ANALYSIS OF UNUSUAL SEED OIL FATTY ACIDS FROM *EXOCARPUS CUPRESSIFORMIS* USING MULTIPLE GC PHASES

Paul Wynne[1], Melissa Vanjek[2], Nicolette Kalafatis[2], Naza Lahoutifard[3], Lynn Hodges[2] and Theo Macrides[2]
[1]SGE Analytical Science, Ringwood, Victoria, Australia [2]School of Medical Sciences, RMIT University, Bundoora, Victoria, Australia [3]SGE Europe, Courtaboeuf, France

Introduction

The Cherry Ballart *(Exocarpos cupressiformis)* is a small semi-parasitic tree that is native to Eastern Australia. The 'fruit' is recognized as a traditional food and also consumed by native animals. Although the seeds are external to the 'fruit' they may be consumed and passed undigested in faeces of mammals. The seed oil has been found to contain a number of unique fatty acids that are of interest for their potential physiological activity. In this presentation, we describe the chromatographic behavior of the fatty acid methyl esters derived from the species in terms of steric barriers to their interaction with different phases. A correlation of single dimension GC experiments is used to assist in structure determination.

Discussion

In addition to the known ximenynic acid (octadec-9-yn-11-trans-enoic acid), four related acetyleneic acids were also tentatively identified on the basis of their mass spectral characteristics (2 shown in Fig 1). Retention times were determined for compounds on each column and correlations were sought between molecular weight and retention time and between paired retention time data to assist in structure assignment.

The increased spread of points away from the saturated FAME line in a plot of retention time versus molecular weight (Fig 2) demonstrates that the isomeric separation of FAME is more effective for the biscyanopropyl (BPX90) and carborane (HT8) phases. The highly polar BPX90 retains FAME almost exclusively on the basis of unsaturation and shows a dominant speciation capability that is not heavily influenced by non-polar effects (such as carbon number). In contrast, the HT8 is a 5 % phenyl substituted PDMS mimicking phase and shows a dominant non-polar separation with the carborane's unique selectivity towards unsaturation.

Retention time pairs for the non-polar versus polar columns were plotted (Fig 3) and familial correlations were noted for structurally related compounds (e.g. saturated FAME, mono-unsaturated cis-enes and trans-ene-acetylenic acids). The familial relationship between the 9-yn, 9-yn-11-trans-ene and 9,11-diyn but not the 9-yn-11-cis-ene modified octadecanoates acids may be attributed primarily to steric factors. While the cis-ene bond reduces the overall steric volume and decreases the unhindered distance between unsaturated sites, both the trans-ene and acetylenic bonds hold the chain in an extended conformation and therefore increase the steric accessibility for multiple sites of unsaturation to simultaneously interact with the GC phase. The same steric effects give the double and triple bonds almost equal capacity to increase polar retention as each bond is sterically capable of allowing only one set of π-orbitals to interact with a phase bonding-moiety at one time. The very polar phase shows a much high degree of selectivity towards the n-electrons on the oxirane acid than the less polar phases and reflects the ability of the bis-cyano phase to interact with n-electrons.

The use of GC phases that are capable of exhibiting different types of interactions with π-electron containing analytes is the basis for this combinatorial gas chromatographic technique. The identification of fractions rich in the acetylenic fatty acids is important for future targeted study of the physiological activity of *Exocarpus* oil.

