Carnosine-synthetase activity has been shown to be present in chick erythrocytes[5]; however, no synthetase activity was detected in rabbit reticulocytes or erythrocytes under our conditions. It may be that the enzyme lost activity prior to the time of assay, since there is a loss in enzyme activity from rat skeletal muscle shipped in this manner.

It was interesting to find higher carnosine levels in rabbit reticulocytes than in rabbit erythrocytes. Mammalian reticulocytes differ from mammalian erythrocytes in many respects[8,9]. Reticulocytes have the ability to synthesize globin, whereas mature erythrocytes have lost all of their ability to synthesize protein[9-11]. It may be that carnosine is somehow involved in RNA and/or protein synthesis in reticulocytes. Studies with inhibitors of carnosine synthesis may be useful in elucidating the physiological function of carnosine in mammalian reticulocytes.

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Hydroxydihydroergosine, a new ergot alkaloid analogue from directed biosynthesis by *Sphacelia sorghi*[1]

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Summary. A new ergot alkaloid, 9′-hydroxydihydroergosine, has been produced by axenic cultures of *Sphacelia sorghi* to which 4-hydroxy-proline was added when biomass accumulation was complete. Evidence for a lack of biosynthetic specificity for the first, second and third amino acid in cyclic tripeptide alkaloids has now been found amongst ergot fungi.

As an ergot fungus parasitic on sorghum *Sphacelia sorghi* produces a group of ergoline alkaloids in the sclerotia[2]. The principal alkaloid is dihydroergosine (DHES), a cyclic tripeptide derivative of dihydrolysergic acid, the tripeptide being composed of proline, leucine and alanine. Other alkaloids, most of which may be regarded as biosynthetic intermediates, such as chanoclavine, festuclavine, pyroclavine and dihydroelymoclavine also occur as minor components. However, when an isolate of *S. sorghi* capable of alkaloid synthesis in vitro is grown in surface liquid culture several other indole alkaloids are evident in trace amounts on chromatograms of culture filtrate extracts[3,4]. Extended attempts to characterise these alkaloids have failed but there is evidence that at least some are artefacts of the cultural and/or work-up conditions.

Laboratory culture of *S. sorghi* for the production of dihydrogenated ergot alkaloids is best achieved using a sucrose/asparagine medium but the fungus in current use gave optimum alkaloid yields, and accumulated the least glucan, after modification of the medium formerly preferred[5]. Therefore cultures were both maintained and grown for alkaloid production at 27 °C on a medium containing sucrose, 100 g/l; asparagine, 10 g/l; $Ca(NO_3)_2 \cdot 4\,H_2O$, 1 g/l; KH_2PO_4, 0.25 g/l; $MgSO_4 \cdot 7\,H_2O$, 0.25 g/l; KCl, 0.125 g/l; $FeSO_4 \cdot 7\,H_2O$, 0.033 g/l; $ZnSO_4 \cdot 7\,H_2O$, 0.027 g/l; yeast extract, 0.1 g/l; L-cysteine hydrochloride, 0.01 g/l; distilled water; presterilisation pH 5.2 adjusted with NaOH; sterilised at 15 psi for 15 min. Erlenmeyer flasks (50 ml or 500 ml) contained 10 ml or 100 ml liquid medium respectively. Solid media contained 15 g/l agar. Surface liquid cultures were established using tissue (circa 0.5 cm^2), from the center of a 3-week agar slant culture, macerated into the production medium using an inoculating spatula.

Autoradiography of the alkaloid produced by cultures given radiolabelled (^{14}C) proline, leucine or alanine showed their incorporation principally into DHES, but at least 2 other alkaloid components were labelled by all 3 amino acids. Following feeding of a 14-day *S. sorghi* culture with the proline analogue allo-4-OH-proline (4 mg ml^{-1}) and incubating until alkaloid biosynthesis was complete (21 days, 0.5 mg alkaloid ml^{-1}), the proportion of one of these minor alkaloids [R_f 0.15 on silica gel chromatograms developed in chloroform/methanol/ammonium hydroxide

Incorporation of amino-acid analogues into ergot alkaloids

Ergot alkaloid	Ergot fungus	Amino acid sequence of peptide side chain	Amino acid analogue incorporated	Reference
Dihydroergosine	*S. sorghi*	*Pro*-Leu-Ala	4-Hydroxy-proline	Present paper
Ergotamine		Pro-*Phe*-Ala	p-Fluorophenylalanine	Beacco et al.[9]
Ergocryptine		Pro-*Leu*-Val	L-Norleucine	Beacco et al.[9]
Ergocornine	*C. purpurea*	Pro-*Val*-*Val*	L-Norvaline	Beacco et al.[9]
Ergotamine		Pro-Phe-*Ala*	α-Aminobutyric acid	Belzecki et al.[10]

