Studies on the Biosynthesis of Striatal-Type Diterpenoids and the Biological Activity of Herical

Timm Anke[a,*], Udo Rabe[a], Peter Schu[a], Thomas Eizenhöfer[b], Monika Schrage[b], and Wolfgang Steglich[b,*]

[a] LB Biotechnologie der Universität, Paul-Ehrlich-Str. 23, D-67663 Kaiserslautern, Germany. Fax: +49-631-205-2999. E-mail: anke@rhrk.uni-kl.de

[b] Department Chemie der Universität München, Butenandtstr. 5–13, D-81377 München, Germany. Fax: +49-89-2180-7756. E-mail: wos@cup.uni-muenchen.de

* Authors for correspondence and reprint requests

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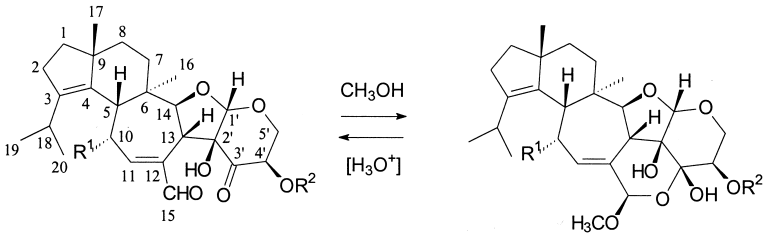
Feeding experiments with specifically ^{13}C-labeled glucose disclosed that the diterpenoid part of the striatals/striatins is formed via the mevalonate pathway, whereas the pentose moiety originates either *via* glucuronic acid (70%) or the pentose phosphate cycle (30%). Application of radioactively labeled herical to cultures of *Hericium ramosum* demonstrates the pivotal role of this cyathane-xyloside in striatal biosynthesis. Herical inhibits a large spectrum of fungi and bacteria and shows cytotoxic and hemolytic properties.

Introduction

The striatals (**1**) and striatins (**2**) are an unusual group of diterpenoids isolated from cultures of the bird's nest fungus *Cyathus striatus* (Basidiomycetes) (Hecht *et al*., 1978; Steglich, 1981) and tropical *Cyathus* species (Anke and Steglich, 1988). The least functionalized member of this group, striatal D (**1d**), is produced by cultures of the agaric *Gerronema fibula* (Anke *et al*., unpublished). The striatins are artifacts, formed by extraction of the mycelia with methanol. On mild treatment with acids, they are re-converted to the corresponding striatals. Both types of compounds possess antibacterial, antifungal (Anke *et al*., 1977; Anke and Steglich, 1988) and leishmanicidal (Inchausti *et al*., 1997) activity and exhibit cytotoxic properties (Anke and Steglich, 1988). In Ehrlich ascites tumor cells the incorporation of thymidine, uridine and leucine in DNA, RNA, and proteins is completely inhibited at concentrations of 2 μg/ml. Positive results were obtained in the treatment of mice with P388 lymphocytic leukemia (Anke and Steglich, 1988).

The striatals (**1**) possess a cyathane skeleton (Ayer *et. al*., 1978; Ayer and Browne, 1981) attached to a modified pentose moiety and are biosynthetically related to several cyathane-xyloside diterpenoids from cultures of *Hericium* species. The first cyathane-xyloside reported was herical (**3**), isolated in 1985 from *H. ramosum* (Bull. ex Mérat) Let. [=*H. clathroides* (Pallas ex Fr.) Pers.]



Striatal A (**1a**)	R^1 = H, R^2 = $COCH_3$	Striatin A (**2a**)
Striatal B (**1b**)	R^1 = OH, R^2 = $COCH_3$	Striatin B (**2b**)
Striatal C (**1c**)	R^1 = OH, R^2 = H	Striatin C (**2c**)
Striatal D (**1d**)	R^1 = R^2 = H	

Fig. 1. Striatals (**1**) and striatins (**2**).

and *H. abietis* (Weir) Harrison (Schu, 1985; Schrage, 1988; Steglich *et al.*, 1993). Subsequently, Japanese scientists (Kawagishi *et al.*, 1994; Kawagishi *et al.*, 1996a; Kawagishi *et al.*, 1996b) isolated several closely related erinacines from the mycelia of *H. erinaceum* (Bull. ex Fr.) Pers., including herical, which was renamed as erinacine P (Kenmoku *et al.*, 2000). Several of these compounds are strong stimulators of nerve growth factor synthesis, and erinacine E (**4**) exhibits activity as a κ opioid receptor agonist (Saito *et al.*, 1998).

