# Fatty Acids From the Scent Glands of the Louisiana Muskrat[1]

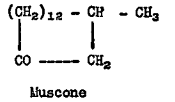
J. L. E. ERICKSON and HOMER B. HIX,[2] Coates Chemical Laboratory, Louisiana State University, Baton Rouge, La.

THE scent glands of a typical North American muskrat *(Ondatra zibethicus rivalicius)* contain fats and complex esters of fatty acids. The unsaponifiable or neutral fraction of these esters has been investigated (1) and found to contain $C_{13}$, $C_{15}$, $C_{17}$, and $C_{19}$ macrocyclic carbinols together with very much smaller quantities of their corresponding cyclic ketones. Simmons and Hills (2) have reported a preliminary examination of the mixed fatty acids present in these glands. The present investigation, however, deals with the quantitative analysis of the component fatty acids contained in the acid fraction of the muskrat musk glands as a preliminary step towards their identification.

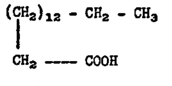
While it is possible to account for the production of macrocyclic ketones from monobasic acids using biological processes, it would seem difficult now to relate the acids found in muskrat scent glands to macrocyclic carbinols, which must be regarded as the parents of the corresponding ketones. This analysis gives no indication of the presence of macrocyclic acids, which might possibly bear a close relationship to the macrocyclic carbinols already identified in muskrat scent glands.

The results of this analysis reveal that the esters of myristic, palmitic, hexadecenoic, oleic, and linoleic acids are present as major components in these glands. It is significant that unsaturated acids of the $C_{24}$ and $C_{26}$ series are also present to the extent of 8.5% of the total fatty acids. The acids present to less than 1%, which include decanoic, dodecanoic, stearic, tetracosanoic, dodecenoic, and tetradecenoic acids, are reported with some reluctance and only as a consequence of the method of calculation used. Since linoleic acid is the only diethenoid fatty acid, which has been identified as being present in appreciable quantities in either plant or animal fats, a more critical method of analysis of the fractions containing these high molecular acids would undoubtedly aid in giving a clearer picture of the true composition of these higher fractions.

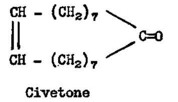
It is conceivable that some of these acids may be progenitors of the macrocyclic compounds already isolated and identified in these glands. In this connection the similarity in structure between palmitic acid and muscone and between oleic acid and civetone has already been pointed out by Ruzicka (3), and various mechanisms for such transformations have been suggested by Stevens (1).



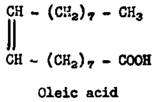
Muscone



Palmitic acid



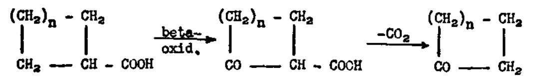
Civetone



Oleic acid

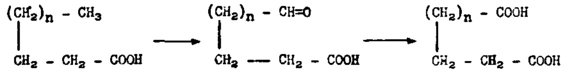
There is nothing to indicate that it is possible to synthesize macrocyclic ketones from saturated monobasic acids; however, if the biological processes of beta oxidation (4) and omega oxidation (5) are recognized, the formation of large ring ketones from these oxidation products can be accounted for on the basis of well known methods of organic chemical synthesis.

It is obvious that beta oxidation of macrocyclic carboxylic acids, if present, would lead to the beta-keto cyclic acids, which readily lose carbon dioxide to yield ketones.



e. g., Exaltone (n = 12)
Dihydro-civetone (n = 14)

Aliphatic monobasic acids may undergo biological omega oxidation in the following manner:



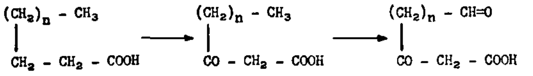
Palmitic acid (n = 12)
Stearic acid (n = 14)

partial omega oxidation product

omega oxidation product

An unsaturated acid, such as oleic acid, should undergo these reactions also to yield corresponding oxidation products.

It is also conceivable that both beta and omega oxidation may occur within the same monobasic acid.

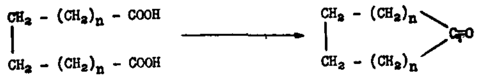


beta oxidation product

beta and omega oxidation product

Assuming the formation of these biological oxidation products, it is now possible to account for the formation of cyclotridecanone from myristic acid, cyclopentadecanone (exaltone) from palmitic acid, cycloheptadecanone (dihydro-civetone) from stearic acid, and cycloheptadecenone (civetone) from oleic acid in a variety of ways. Since 3-methyl-cyclopentadecanone (muscone) is found only in the glands of the male musk deer, it is possible that it may be formed in a different manner.

It is known from the work of Ruzicka (6) that macrocyclic ketones may be obtained from the salts of dibasic acids.



e. g., Exaltone (n = 6)
Dihydro-civetone (n = 7)

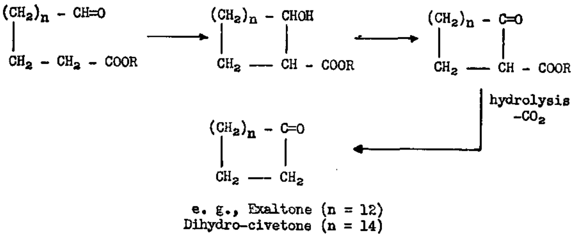
In a similar way it is conceivable that an omega-aldehydo-ester can undergo the aldol condensation at high dilution and cyclize according to the equations below. At this point it is perhaps necessary to men-

---

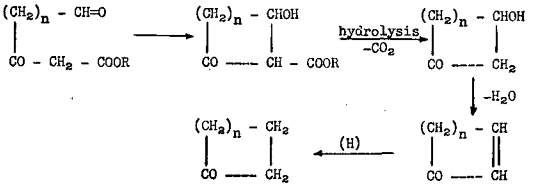
[1] Abstracted from a dissertation submitted by Homer B. Hix to the Faculty of Louisiana State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, May, 1948.

[2] Ethyl Corporation Research Fellow, 1947. Present address: Magnolia Petroleum Company, Field Research Laboratories, Dallas, Texas.

