Anti-Cancer Drugs, 3, pp. 609–614

Clavine alkaloids and derivatives as mutagens detected in the Ames test

Hansruedi Glatt,[CA] Heinz Pertz, Reinhard Kasper and Eckart Eich

H Glatt is at the Department of Toxicology, Johannes Gutenberg-Universität, Obere Zahlbacher Strasse 67, D-6500 Mainz, Germany.
Tel: +49-6131-233662. Fax: +49-6131-230506.
H Pertz, R Kasper and E Eich are at the Department of Pharmaceutical Biology, Freie Universität, Berlin, Germany.

Eight cytostatic clavines were investigated for mutagenicity in *Salmonella typhimurium* (reversion of the *his*$^-$ strains TA98, TA100, TA102 and TA1537), directly and in the presence of a mammalian xenobiotic metabolizing system, S9 (NADPH-fortified postmitochondrial fraction of liver homogenate from Aroclor 1254-treated rats). Four compounds (festuclavine, 17-bromofestuclavine, 1-allylelymoclavine and 1-methyllysergol methyl ether) were direct mutagens, whose activity was enhanced in the presence of S9. The other compounds (1-cyclopentylfestuclavine, 13-bromo-1-cyclopropylmethylfestuclavine, 6-cyano-1-propyl-6-norfestuclavine and 6-allyl-1-propyl-6-norfestuclavine) showed mutagenic effects only in the presence of S9, as previously observed with other clavines (agroclavine and its 1-propyl and 1-pentyl derivatives). Thus, all investigated clavines may be metabolized to mutagenic products by mammalian enzymes. Bacteriotoxic activities did not correlate with mutagenic activities. The bacteriotoxicity of several clavines was reduced in the presence of S9. The results are discussed with regard to the potential therapeutic use of clavine alkaloids as antimicrobial and antineoplastic agents.

Key words: Clavines, elymoclavine, ergolines, festuclavine, lysergol.

Introduction

Clavines are alkaloids of the 8-methyl- or 8-hydroxymethylergoline type which are produced by fungi of the genera *Claviceps, Aspergillus* and *Penicillium*, and by certain genera of the plant family Convolvulaceae.[1] In contrast to the ergot alkaloids of the lysergic acid amide type, clavines have not yet been used therapeutically. Nevertheless, they also interact with dopamine and serotonin receptors and, with lower affinity, with α-adrenoreceptors.[2] Several clavines have shown remarkable antibiotic activity against various bacteria and yeast.[3] Some natural clavines and, especially, certain 1- and 10-substituted derivatives are potent cytostatic agents *in vitro*.[4–7] Compounds like 13-bromo-1-cyclopropylmethylfestuclavine[8] and the corresponding 13-bromo-1-cyclopentyl derivative[9] have already shown pronounced antineoplastic activity *in vivo*. Treatment with these clavines increased the survival time of mice injected with L5178y lymphoma cells more than 2-fold.

Agroclavines and 1-alkylated derivatives showed mutagenic activities in *Salmonella typhimurium*, when tested in the presence of a hepatic xenobiotic-metabolizing system.[10] At the same time, the metabolizing system strongly decreased the antibacterial activities, indicating that the toxicity and mutagenicity of agroclavine and its 1-alkylated derivatives are mediated by different chemical species. In the present study, further clavine alkaloids, which are potentially useful as antimicrobial and/or antineoplastic agents, were therefore investigated for mutagenic and toxic activities in the *Salmonella* assay. The structures of the examined compounds are shown in Figure 1.

Materials and methods

Compounds

Festuclavine (**IV**) was obtained by catalytic transfer hydrogenation of agroclavine (**I**) with Raney nickel in methanol.[11] 17-Bromofestuclavine (**V**) was synthesized by bromination of dihydrolysergol-I,[12] 1-cyclopentylfestuclavine (**VI**) by *N*-1 alkylation of (**IV**),[9] 13-bromo-1-cyclopropylmethylfestucla-

