Growth Inhibitions on Human Cancer Cell Cultures with the Indole Sulphur-Containing Phytoalexins and Their Analogues

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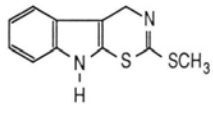
Cell growth inhibitions on human cancer cell cultures were determined for the indole sulphur-containing phytoalexins cyclobrassinin, brassilexin (previously isolated from vegetables of the Cruciferae family) and their synthetic analogues 5-methoxybrassilexin and homocyclobrassinin. The most biologically active of these products is brassilexin (LD_{50} = 8 µg/ml).

A series of tryptophane-derived sulphur-containing phytoalexins was previously isolated from vegetables of the Cruciferae family [1–8]. These substances are characterized by powerful antifungal activities *in vitro* and hence are considered as responsible for the resistance of these Cruciferae against fungi. A hypothesis according to which brassilexin (**2**) could be originated from cyclobrassinin (**1**) was recently argumented by the *in vitro* ring contraction of **1** to **2** promoted by oxidation [9]. The common biological precursor of these substances is very likely tryptophane as shown in particular by the high metabolism of this aminoacid in the cauliflower, leading to the presence of fairly large amounts of indole-3 carboxaldehyde [10].

Cruciferous vegetables were shown to contain products able to modulate carcinogenic processes at least *in vitro*, in some cases *in vivo* [11–16]. Most of these substances were indole-derived compounds from the tryptophane series. Of special interest was indole-3 carbinol, present as the glucosinolate in cabbage, cauliflower and broccoli which inhibits tumorigenesis in rodents exposed to polycyclic aromatic hydrocarbons [17]. Indole-3 carbinol on the other hand was found to inhibit aflatoxin-B_1 induced hepatocarcinogenesis in the rainbow trout [18]. It has been suggested that the capability of the dietary indoles to inhibit DNA binding and carcinogenesis, was due to their potency to promote cytochrome P-448 monooxygenases [14].

It is in this context that we decided to carry out bioassays on growth inhibitions of human cancer cell cultures with some of the indole-derived sulphur-containing phytoalexins and some of their non natural synthetic derivatives. For this purpose, the substances **3** and **4** were synthesized and submitted to parallel bioassays. This publication reports on the results so far observed with this series of substances.

Synthetic cyclobrassinin (**1**) [1], brassilexin (**2**) [8] and the hitherto non natural 5-methoxybrassilexin (**3**) and homocyclobrassinin (**4**) [19] were submitted to bioassays in order to determine growth inhibition. These compounds were dissolved in DMSO which revealed to be non toxic within the experimental concentrations used (less than 1%). The solutions were deposited at the moment the cultures started at concentrations of 0.1, 1, 5, 10, 20, 30, 40 µg/ml and cell proliferation was determined 3 days later. The usual colorimetric



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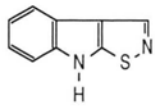

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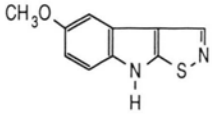

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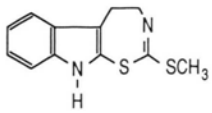

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1, Cyclobrassinin
2, Brassilexin
3, 5-methoxybrassilexin
4, Homocyclobrassinin

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method using neutral red was employed by reference with a standard culture. Both human cancer cells (KB cells) and normal monkey kidney cells (Vero cells) were used for these experiments. Repetitions of these assays in the same conditions gave a similar result. In all cases linear dose-responses could be observed and the corresponding LD_{50} were established upon the average result.

With the human cancer cells KB in culture, brassilexin (**2**) gave the strongest growth inhibition with a LD_{50} at 8 μg/ml (5.7×10^{-6} M). With cyclobrassinin (**1**), the LD_{50} was found to be 22 μg/ml (9.4×10^{-5} M). The non natural analogues 5-methoxybrassilexin (**3**) and homocyclobrassinin (**4**) gave both the same LD_{50} values at 10 μg/ml (respective molar concentrations 4.9×10^{-5} and 4.03×10^{-5} M). Determination of the growth inhibitions for the most active compound brassilexin (**2**) were carried out on the normal monkey kidney cells and were found to be of the same order ($LD_{50} = 8$ μg/ml) than observed with the human cancer cells in culture.

Brassilexin (**2**) was tested for the inhibition of germination of water cress seeds and the 100% inhibition was noticed, after a week at 20 °C, for a concentration of 50 μg/ml.

The conclusion is that growth inhibitions on human cancer cells in this series is noticeable, but of insufficient magnitude to justify an *in vivo* transposition. What is more, the lack of difference between the observed inhibitions on growth of cancer cells and of simian normal kidney cells is of course not in favour of a specificity and rather points out a general cytotoxicity.

As indeed nobody knows what can be the effects of these substances on cell development, care should be taken in the manipulations. They are however present in trace amounts in the healthy plants, their concentrations being considerably increased by fungal, microbial or chemical aggressions. The indole metabolites reported from the edible Cruciferae such as cabbages, cauliflower, broccoli, do not seem to have this kind of cytotoxicity.

[1] M. Takasugi, N. Katsui, and A. Shirata, J. Chem. Soc. Chem. Commun. **1986,** 1077.
[2] M. Takasugi, K. Monde, N. Katsui, and A. Shirata, Chem. Letters **1987,** 1631.
[3] M. Takasugi, K. Monde, N. Katsui, and A. Shirata, Bull. Chem. Soc. Jpn. **61,** 285 (1988).
[4] T. Rouxel, A. Sarniguet, A. Kollmann, and J. F. Bousquet, Physiol. Molec. Plant Pathol. **34,** 507 (1989).
[5] A. Kollmann, T. Rouxel, and J. F. Bousquet, J. Chromatogr. **473,** 293 (1989).
[6] M. Devys, M. Barbier, I. Loiselet, T. Rouxel, A. Sarniguet, A. Kollmann, and J. F. Bousquet, Tetrahedron Lett. **29,** 6447 (1988).
[7] M. Devys, M. Barbier, A. Kollmann, T. Rouxel, and J. F. Bousquet, Phytochemistry **29,** 1087 (1990).
[8] M. Devys and M. Barbier, Synthesis **1990,** 214.
[9] M. Devys and M. Barbier, J. Chem. Soc. Perkin Trans. 1, **1990,** 2856.
[10] M. Devys and M. Barbier, Phytochemistry **30,** 389 (1991).
[11] G. S. Stoewsand, J. G. Babish, and H. C. Wimberley, J. Environ. Path. Toxicol. **2,** 399 (1978).
[12] C. Srisangam, D. G. Hendricks, R. P. Sharma, D. K. Salinkhe, and A. W. Mahoney, J. Food. Saf. **4,** 235 (1980).
[13] J. N. Boyd, J. G. Babish, and G. S. Stoewsand, Food Chem. Toxicol. **20,** 47 (1982).
[14] L. W. Wattenberg, Cancer Res. **43** (Suppl.), 2448 (1983).
[15] K. E. Aspry and L. F. Bjeldanes, Food Chem. Toxicol. **21,** 133 (1083).
[16] S. Hendrich and L. F. Bjeldanes, Food Chem. Toxicol. **21,** 479 (1983).
[17] L. W. Wattenberg and W. D. Loub, Cancer Res. **38,** 1410 (1978).
[18] J. E. Nixon, J. D. Hendricks, N. E. Pawlowski, C. B. Pereira, R. O. Sinnhuber, and G. S. Bailey, Carcinogenesis **5,** 615 (1984).
[19] M. Devys and M. Barbier, unpublished syntheses.