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DEPARTMENT OF THE ARMY  
Fort Detrick  
Frederick, Maryland

HYDROCARBONS CONTAINED IN THE UNSAPONIFIABLE  
FRACTION OF CERTAIN VEGETABLE OILS

Translation of a paper presented at the 6th Congress of the International Society for the Study of Fatty Substances (ISF), London, April 1962. Some of the results reported were obtained from a research project carried out under contract with the U. S. Department of Agriculture (Research Project FG-It-104-60). The authors again thank the Department of Agriculture for permission to publish. This paper appeared in the Italian-language publication, La Rivista Italiana delle Sostanze Grasse, (The Italian Fatty Substances Review) No. 11, 1963, pp. 603-606.

By  
D. P. Capella  
and Others

Hydrocarbon fractions of the unsaponifiable portions of certain vegetable oils are the object of this study. By means of gas chromatography and with the help of model compounds synthetically obtained, it was possible to identify almost all the components of these fractions. The presence in each oil studied of the normal series from  $C_{10}$  to  $C_{30-35}$  was ascertained, as was that of the iso and/or ante-iso series from  $C_{10}$  to  $C_{22}$ .

It is noteworthy that the hydrocarbon present in greatest abundance is  $nC_{29}H_{60}$ .

In certain previously published papers (1) on the composition of the unsaponifiable components of vegetable oils, some of us had observed that it was possible to separate the non-polyoenic hydrocarbons from the polyoenic (squalene) by means of chromatography of the unsaponifiable component on silicic acid. We report here the results of our study on the first type of these natural hydrocarbons, which we isolated from the oils of flax, peanuts, cole-seed, corn and olives. We worked first and most intensively on linseed oil, and made use of the data gathered for the other oils.

### Preparation of the Unsaponifiable Components

In connection with other research on other groups of substances contained in the unsaponifiable components, we were forced to forego the standard laboratory method and to have recourse to other methods, which allowed us to obtain considerable quantities of these substances at a lower cost and in a shorter time. In our linseed oil study, we conducted successive experiments with the following methods: a) continuous extraction of the oil using methanol; b) preparation of dry soaps and their extraction with hexane; c) molecular distillation. In each case we obtained a marked enrichment in unsaponifiable components, and the enriched product was subsequently handled with standard laboratory procedures.

Using method (a), we extracted about 30 g of oil containing 11% unsaponifiable components from 10 kg of oil. With method (b), using autoclave saponification and drying the soaps in the "Niro," we obtained about 16 kg of dry soap from 10 kg of linseed oil, of which 130 g were crude unsaponifiable components. Method (c) proved most practicable (1 - We take this occasion to extend our cordial thanks to SIAI Lerici and to Chief Engineer Narduzzi of the Corman plant for making available to us the molecular distillation equipment that solved our problem.): all told, we distilled 50 kg of linseed oil, obtaining about 500 g of enriched distillate, from which we obtained about 120 g of unsaponifiable components.

The yield in unsaponifiables varied widely according to the method used, as did the quantitative composition of the unsaponifiables obtained. While the various types of unsaponifiables all contained the same groups of substances, the weight ratios of the different groups differed according to whether the component had been obtained with one or the other of the methods described. The method that apparently gives the most complete results is the standard laboratory method, used at most on 50 to 100 g of oil.

The unsaponifiable component, obtained by the usual method from one of the enriched residues, was then fractionated. The application of the chromatography separation method on a silicic column, described in an earlier paper (1), had proven practicable only for small quantities of substances, on the order of one or two grams, we decided on an initial fractionization on an aluminum column, which makes it possible to handle far larger quantities of material far more rapidly. This gave us four fractions, as indicated in Table 1.

Number of waves in  $\text{cm}^{-1}$

Transmittance %

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Wave length (micron)

Figure 1

Table 1

| Fractions | Elutent                        | Substances                                |
|-----------|--------------------------------|---|
| 1         | Petroleum ether                | Hydrocarbons                              |
| 2         | Petroleum ether + benzol (1:1) | Super. alcohols, waxes, terpenic alcohols |
| 3         | Benzol                         | Sterols                                   |
| 4         | Chloroform + methanol (1:1)    | Unidentified colored substances           |

Fraction (1), consisting of the total hydrocarbons in the oil, was then chromatographed on silicilic acid, as explained in the previous reports (1), getting separate readings for the non-polyoenic and squalene hydrocarbons.

