Antimicrobial Compounds from the Shoots of *Arctotis Arctotoides*

Nasim Sultana* a, AJ Afolayan b and Rauf Ahmed Bhuiyan c

*aBCSIR Laboratories Dhaka, Dr. Qudrat-i-Khuda Road, Dhanmondi, Dhaka-1205, Bangladesh, bDepartment of Botany, University of Fort Hare, Alice 5700, South Africa. and cDepartment of Botany, University of Dhaka, Dhaka-1000, Bangladesh.

Abstract

*Arctotis arctotoides* is a perennial herb used medicinally for the treatment of various ailments in the Eastern Cape, South Africa. Different extracts from the shoots of this herb showed antimicrobial activity against some bacterial species. Bioassay guided fractionation of the extracts has led to the isolation of three compounds, glycerol-1-docosanoate, zaluzanin C and perydiscolic acid. The structures were elucidated on the basis of their one- and two-dimensional NMR spectral analysis and by a comparative literature study. The evaluation of antimicrobial activity of the compounds revealed moderate activity against four Gram-positive and two Gram-negative bacteria.

Keywords: *Arctotis arctotoides*, Asteraceae, Antibacterial activity, Glycerol-1-docosanoate, Zaluzanin C, Perydiscolic acid.

Introduction

*Arctotis arctotoides* (L.F.) Hoffm (Asteraceae) (Syn. *Venidium decurrens* Less) locally known as Ubashwa is a perennial herb, commonly found as roadside weed in most costal districts of South Africa. This herb is widely used among the Xhosas for the treatment of various diseases and ailments in the Eastern Cape province (Watt and Breyer-Brandwijk, 1932). Extracts from the plant demonstrated significant activity against bacteria and fungi (Afolayan, 2003). The main constituents of this herb are sesquiterpene lactones and fernesol derivatives (Dahmy et al., 1985; Tsichritzis et al., 1990), nevertheless, little is known about the relationship between the structures and the activities of the components. We reported earlier the isolation and structural elucidation of some bioactive compounds (Sultana and Afolayan, 2003) on this herb. In continuation of our work on the same species, we now report here three more compounds; glycerol-1-docosanoate (1), zaluzanin C (2) and perydiscolic acid (3). These compounds...
were previously reported from *Zaluzania augusta*, *Z. triloba*, *Arcototis venusta*, *Perymenium discolor* (Vivar et al., 1967; Maldonado et al., 1987; Tsichritzis et al., 1990; Spring et al., 1995) but this is the first report of these compounds from *A. arctotoides*. The minimum inhibitory concentration (MIC) of each of these compounds was determined against four Gram-positive and two Gram-negative bacteria by microplate serial dilution method using 96-well microtitre plates (Eloff, 1998).

**Materials and Methods**

**General experimental procedures**

The UV spectra were run in methanol using a Beckman DU-7400 spectrophotometer. The IR data for all compounds were obtained from thin films on NaCl discs using a Perkin Elmer FT-IR spectrometer. $^1$H and $^{13}$C NMR spectra were recorded on Bruker 400 MHz Avance NMR spectrometer using CDCl$_3$ as the solvent reference at $\delta$ 7.25/77.03 ppm from the Chemistry Department, Rhodes University, South Africa. The HREIMS data were acquired from the University of Potchefstroom, South Africa on a Micromass 70-70E spectrometer. General laboratory solvents were distilled using an all-glass distiller before use. Vacuum liquid chromatography (VLC) and column chromatography (CC) were performed using Merck silica gel 60H (15µm) and silica gel (0.063-0.2mm) respectively. Analytical thin layer chromatography (TLC) was performed on precoated Merck F$_{254}$ plates and visualized by spraying with anisaldehyde-H$_2$SO$_4$ reagent. Gel filtration chromatography (GFC) was performed on Sephadex LH-20 (0.25-1mm).

**Plant materials**

The shoots of *A. arctotoides* were collected from the natural population around the University of Fort Hare (UFH) campus and air-dried. A voucher specimen of the plant (Afol. 99/03) was deposited at the Giffen Herbarium, UFH.

