The Latex of Ficus Pumila L.*

I. HISTORY, PROXIMATE COMPOSITION AND ANTHELMINTIC PROPERTIES

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Botany: Ficus pumila L. was first described scientifically by Linne,[1] who bestowed on it its present name. The etymology of the generic name is to be found in the old Latin *ficus,* fig tree, the common name for the type species of the genus, *Ficus Carica* L. The specific name *pumila* is derived from the Latin pumilo, meaning dwarf or pigmy. The original habitat, according to Linne, was China and Japan. King,[2] in a monograph on the species of Ficus of the Indo-Malayan and Chinese countries, published by the Royal Botanical Garden of Calcutta in 1883, describes very critically this species. He mentions the fact that considerable confusion has arisen in the nomenclature of this plant from the dimorphism of its leaves. The synonymy has been very carefully disentangled by Maximowicz[3] in a paper published in the bulletin of the St. Petersburg Academy of Science.

In a recent communication on this species by Cheng,[4] a variety is recorded called *ellipsoidea* Cheng, var. nov. It differs from the type by its oblong ellipsoid receptacles, 4.5-5.5 cm. long, with impressed lenticels. The original species and its variety have been collected, according to that author, in more than half a dozen different localities throughout China.

In Puerto Rico and the Virgin Islands this species has been described by Britton[5] as follows:

Ficus pumila L. Creeping Fig. Asiatic, commonly grown on walls in Puerto Rico and occasionally in the Virgin Islands, is a small-leaved vine, adhering to walls by its numerous aerial roots; it sends out horizontal branches, with large ovate or elliptic leaves 5-9 cm. long and obovoid figs about 5 cm. long.

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Dragendorff[6] mentions the use of the unripe fruit as an external remedy in China, but does not specify for what particular disease. This plant is known in that country by various names: *Mulien, Pi-li, Mu-*

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man-t'ou, and *Kuei-man-t'ou.* The first name given above is also used for *Magnolia,* while the third is equally applied to *Ficus stipulata* and probably also to *Ficus Carica.* Probably the most distinctive name is the second. Quoting from Dr. Stuart:[7]

> The leaves are large and round, and if bruised, exude a white juice, like varnish. This suggests its similarity to *Ficus indica,* the source of gum-lac. The plant is a creeper and bears a hollow "fruit" of red color. This product is much esteemed by the birds, which eat of it with great avidity. The leaves are used in medicine in the treatment of dysentery, hematuria and locally as an application to carbuncle. The juice of the vine is also employed in the treatment of skin diseases. The whole plant is thought to have a beneficial action upon the virile powers, and is therefore used in the treatment of spermatorrhoea, and as a galactogue. The plant when eaten is said to remove pain in the heart.

In the West Indies, so far as we have been able to find out, no part or product of *Ficus pumila* L. has been used as a medicine. As a matter of fact, the only written records found in the literature consulted about the medicinal uses given to this plant are the ones described above.

Latexes of many other species of ficus, however, have been used for centuries as drugs. Dioscorides (Pedacios Dioscorides Anazarbeo)[8] mentions the use of the latex of *Sukon en Kupro* (*Ficus Syncomorus*) and *Suka* (*Ficus Carica*) as remedies. They were used by the ancient Greeks as laxatives, in menses, in gout, in leprosy and skin diseases, in the bites of poisonous animals, in toothache and as milk coagulants.

Pliny the Elder (Caius Plinius Secundus A.D. 23-79)[9] mentions the use of *ficus* (common fig) in medicine.

1826. Ainslie[10] writes in his *Materia India* that the latex of *F. indica* L. is applied in toothache to the tooth or gums, and also to the soles of the feet when cracked or inflamed. The root exudate of *F. racemosa* is considered a powerful tonic.

1861. Peckolt[11] mentions the use of the latex of ficus against ancylostomiasis in Brazil. A chemical study is reported of *Ficus silvestris* St. Hilaire and *Ficus doliaria* Mart. A substance in the crystalline form was obtained by him from the latex of *F. doliaria* Mart. The name of doliarina was assigned to this substance, which was found to be a glycoside. He also reports having obtained from this same latex a vegetable pepsin named by him, urostigma papayotin.

1880. Bouchut[12] investigated the milky juice of the common fig tree, *Ficus Carica* L. He found a digestive ferment present which acted very readily on fibrin.

1880. Bozzolo[13] states that he had learned of the use in Brazil of a potent anthelmintic against hook-worm, named doliarina. According to

him, this remedy was prepared by Peckolt of Rio de Janeiro and marketed under the name of "Powder of Doliarina and Iron—a Specific against Opilaçao," opilaçao being the Portuguese name for the anemic condition resulting from anchylostoma infection. This powder is a mixture of crystals of doliarina, iron (in what form the iron enters into this mixture is not mentioned) and an aromatic vegetable substance. The doliarina is obtained from the juice of *Ficus doliaria* Martius in a crystalline form. He mentions having used this preparation with success in the treatment of ancylostomiasis at his clinic in Turin.

1881. A short unsigned article[14] in the *Paris Médical* discusses the use as an anthelmintic of the juice of *Carica papaya* L. in Reunion and the West Indies, and points to the probable similar mode of action of this juice and the ficoin and doliarina obtained respectively from *Ficus carica* and *Ficus doliaria*. The rest of the note gives an account of Bozzolo's work on the effect of doliarina on ancylostomiasis.

1882. Moncorvo, a physician of Rio de Janeiro, Brazil, is quoted in the *Lancet*[15] as stating that the most effective treatment against the peculiar anemia due to the presence of the *Ancylostoma duodenale*, consisted in the administration of the juices of *Ficus doliaria* or *Ficus gamellaria*. According to him it is an old native remedy for the indigenous disease, and he is inclined to think that its drastic and vermifuge properties are due to the presence of doliarina, a sort of vegetable pepsin, analogous to, or perhaps identical with, the papain of *Carica papaya* L. It is stated that the substance has already been administered with advantage to the St. Gothard sufferers.

1882. Christy,[16] in *New Commercial Plants and Drugs*, a publication printed by the drug firm of Thomas Christy and Co., lists *Ficus doliaria* and quotes the *Lancet's* note.

1885. Hansen[17] states that the latex of the common fig contains a peptonizing ferment which resembles pepsin in its effects upon fibrin and the coagulation of milk, and produces the diastatic reaction of the conversion of starch into sugar. A syrup made from the dried figs has the same peptonizing properties as the latex, which suggests its possible usefulness for children as an adjunct to starchy foods.

