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Synthesis and antiviral activity of scopadulcic acids analogues

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Abstract—The synthesis and antiviral properties of several scopadulcic acid analogues functionalized at C-6/C-7 and C-13 is reported. The preparation of advanced intermediates for the synthesis of scopadulcic acid B/scopadulciol analogues is also described. The biological study revealed the importance of polar groups at C-13, while the stereochemistry at C-8 was not critical for activity.
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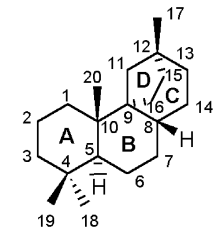
1. Introduction

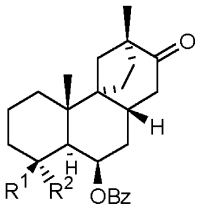
The search for new bioactive compounds from *Scoparia dulcis* L. (Scrophulariaceae) species led to the discovery of three diterpenoids, all possessing a new carbon skeleton (**1**), the scopadulcic acids A (**2**, SDA) and B (**3**, SDB) which were isolated from Paraguayan plants[1] and scopadulciol (**4**, SDC) from plants collected in Taiwan.[2] Scopadulciol (**4**), originally named dulcinol, was described earlier as a constituent of *S. Dulcis* collected in Bangladesh,[3] which is also present in specimens from China and Thailand.[4] During the past decade, Hayashi and co-workers proved that these natural products, and some derivatives, exhibit an amazing pattern of biological activities, including antiviral, antitumour, inhibition of gastric acid secretion and bone resorption as well as potentiation of known antiviral drugs.[5] Recently, SDA (**2**) has shown in vitro activity against the malaria parasite *Plasmodium falciparum*.[6]

The systematic study of phytochemicals from plants of the genus *Calceolaria* (Scrophulariaceae) led by Professor Garbarino has resulted in the isolation of 77 new diterpenes, including thyrsiflorane-type diterpenes having a carbon framework identical to that of scopadulcic acids.[7,8] For instance, thyrsiflorin A (**5**, TA), thyrsiflorin B (**6**, TB), and thyrsiflorin C (**7**, TC) were identified as constituents of *C. thyrsiflora*, where the acids were isolated as their corresponding methyl esters.[9] More recently, several new thyrsiflorins were isolated such as 13-hydroxy-thyrsiflorin **8** and its corresponding acetate **9** from *C. recta* and *C. paposana*.[10] Another C7-functionalized member such as thyrsiflorin B acetate (**10**, TBA) was isolated from *C. dentata*[11] together with isovaleroyl esters **11** and **12**, which were also found in *C. glabrata* (Fig. 1).[12]

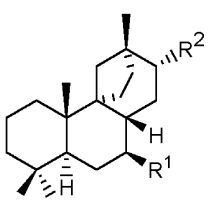
In view of the broad biological activity shown by the scopadulcic acids and some derivatives, their production by leaf culture[13] and chemical synthesis[14] has attracted the attention of biochemists and synthetic chemists. Concurrent to our studies towards the synthesis of TA (**5**),[15] starting from (+)-podocarp-(8)14-en-13-one (**13**) via enone **14**,



1 Scopadulane skeleton



2 R^1= CO_2H; R^2= CH_2OH
3 R^1= Me; R^2= CO_2H
4 R^1= Me; R^2= CH_2OH



5 R^1= H; R^2= $OCOCH_2CO_2H$
6 R^1= OH; R^2= $OCOCH_2CO_2H$
7 R^1= R^2= OH
8 R^1= H; R^2= OH
9 R^1= H; R^2= OAc
10 R^1= OAc; R^2= $OCOCH_2CO_2H$
11 R^1= $OCOCH_2CH(Me)_2$
R^2= OAc
12 R^1= $OCOCH_2CH(Me)_2$
R^2= $OCOCH_2CO_2H$

Figure 1.

Keywords: scopadulcic; diterpenes; thyrsiflorin; thyrsifloranes; antivirals.
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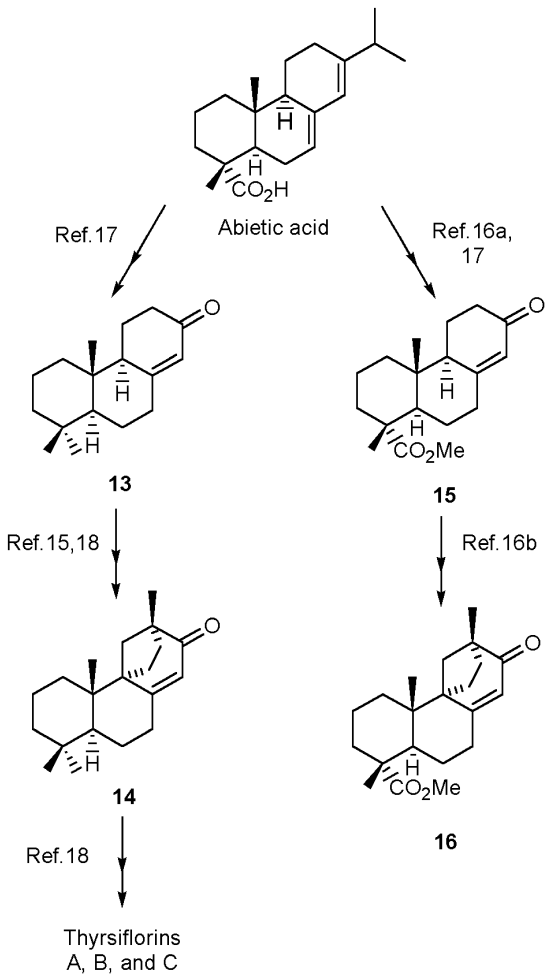


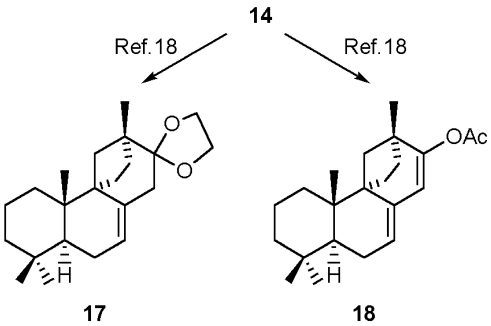
Scheme 1. Conversion of Abietic acid into scopadulenones **14** and **16**, via podocarpenones **13** and **15**.

Tagat and co-workers described the chiral preparation of the enone **16** using a different strategy from podocarpenone **15**,[16] which was prepared from abietic acid using a modification of the protocol developed in our laboratory (Scheme 1).[17] A few years later, we reported the versatility of enone **14** for the synthesis of C7-functionalized thyrsiflorins such as TBA (**10**) and TC (**7**).[18] A lack of reports on the biological activity of the thyrsiflorins prompted us to carry out a biological evaluation of several compounds prepared during the course of our synthetic studies.[19] As a result, four of these compounds showed moderate in vitro antiviral activity against *Herpes simplex* virus type II (HSV-2), likely due to their cytotoxicity. The cytotoxic activity against two human tumour cell lines (HeLa and HEp-2), however, was too weak to prompt further investigation.

In this paper, we report the synthesis of several scopadulcic acids analogues (**21**–**25**, **27**, and **34**) functionalized at C-7 and C-13, and the chemical studies towards functionalization at the C-6 position of enone **14** en route to prepare SDB and SDC. The in vitro anti-HSV-2 activity of these analogues and several C6-functionalized scopadulanes (**35**–**38**, and **41**) is also described.

