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FEEDING EXPERIMENTS RELATIVE TO CATARACT PRODUCTION UTILIZATION OF LACTOSE IN MILK NUTRITIVE VALUE OF LIGANIC ACID and INVESTIGATIONS IN TERPENE CHEMISTRY and PRELIMINARY INVESTIGATION OF PO-YOK SEED KERNELS

THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY at the UNIVERSITY OF WISCONSIN

By
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* Presented in the order in which the work has been performed.

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CHAPTER I

RELATION OF SKIM MILK FEEDING TO CATARACT PRODUCTION

In 1935 Mitchell and Dodge (1) reported that 65 percent of rats fed synthetic diets containing 70 percent lactose developed mature bilateral cataracts and that 27 percent on diets containing 50 percent lactose became similarly afflicted. Since skim milk solids contain about 50 percent lactose it was thought that skim milk might be dangerously high in lactose. However, cataracts have never been observed in this laboratory in rats on a skim milk diet, but they have been observed in rats receiving skim milk containing added galactose. Therefore it was thought advisable to make a study of the relation of added lactose and of added galactose to the causation of nutritional cataract in animals on a skim milk diet, and to feed some synthetic diets high in lactose and others high in galactose, supplementing these diets with skim milk, in order to determine whether or not skim milk had any protective action against the incidence of this type of nutritional cataract.

Since Day (2) and others (3) have reported a type of nutritional cataract caused by flavin deficiency it was considered necessary to feed additional quantities of flavin with the skim milk diet to see if any relation between the flavin content of milk and the incidence of cataract caused either by lactose or by galactose could be discovered.

Experimental Part:

Because young rats could not be raised on mineralized skim milk alone or on rations high in galactose, it was necessary to raise them on

* Published: Krewson, C. F., Schantz, E. J., and Elvehjem, C. A., Proceedings of the Society for Experimental Biology and Medicine 42, 573 (1939)

mineralized whole milk until they weighed 80 to 100 grams each and then change them to the experimental rations of fresh mineralized skim milk containing the dissolved sugars. The fat-free synthetic basal ration had the following composition:

Carbohydrates (see Table II)	70
Purified casein	20
Brewers yeast	6
Salts	4

One microgram of beta-carotene was added to each gram of dry ration and 10 micrograms to each 100 ml. of skim milk and crystalline riboflavin dissolved in water was added to the milk rations. All animals were irradiated for a period of ten minutes each day.

The onset of cataract was determined by the first appearance of white in the lens of the eye. No instruments were used to identify any other changes in the eye structure.

The different skim milk diets and the varied synthetic ones together with a summary of the experimental results obtained are shown in Tables I and II.

It may be observed from the data that a considerable proportion of galactose may be added to the skim milk before cataract formation becomes apparent. Skim milk with sufficient galactose added to be equivalent to 20 percent of the solid material did not cause cataract, while that with sufficient to be equivalent to 30 percent of the solids did result in its formation in all animals in 26 to 30 days. Amounts of galactose above 30 percent resulted in cataract formation in 20 to 25 days, which formation was not delayed by the additional feeding of 100

TABLE I

Skim Milk Diets and Results Obtained with Each Diet

Diet	Number of Rats	Cataract
Skim milk *	4	None within 150 days
Skim milk plus lactose to make lactose content of solids equal 70%	5	None within 90 days
Skim milk plus equal amounts of glucose and galactose to make sugar content 70%	5	None within 90 days
Skim milk plus galactose to make 20 % of solids	5	None within 90 days
Skim milk plus galactose to make 30% of solids	5	Both eyes, 28-30 days
Skim milk plus galactose to make 30% of solids plus 100 micrograms flavin per rat daily	5	Both eyes, 28-30 days
Skim milk plus galactose to make 40% of solids	5	Both eyes, 20-25 days
Skim milk plus galactose to make 40% of solids plus 100 micrograms flavin per rat daily	5	Both eyes, 20-25 days

* Same observation was made on 40 other rats on previous occasions.

TABLE II

Synthetic Rations and Results Obtained with Each Diet

Diet	Number of Rats	Cataract
Basal ration (70% lactose)	4	One in 60 days, none in others within 90 days
Basal ration (70% lactose) plus 15 ml. skim milk per rat daily	4	None within 90 days
Basal ration (70% lactose) 80 parts, lard 20 parts	4	None within 90 days
Basal ration (galactose 35%, dextrin 35%)	4	Both eyes, 25-30 days
Basal ration (galactose 60%, dextrin 10%)	4	Both eyes, 18-20 days
Basal ration (galactose 60%, dextrin 10%) 80 parts and lard 20 parts	4	Both eyes, 18-22 days
Basal ration (70% lactose) (later experiment)	8	None within 90 days

micrograms of flavin daily to each rat. No cataracts became apparent in rats on skim milk during a feeding period of 150 days, and addition of lactose to skim milk, to make the lactose content of the solids equivalent to 70 percent, did not result in cataract formation within a period of 90 days. Similar data were obtained on rats on synthetic diets either of lactose or galactose. Only one animal showed any signs of cataract on the 70 percent lactose ration while on a ration containing 35 percent galactose and 35 percent dextrin all animals developed cataract in 28 to 30 days. No evident beneficial effect was caused by feeding of either skim milk or of fat along with the synthetic rations.

Discussion:

It is apparent from the data that the development of cataract under the conditions of the experiments described is dependent upon the amount of galactose in the diet. However, the animals receiving 20 percent galactose in the diet did not develop cataract within the experimental period of 90 days, due to the fact that the animal is able to utilize some galactose by converting it to glycogen. This utilization together with the excretion by the kidneys of about 40 to 50 percent of the ingested galactose may account for all of the galactose consumed at a 20 percent level. Greater percentages of galactose in the diet always resulted in cataract formation. No cataracts developed in rats on a 70 percent lactose ration, with the exception of one slight case while all animals developed definite cataracts in both eyes in 28 to 30 days on a ration containing 35 percent galactose and 35 percent dextrin. It would appear rational to explain the above observations upon the premise that in the hydrolysis of lactose in the intestional

tract galactose is liberated slowly, but so slowly that the animal can metabolize it, whereas, when free galactose is ingested the system is flooded with galactose and cataract ensues. Mitchell (4) has shown cataract formation of this variety to be paralleled by high blood galactose. Since the onset of cataract was not delayed by the addition of flavin to the milk diets the cataracts produced by the feeding of galactose are undoubtedly different from those resulting from a flavin deficiency. Some delay in the onset of cataract was observed when fat was included in the diet, the fat apparently acting as a diluent in the ration since a diet composed of 80 parts of the ration mixed with 20 parts of the fat gave results similar to those obtained on a ration containing a lower percentage of galactose. The presence of fat both retards the absorption, and probably aids in the metabolism, of galactose which may account for some of the delay.

Since the addition of skim milk to the synthetic galactose rations did not delay the onset of cataract it appears that skim milk contains no principle which prevents cataract formation. Throughout all the experiments the results obtained on skim milk rations and on synthetic rations were similar. The differences between Mitchell's results and those obtained in these experiments may be explained on the basis of a difference in the susceptibility to cataract of different strains of rats, a difference which Mitchell (5) has shown to prevail.

Also, throughout these cataract studies it was observed that the animals made very little if any growth either on lactose or on galactose rations and that on rations high in galactose all animals lost weight and if allowed to remain on the ration for much longer than five or

six
weeks would die. Guha (6) has reported similar results but offers no explanation for them.

Conclusions

It has been shown that the development of cataract in the rat is dependent upon the amount of galactose consumed in the diet and skim milk alone is not sufficiently high in lactose to produce the same effect. It appears, also, that skim milk does not contain any principle capable of counteracting cataract formation.

Bibliography

1. Mitchell, H. S. and Dodge, W. H. Jr., Jour. Nutr. 9, 37 (1935)

2. Day, P. L., Darby, W. J., and Langston, W. C., Jour. Nutr. 13, 389 (1937)

3. O'Brien, C. S., Arch. Opth. 8, 880 (1932)

Yudkin, M. M., J. Am. Med. Assoc. 101, 521 (1933)

Guha, B. C., Nature 135, 395 (1935)

Bourne, M. C. and Pyke, M. A. Biochem. J. 29, 1865 (1935)

4. Mitchell, H. S., Morrison, G. A., and Cook, G. M., Jour. Nutr. 13, 501 (1937)

5. Mitchell, H. S., Jour. Nutr. 12, 447 (1936)

6. Guha, B. C., Biochem. J. 25, 1385 (1931)

CHAPTER II

FURTHER STUDIES ON RELATION OF FAT TO UTILIZATION OF LACTOSE IN MILK

FURTHER STUDIES ON RELATION OF FAT TO UTILIZATION OF LACTOSE IN MILK*

It was found in our laboratory (1) that rats placed on a whole milk diet mineralized with iron, copper, and manganese made very efficient use of the milk sugar but when placed on a mineralized skim milk diet 20-30% of the ingested galactose was lost in the urine. Natural fats such as lard, corn oil, coconut oil, and linseed oil could be substituted for butter fat in the milk without loss of galactose. Synthetic triglycerides such as palmitin and olein were effective while caprein was not effective in preventing the loss of galactose in the urine.

In view of these results it was thought advisable to test the effectiveness of several other triglycerides including odd chain fatty acids. It was also considered advisable to feed salts of the lower fatty acids and high levels of glucose along with skim milk.

Experimental Effect of Even Chain Triglycerides:

The triglycerides of caproic, caprylic, capric, and lauric acids were synthesized from the crude acids obtained from Eastman Kodak Company. These glycerides were homogenized into fresh skim milk at a level of 4%. The metabolism experiments were performed on rats weighing between 100 and 140 grams and which had been raised on mineralized whole milk. The animals were placed on a skim milk diet until they began to lose considerable galactose in the urine, which required 10-12 days. At this time 24 hour metabolism periods were run on the rats receiving the synthetic triglycerides homogenized into the milk. The results obtained show that caprein, caprylin, and caprin were not effective within a 10-day period in preventing any appreciable loss of galactose but laurin was quite effective. These animals stopped losing

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galactose in the urine within 3 or 4 days when corn oil was substituted in the skim milk. The 18 carbon keto acid, licanic,(2) was also fed and found to be effective.

Effect of Feeding Odd Chain Acids:

The glyceride of daturic acid, which had been isolated from "Datura Stramonium"(3) was fed in skim milk, and it was found to be quite effective in preventing the loss of galactose. Since Schriner, Fulton, and Burke (4) have pointed out that an equimolecular mixture of palmitic and stearic may be mistaken for margaric acid and that a mixed melting point is not conclusive evidence for identification, it was considered advisable to feed a synthetic odd chain acid. Pentadecylic acid was synthesized (5) and the triglyceride made and fed with skim milk. A slight response was obtained in some cases but no definite and lasting effect was noticed in preventing the loss of galactose in the urine.

Effect of Various Salts:

Since some difficulty was encountered in feeding the lower triglycerides due to liberation of free acid in the milk , the sodium salts of caproic, caprylic, and capric acids were fed at levels of 4% in skim milk. These salts did not stop the loss of galactose in the urine but in some cases tended to increase the excretion of galactose. Feeding 2% of NaCl or Na_2HPO_4 in the skim milk increased the excretion of galactose as much as 20%. The action of KCl was similar to but not as pronounced as NaCl or Na_2HPO_4. The feeding of these salts with whole milk caused some animals to lose small amounts of sugar in the urine.

Effect of Feeding High Levels of Glucose:

Previously it was reported (1) that feeding glucose along with the skim milk prevented some loss of galactose in the urine of some animals. Later

it was thought advisable to replace the calorific value of the fat by an equivalent amount of glucose which required 8% glucose. Glucose was fed at levels of 8% and 10% in the skim milk and the galactose excretion decreased 10-30%. The sugar excreted in the urine on high levels of glucose was non-fermentable.

A summary of the results obtained with various fatty acids, with salts, and with glucose is shown in Table I.

It is apparent from these experiments that the fatty acids with 12 or more carbon atoms are the most effective in preventing the loss of galactose in the urine. No explanation can be offered for this until more is known about the mechanism concerning fatty acid function in galactose metabolism.

The contradiction between the results obtained with daturic acid and pentadecylic acid may be explained by the fact that daturic acid which was isolated from a natural source may have been a eutectic mixture of palmitic and stearic acids.

The fact that NaCl and Na_2HPO_4 increased the excretion of galactose is surprising in view of the fact that McQuarrie, Thompson, and Anderson (6) found that sodium salts caused a decrease in glycosuria in diabetic children. Crabtree and Longwell (7) found that rats on a high salt diet containing glucose deposited almost twice as much liver glycogen as rats on a low NaCl diet. This phenomenon has not been further investigated by us, but it points again to the difference between the metabolism of galactose and glucose.

Feeding high levels of glucose in skim milk to replace the calorific value of fat apparently does not take the place of fat. It is very likely that the small decrease in the excretion of galactose when glucose was

TABLE I

Effect of Various Fatty Acids, Salts, and Glucose on Galactose Metabolism

Substance	Level Fed, %	Effectiveness
Caproin	4	Very slight, if any
Caprylin	4	Very slight, if any
Caprin	4	Very slight, if any
Lauric acid	4	Good response
Licanic acid	2	Good response
Daturin acid	4	Good response
Pentadecylin	4	Poor response
Sodium salt of caproic acid	4	Poor response
Sodium salt of caprylic acid	4	Poor response
Sodium salt of capric acid	4	Poor response
Sodium chloride	2	Increased excretion of galactose
Disodium phosphate	2	Increased excretion of galactose
Potassium chloride	2	Increased slightly in some cases
Glucose	8	Very slight
Glucose	10	Very slight

fed was due to the retarding action of glucose upon the absorption of galactose.

Conclusions:

Even chain fatty acids containing 12 or more carbon atoms when fed with skim milk to the extent of 3 to 4% were effective in preventing the loss of galactose in the urine while acids with less than 12 carbon atoms were not effective in preventing this loss. An odd chain fatty acid (pentadecylic) was not effective in preventing loss of galactose. Feeding glucose at levels of 8 to 10% in skim milk did not prevent the loss of galactose.

The excretion of galactose was increased as much as 20% by feeding 2% of NaCl or Na_2HPO_4 in the skim milk. Potassium chloride was not as effective in increasing the excretion of galactose as was NaCl or Na_2HPO_4. These salts caused some excretion of galactose on whole milk diets.

BIBLIOGRAPHY

1. Schantz, E. J., Elvehjem, C. A., and Hart, E. B., J. Biol. Chem. 122, 581 (1938)

2. Obtained as a gift from Alfred Rheineck of Davco and Reynolds Company; prepared according to the method of Brown, W. B. and Farmer, E. M., Biochem. J. 29, 651 (1935)

3. Obtained as a gift from R. W. Clark, formerly of the Pharmacy Department, University of Wisconsin, now of Merck and Company

4. Schrimer, R., Fulton, J., and Burke, D., Jr., J. Am. Chem. Soc. 55, 1194 (1933)

5. Krewson, C. F., this thesis and Pharmaceutical Archives 10, 88 (1939)

6. McQuarrie, I., Tompson, W. H., and Anderson, J. A., J. Nutrition 11, 77 (1935)

7. Crabtree, D. G., and Longwell, B. B., Proc. Soc. Exp. Biol. and Med. 34, 705 (1936)

CHAPTER III

THE SYNTHESIS OF N-PENTADECYLIC ACID

THE SYNTHESIS OF n-PENTADECYLIC ACID*

In a study of the relation of fat to the utilization of lactose in milk in animal feeding it was proposed to make use of a synthetic fat of an odd number of carbon atoms. Pentadecylic acid was selected for this purpose because myristic acid and tetradecyl alcohol, two possible starting points for such a synthesis, were available as reasonably priced commercially as any other suitable compounds. In addition there was available for the feeding work some daturic acid, isolated from "Datura stramonium" L., to which the formula $C_{17}H_{34}O_2$ had been assigned (1). As a check to daturic acid, a second odd chain acid seemed desirable, especially since there was the possibility that, as Shriner, Fulton, and Burks (2) have pointed out, an equimolecular mixture of palmitic and stearic acids may be mistaken for margaric acid and that mixed melting points are not conclusive evidence for identification. This doubt as to the individuality of daturic acid is however contrary to the opinion of many investigators (2) who claim to have isolated such an acid of an odd number of carbon atoms from several natural plant sources.

Unsuccessful attempts by Shriner, et al, (2) to prepare daturic acid by use of the Grignard synthesis, by which they obtained over 41% of the hydrocarbon dicetyl, $C_{32}H_{66}$, and considerable cetyl alcohol, but no pure margaric acid, made it undesirable to use this method for the synthesis of pentadecylic acid, and it was planned to use the following general scheme employing the nitrile process for attaching an additional carbon atom to the chain:

Myristic acid	→ Butyl myristate	→ Myristyl alcohol	→ Myristyl bromide or iodide	→ Myristyl cyanide	→ Pentadecylic acid

* Published in abbreviated form: Krewson, C. F., Pharm. Arch., 10, 88 (1939)

This is not a new procedure as the various steps have been referred to in the literature in a variety of connections (2,9,10,11). A report is made here in collective form of experiences with the process as modified; also, of the yields obtained, so a comprehensive view may be gained as to what may be expected of this synthesis on a practical basis when fairly large amounts of an acid such as pentadecylic are desired.

EXPERIMENTAL

I. Preparation of n-Butyl Myristate:

For a period of 20 hours 200 grams of Eastman myristic acid were refluxed with a mixture of 16 cc. of concentrated sulfuric acid and 800 cc. of butyl alcohol in a three liter flask. After cooling, this mixture was washed three times with 333 cc. of a saturated sodium chloride solution. During the final washing a few drops of methyl orange solution were added together with sufficient sodium chloride to neutralize any remaining acid (3). The excess of butyl alcohol was distilled off and the butyl myristate transferred to a 500 cc. Claisen flask for distillation under diminished pressure. After the removal of the remaining traces of butyl alcohol by the aid of a water pump the butyl myristate was distilled under vacuum. The major fraction retained had a boiling point of 163-165° C. at 6 mm. and weighed 226 grams, 90.8% of the theoretical yield.

II. Preparation of n-n-Tetradecyl Alcohol (Myristyl Alcohol):

The method for the preparation of myristyl alcohol was that of Reid, et al, (3) one which these workers employed in their preparation of oleyl alcohol, and the quantities of materials used are listed:

226 grams of butyl myristate
1590 cc. anhydrous butyl alcohol
95 grams of sodium

1000 cc. 20% solution sodium chloride
100 cc. 55% solution calcium chloride
350 cc. toluene

The procedure was modified as suggested in note 11 (3) in order to recover any unreduced myristic acid. Of the various procedures for purification of the myristyl alcohol, the process was selected involving the removal of the toluene by distillation, followed by vacuum distillation of the residual myristyl alcohol. The main fraction of myristyl alcohol, melting sharply at 37.3-37.5° C. (uncorrected) boiled at 131.5-132.5° C. under 6 mm. pressure and weighed 97 grams, 57% of the yield theoretically possible. Thirty-four grams, 17% of the original quantity of myristic acid, were recovered unchanged.

