Short communication

Synthesis and antiviral activity of scopadulane-rearranged diterpenes

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A new bioactive diterpene skeleton resulting from a backbone rearrangement is described. Activity of the rearranged product and several derivatives against Herpes Virus Simplex type 2 is reported.

Scoparia dulcis L. (Scrophulariaceae) is a perennial herb, which grows in tropical and subtropical regions. The roots and leaves of the plant are used as a cure of toothache, blennorrhagia, stomach disorders and diabetes. Several groups of researchers studied the plant and reported the isolation of bioactive diterpenes (Hayashi et al., 1987, 1991, 1993; Ahmed and Jakupovic, 1990). The tetracyclic diterpenoids scopadulcic acids A (1) and B (2), and dulcinol/scopadulciol (3) (Fig. 1) were isolated as the first members of a unique carbon skeleton of diterpenoids which was named “scopadulane” by Hayashi et al. (1987). In recent years, further efforts resulted in the isolation of four new natural metabolites, 4-episcopadulcic acid B (4), dulcidiol (5), isodulcinol (6) and scopadulcic acid C (7) (Fig. 1) (Ahsan et al., 2003; Giang et al., 2006).

During the past decade, Hayashi (2000) proved that these natural products, and some derivatives, exhibit a broad pattern of biological activities, including antiviral, antitumor, inhibition of gastric acid secretion and bone resorption as well as potentiation of known antiviral drugs.

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The systematic study of phytochemicals from plants of the genus Calceolaria (Scrophulariaceae) has resulted in the isolation of many new diterpenes, including thysriflorane-type diterpenes having a carbon framework identical to that of scopadulcic acids. For example, thysriflorin A (8), thysriflorin B (9) and thysriflorin C (10) (Fig. 1) were identified as constituents of Calceolaria thysriflora (Chamy et al., 1991).

The unique carbon skeleton and interesting biological activities of scopadulcic acids together with the absence of reports on the biological activity of the thysriflorins prompted us to carry out the synthesis and biological evaluation of several thysriflorins (Arnó et al., 2000; Betancur-Galvis et al., 2001) as well as several scopadulcic acid analogues (Arnó et al., 2003). Several of these compounds showed moderate in vitro antiviral activity against herpes simplex virus type 2.

During our synthetic studies towards the preparation of thysriflorin C (10) (Arnó et al., 2000), we obtained an unexpected product resulting from a p-toluensulfonic acid-catalyzed rearrangement. This product was tentatively assigned the chemical structure 11 (Fig. 2) on the basis of NMR data. The new compound has been named as “scopadurosane” as it is the result of mixing the scopadulane and the rosane (Connolly and Hill, 1991) carbon skeletons.

In this paper, we report the optimized synthesis of the molecule 11 and several derivatives in order to carry out a biological study of this new carbon framework.

1. Chemistry

Compound 11 was obtained during our studies for the synthesis of thysriflorin C (10) (Scheme 1) (Arnó et al., 2000). Our attempts to optimize the isomerization of the double bond in enone 12 to give ketal 13 led us to increase the amount of p-toluensulfonic acid (PTSA). With a PTSA concentration of 0.04 M in refluxing benzene the reaction followed a different reaction pathway leading to the formation of a new product 14 which was identified, after deketalization, as the rearranged ketone 11. We observed that this new
Fig. 1. Chemical structures of 1–10.

Fig. 2. Chemical structure of 11.

Scheme 1. Unexpected synthesis of scopadurosane 11.

Scheme 2. Synthesis of scopadurosane 11 from cyclopropane 16.

Fig. 1. Chemical structures of 1–10.

Fig. 2. Chemical structure of 11.

