199. Biosynthesis of the Cytochalasans. Biosynthetic Studies on Chaetoglobosin A and 19-*O*-Acetylchaetoglobosin A

by **Alessandro Probst** and **Christoph Tamm**[1])

Institut für Organische Chemie der Universität Basel, St. Johanns-Ring 19, CH-4056 Basel

(26. VI. 81)

Summary

Incorporation of [1-^{13}C]-, [2-^{13}C]- and [1,2-$^{13}C_2$]-acetate, [1-^{13}C]-propionate, [^{13}C–CH_3]-L-methionine and [3-^{14}C]-DL-tryptophan into chaetoglobosin A (**1**) and 19-*O*-acetylchaetoglobosin A (**2**) by *Chaetomium globosum* demonstrated that the building blocks of **1** and **2** are 9 and 10 units of acetate/malonate respectively, 3 units of methionine and 1 unit of tryptophan. Propionate is incorporated indirectly after several biological transformations. Using [2-^{13}C, 2-$^{2}H_3$]-acetate as precursor, the starter unit of the polyketide-chain was identified. Experiments which [^{13}C, $^{2}H_3$-CH_3]-L-methionine demonstrated that the three *C*-methylations occur with retention of all three H-atoms of the methyl group. Incorporation experiments with various ^{14}C- and ^{3}H-labelled tryptophan samples and with [2-^{2}H]- and [2-^{15}N]-L-tryptophan showed that the amino acid is incorporated intact with retention of both the α-H- and the α-N-atom. On the basis of these results a more detailed general scheme of the cytochalasan biogenesis is proposed.

Introduction. – Since the isolation and structure elucidation of the first two cytochalasans, cytochalasin A and B [1] in 1966, the number of members of this class of microbial metabolites has risen to 36[2]). Nevertheless, studies on the biosynthesis of cytochalasans have until now been reported only for cytochalasin B [4] [5a], a phenyl-24-oxa-[14]cytochalasan, and cytochalasin D [5a] [6], a phenyl-[11]cytochalasan. The results of these investigations clearly demonstrate closely related biogenetic pathways from a polyketide-derived chain, with respectively two and three introduced C_1-units, and which is combined with phenylalanine. The great structural similarity of the four basic types of cytochalasans known permitted us to postulate a common biogenetic scheme for all the members of this class of metabolites [7]. It is evident that to establish the validity of this general biogenetic scheme, additional experimental data are required, such as the isolation of intermediates of the biogenetic

[1]) Author to whom correspondence should be addressed.

[2]) Recent additions to this class of compounds include engleromycin [2], 19-*O*-acetylchaetoglobosin B and D [3a] and cytochalasin K, L and M [3b].

0018-019X/81/7/2065-13$01.00/0 © 1981 Schweizerische Chemische Gesellschaft

sequence, discovery of new cytochalasans and biosynthetic studies of cytochalasans potentially derived from amino acids other than phenylalanine. Therefore the investigation of the biosynthetic origin of a 3-indolyl-cytochalasan, which is probably biosynthesized from tryptophan, seemed to be of interest. We have tested the validity of the proposed biogenetic scheme in the case of chaetoglobosin A (**1**) and 19-*O*-acetylchaetoglobosin A (**2**), main metabolites of *Chaetomium globosum* (Strain *Lederle* H-124) [3], by incorporation experiments using radioactive as well as ^{13}C-, ^{2}H- and ^{15}N-labelled precursors. In order to distinguish between the various theoretically possible pathways, sodium-[1-^{14}C]- and -[2-^{14}C]-acetate, sodium-[1-^{14}C]-propionate, [^{14}C–CH_3]-L-methionine, [1-^{14}C]-L-, [3-^{14}C]-L-, [3-^{14}C]-DL- and [5′-^{3}H]-L-tryptophan, sodium-[1-^{13}C]-, -[2-^{13}C]-, -[1,2$^{13}C_2$]- and [2-^{13}C, 2-$^{2}H_3$]-acetate, sodium-[1-^{13}C]-propionate, [^{13}C, $^{2}H_3$-CH_3]-L-methionine, [2-^{2}H]-L- and [2^{15}N]-L-tryptophan were administered to growing cultures of *Chaetomium globosum*. From the signal enhancements in the ^{13}C-, ^{2}H- and ^{15}N-NMR. spectra of the enriched 19-*O*-acetylchaetoglobosin A (**2**) samples, and in the mass spectra of the enriched chaetoglobosin A (**1**)[3]) samples, the labelling pattern could easily be deduced.

Incorporation experiments. – The strain of *Chaetomium globosum* was grown on a molasses/peptone/glucose medium. The production curve revealed that the formation of chaetoglobosins begins after 2–3 days, reaching a maximum after 10–11 days, yielding 200–700 mg 19-*O*-acetylchaetoglobosin A (**2**) and 150–600 mg chaetoglobosin A (**1**) per litre. Therefore, the precursors were administered after 3 days and the cultures were havested after 11 days. The radioactive samples of **2** were recrystallized three times from benzene in order to achieve constant radioactivity. The results obtained after addition of the potential precursors are presented in *Table 1*. All of them are incorporated significantly. The high incorporation rates of acetate, methionine and tryptophan seem to confirm the origin of the chaetoglobosins from an acetate/malonate polyketide and from tryptophan with introduced methyl groups derived from methionine. The incorporation of propionate, as compared with that of the other administered precursors, is very low. Thus the participation of propionate in the biosynthesis of chaetoglobosins – *via* methylmalonate – as a link of the polyketide-chain can be excluded. The measured activity is probably derived from an indirect incorporation after several biological transformations.