Since a considerable quantity of pentadecylic acid was necessary for the animal feeding work it was decided to fortify the myristyl alcohol with an additional quantity of commercial tetradecyl alcohol. On two different occasions a quantity of tetradecyl alcohol was kindly supplied by E. I. du Pont de Nemours and Company, Incorporated. The first lot received, 240 grams melting at 36.0-36.2° C. (uncorrected), was of excellent quality having been twice distilled through a nichrome spiral column before being received.

Since the second lot had a melting point range of 32.0-37.5° C., it was re-distilled twice through an electrically preheated twelve-inch Vigreux column under vacuum of less than 1 mm. pressure. The 357 grams received was cut to 220 grams by these distillations and by two crystallizations, one from petroleum ether (B.P. 40-60° C.) and one from anhydrous alcohol. The melting point of 36.5-37.8° C., although not as sharp, agreed favorably with that of the synthetic product.

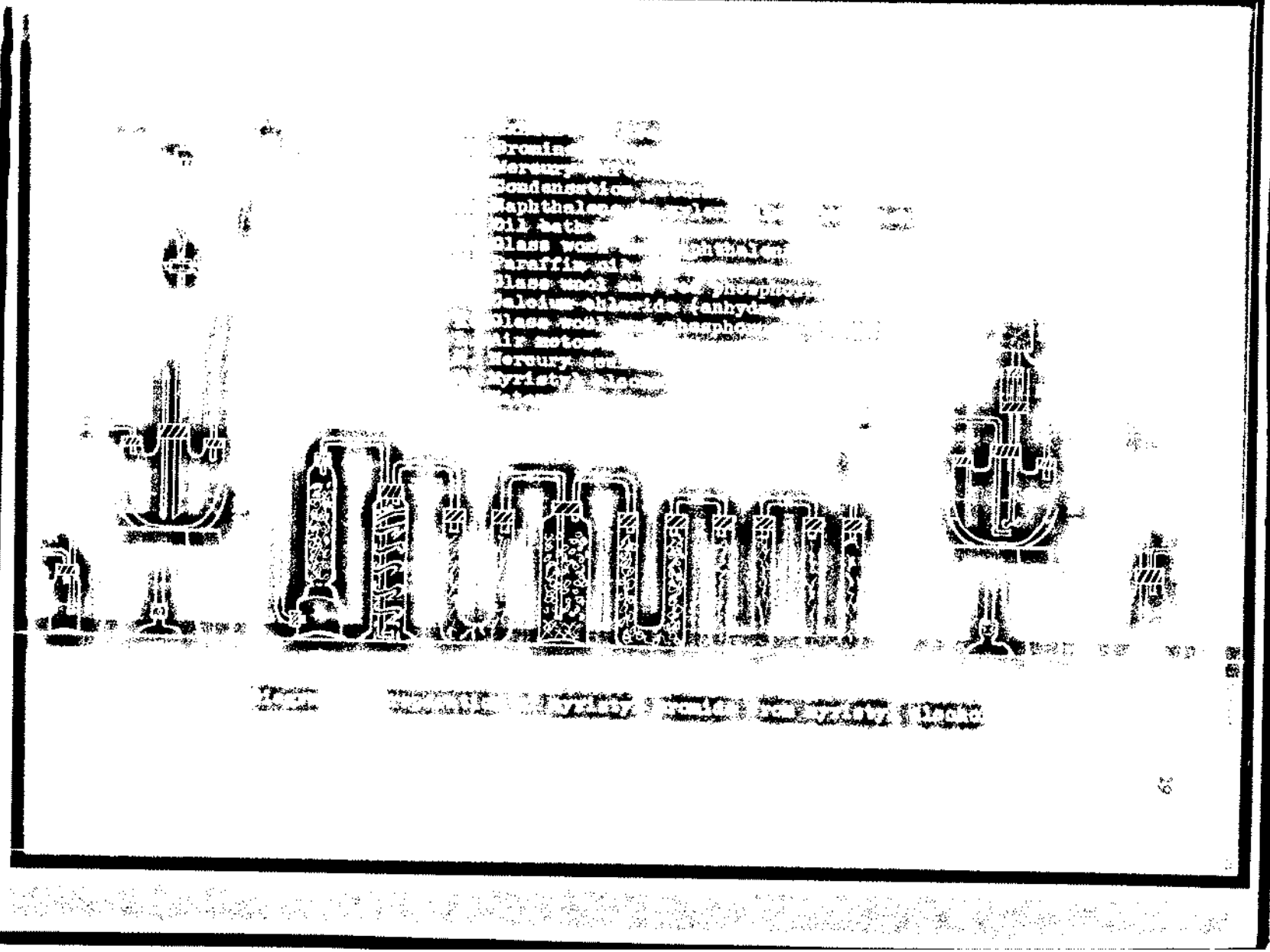
In the next step in the synthesis of pentadecylic acid, i.e., the conversion of the myristyl alcohol to the bromide or to the iodide, three different runs were made. The first was a conversion of the synthetic alcohol to the bromide as a preliminary run; the second was a conversion of the 240 grams of du Pont tetradecyl alcohol into tetradecyl bromide; the third was a conversion of the purified sample of du Pont tetradecyl alcohol into tetradecyl iodide.

III. Preparation of n-Myristyl Bromide:

It was presumed that for maximum yield of myristyl bromide anhydrous hydrogen bromide had best be employed. Of the several methods for the preparation of anhydrous hydrogen bromide described in the literature the Polk and Manson (4) modification of the Kastle and Bullock (5) method was used. This method, as adapted for use in the conversion of myristyl alcohol into myristyl bromide, is herewith described: The quantity of myristyl alcohol to be used (see Table 1) was placed in a three-way flask of suitable size fitted with thermometer, mercury-sealed mechanical stirrer, fritted-glass bubble-tube, and a properly bent exhaust tube whose end nearly touched the surface of the water in an adjoining bottle (see Diagram 1). The hydrogen bromide gas generator consisted of a three-way two liter flask to which was attached a long-stemmed separatory funnel to act as bromine reservoir. The end of this funnel tube was immersed in the reaction mixture consisting of naphthalene, ordinary moth-balls (tetrahydronaphthalene is also satisfactory), dissolved in xylene (kerosene B.P. over 140° C. is satisfactory) and a small quantity of metallic iron to catalyze the reaction. This gas generator was connected by means of a bent glass tube to a small bottle containing mercury to act as a safety in case of blockage in the apparatus train or sudden addition of too much bromine to the generator. A thermometer and a long inclined exit-tube completed the generator's equipment. The

TABLE 1 - DATA CONCERNING PREPARATION OF MYRISTYL BROMIDE

RUN 1 (Using synthetic tetradecyl alcohol)			RUN 2 (Using commercial tetradecyl alcohol)
Tetradecyl alcohol	grams	87.0	240.0
Naphthalene	grams	38.8	109.2
Xylene	cc.	170	475
Bromine(approx.amt.req'd.),	cc.	112	310
Theor. yield myristyl bromide,	grams	105.8	299.3
Actual yield of distld. bromide,	grams	81.0	233.0
Percent yield		74.7	78.0
Boiling Point at 6 mm.,	degrees	130 - 131	129 - 131



purpose of such an exit-tube connecting the generator with the wash bottle train was to serve as a condensation return for the major portion of the volatilized solvent escaping the flask. The purification train consisted of a bottle of glass wool and naphthalene chips to use up escaping bromine, a Milligan gas washing bottle containing paraffin oil to remove traces of xylene carried over mechanically, a U-tube of glass wool and red phosphorus to remove the last traces of bromine, and four drying tubes, two of anhydrous calcium chloride and two of phosphorus pentoxide. During operation the temperature of the generator flask was maintained at 120-125° C., the reaction mixture flask at 110-115° C., by use of oil baths, this temperature of the reaction mixture flask serving to increase the velocity of the reaction and to remove water of formation. The completeness of the conversion of alcohol to bromide was judged by frequent weighings of the water bottle at the end of the apparatus train. A rapid increase in weight, occurring after four and one-half to five hours, was assumed to be indicative of the completeness of the reaction.

In order to remove any uncombined myristyl alcohol as acid sulfate and to rid the myristyl bromide of any hydrogen bromide, the reaction mixture, after a run as described above, was shaken with one-third its volume of concentrated sulfuric acid. Careful separation of the bromide layer from the acid layer was effected at once in a separatory funnel and the acid layer discarded. The bromide was then treated with an equal volume of 20% methyl alcohol and enough aqueous ammonia to render the solution slightly alkaline to phenolphthalein. After washing the bromide again with an equal volume of 20% alcohol it was dried with about five grams of anhydrous calcium chloride, filtered and distilled in vacuo. The results of Runs 1 and 2, together with the quantities of materials used are summarized in Table 1.

A period of several weeks elapsed before it was convenient to convert the myristyl bromide into pentadecylic acid through the nitrile process. Such a marked decomposition had taken place (colorless liquid changed to deep red) that it was necessary to re-distill the bromide, so the products of the two runs were combined and distilled in vacuum, with a yield of 290 grams.

IV. Preparation of n-Tetradecyl Iodide (Myristyl Iodide):

The method used in the preparation of myristyl iodide followed that of Bleyberg and Ulrich in their preparation of cetadecyl iodide (7), a similar synthesis to that of Majima and Nakamura (8). The procedure in this work may be outlined as follows:

One hundred and thirty-nine and one-half grams of iodine and 17.7 grams of red phosphorus were mixed in a one liter round bottom flask provided with a calcium chloride drying tube, heated to 100° C., 220 grams of tetradecyl alcohol added and heating continued at a temperature of 170° C. for an hour. The reaction product when cool was transferred to a separatory funnel and the syrupy phosphorous acid layer, containing some unused red phosphorus, withdrawn. Prior to the attachment of the high vacuum pump the product was subjected to the vacuum of a water pump for the removal of the low-boiling impurities and excess iodine. The major portion obtained by distillation through a twelve-inch electrically preheated Vigreux column boiled at 145.5-144.5° C. under a vacuum of less than 1 mm. pressure with a yield of 307 grams, 92.3% of the theoretical. It will be noted that this is considerably larger than the yield obtained in the tetradecyl bromide runs.

V. Preparation of n- Pentadecylic Acid:

Pentadecylic acid was prepared from tetradecyl bromide and tetradecyl iodide according to the method of Levene and Taylor (9). The cyanide was not independently isolated but directly hydrolyzed and saponified with a large excess of 50% sodium hydroxide solution. Upon the advice of D. H. Woolley (10) the cyanide was subjected to longer refluxing than suggested by Levene and Taylor in order to insure complete hydrolysis. Also, the time of refluxing of the bromide and iodide with potassium cyanide was increased from "over night" to 24 hours. Three separate runs were made, two using tetradecyl bromide and one tetradecyl iodide. The quantities used and the results obtained are summarized in Table 2.

The products of Runs 1 and 2 were combined, crystallized six times from alcohol, once from acetone and once from benzene. The constant melting point obtained was 52.1-52.3° C. (uncorrected). Reported in the literature for pentadecylic acid are 53- 54° C. by Levene and West (11), 52° C. by Majima and Nakamura (8), and 51.5° C. by Eckert and Halla (12). The Neutralization equivalent of the pure recrystallized product was 245.1 and 245.0, agreeing well with the molecular weight 242.2 of pentadecylic acid, $C_{15}H_{30}O_2$.

The pentadecylic acid made from the iodide (Run 3) was purified by two distillations through the Vigreux column under vacuum of less than 1 mm. pressure. In the first distillation a cut of 177 grams was made from the 210 grams of crude pentadecylic acid. Its boiling range was 169-172° C. Subjected to re-distillation this was further cut to 153 grams with boiling range 169.5-170.5° C. The neutralization equivalent of this fraction was 241.5 and 241.7. The silver salt of the acid was prepared according to the

TABLE 2 - DATA CONCERNING PREPARATION OF PENTADECYLIC ACID

	RUN 1	RUN 2	RUN 3
Tetradecyl bromide, grams	150.	140.	307.
Tetradecyl iodide, grams			597.
Potassium cyanide, grams	45.	42.	90.
Absolute alcohol, cc.	1000.	1000.	2000.
50% solution NaOH, grams	100.	100.	160.
Time for hydrolysis, hours	72.	72.	40.
Yield pentadecylic acid, grams	93.0	90.0	210.0
Theor. yield, grams	130.7	122.4	229.0
% yield,	71.2	73.5	91.7

method suggested by Plimmer (15). Upon analysis for silver by ignition in the usual manner the average for two samples was 30.87% silver; theory, 30.91% silver. Calculated to a molecular weight value this gave 242.3 as compared with the theoretical value for pentadecylic acid of 242.2.

Cooling curve data were obtained from a two gram sample by placing the pentadecylic acid in a small test-tube which was held by a cork and inserted in a Dewar flask. The acid was placed at the desired temperature above the melting point by passing a current of warm air into the flask by means of an inlet in the cork. Temperature readings were made every half-minute during cooling by a Beckmann thermometer immersed in the pentadecylic acid. The results obtained by plotting the data received in two separate trials are shown in Figures 1 and 2.

VI. Preparation of Tripentadecylin:

Since animals seem to have a better appetite for glycerides than for their corresponding fatty acids, the pentadecylic acid was fed as a triglyceride. Accordingly, the purified pentadecylic acid fraction, made in Run 3 by the iodide process was converted into triglyceride, using anhydrous glycerine (14) with dry hydrogen chloride gas as catalyst. The details of the procedure are here recorded since this is essentially the method used for the preparation of a considerable number of triglycerides needed for the animal feeding work.

The calculated amounts of anhydrous glycerine, 19.3 grams, and pentadecylic acid, 152 grams, were treated in a 250 cc. Glaisen flask with a current of hydrogen chloride dried by passing in succession through one wash bottle of anhydrous calcium chloride, two of concentrated sulfuric acid and

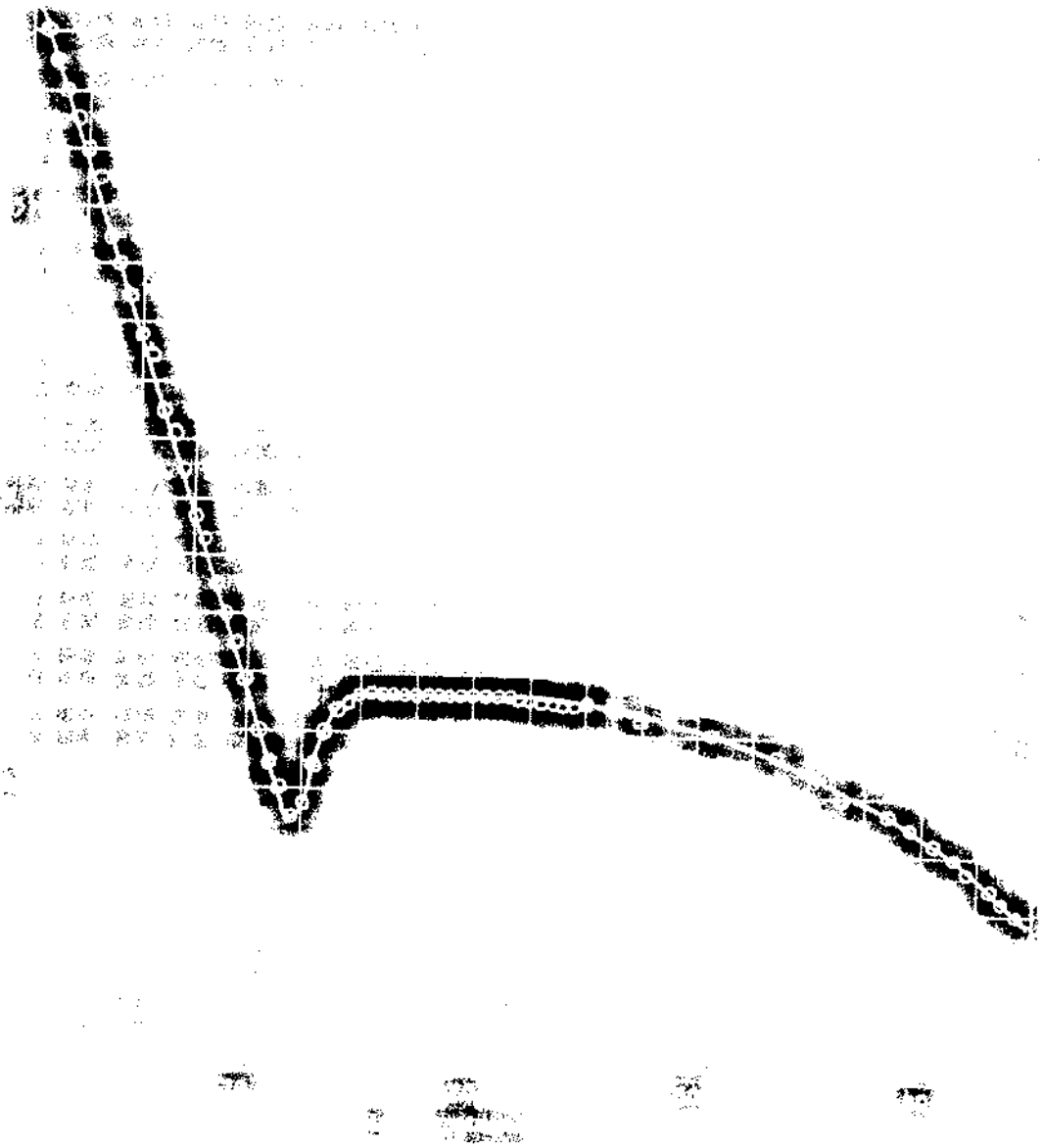
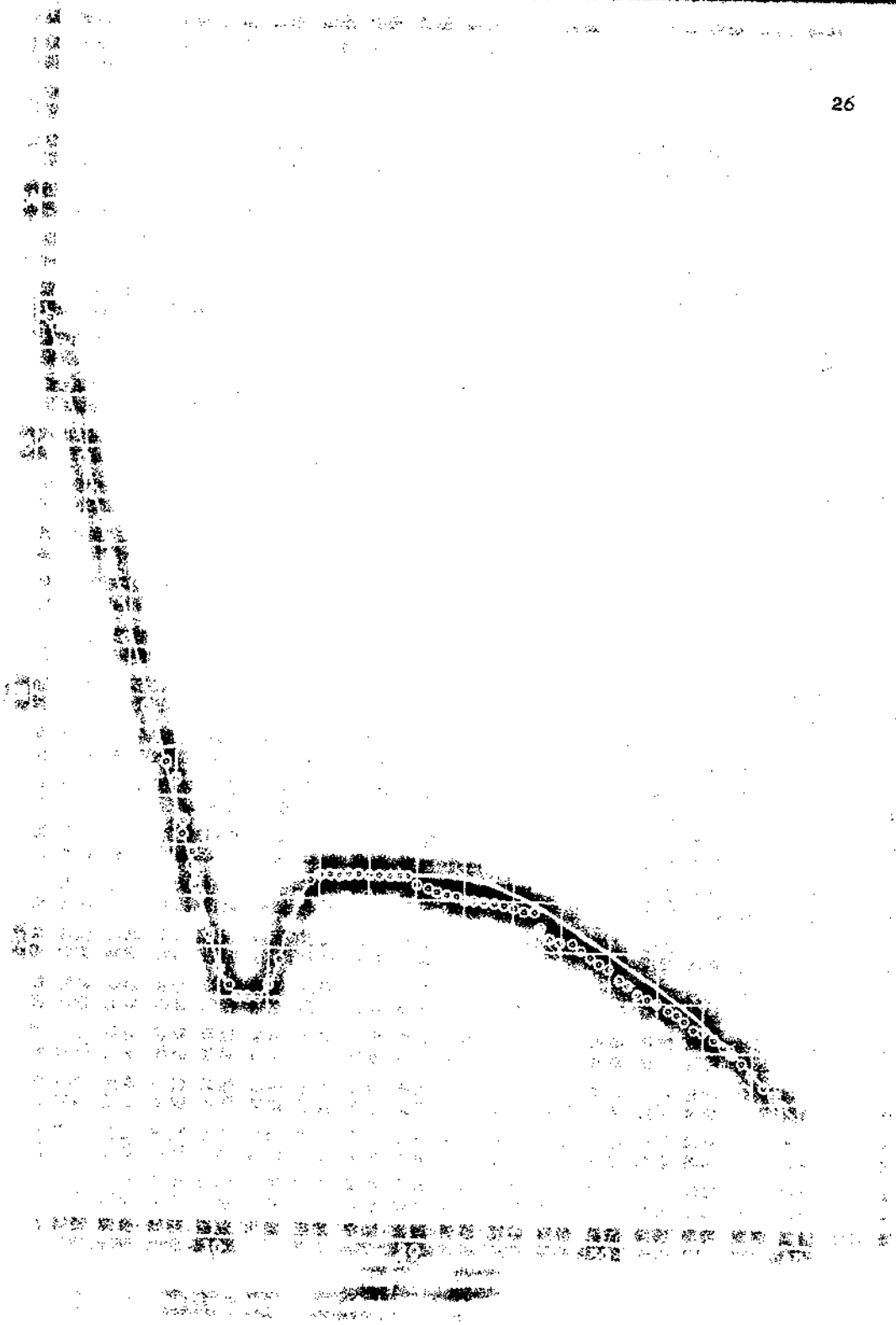


