AN INNOVATIVE TECHNOLOGY FOR THE PRODUCTION OF ACTIVE INGREDIENTS FROM PLANTS

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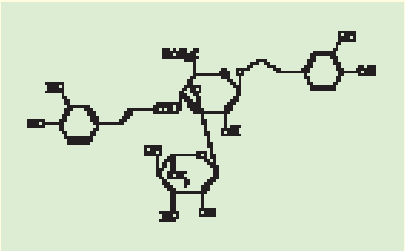
Part Two

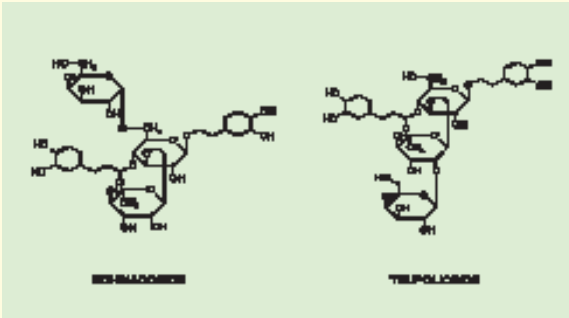
SECONDARY METABOLITES PRODUCED WITH CULTURES OF CELLS IN SUSPENSION

ANTI-OXIDANT MOLECULES

Phenylpropanoids (PP) belong to a very wide class of secondary metabolites also commonly called phenylethanol glucosides. These are natural compounds soluble in water and widely distributed in the organs of superior plants.
From the structural point of view, they are characterized by a derivative of cinnamic acid and a derivative of phenylethanol bonded to the same molecule of _-glucopyranose, respectively by an ester bond and a glycosidic bond.
Other saccharidic molecules, such as rhamnose, xylose and apiose, are often bonded to glucose which acts as a bridge between the two aromatic structures.



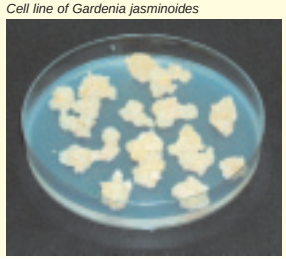
They are classified indifferently as phenylpropanoid glucosides due to the presence in the molecule of a C6-C3 structure such as caffeic, ferulic or cinnamic acid, or phenylethanoids due to the simultaneous presence of a derivative of phenylethanol or one of its analogues. However, as the phenylethanol part is biosynthetically originated from a phenylpropanoid structure, it is preferred to defined these structures as phenylpropanoids, but the two terms can be considered synonyms.
The phenylpropanoid glycosides, according to the number and type of bonded sugars are divided into monosaccharides, disaccharides and trisaccharides.
The monosaccharide glycosides present a molecule of glucopyranose between the phenylethanol chain and the acid bonded with an ester bond to any free oxydryl of the structure of the glucopyranoside. The acids bonded with sugar (cinnamic, caffeic, ferulic, methylcumaric etc.) are almost always in the trans form and rarely in the cis form; the sugars apiose, arabinose, galactose and glucose are always bonded with a b-glycosidic bond, whilst rhamnose and xylose have a a-glycosidic bond..
Disaccharide glucosides derives from monosaccharides and are classified according to the sugar bonded to glucose.
The group most represented in nature remains, however, that of trisaccharides, which contain rhamnose as the second glucidic unit, to which a third, generally glucose, xylose, apiose, galactose, lyxose or rhamnose, is bonded. The aromatic acids most frequently bonded to the C-4 of glucose are caffeic, ferulic and cinnamic.
The first phenylpropanoid glucosides isolated were echinacoside from Echinacea augustifolia (Stoll A. et al. Helv. Chim. Acta. 33:1877 1950) and verbascoside from Verbascum sinuatum (Scarpati M.L. et al.. Ann Chim 53:356 1963). At first, this latter compound was called acteoside by other researchers and it was not until 1982 that it was shown that the structures of acteoside and verbascoside were identical (C. Andary et al.., Phytochemistry 21:11231982,) and corresponded to the structure formula shown.
Experiments were carried out for a long time on both molecules for their biological and pharmacological effects. In addition to anti-oxidant properties, anti-microbial, anti-viral and anti-hepatoxic properties have also been ascertained. Echinacoside enters into the composition of many herbalist and cosmetic products, due to its anti-inflammatory and cicatrizing action.
In the case of verbascoside, analgesic and anti-hypertensive properties have been shown, probably linked to its inhibitory action on the Protein Kinase C (PKC), on aldose reductase and on the formation of 15-hydroxyeicosatetraenoic acid (15-HETE).
Some phenylpropanoid glucosides having a very similar structure to that of echinacoside have been isolated by cultures of cells of Ajuga reptans, a plant belonging to the family of the Labiates, known since the Middle Ages for their cicatrizing properties on cuts.
These include teupolioside, the structure of which is represented alongside that of echinacoside.
Confirming these traditional uses, laboratory tests have shown that many of the complex phenylpropanoids have, molarity being equal, an anti-oxidant capacity that is from 10 to 15 times greater than that of Vitamin E.