**Extraction and isolation**

The dried, ground plant material (492.2g) was extracted by shaking in ethanol (5 L) at room temperature for three days. The extract was filtered and evaporated to a gummy mass in a rotary evaporator under vacuum at a maximum temperature of 40°C. The gummy mass (40.4g) was partitioned between water (300 mL) and n-hexane (1.5 L) and the aqueous part was further portioned between ethyl acetate (1.5 L) and water.

The dried n-hexane extract (8.2g) was fractionated by VLC over silica gel (164 g), eluting with solvents of increasing polarity to give 26 fractions (200 mL each). All fractions were tested for their antibacterial activity using bioautographic assay (Slusarenko et al., 1989) and 16 of them showed activity. The bioactive fractions were further fraction-
ated using different chromatographic techniques. The VLC fraction (750 mg) obtained with 40-45% EtOAc in n-hexane was subsequently subjected to GFC (15 g) eluting with CHCl₃ and then CHCl₃-MeOH mixtures to give chlorophylls free 30 fractions and active fractions 6-16 were combined (500 mg) and finally CC using 2% MeOH in CHCl₃ gave compounds 1 (104 mg) and 2 (20 mg).

The EtOAc extract (12.5 g) was subjected for fractionation following the same fractionation procedure as above. A total of 30 fractions (250 ml each) were collected of which a few fractions showed activity.

The fraction (1.52 g) eluted with 90-100% EtOAc in n-hexane was subsequently subjected to GFC (30.4 g), eluted with CHCl₃ to remove chlorophylls and finally through CC using 10% MeOH in CHCl₃ yielded the compound 3 (6.8 mg).

### Glycerol-1-docosanoate (1):

Amorphous solid, Observed M⁺ 414.11076, C₂₅H₅₀O₄, calculated 414.110733, IR (liquid film) cm⁻¹: 3450, 1715, 1470, 1460, 1430, 1409, 1300, 930, 735, 720, EIMS m/z (rel.int.): 414 [M⁺] (10), 355 (18), 340 (28), 323 (38), 295 (31), 154 (25), 112 (43), 98 (100). ¹H and ¹³C NMR (CDCl₃+CD₃OD) - see Table I.

### Zaluzanin C (2):

M.p. 95-96°C, UV, IR data were in agreement with the literature (Vivar et al., 1967; Spring et al., 1995). ¹H NMR (CDCl₃) d: 2.85 m (H-1), 2.36 (ddd, J= 7.6, 2.0, 12.8 Hz, H-2a), 1.86 (ddd, J= 7.4, 14.0, 6.4 Hz, H-2b), 4.52 (bt, 7.4 Hz, H-3), 2.85 (m, H-5), 4.07 (t, J=9.2 Hz, H-6)), 2.85 (m, H-7), 2.33 (m, H-8a), 1.24 (m, H-8b), 2.43 (dd, J= 8.4, 12.6, H-9a), 2.01 (dd, J= 12.6, 4.8 Hz, H-9b).