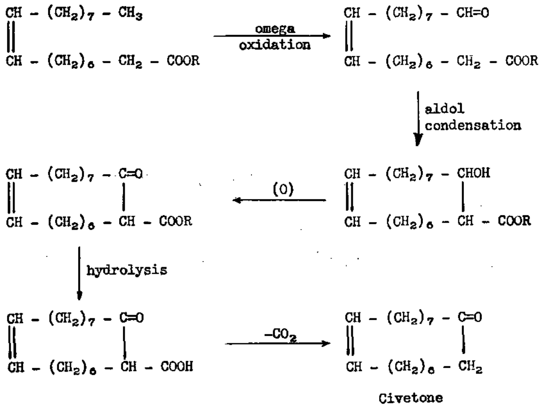
tion that the acids probably occur in nature as the glycerides or esters.



A beta-keto-omega-aldehydo-ester, by similar reactions, may also yield a macrocyclic ketone as shown below.

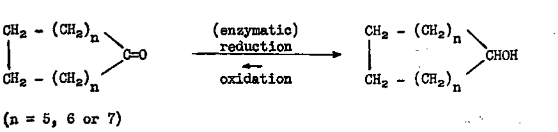


Civetone from oleic esters may also be accounted for by similar mechanisms as shown below.



The utilization of oleic acid for the production of dihydrocivetone may be accounted for by assuming, first, its hydrogenation to stearic acid before undergoing the transformation suggested above. If this be the case, stearic acid might be expected present in larger amounts than a fraction of 1% of the total fatty acids unless it is assumed essentially all of the stearic acid has been used in the transformation to dihydro-civetone.

The preceding discussion which pertains to the possible relation existing between fatty acids and macrocyclic compounds has been limited to possible mechanisms which relate fatty acids to macrocyclic ketones. Since macrocyclic compounds found in muskrat scent glands have been found to contain $C_{13}$, $C_{15}$, $C_{17}$, and $C_{19}$ macrocyclic carbinols together with much smaller quantities of their corresponding cyclic ketones (1), it is perhaps necessary to indicate a possible relationship between these two classes of compounds.



This possible mechanism assumes an equilibrium between these macrocyclic ketones and carbinols in the presence of enzymes with the equilibrium far to the right. It is questionable, however, that any living cell can be said to be in a state of true equilibrium; therefore, it would seem best to consider the above mechanism as the attainment of a steady state.

The presence of the three lower members of this series of cyclic carbinols, which include cyclotridecanol, cyclopentadecanol, cycloheptadecanol and cyclononadecanol, found in the muskrat scent glands, may be accounted for on the basis of the proposed mechanisms. The presence of cyclononadecanol in this mixture of carbinols, however, cannot be accounted for on the basis of this analysis as no eicosanoic acid was found present.

## Experimental

The ester fractionation method of analysis as outlined by Hilditch (7) was used in this investigation. Traces of unsaponifiable matter were removed from the mixed fatty acids by extraction of an aqueous-alcoholic solution of the potassium salts with ether. The methyl esters were then prepared and distilled from a Claisen flask. The residual non-volatile esters were left unanalyzed while the volatile esters were converted to the lead salts of the fatty acids which were further separated by fractional crystallization from alcohol. In this way the lead salts of the fatty acids were separated into three fractions. The 1-S fraction was insoluble in hot alcohol while the 2-S fraction was soluble. The L fraction was soluble in alcohol at 15°C. The lead salt fractions were converted to methyl esters and separated further by fractional distillation. The resulting fractions were then analyzed by determining the iodine number and mean molecular weight (saponification equivlent).

*Removal of Unsaponifiable Matter.* Muskrat scent glands, upon extraction with petroleum ether, yield a fat and complex esters, which yield upon saponification a neutral fraction containing macrocyclic carbinols and an acid fraction consisting of salts of fatty acids. The fatty acids may be liberated from these salts upon treatment with 40% sulfuric acid.

The mixed fatty acids (supplied through the courtesy of Givaudan-Delawanna, Inc., New York) were dissolved in 10% alcoholic potassium hydroxide and refluxed for three hours. This solution was then diluted with water and extracted repeatedly with ether to remove traces of unsaponifiable matter. The soap solution was then cooled, and the fatty acids were liberated from their potassium salts with 40% sulfuric acid. The fatty acids were then extracted with ether and the ether removed by distillation.

*Preliminary Separation of Mixed Fatty Acids.* Preliminary work had shown that 20% of the methyl esters did not distill through an efficient 100-cm. fractionating column as the boiling point of the residual esters was extremely high (above 200°C. under a pressure of 1-2 mm. of Hg.). To eliminate this large residue in the subsequent fractional distillation of the methyl esters, the mixed fatty acids were converted into methyl esters (732 g.) by the usual procedure and distilled from a Claisen flask at a reduced pressure of 1-2 mm. of Hg. A residue of 139 grams (19%) of the original methyl esters remained undistilled and was not analyzed. The distilled methyl esters (593 g.) were then converted into the acids by saponification and acidification as described above.

*Lead Salt-Alcohol Procedure.* In view of the complexity of the mixture of fatty acids under investigation it was necessary to subject the mixture of acids to the lead salt-alcohol procedure which resulted in the subsequent production of three distinct fractions. The mixed fatty acids, obtained from 593 grams of methyl esters, were dissolved in 2800 ml. of 95% alcohol and mixed with a boiling solution of the following composition: lead acetate (395 g.) and 2800 ml. of 95% alcohol containing 1.5% glacial acetic acid. The lead salts were allowed to crystallize at 15°C. for 12 hours, and the solid acid salts were collected by filtration and then recrystallized from alcohol. The first fractional crystallization of the lead salts yielded a mixture of white and yellow crystals. These crystals were filtered from the solution of unsaturated lead salts (L fraction) and placed in a volume of boiling alcohol equal to the volume used for the first crystallization. The yellow lead salts (1-S fraction) of this mixture were found to be insoluble in hot alcohol and formed a separate liquid phase at the bottom of the hot solution.

Upon decanting this solution of saturated lead salts (2-S fraction), the insoluble yellow salts solidified and remained on the walls of the flask. This permitted a clean separation of the two saturated fractions. The decanted solution was then cooled and the white saturated lead salts were again crystallized at 15°C. for 12 hours and filtered from the alcoholic solution. This solution was then combined with the other alcoholic solution which contained the unsaturated acid salts. The acids of each fraction were liberated separately from the salts with 6 N hydrochloric acid and converted to methyl esters by the usual procedure.

