

**Zeitschrift:** Saussurea : journal de la Société botanique de Genève  
**Herausgeber:** Société botanique de Genève  
**Band:** 14 (1983)

**Artikel:** Study of the non isoprenoid hydrocarbons of the latices of the Euphorbia genus  
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**DOI:** <https://doi.org/10.5169/seals-1099234>

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# Study of the non isoprenoid hydrocarbons of the latices of the Euphorbia genus

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## RÉSUMÉ

DESTORDEUR, M., J.-L. RAMAUT & M. E. ROSSI (1983). Etude des hydrocarbures non-isoprénoïdes du latex du genre *Euphorbia*. *Saussurea* 14: 29-38. En anglais, résumé français.

Les hydrocarbures non-isoprénoïdes du latex d'*Euphorbia candelabrum*, *E. dawei* et *E. tirucalli*, trois espèces du Burundi (Afrique) ont été étudiés qualitativement et quantitativement par spectroscopie I.R. et chromatographie en phase gazeuse (C.G.L.).

## ABSTRACT

DESTORDEUR, M., J.-L. RAMAUT & M. E. ROSSI (1983). Study of the non isoprenoid hydrocarbons of the latices of the *Euphorbia* genus. *Saussurea* 14: 29-38. In English, French abstract.

The non isoprenoid hydrocarbons present in the latex of *Euphorbia candelabrum*, *E. dawei* and *E. tirucalli*, three species from Burundi (Africa), were studied qualitatively and quantitatively by I.R. spectroscopy and gas-liquid chromatography (G.L.C.).

Because of the increase of the price of the oil products and the existence of a final limit to the sources of fossil raw material since 1974 numerous research projects have been developed on solar energy and more generally on the sources of raw material and renewable energies, of which biofuels are a facet (1, 2, 3, 4, 5, 6).

By reading some works among which those of CALVIN (2, 4, 6) in California; those of JARVIS (3) in Australia; of VIAUD & TEISSEIRE (1) in Senegal, it was found that the latex plants and more particularly several species of the genus *Euphorbia* are liable to provide energetic materials like hydrocarbons in more or less great quantities.

There are now about 2000 species of plants producing latices containing rubber (= cis-polyisoprene) spread over all latitudes and adapted to all climatic conditions; they are found in several families like the *Moraceae*, *Euphorbiaceae*, *Sapotaceae*, *Apocynaceae*, *Asclepiadaceae* and *Asteraceae* liguliflores. Of these 2000 species only the *Hevea* has been cultivated on an industrial scale, but since a few years *Parthenium argentatum*, guayule, has been studied intensively (5) especially in Mexico or in the U.S.A., its natural habitat.

Nevertheless it has to be specified that most of the works published up to now are especially concerned with culture problems (species of the genus *Euphorbia*), with "oil" yields as indicated by some authors, among whom Calvin, and in the field of hydrocarbons, with the only study of the isoprenoids. Indeed, the study of the "alkanes" fraction of the hydrocarbons of the latices of the genus *Euphorbia* doesn't seem to have had much attention whereas these same alkanes have been studied in several works when it concerns the study of cuticular waxes of several species with taxonomic objectives (7, 8, 9, 10).

The principal cuticular wax hydrocarbons are in general the C<sub>27</sub>, C<sub>29</sub>, C<sub>31</sub>, C<sub>33</sub> and C<sub>35</sub> alkanes with a *n*-alkanes predominance in comparison with the iso-alkanes.

The present work has been limited to the study of the non isoprenoid hydrocarbons of the latex of three African species of the genus *Euphorbia*, i.e.: *E. candelabrum*, *E. dawei* and *E. tirucalli*.

## Equipment and methods

### *Equipment*

This is latex collected after incision in three species of the genus *Euphorbia*: *E. candelabrum* Trem. ex Kotschy, *E. dawei* N. E. Br. and *E. tirucalli* L. which were found in Burundi in the Ruzizi plain in Rukoko in latitude 3°16' South and in longitude 29°18' East.

After collecting, the latices are dessicated in a dessicator, vacuum, at ordinary temperature, during several weeks up to a constant weight. After crushing it, the latex looks like a very irritating white powder, its inhalation causes sneezing and contact with the skin causes burning.

