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PHOSPHOLIPID STUDIES OF MARINE ORGANISMS 9.[1] NEW BROMINATED DEMOSPONGIC ACIDS FROM THE PHOSPHOLIPIDS OF TWO PETROSIA SPECIES

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Abstract: Two Novel long chain fatty acids, (5Z, 9Z)-6-bromo-25-methyl-5,9-hexacosadienoic and (5Z, 9Z)-6-bromo-24-methyl-5,9-hexacosadienoic acids were found in the phospholipids of Petrosia ficiformis and P. hebes. Their structures were elucidated with the help of CI-EI/MS and a homogeneous hydrogenation catalyst.

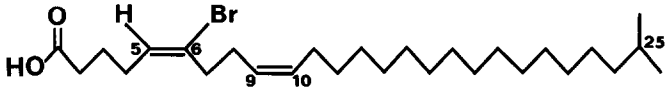
Phospholipids of sponges provide a source of various unique fatty acids[1-3] featuring branching, unconventional pattern of unsaturation, and the presence of different substituents superimposed upon a very long (24-30 C atoms) hydrocarbon chain. We describe now the isolation and structure elucidation of two such "demospongic" acids which were first encountered in the total phospholipid fraction of Petrosia ficiformis in very small amounts and subsequently in larger quantities (app. 8-10 %) in the phospholipids of P. hebes.

The separation of the phospholipid fraction, and the derivatization of the fatty acids from the total lipid extracts were carried out in the usual manner.[1,2] Both P. ficiformis[2] and P. hebes gave 25- and 24-methyl-5,9-hexacosadienoic acids (III and IV) as major components (methyl esters M^+ 420, $M^+ - 32 = 388$, $M^+ - 49 = 371$, $M^+ - 74 = 346$; pyrrolidides M^+ 459, base peak: 113, a very intense peak at m/z 180, due to the presence of a double allylic fragmentation between C-6 and C-9. The pyrrolidide of III produced a weaker signal at m/z 430 with enhanced flanking peaks at m/z 416 and 444 due to iso branching[2] whereas the pyrrolidide of IV gave rise to a weaker signal at 416 together with intense peaks at m/z 402 and 430 due to anteiso branching.[2]) Capillary GC of the methyl esters of acids I and II gave two peaks very close to each other with longer retention times than those of the above mentioned major acids. They also displayed slightly lower Rf values on TLC (silica gel, hexane/ether, 8:2 (v/v)). These two components were purified using preparative TLC, followed by HPLC (two 25 x 10 cm Altex Ultrasphere columns in series, solvent: absolute methanol, flow rate: 3.0 ml/min., refractometric detector). The methyl esters of both I and II showed intense mass spectral peaks at m/z 419, 387, 369 and 345 which, when compared with those (m/z 420, 388, 371 and 346) of the methyl esters of the major components III and IV, implied the presence of nitrogen or of a highly labile group which under electron impact conditions did not produce an observable molecular ion. A similar result was observed with the corresponding pyrrolidides whose mass spectra furnished

m/z 458 as the highest peak. The strong fragment peak at m/z 180 noted with III and IV was also present in the pyrrolidide mass spectra of I and II, but with relatively lower intensitites. Additional peaks at m/z 258 and 260 were also observed. The IR spectra had no prominent peaks at 980-968 cm^{-1}, indicating cis rather than trans unsaturation, as is usual for demospongic acids.[1-3] In addition to the typical IR absorptions for unsaturated fatty acid methyl esters (2840 cm^{-1}: olefinic CH str.; 1740 cm^{-1}: ester C=O str.; 1460 cm^{-1}, 1430 cm^{-1}: C=C str.) a small peak at 1605 cm^{-1} was seen, implying the presence of a substituent.

Catalytic hydrogenation of the methyl esters of I and II using PtO_2 in ethanol yielded two completely saturated products which were identical in all respects with the saturated products from the catalytic hydrogenation of the methyl esters of III and IV. Ozonolysis of I and II in BF_3/methanol afforded the methyl esters of glutaric, succinic, iso-octadecanoic and anteiso-octadecanoic acids. The same degradation products were obtained by ozonolysis of the known acids III and IV confirming that all four have the same carbon skeleton, except for the iso branching in I and III, and the anteiso branching in II and IV.

The 300 MHz ^{1}H-NMR spectra of the methyl esters of I and II gave an expected multiplet at 5.47 ppm (δ) (2H) for vinylic hydrogens, but also an unusual triplet at 5.79 (1H). The presence of this downfield triplet strongly suggests that one of the vinylic carbons is substituted, perhaps by an electronegative group. Chemical ionization mass spectra of I and II revealed the presence of two signals of almost equal intensities at m/z 516 and m/z 518 (adduct ions) in addition to weaker signals at m/z 499 and 501 (protonated ions), indicating the presence of a bromine atom as the substituent on one of the vinylic carbons. Since the chemical ionization mass spectra of the methyl esters did not provide any information on the location of the bromine substitution, the pyrrolidides of I and II were analyzed by CI and EI-CI mass spectrometry using NH_3 as reagent gas. In addition to the adduct ions at m/z 516



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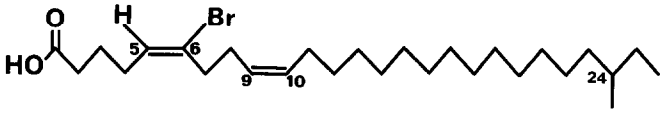

