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INTRICENYNE AND RELATED HALOGENATED COMPOUNDS FROM *LAURENCIA INTRICATA*

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Key Word Index—*Laurencia intricata*; Rhodomelaceae; red alga; marine natural products; halogenated natural products; intricenyne.

Abstract—A total of eleven C_{15} nonterpenoid halogenated compounds were isolated and characterized to varying degrees from the marine red alga *Laurencia intricata*. Based on IR, UV, NMR, MS and chemical data, a structure is proposed for one of these compounds which was assigned the name intricenyne.

INTRODUCTION

A detailed analysis of the lipid extract of the red alga *Laurencia intricata* gave a total of 17 different halogenated compounds [1]. As previously reported for other halogenated compounds from members of the genus *Laurencia* (family Rhodomelaceae), these halogenated compounds were found to belong to two distinct classes, sesquiterpenes and C_{15} nonterpenes [2, 3]. This paper describes the C_{15} nonterpenes found in *Laurencia intricata*. The sesquiterpenes isolated from this alga have been previously reported [4, 5].

RESULTS

The least polar C_{15} nonterpenoid halogenated compound isolated from the lipid extract of this alga was eluted from a Si gel column with ether–hexane (1:99). A total of 22 mg of pure material was isolated from the lipid extract for a yield of 0.25%. This compound was assigned the name intricenyne.

The MS of intricenyne indicated a molecular formula of $C_{15}H_{20}OClBr$, *m/e* M^+ 330, 332, 334; high resolution *m/e* M^+ 330.0920 (calculated for $C_{15}H_{20}OCl^{35}Br^{79}$ 330.0921) and showed fragments at *m/e* $M^+ - C_5H_5$, 265, 267, 269, and *m/e* $M^+ - C_6H_6Cl$, 217, 219. The UV spectrum and IR spectrum indicated that the compound was an ether having an enyne functional group and that it contained neither hydroxyl nor carbonyl groups. Consideration of this information established that carbons 1–6 were linear, that they contained an enyne group and that the chlorine was on C-6. The NMR data confirmed this assignment and showed the enyne double bond to have the *cis* or *Z* configuration ($J = 11.1$ Hz). This was in agreement with the *Z* configuration of the double bond in the enyne side chain of chondriol as confirmed by X-ray crystallography [6]. The *E* configuration has a coupling constant of 15 Hz [7].

Spin decoupling data also support the proton assignments in the enyne side chain. Irradiation of the C-4 proton at 6.17 caused the C-1 proton to collapse to a doublet ($J = 2.0$ Hz) and caused changes in the coupling patterns of the protons on C-3 and C-5. In like manner, irradiation of the C-3 proton at 5.64 caused the C-1 proton to collapse to a broad singlet. Conversely, irradiation of the C-5 protons at 2.86 caused the C-4 proton to change to a broad doublet ($J = 11.1$ Hz) and the C-3 proton to give a quadruplet with $J = 2.0$ and 11.1 Hz.

The NMR data also showed two additional vinyl protons and the presence of a terminal ethyl group. Considering the total degree of unsaturation in the molecule, this would place a double bond and a cyclic ether somewhere between C-7 and C-13. Confirmation of the additional double bond and cyclic ether came from the MS data of the hydrogenated product which showed that four equivalents of hydrogen were consumed. The product had a characteristic fragment at *m/e* $M^+ - C_6H_{12}Cl$, 219, 221, indicating that one mole of hydrogen was consumed in the ring and three moles in the side chain.

