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SHORT REPORTS

(−)-3-BROMOMETHYL-3-CHLORO-7-METHYL-1, 6-OCTADIENE FROM SRI LANKAN *CHONDROCOCCUS HORNEMANNI*

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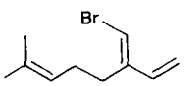
Key Word Index—*Chondrococcus hornemanni*; Rhizophyllidaceae; halogenated monoterpenes; (−)-3-bromomethyl-3-chloro-7-methyl-1,6-octadiene.

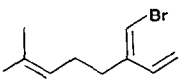
Abstract—(−)-3-Bromomethyl-3-chloro-7-methyl-1,6-octadiene, the proposed precursor of E- and Z-3-bromomethylene-3-methyl-1,6-octadienes from the essential oils of Hawaiian and Japanese *Chondrococcus hornemanni*, is the major halogenated monoterpene in the essential oil of Sri Lankan *C. hornemanni*. Unlike the essential oils of Hawaiian and Japanese (subtropical) *C. hornemanni*, the essential oil of Sri Lankan (tropical) *C. hornemanni* does not contain halogenated myrcenes.

INTRODUCTION

The essential oil of the red alga *Chondrococcus hornemanni* (Mertens) Schmitz from Hawaii [1] and the Amami Islands coasts of Japan [2] is composed of mainly mono- and dihalogenated myrcenes. Generally bromine is found on the C-3 methylene carbon (e.g. in **1** and **2**) and chlorine is attached to the C-2 and C-6



(**1**)

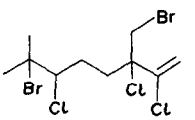


(**2**)

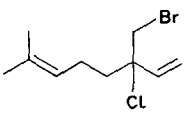
carbons of 7-methyl-3-methylene-1,6-octadiene (myrcene). Z-3-Bromomethylene-7-methyl-1,6-octadiene (**1**) and its E isomer **2** have been proposed to be the biogenetic precursors of two bromochlorodimethylhexahydrobenzofurans, chondrocoles A and B, respectively, from the essential oil of Hawaiian *C. hornemanni* [1]. The isolation of halogenated di- and tetrahydromyrcenes such as **3** from Hawaiian *C. hornemanni* [3] indicates that the halogenated myrcenes are formed by the enzymatic addition of BrCl to myrcene followed by the elimination of HCl and/or HBr. The BrCl addition is predominately Markovnikov to the Δ^1 and C-3 methylene double bonds and anti-Markovnikov to the Δ^6 double bond.

RESULTS AND DISCUSSION

Interestingly we have found that the essential oil of *C. hornemanni* from tropical Sri Lanka (Ceylon) does not contain halogenated myrcenes. In the extract of this seaweed the major halogenated compound is 3-bromomethyl-3-chloro-7-methyl-1,6-octadiene (**4**), the probable biogenetic precursor of **1** and **2**.



(**3**)



(**4**)

Compound 4, a levorotatory oil, $[\alpha]_D$ − 3.6°, was identified from spectral analysis. The mass spectrum of **4** showed a small molecular ion cluster and fragments ions for successive losses of the allylic Cl and HBr from the molecular ion. The base peak at *m/e* 69 for fission of the C(4)–C(5) bond and formation of a isopentenyl cation indicated that halogen was not attached to C-6. The PMR spectrum exhibited a typical AMX pattern for the vinyl group and since the C-2 proton signal was a doublet of doublets, the C-3 position had to be fully substituted. The only other olefinic proton signal was the broad multiplet at δ 5.12 for the C-6 proton which is coupled vicinally to the C-5 methylene, virtually to the C-6 methylene, and allylically to two methyl groups. The PMR spectrum also exhibited an AB quartet for two non-equivalent protons of the bromomethyl substituent at C-3. The carbon-13 magnetic resonance (CMR) spectrum of **4** confirmed the structure. There was a signal at δ 40.1 (a triplet in the single frequency off-resonance spectrum) for a bromomethyl carbon and a singlet at δ 72.4 for the C-3 carbon bearing chlorine [3]. The remaining signals in the CMR spectrum had chemical shifts comparable with those of myrcene and other monoterpenes [4]. Compound **4** was not detected in Hawaiian *C. hornemanni*.

EXPERIMENTAL

PMR spectra were obtained on a Varian HA-100 spectrometer and CMR spectra were obtained on a Varian XL-100

spectrometer equipped with a Digilab Fourier transform system. Single frequency off-resonance decoupling experiments were carried out with the proton decoupler at δ 14. Chemical shifts are reported in δ units (parts per million) relative to TMS ($\delta = 0$) as an internal standard in $CDCl_3$. All chromatographic separations were continuously assayed for UV absorption at 254 and 280 nm.