[CA] Corresponding Author

© *1992 Rapid Communications of Oxford Ltd*

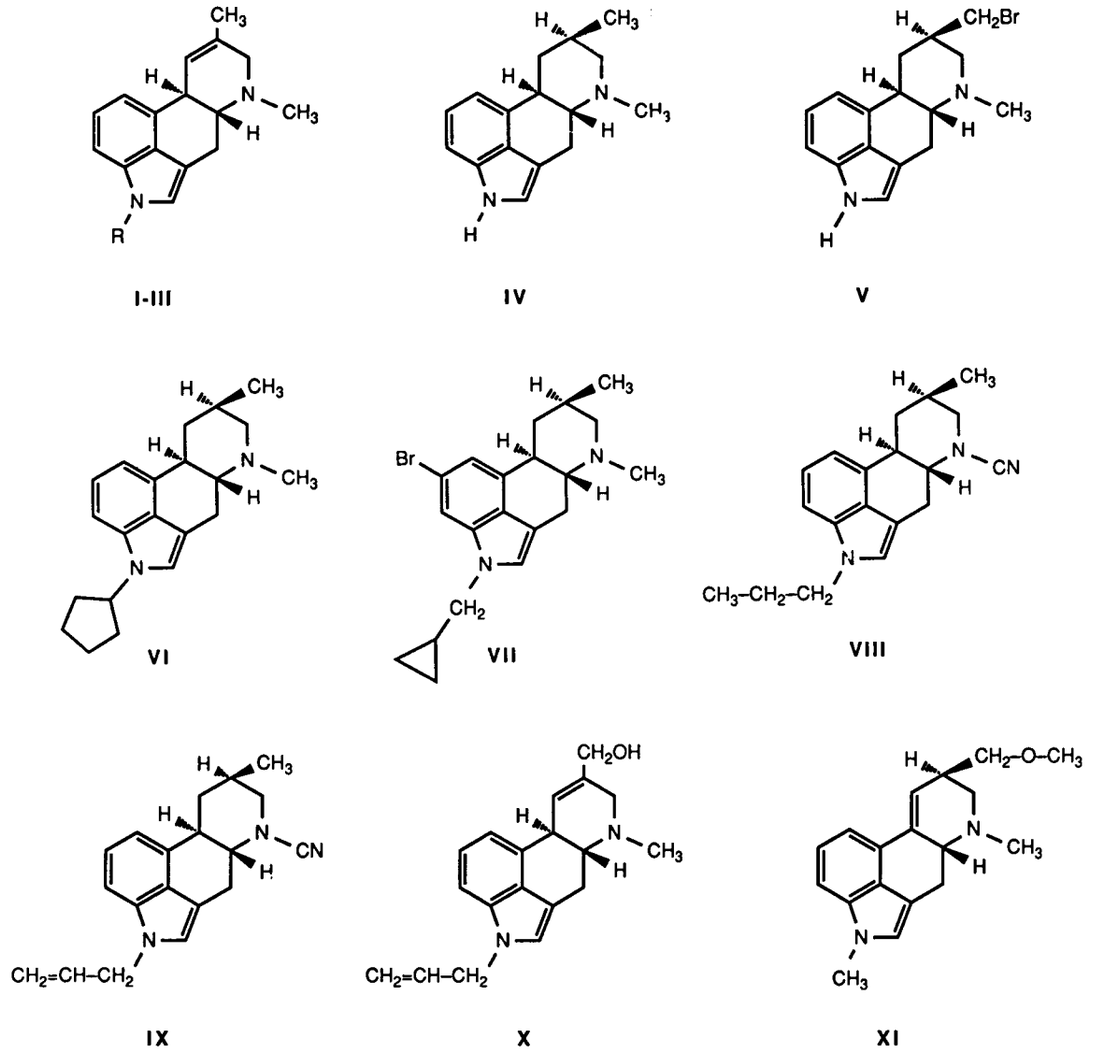

Figure 1. Structural formulas of the investigated clavines. **I**, agroclavine (R = H); **II**, 1-propylagroclavine (R = $(CH_2)_2$–CH_3); **III**, 1-pentylagroclavine (R = $(CH_2)_4$–CH_3); **IV**, festuclavine; **V**, 17-bromofestuclavine; **VI**, 1-cyclopentylfestuclavine; **VII**, 13-bromo-1-cyclopropylmethylfestuclavine; **VIII**, 6-cyano-1-propyl-6-norfestuclavine; **IX**, 1-allyl-6-cyano-6-norfestuclavine; **X**, 1-allylelymoclavine; **XI**, 1-methyllysergol methyl ether.