2. Results and discussion

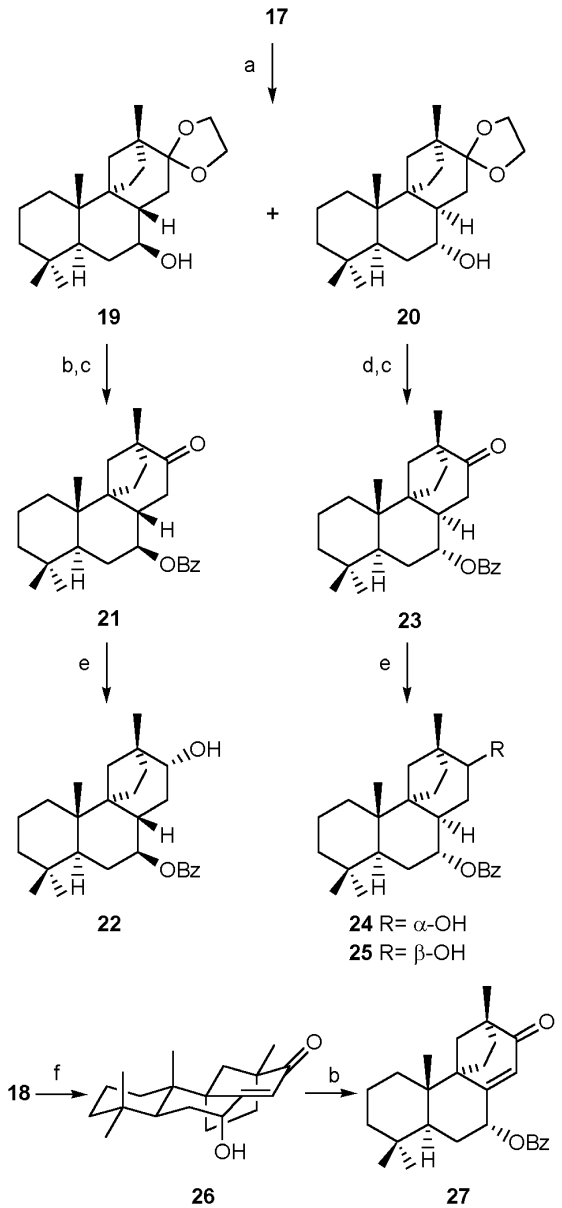
Enone **14**, ketal **17** and 7,13-dienyl acetate **18**, which were used as precursors for the synthesis of the C6- and C7-functionalized scopadulanes, were prepared from podocarpenone (+)-**13** following our reported synthetic strategy (Schemes 1 and 2).[18]



Scheme 2. Synthesis of intermediates **17** and **18**.

The synthesis of several scopadulcic acids analogues functionalized at C-7 and C-13 starting from **17** and **18** is outlined in Scheme 3. First, hydroboration of **17** followed by oxidation with H_2O_2/NaOH gave the 7β-alcohol **19** in 71% yield together with 7α-alcohol **20** (22%). Benzoylation of **19** with freshly distilled BzCl and 4-pyrrolidinopyridine (4-PP) as catalyst in Et_3N followed by hydrolysis with aqueous HCl gave benzoate **21** in 65% overall yield. Reduction of **21** using $NaBH_4$ in MeOH gave 13α-alcohol **22** as the major product in 70% yield. Similarly, the 8α-scopadulane alcohol **20** gave 7α-benzoate **23** (70% over two steps). Reduction of **23** with $NaBH_4$/MeOH gave 13β-alcohol **24** in 62% yield accompanied by a 22% of the 13α-epimer **25**. The stereochemistry at C-13 was determined by NOE experiments. In particular, irradiation of the proton signal H-7 in **25** gave a clear NOE enhancement of the signal assigned to proton H-13. On the other hand, stereoselective epoxidation of dienyl acetate **18** with *m*-chloroperoxybenzoic acid (MCPBA) (see Fig. 2) followed by treatment with $Na_2S_2O_3$/$NaHCO_3$ gave the 7α-hydroxy enone **26** in 67% yield. Assignment of the α-orientation of the 7-OH group in **26** may be inferred from its spectroscopic data, for example the ^{13}C NMR signal due to C-5 which is shifted upfield with respect to the similar signal in enone **14** (41.2 and 47.2, respectively) due to the γ-effect exerted by the OH group on C-7. Also the splitting pattern showed for H-7 (dd, J=3.4, 2.9 Hz) is in agreement with an equatorial orientation. Enone **26** was next benzoylated to afford benzoate **27** in 70% yield.

Following the preparation of scopadulcic acids analogues **22**–**27**, we turned our attention towards the functionalization of compounds **17** and **18** at position C-6 to prepare additional analogues and to explore the introduction of a 6β-benzoyl moiety via allylic oxidation (Scheme 4). We attempted several methods for the allylic oxidation at C-6 in alkene **17** to obtain enone **28**. Reduction of the enone system in **28** would give an equivalent intermediate used by Ziegler and Wallace to synthesize SDB and SDC.[14c] Treatment of **17** with *N*-bromosuccinamide (NBS) and $CaCO_3$ in aqueous dioxane,[20] chromium trioxide/3,5-dimethylpyrazole (CrO_3 3,5-DMP) complex,[21] hexacarbonyl chromium ($Cr(CO)_6$)/



Scheme 3. Synthesis of scopadulcic acids analogues functionalised at C-7, C-8, and C-13. *Reagents and conditions*: (a) (i) BH_3/THF, (ii) H_2O_2/NaOH; (b) 4-PP, BzCl, Et_3N; (c) 9:1 acetone/12 M HCl; (d) BzCl, pyridine; (e) $NaBH_4$/MeOH; (f) (i) *m*-CPBA, (ii) NaS_2O_3/$NaHCO_3$.

tert-butylhydroperoxide (TBHP),[22] and pyridium dichromate (PDC)/TBHP in the presence of celite,[23] led to complex mixtures of products. Among the reaction products of **17** with PDC/TBHP we observed some compounds without the ketal moiety. Therefore, we repeated this oxidation in the presence of pyridine, and under these basic conditions the reaction was cleaner giving as major product the ketal enone **29** in 50% yield, accompanied by other products, including a compound whose ^1H NMR spectrum was assigned to the desired structure **28**. However, **28** was obtained in less than 5% yield. The structure assignment of compound **29** was confirmed after deketalization giving rise to γ-diketone **34** (80%), which was also obtained from enol acetate **18** as we describe later.

Recent reports on the use of catalytic ruthenium, copper and

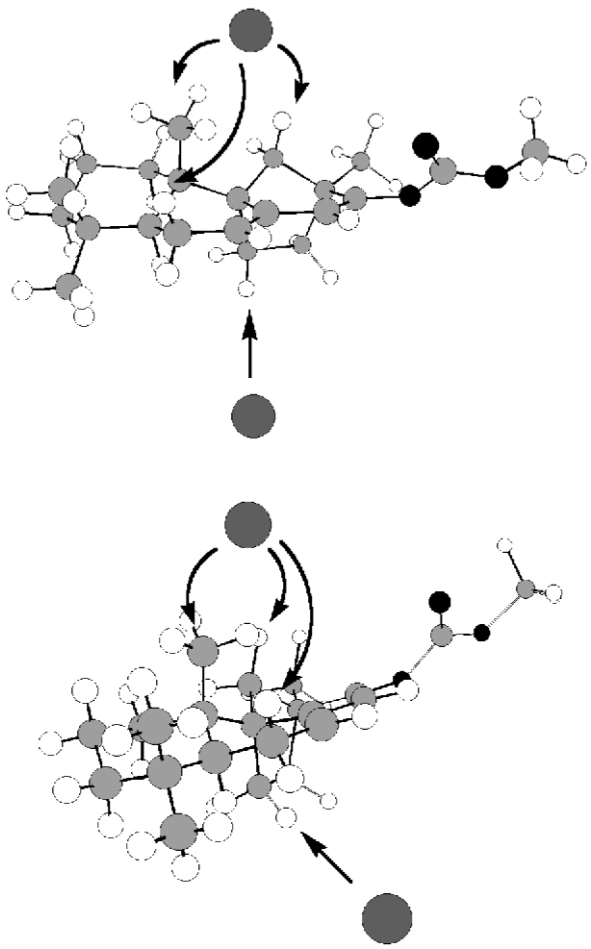
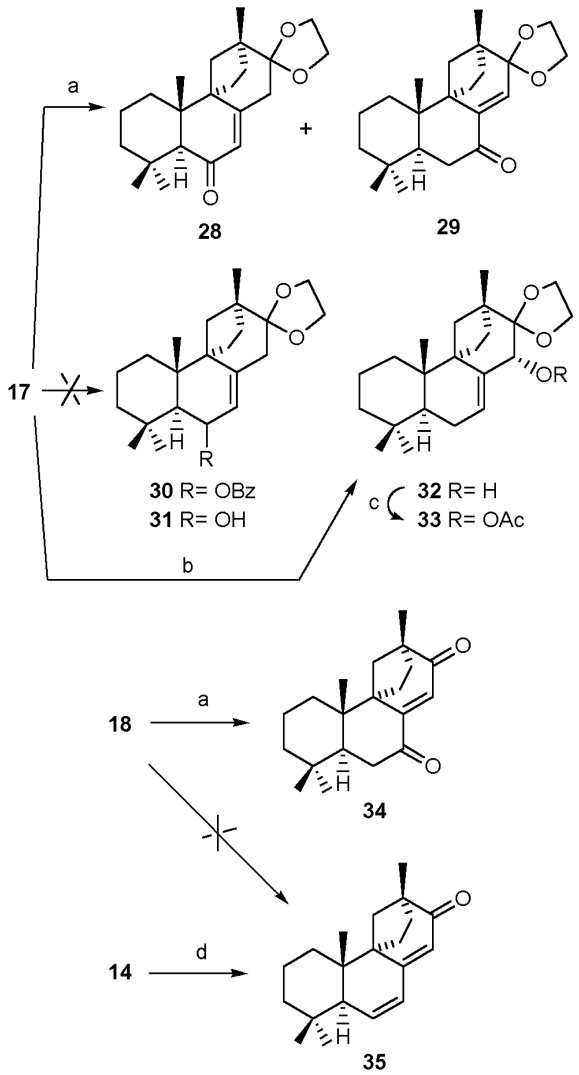