Table 1. *Incorporation of radioactive precursors into 19-O-acetylchaetoglobosin A* (**2**)

Precursor	Absolute incorporation rate %
Sodium-[1-^{14}C]-acetate	2.83
Sodium-[2-^{14}C]-acetate	4.07
Sodium-[1-^{14}C]-propionate	0.13
[^{14}C–CH_3]-L-Methionine	16.9
[3–^{14}C]-DL-Tryptophan	11.7

Having established the basic biogenetic units of **2**, the precursors were also fed as ^{13}C-labelled molecules in order to elucidate the labelling pattern. The expected

[3]) For mass spectroscopic measurements, **1** was preferred to **2** because of the better absolute intensities in the molecular region.

signal enhancement can be estimated, assuming that the absolute incorporation rates are the same in both experiments with ^{14}C- and ^{13}C-labelled precursors [8]. Since the experimental conditions are different, *e.g.* the dilution of the precursor by the natural pools of the media and of the organism, the calculation is not on very safe ground.

The proton noise decoupled ^{13}C-NMR. spectra of unlabelled (natural abundance) *(cf. Fig. 1)* and biologically enriched 19-*O*-acetylchaetoglobosin A (**2**) samples were recorded under identical conditions, in order to avoid any differences in signal height resulting from the differing T_1 the longitudinal relaxation times of the various

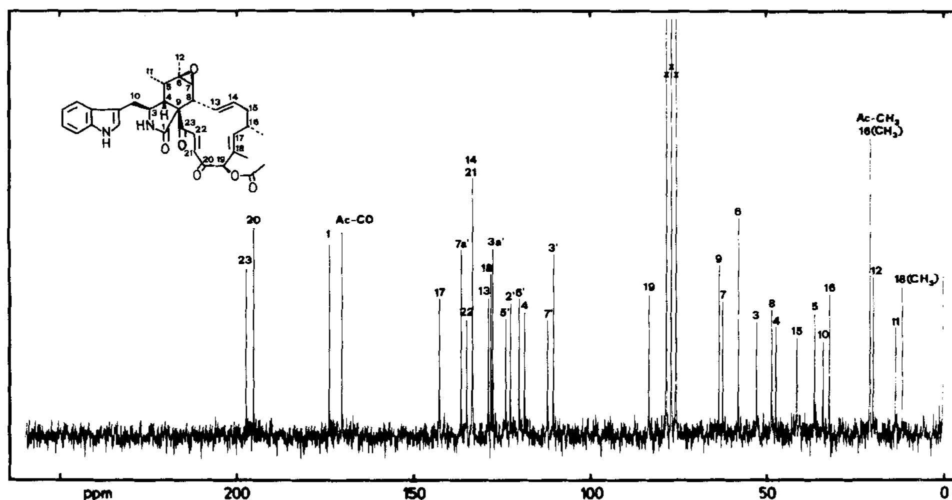


Fig. 1. *^{13}C-NMR. spectrum of unlabelled 19-O-acetylchaetoglobosin A* (**2**) *in $CDCl_3$* (22.63 MHz)

carbon nuclei[4]). The intensities of the signals for C(1), C(5), C(7), C(13), C(15), C(17), C(19), C(21), C(23) and for the carboxyl group of the *O*-acetyl unit in the ^{13}C-NMR. spectrum were enhanced relative to natural abundance in [1-^{13}C]-acetate-enriched **2** and for C(6), C(8), C(9), C(11), C(14), C(16), C(18), C(20), C(22) and for the methyl group of the *O*-acetyl unit in [2-^{13}C]-acetate-enriched **2**, respectively. Additional couplings between two enriched C-atoms from different acetate units were observed between C(19) and C(21) ^2J = 16 Hz), C(8) and C(9) (J = 29 Hz) and C(9) and C(22) (^2J = 10 Hz). Such inter-acetate unit coupling can only occur if there is a high probability of two acetate units being incorporated into adjacent positions in one molecule. Ion intensities in the molecular ion region of the mass spectra of natural abundance, [1-^{13}C]- and [2-^{13}C]-acetate-enriched chaetoglobosin A (**1**) confirmed that a substantial portion of the biosynthetically enriched samples were multiply labelled. There was even a significant number of molecules in which eight of the nine acetate units were derived from the added precursor. Addition of a large amount of exogenous acetate probably represses the production of endogenous acetate by a

4) The assignments of the ^{13}C-NMR. spectra of various chaetoglobosins were previously reported [3].

feedback inhibition mechanism. Consequently until this pool falls below a certain threshold level and endogenous acetate production recommences, most of the metabolite is produced from exogenous precursor. This fact caused problems in feeding experiments using doubly-labelled acetate. The ^{13}C-NMR. spectrum of **2** enriched from [1,2-$^{13}C_2$]-acetate was very complicated. The expected characteristic triplets were visible only for C(1), C(11) and both C-atoms of the *O*-acetyl group. The remaining acetate-derived C-atoms showed complex signals, due to coupling between enriched C-atoms from different acetate units, confirming a large extent of multiply labelled metabolite molecules. This problem could be circumvented by dilution of labelled with unlabelled acetate before feeding [9]. Thus in the ^{13}C-NMR. spectrum of **2** obtained after incorporation of diluted [1,2-$^{13}C_2$]-acetate C(1)–C(9), C(5)–C(11), C(6)–C(7), C(8)–C(13), C(14)–C(15), C(16)–C(17), C(18)–C(19), C(20)–C(21), C(22)–C(23) and the two C-atoms of the *O*-acetyl group showed – in addition to the usual singlets arising for ^{13}C present at natural abundance – a doublet, due to ^{13}C, ^{13}C-coupling in the doubly labelled acetate unit. This result agrees with a biosynthesis *via* a C_{18}-polyketide, which is obtained by head-to-tail condensation of nine intact acetate/malonate units, and a single acetate unit which is introduced as *O*-acetyl function, probably at a later stage of the biosynthesis.

