Phytochemistry, 1975, Vol. 14, pp. 971–973. Pergamon Press. Printed in England.

ENZYMIC ISOPRENYLATION OF TRYPTOPHANYL CYCLIC DIPEPTIDES BY *ASPERGILLUS AMSTELODAMI*

CYNTHIA L. DEYRUP and CHARLES M. ALLEN, JR.*

Department of Biochemistry, University of Florida, Gainesville, Florida 32610, U.S.A.

(*Received* 5 *October* 1974)

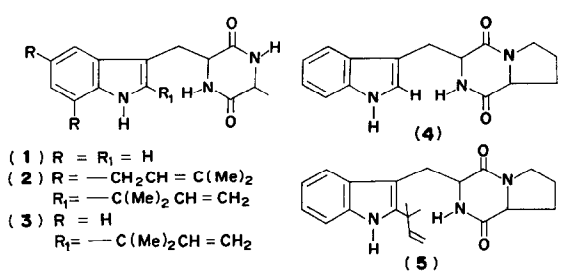
Key Word Index—*Aspergillus amstelodami*; fungi; isoprenylation; *cyclo*-L-prolyl-L-tryptophanyl; *cyclo*-L-alanyl-L-tryptophanyl; enzymic biosynthesis; spectra; cyclopentylideneethyl pyrophosphate.

Abstract—*Cyclo*-L-prolyl-L-tryptophanyl, a component of several metabolites of *Aspergillus ustus* and *cyclo*-L-alanyl-L-tryptophanyl were compared as co-substrates with 3-methyl-2-butenyl-[1-^{3}H]-1-pyrophosphate for an enzyme from *A. amstelodami* which previously had been described to isoprenylate *cyclo*-L-alanyl-L-tryptophanyl. Both compounds were equally active as isoprene acceptors using the *A. amstelodami* enzyme. The mass spectrum of the isoprenylated *cyclo*-L-prolyl-L-tryptophanyl indicated that the product was a monoisoprenylated derivative of the starting cyclic dipeptide. The most probable structure for this enzymic product is *cyclo*-L-prolyl-2(1,1-dimethylallyl)-L-tryptophanyl. The *cyclo*-pentylidene analogue of 3-methyl-2-butenyl-1-pyrophosphate did not serve as an alkylating agent when *cyclo*-L-alanyl-L-tryptophanyl was used as co-substrate.

INTRODUCTION

Biosynthetic studies in *Aspergillus amstelodami* indicate that mevalonic acid [1] and cyclo-L-alanyl-L-tryptophanyl [2] (**1**) are *in vivo* precursors of echinulin (**2**). A partially purified enzyme from this fungus has been described [3] which transfers the isoprene unit from 3-methyl-2-butenyl-1-pyrophosphate to (**1**). The monoisoprenylated (**1**) formed has been tentatively identified as cyclo-L-alanyl-2-(1,1-dimethylallyl)-L-tryptophanyl (**3**). Subsequent studies [4] have established that (**3**) is also an *in vivo* precursor of echinulin.

Analogous isoprenylated cyclic dipeptides derived from *cyclo*-L-prolyl-L-tryptophanyl (**4**) are biosynthesized in *A. ustus* [5] and *Penicillium brevicompactum* [6]. Some of these metabolites (like those isolated from *A. amstelodami*) contain a 2(1,1-dimethylallyl) indole moiety. In fact, 1 metabolite characterized from *A. ustus* is a direct analogue of (**3**), *cyclo*-L-prolyl-2(1,1-dimethylallyl)-L-tryptophanyl (**5**). Furthermore, an apparent rearrangement product of (**5**), austamide, has been isolated from *A. ustus* and shown to cause toxicosis in ducklings. This paper describes the *in vitro* biosynthesis of (**5**) catalyzed by an enzyme from *A. amstelodami*.