Figure 2. Steric interactions during the MCPBA epoxidation of the C7–C8 double bond of dienolacetate **18**. It can be seen a major steric hindrance in the attack from the β-face.

cobalt salts for allylic oxidations with TBHP encouraged us to carry out further experimentation in this line. Thus, we attempted the oxidation of **17** with TBHP in the presence of either ruthenium trichloride ($RuCl_3$) in acetonitrile and dichloroethane,[24] copper iodide (CuI) in acetonitrile,[25] or cobalt acetate ($Co(OAc)_2{\cdot}4H_2O$) in acetonitrile.[26] In spite of observing some reaction using $Co(OAc)_2$, the results did not merit further optimization. We also considered the possibility of carrying out a Karasch–Sosnovsky benzoyloxylation[27] on **17** with the hope that this metal-mediated reaction would favour, at least in some extent, the allylic hydrogen abstraction at C-6. If successful, we could then obtain directly the desired 6β-benzoate group (**30**). Not surprisingly, in all attempts the substrate **17** failed to react under the conditions used.[28]

After our fruitless efforts to oxidize alkene **17** to the unsaturated ketone **28**, or ester **30**, we subjected the alkene **17** to typical allylic hydroxylation conditions, which would lead to the desired enone **28** after oxidation of the alcohol **31**. The starting alkene **17** was recovered after treatment with trimethylamine *N*-oxide and selenium dioxide (SeO_2) in dioxane,[29] however, the use of catalytic SeO_2 and TBHP in DCM[30] gave a single allylic alcohol in excellent yield (95%). Unfortunately, a comparison between the NMR data of the starting material and this alcohol revealed that the

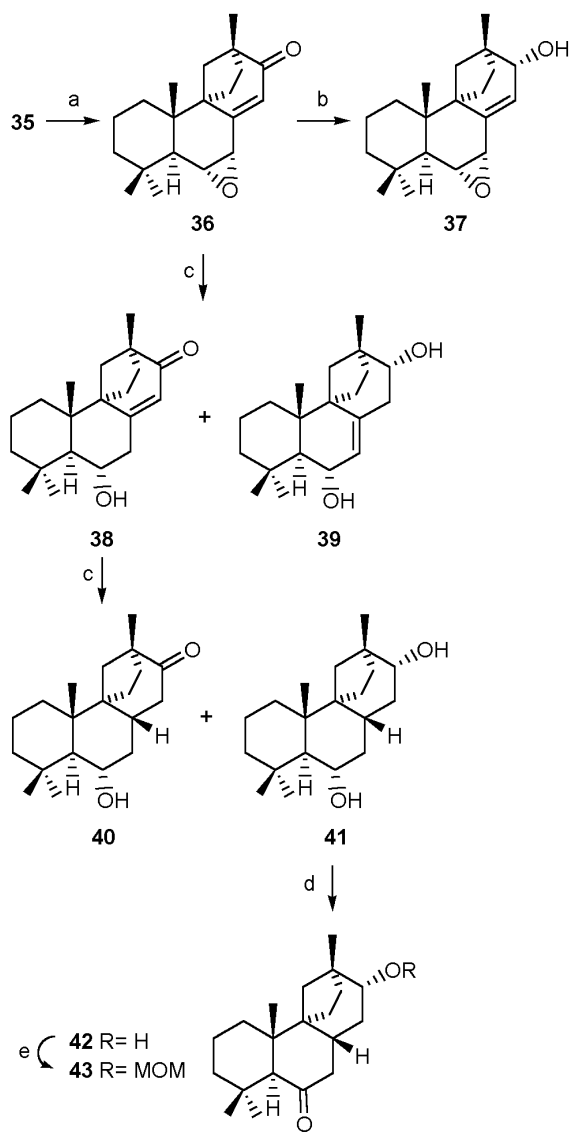


Scheme 4. Allyic oxidation of intermediates **17**, **18** and **14**. *Reagents and conditions*: (a) PDC, TBHP, celite, pyridine, ~5% of **28**, 50% of **29**; (b) cat. SeO_2, TBHP, 95%; (c) Ac_2O, 4-PP, Et_3N; (d) DDQ, PhH, 89%.

structure of the latter was actually **32**. The two H-14 signals of **17** disappeared in the ^1H NMR spectrum of the product, proton H-14 in **32** was shown as a singlet at 3.86 ppm, and the chemical shifts (^{13}C) for carbons C-5, C-6, and C-7 remained unchanged. The stereochemistry of C-14 was determined by NOE difference studies of the corresponding acetate (**33**).[31]

Parallel experiments of allylic oxidation of 7,13-dienyl acetate **18** using the conditions applied to compound **17** were also attempted, but most often gave rise to complex mixtures of products. For example, when compound **18** was treated with PDC/TBHP/celite/pyridine the γ-diketone **34** was obtained in 63% yield. This transformation probably occurs through the enone **14** since treatment of enone **14**, under the same conditions, gave the diketone **34** as major product.

The failure of our own attempts of allylic oxidation to incorporate an oxygenated function at C-6 forced us to abandon this approach and focus our attention on the preparation of dienone **35**, since Overman and co-workers reported the synthesis of SDB from a similar dienone system.[14d] Compounds with C6-hydroxy functionalization can be obtained by selective epoxidation of the C6–C7 double bond in **35** and regioselective opening of the epoxide moiety (Scheme 5). Initial treatment of dienyl acetate **18** using *N*-bromoacetamide in acetone followed by hydrolysis with $CaCO_3$ in DMF[32] failed to give the dienone **35** leading to a mixture of products. Eventually, the treatment of enone **14** with dichlorodicyanoquinone (DDQ) in refluxing benzene gave the desired dienone **35** in high yield (89%). However, epoxidation of the C6–C7 double bond in **35** was low yielding, though highly stereoselective, in agreement with the observations (α-epoxide in 40% yield) by Tagat and co-workers for the epoxidation of the 18-carbomethoxy analogue of dienone **35** with MCPBA/$NaHCO_3$.[16b] They pointed out that this result could arise from partial deprotonation of H-5 leading to by-products of oxidation in ring C. Initial attempts to epoxidise **35** were conducted using *p*-nitroperoxybenzoic acid in chloroform,[33] however,



Scheme 5. Functionalization of scopadulenone **14** at C-6. *Reagents and conditions*: (a) *m*-CPBA, pH=8, DCM, reflux, 50%; (b) NaTeH, EtoH, 60%; (c) Li, NH_3, THF, −35°C; (d) PCC, alumina, 70%; (e) MOMCl, $^iPr_2NEt$, 75%.

the desired epoxide **36** was not obtained. Little improvement was observed when the reaction mixture was buffered at pH=8, so we then changed the oxidizing agent to MCPBA. After some optimization we were able to obtain 50% yield of the epoxide **36**. For this result the MCPBA was added to a mixture of dienone **35** in DCM and pH=8 buffer in several portions (2.4 equiv.) over a period of 48 h heating at 40°C. The stereochemistry of the epoxide **36** was assured by NOE difference experiments, in particular, irradiation of the C-20 methyl signal gave NOE enhancement of the signals due to protons H-6, H-7 and H-19, and therefore confirming the α-orientation of the oxirane ring.

