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Antibacterial compounds from *Carissa lanceolata* R.Br.

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Abstract

The dichloromethane extract of the wood of *Carissa lanceolata* R.Br. (Apocynaceae) afforded the eudesmanes carissone, dehydrocarissone and carindone. This is the first account of carissone being isolated from the wood of *C. lanceolata*, and of carindone being isolated from this *Carissa* species. Dehydrocarissone has not been isolated previously from any *Carissa* species. The antibacterial activity of these natural products were examined against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. All three compounds showed activity, with dehydrocarissone and carindone having a minimum inhibitory concentration less than 0.5 mg/ml against *S. aureus* and *E. coli*.

Keywords: *Carissa lanceolata*; Apocynaceae; Conkerberry; Carissone; Dehydrocarissone; Carindone

1. Introduction

Carissa lanceolata R.Br. or conkerberry, is a poisonous plant that belongs to the order Gentianales, family Apocynaceae (Everist, 1981; Collins et al., 1990). There are thirty to thirty five *Carissa* species worldwide, with three, including *C. lanceolata*, being endemic to Australia (Forster, 1996). The Apocynaceae family, and genus *Carissa*, contain numerous plants that have been used in traditional medicine and/or have yielded pharmacologically active compounds (DeLaveau, 1980; Bentley and Brackett, 1984; Omino and Kokwaro, 1993; Taylor et al., 1996). The roots, leaves, bark and twigs of *C. lanceolata* have been used traditionally by Aboriginal communities of Western Australia, Queensland and the Northern Territory (NT) of Australia for the treatment of various medical conditions, including toothache, respiratory infection and the cleaning of sores, all of which could be bacterial in origin (Webb, 1959; White et al., 1982; Aboriginal Communities of the NT, 1993; Bindon, 1996; Lassak and McCarthy, 1983). Crude extracts of the roots and stems have been reported to show antibacterial activity, but the constituents responsible for activity have not been investigated (Joshi and Boyce, 1957; Aboriginal Communities of the NT, 1993). As part of a program to identify novel antibacterial lead compounds, and to address in part the bacterial resistance problem, we are investigating new bioactive compounds from natural sources. Here we report the isolation of antibacterial compounds from the wood of *C. lanceolata* from a bioassay-directed study.

2. Results and discussion

The wood of *C. lanceolata* was extracted sequentially with hexane, DCM, MeOH and water. The DCM extract exhibited high antibacterial activity and so was investigated further. Successive chromatographic separation of this extract, combined with antibacterial assaying of fractions, led to the isolation of three active compounds. These were identified as carissone (11-hydroxy-4-eudesmen-3-one) (**1**), dehydrocarissone (11-hydroxy-1,4-eudesmadien-3-one) (**2**) and carindone (**3**). The physiochemical data and ^{1}H NMR, ^{13}C NMR and mass spectrometry data obtained for carissone (**1**) were in agreement with the data reported in the literature (Mohr et al., 1954; Joshi and Boyce, 1957; Sathe and Rao, 1971; Achenbach et al., 1985). Carissone has been isolated from the roots of *C. lanceolata* and from other plant species (Mohr et al., 1954; Joshi and Boyce, 1957;

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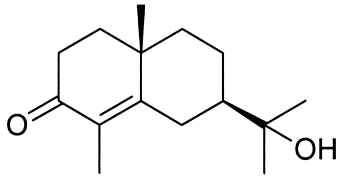
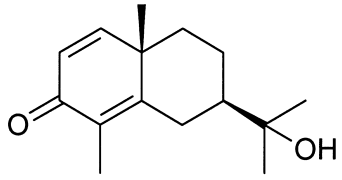
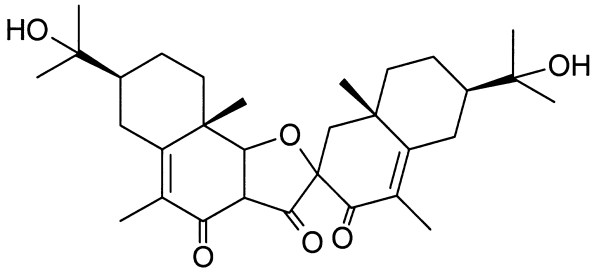
Bohlmann et al., 1977; Achenbach et al., 1985), however, this is the first report of carissone being isolated from the wood. The UV and ^{1}H NMR spectral data obtained for carindone (**3**) were identical to that obtained by Singh and Rastogi (1972). The identification was further assisted by the structural similarities with carissone (**1**) and the confirmation of molecular mass by CIMS ($[MH]^+$, m/z 513). Carindone has been isolated from other *Carissa* species (Singh and Rastogi, 1972; Zaki et al., 1981), however this is the first report of its finding in *C. lanceolata*.