In addition to the side chain cleavage, intricenyne, hydrogenated intricenyne and hydrated intricenyne (see Experimental) each had an important fragment originating from the other end of the molecule. In hydrogenated intricenyne and in hydrated intricenyne this fragment corresponded to the loss of C_3H_6O. The C_3H_6O fragment was weak in intricenyne but a strong $M^+ - C_3H_6$ fragment was observed. Considering the chemical composition of these eliminated fragments, they could only have resulted from carbons 13, 14 and 15. Since C-14 and C-15 form an ethyl group, the ether must be bonded to C-13. This oxygen is also bonded to C-7 based on the chemical shifts of the C-7 proton and the observed coupling constants between the C-6 and C-7 protons. The splitting pattern of the C-7 proton also confirmed that C-8 must have two protons. Spin decoupling established that these two protons must be adjacent to the double bond between C-9 and C-10. Irradiation of the C-9 and C-10 protons at 5.85 caused a pronounced change in the pattern of the C-8 protons at 2.45. In addition, this irradiation caused changes in the splitting patterns of the two resolved protons on C-11.

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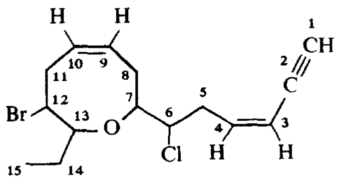
Both of these C-11 protons changed from a coupled multiple line pattern to an uncoupled quadruplet with $J = 3.4$ and 14.0 Hz for one of the protons and $J = 3.5$ and 14.0 Hz for the other. Thus, the only carbon remaining for the bromine to be attached to was C-12. The observed chemical shift and coupling constants for the C-12 proton also supported this assignment.

Based on these data, structure **1** is proposed for intricenyne. Substitution of the chlorine by an acetate group would give the same carbon skeleton as that found in laurencin [7, 8]. Allowing for this substitution and the substitution of an *E* for a *Z* double bond in the side chain of laurencin, the NMR spectra of the two compounds would be identical.



(1)

In addition to intricenyne, several more polar halogenated compounds were identified in subsequent fractions of the lipid extract. From the ether–hexane (1:9) eluent was isolated 1.4 mg of a pure compound with the molecular formula $C_{17}H_{23}O_3ClBr_2$, *m/e* M^+ 468, 470, 472, 474; high resolution *m/e* M^+ —HOAc, 408, 410, 412, 414. From the ether–hexane (1:24) eluent was isolated 40 mg of a TLC pure material that consisted of nine major halogenated compounds; these were shown by GC–MS to be C_{15} compounds containing one oxygen and various combinations of chlorine and bromine. All of these compounds had UV and IR spectra indicating the presence of both ether and enyne groups. Thus, each of these compounds appears to contain the same structural elements common to intricenyne and other C_{15} straight chain derived halogenated compounds from red algae [3].

DISCUSSION

In this paper, a total of eleven isomeric halogenated compounds are described as occurring in *Laurencia intricata*. Combined with the four sesquiterpenes described in previous work, this brings the total number of known halogenated compounds from this alga to 15 [5]. This is in contrast to the normally reported occurrence of from 1 to 4 halogenated compounds from a single species of a red alga. This substantial difference in the number of reported halogenated compounds probably results from the difference in the methods used to fractionate the lipid extract. The standard procedure would involve finding the principal compound or compounds responsible for a specific bioactivity; whereas the methods of fractionation used in this study were based on the isolation of those components with maximum organic halogen.

The fact that so many different compounds were identified makes one wonder if all of these compounds are indeed produced by this single species in its natural, undisturbed habitat. If the alga extracted consisted of several morphologically similar species, then the wide range of compounds could be explained by species variation. This has been observed in the natural product chemistry of *Laurencia pacifica* where algae of the 'same' species were found to have different natural products. This was later attributed to the existence of several new morphologically similar species [9].

The chemical consistancy of several single species of *Laurencia* has been studied by Fenical [10]. Algal halogenated products were found to be largely independent of the habitat or reproductive stage of the alga. This lends further support that *Laurencia intricata* produced all the halogenated products isolated.

Therefore, we would expect that a detailed analysis of previously studied red algae, using the methods described, would reveal the presence of additional unreported halogenated compounds.

