2.44 A^3/dalton. This is near the center of the range of values described by Matthews for other crystalline proteins[11] and is close to those of the beef liver and first deer liver crystal forms. Thus in all of the crystals described here, the asymmetric unit consists of 4 entire protein subunits.

1 Acknowledgment. This research was supported by the National Institutes of Health, grant GM21398 and RR00576. The authors also wish to thank Wardens John Kreider and Jack Schweitzer of the Pennsylvania Game and Fish Commission for providing the deer liver.
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Discussion. The results indicate that the catalase molecules from deer and beef liver are essentially isomorphous. Considering the differences in amino acid composition that must be expected for the 2 species and the surprisingly low residual observed to a rather high resolution, it seems fair to conclude that the overall conformation of these 2 catalases must be almost identical. The differences observed in the diffraction pattern are roughly equivalent to those that might be expected, for example, by the introduction of a single heavy atom into each subunit of the tetramer and would easily be accounted for by the amino acid side chain differences.

Thus in spite of the substantial difference in the animal species involved, it can be stated with considerable certainty that the 3dimensional structure of the catalase molecule has been essentially conserved. One may speculate, therefore, that this hemeprotein, like hemoglobin and myoglobin, very likely exhibits a structural constancy among widely different species. A similar kind of result was obtained by North et al.[12] who showed the close identity of seal and sperm whale myoglobin by direct examination of their respective diffraction patterns.

Beef liver catalase crystals are presently under investigation in 2 laboratories, and will likely result in the 3dimensional structure of that molecule within the next 2 years. Since the deer liver form yields data to high resolution which can probably be phased directly from a beef liver catalase structure, the deer liver catalase molecular structure should follow soon thereafter.

Lysergylpeptides in the course of peptide ergot alkaloid formation

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Summary. Radioactive d-lysergyl-Val-Leu-OMe, d-lysergyl-Val-Val-OMe and d-lysergyl-Val-Val-Pro-OMe were synthetized according to the dicyclohexylcarbodiimide/1-hydroxybenzotriazole procedure. These compounds are not used by intact mycelium of Claviceps as immediate precursors for cyclolalkaloid biosynthesis.

The mechanism of peptide alkaloid formation in Claviceps, especially of the cyclol moiety, is still obscure. Feeding of lysergic acid and appropriate amino acids led to a specific incorporation into ergot alkaloids[1]. Furthermore neither d-lysergyl-L-alanine nor d-lysergyl-L-valine were incorporated as intact units into ergotamine or ergotoxine respectively[2–4]. Also other potential intermediates like dipeptides, diketopiperazines and tripeptides were split prior to incorporation by the ergot fungi[5–7]. For example, Val-Leu-Pro (sequence of ergokryptine) and Val-Val-Pro are no free intermediates in ergotoxine biosynthesis[6,7]. A plausible scheme for cyclol type ergot alkaloid formation was recently developed[7,8]. Apparently the peptide chain formation takes places in a concerted fashion on a multienzyme complex. As a key intermediate, an enzyme-bound lysergyltripeptide was proposed. Concomitantly with releasing this lysergyl derivative from the enzyme, an acylated diketopiperazine could be formed. We tested now whether a lysergyltripeptide (as –OMe derivative) is converted in vivo by the fungus into the corresponding peptide alkaloid. Also 2 appropriate lysergyldipeptides were fed which, after coupling with the 'starter'-molecule proline, might in turn be transformed into alkaloids. Previous experiments[4] revealed that ergot fungi demethylate methyl esters of lysergyl amino acids quite easily.