Fig. 1.- Cooling curve pentadecylic acid.



one U-tube of phosphorus pentoxide. To the side-arm of the Claisen flask was attached an ordinary small distilling flask to serve as a water trap. The reaction mixture was maintained at 110° C. for twenty-four hours, following which the temperature was raised to 160-170° C. and a water pump was attached to the side-arm of the distilling flask. The pump was allowed to operate for several hours in order to remove any water of formation from the reaction flask. Continuously during this heating a slow stream of dry hydrogen chloride was passed through the system. Finally, the hydrogen chloride supply was cut off and the apparatus allowed to draw air in order to sweep out the hydrogen chloride remaining. When cool the product was dissolved in ether, washed with dilute sodium carbonate solution, with water, dried, and the ether removed. The yield was 140 grams of product obtained after two recrystallizations from Skelly Solvent "B", 87.5% of the theoretical amount. The melting point of 52.8-53.0° C. (uncorrected) agrees with that reported for the beta- form of tripentadecylin by Clarkson and Malkin (13) of 54.0° C. However, the property of "triple melting" (13) was not observed in this product although several attempts were made to obtain such alteration. Further analytical data were not obtained for the triglyceride as this hardly seemed necessary in view of those obtained for the pentadecylic acid and in view of the fact that nothing but chemically pure reagents were used in the preparation of the tripentadecylin from purified pentadecylic acid.

Conclusions:

n-Pentadecylic acid has been synthesized starting with both commercial myristic acid and tetradecyl alcohol. Procedures have been tried coming through tetradecyl bromide and tetradecyl iodide as intermediaries. The results obtained indicate that the iodide procedure is more rapid and gives substantially better yields. A general idea of the net yield may be gained

from the figures: 220 grams of highly purified tetradecyl alcohol yielded 153 grams of highly purified pentadecylic acid by way of the iodide to the nitrile to the acid. This is a percentage net yield of 69.6 on the basis of the original amount of pure tetradecyl alcohol used; not based on theoretical yields. The net yield of tripentadecylin from the tetradecyl alcohol is 63.0%.

Acknowledgements:

Expression of appreciation is made to H. H. Baker of the Chemistry Department of the University of Kentucky where this synthesis was effected for his constructive criticism and timely suggestions; to Frank McGee, student at the University, for his assistance in attending the still during the several time-consuming fractionations; and to Professors F. E. Tuttle of the University of Kentucky and Edward Kremers of the University of Wisconsin for editorial assistance.

Bibliography

1. Gerard, E., Compt. rend. 111, 305 (1890); Ann. Chim. Phys. 6, 27, 549 (1892).

2. Shriner, R., Fulton, J., and Burks, D., Jr., J. Am. Chem. Soc. 55, 1494 (1933)

3. Reid, E. E., Cookerille, F. O., Meyer, J. B., Cox, H. N., Jr., and Ruhoff, J. R., Org. Syn. 15, 51 (1935)

4. Polk, M., and Maxson, R., Unpublished work, Dept. of Chem., Univ. of Kentucky, Lexington (1931)

5. Kastle, J. H., and Bullock, J. N., Am. Chem. J. 18, 105 (1896)

6. Reid, E. E., Ruhoff, J. R., and Burnett, R. E., Org. Syn. 15, 24 (1935)

7. Bleyberg, M., and Ulrich, H., B. 64B, 2504 (1931)

8. Majima, R., and Nakamura, I., B. 46, 4094 (1913)

9. Levene, P. A., and Taylor, F. A., J. B. C. 59, 905, 917 (1924)

10. Pooley, D. W., and Sumsin, H. B., J. Am. Chem. Soc. 57, 1078 (1935)

11. Levene, P. A., and West, C. J., J. B. C. 18, 467 (1914)

12. Eckert, A., and Halla, O., Monatshefte für Chemie 34, 1819 (1913)

13. Plimmer, R. H. A., Organic and Biochem., 5th ed., 33, Longmans, Green and Co., New York (1933)

14. Spaeth, C. P., and Hutchison, C. F., Ind. Eng. Chem., Anal. Ed. 8, 29 (1936)

15. Clarkson, C. E., and Malkin, T., J. Chem. Soc., 667 (1934)

CHAPTER IV

LICANIC ACID OF OITICICA FAT AND A STUDY OF ITS NUTRITIVE VALUE AND EFFICIENCY

LICANIC ACID OF OITICICA FAT AND A STUDY OF ITS NUTRITIVE VALUE AND EFFICIENCY*

Introduction:

Within the past few years considerable attention has been devoted to a newly discovered oil bearing the native name "Oiticica" or "Oilizika" extracted on a commercial scale from the nuts of a Northeast Brazilian tree, "Licania rigida" (Bentham) of the Rosaceae family (12, 18). The interest in this oil has centered mainly in the paint and varnish industry where it is used both as a substitute and as a diluent for tung oil and, quite effectively, in the phenolic resin-oiticica oil varnishes to which it gives greater durability than is possessed by similar products made with tung oil. According to the Chemical Division of the Commerce Department oiticica oil is considered one of the leading drying oils and is finding increased use as is indicated by imports from Brazil which during the first five months of the current year (1939) amounted to 5,328,500 pounds valued at more than $400,000, more than was imported during the whole of 1938. However, oiticica fat is of special interest chemically; its nature is unusual due to its exceptionally high content of glycerides of one acid, a new, highly unsaturated ketone fatty acid recently referred to as "licanic acid".

In order to avoid confusion in terminology it is necessary at once to point out the difference between oiticica "fat" and oiticica "oil". As generally used the term "fat" is applied to the material obtained by pressing or by solvent extraction of the kernels of Licania rigida. This material is solid or semi-solid at ordinary temperatures. The oiticica "oil" (a liquid) is the so-called "polymerized" product of commerce obtained by heat treatment of the oiticica fat. As Brown and Farmer (7, 8)

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have recently shown, commercial oiticica fat normally contains alpha-licanic acid, while the liquid (polymerized) oil, and the material obtained from old nuts, Machado (36), contains predominately the stereoisomeric beta-licanic acid. Oiticica fat has already been subjected to some chemical investigation and it is proposed to continue research on one of its principal components, alpha-licanic acid.

Since an exhaustive study of the chemical literature concerning Licania rigida was made (see Bibliography) before beginning actual research, one of the objectives of this paper will be the presentation of a brief survey of the literature on oiticica. The problem of the nutritive value of licanic acid was suggested by Alfred Steinock (Devoe and Raynolds Co.) who kindly supplied for investigation enough of the acid, isolated and carefully protected from oxidation by the Brown and Farmer method (7). A neutralization equivalent of 295.8 obtained in our laboratory was practically identical with the theoretical molecular weight of licanic acid, 295.2.

Concerning Previous Work on Oiticica and Licanic Acid:

The earliest chemical investigation of oiticica fat from a nut oilseed from Brazil was probably made during the first months of 1917 by Bolton and Revis (4). From their constants (Table I, No. 1) little more could be inferred than that the fat was highly unsaturated. There seems to have been no further investigation of oiticica fat until 1929 when articles by Wilborn and Lewa (39), Anon. (2), and by Margaillan (37) appeared giving additional characteristics of the fat (Table I).

Van Loon and Steger (56, 57, 58) apparently were the first to study the fatty acid composition of the oiticica fat and they claimed that the high refractive index and high iodine number of the fat was due to the

TABLE 1 – CHARACTERISTICS OF OITICICA FAT

No.	Name	Date	M.P.	Oil nuts %	Density	Index Refr.	Sap. No.	I_2 No. Hanus	I_2 No. Wijs	I_2 No. True	Acid Val.	R.M. No.	Acetyl No.	Hexabrom. Test	Thiocyanogen No.	Unsap. Matter
1	Bolton and Revis (4)	1918			15.5° 0.9694		188.6	179.8								
2	Wilborn and Löwa (59)	1929				21° 1.5094	186.3	178	1525		3.0					0.91
3	Anon. (2)	1929		64.8	15° 0.9676	40° 1.506	188.5		1419		3.7					0.7
4	Margaillan (37)	1929	50°	60.0		50° 1.517			140							
5	Stock (48)	1931			15° 0.9697	40° 1.5057	192.8		1421		3.9					0.78
6	VanLoon (57)	1931			80° 0.9440	20° 1.5188	188.3			231	0.92	056				0.39
7	Gardner (12)	1934	50–62°	←(Constants of fatty acids) →					1506	218	185.3					
8	Kappelmeier (19,20)	1935			20° 0.9675	20° 1.5160	190.0		145		5		35	neg.		0.40
9	McKinney & Jamieson (34	1936				25° 1.5145	192.6			218					76.2	0.57
10	Machado (36)	1938		48		20.5 1.5158	187.7		1524		5.04		30			0.34

No.	I_2 No. Unsap.	Fatty Acid Sol.Pt.	Water In-Sol.Fat. Acids %	Sat.Fat. Acids % Bertram	Sat.Fat. Acid Glycerides %	Unsat. Acids %	Oleic Acid. %	Licanic Acid %	Licanic Glycerides %	Glycer. Residue %	Volat. Matter %	Hydroxy-Acids %
1												
2							5.7					
3		44.8–47.40										
4												
5												
6			93.85	11.42		82.43				4.23	1.53	
7												
8	111			11.40		82.4						
9				10.7	11.2		5.9	78.2	81.2		1.6	
10				5.2(stear.) 6.1(palm.)			4.21	75.4		6.33		2.4

presence of a geometrical isomer of elaeostearic acid belonging to a double conjugated system of double bonds. This acid they named "Cowpic" from the "Cowpia grandiflora" with which they associated their sample of citicica fat.

Apparently the early workers were much confused as to the correct identity of the tree from whose fruit the citicica fat was obtained. While it was commonly referred to as "Licania rigida" or "Cowpia grandiflora" and less frequently as "Hequilea tomentosa", "Pleragina umbrosissima", or "Arruda camara", finally it has been definitely classified as "Licania rigida" (7, 12). For botanical information concerning the tree and for a discussion of the commercial production of the oil, its properties and utilization in the manufacture of paint and varnish the reader is referred to Gardner's writings (12). Additional information concerning the commercial utilization of the oil may be obtained by consulting the bibliography of this paper with particular attention to the various trade journal headings.

What now appears to be the correct characterization of licanic acid, the chief component of citicica fat, was the work of Brown and Farmer (7), whose method of isolation of the alpha-licanic acid will be described.

It was the intention of these authors to investigate the highly unsaturated, so-called cowpic acid of the oil of Cowpia grandiflora, to which Van Loon and Steger had assigned the conjugated triene acid formula, $CH_3(CH_2)_3(CH{:}CH)_3(CH_2)_7CO_2H$. Van Loon and Steger had contended that their acid was a "cis-trans" form of the two well-known alpha- and beta- forms of elaeostearic acid and had based their deductions upon the reputed conversion of their cowpic acid into stearic acid which by catalytic hydrog-

ensation, followed by oxidation with ozone, they claimed, was converted into valeric acid and azelaic acid. However, Brown and Farmer did not obtain stearic acid in their reduction of licanic acid, but in its stead they obtained gamma-keto-stearic acid. On the other hand oxidation of licanic acid with potassium permanganate gave valeric and gamma-keto-azelaic acids; the latter yielding by oxidation with Beckmann's chromate mixture succinic and adipic acids. Brown and Farmer also compared their gamma-keto-stearic acid with a synthetic sample and were able to reduce the former with zinc amalgam and hydrochloric acid to stearic acid. With the chromate mixture they were able to oxidize the gamma-keto-stearic acid to succinic and myristic acids accompanied by a small amount of pentadecanoic acid. The authors believed their acid to be the same as the oxeopic acid of Van Loon and Steger but were unable to give it the same constitution. Instead they suggested the name "licanic acid" and proposed the formula, $C_{18}H_{28}O_3$, 4-keto-$\Delta^{9,11,13}$octodecatrienoic acid. Confirmation of the work of Brown and Farmer has been offered by Kappelmeier (19) and by Morrell and Davis (40), the latter also giving details for the preparation of alpha- and beta-licanic acids and advising extraction of the fat and its saponification in an inert atmosphere. The highest yield of licanic acid from oiticica fat which Brown and Farmer were able to obtain was 55 per cent. The yields obtained by Alfred Rheineck in preparing the acid for this investigation were 46-48 per cent. However, it will be observed in Table I that recently McKinney and Jamieson (34) and Machado (36) have reported, respectively, calculated values of 78.2 per cent and 78.4 per cent.

Preparation of licanic Acid by the Brown and Farmer Method as Used by Alfred Rheineck:

Each 100 grams of fat is saponified by the use of 30 grams of potassium hydroxide. The potassium hydroxide is first dissolved in a minimum amount of water, then diluted with 200 cc. of alcohol and added to the fat. After saponification in the usual manner the alcoholic solution of potassium salts made up to 500 cc. with cold water and further cooled in ice is cautiously acidified with 100 cc. of sulfuric acid sp. gr. 1.3476, and extracted with 150 cc. of ether. The ethereal solution of fatty acids thus obtained is washed with water, dried with anhydrous sodium sulfate (below 20° C.) and gently warmed to remove the bulk of solvent, the last traces of which are removed under reduced pressure without further heating. The saponification product which sets on cooling to a pale waxy solid is immediatly crystallized from warm petroleum ether (B.P. 60-80° C.) and the highly unsaturated component, the compound sought, is collected by filtering off the crystals which had separated on cooling. The crystals melt at 73-74° C. and are raised to 74-75° C. by repeated recrystallizations from petroleum ether. The crystals rapidly absorb oxygen on exposure to air yielding a sticky liquid; consequently it is highly necessary that they be protected from air which is done by either preserving the acid in petroleum ether until time for using or by storing the acid under carbon dioxide.

Licanic acid is thought to be the first naturally occurring unsaturated ketone fatty acid to be described in the literature. It is of special interest biologically since it offers for the first time direct indication of the occurrence of biological oxidation at the gamma-position of fatty acid side chains. It is also interesting to note that the alpha- form may be converted into the geometrically isomeric beta-form by irradiation in the presence of a trace of iodine or sulfur (8).

Experimental Part

The Nutritive Value and Efficiency of Linoleic Acid:

As the most rational method of approach to this study it was thought advisable to determine by experiment if possible to what extent linoleic acid could replace an ordinarily high quality fat such as butter in the normal diet. Since it had been repeatedly observed in our laboratory (23) that rats fed on whole cow's milk, mineralized with iron, copper, and manganese, not only made excellent growth, but used the milk solids as efficiently as the solids of a good stock ration, mineralized skim milk was selected as the basal for a fat-free ration.

For this investigation young albino rats 19-21 days old were used. The rats were placed in separate cages, fed morning and evening, weighed at regular intervals at the same hour of the day, and upon each rat a consumption record was maintained. To the skim milk required in making up the diets sufficient supplementary ferric pyrophosphate, copper sulfate, and manganous sulfate were added to insure each 100 cc. a content of 1.5 mg. of elemental iron, 0.15 mg. of elemental copper, and 0.15 mg. of elemental manganese. Also, sufficient beta-carotene was added to the skim milk so that each rat received 5 micrograms per day. Each rat was submitted to irradiation for a period not exceeding ten minutes daily.

The method adopted for addition of butter fat to the mineralized fresh skim milk consisted in melting the butter carefully at low heat, weighing out the desired quantity and homogenizing it into the measured amount of warmed skim milk by the use of a small hand homogenizer. In

In preparing the licanic acid ration care had to be exercised in order to prevent oxidation. Portions of the acid as needed were taken rapidly from the original container which was then revealed in an atmosphere of carbon dioxide. The relatively high melting point of 74-75° C. of licanic acid as compared with butter fat, together with its extreme susceptibility to oxidation, made it seem unwise to subject it to any heat operation before adding it to the skim milk. However, due to its soft fat-like texture no difficulty was experienced in directly adding the licanic acid to the warm skim milk and sending it through the homogenizer in the same manner as in emulsifying any two liquid phases. Furthermore, the licanic acid thus emulsified appeared to have formed a more stable emulsion than the cream of the butter fat.