The observation that the fate of H-atoms during biosynthesis of secondary metabolites offers valuable mechanistic insights has led to expanded use of ^{2}H- and ^{3}H-labelled precursors (*cf.* [10]). However, one of the critical problems is the detection of the incorporated label. This problem can be partly circumvented by using ^{13}C/^{2}H doubly-labelled precursors. The ^{2}H-atoms directly bound to ^{13}C are easily detected indirectly in ^{1}H-decoupled ^{13}C-NMR. spectra. Although the application of this methodology to polyketide biogenesis by incorporation of [2-^{13}C, 2-$^{2}H_3$]-acetate is limited because of the considerable hydrogen exchange that can occur during formation of C-chains by acetate/malonate, ^{13}C/^{2}H doubly-labelled acetate can be used to identify starter units [5]. Thus commercially available sodium-[2-^{13}C, 2-$^{2}H_3$]-acetate was administered to growing cultures of *Chaëtomium globosum*. In ^{1}H-decoupled 100 MHz-^{13}C-NMR. spectrum[5]) of the resulting 19-*O*-acetylchaetoglobosin A (**2**), the signals for C(6), C(8), C(9), C(14), C(16), C(18), C(20) and C(22) were strongly enhanced singlets, but the peaks for C(11) and the *O*-acetyl methyl group appeared as natural abundance singlets superimposed on small multiplets. The ^{1}H-decoupled 30.7-MHz-^{2}H-NMR. spectrum confirmed that deuterium was primarily localized at these two C-atoms. Whereas the *O*-acetyl methyl group clearly appeared as a ^{13}C-coupled doublet of $J = 20$ Hz, the coupling constant of C(11) could unfortunately not be exactly determined, because of line broadening and a poor signal-to-noise ratio[6]). Since no other deuterium signals were observed, the other hydrogen-bearing C-atoms originating from C(2) of acetate had exchanged most if not all of their deuterium during biosynthesis. One deuterium can of course be lost from acetate units which are incorporated as malonate during the dehydration step of normal fatty acid biosynthesis. Apparently a non-stereospecific exchange which oc-

---

[5]) We thank *Dr. J. C. Vederas*, University of Alberta, Edmonton, Canada, for the 100-MHz-^{13}C- and 30.7-MHz-^{2}H-NMR. spectra.

[6]) The approximate value is $J = 18–22$ Hz.

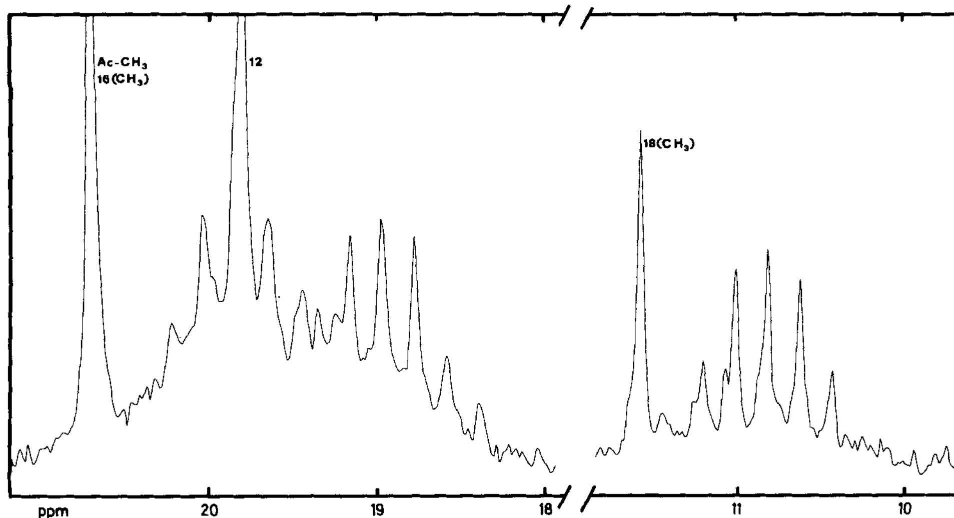


Fig. 2. *^{13}C-NMR. spectrum of 19-O-acetylchaetoglobosin A* (**2**) *enriched with [^{13}C, $^{2}H_3$-CH_3]-L-methionine in the region of the methyl signals, in $CDCl_3$ (100 MHz)*

curs at the malonate or a post-malonate stage may account for most of the remaining deuterium loss. Thus only the starter unit of the acetate/malonate polyketide and the single *O*-acetyl group retain large amounts of hydrogen label.

L-Methionine, which after the feeding experiments with radioactive precursors had been supposed to be the C_1-donor of the remaining methyl groups of **2**, was administered with the methyl group labelled by ^{13}C. In the resulting ^{13}C-NMR. spectrum the intensity of C(12) and of the methyl groups at C(16) and at C(18) were increased about fourteen-fold. Since the unambiguous proof that two different mechanisms for biological *C*-methylations exist [11], many studies have been reported concerning the problem of the stereochemistry of the reaction [12] and the possible pathways [13]. In order to gain more insight into the mechanism of the methylation processes in cytochalasans[7]), [^{13}C, $^{2}H_3$-CH_3]-L-methionine[8]) (isotopic purity 91.5% ^{13}C,98% ^{2}H) was administered to growing cultures of *Chaetomium globosum*. *Figure 2* shows the ^{1}H-decoupled 100-MHz-^{13}C-NMR. spectrum of the resulting 19-*O*-acetylchaetoglobosin A (**2**). The signals of C(12) and of the methyl groups at C(16) and at C(18) are clearly split into a septet with J= 19, 19 and 20 Hz, respectively. Also, the upfield shifts of the resonance frequencies (0.75–0.85 ppm) are consistent with retention of all three ^{2}H-atoms. In addition, the signal of the methyl group at C(18) clearly shows a quintet, due to the presence of $^{13}C^{1}H^{2}H_2$-groups. In the case of C(12) and of the methyl group at C(16), such an additional quintet cannot be detected unambiguously, because of the overlapping of the signals. Owing to quadruple broadening and the different nuclear *Overhauser* effects, the extent of the $^{13}C^{1}H^{2}H_2$-labell-

[7]) Previous studies on cytochalasin B showed that the methylation at C(16) occurs with retention of all three H-atoms, but could not clarify the methylation pathway at C(6) [14].