With the epoxide **36** in hand, we studied the regioselective opening of the oxirane ring at the allylic carbon (Scheme 5). Acid-catalysed opening of the epoxide **36** was attempted with PTSA leading to a mixture of products.[34] Following the precedent of Overman and co-workers,[14d] epoxide **36** was treated with sodium hydrogen telluride (NaTeH),[35] a known reducing agent for the opening of α,β-epoxy-ketones and esters at the α-position. To our surprise, we obtained alcohol **37**[36] as a single stereoisomer in modest yield (60%) instead of the expected alcohol **38**. This anomalous 'Luche-type' reduction could be controlled by the significant steric hindrance of the β-face, preventing the attack of the hydride species to either the oxirane ring or even the enone system.[37] Regioselective epoxide opening of similar epoxy-enone systems such as 6α,7α-epoxy−steroid derivatives using lithium−ammonia is a known method for introducing a hydroxy group at C-7 in the testosterone skeleton.[38] The carbanionic character generated under these conditions at the β-position of the enone system initiates the cleavage of the oxirane ring at the allylic position. Accordingly, treatment of **36** under these conditions led to 40% yield of the desired alcohol **38** accompanied by a product of further reduction, diol **39** (33%).[39] These conditions were then used to reduce the enone system in **38** to give ketone **40** in 26% yield and diol **41** in 55% yield.[39]

Finally, compounds **40** and **41** could be converted into SDB analogues, lacking the carboxylic acid at C-4, using standard functional group manipulation. In fact, diol **41** was oxidized selectively to ketone **42** in 70% yield using pyridinium chlorochromate (PCC) adsorbed on alumina,[40] which was then converted into its corresponding methoxymethyl (MOM) ether **43** (75%). Compound **43** is an equivalent intermediate to those used by Overman and co-workers in the synthesis of SDA and SDB.

The preparation of the scopadulane acid analogues (**21**–**25**, **27**, and **34**) and several C6-functionalized scopadulane derivatives (**35**–**38**, and **41**) enabled us to study the antiviral activities of these compounds through biological testing.

3. Antiviral evaluation

The antiviral activities of the scopadulcic acid analogues (**21**–**25**, **27**, and **34**) including compounds **35**–**38**, and **41** were evaluated against a common sexually transmitted pathogen, *Herpes simplex* virus type 2 (HSV-2). These were investigated at different concentrations depending on cytotoxicity. The antiviral and cytotoxic activities of the compounds and the positive control acyclovir against HSV-2 were determined initially using a modified end-point titration technique (EPPT)[19] and are shown in Table 1. Most of the compounds reduced the virus replication, though very weakly. The examination of this preliminary assay indicates the following: benzoates **21** and **27**, analogues of SDB lacking the carboxylic acid at C-4 and benzoate at C-6, have no significant activity. Among the benzoates in the 8α-scopadulane series, compounds **23**–**25**, only **24** exhibited some activity while **23** showed no activity at all. Among the derivatives of enone **14**, the compounds **27**, **35** and **37** were the only ones that showed slight activity. As can be seen in Table 1, the highest antiviral activity was found in compounds **24**, **27** and **37** (a reduction factor of the virus titer of $10^{1.5}$). More accurate values of anti-HSV-2 activity for compounds **24**, **27** and **37** were obtained by the standard plaque reduction assay and are given in Table 2. Therefore, none of the new derivatives improved the highest inhibitory values previously reported by us for other scopadulanes derivatives, including alcohol **8** and enone **14**. Comparison of the data for **8** with derivatives **22** and **41** suggests that 6α and 7β polar substituents in the scopadulane skeleton reduce the activity. Furthermore, in the series of enone **14** derivatives the 6α-hydroxy enone **38** is much less active.

Table 1. Cytotoxicity and anti-HSV-2 activity of scopadulane-type diterpenes on vero cells determined the end-point titration technique (EPTT)

Compound	CC_{100} (μg/mL)[a]	Viral reduction factor[b]	Antiviral activity (μg/mL)[c]
8[d]	32	$10^{2.0}$	7.5
14[d]	40	$10^{2.0}$	20
21	>50	$10^{0.5}$	12.5
22	25	$10^{0.5}$	12.5
23	>50	NA	NA
24	50	$10^{1.5}$	25
25	50	$10^{1.0}$	25
26[d]	28	$10^{1.0}$	14
27	50	$10^{1.5}$	25
34	35	$10^{1.0}$	17.5
35	>50	$10^{1.0}$	25
36	50	$10^{0.5}$	25
37	50	$10^{1.5}$	25
38	25	$10^{0.5}$	12.5
41	50	$10^{0.5}$	25
Acyclovir	>600	10^{4}	6.0

VERO, *Cercopithecus aethiops* african green monkey kidney cell line ATCC CCL 81.
[a] Minimal toxic dose that detached 100% of the cell monolayer.
[b] Ratio of the virus titer in the absence over virus titer in the presence of the tested compound.
[c] Maximal nontoxic dose that showed the highest viral reduction factor. NA, no activity.
[d] Data taken from Ref. 19.

Table 2. Anti-HSV-2 activity of scopadulane on vero cells determined by plaque reduction assay

Compound	Antiviral activity (ED_{50}) (μg/mL)
24	6.7±0.7
27	5.4±0.4
37	9.3±0.6

VERO, *Cercopithecus aethiops* african green monkey kidney ATCC CCL 81.

In summary, as we described earlier,[19] these results confirm the importance of polar groups at C-13, as well as the finding that the stereochemistry at C-8 is not critical for antiviral activity, though in comparison with the anti-HSV-1 activity of SDB and analogues[41] it is clear that the carboxylic acid at C-4 and 6β polar substituents are necessary for important antiviral properties.

4. Experimental

4.1. General experimental details

Melting points were measured on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were determined using a 5-cm path-length cell, using chloroform as solvent (concentration expressed in g/100 mL). $[\alpha]_D$-values are given in 10^{-1} deg cm^2 g^{-1}. IR spectra were taken as KBr pellets, liquid films on NaCl plates or solutions in chloroform. NMR spectra were recorded on 300 or 400 MHz spectrometers with tetramethylsilane as an internal standard. All spectra were recorded in $CDCl_3$ as solvent. Complete assignments of ^{13}C NMR multiplicities were made on the basis of DEPT experiments. HMQC and NOE experiments were used in some ^1H NMR assignments. *J* values are given in Hz. For all compounds, NMR assignments are given with respect to the numbering scheme shown in structure **1**. Reactions were monitored by thin-layer chromatography (TLC) using Merck silica gel 60 F-254 in 0.25 mm-thick plates. Compounds on TLC plates were detected under UV light at 254 nm and visualized by immersion in a 10% sulfuric acid solution and heating on a hotplate. Purifications were performed by flash chromatography on Merck silica gel (230–400 mesh). All non-aqueous reactions were carried out in an argon atmosphere in oven-dried glassware. Commercial reagent grade solvents and chemicals were used as received unless otherwise noted. THF was freshly distilled from sodium benzophenone ketyl under argon atmosphere. Combined organic extracts were washed with brine, dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. Preparation and spectroscopic data for compounds **14**, and **17**–**20** can be found in Ref. 18.

4.1.1. 7β-Benzyloxyscopadulan-13-one (21). A solution of ketal-alcohol **19** (20.0 mg, 0.057 mmol) and 4-pyrrolidinopyridine (98%, 0.9 mg, 6 μmol) as a catalyst in dry Et_3N (0.6 mL) cooled in an ice-bath was treated with freshly distilled benzoyl chloride (30 μL, 0.265 mmol) dropwise. After being stirred 3 h at room temperature, the reaction mixture was diluted with diethyl ether. Workup as usual gave the crude ketal-benzoate, which was hydrolyzed with a 9:1 mixture of acetone/12 M HCl (1.2 mL). After being stirred for 30 min at room temperature, the mixture was diluted with diethyl ether and washed with 10% aqueous $NaHCO_3$ solution, dried and concentrated. The residue was purified by column chromatography, using hexane–ethyl acetate (from 95:5 to 9:1) as eluent, to give the benzoate **21** (15.2 mg, 65%) as a colourless oil: $[\alpha]_D^{25}$=+64.0 (*c* 1.2, $CHCl_3$); IR (NaCl) 1710, 1275 cm^{-1}; ^1H NMR (300 MHz) δ 8.03 (2H, m, OCO*Ph*), 7.57 (1H, m, OCO*Ph*), 7.44 (2H, m, OCO*Ph*), 5.10 (1H, ddd, *J*=10.9, 10.9, 5.4 Hz, H-7), 2.56 (1H, dd, *J*=15.5, 6.0 Hz), 2.43 (1H, m), 2.23–2.07 (3H, m), 1.11, 1.08, 0.90 and 0.85 (3H each, each s, H-17, H-18, H-19 and H-20); ^{13}C NMR (75 MHz) δ_C 213.3 (s), 166.3 (s), 133.0 (d), 130.2 (s), 129.5×2 (d), 128.3×2 (d), 76.8 (d), 53.8 (s), 52.4 (s), 46.2 (d), 45.1 (t), 44.4 (d), 41.8 (t), 39.9 (t), 38.7 (s), 36.6 (t), 33.5 (q), 33.2 (s), 32.2 (t), 28.0 (t), 25.5 (t), 21.9 (q), 19.6 (q), 18.5 (t), 17.5 (q); HRMS (CI) $C_{27}H_{36}O_3$ requires 408.2664 (M^+), found 408.2607.

