The Thiocyanogen Value of Linolenic Acid*

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ABSTRACT

Ethyl linolenate (Wijs iodine number 247.3; theory 248.5) was prepared by the debromination of purified hexabromostearic acid. The thiocyanogen value determined exactly as recommended by the Committee on Analysis of Commercial Fats and Oils, [Ind. Eng. Chem. Anal. Ed. 8: 234 (1936)] **was found to be 151.2, corresponding to a corrected value of 167.3 for the** free linolenic acid, rather than the theoretical value of 182.5. Data are presented to show the effects of time, excess and concentration of reagent. The thiocyanogen number of linolenic acid is proved to be an empirical value which, however, is reproducible under carefully controlled conditions. Several improvements of technique found to insure reproductibility of results are discussed in detail.

THE quantitative determination of linolenic acid in oils or their mixed fatty acids by the thiocyanometric method of Kaufmann (8) is based upon calculations in which the thiocyanogen value of linolenic acid is assumed to be exactly two-thirds of its iodine number (182.5 and 273.7 respectively). While Kaufmann and Keller (8) and Toyama and Tsuchiya (12) have demonstrated indirectly that thiocyanogen adds to only two of the three double bonds of linolenic acid, the quantitative nature of the addition and the actual thiocyanogen value of this acid have not been determined, largely because of the difficulty of preparing pure linolenic acid as the standard, and the uncertainty regarding the identity of the artificial product with the natural acid present in linseed and similar oils (7).

In the course of an investigation involving the analysis of mixtures of fatty acids containing *alpha* linolenic acid, we found that the thiocyanogen value of this acid was markedly lower than the theoretical value of 182.5, and that it was significantly affected by the concentration and excess of reagent, as well as the reaction period. Although Kaufmann and Keller (8) showed that increasing the excess of reagent over 100 per cent or prolonging the reaction period over 18 hours had no significant effect on the thiocyanogen value of linseed oil, Griffiths and Hilditch (3) observed a slight drop after 24 hours, while Gay (2) noted a distinct rise and, with others (13), questioned the applicability of the thiocyanometric technic to oils containing linolenic acid, since a satisfactory end-point and reproducibility of values could not be obtained. We, therefore, considered it desirable to make a study of the thiocyanogen value of *alpha* linolenic acid under the conditions of the determination described by the F.A.C. (5), which have been adopted by the American Oil Chemists' Society (1).

Experimental

The first series of experiments which dealt with the applicability of the thiocyanometric method to mixtures of fatty acids containing all three commonly found octadecenoic acids (oleic, linoleic, and linolenic) was performed with a sample of linolenic acid prepared exactly according to the directions of Rollet (11). Hexabromostearic acid obtained by the bromination of linseed oil fatty acids in ether was washed copiously with ether, recrystallized once from xylene, and rewashed with ether. It was debrominated with zinc in 7.5 N ethyl alcoholic hydrochloric acid, the resulting ester extracted with ligroin, washed, dried over sodium sulfate, the solvent removed under diminished pressure, and the ester distilled in an alembic flask in a stream of nitrogen. The colorless distillate was saponified in the cold with 7 per cent alcoholic aqueous potassium hydroxide, and the acid, liberated in the usual manner, was redistilled in a molecular still at 10^{-5} mm. pressure. The colorless product had an iodine number (Wijs ½ or 1 hour) of 271.0 (theory 273.7). For subsequent determinations of the actual thiocyanogen value of linolenic acid, a product of greater purity was prepared by omitting the saponification, which results in a slight discoloration that must be removed by distillation, and by further purification of the starting material. A portion of the hexabromostearic acid described previously was recrystallized three times more from xylene, then washed copiously with boiling ethyl and petroleum ethers. Its melting point was 178-178.3° (uncorr.) when the temperature of the heating bath was raised very slowly and from 179° to 181° when heated rapidly. This was debrominated as above, and the resulting ethyl ester, after distillation under nitrogen in the alembic flask at 2 mm. pressure, was a colorless liquid, iodine number 247.3, 247.7 (1 hour Wijs), 247.1, 247.2 [3 minute Wijs-mercuric acetate (4); theory 248.8].

