# The Thiocyanogen Values of the Methyl Esters of Oleic, Linoleic, and Linolenic Acids

## The Application of these Values in the Analysis of Mixtures*

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THE thiocyanogen value has been extensively used in conjunction with the iodine value for the estimation of fatty acid composition dating from the original work of Kaufmann (1). The assumption was made that thiocyanogen is quantitatively absorbed by the double bond of oleic acid, by one of the two double bonds of linoleic acid and by two of the three ethylenic linkages of linolenic acid. Attempts by others (2) (3) (4) (5) to confirm these assumptions have raised some questions as to the extent of addition of thiocyanogen to linoleic and linolenic acids.

In recent work (6) (7) it has been shown that methyl oleate, methyl linoleate, triolein and trilinolein absorb nearly the theoretical amounts of thiocyanogen in 3 to 4 hours, using 0.1 N solutions of thiocyanogen in 100-150 percent actual excess over that absorbed. In 24 hours' absorption time, as used by Kaufmann, the value for methyl linoleate was 2.5 units higher than theory. Application of the 3-hour absorption method to mixtures containing only oleic and linoleic acids as the unsaturated constituents enabled estimation to be made of each constituent to within 1 unit percent of the known value.

The 3-hour absorption period, however, is not satisfactory for those oils or mixtures containing linolenic acid, as shown by the work of Kass and co-workers (8). They found that in 3 to 4 hours, using 0.16 to 0.18 normal solutions, the rate of absorption of thiocyanogen by ethyl linolenate was too rapid to yield a reliable value for use in calculating composition. In 24 hours, the rate of absorption was slow enough to permit good duplication. However, even in 30 hours this ester did not absorb an amount of thiocyanogen equivalent to two of the three double bonds. These authors found that, by the method of the F. A. C. (9), the T. V. for linolenic acid is 167.3; for linoleic acid, 96.3.

Hilditch and Murti (10) determined the T. V. for linolenic acid to be 162.5; for linoleic acid, 95.9. They employed 20 cc. of 0.2 N thiocyanogen solution for each determination, but dissolved the sample in 20 cc. of acetic acid, thus making the effective concentration only 0.1 N. Kass and co-workers (loc. cit.) found the T. V. for linolenic acid to be 163.8 using 0.1 N thiocyanogen solution in 158 percent actual excess over the amount absorbed.

To summarize briefly, it has been shown that the T. V. is an empirical value which is dependent upon the concentration and excess of thiocyanogen and upon the time allowed for absorption.

The present investigation was undertaken, first, to determine whether values comparable to those reported could be confirmed by using the methyl esters of oleic, linoleic, and linolenic acids prepared independently in our laboratory and secondly, to extend the investigation to include analysis of known mixtures of the purified esters.

### Experimental

The methods used in the preparation of methyl oleate and methyl linoleate have been reported in previous work (7) (11). The Wijs iodine numbers were 85.8 and 172.6, respectively; theory, 85.6 and 172.4.

For the preparation of methyl linolenate, hexabromostearic acid was obtained by the bromination of high-iodine number perilla-oil fatty acids in ether solution. The hexabromides were purified by repeated washing with ether under reflux and finally by crystallization from benzene. M. P. 181.5-182.0° (Corr.), when placed in a bath at 175° and the temperature increased at the rate of 1° per minute.

The debromination of hexabromostearic acid was accomplished by a slight modification of the procedure described by Kimura (12). Fifty gm. bromides, 35 gm. zinc dust and 125 c.c of absolute methanol were heated under reflux in an atmosphere of nitrogen until no further reaction was noticeable (about 45 minutes). A solution of 15 cc. of conc. sulfuric acid in 75 cc. of methanol was then added in 10-cc. portions, allowing about five to six minutes reflux between successive additions. Finally, the resultant mixture was refluxed for one hour and cooled. The zinc salts and unreacted zinc were removed by filtration on a small Büchner funnel. The filtrate was poured into two volumes of water and extracted with petroleum ether (30°-60°). After thorough washing with water, the solution was dried over sodium sulfate and the solvent was removed by distillation; the last portion, under vacuum. The methyl linolenate was then distilled at 0.05 to 0.1 mm. pressure using a round bottom flask with a short still head without column. Yield of water-white ester, boiling point 125-128°, 17.7 gm.—92 percent of theory. Wijs iodine number, 260.2. Theory, 260.4.