1



2



3

The final compound purified was identified as (−)-dehydrocarissone (**2**). Low resolution CIMS confirmed a molecular mass of 234, 2 amu less than carissone, with the base peak at m/z 235 $[MH]^+$. The ^{1}H NMR spectrum of compound **2** was very similar to that of carissone with the main difference being the presence of two doublets at δ 6.2 and 6.7 ($J = 10$ Hz), consistent with the olefinic protons of the *cis* double bond in the C-1, C-2 position. The ^{1}H NMR and ^{13}C NMR spectral data were in agreement with that reported by Uegaki et al. (1988) and Liu et al. (1995); the latter paper describes the synthesis of (−)-dehydrocarissone (**2**) from (−)-citral, thus confirming the absolute configuration of **2**. Dehydrocarissone was first isolated as a stress compound from the leaves of the tobacco plant *Nicotiana undulata* (Solanaceae) following viral infection (Uegaki et al., 1988). It has not been isolated previously from any *Carissa* species and this is the first report of its isolation under non-stress related conditions.

Antibacterial testing showed that dehydrocarissone (**2**) was the most active compound, followed by carindone (**3**) and then carissone (**1**). From preliminary studies, dehydrocarissone had an estimated minimum inhibitory concentration (MIC) less than 0.5 mg/ml against *S. aureus*, and *E. coli*, and between 1 and 2 mg/ml against *P. aeruginosa*. The MIC of carindone was less than 0.5 mg/ml against *S. aureus* and *E. coli*, and greater than 1 mg/ml for *P. aeruginosa*. Carissone had a MIC between 0.1 and 0.5 mg/ml for *E. coli* and *S. aureus*, and between 1 and 2 mg/ml for *P. aeruginosa*. Replating on agar, and associated dilution studies, of samples that tested positive in the fluorescein diacetate assay was also undertaken to assess bactericidal or bacteriostatic activity. Generally, all three compounds tended to act bactericidally at high concentrations and bacteriostatically at low concentrations, although no compound was bactericidal against all three bacteria at less than 1 mg/ml. This is the first report of carissone (cf. Joshi and Boyce, 1957), dehydrocarissone and carindone showing antibacterial activity.

Other minor components present in the DCM and MeOH extracts also showed antibacterial activity, and these will be the subject of further investigation. This ethnobotanically targeted study has validated the traditional medicinal usage of *C. lanceolata* by Australian Aborigines.

3. Experimental

3.1. General

CI mass spectra (reactant gas: isobutane) were obtained on a Shimadzu QP-5000 by the direct insertion technique. EI mass spectra were obtained on a Shimadzu QP-5000 at 70 eV. ^{1}H NMR were recorded on a Varian Unity 300 spectrometer and ^{13}C NMR on a Varian Unity 400 spectrometer. UV absorption spectra were recorded on a Shimadzu UV-265 spectrophotometer. Prep. TLC was performed on Merck silica gel 60 F-254, 0.5 mm thick plates, and observed under UV light (254 nm). Column chromatography was perfomed on Merck silica gel 60. Solvent ratios are v/v. M.p.s were uncorrected. The optical rotations were measured on a Jasco Dip-370 Digital Polarimeter.

3.2. Plant material

The wood was collected from 1995 to 1998 at various locations in Northern Queensland. Voucher plant

specimens MEL 2064628 and MEL 2064629) are deposited in the Herbarium at the Royal Botanic Gardens, Melbourne, Australia, and refer to material collected at Millungera Station, near Julia Creek, Queensland, Australia. Chemical component similarity between wood samples was demonstrated by TLC analysis on the hexane and DCM extracts.