The feeding experiments may be divided into three groups, the second and third arising, as will be pointed out, from the findings in the first. Six rats were used in the first experiment; two were fed mineralized skim milk containing 4 per cent licanic acid; two were fed mineralized skim milk containing 4 per cent butter fat (the average amount present in whole milk) as a positive control; and two were fed unsupplemented mineralized skim milk as a negative control. Figure 1 shows the growth curves for these animals and Table for Figure 1[8] presents a summary of data obtained on each rat. At the end of one week Rat 4 had died and since Rats 3, 5, and 6 were on the verge of the same fate the experiment was discontinued. No attempt had been made in any case to restrict the daily food allowance of the animals. It was expected that Rats 5 and 6 would die since from past experience we knew that a rat must have attained an age of six weeks or more before it is possible to make a change to a fat depleted diet with

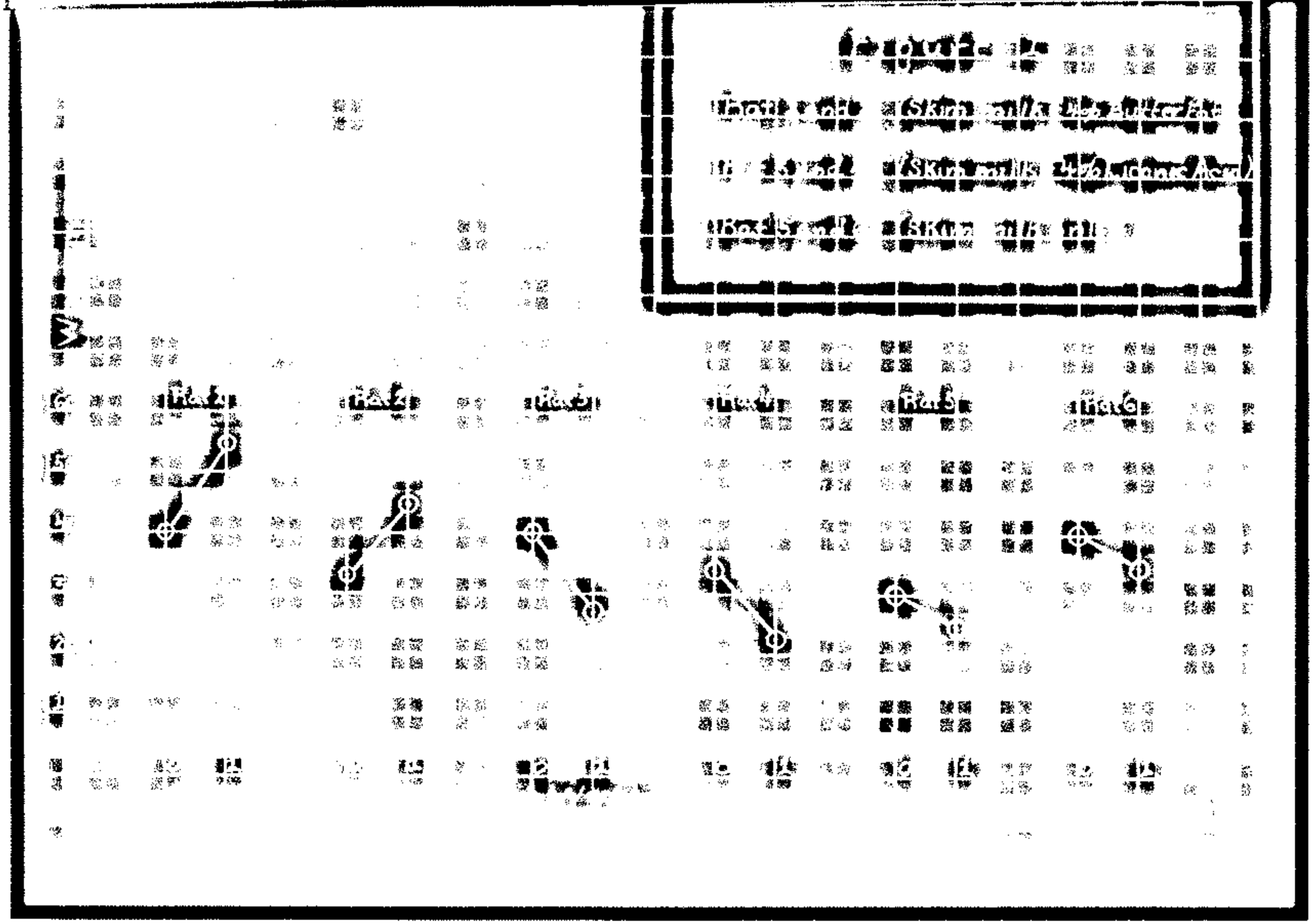


TABLE FOR FIGURE 1

	Mineralized skim milk plus 4% butter fat		Mineralized skim milk plus 4% linoleic acid		Mineralized skim milk only	
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6
Avg. consumed daily, cc.	29	26	16	16	43	43
Avg. total solids daily, Gm.	3.57	3.38	2.08	2.08	3.87	3.87
Avg. gain daily, Gm.	2.1	1.9	-1.7	-1.6	-0.7	-0.7
Avg. gain daily, avg. for each two rats	2.0		-1.65		-0.7	
Gm. milk solids to produce 1 gram gain in weight	1.85		negative		negative	

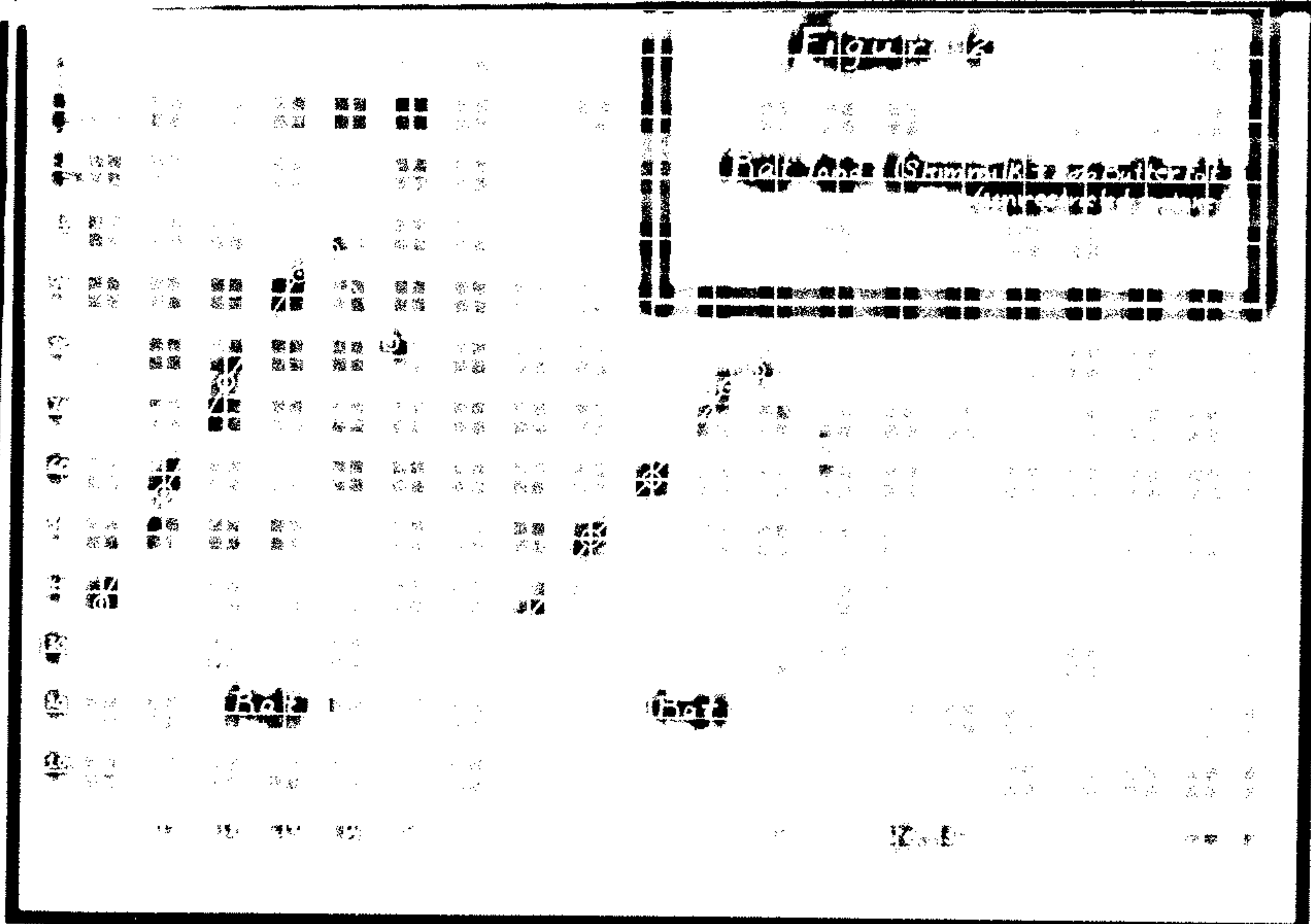
assurance that the animal will survive for any length of time. Although the total solids consumed daily by Rats 3 and 4 on the licanic acid was only little more than half of that consumed by Rats 1 and 2 on butter fat, there still appeared to be sufficient justification for holding the view that licanic acid might possess some toxicity. Attention should be called to the apparently good start toward normal growth of Rats 1 and 2, although their increase in weight of 1 gram for every 1.65 grams of milk solids ingested represents a more rapid development than that of 1 gram gain in weight for every 2.25 grams of milk solids as previously reported by this laboratory (25) for slightly older young rats. However, rats reported in this previous paper had reached a weight of 60 grams compared with our average weight of 35 grams at the beginning of the experiment. Young rats would naturally be expected to show more rapid development and the figure quoted above from previous data represents that accumulated over a much longer growth period in which the animals had shown an average weight increase of 140 grams, i.e., from 60 to 200 grams.

It was apparent from our experience with the rats in the first experiment that there did exist some aversion to the taste of licanic acid which accounted for the fact that the voluntary consumption of the licanic acid ration was not as high as that of the butter fat ration. To avoid such an aversion and to further test the nutritional value of the licanic acid the following experiment was carried out: four rats were fed diets unrestricted as to amount consumed daily. Rats 7 and 8 were fed mineralized skim milk with 2 percent added butter fat; Rats 9 and 10 were fed mineralized skim milk with 2 per cent added butter and 2 per cent added licanic acid in order to determine whether licanic acid had any nutritional value.

On inspection of the growth curves in Figure 2 it will be observed that Rats 7 and 8 on the 2 per cent butter supplement showed favorable development, almost attaining the normal growth gain of about three grams daily. Comparing curves in Figure 2 with those in Figure 3, it is apparent that animals on 2 per cent butter fat supplement grew more rapidly than those on the 2 per cent butter fat plus 2 per cent linoleic acid. However, in "Table for Figures 2 and 3" it will be noted that Rats 9 and 10 required 3.97 grams of total solids to produce 1 gram gain in weight compared with 2.45 grams of total solids to produce 1 gram gain in weight for Rats 7 and 8. These data seem to indicate that butter fat, therefore, is superior to linoleic acid in nutrient growth principle.

There still remained the point to be settled as to whether linoleic acid had any measurable nutrient value. Accordingly, experiment three, an attempt to decide this issue, was begun as follows: Rats 15, 16, 17, and 18 were fed restricted diets, that is, restricted daily to the amount of total solids which Rats 9 and 10, those on 2 per cent butter fat plus 2 per cent linoleic acid supplement, had voluntarily selected. The experiment was in every manner a duplicate of the procedure used with Rats 9 and 10. The diet of Rats 15 and 16 consisted of the restricted amounts of mineralized skim milk with 2 per cent butter fat supplement; the diet of Rats 17 and 18 consisted of the restricted daily amounts of mineralized skim milk with 4 per cent butter fat supplement. The duration of the experiment was 26 days the same as that for Rats 9 and 10.

It seems reasonable to believe that if linoleic acid had possessed the same degree of nutrient efficiency as butter fat it should have given the same performance as the 4 per cent butter supplement. Study of the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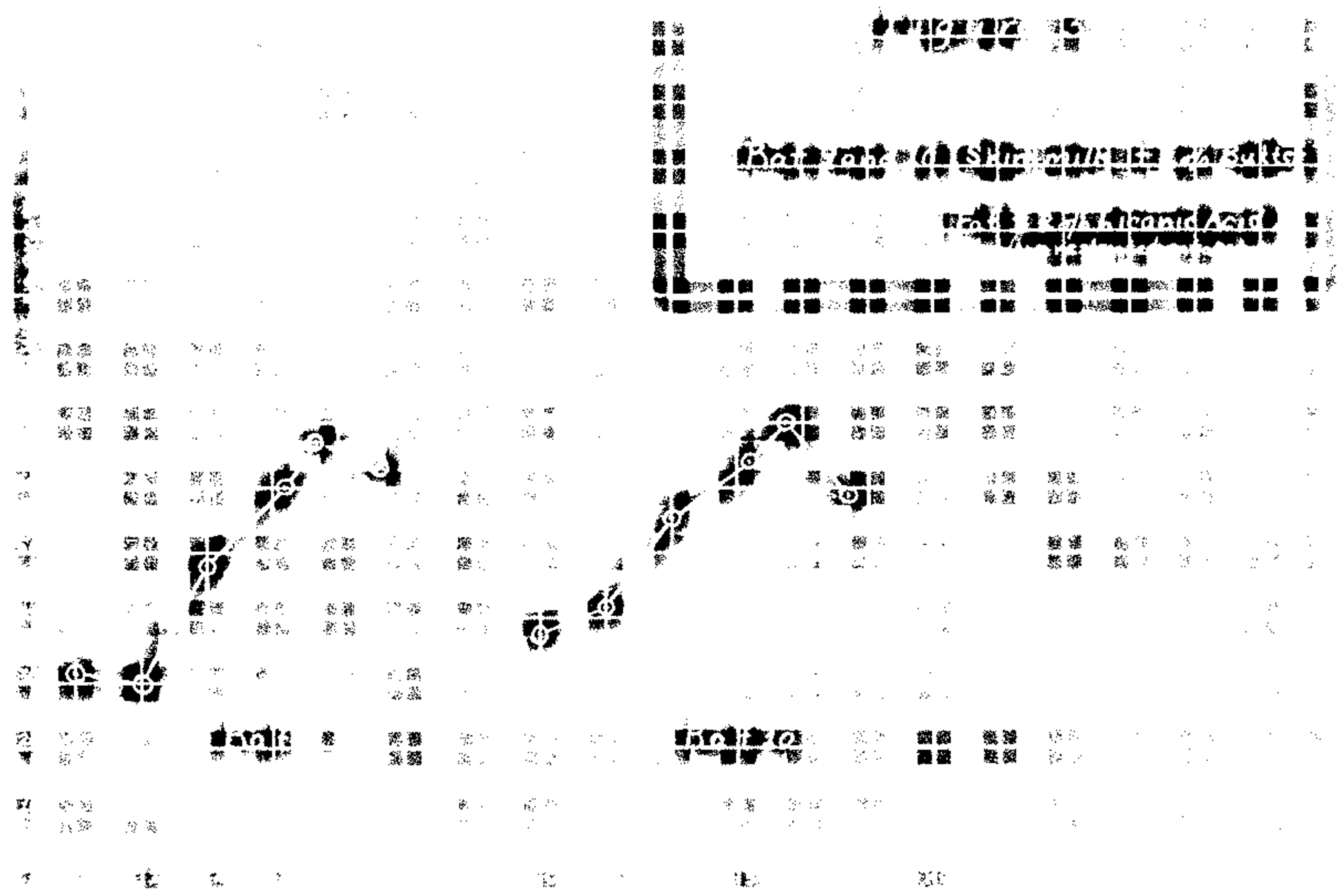

TABLE FOR FIGURES 2 AND 3

	Mineralized skim milk plus 2% butter fat		Mineralized skim milk plus 2 % butter fat plus 2% linoleic acid	
	Rat 7	Rat 8	Rat 9	Rat 10
Avg. consumed daily, cc.	49	40	26	35
Avg. total solids daily, Gm.	5.39	4.40	5.36	4.35
Avg. gain daily, Gm.	2.3	1.6	1.0	1.2
Avg. gain daily, avg. for each 2 rats, Gm.	2.0		1.1	
Gm. milk solids to produce 1 Gm. gain in wt.	2.45		5.97	

records of Rats 17 and 18, those on the restricted 4 per cent butter fat supplement, shows their approximately normal growth with 2.57 grams of milk solids consumed to produce 1 gram gain in weight. This same figure for Rats 9 and 10 on the butter fat plus licanic acid supplement, it will be recalled, was 5.97 grams of total solids required to produce 1 gram gain in weight. These data indicate that licanic acid was greatly inferior to butter fat in nutrient value. Furthermore, when we consider the growth of Rats 15 and 16, those on the restricted 2 per cent butter supplemented diet, it is to be noted that, from the figure of 3.39 grams of total solids required to produce 1 gram gain in weight, these animals on the restricted 2 per cent butter supplemented diet actually out-grew those receiving the additional licanic acid. It would appear then that the licanic acid not only was worthless in so far as possessing nutrient value was concerned but actually under the conditions of this experiment proved slightly deleterious to the growth of the rat. This is indeed a difficult situation to explain in view of the fatty acid constitution of licanic acid and the fact that it should function just like any other unsaturated fatty acid in supplying the animal with energy.

Mention is made in passing of Rats 11-14 which were started on the same diet as used for Rats 15-18. The former were animals averaging around 60 grams at the beginning of the experiment and were too large to show the desired response. In fact they were discontinued at the end of two weeks, after considerable loss in weight, in favor of Rats 15-18 of the proper weight.

[illegible]

TABLE FOR FIGURES 4 AND 5

	Mineralized skim milk plus 2% butter fat (restricted intake)		Mineralized skim milk plus 4% butter fat (restricted intake)	
	Rat 15	Rat 16	Rat 17	Rat 18
Avg. consumed daily, cc.	51	51	51	51
Avg. total solids daily, Gm.	3.41	3.41	4.05	4.05
Avg. gain daily, Gm.	0.9	1.0	1.9	1.8
Avg. gain daily, avg. for each 2 rats, Gm.	0.95		1.7	
Gm. milk solids to produce 1 Gm. gain in wt.	3.59		2.37	

Relation of Linoleic Acid to the Utilization of Lactose in Milk

It has previously been shown in this laboratory (45) that rats placed on a mineralized whole milk diet made very efficient utilization of all the milk sugar, but that this was not the case with animals placed on a mineralized skim milk diet, and that in the latter case, after feeding a few days, sugar, which proved to be galactose, was readily detected in the urine. Further, it was found that the feeding of certain fats, such as butter, corn oil, lard, linseed oil, palmitic acid, oleic acid, etc., in quantities of 3-4 per cent as supplements to mineralized skim milk, prevented this loss in the urine but that lower organic acids as butyric, lactic, caproic, etc., did not prevent the loss.

Since a number of other fats were being tested for their effect on the utilization of lactose, it was thought advisable to make similar use of linoleic acid. Accordingly, as previously arranged Rats 9 and 10, those on 8 per cent butter fat plus 2 per cent linoleic acid supplement, and Rats 7 and 8, those on 2 per cent butter fat supplement, were subjected regularly to confinement in metabolism cages and to urine collections. The data secured, together with the results of the calculations made to determine the percentage of galactose, the percentage of ingested galactose lost, and the percentage of lactose lost, in the urine, may be found in Table II. At the end of the 26th day the four rats were changed to mineralized skim milk in order to determine if those not voiding sugar in the urine would void it. In each case the animals did void sugar. The dotted lines on the growth curves of Figures 2 and 3 show the corresponding loss in weight accompanying the change to fat-free diets.

