

not yield this organism in a single instance. Milk from bottles paraffined at 76.6°C. (170° F.) yielded *Escherichia coli* in but few instances. The bottles which were filled with plain broth were entirely free from the organism. Bearing in mind that in such work much heavier inoculations are used than would be encountered in actual practice, the results indicate that paraffination of paper is distinctly germicidal and a practice with real sanitary advantages.

The aerobic spore-forming bacterium was used because it should have survived the treatment. It was used as a control. However, it was destroyed with almost the same regularity as *Escherichia coli*, showing again that paraffination is a germicidal process.

The authors wish to stress the importance of good technique and especially good logic in experimental work on the effect of hot paraffin on micro-organisms. To use a heavy suspension of cells much heavier than the number which would be found in practice, renders the experiments of little practical value. Quantitative results should be sought, for qualitative results might only show that one cell had survived to start growth in the culture tube.

Another warning should be given. To sterilize paperboard by high pressure steam and then subject it to paraffination, gives results of questionable value. Steam sterilization changes the nature of the board and causes it to accept paraffin in a much different manner than regular untreated board. If results of experiments in our laboratories are to be of help in practice, they must be secured under conditions which obtain in practice.

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Licanic Acid of Oiticica Fat and a Study of Its Nutritive Value and Efficiency

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Introduction

Within the past few years considerable attention has been devoted to a newly discovered oil bearing the native name "oiticica" or "oilzika" which is being extracted on a commercial scale from the nuts of a Northeast Brazilian tree, commonly referred to as *Licania rigida*, Bentham, of the Rosaceae family (13, 20). 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 writings of Gardner (13) should be consulted. This oil is of special chemical interest because of an exceptionally high content of the glycerides of an unsaturated ketone fatty acid recently named "licanic acid."

As generally used the designation "oiticica fat" refers to the material obtained by pressing or by solvent extraction of the kernels in contra-distinction to the term "oiticica oil" which refers to the so-called polymerized product of commerce obtained by heat treatment of the oiticica fat. The fat, as Brown and Farmer (8, 9) have recently shown, normally contains the alpha-licanic acid while the oil, or the extracted material from old nuts (36), contains pre-

dominantly 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.

The problem of the nutritive value of licanic acid was suggested by Alfred Rheineck of Devoe and Reynolds Company who kindly supplied for investigation enough of the acid, isolated and carefully protected from oxidation by the Brown and Farmer method (8). A neutralization equivalent of 295.0 obtained in our laboratory was practically identical with the theoretical molecular weight of licanic acid, 295.2.

Review of Earlier Work on Oiticica and Licanic Acid

The earliest chemical investigation of oiticica fat appears to have been made during the first months of 1917 by Bolton and Revis (5), whose work showed that the fat was highly unsaturated. Additional characteristics were not reported until 1929 (2, 37, 59).

Van Loon and Steger (56, 57, 58) apparently were the first to study the fatty acid composition of oiticica fat. They claimed that the high refractive index and high iodine number were due to the presence of a geometrical isomer of elaeostearic acid belonging to a double conjugated system of double bonds. This acid they named "couepic" from *Couepia grandiflora* with

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which they associated their sample of oiticica fat. For a time much confusion prevailed as to the correct identity of the tree from whose kernels the oiticica fat was obtained, but at present it has been definitely established (8, 13) that the oiticica fat of commerce is a product of *Licima rigida*.

What now appears to be the correct characterization of licanic acid was the work of Brown and Farmer (8) which has been confirmed by Kappelmeier (21) and by Morrell and Davis (40). Brown and Farmer believed their acid the same as the couepic acid of Van Loon and Steger but declared it to be a ketotriene acid with the formula, $C_{18}H_{28}O_3$, and proposed the name "licanic acid," with the configuration suggested by the chemical name 4-keto-octadecatrienoic acid, 9, 11, 13.

Licanic acid is thought to be the first naturally occurring unsaturated ketone fatty acid to be described and 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.

The Nutritive Value and Efficiency of Licanic Acid

As the most rational method of approach to this study it was thought advisable to determine by experiment if possible to what extent licanic acid could replace an ordinarily high quality fat such as butter in the normal diet. Since it had been repeatedly observed in this laboratory (27) that rats fed on whole cow's milk mineralized with iron, copper, and manganese, not only made excellent growth, but assimilated the milk solids as effectively as the solids of a good stock ration, mineralized skim milk was selected as the basal diet for a fat-free ration.

Experimental Part

For this investigation young albino rats 19 to 21 days old were placed in separate cages, fed morning and evening, weighed at regular intervals, and upon each 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. of skim milk 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. All rats were submitted to irradiation for a period of ten minutes each day.

No difficulty was experienced in making up the butter fat rations since the butter could be melted, weighed, and the proper amount added to the warmed skim milk, the use of a small hand homogenizer serving to emulsify the mixture. Since 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 promptly resealed in an atmosphere of carbon dioxide. It was found that the fairly soft texture of the licanic acid permitted its homogenization with the warm skim milk without previous melting and that the emulsion so formed was as permanent as the cream in the butter fat rations.

