m*-CPBA (77 mg, 0.991 mmol) was added. After 5 min, the

solution was warmed to room temperature and stirred for 1 h. Then, the solution was cooled to 0 °C, a 10% solution of Na_2SO_3 (12 mL) was added and the resulted mixture was stirred at room temperature for 1 h. The aqueous layer was extracted with AcOEt, the combined organic layers were washed with $NaHCO_3$, dried (anhydrous Na_2SO_4), filtered, and evaporated in vacuo. The crude product was flash chromatographed (5→40% AcOEt in petroleum ether) to afford 124 mg (78%) of pure **12** as a white solid. [Found: C, 68.61; H, 11.22. $C_{21}H_{40}O_3Si$ requires C, 68.42; H, 10.94]; R_f (20% EtOAc/hexane) 0.49; $[\alpha]_D^{25}$ −11.8 (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ 3.59 (1H, ddd, *J*=10.2, 10.2, 3.3 Hz), 3.40 (1H, dd, *J*=10.4, 10.2 Hz), 3.23–3.21 (1H, m), 3.18 (1H, dd, *J*=3.6, 2.1 Hz), 3.06 (1H, d, *J*=10.2 Hz, OH), 2.70 (1H, d, *J*=3.6 Hz), 2.00–1.92 (1H, m,), 1.87–1.85 (1H, m), 1.85–1.80 (1H, m), 1.70–1.62 (1H, m), 1.61–1.54 (3H, m), 1.43–1.39 (1H, m), 1.34–1.30 (1H, m,), 1.04 (1H, dd, *J*=12.4, 2.3 Hz), 0.93 (3H, s), 0.88 (9H, s), 0.84 (6H, s), 0.76 (3H, s), 0.04 (6H, s) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 78.9 (d), 61.6 (s), 58.8 (t), 54.2 (d), 53.9 (d), 51.7 (t), 39.6 (s), 38.8 (s), 36.7 (t), 36.2 (t), 28.7 (q), 27.6 (t), 25.8 (q), 21.5 (t), 18.1 (s), 16.0 (q), 15.7 (q), −3.8 (q), −5.0 (q) ppm. ES-MS (*m*/*z*)=391 ($M+Na^+$).

*5.2.5. Synthesis of diol **13**.* To a solution of **12** (55 mg, 0.149 mmol) in a 10:1 mixture of CH_3CN (16 mL) and DMSO (1.6 mL), under N_2 atmosphere, at room temperature, was added CSA (34.6 mg, 0.149 mmol) and the solution was stirred at room temperature overnight. The CSA was quenched with saturated $NaHCO_3$ solution and the mixture was concentrated in vacuo. The residue was diluted with H_2O and AcOEt and the aqueous layer was extracted with AcOEt. The combined organic phases were dried (anhydrous Na_2SO_4), filtered, and evaporated in vacuo. The crude product was flash chromatographed (from 15% AcOEt in petroleum ether to pure AcOEt) to afford 32 mg (58%) of **13** as white matt solid, 12.6 mg (23%) of $\Delta^{8,9}$ double bond isomer **14** as a white solid. Compound **13**: [Found: C, 68.67; H, 11.35. $C_{21}H_{40}O_3Si$ requires C, 68.42; H, 10.94]; R_f (40% EtOAc/hexane) 0.41; $[\alpha]_D^{23}$ +1.9 (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ 5.81 (1H, br s); 4.35 (1H, d, *J*=12.2 Hz), 3.99 (1H, d, *J*=12.2 Hz), 3.90 (1H, dd, *J*=11.2, 2.0 Hz), 3.69 (1H, dd, *J*=11.2, 8.2 Hz), 3.21 (1H, dd, *J*=11.1, 4.4 Hz), 2.77 (2H, br s), 2.12–2.06 (1H, m), 2.07–1.99 (2H, m), 1.66–1.57 (2H, m), 1.33–1.21 (3H, m), 0.88 (12H, br s), 0.82 (3H, s), 0.75 (3H, s), 0.05 (3H, s), 0.03 (3H, s) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 136.7 (s), 127.7 (d), 79.2 (d), 67.3 (t), 61.4 (t), 54.4 (d), 48.9 (d), 39.2 (s), 37.4 (t), 35.3 (s), 28.3 (q), 27.7 (t), 25.8 (q×3), 23.4 (t), 18.0 (s), 15.6 (q), 14.6 (q), −3.9 (q), −5.0 (q) ppm. ES-MS (*m*/*z*)=391 ($M+Na^+$), 351; Compound **14**: [Found: C, 68.01; H, 11.16. $C_{21}H_{40}O_3Si$ requires C, 68.42; H, 10.94]; R_f (40% EtOAc/hexane) 0.23. ^1H NMR ($CDCl_3$, 400 MHz): δ 4.22 (1H, d, *J*=11.7 Hz), 4.17 (1H, d, *J*=11.6 Hz), 4.11 (1H, d, *J*=11.7 Hz), 4.01 (1H, d, *J*=11.6 Hz), 3.22 (1H, dd, *J*=11.0, 5.1 Hz), 2.26–2.22 (2H, m), 1.84 (1H, ddd, *J*=13.2, 3.0, 3.0 Hz), 1.79–1.35 (6H, m), 0.99 (3H, s), 0.93 (3H, s), 0.89 (9H, s), 0.78 (3H, s), 0.04 (3H, s), 0.03 (3H, s) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 145.9 (s), 136.2 (s), 79.1 (d), 63.9 (t), 58.0 (t), 50.5 (d), 39.3 (s), 37.8 (s), 34.3 (t), 31.6 (t), 28.4 (q), 27.9 (t), 25.9 (q×3), 20.4 (q), 18.6 (t), 18.1 (s), 15.9 (q), −3.8 (q), −4.9 (q) ppm. ES-MS (*m*/*z*)=391 ($M+Na^+$).