#### Fractionating the Hydrocarbons

The general chemical characteristics of the non-polyoenic hydrocarbons in linseed oil indicate that they are very largely constituted of paraffins; they do not absorb bromine, show a negative reaction to tetranitromethane, show  $n_D$  hydrogen = 0,  $n_D$  J = 7,  $(\alpha_D)^D$  practically = 0, and

an IR spectrum (Figure 1) which rules out the presence of olefins in any considerable quantity.

An initial gas chromatography on isothermic equipment showed a remarkably complex composition (Figure 2). Since it seemed impossible to make any direct identification of the individual chemicals present, we tried various ways to fractionate the hydrocarbon mixture.

At first, we tried boiling distillation in tubes. By this method we got three fractions: one that boiled at 145° C at 20 mm of mercury, one boiling at 170° C at 1 mm of mercury, and a residue.

Of these three fractions, the first is liquid, the second tends to precipitate crystals, and the third is a solid.

Gas chromatographic examination of the three fractions did show, however, that while the first fraction is composed of a modest number of components (ten, to be precise), the other two fractions contain practically all the compounds present in the original mixture.

In order to obtain better fractionation of the heavier components, we then proceeded to crystallization fractionation. By acetone crystallization fractionation. By acetone crystallization we obtained three sections of the mixture.

The first, obtained by crystallization at room temperature, consisted of solid crystalline hydrocarbons, melting at 50-60° C, and representing 20% of the total.

By cooling to 0° C, we produced formation of other crystals, which become a semi-solid mass at room temperature, and constitute 45% of the total.

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Figure 2.

And finally, by evaporation of the residual fluids, we obtained a residue constituting 35% of the total.

The fractions obtained by crystallization at 0° C and by evaporation of the residual fluids, however, showed only quantitative differences under gas chromatography, with both showing differing proportions of the same components.

Thereafter we decided to perform only one crystallization, yielding only two fractions. Obviously, such a separation could not be rigorous; it led merely to a marked enrichment in the lighter extremes of a liquid

fraction (Fraction A<sub>1</sub>), and of the heavier extremes in a solid fraction (fraction A<sub>2</sub>). The A<sub>1</sub> was 80% of the total, the A<sub>2</sub> about 20%.

The two fractions were studied separately under gas chromatography.

Fraction A<sub>1</sub> (Figure 3) was chromatographed on a column 2 m / 2 mm, with Chromosorb W 60-80 mesh and 5% Apiezon L. Column temperature was 200° C, evaporator temperature 280° C, nitrogen 10 ml/min. flame ionisation indicator. The chromatogram shows at least 16 clearly visible elements.

The A<sub>2</sub> fraction (Figure 4) was chromatographed on a 1 m / 4 mm column, with celite 545 and 20% Apiezon L. Column temperature 300° C, evaporator temperature 450° C, helium 25 ml/min, filament indicator, reactor temperature 720° C, indicator temperature 100° C.

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Figure 3

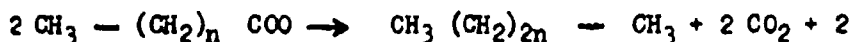
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Figure 4

### Hydrocarbon Identification

We made identification by comparing the chromatography behavior of natural hydrocarbons with that of known hydrocarbons. At first we used a compound made up of n-octane, n-decane, n-dodecane, n-tetradecane, n-hexadecane, n-octadecane, n-docosane, n-hexacosane and n-triacontane.

