Activity of 6-Pentadecylsalicylic Acid from *Ozoroa insignis* Against Marine Crustaceans

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Abstract

Bioassay-guided fractionation of the dichloromethane extract from twigs of *Ozoroa insignis* using *Artemia salina* larvae as a model led to the isolation of 6-pentadecylsalicylic acid as the active principle. This is the first report of this so-called antifouling activity for this compound. In addition, this compound may relate to the traditional use of this plant as a herbal remedy for the treatment of urinary schistosomiasis in Africa.

Keywords: Antifouling activity, *Ozoroa insignis*, Anacardiaceae, 6-pentadecylsalicylic acid.

Introduction

*Ozoroa insignis* Del. (Anacardiaceae) is a 1.5 to 10 m high shrub or tree growing in the eastern part of Africa. A root decoction or bark infusion is employed for kidney trouble and diarrhoea in Kenya (Beentje, 1994). Distinctly, *O. insignis* was selected as one of the most promising plants for the treatment of Bilharziasis (*Schistosoma haematobium*) in Zimbabwe, because the water extract of roots and fruits showed a level of activity comparable to praziquantel (Ndamba et al., 1994). No study has been done so far, neither of active principles nor of any other chemical constituents of this plant.

The marine crustacean *Artemia salina* is ideally suited as a bioassay organism for detecting toxicity in plant extracts (Meyer et al., 1982; Sam, 1993; Lewis, 1995). Moreover, toxicity against *Artemia salina* may be an indication of potency against marine fouling organisms, more specifically, crustaceous foulers like barnacles (Mawatari 1973; Persoone & Castritsi-Catharios, 1989). In addition, the assay can be conveniently executed in microplates (Solis et al., 1993). In a preliminary screening of medicinal plants from Africa, the dichloromethane extract of dried *O. insignis* twigs was moderately active against *Artemia salina* larvae. Further bioassay-guided fractionation of the dichloromethane extract of the twigs by medium pressure liquid chromatography (MPLC) followed by centrifugal countercurrent partition chromatography (CPC) led to the isolation of 6-pentadecylsalicylic acid I which is responsible for the activity against *Artemia salina*.

Twigs of *Ozoroa insignis* Del. were collected at Munyori, Embu, Kenya in July, 1995. The plant was identified by Mathenge, SG and Mudida FP. The voucher herbarium specimen (No. 917-95) was deposited at the Herbarium of the Department of Botany of Ghent University.

Materials and methods

Air-dried twigs (94 g) were powdered mechanically and extracted in a percolator with dichloromethane. The crude extract was then filtered over Schleicher & Schuell 5951/2 filter paper and concentrated in vacuo to a dark-brown residue (3.17 g) using a rotavapor (Büchi) at 40 °C. The extract was adsorbed on 30 g of silica gel (15–40 μm, Merck) and subjected to medium pressure liquid chromatography (consisting of a 688 chromatography pump, a 687 gradient former, a 684 fraction collector, Büchi, Switzerland) over a column packed with silica gel (15–40 μm, 460 × 49 mm i.d.).
Gradient elution was applied using CH$_2$Cl$_2$ for 60 min, CH$_3$Cl$_2$-MeOH 100:0 to 95:5 in 250 min, 95:5 to 90:10 in 40 min, 90:10 to 50:50 in 60 min, 50:50 to 0:100 in 30 min, and MeOH for 15 min with a flow rate of 40 ml/min, and the eluates were monitored by a SEDEX 55 evaporative light scattering detector (LSD) (SEDERE, France) and collected every 1.5 min resulting in 22 fractions on the basis of LSD profiles. One of these fractions (F19, 742 mg) killed Artemia larvae at 25 mg/L while all the other fractions were inactive. A portion of F19 (324 mg) was injected to a centrifugal countercurrent partition chromatography (CCC-1000 High Speed Countercurrent Chromatograph, Pharma-Tech Research Corp. USA) equipped with a SSI 300 pump, and a Retriever II fraction collector. The CPC conditions were: coiled column, 3 × 120 ml; solvent, CHCl$_3$/MeOH/H$_2$O (5:6:4), the lower phase as mobile phase, the upper phase as stationary phase, column filled by pumping both phases simultaneously with the same proportion at 5 ml/min; revolution speed, 1080 rpm; head pressure, 100 psi; flow rate, 1 ml/ml; collection, 6 ml per fraction; elution mode, head to tail for 840 min and push-out. After TLC check-up, 12 fractions were obtained.

The active fractions (F3 ~ F6) were pooled (60 mg) and submitted again to CPC using a solvent system: hexane/ethyl acetate/methanol/water (1:1:1:1), the upper phase as stationary phase and the lower phase as mobile phase, head to tail mode, 2 ml/min flow rate resulting in an equilibrium volume of 82 ml. During the 360 min run time and the 90 min push-out (4 ml/min), the eluates were collected every 4 min. The last few fractions, showing activity, were pooled (21.5 mg), evaporated and the residue crystallised from hexane to yield 6.9 mg of fine white needles. The structural elucidation of this solid compound revealed it to be 6-pen-tadecylsalicylic acid 1. Most of $^1$H NMR spectral data of 1 were in good agreement with that published, although the aromatic protons were not clearly assigned in the literature (Yamagiwa et al., 1987). Protons H-3 and H-5 appear individually as doublets at $\delta$ 6.77 and 6.86 in correlation with carbons at $\delta$ 122.71 and 115.85, respectively (HETCOR). According to calculations, however, the chemical shifts should pair as 6.77/115.85 and 6.86/122.71. It could be observed in a DIFNOE experiment that the proton at $\delta$ 6.77 was proximate with the protons of the methylene function ($\delta$H 2.97), and vice versa. It should be mentioned that no effect was observed between H-3 and H-5. The $^{13}$C NMR spectra were assigned on the basis of HETCOR and by comparison with that reported for a similar compound 6-tridecysalicylic acid 2 (Kazlauskas et al., 1980).

