The structure of japonene, a hopane triterpene from *Heterodermia* lichens (Physciaceae, Ascomycota)

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Abstract

The triterpene japonene [hopane-6α,16α,22-triol] (1) has been isolated from the lichen *Heterodermia propaguligera*, and its structure established by mass spectrometry and NMR spectroscopy.

Introduction

Japonene was first detected in the lichen *Heterodermia japonica* (Sato) Swinscow & Krog (Elix 2011a), but has since been found to be widely distributed in the genus *Heterodermia* (Elix 2011b; Mongkolsuk *et al.* 2015). It was initially called japonin (Elix 2011a), but that name had to be abandoned because it had been introduced earlier for an alkaloid present in the higher plant *Orixa japonica* (Rutaceae) by Ha-Huy-Kê & Luckner (1970). Although japonene could readily be characterized by thin-layer chromatography (Elix 2011a; Mongkolsuk *et al.* 2015), its structure remained unknown. This paper describes the elucidation of its structure.

Methods

All nuclear magnetic resonance spectra (NMR) were recorded on a Bruker Biospin GmbH spectrometer at 300 K with probe 5 mm PABBO BB/19F-1H/D Z-GRD Z116098/0258. ^{1}H and ^{13}C NMR experiments were undertaken at 400 MHz and 100 MHz respectively. Low-resolution electrospray mass spectra (LRESIMS) were recorded on a Waters Micromass ZMD single quadrupole mass spectrometer using an ionization field of 3500V, source temperature 100°C, desolvation temperature 150°C, coupled to a Waters Alliance 2995 HPLC system. High resolution electrospray mass spectra (HRESIMS) were recorded on a Waters LCT Premier mass spectrometer using an ionization field of 2500 V, source temperature 100°C, desolvation temperature 150°C, coupled to a Waters Alliance 2995 HPLC system. All MS used positive electron ionization mode.

Fluorescent active thin-layer chromatographic (TLC) plates (silica gel 60 F_{254}) and silica gel (230–400 mesh) for flash chromatography were supplied by Merck Millipore Pty Ltd, Bayswater, Victoria 3153, Australia.

Extraction of *Heterodermia propagulifera* (Vain.) Dey

The lichen *Heterodermia propaguligera* was collected on shaded granite rocks at Mount Chudalup, 17 km SSE of Northcliffe, Western Australia, 34°46'S, 116°05'E, 165 m alt., *J.A. Elix 41219, H.T. Lumbsch & H. Streimann*, 14.ix.1994 (CANB). The dried lichen thallus (19.90 g) was extracted in a Soxhlet extractor with anhydrous diethyl ether (350 mL) for 48 h. The ether extract was filtered, the filtrate concentrated to 30 mL, and then filtered again. The final filtrate was concentrated to dryness to yield 770 mg of a colourless solid. A portion of that solid (66 g) was purified by sequential column chromatography over silica gel using ethyl acetate and then 70% ethyl acetate / light petroleum as eluant. Three major bands developed, and they were collected and concentrated in turn. The faster moving band afforded zeorin [hopane-6α,22-diol] (2), and the subsequent band yielded 16β-acetoxyhopane-6α,22-diol (3) with m/z $[M+Na]^+$ 467.4 and m/z $[M+Na]^+$ 525.5 on ESIMS respectively. The TLC behaviour of those two compounds was identical with that of authentic material. The third band from the chromatographic column afforded japonene [hopane-6α,16α,22-triol] (1) as a white solid, $[\alpha]_D^{24}$ +28.0 (c = 0.16, $CDCl_3$), with chromatographic properties identical to that reported previously (Elix 2011a; Mongkolsuk *et al.* 2015).

Structural elucidation of japonene

Japonene [hopane-6α,16α,22-triol] (1) was obtained as a colourless, crystalline solid with m/z $[M+Na]^+$ 483.3814 on high-resolution ESIMS with a sodiated adduct ion corresponding to $C_{30}H_{52}O_3Na$, thus establishing the molecular formula of japonene as $C_{30}H_{52}O_3$. The spectroscopic properties of japonene were consistent with its being a hopanetriol, but its Rf values differed significantly from those of leucotylin [hopane-6α,16β,22-triol] (4) on TLC. Leucotylin (4) is also widely distributed in *Heterodermia* species. Assignments in the ^{1}H-NMR spectrum of japonene are summarized in Table 1. As expected, the ^{13}C-NMR spectrum of japonene (Table 2) exhibited thirty carbon signals. In the HSQC spectrum, carbon signals assigned to C6 (δ 69.45) and C16 (δ 69.52) were associated with the corresponding oxymethine proton signals due to H6 (δ 3.96) and H16 (δ 4.69) respectively, which indicates that two secondary hydroxy groups are present in japonene.