3.3. Extraction and isolation

Air dried and finely powdered wood (400 g) was extracted twice with hexane (2×800 ml) at room temperature for 24–48 h. This procedure was repeated on the same material sequentially with DCM, MeOH and water. The water was heated to 80–85°C before pouring over the material. In each case the solvent was evaporated to give total crude extract yields of 15 g (hexane), 27 g (DCM), 31 g (MeOH), and 2 g (H_2O). As high antibacterial activity was shown by the DCM extract, this was investigated in detail. A portion (2 g) of the concentrated DCM extracts was chromatographed successively on silica gel columns (1:100 ratio of sample:silica gel), under medium pressure, eluting with an increasing polarity gradient from DCM to EtOAc to MeOH, to produce several active fractions including A (530 mg) and B (23 mg). Further small scale column chromatography of fraction A, eluting with DCM, afforded a mixed fraction (54 mg), and impure carissone (**1**) (29 mg). The mixed fraction was active in the antibacterial assay and consequently further chromatographed with DCM–EtOAc (9:1) to afford dehydrocarissone (**2**) (33 mg, 0.11% w/w of wood), and more impure carissone (15 mg). Small scale column chromatography of the combined impure carissone fractions, eluting with DCM, afforded pure carissone (**1**) (40 mg, 0.13% w/w of wood). Fraction B was further fractionated by small scale column chromatography using gradient elution, from DCM to EtOAc, and then by prep. TLC. The prep. TLC plate was developed twice in DCM–EtOAc (2:1), and the major bands removed and washed thoroughly with DCM. Evaporation of the filtrate yielded carindone (**3**) (13 mg, 0.04% w/w of wood). In later work on the plant, carissone was obtained in crystalline form by passing the impure carissone fractions through a neutral alumina column as described by Joshi and Boyce (1957).

3.3.1. Carissone (11-hydroxy-4-eudesmen-3-one) (*1*)

Colourless solid, m.p. 74–75°C (lit. m.p. 77–78°C, Joshi and Boyce, 1957; lit. m.p. 78–79°C, Achenbach et al., 1985), R_f 0.65–0.68 (pet. ether 40–60–EtOAc, 3:7). $[\alpha]_D^{25}+125.0$ (*c*, 0.01 in $CHCl_3$) (Mohr et al., 1954: $[\alpha]_D^{19}+136.6$ (*c*, 1.025 in $CHCl_3$)). Spectral data (EIMS, ^1H NMR and ^{13}C NMR) were consistent with data reported in the literature (Sathe and Rao, 1971; Achenbach et al., 1985).

3.3.2. Dehydrocarissone (11-hydroxy-1,4-eudesmadien-3-one) (*2*)

Yellow oil, R_f 0.44–0.48 (pet. ether 40–60–EtOAc, 3:7). $[\alpha]_D^{25}-79.2$ (*c*, 0.0197 in $CHCl_3$) (Liu et al., 1995: $[\alpha]_D^{15}-84$ (*c*, 0.33 in $CHCl_3$)). ^1H and ^{13}C NMR spectral data were consistent with data reported in the literature (Uegaki et al., 1988; Liu et al., 1995). UV λ_{max} (MeOH): 245 nm (ε_{max} 6.2×10^5). CIMS *m*/*z* (rel. int.): 235 $[MH]^+$ (100), 217 $[MH\text{-}H_2O]^+$ (59).

3.3.3. Carindone (*3*)

Pale yellow solid, m.p. 266–268°C (lit. m.p. 262°C, Singh and Rastogi, 1972), R_f 0.52–0.58 (pet. ether 40–60–EtOAc, 3:7). UV and ^1H NMR spectral data were consistent with the data reported by Singh and Rastogi, 1972. CIMS *m*/*z* (rel. int.): 513 $[MH]^+$ (65), 495 $[MH\text{-}H_2O]^+$ (20).

3.4. Antibacterial assay

The bacterial cultures used were *S. aureus* ACM844, *E. coli* ACM845 and *P. aeruginosa* ACM846. These were originally obtained from the Culture Collection of the University of Queensland, Brisbane, Australia. Antibacterial activity was determined by a fluorescein diacetate assay following the method of Chand et al. (1994). Samples were tested at 4, 2, 1, 0.6, 0.5 and/or 0.1 mg/ml (in acetone) in triplicate. Samples that tested positive to this assay were then assayed for bactericidal activity. Initial test solns (20 μl of each) were plated onto agar in duplicate, incubated for 12 h at 37°C, and visible colonies counted. This was accompanied by serial dilutions of each bacterial broth, for comparison of colony counts.

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