4.1.2. 7β-Benzyloxyscopadulan-13α-ol (22). To a solution of ketone **21** (8.3 mg, 0.020 mmol) in MeOH (0.8 mL) at 0°C, $NaBH_4$ (98%, 8 mg, 0.204 mmol) was added in one portion. The reaction mixture was stirred for 50 min and then diluted with diethyl ether. Usual workup followed by column chromatography, using hexane–ethyl acetate (from 95:5 to 9:1) as eluent, yielded the α-alcohol **22** (5.8 mg, 70%) as a white solid: mp 176–178°C (from hexane–ethyl acetate); $[\alpha]_D^{26}$=+30.2 (*c* 1.1, $CHCl_3$); IR (NaCl) 3600–3300, 1715, 1451, 1280 cm^{-1}; ^1H NMR (300 MHz) δ 8.05 (2H, m, OCO*Ph*), 7.56 (1H, m, OCO*Ph*), 7.44 (2H, m, OCO*Ph*), 4.96 (1H, ddd, *J*=10.9, 10.9, 5.5 Hz, H-7), 3.39 (1H, dd, *J*=10.9, 5.6 Hz, H-13), 1.07, 1.02, 0.86 and 0.82 (3H each, each s, H-17, H-18, H-19 and H-20); ^{13}C NMR (75 MHz) δ_C 166.3 (s), 132.8 (d), 130.6 (s), 129.5×2 (d), 128.3×2 (d), 77.0 (d), 76.0 (d), 53.2 (s), 46.0 (d), 44.6 (t), 44.1 (s), 42.2 (d), 42.0 (t), 38.4 (s), 34.2 (t), 33.5 (q), 33.2 (s), 32.3 (t), 30.6 (t), 28.0 (t), 25.9 (t), 23.1 (q), 22.0 (q), 18.6 (t), 17.6 (q); HRMS (CI) $C_{27}H_{38}O_3$ requires 410.2812 (M^+), found 410.2846.

4.1.3. 7α-Benzyloxy-8α-scopadulan-13-one (23). A solution of ketal–alcohol **20** (12.0 mg, 0.034 mmol) in dry pyridine (1.6 mL) cooled in an ice-bath was treated with freshly distilled benzoyl chloride (120 μL, 1.06 mmol). After being stirred for 15 h at room temperature, the reaction mixture was diluted with diethyl ether, washed with 5% aqueous HCl and brine, dried and concentrated. The crude benzoate was dissolved in acetone/HCl 12 M (9:1, 2.0 mL) and stirred for 30 min. Then, the reaction mixture was diluted with diethyl ether, washed with 10% aqueous $NaHCO_3$ and brine, dried and concentrated. The obtained residue was purified by column chromatography, using hexane–ethyl acetate (from 95:5 to 9:1) as eluent, yielded the benzoate **23** (9.6 mg, 70%) as a colourless oil: $[\alpha]_D^{25}$=−102.8 (*c* 0.9, $CHCl_3$); IR (NaCl) 1714, 1451, 1275 cm^{-1}; ^1H NMR (300 MHz) δ 8.02 (2H, m, OCO*Ph*), 7.56 (1H, m, OCO*Ph*), 7.44 (2H, m, OCO*Ph*), 5.29 (1H, m, H-7), 2.63 (1H, dd, *J*=16.7, 5.9 Hz), 1.15, 1.09, 0.97 and 0.83 (3H each, each s, H-17, H-18, H-19 and H-20); ^{13}C NMR (75 MHz) δ_C 214.4 (s), 166.3 (s), 132.9 (d), 130.3 (s), 129.5×2 (d), 128.3×2 (d), 75.4 (d), 52.8 (s), 51.2 (s), 45.1 (d), 42.9 (d), 42.0 (t), 40.7 (t), 40.2 (t), 38.2 (s), 35.6 (t), 35.5 (t), 34.0 (t), 33.9 (s), 33.1 (q), 27.8 (t), 22.3 (q), 21.2 (q), 20.1 (q), 18.9 (t); HRMS (CI) $C_{27}H_{36}O_3$ requires 408.2664 (M^+), found 408.2622.

4.1.4. 7α-Benzyloxy-8α-scopadulan-13β-ol (24) and 7α-Benzyloxy-8α-scopadulan-13α-ol (25). To a solution of ketone **23** (12.7 mg, 0.031 mmol) in MeOH (1.2 mL) and THF (0.3 mL) at 0°C, $NaBH_4$ (98%, 12 mg, 0.31 mmol) was added in one portion. The reaction mixture was stirred for 50 min and then diluted with diethyl ether. Usual workup followed by column chromatography, using hexane–ethyl acetate (from 9:1 to 8:2) as eluent, gave the β-alcohol **24**

(7.9 mg, 62%) followed by the α-alcohol **25** (2.8 mg, 22%) as colourless oils. For compound **24**: $[\alpha]_D^{26}=-3.5$ (*c* 1.1, $CHCl_3$); IR (NaCl) 3600–3370, 1699, 1451, 1283 cm^{-1}; ^1H NMR (300 MHz) δ 8.05 (2H, m, OCO*Ph*), 7.55 (1H, m, OCO*Ph*), 7.44 (2H, m, OCO*Ph*), 5.90 (1H, ddd, *J*=9.0, 6.8, 1.3 Hz, H-7), 3.46 (1H, br s, $W_{h/2}$=7 Hz, H-13), 1.07, 0.95, 0.87×2 (3H each, each s, H-17, H-18, H-19 and H-20); ^{13}C NMR (75 MHz) δ_C 167.1 (s), 132.8 (d), 130.9 (s), 129.6×2 (d), 128.3×2 (d), 75.5 (d), 74.9 (d), 50.8 (s), 45.6 (d), 45.1 (s), 41.0 (t), 40.0 (d), 38.8 (s), 38.2 (t), 36.0 (t), 34.3 (t), 33.9 (q), 33.2 (s), 31.2 (t), 29.7 (t), 26.4 (t), 24.4 (q), 22.4 (q), 20.2 (t), 17.6 (q); HRMS (EI) $C_{27}H_{38}O_3$ requires 410.2812 (M^+), found 410.2821. For compound **25**: $[\alpha]_D^{26}=-31.1$ (*c* 0.4, $CHCl_3$); IR (NaCl) 3550–3350, 1712, 1596, 1452, 1278 cm^{-1}; ^1H NMR (300 MHz) δ 8.04 (2H, m, OCO*Ph*), 7.57 (1H, m, OCO*Ph*), 7.44 (2H, m, OCO*Ph*), 5.22 (1H, ddd, *J*=9.0, 6.1, 1.1 Hz, H-7), 3.62 (1H, dd, *J*=10.2, 5.5 Hz, H-13), 1.05, 0.94, 0.84, 0.80 (3H each, each s, H-17, H-18, H-19 and H-20); ^{13}C NMR (75 MHz) δ_C 166.7 (s), 132.8 (d), 130.7 (s), 129.5×2 (d), 128.3×2 (d), 74.9 (d), 73.9 (d), 49.9 (s), 46.0 (d), 45.2 (s), 44.8 (t), 41.3 (d), 40.8 (t), 38.6 (s), 35.8 (t), 33.8 (q), 33.2 (s), 32.3 (t), 31.3 (t), 28.0 (t), 26.1 (t), 23.6 (q), 22.3 (q), 20.2 (t), 17.7 (q): HRMS (EI) $C_{27}H_{38}O_3$ requires 410.2812 (M^+), found 410.2825.