In addition, eight carbon signals (δ 17.22–29.23) were strongly correlated with eight singlet CH_3 proton signals (δ 0.87, 1.01, 1.02, 1.11, 1.16, 1.18, 1.34, 1.39), indicating that the methyl groups are bonded to quaternary carbon atoms. Because japonene is isomeric with leucotylin (4), their respective ^{13}C-NMR spectral data have been compared in Table 2. The carbon chemical shifts of those two compounds are well matched except for C15, 16, 17, 18, 19, 21, 22, 23 and 28, the majority of which are located in rings D and E and are centred around C16, suggesting that their structural differences most likely occur in that region.

Correlations in the gHMBC spectrum are illustrated in Figure 2. All the observations are consistent with structure (1) for japonene, with the same carbon skeleton as leucotylin (4) but with a 16α- rather than a 16β-hydroxy group.

In the NOESY spectrum of japonene, H24 (δ 1.02), H25 (δ 0.87) and H26 (δ 1.01), protons are correlated with one another, whereas the H23 (δ 1.16) proton exhibits no association with H25 and H26. In addition, there is no correlation between the H26 and H27 (δ 1.34) protons, but the methine proton signal H6 (δ 3.96) is correlated with the H24, H25 and H26 protons (Figure A). Those correlations confirm that C24, C25 and C26 have β-stereochemistry, whereas C23, C27 and the 6-OH have α-configuration. This established that japonene (1) possessed the same stereochemistry in rings A, B and C as does leucotlyin (4). Further, the methine proton signal H16 (δ 4.69) was correlated with H30 (δ 1.39), and in addition the H27 (δ 1.34), H28 (δ 1.11) and H29 (δ 1.18) signals were correlated with one another. Similarly, protons H29 and H30 were correlated with each other (Figure 3B). Those observations confirm that C30 has β-configuration, whereas C28, C29 and the 16-OH have α-configurations. The configuration of C22 in japonene (1) was confirmed by the comparison of the ^{13}C-NMR data for compounds 1 and 4, with 22-hydroxyhopane (5) and 22-hydroxyisohopane (6) (Table 2). Because the C30 chemical shift changes significantly from approximately δ 30 (α-side chain at C21 in 5) to *c.* δ 26 (β-side chain at C21 in 6) (Ageta *et al.* 1993), both japonene (1) (δ 31.2) and leucotylin (4) (δ 30.2) have α-side chains at C21. The apparent difference in chemical shift between C23 in japonene (δ 36.9) and leucotylin (δ 29.8) was found to be due to the incorrect assignment of the latter (Brahmachari & Chatterjee 2002), and was confirmed by the comparison with compound 2 (δ 36.8) (Table 2) (Elix *et al.* 1982).

References

Ageta, H; Shiojima, K; Suzuki, H; Nakamura, S (1993): NMR spectra of triterpenoids. I. Conformation of the side chain of hopane and isohopane, and their derivatives. *Chemical and Pharmaceutical Bulletin* (Tokyo) **41**, 1939–1943.

Brahmachari, G; Chatterjee, D (2002): Triterpenes from *Adiantum lunulactum*. *Fitoterapia* **73**, 363–368.

Elix, JA (2011a): Three new species of *Heterodermia* (Physciaceae: Ascomycota) from Australia. *Australasian Lichenology* **68**, 16–21.

Elix, JA (2011b): *Australian Physciaceae* (*Lichenised Ascomycota*). Australian Biological Resources Study, Canberra. Version 18 October, 2011. http://www.anbg.gov.au/abrs/lichenlist/PHYSCIACEAE.html

Elix, JA; Whitton, AA; Jones, AJ (1982): Triterpenes from the lichen genus *Physcia*. *Australian Journal of Chemistry* **35**, 641–647.

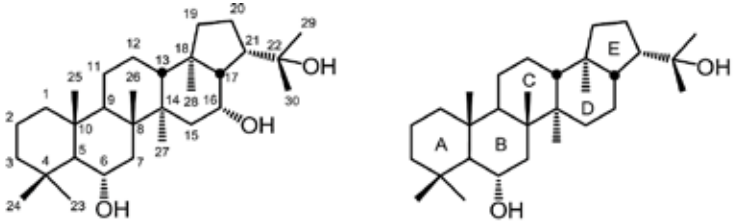
Ha-Huy-Kê; Luckner, M (1970): Japonin, 1-methyl-2-phenyl-3,6-dimethoxy-chinolon-(4), ein neues alkaloid aus *Orixa japonica*. *Phytochemistry* **9**, 2199–2208.
Mongkolsuk, P; Meesim, S; Poengsungnoen, V; Buaruang, K; Schumm, F; Kalb, K (2015): The lichen family Physciaceae in Thailand - II. The genus *Heterodermia* sensu lato. *Phytotaxa* **235**, 1–66.

