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60. Radiolabelled Peptide Ergot Alkaloids

83. Mitteilung über Mutterkornalkaloide [1]

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Herrn Dr. *Albert Hofmann* zum 70. Geburtstag gewidmet

(18. XII. 75)

Summary. Paspalic acid (**11**), labelled at different positions with ^{3}H or ^{14}C and at specific activities up to 1 Ci/mmol (^{3}H) and 2 mCi/mmol (^{14}C), has been prepared biosynthetically in a scale of 2–5 mmol in submerged cultures of a selected strain of *Claviceps paspali* by incorporation of DL-[5-^{3}H]-tryptophan, DL-[6-^{3}H]-tryptophan, DL-[alanine-2,3-^{3}H]-tryptophan, or DL-[alanine-3-^{14}C]-tryptophan. Radioactive lysergic acid (**12**) was obtained from paspalic acid by base catalysed rearrangement.

The procedures for labelling the precursors at high specific activity are described.

The ergolene carboxylic acids **11** and **12** were used as key intermediates for the chemical synthesis of radiolabelled peptide ergot alkaloids **14** required for pharmacokinetic and metabolic studies. Linking of the aminocyclols **13** (peptide parts) of the ergotamine (R^1 = methyl), ergoxine (R^1 = ethyl) and ergotoxine (R^1 = isopropyl) series with a reactive derivative of either lysergic acid or its mixture with paspalic acid was accomplished by standard procedures. 1-Methyl-[13-^{3}H]-ergotamine (MY 25) (**16**) and 2-bromo-α-ergocryptine (bromocriptine, CB 154) (**17**), labelled with ^{3}H at position 12 and with ^{14}C in position 4, were obtained by alkylating ^{3}H-ergotamine and by brominating appropriately labelled α-ergocryptines.

Radioactive peptide ergot alkaloids labelled by the present method proved suitable for the use in biological tracer studies.

Introduction. – The use of isotopically labelled compounds is the most efficient approach for the elucidation of the metabolic fate and the quantitative behaviour of a drug in the organism, especially for the drug substances belonging to the class of peptide ergot alkaloids which are medicated in milligram doses. The main excretion route of these substances is the bile, thus leading to very low concentrations in plasma and urine. In addition, taking into consideration the extensive rate of biotransformation and the high tissue affinity, the maximum plasma concentration of the parent drugs in man never exceeds 1 ng/ml, even after multiple dosing in the steady state. Most of the bioanalytical methods which are in current use, therefore are not applicable to this class of compounds because they are lacking in sensitivity. The compounds are also not suitable to be determined by the highly sensitive gas chromatography/mass spectrometry method due to their low volatility and thermal instability.

Consequently, the availability of radioactive ergot alkaloids became an absolute requirement.

This publication which is the first of a series dealing with the radioactive labelling of ergot alkaloids and related compounds outlines a general approach to the labelling of the tetracyclic ergoline skeleton at different sites with ^{3}H or ^{14}C. The method of choice makes use of a combination of different labelling procedures, namely direct chemical synthesis, biosynthesis and organic chemical conversion of labelled intermediates.

The general pathway applied to label peptide ergot alkaloids may be characterized by the following main steps: 1) direct chemical synthesis of DL-tryptophan labelled with ^{3}H or ^{14}C at high molar specific activities sufficient for use as precursors, 2) incorporation of the labelled precursors into paspalic acid by a biochemical method using a strain of *Claviceps paspali* in submerged culture, 3) chemical linking of the labelled paspalic acid isolated from the culture filtrate either directly or after rearrangement to lysergic acid with aminocyclols (peptide parts) of the ergot alkaloid series to obtain the lysergic acid and isolysergic acid derived peptide alkaloids, and 4) further chemical modification of some of the labelled peptide ergot alkaloids by chemical procedures to derivatives particularly required for biological tracer studies, *e. g.* MY 25, CB 154.

Other methods for tritium labelling of lysergic acid, paspalic acid or peptide alkaloids have been investigated. Although in some cases labelling was possible, the products were only of limited use for biological studies or as starting materials for the synthesis of peptide ergot alkaloids on a preparative scale. The low molar specific

activities attained, due to low isotope incorporation, or the instability of the labelled position to chemical exchange or to biological transformations are the limiting factors.

Lysergic acid could be labelled directly with tritium by sodium hydroxide or potassium *t*-butoxide catalysed exchange of the corresponding salt in tritiated water at 120–140°, or by heterogeneous platinum catalysed exchange of the sodium salt in tritiated water at 120° for 16–20 h [2]. [8-^{3}H]-Lysergic acid has been obtained by heating paspalic acid in tritiated water, 0.1 N in NaOH, for 1 h at 100° [3]. Tritium incorporation rates of 0.03–3% were achieved by these procedures, thus yielding products of unsatisfactory molar specific activities.

In another attempt to label peptide ergot alkaloids directly with tritium, [2-^{3}H]-ergostine was successfully prepared at high specific activity by palladium-black catalysed halogen-tritium exchange in 2-bromo-ergostine [2]. The tritium label at position 2 of ergostine, however, was found to be only moderately stable to chemical exchange in 0.1 N HCl and highly susceptible to biochemical transformation in pharmacokinetic studies with experimental animals (dog, rhesus-monkey) [4]. As ergot alkaloids brominated in positions other than 2 are not available this labelling procedure was therefore discontinued.

Except for the labelling of the methyl group at position 6, which will be the subject of a further publication, no rational chemical method exists at present to introduce carbon isotopes into the ergoline skeleton.

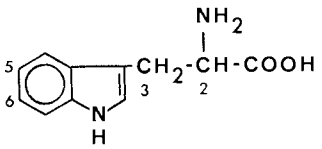
Radiolabelled precursors. – Tryptophan has been clearly established as a biosynthetic precursor in various ergot alkaloid producing strains of *ergot fungi* [5]. This fact prompted us to investigate the incorporation of labelled tryptophan into paspalic acid, the main metabolite produced by a selected strain of *Claviceps paspali*. Preliminary incorporation experiments with radioactively labelled tryptophans and other potential precursors, *e. g.* sodium [1-^{14}C]-acetate, [^{14}C]-mevalonic acid, [2,3-^{14}C]-succinic acid, confirmed that tryptophan was favored in the biosynthesis of paspalic acid.

Tryptophan labelled with carbon isotopes at any carbon atom other than the carboxyl group should prove a useful substrate, as tryptophan is believed to provide biosynthetically the tryptamine unit of the ergoline skeleton after loss of only the carboxyl group. Tritium atoms at positions 5 to 7 of the indole nucleus, or at the α or β position of the alanine side chain are considered to be located at positions admissible to give appropriately labelled tryptophans.

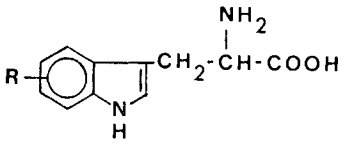
Relative costs, the feasibility of the synthetic route, the availability of labelled and unlabelled starting materials, the position of label and the high molar specific activities required have been the criteria for the choice of DL-[5-^{3}H]-tryptophan, DL-[6-^{3}H]-tryptophan, DL-[alanine-2,3-^{3}H]-tryptophan and DL-[alanine-3-^{14}C]-tryptophan as radiolabelled precursors.

A general tritiation procedure which involves the replacement of a halogen atom by a tritium atom under catalytic conditions was used for preparing DL-tryptophan labelled in the indole nucleus at positions 5 or 6, **1a** and **1b**. In this type of reaction the tritium atom is specifically introduced at the position where the halogen atom was located in the unlabelled starting material, recently confirmed in the case of [5-^{3}H]-tryptophan by ^{3}H-NMR. spectrometry [6].

Commercial DL-5-bromo-tryptophan (**2**), and DL-6-bromo-tryptophan (**3**) prepared from 6-bromo-indole-2-carboxylic acid, have been used as unlabelled, and carrier free tritium gas as the radioisotopic starting material. The dehalogenation reaction was performed according to known procedures [7–9] with palladium supported on alu-



1 DL-Tryptophan

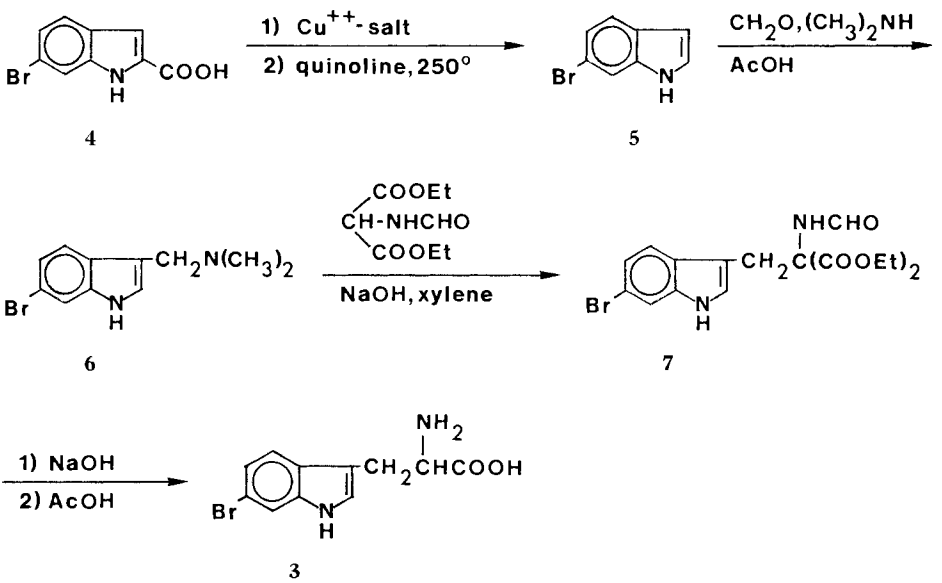


2 R: 5-bromo
3 R: 6-bromo

No.	Label Position
1a	[5-^{3}H]-indolyl-alanine
1b	[6-^{3}H]-indolyl-alanine
1c	indolyl-[2,3-^{3}H]-alanine
1d	indolyl-[3-^{14}C]-alanine

minium oxide as the catalyst in a methanol solution containing a slight excess of potassium hydroxide, which neutralizes the tritium bromide formed during the reaction. In the absence of the base the tritium halide may poison the catalyst and slow down the dehalogenation process. The labelled tryptophan was isolated after removal of the catalyst and any labile tritium, as tritiated water, by filtration on a cation exchange resin followed by elution of the washed column with dilute ammonia. Tryptophan labelled by this procedure had a specific activity of about 20–25 Ci/mmol, 70–85% of the theoretical activity taking into account that in the catalytic dehalogenation reaction half of the tritium is lost as tritium bromide.