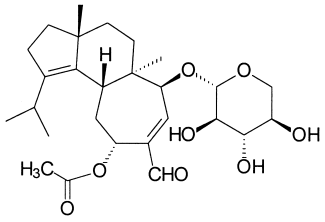
In this publication we report on studies of the biosynthesis of striatal A (**1a**) and describe the production and antibiotic activity of herical (**3**).

Results and Discussion

Biosynthetic studies

The biosynthesis of striatal A (**1a**) was studied by adding [1-^{13}C]- and [2-^{13}C]-D-glucose respectively, to resting cells of *C. striatus*. The optimal glucose concentration in the incubation solution (0.5%) was determined in preliminary experiments. Under the conditions described in the experimental section approximately 7 mg of striatin A were produced within 16 h. In the feeding experiments with ^{13}C-labeled glucoses the incubation was stopped when the glucose was completely used up (2 days). From the mycelia fed with [1-^{13}C]- or [2-^{13}C]-D-glucose 18–24 mg of striatin A (**2a**) and 20–22 mg of striatin B (**2b**) were obtained after extraction and preparative TLC. The striatins were converted to the corresponding striatals by treatment in THF with a few drops of 1 N HCl (Hecht *et al.*, 1978).

From an integration of the normalized ^{13}C NMR signals the ^{13}C-enrichments given in Fig. 3 and Table I were determined for **1a**. In the experiment with [1-^{13}C]-D-glucose, 12 carbon atoms of the diterpene skeleton show a ^{13}C-enrichment of about 6% (Fig. 3A). As expected, the labeling pattern of the diterpene part after feeding of [2-^{13}C]-glucose is complementary (Fig. 3B). These results can be explained by a degradation of glucose



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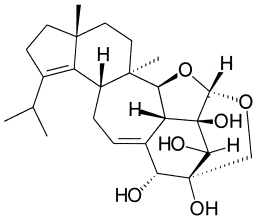

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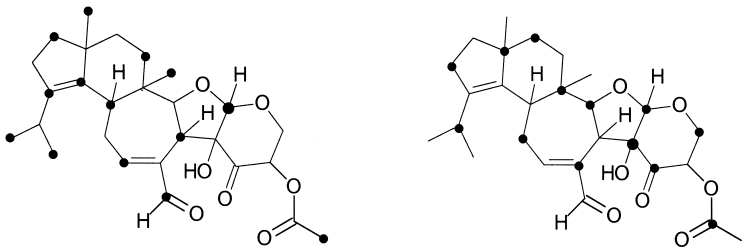
Fig. 2. Herical (**3**) and erinacine E (**4**).

Table I. ^{13}C-enrichments in striatal A (**1a**) after feeding of [1-^{13}C]- (A) and [2-^{13}C]-D-glucose (B) (99%), respectively.

C-atom	δ (ppm)	^{13}C-enrichment[a] (%)		C-atom	δ (ppm)	^{13}C-enrichment (%)	
		A	B			A	B
C-1	38.2	6.5	–	C-15	195.9	4.9	–
C-2	28.3	–	2.1	C-16	17.4	6.8	–
C-3	139.9	6.9	–	C-17	24.5	6.9	–
C-4	136.0	4.6	–	C-18	27.0	–	1.9
C-5	42.7	6.0	–	C-19	21.4[b]	7.4	–
C-6	41.9	–	1.5	C-20	21.8[b]	7.3	–
C-7	26.8	7.1	–	C-1′	108.2	27.7	2.4
C-8	36.3	–	1.7	C-2′	84.0	–	8.2
C-9	49.7	–	1.3	C-3′	200.9	–	1.2
C-10	29.2	–	1.9	C-4′	75.1	–	–
C-11	158.2	6.3	–	C-5′	65.0	–	1.0
C-12	142.2	–	2.0	H_3CCOO	170.5	–	2.6
C-13	46.4	5.0	–	H_3CCOO	20.4	10.4	–
C-14	87.3	–	1.6				

[a] ^{13}C-enrichment (%) = integral of labeled C signal/integral natural abundance C signal × 1.1 – 1.1.
[b] Assignments may be exchanged.



A B

Fig. 3. ^{13}C-enrichments of striatal A (**1a**) after feeding of [1-^{13}C]- (A) and [2-^{13}C]-D-glucose (B), respectively.

to [2-^{13}C]- and [1-^{13}C]acetate, respectively, which then form the diterpene system *via* [2,4,6-$^{13}C_3$]- or [1,3,5-$^{13}C_3$]mevalonate. In accordance with this proposal is the high specific incorporation of ^{13}C in C-2 and C-1 of the respective acetate residues. The distribution of the labeled carbons in the cyathane skeleton follows from the mechanism postulated by Ayer for the biosynthesis of these diterpenoids (Ayer *et al.*, 1978; Ayer *et al.*, 1979) (see Fig. 4, below).