1890. Mussi[18] reports the isolation of the ferment responsible for the digesting properties of the latex *Ficus Carica*. He precipitated from the aqueous portion of the latex, on the addition of absolute alcohol, a yellow amorphous mass. This, when treated with water, swelled up and imparted a milky appearance to the liquid. The residue left on filtration of this suspension is very active, while the filtrate is inactive. This

residue is insoluble in water but dissolves readily on the addition of traces of alkali or acid. Mussi named this ferment, cradina. It contains nitrogen. This amber yellow powder differs from pepsin in maintaining its digestive power in alkaline solution and from papain, in being insoluble in water. In neutral liquid it is devoid of digestive power and it has no action on starch.

1892. Millspaugh[19] reports that *F. daemona* Vahl. and *F. toxicara* L. yield extremely virulent juices.

1893. Theodoro and Gustavo Peckolt[20] in their interesting book, *Plantes Medicinales do Brazil,* give a complete account of the medicinal uses accorded the latex of different species of ficus in that country. They quote the names of several doctors in Brazil who have used the latexes of *Ficus anthelmintica* Mart., *Ficus glabra, Ficus doliaria* Mart. against ancylostomiasis and ascariasis. They also report in this book the isolation of a crystalline substance from the latex of *Ficus doliaria* Mart., to which they give the name of doliarina which is, according to them, a glycoside. The elementary analysis was performed, according to Peckolt, by Dr. Nortmann of the University of Vienna, who assigned to it the following empirical formula, $C_{28}H_{20}O_2$. Besides this substance they found the following others present:

Water	60.00
Urostigma-papayotin (vegetable pepsin)	1.66
Crystalline doliarina	5.65
Caoutchouc	11.13
Sugars	5.00
Wax and resin	0.31
Soft resin	1.16
Bitter principle	0.21
Albuminoids, gums, tannin, etc.	14.37

As this particular latex is used very widely against ancylostomiasis, they had developed since 1863 a pharmaceutical preparation in which the principal constituents were the crystalline doliarina and the vegetable pepsin of the latex. These substances were mixed with an assimilable form of iron (they do not specify which particular iron compound) and the preparation was sold under the name of "Powders of Doliarina and Iron." The dose for this powder is 0.05 to 0.10 grams per day. They do not seem to know exactly which one of the constituents of their latex is the one responsible for the anthelmintic activity. In their pharmaceutical preparation they included the crystalline doliarina as well as the vegetable pepsin; the latter one being the active principle.

1913. Tschirch[21] mentioned the latex of various species of ficus used as anthelmintics.

1913. Gerber[22] reports that the latex of *Ficus coronata* contains a vegetable pancreatic juice containing a proteolytic enzyme, but no amylase.

1914. Monat-Biggs[23] reported on the treatment of ancylostomiasis in Venezuela. This consists simply in taking a spoonful of the milk of the *higueron* (*Ficus laurifolia*) in the morning, at mid-day and night, for three consecutive days. On the morning of the fourth day the patient must take 30 gm. of Glauber's salt. The dose for adults amounts to about 30 gm. of milk of *higueron*. For children, half the adult dose is used. This treatment was adopted by the Venezuelan Government in the suppression of ancylostomiasis in the Republic. It is also good for trichuriasis, and is used in many other countries in Tropical America.

1914. Paez,[24] a Venezuelan physician, recommends the drug as an anthelmintic useful against ancylostomiasis, but more powerful against trichuriasis. The best plan, according to him, is to give a saline purge on the evening before; then, early next morning, the latex, which is then followed two or three hours later by another purgative. Three or four treatments are generally sufficient. He leaves the question of the active principle open. According to him, the species most used is *Ficus glabrata,* although he agrees that the latex of many other trees of the same genus have the same properties.

1914. Sandwith[25] states that the latex of *Ficus doliaria* Mart. has been used in Brazil for dozens of years against trichuriasis and ancylostomiasis.

1925. Schapiro,[26] in Panama, administered the latex of *Ficus elastica* to five cases of trichuriasis with very successful results.

1927. Hall[27] treated a small group of patients in Nicaragua with *leche de higueron* and observed that it was the only drug that could be depended upon to remove whipworms almost every time (*Trichuris trichiura* L.)

Fernan Nuñez[28] writes:

> For centuries the indigenous of South America have employed the sap of the tree, *Ficus laurifolia,* known locally as *leche de higueron* in the treatment of all classes of intestinal parasites. I heard it discussed by the peons of Colombia, and used with very good results against tricocephalus. It is not generally employed by physicians there, because the sap must be fresh, and it is not easily obtained outside the river valleys. Its active principle has not been marketed; indeed, so far as I know, the chemistry and pharmacology of the drug have scarcely been studied.

1927. Nadkarni[29] in his *Indian Materia Medica* reports that *F. indica* is used externally in bruises, rheumatism, lumbago, toothache, cracked

hands and feet, and internally in dysentery and diarrhea. The concentrated juice is also used as an aphrodisiac. The milky juice of *F. oppositifolia,* according to this writer, is poisonous.

1929. Caldwell and Caldwell[30] studied the effect of *higuero* latex in the treatment of trichuriasis. A preparation of the sap of *Ficus laurifolia,* higuerolatex, kept cool in a dark bottle for almost a year before use was found to be highly efficient in the treatment of both trichuriasis and ascariasis.

1929. Hall and Agustin[31] administered various doses of the sap of *Ficus laurifolia* to Colombian soldiers. It was either fresh or else sterilized at 60° C. an hour a day for three successive days and an alcoholic solution of thymol added as a preservative to make 1:1000 solution. The preserved latex remained, according to their account, in good condition during the time the investigation lasted. They found that the drug apparently has limited usefulness in combating hookworm.

1930. Robbins[32] made very interesting and valuable experiments with the crude sap of *Ficus laurifolia.* He found the active principle responsible for the anthelmintic activity of the latex to be a substance of protein-like nature. The latex contained 25 per cent by weight of this substance, which can be precipitated with $HgCl_2$ or $MgSO_4$, or as a waxy mass when 3 parts of acetone or 5 parts of alcohol are added. After dissolving and reprecipitating various times he obtained a light yellowish powder containing the active anthelmintic principle. The purified protein-like substance is about 12 per cent of the latex. This substance gives the positive test for free amino groups and sulphur, containing respectively 16 per cent of the first and 1.5 per cent of the latter, values which are well within the normal range for proteins. Also this substance gives all the usual color tests for proteins.

The name of ficin, from Ficus, the generic name of the tree from which *leche de higueron* is obtained, has been given by Robbins to this fraction of the latex containing the active principle.

