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ABOUT THE MIRACLE OF NATURE FROM RAPESEED POLLEN

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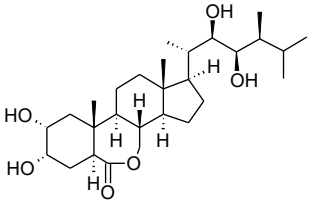
Brassinosteroids (brassinolides, BS) are specific plant polyhydroxylated derivatives of 5α-cholestane, structurally similar to cholesterol-derived animal steroid hormones, polyhydroxylated bile acids[1] and insect ecdysteroids[2]. They are characterized by a *cis*-diol on ring A at the C-2 and C-3 position, a hydroxylated side chain, all-*trans* annulation, and oxidation on ring B. Ring B contains either a seven-membered lactone (e.g., brassinolide) or a six-membered ketone (e.g., castasterone). BS are found in very small concentrations in virtually all parts of plants, especially in pollen, seeds, leaves and young vegetative tissues throughout the plant kingdom[3]. The first biologically active plant BS was isolated[4] in an amount of 4 mg from 40 kg of rapeseed (*Brassica napus*) pollen in 1979. The idea arose from the ancient practice of growers watering their cultures with water containing plant pollen to improve growing results. Over time, it has been found that similar substances from the group of these polyhydroxysteroids are found in virtually all plants and that the group contains more than a hundred related substances. Extensive study, even in field trials, has shown that these substances in small concentrations (almost "homeopathic") have a significant and positive effect on yields, both by improving growth, yield, but also plant resistance.

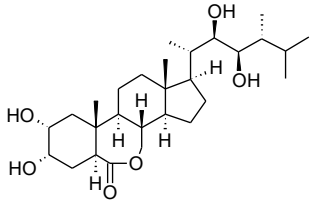
BS research has always been limited by their low availability in nature and synthetic difficulty, but operational scale synthesis has been resolved either by the use of cell cultures[5] or by partial synthesis[6]. Interest in this group of substances is still growing[7]. Web of Science answered the search on "brassinolide" with 1309 and on "brassinosteroid" with 4566 citations. When Pavel Kočovský, in the early 1980s, as a "young soldier", suggested to his boss that it would be useful to synthesize such substances, he was abruptly rejected.

Exogenous application of BS to plants at nanomolar to micromolar concentrations has a wide range of growth responses, such as stem elongation, inhibition of root growth, promotion of cell division, and enhancement of stress tolerance due to changes in enzyme activity and gene expression; however, in some cases, it depends on the concentration and plant species; low concentrations, on the other hand, may support root growth.

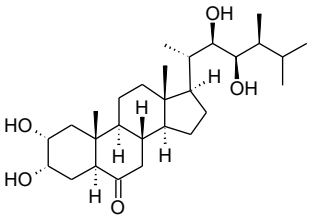
In plants, BS increase the synthesis of other phytohormones, promote individual cell expansion and protein synthesis, activate photosynthesis through key enzymatic reactions, and thereby enhance plant growth, development and stability. In agriculture, they are applied in extremely



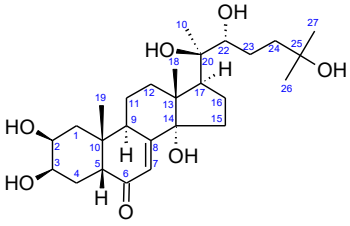
brassinolide



24-epibrassinolide



castasterone



20-hydroxyecdysone with carbon numbering

low, almost "homeopathic" doses (e.g. 5–50 mg/ha), are environmentally safe, non-toxic to humans, mammals, beneficial insects and fish.

In recent years, biochemical and genetic analysis has provided compelling evidence for the essential role of BS in plant development. As a biologically active compound for agricultural use, the first brassinosteroid, 24-epibrassinolide, was only authorised[8] in the EU in 2021 on the grounds that '*It has been established with respect to one or more representative uses of at least one plant protection product containing the active substance, and in particular the uses which were examined and detailed in the review report, that the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009 are satisfied. The Commission further considers that 24-epibrassinolide is a low-risk active substance ... it is therefore appropriate to approve 24-epibrassinolide as a low-risk substance*'. In the Czech Republic, BS are not yet authorised for agricultural use because they are assessed as fertilisers and have failed to demonstrate that they have the active properties of this group of substances, although, according to the legal provisions, the concentrations of arsenic, cadmium, chromium, mercury and lead in the samples submitted were shown to be 'below' the required limits, which is quite logical since there are no heavy metals in BS.