vine (**VI**) by bromination and *N*-1 alkylation of **IV**.[8] 6-Cyano-6-norfestuclavine was converted to its derivatives **VIII** and **IX** with potassium and the corresponding alkyl iodide in ammonia.[13] 1-Allylelymoclavine (**X**) was prepared by this method from elymoclavine.[14] 1-Methyllysergol methyl ether (**XI**) was obtained by dialkylation of lysergol.[15]

Compounds **VI** and **VII** were applied as hydrogen maleates. The other bases were used in their free form. 1-Propylagroclavine (**II**) had given similar results in previous investigations, when used as free base, hydrogen maleate or hydrogen (2R,3R)-tartrate.

The purity of all compounds was greater than 98%, as determined by chromatographic as well as elementary analyses.

Tissue preparations

Male Sprague-Dawley rats (200–300 g) were treated with Aroclor 1254 in order to induce xenobiotic-metabolizing enzymes.[16,17] Aroclor 1254 was diluted with sunflower oil (1:5, v/v) and i.p. injected at a dose of 500 mg/kg body weight on the sixth day before the rats were killed. The livers were

homogenized in 3 volumes of a sterile, cold solution of 150 mM KCl in 10 mM sodium phosphate buffer, pH 7.4. The homogenate was centrifuged at 10 000 *g* for 10 min. One volume of the resulting supernatant was mixed with 2 volumes of a solution which contained 12 mM $MgCl_2$, 50 mM KCl, 6 mM NADP and 7.5 mM glucose 6-phosphate in 75 mM sodium phosphate buffer, pH 7.4. This preparation is termed S9. Liver homogenate was always prepared on the day of the mutagenicity experiment and used immediately.

Bacteria

The histidine-auxotrophic strains of *S. typhimurium* were kindly provided by Dr BN Ames (Berkley, CA). Strains TA100 and TA102 are derived from prototrophic bacteria by substitution mutations.[18] The auxotrophy of strains TA98 and TA1537 is due to frameshift mutations.[18] The bacteria were grown overnight in Nutrient Broth No. 2 (Oxoid GmbH, Wesel, Germany). Stock cultures that were stored at −70°C were used for inoculation. The overnight cultures were centrifuged, resuspended in medium A (1.6 g/l Bacto Nutrient Broth and 5 g/l NaCl), adjusted nephelometrically to a titer of 1–2 × 10^9 bacteria (colony-forming units)/ml and kept on ice. In each experiment the presence of the R-factor pKM101 was ascertained by growing diluted cultures in ampicillin-containing and ampicillin-free, histidine-supplemented agar plates. With strains TA98, TA100 and TA102, the number of colonies was always virtually identical under the two culture conditions, whereas the plasmid-free strain TA1537 grew only in the absence of ampicillin.

Bacterial mutagenicity assay

Mutagenicity in *S. typhimurium* was determined using the plate-incorporation assay described by Maron and Ames[18] with minor modifications. The test compound (in 10–30 µl dimethylsulfoxide), 500 µl of S9 or buffer (150 mM KCl, 10 mM sodium phosphate buffer, pH 7.4), 100 µl of the bacterial suspension and 2 ml of 45°C warm soft agar (0.55% agar, 0.55% NaCl, 50 µM biotin, 50 µM histidine, 50 µM tryptophan, 25 mM sodium phosphate buffer, pH 7.4) were mixed in a 10 ml glass tube and poured onto a Petri dish containing 24 ml minimal agar (1.5% agar in Vogel–Bonner E medium with 2% glucose). After incubation for 3 days in the dark, colonies (*his*$^+$ revertants) were counted.