*Preparation of the Methyl Esters.* Each group of acids, which resulted from the lead salt-alcohol procedure, was dissolved in four times its weight of methyl alcohol and the acids converted to their methyl esters by refluxing for three hours in the presence of 2% sulfuric acid. After the esterification was completed, 70-80% of the solvent methyl alcohol was distilled from the esters. They were then dissolved in ether and extracted repeatedly with potassium carbonate solution. The unesterified acids were recovered, converted to methyl esters, and combined with the main fraction. The ether was then distilled, leaving the pure neutral esters, which were ready for fractional distillation.

*Ester Fractionation.* The 1-S esters (185.24 g.), 2-S esters (128.83 g.), and L esters (276.13 g.) were distilled under reduced pressure through an "E. H. P. Column," similar to the one described by Longenecker (8), into 15, 14, and 21 fractions, respectively. Each fraction was analyzed by determination of the iodine number (Hanus) and mean molecular weight (saponification equivalent). The distillation and analytical data are recorded in Tables I, II, and III.

## Calculations

Charnley (9) has given equations relating total iodine number and equivalents of mixtures of esters of the fatty acids to those of the individual esters; however, the method of calculation used in this work is essentially the method used by Hilditch (7). Application of the method of determinants for solution of three simultaneous equations, however, greatly simplifies the calculations necessary in this type of work. The assumption was made that any one distillation fraction contains not more than three esters of the following types:

1. Two adjacent homologous saturated esters and one unsaturated ester of the same carbon series as the higher saturated ester, e. g., $C^{s}_{16}$, $C^{s}_{18}$ and $C^{u}_{18}$.
2. Two adjacent homologous saturated esters and one unsaturated ester of a higher series, e. g., $C^{s}_{14}$, $C^{s}_{16}$ and $C^{u}_{18}$.
3. One saturated ester and two adjacent homologous unsaturated esters, the lower member of which is of the same carbon series as the saturated ester, e. g., $C^{s}_{16}$, $C^{u}_{16}$ and $C^{u}_{18}$.
4. One saturated ester and two unsaturated esters of the next higher series, e. g., $C^{s}_{16}$, $C^{u}_{18}$ and $C^{2u}_{18}$.
5. Three unsaturated esters, two of which belong to the same carbon series and the other belongs to either a higher or a lower series, e. g., $C^{u}_{16}$, $C^{2u}_{18}$ and $C^{u}_{24}$, or $C^{2u}_{24}$, $C^{u}_{26}$ and $C^{2u}_{26}$.

The composition of fractions of these types was calculated directly from their mean molecular weight and iodine number by use of the following equations:

$$x + y + z = w \qquad (1)$$

$$x/E_x + y/E_y + z/E_z = w/E_w \qquad (2)$$

$$xI_x + yI_y + zI_z = wI_w \qquad (3)$$

where x, y, and z are the respective weights of the component esters in a fraction of weight w, $E_x$ $E_y$, and $E_z$ are the corresponding theoretical molecular weights and $E_w$ the observed mean molecular weight of the fraction; $I_x$, $I_y$, and $I_z$ are the corresponding theoretical iodine numbers and $I_w$ the observed iodine number of the fraction.

Using determinants for the solution of the above system of equations, the expressions yielding values of x/w, y/w, and z/w are as follows:

$$x/w = \frac{(I_z/E_y) + (I_w/E_z) + (I_y/E_w) - (I_y/E_z) - (I_z/E_w) - (I_w/E_y)}{(I_z/E_y) + (I_x/E_z) + (I_y/E_x) - (I_y/E_z) - (I_z/E_x) - (I_x/E_y)} \qquad (4)$$

$$y/w = \frac{(I_z/E_w) + (I_x/E_z) + (I_w/E_x) - (I_w/E_z) - (I_z/E_x) - (I_x/E_w)}{(I_z/E_y) + (I_x/E_z) + (I_y/E_x) - (I_y/E_z) - (I_z/E_x) - (I_x/E_y)} \qquad (5)$$

$$z/w = \frac{(I_w/E_y) + (I_x/E_w) + (I_y/E_x) - (I_y/E_w) - (I_w/E_x) - (I_x/E_y)}{(I_z/E_y) + (I_x/E_z) + (I_y/E_x) - (I_y/E_z) - (I_z/E_x) - (I_x/E_y)} \qquad (6)$$

The denominator of equations (4), (5), and (6) is the same for the calculation of any one combination of esters.

*Sample Calculation.* The calculation of Fraction L-07 (Tables Va and Vb) yields positive values when calculated as palmitic, hexadecenoic and oleic esters.