Material and methods. L-Valyl-L-valin-(U-^{14}C)methyl ester, L-valyl-L-leucine-(1-^{14}C)methyl ester were synthetized by the mixed anhydride method with isobutyl chloroformate[9] and triethylamine. L-Valyl-L-valyl-(U-^{14}C)-L-proline

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Table 1. Incorporation of lysergyldipeptides into ergot alkaloids of the ergotoxine group by Claviceps purpurea

	A	*B*	*C*	*D*
Culture broth				
Total radioactivity	$9.6 \cdot 10^5$ dpm	$1.4 \cdot 10^6$ dpm	$8.1 \cdot 10^5$ dpm	$8.8 \cdot 10^5$ dpm
$CHCl_3$-extract	$3.8 \cdot 10^5$ dpm (14.1)*	$5.3 \cdot 10^5$ dpm (17.7)	$4.8 \cdot 10^5$ dpm (25.9)	$4.8 \cdot 10^5$ dpm (30.0)
Ergocornine	$1.0 \cdot 10^4$ dpm/mg	$1.5 \cdot 10^4$ dpm/mg	$1.0 \cdot 10^4$ dpm/mg	$3.3 \cdot 10^4$ dpm/mg
Ergokryptine	$1.6 \cdot 10^4$ dpm/mg	$2.0 \cdot 10^4$ dpm/mg	$1.1 \cdot 10^4$ dpm/mg	$3.0 \cdot 10^4$ dpm/mg
Aqueous phase	$2.6 \cdot 10^5$ dpm (9.6) (leucine-^{14}C)	$3.9 \cdot 10^5$ dpm (13.0) (leucine-^{14}C)	$2.8 \cdot 10^5$ dpm (15.1) (valine-^{14}C)	$3.8 \cdot 10^5$ dpm (23.8) (valine-^{14}C)
Mycelium				
$CHCl_3$-extract	$2.1 \cdot 10^5$ dpm (7.8)	$2.3 \cdot 10^5$ dpm (7.7)	$1.65 \cdot 10^5$ dpm (8.9)	$6.6 \cdot 10^4$ dpm (4.1)
EtOH-extract	$6.1 \cdot 10^5$ dpm (22.6) (leucine-^{14}C)	$6.4 \cdot 10^5$ dpm (21.3) (leucine-^{14}C)	$4.5 \cdot 10^5$ dpm (24.3) (valine-^{14}C)	$3.5 \cdot 10^5$ dpm (21.9) (valine-^{14}C)
Proteinfraction	$9.0 \cdot 10^5$ dpm (33.3)	$7.5 \cdot 10^5$ dpm (25.0)	$4.2 \cdot 10^5$ dpm (22.7)	$3.0 \cdot 10^5$ dpm (18.8)

Administered compounds: *A* 10 mg d-lysergyl-Val-Leu-(1-^{14}C)–OMe ($8 \cdot 10^6$ dpm). *B* 10 mg d-isolysergyl-Val-Leu-(1-^{14}C)–OMe ($8 \cdot 10^6$ dpm). *A* and *B* The precursors were fed to 100 ml culture broth. *C* 5 mg d-lysergyl-Val-Val-(U-^{14}C)–OMe ($3 \cdot 10^6$ dpm). *D* 5 mg d-isolysergyl-Val-Val-(U-^{14}C)–OMe ($3 \cdot 10^6$ dpm). *C* and *D* The precursors were fed to 50 ml culture broth. Feeding period (*A–D*) 72 h. Ergotoxine mixture of *A* and *B* each ~ 25 mg and of *C* and *D* each ~ 15 mg.
*% of recovered radioactivity in culture broth and mycelium.

methyl ester was prepared stepwise starting from Pro–OMe using the mixed anhydride method. The t-butyloxycarbonyl and benzyloxycarbonyl groups were used for α-amino protection. We tested a number of methods for coupling the above-mentioned peptide esters with lysergic acid. Best results were obtained using the dicyclohexylcarbodiimide/1-hydroxybenzotriazole procedure[10] according to Losse et al.[11]. The d-lysergyl- and d-isolysergylpeptide esters were separated by TLC. The lysergylpeptides and peptide derivatives were characterized by m.p., optical rotation, hydrolysis, UV, MS and chromatographical methods.

The precursors were fed to submerged cultures of the ergocornine/ergokryptine producing Claviceps purpurea strain, Pepty 695 during the idiophase. After harvesting the culture, broth and mycelium were separated. Ergocornine and ergokryptine were purified to constant specific radioactivity[12].