Scheme 1

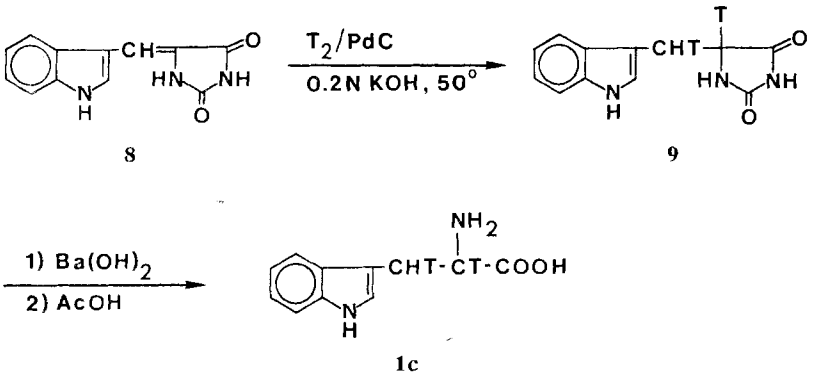


4 **5** **6** **7** **3**

DL-6-Bromo-tryptophan (**3**), the starting material for DL-[6-^{3}H]-tryptophan, has been prepared as outlined in *Scheme 1*. Decarboxylation of the copper salt of 6-bromo-indole-2-carboxylic acid (**4**) [10] [11] in boiling quinoline according to *Piers* & *Brown* [12] led to 6-bromo-indole (**5**). Conversion of this intermediate to DL-6-bromo-tryptophan was accomplished as described by *Weygand* & *Linden* [13] for the synthesis of DL-tryptophan from indole. By *Mannich* reaction of 6-bromo-indole (**5**) with formaldehyde and dimethylamine in acetic acid, and alkylation of ethyl formamido-malonate with the obtained 6-bromo-gramine (**6**) in the presence of sodium hydroxide the skatyl derivate **7** is obtained. After hydrolytic cleavage of the formamido and the ester group by treatment of **7** with sodium hydroxide, and the decarboxylation of the intermediate malonic acid derivative in a slightly acidic medium, DL-6-bromo-tryptophan (**3**) resulted.

DL-[Alanine-2,3-^{3}H]-tryptophan (**1c**) has been synthesized according to [14] [15] (*Scheme 2*) by catalytic addition of tritium gas to the exocyclic double bond of skatylidene-hydantoin (**8**), prepared from indole-3-carbaldehyde by condensation with hydantoin according to [16] [17], and subsequent barium hydroxide hydrolysis of the labelled intermediate indolylmethyl-hydantoin (**9**). According to [14], tritiation with excess carrier free tritium gas and *Raney*-nickel in aqueous sodium hydroxide provided DL-[alanine-2,3-^{3}H]-tryptophan having a specific activity of 15 Ci/mmol, which is about 26% of the theoretical value. Own experiments with a tritium/hydrogen gas mixture and palladium on carbon in aqueous potassium hydroxide solution,

Scheme 2



8 **9**

1c

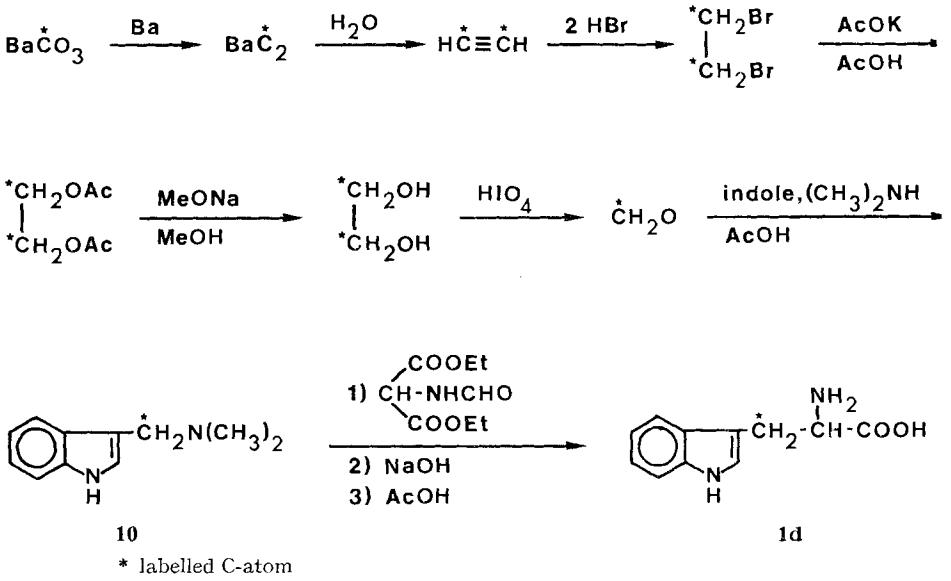
however, yielded much lower activities. This labelling procedure therefore offers no advantages compared to the halogen-tritium replacement method.

^{14}C-labelled tryptophan is commercially available, but in view of the large quantities (100 mCi and more) needed as substrate in the biosynthetic preparation of [^{14}C]-paspalic acid, the synthesis starting from barium [^{14}C]-carbonate was thought to be more economical than the direct purchase.

The variety of reported tryprophan syntheses reflects the difficulty experienced in reproducing some of them. A critical survey of the numerous methods resulted in only a few reaction sequences which appeared praticable for the preparation of

appropriately ^{14}C-labelled DL-tryptophan. The approach finally chosen and d played in *Scheme 3* is based on the procedure of *Weygand et al.* [13]. Starting fr barium [^{14}C]-carbonate, the reported synthetic pathway providing DL-[alanir

Scheme 3



10 **1d**

* labelled C-atom

3-^{14}C]-tryptophan (**1d**) in a 30% yield involves *Mannich* aminoalkylation of indol with [^{14}C]-formaldehyde and dimethylamine, and subsequent conversion of the labelled gramine (**10**) to **1d**.

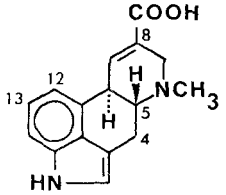
Attempts to prepare [^{14}C]-formaldehyde, the radioactive key intermediate, in accordance to *Weygand*, by simple partial reduction of [^{14}C]-carbon dioxide with lithium aluminium hydride in tetrahydrofuran, failed to give reproducible results on the 5–10 mmol scale. Further methods comprising partial $LiAlH_4$ reduction of suitably disubstituted [^{14}C]-formamides [18], hydrolysis of chloro-[^{14}C]-methyl acetate [19] [20], or catalytic oxidation of [^{14}C]-methanol [21] [22] of high specific activity were judged either too troublesome or not suitable for the sporadic and batchwise preparation of [^{14}C]-formaldehyde. In order to avoid any risk, the method of *Leitch et al.* [23] developed for the synthesis of deuteriated formaldehyde was suitably modified and employed for the preparation of [^{14}C]-formaldehyde. It involved addition of two equivalents of dry hydrogen bromide to [1,2-^{14}C]-acetylene, prepared from barium [^{14}C]-carbonate by a standard procedure, solvolysis of the intermediate 1,2-dibromo-ethane to ethylene diacetate by heating in glacial acetic acid containing potassium acetate, subsequent hydrolysis to [1,2-^{14}C]-glycol, and finally periodic acid oxidation of the glycol in aqueous solution.

The present method has the advantage of allowing the preparation of anhydrous [^{14}C]-paraformaldehyde or aqueous [^{14}C]-formalin solutions by oxidation of [1,2-^{14}C]-glycol with lead tetraacetate in benzene or with aqueous periodic acid, respectively.

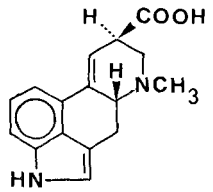
The radiochemical yield based on barium [^{14}C]-carbonate was about 70%. Since [^{14}C]-formaldehyde has recently become commercially available in large quantities at high specific activities and at acceptable price, direct purchase is recommended.

After improving the final steps of the tryptophan synthesis, an overall radiochemical yield of 42–45% was attained based on barium [^{14}C]-carbonate.

Biosynthetic labelling of ergolene carboxylic acids (paspalic acid and lysergic acid). – Incorporation of radiolabelled tryptophan into paspalic acid and lysergic acid was accomplished using the strain (NRRL 3080) of *Claviceps paspali* STEVENS ET HALL. This strain has been isolated from the *sclerotia* grown parasitically on the grass *Paspalum dilatatum* collected near Coimbra in Portugal and was cultivated as described by *Kobel et al.* [24]. It has been demonstrated by the same study group that approximately 90% of the alkaloids produced in submerged culture of this selected strain can be attributed to an ergolene carboxylic acid mixture consisting of more than 70% of paspalic acid (**11**) (6-methyl-$\Delta^{8,9}$-ergolene-8-carboxylic acid) and up to 30% of lysergic acid (**12**).



11



12

Administration of DL-tryptophan in quantities up to 1 g per litre of culture medium did not influence the alkaloid production. The total amount of ergot alkaloids produced averaged 1.5 g/l as was found in experiments without precursors.

In order to label the ergolene carboxylic acids, five days old alkaloid production broths were fed with radioactive DL-tryptophan and incubated at room temperature for ten days. After removal of the mycelium the labelled amino acids were isolated from the harvested cultures by absorption on a cation exchange resin followed by elution of the washed ion exchanger with dilute ammonia. The effluent contained paspalic- and lysergic acid which were purified by crystallization and recrystallization.

Detailed characteristic fermentation data [5a] from the individual experiments with ^{3}H- and ^{14}C-labelled-tryptophans are given in the Exper. Part (*cf.* Tables 4 and 5). The amount (mmol) of ergolene carboxylic acids produced was calculated from the molar specific activity of the ergolene carboxylic acid mixture isolated and the total radioactivity incorporated. In samples taken either from the culture filtrate or from the pooled amino acid fractions of the ion exchanger, the percentage of total radioactivity residing in the ergolene carboxylic acids was determined by thin-layer chromatographic separation of the main constituents, paspalic acid and tryptophan, followed by scanning for radioactivity.

The efficiency of incorporation of a radioactive precursor is best characterized by the incorporation rate and the specific incorporation [5a]. The incorporation rate of

DL-tryptophan into the ergolene carboxylic acids is calculated by relating the total radioactivity incorporated to the total radioactivity of the precursors fed. The specific incorporation is defined as the percentage of the molar specific radioactivity of product to the molar specific radioactivity of precursors.