## Methods

### *Extraction of the hydrocarbon fraction*

This was realized by solubilizing the latex in a *n*-hexane/methanol (2/1) mixture during 24 hours. After that the solution is washed three times in distilled water and the hexane phase is collected for vacuum evaporation at ordinary temperature. The residue is then backflow hydrolysed during two hours in a solution of methanolic KOH.

The hydrolysate is taken back by the hexane, washed three times in distilled water and finally the hexane phase is chromatographed on a  $Al_2O_3$  column (neutral aluminium oxide, Brockman activity II-III, 70-230 mesh.).

### *Recording of I.R. spectra*

The equipment used is a Perkin Elmer model 21. The recording of the spectra is done by placing the samples in sandwich between two KBr-pellets.

### *Gas liquid chromatography (G.L.C.)*

The equipment used is a Perkin Elmer type F22. It is a flame ionization detector. The carrier gas is nitrogen. The column in stainless steel has a length of 10 m and an inner diameter of 0.25 mm.

The liquid phase is of the OV - 1 type. The used flows are: 42 cc/min for hydrogen, 420 cc/min for air and 2 cc/min for nitrogen.

The split ratio is 1/8.

The temperature programming for the alkanes separation from  $C_{17}$  to  $C_{36}$  is as follows: 150°C during 2 minutes; passage from 150 to 280°C with an increase of 4°C/min and a level at 280°C during 2 minutes.

Temperature of the injector: 350°C.

Temperature of the detector: 360°C.

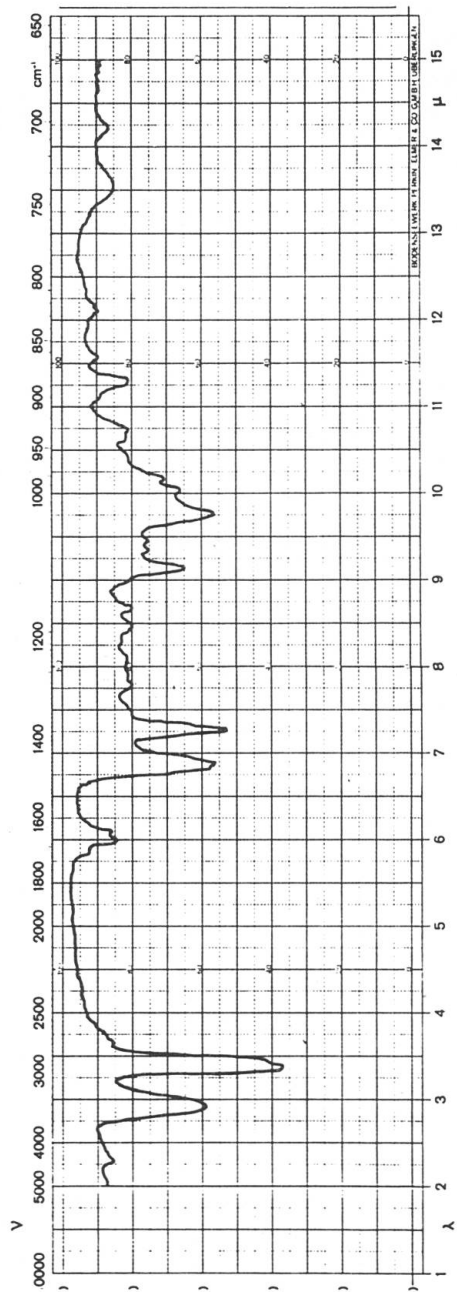
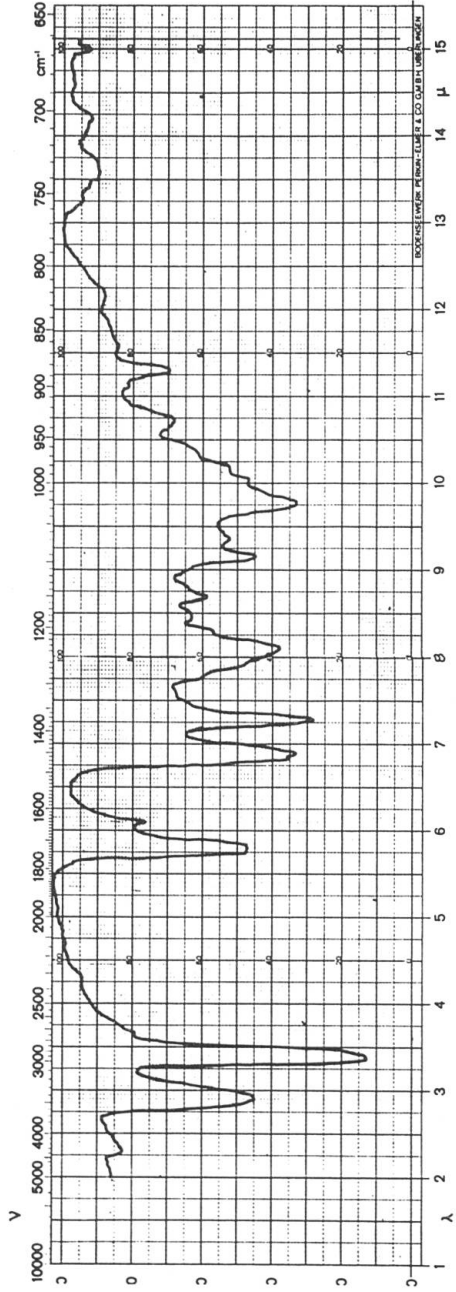
The temperature programming for the alkanes separation from  $C_{11}$  to  $C_{25}$  is as follows: 90°C during 8 minutes; passage from 90 to 200°C with an increase of 4°C/min and a level at 200°C during 2 minutes.