In concentrations of 0.1 to 0.2 per cent in Ringer's solution, ficin will digest living ascaris in about two hours. A temperature of 75°C. or above inactivates this substance. The optimum hydrogen-ion concentration for ficin lies somewhere between 4 and 8.5. The enzymatic activity is destroyed at pH below 4. This may explain why an enormous anthelmintic dose of crude material is given, as a large portion may be destroyed on passing through the stomach, which has a pH around 2 to 3. This indicates also that the enzyme is not of the pepsin, but rather of the trypsin class, as it will not act in a highly acidic medium. The possibility is also suggested of two agents present in ficin, one which

kills the tissue and another which causes digestion of the dead material.

1931. Hoffman[33] suggested the use of enteric-coated capsules in the administration of the active principle of these latexes, to protect it from the low pH during the passage through the stomach.

1934. Robbins and Lamson[34] studied the sap from sixteen species of the genus *Ficus* and found proteolytic enzyme in three cases, but in two only was the concentration high enough to digest ascaris in 2 per cent solution of sap:

Ficus aurea	from S. A.	inactive
Ficus benghalensis	from S. A.	inactive
Ficus benjamina	from S. A.	inactive
Ficus brevifolia	from S. A.	inactive
Ficus carpensis	from S. A.	inactive
Ficus crassinervia	from S. A.	inactive
Ficus elastica	from S. A.	inactive
Ficus glabrella	from S. A.	inactive
Ficus glamarata	from S. A.	inactive
Ficus nota	from S. A.	inactive
Ficus religiosa	from S. A.	inactive
Ficus sprageana	from S. A.	inactive
Ficus vogelii	from S. A.	inactive
Ficus nitida	from S. A.	low conc.
Ficus carica	Alabama	conc. high enough to digest ascaris
Ficus glabrata	S. A.	conc. high enough to digest ascaris

1935. In the Republic of Colombia, South America, milk of *higueron* has been put on the market by two different concerns[35] under the trade names of Tricosan and Ficosan respectively. The dose given of these stabilized forms of latex is as follows:

For children between 2 and 3 years,	3 cc.
For children between 4 and 5 years,	6 cc.
For children between 6 and 15 years,	10 cc.
For adults	20 cc.

It is recommended against Ascaris, Trichuris and Necator, and also against association of these parasites.

1936. Robbins[36] has found that the optimum hydrogen-ion concentration for the ficin-gelatin proteolysis is pH 5. He has also determined the seasonal variation in ficin content in the latex of *F. Carica* L., finding it to be highest in the winter and lowest in the summer.

1936. Williams[37] in a paper on the flora of the woods of northeastern Peru mentions the use given by the natives to the latex of some of the

Ficus collected. He mentions the following: *F. anthelmintica* Mart., a creamy latex used in native medicine as a remedy for anemia; *F. caballina* Standl., exudates a fair quantity of insipid latex which coagulates readily and is used locally to heal wounds and skin infections; and, *F. glabrata,* a latex astringent. Highly esteemed locally as a vermifuge.

1937. *The United States Dispensatory,* 22nd Edition,[38] says:

> Under the name of "leche de higueron" the latex of *F. laurifolia* and probably also *F. glabrata* is used by the native of South America and Panama as vermifuge . . . it is a remedy of real value, especially against trichuris and also against ascaris.

1937. Walti[39] from the research laboratories of Merck and Co., announced at a meeting of the Society of Biological Chemistry that he had obtained crystalline ficin. According to his report the crystalline substance has a sulphur content of 1.6 per cent. This work is still unpublished. Recently this investigator has published a short note on his method.[40] He adjusted the pH of his clarified latex to 5, using NaOH, and then left the solution in an ice box at 50°C. After several weeks, crystals appeared. These crystals had the activity manifested by the original latex. No details are given, however, in this note as to the method of clarification or as to the general technic followed.

Recently Faust[41] has treated several hundred cases of tricocephaliasis (*Trichuris trichiura* L.) using the crude latex from a South American species, obtaining excellent results and having no cases of intoxication.

The pharmacopoeias in which Ficus latexes are official are listed as follows:

Country	*Edition*	*Year*	*Ficus species*
Mexico	1	1874	*Ficus complicata* H.B.K.
Mexico	2	1884	*Ficus complicata* H.B.K.
France	3	1866	*Ficus elastica* Roxb.
Spain	7	1905	*Ficus elastica* Roxb.
France	3	1866	*Ficus indica* L.
Spain	7	1905	*Ficus religiosa* L.

These latexes are mentioned in these pharmacopoeias probably because they were used medically in these countries as adhesives rather than as anthelmintics. Besides the pharmacochemical investigations reported in the preceding perusal of the literature the following purely chemical investigations on the composition of the latexes of several species of *Ficus* are tabulated here following the alphabetic order of the specific name.

TABLE I

Chemical investigations on the latexes of several species of ficus

Genus and Species	*Investigator*	*Year*	*Ref. No.*	*Chemical Constituents Reported*
Ficus alba Reinw.	Ultée	1922	42	A wax present in large amounts, m.p. 60°. On hydrolysis it yields stearic acid, β amyrin and lupeol, free, as the acetate and also as the benzoate.
Ficus benjamina L.	Ultée	1923	43	Cerotic acid present in the wax.
Ficus callosa Willd.	Ultée	1923	43	4.25% nitrogen. Contains little or no rubber.
Ficus Carica L.	Mussi	1893	18	Caoutchouc, cerotic acid, and a white substance insoluble in water and in ordinary organic solvents.
Ficus ceriflua Jungh.	Kessel	1878	44	Wax from the latex consists of two substances easily soluble in ether $C_{15}H_{30}O$ m.p. 73°, and $C_{27}H_{56}O$ m.p. 62°. Slightly soluble in ether. The purified wax yields on dry distillation $C_6H_{12}O$ m.p. 62°, b.p. 345–354°.
Ficus ceriflua Jungh.	Greshoff and Sack	1901	45	Wax from the latex yields principally a crystalline compound $C_{30}H_{52}O$ m.p. 61°. On hydrolysis it yields ficoceryl alcohol $C_{17}H_{28}O$ m.p. 198°, and fico-ceroic acid.
Ficus ceriflua Jungh.	Greshoff and Sack	1901	45	$C_{13}H_{26}O_2$ m.p. 57°. When the wax is subjected to dry distillation it yields acetic and propionic acids, a hydrocarbon $C_{14}H_{26}$, b.p. 220°, and two crystalline acids $C_{44}H_{88}O_2$ m.p. 51° and $C_{12}H_{24}O_2$ m.p. 54°.
Ficus ceriflua Jungh.	Ultée	1915	46	No caoutchouc. A wax m.p. 60–64° present containing 94% of resin. This wax yields b-amyrin m.p. 197° and lupeol acetate.