### Thiocyanogen Values of Methyl Oleate, Linoleate and Linolenate

The essential difference between the A.O.A.C. (13) and the F.A.C. (9) methods is that the former requires 0.1 N thiocyanogen solution and the latter 0.2 N.

In this investigation, lead nitrate and potassium thiocyanate were used to prepare lead thiocyanate. Carbon tetrachloride was also used, as in the A.O.A.C. method. To show the effect of different concentration and excess of thiocyanogen reagent, a series of thiocyanogen values of pure esters were determined with 0.1 N and also 0.2 N thiocyanogen solutions. The normality of the solutions was adjusted to within (±) 3 percent of 0.1 N or 0.2 N.

The thiocyanogen values found for methyl oleate,

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linoleate and linolenate and six different mixtures of these esters, using 0.1 N solutions, are given in Tables I to V.

TABLE I.

Thiocyanogen Values of Methyl Oleate (0.1 N solution, 24 hours). Theory T. V. = 85.6.

| Temp. | Actual Excess Thiocyanogen | Found T. V. |
|---|---|---|
| ±1° C. | Percent | |
| 0 | 194 | 85.3 |
| 0 | 206 | 85.9 |
| 20 | 110 | 85.3 (b) |
| 20 | 127 | 85.1 (c) |
| 20 | 145 | 85.2 (c) |
| 20 | 157 | 85.4 (b) |
| 20 | 166 | 85.3 (a) |
| 20 | 224 | 85.4 (a) |
| Average at 20° | | 85.3 |

TABLE II

Thiocyanogen Values of Methyl Linoleate (0.1 N solution) (Kaufmann) Theory T. V. = 86.2

| Temp. | Time | Theory Excess Thiocyanogen | Actual Excess Thiocyanogen | Found T. V. |
|---|---|---|---|---|
| ±1° C. | Hours | Percent | Percent | |
| 0 | 45 | 213 | 205 | 88.3 |
| 0 | 45 | 253 | 245 | 88.1 |
| 20 | 24 | 153 | 145 | 88.9 (c) |
| 20 | 24 | 156 | 147 | 89.4 (b) |
| 20 | 24 | 157 | 148 | 89.2 (b) |
| 20 | 24 | 170 | 161 | 89.3 (c) |
| 20 | 24 | 237 | 223 | 89.8 (a) |
| 20 | 24 | 243 | 230 | 89.5 (a) |
| 24 | 18 | 167 | 157 | 89.6 |
| 24 | 18 | 172 | 162 | 89.6 |
| Average at 20° | 24 | | | 89.4 |

TABLE III.

Thiocyanogen Values of Methyl Linolenate (0.1 N solution) (Kaufmann) Theory T. V. = 173.6.

| Temp. | Time | Theory Excess Thiocyanogen | Actual Excess Thiocyanogen | Found T. V. |
|---|---|---|---|---|
| ±1° C. | Hours | Percent | Percent | |
| 0 | 45 | 63 | 93 | 146.2 |
| 0 | 45 | 73 | 102 | 146.8 |
| 20 | 24 | 49 | 72 | 150.6 (a) |
| 20 | 24 | 70 | 95 | 151.1 (a) |
| 20 | 24 | 112 | 140 | *153.7 (b) |
| 20 | 24 | 137 | 166 | *154.5 (b) |
| 20 | 24 | 144 | 177 | *153.1 (c) |
| 20 | 24 | 184 | 221 | *153.4 (c) |
| 24 | 18 | 122 | 153 | 152.1 |
| 24 | 18 | 138 | 171 | 152.4 |
| 24 | 24 | 111 | 137 | 154.3 (a) |
| 24 | 24 | 146 | 176 | 154.7 (a) |
| 24 | 24 | 166 | 197 | 155.4 (b) |
| 24 | 24 | 172 | 206 | 154.6 (b) |
| Average at 20° | 24 | | | *153.7 |

* Obtained with 140-221 per cent excess.

TABLE IV.