The results of the preparative fermentation runs with DL-[5-^{3}H]-tryptophan, DL-[6-^{3}H]-tryptophan, DL-[alanine-2,3-^{3}H]-tryptophan and DL-[alanine-3-^{14}C]-tryptophan are given in Table 1. They demonstrate clearly that all chosen radiolabelled substrates were readily incorporated into paspalic and lysergic acid. No significant differences between the incorporation rates of ^{3}H- and ^{14}C-labelled substrates were observed. Since the mean specific incorporation ranges from 5 to 6%, the specific radioactivity of the products is by a factor of 16 to 20 lower than that of the added precursors. The specific incorporation is strongly dependent on the amount of precursor fed and the amount of alkaloids produced by the organism. High specific incorporation is obtained by addition of large amounts of precursors which can be seen from the data of the individual experiments (Tables 4 and 5, Exper. Part).

Incorporation experiments performed with 0.5 mmol or mostly 1 mmol of radiolabelled DL-tryptophans per litre of culture medium revealed that, under optimum conditions, using appropriate ^{3}H-substrates of 25 Ci/mmol or ^{14}C-substrates of 50 mCi/mmol, the preparation of ^{3}H- or ^{14}C-paspalic acid on a scale of 3–5 mmol

Table 1. *Incorporation of differently radiolabelled* DL-*tryptophans into paspalic and lysergic acid (ergolene carboxy acids) by* Claviceps paspali *in submerged cultures* (characteristic fermentation data)

Precursor Label and position		Radiolabelled DL-tryptophan					Summary ^{3}H and ^{14} experime
		5-^{3}H	6-^{3}H	alanine-2,3-^{3}H	all-^{3}H experiments	alanine-3-^{14}C	
Amount fed [a]		0.17–4.90	0.24–1.96	0.49–2.45	0.17–4.90	0.24–4.90	0.17–4.90
Number of experiments		7	8	3	18	13	31
Ergolene carboxylic acids produced [a]	mean	4.7	4.2	6.3	*4.8*	*5.4*	*5.0*
	s.d. [b]	1.2	1.3	0.5	1.3	1.6	1.4
	range	2.8–6.2	2.6–6.0	6.0–6.8	2.6–6.8	3.2–7.8	2.6–7.8
Paspalic acid isolated [a]	mean	3.6	3.2	5.8	*3.8*	*4.1*	*4.0*
	s.d. [b]	0.7	1.3	0.4	1.3	1.1	1.2
	range	2.3–4.3	2.0–4.0	5.4–6.2	2.0–6.2	2.5–5.7	2.0–6.2
Incorporation rate %	mean	22.5	24.6	27.1	*24.2*	*28.6*	*26.1*
	s.d. [b]	3.3	5.5	2.7	4.5	7.2	6.1
	range	16.6–26.9	21.0–37.2	24.0–29.2	16.6–37.2	16.7–40.9	16.6–40.9
% Specific incorporation	mean	7.0	5.2	5.4	*5.9*	*5.7*	*5.85*
	s.d. [b]	5.9	5.1	3.9	5.0	5.1	5.0
	range	1.5–19.0	1.5–17.0	2.4–9.8	1.5–19.0	1.3–18.8	1.3–19.0
Radiochemical yield %	mean	17.7	19.0	24.8	*19.4*	*21.5*	*20.3*
	s.d. [b]	3.9	5.0	2.7	4.8	3.6	4.4

[a] In mmol/l.
[b] s.d. = standard deviation

and at satisfactory specific radioactivities (about 1 Ci/mmol or 2.5 mCi/mmol, respectively) could be achieved; this with radiochemical yields of about 20% based on the total radioactivity fed into the system.

Paspalic acid and lysergic acid prepared by the present biosynthetic method are supposed to be specifically labelled at the sites related to the positions of the label in the substrates as shown in Table 2.

Table 2. *Supposed location of the label in the products biosynthesized by* Claviceps paspali *from differently labelled tryptophans*

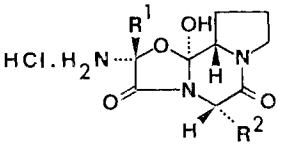
Substrate and Products	Isotope and Location			
	a	**b**	**c**	**d**
DL-Tryptophan (**1**)	5-^{3}H	6-^{3}H	alanine-2,3-^{3}H	alanine-3-^{14}C
Paspalic acid (**11**)	12-^{3}H	13-^{3}H	4,5-^{3}H	4-^{14}C
Lysergic acid (**12**)	12-^{3}H	13-^{3}H	4,5-^{3}H	4-^{14}C

Although the location of the label in tryptophan derived ergot alkaloids has never been established due to the difficulties in chemical degradation of the tetracyclic ergoline system, the accuracy of the proposed labelling pattern is strongly supported by the results of numerous investigations on the biogenesis of the ergoline skeleton [25–27]. These indicate the indole nucleus of tryptophan to be incorporated most likely unchanged. In addition it was proven by work of *Floss et al.* [28] that, with the exception of the carboxyl group, all carbon and hydrogen atoms of the alanine side chain are incorporated into the alkaloids from L-tryptophan. Interesting to note is the fact that only in the case of D-tryptophan the hydrogen atom at the α-carbon of the side chain is lost, because prior to incorporation the D-form is converted to the L-form by transamination *via* the keto acid.

Radiolabelled lysergic acid (**12**) was available from the biosynthetic ergolene carboxylic acid mixture by mild treatment with aqueous alkali. This reaction which involves migration of the ergolene 8,9-double-bond into conjugation with the aromatic indole system was used earlier for the structure elucidation of paspalic acid [24]. The radioactive lysergic acid thus abtained proved to be chemically uniform and identical with unlabelled reference material as judged by UV. and IR. spectroscopy, TLC. and optical rotation.

Transformation of the radiolabelled ergolene carboxylic acids to peptide ergot alkaloids. – The last step in the synthesis of peptide ergot alkaloids consisted in the linking of either radioactive paspalic acid or lysergic acid with the aminocyclols (peptide parts) of the desired alkaloids. This linking which involves the formation of an amide bond was accomplished by a known method of peptide chemistry. However, in order to achieve satisfactory yields on the mmol scale, the procedure had to be improved and specifically adapted to the peculiar properties of these sensitive structures.

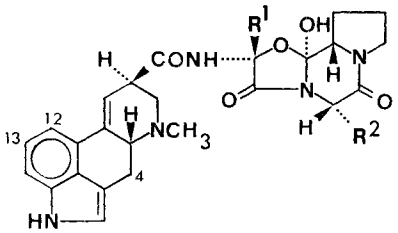
In a two step one pot reaction, lysergic acid or preferentially its mixture with paspalic acid as isolated directly from the culture filtrate, was first transformed to the mixed anhydride by treatment with trifluoroacetic acid anhydride in acetonitrile.



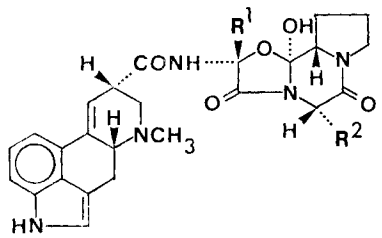
13 Aminocyclols

No.	R^1	R^2
13a	CH_3-	$C_6H_5CH_2-$
13b	CH_3-	CH_3-
13c	C_2H_5-	$C_6H_5CH_2-$
13d	$(CH_3)_2CH-$	$(CH_3)_2CHCH_2-$

This reactive intermediate was then condensed with the hydrochloride of selected aminocyclols **13** of the ergotamine (R^1 = methyl), ergoxine (R^1 = ethyl) or ergotoxine (R^1 = isopropyl) series under very mild conditions in the presence of pyridine. A mix-



14



15

ture of two stereoisomeric peptides derived from lysergic acid and isolysergic acid of the general formulae **14** and **15** was obtained which was separated into its constituents by column chromatography. Under the slighly alkaline reaction conditions of the condensation the double bond at 8,9-position of paspalic acid completely rearranges to 9,10-position which is in conjugation with the indole nucleus, thus leading to the

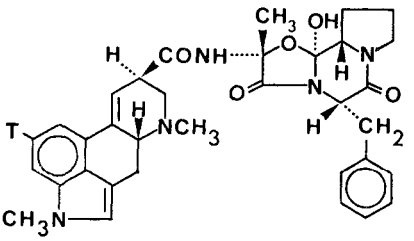
Table 3. *Radiolabelled peptide ergot alkaloids prepared from radioactive paspalic acid or lysergic acid*

General formula		Substitution		Labelling			Exper. Part Section
14	**15**	R^1	R^2		Isotope	Position	
Ergotamine	Ergotaminine	CH_3-	$C_6H_5CH_2-$	**a**	^3H	13	4.1.1.
				b	^{14}C	4	4.1.2.
5′-Methyl-ergo-alanine (MD 121)	–	CH_3-	CH_3-	**c**	^3H	12	4.1.3.
Ergostine	Ergostinine	C_2H_5-	$C_6H_5CH_2-$	**d**	^3H	12	4.1.4.
				e	^3H	13	4.1.5.
α-Ergocryptine	α-Ergo-cryptinine	$(CH_3)_2CH-$	$(CH_3)_2CHCH_2-$	**f**	^3H	12	4.1.6.
				g	^3H	13	4.1.7.
				h	^{14}C	4	4.1.8.

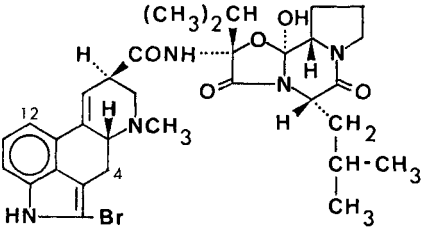
desired lysergic acid derived peptides. All individual radiolabelled peptides prepared by this method are listed in Table 3.

Finally, some of the labelled peptide ergot alkaloids served as starting material for further chemical transformations to labelled derivatives needed for biological tracer investigations in the drug evaluation process.

^{3}H-labelled MY 25 (1-methyl-ergotamine) **16** was prepared from [13-^{3}H]-ergotamine (**14a**) according to [29] by alkylation with methyliodide and potassium ethoxide in liquid ammonia.



16



17a [12-^{3}H]- **17b** [4-^{14}C]-

^{3}H- and ^{14}C-labelled CB 154 (2-bromo-α-ergocryptine[1]) [30]) **17a** and **17b** were obtained from [12-^{3}H]-α-ergocryptine (**14f**) and [4-^{14}C]-α-ergocryptine (**14h**), respectively, by bromination either with N-bromocaprolactam in dioxane or with N-bromosuccinimide in chloroform, procedures used by *Troxler & Hofmann* [31] for the preparation of closely related 2-bromo ergot alkaloids.