4.1.5. 7α-Hydroxy-8(14)-scopadulen-13-one (26). Solid 85% *m*-chloroperoxybenzoic acid (6.5 mg, 0.032 mmol) was added to a stirred solution of dienol acetate **18** (6.8 mg, 0.020 mmol) in 95% ethanol (0.16 mL) at 0°C. The reaction mixture was stirred for 6 h at room temperature, treated with a solution of sodium thiosulphate (10 mg) and sodium hydrogen carbonate (5 mg) in the minimum amount of water and the stirring was continued for a further hour. The solution was poured into cold water and extracted with dichloromethane. The extracts were washed with sodium hydrogen carbonate and brine. Workup as usual followed by column chromatography, using hexane–ethyl acetate (6:4) as eluent, yielded the alcohol **26** (4.2 mg, 67%) as a white solid: $[\alpha]_D^{20}=+15.3$ (*c* 0.2, $CHCl_3$); IR (NaCl) 3600–3100, 1705, 1666, 1596, 1461 cm^{-1}; ^1H NMR (300 MHz) δ 5.96 (1H, s, H-14), 4.50 (1H, dd, *J*=3.4, 2.9 Hz, H-7), 1.17, 0.93 and 0.90×2 (3H each, each s, H-17, H-18, H-19 and H-20); ^{13}C NMR (75 MHz) δ_C 205.3 (s), 169.5 (s), 127.8 (d), 70.8 (d), 58.0 (s), 45.0 (t), 42.0 (t), 41.2 (d), 37.5 (s), 34.1 (t), 33.4 (q), 33.1 (s), 32.9 (t), 30.4 (t), 29.7 (t), 22.6 (q), 21.1 (q), 20.1 (q), 18.3 (t); HRMS (EI) $C_{20}H_{30}O_2$ requires 302.2246 (M^+), found 302.2253. Due to sample size one quaternary carbon signal is not observed.

4.1.6. 7α-Benzyloxy-8(14)-scopadulen-13-one (27). To a solution of alcohol **26** (3.0 mg, 9.8 μmol) and 4-pyrrolidinopyridine (98%, 0.02 mg, 0.13 μmol) as a catalyst in dry Et_3N (0.2 mL) cooled in an ice-bath, freshly distilled benzoyl chloride (10 μL, 0.088 mmol) was added dropwise. After being stirred 2 h at room temperature, the reaction mixture was diluted with diethyl ether. Workup as usual followed by column chromatography, using hexane–ethyl acetate (from 98:2 to 9:1) as eluent, yielded the benzoate **27** (2.8 mg, 70%) as a colourless oil: $[\alpha]_D^{20}=+29.0$ (*c* 0.2, $CHCl_3$); IR (NaCl) 1713, 1677, 1596, 1264, 1107 cm^{-1}; ^1H NMR (300 MHz) δ 7.94 (2H, m, –OCO*Ph*), 7.56 (1H, m, –OCO*Ph*), 7.44 (2H, m, –OCO*Ph*), 6.14 (1H, br s, H-14), 5.98 (1H, dd, *J*=3.2, 3.2 Hz), 1.20, 1.01, 0.94 and 0.90 (3H each, each s, H-17, H-18, H-19 and H-20); ^{13}C NMR (75 MHz) δ_C 204.9 (s), 163.2 (s), 133.0 (d), 130.3 (d), 129.8 (s), 129.5×2 (d), 128.5×2 (d), 72.7 (d), 58.2 (s), 50.7 (s), 45.0 (t), 42.5 (d), 41.9 (t), 37.3 (s), 33.8 (t), 33.4 (q), 33.1 (s), 32.9 (t), 29.7 (t), 29.6 (t), 22.4 (q), 21.1 (q), 20.1 (q), 18.3 (t); HRMS (EI) $C_{27}H_{34}O_3$ requires 406.2508 (M^+), found 406.2511. Due to sample size one quaternary carbon signal is not observed.

4.1.7. 13,13-Ethylenedioxy-8(14)-scopadulen-7-one (29). To a mixture of alkene **17** (20 mg, 0.060 mmol) and oven-dried celite (77 mg) under Argon, benzene (0.73 mL), pyridine (29 μL, 0.363 mmol), PDC (114 mg, 0.303 mmol) and *t*-BuOOH (80% in *t*-BuOH, 59 μL, 0.303 mmol) were sequentially added. After being stirred for 32 h the reaction mixture was directly chromatographed on silica gel, using hexane–ethyl acetate (from 95:5 to 9:1) as eluent, to afford the enone **29** (10.4 mg, 50%) as an amorphous white solid: $[\alpha]_D^{25}=-40.0$ (*c* 0.4); IR (NaCl) 1685, 1558, 1118 cm^{-1}; ^1H NMR (300 MHz) δ 6.51 (1H, s, H-14), 4.15–4.05 (3H, m), 3.85 (1H, m), 2.51 (1H, dd, *J*=19.4, 6.3 Hz, H-6), 2.36 (1H, dd, *J*=19.4, 12.5 Hz, H-6′), 1.08, 0.96, 0.92 and 0.85 (3H each, each s, H-17, H-18, H-19 and H-20); ^{13}C NMR (75 MHz) δ_C 200.6 (s), 143.3 (s), 131.9 (d), 110.9 (s), 65.7 (t), 65.3 (t), 55.2 (s), 46.1 (s), 44.0 (d), 41.8 (t), 41.7 (t), 37.1 (t), 36.4 (s), 33.2 (s), 32.7 (q), 31.7 (t), 31.7 (t), 31.3 (t), 21.5 (q), 19.8 (q), 18.9 (q), 18.3 (t); HRMS (EI) $C_{22}H_{32}O_3$ requires 344.2351 (M^+), found 344.2344. Deketalization of **29**: enone **29** (6 mg, 0.017 mmol) was dissolved in a 9:1 mixture of acetone–HCl (0.6 mL) and stirred for 30 min at room temperature. After this time the reaction mixture was then diluted with diethyl ether, washed with 10% aqueous $NaHCO_3$ and brine. Workup (Na_2SO_4) and flash chromatography, using 9:1 hexane–ethyl acetate as eluent, gave endione **34** (4.2 mg, 80%). Data: see below.

4.1.8. 13,13-Ethylenedioxy-7-scopadulen-14α-ol (32). To a stirred mixture of SeO_2 (1.7 mg, 0.015 mmol) in DCM (0.25 mL) and *t*-BuOOH (80% in *t*-butylperoxide; 11.0 μL, 0.09 mmol) during 20 min at room temperature, the ketal **17** (10 mg, 0.03 mmol) in DCM (0.5 mL) was added. After being stirred for 1 h the solvent was removed and 1 mL of MeOH and 2 mg of $NaBH_4$ were added. After 5 min 1 mL of water was added and the resulting mixture was diluted with ethyl acetate, washed with brine and workup as usual. The residue thus obtained was chromatographed on silica gel, using 8:2 hexane–ethyl acetate as eluent, to give the alcohol **32** (10.0 mg, 95%) as a colourless oil: $[\alpha]_D^{23}=-77.5$ (*c* 0.8); IR (NaCl) 3600–3400, 1456, 1118 cm^{-1}; ^1H NMR (300 MHz) δ5.72 (1H, dd, *J*=5.3, 2.2 Hz, H-7), 4.18–3.85 (4H, m), 3.86 (1H, s, H-14), 1.01, 0.92×2 and 0.84 (3H each, each s, H-17, H-18, H-19 and H-20); ^{13}C NMR (75 MHz) δ_C 142.2 (s), 126.7 (d), 111.8 (s), 74.9 (d), 66.1 (t), 65.4 (t), 54.0 (s), 45.8 (s), 44.5 (d), 42.3 (t), 40.7 (t), 37.1 (s), 33.5 (q), 32.9 (s), 32.5 (t), 32.2 (t), 31.2 (t), 24.6 (t), 22.9 (q), 20.3 (q), 18.7 (t), 18.5 (q); HRMS (EI) $C_{22}H_{34}O_3$ requires 346.2508 (M^+), found 346.2505.