The first six hydrocarbons are commercial products (BDH), but n-docosane, n-hexacosane and n-triacontane were prepared by synthesis according to Peterson's method, by electrolysis of an alcoholic solution of potassium soap in a fatty acid of suitable length:



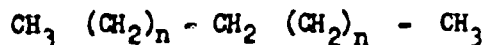
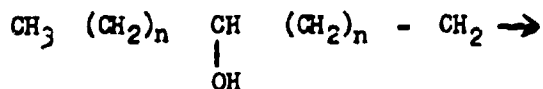
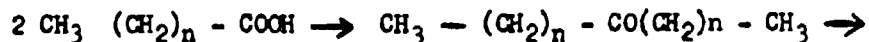
Beginning with an acid containing n carbon atoms, you thus obtain the normal paraffinic hydrocarbon with 2 n-2 atoms.

By plotting on the diagram the logs of the retention times of these hydrocarbons and the number of carbon atoms in each, we obtained a pattern line which, by interpolation and extrapolation, enables us to identify all the natural linear paraffinic hydrocarbons from C<sub>10</sub> to C<sub>35</sub>.

The A<sub>2</sub> fraction was invariably found to contain only linear paraffinic hydrocarbons, while the A<sub>1</sub> fraction, in addition to the linear paraffinic hydrocarbons, contained other substances, albeit in smaller quantities.

We have found no mention in the literature of the composition of the hydrocarbon mixtures in vegetable oils. Even the wax hydrocarbons, which also contain them in large quantities, seem to have received little attention.

It seems, however, fairly well substantiated that the biogenesis of hydrocarbons in plants takes place through the formation of a ketone by elimination of one CO<sub>2</sub> molecule between two fatty acid molecules and subsequent reduction of the ketone group, with probable intermediate formation of the following secondary alcohol:





Now, since secondary acids and alcohols have recently been isolated from waxes, and particularly from wool waxes, and it has been shown that there are always present, in addition to the members of the normal series, those of the iso- and ante-iso-series as well, it seemed plausible to us that the unsaponifiable hydrocarbons would also contain the iso- and ante-iso terms along with the linear.

In order to test this hypothesis, we synthesized several members of these two series and compared their chromatographic behavior with that of the natural hydrocarbon mixtures.

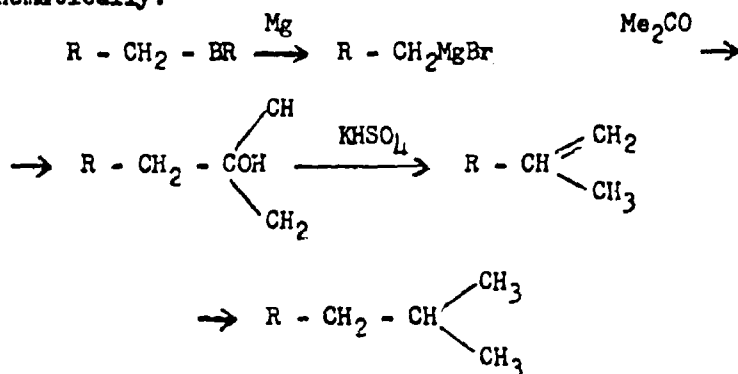
We prepared as our iso-paraffines C<sub>11</sub>, C<sub>15</sub>, C<sub>21</sub>, while our ante-iso-paraffines were C<sub>12</sub>, C<sub>16</sub>, and C<sub>22</sub>.

Paraffine synthesis was conducted beginning with the alkylic halogen R-CH<sub>2</sub>-ER (where R is, respectively, n-C<sub>7</sub>H<sub>15</sub>, n-C<sub>21</sub>H<sub>43</sub>, and n-C<sub>17</sub>H<sub>35</sub>), and obtaining the corresponding Grignard.

We caused this to react with acetone, and through subsequent hydrolysis, we obtained the corresponding tertiary carbinols.

We then proceeded to dehydrate them by boiling distillation in the presence of KHSO<sub>4</sub>. The mixture of two isomeric olefins resulting from each carbinol was then reduced and catalytically hydrogenated with (PtO<sub>2</sub>) to hexane, thus arriving at the corresponding iso-paraffine.