EXPERIMENTAL

The alga (2 kg wet wt) was collected in shallow water in the vicinity of Key Largo, Florida, in March 1973. Pressed samples of this alga were identified as *Laurencia intricata* by Professor Randolph Taylor of the Botany Department at Michigan State University. The fr. alga was extracted with C_6H_6–MeOH (2:1) to give an oil (9 g) which contained 2.1 % Cl and 3.1 % Br. This oil was fractionated on a 6 × 35 cm column of Si gel and the fractions with the highest content of organic halogen were further purified by PLC. The detailed extraction, chromatographic and analytical procedures have been previously described [5, 11].

Intricenyne. UV (λ_{max}^{EtOH} 222.6 nm, $\varepsilon = 13200$, λ_{infl} 231.0 nm, $\varepsilon = 8600$); IR ν_{max} cm^{-1}: 3300, 3050, 2100, 1100 and 1070); PMR (100 MHz, $CDCl_3$) δ 6.09 (1H, *m*, $J = \sim 1.0$, 6.8 and 11.1 Hz, C-4), 5.90 (2H, *m*, C-9/10), 5.56 (1H, *m*. $J = 1.6$, 2.0 and 11.1 Hz, C-3), 4.08 (1H, *m*, $J = 3.5$ and 6.4 Hz, C-12), 3.97 (1H, *m*, $J = 4.2$ and 4.9, C-6), 3.50 (1H, *m*, $J = 4.9$, 6.4 and 7.2 Hz, C-13), 3.47 (1H, *m*, $J = 2.6$, 3.3 and 4.2 Hz, C-7), 3.20 (1H, *m*, $J = 3.5$, 8.0 and 14.0 Hz, C-11), 3.11 (1H, *q*, $J = 1.0$ and 2.0 Hz, C-1), ~2.85 (2H, *m*, C-5), ~2.45 (2H, *m*, C-8), 2.26 (1H, *m*, $J = \sim 3.0$, 3.5 and 14.0 Hz, C-11), 1.95 (1H, *m*, $J = 4.9$, 7.2 and 14.6 Hz, C-14), 1.62 (1H, *m*, $J = 7.2$ and 14.6, C-14), and 0.94 (3H, *t*, $J = 7.2$ Hz, C-15).

Hydrogenation of intricenyne. Intricenyne consumed 4 equivalents of H_2 over Pt (1 atm, 3 min 20″) in EtOH to give the hydrogenated product $C_{15}H_{28}OClBr$, MS *m/e*: M^+ 338, 340, 342; high resolution *m/e* M^+ 338.1027 (calcd for $C_{15}H_{28}OCl^{35}$-Br^{79} 338.1013), which has a characteristic fragment at *m/e* M^+ —$C_6H_{12}Cl$, 219, 221. This material had IR maxima at 1100 and 1070 cm^{-1} indicating that only the ether function remained. PMR (100 MHz, $CDCl_3$): δ 4.05 (1H, *m*, H—C—Br), 3.90 (1H, *m*, H—C—Cl), 3.16 (2H, *m*, H—C—O), 2.10–1.40 (12H), 1.40–1.10 (6H), and 2 superimposed methyl triplets at 0.96.

Hydration of intricenyne. **1** (7 mg) was heated at 60° for 55 min in 1 ml HOAc containing 10 % H_2O and 0.5 % H_2SO_4 satd with $HgSO_4$ to produce a new compound UV λ_{max}^{EtOH}: 220.0 nm. IR ν_{max} cm^{-1}: 3020, 1675, 1100, 1070 indicating the presence of an α,β unsaturated ketone and an ether functional group. The MS gave $C_{15}H_{22}O_2ClBr$, *m/e* M^+ 348, 350, 352; a fragment at *m/e* M^+ —C_6H_8OCl, 217, 219 confirmed the addition of H_2O to the side chain. Reaction of the hydrated material with dinitrophenylhydrazine in 85 % phosphoric acid in EtOH gives the DNP derivative UV λ_{max}^{EtOH} 272 nm which is characteristic of an α,β unsaturated ketone DNP derivative.

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