The results of this work prove that linoleic acid acts as a prophy-

TABLE XI

Date	Milk cc.	Urine cc.	Galactose in urine %	Ingested lactose lost in urine %	Ingested galactose lost in urine %
Rat 7					
Mineralized Skim Milk Plus 2 Per Cent Butter Fat (began Aug. 18, 1938)					
Aug. 30	80	36	0.78	10.8	21.6
Sept. 13	66	28	0.33	1.8	3.6
Sept. 13th changed to skim milk					
Sept. 20	70	54	0.55	8.7	17.4
Rat 8					
Mineralized Skim Milk Plus 2 Per Cent Butter Fat (began Aug. 18, 1938)					
Aug. 30	40	18	0.49	4.8	9.0
Sept. 13	35	38	0.45	8.9	17.9
Sept. 13th changed to skim milk					
Sept. 20	80	40	0.58	9.8	17.6
Rat 9					
Mineralized Skim Milk Plus 2 Per Cent Linoleic Acid Plus 2 Per Cent Butter Fat					
Aug. 30	51	31	None	None	None
Sept. 1	53	30	None	None	None
Sept. 13	35	14	None	None	None
Sept. 13th changed to skim milk					
Sept. 20	70	55	0.65	10.3	20.6
Rat 10					
Mineralized Skim Milk Plus 2 Per Cent Linoleic Acid Plus 2 Per Cent Butter Fat					
Aug. 30	39	27	None	None	None
Sept. 1	39	30	None	None	None
Sept. 13	70	36	None	None	None
Sept. 13th changed to skim milk					
Sept. 20	80	51	0.37	8.5	16.6

lactic in preventing elimination of sugar in the urine, i.e., that the ingested lactose in milk was completely utilized. This is a positive action particularily in view of the fact that the rats fed the mineralized skim milk plus the 2 per cent butter fat supplement always showed a slight sugar loss in the urine and on several occasions a considerable amount. This is an especially interesting observation in consideration of the facts already presented above that lissmic acid possessed no apparent energy value and actually appeared to have a deleterious effect on the growth of the rat.

Summary:

1. A survey of the literature on lissmia rigida has been made and the more important chemical contributions reviewed. Attempt has been made to include a complete bibliography with this paper.

2. It appears from the experimental evidence obtained in this work that lissmic acid possesses no apparent energy value and that under the conditions described is slightly deleterious to the growth of young rats.

3. Lissmic acid has some efficiency in the animal. It is capable, in the rat, of assisting in the utilization of lactose in milk as indicated by its prophylactic action in preventing sugar loss via the urine.

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BIBLIOGRAPHY

The bibliography which is presented with this paper has been obtained by exhaustive survey, under the headings "Oiticica" and "Licania rigida" of the following abstract journals and journals with abstract sections:

Berichte der Deutschen Chemischen Gesellschaft, Referate, (1880-1896)

Chemical Abstracts, (1907 to date)

Chemisches Zentralblatt, (1830 to date)

Jahresbericht der Pharmacie, (1841 to date)

Journal of the American Pharmaceutical Association, Abstracts, (1851 to date)

Journal of the Chemical Society, Abstracts, (1871 to date)

Quarterly Journal Pharmacy and Pharmacology, (Year-book of Pharmacy), (1865 to date)

---

1. Alvarado, A. M., U.S Patent No. 2,112,584, March 29, 1938, (to E. I. du Pont de Nemours and Company)

2. Anon., Bull. Imp. Inst. 27, 277 (1929)

3. Berlinger, E. F. T., U.S. Patent No. 2,121,580, June 21, 1938, (to E. I. du Pont de Nemours and Company)

4. Bolton, H. R., and Revis, C., Analyst 43, 251 (1918)

5. Bontenbal, J., Chem. Weekblad 33, 717 (1936)

6. Brooks, H. F. D., Paint Mfg. 6, 36 (February, 1936)

7. Brown, W. B., and Farmer, E. H., Biochem. J. 29, 651 (1935)

8. Brown, W. B., and Farmer, E. H., J. Chem. Soc. 1652 (1935)

9. Derpf, Karl, Farbe u. Lack 550 (1935).

10. Eisenschiml, Otto and Gerald, Paint Var. Prod. Mgr. 18, 18 (May, 1937)

11. Eisenstein, Alfred, Öle, Fette, Wachse 15, 12 (1936)

12. Gardner, H. A., Natl. Paint Var. Lacq. Assoc., Circ.470, 215 (1954); Ibid, Circ. 481, 158 (1955)

13. Gardner, H. A., Paint Var. Prod. Mgr. 14, No.6, 16, 18, 30 (1956)

14. Hausman, Margaret, Am. Ink Maker 15, No.1, 16 (1957)

15. Heldt, Chas., Drugs, Oils, Paints, 51, 240 (1936); Ibid, 51, 64, 191, 194, 196, 198 (1936); Ibid, 52, 516 (1937)

16. Heldt, Chas., Natl. Paint Bull. 1, 10, 18 (March, 1937)

17. Heldt, Chas., Rev. brasil. chim. (São Paulo) 3, 22 (1937)

18. Hooker, W. J., Hooker's J. Bot. 2, 215, 220 (1840)

19. Kappelmeier, C. P. A., Fettchem. Umschau 42, 145 (1935); Verfkronick 8, 279 (1935)

20. Kappelmeier, C. P. A., Drugs, Oils, Paints 51, 257 (1936)

21. Kappelmeier, C. P. A., Paint Var. Prod. Mgr. 14, 7, 10 (June, 1936)

22. Kaufmann, H. P., and Ostringhaus, H. G., Ber. 69B, 2679 (1936)

23. Kaufmann, H. P., and Baltes, J., Ber. 70B, 2557 (1937); Fette u. Seifen 44, 289 (1937)

24. Kaufmann, H. P., and Baltes, J., Paint Var. Prod. Mgr. 16, 52, 54 (April, 1937)

25. Kemmerer, A. R., Hlvehjem, C. A., Hart, E. B., and Fargo, J. M., Am. J. Physiol. 102, 319 (1932)

26. Kemmer, H., Golden Gate Paint Var. Prod. Club, Paint, Oil and Chem. Rev. 97, No. 25, 70; Paint, Oil, Drug Reptr. 128, No. 22, 54 (1935)

27. Kemmer, H., Balt. Paint Var. Prod. Club, Paint, Oil and Chem. Rev. 97, No. 25, 68; Oil, Paint, Drug Reptr. 128, No. 22, 53 (1935)

28. Kemmer, H., Louisville Paint Var. Prod. Club, Paint, Oil and Chem. Rev. 97, No. 25, 74; Oil, Paint, Drug Reptr. 128, No. 22, 54 (1935)

29. Kemmer, H., Farbe u. Lack 545 (1935)

30. Kittredge, H. G., U.S. Patent No. 2,111,427, March 15, 1938 (to Kay and Ess Chem. Corp.)

31. Kleve, R., Paint Technol. 1, 14, 54 (February, 1936)

32. Little, A. D., Ins., Drugs, Oils, Paints 51, 247 (1936)

33. Lüde, R., Fettchem. Umschau 42, 4 (1935)

54. McKinney, R. S., and Jamieson, G. S., Oil and Soap 13, No. 1, 10 (1936)

55. Machado, A., Peixoto, A., Rev. chim. ind. (Rio de Janeiro) 6, 464 (1937)

56. Machado, A., Rev. soc. brasil. quim. 7, 75, (1938)

57. Margaillan, L., Ann. musée colonial Marseille 7, No. 3, 5 (1939)

58. Meinel, Karl, Fette u. Seifen 43, 250 (1936)

59. Meyer, F., Farbe u. Lack 545 (1937)

60. Morrell, R. S., and Davis, W. R., J. Chem. Soc. 1481 (1936)

61. Morrell, R. S., and Davis, W. R., Paint Var. Prod. Mgr. 15, 15, 18,20 (December, 1936)

62. Morrell, R. S., and Davis, W. R., J. Oil Col. Chem. Assoc. 19, 264 (1936)

63. Panelsik, H., Öle, Fette, Wachse 7, 7 (1937)

64. Rinse, J., and Nichols, M. S., Ind. Eng. Chem. 29, 1149 (1937)

65. Schantz, E. J., Elvehjem, C. A., and Hart, E. B., J. B. C. 122, No. 2,381 (1938)

66. Scofield, F., Natl. Paint Var. Lacq. Assoc., Circ. 519, 271 (1936)

67. Sörenson, S. O., Schumann, C. J., Schumann, J. H., and Mathiella, J., Ind. Eng. Chem. 30, 211 (1938)

68. Stock, E., Seifen.-Ztg. 58, 387 (1931)

69. Stock, E., Farben. Chem. 7, 45 (1936); Ibid 7, 365 (1936)

70. Stock, E., Paint Var. Prod. Mgr., 14, No. 14 (June, 1936)

71. Stock, E., Farbe u. Lack 449, 465, 545 (1937); Farben-Ztg. 42, 889 (1937)

72. Stewart, J. R., Drugs, Oils, Paints 52, 404 (1937)

73. Sverige, G., Paint. Pig. Var. 14, 250 (1937)

74. Sward, G. S., Am. Paint Var. Mfrs. Assoc., Circ. 377, 120 (1951); Rec. trav. chim. 50, 936 (1951)

75. Van Houtteroth, A. W., Natl. Paint Var. Assoc., Circ. 512, 228 (Aug., 1936)

76. Van Loon, J., and Steger, A., Chem. Umschau Fette, Öle, Wachse, Harze 37, 357 (1930)

77. Van Loon, J., Verfkroniek 4, No. 10, 7 (1931)

78. Van Loon, J., and Steger, A., Rec. Trav. Chim. Pays-Bas, 50, 936 (1931)

79. Wilborn, F., and Löwa, A., Farben-Ztg. 35, 388 (1929)

CHAPTER V

INVESTIGATIONS IN TERPENE CHEMISTRY

I. NITROLAMINES OF 1-CHLORO-2-NITROSO-$\Delta^{8(9)}$-MENTHENE

A. Limonene Nitrosochloride and Amino Acetic Acid

The behavior of compounds of the types nitrosochlorides, nitrosonitrite, and nitrosonitrate towards a variety of basic substances has been studied and the following reactions have been observed:

I. $C_{10}H_{16}\begin{cases}NO\\Cl\end{cases} \xrightarrow{-HCl} C_{10}H_{15}NO \longrightarrow C_{10}H_{14}NOH$

"Nitroso" terpene — "Isonitroso" terpene (or oxime)

II. $C_{10}H_{16}\begin{cases}NO\\Cl\end{cases} + HNR^1R^2 \longrightarrow C_{10}H_{16}\begin{cases}NO\\NR^1R^2\end{cases}$

Nitrolamine

III. $C_{10}H_{16}\begin{cases}NO\\Cl\end{cases} \xrightarrow{-NOCl} C_{10}H_{16}$

Regenerated terpene

For a better understanding of these reactions one or more specific illustrations are given:

Ad. I. In 1877 Tilden and Shenstone (1) not only prepared the nitrosochlorides of pinene and limonene, but showed that when these compounds are acted upon by alcoholic potash and, as in the case of limonene nitrosochloride, by heat, hydrogen chloride is split off and compounds which they called nitroso-terpenes resulted, each nitroso-compound being named for the volatile oil from which it was prepared, e.g., nitrosoaustralene from australene of ordinary American dextrogyrate turpentine oil. In 1885 Goldschmidt and Zürrer (2) showed that the so-called nitroso- limonene was not a nitroso-compound, but was an isonitroso-compound, viz., the oxime of

carvone. For a time Wallach (3) was inclined to regard the so-called nitroso-pinene as having the constitution of a true nitroso-compound, especially in view of the fact that nitroso-pinene had failed to submit to hydrolysis by means of sulfuric acid as did nitroso-limonene in its conversion to carvone by this procedure. However, Urban and Kremers (4) were able to show that nitroso-pinene must be regarded as an isonitroso-compound since it could be hydrolysed provided hydrochloric acid was used as the hydrolytic agent. This hydrolysis was accompanied by the formation of a ketone, $C_{10}H_{14}O$ (5,6), not of the bicyclic pinene type, but of the monocyclic type corresponding to carvacrol, $C_{10}H_{13}OH$, with which it is isomeric, the latter being an enolic form of the ketone. As concluded by Kremers, et al. (7) then "In as much as carvone can be converted quantitatively into carvacrol by means of hydrogen chloride, this intramolecular rearrangement is not at all surprising. Hence, so-called nitroso-pinene may also be regarded as an isonitroso-compound." And as demonstrated by other investigators this form of intramolecular rearrangement is not uncommon, e.g., Schmidt (8) has called attention to a similar relation between amylene nitrosate and the oxime:

$C_5H_{10}\begin{cases}-NO\\-ONO_2\end{cases}$ $C_5H_9\begin{cases}=NOH\\-ONO_2\end{cases}$

Amylene nitrosate (Trimethylethylnitrosate) — Amylene nitrate oxime

As will be shown later an amphoteric organic compound, i.e., aminoacetic acid, may also bring about this splitting off of hydrogen chloride although the process is by no means quantitative since reaction II takes place simultaneously with reaction I. In the case of menthone nitrosochloride and piperidine (9) the reaction is also a mixed one.

Ad. II. After Wallach had improved greatly Tilden's method for the preparation of nitrosochlorides, these, and also nitrosites, $C_{10}H_{16}\begin{cases}-NO\\-ONO\end{cases}$, and nitrosates, $C_{10}H_{16}\begin{cases}-NO\\-ONO_2\end{cases}$, became readily available for various experimental studies principal among which were the reactions of nitrosochlorides with primary and secondary organic bases to give well-defined crystalline "nitrolamines", which have served for a ready identification and characterization of the underlying terpenes. Using alcoholic ammonia simple nitrolamines of the type, $C_{10}H_{15}\begin{cases}=NOH\\-NH_2\end{cases}$, were obtained (10). The aliphatic bases methylamine, ethylamine, amylamine, dimethylamine, and diethylamine were used to produce corresponding nitrolamines,

$C_{10}H_{15}\begin{cases}=NOH\\-NHCH_3\end{cases}$, $C_{10}H_{15}\begin{cases}=NOH\\-NHC_2H_5\end{cases}$, $C_{10}H_{15}\begin{cases}=NOH\\-NHC_5H_{11}\end{cases}$, $C_{10}H_{15}\begin{cases}=NOH\\-N(CH_3)_2\end{cases}$,

$C_{10}H_{15}\begin{cases}=NOH\\-N(C_2H_5)_2\end{cases}$, and such aromatic bases as aniline, benzylamine, piperidine (10,11), and morpholine (33) were employed to produce corresponding aromatic nitrolamines,

$C_{10}H_{15}\begin{cases}=NOH\\-NHC_6H_5\end{cases}$, $C_{10}H_{15}\begin{cases}=NOH\\-NHC_7H_7\end{cases}$, $C_{10}H_{15}\begin{cases}=NOH\\-NC_5H_{10}\end{cases}$, $C_{10}H_{15}\begin{cases}=NOH\\-NC_4H_8O\end{cases}$.

Ad. III. Although aniline reacts with limonene nitrosochloride to form limonene nitrolanilide, when allowed to react with pinene nitrosochloride, the hydrocarbon is regenerated (12) according to the reaction:

$$C_{10}H_{16}NOCl + 2C_6H_5NH_2 \longrightarrow C_{10}H_{16} + C_6H_5N{:}NC_6H_4NH_2 + HCl + H_2O .$$

Having both limonene nitrosochloride and aminoacetic acid available, it seemed desirable to find out what action an amphoteric base of this nature might exert on the former compound.

Assuming that the reaction might take place in accordance with reaction II, the one most common for organic bases, two molecules of aminoacetic acid were employed for each molecule of nitrosochloride using the conventional method for the preparation of nitrolamines with the following reaction in view:

$$C_{10}H_{16}\begin{cases}NO\\Cl\end{cases} + 2CH_2NH_2COOH \longrightarrow C_{10}H_{16}\begin{cases}NO\\NHCH_2COOH\end{cases} + \underset{COOH}{CH_2NH_2}.HCl .$$

Several preliminary experiments, however, indicated that this reaction, if taking place at all, did not run its course quantitatively, but that aminoacetic acid seemed to react like an inorganic base splitting off hydrogen chloride, viz.,

$$(A)\ C_{10}H_{16}\begin{cases}NO\\Cl\end{cases} + CH_2NH_2COOH \longrightarrow C_{10}H_{15}NO + \underset{COOH}{CH_2NH_2}.HCl$$

$$(B)\ C_{10}H_{15}NO \longrightarrow C_{10}H_{14}NOH .$$

The correctness of this assumption was demonstrated by the isolation of carvoxime in crystalline form. That still another reaction took place was indicated by the fact that not all of the reaction product could be isolated as glycine hydrochloride and carvoxime, but that an oily product also resulted.

Since the results obtained in these preliminary experiments indicated that no appreciable quantity of a derivative of aminoacetic acid and limonene nitrosochloride could be expected from the use of two moles of the former to one of the latter, it was thought advisable to try to effect condensation by use of "<u>equimolecular</u>" quantities of the reactants, hoping that in addition to reactions (A) and (B) the following reaction (C) might take place:

$$(c)\quad C_{10}H_{16}\begin{cases}NO\\Cl\end{cases} + CH_2NH_2COOH \longrightarrow C_{10}H_{16}\begin{cases}NO\\NH.HCl.CH_2COOH\end{cases}$$

Exhaustive studies of the reaction products obtained from several independent experiments where "equimolecular" quantities of reactants were used resulted in the isolation and characterization of the compound sought in reaction (c), l-aminoacetic-acid-hydrochloride-2-oxime-$\Delta^{8(9)}$-menthene (limonene-nitrosaminoacetic-acid-hydrochloride). Examination of the volatile oil obtained by the steam distillation of the reaction products showed the presence, not only of a relatively large amount of carvoxime, but also a large quantity of carvone. A fourth equation,

$$(d)\quad \underset{\text{Carvoxime}}{C_{10}H_{14}NOH} + H_2O \longrightarrow \underset{\text{Carvone}}{C_{10}H_{14}O} + \underset{\text{Hydroxylamine}}{NH_2OH}$$

is offered to explain the formation of carvone as a result of the hydrolysis of carvoxime, the reverse of which is the well-known method for the preparation of carvoxime (13). This reaction also accounts for the presence of the oily product received in the preliminary experiments. That this is not the complete picture of what takes place between aminoacetic acid and limonene nitrosochloride is shown by the fact that there yet remains another substance from the volatile oil to be characterized which at the present writing is under investigation. Sufficient evidence has not been obtained, as yet, to state that this substance is definitely carvacrol, but there is some indication that it may be this compound. Carvone is known to yield carvacrol with hydrogen chloride (14), especially in the presence of catalysts, an isomerization which Schweizer (15) demonstrated at an early date. Such a rearrangement of carvone into

carvacrol, its enolic form, may therefore be rationally anticipated.

Experimental Part

Run I

Small quantities of reagents were selected for this first, a trial run, using five grams of limonene nitrosochloride. The limonene nitrosochloride and the aminoacetic acid were dissolved in sufficient 60 percent alcohol to maintain solution at the temperature of the steam-bath and refluxed for one and one-half hours. A crystalline solid, melting point 175-179° C., amounting to 5.8 grams was obtained by cooling the reaction mixture in an ice-bath, filtering, washing with several portions of cold 95 percent alcohol, and drying. On recrystallization from 50 percent alcohol this solid gave a melting point of 177.2-177.9° C. Aminoacetic acid hydrochloride prepared from a portion of the same aminoacetic acid as used in the reaction above gave, after several recrystallizations from 50 percent alcohol, a melting point of 183.7-183.9° C. Both these melting points were run simultaneously. Another sample of aminoacetic acid hydrochloride prepared from analytically pure reagents and recrystallized seven times gave a melting point of 182.4-183.2° C. Heilbron (16) gives 185° C. Attempts to determine the neutralization equivalent of this solid and also that of the prepared aminoacetic acid hydrochloride were unsuccessful yielding values in the neighborhood of 200 as compared with 111.6, the theoretical molecular weight of aminoacetic acid hydrochloride. The use of the "formol" titration would undoubtedly have eliminated this difficulty by protecting the amino group during the titration. The solid obtained from the reaction and the prepared aminoacetic acid hydrochloride both gave positive chloride tests with silver nitrate reagent and positive ferric chloride reagent tests for aminoacetic acid. The above information seems to indicate

this isolated solid from Run I to be nothing more than aminoacetic acid recovered as the hydrochloride.