In biological use a tritium atom in labelled ergot alkaloids functions as tracer for the carbon atom to which it is bound. Since C–H bonds may vary in their stability depending on their position in the molecule, the tritium atom in the labelled ergot alkaloids was tested for its stability to chemical exchange under acidic and alkaline conditions at 50°. In order to get a measure of the stability, the percentage of tritium exchange was determined after 24 h by assaying the tritiated water in the condensates of freeze-dried aliquots of test solutions. Under alkaline conditions (pH ~ 13) a tritium/hydrogen exchange rate of up to 12% for the alkaloids labelled at position 12, and of less than 5% for those labelled at position 13 has been observed. In acidic media (pH ~ 1) the percentage of tritium exchange never exceeded 2%, regardless of the tritium location. A tritium atom at the benzene carbon atom *para* to the indole nitrogen atom seems to be more susceptible to base catalysed exchange than the one at the adjacent 13-position. Although the stability of the label to chemical exchange was not very satisfactory, the tritiated compounds nevertheless proved useful in biological experiments. On the other hand, even tritium atoms chemically tested to be stable may become labile under biological conditions due to enzyme reactions at the labelled positions.

Another feature commonly encountered with radiochemicals is the problem of stability on storage. Tritium labelled ergot alkaloids were found to be unstable due to self-radiolysis. Dispersion of the radioactive molecules by dissolution was not

[1]) Generic name: bromocriptine; active substance of PARLODEL®, trademark of a *Sandoz* speciality.

applicable for controlling the self-decomposition because of the chemical instability of the compounds in the most commonly used solvents. The best conditions for minimizing damage by radiolytical self-decomposition were sealing under vacuum and storage at –20° in the dry solid state. All tritium labelled preparations of high specific radioactivity were regularly examined for radiochemical purity before use.

Experimental part

General remarks. – Melting points (m.p.) were determined in sealed capillaries on a *Tottoli* melting point apparatus (*Büchi*, Flawil) and are uncorrected. UV. spectra were recorded on a *Beckman* model DK 2 spectrophotometer and IR. spectra on a *Perkin Elmer* model 21 spectrophotometer. Thin-layer chromatography (TLC.) was performed unless otherwise stated on $5 \times 20 \times 0.025$ cm *Merck* precoated silica gel 60 F 254 plates. The following solvent systems were used: I: chloroform, II: chloroform/methanol 95:5, III: chloroform/methanol 9:1, IV: *n*-hexane/acetone 4:1, V: *n*-butanol/glacial acetic acid/water 4:1:5 (upper-layer), VI: chloroform/methanol/glacial acetic acid 4:3:3, VII: ethanol/25 perc. ammonia 95:5, VIII: benzene/ethanol/25 perc. ammonia 84:15:1, IX: acetone/25 perc. ammonia 99:1. For column chromatography, silica gel 0.05–0.2 mm or 0.064–0.2 mm (E. *Merck* A.G., Darmstadt) was employed.

Abreviations: RT. = room temperature; i.V. = in the vacuum; i.HV. = in high vacuum.

General procedures. – *Measurement of radioactivity.* Duplicate aliquots (0.1 ml or 0.5 ml) of test solutions prepared by dissolving weighed amounts of ^{3}H- or ^{14}C-compounds in organic solvents (*e.g.* methanol, ethanol and mixtures with chloroform) were added to 15 ml of a liquid scintillation cocktail, *e.g.* Instagel® (*Packard*) or a solution of 0.6% butyl-P.B.D. in ethanol/toluene 6:4 (*v/v*), and directly assayed for radioactivity by means of a liquid scintillation spectrometer TRI-CARB model 3375 (*Packard*). Counting data were corrected for quenching by the external standard ratio method and processed by an on-line connected *Hewlett-Packard* minicomputer model 2114 A.

Purity and identity determination of labelled compounds. For determination of radiochemical purity, samples equal to 0.05–0.1 μCi (^{14}C-compounds) and 1–2 μCi (^{3}H-compounds) as solutions in methanol or chloroform were chromatographed on TLC. plates and assayed for radioactivity with a *Berthold* thin-layer scanner model II (*Berthold/Frieseke*, Karlsruhe). Quantitative values were obtained by electronic peak integration or by scraping 5 mm wide zones of the plates and assaying for radioactivity by liquid scintillation counting.

Chemical purity and identity of labelled ergot alkaloids with unlabelled reference compounds was checked by UV. and IR. spectroscopy and by co-chromatography on TLC. plates using 50 μg samples. The spots were detected by UV. (254 nm) and by spraying the plates with ***van Urk*** or *Dragendorff* reagent.

Stability determination of the ^{3}H-label. The stability of the label in tritiated ergot alkaloids to chemical exchange was checked by treating a 0.1–0.5 mCi sample of the tritium labelled alkaloid added by the 10fold amount of carrier in 10 ml of an aqueous 0.1 N HCl or 0.1 N NaOH medium at 50° for 24 h. The tritium/hydrogen-exchange rate was determined by measuring the radioactivity in the condensates of freeze-dried 2 ml test solution samples.

1. Syntheses of radiolabelled precursors. – 1.1. DL-[*5-^{3}H*]-*Tryptophan* (**1a**). 300 mg (1.06 mmol) of DL-5-bromo-tryptophan (**2**), 200 mg of 5% Pd/Al_2O_3 and 4 ml of 0.5 N methanolic potassium hydroxide solution were combined and carrier free tritium gas (30 ml, approx. 75 Ci) was introduced. The mixture was stirred at RT. After the uptake of tritium had ceased, the remaining tritium gas i.V. manifold was transferred by means of a *Toepler* pump into a glass ampoule and sealed. To remove labile bound tritium the mixture was evaporated i.V., the resulting residue taken up in 2 ml of water, and the solution freeze-dried. This procedure was repeated twice. The residue was then taken up in water, the catalyst filtered off, and the filter thoroughly rinsed with water. The filtrate was adjusted to 25 ml with water (total activity 26 Ci) and passed through a column of 40 ml of Amberlite IR 120 (H^+-form) at a flow rate of 50 ml/h. After washing the column with 250 ml of water, the ^{3}H-tryptophan adsorbed was eluted with 1.5 l of 0.5% ammonia, the effluent evaporated at 50°/12 Torr to dryness, and the resulting residue (200 mg)

of DL-[5-^{3}H]-tryptophan (**1a**) dissolved in 50 ml of deionized water. This solution was sterilized for 20 min at 120° and immediately used in the fermentation run.

The product was shown to be chemically pure by TLC. (solvent system V, detection by exposure to iodine vapor). The content as determined radiometrically using the same TLC. system was greater than 95%. A total activity of 25 Ci was measured for the ^{3}H-tryptophan solution, having a specific activity of 25.5 Ci/mmol (87.9% of theory).

Crystalline **1a** at specific radioactivity of ~5 Ci/mmol was prepared by the same procedure using 0.5 mmol of DL-5-bromo-tryptophan and about 15 Ci (6 ml) of tritium gas. The tritiation was terminated by adding 10 ml of hydrogen gas and stirring 1 h. After removal of the catalyst and labile bound tritium, the product was isolated from the aqueous alkaline solution by precipitating with 5% acetic acid at pH 5.5 and 5°. Then 1 mmol of unlabelled DL-tryptophan was added as carrier. Recrystallization from glacial acetic acid, and drying i.V. at 100° for 4 h gave 255 mg of **1a**, 4.8 Ci/mmol, in a radiochemical yield of 79.9%.

Samples of lower specific activity were prepared by diluting this material with appropriate amounts of unlabelled carrier tryptophan followed by crystallization from acetic acid.

1.2. DL-[*6-^{3}H*]-*Tryptophan* (**1b**) (*Scheme 1*). 1.2.1. *Copper-salt of 6-bromo-indole-2-carboxylic acid.* To a hot solution of 2.4 g (10 mmol) of 6-bromo-indole-2-carboxylic acid (**4**) and 530 mg (6.4 mmol) of Na_2CO_3 in 100 ml of water was added under stirring a solution of 1.5 g (6 mmol) of copper(II)sulfate · 5 H_2O in 50 ml of water. After cooling to RT. the precipitated copper-salt was filtered off, thoroughly rinsed with water, and dried i.V. at 75° for 16 h: 2.4 g.

1.2.2. *6-Bromo-indole* (**5**). To a boiling solution of 9.6 g (40 mmol) of 6-bromo-indole-2-carboxylic acid in 40 ml of quinoline (bath temp. 250°) were added 2.4 g (~8 mmol) of copper-salt of 6-bromo-indole-2-carboxylic acid in six portions of 0.4 g at intervals of 1 h. After addition of the last portion the mixture was refluxed for 1 h, cooled to 80°, and dropped into 600 ml of diethyl ether. The precipitate was filtered, the filtrate extracted with 1N HCl (1 × 400 ml, 3 × 100 ml), water (2 × 100 ml), 2N Na_2CO_3 (2 × 100 ml) and again with water (2 × 100 ml), dried over anhydrous Na_2SO_4, and evaporated to dryness at 30°/12 Torr. *Kugelrohr*-distillation at 70–75°/0.01 Torr, crystallization of the distillate from 50 ml of cyclohexane and redistillation yielded 6.6 g (70%) of **5**, m.p. 92°, purity > 98% as determined by TLC. (system I, detection with *van Urk* reagent).

1.2.3. *6-Bromo-gramine* (**6**). A mixture of 10 ml of glacial acetic acid, 5 ml of 40% diethylamine and 1 g of *para*formaldehyde was kept at 60° for 3 h, cooled to RT. and after the addition of 4.9 g (25 mmol) of 6-bromo-indole (**5**) stirred for 20 h at RT. The red coloured solution was then made alkaline with 2N NaOH and thoroughly extracted with diethyl ether. The basic components were extracted from the ether layer with 2N HCl, the aqueous phases combined, made alkaline at 5° with 40% NaOH, and reextracted with diethyl ether. The combined ethereal extracts were washed with water, dried over anhydrous K_2CO_3 and evaporated to a small volume. Dilution with cyclohexane yielded crystalline **6** which was collected, washed with light petroleum, and dried i.V. at 80° for 4 h: 5.05 g (80%) of **6**, m.p. 138–140°, uniform as shown by TLC. (system IX, detection with *van Urk* reagent).

1.2.4. *Diethyl(6-bromo-skatyl)formamido-malonate* (**7**). The mixture of 4.75 g (18.8 mmol) of 6-bromo-gramine (**6**), 5 g (24.6 mmol) of diethylformamido-malonate (*Fluka AG.*, Buchs) and 0.25 g of finely powdered NaOH in 50 ml of xylene was refluxed under nitrogen for 7 h (bath temp. 150°). The reaction was terminated by cooling to RT., addition of water and extraction of the aqueous phase with dichloromethane. The organic extract was washed with water and dried over anhydrous Na_2SO_4. After removal of dichloromethane by distillation, pure crystalline (6-bromo-skatyl)-malonic ester separated from the resulting xylene solution on cooling. The product was collected, washed with xylene and light petroleum, and dried i.V. at 80° for 4 h: 7.25 g (93.7%) of **7**, m.p. 173–175°. TLC.: system IX, detection with *van Urk* reagent.