[8]) We are indebted to Mr. *R. Wyss* for the gift of doubly-labelled methionine.

ing of the metabolite could not be calculated by comparing the intensities of the septet and of the quintet. However, it could be determined by comparison of the ion intensities in the molecular region of natural abundance and enriched chaetoglobosin A (**1**). The calculation showed that the majority – probably all – of the $^{13}C^{1}H^{2}H_2$-labelling present in the metabolite is due to the $^{13}C^{1}H^{2}H_2$ groups of the precursor[9]). Finally the ^{1}H-decoupled 30.7-MHz-^{2}H-NMR. spectrum of the enriched **2** confirmed that deuterium was primarily localized at these three positions. The C-atom C(12) and the methyl groups at C(16) and at C(18) appeared as ^{13}C-coupled doublets with J = 19, 19 and 20 Hz, respectively. These findings agree with the assumption that the three *C*-methylations take place at an early stage of the biosynthesis, when *S*-adenosylmethionine can react with the enolic double bonds of the polyketide-chain.

The incorporation experiments discussed above clearly preclude the participation of propionate – or 2-methylmalonate – as a link of the polyketide-chain during biosynthesis of chaetoglobosins. Although biosynthetic studies on cytochalasin D showed that propionate can be incorporated indirectly, either by degradation to acetate or by transformation first to phosphoenol pyruvate and then, *via* the shikimic acid pathway, to phenylalanine [4c], none of these pathways can account for the activity measured after feeding [1-^{14}C]-propionate, because of the expected loss of $^{14}CO_2$ in the first case and of the different biosynthetic pathways for phenylalanine and for tryptophan in the latter case. Thus at least one additional pathway should exist, accounting for the incorporation of propionate in cytochalasans. In order to detect this pathway, [1-^{13}C]-propionate was administered to cultures of *Chaetomium globosum* and the ^{13}C-NMR. spectrum of the resulting 19-*O*-acetylchaeotoglobosin A (**2**) compared with that which was obtained from natural **2**. For the unequivocal determination of the labelled site we applied the method of *Hanson* [15] by normalizing the peak intensities with C(11), a C-atom which with high probability is not labelled even indirectly by [1-^{13}C]-propionate. Surprisingly only C(4) showed a significant enhancement of 80%, as was the case in analogous experiments with cytochalasin D. This can be explained, assuming that [1-^{13}C]-propionate is transformed *via* phosphoenol pyruvate, phospho-D-glycerate and phosphohydroxypyruvate to [1-^{13}C]-L-serine, which is combined by tryptophan-synthetase with indol-3-glycerolphosphate yielding [1-^{13}C]-L-tryptophan. Incorporation of the latter in **2** led to the observed signal enhancement.

The mode of incorporation of tryptophan. – The high incorporation rate of radioactive tryptophan in the previous experiments showed that this amino acid is a specific precursor of 19-*O*-acetylchaetoglobosin A (**2**). Although **2** possesses the *(S)*-configuration at C(3) corresponding to the naturally predominant L-tryptophan ((2*S*)-configuration), incorporation pathways leading to symmetrical degradation products of tryptophan could not be excluded *a priori*. Difficulties with the establishment of the incorporation pathway in biogenetic studies with amino acids are often due to the presence in the organism of transaminases, which convert α-amino acids reversibly into the corresponding α-keto acids, and of racemases. Therefore

[9]) The enrichment data of the precursor shows that only 86.1% of the molecules are $^{13}C^{2}H_3$-labelled; 8.3% are $^{12}C^{2}H_3$- and 5.3% are $^{13}C^{1}H^{2}H_2$-labelled.

incorporation of both enantiomers of the amino acid, or a negative result after feeding ^{15}N-labelled precursor, do not allow reliable conclusions. Finally, the possibility of more than one pathway existing for the incorporation of the amino acid [6c] can cause additional difficulties.

In order to elucidate the mechanism of incorporation of tryptophan in chaetoglobosin biosynthesis, an approach was made by the feeding in the usual fashion (2*S*)-[1-^{14}C]-, (2*S*)-[3-^{14}C]- and (2 *RS*)-[3-^{14}C]-tryptophan, each of which was mixed with (2*S*)-[5'-^{3}H]-tryptophan (as internal standard). The composition of the precursor mixtures which were fed, and the results of the incorporation experiments are listed in *Table 2*.

Table 2. *Incorporation of ^{3}H- and ^{14}C-labelled tryptophan into 19-O-acetylchaetoglobosin A* (**2**)

Exp. No.	Precursor	Absolute incorporation rate %	$^{3}H/^{14}C$ ratio	
			Precursor	**2**
1	Tryptophan (2*S*)-[5'-^{3}H] (2*S*)-[3-^{14}C]	 7.6 8.9	5.00	4.00
2	(2*S*)-[5'-^{3}H] (2*S*)-[1-^{14}C]	10.5 13.4	5.16	4.07
3	(2*S*)-[5'-^{3}H] (2*RS*)-[3-^{14}C]	6.6 4.1	5.16	8.2
4	(2*S*)-[5'-^{3}H] (2*RS*)-[3-^{14}C] (50 mg/l inact. tryptophan)	6.3 3.9	5.16	8.6
5	(2*S*)-[5'-^{3}H] (2*RS*)-[3-^{14}C] (100 mg/l inact. tryptophan)	6.5 3.9	6.9	11.4
6	(2*S*)-[5'-^{3}H] (2*RS*)-[3-^{14}C] (300 mg/l inact. tryptophan)	9.4 2.4	6.25	24.8
7	(2*S*)-[5'-^{3}H] (2*RS*)-[3-^{14}C] (1000 mg/l inact. tryptophan)	6.6 1.6	6.9	27.8

