Squalene and other unsaturated hydrocarbons in *Bryocladia cuspidata*

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Summary. By gas chromatography-electron impact mass spectrometry the red alga *Bryocladia cuspidata* was found to contain n-pentadecane and n-heptadecane. Gas chromatography-chemical ionization mass spectrometry indicated the presence of squalene as well as a C_{21} pentaene and a C_{21} hexaene, tentatively identified as 1,6,9,12,15-heneicosapentaene and 1,6,9,12,15,18-heneicosahexaene.

We wish to report here a simple application of coupled gas chromatography-chemical ionization mass spectrometry (GC-CIMS) which allowed identification of a C_{21} pentaene, a C_{21} hexaene and squalene in the marine alga *Bryocladia cuspidata* (J. Agardh) De Toni (phylum Rhodophyta, order Ceramiales, family Rhodomelaceae)[2]. Extraction[3] of the alga, collected intertidally in March, 1975 near St. Augustine, Florida[4], gave a dark tar, which was chromatographed over silica gel, employing hexane as eluting solvent. The eluate was concentrated and characterized by gas chromatography-electron impact mass spectrometry (GC-EIMS). The gas chromatogram of this fraction (12 ft × 2 mm inner diameter glass column packed with 3% OV-17 on 100-120 mesh GCQ, helium flow 20 ml/min, temperature programmed at 10 °C/min from 120-280 °C then held at 280 °C) showed 5 peaks (A-E) in the relative intensities 1:59:2:10:3 at 5.5, 8.0, 13.3, 13.5 and 21.3 min retention times. Component A was identified by GC-EIMS as n-pentadecane, and the most abundant component, B, was identified as n-heptadecane. The latter has also been observed to predominate in Rhodophytae from other geographical areas[5-8], while the former is more abundant in Phaeophytae.

Components C, D and E gave only very weak molecular ions (<0.5%) at m/e 286, 284 and 410, respectively, and nonspecific fragmentation patterns typical of unsaturated hydrocarbons by EIMS. In order to gain further insight into their structures, components C, D and E were also studied by GC-CIMS[9]. The mol. wts 286 and 284 assigned tentatively by EIMS were confirmed by the intense ions at m/e 287 and 285 (M+H), at 285 and 283 (M−H), and at 343 and 341 (M+C$_4$H$_9$) in the isobutane CI mass-spectra for peaks C and D, respectively. These mol. wts correspond to those for a heneicosapentaene ($C_{21}H_{34}$) and a heneicosahexaene ($C_{21}H_{32}$), respectively. All-cis-1,6,9,12,15-heneicosapentaene and all-cis-1,6,9,12,15,18-heneicosahexaene have been isolated and characterized from *Fucus distichus*[10] and brown algae[5], while their isomers all-cis-3,6,9,12,15-heneicosapentaene and all-cis-3,6,9,12,15,18-heneicosahexaene have been identified in diatoms[11], copepods[12], and planktonic[6] and green algae[5,13], and an unidentified C_{21}-hexaene was recently identified by GC-CIMS in an extract of abalone viscera[14].

Assuming one or the other known pentaene and hexaene structures, a possible means of distinguishing between the 1- and 3-isomers is provided by the presence of ions at m/e 273 and 271 (M − CH) in the isobutane CI spectra of peaks C and D, respectively. These unusual peaks can be explained as arising from the addition of $C_3H_7^{+15}$ or $C_4H_9^{+}$ ions to C-1 of a 1-alkene followed by hydride transfer followed by loss of C_4H_8 (after $C_3H_7^{+}$ addition), as illustrated below, involving a mechanism similar to that proposed by Field for the presence of M–CH peaks in the CI (methane) spectra of di- and triolefins[16]. This fragmentation process would be represented, in the case of the heneicosahexaene, by the sequence

$$M + C_3H_7^{+} \longrightarrow m/e\ 327 \xrightarrow{-C_4H_8\ (56\ amu)} (M-CH)^{+}.$$
$$\begin{array}{ccc} 284 & m/e\ 43 & m/e\ 271 \\ amu & & \end{array}$$

(A similar but slightly more complex process can be written involving initial addition of $C_4H_9^{+}$.) The occurrence of M − CH peaks supports the hypothesis that compounds C and D have a terminal double bond, thus suggesting them to be the known all-cis-1,6,9,12,15-heneicosapentaene and all-cis-1,6,9,12,15,18-heneicosahexaene.

A weak molecular ion at m/e 410 in the EI mass-spectrum of component E suggests the molecular formula $C_{30}H_{50}$. This mol. wt was confirmed in the CI (isobutane) spectrum, which contains intense M+H and M−H peaks at m/e 411 and 409, respectively, as well as major ions at m/e 137 ($C_{10}H_{17}$), 219 ($C_{16}H_{27}$), 261 ($C_{19}H_{33}$) and 329 ($C_{24}H_{41}$) indicating regularly spaced olefinic linkages. The triterpene squalene ($C_{30}H_{50}$) thus appeared to be a reasonable candidate for component E and this conjecture was confirmed by GC coinjection of an authentic sample of squalene with the hydrocarbon fraction from *B. cuspidata*, which enhanced peak E. Authentic squalene also gave the same EI and CI mass-spectra as those for component E.