TABLE I—*Continued*

Genus and Species	*Investigator*	*Year*	*Ref. No.*	*Chemical Constituents Reported*
Ficus ceriflua Jungh.	Wood and LaWall	1937	47	Pisang wax m.p. 79–81°. Pisang cerylic acid $C_{24}H_{48}O_2$ m.p. 71°. Pisang cerylic alcohol $C_{13}H_{18}O$ m.p. 78°.
Ficus elastica	Harries	1904	48	$C_{20}H_{32}O_2$ sinters at 185°, melts at 195°. Soluble in ether.
Ficus fulva	Ultée	1922	49	Large quantities of a wax m.p. 52–58° which on hydrolysis yields stearic acid and a phytosterol m.p. 199–203°.
Ficus glabella Bl.	Ultée	1923	43	Cerotic acid in the wax.
Ficus glomerata Roxb.	Ultée	1923	43	Cerotic acid in the wax.
Ficus magnolooides Borc.	Harries	1904	48	A compound $C_{30}H_{48}O_3$ present and also para caoutchouc $C_{10}H_{16}$.
Ficus rubiginosa Desf.	Warren de la Rue and Müller	1862	50	Resin cycoceritin. Acetic acid compound $C_{20}H_{16}O_2$which yields ficoceryl alcohol.
Ficus rubiginosa Desf.	Rennie and Goyder	1892	51	$C_{34}H_{56}O_2$. $C_{32}H_{54}O$.
Ficus variegata				See *Ficus ceriflua*.
Ficus Vogelii Miq.	Spence	1907	52	Two isomeric substances $C_{16}H_{26}O$ m.p. 201–205° and m.p. 154°. Not acid or alkalies. Named α and β alban respectively.
Ficus Vogelii Miq.	Ultée	1921	53	The α and β albans are amyrin acetate and lupeol respectively.

SUMMARY

The latex of various species of ficus according to this review, seems to have been a favorite remedy against parasitic diseases among the population of Central and South America. In no other part of the world is it mentioned as having been used for this purpose.

Its efficacy seems to be specific against certain species of parasites; especially is it valuable against such diseases as trichuriasis and ascariasis. On the other hand, although it has been used against hookworm very widely, the results reported are conflicting and they seem to point to the fact that it does not act so efficiently against this parasite.

These latexes do not seem to contain any powerful toxic substances with the exception of *F. doemona* Vahl., *F. toxicaria* L. and *F. oppositofolia.*

The latexes of the species used as anthelmintics are usually given in doses of 20 to 30 cc. There is no report in the literature consulted on toxic effects having been observed. Nevertheless, the pharmacological phase of the question is still open to investigation, as nothing is known definitely about the action that the active principle present in these latexes might have on the intestinal wall. In the long run they might produce lesions and ulcers of similar character as those produced on the parasites lodging in this tract. Further research along this line may answer this question definitely.

It has been quite conclusively demonstrated that the active principle of these latexes resides in the protein fraction. The isolation of the active principle in a crystalline form will greatly help in the final evaluation and standardization of this substance as a drug. The crude latexes will never be a reliable medicine, as too many variable factors affect the amount of active principle present in them. On the other hand, isolating the active principle responsible for the anthelmintic activity will insure a reliable uniformity in the drug which will make it possible to adjust their efficiency to a definite strength. Recently, the isolation of the active principle in a crystalline form has been announced. This substance is a proteolytic enzyme.

The chemical constitution of the latex of different species of *Ficus* is by no means clear. The presence in various species of a waxy substance with reported melting points ranging from 60 to 81°C. is indicated in the literature, which wax in all probability is a mixture of several substances. Also the presence of amyrin and lupeol has been established in various latexes as well as palmitic and cerotic acid. Caoutchouc is

present in all of them in various amounts. The great majority of the other compounds reported are still in the process of identification.

EXPERIMENTAL PART

I. Preliminary Examination

The preliminary examination was carried out in connection with the latex obtained from a *Ficus pumila* L. vein. The plant contains a milky sap or latex, which is most abundant at the junction of the figs and the stem.

Moisture content. Duplicate determinations were made by the xylene method.[54] In each determination 7.3 g. of latex were used. Yield, 70.0 per cent and 69.0 per cent respectively.

Upon boiling with xylene the white latex became yellowish and granular. When separated, this weighed 0.6 g. The xylene solution, after having been separated from the granular deposit, was allowed to evaporate at room temperature. The caoutchouc-like residue weighed 1.6. grams.

Ash determination. The determinations were made according to the directions in the U.S.P. XI.[55]

I. 2.0634 g. latex yielded 0.0210 g. ash = 1.02%
II. 2.0630 g. latex yielded 0.0211 g. ash = 1.02%

The percentages of water-soluble and acid-soluble ash are herewith tabulated:

Water-soluble ash	0.14%	0.15%
Water-insoluble ash	0.88%	0.87%
Total ash	1.02%	1.02%
Acid-soluble ash	0.97%	0.96%
Acid-insoluble ash	0.05%	0.06%
Total ash	1.02%	1.02%

Test for cyanogenetic glucoside. The possibility of a cyanogenetic glucoside having suggested itself, duplicate tests were made according to the method described by Dohme and Herman.[56] Both tests gave negative results.

Test for oxidases. The latex gives positive reaction for oxidases. When guaiac reagent is added to the latex, a very deep coloration is obtained immediately, showing the presence of oxidases.

Action of latex on milk. As latexes from other species of *Ficus* have been reported to coagulate milk, some fresh milk was subjected to the

TABLE II

Action of solvent on the latex

Solvent	*When added*	*On shaking*	*Residue after evaporation of solvent*	*Remarks*
Hydrocarbons				
1. Petroleum ether	Immiscible. Latex at the bottom.	Immiscible.	Crystals deposited. Needles.	
2. Benzene	Immiscible. Latex at the bottom.	Emulsion formed. Separates after a while.	No crystals. A pattern-like system of crevices.	
3. Toluene	Immiscible. Latex at the bottom.	Emulsion formed. Separates after a few minutes.	Crystal bundles present. Not very many.	
4. Heptane	Latex at the bottom.	Emulsion formed. Separates slowly. Latex at the bottom.	Crystals deposited. Needles.	
5. Kerosene	Latex at the bottom.	Emulsion formed. Separates slowly. Latex at the bottom.		
Halogen substitution products				
1. Chloroform tetrachloride	Immiscible. Latex on the surface.	Emulsion formed. Separates quite rapidly.	Thread-like bundles of crystals?	