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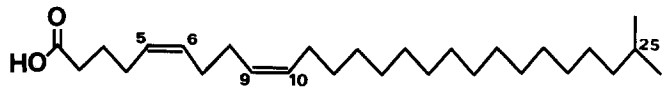

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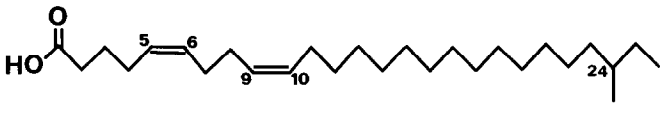

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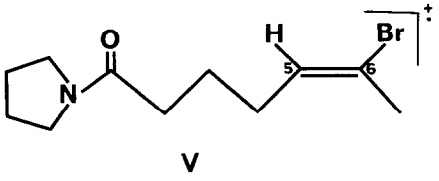

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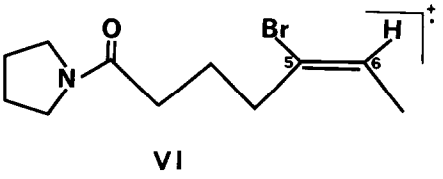
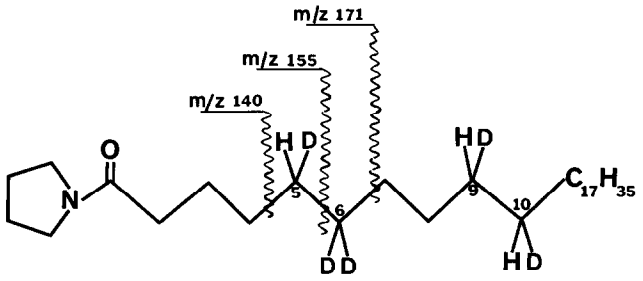


VI



VII

and 518, we were able to see two strong signals at m/z 258 and 260 which may be caused by one of the two ions (V or VI)—products of a double allylic cleavage. Therefore it is evident that the bromine is attached either to C-5 or to C-6, rather than to C-9 or C-10. 300 MHz ^{1}H-NMR decoupling of the methyl esters of I and II in benzene-d_6 were carried out to verify this conclusion. The use of benzene-d_6 enhanced the resolution in the vinylic region, thus causing the C-9 proton to appear at 5.48 ppm and C-10 proton at 5.36 ppm.[3] Irradiation of the C-10 proton caused a significant change in the multiplicity of the signal at 5.36 ppm, but the triplet at 5.79 ppm remained unchanged, confirming that both C-9 and C-10 were unsubstituted. Further decoupling experiments aimed at proving the location of the bromine at either C-5 or C-6 were inconclusive.

A successful approach consisted of replacing the bromine with a more easily locatable substituent without drastically disturbing the rest of the molecule, specifically to deuterate the methyl esters of I and II using a homogeneous catalyst[4] (tristriphenylphosphinerhodium chloride, D_2, 1 atm, rt, benzene). While 3 hr led only to partial reduction, extension to 18 hr resulted in complete reduction[5] along with the replacement of bromine by deuterium. After isolating the methyl esters, they were converted to pyrrolidides and subjected to capillary GC/MS analysis. A difference of 15 amu was observed between C-4 and C-5, between C-8 and C-9, and between C-9 and C-10, indicating the addition of deuterium atoms to the double bonds. On the other hand, a difference of 16 amu was seen (VII) between C-5 (m/z 155) and C-6 (m/z 171), clearly demonstrating an additional replacement of bromine with deuterium at C-6.[6] The pyrrolidide of deuterated I (M^+ 468) gave rise to a weaker signal at C-25 (m/z 439), with enhanced flanking peaks at C-24 (m/z 425) and C-26 (m/z 453) showing an iso-branching, whereas

the pyrrolidide of deuterated II (M^+ 468) produced a weaker signal at C-24 (m/z 425) with fragment peaks of strong intensities at C-23 (m/z 411) and at C-25 (m/z 439) consistent with an anteiso-branching.[6] All these results prove the structures of I as (5Z, 9Z)-6-bromo-25-methyl-5,9-hexacosadienoic and II as (5Z, 9Z)-6-bromo-24-methyl-5,9-hexacosadienoic acids. Petrosia ficiformis and P. hebes contain very small amounts of two more brominated fatty acids with longer retention times than those of I and II on capillary GC. Unfortunately, the low concentrations of these acids prevented further purification and complete analysis. However, their gas chromatographic and mass spectral (the presence of $M + NH_4^+$ at m/z 530-532 in the CI; and of m/z 433, 401, 383 and 369 in the EI mass spectra of the methyl esters, as well as the presence of m/z 472, 260, 258 and 180 in the EI mass spectra of the pyrrolidides) data strongly suggest these acids may be C-28 homologs of I and II.

The biosynthetic pathways leading to the formation of vinylic halogenated fatty acids are not known and raise interesting questions. Some halogenated fatty acids with conventional chain lengths have been found before in marine organisms,[7,8] but this is the first report of the occurence of brominated demospongic acids in the phospholipids of a living organism. Investigations of other unusual features regarding membrane phospholipids from marine organisms are currently under way in our laboratory.

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