<table>
<thead>
<tr>
<th>Position</th>
<th>¹H</th>
<th>¹³C</th>
<th>²J</th>
<th>³J</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Ha</td>
<td>4.08 (dd, J=4.8, 11.4 Hz)</td>
<td>65.0</td>
<td>C-2</td>
<td>C-1', C-3</td>
</tr>
<tr>
<td>1-Hb</td>
<td>4.04 (dd, J=6.0, 11.4 Hz)</td>
<td>69.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3.80 (q, J=4.8 Hz)</td>
<td>63.1</td>
<td>C-2</td>
<td></td>
</tr>
<tr>
<td>3-Ha</td>
<td>3.58 (dd, J= 3.6, 11.2 Hz)</td>
<td>174.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Hb</td>
<td>3.48 (dd, J=6, 11.2 Hz)</td>
<td>34.0</td>
<td>C-1'</td>
<td>C-3'</td>
</tr>
<tr>
<td>1'</td>
<td>2.28 (t, J=7.6 Hz)</td>
<td>34.0</td>
<td>C-2'</td>
<td></td>
</tr>
<tr>
<td>3'</td>
<td>1.55 (q, J=7.1 Hz)</td>
<td>24.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4'-20'</td>
<td>1.18 (br)</td>
<td>29.5-30.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21'</td>
<td>1.18 (br)</td>
<td>22.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22'</td>
<td>0.81 (t, J=6.8 Hz)</td>
<td>13.9</td>
<td>C-21'</td>
<td></td>
</tr>
</tbody>
</table>
H-9b), 6.25 (d, J=3.2 Hz, H-13a), 5.42 (d, J=3.2 Hz, H-13b), 4.97 (s, H-14a), 4.92 (s, H-14b), 5.53 (d, J=3.2 Hz, H-15a), 5.39 (d, J=3.2 Hz, H-15b). $^{13}$C NMR (CDCl$_3$) δ: 45.5 (C-1), 38.6 (C-2), 72.9 (C-3), 152.5 (C-4), 49.6 (C-5), 83.9 (C-6), 43.9 (C-7), 30.4 (C-8), 34.9 (C-9), 147.8 (C-10), 139.6 (C-11), 170.5 (C-12), 120.3 (C-13), 114.2 (C-14), 110.7 (C-15).

**Perydiscolic acid (3):**

UV, IR, EI MS (m/z (rel.int.): 262 [M]$^+$ (10), 248 [M-Me]$^+$, 244 [M-H$_2$O]$^+$, 206 [M-HCO$_2$H]$^+$ were in agreement with the literature (Maldonado et al., 1987). $^1$H NMR (CDCl$_3$) d: 6.67 (d, J=10.4 Hz, H-1), 2.36 (d, J=10.4 Hz, H-2), 2.74 (d, J=11.3 Hz, H-3a), 2.21 (dd, J=12.4, 7.5 Hz, H-3b), 5.13 (t, J=7.6 Hz, H-5), 4.68 (t, J=8.8 Hz, H-6), 2.72 (m, H-7), 2.05/1.90 (m, H-8a/H-8b), 2.48/2.36 (m, H-9a/H-9b), 6.38 (d, J=2.4 Hz, H-13a), 5.72 (d, J=2.4 Hz, H-13b), 1.71 (s, 15-CH$_3$). $^{13}$C NMR (CDCl$_3$) δ: 140.4 (C-1), 34.5 (C-2), 25.6 (C-3), 135.1 (C-4), 124.1 (C-5), 80.0 (C-6), 45.5 (C-7), 25.7 (C-8), 29.7 (C-9), 135.1 (C-10), 139.0 (C-11), 171.5 (C-12), 124.4 (C-13), 169.7 (C-14), 15.9 (C-15).

Fig. 1. The structure of compounds 1-3 isolated from *A. arctotoides*
Antibacterial activity

Laboratory strains of *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, *Shigella sonnei* were obtained from the Microbiology Department, Rhodes University, South Africa. During the extraction and purification procedure, bioautographic assay (Sultana and Afolayan, 2003; Slusarenko et al., 1989) was performed on TLC plates using *B. subtilis*. An inoculated layer of agar was sprayed with fresh culture bacteria over a developed TLC plate and incubated for 24h at 37°C. As an indicator of bacterial growth, 0.2 mg/mL p-iodonitrotetrazolium (INT) solution was sprayed over the plate and incubated at 37°C for 30 min. The inhibition of bacterial growth by compounds separated on the TLC plate was visible as white spots against a deep red background. The MIC values of the pure compounds were determined with a microplate dilution method against four Gram-positive (*B. cereus*, *B. subtilis*, *S. aureus*, *S. epidermidis*) and two Gram-negative (*E. coli*, *S. sonnei*) bacteria using 96-well microtiter plates. Each test organism was prepared by diluting 24 hrs old broth culture with sterile nutrient broth. The culture was then diluted 100-fold to give approximately 10^6 bacteria mL^-1. The microtiter plates were prepared using serial dilution (Eloff, 1998) and incubated for 24-48h at 37°C. As an incubator of bacterial growth, 40 μL of 0.2 mg/ml p-iodonitrotetrazolium (INT) solution was added to each well and incubated at 37°C for 30 min. The colourless tetrazolium salt was reduced to a red-coloured product by biological activity of the organisms, thereby making the inhibition of bacterial growth visible as clear wells. MIC values were recorded as the lowest concentration resulting in complete inhibition of bacterial growth. Each treatment was replicated thrice. Streptomycin, solvents and sample free solutions were used as standard and blank controls.

