

totally superimposable on that of authentic pyrethrin I (1, R = Me, R¹ = vinyl).

Not for the first time, these studies emphasise that unorganised tissue cultures often do not have the secondary metabolism characteristic of the corresponding intact plant. The studies also demonstrate that undifferentiated cultures of *T. cinerariifolium* are of limited value in studying the biosynthesis of the pyrethrins which accumulate in the intact plant; it is interesting that pyrethrins have recently been detected in callus cultures of *Tagetes erecta* [9] and *T. minuta* [10]. Since anomalous secondary metabolic pathways have been a feature in studies of terpenes with other plant tissue cultures [11], we are currently examining cultures of *Tanacetum cinerariifolium* for alternative, and biosynthetically significant, metabolites.

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REVISED STRUCTURE OF BEAUVELLIDE, A CYCLODEPSIPEPTIDE FROM *BEAUVERIA TENELLA*

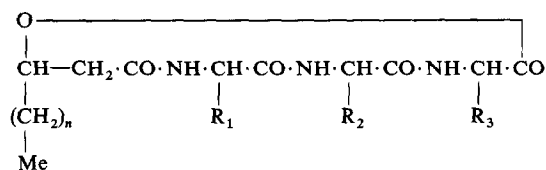
FRANCOIS FRAPPIER*, MARY PAIS*, JOHN F. ELSWORTH† and JOHN FREDERICK GROVE‡

*Institut de Chimie des Substances Naturelles, C.N.R.S. 91190-Gif/Yvette, France; †Department of Organic Chemistry, University of Cape Town, Rondebosch, South Africa 7700; ‡A.R.C., Unit of Invertebrate Chemistry and Physiology, School of Molecular Sciences, University of Sussex, Brighton, BN1 9QJ, U.K.

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Key Word Index—*Beauveria tenella*; *B. bassiana*; Moniliales; cyclodepsipeptides; beauvellide; beauverolides.

Structure 1 has been proposed for a cyclodepsipeptide named beauvellide, isolated from a strain of *Beauveria tenella*, on the basis of spectral data, essentially low resolution MS ($M^+ 515$) [1]. A closely related cyclodepsipeptide from a strain of *Beauveria bassiana*, beauverolide H [2], was assigned the structure 2. This structure was deduced from high resolution MS analysis ($M^+ 487$) and acidic hydrolysis giving L-phenylalanine, L-alanine D-leucine and R- β -hydroxynonanoic acid [3]. MS also showed the presence of a small amount of a homologue 3 derived from β -hydroxyundecanoic acid, beauverolide I ($M^+ 515$).



- 1 R₁ = CH₂Ph, R₂ = Me, R₃ = CH(Me) Et. n = 7
- 2 R₁ = CH₂Ph, R₂ = Me, R₃ = CH₂-CHMe₂, n = 5
- 3 R₁ = CH₂Ph, R₂ = Me, R₃ = CH₂-CHMe₂, n = 7

The low resolution MS of beauvellide showed an intense M-28 peak ($m/e 487$) attributed to the elimination of CO [4]. However, as the elimination of CO was not observed in the high resolution MS of beauverolide H, it appeared that beauvellide could be a mixture of two depsipeptides of respective MW 487 and 515, identical to the mixture of beauverolides H and I.

A high resolution MS [3] was run on beauvellide and was identical to the spectrum of the mixture of beauverolides H and I [3]. An aminoacid analysis performed on a small amount of product after acid hydrolysis confirmed the presence of the aminoacids phenylalanine, alanine and leucine. No isoleucine [1] was detected.

Beauvellide is thus a mixture containing principally beauverolide H (or an isomer thereof in which one or more of the aminoacid and/or hydroxyacid units have the opposite configuration), the minor constituent being beauverolide I (or an isomer).

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AMINOPEPTIDASE ACTIVITY ASSOCIATED WITH α_1 -CONARACHIN (PEANUT PROTEIN)

NAVIN J. NEUCERE

Southern Regional Research Center,* Oilseed and Food Laboratory, P.O. Box 19687, New Orleans, LA 70179, U.S.A.

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Key Word Index—*Arachis hypogaea*, Leguminosae; peanut, aminopeptidase; enzyme adsorption, immunochemistry; germination.

Abstract—Aminopeptidases were investigated in protein extracts of dormant and germinated peanut cotyledons by electrophoretic immunochemical techniques. Considerable activity was observed in protein zones that migrated toward the anode after simple electrophoresis. Of the immunogenic proteins in dormant seed, aminopeptidase activity was associated only with the immunocomplex of α_1 -conarachin, a globular protein in peanuts. The specific aminopeptidase activity of total extracts was marginally higher than that of purified α -conarachin. Specific isoenzyme(s) adsorption on the antigen-antibody complex of α_1 -conarachin might be attributed in part, to these phenomena. Reactions of protein extracts from germinated cotyledons with immune sera made against protein in germinated tissues—roots and cotyledons—showed the identical associative interaction. Some of the determinant groups on α_1 -conarachin in the germ and cotyledon were apparently maintained during early phases of germination.

INTRODUCTION

Aminopeptides in dormant seeds of peanuts have been investigated in our laboratory during the last few years [1, 2]. With starch gel electrophoresis, 4 isoenzymes that hydrolyzed L-leucyl-beta-naphthylamide-HCl were detected at the anode [2]. With polyacrylamide disc electrophoresis, 5 similar enzyme bands that hydrolyzed the same substrate were observed [1]. In these studies, simple electrophoresis was employed on whole extracts that contained mixtures of albumins and globulins of the peanut seed. The objective of the present study was to further investigate this enzyme system in both dormant seed and in tissues of the growing plant by the more specific immunochemical methods.

RESULTS AND DISCUSSION

The immunoelectrophoretic analysis (IEA) of peanut proteins soluble in buffered aqueous media, reported by Daussant *et al.* [3], showed that at least 14 precipitin arcs were detected with immune serum made from a total cotyledonary extract. Alpha-conarachin, the major component in the classic conarachin fraction isolated by ion-exchange chromatography, showed 2 serologically distinct proteins that were named α_1 - and α_2 -conarachin. Alpha₁-Conarachin migrated toward the anode and α_2 -conarachin moved toward the cathode. In part, the

present data are based on these results.

Preliminary analyses by simple electrophoresis in agar showed considerable aminopeptidase staining on poorly separated protein zones (not shown). Further analyses by IEA, however, showed activity only on one precipitin line that was located in the region where α_1 -conarachin migrated. Therefore, simultaneous analyses using anti- α -conarachin and anti-cotyledonary proteins were conducted with purified α -conarachin and with total cotyledonary extracts (Fig. 1). These results showed enzyme activity associated only with the immunocomplex of α_1 -conarachin (a), as evidenced by the coalescence of the 2 arcs. With purified α -conarachin (Con) as antigen, identical results were noted (lower Fig. 1a).

To further investigate this system, sera made against protein from germinated tissues—cotyledons, roots and leaves—were employed for qualitative and semi-quantitative analyses. In Fig. 1b, (double diffusion) immune sera from 5-day germinated cotyledons and roots, mature leaves, and α -conarachin were reacted with proteins of 7-day germinated cotyledons in series. The characteristic aminopeptidase reaction of identity was observed in all tissues except for the leaves. This was expected for leaves, since α_1 -conarachin is not present in that tissue.

Enzyme staining on the immunochemical complexes of α_1 -conarachin after progressive germination showed about the same intensity (Fig. 1c), however, some

* One of the facilities of the Southern Region, Agricultural Research Service, U.S. Department of Agriculture.