The combined filtrates from Run I gave upon spontaneous evaporation about 3 cc. of an oil from which on standing there separated about one-half gram of crystals of melting point 145-160° C. In view of information gained by repeated runs, this material although not further characterized, probably consisted of a mixture of more of the aminoacetic acid hydrochloride and possibly some of a new derivative similar to what would be a hydrochloride of such a compound as speculated upon in equation (6).

Run II

In this attempt the procedure of Run I was duplicated with the exception that double the quantities of materials were used and the heating time was cut to one-half hour. The aminoacetic acid hydrochloride was again recovered to the extent of 5 grams, melting point 174-184° C., for the first portion crystallized, and 2 grams, melting point 175-176° C., for the second portion obtained by concentration of the filtrate. A copper salt was made of this product by boiling an 80 cc. solution containing 2 grams with 1.5 grams of chemically pure $CuCO_3.Cu(OH)_2$ in order to obtain material for a copper analysis to furnish additional evidence of its composition. The excess of copper reagent was removed by filtering and the solution was evaporated until crystals of the derivative appeared. These were filtered from a cold solution, dried in vacuum, and analyzed for copper in the usual manner by ignition using a pinch of mercuric oxide to insure complete oxidation of the copper to copper oxide. A 0.1427 gram sample gave 0.0475 gram of copper oxide, i.e., 26.65 percent copper and a 0.1237 gram sample gave 0.0405 gram of copper oxide, i.e., 26.17 percent copper. Another portion of this copper derivative previously

recrystallized twice from 70 percent alcohol and analyzed for copper by the same procedure gave: a 0.0545 gram sample formed 0.0188 gram of copper oxide, i.e., 27.72 percent copper and a 0.0683 gram sample formed 0.0239 gram of copper oxide, i.e., 28.00 percent copper. The theoretical value for the percentage copper in $Cu(C_2NH_2COO)_2.H_2O$ is 27.66. An analysis of the original aminoacetic acid used in this work was made by Mr. A. A. Dodge* who prepared the copper salt in the manner described, and using samples of approximately the same size, obtained a value of 27.78 percent copper.

The filtrate and washings of Run II upon spontaneous evaporation in vacuum yielded a volatile oil amounting to about 6 cc. which upon steam distillation and extraction of the distillate with ether, followed by spontaneous evaporation of the ether, gave an oil weighing 4.5 grams. The water residue of the flask upon evaporation left only a trace of oil film after 2 grams of crystals, melting point 225-226° C. with charring, had been obtained. A "formol" titration upon a sample of these crystals which were purified by one crystallization from 50 percent alcohol gave a neutralization equivalent of 72.75 as compared with 75.04 the theoretical for aminoacetic acid. The end-point of this titration is difficult to observe and success resulted only by titrating the hot solution. The melting point of the crude material above compares favorably with that reported in the literature, i.e., 233° C. with charring, for aminoacetic acid. The 4.5 grams of oil mentioned above has not been further characterized since a larger sample, obtained in subsequent runs, has been used for this purpose.

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Run III

Run III represents the first attempt to effect condensation by the use of "equimolecular" quantities of the reactants and the procedure for this run is here summarized:

Fifteen grams of aminoacetic acid dissolved in 300 cc. of alcohol were warmed on a steam-bath for one and one-half hours as 40.0 grams of limonene nitrosochloride were added gradually and with agitation. To effect complete solution it was necessary to add 50 cc. of water while heating was continued for another period of similar duration. The reaction mixture was then filtered and allowed to remain in the ice-box over night. Since no crystals appeared with reasonable standing in the cold a concentration of the liquor was effected by distilling off about 200 cc. of the 400 cc. volume. (The distillate thus obtained was later added to the steam distillate of the volatile oil.) During this concentration an oil separated which upon cooling was removed by a separatory funnel. The wet oil weighing 46 grams was subjected to steam distillation and the alcohol-water solution from which the oil had been removed was allowed to evaporate spontaneously, crops of crystals being removed from time to time and the melting point of each determined.

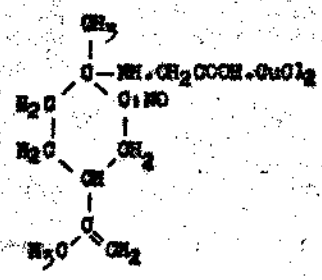
Characteristics of Crystalline Products Removed from Alcohol-Water Solution:

Four different crops taken from this alcohol-water solution gave melting points checking with that of aminoacetic acid hydrochloride and in the light of evidence pointed out in Run I and Run II may be considered to be this compound. The amount of recovered aminoacetic acid hydrochloride was 5.9 grams.

One-half gram of crystals checking as to melting point with those of d- or l- carvoxime (melting point 71.0° C.) were also obtained from the

water-alcohol solution.

By further concentration of the alcohol-water solution 1.7 grams of a new derivative of limonene nitrosochloride and aminoacetic acid was obtained which apparently was not prepared in any appreciable quantity by following the procedure of Run I and Run II. The melting point of these clear needle crystals was found to be 141.0-141.5° C. (uncorrected). They gave a positive test for chloride with silver nitrate reagent and a positive test for nitrogen using the sodium fusion method. They failed to give the qualitative ferric chloride test for aminoacetic acid. Chemical equation (O) has already been suggested for an explanation of the formation of this derivative and analytical data are presented in support of the formation of this compound, structural configuration for the molecule resting upon the strength of the correctness of limonene nitrosochloride and aminoacetic acid structures. The results of a Kjeldahl nitrogen determination are in close accord with the calculated value of 10.14 percent for the compound limonene-nitrolaminoacetic-acid-hydrochloride. Those found were 10.14 percent and 10.39 percent, using respectively 0.2025 and 0.2040 gram samples in the analysis. A copper salt derivative was prepared from the limonene-nitrolaminoacetic-acid-hydrochloride by use of $CuCO_3.Cu(OH)_2$ reagent, in the method described elsewhere in this paper. The analyses of this copper derivative gave a copper value of 16.96 percent compared with the calculated theoretical value of 16.98 percent for the molecule:



The structure of such a salt was assumed on the strength of the fact that aminoacetic acid forms a copper salt, e.g., (a) $NH_2CH_2COOH.CuCl_2$. It is also true that aminoacetic acid forms in addition to a complex of the type referred to, a salt of the formula, (b) $(NH_2CH_2COOH)_2Cu.H_2O$. It is reasonable to believe that under the working conditions here involved a copper salt of type (a) was formed by the limonene-nitrosamine, acetic-acid-hydrochloride since a copper salt of type (b) would give a calculated theoretical copper value of 11.34 percent, a value not in accord with the found value of 16.96 percent as cited above.

The filtrate from this alcohol-water solution on complete evaporation yielded several drops of an oil and a mere trace of solid. The melting point of 180-240° C. indicates this solid in the range of 233° C., that of aminoacetic acid.

Consideration of the Volatile Oil from Run III:

This oil was obtained by steam distillation of the 46 grams of oil that separated when the original alcohol-water solution of Run III was concentrated. To obtain the oil the steam distillate was extracted with ether and the ether allowed to evaporate spontaneously. The water obtained from this procedure yielded nothing upon spontaneous evaporation. During the steam distillation 3.2 grams of crystals were taken from the condenser tube. The melting point of these after one crystallization from about 70 percent alcohol was 71.5-72.5° C. in close agreement with those of d- or l- carvoxime, melting point 71.0° C.

Suspecting this volatile oil of containing additional carvoxime an attempt was made to remove the latter by shaking the oil which had been dissolved in petroleum-ether with a 5 percent hydrochloric acid solution, a solvent in which carvoxime appeared to be soluble. Neutralization of the water-acid layer with sodium carbonate failed to liberate

carvoxime or any other water-insoluble material. After several days, during which time the petroleum-ether evaporated spontaneously, the volatile oil was subjected to diminished pressure distillation. Data received in this distillation are recorded in the table below:

Distillation of 12.3 grams of volatile oil from Run III

Fraction	Weight	Boiling Range	Oil Bath Temp.	Pressure, mm.
I	2.7	-104.0°	135-140°	less than 1
II	2.3	104.0-120.5°	157-185°	less than 1
III	5.2	120.5-125.0°	192-195°	less than 1
Residue	2.1			

The wide boiling range indicated that Fraction II was a mixture. It gave a characteristic nitrosochloride blue color when treated with ethyl nitrite and hydrochloric acid in the usual manner although no crystalline derivative was obtained by repeated trials and chilling with dry-ice. The presence of traces of limonene was thus indicated but there was not sufficient material to allow redistillation of the fraction and further characterization.

Fraction III on standing solidified and after recrystallizing twice from water-acetone solvent gave a melting point corresponding to that of d- or l- carvoxime, 71.0-72.5° C. No consideration was given to the resinified mass left in the flask after the diminished pressure distillation of the volatile oil.

Also, no attempt was made to characterize the flask residue from the steam distillation of the volatile oil from Run III since it was a very heavy resinous mass probably consisting of polymerized material produced by the prolonged steam distillation (60 hours) which was required for the removal of the volatile oil from the original reaction mixture.

Summary of Yields for Run III

Carvoxime	8.9 grams	27.2 percent based on original limonene nitrosochloride used
Aminoacetic acid recovered as hydrochloride	8.9 grams	39.9 percent based on aminoacetic acid used, percent recovered
Volatile oil, carvoxime deducted	7.1 grams	
Flask residue from steam distillation	7.5 grams	
Limonene-nitrolaminoacetic-acid-hydrochloride	1.7 grams	3.1 percent based on original limonene nitrosochloride used
Total	34.1	
Total reactants used	55.0	
Unaccounted for, gaseous and possibly volatile oil loss	20.9	38.9 percent of total reactants

Although these figures do not show the recovery that might be expected a more rational consideration of the problem suggests several explanations for the high loss listed as "unaccounted for" in the table above. During the original heating process of the reaction mixture considerable gas evolution was noticed, the gas possessing a brown color, suggesting nitrosyl chloride from the decomposition of limonene nitrosochloride. The maximum loss of this kind if all the limonene nitrosochloride was decomposed in this fashion, however, would be 13.1 grams, 23.7 percent of the total reactants used. During the various heating operations and evaporations as outlined there is no doubt that considerable loss of volatile oil occurred. It is suggested therefore that the figure for the yield of carvoxime and other volatile oil constituents will be considerably lower than the actual amount produced by the action of aminoacetic acid and limonene nitrosochloride.

Run IV

The main purpose in conducting this run was to ascertain whether any change in procedure would effect an increase in yield of the compound limonene-nitrolaminoacetic-acid-hydrochloride, and incidently to prepare more of the compound for experimentation. About simultaneously with the time of this run another, Run V, was started using larger quantities of reagents in order to obtain good workable amounts of limonene-nitrolaminoacetic-acid-hydrochloride and volatile oil. The fourth run was conducted along the lines of the procedure used in the third and may be summarized as follows:

Equimolecular quantities (50 grams of limonene nitrosochloride and 19 grams of aminoacetic acid) were used; all of the aminoacetic acid and half the limonene nitrosochloride were placed in 100 cc. of 95 percent alcohol and 20 cc. of water and allowed to stand over night in the cold. The remainder of the nitrosochloride, together with 100 cc. more alcohol and 20 cc. of water, was added and the mixture allowed to stand in the cold for 48 hours. The mixture was then warmed on the water-bath to a temperature not exceeding 50° C. for 40 hours.

The reaction mixture was steam distilled directly instead of concentrating to smaller volume as in Run III. By the same procedure as in the latter 24 grams of a volatile oil were obtained, but possibly owing to considerably warmer weather no carvoxime crystals were obtained from the condenser during the steam distillation. This oil has not been subjected to further consideration.

The residue from the steam distillation having gone nearly to dryness was extracted with water, with ether, and then with alcohol. Both the ether and the alcohol extracts failed to give any crystals upon evaporation;

the ether extract yielded 4.5 grams of resin, the alcohol extract 7.1 grams, a total of 11.6 grams of very resinous polymerized material corresponding to the material obtained in the third run. The flask residue residue was completely dissolved by use of these three solvents.

The water soluble extract yielded several crops of crystals by effecting concentrations and addition of acetone as a precipating agent. The crude yield of limonene-nitrolaminoacetic-acid-hydrochloride was 2.0 grams, 3.03 percent on the basis of the original limonene nitrosochloride used, an amount duplicating that obtained in Run III.

Run V

The procedure followed here was in duplicate of that used in Run III. The quantities of reactants were 150 grams of limonene nitrosochloride, 60 grams of aminoacetic acid, 600 cc. of 95 percent alcohol, and 60 cc. of water. Very noticeable evolution of gas was discernible with the use of this large amount of material, a process which continued during the warming for a period of one-half hour. The steam distillation required about sixty hours.

The 80 grams of volatile oil obtained in this run have been fractionally distilled under diminished pressure and the results obtained in this distillation are here recorded:

Fractional Distillation of Volatile Oil of Run V (80 grams)

Fraction	Wt. in Gm.	Boiling Range	Bath Temp.	Pressure in mm.
I	1.4	- 85°		3
II	5.0	85 - 90°		3
III	34.0	90 - 95°	138° avg.	3 to 1
IV	9.1	101.5-104.0°	145° avg.	less than 1
V	27.6	110.0-120.0°	150-170°	less than 1
Residue	6.6			
Total	81.7			

Consideration of Fraction III of the Volatile Oil from Run V:

Fraction III was redistilled at atmospheric pressure using an ordinary Claisen flask and the following data were received:

Data for Redistilled Fraction III

Fraction	Weight	Boil.Range	mm.	Index Refr. 20°
1	4.8	-229°	742.6	1.4968
2	19.0	229-233°	742.6	1.4969
3	5.0	233° +	742.6	1.4985
Residue	5.2			

From a consideration of the indices of refraction it is apparent that no separation of consequence was effected by this distillation and Fractions 1 and 2 having the same composition were combined in order to be subjected to redistillation through a five foot nichrome spiral column under diminished pressure in anticipation of bringing about a separation. A summary of the results obtained in this distillation is here recorded:

Data for Redistilled Fractions 1 and 2

Fraction	Weight	Boil. Range	mm.	Index Refr. 20°
C	3.6	-91.5°	1.5	1.4960
D	15.7	91.0-91.5°	1.5	1.4959
E	2.5	91.5-98.0°	1.5	1.4919
Residue	2.0			

Fraction D of this distillation was selected for characterization and was found to have the following physical constants: b.p. 231° C./745 mm., $d^{20°}$ 0.9612, $n_D^{20°}$ 1.4959, $(\alpha)_D^{20°}$ +45.25° compared with b.p. 230° C./755 mm., $d^{20°}$ 0.9611, $n_D^{20°}$ 1.49938, $(\alpha)_D^{20°}$ +62.32° for d-carvone as reported by Simonsen (17). Carvone phenylhydrazone, the hydrogen sulfide addition compound of carvone, and cyanodihydrocarvone were the derivatives made to identify Fraction D and a quantitative analysis for carvone was also made upon this fraction. The details of this examination follow:

The Phenylhydrazone of Carvone :

0.50 gram of Fraction D was dissolved in 2 cc. of ethyl alcohol and sufficient water added drop by drop until the precipitate barely redissolved. To this 0.04 gram of phenylhydrazine base (an equimolecular quantity) was added but no derivative crystallized out on cooling until a drop of acetic acid was added. Recrystallized from hot alcohol the phenylhydrazone melted at 105.0-105.5° C. and upon a second recrystallization the melting point obtained was 105.5-106.0° C., comparing favorably with that reported by Goldschmidt (18) of 106° C. for the phenylhydrazone of carvone.

The Hydrogen Sulfide Carvone Derivative:

A solution containing 5.2 grams of Fraction D, 0.8 gram of alcohol and three drops of ammonium hydroxide (sp.gr.$^{15°}$0.96) was saturated with hydrogen sulfide. On standing over night the addition product crystallized yielding, after filtering and drying on a porous plate, 2.1 grams, 35.9 percent of that theoretically possible. The filtrate, following evaporation of the alcohol solvent, weighed 1.2 grams and probably consisted of unchanged carvone. The derivative recrystallized once from a mixture of three parts of chloroform and one part of alcohol melted at 186.0° C. After another crystallization the melting point was 187.5-188.5° C. comparing favorably with that of 187° C. as reported by Heusler (19). An alcohol solution of this derivative 0.1274 gram/ 25 cc. proved to be optically inactive although Baeyer (20) reports that carvone hydrogen sulfide made from d-carvone has a specific rotation of +5.55°.

Carvone was regenerated from the hydrogen sulfide derivative by

dissolving 1.000 gram of the latter in a solution containing 1.6 grams of potassium hydroxide to 8 grams of water heated on a water-bath for several hours with occasional shaking. The alkali solution when cold was extracted with ether, washed with several portions of water and the ether removed with the aid of the pump. The regenerated carvone was distilled at ordinary temperature. Its index of refraction of 1.4966 at 20° C. agreed favorably with that of 1.4959 at 20° C. of original Fraction D. This regenerated carvone was also optically inactive in an alcohol solution containing 0.1865 gram of carvone/ 25 cc. of alcohol.

Cyanodihydrocarvone:

This derivative was prepared from Fraction D according to the method of Lapworth (21) using one-tenth the quantities suggested by him. Some difficulty was experienced in getting the cyanide addition and the mixture was allowed to stand for about a week. The solution was then diluted as instructed and the product received after filtering and drying on a porous plate melting at 92-94° C. weighed 1.6 grams, 54.5 per-cent of the yield theoretically possible. One recrystallization from dilute alcohol yielded a product melting at 93.2-94.0° C. which agrees favorably with that of 93.5-94.5° C. reported by Lapworth (21).

Analysis of Fraction D for Carvone:

The sulfite method as modified by Burgess (22) was used with the amount of material subjected to analysis reduced from 5 cc. to 3 cc., a procedure which could be expected to reduce the accuracy of the method, but which was necessary in order to conserve Fraction D. The accuracy of the method with a 10 gram sample is stated to be 1-2 percent. The percent of carvone found in Fraction D by this method was 96.7, an

amount quite satisfactory considering the size of the sample used in the analysis.

Beyond all questionable doubt Fraction B may be declared to be carvone.

From the fairly close agreement of the indices of refraction of Fractions I, II, III, and IV of Run V it would appear that all may be considered to be largely carvone. Their total weight of 46.5 grams represents a yield of 39.4 percent of the theoretical amount possible based on the conversion of all the limonene nitrosochloride to carvone.