TABLE I

Fraction Analysis Data

| Fraction | B. pt. °C. 2 mm. | Mol. Wt. | I. No. | Weight g. | $C^{s}_{14}$ | $C^{s}_{16}$ | $C^{s}_{18}$ | $C^{u}_{16}$ | $C^{u}_{18}$ | $C^{u}_{24}$ | $C^{2u}_{24}$ | $C^{u}_{26}$ | $C^{2u}_{26}$ | Residue |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1-S-01 | 135-149 | 247.6 | 11.72 | 2.05 | 1.61 | 0.19 | ---- | 0.25 | ---- | ---- | ---- | ---- | ---- | ---- |
| 1-S-02 | 149-149 | 264.4 | 4.63 | 24.92 | 5.94 | 17.61 | ---- | ---- | 1.37 | ---- | ---- | ---- | ---- | ---- |
| 1-S-03 | 149-151 | 264.4 | 6.81 | 22.09 | 5.08 | 15.25 | ---- | ---- | 1.76 | ---- | ---- | ---- | ---- | ---- |
| 1-S-04 | 151-154 | 269.5 | 13.92 | 24.03 | 3.69 | 16.46 | ---- | ---- | 3.88 | ---- | ---- | ---- | ---- | ---- |
| 1-S-05 | 154-155 | 270.8 | 16.70 | 26.88 | 4.42 | 17.97 | ---- | ---- | 4.49 | ---- | ---- | ---- | ---- | ---- |
| 1-S-06 | 155-159 | 271.8 | 19.25 | 28.60 | 3.60 | 18.57 | ---- | ---- | 6.43 | ---- | ---- | ---- | ---- | ---- |
| 1-S-07 | 159-165 | 274.7 | 28.73 | 19.24 | 2.32 | 10.49 | ---- | ---- | 6.43 | ---- | ---- | ---- | ---- | ---- |
| 1-S-08 | 165-186 | 282.2 | 48.64 | 2.08 | 0.11 | 0.84 | ---- | ---- | 1.13 | ---- | ---- | ---- | ---- | ---- |
| 1-S-09 | 186-192 | 292.7 | 56.39 | 3.95 | ---- | 0.57 | 0.78 | ---- | 2.60 | ---- | ---- | ---- | ---- | ---- |
| 1-S-10 | 192-204 | 304.6 | 61.31 | 1.31 | ---- | ---- | 0.34 | ---- | 0.82 | 0.15 | ---- | ---- | ---- | ---- |
| 1-S-11 | 204-214 | 342.8 | 72.68 | 1.34 | ---- | ---- | 0.52 | ---- | ---- | 0.18 | 0.64 | ---- | ---- | ---- |
| 1-S-12 | 214-230 | 380.1 | 79.76 | 5.56 | ---- | ---- | 0.01 | ---- | ---- | 4.47 | 1.08 | ---- | ---- | ---- |
| 1-S-13 | 230-240 | 400.5 | 81.92 | 9.78 | ---- | ---- | ---- | ---- | ---- | ---- | 2.49 | 6.95 | 0.34 | ---- |
| 1-S-14 | 240-244 | 403.1 | 81.76 | 5.24 | ---- | ---- | ---- | ---- | ---- | ---- | 0.87 | 3.63 | 0.74 | ---- |
| 1-S-15 | 244 | 408.4 | 80.53 | 1.41 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 1.21 | 0.20 | ---- |
| Residue | ------- | ----- | ----- | 6.76 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 6.76 |
| Total |  |  |  | 185.24 | 26.77 | 97.95 | 1.65 | 0.25 | 28.91 | 4.80 | 5.08 | 11.79 | 1.28 | 6.76 |
| Weight per cent |  |  |  |  | 14.45 | 52.88 | 0.89 | 0.13 | 15.61 | 2.59 | 2.74 | 6.37 | 0.69 | 3.65 |

TABLE II

Fraction Analysis Data

| Fraction | B. pt. °C. 2 mm. | Mol. Wt. | I. No. | Weight g. | $C^{s}_{14}$ | $C^{s}_{16}$ | $C^{s}_{24}$ | $C^{u}_{18}$ | $C^{2u}_{24}$ | $C^{u}_{26}$ | $C^{2u}_{26}$ | Residue |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2-S-01 | 121-139 | 252.7 | 15.63 | 5.49 | 4.09 | 0.39 | ---- | 1.01 | ---- | ---- | ---- | ---- |
| 2-S-02 | 139-142 | 267.1 | 10.64 | 20.73 | 4.15 | 13.97 | ---- | 2.61 | ---- | ---- | ---- | ---- |
| 2-S-03 | 142-145 | 268.1 | 12.49 | 21.74 | 4.05 | 14.53 | ---- | 3.16 | ---- | ---- | ---- | ---- |
| 2-S-04 | 145-152 | 272.5 | 26.60 | 16.17 | 2.75 | 8.42 | ---- | 5.00 | ---- | ---- | ---- | ---- |
| 2-S-05 | 152-159 | 281.6 | 53.08 | 19.81 | 2.55 | 4.94 | ---- | 12.32 | ---- | ---- | ---- | ---- |
| 2-S-06 | 159-164 | 288.5 | 72.00 | 13.86 | 1.37 | 0.83 | ---- | 11.66 | ---- | ---- | ---- | ---- |
| 2-S-07 | 164-190 | 297.2 | 74.44 | 5.67 | ---- | 0.34 | 0.40 | 4.93 | ---- | ---- | ---- | ---- |
| 2-S-08 | 190-211 | 309.7 | 72.08 | 2.48 | ---- | ----- | 0.42 | 2.01 | 0.05 | ---- | ---- | ---- |
| 2-S-09 | 211-215 | 380.9 | 98.21 | 1.64 | ---- | ---- | 0.40 | ---- | 1.16 | 0.08 | ---- | ---- |
| 2-S-10 | 215-216 | 384.6 | 98.96 | 1.65 | ---- | ---- | 0.25 | ---- | 1.08 | 0.32 | ---- | ---- |
| 2-S-11 | 216-224 | 389.4 | 99.27 | 1.82 | ---- | ---- | 0.11 | ---- | 1.04 | 0.67 | ---- | ---- |
| 2-S-12 | 224-227 | 394.6 | 100.63 | 5.64 | ---- | ---- | ---- | ---- | 2.52 | 2.48 | 0.64 | ---- |
| 2-S-13 | 227-227 | 405.1 | 101.50 | 5.63 | ---- | ---- | ---- | ---- | 0.40 | 2.12 | 3.11 | ---- |
| 2-S-14 | 227 | 401.6 | 100.45 | 1.53 | ---- | ---- | ---- | ---- | 0.31 | 0.63 | 0.59 | ---- |
| Residue | ------- | ----- | ------ | 4.97 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 4.97 |
| Total |  |  |  | 128.83 | 18.96 | 43.42 | 1.58 | 42.70 | 6.56 | 6.30 | 4.34 | 4.97 |
| Weight per cent |  |  |  |  | 14.72 | 33.70 | 1.23 | 33.14 | 5.09 | 4.89 | 3.37 | 3.86 |

