3. Tanaka, Y., Hashimoto, Y., Yanagisawa, K., Abe, H. and Uchiyama, M. (1975) *Agric. Biol Chem. (Japan)* **39**, 1929
4. Abe, H., Uchiyama, M., Tanaka, Y. and Saito, H (1976) *Tetrahedron Letters* 3807.
5. Nomura, T., Tanaka, Y., Abe, H. and Uchiyama, M. (1977) *Phytochemistry* **66**, 1819.
6. Taya, Y., Tanaka, Y. and Nishimura, S., in preparation
7. Hall, R H (1973) *Ann. Rev. Plant. Physiol.* **24**, 415.
8. Schlee, D. (1975) *Pharmazie* **30**, 345.
9. Hahn, H., Heitman, I. and Blumbach, M. (1976) *Z. Pflanzenphysiol.* **79**, 143.
10. Helgeson, J. P. and Leonard, N. J. (1966) *Proc. Natl. Acad Sci., U.S.* **56**, 60.
11 Sussman, M. (1961) *J Gen. Microbiol.* **25**, 375.

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

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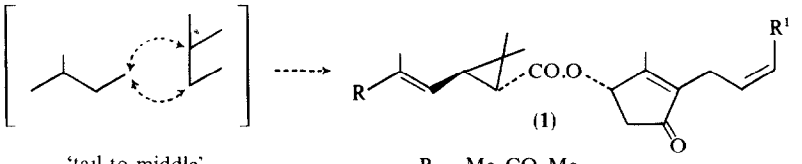
Key Word Index—*Tanacetum cinerariifolium*; Compositae; pyrethrins; biosynthesis; tissue cultures.

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

totally superimposable on that of authentic pyrethrin I (**1**, R = Me, R^1 = 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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REFERENCES

1. Casida, J. E. (1973) *Pyrethrum, the Natural Insecticide.* Academic Press, London.
2. Pattenden G. and Storer, R. (1973) *Tetrahedron Letters* 3473.
3. Pattenden, G., Popplestone, C. R. and Storer, R. (1975) *J. Chem. Soc. Chem. Commun.* 290.
4. Murashige, T. and Skoog, F. (1962) *Physiol. Plantarum.* **15**, 473.
5. Chumsri, P. and Staba, E. J. (1975) *Acad. Pharm. Sci. Abs.* **5**, 169.
6. Pattenden, G., Crombie, L. and Hemesley, P. (1973) *Org. Mass Spectrum* **7**, 719.
7. Bramwell, A. F., Crombie, L., Hemesley, P., Pattenden, G., Elliott, M. and Janes, N. F. (1969) *Tetrahedron* **25**, 1727.
8. Crombie, L., Pattenden, G. and Simonds, D. J. (1976) *Pestic. Sci.* **7**, 225.
9. Khanna, P., Sharma, R. and Khanna, R. (1975) *Indian J. Exp. Biol.* **13**, 508; Khanna, P. and Khanna, R. (1976) *Indian J. Exp. Biol.* **14**, 630.
10. Jain, S. C. (1977) *Planta Med.* **31**, 68.
11. Overton, K. H. and Picken, D. J. (1976) *J. Chem. Soc. Chem. Commun.* 105 and refs cited therein.

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

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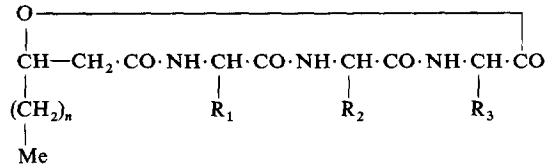
Key Word Index—*Beauveria tenella*; *B. bassiana*; Moniliales; cyclodepsipeptides; beauvellide; beauverolides.

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 the 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 R_1 = CH_2Ph, R_2 = Me, R_3 = CH(Me) Et, n = 7
2 R_1 = CH_2Ph, R_2 = Me, R_3 = CH_2·$CHMe_2$, n = 5
3 R_1 = CH_2Ph, R_2 = Me, R_3 = CH_2·$CHMe_2$, 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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REFERENCES

1. Frappier, F., Ferron, P. and Pais, M. (1975) *Phytochemistry* **14**, 2703.