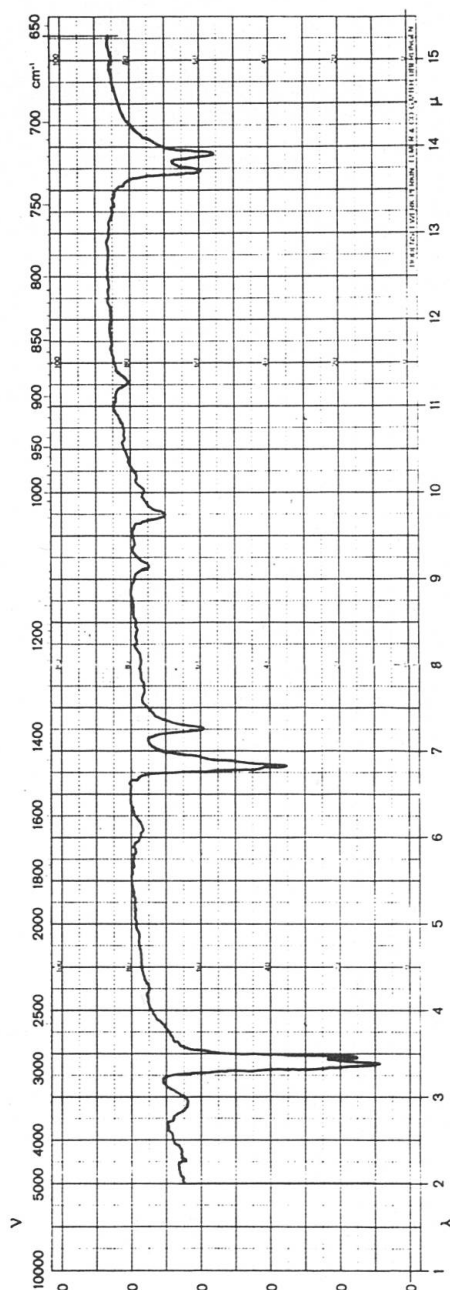
Temperature of the injector: 150°C.

Temperature of the detector: 200°C.

### *Determination of the upper calorific value at constant volume*

It was determined on the basis of 1 g dry latex, according to the Belgian norm NBN M 02 - 010.





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Fig. 1-3. - I.R. spectra of *E. candelabrum* at different stages of the "alkanes" fraction purification.

