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Alessandro Ballio, Rodolfo Federico, Daniela Scalorbi

Metabolic stability of fusicoccin in plant tissues

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Fisiologia. — *Metabolic stability of fusicoccin in plant tissues* (*). Nota di ALESSANDRO BALLIO, RODOLFO FEDERICO e DANIELA SCALORBI, presentata (**) dal Corrisp. E. MARRÈ.

RIASSUNTO. — L'incubazione di fusicoccina con coleottili di mais, segmenti di internodio di piselli, foglie di spinacio, apici radicali di mais, in condizioni usate precedentemente per studiarne gli effetti fisiologici, non provoca modificazioni rilevanti del composto, tranne per una lieve idrolisi dei gruppi O–acetilici che è più marcata nel tessuto fogliare.

INTRODUCTION

In recent years fusicoccin (FC), a carbotricyclic diterpenoid glucoside produced by *Fusicoccum amygdali* Del. [1, 2, 3] has become a useful tool for the study in higher plants of several physiological processes [4]. Experiments with FC have been performed in a number of materials, such as sections of stem, coleoptile and root, leaf discs, isolated cotyledons and whole seeds. According to the material used and the type of response sought, incubation with FC lasts long enough (from 30′ to several hours) to suggest that the compound might be modified by enzymes of the plant tissues, or of microbial contaminants, with a concomitant change in its activity.

The present paper reports the results of experiments performed to provide this type of information.

MATERIALS AND METHODS

Chemicals. FC, monodeacetyl-FC and dideacetyl-FC were prepared according to published procedures [5, 6]. Solvents and salts were purchased from E. Merck, Darmstadt.

Radiochemicals. [^{3}H]-Dihydro-FC (1 mCi/μmole) was prepared by catalytic hydrogenation of FC [2] with $^3H_2$. All of the label was present in the *t*-pentyl moiety. The radioactivity was measured with a Packard scintillation counter in Bray's solution.

Preparation of plant material and incubation conditions. Pea (*Pisum sativum* L., cv. Alaska) seeds were grown on sawdust for 6-8 days in the dark at 25 °C. Segments 10 mm long were cut from the apical part of the distal internode.

(*) Istituto di Chimica Biologica, Università di Roma. Lavoro eseguito nell'ambito del progetto Finalizzato C.N.R. « Fitofarmaci e Fitoregolatori ».

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Maize (*Zea mays* L., cv. Dekalb XL 342) coleoptiles and roots were obtained from seedlings grown on sawdust for 5-6 days at 28° C; maize root segments 3 cm long were excised from the subapical part of the main root.

Spinach (*Spinacia oleracea*) leaf fragments were prepared from commercial plants.

Tissues (400 mg) were preincubated for 2 h at 25° C in 10^{-3} M phosphate buffer, pH 7.0 (10 ml), then washed with distilled water and incubated at 25° C in 10^{-3} M KCl until the pH became constant (approximately 1 h). After addition of FC (10^{-5} M final concentration) and [^{3}H]-dihydro-FC (0.1 μCi in 10 ml final volume) the samples were kept 3 h at 25° C.

Measurements of pH (Radiometer pH-meter 28) were performed immediately after addition of FC and at the end of the 3 hour incubation period. The difference between initial and final values is indicated as Δ pH.

Extraction of radioactive compounds after incubation with plant tissues. At the end of incubation each sample was filtered and thoroughly washed with water. After blotting on filter paper, the tissue was homogenized in Potter at 4° C with 2 ml of 1 M perchloric acid, the homogenate was centrifuged and the pellet was twice extracted with the same amount of perchloric acid. All radioactivity was removed by this treatment, as shown by the measurement of radioactivity of the pellet solubilized in Soluene-350 (Packard Instruments Co., U.S.A.). The pooled perchloric supernatants were extracted three times with 3 ml of chloroform and the pooled chloroform solutions washed with 3 ml of 0.1 M citrate buffer, pH 5.5. The washed chloroform solution was concentrated at reduced pressure and quantitatively transferred to a silica gel plate (DC-Fertigplatten Kieselgel 60, E. Merck, Darmstadt) to which reference samples of [^{3}H]-dihydro-FC, FC and its mono- and di-deacetyl derivatives were also applied. The plate was developed with chloroform/2-propanol (9 : 1, v/v), dried and the whole area corresponding to the path run by the extract of the incubated samples was sectioned in one cm long zones, each being then separately scraped away and counted in Bray's solution. Results are expressed as the percentage of total radioactivity recovered after chromatography, which was always more than 95 %. Finally the plate was sprayed with 5 % sulphuric acid in methanol and heated at 110° C in order to locate the reference compounds. The same overall procedure was used for the analysis of samples of [^{3}H]-dihydro-FC incubated without tissue or with boiled (5') tissues.

RESULTS AND DISCUSSION

In this paper the fate of FC in a number of plant tissues has been investigated by using dihydro-FC labelled with tritium in the *t*-pentyl moiety. This FC derivative is slightly more phytotoxic than FC [7], but otherwise is as active as the parent compound in influencing various physiological processes [8, 9, 10]. In order to check that such processes were properly functioning

TABLE

Fate of radioactivity taken up by some plant tissues on incubation with [^{3}H]-*dihydro*-FC.