After feeding of [1-^{13}C]-D-glucose, only C-1′ of the pentose part in **1a** shows ^{13}C-enrichment. The high incorporation of 27.7% can be explained by the usual oxidation of UDP-[1-^{13}C]-D-glucose to UDP-[1-^{13}C]-D-glucuronic acid, which is then decarboxylated to UDP-[1-^{13}C]-D-xylose. In agreement with this proposal, C-2′ of the pentose unit exhibits the highest ^{13}C-incorporation (8.2%) after application of [2-^{13}C]-D-glucose. In this experiment C-1′ (2.4%) and to a lesser degree C-

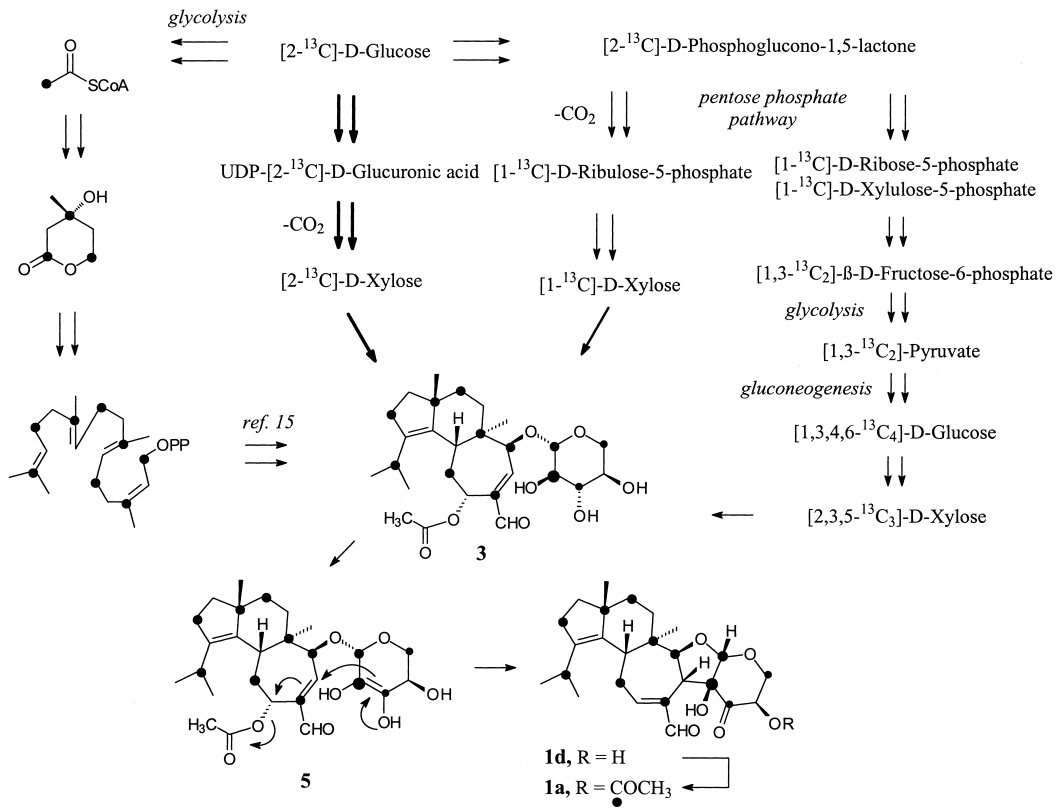


Fig. 4. Labeling of striatal A (**1a**) after feeding [2-^{13}C]glucose to cultures of *Striatus cyathus.*

3′ and C-5′ (~1%) are also labeled, which can be explained by conversion of glucose into D-xylose *via* the pentose phosphate pathway (Fig. 4, below). [2-^{13}C]-D-glucose is thereby transformed into [2-^{13}C]-D-phosphoglucono-1,5-lactone that can either yield [1-^{13}C]-D-xylose *via* [1-^{13}C]-D-ribulose-5-phosphate or [2,3,5-$^{13}C_3$]-D-xylose *via* the nonoxidative part of the pathway. In the latter case [1,3-$^{13}C_2$]-β-D-fructose-6-phosphate is formed, which is then degraded to [1,3-$^{13}C_2$]pyruvate. Gluconeogenesis and oxidative degradation of the glucose formed *via* glucuronic acid yields [2,3,5-$^{13}C_3$]-D-xylose (Fig. 4). The signal enhancements given in Table I suggest that ~70% of the D-xylose are formed *via* glucuronic acid and ~30% *via* the pentose phosphate cycle.