RESULTS AND DISCUSSION

Cyclic dipeptides (**1**) and (**4**) were compared as isoprene acceptors using an enzyme prepared from *A. amstelodami* and 3-methyl 2-butenyl-[1-^{3}H]-1-pyrophosphate as the isoprene donor. Both cyclic dipeptides are highly active as isoprene acceptors. The dependence of product formation on the concentration of cyclic dipeptide is illustrated in Fig. 1. The chromatographic mobilities of the Et_2O–EtOH extracted products were compared by TLC on Si gel in C_6H_6–BuOH. Isoprenylated (**1**) gave an R_f 0·43 corresponding to that described for

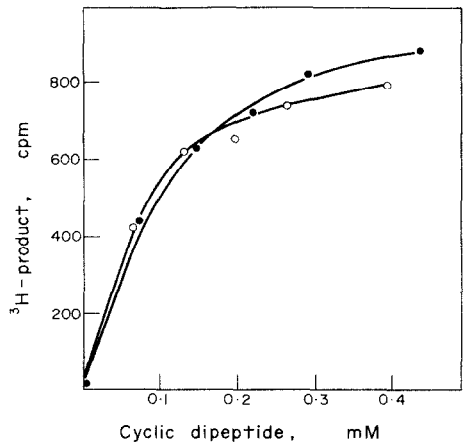


Fig. 1. Dependence of product formation on cyclic dipeptide concentration. *Cyclo*-L-alanyl-L-tryptophanyl (●——●) and *cyclo*-L-prolyl-L-tryptophanyl (○——○) were incubated with 3-methyl-2-butenyl-[1-^{3}H]-1 pyrophosphate (0·03 mM) and enzyme in 0·1 M tris buffer pH 8 for 30 min at 30°.

compound (**3**) [3]. Isoprenylated (**4**) chromatographed as a single radioactive peak in the same solvent system at R_f 0·75.

Spectral analyses were made on isoprenylated (**4**) which had been purified by TLC. The UV spectrum showed maxima at 223, 275 (sh), 283, and 291 nm and thus represented a slight hypsochromic shift compared to that of the starting cyclic dipeptide (maxima at 221, 274, 281 and 290 nm). A similar spectral difference was described previously for (**1**) and (**3**) [3]. The spectrum of this metabolite is essentially the same as that described for (**5**) [5]. High resolution MS analysis of the compound gave the M^+ and principal peaks given in Table 1. The MW is consistent with (**5**). The base peak at M^+-153, $C_{14}H_{16}N_1$, indicates an isoprenylated indole nucleus and the presence of a M^+-198, $C_7H_9N_2O_2$ confirms the presence of a non-isoprenylated diketopiperazine moiety.

A comparison of other spectral features with similar data obtained from (**3**) [3] shows the loss of an isoprene unit from the indole nucleus as demonstrated by the appearance of M^+-153 ($C_{14}H_{16}N_1$) and M^+-221 ($C_9H_8N_1$) for the enzymic product and M^+-127 and M^+-195 for compound (**3**) [3, 7]. This is confirmed by the presence of an ion *m/e* 69 (C_5H_9). A similar fragmentation resulting in an isoprene unit loss is seen with the 2(1,1-dimethylallyl) substituted indoles echinulin [3] and neoechinulin [8]. A loss of one or two methyl groups from the isoprene substituted indole is also observed with the appearance of M^+-168 ($C_{13}H_{13}N_1$) and M^+-183 ($C_{12}H_{10}N_1$). This fragmentation pattern has been reported for the 2 substituted 1,1-dimethylallyl indoles (**3**), echinulin [3] and neoechinulin [8].

In an attempt to determine whether a bulkier structural analogue of 3-methyl-2-butenyl-1-pyrophosphate could serve as an alkyl donor, *cyclo*-L-alanyl-L-tryptophanyl-[3-^{14}C] (0·5 μM) was incubated with cyclopentylideneethyl pyrophosphate (**6**) (0·31 μM) and enzyme. No alkylation was detected, since the only radiochemical compound observed, following TLC of the reaction components, was starting material. Also no alkylation was observed in a similar reaction where the concentrations of cyclic dipeptide and pyrophosphate