Results and Discussion

Through bioactivity-guided partitioning of extracts from *A. arctotoides*, three compounds, glycerol-1-docosanoate (1), zaluzanin C (2) and perydiscolic acid (3) were isolated.

The HREI mass spectrum of 1 showed a molecular ion m/z 414 which solved for C_{25}H_{50}O_{4}, with an intense fragment at m/z 295 due to the loss of C_{4}H_{7}O_{4}. The 1H NMR spectrum showed oxymethylene proton signals for an -O-CH_{2}-CH-(O)-CH_{2}-O- spin system attributable to glycerol and a long alkyl chain. The 13C NMR and DEPT spectra supported this hypothesis and showed an ester carbonyl signal at δ 174.4. In the HMBC experiment (Table I) the methylene protons (H-1) at 4.08/4.04 showed a 3J correlation with the carbonyl carbon (C-1), thereby revealing the single point of esterification. As the NMR spectra revealed a single linear alkyl chain, this must be C-22 and on this basis, compound 1 was identified as
glycerol-1-docosanoate. The Dictionary of Natural Products CD-ROM (2001, version 10:1) revealed a record of this compound but no information was available about its source.

The known compound 2 was identified as zaluzanin C by comparison of the NMR, IR and UV data with those which were previously reported from Z. augusta, Z. triloba (Vivar et al., 1967), Arctotis revoluta (Tsichritzis et al., 1990) and Zaluzania grayan (Spring et al., 1995).

The IR, EIMS and $^1$H NMR spectral data of compound 3 were shown similarities with the structure of perydiscolic acid which was previously reported from Perymenium discolor (Maldonado et al., 1987). The $^{13}$C NMR spectrum supported this and exhibited 15 chemical shifts including two carbonyl groups at 171.5 (C-12) and 169.7 (C-14). The positions of methyl and carboxyl groups were established from the HMBC experiment. The methyl group position was also confirmed by phase sensitive NOESY experiment. On this basis, compound 3 with a melampolide skeleton, was identified as perydiscolic acid. Since the $^{13}$C NMR data of 2 and 3 were not published before, these are shown in the extraction and isolation section. The $^{13}$C NMR data were made based on HMBC analysis.

### Conclusions

Many of the sesquiterpenoids have been reported to exhibit anti-tumor, antinociceptive and anti-malarial activities (Takaya et al., 1998; 2000; MacMorris et al., 2002). The extracts of A. arctotoides showed signif-

---

**Table II. Antibacterial activity of glycerol-1-docosanoate (1), zaluzanin C (2) and perydiscolic acid (3) isolated from A. arctotoides**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Minimum inhibitory concentration (MIC) of compounds (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Gram (+)</td>
<td></td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>125</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>250</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>250</td>
</tr>
<tr>
<td>S. epidermides</td>
<td>125</td>
</tr>
<tr>
<td>Gram (-)</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>250</td>
</tr>
<tr>
<td>Shigella sonnei</td>
<td>250</td>
</tr>
</tbody>
</table>
icant activity against bacteria and fungi (Afolayan, 2003). The antimicrobial activities of compounds 1, 2 and 3 were moderate against four Gram-positive and two Gram-negative bacteria (Table II). The present results may provide some explanation for the medicinal uses of this plant.

Acknowledgements

The authors are grateful to the National Research Foundation of South Africa and Govan Mbeki Research and Development Centre of the University of Fort Hare for financial support.

References


Takaya, Y., Takeuji, Y. Akasaka, M. Nakagawasai, O. Tadano, T. Kisara, K. Kim, H.-S., Wataya, Y., Niwa, M. and


*Received: June, 21, 2007;*  
*Accepted: November 06, 2007*