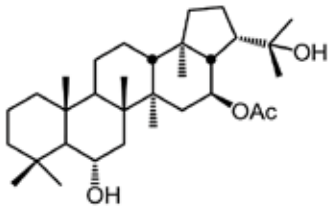
Table 1 ^{1}H-NMR data (400MHz, $CDCl_3$) of japonene (1)

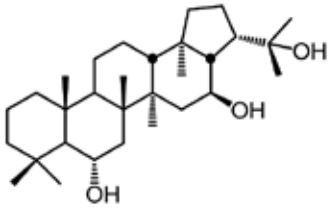
H-5	0.84
H-6	3.96
H-7	1.54
H-15	1.54, 1.75
H-16	4.69
H-17	1.55
H-23	1.16
H-24	1.02
H-25	0.87
H-26	1.01
H-27	1.34
H-28	1.11
H-29	1.18
H-30	1.39

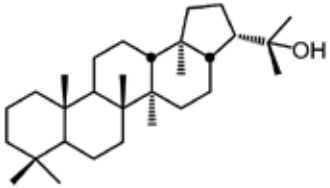
Table 2 ^{13}C-NMR data (100MHz, $CDCl_3$) of compounds 1,2,4,5 and 6

	1	4	5	6	2
C-1	40.44	40.4	40.29	40.31	40.4
C-2	18.67	18.4	18.69	18.69	18.5
C-3	43.93	43.6	42.10	42.10	43.8
C-4	33.78	33.4	33.25	33.25	33.6
C-5	61.27	61.1	56.08	56.11	61.1
C-6	69.45	69.2	18.69	18.69	69.3
C-7	45.6	45.3	33.21	33.25	45.5
C-8	43.27	42.8	41.90	41.87	42.9
C-9	48.8	49.5	50.34	50.42	49.5
C-10	39.47	39.1	37.38	37.39	39.4
C-11	21.21	20.9	20.89	20.91	21.1
C-12	23.93	23.9	24.13	23.80	24.0
C-13	50.09	49.5	49.83	48.56	49.8
C-14	41.7	41.6	41.84	41.72	41.9
C-15	40.65	44.9	34.37	32.67	34.3
C-16	69.52	66.8	21.95	23.21	21.9
C-17	55.93	57.4	53.91	52.07	54.0
C-18	43.35	46.2	44.09	44.90	44.0
C-19	42.59	39.3	41.23	39.50	41.3
C-20	26.39	25.5	26.60	24.82	26.6
C-21	50.21	51.4	51.11	51.05	51.1
C-22	72.31	70.7	73.96	73.62	73.9
C-23	36.89	29.8	33.41	33.41	36.8
C-24	22.27	21.8	21.60	21.60	22.1
C-25	17.22	17.1	15.83	15.88	17.1
C-26	18.62	17.7	16.71	16.73	18.3
C-27	18.05	17.3	17.03	16.70	17.1
C-28	17.41	15.6	16.14	15.37	16.1
C-29	29.23	28.2	28.72	29.45	28.7
C-30	31.15	30.2	30.85	26.49	30.9









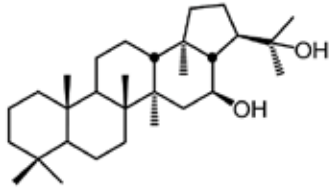


Figure 1. Structure of triterpenes

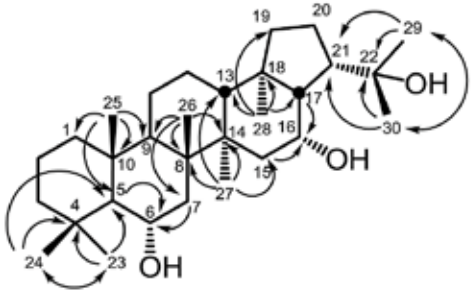


Figure 2. HMBC correlations of japonene (1)