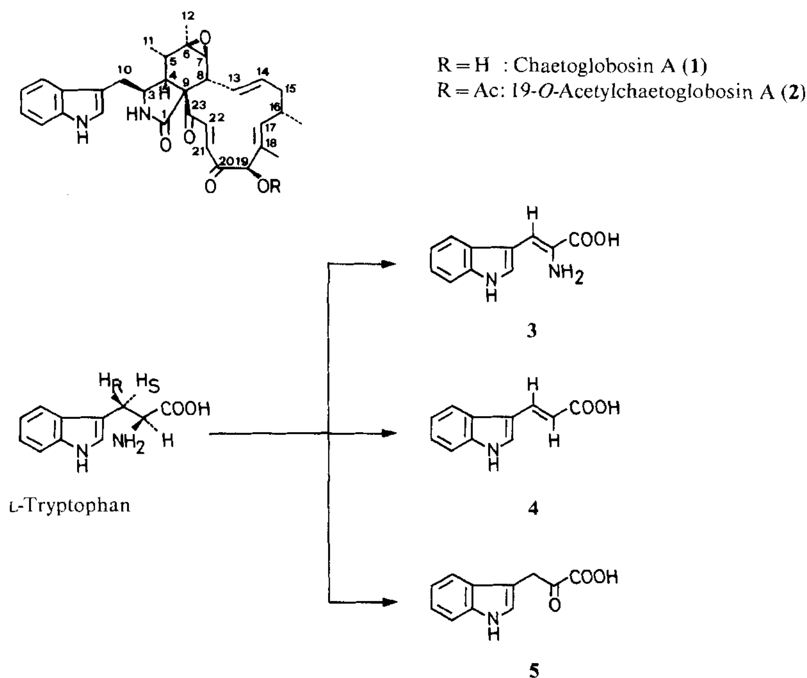
The $^{3}H/^{14}C$ ratios of **2** resulting from the feeding experiments No. 1–5 clearly show that only the naturally predominant L-tryptophan (with intact C-skeleton) is incorporated. The loss of tritium observable in all of these experiments is probably due to a reversible hydroxylation of C(5') of tryptophan, analogous to that known for the transformation of tryptophan to serotonin [16]. In the case of experiments No. 6 and 7, where the radioactive precursors were diluted with large amounts of tryptophan, an anomalous increase of the $^{3}H/^{14}C$ ratio was observed. It is highly

probable that the organism possesses a small constant tryptophan pool, and in the case of a large supply of exogenous amino acid activates the tryptophanase [17], to prevent toxic damage. The fact that the ^{3}H-labelled indole formed by action of the tryptophanase can be recycled into tryptophan biosynthesis, while the ^{14}C-activity is lost as pyruvate, explains the observed ^{3}H/^{14}C ratios of the metabolites.

These results exclude D-tryptophan as an exogenous precursor in chaetoglobosin biosynthesis. However, the degradation of tryptophan to symmetrical derivatives cannot yet be ruled out. The naturally predominant L-amino acids are often more efficiently incorporated than their enantiomers, even in cases where the end product possesses the configuration corresponding to the D-amino acid. This may be due to enhanced transport across the cell membrane by permeases [18], or to racemization after biochemical elaboration.

The results obtained so far are in agreement with the participation of L-tryptophan, dehydrotryptophan (**3**), 3-(3′-indolyl)pyruvic acid (**5**) or 3-(3′-indolyl)acrylic acid (**4**) (s. *Scheme 1*). Dehydrotryptophan (**3**) is synthesized by enzymatic *cis*-dehydration of L-tryptophan with loss of the 3-*pro(S)*-hydrogen [19]. 3-(3′-indolyl)acrylic acid (**4**) is synthesized in higher plants from tryptophan by ammonium lyases [20], and 3-(3′-indolyl)pyruvic acid (**5**) is derived from the amino acid by the action of aminotransferases and amino acid oxidases [21]. Amino acid oxidases normally do not exchange the β-H-atoms of their substrates, while transaminases in some cases exchange both the α- and β-H-atoms [22].

Scheme 1



In order to distinguish between the participation of L-tryptophan or the acrylic acid **4** on one hand and of dehydrotryptophan (**3**) or the pyruvic acid **5** on the other, [2-^{2}H]-L-tryptophan (isotopic purity 80% ^{2}H) was synthesized using the method of *Matsuo et al.* [23], mixed with [3-^{14}C]-L-tryptophan as internal standard, and administered to growing cultures or *Chaetomium globosum*. The activation of tryptophanase was prevented by pulse feeding of the precursor (204 mg/l) [8] from the third to the seventh day. Measurements of the radioactivity of the resulting 19-*O*-acetylchaetoglobosin A (**2**) showed a specific ^{14}C-incorporation rate of 20.1%. The specific ^{2}H-incorporation rate, determined by mass spectrometry of enriched chaetoglobosin A (**1**) was 19.1%. Furthermore a ^{2}H-NMR. spectrum[10]) of enriched **2** showed that all deuterium activity was located at C(3) of the metabolite, demonstrating that no deuterium loss from L-tryptophan had occurred during biosynthesis within experimental error. Thus the participation of intermediates like **3** or **4** can be excluded.

This result encouraged us to use ^{15}N-labelled tryptophan for an incorporation experiment. In contrast to biogenetic studies on cytochalasin D [6c] and pseurotin A [24], where the administered ^{15}N activity of phenylalanine was almost completely removed by transaminases, we expected the results of the incorporation of [2-^{15}N]-L-tryptophan would elucidate unambiguously the origin of the lactam N-atom.