The feeding experiments may be divided into three groups, the second and third arising, as will be pointed out, from the findings with the first group. Six rats were used in the first experiment; two were fed

mineralized skim milk containing 4 per cent butter fat (the average amount present in whole milk) as a positive control; two were fed a diet of mineralized skim milk containing 4 per cent licanic acid; and two were fed unsupplemented skim milk as a negative control. In Table 1 will be found a summary of the data obtained. At the end of one week one of the rats on the licanic acid diet had died and since the other rat on the same diet and those on the unsupplemented skim milk diet 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 and it was expected that the rats on the unsupplemented skim milk ration would die since from past experience it was known that rats must have attained an age of six weeks or more before they can be subjected to a fat depleted diet with assurance that they will survive. Although the total solids consumed by the rats on licanic acid was only little more than half of that consumed by those 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 the rats on butter fat, although their increase in weight of 1 gram for every 1.85 grams of milk solids ingested is 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 (27) for slightly older young rats. However, rats reported in the previous paper had reached a weight of 60 grams compared with the average weight of 35 grams at the beginning of this experiment. Young rats would naturally be expected to show more rapid development and the figure quoted above from previous data covers a much longer growth period in which the animals had shown an average increase of 140 grams, i.e., from 60 to 200 grams.

It was apparent from experience with rats in the first experiment that there did exist some aversion for 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 licanic acid the following experiment was carried out upon four rats fed diets unrestricted as to the amount consumed daily. The diets of two of these consisted of mineralized skim milk with 2 per cent added butter fat; the diets of the other two consisted of mineralized skim milk with the addition of 2 per cent each of butter fat and licanic acid. It was obvious that with this arrangement if the licanic acid had any nutritional value in so far as the growth principle was concerned it would become apparent. In Table 1 will be found a summary of the data obtained on these animals. Rats on the 2 per cent butter fat supplement made good growth and nearly attained a normal gain of about three grams daily. It will be noted from the data that rats on the 2 per cent licanic acid plus 2 per cent butter fat supplement 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 on the diet containing 2 per cent butter fat. These data seem to indicate that butter fat is superior to licanic acid in nutrient value.

Since there still remained the doubt as to whether licanic acid had any measurable nutritional value the following experiment was begun in an attempt to

decide this issue: four rats were fed restricted diets, that is, restricted daily to the amount of total solids which rats on the 2 per cent licanic acid plus 2 per cent butter fat diet had voluntarily selected. This third experiment was in every particular a duplicate of the procedure used in the second. The diets of two rats consisted of the restricted daily amounts of mineralized skim milk containing 2 per cent butter fat; the diets of the other two rats consisted of the restricted daily amounts of mineralized skim milk containing 4 per cent butter fat. The duration of the experiment was 26 days the same as that of the second.

It seems reasonable to believe that if licanic acid possessed the same degree of nutrient efficiency as butter fat it should have given the same results as the ration supplemented by 4 per cent butter fat. Study of the records (Table 1) of rats on the restricted diet containing 4 per cent butter fat shows their approximately normal growth, 2.37 grams of milk solids being required to produce 1 gram gain in weight compared with 3.97 grams of total solids required to produce the same result with the rats on the licanic acid-butter fat ration. These data indicate licanic acid to be greatly inferior to butter fat in nutrient value. Furthermore, when the performance of rats on the restricted diet supplemented with 2 per cent butter fat is considered, it is to be noted that with 3.59 grams of

total solids required to produce 1 gram gain in weight, these animals actually outgrew those receiving the additional licanic acid. It would appear then that the licanic acid not only was worthless as a food but actually under the conditions of this experiment proved slightly deleterious to the growth of the rat, a result difficult to explain in view of the constitution of licanic acid and the expectation that it would function just like any other unsaturated fatty acid in supplying the animal with energy.

Relation of Licanic Acid to the Utilization of Lactose in Milk

It has been previously shown in this laboratory (45) that rats fed a mineralized whole milk diet made very efficient utilization of all the milk sugar, but that this was not the case with animals fed 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 demonstrated that the feeding of certain fats such as butter, lard, linseed oil, palmitic acid, oleic acid, etc., in quantities of 3 to 4 per cent as supplements to mineralized skim milk, prevented this loss in the urine, which the lower organic acids such as butyric, lactic, caproic, etc., failed to do.

Since a number of other fats were being tested for their effect on the utilization of lactose, licanic acid was included in the list. Accordingly, as previously arranged, rats on the diet of 2 per cent licanic acid plus 2 per cent butter fat and rats on the diet of 2 per cent butter fat were subjected regularly to confinement in metabolism cages and to urine collections. The data secured, together with the results of calculations made to determine the percentage of galactose, the percentage of ingested lactose lost, and the percentage of ingested galactose lost, in the urine, may be found in Table 2. At the end of the 26th day the diet of the rats was changed to mineralized skim milk in order to determine if those not eliminating sugar in their urine would void it. In each case the animals did void sugar and also showed an immediate and rapid loss in weight from this change to a fat-free diet.