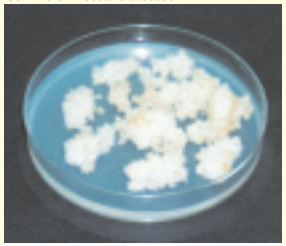
ANTIBLASTIC MOLECULES

Similarly to the systems developed to protect themselves from the harmful effects of the free radicals in oxygen, plants have also had to develop systems to protect themselves from the aggression of parasites such as animals, insects, fungi, bacteria and viruses.
In this sector, each botanical family has developed its own defensive strategy represented either by physical obstacles or by chemical barriers. The latter include many secondary metabolites that present a broad spectrum of biological actions, many of which have a pharmacological use. In particular, some plants have developed molecules that are effective in blocking the mitotic mechanisms of the cell by means of a specific interaction with tubulin, the main constituent protein of the microtubules of the mitotic spindle (Rao, S. et al. (1994) J. Biol. Chem. 269, 3132–3134; Rao, S. et al. (1999) J. Biol. Chem. 274, 37990-37994). Due to the action of these molecules. the cells in the progress of proliferation are blocked in the late phase G2 of the cell cycle. The activity carried out means that these secondary metabolites are used as anti-tumorigenic agents.
The best known example is that of taxol, extracted from Taxus brevifolia. Taxol, marketed under the name of paclitaxel, and its semi-synthetic derivative docetaxel, are today considered two of the most promising treatments for breast and ovarian cancer and are currently at an advanced stage of study which shows their therapeutic efficacy in other tumours as well, such as lung cancer and melanoma. The practicability of tumour thera-

Cell line of Gardenia jasminoides



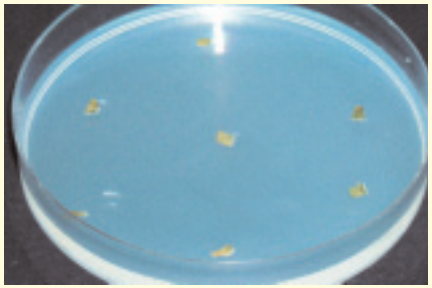
Cell line of Nicotiana tabaccum



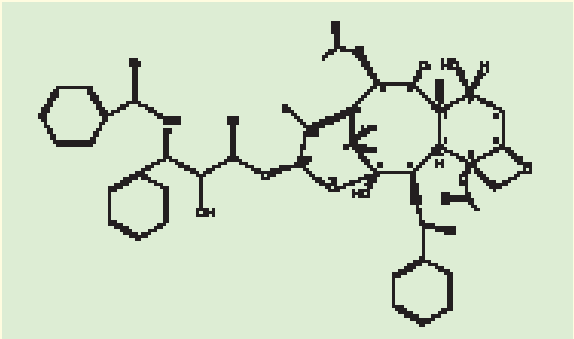
pies based on taxane derivatives is however limited by the difficulty of obtaining, by extraction from the plant, sufficient quantities of products.
In fact, from about 13 tons of yew bark, 1 kg of active ingredient is obtained with the processes currently in use. At present, the world market requires at least 600 kg per year and annual demand is expected to increase by 10% in the next ten years. As can easily be imagined, this is a quantity that is incompatible with the long-term survival of the yew.

PACLITAXEL

Many attempts have been made to date to find alternative sources of taxanes, including production by endophyte microorganisms of the yew (Stierle A, et al. (1995) J Nat Prod 58(9):1315-1324), but so far all have been shown to be of scarce use due to the low capacity of synthesis and because they are not very economically viable.
Other methods currently under study consider cloning and the reconstruction in appropriate micro-organisms of the entire metabolic itinerary, made up of eight enzymatic passages which have still not been completely clarified (Schoendorf et al. (2001) PNAS 98(4):1503).
At present, given the structural complexity of the molecule (see the structure of paclitaxel) which makes chemical synthesis impracticable, the only alternative source remains the culture in suspension of yew cells.



Examples of explants: Explants of Echinacea angustifolia immediately after sowing on solid nutritive medium.



Conclusions

The cultures of plant cells represent an economically advantageous alternative compared to the direct collection of plants in the field, when in nature there exist conditions of poor availability and slow renewal of the sources, associated with difficulties of chemical synthesis of the active ingredient.
Given the structural complexity of many plant molecules of pharmacological, dietetic and cosmetic interest, the technology of plant cell cultures presents a wide potential of use.
If their eco-compatibility and the biological safety inborn in the entire process is added to this, the usefulness and advantages that the use of this technology makes possible appear obvious.

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