Thiocyanogen Values of Mixtures of Methyl Oleate, Linoleate, and Linolenate (0.1 N solution, 20°, 24 hours).

| Mixture | Theory Excess Thiocyanogen Percent | Actual Excess Thiocyanogen Percent | Found T. V.* |
|---|---|---|---|
| A | 135 | 159 | 133.4 (b) |
| A | 135 | 160 | 133.1 (a) |
| A | 140 | 164 | 133.7 (b) |
| A | 148 | 175 | 133.0 (a) |
| A | | | 133.3 (Av.) |
| B | 188 | 187 | 95.0 (a) |
| B | 201 | 200 | 95.2 (a) |
| B | 220 | 217 | 95.7 (b) |
| B | 256 | 249 | 96.6 (b) |
| B | | | 95.6 (Av.) |
| C | 160 | 171 | 107.8 |
| C | 216 | 229 | 107.7 |
| C | | | 107.8 (Av.) |
| D | 105 | 128 | 132.2 (a) |
| D | 108 | 132 | 132.2 (a) |
| D | 125 | 153 | 131.2 (b) |
| D | 128 | 155 | 131.5 (b) |
| D | | | 131.8 (Av.) |
| E | 144 | 156 | 105.2 |
| E | 152 | 165 | 105.1 |
| E | | | 105.2 (Av.) |
| F | 158 | 160 | 94.4 (a) |
| F | 161 | 166 | 93.4 (a) |
| F | 176 | 177 | 95.0 (b) |
| F | 183 | 185 | 94.5 (b) |
| F | | | 94.3 (Av.) |

* For calculated thiocyanogen values see Table V.

TABLE V

Analysis of Mixtures of Methyl Esters Using Thiocyanogen Values (0.1 N, 20°, 24 hrs.) and Wijs Iodine Values

| Mixture | Iodine Values | | Thiocyanogen Values Calculated | | Thiocyanogen Values Found | Composition | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Calc. | Found (Av.) | From K* values | From F+ values | Av. | | Methyl Oleate Percent | Methyl Linoleate Percent | Methyl Linolenate Percent |
| A | 216.4 | 216.8 | 147.2 | 133.5 | 133.3 | Known | 20.2 | 9.9 | 69.9 |
| | | | | | | F+ | 19.3 | 11.2 | 69.5 |
| | | | | | | K* | 3.5 | 42.6 | 53.9 |
| B | 163.8 | 164.5 | 94.8 | 95.0 | 95.6 | Known | 20.1 | 69.9 | 10.0 |
| | | | | | | F | 20.1 | 69.0 | 10.9 |
| | | | | | | K | 20.1 | 69.0 | 10.9 |
| C | 164.1 | 164.4 | 112.2 | 107.1 | 107.8 | Known | 40.1 | 29.9 | 30.0 |
| | | | | | | F | 40.9 | 27.9 | 31.2 |
| | | | | | | K | 34.5 | 40.5 | 25.0 |
| D | 216.3 | 216.0 | 147.2 | 133.5 | 131.8 | Known | 20.2 | 9.9 | 69.8 |
| | | | | | | F | 17.8 | 15.1 | 67.1 |
| | | | | | | K | 2.7 | 45.1 | 52.2 |
| E | 161.0 | 161.0 | 110.3 | 105.6 | 105.2 | Known | 41.3 | 31.0 | 27.8 |
| | | | | | | F | 40.7 | 32.1 | 27.2 |
| | | | | | | K | 35.4 | 42.6 | 22.0 |
| F | 164.2 | 164.3 | 95.2 | 95.2 | 94.3 | Known | 19.9 | 69.8 | 10.3 |
| | | | | | | F | 18.2 | 73.1 | 8.7 |
| | | | | | | K | 18.9 | 71.8 | 9.4 |

* Calculated from Kaufmann's theory values.

\+ Calculated from Found values reported in Tables I to III.

The thiocyanogen values of these esters and mixtures, using 0.2 N solutions are given in Tables VI to X. Duplicate determinations are identified by corresponding letters after the values, except where it is obvious.

TABLE VI.