Scopadulane skeleton Rosane skeleton

11 Scopadurosane

Scopadurosane 11 was initially obtained as colorless oil but after several crystallizations attempts the compound crystallized in MeOH–H2O at −30 °C giving white crystals (m.p. 87–89 °C). The molecular formula C20H30O was established by HRMS ([M]+, m/z 286.2299, calcd. 286.2297). The IR spectrum showed absorption peaks at 2924 and 1721 cm−1 (carbonyl group). The 1H and 13C NMR signals of 11 were assigned by different 2D NMR, NOE difference experiments and comparison with reported data (González and Zaragozá, 2005). The 1H NMR spectrum indicated the presence of four tertiary methyl groups (δ 0.99, 0.98, 0.94, 0.89), two of which are geminal, and one methylene group (δ 2.21, dd, J = 18.3, 3.0 and δ 2.00, d, J = 18.3) next to a carbonyl group (C-13). The 13C NMR and DEPT spectra indicated the presence of 20 carbons which were assigned to seven quaternary carbons, of which one is the carbonyl group and two are olefinic (tetrasubstituted olefin), nine methylene carbons and the absence of methine carbons. NOE correlations in the NOE difference spectrum of 11 irradiating the methyl group at C-12 established the β-axial orientation of the methyl group at C-9.

Now, we have also prepared rearranged ketone 11 from cyclopropane 16 (Scheme 2). We have also confirmed that other acidic conditions as refluxing 9:1 acetone/HCl and long reaction time leads to the same result, thus, confirming that is the thermodynamically more stable product.

2. Antiviral evaluation

With the rearranged ketone 11 in hand, we have synthesized three derivatives for their biological evaluation (Scheme 3). Thus, we firstly did the epoxidation of the double bond present in 11 to give compound 17, which was subsequently reduced to afford alcohol 18. Scopadurosane 11 was also directly reduced to alcohol 19.

The antiviral activities of scopadurosanes 11, and 17–19 were evaluated against a common sexually transmitted pathogen, herpes simplex virus type 2 (HSV-2). These compounds 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) (Vlietinck et al., 1995) and are shown in Table 1.

Most of the compounds reduced moderately the virus replication, without significant cytotoxicity, except for the parent structure 11 which showed no activity. The examination of this preliminary assay indicates that the presence of an hydroxyl group at C-13 provides this skeleton with antiviral activity. More accurate values of anti-HSV-2 activity for compounds 17–19 were obtained ketone 11 could be obtained directly from enone 12, using the same acid concentration, without the presence of ethylene glycol.
3.2.1. Compound 11 assay. Cytotoxicity and anti-HSV-2 activity of scopadurosane-type diterpenes on VEROa cells determined by the end-point titration technique (EPPT) and the plaque reduction assay. Table 1: new derivative by the standard plaque reduction assay (Table 1). As a result, the new derivative 19 displayed the highest inhibitory value improving those previously reported by us for other scopadulane-type diterpenes (Betancur-Galvis et al., 2001; Arnó et al., 2003).

In summary, as we described earlier (Betancur-Galvis et al., 2001; Arnó et al., 2003), these results confirm the importance of polar groups at C-13 in scopadulane-based skeletons for anti-HSV-2 activity. The antiviral activity found in some new scopadurosanes improves the activities reported by us for scopadulane-type diterpenes; however, their effect on other viruses as well as the mechanism of action remains unknown, and needs further investigation.

3. Experimental

3.1. General experimental details

General details as reported previously (Arnó et al., 2003). All compounds prepared in this work exhibit spectroscopic data in agreement with the proposed structures.

3.2. Preparation and data for compounds 11, 16 and 17–19

3.2.1. Compound 11

A solution of ketal 15 (Arnó et al., 2000) [200 mg, 0.606 mmol] in a 9:1 mixture of acetonitrile/concentrated HCl was stirred at rt for 30 min. Then, it was diluted with diethyl ether, washed with 10% sodium bicarbonate, brine and concentrated. The residue was chromatographed on silica eluting with 95:5 hexane–ethyl acetate to give 70 mg of ketone 11 as colorless oil. The spectroscopic data were in agreement with the reported data (Arnó et al., 2000).