TABLE III

Fraction Analysis Data

| Fraction | B. pt. °C. 2 mm. | Mol. Wt. | I. No. | Weight g. | $C_{10}^{s}$ | $C_{12}^{s}$ | $C_{14}^{s}$ | $C_{16}^{s}$ | $C_{12}^{u}$ | $C_{14}^{u}$ | $C_{16}^{u}$ | $C_{18}^{u}$ | $C_{18}^{2u}$ | $C_{24}^{u}$ | $C_{24}^{2u}$ | $C_{26}^{u}$ | $C_{26}^{2u}$ | Residue |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| L-01 | 82-98 | 238.4 | 39.07 | 1.76 | ---- | 0.76 | 0.27 | ---- | ---- | ---- | 0.73 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| L-02 | 98-I21 | 195.4 | 43.04 | 2.79 | 1.71 | 0.09 | ---- | ---- | 0.99 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| L-03 | 121-141 | 216.1 | 59.74 | 2.89 | ---- | 1.13 | 0.32 | ---- | 1.44 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| L-04 | 141-148 | 260.6 | 75.48 | 16.18 | ---- | ---- | 3.38 | ---- | ---- | 1.42 | 11.38 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| L-05 | 148-158 | 269.6 | 78.91 | 18.37 | ---- | ---- | ---- | 2.95 | ---- | ---- | 14.81 | 0.61 | ---- | ---- | ---- | ---- | ---- | ---- |
| L-06 | 158-162 | 281.4 | 87.24 | 38.61 | ---- | ---- | ---- | 1.17 | ---- | ---- | 18.66 | 18.78 | ---- | ---- | ---- | ---- | ---- | ---- |
| L-07 | 162-166 | 282.2 | 88.54 | 29.19 | ---- | ---- | ---- | 0.35 | ---- | ---- | 13.71 | 15.13 | ---- | ---- | ---- | ---- | ---- | ---- |
| L-08 | 166-167 | 288.0 | 90.66 | 29.51 | ---- | ---- | ---- | 8.40 | ---- | ---- | ---- | 11.24 | 9.87 | ---- | ---- | ---- | ---- | ---- |
| L-09 | 167-168 | 289.0 | 91.96 | 28.95 | ---- | ---- | ---- | 7.19 | ---- | ---- | ---- | 12.65 | 9.11 | ---- | ---- | ---- | ---- | ---- |
| L-10 | 168-169 | 292.2 | 93.45 | 29.76 | ---- | ---- | ---- | 3.05 | ---- | ---- | ---- | 19.02 | 7.69 | ---- | ---- | ---- | ---- | ---- |
| L-11 | 169-171 | 292.2 | 94.33 | 21.60 | ---- | ---- | ---- | 2.15 | ---- | ---- | ---- | 13.59 | 5.86 | ---- | ---- | ---- | ---- | ---- |
| L-12 | 171-183 | 289.8 | 93.74 | 9.59 | ---- | ---- | ---- | 2.10 | ---- | ---- | ---- | 4.54 | 2.95 | ---- | ---- | ---- | ---- | ---- |
| L-13 | 183-185 | 294.2 | 90.47 | 2.08 | ---- | ---- | ---- | 0.06 | ---- | ---- | ---- | 1.83 | 0.19 | ---- | ---- | ---- | ---- | ---- |
| L-14 | 185-187 | 305.3 | 96.94 | 7.61 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 5.36 | 1.21 | 1.04 | ---- | ---- | ---- | ---- |
| L-15 | 187-191 | 319.7 | 92.27 | 2.39 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 1.23 | 0.36 | 0.80 | ---- | ---- | ---- | ---- |
| L-16 | 191-197 | 334.2 | 102.33 | 2.37 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0.42 | 0.72 | 1.23 | ---- | ---- | ---- | ---- |
| L-17 | 197-208 | 346.6 | 111.22 | 1.63 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0.54 | 0.86 | 0.23 | ---- | ---- | ---- |
| L-18 | 208-218 | 363.4 | 114.46 | 3.47 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0.53 | 1.30 | 1.64 | ---- | ---- | ---- |
| L-19 | 218-224 | 389.1 | 117.19 | 7.87 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 4.90 | 1.66 | 1.31 | ---- |
| L-20 | 224-229 | 389.1 | 113.74 | 4.25 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 2.65 | 1.15 | 0.45 | ---- |
| L-21 | 229 | 382.0 | 107.78 | 1.78 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 1.13 | 0.65 | ---- | ---- |
| Residue | ------- | ----- | ------ | 13.48 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 13.48 |
|  |  | Total |  | 276.13 | 1.71 | 1.98 | 3.97 | 27.42 | 2.43 | 1.42 | 59.29 | 104.40 | 39.03 | 5.23 | 10.55 | 3.46 | 1.76 | 13.48 |
|  |  | Weight per cent |  |  | 0.62 | 0.72 | 1.44 | 9.93 | 0.88 | 0.51 | 21.47 | 37.81 | 14.14 | 1.89 | 3.82 | 1.25 | 0.64 | 4.88 |

TABLE IV

Fraction Analysis Summary

| Acid | wt. ester 1-S | wt. ester 2-S | wt. ester L | wt. acid 1-S | wt. acid 2-S | wt. acid L | Total wt. acid | wt. per cent acid |
|---|---|---|---|---|---|---|---|---|
| $C_{10}^{s}$ | 0 | 0 | 1.71 | 0 | 0 | 1.58 | 1.58 | 0.23 |
| $C_{12}^{s}$ | 0 | 0 | 1.98 | 0 | 0 | 1.85 | 1.85 | 0.27 |
| $C_{14}^{s}$ | 26.77 | 18.96 | 3.97 | 25.22 | 17.86 | 3.74 | 46.82 | 6.76 |
| $C_{16}^{s}$ | 97.95 | 43.42 | 27.42 | 92.87 | 41.17 | 26.00 | 160.04 | 23.10 |
| $C_{18}^{s}$ | 1.65 | 0 | 0 | 1.57 | 0 | 0 | 1.57 | 0.23 |
| $C_{24}^{s}$ | 0 | 1.58 | 0 | 0 | 1.52 | 0 | 1.52 | 0.22 |
| $C_{12}^{u}$ | 0 | 0 | 2.43 | 0 | 0 | 2.27 | 2.27 | 0.33 |
| $C_{14}^{u}$ | 0 | 0 | 1.42 | 0 | 0 | 1.34 | 1.34 | 0.19 |
| $C_{16}^{u}$ | 0.25 | 0 | 59.29 | 0.24 | 0 | 56.19 | 56.43 | 8.14 |
| $C_{18}^{u}$ | 28.91 | 42.70 | 104.40 | 27.54 | 40.68 | 99.46 | 167.68 | 24.20 |
| $C_{24}^{u}$ | 4.80 | 0 | 5.23 | 4.62 | 0 | 5.04 | 9.66 | 1.39 |
| $C_{26}^{u}$ | 11.79 | 6.30 | 3.46 | 11.39 | 6.08 | 3.34 | 20.81 | 3.00 |
| $C_{18}^{2u}$ | 0 | 0 | 39.03 | 0 | 0 | 37.17 | 37.17 | 5.37 |
| $C_{24}^{2u}$ | 5.08 | 6.56 | 10.55 | 4.89 | 6.32 | 10.16 | 21.37 | 3.08 |
| $C_{26}^{2u}$ | 1.28 | 4.34 | 1.76 | 1.24 | 4.19 | 1.70 | 7.13 | 1.03 |
| Residue | 6.76 | 4.97 | 13.48 | 6.49 | 4.77 | 12.94 | 24.20 | 3.49 |
| Unanalyzed | ----- | ----- | ----- | ----- | ----- | ----- | 131.43 | 18.97 |
| Total | 185.24 | 128.83 | 276.13 | 176.07 | 122.59 | 262.78 | 692.87 | 100.00 |

