

product titrated for ML-1 might have stabilized the patients' quality of life, even though it didn't alter the tumor development at all. Other authors also observed this modest improvement in quality of life, and an increase in β -endorphin level.

Other Proteins. Other vegetable proteins have pharmacological potentials that probably deserve to be studied thoroughly, for example trichosanthin, a 234-amino acid protein isolated from the subtterranean parts of a Chinese Cucurbitaceae, *Tian Hua Fen*, *Trichosanthes kirilowii* Maxim or *T. japonica* Regel. This drug from the traditional Chinese Pharmacopoeia is known and still used for its abortifacient properties due to trichosanthin, which acts directly on the placental villi: 1.2 mg of the protein (IM) induces abortion in 4 to 7 days in 98% of test subjects. Like the A chain of ricin, this protein inactivates protein synthesis at the ribosomal level. *In vitro*, it inhibits the replication of the HIV virus.

Trichosanthes is not the only Cucurbitaceae genus to contain biologically active proteins: in the genera *Momordica* and *Luffa*, several species are used by traditional Chinese medicine, and there are several glycoproteins of molecular weight near 30,000: momorcharins, momorcochin, luffaculin, luffins... (e.g., *M. cochinchinensis* [Lour.] Sreng [tubercles], *M. charantia* L. [= bitter-melon], *L. acutangula* [L.] Roxb., *L. cylindrica* [L.] Roem [seeds]). Like trichosanthin, momorcharins have abortifacient and antitumor properties (on choriocarcinomas and melanomas), and inhibit protein synthesis. Similarly, they inhibit the replication of the HIV virus *in vitro*; their activity on the humoral and cellular immunity is complex: they are allergens, but can inhibit the reactions induced by other allergens.

BIBLIOGRAPHY

- Friess, H., Begeer, H.G., Kunz, J., Funk, N., Schilling, M. and Buehler, M.W. (1996). Treatment of Advanced Pancreatic Cancer with Mistletoe: Results of a Pilot Trial, *Anticancer Res.*, **16**, 915-920.
- Gottstein, C., Winkler, U., Bohlen, H., Diehl, V. and Engert, A. (1994). Immunotoxins: Is there a Clinical Value? *Ann. Oncol.*, **5**, suppl. 1, S97-S103.
- Heiny, B.-M. and Beuth, J. (1994). Mistletoe Extract Standardized for the Galactoside-specific Lectin (ML-1) Induces β -endorphin Release and Immunopotentialiation in Breast Cancer Patients, *Anticancer Res.*, **14**, 1339-1342.
- Pusztaï, A. (1991). *Plant Lectins*, University Press, Cambridge.
- Yang, S.-L. and Walters, T.W. (1992). Ethnobotany and the Economic Role of the Cucurbitaceae in China, *Econ. Bot.*, **46**, 349-367.

Enzymes

● PAPAYA TREE, *Carica papaya* L., Caricaceae

The fruit of this species, the papaya, is rich in sugars, vitamins, and volatile compounds. It is widely consumed in tropical countries. The 10th edition of the French Pharmacopoeia devotes a monograph to the "mixture of enzymes possessing proteolytic and esterase activities from the thickened latex obtained by incision of the fruits shortly before maturity", designated *suc de papayer* or "papaya latex". In therapeutics, a purified fraction, chymopapain, is often used for the treatment of sciatica due to a herniated lumbar disc.

The Plant, The Drug. The papaya tree belongs to a small family reduced to four genera. It is a tree reaching 3 to 10 m in height, with the habit of a palm: the fleshy stem, marked by scars where leaves have fallen off, is surmounted by a terminal panache of large leaves on long petioles and with 5-7 lobes. A dioecious species, the papaya tree bears ovoid berries of variable size: they can reach 20-30 cm in diameter and weigh 5 kg. When ripe, papayas are yellowish-green, their flesh juicy and orangy-yellow, and their central cavity is filled with black seeds surrounded by mucilage. Within the fruit pericarp and the leaf mesophyll runs a network of anastomosed laticiferous ducts.

The species is native to Central America, and is cultivated in virtually all of the intertropical regions (Brazil, Sri Lanka, Thailand, India, but also on the African continent: Tanzania, Uganda, Zaire).

The drug, that is the latex, is collected after incision of the unripe fruits; the

artificially, at a temperature lower than 50 °C. The papaya latex thus obtained occurs as small, clear, white or brown fragments, smelling "somewhat like grilled meat" (Fr. Ph.), and tasting mildly salty, sometimes bitter.

Chemical Composition. Crude papain is commonly purified by the normal protein separation techniques (alternating precipitations and dissolutions) and by the classic techniques of affinity chromatography. It consists of a mixture of papain, chymopapain, and papayaproteinase Ω . Papain is a protein of 212 amino acids with a molecular weight of about 23,000 daltons; the chain is folded in two lobes at the junction of which is the active site. It is an endopeptidase activated by thiols and reducing moieties (cysteine, thiosulfate, glutathione), resistant to heat, with an optimal pH ranging from 5 to 7; it is inactivated by metal ions, oxidants, and reagents which react with thiols.

Pure chymopapain is a protein of 218 amino acids of structure and properties closely resembling those of papain.

Tests. Identification of papaya latex consists of observing its action on a gelatin solution in the presence of an activating cysteine chlorohydrate solution, at 80°C, for an hour; following prolonged cooling to 4°C, the treated solution must not regel (in contrast to a blank treated in the same conditions).