Commercially available [2-^{15}N]-L-tryptophan (310 mg/l) was mixed with [3-^{14}C]-L-tryptophan (as internal standard) and administered in the same way as described for the deuteriated amino acid to growing cultures of *Chaetomium globosum*. Analysis of the resulting **2** and **1** revealed specific incorporation rates of 31.2% for ^{14}C and

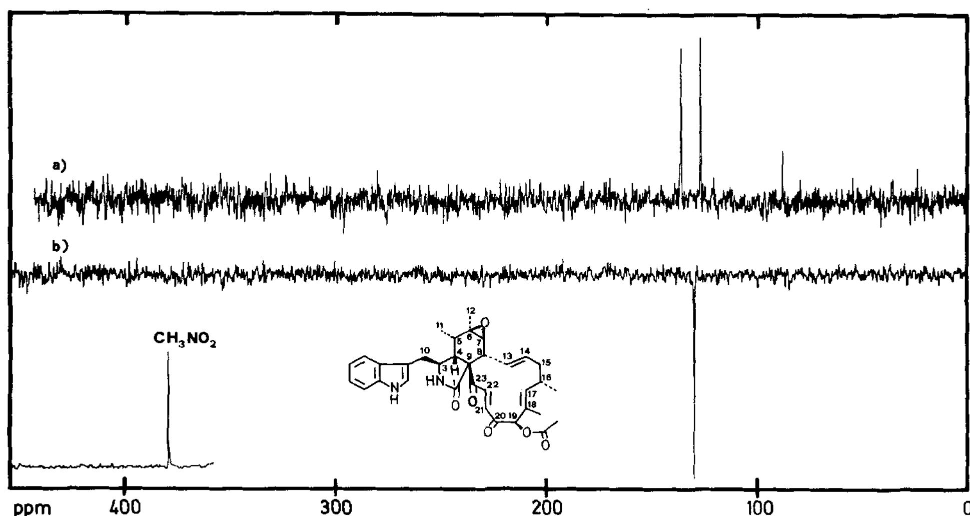


Fig. 3. *^{15}N-NMR. spectrum of 19-O-acetylchaetoglobosin A* (**2**) *enriched with [2-^{15}N]-L-tryptophan in $(CD_3)_2SO$ with CH_3NO_2* (δ=380.2 ppm, relative to liquid NH_3 at 25°) *as external reference.* a) Without, b) with ^{1}H-decoupling.

[10]) We thank *Prof. Dr. J. Seelig*, Biophysikalische Abteilung des Biozentrums der Universität Basel, for the measurement of this spectrum.

34.2% for ^{15}N. Although this result points to an intact incorporation of the amino acid, further efforts were made to determine unambiguously the labelled site; mass spectrometric measurements in the region of *m/z* 130, due to the indolylfragment, and the 10.1-MHz-^{15}N-NMR.[11]) spectrum of enriched **2** *(cf. Fig. 3)* confirmed, within experimental error, that all the ^{15}N-label was present only at the lactam N-atom. Thus the configuration at C(3) of chaetoglobosins is directly derived from the (2*S*)-configuration of the naturally predominant L-tryptophan, which is incorporated with intact chirality.

Conclusion. – Using radioactive as well as ^{13}C-, ^{2}H- and ^{15}N-labelled precursors, the biosynthetic origin of 19-O-acetylchaetoglobosin A (**2**) and chaetoglobosin A (**1**) has been established. Comparison of these results with those of the biosynthetic studies on cytochalasin B [4] [5a] and D [5a] [6] clearly demonstrates closely related biogenetic pathways. On the basis of our results a more detailed general biogenetic scheme [7] for the cytochalasans[12]) is proposed. It is outlined for **1** in the *Scheme 2*. It is also applicable to the other carbocyclic cytochalasans by varying the length of the polyketide, the number of *C*-methylations and the amino acid. However, not all the postulated intermediates and only part of the reaction sequence, are based on experimental proof.

Acetyl-coenzyme A acts as starter unit. It is condensed in successive reactions with eight malonate units to form a C_{18}-polyketide. The *C*-methylations occur before stabilization of the polyketide or its separation from the enzyme take place, perhaps even during its formation [26]. In the next steps the polyketide is combined with L-tryptophan, probably forming first the amide linkage and subsequently closing the lactam ring to yield the tetramic acid derivative **6**. For the construction of the isoindolone unit, **6** is transformed to the substituted pyrrolinone **7** by reductions, dehydrations and allylic oxidation. The latter compound can undergo an internal *Diels-Alder*-type cyclization to form the pentacyclic system **8**, identical with chaetoglobosin J, possessing the required configuration at C(4), C(5), C(8) and C(9). Epoxidation of the cyclohexene ring yields **1**, which may be the biogenetic precursor of the unsaturated alcohol systems present in several chaetoglobosins and of the 19-*O*-acetylchaetoglobosins.

Finally, cytochalasans possessing a macrocyclic lactone system, as represented by cytochalasin B, are obtained from a carbocyclic precursor by a *Baeyer-Villiger*-type oxidation [4d]. The third type of large ring, a cyclic carbonate group, as represented by cytochalasin E [27] might also arise from a carbocyclic precursor, the carbonate group resulting from a second *Baeyer-Villiger*-type insertion of an O-atom into a lactone ring.

All steps of the biogenetic sequence, the order of some of which are interchangeable, correspond to known reactions.