Fraction V from Volatile Oil of Run V:

On chilling Fraction V crystals were obtained which after filtering and drying on a porous plate weighed 12.5 grams and melted at 71.5-72.0° C., a melting point in accord with that of carvoxime. The oil-filtrate from these crystals awaits redistillation and further characterization. However, this much may be stated concerning it; its index of refraction of 1.5142 at 20° C. is entirely too high for it to be carvone, nor were attempts to prepare a phenylhydrazone from it successful. This index of refraction is in close agreement with that of carvacrol although the material does not seem to give characteristic green coloration with alcoholic ferric chloride as does carvacrol.

In order to complete the characterization of the carvoxime from Fraction V several derivatives were prepared, benzoylcarvoximes and hydrochlorcarvoximes, the details of which follow.

Benzoylcarvoximes:

An ethereal solution of 5 grams of the crystalline material from Fraction V was treated with 4.2 grams of benzoyl chloride (equimolecular

quantities) according to the method of Goldschmidt as described by Heusler (23). Following one recrystallization from petroleum-ether and one from alcohol the derivative melted at 95.5-96.0° C. in close agreement with 96° C. the melting point reported by Heusler (23) for benzoylcarvoxime.

Hydrochlorcarvoxime:

Three grams of the crystalline material from Fraction V was dissolved in about 20 cc. of methyl alcohol and the solution saturated with hydrogen chloride by passing a stream of the latter into the solution for several hours. Some difficulty was experienced in getting the hydrochlorcarvoxime to crystalline even after allowing the solution to stand for ten days. Following the addition of chloroform a small amount of crystalline solid was obtained which melted at 156° C. comparing favorably with 155° C. reported by Heusler (23) for hydrochlorcarvoxime.

Limonene-Nitrol-Amino-Acetic-Acid-Hydrochloride from Run V:

The yield of limonene-nitrolaminoacetic-acid-hydrochloride obtained by fractional crystallization of the water residue following steam distillation of the reaction mixture from Run V was 6.1 grams, a figure representing 2.9 percent of that theoretically possible from the amount of nitrosochloride of limonene used. This yield is in agreement with that of 3.1 percent received in Run III and 3.05 percent received in Run IV.

One molecular weight determination was performed on the limonene-nitrolaminoacetic-acid-hydrochloride using the Rast method as described by Shriner and Fuson (25a) with borneol substituted for camphor. The value received by this method was 1122.4 corresponding to 276.6 the theoretical value for the formula $C_{10}H_{16}\begin{cases}NO\\NH.HCl.CH_2COOH\end{cases}$. If the value 1122.4 is divided by 4 a value of 280.6 is received which agrees favorably with the

theoretical value of 276.6. This would seem to indicate that this compound is probably tetramolecular in structure.

Summary Limonene Nitrosochloride and Amino Acetic Acid

While the nitrolamine of 1-chloro-2nitroso-$\Delta^{8(9)}$-menthene (limonene nitrosochloride) with aminoacetic acid has been prepared the yield has not been appreciable and the reaction seems to favor equation I as illustrated in the introductory part of this paper rather than equation II. Although an increase in concentration of the aminoacetic acid might have been expected to increase the yield since 1 molecular equivalent would theoretically take care of all the hydrogen chloride liberated by one equivalent of nitrosochloride experience has shown that this was not the case, i.e., no derivative of this kind was obtained when 2 molecular equivalents of aminoacetic acid were used to 1 equivalent of limonene nitrosochloride. The larger quantity of aminoacetic acid apparently served to assist in the removal of hydrogen chloride from nitrosochloride of limonene as aminoacetic acid hydrochloride and gave results corresponding to those shown by equation I.

The successful isolation and characterization of carvoxime in good yield, together with the large percentage of carvone found, demonstrates that the main reaction between limonene nitrosochloride and aminoacetic acid under the conditions of experimentation described is definitely of the order of equation I. Furthermore, a considerable amount of carvoxime so formed undergoes hydrolysis to produce carvone. That some of the latter enolizes to isomeric carvacrol might rationally be anticipated and although the evidence is not sufficient, as yet, to be convincing it appears to give this anticipation some support.

A. Preparation of Δ^1-p-Menthene

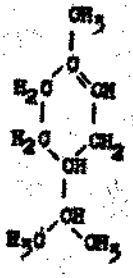
The studies in menthene chemistry reported in this section and those which are now in progress are concerned with but one of the six compounds directly referable to the double linkage isomers of position of the para menthenes, namely Δ^1-p-menthene. Such studies might logically be limited to a consideration of these six menthenes since such limitation is in accord with the original designation of the term menthene as rationally applied to the dehydration product of menthol, that is, the compound derived by the dehydrogenation of menthene, a para compound, and the underlying hydrocarbon of menthol. These six isomers of position, disregarding optical isomerism and cis-, trans-isomerism, with reference to location of the double bond between carbon atoms may be named and structurally diagramed as follows:

The Para Menthenes

C_nH_{2n-2}

$C_{10}H_{18}$

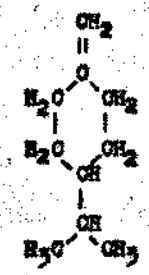
F, Δ



Δ^1-Menthene

Δ^2-Menthene

Δ^3-Menthene



$\Delta^{1(7)}$-Menthene

$\Delta^{4(8)}$-Menthene

$\Delta^{(8-9)}$-Menthene

From this structural viewpoint it is clear that such familar addition products as nitrosochlorides, nitrosites, nitrosates, and the well-known nitrolamines, etc., of the terpenes may be considered substitution products of the menthenes, e.g., in the case of limonene addition compounds, substituted $\Delta^{8(9)}$-p-menthene.

By the separation of the elements of water from carvomenthol, $C_{10}H_{19}OH$, it may be converted into the hydrocarbon Δ^{1}-p-menthene (carvomenthene). Baeyer (24) has converted carvomenthol into carvomenthene by converting the former into the bromide with hydrobromic acid and treating the resulting bromide with quinoline, whereas Wallach (25) has accomplished the same result by treating carvomenthol with potassium acid sulfate. Kondakov and Lutschinin (26) have been able to prepare Δ^{1}-p-menthene from carvomenthyl chloride or bromide by heating them with alcoholic potassium hydroxide obtaining two hydrocarbon fractions by distillation, the one representing about 90 percent of the whole boiling at 172.0-174.5° C. the remainder having a boiling point of 174.5-176.0° C. The properties of these two fractions are given in Table II.

Sabatier and Senderens (27) using copper as a catalyst prepared Δ^{1}-p-menthene from limonene by reduction with hydrogen at 150° C. Vavon

(28) has carefully investigated the hydrogenation of limonene with platinum black as the catalyst and claims to have obtained Δ^1-p-menthene in a high state of purity (characteristics given in Table II). Other investigators who have prepared the menthene by catalytic reduction include Ipatiev (29), Zelinski (30), Willstätter and Waldschmidt-Leitz (31), Escourrou (32), and Windaus (33), and Dupont (34).

The Δ^1-p-menthene used in this work was prepared by the method of Vavon (28), that of the reduction of limonene with molecular hydrogen at approximately 1.5 to 2 atmospheres pressure in the presence of platinum black as catalyst. To condition the limonene about 6 liters of commercial material was rectified by shaking with one liter of 10 percent sodium hydroxide solution and subjecting the mixture to steam distillation. By this process 2500 cc. of limonene were obtained requiring about 36 hours to complete the operation. Subjected to redistillation this entire bulk of rectified limonene gave, excluding a low cut containing a small amount of water and the flask residue, four main fractions, the amounts and physical characteristics of which, together with characteristics found by other workers, are listed in Table I.

The platinum black for the reduction was prepared by adding to a mixture of 6.5 cc. of formalin and 6 cc. of water the material contained in 5 vials (15 grains each) of chloroplatinic acid, i.e., 4.6667 grams of $H_2PtCl_6.6H_2O$. Using a mechanical stirrer and not allowing the temperature to exceed 50° C. (35), a solution of 4.7 grams of sodium hydroxide in 4.7 grams of water was cautiously added and the stirring continued for about an hour after which the reaction mixture was allowed to stand overnight, filtered, washed, and the platinum black dried over concentrated sulfuric acid. According to the equation,

TABLE I

PHYSICAL CHARACTERISTICS OBTAINED ON LIMONENE FRACTIONS AND THOSE OBTAINED BY OTHER INVESTIGATORS

Fraction	Amount cc.	Boiling Range ° C.	Pressure of Distl. mm.	Refractive Index 20° C.	Density 20° C.	Specific Rotation 20°C. ± °
1	500	175.0-176.0	737.8	1.4680	0.8470	119.9
2	500	175.5-176.0	745.2	1.4679	0.8467	119.6
3	160	176.0-177.7	745.2	1.4685	0.8486	119.3
4	800	176.0-189.0	745.2	1.4692	0.8618	106.7
Braun and Lemke (36)		176.0-176.4			0.8411	126.84
Brühl (37)		175.5-176.0	763	$1.47428^{21^{\circ}}$	$0.8402^{21^{\circ}}$	$125.8^{19.5^{\circ}}$
Richter and Wolff (38)		177.6-177.8	755	$1.4727^{17^{\circ}}$	$0.8417^{20.6}$	124.0
Escourrou (34)		175.0-176.0	769	$1.4717^{25^{\circ}}$	$0.847^{14^{\circ}}$	$116.1^{14^{\circ}}$
Kremers (39)				1.47459	0.846	121.3

$$H_2PtCl_6 \cdot 6H_2O + HCHO + 6NaOH \longrightarrow Pt + CO_2 + 6NaCl + 11H_2O,$$

the theoretical yield of platinum black is 1.7582 grams and that received was 1.6918 grams, i.e., 96.22 percent of the theoretical value.

The ratio of platinum black to limonene recommended by Vavon (28) is 2 to 35 parts by weight so that for the amount of platinum black prepared 29 grams of limonene were used in each reduction-run. A total of 16 separate runs were made in order to effect the reduction of 464.0 grams of limonene having the physical characteristics of Fraction I, Table I, to Δ^1-p-menthene. For these reductions an ordinary motor driven low-pressure hydrogenation apparatus (Parr Instrument Company) of approximately 4 liter hydrogen reservoir capacity was used. A narrow-mouth pint bottle was used to hold weighed amounts of catalyst and limonene, this bottle being connected to the delivery tube of the hydrogenation outfit by a one-hole rubber stopper. From Vavon's work (28) it was noted that the rate of hydrogen absorption was fairly constant during the process of the addition of 2 hydrogen atoms in the conversion of limonene to Δ^1-p-menthene. At the completion of this operation there is a sharp decline in the absorption rate and if the process is interrupted at this point the product consists almost wholly of menthene, while continued reduction beyond this stage produces menthane by the addition of 2 additional hydrogen atoms. By following the rate of absorption during several runs the time required for the addition of 2 hydrogen atoms was established and in every run thereafter a time-pressure loss record was maintained in order to make sure that the catalyst was not becoming inactivated. It was also noticed that at the beginning of each reduction there was a slight lag in the rate of absorption. A typical set of data as obtained upon 29 grams of limonene for what will

be called a preliminary run is here recorded:

Time	Pressure lbs./sq.in.	Rate of Absorption (pressure loss/minute)	Room Temp. ° C.
10.15	26.0	0.00	21.0
10.20	23.5	0.50	"
10.25	20.2	0.66	"
10.30	17.0	0.64	"
10.35	13.9	0.62	"
10.40	11.1	0.56	"
10.45	9.0	0.42	"
10.50	7.1	0.38	"

From this data it will be observed that the rate of absorption is nearly constant for the first twenty-five minutes after which it begins to decrease rapidly. Eight preliminary runs were made, each limited to a thirty-five minute period, and giving results similar to the one recorded. Following this, eight additional reduction operations were performed using the correct time (twenty-five minutes) periods as determined in the preliminary experiments. As a check to determine if the theoretical amount of hydrogen was being absorbed in this twenty-five minute period the delivery tank of hydrogen was calibrated as follows: The tank was filled until its pressure gauge registered 26 lbs./sq.in., the initial pressure used in each of the experimental runs. By displacement of water from a graduated cylinder the volume of hydrogen was progressively measured until the pressure gauge registered 7 lbs./sq.in. The volume of hydrogen delivered proportionally followed this pressure drop and the volume of hydrogen delivered was found to be 0.326 liters for every lb./sq.in. drop as registered on the pressure gauge. In the twenty-five minute period runs the pressure drop on the average was 14.7 lbs./sq.in. so that the volume of hydrogen delivered was 14.7 X 0.326 liters = 4.792 liters. The theoretical amount of hydrogen required to reduce 29 grams of limonene, the amount used

in each experimental run, to Δ^1-p-menthene is 4.770 liters. It will be seen then that the twenty-five minute period, provided the catalyst retains its activity, is the time required to deliver just 22cc. in excess of the theoretical requirement for the addition of 2 hydrogen atoms.

The reduction products of the first eight runs (these designated preliminary) were combined, distilled, and the physical characteristics determined, and these characteristics compared with those obtained from the combined distilled products of the main eight runs where the time factor was twenty-five minutes instead of thirty-five. These characteristics are here recorded in Table II together with those found for carvomenthene by other investigators for the purpose of comparison. The physical constants for the product of the combined runs 9-16 are in close agreement with those of Varon and with those of Windaus, and with the exception of specific rotation, are in close agreement with those of Kondakov and Lutschinin for Δ^1-p-menthene prepared from carvomenthylchloride and alcoholic potassium hydroxide.

TABLE II

PHYSICAL CHARACTERISTICS OBTAINED FOR Δ^1-p-MENTHENE AND THOSE OBTAINED BY OTHER INVESTIGATORS

Fraction	Yield Gm.	Boiling Range ° C.	P. of distl. mm.	Refr. Index 20° C.	Density 20° C.	Angle of Rotation	Specific Rotation 20° C.
Combined preliminary Runs 1-8 before distl.	231.8					+ 78.75	+
Combined main Runs 9-16 before distl.	222.6					87.16	
Runs 1-8 after distillation (best fraction)	200.7	175.8-175.5	749.3	1.4517	0.8235	79.16	95.96
Runs 9-16 after distillation (best fraction)	166.1	175.5-175.5	759.1	1.4550	0.8279	87.86	106.1 *
Kremers and Brennan[4]	17.5	174.0-176.0	737.4	1.4589	0.8558	87.37	104.5
Yavon (28)		175.0-177.0		1.4565	$0.8246^{18°}$		$(\alpha)_{234}^{436}$
Windemuth (33) 1.		174.0-175.0		1.4540	0.8211		88.80**
2.		170.0-174.0		1.4540	0.8217		90.10**
Kondakov and Lutschinin (26)*** 1.		172.6-174.5		1.45979	$0.8250^{16.5}$		-2.4
2.		174.5-178.0		1.46108	$0.8230^{19.4}$		-1.28

* Performed at another date upon limonene having the following specifications: Boiling Range 178-180° C., index of refraction at 20°C. 1.4750, density at 20° C. 0.8565, specific rotation at 20° C. + 108.1°.

** These values appear to be angle of rotation measurements which were not calculated to specific rotation.

***Prepared from carvomenthylchloride and alcoholic potash.

B. Δ^1-p-Menthene Nitrosochloride

Using Wallach's method (40) 8 cc. of Δ^1-p-menthene with the characteristics of Fraction, Runs 9-16 distilled, Table II, were mixed with 11 cc. of freshly prepared ethyl nitrite (41) and 12 cc. of glacial acetic acid and the mixture well cooled with ice and salt. Added to this slowly and in small portions with agitation was a mixture of 6 cc. of crude concentrated hydrochloric acid and 6 cc. of glacial acetic acid. The temperature was retained at approximately 0° C. during the several hours required to add the hydrochloric acid mixture. Following the addition of all the hydrochloric acid mixture, 5 cc. of ethyl alcohol were added and the entire mixture allowed to stand for several hours at low temperature. The mass of nitrosochloride crystals that separated was then filtered and thoroughly washed at the pump with cold alcohol. The yield of crude Δ^1-p-menthene nitrosochloride, melting point 84-86° C., was 2 grams, 25 percent of the theoretical, obtained after thorough washing with cold alcohol in which it is slightly soluble. Vavon (42) reported the melting point of carvomenthene nitrosochloride prepared by catalytic reduction as 95-96° C.

C. Δ^1-p-Menthene Nitrolamine with Morpholine

To 2 grams (1 molecular equivalent) of Δ^1-p-menthene nitrosochloride 1.7 grams (2 molecular equivalents) of morpholine were added drop by drop as a vigorous reaction ensued. After the addition of all the morpholine 5 cc. of hot alcohol were required in order to dissolve the solid mass and the solution was allowed to reflux for one-half hour. Chilling and filtering yielded two crops of crystals totaling 1.4 grams, a yield of 58.3 percent, with a melting point of 171.8-173.0° C. Recrystallized from ether-alcohol solvent these crystals melted at 171-172° C.

Using the Kjeldahl method Mr. M. O. Isaacs and Mr. Frank O'Brien* have been unable to obtain check results in analyzing the Δ^1-p-menthene nitrolamine of morpholine for nitrogen, receiving values of 7.26, 8.30, 9.09, and 6.54 percent. The theoretical percentage nitrogen in this compound, $C_{10}H_{18}\begin{Bmatrix}NO\\NC_4H_8O\end{Bmatrix}$, is 11.02 percent and in its hydrochloride, 9.63 percent. As might have been expected the nitrogen in the morpholine ring apparently requires more energetic treatment for its release. Combustion analysis for nitrogen is now in progress.

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III. LIMONENE DERIVATIVES

A. Limonene with Amino Acetic Acid

Interesting observations have been made upon the behavior of aminoacetic acid when refluxed in limonene, and although the reaction products have not been thoroughly characterized, the progress that has been made is here recorded:

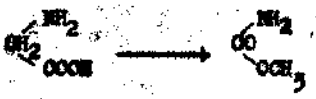
Two grams of aminoacetic acid were added to 5.4 grams of limonene (Fraction 1, Table I), but for equimolecular quantities, 3.6 grams of limonene would have been the correct quantity. Refluxing was effected for a period of about twelve hours and crystals appeared in the condenser tube which would if weighed have amounted to about one-half gram. The contents of the flask gave off a strong ammoniacal odor and the vapors turned moist red litmus blue. The crystals from the reflux tube were collected on a watch glass, and although covered with another watch glass, with the exception of a very small portion, disappeared overnight. Addition of hydrochloric acid to this crystalline product produced an effervescense and the gas proved to be carbon dioxide since it gave a white precipitate when poured downward into limewater; also, a portion of the crystalline material when placed directly in barium hydroxide solution gave a white precipitate indicative of carbonate.

The flask content was steam distilled and the distillate extracted with ether. The water residue of this distillation upon evaporation gave nothing. The ether extract was subjected to distillation after the ether had been removed and the characteristics of the product so obtained were: Boiling range 176.0-176.5° C., index of refraction at 20° C. 1.4752 which compare favorably with those of the original limonene, boiling range

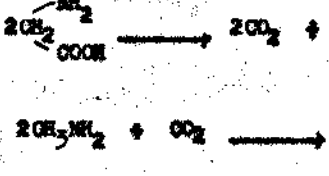
175.5-176.0° C., index of refraction at 20° C. 1.4732. Three and one-half grams of limonene were thus recovered indicating that the limonene took little, if any, part in the reaction and probably only served to catalyze the decomposition of the aminoacetic acid.