3.2.2. Compound 17

To a stirred solution of rearranged ketone 11 (10.4 mg, 0.036 mmol) in DCM (0.5 mL) at 0°C, m-CPBA (85%; 15 mg, 0.087 mmol) was added in one portion. After being stirred for 3 h, the reaction mixture was diluted with diethyl ether. The organic phase was washed with saturated aqueous Na2SO4, 10% NaHCO3 and brine. Workup as usual of the resulting organic extract gave a white, glassy solid which was purified by flash chromatography with 9:1 hexane–ethyl acetate to afford pure epoxide 17 (7.0 mg, 65%) as a colorless oil which solidified upon standing: \( [\alpha]D^20 = +122.9 (c 0.7) \); IR (NaCl) 2932, 2865, 1721 cm\(^{-1} \); \(^1\)H NMR (300 MHz) \( \delta 2.14 \) (1H, dd, \( J = 18.3, 2.8, \) H-14); 1.06, 1.00, 0.98 and 0.86 (3H each, s, H-17, H-18, H-19 and H-20); \(^13\)C NMR (75 MHz) \( \delta C_217.6 (s), 69.65 (s), 68.09 (s), 46.22 (t), 45.81 (t), 43.87 (s), 38.37 (t), 38.29 (s), 35.79 (s), 33.09 (s), 31.12 (t), 27.57 (q), 24.75 (t), 26.30 (t), 26.14 (t), 24.51 (q), 21.13 (t), 20.12 (q), 19.91 (q), 17.23 (t); MS (El) \( m/z \) 302 (M+, 100), 287 (50), 233 (100); HRMS C20H30O2 requires 302.2246, found 302.2256.

3.2.3. Compound 18

To a solution of rearranged ketone-epoxide 17 (4.2 mg, 0.014 mmol) in MeOH (0.55 mL) at 0°C, NaBH4 (6 mg, 0.158 mmol) was added in one portion. After being stirred for 40 min, the reaction mixture was diluted with diethyl ether. The organic phase was washed with water and brine, dried (Na2SO4) and concentrated. The obtained residue was chromatographed with 8:2 hexane–ethyl acetate to give epoxy-alcohol 18 (4.0 mg, 94%) as a colorless oil: \( [\alpha]D^20 = +97.5 (c 0.8) \); IR (NaCl) 3600–3400, 2930, 2860, 1453 cm\(^{-1} \); \(^1\)H NMR (300 MHz) \( \delta 3.54 \) (1H, dd, \( J = 9.0, 6.0 \), H-15); 1.91, 1.01, 0.98 and 0.87 (3H each, s, H-17, H-18, H-19 and H-20); \(^13\)C NMR (75 MHz) \( \delta C_217.6 (s), 73.90 (d), 69.85 (s), 69.69 (s), 39.62 (t), 39.34 (t), 38.78 (t), 37.28 (s), 34.11 (s), 33.16 (s), 33.00 (s), 32.91 (t), 27.99 (t), 27.90 (q), 26.97 (t), 26.47 (t), 24.62 (q), 24.51 (q), 21.78 (t), 20.60 (q), 17.44 (t); MS (El) \( m/z \) 302 (M+, 12), 286 (67), 271 (80), 253 (68); 235 (100); HRMS C20H32O2 requires 304.2246, found 304.2247.

Table 1: Cytotoxicity and anti-HSV-2 activity of scopadurosane-type diterpenes on VEROa cells determined by the end-point titration technique (EPPT) and the plaque reduction assay.

<table>
<thead>
<tr>
<th>Compound</th>
<th>CC50 (µg/mL)*</th>
<th>Antiviral activity (µg/mL)+</th>
<th>Antiviral activity (EC50) (µg/mL)|</th>
</tr>
</thead>
<tbody>
<tr>
<td>11(^a)</td>
<td>35</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>17</td>
<td>&gt;50</td>
<td>25</td>
<td>78 ± 0.3</td>
</tr>
<tr>
<td>18</td>
<td>&gt;50</td>
<td>25</td>
<td>9.1 ± 0.3</td>
</tr>
<tr>
<td>19</td>
<td>50</td>
<td>25</td>
<td>2.5 ± 0.7</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>&gt;600</td>
<td>6.0</td>
<td>–</td>
</tr>
</tbody>
</table>

\(^a\) VERO, Cercopithecus aethiops African green monkey kidney cell line ATCC CCL 81.