$w = 29.19$ g.

$$x/w = \frac{0.3189+0.2986+0.3351-0.3190-0.3033-0.3299}{0.3189+0+0.3497-0.3190-0.3166-0}$$

$$= \frac{0.0004}{0.0330} = 0.0121$$

Then,

$x = x/w \cdot w = 0.0121 \cdot 29.19 = 0.35$ g.

Similarly,

$$y/w = \frac{0.3033+0+0.3274-0.2986-0.3166-0}{0.0330}$$

$$= \frac{0.0155}{0.0330} = 0.4697$$

Then,

$y = y/w \cdot w = 0.4697 \cdot 29.19 = 13.71$ g.

Similarly,

$$z/w = \frac{0.3299+0+0.3497-0.3351-0.3274-0}{0.0330}$$

$$= \frac{0.0171}{0.0330} = 0.5182$$

Then,

$z = z/w \cdot w = 0.5182 \cdot 29.19 = 15.13$ g.

TABLE VI

Analysis of Fatty Acids From Muskrat Scent Glands

| Carbon content | Name of acid | Weight per cent (calculated) |
|---|---|---|
| $C^{s}_{10}$ | Decanoic | 0.2 |
| $C^{s}_{12}$ | Dodecanoic | 0.3 |
| $C^{s}_{14}$ | Myristic | 6.8 |
| $C^{s}_{16}$ | Palmitic | 23.1 |
| $C^{s}_{18}$ | Stearic | 0.2 |
| $C^{s}_{24}$ | Tetracosanoic | 0.2 |
| $C^{u}_{12}$ | Dodecenoic | 0.3 |
| $C^{u}_{14}$ | Tetradecenoic | 0.2 |
| $C^{u}_{16}$ | Hexadecenoic | 8.1 |
| $C^{u}_{18}$ | Oleic | 24.2 |
| $C^{u}_{24}$ | Tetracosenoic | 1.4 |
| $C^{u}_{26}$ | Hexacosenoic | 3.0 |
| $C^{2u}_{18}$ | Linoleic | 5.4 |
| $C^{2u}_{24}$ | Tetracosadienoic | 3.1 |
| $C^{2u}_{26}$ | Hexacosadienoic | 1.0 |
| Residue | | 3.5 |
| Unanalyzed | | 19.0 |
| | Total | 100.0 |

TABLE Va

Calculation Data

| Fraction | Component Esters | w | Ix | Iy | Iz | Iw | Ex | Ey | Ez | Ew |
|---|---|---|---|---|---|---|---|---|---|---|
| L-01 | $C^{s}_{12}$ $C^{s}_{14}$ $C^{u}_{16}$ | 1.76 | 0 | 0 | 94.57 | 39.07 | 214.3 | 242.4 | 268.4 | 238.4 |
| L-02 | $C^{s}_{10}$ $C^{s}_{12}$ $C^{u}_{12}$ | 2.79 | 0 | 0 | 119.56 | 43.04 | 186.3 | 214.3 | 212.3 | 195.4 |
| L-03 | $C^{s}_{12}$ $C^{s}_{14}$ $C^{u}_{12}$ | 2.89 | 0 | 0 | 119.56 | 59.74 | 214.3 | 242.4 | 212.3 | 216.1 |
| L-04 | $C^{s}_{14}$ $C^{u}_{14}$ $C^{u}_{16}$ | 16.18 | 0 | 105.60 | 94.57 | 75.48 | 242.4 | 240.4 | 268.4 | 260.6 |
| L-05 | $C^{s}_{16}$ $C^{u}_{16}$ $C^{u}_{18}$ | 18.37 | 0 | 94.57 | 85.60 | 78.91 | 270.4 | 268.4 | 296.5 | 269.6 |
| L-06 | $C^{s}_{16}$ $C^{u}_{16}$ $C^{u}_{18}$ | 38.61 | 0 | 94.57 | 85.60 | 87.24 | 270.4 | 268.4 | 296.5 | 281.4 |
| L-07 | $C^{s}_{16}$ $C^{u}_{16}$ $C^{u}_{18}$ | 29.19 | 0 | 94.57 | 85.60 | 88.54 | 270.4 | 268.4 | 296.5 | 282.2 |
| L-08 | $C^{s}_{16}$ $C^{u}_{18}$ $C^{2u}_{18}$ | 29.51 | 0 | 85.60 | 172.41 | 90.66 | 270.4 | 296.5 | 294.5 | 288.0 |
| L-09 | $C^{s}_{16}$ $C^{u}_{18}$ $C^{2u}_{18}$ | 28.95 | 0 | 85.60 | 172.41 | 91.96 | 270.4 | 296.5 | 294.5 | 289.0 |
| L-10 | $C^{s}_{16}$ $C^{u}_{18}$ $C^{2u}_{18}$ | 29.76 | 0 | 85.60 | 172.41 | 93.45 | 270.4 | 296.5 | 294.5 | 292.2 |
| L-11 | $C^{s}_{16}$ $C^{u}_{18}$ $C^{2u}_{18}$ | 21.60 | 0 | 85.60 | 172.41 | 94.33 | 270.4 | 296.5 | 294.5 | 292.2 |
| L-12 | $C^{s}_{16}$ $C^{u}_{18}$ $C^{2u}_{18}$ | 9.59 | 0 | 85.60 | 172.41 | 93.74 | 270.4 | 296.5 | 294.5 | 289.8 |
| L-13 | $C^{s}_{16}$ $C^{u}_{18}$ $C^{2u}_{18}$ | 2.08 | 0 | 85.60 | 172.41 | 90.47 | 242.4 | 296.5 | 294.5 | 294.2 |
| L-14 | $C^{u}_{18}$ $C^{2u}_{18}$ $C^{u}_{24}$ | 7.61 | 85.60 | 172.41 | 66.68 | 96.94 | 296.5 | 294.5 | 380.6 | 305.3 |
| L-15 | $C^{u}_{18}$ $C^{2u}_{18}$ $C^{u}_{24}$ | 2.39 | 85.60 | 172.41 | 66.68 | 92.27 | 296.5 | 294.5 | 380.6 | 319.7 |
| L-16 | $C^{u}_{18}$ $C^{2u}_{18}$ $C^{u}_{24}$ | 2.37 | 85.60 | 172.41 | 66.68 | 102.33 | 296.5 | 294.5 | 380.6 | 334.2 |
| L-17 | $C^{2u}_{18}$ $C^{u}_{24}$ $C^{2u}_{24}$ | 1.63 | 172.41 | 66.68 | 134.09 | 111.22 | 294.5 | 380.6 | 378.6 | 346.6 |
| L-18 | $C^{2u}_{18}$ $C^{u}_{24}$ $C^{2u}_{24}$ | 3.47 | 172.41 | 66.68 | 134.09 | 114.46 | 294.5 | 380.6 | 378.6 | 363.4 |
| L-19 | $C^{2u}_{24}$ $C^{u}_{26}$ $C^{2u}_{26}$ | 7.87 | 134.09 | 61.80 | 124.84 | 117.19 | 378.6 | 408.7 | 406.7 | 389.1 |
| L-20 | $C^{2u}_{24}$ $C^{u}_{26}$ $C^{2u}_{26}$ | 4.25 | 134.09 | 61.80 | 124.84 | 113.74 | 378.6 | 408.7 | 406.7 | 389.1 |
| L-21 | $C^{2u}_{24}$ $C^{u}_{26}$ | 1.78 | 134.09 | 61.80 | ------ | 107.78 | 378.6 | 408.7 | ----- | 382.0 |