As stated previously, the preparation of the reagents and the method of the determinations followed the recommendations of the F.A.C. (5), in which the excess thiocyanogen is reacted with dry potassium iodide prior to the thiosulfate titration. We found, however, that this operation occasionally produced erratic results unless the following precautions were observed: during the two-minute period in which the reaction mixture is swirled with the dry KI, the agitation must be sufficiently vigorous to insure thorough mixing of the solution in order to effect the complete conversion of the excess thiocyanogen to iodine. The glass stopper and the sides of the flask should then be washed down with several ml. of a 15 per cent solution of potassium iodide, the flask shaken momentarily, and the addition of the 30 ml. of water should again be followed by shaking for at least one-half minute or until thorough mixing is assured.

The thiocyanogen solutions, which varied in concentration from 0.16 to 0.18 N, were freshly prepared for each series of determinations and were dispensed from a 25 ml. automatic pipette protected with a calcium chloride tube. All glassware was dried for one hour at 105°C., and the reaction temperature was maintained at 19° ± 1°C. by keeping the reagents and reaction mixtures in a large water-jacketed incubator. The determinations were made in duplicate and appropriate blank determinations were made in every case where the reaction time was varied. These blanks never differed from th original blank by more than ± 0.2 ml. of 0.1 N thiosulfate, and duplicate determinations invariably agreed within one unit.

Results

A series of mixtures of known composition, containing corn oil (Mazola) fatty acids and linolenic acid, were prepared by weighing, and their iodine numbers (Wijs, 1 hour) and thiocyanogen values (28-30 hours, 0.16 N solution) were determined. The results, summarized in Table I, show that the thiocyanogen value of linolenic acid is about fifteen points lower than the theoretical 182.5, but that the thiocyanogen values of the mixture calculated on the basis of this empirical value may be experimentally reproduced with prac-

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tically the precision of iodine numbers. Deviations from the calculated values appear to follow increasing concentrations of linolenic acid when the excess of reagent is constant. As expected from the data of McKinney (9), the T.V. of a mixture of corn oil fatty acids with only a small amount of linolenic acid is high in the presence of more than 150 per cent excess of thiocyanogen.

TABLE I
Comparison of Calculated and Experimental I.V. and T.V. of Mixtures of *alpha* Linolenic Acid with Corn Oil Fatty Acids

Linolenic Acid %	I.V. (Wijs 1 hour) Calc.	Found	T.V. (28-30 hours) Excess	Calc.	Found	Deviation
100		271.0	215		166.7	
0 (corn oil f.a.)		133.0	170		84.1	
5.60	140.6	140.3	200	88.7	89.8	1.1
10.70	147.8	148.0	200	92.9	92.5	—0.4
20.46	161.4	160.6	155	101.1	100.8	—0.3
31.12	175.9	175.6	160	109.8	109.1	—0.7
40.63	189.0	187.8	160	117.7	116.5	—1.2
51.11	203.4	203.0	160	126.4	125.1	—1.3

The effect of the excess of thiocyanogen on the T.V. of ethyl linolenate (I.V. 247.3; theory 248.8) is shown by the data in Table II, in which the actual excess of reagent was calculated in the usual manner as 100 times the ratio of the quantity of unconsumed reagent to the reagent consumed after the completion of the stated reaction period. Since the T.V. varies with the excess of reagent, comparisons must be drawn on the basis of the "theoretical excess" calculated on the assumption that the T.V. of ethyl linolenate is 165.8, as required by complete saturation of two double bonds. It is evident that under the conditions of the determination specified by the F.A.C., the T.V. of linolenic acid corrected to its theoretical I.V. is only 167.3, while increasing the excess over 200 per cent as recommended by Kaufmann (8) raises this value to 170.2. This is in good agreement with the value of 171 we obtained previously (6) with a 250 per cent excess of 0.2 N reagent. As may be expected, increasing the excess of reagent decreases the time at which equivalent values are obtained. On the other hand, reducing the concentration of the reagent to 0.1 N but maintaining the relative excess of thiocyanogen by reducing the size of the sample, lowered the T.V. by the linolenic acid to 163.8 (excess: 158 per cent actual, 131 per cent theoretical; time, 24 hours).

