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SHORT REPORTS

ISOPENTENYLADENINE FROM *DICTYOSTELIUM DISCOIDEUM*

YOSHIMASA TANAKA*, HIROSHI ABE†, MASAAKI UCHIYAMA†, YOICHI TAYA‡ and SUSUMU NISHIMURA‡

* Institute of Biological Sciences, The University of Tsukuba, Ibaraki, 300–31; † Department of Plant Protection, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, 183; ‡ Biology Division, National Cancer Center Research Institute, Tokyo, 104, Japan

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Key Word Index—*Dictyostelium discoideum*; Acrasiaceae; isopentenyladenine; cellular slime mold; discadenine biosynthesis.

INTRODUCTION

Spore germination in *Dictyostelium discoideum* [1] is known to be controlled by a water soluble, low molecular weight inhibitor(s) [2]. We have isolated a compound, called discadenine, which causes 100% inhibition of spore germination at a concentration of 0.04 μg/ml [3]. More recent work has shown that discadenine is 3-(3-amino-3-carboxypropyl)-6-(3-methyl-2-butenylamino) purine [4], and that it has considerable cytokinin activity [5]. Two possible biosynthetic pathways for discadenine are (1) introduction of the 3-amino-3-carboxypropyl group into 6-(3-methyl-2-butenylamino)-purine [ip^{6}Ade] and (2) introduction of the isopentenyl group into 3-(3-amino-3-carboxypropyl)adenine [acp^{3}Ade]. Further studies have shown that discadenine may be synthesized enzymatically from ip^{6}Ade when the latter is incubated with an extract of the cells of *D. discoideum* and *S*-adenosylmethionine as donor of the 3-amino-3-carboxypropyl group [6]. It has been suggested that *D. discoideum* cells may synthesise ip^{6}Ade as a precursor of discadenine. Free ip^{6}Ade has been found in various organisms [7, 8] but not previously in *D. discoideum*. In the present work we have demonstrated the existence of ip^{6}Ade in *D. discoideum* cells in the culmination stage.

RESULTS

The purified compound, isolated from *D. discoideum* cells, showed a UV absorption similar to that of synthetic ip^{6}Ade: 273 nm in 0.1 N HCl, 268 nm in EtOH, and 275 nm in 0.1 N NaOH. Also the R_f values of 0.50, 0.87 and 0.76 obtained with solvents (A), (B) and (C) respectively (see Experimental), agreed with those for the synthetic compound. Finally low resolution MS gave peaks at *m/e* 203, 188, 160, 148, 135, 119 and 108 which are characteristic of the naturally occurring [10] and synthetic ip^{6}Ade. From this evidence it may be concluded that the cellular slime mold, *D. discoideum*, does synthesise ip^{6}Ade in the culmination stage of its development.

The amount of ip^{6}Ade present in the cells was estimated by measuring the total UV absorbance of the material separated by preparative PC. Thus, taking ε for ip^{6}Ade as 19400 (269 nm) at pH 7 as a minimum estimate, the content of ip^{6}Ade in the culmination state cells of *D. discoideum* was 3 μg/g wet wt. We obtained about 200 g wet wt of cells and 0.57 mg of ip^{6}Ade from 2500 plates. This amount of ip^{6}Ade is consistent with our finding of 1.2–2.4 mg of discadenine in the sorus of 2500 plates [4]. This high content of ip^{6}Ade is more than 20 times that in *Agrobacterium tumefaciens* strain B6 (i.e. 2.3 μg/17 g bacteria [9]).

Ip^{6}Ade synthesized in the cells of *D. discoideum* therefore must be utilized as a substrate for the synthesis of discadenine.

EXPERIMENTAL

Isolation of ip^{6}Ade. D. discoideum, strain NC-4 was grown in association with *Aerobacter aerogenes* on nutrient agar plates (diameter; 9 cm) containing A medium [11]. The individuals in the culmination phase (10–20 hr after the beginning of morphogenesis) were scraped from the plates with a glass rod and the cells (*ca* 200 g wet wt) suspended in 80% EtOH, centrifuged and the supernatant evapd to dryness *in vacuo* at 45°. The residue was dissolved in 100 ml H_2O and the soln extracted ×3 with an equal vol. of EtOAc. The EtOAc extracts were combined, washed with 100 ml H_2O and evapd to dryness *in vacuo*. The residue was dissolved in a small vol. of EtOH and separated by ascending prep PC in H_2O. Two bands showing UV absorption were obtained; the slower one, which coincided with that of authentic ip^{6}Ade, was eluted with (1) 80% EtOH and (2) EtOAc and the combined eluates evapd to dryness. The dried substance was further purified by TLC on Si gel using the solvent, $CHCl_3$–MeOH (4:1). The following solvents were used for R_f values of the pure compound: (A) H_2O; (B) *n*-BuOH–NH_4OH (28%) (4:1); (C) *iso*-PrOH–conc HCl–H_2O (340:85:72).