Radioactive compounds in the chromatogram	Radioactivity (% of total)												
	Maize coleoptiles			Maize roots			Spinach leaves			Pea stem segments			Without tissues
	Fresh	Boiled	Δ (*)	Fresh	Boiled	Δ (*)	Fresh	Boiled	Δ (*)	Fresh	Boiled	Δ (*)	
Dihydro-FC	57.5	68.5	—11	56	65	—9	40	68	—28	52	64	—12	66.5
Deacetylated derivatives (**)	42.5	31.5	+11	44	35	+9	60	32	+28	48	36	+12	33.5

(*) Value of "fresh" minus that of "boiled".

(**) Sum of mono- and dideacetyldihydro-FC.

in the tissues chosen for this investigation, proton secretion was measured in each experiment. All results reported in the Table were obtained from experiments giving Δ pH values not less than 0.8.

The procedure used to extract the labelled compounds from tissues incubated with [^{3}H]-dihydro-FC is quite effective, as shown by the negligible radioactivity left in the material after perchloric acid treatment. The extracted radioactive compounds must still contain the *t*-pentyl moiety, as the label of [^{3}H]-dihydro-FC is confined to that part of the molecule. Thin-layer chromatography of the extracts consistently showed, besides the starting compound,

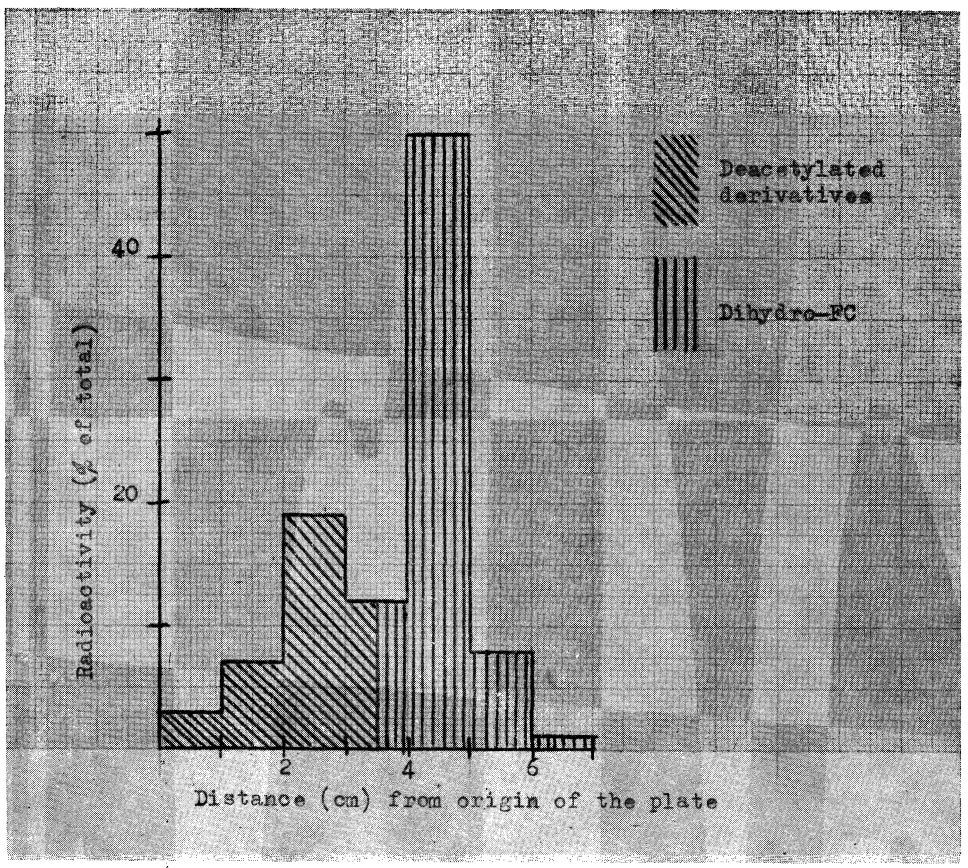

Fig. 1. – Distribution of radioactivity in a thin-layer chromatogram run with an extract of boiled pea stem segments incubated 3 h with [^{3}H]-dihydro-FC.

more polar radioactive substances running like the deacetylated derivatives of FC (these have R_F values identical to those of the corresponding derivatives of dihydro-FC). In fact, the extracts always gave a qualitative pattern identical to that of [^{3}H]-dihydro-FC incubated either without tissue or with boiled tissues (fig. 1). This was characterized by a 30-35 % content of deacetylated

derivatives which arise through chemical hydrolysis of the O-acetyl groups at the pH used for incubation. The lability of these groups is well documented [5, 6, 11, 12, 13]. Labelled compounds less polar than dihydro-FC were never observed.

The results of the experiments performed with pea stems, maize coleoptiles and roots, and with spinach leaves are reported in the Table. The figures are averages of at least two separate experiments. Hydrolysis of the O-acetyl groups, to give the monodeacetyl and/or the diacetylderivative of dihydro-FC, is slight when compared to the controls with boiled tissues, except for spinach leaves which apparently display a marked esterase activity. In any case this finding is of negligible relevance to the purpose of the present investigation since the effects of the partially or totally deacetylated derivatives of FC are still very high [8, 9, 10].

In conclusion, the results reported in this paper demonstrate that FC has a remarkable metabolic stability in plant tissues commonly used for the study of its effect on physiological processes, the only change observed being a partial hydrolysis of O-acetyl groups, which are known to represent structural features irrelevant to the display of biological activity.

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