*5.2.6. Synthesis of compound **15**.* To a stirring solution of oxalyl chloride (84.0 mg, 0.66 mmol) in CH_2Cl_2 (0.4 mL), under N_2 atmosphere, cooled to −78 °C, DMSO (103 mg, 1.32 mmol) was added dropwise. After 5 min, a solution of **13** (30.5 mg, 0.0827 mmol) in a 3/1 mixture of CH_2Cl_2 (0.6 mL) and DMSO (0.2 mL) was added via cannula and the solution was stirred for 2 h. Dry NEt_3 (309 mg, 3.06 mmol) was added and the resulting mixture was allowed to stir at −78 °C for 10 min. The reaction mixture was warmed to room temperature and stirred for further 45 min. Then the mixture was passed through a short pad of silica gel (particle size 0.040–0.263 mm) eluting with ethyl acetate, under N_2, and the eluent was concentrated to give a yellow oil. The oil was purified by flash chromatography (20→40% diethyl ether in petroleum ether) under N_2 to give 23 mg (76%) of **15** as a white amorphous solid. [Found: C, 68.82; H, 10.34. $C_{21}H_{36}O_3Si$ requires C, 69.18; H, 9.95]; R_f (60% Et_2O/hexane) 0.72; $[\alpha]_D^{25}$ −41.4 (*c* 0.5, $CHCl_3$). IR ($CHCl_3$, cm^{-1}) ν 2938, 2860, 1718, 1682. ^1H NMR ($CDCl_3$, 400 MHz): δ 9.51 (1H, d, *J*=4.4 Hz), 9.46 (1H, br s), 7.14–7.12 (1H, m), 3.23 (1H, dd, *J*=9.8, 5.2 Hz), 2.78–2.74 (1H, m), 2.56–2.35 (2H, m), 1.82 (1H, ddd, *J*=13.2, 3.0, 3.2 Hz), 1.65–1.53 (2H, m), 1.49 (1H, ddd, *J*=13.2, 12.6, 4.6 Hz), 1.25–1.21 (1H, m), 0.94 (3H, s), 0.93 (3H, s), 0.89 (3H, s), 0.88 (9H, s), 0.05 (3H,s), 0.03 (3H, s) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 201.7 (d), 193.1 (d), 154.3 (d), 138.1(s), 78.8 (d), 60.2 (d), 48.5 (d), 39.4 (s), 37.3 (t), 36.5 (s), 28.4 (q), 27.0 (t), 25.8 (q×3), 25.2(t), 18.0 (s), 15.8 (q), 15.3 (q), −3.9 (q), −5.0 (q) ppm. ES-MS (*m*/*z*)=365 ($M+H^+$), 233.