1. - After washing the soluble latex fraction in hexane with water, it is observed that this fraction contains respectively keton groups molecules ( $1800-1600\text{ cm}^{-1}$ ), esters ( $1300-900\text{ cm}^{-1}$ ) and hydroxyle ( $3300-3700\text{ cm}^{-1}$ ).
2. - After hydrolysis of the hexane fraction first washed with water the spectrum shows a diminution of absorbance corresponding to oxidized groups with regard to the characteristic absorbance of hydrocarbon groups ( $3000-2840\text{ cm}^{-1}$ ), ( $1375^{-1}$ ), ( $1450\text{ cm}^{-1}$ ).
3. - After passing on the  $\text{Al}_2\text{O}_3$  column of the hexane fraction previously hydrolysed, the absorption bands typical of the oxidized groups disappear almost totally. Only two weak bands situated at  $1080-1105\text{ cm}^{-1}$  and at  $1015-1030\text{ cm}^{-1}$ , correspond to ester groups which are not retained by  $\text{Al}_2\text{O}_3$ . The absorption band situated from  $710$  to  $730\text{ cm}^{-1}$  corresponds to the presence of long hydrocarbon chain ( $\text{CH}_2$ )<sub>n</sub>. With regard to Fig. 2, a relative proportion change is noted between the absorption band corresponding to the  $\text{CH}_2$  groups ( $1450\text{ cm}^{-1}$ ) and those corresponding to  $\text{CH}_3$  groups ( $1375\text{ cm}^{-1}$ ). The chromatography on the  $\text{Al}_2\text{O}_3$  column has thus eliminated the strongly methylized oxidized molecules. The presence of the absorption band typical of hydroxyl groups ( $3300-3700\text{ cm}^{-1}$ ) can be due to the presence of water molecules in the KBr which serves as a support to the I.R. spectra recording.

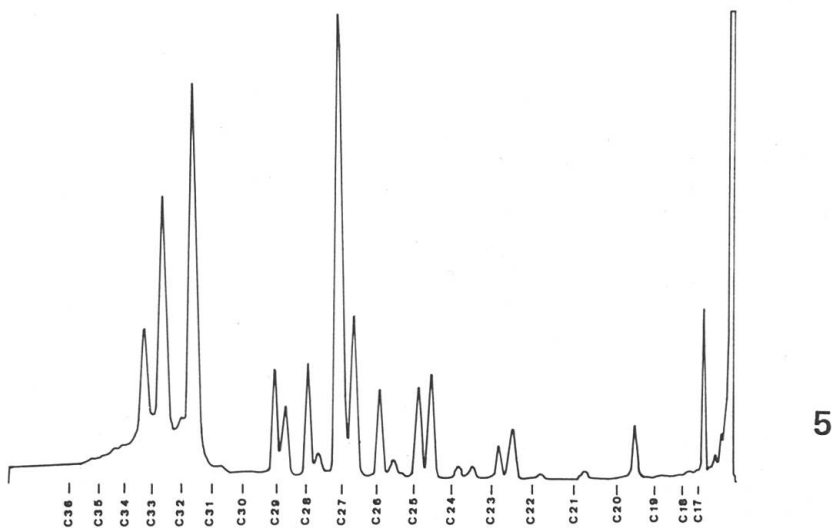
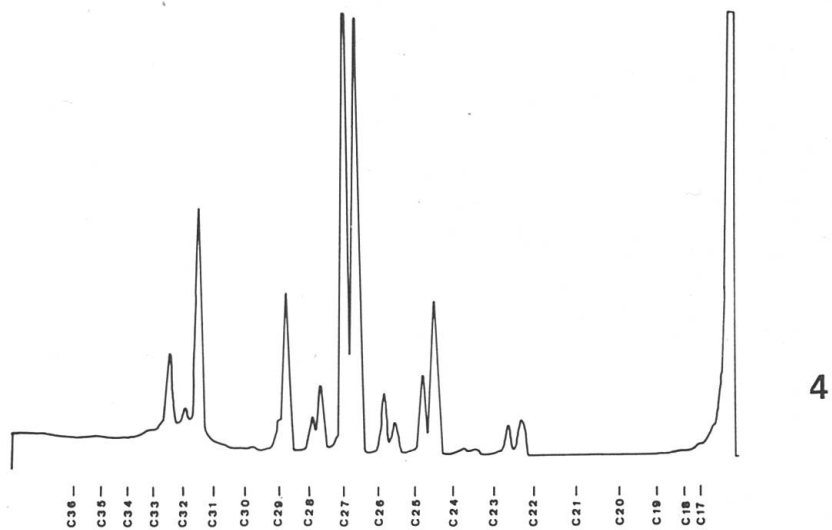


Fig. 4. - Gas-liquid chromatogram of *E. candelabrum* latices hydrocarbons.

Fig. 5. - Gas-liquid chromatogram of *E. dawei* latices hydrocarbons.