Each experiment contained solvent controls and positive controls (benzo[*a*]pyrene 4,5-oxide, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine and *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine in the direct tests, benzo[*a*]pyrene, benzo[*e*]pyrene, 3-methylcholanthrene and 2-aminoanthracene in the S9-mediated tests). The numbers of mutants on these control plates were within the range of previously published figures.[10,19,20]

Specific mutagenicities, as a measure for the mutagenic activity, were calculated as described elsewhere in detail.[21] Briefly, mean numbers of colonies were calculated from all plates belonging to the same treatment group. Treatment groups in which these values were less than twice the value of the solvent control were excluded from further processing. In the remaining groups, the number of colonies of the solvent control plates was subtracted. The resulting value was then divided by the dose. For a perfectly linear curve, the quotients obtained with the different doses are identical and correspond to the slope of the curve. In practice this is seldom exactly true and, as explained elsewhere,[21] we therefore use the highest quotient as a measure of the potency and term it specific mutagenicity. It reflects the number of mutations induced per dose unit at distribution of the material on an optimal number of plates.

Toxicity experiments

To estimate toxicity under the incubation conditions of the mutagenicity experiment, *his*$^+$ bacteria (about 600 colony-forming units) were added as an internal standard to otherwise normal mutagenicity plates. The *his*$^+$ bacteria used were spontaneous revertants from strain TA1537. They were added to plates together with the strain that gives the lowest numbers of revertants, TA1537. The difference in the number of colonies on plates with and without added *his*$^+$ bacteria, in the presence of the compound, was compared with the value obtained with solvent controls. The ratio of these two values gives the surviving fraction.

Results

All clavines showed mutagenic effects in *S. typhimurium* TA100, when tested in the presence of liver S9. Dose–response curves are presented in

Figure 2. In the direct test and in the other bacterial strains, only some compounds were active. The results of the mutagenicity and toxicity experiments are summarized in Table 1.

The results obtained previously with agroclavine (**I**) and 1-alkylated derivatives (**II, III**) are included in the summary table to facilitate comparisons. These compounds showed toxicity, but no mutagenicity, in the direct test. Addition of liver S9 substantially decreased the cytotoxicity and led to the formation of mutagens. Both cytotoxic and mutagenic activities increased with the size of the alkyl substituent.

Festuclavine (**IV**) and 17-bromofestuclavine (**V**) showed direct mutagenic effects. Addition of liver S9 had virtually no influence on the effects in strain TA100, but enhanced those in other strains, suggesting that the parent compounds (or bacterial metabolites) were the major active species in strain TA100, whereas metabolites formed by hepatic enzymes significantly contributed to the effects in other strains.

1-Cyclopentylfestuclavine (**VI**) and 13-bromo-1-cyclopropylmethylfestuclavine (**VII**) differed from festuclavine (**IV**) in that they were strongly cytotoxic, but not mutagenic in the direct test. Addition of S9 substantially mitigated the toxicity, but led to the formation of mutagens. This S9-mediated mutagenicity, in particular that of 1-cyclopentylfestuclavine, was stronger than that of unsubstituted festuclavine. Thus, the structure–activity relationships in the festuclavine series were similar to those in the agroclavine series (**I–III**). The presence of a relatively large alkyl substituent in the 1-position enhanced toxicity (prominent in the direct test) and mutagenicity (found only in the presence of S9, with the exception of festuclavine).

The festuclavine derivatives in which the 6-methyl group was replaced by a cyano group, 6-cyano-1-propyl-6-norfestuclavine (**VIII**) and 1-allyl-6-cyano-6-norfestuclavine (**IX**), showed no toxicity in spite of their 1-alkylation. In contrast to festuclavine, they did not induce mutations in the direct test. In the presence of liver S9, however, they were stronger mutagens than festuclavine in strain TA100. In the other strains they were inactive.