Isolation. Dried plants of *Chondrococcus hornemanni* (100 g) were collected at Trincomalee (Foul Point), Sri Lanka and extracted with Et_2O. The Et_2O extract (2.0 g) was applied to a 1 m × 1.5 cm Si gel column and eluted with hexane. Two main fractions were collected. Fraction 1 (520 mg), an oil, was rechromatographed on a 140 × 10 mm column of Si gel G. Elution with hexane afforded 270 mg (13.5%) of 3-bromomethyl-3-chloro-7-methyl-1,6-octadiene (**4**) as a colorless oil: $[\alpha]_D^{25} = -3.7°$ (CH_2Cl_2, $c = 14.69$); PMR δ 1.63 (*s*, allylic Me), 1.69 (*s*, allylic CH_3), 1.9–2.3 (complex *m*, C-4 CH_2 and C-5 CH_2), 3.68 (AB quartet, $\Delta\nu = 6.6$ Hz, $J_{gem} = 10.0$ Hz, $-CH_2Br$), 5.12 (broad *m*, C-6 CH), 5.21 (*dd*, $J_{cis} = 10.0$ Hz, $J_{gem} = 1.0$ Hz, C-1 H *cis* to C-2 H), 5.40 (*dd*, $J_{trans} = 16.5$ Hz, and $J_{gem} = 1.0$ Hz, C-1 H *trans* to C-2 H), 5.94 (*dd*, $J_{trans} = 16.5$ Hz, $J_{cis} = 10.0$ Hz, C-2 CH); CMR δ 138.6 (*d*, C-2), 132.6 (*s*, C-7), 122.5 (*d*, C-6), 116.9 (*t*, C-1), 72.4 (*s*, C-3), 40.1 (*t*, bromomethyl carbon), 39.2 (*t*, C-4), 25.6 (*q*, E-Me), 23.0 (*t*, C-5), 17.7 (*q*, Z-Me); IR (neat) 2960 (*s*), 2915 (*s*), 2850 (*s*), 1644 (*m*), 1440 (*s*), 1410 (*m*), 1379 (*m*), 1230 (*m*), 1105 (*m*), 980 (*s*), 929 cm^{-1} (*s*); MS *m/e* (rel. intensity) 250, 252, 254, (0.8:1:0.2 molecular ion cluster, <1), 215 (4), 217 (4), 135 (73), 93 (63), 91 (29), 69 (100), 41 (84). Fraction 2 (340 mg), a pale yellow waxy solid, was identified as a mixture of saturated fatty acids by PMR analysis and was not investigated further.

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A NEW CRYPTIC IRRITANT AND COCARCINOGEN FROM SEEDS OF *CROTON SPARCIFLORUS*

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Key Word Index—*Croton sparciflorus*; Euphorbiaceae; cryptic irritant; cryptic cocarcinogen; diterpene esters; 12-*O*-dodecanoylphorbol 13-acetate, 12-*O*-dodecanoylphorbol-13-acetate-20-linolenate.

Abstract—In recent years the genus Croton has been of particular interest following the isolation of 11 different 12,13-diesters of the polyfunctional diterpene phorbol (**1a**), from the seed oil of *Croton tiglium*; these compounds represent its toxic, irritant and cocarcinogenic principles [1]. In addition, croton oil contains what is called "cryptic" cocarcinogens of the phorbol-12,13,20-triester type [1]. The structurally related but non-irritant diterpene ester 20-acetoxy-9-hydroxy-13,15-seco-4α-tigliatriene-(1,6,14)-dione-(3,13) [2] and crotofolin A have been isolated from *C. rhamnifolius* [3] and from *C. corylifolious* L. [4], respectively.

In order to further study these irritant and cocarcinogenic compounds (croton factors), the seed oil of *Croton sparciflorus*, a herb native to Paraguay, was investigated. This plant is used in India as an antiseptic and styptic and is considered to be a troublesome weed [5]. Following the separation procedure developed for *Croton tiglium* seed oil coupled with the mouse ear irritation assay [1], a 12,13-diester and the corresponding 12,13,20-triester of phorbol were isolated, the latter representing a new "cryptic" irritant and cocarcinogen. Various alkaloids and other unrelated chemical constituents have been previously isolated from this species [5].

By comparison with *C. tiglium* seed oil (ID_{50}: 0.5 μg/ear [1]), the seed oil of *C. sparciflorus* is less irritant (ID_{50}: 17 μg/ear). By extracting the oil with methanol, which removes less irritant material as the hydrophobic fraction, the irritant activity is increased in the remaining hydrophilic fraction. On further extraction of the latter fraction with alkali, the activity is concentrated in the neutral fraction. By a Craig distribution [1] of this fraction further inactive material is removed to yield the active fraction, column chromatography of which gave an irritant and a less irritant fraction. From these fractions by PLC, two esters of phorbol, exhibiting different R_f and ID_{50} values were obtained. Similar differences in R_f values, and irritant activities are well known for phorbol-12,13-di- and phorbol-12,13,20-triesters [1]. From the NMR and mass spectra (molecular ion *m/e* = 588), the compound with the lower R_f is a phorbol ester containing a dodecanoic and acetic acid ester function. It has been reported [1] that in the MS of phorbol-12,13-diesters containing long and short chain fatty acyl residues, the long chain acid moiety is fragmented as a acyloxy-radical if it is in position 12. Also in such

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