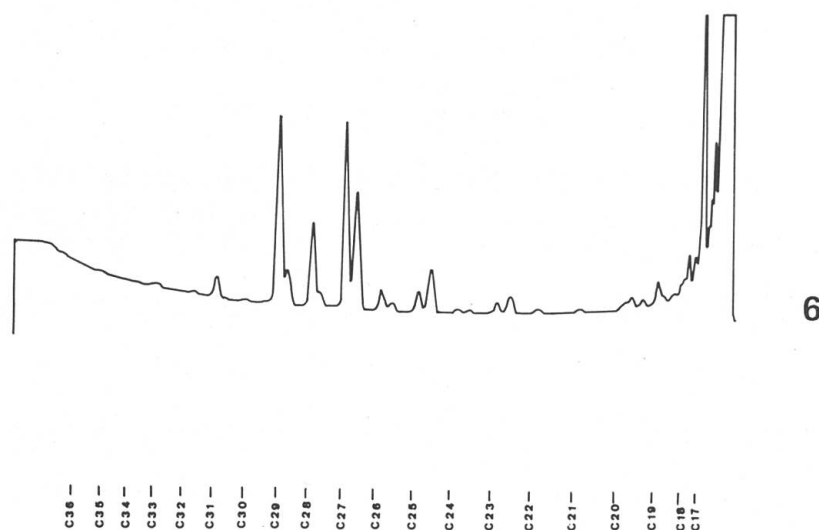


Fig. 6. - Gas-liquid chromatogram of *E. tirucalli* latices hydrocarbons.

## Results

### *Qualificatives*

The stages of the purification of the hexane fraction of the latices *E. candelabrum*, *E. dawei* and *E. tirucalli*, with a view to obtaining pure hydrocarbon fraction, were followed by I.R. spectroscopy.

The I.R. spectra recorded at the same purification stages were identical for the 3 studied species.

Figures 1, 2, and 3 show the I.R. spectra recorded on *E. candelabrum* at the following purification stages.

The used purification method seemed thus efficient since a practically pure hydrocarbon fraction for the 3 studied *Euphorbia* species was obtained. In all the cases this fraction was composed of alkanes as well as olefines as the presence of absorption bands at  $995-985\text{ cm}^{-1}$ , at  $855-885\text{ cm}^{-1}$  and  $1665-1625\text{ cm}^{-1}$  shows (bands corresponding to the presence of double linking). These weak bands coincide with hydrocarbon of the polyethylene type.

Figures 4, 5, and 6 are meant for pure hydrocarbons chromatograms (corresponding to the I.R. spectrum of Fig. 3) extracted from *E. candelabrum* (Fig. 4), from *E. dawei* (Fig. 5) and from *E. tirucalli* (Fig. 6) in solution in hexane.

In the three species peaks are found corresponding to *n*-alkanes (peaks with the same position on the chromatogram as the reference *n*-alkanes) as well as the peaks situated between these *n*-alkanes and corresponding to the presence of *br*-alkanes. The polyolefine with a chain length of more than C<sub>36</sub> are not detectable in the used conditions of separation.

By applying the separation conditions of hydrocarbons from C<sub>11</sub> to C<sub>25</sub> (see equipment and methods) to the studied latices, the chromatograms show for three species the presence of a principal peak situated between n-C<sub>11</sub> and n-C<sub>12</sub>.

This peak is often mistaken with the hexane peak in the case of Fig. 4, 5, and 6 (separation conditions for hydrocarbons ranging from C<sub>17</sub> to C<sub>36</sub>).

The relative height of this peak in comparison to those of the peaks corresponding to alkanes superior to C<sub>17</sub> is higher with *E. tirucalli* than with *E. candelabrum* and *E. dawei*. Moreover with *E. tirucalli* practically no hydrocarbons higher than C<sub>31</sub> are found whereas for the other two species the presence of alkanes superior to n-C<sub>31</sub> is noted.

In conclusion, the three studied species reveal a biomodal alkane repartition in their latex i.e. on the one hand the presence of a short alkane (C<sub>11</sub>-C<sub>12</sub>) and on the other hand the presence of an alkane mixture (*n*-alkanes and *br*-alkanes) longer and localized in the neighbourhood of n-C<sub>27</sub>.

### Quantitatives

The hydrocarbon yield of the latices determined by weight is 0.70% for *E. candelabrum*, 0.75% for *E. dawei* and 0.08% for *E. tirucalli*.

The hydrocarbon losses caused by different purification stages are estimated by submitting 100 mg standard hydrocarbons to these operations and by weighing it again after the treatment.