The $^1$H, $^{13}$C, DEPT, COSY, HETCOR and DIFNOE NMR spectra were obtained with a JNM-EX270 FT NMR system (JEOL, Japan). Chemical shift values ($\delta$) were expressed in ppm from TMS as internal standard. Mass spectra were measured with a MAT-112S (70eV) Mass spectrometer (Varian, USA). IR spectra were recorded on a Nicolet Impact 410 FT-IR spectrometer (Thermo Optec, USA). Melting points were determined on a Büchi 535 apparatus (Büchi, Switzerland).

### Results and discussion

**Compound 1:** mp 88.0–88.6°C [lit.: 90.2–91.5°C (hexane) (Yamagiwa et al., 1987)]; IR (KBr) $\nu_{max}$ 2910, 2860, 1650, 1600, 1440, 1300, 1245, 1215 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 270 MHz) $\delta$ 11.17 (1H, s, COOH), 7.36 (1H, dd, $J = 7.58, 8.09$ Hz, H-4), 6.86 (1H, d, $J = 8.09$ Hz, H-3); 6.77 (1H, d, $J = 7.58$ Hz, H-5), 2.97 (2H, t, $J = 7.59$ Hz, H-1’), 1.58 (2H, m, H-2’), 1.29 (24H, m, H-3’ up to H-14’), 0.88 (3H, t, $J = 6.60$ Hz, H-15’); $^{13}$C NMR (CDCl$_3$, 67.5 MHz) $\delta$ 175.16 (s, COOH), 163.65 (s, C-2), 147.71 (s, C-6), 135.36 (d, C-4), 122.71 (d, C-5), 115.85 (d, C-3), 110.37 (s, C-1), 36.51 (t, C-1’), 32.06 (t, C-2’), 31.93, 29.83, 29.70, 29.70, 29.70, 29.70, 29.51, 29.38 (t, C-3’ – 13’), 22.70 (t, C-14’), 14.12 (q, C-15’); MS m/z 348 [M]+(73), 330 (62), 161 (100), 152 (69), 147 (90), 134 (69); Anal. C 73.53%, H 10.30%, O 13.59%, calcd for C$_{22}$H$_{36}$O$_3$, C 75.81%, H 10.33%, O 13.76%.

**Anti-crustacean activity test**

*Artemia salina* cysts were allowed to hatch in aerated artificial seawater according to Probst, with the following composition (mg/l): NaCl: 27187; MgSO$_4$·7H$_2$O: 6931; MgCl$_2$·6H$_2$O: 5112; CaCl$_2$·2H$_2$O: 1529; KCl: 681; KBr: 98; KF: 4; KI: 0.06; NaHCO$_3$: 200; Na$_2$CO$_3$·10H$_2$O: 111; H$_2$BO$_3$: 28; Sr(NO$_3$)$_2$: 15; C$_6$H$_5$Li$_3$O$_7$·4H$_2$O (lithium citrate): 2.8; PVP (polyvinylpyrrolidone): 2.5; C$_6$H$_5$FeO$_7$·5H$_2$O (iron citrate): 0.3; RbCl: 0.3. Approximately 30 *Artemia salina* instar II larvae obtained in this way were incubated in the laboratory at 25°C in 24-well microplates in 2 ml of the same medium containing different concentrations of the test extracts. Survival of the organisms was evaluated visually after 24 h and scored as follows: 3: >90% kill; 2: 10–90% kill; 1: <10% kill.

The bioassay was carried out during the whole isolation procedure, which revealed that compound 1 was the major contributor to the antifouling activity (Table 1), although less potent than TBTO (tributyltin oxide), an environmentally unfriendly heavy metal compound often used in antifouling paints.
The family Anacardiaceae is considered as an important source of various phenolic lipids including alkylphenols, alkylresorcinols and alkylcatechols (Kozubek & Tyman, 1999), which may cause strong allergenic contact dermatitis (Asakawa et al., 1987). The present findings from O. insig- nis confirm this chemotaxonomic pattern and also reveal a new kind of bioactivity for this type of compounds. The chemical constitution of O. insignis has never been thoroughly studied, although Oketch-Rabah & Dossaji (1998) had tried to unravel the chemical principles related to the molluscicidal activity of O. insignis. The latter research work failed to discover the active principle “due to technical reasons”. However “the active mixture was indicated to contain pentadecyl phenol compounds, salicylic acid and androstan derivatives”. Since 6-pentadecylsalicylic acid derivatives had been proved to have strong molluscicidal activity (Kubo et al., 1986), the report about the use of O. insignis as herbal remedy for the treatment of urinary schistosomiasis in Zimbabwe (Nyazema et al., 1994), stating tannins as the key components, is questionable.

Acknowledgement

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References


Table 1. Antifouling activity of 6-pentadecysalicylic acid against Artemia salina.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>50 ppm</th>
<th>25 ppm</th>
<th>10 ppm</th>
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<tr>
<td>6-pentadecysalicylic acid</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>tributyltin oxide</td>
<td>3</td>
<td>3</td>
<td>3</td>
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Evaluation criterion: 1: toxic to <10%, 2: to ±50% and 3: to >90% of the organisms.