The calculations are easily checked from the data tables at this point either by summing the values of x/w, y/w, and z/w, which should equal 1.0000, or by summing the weights of x, y, and z, which should equal w (Table Vb).

If this fraction is calculated for combinations of esters, other than the one above, negative values result in equations (4), (5), or (6), indicating that such combinations cannot correspond to the analytical data obtained. The following combinations of esters give negative values when calculated to fraction L-07:

1. hexadecenoic, oleic, and linoleic esters
2. palmitic, oleic, and linoleic esters.

All other fractions were calculated in a similar manner. The boiling point, iodine number, and mean molecular weight of each fraction served as guides in the choice of the combination of esters present in any one fraction. In the event that two different combinations of esters give positive values for any one fraction, the combination corresponding to the preceding fraction is chosen. The calculated weights of esters present in each fraction are recorded in Tables I, II, and III.

It should be pointed out that fourth decimal accuracy of the I/E ratios is essential to obtain consistent results for the calculations using any one set of I and E values. A Friden Automatic Calculator was used in this work for all calculations.

The weights of each component ester in the 1-S, 2-S, and L fractions were converted to the weights of corresponding acids present in the original mixture of fatty acids. From these weights of individual acids the percentage composition of each component acid was calculated. These results are recorded in Tables IV and VI.

Other equations containing the refractive index, thiocyanogen number, and other measurable physical properties of the esters may be substituted for equations (2) or (3) and used in this method of calculation of the composition of fatty acid ester mixtures.

## Summary

1. The mixture of fatty acids from muskrat scent glands has been analyzed by the ester fractionation method.

2. The esters of myristic, palmitic, hexadecenoic, oleic, and linoleic acids were found present as major components in these glands. Unsaturated acids of the $C_{24}$ and $C_{26}$ series are also present to the extent of 8.5% of the total fatty acids. Acids present to less than 1% each include decanoic, dodecanoic. stearic, tetracosanoic, dodecenoic, and tetradecenoic acids.

3. Application of determinants for the solution of simultaneous equations has been presented as a method which greatly simplifies and facilitates the calculations necessary to this type of work.

4. The possible relationship existing between fatty acids and macrocyclic carbinols has been considered and possible mechanisms for such transformations have been presented.

### REFERENCES

1. Stevens, P. G., and Erickson, J. L. E., J. Am. Chem. Soc., *64*, 144 (1942); Stevens, P. G., J. Am. Chem. Soc., *67*, 907 (1945).
2. Simmons, W. H., and Hills, C. A., Analyst, *58*, 154 (1933).
3. Ruzicka, L., Helv. Chim. Acta, *9*, 230 (1926).
4. Knoop, F., Beitr. chem. Physiol. u. Pathol., *6*, 150 (1905).
5. Verkade, P. E., and Van der Lee, J., Biochem. J., *28*, 31 (1934).
6. Ruzicka, L., Stoll, M., and Schinz, H., Helv. Chim. Acta, *9*, 249 (1926).
7. Hilditch, T. P., Chemical Constitution of Natural Fats, New York, J. Wiley and Sons, Inc., (1941).
8. Longenecker, H. E., J. Soc. Chem. Ind., *56*, 199T (1937).
9. Charnley, F., J. Biol. Bd. Can. *2* (3), 285 (1936).