1.2.5. DL-*6-Bromo-tryptophan* (**3**). 7.15 g (17.4 mmol) of diethyl (6-bromo-skatyl)formamido-malonate (**7**) were hydrolyzed by refluxing in 50 ml of 2N NaOH for 6 h (bath temp. 130°). To the solution were then added 7.5 ml of glacial acetic acid, and boiling was continued for 3 h. On cooling crude **3** separated. The crystals were collected, washed with cold water, and purified by precipitation with acetic acid at pH 5.5 from a filtered solution in 3 ml of alcohol and 10 ml of 2N NaOH. The product was washed with water, alcohol and diethyl ether, and dried i.V. at 100° for 4 h: 3.95 g (80.1%) of **3**, m.p. 275–280° (dec.), uniform as determined by TLC. (system V, detection with iodine vapor).

1.2.6. DL-[*6-^{3}H*]-*Tryptophan* (**1b**). Material of high specific activity was prepared from DL-6-bromo-tryptophan (**3**) (300 mg, 1.03 mmol) by catalytic halogen replacement with an excess of carrier free tritium gas (70 Ci) and 5% Pd/Al_2O_3 in 0.5N methanolic potassium hydroxide according to the procedure described for the preparation of DL-[5-^{3}H]-tryptophan (**1a**) (section 1.1.). **1b** was obtained in 95% yield, was radiochemically pure, and had a specific activity of 22 Ci/mmol (75.8% of theory).

1.3. DL-[*Alanine-2,3-^{3}H*]-*tryptophan* (**1c**) (*Scheme 2*). ^{3}H-labelled material at 100–500 mCi/mmol, sufficient for the use as substrate in preliminary fermentation runs with *Claviceps paspali*, was prepared according to [15] by catalytic hydrogenation of skatylidene-hydantoin (**8**) [16] [17] with a hydrogen/tritium gas mixture and 10% Pd/C in 0.2N KOH. Subsequent hydrolysis of the intermediate ^{3}H-indolylmethyl-hydantoin (**9**) by treatment with barium hydroxide according to [32], and work-up as usual gave radiochemically pure **1c** in 70% yield.

1.4. DL-[*Alanine-3-^{14}C*]-*tryptophan* (**1d**) (*Scheme 3*). 1.4.1. [*1,2-^{14}C*]-*Acetylene*. 990 mg (5 mmol) of barium [^{14}C]-carbonate (46 mCi/mmol, 230 mCi total) were mixed with 5 g of finely shredded barium metal in a Pyrex glass tube (diameter: 2 cm, length: 12 cm) and covered with 2.5 g barium. The tube was heated under argon in a *Bunsen* flame until the mixture became incandescent and set to a black mass (regulus) which was then cooled in a dessicator. The tube was broken off and the barium ^{14}C-carbide quickly transferred to a dry flask connected *via* the condenser to traps cooled with dry ice/methanol (–70°) and with liquid nitrogen. Air was swept from the system with helium, and 10 ml of water were added slowly to the barium [^{14}C]-carbide. After the vigorous reaction had ceased the mixture was heated to boiling (bath temp. 130°), and the liberated ^{14}C-acetylene was removed by a continuous stream of helium and collected in the cold trap at –180°. Refluxing was continued for 1 h. The cooled trap containing the product was attached to the vacuum manifold and evacuated to remove carrier gas. Then the temp. of the trap was raised to 20° and the product distilled into a 100 ml long necked flask cooled to –180° and containing 5 ml of conc. sulfuric acid. After stirring for 15 min at 20°, the dried [^{14}C]-acetylene was distilled i.V. into a receiver cooled with liquid nitrogen. Yield as determined by manometrical measurement was 2.38 mmol (95%).

1.4.2. *1,2-Dibromo-[1,2-^{14}C]-ethane*. ^{14}C-Dibromoethane was prepared from ^{14}C-acetylene (2.38 mmol) and dry hydrogen bromide (5.2 mmol) in the presence of a catalytic amount of oxygen under UV. illumination according to [23]. The product was isolated in 91% yield (406 mg) by fractional vacuum distillation (10^{-3} Torr) first at –55° (removal of hydrogen bromide and small amounts of unreacted acetylene) and then at 20–30° (dibromoethane), and was collected in a trap maintained at –180°.

1.4.3. *Ethylene[1,2-^{14}C]diacetate*. 406 mg (2.16 mmol) of 1,2-dibromo-[1,2-^{14}C]-ethane were vacuum transferred into a flask containing 600 mg (*ca.* 6 mmol) of fused potassium acetate and 0.2 ml of glacial acetic acid (*ca.* 3.5 mmol). The mixture was heated over night in a bath maintained at 190°, then the volatile products were removed at 90–120°/11 Torr. The distillate was dissolved in 20 ml of dichloromethane, the solution washed free from acetic acid with water and 2N $KHCO_3$, dried over anhydrous Na_2SO_4 and the solvent distilled off at atmospheric pressure through a *Vigreux* column. The concentrate was then fractionated in a *Kugelrohr* to obtain 306 mg (97%) of ethylene [1,2-^{14}C]-diacetate as colourless liquid, b.p. 80–90°/11 Torr. Analysis by GC. showed the product to be practically pure.

1.4.4. [*1,2-^{14}C*]-*1,2-Ethanediol*. To the solution of 305 mg (2.1 mmol) of ethylene[1,2-^{14}C]-diacetate in 4 ml of methanol was added 1 ml of 0.05N sodium methoxide in methanol. The mixture was refluxed for 2 h at a bath temp. of 110°, the solvent distilled off at atmospheric pressure through a *Vigreux* column, and the residue fractionated in a *Kugelrohr* to yield 120 mg (92%) of colourless [^{14}C]-ethanediol, b.p. 90–100°/11 Torr. Analysis by GC. and radio-TLC. (system IV) indicated the product to be pure.

1.4.5. [^{14}*C*]-*Formaldehyde*. To the solution of 120 mg (1.93 mmol) of [^{14}C]-ethanediol in 2 ml of water were added at 0° 435 mg (1.93 mmol) of periodic acid ($HIO_4 \cdot 2\,H_2O$), and the mixture was stirred for 10 min at 0–5°. This [^{14}C]-formaldehyde solution was immediately used for the *Mannich* reaction.

1.4.6. ^{14}*C-Gramine* (**10**). To the aqueous [^{14}C]-formaldehyde solution (*ca.* 3.86 mmol) were added at 0° 1.5 ml of 40% dimethylamine, 3 ml of glacial acetic acid and 515 mg (4.4 mmol) of indole.

The mixture was stirred for 20 h at room temp. The red solution was then made alkaline with 2N NaOH and extracted with diethyl ether. The basic components were extracted from the ether layer with 2N HCl, the aqueous phases combined, made alkaline at 5° with 40% NaOH and extracted again with diethyl ether. After the organic extract had been washed with water, it was dried over anhydrous Na_2SO_4, and evaporated i.V. to yield 592 mg (88%) of crystalline **10**, m.p. 130–132°, 258 μCi/mg = 45 mCi/mmol. The radiochemical purity was >97% as determined by TLC. (system VII).

1.4.7. *Diethyl ^{14}C-skatyl-formamido-malonate.* 590 mg of ^{14}C-gramine (**10**), 110 mg of non-labelled gramine (total: 700 mg, 4 mmol; 38 mCi/mmol), and 1.2 g (6 mmol) of diethyl formamido-malonate were dissolved in 8 ml of xylene, treated with 100 mg of finely powdered NaOH for 6 h at reflux (bath temp. 135°), then cooled to RT. and diluted with water and chloroform. The aqueous layer was extracted 3× with chloroform/2-propanol 95:5, the organic extract washed with water, dried over anhydrous Na_2SO_4, and evaporated i.V. to afford 1.2 g (90%) of crystalline ^{14}C-skatyl-formamido-malonic ester, m.p. 175–177°.

1.4.8. DL-[*Alanine-3-^{14}C*]-*tryptophan* (**1d**). The suspension of 1.2 g (3.6 mmol) of diethyl ^{14}C-skatyl-formamido-malonate in 10 ml of 2N NaOH was refluxed for 6 h, 2 ml of glacial acetic acid were added, and boiling was continued for 3 h. The solid which separated on standing at RT. was collected, washed with cold water, and dried i.V. for 2 h at 100° to give 0.8 g of crude **1d** which was dissolved by heating in 100 ml of water and freed from insoluble, amorphous by-products by filtration through hyflo. The filtrate was concentrated i.V. to 50–60 ml, and passed through a column of 50 ml of Amberlite IR 120 (H^+-form) at a flow rate of 50 ml/h. After washing the column with 300 ml of deionized water the tryptophan was eluted with 1 l of 0.3% ammonia, recovered by evaporation at 30°/12 Torr, and crystallized twice from glacial acetic acid to yield after drying in vacuum at 100° for 15 h 460 mg (62.5%) of **1d**, 186 μCi/mg = 38.0 mCi/mmol. Radiochemical purity >99% as determined by TLC. (system V).

From the concentrated mother liquor a second crop of pure **1d** was obtained: 80 mg (10.8%), 186.5 μCi/mg = 38.1 mCi/mmol, thus increasing the overall radiochemical yield to 100.5 mCi (43.7%) based on barium [^{14}C]-carbonate.

2. Preparation of [12-^{3}H], [13-^{3}H], [4,5-^{3}H], and [4-^{14}C]-paspalic acid by *Claviceps paspali* cultures using radiolabelled DL-tryptophans as precursors. – The experiments performed with DL-[5-^{3}H]-tryptophan, DL-[6-^{3}H]-tryptophan, DL-[alanine-2,3-^{3}H]-tryptophan, and DL-[alanine-3-^{14}C]-tryptophan as precursors are listed in Tables 4 and 5. As a representative example of the preparation of radiolabelled paspalic acid, the procedure for the fermentation, the isolation and purification of [12-^{3}H]-paspalic acid is described in detail.