*5.2.7. Synthesis of 3(S)-hydroxypolygodial (**16**).* To a solution of **15** (23 mg, 0.063 mmol) in CH_3CN (2.3 mL), in a silicon vessel, 48% aqueous hydrofluoric acid (0.64 mL) was added at room temperature. The mixture was stirred at room temperature for 4 h and then $NaHCO_3$ was carefully added until the mixture was neutralized. The organic solvent was removed in vacuo and the residual aqueous layer was extracted with ethyl acetate. The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated in vacuo. Flash chromatography of the crude (30→50% ethyl acetate in petroleum ether) under N_2 afforded 11 mg of **16** (70%) as a white amorphous solid. [Found: C, 71.82; H, 8.98. $C_{15}H_{22}O_3$ requires C, 71.97; H, 8.86]; R_f (50% EtOAc/hexane) 0.23; $[\alpha]_D^{25}$ −77.7 (*c* 0.5, $CHCl_3$). IR ($CHCl_3$, cm^{-1}) ν 3508, 2968, 2938, 2860, 2732, 1722, 1681. ^1H NMR ($CDCl_3$, 400 MHz): δ 9.51 (1H, d, *J*=4.4 Hz), 9.47 (1H, br s), 7.16–7.14 (1H, m), 3.30 (1H, dd, *J*=11.0, 4.1 Hz), 2.80–2.76 (1H, m), 2.57–2.49 (1H, m), 2.46–2.36 (1H, m), 1.88–1.84 (1H, m), 1.73–1.59 (2H, m), 1.52–1.49 (1H, m), 1.27–1.23 (1H, m), 1.04 (3H, s), 0.94 (3H, s), 0.93 (3H, s) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 201.6 (d), 193.0 (d), 154.0 (d), 138.1 (s), 78.3 (d), 60.0 (d), 48.4 (d), 38.8 (s), 37.3 (t), 36.5 (s), 27.9 (q), 26.7 (t), 25.0 (t), 15.4 (q), 15.2 (q) ppm. ES-MS (*m*/*z*)=289 ($M+K^+$), 273 ($M+Na^+$), 251 ($M+H^+$).

*5.2.8. Synthesis of 3β-acetoxypolygodial (**5**).* To a solution of **16** (5.8 mg, 0.023 mmol) in CH_2Cl_2 (1 mL) acetic anhydride (2.8 μL, 0.030 mmol) was added. The solution was cooled to 0 °C and dry NEt_3 (3.8 μL, 0.028 mmol) and DMAP, in catalytic amount, were sequentially added. The solution was warmed to room temperature and stirred for 1 h. The reaction mixture was quenched with HCl 0.2 N (1 mL) and stirred for few minutes. The organic and aqueous layers were separated and the last one was extracted with CH_2Cl_2. The resulting organic layer was washed with H_2O and the resulting aqueous layer was extracted again with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatography of the crude (20–70% di AcOEt/petroleum ether) under N_2 afforded 4.2 mg of **5** (62%) as a white amorphous solid. [Found: C, 69.98; H, 8.39. $C_{17}H_{24}O_4$ requires C, 69.84; H, 8.27]; R_f (50% EtOAc/hexane) 0.60; $[\alpha]_D^{21}$ −17.0 (*c* 0.2, $CHCl_3$). IR ($CHCl_3$, cm^{-1}) ν 2967, 1726, 1682, 1644, 1243. ^1H NMR ($CDCl_3$, 400 MHz): δ 9.53 (1H, d, *J*=4.3 Hz), 9.48 (1H, s), 7.14–7.12 (1H, m), 4.55 (1H, dd, *J*=11.2, 4.3 Hz), 2.82–2.80 (1H, m,), 2.56–2.48 (1H, m), 2.46–2.40 (1H, m), 2.06 (3H, s), 1.91–1.88 (1H, m), 1.78–1.58 (3H, m), 1.35 (1H, dd, *J*=11.6, 4.9 Hz), 1.00 (3H, s), 0.96 (3H, s), 0.92 (3H, s) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 201.3 (d), 192.9 (d), 170.7 (s), 153.3 (d), 138.2 (s), 79.7 (d), 59.9 (d), 48.6 (d), 37.7 (s), 37.0 (t), 36.4 (s), 27.9 (q), 24.7(t), 23.2 (t), 21.2 (q), 16.5 (q), 15.2 (q) ppm. ES-MS (*m*/*z*)=315 ($M+Na^+$), 293 ($M+H^+$).