1-Allylelymoclavine (**X**) and 1-methyllysergol methyl ether (**XI**) were only weakly toxic. 1-Allylelymoclavine was more toxic in the direct test than in the presence of liver S9, while the reverse was the case with 1-methyllysergol methyl ether. Thus, the compounds appear to be

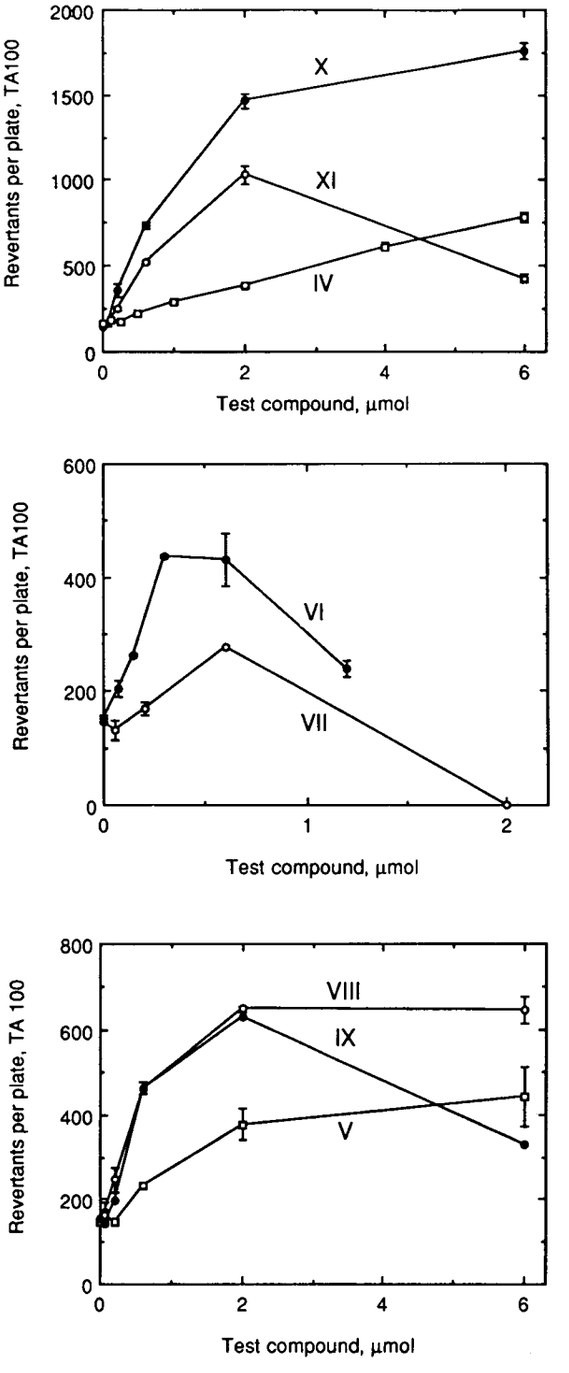

Figure 2. Dose–response curves of the mutagenicity of clavine alkaloids in *S. typhimurium* TA 100 in the presence of rat liver S9: **IV**, festuclavine; **V**, 17-bromofestuclavine; **VI**, 1-cyclopentylfestuclavine; **VII**, 13-bromo-1-cyclopropylmethylfestuclavine; **VIII**, 6-cyano-1-propyl-6-norfestuclavine; **IX**, 1-allyl-6-cyano-6-norfestuclavine; **X**, 1-allylelymoclavine; **XI**, 1-methyllysergol methyl ether. Values are means and SE from two to four plates. Similar results were obtained in a second experiment.

Table 1. Summary of the toxicity and mutagenicity results for clavine alkaloids in *S. typhimurium*

Substance		LD_{50} (μmol/plate)		Specific mutagenicity (revertants/μmol)							
				TA98		TA100		TA102		TA1537	
		direct	+S9	direct	+S9	direct	+S9	direct	+S9	direct	+S9
I	Agroclavine	1.5	>15	<30	60	<60	100	NT	NT	<30	10
II	1-Propylagroclavine	0.5	2	<200	50	<400	300	NT	NT	<200	30
III	1-Pentylagroclavine	0.04	1.5	<500	140	<1000	780	NT	NT	<500	850
IV	Festuclavine	>2	4	5	10	80	100	60	170	<7	2
V	17-Bromofestuclavine	>2	>6	<10	60	90	100	<20	<20	<7	80
VI	1-Cyclopentylfestuclavine	0.2	2	<200	100	<400	950	<1000	550	<200	80
VII	13-Bromo-1-cyclopropyl-methylfestuclavine	0.03	0.8	<1000	<60	<2000	210	<5000	<200	<700	<40
VIII	6-Cyano-1-propyl-6-norfestuclavine	>2	>6	<10	<5	<20	500	<50	<20	<7	<4
IX	1-Allyl-6-cyano-6-norfestuclavine	>2	>6	<10	<5	<20	450	<50	<20	<7	<4
X	1-Allylelymoclavine	6	>6	4	30	10	1050	<40	<20	<6	<4
XI	1-Methyllysergol methyl ether	>6	4	<4	130	20	570	<20	390	<4	<10