Thiocyanogen Values of Methyl Oleate. (0.2 N solution, 24 hours), Theory T. V. = 85.6.

| Temp. ±1° C. | Actual Excess Thiocyanogen Percent | Found T. V. |
|---|---|---|
| 0 | 306 | 85.5 |
| 0 | 405 | 85.5 |
| 20 | 113 | 85.1 (b) |
| 20 | 122 | 85.5 (a) |
| 20 | 139 | 85.4 (a) |
| 20 | 162 | 85.3 (b) |
| Average at 20° | | 85.3 |

TABLE VII.

Thiocyanogen Values of Methyl Linoleate (0.2 N solution) (Kaufman) Theory T. V. = 86.2.

| Temp. ±1° C. | Time Hours | Theory Excess Thiocyanogen Percent | Actual Excess Thiocyanogen Percent | Found T. V. |
|---|---|---|---|---|
| 0 | 24 | 374 | 358 | 89.2 |
| 0 | 24 | 412 | 395 | 89.2 |
| 0 | 48 | 376 | 350 | 91.2 |
| 0 | 48 | 404 | 376 | 91.2 |
| 8 | 24 | 328 | 303 | 91.5 |
| 8 | 24 | 342 | 316 | 91.5 |
| 8 | 48 | 281 | 252 | 93.3 |
| 8 | 48 | 335 | 300 | 93.8 |
| 20 | 24 | 140 | 126 | 91.5 (c) |
| 20 | 24 | 162 | 141 | 93.8 (b) |
| 20 | 24 | 163 | 142 | 93.8 (b) |
| 20 | 24 | 167 | 150 | 91.9 (c) |
| 20 | 24 | 174 | 155 | 92.5 (d) |
| 20 | 24 | 177 | 159 | 92.3 (d) |
| 20 | 24 | 242 | 219 | 92.4 (a) |
| 20 | 24 | 324 | 293 | 93.8 (a) |
| 20 | 44 | 228 | 194 | 96.3 |
| 20 | 44 | 334 | 285 | 97.2 |
| 24 | 24 | 133 | 116 | 93.0 (b) |
| 24 | 24 | 138 | 119 | 93.8 (b) |
| 24 | 24 | 148 | 126 | 94.6 (c) |
| 24 | 24 | 165 | 140 | 95.0 (c) |
| 24 | 24 | 275 | 240 | 95.0 (a) |
| 24 | 24 | 372 | 323 | 96.1 (a) |
| 24 | 48 | 145 | 117 | 97.3 |
| 24 | 48 | 276 | 228 | 98.9 |
| Average at 20° | 24 | | | 92.7 |

TABLE VIII.

Thiocyanogen Values of Methyl Linolenate (0.2 N solution) (Kaufmann) Theory T. V. = 173.6.

| Temp. ±1° C. | Time Hours | Theory Excess Thiocyanogen Percent | Actual Excess Thiocyanogen Percent | Found T. V. |
|---|---|---|---|---|
| 0 | 24 | 145 | 181 | 151.0 |
| 0 | 24 | 156 | 194 | 150.9 |
| 0 | 45 | 127 | 156 | 153.9 |
| 0 | 45 | 148 | 180 | 153.8 |
| 8 | 24 | 184 | 217 | 155.5 |
| 8 | 24 | 215 | 250 | 156.1 |
| 8 | 48 | 201 | 227 | 159.8 |
| 8 | 48 | 235 | 264 | 160.0 |
| 20 | 24 | 122 | 143 | 158.3 (d) |
| 20 | 24 | 128 | 148 | 159.3 (e) |
| 20 | 24 | 135 | 158 | 158.0 (d) |
| 20 | 24 | 140 | 160 | 160.2 (a) |
| 20 | 24 | 138 | 161 | 158.3 (e) |
| 20 | 24 | 138 | 162 | 158.1 (e) |
| 20 | 24 | 151 | 168 | 162.8 (c) |
| 20 | 24 | 151 | 171 | 160.8 (a) |
| 20 | 24 | 159 | 176 | 162.7 (c) |
| 20 | 24 | 218 | 248 | 158.5 (b) |
| 20 | 24 | 225 | 256 | 158.7 (b) |
| 20 | 44 | 178 | 196 | 163.2 (a) |
| 20 | 44 | 225 | 244 | 164.0 (a) |
| 20 | 44 | 227 | 248 | 163.1 (a) |
| 24 | 24 | 132 | 138 | 162.4 (c) |
| 24 | 24 | 131 | 138 | 161.8 (c) |
| 24 | 24 | 128 | 138 | 159.8 (b) |
| 24 | 24 | 144 | 163 | 161.2 (b) |
| 24 | 24 | 232 | 255 | 162.2 (a) |
| 24 | 24 | 246 | 271 | 162.1 (a) |
| Average at 20° | 24 | | | 159.6 |

