Studies on the polyacetylenes from the tissue culture of *Ambrosia maritima*, an Egyptian medicinal plant

 平成 12 年度進学
 AbouZid, Sameh

 指導教官
 折原裕

Many species of the family Asteraceae, the largest family of the higher plants, is known to produce a wide variety of structural types of polyacetylenes (PA). These compounds possess significant antibiotic, antiviral and nematicidal activities. In this research work, we have developed a transformed root culture and a callus culture systems from the plant *Ambrosia maritima*, a weed growing on the Nile banks. We have cleared some of the important points in the pathway of biosynthesis of PA produced by these systems. Additionally, we have discovered a new biological activity for PA as molluscicidal agents.

1- Establishment of the plant tissue culture system

For the root culture 3 lines were established from the sterile plant infected with *Agrobacterium rhizogenes* ATCC15834. These lines, continuously subcultured on Murashige and Skoog (MS) agar basal media at 25 °C in the dark, were inoculated in MS liquid media and cultured on 50 rpm in the dark. Growth time curve showed significant difference between the three lines. Callus culture was initiated by cutting the sterile plant material and putting it on MS agar media contianing 2,4-dicholorophenoxyacetic acid. The callus was maintained on MS agar media containing naphthalene acetic acid (1 ppm) and kintein (0.1 ppm) (NK media). Cell suspension culture was initiated by inoculating callus tissues into NK liquid media and was cultured on 100 rpm in the dark.

2- Biosynthetic studies

A- Determination of the optimum yield conditions

An elicitation strategy was applied to maximize the yield of PA. The elicitor used was methyl jasmonate (MeJ), the common signal molecule in plant stress and development. Optimum conditions were obtained by eliciting the root tissues for 72 h in the early log phase of growth with 40μ M MeJ. Metabolic profiling for the biomass obtianed after elicitation showed thiarubrine A (1), its epoxide (2) and diol (3) as well as its precursor pentaynene (4) (Fig. 1) as the main PA produced by the hairy root. Thiophene A (5) and its diol (6) (Fig. 1), the photodegradative products of their corresponding thiarubrines, were isolated from hairy root cultured under continuous light illumination. When the cell suspension culture was challenged with MeJ, only 4 could be found.

B- Structure elucidation and assignment of the ¹³C-NMR signals

The structure elucidation and assignment of the each carbon signal to its corresponding position was based on MS, 1D- and 2D-NMR spectroscopic analysis.



Fig. 1. Chemical structure of the isolated polyacetylenes

C-Feeding experiments

Our biosynthetic study for **1** and **5**, using $[1^{-13}C]^-$, $[2^{-13}C]^-$ and $[1,2^{-13}C_2]^-$ acetates suggested either anabolic or catabolic pathways. The key steps leading from acetates to **4** are supposed to proceed through unsaturated fatty acid, based mainly on co-occurrence and structural similarity. However, not much is known about the order of these reactions or the specificity of the enzyme involved. When we studied the global effects of MeJ on the root tissues (Fig. 2), we found an increase in linoleic acid (LA) (peak at 120 h) along with that obsverved for **1** and its precursor **4** (peak at 72 h).



Fig. 2. Effects of MeJ on metabolite biosynthesis and LOX activity

Complementary to the above results, incubation of root cultures with linoleic acid- ${}^{13}C_{18}$ resulted in the production of labelled 1. The pattern of enrichment suggested a biosynthetic pathway by chain length reduction and desaturation of the fatty acid precursor. The involvement of LA in polyacetylene biosynthesis suggested a pathway that may include lipoxygenase (LOX), an enzyme which catalyzes the hydroperoxidation of polyunsaturated fatty acids. LOX activity was found to peak 24 h after elicitor addition and before the increase in polyacetylene production (Fig. 2). *N*-propyl gallate, a LOX inhibitor, had significantly reduced the polyacetylene production at 100µM.

In conclusion, our results suggest a catabolic pathway for compound **1** biosynthesis from LA with a possible involvement of LOX at an early stage of the pathway (Fig. 3).



Fig. 3 Hypothesis for PA biosynthesis in the root culture of A. maritima

3- The effect of PA on the viability of *Biomphalaria glabrata*, schistosome intermediate host

Schistosomiasis is a parasitic disease representing a major health risk in the rural areas 74 tropical developing countries. It is estimated that 200 million people are infected with this parasite. One of the effective methods to control of the disease is to control the intermediate host (freshwater snail = mollusc) using molluscicidal agents. Those agents of plant origin are less toxic as they are part of the local ecosystem.

The molluscicidal activities of both *A. maritima* transformed root extract and individual PA, were studied against schistosome intermediate host *Biomphalaria glabrata*. The chloroform extract of the MeJ treated root was effective in killing 100% of the snails at concentration of 50 ppm after 5 days of exposure. While compound **1** showed strong activity (killing 93% of the snails at a concentration of 5 ppm with a LC50 of 2.64 ppm), when compared to that of ambrosin, a sesquiterpene lactone that long considered as the active molluscicidal agent from the aerial parts of the plant. When the other PA were tested, the order of activity was as such 4 < 1 < 2 < 3. Compound **3**, which showed the highest activity, was the only form secreted to the media from these PA.

As such, we clear the ecological picture for the role of these metabolites. The plant root can synthesize them from primary metabolites, then increase their polarity by epoxide and diol formation. The diol is then secreted to the media to act as an allelopathic agent in the surrounding environment.