TABLE II
Effect of Excess of 0.16 N Thiocyanogen on the T.V. of Ethyl Linolenate (I.V. 247.3)

Time (hrs.)	Excess Theoretical %	Actual %	T.V. Found	Calc. to Linolenic Acid of theoretical I.V. 273.7
20	129	153	150.1	
	161	188	149.6	
	225	255	151.1	
	260	290	153.0	
24	139	164	151.2	167.3
	154	177	152.2	168.4
	248	274	153.9	170.2
	260	287	154.1	170.5
28	132	152	152.7	
	144	163	153.8	

The rate of addition of thiocyanogen to the ethyl linoleate at a practically constant theoretical excess of 150 per cent of 0.18 N solution is shown graphically in Fig. 1. No definite end-point appears to be attained in a convenient period, although at the end of eighteen hours the curve has flattened out sufficiently to indicate a rise of only four points in the succeeding twelve hours, with a maximum T.V. still about twelve points below the theoretical value required for two-thirds saturation.

Discussion

In essential agreement with similar studies of linoleic acid (6, 10), the data presented in this paper show that the thiocyanogen number of linolenic acid is an empirical value which may vary by a total of approximately 4 points under the ordinary differences of procedure where the excess of the 0.2 N reagent is varied between 150-250 per cent and the time of reaction between 18-28 hours, although a given value is easily reproducible under identical conditions of determination, and in the case of oils containing less than 50 per cent of linolenic acid, the proportionately smaller deviation due to slight variation of conditions may fall within the acceptable limits of experimental error. However, there can be little doubt that the thiocyanometric determination of the composition of drying and semi-drying oils has only a very limited significance unless the calculations are based upon the empirical rather than the theoretical thiocyanogen values of linoleic and linolenic acids. With no other criterion of purity beside the iodine number available at present, it must be assumed that the linolenic acid and its ethyl ester used in our study are acceptable standards, and that the T.V. of at least *alpha* linolenic acid determined according to the method of the F.A.C. (5) is 167.3. Since *alpha* linolenic acid and the linolenic acid present in linseed and similar oils form identical hexobromostearic and linusic acids, we must further assume that, until contrary proof is presented, the empirical T.V. of natural linolenic acid is also 167.3, or a close approximation to this value as indicated in the experimental data. Under similar conditions, the T.V. of linoleic acid is 96.3 (6), and calculations with thiocyanogen values obtained under conditions significantly different from those specified by the method adopted in this study must necessarily be based upon other empirical constants determined in a similar manner.

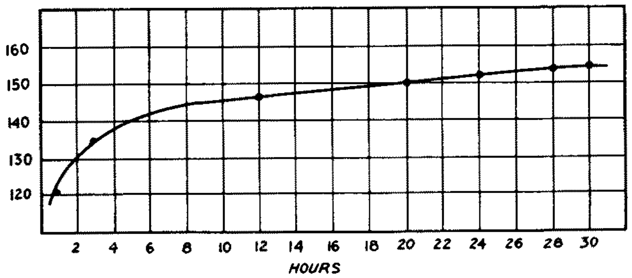

Fig. 1 Rate of addition of Thiocyanogen (0.18) to Ethyl Linolenate

Conclusion

The thiocyanogen value of *alpha* linolenic acid determined according to the method of the F.A.C. and the A.O.C.S. was found to be 167.3 rather than the 182.5 required by theory.

The empirical value was shown to vary significantly with the concentration and excess of reagent and the period of reaction.

While the thiocyanometric technic was found to be perfectly applicable to oils containing linolenic acid, substitution of empirical constants in the equations used in the calculations was suggested.

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