TABLE IX.

Thiocyanogen Values of Mixtures of Methyl Oleate, Linoleate, and Linolenate (0.2 N solution, 20°, 24 hours).

| Mixture | Theory Excess Thiocyanogen Percent | Actual Excess Thiocyanogen Percent | Found T. V. * |
|---|---|---|---|
| A | 139 | 153 | 139.2 |
| A | 152 | 166 | 139.6 |
| A | | | 139.4 (Av.) |
| B | 168 | 155 | 99.7 |
| B | 189 | 176 | 99.3 |
| B | | | 99.5 (Av.) |
| C | 136 | 139 | 110.7 |
| C | 146 | 149 | 110.8 |
| C | | | 110.8 (Av.) |
| D | 127 | 147 | 135.2 (a) |
| D | 130 | 149 | 135.9 (a) |
| D | 137 | 152 | 138.5 (b) |
| D | 151 | 168 | 138.1 (b) |
| D | | | 136.9 (Av.) |
| E | 117 | 124 | 106.7 (a) |
| E | 132 | 137 | 108.0 (a) |
| E | 153 | 157 | 108.5 (b) |
| E | 162 | 167 | 108.3 (b) |
| E | | | 107.9 (Av.) |
| F | 161 | 156 | 97.2 (a) |
| F | 162 | 156 | 97.3 (a) |
| F | 165 | 156 | 98.4 (b) |
| F | 188 | 179 | 98.4 (b) |
| F | 188 | 182 | 97.3 (a) |
| F | | | 97.7 (Av.) |

* For calculated thiocyanogen values see Table X.

TABLE X.

Analysis of Mixtures of Methyl Esters Using Thiocyanogen Values (0.2 N, 20°, 24 Hrs.) and Wijs Values.

| | Iodine Values | | Thiocyanogen Values Calculated | | Found | Composition | | | |
|---|---|---|---|---|---|---|---|---|---|
| Mixture | Calc. | Found (Av.) | From K* values | From F+ values | Av. | | Methyl Oleate Percent | Methyl Linoleate Percent | Methyl Linolenate Percent |
| A | 216.4 | 216.8 | 147.2 | 138.0 | 139.4 | Known | 20.2 | 9.9 | 69.9 |
| | | | | | | F+ | 22.0 | 5.8 | 72.2 |
| | | | | | | K* | 10.6 | 28.4 | 60.9 |
| B | 163.8 | 164,5 | 94.8 | 97.9 | 99.5 | Known | 20.1 | 69.9 | 10.0 |
| | | | | | | F | 21.9 | 65.5 | 12.6 |
| | | | | | | K | 24.7 | 59.9 | 15.4 |
| C | 164.1 | 164.4 | 112.2 | 109.8 | 110.8 | Known | 40.1 | 29.9 | 30.0 |
| | | | | | | F | 41.2 | 27.2 | 31.6 |
| | | | | | | K | 43.0 | 28.6 | 28.4 |
| D | 216.3 | 216.0 | 147.2 | 137.9 | 136.9 | Known | 20.2 | 9.9 | 69.8 |
| | | | | | | F | 18.3 | 13.7 | 68.0 |
| | | | | | | K | 38.6 | 3.3 | 58.1 |
| E | 161.0 | 161.0 | 110.3 | 108.2 | 107.9 | Known | 41.3 | 31.0 | 27.8 |
| | | | | | | F | 40.7 | 32.0 | 27.2 |
| | | | | | | K | 38.6 | 36.3 | 25.1 |
| F | 164.2 | 164.3 | 95.2 | 98.1 | 97.7 | Known | 19.9 | 69.8 | 10.3 |
| | | | | | | F | 19.1 | 71.3 | 9.6 |
| | | | | | | K | 22.8 | 63.9 | 13.3 |

* Calculated from Kaufmann's theory values.
\+ Calculated from Found values reported in Tables VI to VIII.