TABLE Vb
Calculation Data

| Fraction | Ix/Ey | Ix/Ez | Ix/Ew | Iy/Ex | Iy/Ez | Iy/Ew | Iz/Ex | Iz/Ey | Iz/Ew | Iw/Ex | Iw/Ey | Iw/Ez | x/w | y/w | z/w | x | y | z |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| L-01 | 0 | 0 | 0 | 0 | 0 | 0 | 0.4413 | 0.3901 | 0.3967 | 0.1823 | 0.1612 | 0.1456 | 0.4336 | 0.1543 | 0.4121 | 0.76 | 0.27 | 0.73 |
| L-02 | 0 | 0 | 0 | 0 | 0 | 0 | 0.6428 | 0.5579 | 0.6119 | 0.2310 | 0.2008 | 0.2027 | 0.6137 | 0.0306 | 0.3557 | 1.71 | 0.09 | 0.99 |
| L-03 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5579 | 0.4932 | 0.5533 | 0.2788 | 0.2465 | 0.2814 | 0.3895 | 0.1113 | 0.4992 | 1.13 | 0.32 | 1.44 |
| L-04 | 0 | 0 | 0 | 0.4356 | 0.3934 | 0.4052 | 0.3901 | 0.3934 | 0.3629 | 0.3124 | 0.3140 | 0.2812 | 0.2088 | 0.0879 | 0.7033 | 3.38 | 1.42 | 11.38 |
| L-05 | 0 | 0 | 0 | 0.3497 | 0.3190 | 0.3508 | 0.3166 | 0.3189 | 0.3175 | 0.2918 | 0.2940 | 0.2661 | 0.1606 | 0.8061 | 0.0333 | 2.95 | 14.81 | 0.61 |
| L-06 | 0 | 0 | 0 | 0.3497 | 0.3190 | 0.3361 | 0.3166 | 0.3189 | 0.3042 | 0.3226 | 0.3250 | 0.2943 | 0.0304 | 0.4833 | 0.4863 | 1.17 | 18.66 | 18.78 |
| L-07 | 0 | 0 | 0 | 0.3497 | 0.3190 | 0.3351 | 0.3166 | 0.3189 | 0.3033 | 0.3274 | 0.3299 | 0.2986 | 0.0121 | 0.4697 | 0.5182 | 0.35 | 13.71 | 15.13 |
| L-08 | 0 | 0 | 0 | 0.3166 | 0.2907 | 0.2972 | 0.6376 | 0.5815 | 0.5986 | 0.3353 | 0.3058 | 0.3078 | 0.2848 | 0.3808 | 0.3344 | 8.40 | 11.24 | 9.87 |
| L-09 | 0 | 0 | 0 | 0.3166 | 0.2907 | 0.2962 | 0.6376 | 0.5815 | 0.5966 | 0.3401 | 0.3102 | 0.3123 | 0.2483 | 0.4371 | 0.3146 | 7.19 | 12.65 | 9.11 |
| L-10 | 0 | 0 | 0 | 0.3166 | 0.2907 | 0.2940 | 0.6376 | 0.5815 | 0.5900 | 0.3456 | 0.3152 | 0.3173 | 0.1026 | 0.6391 | 0.2583 | 3.05 | 19.02 | 7.69 |
| L-11 | 0 | 0 | 0 | 0.3166 | 0.2907 | 0.2940 | 0.6376 | 0.5815 | 0.5900 | 0.3489 | 0.3181 | 0.3203 | 0.0993 | 0.6291 | 0.2715 | 2.15 | 13.59 | 5.86 |
| L-12 | 0 | 0 | 0 | 0.3166 | 0.2907 | 0.2954 | 0.6376 | 0.5815 | 0.5949 | 0.3467 | 0.3162 | 0.3183 | 0.2185 | 0.4735 | 0.3079 | 2.10 | 4.54 | 2.95 |
| L-13 | 0 | 0 | 0 | 0.3531 | 0.2907 | 0.2910 | 0.7113 | 0.5815 | 0.5860 | 0.3732 | 0.3051 | 0.3072 | 0.0312 | 0.8798 | 0.0890 | 0.06 | 1.33 | 0.19 |
| L-14 | 0.2907 | 0.2249 | 0.2804 | 0.5815 | 0.4530 | 0.5647 | 0.2249 | 0.2264 | 0.2184 | 0.3269 | 0.3292 | 0.2547 | 0.7040 | 0.1589 | 0.1371 | 5.36 | 1.21 | 1.04 |
| L-15 | 0.2907 | 0.2249 | 0.2678 | 0.5815 | 0.4530 | 0.5393 | 0.2249 | 0.2264 | 0.2086 | 0.3112 | 0.3133 | 0.2424 | 0.5171 | 0.1495 | 0.3334 | 1.23 | 0.36 | 0.80 |
| L-16 | 0.2907 | 0.2249 | 0.2561 | 0.5815 | 0.4530 | 0.5159 | 0.2249 | 0.2264 | 0.1995 | 0.3451 | 0.3475 | 0.2689 | 0.1745 | 0.3053 | 0.5202 | 0.42 | 0.72 | 1.23 |
| L-17 | 0.4530 | 0.4554 | 0.4974 | 0.2264 | 0.1761 | 0.1924 | 0.4553 | 0.3523 | 0.3869 | 0.3777 | 0.2922 | 0.2938 | 0.3320 | 0.5268 | 0.1412 | 0.54 | 0.86 | 0.23 |
| L-18 | 0.4530 | 0.4554 | 0.4744 | 0.2264 | 0.1761 | 0.1835 | 0.4553 | 0.3523 | 0.3690 | 0.3887 | 0.3007 | 0.3023 | 0.1531 | 0.3757 | 0.4712 | 0.53 | 1.30 | 1.64 |
| L-19 | 0.3281 | 0.3297 | 0.3446 | 0.1632 | 0.1520 | 0.1588 | 0.3297 | 0.3055 | 0.3208 | 0.3095 | 0.2867 | 0.2881 | 0.6228 | 0.2105 | 0.1667 | 4.90 | 1.66 | 1.31 |
| L-20 | 0.3281 | 0.3297 | 0.3446 | 0.1632 | 0.1520 | 0.1588 | 0.3297 | 0.3055 | 0.3208 | 0.3004 | 0.2783 | 0.2797 | 0.6228 | 0.2719 | 0.1053 | 2.65 | 1.15 | 0.46 |
| L-21 | | | | | | | | | | | | | 0.6348 | 0.3652 | | 1.13 | 0.65 | |