2.1. *[12-^{3}H]-Paspalic acid* (**11a**). The culture of *Claviceps paspali* was first grown on a wort/potato/agar medium for mycelial propagation and then on a malt/cornsteep/agar medium for *conidia* formation. The *conidia* were then aseptically transferred to a malt extract medium and incubated on a shaker. Within 3 days a dense suspension of mycelial particles was formed but no alkaloids were produced. The *mycelium* of this preculture was then used to inoculate the production medium (ten 500 ml *Erlenmeyer* flasks, each containing 100 ml of medium) consisting of sorbitol, ammonium succinate, mineral salts, and iron and zinc as essential trace elements. The small scale cultures were then incubated on a reciprocal shaker at 22–24°. 5 days later, by which time alkaloid synthesis had begun, 5 ml (= 2.5 Ci) aliquots of the sterilized DL-[5-^{3}H]-tryptophan (**1a**) solution (200 mg/50 ml; 25.5 Ci/mmol) were introduced into each *Erlenmeyer* flask. 10 days after the addition of precursor the cultures were harvested, combined, and filtered through hyflo on a sintered glass funnel. The *mycelium* was washed with 200 ml of water, the filtrate assayed spectrophotometrically according to [33] for total ergolene alkaloids (1.75 g, calculated for mol-wt. 270) and radiometrically for total activity (21.5 Ci). By radio-TLC. on silica gel G with H_2O as solvent approximately 27% (= 5.8 Ci) of the total radioactivity found in the culture filtrate could be assigned to the mixture of paspalic acid and lysergic acid.

The culture filtrate (*ca.* 1 l; pH 5.5) was passed through a column (diameter: 2 cm) of 250 ml of Amberlite IR 120 (H^+-form) at a flow rate of 200 ml/h. After washing the column with 800 ml of deionized water, the adsorbed amino acids were eluted over night with 2 l of 0.5% ammonia (flow rate 100–150 ml/h) and the effluent assayed as described above: total activity 12.8 Ci;

Table 4. *Incorporation of* tritium labelled DL-tryptophans *into paspalic and lysergic acid (ergolene carboxylic aci by a strain of* Claviceps paspali *in submerged culture*

^{3}H-DL-Tryptophan				^{3}H-Ergolene carboxylic acids			Fermentation characteristics		
Position of label	Amount fed mg/l	Amount fed mCi/l	Spec. Act. mCi/mmol	Produced mmol	Isolated mmol	Spec. Act. mCi/mmol	Incorporation rate %	% Specific incorporation	Radiochemi yield %
5	35	720	4200	2.80	2.28	62	24.1	1.48	19.6
	200	4.9	5	4.48	3.84	0.24	21.9	4.80	18.8
	200	4700	4800	4.37	4.03	290	26.9	6.04	24.9
	200	16060	16400	6.24	3.40	625	24.3	3.81	13.2
	200	24970	25500	5.95	4.26	975	23.2	3.82	16.6
	500	4.9	2	5.04	3.92	0.20	20.6	10.0	16.0
	1000	4.9	1	4.29	3.81	0.19	16.6	19.0	14.8
6	50	10	40.8	2.78	2.08	0.80	22.2	1.96	16.6
	50	320	1300	3.66	2.80	19	21.7	1.46	16.6
	90	3800	8620	6.04	5.86	133	21.1	1.54	20.5
	100	10	20.4	2.63	2.01	0.80	21.0	3.92	16.1
	200	10	10.2	3.32	2.39	0.65	21.6	6.37	15.5
	200	17140	17500	5.09	3.97	865	25.7	4.94	20.0
	200	21500	21950	5.61	3.36	1016	26.5	4.63	15.9
	400	5250	2680	4.29	3.51	455	37.2	16.98	30.4
alanine--2,3	100	10	20.4	6.08	5.63	0.48	29.2	2.35	27.0
	200	10	10.2	6.82	6.21	0.41	28.0	4.02	25.5
	500	10	4.1	6.00	5.45	0.40	24.0	9.80	21.8

Table 5. *Incorporation of* DL-[alanine-3-^{14}C]-tryptophan *into paspalic and lysergic acid (ergolene carboxylic acid by a strain of* Claviceps paspali *in submerged culture*

^{14}C-DL-Tryptophan			^{14}C-Ergolene carboxylic acids			Fermentation characteristics		
Amount fed mg/l	Amount fed mCi/l	Spec. Act. mCi/mmol	Produced mmol	Isolated mmol	Spec. Act. μCi/mmol	Incorporation rate %	% Specific incorporation	Radiochemic yield %
12	2.0	34.0	4.87	2.12	150	36.5	0.44	15.9
50	0.25	1.02	3.28	2.74	18.3	24.0	1.79	20.1
50	0.25	1.04	5.93	5.26	13.5	32.0	1.30	28.4
100	0.20	0.40	5.93		13.5	40.0	3.38	
100	7.25	0.51	3.21	2.49	19.5	25.0	3.82	19.4
100	0.30	0.62	6.20	5.44	12.9	26.7	2.08	23.4
100	18.6	38.0	7.76	4.24	980	40.9	2.58	22.3
200	0.10	0.10	3.60	3.21	6.6	23.8	6.60	21.2
200	0.20	0.20	5.05	3.41	12.0	30.0	6.00	20.5
200	0.30	0.31	6.60	5.71	10.6	23.3	3.42	20.2
200	37.2	38.0	6.57	5.15	1980	35.0	5.21	27.4
250	46.5	38.0	7.50	4.60	2000	32.3	5.26	19.8
500	0.17	0.07	3.84	3.43	10.0	22.6	14.29	20.2
1000	0.20	0.04	4.45	4.05	7.5	16.7	18.75	15.2

distribution of the radioactivity on the components: ergolene carboxylic acids (Rf 0.35) 45% (= 5.75 Ci), tryptophan (Rf 0.75) 47% (= 6 Ci). The ammoniacal effluent was concentrated at 30°/12 Torr to about 20 ml, adjusted to pH 5.5 with acetic acid, diluted with an equal volume of methanol, boiled briefly, kept several hours at 5°, filtered, and the crude crystalline ergolene carboxylic acid mixture (1.3 g) reprecipitated from 5% ammonia with 2N acetic acid at pH 5.0. Drying i.V. at 110° for 4 h yielded a 1.15 g (4.26 mmol) mixture consisting of about 75% paspalic acid **11a** and 25% lysergic acid **12a**; 3.6 mCi/mg = 975 mCi/mmol; radiochemical yield (purified product) 4.15 Ci (16.6%); ^{3}H-tryptophan incorporation rate 23.2%, specific incorporation 3.82%; radiochemical purity 90–93%, established by radio-TLC. (systems V and VII). By TLC. on silica gel G with water the product was shown to be free of ^{3}H-tryptophan. The amount of lysergic acid in the mixture was approximately determined by comparing its molar extinction at 310 nm with that of the pure components.

2.2. *Storage of radioactive paspalic acid and purification of stored material.* After one year's storage as crystalline solid (initial specific radioactivity 0.5–1 Ci/mmol) in sealed evacuated borosilicate glass tubes at $-20°$ to $+4°$ it was necessary to repurify the material before further use.

Purification of stored material. The 5 ml methanol/25% ammonia 9:1 solution of 400 mg of [12-^{3}H]-paspalic acid (**11a**) (3.6 mCi/mg) of *ca.* 60% radiochemical purity was filtered on a column of 20 g of silica gel. The column was then eluted with methanol/25% ammonia 98:2. The fractions containing the paspalic acid were combined and evaporated i.V. After addition of 400 mg of unlabelled paspalic acid as carrier, the mixture was crystallized from H_2O/2N acetic acid at pH 5 to yield ^{3}H-paspalic acid of > 85% radiochemical purity: 560 mg, 1.25 mCi/mg = 335 mCi/mmol.

3. Rearrangement of labelled paspalic acid to lysergic acid. – As an illustrative example the preparation of [4-^{14}C]-lysergic acid is described. [12-^{3}H]-Lysergic acid and [13-^{3}H]-lysergic acid were similarly prepared from the corresponding ^{3}H-paspalic acids.

[4-^{14}C]-Lysergic acid (**12d**). A solution of 4.5 g (5.6 mmol) of [4-^{14}C]-paspalic acid (**11d**) (1.98 mCi/mmol) in 25 ml of 0.5N NaOH was heated at 100° for 1 h. The crystals obtained upon acidification, first to pH 7 with 2N HCl, then with acetic acid to pH 5.5, were twice recrystallized from methanol containing 5–10% ammonia. The crystals were washed with H_2O and methanol, and dried i. HV. at 110° for 4 h: 1.1 g (73%) of pure [4-^{14}C]-lysergic acid (**12d**), 7.46 μCi/mg = 2.0 mCi/mmol, m.p. 242–247° (dec.), $[\alpha]_D = +98°$ (c = 0.5, 0.1N NaOH). – UV. (0.1N NaOH): λ_{max} (log ε) = 224 (4.32), 238 (4.32) and 310 (3.94), minimum at 268 nm.

The labelled product was identical with unlabelled reference lysergic acid, as judged by UV., optical rotation and TLC. (systems V–VII).

4. Radiolabelled peptide ergot alkaloids. – 4.1. *Condensation of ^{3}H- and ^{14}C-lysergic acid or its mixture with radioactive paspalic acid with aminocyclols of the ergotamine, ergoxine and ergotoxine series. General procedure.* Radiolabelled lysergic acid or its mixture with paspalic acid obtained directly by fermentation of *Claviceps paspali* was reacted at $-20°$ to $-10°$ with 1.3–1.5 mol-equiv. of trifluoroacetic acid anhydride in an inert solvent (preferably acetonitrile) to give the mixed anhydride. The latter was condensed at $-20°$ to 0° in the presence of a weak base, such as pyridine, with 1.1–1.3 mol-equiv. of aminocyclol (peptide part) in the form of its hydrochloride to yield the corresponding peptide alkaloids as a mixture of the two stereoisomers at C(8). The reactions were carried out under dry nitrogen and anhydrous solvents of high quality were used.

As representative examples, the preparation of [13-^{3}H]-ergotamine- (**14a**) and [4-^{14}C]-α-ergocryptine (**14h**) is described in detail.

4.1.1. *[13-^{3}H]-Ergotamine* (**14a**) *(sulfate, free base, and tartrate) and [13-^{3}H]-ergotaminine* (**15a**) *(base).* The mixture of tritiated paspalic acid and lysergic acid (purity *ca.* 90%, paspalic acid/lysergic acid 7:3) isolated as crystalline product from the culture filtrate of a fermentation with DL-[6-^{3}H]-tryptophan (**1b**) was used as starting material for the preparation of [13-^{3}H]-ergotamine and [13-^{3}H]-ergotaminine.