## Discussion of Results

It is apparent from the data that the temperature, time allowed for absorption, concentration and excess of thiocyanogen are important factors.

It should be pointed out that in these series of analysis, duplicate determinations were made on different days extending over a period of about six months. Fresh thiocyanogen solutions were prepared for each set of duplicates. Two preparations of methyl linoleate and linolenate were used during the course of the work. All samples were stored at 0° to -5° in glass ampoules under nitrogen.

Duplicate determinations made on the same day usually agreed within 0.5 unit but occasionally agreed no better than within 1 unit. However, the averages of duplicate determinations on methyl linolenate run on different days with different preparations of 0.2 N thiocyanogen varied as much as 4.8 units during the course of the investigations.

In general, the data indicate that at 20° C. in 24 hours absorption time, an excess of about 140 to 200 percent of thiocyanogen is desirable. Less fluctuation in values was obtained with 0.1 N than with 0.2 N solutions.

When it is considered that in the estimation of the composition of mixtures, there are involved normal errors in establishing empirical thiocyanogen values for each unsaturated constituent and in determining the iodine numbers and thiocyanogen values of the mixtures, it may reasonably be expected that, occasionally, considerable variation from the true composition will be observed, especially in cases where the errors happen to be additive. It is apparent from these results that this procedure for determining the composition of mixtures containing three unsaturated fatty acids leaves much to be desired as to precision. However, it is also true that, to date, a more satisfactory method has not been developed.

For comparison with thiocyanogen values reported by others, the following data are tabulated. Reasonably good agreement is noted.

TABLE XI.

Thiocyanogen Values of Oleic, Linoleic and Linolenic Acids—Found in Literature.

| Observers | Oleic Acid 0.1 N | Oleic Acid 0.2 N | Linoleic Acid 0.1 N | Linoleic Acid 0.2 N | Linolenic Acid 0.1 N | Linolenic Acid 0.2 N |
|---|---|---|---|---|---|---|
| Waterman, Bertram and van Westen (14)........... | | | 92.9 | | | |
| Kimura (15) .................... | (Elaidic)<br>89.5 | | 94.4* | | | |
| Shinowara and Brown (5) | | | | | 161.0 | |
| Kass and co-workers (8) (16) ............................ | | (Elaidic)<br>89.1 | 93.0 | 96.3 | 163.8<br>161.4† | 167.3† |
| Hilditch and Murti (10).. | 89.0 | | 95.9 | | 162.5 | |
| Riemenschneider and Wheeler (7) ................ | 89.6* | | 93.4* | | | |
| Present work..................... | 89.5* | 89.5* | 93.9* | 97.3* | 161.4* | 167.6* |
| Average ............................ | 89.4 | 89.3 | 93.9 | 96.8 | 162.0 | 167.5 |

* Calculated from methyl ester values.
† Calculated from ethyl ester values.

To facilitate the determination of thiocyanogen numbers of oils containing all three of the common unsaturated acids, oleic, linoleic and linolenic, it has been found convenient to use the following tabulation as a guide in adjusting the sample weights. These suggested weight ranges apply only when 25 ml. of 0.1 N or 0.2 N thiocyanogen solution is employed for the determination to give actual excess of 140 to 200 percent over the amount absorbed.

| Iodine Value (I. V.) Range | Appropriate weight of sample in grams— 0.1 N Thiocyanogen Sol. | 0.2 N Thiocyanogen Sol. |
|---|---|---|
| 275-250 | 0.07-0.08 | 0.14 -0.155 |
| 250-225 | 0.08-0.09 | 0.155-0.17 |
| 225-200 | 0,09-0.10 | 0.17 -0.185 |
| 200-175 | 0.11-0.12 | 0.185-0.215 |
| 175-150 | 0.12-0.13 | 0.215-0.24 |
| 150-125 | 0.13-0.14 | 0.24 -0.26 |
| 125-100 | 0.14-0.15 | 0.26 -0.28 |
| 100- 75 | 0.15-0.16 | 0.28 -0.30 |

*Composition of perilla oil*: In the course of isolating and purifying hexabromostearic acid from the brominated mixture of fatty acids of high-iodine number perilla oil*, the absence of any contaminating tetrabromostearic acid was noticed. A careful examination of the brominated products from a kg. of perilla oil fatty acids failed to give any trace of the characteristic crystalline tetrabromostearic acid. It was tentatively concluded that the amount of linoleic acid present was too small to be detected by bromination.

