Unidimensional NMR, Very Simple but Important Tool for Plant Populational Studies: *Croton sonderianus* Muell. Arg.

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Abstract: *Unidimensional ^{1}H and ^{13}C NMR was used to study the volatile chemical composition variation of a plant population from "caatinga", the characteristic flora of the northeastern Brazil, composed mainly by Croton sonderianus Muell. Arg. (marmeleiro preto). The NMR study, combined with GC/MS analysis, revealed that unidimensional NMR is a fast and reliable tool for the characterization of a chemical profile of seven chemotypes (não sei se o corte altera o sentido; creio que não. A frase "tal qual" está excessivamente longa e torna a leitura complicada): group **A** (germacratetraene/germacrone, 2 specimens); group **B** (α-pinene/1,8-cineole, 6 specimens); group **C** (limonene/1,8-cineole, 11 specimens); group **D** (spathulenol, 2 specimens); group **E** (limonene/spathulenol, 8 specimens); group **F** (β-phellandrene/byciclogermacrene, 3 specimens); group **G** (1,8-cineole/α-pinene, 6 specimens) and group **H** (1,8-cineole/spathulenol, 2 specimens). The NMR analysis was also used to detect the misidentification of germacratetraene as curzerene, probably an artefact generated during GC/MS analysis.*

For almost two decades we have been studying *Croton sonderianus* Muell. Arg.,a shrub, sometimes a small tree, very common in the "caatinga", the characteristic flora of the northeastern Brazil. Popularly known as "marmeleiro preto", due to the dark brown color of its branches, it is used by the peasants for gastric problems. We performed a circadian analysis of the leave oil composition of this species, rich in volatile oils from the roots to the leaves, by using ^{1}H NMR to monitor the major compounds variation. Thus, 6.0 mL $CDCl_3$ solution of 50 mg of oil, in 5 mm NMR tubes were run on a Bruker Avance DRX spectrometer. The equipment operated at 11.7 T at room temperature, *observing?* ^{1}H at 500.13 and ^{13}C at 125.77 MHz, respectively. Two specimens of *C. sonderianus* (CS-1 and CS-2) were harvested (about 200 g) at every three hours, starting at 6:00 am. Even though the ^{1}H NMR profile showed to be similar during the experiment for both specimens, they indicated different chemical compositions and were characterized by absorptions at the range δ 0.8-1.7 and δ 4.0-6.5 (Fig. 1 and 2). GC/MS (Hewlett-Packard 5971 A) was used to determine the relative composition of individual constituents, confirming NMR prediction.

These data led us to analyze a more complex population. Leaves of forty randomly chosen plants of the same geographic site were collected and extracted by hydrodestillation to yield the correspondent volatile oils.

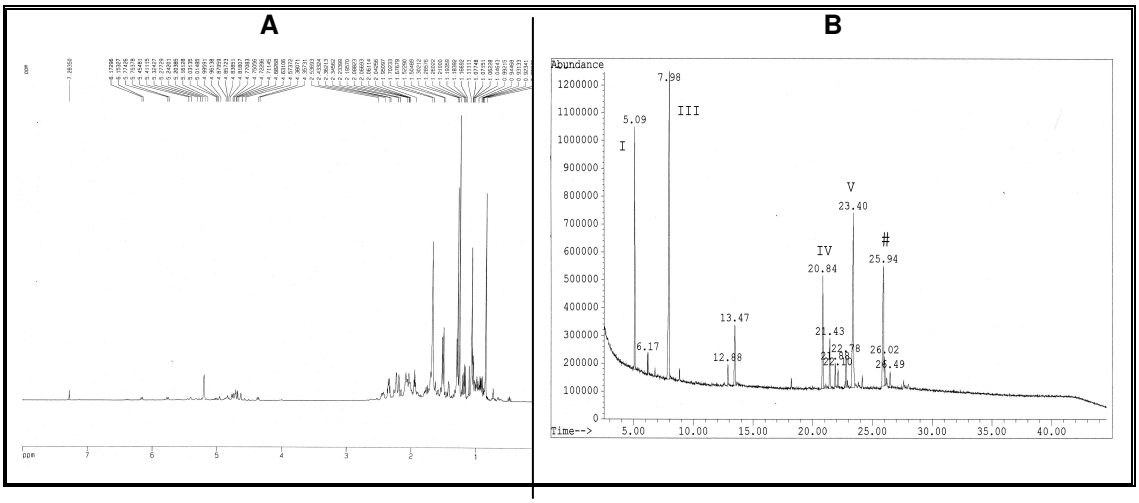

Figure 1. (A) ^{1}H NMR spectrum (500 MHz, $CDCl_3$) and (B) gas-liquid chromatogram of CS-1(6:00 am)