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2 The alga was tentatively identified by reference to W.R. Taylor, in: Marine Algae of the Eastern Tropical and Subtropical Coasts of the Americas, p. 586. Univ. of Michigan Press, Ann Arbor 1972. The identification was confirmed by R.B. Setzer, Allan Hancock Foundation Herbarium, University of Southern California, Los Angeles, Cal. 90007, USA.

3 *B. cuspidata* was extracted by the previously described procedure. The wet alga (5.0 g) was homogenized in a mixture of methanol-toluene (3:1) and centrifuged. Sodium nitrate solution was added and the resulting toluene layer was separated by centrifugation and dried to give 0.7% of lipid; L.P. Hager, R.H. White, P.F. Hollenberg, D.L. Doubek, R.C. Brusca and R. Guerrero, in: Food-Drugs from the Sea Proceedings 1974, p. 421. Ed. H.H. Webber and G.D. Ruggieri. Marine Technology Society, Washington 1976.

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Effect of seasonal variations and cold acclimation on serum transaminase activity of common Indian frog *Rana tigrina*[1]

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Summary. A definite seasonal change is observed in 2 transaminases, SGOT and SGPT of *R. tigrina*. Cold acclimation significantly depresses transaminase activity of serum.

Although transamination reactions have been well-documented and a number of studies have been made on the kinetics and properties of transaminases in mammalian[2-4] and nonmammalian tissues[5-9], very little information is available on the effect of cold acclimation and seasonal variations on the transaminase activity. Almost nothing is known about these factors in amphibians. The present work is an attempt in this direction to fill the void.

Materials and methods. The frogs of both the sexes ranging from 200 to 500 g in weight were obtained from the local suppliers and kept in a vivarium maintained outside the laboratory in perfectly natural conditions. The blood was always obtained by aortic puncture from pithed and dissected frogs. Transaminase activities were recorded for each month during 1976 to 1977. The influence of cold acclimation was studied by keeping the frogs at 13 °C (inside the refrigerator), whereas the room temperature was 38 °C at the same time. The frogs were taken out at regular intervals and their transaminase activity was determined. Appropriate controls were run simultaneously. Transaminase activities were determined according to Reitman and Frankel[10] using Spectronic-20 Spectrophotometer, at a temperature of 37 °C and pH 7.5. The results are expressed in RF-units. 1 RF-unit is equivalent to the formation of 4.82×10^{-4} µM glutamate/min. The points in the figures represent an average of 5-6 readings±SEM. 2 transaminases aspartate aminotransferase (EC 2.6, 1.1) and L-alanine aminotransferase (EC 2.6, 1.2) would be referred here after as SGOT and SGPT respectively.

Results. The values of SGOT and SGPT observed throughout the year are shown in figure 1. SGOT has been found much more active than SGPT. The serum transaminase activity is lowest in January (SGOT 45.7±1.0 and SGPT 12.5±0.99), rise gradually till the peak values are observed in August (SGOT 133.6±2.0 and SGPT 28.8±1.35). Thereafter the activity again shows a gradual fall till almost the same level is reached as in January. The effect of cold acclimation of the frogs kept at 13 °C for various periods has been shown in figure 2. A steep fall was recorded in transaminase activity from 2nd day onwards.

Discussion. It is well-known that transaminases are widely distributed in plant and animal tissues[11] and establish a link between the metabolism of amino acids, carbohydrates and fats. The normal values of SGOT and SGPT recorded in *R. tigrina* compare favourably with the values recorded for other vertebrates[12,13] and invertebrates[6-9], where GOT activity has been observed to be higher than GPT.
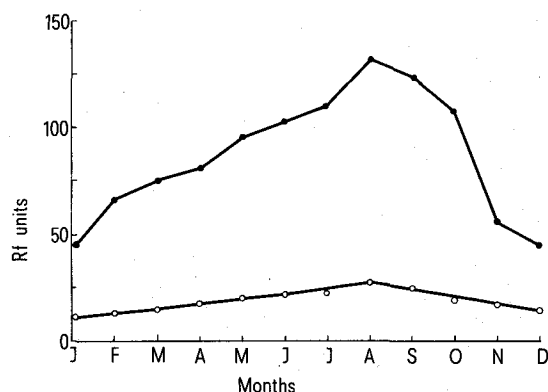

Fig. 1. Monthly average SGOT (●———●) and SGPT (○———○) activity showing seasonal change in *Rana tigrina*. (Average values of the whole year: SGOT 87.6±6.0; SGPT 20.0±2.0 RF-units.)
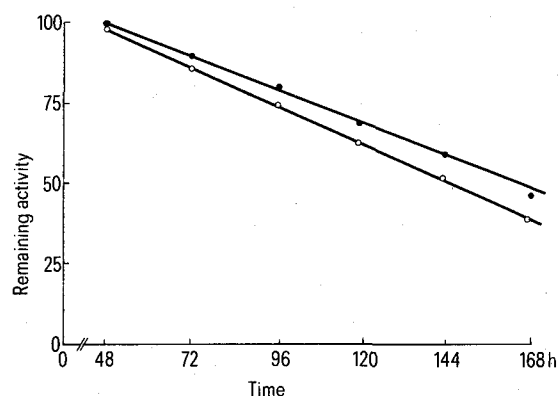

Fig. 2. Effect of cold acclimation on SGOT (●———●) and SGPT (○———○) activity in *Rana tigrina*.