5.3. Assays on TRPV1 receptors

All prepared compounds have been assayed for TRPV1 sensitivity using fluorometric measurements of changes in intracellular

calcium concentration. HEK-293 (human embryonic kidney) cells were grown as monolayers in minimum essential medium supplemented with non-essential amino acids, 10% fetal calf serum and 2 mM glutamine, and maintained under 95%/5% O_2/CO_2 at 37 °C and stably transfected by using TRPV1 plasmids. Cells have been loaded for 1 h at 25 °C with 4 μM Fluo-4 methyl ester (Molecular Probes) in DMSO. $[Ca^{2+}]_i$ was determined before and after the addition of various concentrations of test compounds. After the loading, cells were washed with Tyrode pH 7.4, resuspended in Tyrode and transferred to the quartz cuvette of the fluorescence detector (Perkin–Elmer LS50B) under continuous stirring. Experiments were carried out by measuring cell fluorescence at 25 °C (λ_{EX}=488 nm, λ_{EM}=516 nm).

Acknowledgements

Financial support from Università di Salerno is gratefully acknowledged.

References and notes

1. Calixto, J. B.; Beirith, A.; Ferreira, J.; Santos, A. R. S.; Cechinel-Filho, V.; Yunes, R. A. *Phytother. Res.* **2000**, *14*, 401–418.
2. Caterina, M. J.; Schumacher, M. A.; Tominaga, M.; Rosen, T. A.; Levine, J. D.; Julius, D. *Nature* **1997**, *389*, 816–824.
3. Jansen, B. J. M.; de Groot, A. *Nat. Prod. Rep.* **2004**, *21*, 449–477 and references therein.
4. Szallasi, A.; Blumberg, P. M. *Pharmacol. Rev.* **1999**, *51*, 159–212.
5. Szallasi, A.; Biro, T.; Modarres, S.; Garlaschelli, L.; Petersen, M.; Klusch, A.; Vidari, G.; Jonassohn, M.; De Rosa, S.; Sterner, O.; Blumberg, P. M.; Krause, J. E. *Eur. J. Pharmacol.* **1998**, *356*, 81–89.
6. (a) Taniguchi, M.; Adachi, T.; Oi, S.; Kimura, A.; Katsumura, S.; Isoe, S.; Kubo, I. *Agric. Biol. Chem.* **1984**, *48*, 73–78; (b) Cimino, G.; Spinella, A.; Sodano, G. *Tetrahedron Lett.* **1984**, *25*, 4151–4152.
7. Cimino, G.; Sodano, G.; Spinella, A. *Tetrahedron* **1987**, *43*, 5401–5410.
8. De Rosa, S.; Puliti, R.; Crispino, A.; De Giulio, A.; Mattia, C. A.; Mazzarella, L. *J Nat. Prod.* **1994**, *57*, 256–262.
9. Della Monica, C.; Della Sala, G.; D'Urso, D.; Izzo, I.; Spinella, A. *Tetrahedron* **2007**, *63*, 6866–6873.
10. Al-Said, M. S.; El-Khawaja, S. M.; El-Feraly, F. S.; Hufford, C. D. *Phytochemistry* **1990**, *29*, 975–977.
11. Barrero, A. F.; Quilez del Moral, J. F.; Sànchez, E. M.; Arteaga, J. F. *Eur. J. Org. Chem.* **2006**, 1627–1641.
12. Vidari, G.; Dapiaggio, A.; Zanoni, G.; Garlaschelli, L. *Tetrahedron Lett.* **1993**, *34*, 6485–6488.
13. Brennan, N. K.; Guo, X.; Paquette, L. A. *J. Org. Chem.* **2005**, *70*, 732–734.
14. Mohapatra, D. P.; Naus, C. *J. Biol. Chem.* **2005**, *280*, 13424–13432; Huang, S. M.; Bisogno, T.; Trevisani, M.; Al-Hayani, A.; De Petrocellis, L.; Fezza, F.; Tognetto, M.; Petros, T. J.; Krey, J. F.; Chu, C. J.; Miller, J. D.; Davies, S. N.; Geppetti, P.; Di Marzo, V. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 8400–8405; Lizanecz, E.; Bagi, Z.; Pasztor, E. T.; Papp, Z.; Edes, I.; Kedei, N.; Blumberg, P. M.; Toth, A. *Mol. Pharmacol.* **2006**, *69*, 1015–1023.
15. Huang, A. X.; Xiong, Z.; Corey, E. J. *J. Am. Chem. Soc.* **1999**, *121*, 9999–10003.