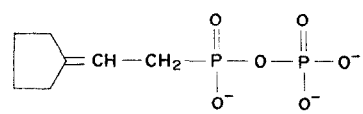


Table 1. Mass spectra of (**4**) and its isoprenylated product

	Molecular formula	Required mass	Observed mass	Relative intensity (%)
Compound (**4**)				
M^+	$C_{16}H_{17}N_3O_2$	283·1320	283·1312	8
M^+-153	$C_9H_8N_1$	130·0656	130·0652	100
M^+-130	$C_7H_9N_2O_2$	153·0663	153·0658	1
Isoprenylated (**4**)				
M^+	$C_{21}H_{25}N_3O_2$	351·1946	351·1940	6
M^+-153	$C_{14}H_{16}N_1$	198·1282	198·1307	100
M^+-168	$C_{13}H_{13}N_1$	183·1047	183·1041	17
M^+-183	$C_{12}H_{10}N_1$	168·0812	168·0806	11
M^+-198	$C_7H_9N_2O_2$	153·0663	153·0641	2
M^+-221	$C_9H_8N_1$	130·0656	130·0676	6
69	C_5H_9	69·0704	69·0696	11

were 0·58 and 0·032 μM respectively. Apparently cyclopentylidineethyl pyrophosphate, which is active as a substrate for pig liver prenyl transferase [9], cannot serve as a substrate for the alkylating enzyme in spite of the close structural resemblance to 3-methyl-2-butenyl-1-pyrophosphate.

EXPERIMENTAL

Cyclo-L-prolyl-L-tryptophanyl was prepared from L-prolyl-L-tryptophan Me ester by treatment with methanolic NH_3 according to Ref. [3]. The product was recrystallized from EtOH, mp 175–176°. *Cyclo*-L-alanyl-L-tryptophanyl [3-^{14}C], 3-methyl-2-butenyl-1-pyrophosphate and 3-methyl-2-butenyl-[1-^{3}H]-1-pyrophosphate were prepared as previously described [3]. Cyclopentylideneethyl pyrophosphate was the generous gift of Dr. Kyozo Ogura.

The enzyme was prepared from a 3-day-old culture of *A. amstelodami* grown at 30° on Czapek Dox medium supplemented with sucrose (30%) and molasses (5%) [3]. Enzymic activity was observed in 2 protein fractions precipitating between 0–40% and 40–70% saturation in $(NH_4)_2SO_4$. The fraction precipitating between 40–70% saturation was used in each expt except the studies with the cyclopentylidene analogue where the 0–40% fraction was used. The isoprenylated products were extracted with Et_2O–EtOH and analyzed as previously described [3]. TLC was carried out on Si gel layers on plastic sheets. The position of migration of radiochemical compounds was determined with a radio-chromatogram scanner. Sufficiently pure isoprenylated (**4**) for spectral analyses was prepared by multiple development TLC on EtOH washed Si gel G in C_6H_6–BuOH (95:5). The radioactive product was removed from the layers and dissolved in EtOH for analysis. The high resolution MS gave the required analyses for $C_{16}H_{17}N_3O_2$.

Acknowledgements—This work has been supported in part by the Research Corporation and the University of Florida, Division of Sponsored Research. We also wish to thank Dr. Roy King for carrying out the MS analyses.

REFERENCES

1. Birch, A. J., Blance, G. E., David, S. and Smith, H. (1961) *J. Chem. Soc.* 3128.
2. Slater, G. P., MacDonald, J. C. and Nakashima, R. (1970) *Biochemistry* **9**, 2886.
3. Allen, C. M., Jr. (1972) *Biochemistry* **11**, 2154.
4. Allen, C. M., Jr. (1973) *J. Am. Chem. Soc.* **95**, 2386.
5. Steyn, P. S. (1973) *Tetrahedron* **29**, 107.
6. Baldas, J., Birch, A. J. and Russell, R. A. (1974) *J. Chem. Soc. Perkin* **I**, 50.
7. Saxton, J. E. Personal communication.
8. Barbetta, M., Casnati, G., Pochini, A. and Selva, A. (1969) *Tetrahedron Letters* 4457.
9. Nishino, T., Ogura, K. and Seto, S. (1971) *Biochim. Biophys. Acta* **235**, 322.