In further support of this conclusion, the composition of the perilla oil was determined from calculations based on the Wijs I. V., 203.9; T. V., 127.1 (0.1 N, 20°, 24 hrs.); and the (Bertram) saturated acid content, 9.09 percent. The T. V.'s for triolein (85.7), for trilinolein (89.8), and for trilinolenin (154.2) were used in the equations for the calculations. These values were computed from the found thiocyanogen values for the methyl esters reported in Tables I to III. The fatty acid composition, computed to the glycerides, was found to be oleic 17.97, linoleic, 0.00, linolenic, 72.47, and saturated, 9.56 percent.

For comparison, the composition calculated from Kaufmann theory values was found to be: oleic 2.08, linoleic, 32.68, linolenic, 55.68, and saturated, 9.56 percent.

It is apparent from the work of others and from the data in this paper that revision of "Kaufmann theory" values is necessary. It is suggested that since reasonably good agreement was obtained between various workers (Table XI), the average of the found values for oleic, linoleic and linolenic acids be used in the equations for calculation of composition in place of the Kaufmann-theory values.

## Summary

Thiocyanogen values were determined on the methyl esters of oleic, linoleic and linolenic acids and on six different mixtures of these esters, using 0.1 and 0.2 normal thiocyanogen solutions. The values determined with 0.1 N solutions showed less variation than those determined with 0.2 N.

The composition of the mixtures calculated from equations based on the found thiocyanogen values of the esters agreed with the known composition within reasonable limits. Comparisons were made with the composition calculated with the Kaufmann-theory values.

It is suggested that the F.A.C. consider adopting tentatively the values 89.4 for oleic acid, 93.9 for linoleic acid, and 162.0 for linolenic acid when 0.1 N thiocyanogen solutions are used; the values 89.4, 96.8, and 167.5 when 0.2 N solutions are employed. These represent the average of the values for these acids which have been reported in the literature.

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# An Investigation of Oil from Seed of Hygrophila Spinosa*

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HYGROPHILA SPINOSA (Indian name—Tal makhana) of natural-order acanthaceae, is a little annual plant found in abundance throughout India and Ceylon in moist places or on the banks of ponds. Its seeds, which are very small (diameter approximately 1 mm.) in size and deep brown in color, are very light, 100 seeds on an average weighing about 1 gram.

The root, leaves and seeds of this plant find a prominent place in Hindu medicine. The seeds are diuretic and aphrodisiac and are used by Unani physicians in the treatment of gonorrhea, impotence, spermatorrhoea, and general debility.

In the present communication, a quantitative statement of the component acids of the hygrophila spinosa seed oil is given.

Phalnikar et al. (1) have analyzed the oil from the seeds of hygrophila spinosa; the results of their analysis, along with those of the present study, are given in Table I below, from which it will be seen that the present fat differs markedly from the one studied by the above authors.

TABLE I.

| | Phalnikar et al. % | Present work % |
|---|---|---|
| Myristic acid | 1.4 | 1.4 |
| Palmitic acid | 18.2 | 5.4 |
| Stearic acid | 5.4 | 11.9 |
| Oleic acid | 75.0* | 9.8 |
| Linoleic acid | | 71.5 |
| Unsaponifiables (% on oil) | 7.5 | 2.3 |

*Proportions of oleic and linoleic acids are not given.

The seeds, obtained locally (the United Provinces, India), were freed from dust and other foreign impurities and extracted with petroleum ether (40°-60°C.)

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* From the thesis of P. D. Srivastava, approved for D.Sc. of Benares Hindu University, Benares, India.