The feeding experiments with specifically ^{13}C-labeled glucoses, point to a pivotal role of herical (**3**) in striatal biosynthesis. In enediol **5**, obtained from **3** by oxidation at C-2′ or C-3′, the functional groups are well placed to form the C-C-bond between the diterpene and sugar part through S_N2'-displacement of acetate by the enediol moiety. The resulting striatal D (**1d**) is then acetylated to striatal A (**1a**). To prove this idea experimentally, ^{14}C-labeled herical was produced by the "resting cell" technique by feeding [2-^{14}C]glucose to cultures of *H. ramosum*. The ^{14}C-labeled **3** was purified by chromatography (8 mg, 337 Bq) and then applied to resting cells of *C. striatus* in a 0.5% solution of glucose. The fraction of striatins A (**2a**) and B (**2b**) (20 mg, 78 Bq), isolated from the mycelium after 43 h by extraction with MeOH and TLC exhibited 23.5% of the original radioactivity. In the TLC-analyzer radioactivity could only be detected in the striatin-containing bands.

Since herical (**3**) contains D-xylose (Schrage, 1988) and the relative configuration of striatin A (**2a**) is known from an X-ray crystal structure analysis (Hecht *et al.*, 1978), the conversion of **3** in **2a** defines the absolute configuration of the striatins and striatals as given in the formulas.

Production and biological activity of herical

Herical (**3**) was produced by fermentation of *Hericium ramosum* as described in the Experimental Section. Antibiotic production started three days after inoculation and paralleled mycelial growth until the glucose in the medium was used up (10th day). Herical was isolated from the culture fluid and the mycelia as described in the Experimental Section. Compared to the striatins/striatals the yields (1 mg/l) are much lower (*Anke et al.*, 1977). Besides herical, several other minor compounds with antibiotic activity could be detected in the crude extracts.

When grown in 150 ml batches in Erlenmeyer flasks on a rotary shaker with 150 rpm at 22 °C in BAF1 medium *H. abietinis* was also found to produce herical in amounts comparable to *H. ramosum*.

The antifungal activity of herical (**3**) in the plate diffusion assay is shown in Table II. At 100 μg/disc all tested fungi were inhibited by the antibiotic, *Mucorales* appearing to be most sensitive. The antifungal activities of **3** are comparable to those of the striatals (**1**) and striatins (**2**) (Anke *et al.*, 1977, 1986).

Table II. Antifungal spectrum of herical (**3**) in the plate diffusions assay.

Test organism	Diameter inhibition zone [mm] 100 μg/disc
Absidia glauca (+)	12
Absidia glauca (−)	20
Alternaria porri	28
Aspergillus ochraceus	15
Epicoccum purpurascens	10
Fusarium fujikoroi	12
Mucor miehei	31
Nematospora coryli	10
Neurospora crassa	17
Paecilomyces varioti	13
Penicillium notatum	15
Pythium debaryanum	13
Saccharomyces cerevisiae	12
Ustilago nuda	13
Verticillium spec.	30i[a]

[a] Incomplete.

Among the bacteria tested in a serial dilution test Gram-positive bacteria were most sensitive (Table III). The spectrum of activity is very similar to that of the striatins while the minimal inhibitory concentrations of **3** are generally somewhat higher.

The cytotoxic activity of herical is shown in Table IV. Pronounced effects on the different cells could be observed after 24 h of incubation. Balb/3T3 cells showed the highest sensitivity against herical, whereas cells of the ascitic form of Ehrlich

Table III. Antibacterial spectrum of herical (**3**) in the serial dilution assay.

Test organism	Minimal inhibition concentration MIC [μg/ml]	[nmol/ml]
Gram-negative		
Escherichia coli	>100	>200
Gram-positive		
Arthrobacter citreus	10–20	20–40
Bacillus brevis	2–5	4–10
Bacillus subtilis	5–10	10–20
Corynebacterium insidiosum	10–20	20–40
Sarcina lutea	10–20	20–40
Streptomyces spec.	20–50	40–100

Table IV. Cytotoxic activity of herical (**3**).

Cell line	Concentration inducing lysis of 90% of the cells [μg/ml]	[nmol/ml]
Ehrlich ascites tumor cells (mouse)	20–50	40–100
BALB/3T3 (mouse embryonic)	0.5–1	1–2
L-1210 (lymphocytic leukemia, mouse)	5–10	10–20
HeLa-S3 (epitheloid carcinoma, cervix, human)	2–5	4–10
KB cells (epidermoid carcinoma, oral, human)	1–2	2–4

carcinoma were not or only weakly affected up to concentrations of 20 μg/ml.