4.1.9. 8(14)-Scopadulen-7,13-dione (34). A mixture of dienolacetate **18** (6.5 mg, 0.02 mmol), celite (25 mg) in benzene (0.25 mL) and pyridine (7 μL, 0.08 mmol) was treated with PDC (32 mg, 0.08 mmol) and *t*-BuOOH (5.5 M

in nonane, 15 μL, 0.08 mmol). After being stirred for 8 h the reaction mixture was directly purified by flash chromatography, using 95:5 hexane–ethyl acetate as eluent, to give endione **34** (3.7 mg, 63%) as a yellow oil: $[\alpha]_D^{25}=-30.0$ (*c* 0.8); IR (NaCl) 1681, 1458 cm^{-1}; ^1H NMR (300 MHz) δ 6.64 (1H, s, H-14), 2.62 (1H, dd, *J*=19.0, 7.0 Hz, H-6), 2.51 (1H, dd, *J*=19.0, 12.5 Hz, H-6′), 1.24, 1.00, 0.96 and 0.89 (3H each, each s, H-17, H-18, H-19 and H-20); ^{13}C NMR (75 MHz) δ_C 204.5 (s), 200.1 (s), 158.0 (s), 128.3 (d), 57.4 (s), 52.2 (s), 44.9 (t), 44.0 (d), 41.5 (t), 37.0 (t), 36.6 (s), 33.3 (s), 32.9 (t), 32.6 (q), 31.6 (t), 31.2 (t), 21.5 (q), 20.1 (q), 19.5 (q), 18.1 (t); HRMS (EI) $C_{20}H_{28}O_2$ requires 300.2089 (M^+), found 300.2086.

4.1.10. 6,8(14)-Scopaduladien-13-one (35). To a solution of enone **14** (67.5 mg, 0.236 mmol) in benzene (2.7 mL) at reflux was added DDQ (202 mg, 0.89 mmol) and reflux continued for 24 h. The reaction mixture was then cooled and directly chromatographed on silica gel, using 9:1 hexane–ethyl acetate as eluent, to give the dienone **35** (59.8 mg, 89%) as a colourless oil which solidified on standing: mp 104–105°C (from hexane); $[\alpha]_D^{23}=-119.7$ (*c* 1.3); IR (NaCl) 3033, 3009, 1665, 1616, 1191 cm^{-1}; ^1H NMR (300 MHz) δ 6.23 (1H, dd, *J*=9.9, 2.3 Hz), 6.18 (1H, dd, *J*=9.9, 1.6 Hz), 5.68 (1H, s, H-14), 2.20 (1H, m, H-16), 2.04 (1H, br s, H-5), 1.22, 0.99 and 0.95×2 (3H each, each s, H-17, H-18, H-19 and H-20); ^{13}C NMR (75 MHz) δ_C 205.3 (s), 163.0 (s), 138.4 (d), 127.2 (d), 122.5 (d), 56.6 (s), 51.0 (s), 50.0 (d), 43.1 (t), 41.1 (t), 38.1 (s), 33.1 (t), 33.0 (q), 32.8 (s), 31.2 (t), 29.1 (t), 22.7 (q), 20.3 (q), 18.4 (q), 18.2 (t); HRMS (EI) $C_{20}H_{28}O$ requires 284.2140 (M^+), found 284.2144.

4.1.11. 6α,7α-Epoxy-8(14)-scopadulen-13-one (36). To a refluxing mixture of dienone **35** (57 mg, 0.20 mmol) in DCM (6 mL) and a buffer solution (pH=8) of Na_2HPO_4/KH_2PO_4 (6 mL), MCPBA (ca. 85%, 96 mg, 0.48 mmol) was added in four portions over 48 h. The reaction was carried out at 40°C in the dark, and after two days the mixture was then cooled, diluted with diethyl ether, washed with $Na_2S_2O_3$, 10% aqueous $NaHCO_3$ and brine. The resulting organic extract was dried (Na_2SO_4) and concentrated to afford a residue which was chromatographed on silica gel, using 8:2 hexane–ethyl acetate as eluent, to give 4.0 mg of recovered starting material followed by the epoxyenone **36** (30.7 mg, 51%) as a colourless oil which solidified on standing: $[\alpha]_D^{22}=-67.8$ (*c* 1.8); IR (NaCl) 1712, 1680, 1458 cm^{-1}; ^1H NMR (400 MHz) δ6.27 (1H, s, H-14), 3.58 (1H, d, *J*=3.8 Hz, H-7), 3.38 (1H, dd, *J*=3.8, 2.4 Hz, H-6), 1.50 (1H, br s, H-5), 1.20 (3H, s, H-17), 1.11 (3H, s, H-18), 1.08 (3H, s, H-19), 1.02 (3H, s, H-20); ^{13}C NMR (75 MHz) δ_C 203.9 (s), 162.2 (s), 131.0 (d), 56.3 (s), 56.1 (d), 53.3 (d), 51.2 (s), 48.6 (d), 44.7 (t), 41.0 (t), 37.9 (s), 33.8 (t), 33.2 (s), 32.9 (q), 30.8 (t), 30.0 (t), 22.7 (q), 20.3 (q), 20.1 (q), 18.1 (t); HRMS (EI) $C_{20}H_{28}O_2$ requires 300.2089 (M^+), found 300.2086.

4.1.12. 6α,7α-Epoxy-8(14)-scopadulen-13α-ol (37). Tellurium powder (9 mg, 0.068 mmol) and $NaBH_4$ (5 mg, 0.132 mmol) were added to degasified EtOH (0.2 mL) and the resulting solution was heated at 80°C with vigorous stirring. After 1 h, the pale purple suspension was cooled to 0°C, and a solution of epoxide **36** (5 mg, 0.017 mmol) in EtOH (0.2 mL) was added dropwise. After being stirred for 1 h 30 min, 0.1 mL of water were added and stirring continued for 30 min more, then the reaction mixture was filtered through celite. After concentration the obtained residue was purified by chromatography, using 7:3 hexane–ethyl acetate as eluent, to give 1.0 mg of recovered starting material followed by the epoxyalcohol **37** (3.0 mg, 60%) as a white solid: mp 108–110°C (hexane–ethyl acetate); $[\alpha]_D^{25}=-66.7$ (*c* 0.3); IR (NaCl) 3500–3300, 1381 cm^{-1}; ^1H NMR (300 MHz) δ5.80 (1H, d, *J*=1.9 Hz, H-14), 4.20 (1H, br s, H-13), 3.42 (1H, d, *J*=4.1 Hz, H-7), 3.22 (1H, dd, *J*=4.1, 2.3 Hz, H-6), 1.13, 1.07, 1.03 and 0.93 (3H each, each s, H-17, H-18, H-19 and H-20); ^{13}C NMR (75 MHz) δ_C 141.6 (s), 132.8 (d), 77.7 (d), 55.6 (d), 54.4 (d), 53.3 (s), 48.6 (d), 43.5 (s), 43.4 (t), 41.3 (t), 37.6 (s), 33.2 (s), 32.9 (q), 31.8 (t), 31.0 (t), 28.8 (t), 24.7 (q), 22.7 (q), 19.4 (q), 18.4 (t); HRMS (EI) $C_{20}H_{30}O_2$ requires 302.2246 (M^+), found 302.2251.