The results of this work prove that licanic acid acts as a prophylactic in preventing elimination of sugar in the urine, i.e., the complete utilization of the ingested lactose in milk. This is a positive action particularly in view of the fact that the rats fed the mineralized skim milk plus 2 per cent butter fat supplement always showed a slight amount (and on several occasions considerable) of sugar in the urine. This is an interesting observation in consideration of the facts already presented that licanic 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 *Licania rigida* has been made and the more important chemical contributions briefly 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 licanic acid possesses no apparent energy value and that under the conditions described is slightly deleterious to the growth of young rats.

3. Licanic 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.

TABLE 1

	Average figures for rats in first experiment			Average figures second experiment		Average figures for rats in third experiment	
	Mineralized skim milk + 4% butter fat	Mineralized skim milk + 4% licanic acid	Mineralized skim milk only	Mineralized skim milk + 2% butter fat	Mineralized skim milk + 2% butter + 2% licanic acid	Mineralized skim milk + 2% butter (restricted intake)	Mineralized skim milk + 4% butter (restricted intake)
Avg. daily consumption cc.	28	16	43	45	31	31	31
Avg. total solids daily, gm.	3.43	2.08	3.87	4.90	3.97	3.41	4.03
Avg. daily gain in wt., gm.	2.0	-1.65	-0.7	2.0	1.1	0.95	1.7
Gm. milk solids to prod. 1 gm. gain	1.85	negative	negative	2.45	3.97	3.59	2.37

TABLE 2

Rat 8 (Representative of several animals)					
Mineralized skim milk plus 2% butter fat (started Aug. 18, 1938)					
Date	Milk cc.	Urine cc.	Galactose in urine %	Ingested lactose lost in urine %	Ingested galactose lost in urine %
Aug. 30	40	18	0.49	4.5	9.0
Sept. 13	35	35	0.45	8.9	17.8
Sept. 13 changed to skim milk					
Sept. 20	50	40	0.55	8.8	17.6
Rat 10 (Representative of several animals)					
Mineralized skim milk plus 2% butter fat plus 2% licanic acid (Aug. 18, 1938)					
Aug. 30	39	27	none	none	none
Sept. 1	39	30	none	none	none
Sept. 13	70	36	none	none	none
Sept. 13 changed to skim milk					
Sept. 20	70	51	0.57	8.3	16.6

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Disposition of Soap in Detergent Operations

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DETERGENT operations usually involve the presence of fabrics, soil in intimate contact with the fabrics, and water containing more or less hardness in the form of calcium and magnesium salts. The object of the operation, of course, is to remove the soil from the fabric and to maintain the soil in a state of deflocculation. In accomplishing this result there are four principal dispositions made of the soap. A portion of the soap is used in satisfying the demands of the calcium and magnesium ions present. A second portion of the soap is adsorbed from the solution by the fabric. The soil removed from the fabric during the detergent operation requires a third portion of the soap to effect deflocculation, and finally, a definite effective concentration of soap solution must remain after satisfying the three forementioned demands to provide the desired degree of lathering power and detergency, this concentration being determined by the composition of the soap and the temperature of the detergent operation.

When suds and detergency tests are determined as a function of the concentration of the soap solution, a suds or a detergency curve is obtained that has the characteristics of Figure I. A certain minimum value of the concentration is necessary before sudsing action appears and there is a maximum concentration beyond which improvement in sudsing action does not result; similarly with the detergency curve but the location of the curve along the concentration axis does not necessarily correspond to the location of the curve for the sudsing action. The position of the two curves shifts to the left or right along the concentration axis

depending on such factors as the fatty acid composition of the soap, the amount and nature of alkaline builders, the degree of hardness of the water, the temperature of the solution, the kind and amount of the fabric load, and the kind and amount of soil present. The interesting point is that each combination of conditions results in a fairly definite concentration at which sudsing action and detergent action become effective. The minimum concentration at which good detergency is obtained in the absence of water hardness, fabric load, and soil load, may be considered the effective concentration mentioned earlier as being required to produce adequate detergency after satisfying the other three factors.

Attention has been called to the fact that the fabric load affects the concentration required to produce satisfactory sudsing and detergent properties. This fact has been studied by Neville & Harris (1) and by Williams, Brown and Oakley (2) who noted that fabrics selectively adsorb alkali in greater degree than the fatty acid. Acharya and Wheeler (3) expressed their belief that an association exists between adsorption and cleansing power. Adams (4) showed that the amount adsorbed varies considerably with the different detergents and that this adsorption is sufficient to reduce the effective concentration of the detergent. If fabrics adsorb alkali to an extent in excess of the fatty acid radical, as stated by Williams and others, it follows that definite disturbances must result in the nature of the soap remaining in solution. Investigators which include McBain (5, 6, 7, 8, 9) Hartley (10), Rebinder and Petrova (11), McBain & Salmon