The fact that after application of BS plants resist drought, excessive moisture, soil salinization and that it significantly increases the yield of agriculturally and forestry important plants did not find a hearing even in the Ministry of Agriculture of the Czech Republic. However, the use of 24-epibrassinolide in agriculture is now already allowed in a number of countries, including the USA[9]. In Belarus, where the producer encountered a similar official procedure as in the Czech Republic, the substance was finally registered as a fertiliser in accordance with the relevant regulations[10].

Products with BS can be considered as representative representatives of a new generation of biorational agrochemicals, which have no impact on the environment, act in natural doses and in a natural way[11]. They are traditionally involved in the food chains of humans and mammals or their metabolic pathway is evolutionarily normal for them. The BS content in plants increases during the first 12 to 14 days after treatment, then the product is completely metabolised during the following 10 days. The main metabolites are steroidal glycosides. Plants must be treated at the appropriate stage of development; application to young plants gives good results. Two modes of application are available: seed soaking, cutting and foliar spraying. On the whole, BS is responsible for the long-term results in plant production:

- Increased yield (Fig. 1), yield and improved production quality (commercial appearance, ornamental value of flowers), flower stalk formation, enhanced seedling stability, root growth, accelerated rooting of cuttings and improved cuttings quality, improved nitrogen utilization, increased seed and tuber yields, protein, gluten, essential amino acids, etc., oils, sugars and vitamins, fibre quality, number of annual shoots and photosynthetic capacity,
- acceleration and improvement of germination, formation of uniform stems, reduction in the formation of defective plants, shortening of the growing season,
- reduction of damage caused by diseases, fungi and rot,
- increasing fertiliser efficiency, reducing nitrate accumulation, reducing pesticide damage,
- increasing plant resistance to drought, frost and excess water, salinity.

Under drought conditions, BS application can be critically important for productivity. An example of this is the use of the 24-epibrassinolide product EPIN in Moldova in 2007 (see Fig. 2).

The popularity of BS products for both agriculture and gardeners can be easily seen by typing "эпин" (epin in Cyrillic) into an internet search engine.

BSs have a number of biological properties and effects beyond the plant host. The fact that BS have positive effects on human health has been documented many times[12–14] even when they come from normal food[15]. In the

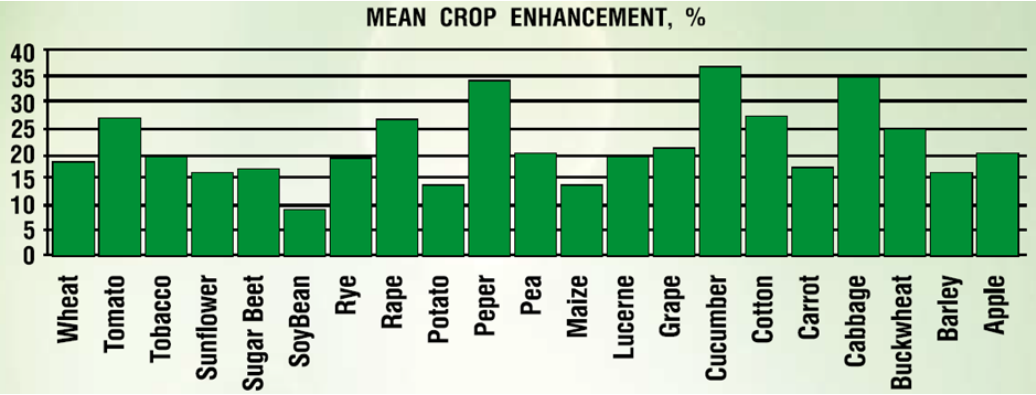


Fig. 1. **Yield increase of some crops after BS application** (ref.[16])

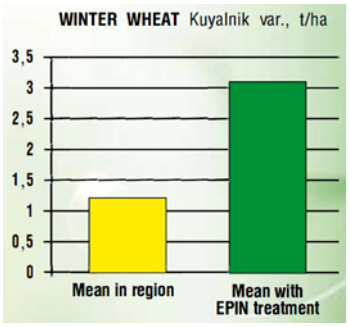


Fig. 2. **Wheat yield increase under catastrophic drought in Moldavia 2007 after BS treatment** (ref.[16])

countries of the former USSR, 24-epibrassinolide is even registered as an "herbal stimulant for elite athletes" and a means of inducing "well-being"[16] with the proviso that it is not considered by the anti-doping agency as a doping agent[17]; it is widely distributed under the trade name Фитонол (Fitonol)[18] with a content of 25–40 μg per capsule. A maximum of 75 μg per day is recommended (Fig. 3).

