Biosynthesis of Neoechinulin by *Aspergillus amstelodami* from *cyclo*-L-[*U*-^{14}C]Alanyl-L-[5,7-$^{3}H_2$]tryptophyl

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Summary Doubly labelled *cyclo*-L-alanyl-L-tryptophyl (**1**) is incorporated as a unit into neoechinulins A (**2**), B (**4**), C (**5**), and D (**3**), and appears to be an intermediate in the biosynthesis of neoechinulin (**6**).

A NUMBER of neoechinulin[1]-type metabolites, characterized by a dehydrotryptophan unit, all containing a 'reversed' isoprenic chain in the 2-position of the indole nucleus, have been recently isolated from moulds of the genus *Aspergillus*:[2] neoechinulin A (**2**), B (**4**), C (**5**), and D (**3**). The close structural similarity between these compounds, which are probable biogenetic intermediates in the biosynthesis of neoechinulin (**6**), may allow the elucidation of interesting natural processes such as the prenylation and dehydrogenation of tryptophan systems.

We report now the results of feeding experiments designed to examine the possibility that neoechinulins may be derived from a preformed cyclic dipeptide, *cyclo*-alanyltryptophyl (CAT) (**1**).

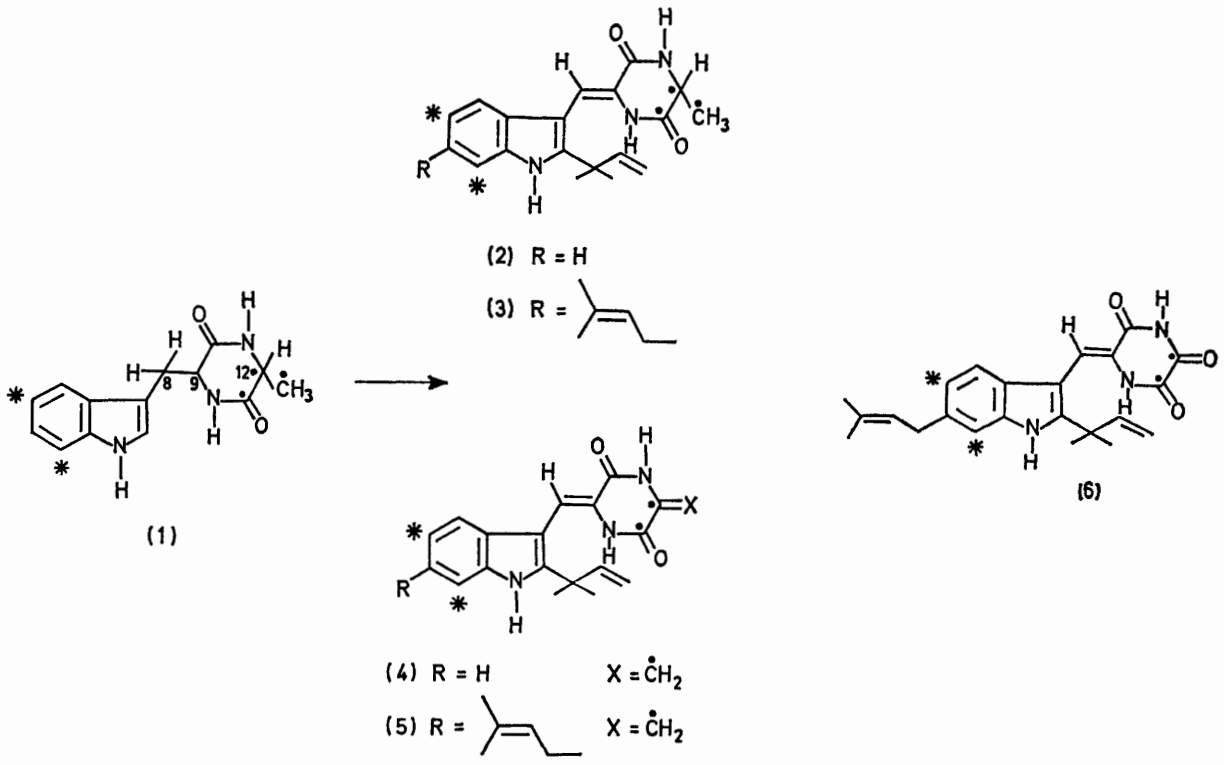
cyclo-L-[*U*-^{14}C]Alanyl-L-[5,7-$^{3}H_2$]tryptophyl and *cyclo*-L-[*U*-^{14}C]alanyl-D-[5,7-$^{3}H_2$]tryptophyl were synthesized from L-[*U*-^{14}C]alanine and DL-[5,7-$^{3}H_2$]tryptophan, by a known procedure,[3] separated by preparative t.l.c., and recrystallized to constant activity from EtOH–EtOAc (1:1). The two precursors LL-CAT (60·36 μCi mmol^{-1}, 50 mg, ^{3}H:^{14}C 6·30), and LD-CAT (27·8 μCi mmol^{-1}, 28mg, ^{3}H:^{14}C 8·7) were independently fed to molasses cultures of *Aspergillus amstelodami*. The isolated neoechinulins, purified to constant activity, showed that incorporation of LL-CAT had occurred to different extents, though less efficiently than into echinulin[4] (neoechinulins A 88%, B 43%, C 43%, D 34%, and neoechinulin 43% respectively of the radioactivity recovered in echinulin). Moreover, the ^{3}H:^{14}C ratio was kept constant in all neoechinulins (A 6·45, B 6·75, C 6·25, D 5·75, and neoechinulin 7·20).† In contrast, in the parallel experiment, LD-CAT was poorly incorporated and is unlikely to be a direct precursor of these metabolites since the original ^{3}H:^{14}C ratio was lost completely.

Thus, LL-CAT seems to be a likely intermediate in the biosynthesis of neoechinulins. It is not certain whether, in the biogenetic pathway, the step following cyclisation of the dipeptide is dehydrogenation at C(8)–C(9) or prenylation at the 2-position of the indole nucleus. Isolation of *cyclo*-L-alanyl-2-$\alpha\alpha$-dimethyl-allyl-L-tryptophyl in an *in vitro* experiment performed by Allen[5] supports the latter hypothesis. On the other hand, the prenylation site

† The ^{3}H:^{14}C ratio for neoechinulin, which has lost one carbon atom derived from the alanine unit of the precursor, has only slightly increased. Owing to the paucity of the isolated material, it has not been possible so far to obtain a more satisfactory result. However, we do not know how uniform was the original label in the commercially obtained (Amersham) L-[*U*-^{14}C]alanine.

in the benzene unit seems to be independent of the structure of the dioxopiperazine ring and to depend mainly upon the presence of the C(8)–C(9) unsaturation.

hypothesis[2] that the carbonyl group [at C(12)] in neoechinulin may be formed by oxidation of the alanyl unit present in a preformed dioxopiperazine ring.



Moreover, the *Z*-stereochemistry around the C(8)–C(9) double bond for all these compounds has been tentatively assigned by ^{1}H (solvent effect and simulated spectra)[6] and ^{13}C n.m.r. spectroscopy.[7]

In conclusion, the data obtained support our previous

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