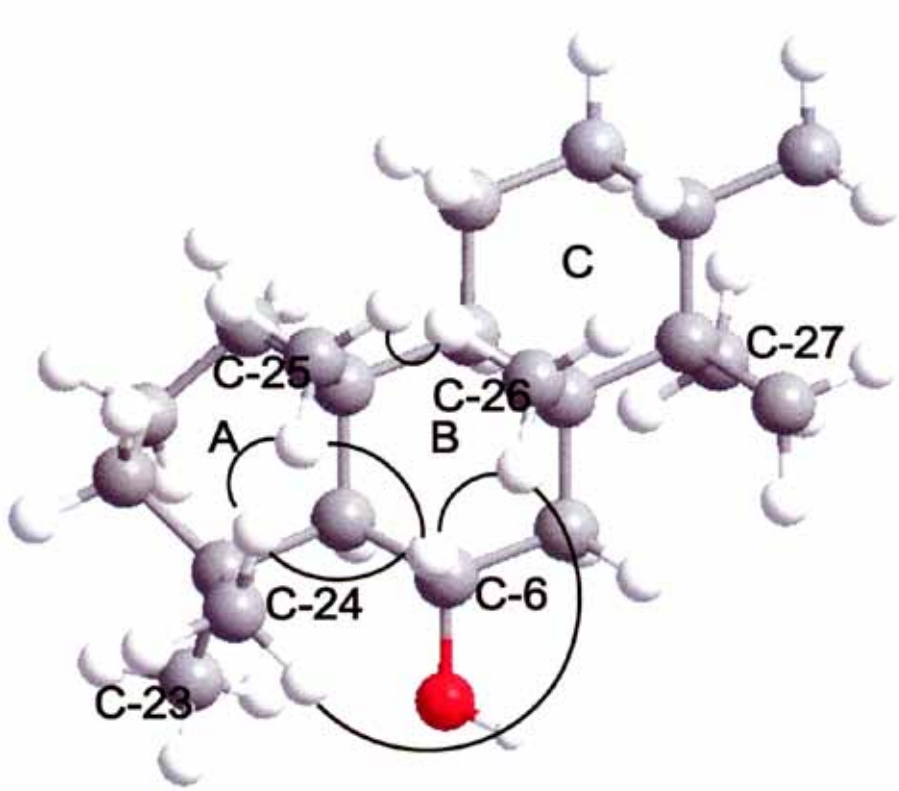


Figure 3A. NOESY associations of compound 1 in ring A, B and C

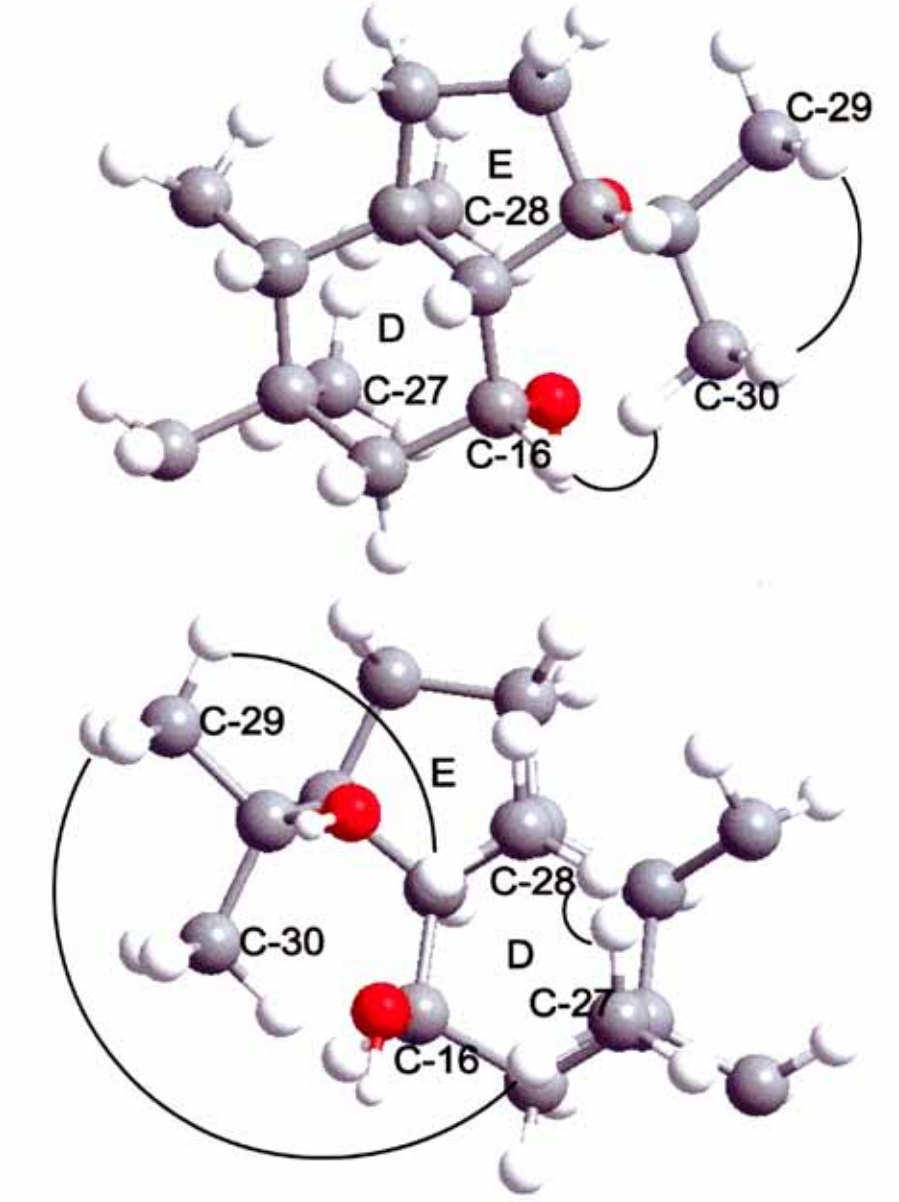


Figure 3B. NOESY associations of compound 1 in ring D and E (upper is frontal, lower is rear)

Maritime species of the genus *Verrucaria* in Kerguelia

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Abstract

Twenty maritime species of *Verrucaria* are reported from Kerguelia (Kerguelen, Heard Island and Prince Edward Islands), including the new species *V. placodioides* Øvstedal. Taxonomy, ecology and distribution are discussed.

Introduction

The term Kerguelia was first introduced by Tuckerman (1875) for the island of Kerguelen. Dodge later (1948) added Crozet Island, Heard Island and the Prince Edward Islands (no material from Crozet Island was examined in the present work).

Kerguelen is situated in the Southern Indian Ocean at 48°27'–50°01'S, 68°25'–70°33'E. The main island, Grande Terre, is surrounded by many smaller islands. It is among the remotest places on earth, 450 km from Heard Island and 2400 km from the Prince Edward Islands. Grande Terre covers *c.* 6680 km², and with the smaller islands has an extensive coastline. The origin of the bedrock is volcanic, at least 30 Ma old (Wallace *et al.* 2002). The climate is oceanic and subantarctic, with strong winds throughout the year.

Heard Island is situated at 52°05'S, 73°30'E, and is a small island of 368 km². The continent nearest to it is Antarctica, 1650 km to the south, with Australia 3500 km to the north-west, and Africa 4800 km to the north-east. The island consists of a relatively young volcanic complex, less than 1 million years old, on a basement of much older formations (Quilty & Wheller 2000). The climate is cold and oceanic, usually with very strong winds.