TABLE II—*Continued*

Solvent	*When added*	*On shaking*	*Residue after evaporation of solvent*	*Remarks*
2. Carbon tetrachloride	Immiscible. Latex on the surface.	Emulsion formed. Separates after a while.	A pattern-like system of crevices produced on drying or else crystals at a very early stage of formation.	
3. Ethylene dichloride	Emulsion formed. Separates slowly. Latex rises to the top.	Emulsion formed. Separates slowly.		
Alcohols				
1. Methyl	Latex coagulates at the bottom.	Emulsion formed. Separates slowly.	Very small bundles of crystals.	
2. Ethyl	Immiscible. Latex at the bottom.	Emulsion formed. Does not separate immediately.	Few crystals.	After 24 hrs. a strong odor of acetaldehyde is noticeable. The upper part of the latex has acquired a fleshy color.
3. Isopropyl	Immiscible. Latex at the bottom.	Latex coagulates and becomes brown after 24 hours.	No crystal formation. Amorphous granules only.	

TABLE II—*Continued*

Solvent	*When added*	*On shaking*	*Residue after evaporation of solvent*	*Remarks*
4. Butyl	Immiscible. Latex at the bottom.	Latex becomes flocculent. Settles on the bottom.	No crystals.	
5. Amyl	Diffusion of the latex into the amyl alcohol takes place immediately.	Even emulsion formed. After standing for a few hours three distinct layers formed.	No crystals.	
6. Phenol	Coagulates latex.	Emulsion formed.	No crystals.	
Aldehydes and Ketones				
1. Formaldehyde	Latex becomes flocculent and rises to the surface.	Emulsion formed but it separates and flocculency reappears and rises to the surface.	No crystals.	
2. Acetaldehyde	Immiscible. Latex at the bottom.	Latex coagulates. Rises to the surface.	No crystals.	
3. Benzaldehyde	Partly miscible.	Emulsion formed. Turns violet in color.		
4. Acetone	Immiscible. Latex coagulates.	Immiscible.	A few crystals.	

TABLE II—*Continued*

Solvent	*When added*	*On shaking*	*Residue after evaporation of solvent*	*Remarks*
Acids				
Glacial cetic	Latex coagulates on the surface.	Emulsion formed. Separates slowly.	No crystals.	
Ethers				
Ethyl	Immiscible. Latex at bottom.	Emulsion formed but separates out immediately.	A few crystals.	After 24 hrs. the latex becomes yellowish and there seems to be taking place a very slow reaction.
Isopropyl	As soon as the isopropyl ether was added the latex changed to a fleshy color. After a while the whole latex became yellow. Gas evolution.	On shaking an emulsion is formed which separates very rapidly. The latex acquires a very fleshy color.	No crystals.	A strong evolution of a gas took place right after the addition of the solvent.
Bases				
1. Anilin	Immiscible. Latex at the bottom.	Emulsion formed which separates after a few seconds. Part of the latex went to the surface and part to the bottom.	A few crystals.	On addition of acid to the decanted solvent no precipitate is formed.

TABLE II—*Continued*

Solvent	*When added*	*On shaking*	*Residue after evaporation of solvent*	*Remarks*
2. Pyridine	Latex diffuses through the pyridine right away, forming an emulsion. The latex acquires a yellowish color.	Emulsion separates after a long while.	A few crystal bundles present.	On addition of acid to the decanted solvent no precipitate is formed.
Miscellaneous				
1. Dioxan	Latex at the bottom. Turns brown and becomes flocculent.	Latex rises to the top. Very flocculent. No emulsion formed. After $\frac{1}{4}$ hr. settled to bottom again.	No crystals.	
2. Deo Base	Latex at the bottom.	Emulsion formed. Separates slowly. Latex at bottom.	No crystals.	
3. Carbon bisulfide	Latex at bottom.	Emulsion formed which separates on standing.	Crystals deposited.	

action of this latex. To 20 cc. of milk 5 cc. of latex were added. After standing for 24 hours no coagulation was observed.

Test for sugars. A very small amount of reducing sugars seems to be present in the latex, for when tested with Fehling's solution, a very slight precipitate is formed. On boiling the latex with 5 per cent HCl and neutralizing, no noticeable increase is observed.

Test for protein. To 2 cc. of Millon's reagent a few drops of latex were added and the mixture slightly heated. An intense brick-red coloration developed, showing that proteins are present in the raw latex.

Test for starch. When treated with iodine solution, no blue coloration develops.

Action of solvents. Several of the preliminary experiments having suggested the desirability of a better understanding of the action of various solvents upon the latex, a somewhat more systematic study of this aspect was undertaken. In Table II (pp. 153-157 incl.) the solvents are grouped roughly according to their chemical characteristics:

Proteolytic action of the latex. The proteolytic action of the crude latex was tested by measuring its hydrolytic action on a 2 per cent solution of gelatin, using the Sörensen formol titration as modified by Northrop.[57]

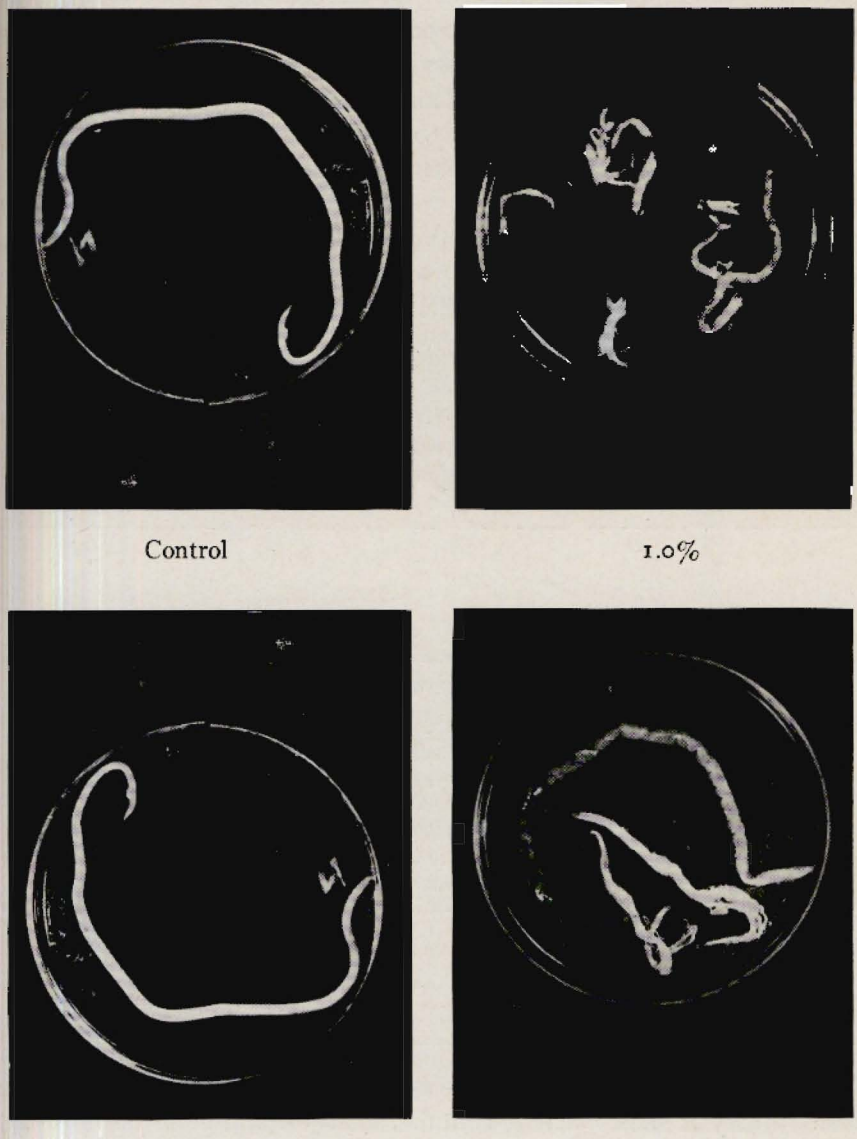
The action of the crude latex was tested on *Ascaris* obtained from a hog. A 15 per cent concentration of the latex using saline solution as a solvent digested the parasites completely in a period of 24 to 48 hours.

