Short Communications

Effect of Substituted Uracil Derivatives on Induction of Nitrate Reductase in Plants

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Substituted uracil derivatives including 5-bromo-3-*sec.*-butyl-6-methyluracil (bromacil) and 5-bromo-3-isopropyl-6-methyluracil (isocil) were found by Bucha, Cupery, Harrod, Loux & Ellis (1962) to possess severe phytotoxic properties, resembling those of other herbicides found to inhibit photosynthesis in independent experiments by Hoffman, McGahen & Sweetster (1964) and by Hilton, Monaco, Moreland & Gentner (1964). The compounds were effective at about micromolar concentrations with chloroplasts from several sources. Following the work of Hewitt & Afridi (1959) and Afridi & Hewitt (1962, 1965), who tested effects of several potential antimetabolites on the induction of nitrate reductase in excised plant tissues when nitrate or molybdenum was present as inducer, similar experiments with the substituted uracils revealed unexpectedly large effects on enzyme production.

Methods. The methods used to demonstrate the formation of nitrate reductase in excised tissues from plants grown in sand cultures, together with reports of the effects of time, temperature and concentration of inducers and inhibitors on the activities produced, were fully described by Afridi & Hewitt (1964, 1965). In the present work induction by nitrate was carried out with leaf tissues from plants deprived of nitrate for about 5 days until endogenous enzyme activity had decayed to low levels (Afridi & Hewitt, 1964), and induction by molybdenum was tested on tissues from plants grown with nitrate in molybdenum-deficient culture media (Hewitt, 1966). Solutions of sodium nitrate (4mM) or sodium molybdate (3 μ M) alone or with the inhibitors were vacuum-infiltrated into well-mixed 1g. samples of small fragments of tissues of cauliflower (*Brassica oleracea* var. *botrytis*) or radish (*Raphanus sativus*). The tissues were either incubated for 5hr. at 25° in 11000-lux white fluorescent light or were processed at once (0hr.). The conditions were those previously shown to be most suitable for recording changes in enzyme content formed in response to treatments, when compared with control treatments with water only or at 0hr. Activity was assayed in 0.5ml. of centri-

fuged extract obtained by grinding 1g. of leaf tissue in 3ml. of 0.1M-potassium phosphate buffer, pH 8.5, in a frozen mortar. Incubation was for 15min. at 25° in the presence of NADH (0.2mM), nitrate (6mM) and potassium phosphate buffer, pH 7.2 (0.06M), in a volume of 4ml. The reaction was stopped by oxidative removal of NADH with acetaldehyde and alcohol dehydrogenase and mixing with the Griess Ilosvay reagents to determine nitrite produced by measurement of $E_{540m\mu}$ (Hewitt & Nicholas, 1964). Activity was recorded as increase in nitrite produced compared with controls from which NADH was omitted during the incubation. Neither bromacil nor isocil at the concentrations used had any effect on the assay *in vitro* and they did not inhibit endogenous enzyme activity as manifested by nitrite accumulation of tissues kept at low light-intensities (about 500 lux) after infiltration with nitrate.

Results. Effects of bromacil and isocil on the induction of nitrate reductase by nitrate or molybdenum in two species are shown in Table 1. The inhibition was severe at the lowest concentration tested (1 μ g./ml. in the infiltrating solutions). These probably resulted in internal (cellular) concentrations not exceeding 7–10% of those used (Afridi & Hewitt, 1964) and could therefore have been about 0.4 μ M. Inhibition was similar in extent with both compounds in both cauliflower and radish tissues and was comparable with respect to induction by either nitrate or molybdenum. On a concentration basis the activities of bromacil and isocil were more inhibitory than several antimetabolites tested in previous work (Afridi & Hewitt, 1965). The most inhibitory compound then reported was cycloheximide (Actidione), which produced 46–79% inhibition at 20 μ M in the infiltrating solutions, whereas bromacil and isocil produced 35–94% inhibition at 4 μ M (1 μ g./ml.) and 70–95% inhibition at 20 μ M (5 μ g./ml.), and in one test inhibition by isocil reached 98% at 100 μ g./ml. for cauliflower in the presence of molybdenum.

The relative inertness of several thio and azo analogues of purines and pyrimidines when tested as inhibitors of nitrate reductase formation in

Table 1. *Effect of isocil and bromacil on induction of nitrate reductase by molybdenum or nitrate in cauliflower and radish leaf tissue*

Activities were measured as increase in nitrite produced proportional to $E_{540m\mu}$ during a 15 min. assay period after grinding 1 g. sub-samples of leaf material in phosphate buffer as described in the text. Induction of enzyme is indicated by increase over activity at 0 hr. resulting from infiltration with molybdenum or nitrate with or without the inhibitor and incubating at 25° in the light for 5 hr.

Inducer	Inhibitor	Species	Endogenous enzyme activity at 0 hr.	Concn. of inhibitor ($\mu\text{g./ml.}$) ...	Enzyme activity after incubation for 5 hr. with inducer and different concentrations of inhibitor			
					0	1	5	10
Molybdenum	Isocil	Cauliflower	0.009		0.543	—	—	0.044
Molybdenum	Isocil	Radish	0.091		0.474	0.180	0.102	0.063
Molybdenum	Bromacil	Cauliflower	0.019		0.273	0.020	0.057	0.021
Molybdenum	Bromacil	Radish	0.053		0.165	0.055	0.033	0.016
Nitrate	Isocil	Cauliflower	0.038		0.151	0.098	0.038	0.046
Nitrate	Bromacil	Cauliflower	0.024		0.174	0.081	0.053	0.041

leaves at concentrations between 0.4 and 16 mm (Afridi & Hewitt, 1965), and the negligible effects of isocil or bromacil on DNA metabolism reported by McGahen & Hoffman (1963*a,b*, 1966), suggests that the effects of bromacil and isocil now reported may relate to some other and quite different mechanism of action. It is possible that nitrate reductase in leaves, like nitrite reductase (Betts & Hewitt, 1966), may be located in chloroplasts. If so, a common site or mechanism for inhibition of some aspect of photosynthesis and formation of nitrate reductase by these compounds may be involved.

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A Novel Method for Evaluating K_i/K_m and its Application to the Competitive Inhibition of Staphylococcal Penicillinase by Cephalosporins

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During a study of the competitive inhibition of staphylococcal penicillinase (EC 3.5.2.6) by the semisynthetic cephalosporins cephaloram, cephalo-

thin and cephaloridine, a rapid method was needed for calculating the ratio K_i/K_m . This ratio is conventionally obtained by plotting data by the method