A suspension of 600 mg of the above ^{3}H-ergolene carboxylic acid mixture (corresponding to 2 mmol of pure product; 3.8 mCi/mg = 1 Ci/mmol, vacuum dried at 110° for 20 h) in 12 ml of acetonitrile was cooled to $-20°$ and 0.35 ml (2.5 mmol) of trifluoroacetic anhydride in 2 ml of acetonitrile were added. After stirring for 10 min at $-20°$ a suspension of 920 mg (2.5 mmol) of aminocyclol hydrochloride **13a** [34] of ergotamine in 5 ml of dry dichloromethane was added at

once, followed immediately by 4 ml of pyridine keeping the temp. at $-20°$. The mixture was stirred for 1 h at $-10°$ to 0°, poured into 50 ml of dichloromethane, and 20 ml of ice-cold 2N Na_2CO_3 were added with shaking. The aqueous phase was separated, extracted with dichloromethane (4×20 ml), the organic extract washed with aqueous Na_2CO_3 and H_2O, dried with anhydrous Na_2SO_4, and the combined filtrate evaporated at 40°/12 Torr. The crude product was dissolved in a small amount of methanol and partitioned between ethyl acetate and 10% aqueous tartaric acid. After repeated extraction of the organic phase with tartaric acid, the aqueous layers were combined, cooled, and made alkaline with 40% NaOH. The bases were extracted from that with dichloromethane, the organic extracts washed with NaCl solution, dried over anhydrous Na_2SO_4, and evaporated i.V. to yield 905 mg (78%) of a mixture of bases which was shown by radio-TLC. (system III) to consist of about 65% of ^{3}H-ergotamine (**14a**) and 35% of ^{3}H-ergotaminine (**15a**).

[13-^{3}H]-Ergotamine sulfate. To the mixture of the stereoisomeric bases (1.55 mmol) dissolved in 15 ml of 10% acetic acid in methanol were added at RT. 15 ml of 0.1N H_2SO_4 in methanol and the solution was seeded with crystalline ergotamine sulfate. After 2 days at 5° the crystals were collected, washed with cold methanol and ether, and dried i.HV. at 100° for 2 h to give 815 mg (83%) of ^{3}H-ergotamine sulfate, m.p. 198–200° (dec.).

[13-^{3}H]-Ergotamine (**14a**). The free base was liberated partitioning 630 mg (1.0 mmol) of ^{3}H-ergotamine sulfate between dichloromethane and 5% ammonia. The organic phase was separated, washed with NaCl solution and water, dried over anhydrous Na_2SO_4, and evaporated i.V. The residue was crystallized from methanol/ethyl acetate and dried at 60° for 4 h to yield 480 mg (82%) of **14a**, m.p. 180–185° (dec.), 1.75 mCi/mg = 1.02 Ci/mmol. The product was shown to be identical with an unlabelled reference sample by TLC. and by comparing the UV. (methanol) and IR. (CH_2Cl_2) spectra. Radiochemical purity was 98% as determined by radio-TLC. (system III). Less than 2% of ^{3}H-ergotaminine was detected as byproduct.

[13-^{3}H]-Ergotamine tartrate. Material of 1 mCi/mg, suitable for pharmacokinetic experiments in man, was prepared by dissolving 200 mg of ^{3}H-ergotamine (1.75 mCi/mg) and 100 mg of ergotamine as carrier (total: 0.52 mmol) in 2 ml of methanol and adding a solution of 40 mg (0.265 mmol) of L-(+)-tartaric acid in 1 ml of methanol. The mixture was boiled briefly, cooled to 5°, the crystals were collected after 2 h, washed with methanol/diethyl ether 1:1 and dried i.HV. at 60° for 2 h to give 265 mg (77%) of ^{3}H-ergotamine tartrate, m.p. 185–190° (dec.), 1.045 mCi/mg = 686 mCi/mmol.

[13-^{3}H]-Ergotaminine. The mother liquor of the ^{3}H-ergotamine sulfate preparation was evaporated to dryness i.V. and the residue partitioned between dichloromethane and 10% ammonia. The organic layer was separated, washed with NaCl solution, dried over anhydrous Na_2SO_4, and the solvent removed under reduced pressure. The residue (135 mg) was taken up in dichloromethane and chromatographed on a column of 10 g of silica gel with dichloromethane/methanol 98:2. The fractions containing the ergotaminine were combined, evaporated to dryness, and the residue was crystallized from ethanol to give 85 mg of pure ^{3}H-ergotaminine, m.p. 230–232° (dec.), 1.81 mCi/mg = 1.05 Ci/mmol, $[\alpha]_D^{20} = +375°$ ($c = 0.2$, $CHCl_3$). The product proved identical with the unlabelled reference substance as shown by TLC. (system III) and mixed m.p. determination.

Chemical stability of the ^{3}H-label. Test solutions of [13-^{3}H]-ergotamine (**14a**) showed tritium/hydrogen exchange rates of less than 1% and less than 5% on treatment with ethanol/0.2N HCl 1:1 and ethanol/0.2N NaOH 1:1, respectively.

4.1.2. *[4-^{14}C]-Ergotamine* (**14b**) *and [4-^{14}C]-ergotaminine* (**15b**). ^{14}C-Ergotamine and ergotaminine were similary prepared from a 1.8 mmol mixture of ^{14}C-paspalic acid and lysergic acid (2 mCi/mmol) obtained by culturing *Claviceps paspali* fed with DL-[alanine-3-^{14}C]-tryptophan (**1d**). The crude mixture of bases which resulted in 68% yield by condensation with the aminocyclol **13a** consisted of 60% of ^{14}C-ergotamine and 40% of ^{14}C-ergotaminine. Epimerisation with sulfuric and acetic acid in methanol gave 88% crystalline ^{14}C-ergotamine sulfate, from which the free base [4-^{14}C]-ergotamine (**14b**), and finally [4-^{14}C]-ergotamine tartrate, 2.95 μCi/mg = 1.94 mCi/mmol, were prepared.

^{14}C-Ergotaminine (**15b**) (m.p. 228–230° (dec.), 3.52 μCi/mg = 2.05 mCi/mmol) was obtained from the mother liquor of the ergotamine sulfate as described above for ^{3}H-ergotaminine.

4.1.3. *5'-Methyl-[12-^{3}H]-ergoalanine* (**14c**) (*MD 121* [35]). Upon condensation as above of 2 mmol of aminocyclol **13b** [35] with a 1.35 mmol mixture of tritiated paspalic acid and lysergic acid (290 mCi/mmol, isolated from a fermentation with DL-[5-^{3}H]-tryptophan (**1a**), the crude bases were obtained in 65% yield. They were purified by column chromatography on silica gel (20 g) with dichloromethane/methanol 99:1 as eluant and treated with an equivalent amount of 0.1 N H_2SO_4 in ethanol in the presence of acetic acid at 80° for 1 h. The product which separated after seeding with unlabelled 5'-methyl-ergoalanine sulfate and standing at 5° for 20 h was collected, recrystallized from acetone/water and dried i.HV. at 40° for 2 h to give 0.64 mmol (73%) of 5'-methyl-[12-^{3}H]-ergoalanine sulfate, m.p. 208–210° (dec.), 428 μCi/mg = 257 mCi/mmol.

Chemical stability of the ^{3}H-label. By treating test solutions of the labelled product in 0.1 N HCl and in ethanol/0.2 N NaOH 1:1 tritium/hydrogen-exchange rates of 1.8 and 11.9%, respectively, were found.

4.1.4. *[12-^{3}H]-Ergostine* (**14d**) *and [12-^{3}H]-ergostinine* (**15d**). The mixture of isomeric bases obtained in 75% yield upon condensation of 1.5 mmol of the aminocyclol **13c** [36] with 1.35 mmol of [12-^{3}H]-ergolene carboxylic acid mixture (70% paspalic acid and 30% lysergic acid, 290 mCi/mmol) consisted of 42% [12-^{3}H]-ergostine and 58% [12-^{3}H]-ergostinine. The mixture was separated on a column of 20 g of silica gel with dichloromethane/methanol 99:1 and 98:2 as eluants.

The dichloromethane/methanol 99:1 fractions gave on evaporation, and crystallization of the residue from methanol 305 mg (38%) of pure [12-^{3}H]-ergostinine (**15d**), m.p. 213–215° (dec.), $[\alpha]_D^{20} = +355°$ ($c = 0.25$, $CHCl_3$), 492 μCi/mg = 293 mCi/mmol.

The fractions of dichloromethane/methanol 98:2 which were shown by TLC. (system III) to be uniform ergostine yielded 245 mg (30%) of [12-^{3}H]-ergostine (**14d**). The free base (0.41 mmol) was dissolved in a small amount of methanol, and a solution of 55 mg (0.47 mmol) of maleic acid in 1 ml of methanol was added. The solid which separated on standing at 5° was collected, washed with cold methanol, dried in vacuum at 80° for 1 h to give 275 mg (94%) of [12-^{3}H]-ergostine hydrogenmaleate, m.p. 175° (dec.), 402 μCi/mg = 286 mCi/mmol. Overall radiochemical yield: 28.3% (110 mCi).

Chemical stability of the ^{3}H-label. A tritium/hydrogen exchange of 1.7 and 8.5% was found on treating solutions of [12-^{3}H]-ergostine (**14d**) in ethanol/0.2 N HCl 1:1 and in ethanol/0.2 N NaOH 1:1, respectively.

4.1.5. *[13-^{3}H]-Ergostine* (**14e**) *and [13-^{3}H]-ergostinine* (**15e**). The procedure for the preparation of [13-^{3}H]-ergostine hydrogenmaleate (190 μCi/mg = 136 mCi/mmol) and of the free base [13-^{3}H]-ergostinine (232 μCi/mg = 138 mCi/mmol) was essentially the same as that described for [12-^{3}H]-ergostine (*cf.* above). As radioactive starting material the ^{3}H-ergolene carboxylic acid mixture obtained biosynthetically by fermentation with DL-[6-^{3}H]-tryptophan (**1b**) was used.

Chemical stability of the ^{3}H-label. Test solutions of [13-^{3}H]-ergostine (**14e**) showed a tritium/hydrogen exchange of 0.5 and 6.9% in ethanol/0.2 N HCl 1:1 and in ethanol/0.2 N NaOH 1:1, respectively.

4.1.6. *[12-^{3}H]-α-Ergocryptine* (**14f**) *and [12-^{3}H]-α-ergocryptinine* (**15f**). The condensation of 1.64 mmol of the ^{3}H-ergolene carboxylic acid mixture (70% [12-^{3}H]-paspalic acid and 30% [12-^{3}H]-lysergic acid, 335 mCi/mmol) with 2 mmol of aminocyclol **13d** according to the procedure described for [4-^{14}C]-α-ergocryptine (section 4.1.8.) yielded 63% of a mixture of ^{3}H-α-ergocryptine (55%) and ^{3}H-α-ergocryptinine (45%). Separation by column chromatography gave 283 mg (30%) of pure, crystalline [12-^{3}H]-α-ergocryptine (**14f**), 580 μCi/mg = 334 mCi/mmol, and 227 mg (24%) of pure [12-^{3}H]-α-ergocryptinine (**15f**), 585 μCi/mg = 337 mCi/mmol.