SYSTEMATIC INVESTIGATION

Separation of the latex into its proximate constituents

In the experiment which follows, the latex was treated by different physical and chemical methods. The object was to separate the proximate constituents which make up this complex mixture.

One hundred grams of latex having a water content of 70 per cent was dialyzed against distilled water using a membrane of collodion. After a few hours the water outside the dialyzer acquired a yellow color which deepened with time. After four days the outside water was replaced by fresh. This also became yellow, but not so dark as the first one. On dialyzing for a third time only a trace of green was visible. The dialyzates were mixed and evaporated at a temperature not exceeding 50°C. This yielded 7 grams of a horny, yellow, aromatic substance. When a similar dialyzate was evaporated under diminished pressure in a desiccator, instead of the horny yellow substance a flaky green one



Control 1.0%

Control 0.125%

FIG. 2. Action on *Ascaris lumbricoides* of different dilutions of the protein fraction (ficin) obtained from the latex of *Ficus pumila* L.

was obtained; in both cases the substance was fluorescent in alkaline solution. The two forms in which the substance is recovered from solution when treated in either one of the ways described above may be

attributed either to the presence of an enzyme which is destroyed on evaporating the solution at 50°C. or to the fact that such a temperature may act in some unknown physical or chemical way on this substance, changing its appearance. This point is worthy of consideration.

The substance obtained by evaporating the dialyzate at 50°C. was found to be insoluble in all the common organic solvents. It is ampho-

TABLE III

Latex-Gelatin Proteolysis

0.25 cc. of latex were added to 25 cc. of 2 per cent gelatin. Incubated at 37.5°C. Initial pH of 2 per cent gelatin 8.4–8.5. 5 cc. samples were taken at different times for titration.

Incubation period		*cc. of 0.01N NaOH taken for titration at 0 time*	*cc. of 0.01N NaOH taken at different times*	*Difference cc. of 0.01N NaOH*
Hrs.	*Mins.*			
0	5	5.00	6.00	1.00
0	40	5.00	7.60	2.60
1	00	5.00	7.60	2.60
14	00	5.00	11.54	6.54

Control

25 cc. of per cent gelatin. Incubated at 37.5°C. Initial pH of 2 per cent gelatin 8.4–8.5. 5 cc. samples taken at different times for titration.

Incubation period		*cc. of 0.01N NaOH taken for titration at a time*	*cc. of 0.01N NaOH taken for titration at different times*	*Difference cc. of 0.01N NaOH*
Hrs.	*Mins.*			
0	35	4.00	4.30	0.30
0	35	4.00	4.30	0.30
1	35	4.00	4.30	0.30
14	30	4.00	4.30	0.30

teric as it is soluble both in dilute alkaline and acid solutions. Sodium fusion analysis shows that it contains nitrogen and chlorine. It reacts in the cold with concentrated nitric acid liberating nitrous oxide fumes. A few radial crystals were deposited after the reaction took place, but they soon disappeared again. Well-shaped crystals were, however, obtained from the solution of this substance in concentrated sulphuric acid (see photograph 3). These crystals are very deliquescent and cannot be kept in contact with the atmosphere for any length of time. Their melting point is very indefinite, somewhere between 125-140°C.

The yellow dialyzable substance can be precipitated out of aqueous solution in the form of a brownish, gummy substance, by the addition of acetone. This substance, after being washed with alcohol, changes into a white amorphous powder, showing when dissolved in an alkaline solution the same fluorescence as the original yellow substance. The powder does not have a melting point although it burns on heating, leaving a white ash which is 11.3 per cent of the total powder.

The yellow dialyzable substance therefore is probably a mixture of

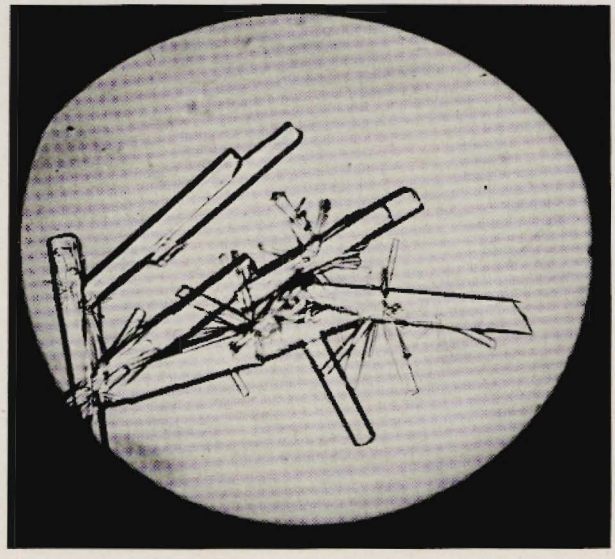


FIG. 3. Crystals obtained by treating the yellow dialyzable substance with concentrated sulfuric acid.

various substances. The fluorescence shown by this substance in alkaline solution suggests, however, the presence of a flavin among the different constituents of this fraction.

Non-dialyzable fraction of the latex. The non-dialyzable fraction of the latex was poured into 300 cc. of water and set aside. After standing over night the water acquired a brownish color and the white portion of the latex was deposited at the bottom of the beaker. The aqueous solution was decanted and filtered. Three times its volume of acetone was added to the filtrate, resulting in a flocculent, light brown precipitate. The yield was 0.6 grams. This precipitate gives a positive test for proteins with Millon's reagent and was found to hydrolyze gelatin.