Speculation as to what the crystalline decomposition product of the aminoacetic acid might have been leads to several possibilities:

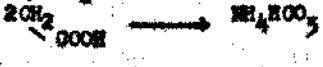
1. Limonene may catalyze the rearrangement of aminoacetic acid to methylcarbamate (a urethane).



2. Limonene may catalyze decomposition of aminoacetic acid to ammonia, carbon dioxide, and methylamine methylcarbamate.



3. Limonene may catalyze decomposition of aminoacetic acid to ammonium acid carbonate, methyl cyanide, and formaldehyde.



No further experimentation has been carried out relative to this problem.

B. Limonene with Monochloracetic Acid

Ten grams of limonene having the following characteristics: boiling point 175.5-176.0° C., specific gravity at 20° C. 0.8467, index of refraction at 20° C. 1.4679, and specific rotation at 20° C. + 119.6°; were added to an equimolecular quantity, 7 grams of melted chemically pure monochloracetic acid in a small round bottom flask and the mixture refluxed. After cooling, following several hours refluxing, the crystalline material remaining was filtered off and twice recrystallized from Skelly Solvent "B" (boiling point 60-68° C.) and finally given a thorough washing with cold solvent. The melting point of 62-63° C. indicates that the monochloracetic acid underwent no change.

The filtrate from the above was washed with dilute barium hydroxide solution to remove any free acid that might have resulted in the heating, and filtered to break a slight emulsion. A saponification number was run upon this oil in order to determine if any acetylation had taken place. The two samples gave an average acid back-titration value of 25.78 cc., the exact value obtained by use of two blanks, indicating that no measurable acetylation had taken place under the conditions of reaction.

Due to the highly volatile nature of monochloracetic acid no attempt was made to weigh the recovered quantity of this reagent.

BIBLIOGRAPHY

1. Tilden, W. A., and Shenstone, W. A., J. Chem. Soc. 31, 554 (1877)

2. Goldschmidt, H., and Zürrer, R., Ber. 18, 1729, 2223 (1885)

3. Wallach, Otto, Ber. 24, 1547 (1891)

4. Urban, L. C., and Kremers, Ed., Am. Chem. J. 16, 404 (1894)

5. Mond, J. L., and Kremers, Ed., Am. Chem. J. 17, 607 (1895)

6. Baeyer, Adolf, Ber. 28, 646 (1895)

7. Kremers, Ed., et al, Bull. Univ. Wisc., Series No. 1813, 51, Gen. Sc. No. 1597 (1952)

8. Schmidt, Julius, Ber. 35, 2556 (1902)

9. Urban, L. C., and Kremers, Ed., Proc. Am. Pharm. Assoc. 103 (1893)

10. Wallach, Otto, Ann. 241, 318 (1887)

11. Wallach, Otto, Ann. 252, 106 (1889)

12. Wallach, Otto, Ann. 252, 152 (1889); 258, 348 (1890)

13. Wallach, Otto, Ann. 275, 118 (1893); 277, 153 (1894)

14. Goldschmidt, H., and Kisser, E., Ber. 20, 488 (1887); Wallach, Otto, Ann. 305, 255 (1899)

15. Schweizer, P., J. pr. Chem. 24, 257 (1841)

16. Heilbron, Dictionary of Organic Chemistry, 2, 643, Eyre and Spottiswoode, London (1936)

17. Simonsen, J. L., The Terpenes, 1, 351, Cambridge Univ. Press (1931)

18. Goldschmidt, H., Ber. 17, 811 (1884)

19. Heusler, F., and Pond, F. J., The Chemistry of the Terpenes, 180, P. Blakiston's Son and Co., Phila. (1902)

20. Baeyer, Adolf, Arch. Pharm. 221, 285

21. Lapworth, Arthur, J. Chem. Soc. 89, 948 (1906)

22. Burgess, H. E., Analyst 29, 78 (1904)

23. Heusler, F., and Pond, F. J., The Chemistry of the Terpenes, 191, P. Blakiston's Son and Co., Phila. (1902); Wallach, Otto, Ann. 252,149 (1889); Goldschmidt, H., Ber. 18, 1752 (1885)

23a. Ehrimer, R. L., and Fuson, R. C., The Systematic Identification of Organic Compounds, 67, John Wiley and Sons, N.Y. (1935)

24. Baeyer, Adolf, Ber. 26, 824 (1893)

25. Wallach, Otto, Ann. 277, 150 (1893)

26. Kondakov, I. L., and Lutschinin, E. J., J. pr. Chem. 60, (II), 257 (1899)

27. Sabatier, P., and Sanderens, J., Compt. rend. 132, 1256 (1901)

28. Vavon, G., Bull. soc. chim. (IV) 15, 282 (1914); Compt. rend. 152, 1675 (1911)

29. Ipatiev, V. N., Ber. 43, 3547 (1910)

30. Kolinski, M. S., Ber. 57, 2058 (1924)

31. Willstätter, R., and Waldschmidt-Leitz, E., Ber. 54, 125 (1921)

32. Escourrou, René, Parfums de France 26, 86 (1925)

33. Hindsmith, Nevada, Doctorate Thesis, University of Wisconsin (1938)

34. Dupont, Georges, Bull soc. chim. (V), 3, 1021 (1936)

35. Talpail, E. A., Chem. Abstr. 20, 3282 (1926); J. Russ. Phys.-Chem. Soc., Chem. Part 57, 467 (1925)

36. Braun, J., and Lemke, E., Ber. 56, 1652 (1923)

37. Brühl, J., J. Chem. Soc. 91, 121 (1907)

38. Richter, F., and Wolff, W., Ber. 63, 1724 (1930)

39. Kremers, Ed., Am. Chem. J. 17, 692 (1895)

40. Wallach, Otto, Ann. 252, 106 (1889); 279, 174 (1892)

41. The Pharmacopoeia of the United States of America VIII (1905)

42. Vavon, G., Compt. rend. 152, 1675 (1911)

CHAPTER VI

A PRELIMINARY REPORT OF THE PHYTO-CHEMICAL INVESTIGATION OF PO-YOK SEED KERNEL OIL (AFROLICANIA ELAEOSPERMA)

A PHYTO-CHEMICAL INVESTIGATION OF PO-YOK (AFROLICANIA ELAEOSPERMA)

Introduction:

Recent interest in Po-yok (or Po-yoak) oil imported from West Africa has been aroused because of its resemblance to tung oil and its possible use in the paint and varnish industry and although this interest is justified by its physical and chemical properties there appears to be little possibility of supplying the demand that might be created for it owing to the slow growth of the tree bearing the fruit from which the oil is secured. At present, the most complete and authentic reports on the Po-yok tree are those appearing from time to time in the Bulletin of the Imperial Institute (1) of which the 1935 article on "Some African Oil Seeds" states that seedlings at least five years old were only eight feet high and showed no signs of fruiting, that twenty-year old trees were only twenty feet high, and that a good tree yields one to two bushels of fruit but may not bear each year. The director of agriculture at Sierra Leone, West Africa reports that in the hope that under cultivation the trees would grow more rapidly and fruit much earlier, seedlings have been planted and are under observation at Njala. Opinions of various writers seem to be that for the time being the oil will be without practical significance.

Since the fruit of the Po-yok tree so closely resembles that of certain species of "Parinarium" it was first regarded as belonging to that Genus, and the literature still appears to describe it as Parinarium Mobaba (2), Parinarium glaberrimum (3), Parinarium paleuense (3), Parinarium pajura (4), Parinarium jabety (4), Parinarium senegalense(5), Parinarium macrophyllum (6,7), Parinarium laurinum (8), and Parinarium

sherbreense (9). The Po-yok tree, however, has been definitely identified by Mildbraed (10), who gives a complete description of the tree together with information concerning its habitat, as "Afrolicania elaeosperma" of the Rosaceae Family.

The seeds are described in the Imperial Institute Bulletin for 1955 (1) as being grey and warty on the outside, ovoid, length 2 cm., and greatest width 2.75-3 cm. The thickness of the easily cracked thin shell is only 1 mm. and this is covered with a tough outer layer of about the same thickness which decays fairly readily when the fruit is on the ground. The fruit contains a large, very oily kernel, brown or reddish brown on the outside fading to a lighter shade in the interior and possessing the odor of tung oil which becomes stronger with age.

The Po-yok tree appears to be common in Sierra Leone, Benthe Island, Liberia, Nigeria, and the French Cameroun, its habitat, apparently limited, to West Tropical Africa. The natives are said to be familar with the oil, Bundu women using it either mixed with white clay as a body perfume or alone as hair oil. To prepare it the fruits are dried over a fire or preferably in the sun which shrinks the kernels and facilitates the cracking of the shells which is done by beating with sticks. The kernels are heated and pounded in a mortar, the resulting mass subsequently boiled with water and the oil skimmed off the surface (1).

Previous Work on Po-yok Seed and Its Oil:

A first investigation of Po-yok seed oil from Sierra Leone at the Imperial Institute in 1917 and published in its Bulletin of January 29, 1918 reports the oil to be one of the drying type with properties somewhat similar to those of tung oil. The results of later research have appeared

in the same Bulletin (1). In Table I will be found the results of examination of Fa-yok seed and its oil by the Imperial Institute (1), by Bray and Zelip (11), and Steger and van Loon (9). The oil extracted with petroleum-ether by Bray and Zelip (11) is described as being an ill-smelling dark brown liquid which on keeping becomes a soft, semi-solid fat, while it is described in the Bulletin of the Imperial Institute of 1913 as a golden yellow oil which deposits considerable stearin on standing.

Rheineck (12) has found one of the active acids of Fa-yok seed oil to be an 18 carbon unsaturated ketone acid resembling the licanic acid from citicica oil, probably an isomer, which he oxidized fully breaking it down into valeric, oxalic, and keto-azelaic acids. As the latter he has not established the position of the ketone group. In a later communication he states that there are two other fatty acids in the oil which seem to be unusual. More recently Steger and van Loon (9) state that tests on the fatty acids, i.e., fractional crystallization, spectroscopic analysis, and analysis of ozone-oxidized products, indicated the presence of alpha- and beta- eleostearic and alpha- and beta- oxyepic acids.

Experimental Part

Alfred Rheineck of Devoe and Raynolds Company, Inc., Louisville, Kentucky, has kindly provided for this investigation 1159.4 grams of seed which, after being shelled yielded 38.36 percent pericarp and 61.44 percent kernel. The average weight of each seed was found to be 8.1 grams and the average weight of each kernel, 5.0 grams.

Moisture Determination:

Inasmuch as proper comminution of the kernels for extraction with

TABLE I - CHARACTERISTICS OF PO-YOK SEED AND OIL

Seed Characteristics	From Sierra Leone (1) 6 samples	From Sierra Leone (11)	From South America (11)	Parinarium sherbroense (9)
Average weight of each seed	7.3-11.8			9.5
Average weight of each seed kernel	4.6- 7.2			5.5
Amount of shell on seed % by Weight	31.4-44.0			
Amount of kernel in seed % by weight	56.0-68.6			
Water in kernels % by weight	5.9- 9.0	8.4	3.4	
Oil in kernels as received %	41.7-68.6	58.3	74.2	
Oil in Water-free kernels %	45.8-63.8			
Oil on fruits as received %	32.6-39.4			
Oil Characteristics				
Density	15.6° 0.9535-0.9690	15° 0.969	15° 0.905	78° 0.9250
Index of refraction	40° 1.5020-1.5110	40° 1.469	40° 1.469	46° 1.5119
Solidifying point of fatty acids	39.0-50.3°	48.3°	41.6°	
Acid value	0.4-19.3			
Saponification value	188.9-192.3	192.3	200.5	
Iodine value	Wijs, 5 hrs. 139.9-157.1	157.1	77.3	207.5 Calc.by hydrogenation
Unsaponifiable matter %	0.3-1.0	0.7	0.76	0.58
Soluble volatile acids %		0.2	2.68	
Insoluble volatile acids %		0.4	0.52	
Thiocyanogen number				76.1
Diene number (6 mo. old kernels)				60.0
Saturated acids %				12
Oleic acid %				9-10
Seed Meal Analysis				
Water %		12.2	7.4	
Crude proteins %		12.1	24.7	
Fat %		7.0	7.0	
Carbohydrates %		56.1	46.6	
Crude fiber %		8.9	8.2	
Ash %		3.7	6.1	

selective solvents was unsatisfactory because of their oily character, they were crushed in a mortar and distilled with hydrocarbon, Skelly Solvent "C". The Skelly Solvent "C" used in this work was previously shaken with portions of concentrated sulfuric acid over a period of several days, washed with portions of dilute sodium bicarbonate solution, with water, and distilled through a 2 foot Vigreux column, the fraction boiling at 80-86° C. being retained. Two determinations for moisture were made using 100 gram samples and ordinary distillation apparatus collecting the distillates in cylinders graduated in tenths of a cc. and four determinations were made with 100 gram samples using Bidwell-Sterling moisture tubes. The following results were obtained, viz., 3.7 cc., 3.5 cc., 3.8 cc., 3.7 cc., 3.6 cc., and 3.2 cc., an average of 3.5 percent moisture in the seeds.

Treatment of Aqueous Distillates from Moisture Determinations:

The aqueous distillates were separated from the Skelly Solvent "C" used in their extraction, combined, and found to give an acid reaction with litmus, whereupon the solution was diluted with about 50 cc. of water containing several grams of barium carbonate. After gentle warming the suspension was filtered and the filtrate placed in a vacuum desiccator over potassium hydroxide. Following the evaporation of water by this procedure only a mere trace of semisolid, fat-like in character, remained, indicating the absence of free volatile water-soluble acids in the Po-yok seed kernels.

Selective Solvent Extractions:

All of the mare from the moisture determinations was placed in the combined portions of Skelly Solvent "C" used for water extractions and the whole warmed with continuous stirring for about one-half hour. After standing the hot solvent was decanted through a filter and this process repeated five times with the aid of fresh solvent after each decantation, at the

completion of which the mare was subjected to filtration. Following this treatment the mare was given selective solvent extraction using an extractor of the Soxhlet type, with ether (30 hours), with chloroform (48hours), and with 95 percent alcohol (48 hours).

Treatment of the Skelly Solvent "O" Extract:

The Skelly Solvent "O" portions used to extract the mare were combined and the hydrocarbon solvent removed under diminished pressure in an atmosphere of carbon dioxide. The solvent thus obtained awaits further investigation. The bulk of this carbon dioxide charged yellow colored oil has not become solid or semisolid although it has precipated a small amount of stearin-like material while standing in a stoppered bottle in the dark for a period of 14 months.

A portion of this oil from which the carbon dioxide was removed in a vacuum desiccator over potassium hydroxide has been subjected to analysis in order to determine some of its physical and chemical characteristics. Table II obntains the results of these analyses according to the methods of The Pharmacopoeia of the United States of America XI.

The characteristics of the oil obtained in this laboratory by petroleum-ether extraction of Pe-yok seed kernels agree favorably [illegible] with those of other investigators (see Table I) with the exception of the iodine number which appears to be low for Pe-yok oil of West African variety, and too high to agree with that reported by Bray and Lalip (11) for the South American variety. No further attention has been given this Skelly Solvent "O" extract.

Treatment of the Ether Extract:

Other than the hydrocarbon extract the only extract from the continuous solvent extraction which has received attention to date is the ether

TABLE II

CHARACTERISTICS OF SHELLY SOLVENT "C" EXTRACTED OIL OF PO-YOK SEED KERNELS

Weight of ground seed kernels	600 grams
Weight of Shelly Solvent "C" extract	245 grams
Shelly Solvent "C" extract %	40.1
Specific gravity at 20° C.	0.9694
Index of refraction at 20° C.	1.5114
Acid value	7.575
Saponification value	187.5
Iodine number (Hanus)	99.55
Unsaponifiable matter %	0.47

extract. From it the ether has been removed under vacuum in an atmosphere of carbon dioxide and the pale yellow oil obtained weighed 70.5 grams, 11.8 percent , based on the original weight of the seed kernels. This oil was treated with a dilute solution of sodium carbonate and gently warmed. The resulting soap solution could not be filtered rapidly and during the operation a film was obtained, insoluble in water and ether, amounting, when dried, to 3 grams. This material appears to have been produced as a result of the rapid oxidation of unsaturates and awaits further investigation. The soap solution filtrate was extracted with several portions of some of the same ether previously distilled from the ether extracted oil and the water was removed from this filtrate leaving the sodium salts of the free acids of the ether extract. The amount of these salts was 32.5 grams, a value which bears no significance until these salts are converted into their respective acids since an excess of sodium carbonate was not avoided in this procedure. Further investigation of these salts will be continued.

Conclusions:

A phyto-chemical investigation of Pe-yak seed native to West Africa has been started. Moisture determinations and continuous solvent extractions have been made upon the crushed seeds. The water distillate from the moisture determinations performed upon 600 grams of ground seed kernels failed to disclose the presence of any free water-soluble volatile acids. The Skelly Solvent "C" (petroleum-ether) extract has been examined and its characteristics found to be in agreement with those of other investigators although its iodine number is somewhat low. The ether extract of the mare has been treated with sodium carbonate solution and the resulting products separated into (1) water soluble, (2) ether soluble, and (3) water-ether insoluble portions. The air-dried seed kernel meal also awaits investigation.

BIBLIOGRAPHY

1. Bulletin Imperial Institute 16, 55 (1918); 17, (July 30, 1919); 20, 1 (1922); 30, (May 27, 1932); 33, 271 (1935)

2. Pharm. Era 52, 72 (1919) seen in abstract only in Year Book Am. Pharm. Assoc. 8, 471 (1919)

3. Matsuo, Hodero, Repts. Imp. Ind.Research. Inst., Osaka, Japan 17, No. 12, 1 (1937)

4. Bolton, E. R., and Hower, D. G., Analyst 47, 282 (1922)

5. Bulletin Imperial Institute 20, 1 (1922)

6. Brown, H. B., and Farmer, E. H., J. Chem. Soc. 761 (1935)

7. Steger, A., and van Loon, J., Rec. trav. chim. 53, 197 (1934)

8. Farmer, E. H., and Sunderland, E., J. Chem. Soc. 759 (1935)

9. Steger, A., and van Loon, J., Rec. trav. chim. 57, 620 (1938)

10. Mildbraed, J., Notizblatt des Botanischen Gartens und Museums zu Berlin 7, 485 (1921). Dahlem sowie der Botanischen Zentralstelle für Nutzpflanzen

11. Bray, G. T., and Islip, H. T., Analyst 46, 325 (1921)

12. Rheineck, A. E., Communications, April 11, 1938 and June 7, 1938

13. Anon., J. Ind. Eng. Chem. 11, 156 (1919)

14. Anon., Oil and Color Trades Journ. 88, 1709 (1935)

15. Exper. Sta. Rec. 39, (1919)

16. Van Loon, J., Farben-Ztg. 37, 485 (1931)

17. Jeffery, I., and Stewart, J. R., Paint Var. Prod. Mgr. 14, 28 (1936)

18. Rheineck, A. E., Paint, Oil Chem. Rev. 99, No. 9, 7 (1937)