Marion and Prince Edward Islands (47°S, 38°E) are situated in the Southern Indian Ocean some 2000 km southeast of the southernmost point of Africa. The nearest land is the Crozet archipelago. Marion Island covers 290 km², and reaches an elevation of 1230 m. It is situated 22 km from Prince Edward Island, which covers 90 km² and reaches an elevation of 672 m. The islands represent the summits of shield volcanoes rising from the West Indian Ocean Ridge (Verwoerd 1971), and geologically are relatively young. The climate is oceanic, with strong winds.

Material and methods

The collections by Imshaug and his collaborators (Imshaug 1971; Fryday & Prather 2001) studied for the present work are lodged in MSC. They were examined using a Zeiss Stemi 2000C microscope and a Zeiss Axiolab compound microscope. No lichen substances were detected in the material. Recent molecular data have supported separating several small genera from *Verrucaria*. However, because many of the species in the present work have not been sequenced, I have taken a conservative approach and retained them all in *Verrucaria* sens. lat.

The species

1. Verrucaria aethioboliza Nyl., *C. r. hebd. Séanc. Acad. Sci., Paris* **81**, 726 (1875)
Thallus thin, gelatinous, non-rimose, violet-brown. Fertile portions black, up to 0.8 mm wide, slightly thicker than the non-fertile portions, stroma-like, with 1–3 protruding ostioles. Involucrellum in only the upper part, spreading laterally. Exciple entire, brown. Ascospores 10–15 × 4–6 µm.
Ecology: upper littoral zone?
Distribution: St. Paul Island, Heard Island (Øvstedal & Gremmen 2006).

Remarks

Described from St. Paul Island. The extended black, stroma-like areas laterally surrounding the perithecia are characteristic.