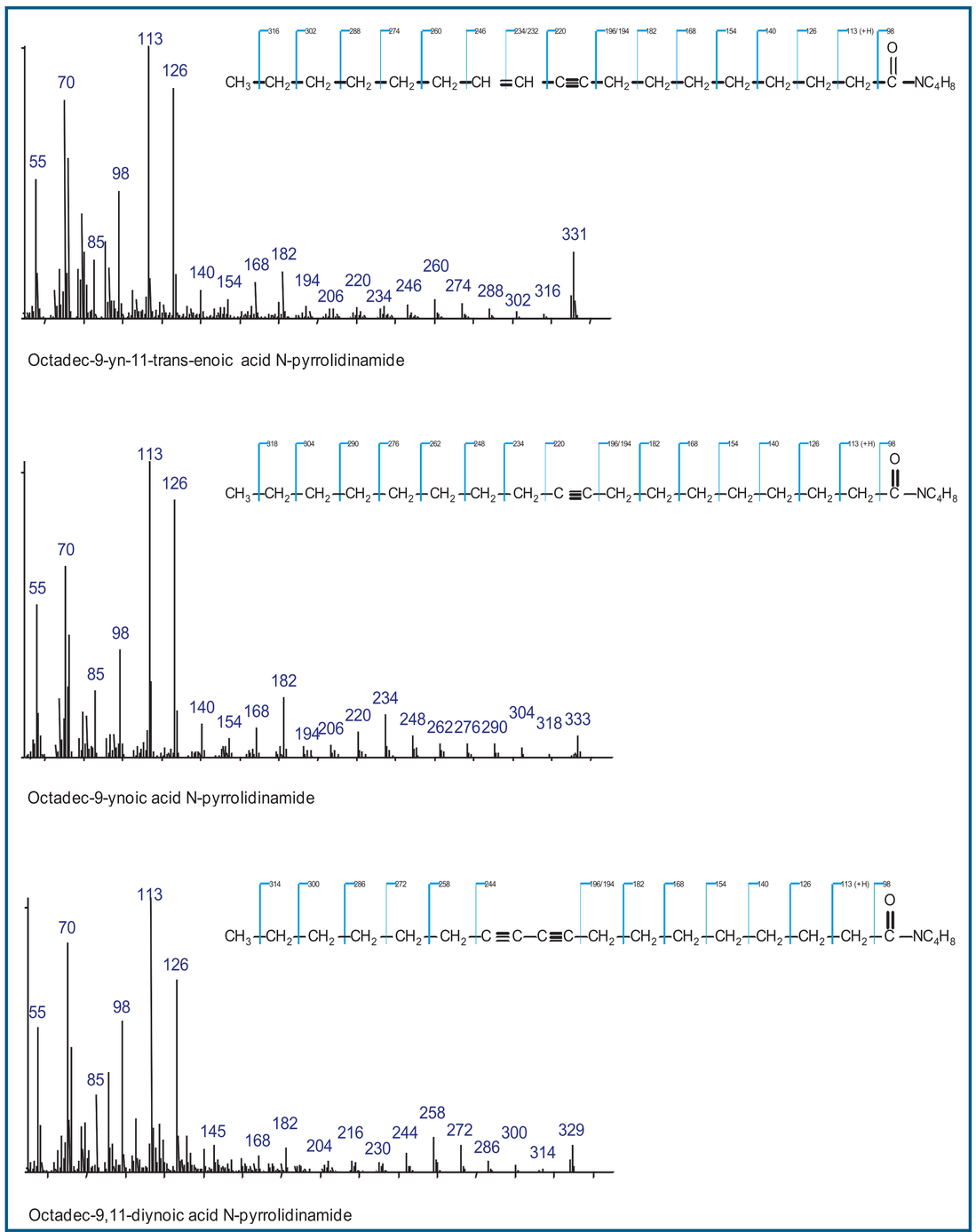

Figure 1: The structures and EI mass spectra for unsaturated C18 fatty acid pyrrolidinamides identified in the seed oil of *Exocarpus cupressiformis*.

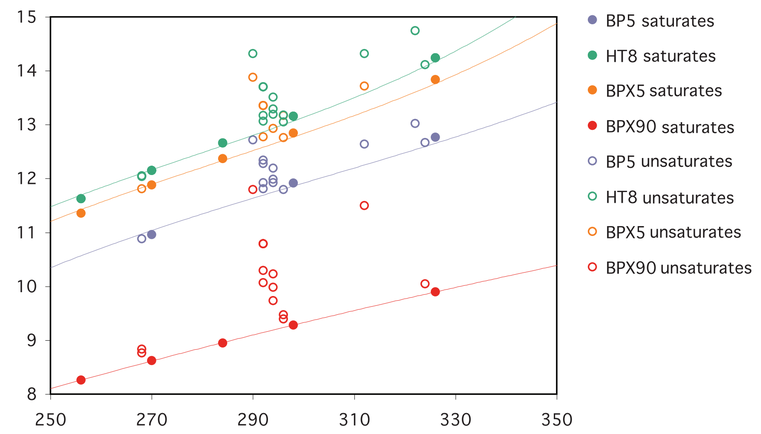

Figure 2: Correlation of retention time on different columns with molecular weight of FAME.

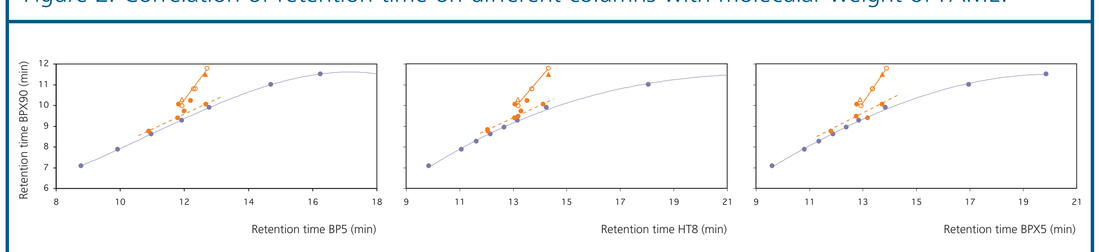

Figure 3: Correlation plots of the retention time for fatty acid methyl esters in the seed oil of *Exocarpus cupressiformis* chromatographed on BP5 (left), HT8 (middle) and BPX5 (right) versus retention under identical conditions on a BPX90 column. Blue closed circles are saturated FAME, orange closed circles are unsaturated normal FAME, orange open circles are acetyleneic and trans-ene-acetyleneic FAME, orange open triangles are cis-ene-acetyleneic FAME and orange close triangle is oxirane modified C18 FAME.

Experimental conditions:

Free and bound fatty acids were isolated, speciated by preparative thin-layer chromatography and released by hydrolysis from dried green seeds of Exocarpus cupressiformis. The residues were converted to the corresponding methyl ester and pyrrolidinamide derivatives and total residues from each fraction were reconstituted in hexane at a concentration of 25-35 mg/mL. The mixtures were analyzed by GCMS using capillary columns of identical dimensions but containing different phases (HT8, BPX90, BPX5 and BP5) operated under identical conditions. All columns were 30 m x 0.25 mm i.d. with a 0.25 µm film thickness. Analysis was performed on a 6890 GC-5973N MSD (Agilent Technologies) fitted with an ETP 14642 electron multiplier. Injection was split 50:1 with a split flow of 65 mL/min at a temperature of 250 °C. The carrier gas was helium with a nominal flowrate of 1.3 mL/min in constant flowrate mode and a nominal inlet pressure of 10.8 psi. The oven temperature was programmed from 50 °C (held for 2 minutes) to 270 °C (held for 15 minutes) at 20 °C/min. The transfer line was at 280 °C. The MS scanned from 50-550 Da at 2.9 scan/sec.

Fatty acids were identified by comparison of their EI mass spectral characteristics and retention times for both derivatives to the same characteristics for known standards or for compounds previously reported. New compounds were tentatively identified by interpretive mass fragmentography.

RMIT UNIVERSITY

SGE Analytical Science
www.sge.com