\(^b\) Minimal toxic concentration that detached 100% of the cell monolayer.

\(^c\) EPPT results obtained at 48 h, maximal non-toxic dose that showed the highest viral reduction factor. N.A., no activity.

\(^d\) 50% effective concentration based on plaque reduction assay results obtained at 72 h.

\(^e\) Data taken from Betancur-Galvis et al. (2001).
was added in one portion. After being stirred for 30 min, the reaction mixture was diluted with diethyl ether. The organic phase was washed with brine, dried (Na$_2$SO$_4$) and concentrated. The obtained residue was chromatographed with 98:2 hexane–ethyl acetate to afford pure alcohol 19 (6.0 mg, 85%) as a colorless oil: [α]$_D^{20}$ = 96.0 (c 0.5); HRMS (300 MHz) δ 3.57 (1H, br d, J = 7.0, H-13α), 1.14, 0.97, 0.94 and 0.83 (3H each, s, H-17, H-18, H-19 and H-20); 13C NMR (75 MHz) δC 135.07 (s), 131.40 (s), 74.22 (d), 41.03 (t), 40.32 (t), 38.77 (t), 38.32 (s), 34.64 (s), 34.05 (s), 33.48 (s), 32.47 (t), 29.51 (t), 29.01 (q), 28.00 (q), 27.59 (t), 26.29 (t), 25.54 (q), 24.81 (q), 21.53 (t), 20.25 (t); MS (EI) m/z 288 (M$^+$, 16), 273 (50), 255 (100); HRMS C$_{20}$H$_{32}$O requires 288.2453, found 288.2449.

3.3. Anti-HSV-2 assay

3.3.1. Cell culture and virus

The cell line was Cercocephalites aethiops African green monkey kidney cells (VERO cell line ATCC CCL-81). Cells were grown in MEM supplemented with 10% FBS, 100 units mL$^{-1}$ of penicillin, 100 mg mL$^{-1}$ of streptomycin, 2 mM L-glutamine, 0.07% NaHCO$_3$, 1% non-essential amino acids and vitamin solution. The cultures were maintained at 37 $^\circ$C in humidified 5% CO$_2$ atmosphere.

HSV-2 was obtained from the Center for Disease Control (Atlanta, GA). The virus stock was prepared from HSV-2-infected VERO cell cultures. The infected cultures were subjected to three cycles of freezing–thawing and centrifuged at 2000 rpm for 10 min. The supernatant was collected, titrated, and stored at −170 $^\circ$C in 1 mL aliquots. To titrate the virus suspension, confluent monolayer VERO cells were grown in 96-well flat-bottomed plates, infected with 0.1 mL of serial 10-fold dilutions of the virus suspension in quadruplicate and incubated for 48 h.

All compounds were dissolved in dimethyl sulfoxide (DMSO, Sigma) at 30 $^\circ$C. Stock solutions of diterpenes were prepared in DMSO and frozen at −70 $^\circ$C. The concentration of DMSO in biological assays was of 0.05%. Cell controls with DMSO at 0.05% were used.

3.3.2. End-point titration technique (EPTT)

The virus titer was obtained (the dilution of the virus required to obtained 50% lytic effect of the culture in each well in 100 mL of viral suspension, CCID$_{50}$/0.1 mL) using the Spearman–Käber formula. The technique EPTT (Vlietinck et al., 1995), with few modifications was used. Confluent monolayer VERO cells were grown in 96-well flat-bottomed plates. Two-fold dilutions of the compounds in maintenance medium (MM), identical to growth medium except 1% non-essential amino acids and vitamin solution, were added and the cells well incubated again at 37 $^\circ$C in humidified 5% CO$_2$ atmosphere for 72 h. At least two assays were carried out in duplicate with four concentrations of compound and reproducible results were obtained. The 50% effective concentration (EC$_{50}$) was defined as the concentration that reduced by 50% plaque forming units. EC$_{50}$ for each compound were obtained from dose–effect curves for linear regression methods and EC$_{50}$ values are expressed as the mean ± S.E.M. of at least four quadruplicate dilutions.

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References