In ECA cells herical inhibits DNA, RNA, and protein syntheses by interfering strongly with the uptake of the appropriate radioactive precursors (Table V) leading to a reduced incorporation into the macromolecules. Very similar effects are exhibited by the striatals and striatins which interfere with the uptake of other precursors as well (Lee and Anke 1979).

Herical (**3**) exhibits hemolytic properties on porcine erythrocytes when tested according to Kupka *et al.* (1979). At 50 μg/ml more than 50% of the porcine erythrocytes were hemolysed. These findings together with the inhibition of the uptake of the precursors of DNA, RNA and proteins, suggest an interference with the cytoplasmatic membrane of the cells.

Experimental

General

NMR: Bruker WM-400 spectrometer (^{1}H at 400, ^{13}C at 100.6 MHz), chemical shifts in δ rel. to $CDCl_3$ (δ_H 7.26, δ_C 77.7) as internal standard. MS: AEI MS 50 instrument using EI at 70 eV.

Hericium ramosum, H. abietis, and Cyathus striatus

Hericium ramosum strain 8377 was derived from the spore print of a fruit body, collected in the Great Smoky Mountains (USA). *H. abietis* CBS 243.48 was purchased from the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. Both fungi were maintained in a yeast extract – malt extract – glucose (YMG) medium containing (g/l): yeast extract 4; malt extract 10; glucose 4; 20 g/l of agar. *Cyathus striatus* was cultivated from fruit bodies collected near Bad Königshofen, Bavaria.

Fermentation and isolation of herical (**3**)

H. ramosum 8327 was grown in a medium (BAF1) composed of (g/l): Maltose 20, glucose 10, peptone 2, yeast extract 0.2, KH_2PO_4 0.5, $MgSO_4 \times 7\ H_2O$ 1, $FeCl_3$ 0.01, $ZnSO_4$ 0.001, $CaCl_2$ 0.068 and (μg/ml): thiaminium dichloride 50, biotin 1, folic acid 100, *myo*-inositol 50. For fermentation 20 l of medium in a Biolafitte C-6 fermenter were

Table V. Effect of herical (**3**) on uptake (Upt.: total radioactivity consisting of the acid-soluble and acid-insoluble fraction of cells) and incorporation (Inc.: TCA-precipitable material) of ^{14}C-leucine, ^{14}C-uridine, and ^{14}C-thymidine in ECA cells.

Herical (**3**)	Leucine [pmol]		Uridine [pmol]		Thymidine [pmol]	
$\mu g/10^6$ cells	Upt.	Inc.	Upt.	Inc.	Upt.	Inc.
0	160.6	112.1	129.0	30.1	23.9	18.2
2	113.1	80.9	125.2	31.4	17.6	13.4
5	37.2	27.5	72.1	18.3	14.3	11.8
10	29.8	21.5	44.8	12.9	12.3	10.2
20	15.1	11.0	31.5	9.9	10.7	8.7

inoculated with 200 ml of a well grown culture and grown with stirring (200 rpm) and aeration (2 l/min) at 23 °C. Antibiotic production was followed using the paper disc/agar diffusion assay with *Bacillus brevis* as test organism. After 10 days, the mycelia (24 g wet weight) were separated from the culture fluid by filtration and extracted with 600 ml of MeOH. Chromatography of the crude extract (795 mg) on silica gel (Merck 60; column 20 × 2.1 cm, CH_2Cl_2-MeOH, 98:2 v/v) yielded 66 mg, which were purified by chromatography on Sephadex LH-20 (column 200 × 1.5 cm, eluent MeOH) to afford 26.5 mg of impure **3**. Preparative HPLC (Lichrosorb RP-8, 25 × 2.5 cm; MeOH-H_2O, 8:2 v/v) yielded 11 mg of herical (**3**). The culture fluid (19 l) was extracted with EtOAc (4 × 2 l). After evaporation of the solvent, the crude extract (1.8 g) was purified as described above to yield 9 mg of **3**.