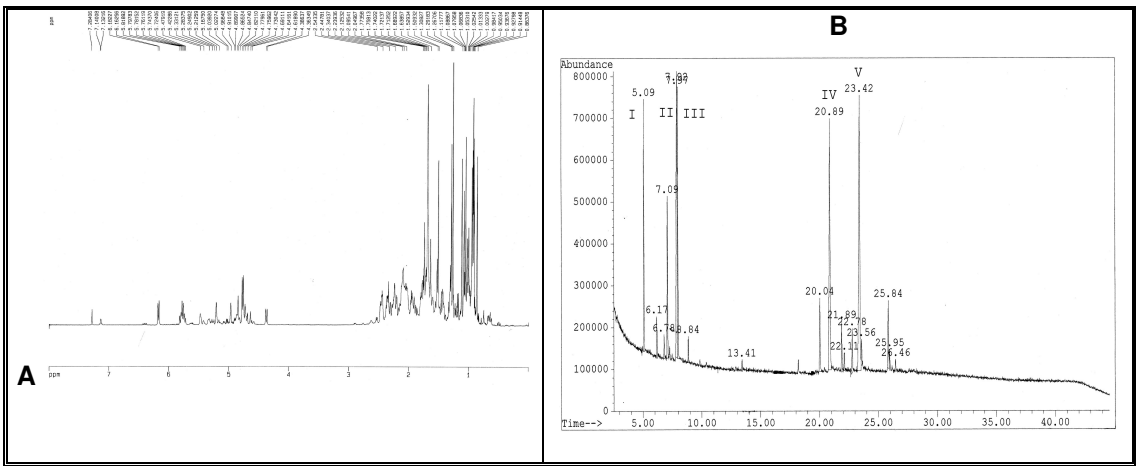

Figure 2. (A) ^{1}H NMR spectrum (500 MHz, $CDCl_3$) and (B) gas-liquid chromatogram of CS-2 (6:00 am)

Analysis by ^{1}H NMR allowed the characterization of 7 different spectral profiles by theirmajor components: group **A** (germacratetraene/germacrone, 2 specimens); group **B** (α-pinene/1.8-cineole, 6 specimens); group **C** (limonene/1.8-cineole, 11 specimens); group **D** (spathulenol, 2 specimens); group **E** (limonene/ spathulenol, 8 specimens); group **F** (β-phellandrene/ byciclogermacrene, 3 specimens); group **G** (1.8-cineole/ α-pinene, 6 specimens) and group **H** (1.8-cineole/spathulenol, 2 specimens). GC/MS analysis was used to determine the relative composition of the individual constituents.[1]

Seven months later, 10 plants from the group previously chosen were re-analyzed and showed the same profile as observed before, revealing that no seasonal changes affect the chemical composition of *C. sonderianus* volatile oils. On the other hand, the unexpected variation, but similar chemical composition, can be explained by anthropic influences (fire, cropping, etc) or by a kind of "evolution" associated with the propagation method of *C. sonderianus* by seed germination or clonization (a daughter plant from the root of an adult plant), etc.

The new generation should be better adapted to the drastic climatic conditions of the northeast region, or yet to the attack of herbivores, etc. Further experiments and statistic analysis will be carried out at a region where no anthropic devastation has been imposed yet.

It is worth noticing that curzerene was identified, by GCMS search library, as the major component of the plant oil. However, ^{1}H and ^{13}C NMR analyses of this oil did not reveal any characteristic absorption for a vinyl group, suggesting that curzerene is indeed an artifact generated by Cope rearrangement of germacratetraene at the MS conditions. This finding points therefore to the importance of conducting NMR for routine analyses of volatile oils, prior to carrying out GC/MS analysis.

Acknowledgements

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References

1. G. T. Malcher, *MSc. Dissertation*, Universidade Federal do Ceará, Brazil, 2003.