The assay consists mainly of titrating the esterase activity by comparing the hydrolysis rates of the ethyl ester of benzoylarginine by the latex to be tested and by a reference latex. This is done with an aqueous suspension of the latex to be titrated (and of the reference latex) at pH 7 and at 25°C, in the presence of cysteine chlorohydrate and under nitrogen. The pH is maintained at 7 with an automatic titrator, by adding sodium hydroxide and titrating it while the enzyme releases acid. From these data is deduced the activity of the preparation being tested, which must be not less than 15 nanokatal/mg*. Papaya latex must also pass a microbial contamination test (total viable aerobic germs, *Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*).

To estimate the enzymatic activity, it is possible to use *N*-alphanbenzoyl-DL-arginine *p*-nitroanilide and to titrate the *p*-nitroaniline which is released. For chymopapain the correspondence is as follows: 1 unit = 1 picokatal = 10-3 nanokatal.

Properties and Uses.

1. Chymopapain

Because of its proteolytic properties, chymopapain can be injected into an intervertebral disc to cleave the proteoglycans that constitute the nucleus pulposus: this is chemonucleolysis, a form of therapy for a herniated lumbar disc with root compression that is refractory to proper conventional medical treatment. The

* One nanokatal corresponds to the transformation of one nanomole of substrate per second.

contraindications, the risk of anaphylactic shock, the neurotoxicity of the enzyme in case of intrathecal leakage subsequent to a technical error, and the requirement for strict asepsis all explain why the technique may only be applied by highly trained personnel and only in the hospital setting (neuroleptanesthesia, radiological monitoring of the position of the needles, observation of the patient, etc.); the efficacy of this technique is similar to that of surgery but the risk of complications may be lower. Controlled clinical studies have shown an efficacy greater than that of a placebo and about 75% of patients were cured; the cost of the treatment is much lower than that of a surgical discectomy. Chymopapain is currently available lyophilized in combination with sodium cysteine.

2. Papain

Papain, alone or in combination, is promoted as therapy for digestive disorders, and in dietetics, as a substitute enzyme to relieve gastric or duodenal insufficiency and for the symptomatic treatment of dyspepsia. It is used locally in the formulation of adjunct treatment products for disorders limited to the buccal and oropharynx mucous membranes, for post-operative care, and for accidental buccal lesions; as a wound healing and cleansing agent, it is often combined with an antibiotic or with lysozyme. It can also be used in contact lens cleaning liquids.

3. Papaya

Papaya leaf and papaya latex may be used in the composition of phytomedicines traditionally used in the symptomatic treatment of digestive troubles such as epigastric bloating, impaired digestion, eructations, and flatulence [French Expl. Note, 1998].

● PINEAPPLE, *Ananas comosus* (L.) Merr., Bromeliaceae

Pineapple is a herbaceous plant native to Central America, widely cultivated in all of the tropical regions of the world. The species is characteristic: rosette of dentate thorny leaves, spike of trimerous purplish-blue flowers leading to a peculiar fruit, a ctenocarpium, formed by the concrecence of the berries of a given inflorescence together with the axis and the bracts turned fleshy.

Chemical Composition. The pineapple fruit is rich in soluble mono- and disaccharides (up to 15%), in organic acids, and in vitamins. Its color is due to carotenoids and its flavor to a complex mixture in which oxygenated aliphatic compounds predominate. The ripe fruit and the stem contain a proteolytic enzyme, bromelain (the commercial product is in fact a mixture of proteases). The bromelain from the stems is a mixture of basic glycoproteins of molecular weight between 10 000 and 28 000 daltons in which the protein moiety is not very different from

that of papain. The bromelain from the fruits is an acidic protease. Bromelains are proteases with thiol groups, activated by reducing agents (cysteine), and inhibited by oxidants and metals.

Tests. The proteolytic activity of bromelains can be evaluated with natural or synthetic substrates, for example by spectrophotometrically measuring the hydrolysis products of casein in specific conditions, and by comparison to an international standard (Rorer units).

Pharmacological Activity. The antiinflammatory and antiexudative properties of bromelain on various experimental models have been the subject of numerous publications: they might be linked to an interaction of the enzyme with the metabolism of eicosanoids (inhibition of the synthesis of inflammatory and vasoconstricting products); note also an anti-platelet aggregation and fibrinolytic activity. Although multiple clinical observations tend to establish the applicability of this enzyme for the treatment of edemas of various origins in stomatology or dermatology, experiments designed according to currently accepted standards have yet to be conducted.

Bromelains are promoted as a treatment for post-traumatic and post-operative edemas (*per os*, 500,000 U/day, enteric coated tablets). They are sometimes combined with antibiotics. Until the mid 1990s, they were used as ingredients of pharmaceuticals for the symptomatic treatment of dyspepsia.

- **FIGUS,**
Ficus spp., Moraceae

A certain number of species of the genus *Ficus* provide ficin, a proteolytic enzyme closely related to the previous ones. These species (*F. carica* L., *F. insipida* Willd., and more) are trees with palmate leaves characterized by a syconium, in other words a compound fruit formed of akenes gathered into a fleshy receptacle. By incising the trunk a latex is collected and coagulates rapidly; filtered and dried, it constitutes crude ficin. Ficin is a mixture of proteases which possesses an activity close to that of papain. It is used in food technology (meat tenderizing). The ficus pseudo-fruit may be used in the symptomatic treatment of constipation [French Expl. Note, 1998].

BIBLIOGRAPHY

- Benoist, M. (1996). Vingt ans de chymonucléolyse lombaire, *Presse Méd.*, **25**, 743-745.
Lotz-Winter, H. (1990). On the Pharmacology of Bromelaine: An Update with Special Regard to Animal Studies on Dose-dependent Effects, *Planta Med.*, **56**, 249-253.

PHENOLICS

Shikimates Acetates