Feeding experiments

Cyathus striatus 7770 was grown in YMG medium composed of (g/l): Glucose 4, malt extract 10, yeast extract 4, pH 5.5. Fermentations were carried out in Biolafitte C-6 fermenters containing 20 l of YMG medium with stirring (230 rpm) and aeration (3.3 l/min) at 25 °C. 200 ml of a well grown culture in the same medium served as inoculum. For the feeding experiments using ^{14}C- or ^{13}C-labeled precursors, 150 ml of the culture was removed from the fermentation just at the onset of striatal production (detection by TLC or HPLC). The mycelia were collected on a Büchner funnel, washed with water and re-suspended in 100 ml of a solution containing 500 mg [1-^{13}C]-D-glucose, [2-^{13}C]-D-glucose (each 99% ^{13}C) or 8 mg ^{14}C-herical (337 Bq) together with 500 mg unlabeled glucose. These resting cell cultures were incubated in 500 ml Erlenmeyer flasks at 24 °C and 110 rpm on a New Brunswick rotary shaker. After 16–48 h, the mycelia were separated from the culture fluid by filtration, washed with water and extracted with MeOH (2 × 100 ml). The combined extracts were concentrated in a rotary evaporator and the striatins separated by preparative TLC [Alugram silica gel TLC plates; toluene-acetone-acetic acid, 70:30:1 v/v]. The bands containing the striatins were extracted with MeOH and treated with 1N HCl in THF to yield the corresponding striatals for NMR analysis. The ^{14}C-labeled striatins derived from [^{14}C]herical were located by a TLC-analyzer (Berthold), and the radioactivity incorporated into the striatins A and B was determined in a liquid scintillation counter.

^{14}C-labeled herical was obtained by incubation of the mycelia of 150 ml batches taken from fermentation of *H. ramosum* at the onset of antibiotic production. After re-suspension and incubation of the mycelia in 100 ml of a solution of 300 mg [2-^{14}C]-glucose (248 Bq/mg) for 46.5 h as described above, the labeled herical was isolated from the culture fluid and mycelia by extraction with EtOAc and MeOH, respectively. The extracts were purified by preparative TLC (Alugram Sil G/UV_{254}, Macherey & Nagel; toluene-acetone-acetic acid, 70:30:1 v/v). Column chromatography on Sephadex LH-20 (MeOH) yielded 8 mg of herical (**3**) (42 Bq/mg) from two batches.

Spectroscopic data

Striatal A (**1a**): ^1H NMR ($CDCl_3$): δ (ppm): 1.10 (br., 12H, 16-H, 17-H, 19-H, 20-H), 1.58 (br. m, 6H, 1-H, 7-H, 8-H), 2.27 (m, 2H, 2-H), 2.33 (s, 3H, CH_3CO_2), 2.40 (d, J = 11 Hz, 1H, 5-H), 2.75 (m, 2H, 10_a-H, 18-H), 2.84(m, 1H, 10_b-H), 3.43 (dm, J = 11 Hz, 1H, 13-H), 4.08 (dd, J = 12, 4.5 Hz, 1H, 5_b'-H), 4.22 (d, J = 11 Hz, 14-H), 4.41 (dd, J = 12, 4.5 Hz, 1H, 5_a'-H), 4.92 (t, J = 4.5 Hz, 1H, 4′-H), 5.38 (s, 1H, 1′-H), 5.86 (s, 1H, 2′-OH), 7.04 (dt, J = 9, 3 Hz, 1H, 11-H), 9.34 (s, 1H, H-15). – ^{13}C NMR ($CDCl_3$): δ (ppm): 17.4 (C-16), 20.4 (CH_3CO_2), 21.4 (C-19 or C-20), 21.8 (C-19 or C-20), 24.5 (C-17), 26.8 (C-7), 27.0 (C-18), 28.3 (C-2), 29.2 (C-10), 36.3 (C-8), 38.2 (C-1), 41.9 (C-6), 42.7 (C-5), 46.4 (C-13), 49.7 (C-9), 65.0 (C-5′), 75.1 (C-4′), 84.0 (C-2′), 87.3 (C-14), 108.2 (C-1′), 136.0 (C-4), 139.9 (C-3), 142.2 (C-12), 158.2 (C-11), 170.5 (CH_3CO_2), 195.9 (C-15), 200.9 (C-3′). – EI-MS: *m/z* (rel. int.): 472 (24) [M^+], 457 (21), 444 (52), 430 (25), 429 (100), 366 (21), 342 (14), 341 (17), 323 (18), 284 (12), 283 (27), 204 (20), 203 (84), 199 (15), 175 (22), 147 (25), 145 (23), 135 (21), 133 (26), 131 (24), 123 (23), 121 (31), 119 (47), 109 (25), 107 (31), 105 (58), 95 (44), 93 (37), 91 (49), 81 (45), 79 (32), 69 (51), 67 (44), 57 (24), 55 (72), 43 (90), 41 (69). – HR EI-MS: *m/z* 472.2432, $C_{27}H_{36}O_7$ requires 472.2461.