The compounds were tested directly and in the presence of S9 (NADPH-fortified postmitochondrial supernatant fraction of liver homogenate from Aroclor 1254-treated rats). The data for compounds **I–III** are from a previous publication.[10] For the other compounds, dose–mutagenicity curves with strain TA100 are presented in Figure 2. In the toxicity experiments, the lowest dose at which a precipitate was macroscopically seen was used as the limit of detection. In the mutagenicity experiment, the detection limit was estimated from the solubility and toxicity of the compound and the number of spontaneous mutants under the corresponding experimental condition. NT, not tested.

metabolized to less and more toxic products, respectively. Both compounds were weak direct mutagens, whose activity was substantially enhanced in the presence of liver enzymes.

Discussion

Clavine alkaloids are potentially useful in the therapy of infections and tumors. However, as demonstrated in the present study, mutagenicity is a common property of this class of chemicals. It is therefore important to understand the relationships between these activities.

The ratio of bacteriotoxic and mutagenic activities varied greatly in the investigated series of clavines, and was strongly influenced by the addition of a hepatic enzyme preparation. In several cases, only one activity, either toxicity or mutagenicity, was observed. Thus these activities are not intrinsically linked.

Particularly strong bacteriotoxic activity was found with 1-alkylated derivatives of agroclavine and festuclavine. While these compounds were not mutagenic themselves, they were converted to mutagens in the presence of liver enzymes. Concurrently, the bacteriotoxic activity was decreased efficiently. Both of these effects are undesirable in the therapy of infections. It will be therefore necessary to find analogs with a more favorable metabolism.

It is unlikely that a genotoxic mechanism is involved in the antineoplastic activity of the clavines, since they are effective against mouse L5178y lymphoma cells in culture[5,6,8,9,22] and since these cells express extremely low levels of cytochromes P450,[23] which are the major active enzymes contained in the liver S9 preparation. This notion is supported by the lack of a correlation between the mutagenic activities observed in the present study and the EC_{50} of compounds **I–XI** for the cytostatic activities in L5178y cells (6.3, 1.3, 0.9, 10.6, 5.6, 3.8, 4.1, 10.6, 10.3, 4.0 and 3.6 μM, respectively).[5,6,8,9,22] Neither was there any obvious correlation between the cytostatic activities in L5178y cells and the bacteriotoxic activities in *S. typhimurium*. It is not clear therefore whether cytostatic and bacteriotoxic activities are due to independent mechanisms or are mediated by orthologous receptors differing in their affinity spectrum for ligands.

The dissociation between cytostatic, bacteriotoxic and mutagenic activities suggests that it may be possible to develop derivatives that are antimicrobial but not mutagenic and cytostatic, or cytostatic but not mutagenic and antimicrobial.