The latex sediment collected in the funnel was washed several times

a day with water. It was very sticky and had an aromatic odor. After having been dried in a desiccator over night, the yield was 22 grams. Upon prolonged stirring with cold petroleum ether, the sticky portion thereof was removed and recovered upon evaporation of the solvent. The residue was then refluxed with hot petroleum ether which, upon cooling, deposited crystals, apparently identical with those obtained previously during the solubility test. A second hot extraction yielded more crystals,

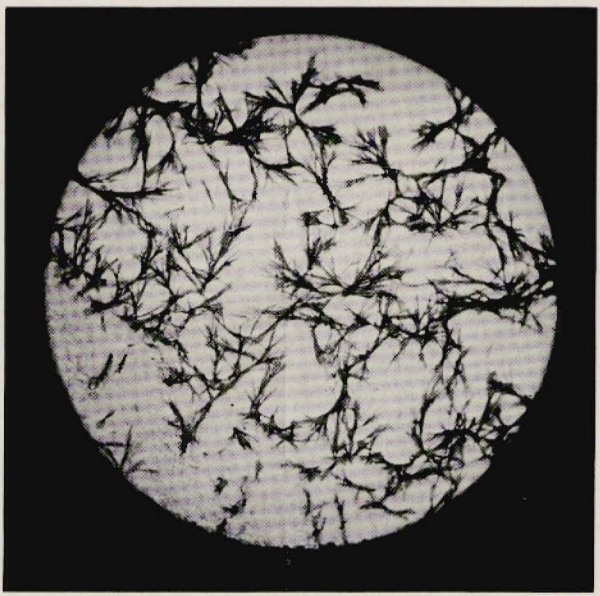


FIG. 4. Crystalline products obtained from the petroleum ether extract. Before sublimation.

but a third extraction yielded nothing further. The hot petroleum ether also extracted in addition a small amount of the sticky substance which could not be washed out with the cold petroleum ether. The crystalline precipitate was found after some trials, to be a mixture of two crystalline substances which could be readily separated by sublimation, the one subliming melting at 184-185°C. and the other which did not sublime at 68-69°C. This latter is of a waxy nature.

These two substances contain only carbon, hydrogen and oxygen. Their elementary analysis* yielded the following percentages of substances:

* Performed by Mr. H. A. Campbell, assistant at Professor Karl P. Link laboratory, Department of Biochemistry, University of Wisconsin.

TABLE IV

Digestive Action of Ficin Compared with that of a Supposedly High Grade Pancreatin

Enzyme and Substrate	*Incubation period*		*cc. of 0.01N NaOH taken at 0 time*	*cc. of 0.01N NaOH taken at different times*	*Difference*
	Hrs.	*Min.*			
0.1649 gm. of ficin added to 25 cc. 2% gelatin.	2	5	8.43	11.59	3.16
	17	30	8.43	15.73	7.30
	66	00	8.43	28.83	20.40
0.1649 gm. of Pancreatin added to 25 cc. of 2% gelatin.	1	30	8.00	11.76	3.76
	16	35	8.00	22.36	14.36
	66	00	8.00	44.27	36.27
Control (1) 0.1649 gm. ficin in 25 cc. H_2O.	2	5	2.44	2.57	0.13
	17	40	2.44	2.47	0.03
	66	00	2.44	2.76	0.32
Control (2) 0.1649 gm. pancreatin in 25 cc. H_2O.	1	25	2.91	3.10	0.19
	17	00	2.91	3.58	0.67
	66	00	2.91	4.32	1.41
Control (3) 25 cc. of 2% gelatin alone.	2	40	5.45	5.60	0.15
	18	00	5.45	5.41	−0.04
	66	00	5.45	7.32	1.87

Incubation carried at 37.5°C.
Initial pH of 2 per cent gelatin solution 9.
Five cc. taken at a time for titration.

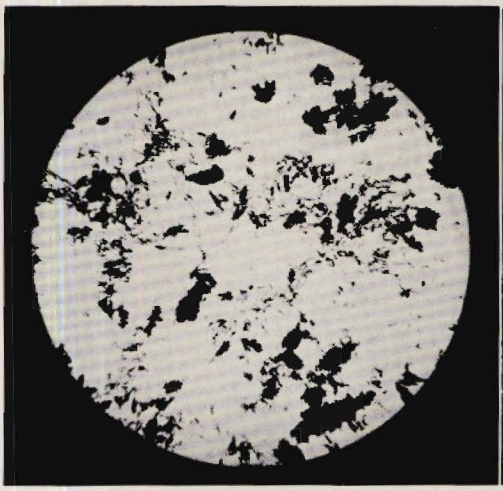


FIG. 5. Crystalline products obtained from the petroleum ether extract. Fraction that sublimes, m.p. 184-185°C.

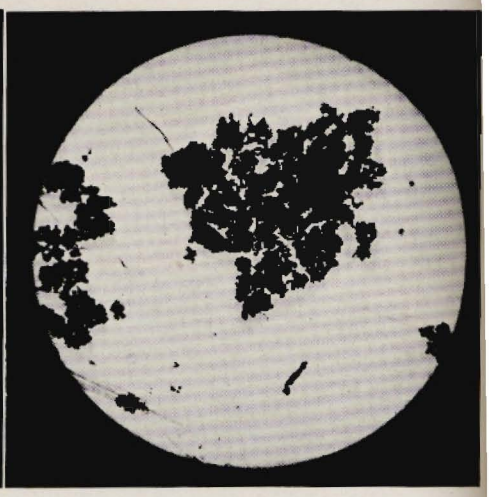


FIG. 6. Crystalline products obtained from the petroleum ether extract. Fraction that does not sublime (waxy), m.p. 68-69°C.

	%C	%H	%O
Substance with m.p. 184-185°C.	67.40	4.14	28.46
	66.20	4.06	29.74
	66.80	4.10	29.10
Substance with m.p. 68-69°C. (waxy)	77.50	12.37	10.13
	78.00	12.45	9.55
	77.75	12.41	9.84

It was found out after performing the above analysis, that Halden and Grün[58] report the presence of a wax in *F. ceriflua* which melts at 69.5°C. and is a mixture consisting of β amyrin, lupeol and palmitic acid.

Protein Fraction

The protein fraction was found to carry with it the proteolytic activity observed in the original latex. We shall adopt the name of ficin for this substance. This name was coined by Robbins[32] for a similar fraction obtained by him from other ficus latexes. This fraction which is a mixture of several substances gives positive tests for nitrogen, sulphur, and chlorine. It also gives positive Millon's test for proteins.