Striatin A (**2a**): ^{13}C NMR ($CDCl_3$): δ (ppm): 17.4 (C-16), 20.8 (CH_3CO_2), 21.6 (C-19 or C-20),

21.9 (C-19 or C-20), 24.7 (C-17), 26.3 (C-18), 27.2 (C-7), 28.3 (C-2), 29.4 (C-10), 36.6 (C-8), 38.5 (C-1), 40.0 (C-6), 43.6 (C-5), 46.0 (C-13), 49.7 (C-9), 60.4 (C-5′), 71.2 (C-4′), 80.3 (C-2′), 90.9 (C-14), 95.2 (C-3′), 98.9 (C-15), 105.7 (C-1′), 131.2 (C-12), 132.4 (C-11), 137.0 (C-4), 139.3 (C-3), 169.4 (CH_3CO_2).

Striatal B (**1b**): ^{1}H NMR ($CDCl_3$): δ (ppm): 0.95 (s, 3H, 17-H), 0.99 (d, *J* = 6.5 Hz, 3H, 20-H), 1.01 (d, *J* = 6.5 Hz, 3H, 20-H), 1.04 (s, 3H, 16-H), 1.62 (br. m, 6H, 1-H, 7-H, 8-H), 2.15 (m, 1H, 5-H), 2.16 (s, 3H, CH_3CO_2), 2.29 (m, 2H, 2-H), 3.02 (sept, *J* = 6.5 Hz, 1H, 18-H), 3.33 (dd, *J* = 10.5, 2.8 Hz, 1H, 13-H), 4.04 (dd, *J* = 12, 4.5 Hz, 1H, 5_b'-H), 4.43 (dd, *J* = 12, 4.5 Hz, 1H, 5_a'-H), 4.68 (d, *J* = 10.5 Hz, 14-H), 4.90 (t, *J* = 4.5 Hz, 1H, 4′-H), 4.93(d, *J* = 7.5, 1H, 10-H), 5.39 (s, 1H, 1′-H), 5.77 (s, 1H, 2′-OH), 6.91 (dd, *J* = 7.5, 2.8 Hz, 1H, 11-H), 9.31 (s, 1H, H-15). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ (ppm): 20.2 (CH_3CO_2), 20.6 (C-16), 21.5 (C-19 or C-20), 21.8 (C-19 or C-20), 23.8 (C-17), 25.1 (C-18), 28.1 (C-2), 28.3 (C-7), 36.7 (C-8), 39.1 (C-1), 41.1 (C-6), 45.5 (C-5), 48.6 (C-13), 48.7 (C-9), 64.0 (C-5′), 67.9 (C-10), 73.9 (C-4′), 86.6 (C-2′), 87.2 (C-14), 108.6 (C-1′), 135.8 (C-4), 138.7 (C-3), 141.4 (C-12), 154.8 (C-11), 169.2 (CH_3CO_2), 195.0 (C-15), 199.9 (C-3′). – EI-MS: *m/z* (rel. int.): 488 (18.5) [M^+], 473 (22), 445 (27), 204 (18), 203 (100), 202 (12), 190 (13), 175 (12), 147 (13), 121 (18), 119 (31), 105 (36), 95 (16), 93 (17), 91 (21), 81 (15), 79 (12), 77 (9), 69 (25), 55 (26), 43 (66), 41 (26). – HR EI-MS: *m/z* 488.2413, $C_{27}H_{36}O_8$ requires 488.2410.

Striatal C (**1c**): ^{13}C NMR (50.3 MHz, $CDCl_3$): δ (ppm): 21.1 (C-16), 21.7 (C-19 or C-20), 21.9 (C-19 or C-20), 24.0 (C-17), 26.3 (C-18), 28.8 (C-2), 28.9 (C-7), 36.7 (C-8), 39.3 (C-1), 42.1 (C-6), 45.9 (C-5), 46.4 (C-13), 48.6 (C-10), 49.2 (C-9), 68.5 (C-5′), 74.2 (C-4′), 83.9 (C-2′), 86.8 (C-14), 108.1 (C-1′), 135.0 (C-4), 140.2 (C-3), 142.9 (C-12), 155.6 (C-11), 197.3 (C-15), 204.5 (C-3′). – EI-MS: *m/z* (rel. int.): 447 (16) [M^++1], 446 (59) [M^+], 432 (20), 431 (83), 430 (20), 428 (20), 415 (38), 413 (25), 403 (12), 387 (13), 358 (10), 219 (26), 204 (18), 203 (100), 201 (29), 189 (19), 175 (19), 161 (18), 159 (17), 147 (23), 145 (18), 135 (30), 133 (20), 131 (15), 123 (21), 121 (31), 119 (39), 109 (16), 107 (28), 105 (51), 95 (27), 93 (29), 91 (35), 81 (31), 79 (34), 77 (19), 69 (36), 67 (18), 55 (43), 43 (37), 41 (50). – HR EI-MS: *m/z* 446.2335, $C_{25}H_{34}O_7$ requires 446.2305.