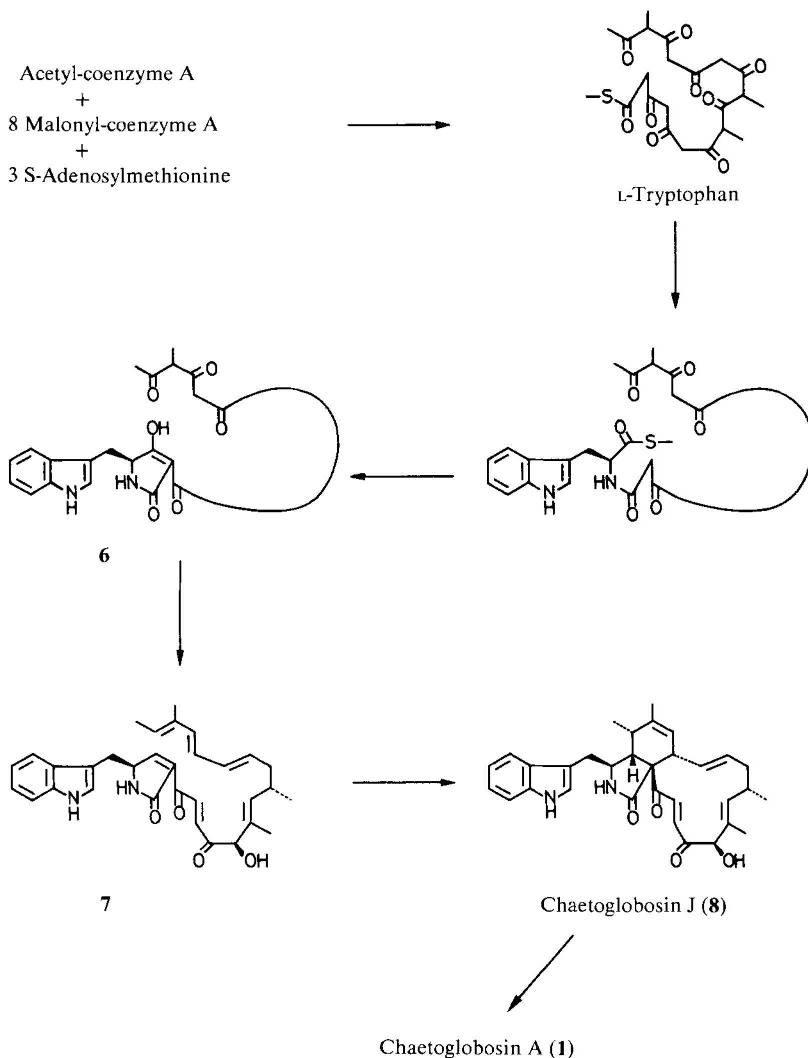
The support of these investigations by the *Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung* is gratefully acknowledged.

---

[11]) We thank *Prof. Dr. W. von Philipsborn*, Universität Zürich, Switzerland, for the measurement of this spectrum.

[12]) Chaetoglobosin K [25] should be excluded of course from this scheme.

Scheme 2



Experimental Part

General methods. See [28]. Radioactivity of precursors and 19-*O*-acetylchaetoglobosin A (**1**) was determined in the isotopic laboratories of *Sandoz AG.*, Basel (Mr. *Marbach*) and the Institute for Inorganic Chemistry of the University of Basel, radioactive precursors were purchased from *Amersham Radiochemical Centre* (Buckinghamshire, England) and *New England Nuclear* (Dreieich, BRD), ^{13}C-, ^{2}H- and ^{15}N-labelled compounds were obtained from *Radium Chemie* (Teufen, AR, Switzerland), *Sharp & Dohme GmbH* (München, BRD), *Prochem* (London, England) and *Ciba-Geigy* (Basel, Switzerland).

Production of chaetoglobosin A (**1**) *and 19-O-acetylchaetoglobosin A* (**2**). *Chaetomium globosum* was grown in the manner previously reported (*cf.* [3]). Aqueous solutions of the precursors were sterilized and added aseptically after 3 days, or in the case of [2-^{2}H]- and [2-^{15}N]-L-tryptophan sequentially from the third to the seventh day. The CH_2Cl_2 extract of the culture broth (*cf.* [3]) was dried (Na_2SO_4) and evaporated *i. V.* (40°). The brown residue was purified on a silica gel column (100:1), using increasing amounts of methanol in CH_2Cl_2, to give 150–600 mg/l chaetoglobosin A (**1**) and 200–700 mg/l 19-*O*-acetylchaetoglobosin A (**2**). **1** and **2** could be recrystallized from acetone/diisopropyl ether and from benzene respectively.

[2-^{2}H]-L-Tryptophan. According to *Matsuo* [23], 1.23 g (5 mmol) of *N*-acetyl-DL-tryptophan were converted to the corresponding azlactone and then treated with 99.8% D_2O to yield 975 mg (3.95 mmol) *N*-acetyl-[2-^{2}H]-DL-tryptophan. *N*-Acetyl[2-^{2}H]-L-tryptophan was obtained from the mixture with (–)-1-phenylethylamine by the method of *Overby* [29]. The above hydrolysis procedure allowed isolation of 205 mg (1.0 mmol) of [2-^{2}H]-L-tryptophan. No signal for H–C(2) remained in the ^{1}H-NMR. spectrum (60 MHz, $D_2O + DCl$) of this product. Comparison of the intensities in the molecular region of the mass spectrum of [2-^{2}H]-L-tryptophan and unlabelled amino acid showed that 80% of the molecules were labelled with deuterium.

REFERENCES

[1] *W. Rothweiler & Ch. Tamm*, Experientia *22*, 750 (1966); *W. Rothweiler & Ch. Tamm*, Helv. Chim. Acta *53*, 696 (1970); *D. C. Aldridge, J. J. Armstrong, R. W. Speake & W. B. Turner*, J. Chem. Soc., Chem. Commun. *1967*, 26; *D. C. Aldridge, J. J. Armstrong, R. W. Speake & W. B. Turner*, J. Chem. Soc. (C), *1967*, 1667.

[2] *E. J. Pedersen, P. Larsen & P. M. Boll*, Tetrahedron Lett. *1980*, 5079.

[3] a) *A. Probst & Ch. Tamm*, Helv. Chim. Acta *64*, 2056 (1981); b) *T. Fex*, Tetrahedron Letters *22*, 2703 (1981).

[4] a) *M. Binder, J. R. Kiechel & Ch. Tamm*, Helv. Chim. Acta *53*, 1797 (1970); b) *W. Graf, J. L. Robert, J. C. Vederas, Ch. Tamm, P. H. Solomon, I. Miura & K. Nakanishi*, Helv. Chim. Acta *57*, 1801 (1974); c) *J. L. Robert*, Dissertation, Basel 1977; d) *J. L. Robert & Ch. Tamm*, Helv. Chim. Acta *58*, 2501 (1975).