A US patent provides a discovery using BS to induce an anabolically favourable state for growth, repair and maintenance of skeletal muscle and skin[19] in concentrations as low as 0.01 μM and doses as low as 0.1 mg kg^{-1} per week. It has also been patented that BS may be useful in cholesterol control by lowering LDL levels without lowering HDL levels[20]. A Japanese patent claims a preventive effect of BS against arteriosclerosis at a concentration of 20 μM (ref.[21]). Similar to structurally related ecdysteroids[2], BS also act as anabolics and adaptogens[22,23] and naturally, have an effect on insect development[14]. BS has also been shown to have a very positive role on fish farming[14,24].

In addition to the properties cited above, antiviral activity of these compounds against RNA and DNA viruses has also been found. Some of the compounds showed 10 to 18 times higher selectivity index (SI) than ribavirin, a broad-spectrum antiviral compound, when tested against Junin virus (JV) (*Arenaviridae*); good antiviral activity against measles virus (MV) (*Paramixoviridae*), with SI

Obr. 3. **Fitonol** (ref.[18])

values also higher than ribavirin used as a reference drug, and similar or lower activity against herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) (Herpesviridae) compared to foscarnet or acyclovir[25]. They have been found to have the potential to reverse multidrug resistance[26], e.g. in the human T lymphoblastoid cell line CCRF-VCR1,000.

It has been widely described that BS have suitable therapeutic properties against cancer development and show potential for the development of new anticancer drugs[27,28]. However, BS with a fluorinated side chain have negligible anticancer activity[29], similar to that of fluorinated skeletal derivatives[30]. Non-fluorinated BS have shown, at micromolar concentrations, the ability to inhibit the growth of several human cancer cell lines without affecting the growth of normal cells[27]. Notably, brassinolide itself was able to induce apoptosis in the androgen-independent prostate cell line PC-3 at concentrations as low as 20 μM and has therefore been proposed for the prevention or treatment of advanced prostate cancer[31] and drug-resistant small cell lung cancer[32]. 24-Epibrassinolide alters the PI3K/MAPK signalling axis by activating Foxo3-induced mitochondria-mediated apoptosis in colon cancer cells[33]. BS has demonstrated the ability to induce apoptosis in prostate[34] and breast cancers[35]. There are indications that BS may be used in the treatment of androgen-related conditions such as benign prostatic hyperplasia and androgenic alopecia[36].

Interesting findings include that BS are associated with the ability to induce resistance against viruses[37], bacteria, ciliates (oomycetes) as well as fungi and moulds[12,38]. BS are considered serious candidates for agents with antiviral activity and in the treatment of patients suffering from herpetic and viral diseases[39], including HIV[40].

It has been found through inhibition of $^{86}Rb^{+}$ incorporation into human erythrocytes that BS inhibit $Na^{+}K^{+}$-dependent ATPase, but to a lesser extent than digitoxin[41]. BS with a fluorinated side chain have also been described to exhibit GABAA activity comparable to the endogenous neurosteroid allopregnanolone[29].

BS have been described to exhibit antiglycaemic activity in diabetic rats, improve RBC, WBC, platelet, haemoglobin levels and reduce cell damage in diseases such as inflammatory bowel disease, allergic reactions and leukaemia or myeloproliferative neoplasms in humans[42].

We present this paper as a teaching text describing various interesting aspects of the chemistry of natural compounds[43–45] and because we want to respond to the many fabrications, half-truths and nonsense that are being spread about natural compounds today. It is clear that the study of natural substances as substances from renewable sources is one way of contributing to the general good in a cheap and effective way[46,47]. If this is done with a natural substance that also has negligible toxicity, almost zero contraindications and has been used by mankind for thousands of years, it is a good thing.

Secondary metabolites such as BS from renewable sources having broad biological efficacy and minimal toxicity (LD_{50} for 24-epibrassinolide is higher[14] than 5000 mg kg^{-1}) are a well of possibilities for future use in human,

agricultural and forestry practices. Brassinosteroids are the first group of steroid hormone compounds isolated from plants and acting in plants. Among the numerous physiological effects, the growth stimulation and adaptogenic activity affected by BS are particularly noteworthy. Nowadays, there is much evidence that BS have similar types of activity outside the plant kingdom and even in human bioregulation[48] and this article was intended to contribute to such awareness.

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Abstract

Secondary metabolites such as brassinosteroids (BS) from renewable sources having broad biological efficacy

and minimal toxicity represent a rich well of possibilities for future use in human, agricultural and forestry practices. BS are the first group of steroid hormone compounds isolated from plants and acting in plants. Among the numerous physiological effects, the growth stimulation and adaptogenic activity affected by BS are particularly noteworthy. Nowadays, there is much evidence that BS have similar types of activity outside the plant kingdom and even in human bioregulation.

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