Comparative study of the digestive action of ficin with that of pancreatin. By means of the Sörensen formal titration,[57] the action of ficin and pancreatin on a 2 per cent solution of gelatin was studied. See Table IV.

The pancreatin used proved to have nearly twice as much more proteolytic activity on the gelatin solution than an equal amount of ficin.

Action on parasites in vitro. The action of different concentrations of ficin on *Ascaris lumbricoides* was tested by putting the parasites in contact with different concentration of this substance and running at the same time a control group in pure saline solution. Observations were taken at 6, 15, and 24 hours. Saline solution was used as the solvent for the ficin. The experiment was run at 37.5°C.

TABLE V

Action of Different Concentrations of Ficin on Ascaris.

Time hours	*1.00% Ficin solution*	*0.50% Ficin solution*	*0.25% Ficin solution*	*0.125% Ficin solution*
0	Normal and very active.	Normal and very active.	Normal and very active.	Normal and very active.
6	Wrinkled small lesion.	Wrinkled small lesions.	Wrinkled.	Wrinkled.
15	All dead and partly digested.	All dead and well developed lesions.	All dead and well developed lesions.	All dead and well developed lesions.
24	Fully digested.	Fully digested.	Digested.	Digested.

Control groups were normal and very active at the end of the experiment. The controls lived in the saline solution for nine consecutive days.

The action of 0.125 per cent of ficin was tried on various parasites.

TABLE VI

Action of a Solution Containing 0.125 per cent F. pumila Ficin on Various Parasites

Parasites	*Results*
Ascaris sp. (from hog)	Small lesions 5–8 hours. Digested in 24 hours.
Fasciola hepatica	Digested in 12 hours.
Ancylostoma sp. (from dog)	Slightly ulcerated after 24 hours.
Ancylostoma sp. (from cat)	Slightly ulcerated after 24 hours.
Trichina larvae	Became encysted after 24 hours.

Hemolytic test. The action of different concentrations of the ficin on a 3 per cent suspension of rabbit erythrocytes was studied following the usual method.[59] The concentrations of ficin used were the follow-

TABLE VII

1 cc. of 0.5% ficin sol. heated for 5 min. to:	*Millimeters of 3% gelatin gel digested after:*			
	3 days	*5 days*	*7 days*	*9 days*
25°C.	11	16	21	24
37	11	16	20	24
41	11	15	19	22
46	10	15	19	23
56	10	15	18	22
65	9	14	17	19
75	8	10	13	16
80	6	8	11	13
85	2	3	5	6
90	0	0	0	0
100	0	0	0	0
Control (H_2O)	0	0	0	0

ing: 2 p.c., 1 p.c., 0.5 p.c., 0.25 p.c., 0.13 p.c., 0.07 p.c., 0.035 p.c., 0.018 p.c., 0.009 p.c., 0.0045 p.c., and a control. In no case was hemolysis observed after twelve hours.

Purification of the ficin. The experiments previously described were performed with the unpurified protein concentrate. This substance has a light brown color and is an amorphous powder.

In an attempt to obtain a purer product, it was dissolved in water and reprecipitated by the addition of acetone. This was repeated three times. The final product was composed of dark brown, shiny flakes. On testing its action on *Ascaris* it was found that the activity was gone. The *Ascaris* remained alive even after a week in contact with different concentrations of ficin used (1.0 p.c. and 0.1 p.c.). *Ascaris* incubated with the crude protein fraction under similar conditions were digested in 24 to 48 hours.

These results seem to point to the fact that repeated purification using acetone as a precipitant inhibits the proteolytic activity of the ficin in some way.

Effect of heat on the proteolytic action of ficin. This was determined on a 3 per cent gelatin gel, equal amounts of which had been transferred to test tubes. The active protein fraction obtained as previously described was used in the form of a 0.5 per cent aqueous solution, one c.c. being taken for each test. Before adding to the gelatin gel the 1 c.c. of 0.5 per cent ficin solution, each c.c. was heated respectively to 37°, 41°, 46°, 56°, 65°, 75°, 80°, 85°, 90°, and 100°C. for five minutes and then it was immediately cooled to room temperature. To one tube of gel one c.c.

TABLE VIII

Proximate Constitution of the Latex of F. pumila L.

Products obtained	*Yield computed with reference to*	
	Fresh latex	*Latex deprived of H_2O*
Yellow-green dialyzable substance	7.1 p.c.	24.0 p.c.
Crude protein fraction	0.6 p.c.	2.0 p.c.
Inert powder	1.0 p.c.	3.3 p.c.
Sticky transparent substance soluble in petrol. ether and benzene (caoutchouc)	21.0 p.c.	70.0 p.c.
Crystals m.p. 184–185°	0.1 p.c.	0.3 p.c.
Crystals m.p. 68–69° (waxy)	0.2 p.c.	0.6 p.c.
Water	70.0 p.c.	0.0 p.c.

of the ficin solution was added without previously heating. To another, one c.c. of water was added as a control test.

For two days no appreciable digestion of gelatin was apparent, but after five days the amount of gelatin digested was very noticeable. The greatest amount of digestion had taken place in the tube containing the ficin solution that had not been heated. The least amount of digestion had taken place in the tube containing the ficin solution heated to 85°C. Those containing the ficin solutions heated to 90° and 100° respectively showed no digestion whatever.

It thus becomes apparent that *F. pumila* L. ficin is gradually inactivated at temperatures above room temperature and that the thermal death point of the enzyme is in the neighborhood of 85° to 90°C. The tubes containing the gelatin and ficin were incubated at room temperature (about 25°C.).

Sticky Substance (Caoutchouc)

This substance is very soluble in benzene and to a less extent in petroleum ether and ethyl ether. It can be purified by dissolving it in benzene and recovering on the addition of alcohol. It is very sticky and has a faint aromatic odor. It absorbs bromine, showing its unsaturated nature. It has not been further examined.

Inert Powder

This substance is insoluble in all the common organic solvents and does not have a melting point. It has not been further examined.

Summary

1. A review of all the pharmacological and pharmacochemical literature on the latexes of the genus Ficus is given.

2. It was shown that the latex of *Ficus pumila* L. possesses proteolitic activity.

3. The latex has been resolved into six proximate constituents besides water. Of these, two are crystalline substances with sharp melting points.

4. The proteolitic activity of the latex is present in its protein fraction and is totally destroyed by a temperature of 85-90°C. for 5 minutes.

5. The crude latex and also the protein fraction obtained from it digests certain species of parasites.

6. The active principle present in the protein fraction does not hemolyze rabbit's blood.

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