References

1. Floss HG. Biosynthesis of ergot alkaloids and related compounds. *Tetrahedron* 1976; **32**: 873–912.
2. Berde B, Schild HO, eds. *Ergot alkaloids and related compounds. Handbook of experimental pharmacology, Vol. 49.* Berlin: Springer-Verlag, 1978.
3. Eich E, Eichberg D, Schwarz G, *et al.* Antimicrobial activity of clavines. *Arzneim-Forsch/Drug Res* 1985; **35**: 1760–2.
4. Eich E, Eichberg D, Müller WEG. Clavines—new antibiotics with cytostatic activity. *Biochem Pharmacol* 1984; **33**: 523–6.
5. Eich E, Becker C, Mayer K, *et al.* Clavines as antitumor agents, 2: natural 8-hydroxymethyl-ergoline type clavines and their derivatives. *Planta Med* 1986; **52**: 290–4.
6. Eich E, Becker C, Sieben R, *et al.* Clavines as antitumor agents, 3: cytostatic activity and structure/activity relationships of 1-alkyl agroclavines and 6-alkyl 6-noragroclavines. *J Antibiot* 1986; **39**: 804–12.
7. Faatz W, Pertz H, Maidhof A, *et al.* Zytostatische Wirkung neuer partialsynthetischer Agroclavine. *Arch Pharm (Weinheim)* 1990; **323**: 762.
8. Pertz H, Maidhof A, Müller WEG, *et al.* Bromierte 1-Alkylfestuclavine mit Antitumor-Wirkung, *Arch Pharm (Weinheim)* 1987; **320**: 923.
9. Kasper R, Pertz H, Maidhof A, *et al.* Struktur-Aktivitäts-Beziehung zytostatisch wirkender 1-Alkylfestuclavinderivate. *Arch Pharm (Weinheim)* 1989; **322**: 748.
10. Glatt HR, Eich E, Pertz H, *et al.* Mutagenicity experiments on agroclavines, new natural antineoplastic compounds. *Cancer Res* 1987; **47**: 1811–4.
11. Mayer K, Eich E. Raney-Nickel-katalysierte Transferhydrierung: Eine Methode zur Darstellung Ring D-gesättigter Ergot-Alkaloide. *Pharmazie* 1984; **39**: 537–8.
12. Mayer K. *Zur C-17-Oxidation von Clavinalkaloiden mit primärer alkoholischer Hydroxylgruppe.* PhD Thesis, University of Mainz 1975.
13. Eichberg D, Eich E. Partialsynthese neuer Ergolinderivate aus Clavinalkaloiden, 5: *N*-1- und *N*-5-mono- und disubstituierte Festuclavine. *Arch Pharm (Weinheim)* 1985; **318**: 621–4.
14. Sieben R, Philippi U, Eich E. Microbiological *C*-17 oxidation of clavine alkaloids, 1: substrate specificity of agroclavine hydroxylase of *Claviceps fusiformis*. *J Nat Prod* 1984; **47**: 433–8.
15. Eich E. Partialsynthese neuer Ergolinderivate aus Clavinalkaloiden, 3: *O*-Acyl- und *O*-Alkyl-1,6-dimethyl-8-hydroxymethylergol-9-ene. *Arch Pharm (Weinheim)* 1983; **316**: 781–8.
16. Alvares AP, Bickers DR, Kappas A. Polychlorinated biphenyls: a new type of inducer of cytochrome P-448 in the liver. *Proc Natl Acad Sci USA* 1973; **70**: 1321–5.
17. Parkinson A, Thomas PE, Ryan DE, *et al.* Immunochemical quantitation of cytochrome P-450 isozymes and epoxide hydrolase in liver microsomes from polychlorinated and polybrominated biphenyls: a study of structure activity relationships. *J Biol Chem* 1983; **258**: 5967–76.
18. Maron DM, Ames BN. Revised methods for the *Salmonella* mutagenicity test. *Mutat Res* 1983; **113**: 173–215.
19. Glatt HR, Metzler M, Oesch F. Diethylstilbestrol and 11 derivatives: a mutagenicity study with *Salmonella typhimurium*. *Mutat Res* 1979; **69**: 113–121.
20. Glatt HR, Witz G. Benzene metabolites, *trans,trans*-muconaldehyde and *trans,trans*-muconic acid tested on induction of gene mutations in bacterial and mammalian cells. *Mutagenesis* 1990; **5**: 263–6.
21. Glatt HR. Mutagenicity spectra in *Salmonella typhimurium* strains of glutathione, L-cysteine and active oxygen species. *Mutagenesis* 1989; **4**: 221–7.
22. Becker C. *Zur zytostatischen Wirkung natürlicher ond partialsynthetischer Ergoline.* PhD Thesis, University of Mainz 1985.
23. McGregor DB, Edwards C, Wolf CR, *et al.* Endogenous xenobiotic enzyme levels in mammalian cells. *Mutat Res* 1991; **261**: 29–39.

(Received 1 October 1992; accepted 12 October 1992)