4.1.13. 6α-Hydroxy-8(14)-scopadulen-13-one (38) and 7-scopadulen-6α,13α-diol (39). To a solution of lithium (12 mg, 1.72 mmol) in NH_3 (7 mL) at −35°C under argon, epoxide **36** (22 mg, 0.073 mmol) in THF (1.2 mL) was added. After being stirred for 30 min, solid NH_4Cl was slowly added and the excess of NH_3 was evaporated under a stream of Argon. The reaction mixture was then diluted with diethyl ether and water (2 mL), washed with brine, dried and concentrated. The white solid residue was chromatographed on silica gel using hexane–ethyl acetate (from 7:3 to 5:5) eluent, to furnish alcohol **38** (9.0 mg, 41%) and the diol **39** (7.4 mg, 33%) as white solids. Compound **38**: mp 71–73°C (hexanes–ethyl acetate); $[\alpha]_D^{25}=+72.7$ (*c* 0.9); IR (NaCl) 3600–3200, 1656, 1193 cm^{-1}; ^1H NMR (300 MHz) δ5.78 (1H, dd, *J*=1.7, 1.6 Hz, H-14), 4.10 (1H, m, H-6), 3.02 (1H, ddd, *J*=17.9, 5.8, 1.6 Hz, H-7), 2.52 (1H, ddd, *J*=17.9, 6.6, 1.7 Hz, H-7′), 1.20, 1.12, 1.07 and 0.99 (3H each, each s, H-17, H-18, H-19 and H-20); ^{13}C NMR (75 MHz) δ_C 204.3 (s), 169.7 (s), 124.6 (d), 67.5 (d), 58.1 (s), 54.3 (d), 51.0 (s), 45.0 (t), 42.8 (t), 40.3 (t), 37.8 (s), 35.3 (q), 34.6 (t), 33.8 (s), 33.3 (t), 29.8 (t), 22.8 (q), 21.8 (q), 20.3 (q), 18.2 (t); HRMS (EI) $C_{20}H_{30}O_2$ requires 302.2246 (M^+), found 302.2253. Compound **39**: ^1H NMR (300 MHz) δ 5.17 (1H, br s, H-7), 4.18 (1H, br d, *J*=8.7 Hz, H-6), 3.47 (1H, dd, *J*=10.4, 6.8 Hz, H-13), 1.13, 1.09, 1.06 and 0.96 (3H each, each s, H-17, H-18, H-19 and H-20); ^{13}C NMR (75 MHz) δ_C 142.2 (s), 122.3 (d), 76.0 (d), 69.7 (d), 54.6 (s), 52.4 (d), 44.0 (t), 43.5 (s), 42.4 (t), 38.5 (s), 37.8 (t), 36.6 (q), 33.9 (t), 33.1 (s), 31.6 (t), 29.1 (t), 23.5 (q), 23.5 (q), 19.7 (q), 18.6 (t); HRMS (EI) $C_{20}H_{32}O_2$ requires 304.2402 (M^+), found 304.2410.

4.1.14. 6α-Hydroxy-13-scopadulanone (40) and 6α,13α-scopadulandiol (41). To a solution of lithium (10 mg, 1.44 mmol) in NH_3 (4 mL) at −35°C under Argon, enone **38** (8.8 mg, 0.029 mmol) in THF (1.0 mL) was slowly added dropwise. After being stirred for 8 min, the reaction mixture was cooled to −78°C and solid NH_4Cl was slowly added. The excess of NH_3 was evaporated under a stream of Argon while warming to room temperature. The reaction mixture was then diluted with diethyl ether and water (1 mL), washed with brine, dried and concentrated. The white solid residue was chromatographed on silica gel using hexane–ethyl acetate (from 7:3 to 5:5) eluent, to furnish alcohol **40** (2.3 mg, 26%) as a colourless oil and the diol **41** (4.9 mg,

55%) as white solid. Compound **40**: $[\alpha]_D^{25}=+55.0$ (*c* 0.4); ^{1}H NMR (300 MHz) δ 3.94 (1H, ddd, *J*=10.8, 10.8, 5.0 Hz, H-6), 1.15, 1.07, 1.04 and 1.00 (3H each, each s, H-17, H-18, H-19 and H-20); ^{13}C NMR (75 MHz) δ_C 214.0 (s), 69.7 (d), 53.3 (d), 52.5 (s), 52.4 (s), 45.1 (t), 43.8 (t), 43.1 (t), 41.3 (t), 40.1 (s), 39.4 (d), 36.9 (q), 36.6 (t), 33.6 (t), 33.3 (s), 24.4 (t), 22.5 (q), 19.8 (q), 18.6 (t), 18.5 (q); HRMS (EI) $C_{20}H_{32}O_2$ requires 304.2402 (M^+), found 304.2408. Compound **41**: ^{1}H NMR (300 MHz) δ 3.90 (1H, ddd, *J*=11.1, 11.1, 5.6 Hz, H-6), 3.40 (1H, dd, *J*=10.6, 5.6 Hz, H-13), 1.12, 1.02, 1.01 and 0.96 (3H each, each s, H-17, H-18, H-19 and H-20); ^{13}C NMR (75 MHz) δ_C 76.1 (d), 70.1 (d), 53.3 (d), 51.8 (s), 44.3 (t), 44.2 (s), 43.9 (t), 41.4 (t), 40.0 (s), 37.6 (t), 37.0 (d), 37.0 (q), 33.6 (t), 33.3 (s), 30.5 (t), 24.7 (t), 23.3 (q), 22.5 (q), 18.7 (t), 18.6 (q); HRMS (EI) $C_{20}H_{34}O_2$ requires 306.2559 (M^+), found 306.2564.

4.1.15. 13α-Hydroxy-6-scopadulanone (42) and 13α-methoxymethyloxy-6-scopadulanone (43). To a solution of diol **41** (4 mg, 0.013 mmol) in DCM (1.0 mL) at 0°C, PCC over alumina (35 mg; 0.8 mmol CrO_3/g) was added. After 2 h the reaction mixture was allowed to warm to room temperature and stirred for a further 1 h 30 min. Then, the reaction mixture was directly filtered on silica using fresh DCM and concentrated to give a white solid residue. This residue was chromatographed on silica using 7:3 hexane–ethyl acetate as eluent to afford the ketone **42** (2.8 mg, 70%) as a white solid: ^{1}H NMR (300 MHz) δ3.44 (1H, dd, *J*=10.4, 5.7 Hz, H-13), 1.21, 1.05, 0.93, and 0.93 (3H each, each s, H-17, H-18, H-19 and H-20); ^{13}C NMR (75 MHz) δ_C 211.1 (s), 75.7 (d), 60.9 (d), 52.3 (s), 46.8 (t), 44.5 (s), 43.7 (t), 42.7 (t), 42.5 (s), 39.2 (d), 37.6 (t), 33.0 (q), 32.1 (t), 32.1 (s), 30.6 (t), 25.6 (t), 23.2 (q), 21.8 (q), 19.9 (q), 18.2 (t). Compound **42** (2.7 mg, 0.009 mmol) in DCM (0.3 mL) at 0°C under Argon was treated with ^{i}Pr$_2$NEt (15 μL, 0.072 mmol) and MOMCl (4 μL, 0.054 mmol). The reaction mixture was allowed to warm to room temperature and stirred overnight. After being stirred for 14 h, the reaction mixture was diluted with diethyl ether, washed with 5% aqueous HCl, 10% $NaHCO_3$, and brine. Workup as usual of the resulting organic extract gave a residue which was purified by flash chromatography with 8:2 hexane–ethyl acetate as eluent to furnish methoxy methyl ether **43** (2.3 mg, 75%) as a colourless oil: ^{1}H NMR (300 MHz) δ 4.69 (1Hd *J*=6.8 Hz, MeOC*H*$_2$-), 4581Hd *J*=6.8 Hz, MeOC*H*$_2$-), 3.36 (3H, s, *Me*OCH$_2$–), 331 (1H, dd, *J*=10.3, 5.6 Hz, H-13), 1.21, 1.05, 0.93, and 0.92 (3H each, each s, H-17, H-18, H-19 and H-20); ^{13}C NMR (75 MHz) δ_C 211.1 (s), 95.6 (t), 81.1 (q), 60.8 (d), 55.4 (d), 52.3 (s), 46.9 (t), 44.2 (t), 44.1 (s), 42.8 (t), 42.5 (s), 39.1 (d), 37.5 (t), 33.0 (q), 32.1 (t), 32.0 (s), 31.6 (t), 25.7 (t), 23.8 (q), 21.8 (q), 19.9 (q), 18.2 (t). HRMS (EI) $C_{22}H_{36}O_3$ requires 348.2664 (M^+), found 348.2669.

4.2. Anti-HSV-2 assay

The antiviral tests have been carried out using the reported protocol,[19] with the following amendments:

1. To titre the virus suspension, confluent monolayer VERO cells were grown in 96-well flat-bottomed plates and were infected with 0.1 mL of serial tenfold dilutions of the virus suspension by quadruplicated for a period of 48 h.
2. *Plaque reduction assay*. Confluent monolayer VERO cells were grown in 24-well flat-bottomed plates. 500 μL of two-fold dilutions of the compound in MM were added 1 h before viral infection. Treated cells were inoculated with 100 μL of approximately 100 PFU (plaque forming units) of virus; after 1 h 400 μL of MM with 2% carboxymethylcellulose was added and finally incubated again at 37°C in humidified 5% CO_2 atmosphere for 72 h. At least two assays were carried out in duplicated with four concentrations on separate occasion and reproducible results were obtained. The ED_{50} concentration is the dilution tested that 50% eliminated macroscopic plaque formation without overt toxicity to cell monolayers.

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