The results are the following:

Test number	Weight before treatment (mg)	Weight after treatment (mg)
<sup>1</sup> Reference .....	100	88
<sup>2</sup> Alkanes .....	100	45
<sup>3</sup> .....	100	47
<sup>4</sup> Reference olefines .....	100	82

It appears that the hydrocarbon losses lie between 12 and 55%. This important variation is in fact function of the care with which the separation of hexane/water is fundamentally done.

This test shows also that an olefine submitted to the method used for the purification of the hydrocarbon fraction of the latices is found

intact after this treatment. The gas liquid chromatography of this olefine before and after the treatment shows indeed a peak on the chromatogram situated at the same distance in comparison to the one of the hexane. There is only one difference in peak heights due to the injection variability as well as to losses during the treatment.

#### *Upper calorific value at constant volume of the latices*

The upper calorific value at constant volume estimated on dry latices is 7540 kcal/kg (i.e.  $\pm 31 \cdot 10^6$  J/kg) for the latex from *E. candelabrum*; 6663 kcal/kg (i.e.  $\pm 21 \cdot 20^6$  J/kg) for the latex from *E. dawei* and 6926 kcal/kg (i.e.  $29 \cdot 10^6$  J/kg) for the one from *E. tirucalli*.

In comparison, the upper calorific value determined in the same conditions is average 4700 kcal/kg (i.e.  $19 \cdot 10^6$  J/kg) for wood and 8500 kcal/kg (i.e.  $37 \cdot 10^6$  J/kg) for coal.

Thus it appears that the upper calorific value of the latices of the studied *Euphorbia* is not negligible since it is situated between that of wood and that of coal.

### Discussion

The studied hydrocarbon fraction concerns the hydrocarbons situated between  $C_{11}$  and  $C_{36}$ ; the hydrocarbons shorter than  $C_{11}$  or longer than  $C_{36}$  are not perceptible in G.L.C. in the utilized separation conditions.

The polyisoprenes being insoluble, in the utilized solvents their contents could not be estimated.

NIELSEN & al. (6) found in *E. tirucalli* a rubber content of about 1% with regard to the dry weight of the latex.

According to CALVIN (2), the molecular weight of this rubber is lower than that of *Hevea brasiliensis*.

As far as we know, no works were written on the rubber contents of the latices *E. candelabrum* and *E. dawei*.

VIAUD & TEISSEIRE (1) studying the composition of a meal composed of dried and crushed aerial parts of *E. tirucalli* note the presence of hydrocarbons in  $C_{12}$ ,  $C_{14}$ ,  $C_{16}$ ,  $C_{18}$ , terpenes, euphol, tirucallol and  $C_{12}$ ,  $C_{14}$ ,  $C_{16}$ ,  $C_{18}$ ,  $C_{20}$ ,  $C_{22}$  fatty acids. Thereby it is difficult to compare our results concerning hydrocarbons of *E. tirucalli* to those of these authors since they studied the composition of the whole plant and not of their latex.

Moreover they indicate the existence of hydrocarbons inferior to  $C_{18}$ , but on the other hand they do not find longer hydrocarbons. The conditions used for the separation of these hydrocarbons being not mentioned in the article, it is likely that the analysis method can not

separate longer hydrocarbons. Indeed, working on the whole plant, the hydrocarbons of the cuticular waxes should be detected and these are generally longer, ranging from  $C_{27}$  to  $C_{35}$  (7-10).

So concerning the possible use of the isolated *Euphorbia* latices hydrocarbons, our qualitative results show that it cannot be considered as a fuel without preliminary modifications (cracking to obtain a greater proportion of light hydrocarbons) or as raw material for the plastic industry without separating the alkanes from the olefines.

From a qualitative point of view the amelioration of the species should allow a selection of strains with a higher hydrocarbon content (isoprenoids).

The relatively high calorific value of dry latices is probably due to the presence of isoprenoids like sterols.

Indeed, NIELSEN & al. (2) note a sterol content of 50% (in comparison to the dry weight) in the latex of *E. tirucalli*. These sterols were identified (G.L.C., GC – MS) like euphol, euphorbol and tirucallol.

To our knowledge no comparable study was realized on the latex of *E. candelabrum* and of *E. dawei*.

Before considering a large scale culture of these species it is necessary to have a better knowledge of the latices composition of *Euphorbiae*, of their seasonal variation and to select strains with high hydrocarbon and/or sterol yield.

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