4.1.7. *[13-^{3}H]-α-Ergocryptine* (**14g**) *and [13-^{3}H]-α-ergocryptinine* (**15g**). ^{3}H-α-Ergocryptine and ^{3}H-α-ergocryptinine labelled at position 13 of the lysergic acid part and at a specific activity of 986 μCi/mg = 568 mCi/mmol were prepared by the standard method from a mixture of equal parts of [13-^{3}H]-paspalic acid and [13-^{3}H]-lysergic acid biosynthesized from DL-[6-^{3}H]-tryptophan.

Chemical stability of the ^{3}H-label. Tritium/hydrogen exchange in test solutions of [13-^{3}H]-α-ergocryptine (**14g**) was less than 1% in ethanol/0.2 N HCl 1:1 and less than 5% in ethanol/0.2 N NaOH 1:1.

4.1.8. *[4-^{14}C]-α-Ergocryptine* (**14h**) *and [4-^{14}C]-α-ergocryptinine* (**15h**). To 1.05 g (3.9 mmol) of [4-^{14}C]-lysergic acid (7.5 μCi/mg, vacuum dried at 110°) suspended in 20 ml of dry acetonitrile and maintained at −20° under dry nitrogen were added with vigorous stirring 0.7 ml of trifluoroacetic anhydride (*ca.* 5 mmol). 10 min later a 10 ml dry dichloromethane suspension of 1.6 g (4.3 mmol) of the aminocyclol hydrochloride **13d** [37] of α-ergocryptine was added at once followed by 5 ml of dry pyridine added dropwise at −20° over a period of 10 min. The mixture was stirred 15 min at −20° and 1 h at 0°. After the addition of 2 ml of water, the solution was diluted with 100 ml dichloromethane, washed with water and 2N Na_2CO_3, dried, and evaporated at 40°/12 Torr to yield 1.74 g (78%) of mixture of crude bases. Analysis by radio-TLC. (system III) showed the mixture to consist of about 55% of ^{14}C-α-ergocryptine, 40% of ^{14}C-α-ergocryptinine and 5% of non identified radioactive byproducts. It was separated by column chromatography on 100 g of silica gel. With dichloromethane/methanol 98:2 α-ergocryptinine was first eluted followed by α-ergocryptine. The fractions containing ^{14}C-α-ergocryptinine were combined and evaporated. The product was crystallized from ethanol: 606 mg (27%) **15h**, m.p. 238° (dec.), $[\alpha]_D^{20} = +482°$ (*c* = 0.5, pyridine), 3.5 μCi/mg = 2.0 mCi/mmol.

The fractions which were shown by TLC. (system III) and on aluminium oxide F 254 (system II) to be uniform α-ergocryptine yielded after evaporation 1.0 g (44.5%) ^{14}C-α-ergocryptine. It was crystallized from benzene and vacuum dried at 110° for 2 h to give 825 mg (36.7%) of pure **14h**. 3.5 μCi/mg = 2 mCi/mmol. – UV. (MeOH): λ_{max} (log ε) = 240 (4.28) and 312 (3.92) nm, identical with the spectrum of an unlabelled reference sample.

4.2. *1-Methyl-[13-^{3}H]-ergotamine* (**16**) (*MY 25*) (*free base and hydrogen tartrate*). In an atmosphere of dry nitrogen 200 mg (5 mmol) of potassium were dissolved in 5 ml of a mixture of ethanol/toluene 1:4 with stirring and heating. The potassium ethoxide solution was cooled to −80° and 15 ml of dry liquid ammonia was condensed into the flask followed at once by 450 mg (0.775 mmol) of [13-^{3}H]-ergotamine (**14a**) (0.94 mCi/mg ~547 mCi/mmol, high vacuum dried at 100° for 20 h). The mixture was allowed to react at −50° to −40° for 15 min by which time almost all of the ergotamine had dissolved. Then 1 ml of a solution of 30% methyliodide in diethyl ether was added and the mixture stirred at −40° for 30 min. The solution was reduced to a small volume under vacuum at −40°, ice and a saturated solution of $KHCO_3$ were added, and the bases extracted with dichloromethane, the organic layers washed with water, dried over anhydrous Na_2SO_4, and evaporated i.V. at 30°. The crude alkylated product which was shown by radio-TLC. (system III) to be a mixture of the stereoisomers methyl-ergotamine and methyl-ergotaminine was subjected to column chromatography on 20 g of silica gel by eluting with dichloromethane followed by dichloromethane/methanol 99:1 and 95:5. The fractions eluted with dichloromethane/methanol 95:5 and shown by TLC. to be uniform 1-methyl-ergotamine yielded after evaporation 352 mg (76%) of crystalline **16**.

Labelled **16** (0.59 mmol) in methanol was converted with 90 mg (0.60 mmol) of L-(+)-tartaric acid in methanol to the hydrogentartrate salt which was crystallized from ethanol and dried i.HV. at 80° for 2 h: 341 mg (77.5%), m.p. 170–173° (dec.), 721 μCi/mg = 537 mCi/mmol. Radiochemical purity was 98% as established by radio-TLC. (system III and VIII) and on aluminium oxide F 254 (system II). Less than 2% of ^{3}H-1-methyl-ergotaminine was detected as byproduct.

Chemical stability of the ^{3}H-label. Tritium/hydrogen exchange of test solutions of 1-methyl-[13-^{3}H]-ergotamine hydrogentartrate was less than 2% in ethanol/0.2N HCl 1:1 and 1% in ethanol/0.2N NaOH 1:1.

4.3. *^{3}H- and ^{14}C-labelled 2-bromo-α-ergocryptine* (*CB 154, bromocriptine,* PARLODEL®). 4.3.1. *2-Bromo-[12-^{3}H]-α-ergocryptine* (**17a**) (*free base and methane sulfonate*). To a stirred solution containing 280 mg of [12-^{3}H]-α-ergocryptine (**14f**) (580 μCi/mg) and 160 mg of unlabelled α-ergocryptine (total: 0.76 mmol) in 15 ml of dioxane was added at RT. a solution of 250 mg (1.3 mmol) of N-bromocaprolactam in 10 ml of dioxane. The mixture was kept for 5 h at RT., and evaporated at 40°/12 Torr. The residue was dissolved in dichloromethane, the solution washed with 2N Na_2CO_3 and water, dried, and the solvent removed under reduced pressure. The crude bromo compound was taken up in chloroform and chromatographed on 30 g of silica gel. The main fraction of the chloroform/methanol 98:2 eluate yielded after evaporation 280 mg (55%) of uniform ^{3}H-2-bromo-α-ergocryptine (**17a**) as a foam: 305 μCi/mg = 210 mCi/mmol.

The solution of 280 mg (0.427 mmol) of **17a** in 1.3 ml of 2-butanone was treated with a freshly prepared solution (0.42 ml) of 1N methane sulfonic acid in 2-butanone. The mixture was kept for

several h at 5°. The crystalline solid was collected, washed with small amounts of 2-butanone and diethyl ether, and dried i.V. at 60° for 2 h to give 290 mg (87%) of ^{3}H-2-bromo-α-ergocryptine methane sulfonate, *243* μCi/mg = *192* mCi/mmol. – UV. (MeOH): λ_{max} = 312 nm (log ε = 4.0).

The purity of the products (base and methane sulfonate) was examined by TLC. (system III) and on aluminium oxide F 254 (system II).

Crystalline ^{3}H-labelled product proved to be unstable on storage at RT. The radiochemical purity found after one year's storage amounted to about 80%. Better results were obtained storing the solid in sealed evacuated glass tubes at –20° and below. Stored methane sulfonate was purified as the free base by column chromatography on silica gel with dichloromethane and dichloromethane/methanol 98:2 as eluant, the free base having been liberated by partition between 2N Na_2CO_3 and dichloromethane.

Chemical stability of the ^{3}H-label. Tritium/hydrogen exchange of test solutions of 2-bromo-[12-^{3}H]-α-ergocryptine methane sulfonate was less than 0.2% in ethanol/0.2N HCl 1:1, less than 2% in ethanol/triethylamine 7:3 at pH 9, and less than 10% in ethanol/0.2N NaOH 1:1.

4.3.2. *2-Bromo-[4-^{14}C]-α-ergocryptine* (**17b**) (*base and methane sulfonate*). To 824 mg (1.43 mmol) [4-^{14}C]-α-ergocryptine (3.5 μCi/mg) in 30 ml of chloroform were added at 60° and with stirring 350 mg (1.96 mmol) of N-bromo-succinimide. The mixture was kept at 60° for 30 min, cooled to RT., and then extracted with 2N Na_2CO_3 and water. The dried chloroform solution was evaporated, the crude bromo compound taken up in chloroform, and chromatographed on a column of 50 g of silica gel. The main fraction of the chloroform/methanol 98:2 eluate yielded after evaporation 675 mg (72%) of uniform ^{14}C-2-bromo-α-ergocryptine (**17b**).

The solution of 675 mg (1.03 mmol) of **17b** in 6 ml of chloroform was treated at 0° with 2 ml of a freshly prepared solution of 0.5N methane sulphonic acid in chloroform/diethyl ether 3:1, and the mixture kept over night at 5° to 10°. The crystalline solid was collected, washed with small amounts of chloroform, and dried i.HV. at 60° for 4 h to give 600 mg (78%) of methane sulfonate: 2.7 μCi/mg = 2.0 mCi/mmol.

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61. Metall-Bis(silyl)chelat-Komplexe

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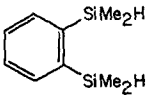
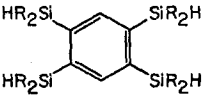
(11. VIII. 75)

Summary. The reaction of poly(silyl)benzenes with $Co_2(CO)_8$, $Fe_2(CO)_9$, $Ru_3(CO)_{12}$ or $(Ph_3P)_2Pt \cdot C_2H_4$ affords the bis(silyl)chelate complexes of Co, Fe, Ru and Pt. Infrared, proton magnetic resonance and mass spectra are reported.

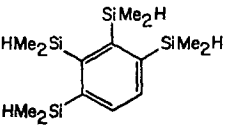
Es wurde kürzlich u.a. über die Poly(dimethylsilyl)benzole **1**, **2** und **3** berichtet [1]. Diese Verbindungen, sowie das bisher nicht beschriebene Tetrakis(diphenylsilyl)-benzol **4**, tendieren aufgrund ihrer *ortho*-ständigen, reaktiven Diorganosilylgruppen (Knüpfung von Silicium-Metall-Bindungen durch oxydative SiH-Metall-Anlagerung)



1



2 R = Me
4 R = Ph



3

[1]) Experimentell mitbearbeitet von den Herren *A. Wenger* und *H. U. Kellenberger*.