Table 2. Incorporation of lysergyltripeptides into ergot alkaloids of the ergotoxine group by Claviceps purpurea

Experiment	*A*	*B*
Culture broth		
Total radioactivity	$3.3 \cdot 10^6$ dpm	$3.2 \cdot 10^6$ dpm
$CHCl_3$-extract	$1.9 \cdot 10^6$ dpm (53.4)*	$2.1 \cdot 10^6$ dpm (60.7)
LVVP resp. IsoLVVP	$1.5 \cdot 10^6$ dpm	$1.3 \cdot 10^6$ dpm
Ergocornine	$8 \cdot 10^3$ dpm/mg	$1.74 \cdot 10^4$ dpm/mg
Ergokryptine	$9.1 \cdot 10^3$ dpm/mg	$9.3 \cdot 10^3$ dpm/mg
Aqueous phase (valine-^{14}C)	$1.3 \cdot 10^6$ dpm (36.5)	$8.7 \cdot 10^5$ dpm (25.1)
Mycelium		
$CHCl_3$-extract	$6.1 \cdot 10^4$ dpm (1.7) (LVVP)	$6.9 \cdot 10^4$ dpm (2.0) (IsoLVVP)
EtOH-extract (valine-^{14}C)	$8.4 \cdot 10^4$ dpm (2.4)	$6.0 \cdot 10^4$ dpm (1.7)
Protein fraction	$1.2 \cdot 10^5$ dpm (3.4)	$1.3 \cdot 10^5$ dpm (3.8)

The following compounds were administered: *A* 10 mg d-lysergyl-Val-Val-(U-^{14}C)-Pro–OMe (LVVP) ($6 \cdot 10^6$ dpm). *B* 10 mg d-isolysergyl-Val-Val-(U-^{14}C)-Pro–OMe (Iso LVVP) ($6 \cdot 10^6$ dpm). The precursors were added to single shake flask cultures (100 ml). Feeding period 72 h. Amount of ergotoxine mixture ~ 28 mg.
* % of recovered radioactivity in culture broth and mycelium.

After extraction with chloroform, the aqueous phase of the culture broth was chromatographed on a Dowex 50 (H^+) column and the amino acids eluted with 5% NH_4OH and separated on TLC plates or by paper chromatography. The damp dry mycelium was crushed with dry ice and exhaustively extracted with chloroform and ethanol successively. Afterwards a protein fraction was prepared. Both the $CHCl_3$– and EtOH fraction were subjected to various chromatographical procedures. The radiocative profiles of the chromatograms were checked with the thin layer scanner II (Berthold, Wildbad, Federal Republic of Germany).

Results and discussion. The results (tables 1 and 2) demonstrate clearly that radioactivity from exogenously applied d-lysergyl-Val-Val-(U-^{14}C)–OMe, d-lysergyl-Val-Leu-(1-^{14}C)–OMe and d-lysergyl-Val-Val-(U-^{14}C)-Pro–OMe is incorporated only after splitting the precursors into its components. d-Lysergyl- and d-isolysergylpeptides are likewise degraded and gave practically the same results. In case of a specific incorporation d-lysergyl-Val-Val and d-lysergyl-Val-Val-Pro should label only ergocornine, and d-lysergyl-Val-Leu only ergokryptine. But in each experiment both ergotoxine alkaloids were radioactive. Furthermore radioscans of the free amino acid fraction of the mycelium, as well as of the culture broth, revealed the presence of radioactive leucine and valine. Also a heavy labelling of the protein fraction was found (table 2). Apparently free lysergyl peptides are not converted to the corresponding acylated diketopiperazines, and in turn to cyclol alkaloids; rather peptidases catalyze the breakdown into its components. Because appropriate lysergyltripeptides do not reach the site of alkaloid biosynthesis in intact mycelium of Claviceps, experiments with cell-free preparations should give more promising results.

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