Mass spectrometry. MS were measured with an ionizing electron energy of 70 eV and an ion source temp. of 290°.

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IN VITRO SYNTHESIS OF PYRETHRINS FROM TISSUE CULTURES OF *TANACETUM CINERARIIFOLIUM*

M. M. CASHYAP, J. S. H. KUEH, I. A. MACKENZIE and G. PATTENDEN
Departments of Botany and Chemistry, The University of Nottingham, Nottingham NG7 2RD, U.K.

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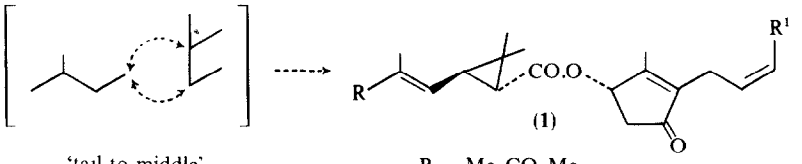
Key Word Index—*Tanacetum cinerariifolium*; Compositae; pyrethrins; biosynthesis; tissue cultures.

The 'pyrethrins' comprise a group of six closely related insecticidal esters, and are found in the flower heads of pyrethrum, *Tanacetum cinerariifolium* (syn. *Chrysanthemum cinerariaefolium*) [1]. They are based on the common structure (**1**). The esters command considerable interest from the point of view of their biosynthesis, not least because of the unusual 'tail-to-middle' combination of isopentane units found in the cyclopropane ring ('chrysanthemyl') portions of their structures. A similar combination of isopentane units is found in 'presqualene' and 'prephytoene', intermediates in the biosynthesis of squalene and phytoene respectively. Biosynthetic studies directed towards understanding the mechanism whereby this unusual combination of isopentane units is accomplished in the pyrethrins have been frustrated by low incorporations and seasonal variations of administered radiolabelled precursor molecules to intact plants of *T. cinerariifolium* [2, 3]. Accordingly we have examined the suitability of tissue cultures of this species for conducting more refined biosynthetic experiments.

Undifferentiated callus tissue was initiated from sterile six week old seedlings of *T. cinerariifolium* and grown statically at 27° under fluorescent lights of *ca* 1500 lux intensity, on a modified Murashige and Skoog's medium [4] supplemented with 2,4-dichlorophenoxyacetic acid (0.5 mg/l.), kinetin (0.75 mg/l.) and casein hydrolysate (1 g/l.) solidified with Agar (0.7%). Frequent sub-culturing provided a 4 kg sample of the callus for pyrethrin analysis. Newly isolated callus differentiated roots when the 2,4-dichlorophenoxyacetic acid and kinetin were omitted from the medium, and naphthalene–acetic acid (1 mg/l.) was added and the cultures maintained in darkness. Shoot formation was accomplished only at low and irregular frequency when newly isolated callus was placed on a medium containing β-indolylacetic acid (0.01 mg/l.) and kinetin (3 mg/l.) in darkness. After one month, cultures which had produced shoots were grown under light prior to collection for pyrethrin analysis; those cultures having either roots or shoots present were also analysed for insecticidal pyrethrins.

TLC and GLC analysis of the petroleum extract of undifferentiated callus tissue, and comparison with authentic specimens, failed to reveal the presence of pyrethrins in the callus [5] or in the medium in which the callus was grown. Although a peak coincident with authentic cinerin I (**1**, R = R^1 = Me) was found in GLC analysis, inspection of the MS of the peak from a GC–MS analysis failed to support its authenticity (cf. ref. [5]). Attempts to detect pyrethrins in callus which had differentiated only roots were also unsuccessful.

GLC analysis of shoots which had been differentiated from callus revealed the presence of the six pyrethrin esters, cinerin I, jasmolin I, pyrethrin I, cinerin II, jasmalin II and pyrethrin II in the *ca* proportions 3:14:76:0.2:0.8:6; a similar ratio of pyrethrins was found in four week old seedlings. The authenticity of the pyrethrins in the shoots was indicated by GC–MS of extracts and comparison with mass spectra from authentic specimens [6]. In addition a combined 'pyrethrin I' fraction (~1.5 mg) (containing ~85% pyrethrin I) from fifteen shoots was separated by TLC and analysed by PMR [7, 8]. The resultant spectrum was almost



'tail-to-middle'

(1)

R = Me, CO_2Me
R^1 = Me, Et, vinyl