[5] a) *R. Wyss, Ch. Tamm & J. C. Vederas*, Helv. Chim. Acta *63*, 1538 (1980); b) *U. Sankawa, H. Shimada, T. Sato, T. Kinoshita & K. Yamasaki*, Tetrahedron Lett. *1977*, 483; c) *M. J. Garson, R. A. Hill & J. Staunton*, J. Chem. Soc., Chem. Commun. *1977*, 624, 921.

[6] a) *C. R. Lebet & Ch. Tamm*, Helv. Chim. Acta *57*, 1785 (1974); b) *J. C. Vederas, W. Graf, L. David & Ch. Tamm*, Helv. Chim. Acta *58*, 1886 (1975); c) *J. C. Vederas & Ch. Tamm*, Helv. Chim. Acta *59*, 558 (1976).

[7] a) S. W. Tanenbaum (Ed.) "Cytochalasins – Biochemical and Cell Biological Aspects", Elsevier/North-Holland Biochemical Press, Amsterdam, New York, Oxford 1978, Ch. 2; b) P. S. Steyn (Ed.), "The Biosynthesis of Mycotoxins", Academic Press, New York, London, Toronto, Sydney, San Francisco 1980, Ch. 8.

[8] *U. Séquin & I. Scott*, Heterocycles *5*, 525 (1976).

[9] *M. Tanabe & K. T. Suzuki*, Tetrahedron Lett. *1974*, 4417; *H. Seto, L. W. Cary & M. Tanabe*, Tetrahedron Lett. *1975*, 4491.

[10] *M. J. Garson & J. Staunton*, Chem. Soc. Rev. *8*, 539 (1979).

[11] *E. Lederer*, Biochem. J. *93*, 449 (1964); *G. Jauréguiberry, J. H. Law, J. A. McCloskey & E. Lederer*, Biochemistry *4*, 347 (1965); *G. Jauréguiberry, M. Lenfant, B. C. Das & E. Lederer*, Tetrahedron Suppl. *8*, Part I, 27 (1966); *E. Lederer*, Quart. Rev. *23*, 453 (1969); *M. Lenfant, H. Audier & E. Lederer*, Bull. Soc. Chim. Fr. *1966, 2775.*

[12] *L. Mascaro, R. Höhrhammer, S. Eisenstein, L. K. Sellers, K. Mascaro & H. G. Floss*, J. Am. Chem. Soc. *99*, 273 (1977); *H. F. Hegazi, R. T. Borchardt & R. L. Schowen*, J. Am. Chem. Soc. *98*, 3048 (1976).

[13] *M. Imfeld, C. A. Townsend & D. Arigoni*, J. Chem. Soc., Chem. Commun. *1976*, 541; *A. Battersby, R. Hollenstein, E. McDonald & D. C. Williams*, J. Chem. Soc., Chem. Commun. *1976*, 543; *A. I. Scott, M. Kajiwara, T. Takahashi, I. M. Armitage, P. Demon & D. Petrocine*, J. Chem. Soc., Chem. Commun. *1976*, 544.

[14] *R. Wyss & Ch. Tamm,* unpublished results.
[15] *J. M. Hanson, T. Marten & M. Silverns,* J. Chem. Soc. Perkin I, *1974,* 1033.
[16] *S. Udenfriend, E. Titus, H. Weissbach & R. E. Peterson,* J. Biol. Chem. *219,* 335 (1956).
[17] *F. C. Happold & L. Hoyle,* Biochem. J. *29,* 1218 (1935); *W. A. Wood, I. C. Gunsalus & W. W. Umbreit,* J. Biol. Chem. *170,* 313 (1947); *W. A. Newton, Y. Morino & E. E. Snell,* J. Biol. Chem. *240,* 1211 (1965); *E. Morino & E. E. Snell,* J. Biol. Chem. *242,* 2793, 2800 (1967).
[18] *J. Kuhn & R. l. Somerville,* Biochem. Biophys. Acta *332,* 298 (1974).
[19] *B. W. Bycroft,* Nature *224,* 595 (1969); *R. Cardillo, C. Fuganti, D. Ghiringhelli, P. Grasselli & C. Gatti,* J. Chem. Soc., Chem. Commun. *1975,* 778; *M. E. Gustafson, D. Miller, P. J. Davis, J. P. Rosazza, C.-J. Chang & H. G. Floss,* J. Chem. Soc., Chem. Commun. *1977,* 842.
[20] *H. Ruis & H. Kindl,* Phytochemistry *10,* 2627 (1971).
[21] *A. E. Braunstein,* "The Enzymes", P. D. Boyer (Ed.), Vol. 9, Academic Press, New York 1973, Chapter 10.
[22] *D. J. Whelan & G. J. Long,* Aust. J. Chem. *22,* 1779 (1969); *T. Oshima & N. Tamiya,* Biochem. J. *78,* 116 (1961); *E. H. Abbott & A. E. Martell,* J. Chem. Soc., Chem. Commun. *1968,* 1501.
[23] *H. Matsuo, Y. Fujimoto & T. Tatsuno,* Tetrahedron Lett. *1965,* 3465.
[24] *P. Mohr & Ch. Tamm,* Tetrahedron, Suppl. *9,* 201 (1981).
[25] *J. P. Springer, R. M. Cox, H. G. Cutler & F. G. Crumley,* Tetrahedron Lett. *1980,* 1905.
[26] *W. B. Turner, "Fungal Metabolites", Academic Press, London & New York 1971, p. 82.*
[27] *D. C. Aldridge, B. F. Burrows & W. B. Turner,* J. Chem. Soc., Chem. Commun. *1972,* 148.
[28] *T. Schmidlin & Ch. Tamm,* Helv. Chim. Acta *63,* 121 (1980).
[29] *L. R. Overby,* J. Org. Chem. *23,* 1393 (1958).