(95:7:5)], which from radioisotope studies is probably structurally closely related to DHES, seemed considerably increased. Alternatively, a new alkaloid with identical chromatographic characteristics had been superimposed. The procedure was repeated and 2.4 mg alkaloid (R_f 0.15), which represented approximately 5% of the total alkaloids, was obtained. Field desorption mass spectrometry showed m/z 565, interpreted as the addition of 16 mass units (1 oxygen) to the molecular ion of DHES (m/z 549)[6]. The electron impact mass spectrum showed, in addition, m/z 269 (base peak) which corresponds to the dihydrolysergamide fragment of DHES. The spectrum also showed an important ion (m/z 170) and a weak ion (m/z 154); the latter could represent a fragment of the ergoline nucleus[7], or a proline-containing fragment of the cyclic tripeptide of DHES[6] in which case m/z 170 would represent the OH-prolyl analogue. DL-hydroxy [2-^{14}C] proline (10 μCi) was administered to a 12-day 10-ml culture of *S. sorghi*. Autoradiography of a chromatogram of the alkaloids subsequently produced showed intense radioactivity exactly coincident with the new alkaloid. A similar result was obtained when the radioisotope was given at 5, 8 and 10 days after inoculation. Radioisotopic and mass spectral evidence thus indicated that *S. sorghi* can use OH-Pro to biosynthesize the novel alkaloid 9′-hydroxy-dihydroergosine (OH-DHES), following the conventional nomenclature[8]. The yield was relatively low but a pro$^-$ auxotroph might be expected to give improved yield[9]. Other proline analogues were tested at concentrations of 6–12 mg ml^{-1} for their ability to become incorporated, but only cis-4-OH-Pro gave rise to a new alkaloid having a slightly lower chromatographic R_f-value than the OH-DHES derived from the allo-form. D-proline, allo-4-OH-D-proline, L-azetidine-2-carboxylic acid, 3,4 dehydro DL-proline, DL-pipecolic acid, L-pipecolic acid, OH-L-pipecolic acid, L-baikain, thioproline and S-piperazic acid were not incorporated.

Addition of OH-Pro (1–10 mg ml^{-1}) to cultures after growth at the 12-, 14-, 16- or 18-day stage did not affect total alkaloid yield. Similarly, when OH-Pro was administered (0–0.5 mg ml^{-1}) during growth at 5, 8 or 10 days alkaloid yield was not affected and up to 1.5 mg ml^{-1} at the same stages did not affect biomass accumulation. Total alkaloid yield was only diminished when > 0.5 mg ml^{-1} was given at 5 days; at less than 0.5 mg ml^{-1} there was a dose dependant effect on the proportion of OH-DHES formed but at greater concentrations the proportion of OH-DHES was not increased. On agar media containing OH-Pro (0.4 mg ml^{-1}) colony growth was reduced by 50%. Maximum growth suppression occurred at 4 mg ml^{-1} but complete fungistasis was not achieved even at 10 mg ml^{-1}. Since in this fungus alkaloid synthesis commences only after growth has ceased, optimum directed biosynthesis using OH-Pro could conveniently be achieved by administration to surface cultures at about day 12.

Clearly, in ergot fungi the multi-enzyme complex forming the cyclic tripeptide alkaloid moiety is not always specific (table) and this now applies to all 3 amino acid components. However, it does not necessarily mean that analogues can be accepted for all of the 3 amino acids by any one fungus. In the present biosynthetic studies *S. sorghi* would not accept iso-leucine or nor-leucine to replace leucine, and a *Claviceps purpurea* strain typically producing ergotamine[11] nevertheless failed to accept OH-Pro although, as in *S. sorghi*, it was completely taken up by the mycelium. Also OH-Pro did not substitute for proline in the biosynthesis of the tremorgenic mycotoxin verruculogen when tested in the system described elsewhere[10]. *S. sorghi* appears therefore to be unique in accepting OH-Pro in the biosynthesis of a fungal secondary metabolite, the only others containing 4-OH-Pro are the *Amanita* toxins which can be considered to be biosynthesized initially from proline, oxygen being inserted later.

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In vivo inhibition of citrate cleavage enzyme by polychlorinated biphenyls[1]

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Summary. Polychlorinated biphenyls (Aroclor 1254, PCB) administered in the diet (0.01%, w/v) to rats inhibited citrate cleavage enzyme. The results suggest that the decreased activity might in part account for decreased fatty acid synthesis in the livers of PCB-treated rats.

Alterations in lipid metabolism by polychlorinated biphenyls have been reported by several investigators[2-5]. Nagai and coworkers[6] found that the oral administration of PCB reduced the free fatty acids and triglyceride in the skin lipids of the rat. Incorporation of acetate into triglyceride and free fatty acids was decreased. Kling et al.[7] previously reported that PCB, in vitro, had no effect on acetyl CoA carboxylase, the enzyme which catalyzes the rate limiting step in the biosynthesis of fatty acids or on fatty acid synthase. Citrate cleavage enzyme was inhibited in vitro by polychlorinated biphenyls. The inhibition was noncompetitive. The enzyme occupies a key position in the biosynthesis of fatty acids. Firstly, it directly provides the precursor acetyl CoA. Secondly, it indirectly participates in the for-