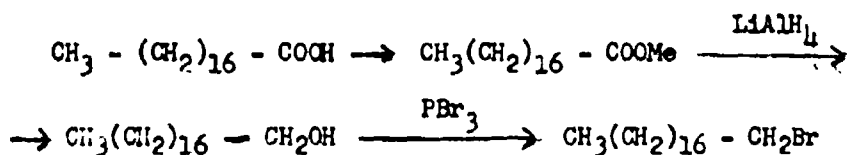
Schematically:



We conducted an analogous synthesis of the ante-iso paraffines, using methyl-ethyl-ketone in place of acetone.

For the C<sub>11</sub> iso, C<sub>12</sub> ante-iso, and C<sub>15</sub> iso and C<sub>16</sub> ante-iso, we began respectively with bromides of n-octyl and n-dodecyl (BDH commercial)

For the C<sub>21</sub> iso and the C<sub>22</sub> ante-iso, we began with stearyl bromide obtained from stearic acid by the following process:



The retention times of all the hydrocarbons prepared in this manner helped identify the great majority of the natural hydrocarbons. Two points still remain obscure: it is impossible to distinguish between the iso series |R-CH(CH<sub>3</sub>)<sub>2</sub>| and the ante-iso series |R-CH(CH<sub>3</sub>)(C<sub>2</sub>H<sub>5</sub>)| of the hydrocarbons, since the retention times of the two series, when compared with the pattern, are almost identical; likewise still unknown is a third category of hydrocarbons, and it is by no means impossible that they will prove to be olefins, in view of the low, though not quite nil n. iodine in the total hydrocarbons of all the oils examined.

Figures 5 and 6 show the diagrams that enabled us to identify almost all the hydrocarbons in flax. They refer to the A<sub>1</sub> and A<sub>2</sub> fractions, whose gas chromatograms are also reported. The ordinates show the logs of the retention times for the natural hydrocarbons, and the abscissas the number of carbon atoms. Similar diagrams were worked out for the other oils.

It was shown therefore that the flaxseed oil we studied contains: 35 normal paraffinic hydrocarbons, the complete series from C<sub>10</sub>H<sub>22</sub> to C<sub>35</sub>H<sub>72</sub>, eleven paraffinic hydrocarbons belonging to the iso and/or ante-iso series, all the terms from C<sub>11</sub>H<sub>24</sub> to C<sub>22</sub>H<sub>46</sub>, and six or seven hydrocarbons belonging to an unidentified series, probably from C<sub>14</sub> to C<sub>20</sub>.

Figure 5

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Figure 6

Present in greatest abundance is the saturated hydrocarbon  $C_{29}H_{60}$ . It alone constitutes eight to ten percent of the total. It would appear that this hydrocarbon is the only one actually identified by previous workers using traditional methods.

Using altogether analogous methods, we studied the hydrocarbons of several other oils. Table 2 summarized our data.

Table 2

| Oils studied | Paraffine no.           | Iso and/or ante-iso paraffines       | Number of unidentified elements | Total number of elements |
|--------------|-------------------------|--------------------------------------|---------------------------------|--------------------------|
| Corn         | $C_{11}-C_{31}$         | $C_{11}-C_{21}$                      | 8                               | 40                       |
| Peanut       | $C_{11}-C_{30}$         | $C_{11}-C_{23}$                      | 7                               | 40                       |
| Cole-seed    | $C_{11}-C_{31}$         | $C_{11}-C_{17}$ ,<br>$C_{19}-C_{21}$ | 6                               | 36                       |
| Olive        | $C_{11}, C_{13}-C_{30}$ |                                      | 6                               | 29                       |
| Linseed      | $C_{11}-C_{35}$         | $C_{11}-C_{21}$                      | 7                               | 43-45                    |

The hydrocarbon group in an oil is far less characteristic than are its terpenic alcohols. Such a study might prove rewarding for inquiry into the basic biogenesis of vegetable oils. It is particularly interesting to note that the uneven subscriptions of C are more abundant than the even ones, and that the single fruit oil (olive) we studied contains considerably fewer hydrocarbons than do the seed oils.

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- (1) D. P. Capella, G. de Zotti, G. S. Ricca, A. F. Valentini, and G. Jacini: J.A.O.C.S., 37 (1960) 564-67.