Herical ("erinacin P") (**3**): Colorless oil, $[\alpha]_D^{20}$ −35.9 (c 2.68, $CHCl_3$). CD (MeOH): θ_{234} = $+19.19 \times 10^3$, $\theta_{256} = 0$, $\theta_{267} = -1.68 \times 10^3$, $\theta_{299} = 0$, $\theta_{338} = +1.01 \times 10^3$. UV (MeOH): λ_{max} (nm): 230, 277, 320. IR (KBr): $\tilde{\nu}$ (cm^{-1}): 1735, 1680. ^{1}H NMR ($CDCl_3$): δ (ppm): 0.97 (s, 3H, 17-H), 1.00 (s, 3H, 16-H), 1.00 (d, *J* = 6.5 Hz, 6H, 19-H, 20-H), 1.42–1.70 (br. m, 6H, 1-H, 7-H, 8-H), 1.86 (dd, *J* = 14, 8.5 Hz, 10_a-H), 2.06 (s, 3H, CH_3CO), 2.16 (m, 2H, 5-H), 2.29 (t, *J* = 7.5 Hz, 2H, 2-H), 2.60 (dd, *J* = 14, 8.5 Hz, 10_b-H), 2.78 (sept, *J* = 6.5 Hz, 1H, 18-H), 3.33 (dd, *J* = 12, 9 Hz, 1H, 5′-H_a), 3.51 (dd, *J* = 8, 6.5 Hz, 1H, 2′-H), 3.59 (t, *J* = 8 Hz, 1H, 3′-H), 3.77 (ddd, *J* = 9, 8, 5 Hz, 1H, 4′-H), 4.04 (dd, *J* = 12, 5 Hz, 1H, 5′-H_b), 4.44 (d, *J* = 5.5 Hz, 1H, 14-H), 4.42, d, *J* = 6.5 Hz, 1H, 1′-H). 5.90 (t, *J* = 8.5 Hz, 1H, 11-H), 6.92 (d, *J* = 5.5 Hz, 1H, 13-H), 9.45 (s, 1H, 15-H). ^{13}C NMR ($CDCl_3$): δ (ppm): 16.7 (Qm, *J* = 127 Hz, C-16), 21.1 (Q, *J* = 130 Hz, CH_3CO), 21.47, 21.52 (each Qquin, *J* = 125, 5 Hz, C-19, −20), 24.5 (Q, *J* = 124 Hz, C-17), 27.0 (D, *J* = 126 Hz, C-18), 28.5 (Tm, *J* = 127 Hz, C-2), 29.6 (Tm, *J* = 124 Hz, C-10), 31.0 (Tm, *J* = 128 Hz, C-7), 36.9 (Tm, *J* = 130 Hz, C-8), 38.3 (Tm, *J* = 132 Hz, C-1), 39.9 (Dm, *J* = 121 Hz, C-5), 44.0 (m, C-6), 49.2 (m, C-9), 65.3, (DD, *J* = 149, 140, C-5′), 68.2 (Dm, *J* = 146 Hz, C-11), 69.6 (D, *J* = 142 Hz, C-4′), 73.4 (Dd, *J* = 146, 5 Hz, C-2′), 75.8 (Dm, *J* = 140 Hz, C-3′), 84.8 (Dm, *J* = 142 Hz, C-14), 105.3 (Dm, *J* = 160 Hz, C-1′), 136.3 (m, C-4), 138.3 (dm, *J* = 20, C-12), 140.2 (m, C-3), 156.2 (Dm, *J* = 161 Hz, C-13), 170.4 (m, CH_3CO), 191.8 (Dd, *J* = 176, 9 Hz, C-15). Difference-NOE's: δ 2.16 ↔ 5.90; 1.86 ↔ 4.44; 1.86 ↔ 1.00; 2.60 ↔ 5.90; 4.44 ↔ 1.00. – HR EI-MS: *m/z* (rel. int.): 432.2504 (53) [M^+ – AcOH, $C_{25}H_{36}O_6$ requires 432.2512], 417 (100, $C_{24}H_{33}O_6$), 389 (15, $C_{22}H_{29}O_6$), 300 (34, $C_{20}H_{28}O_2$), 298 (13, $C_{20}H_{26}O_2$), 287 (21, $C_{19}H_{27}O_2$), 285 (35, $C_{19}H_{25}O_2$), 282 (31, $C_{20}H_{26}O$), 271 (5, $C_{19}H_{27}O$), 269 (15, $C_{19}H_{25}O$), 267 (44, $C_{19}H_{23}O$), 257 (44, $C_{17}H_{21}O_2$), 239 (57), 228 (26), 175 (31), 169 (24), 145 (26), 131 (26), 121 (33), 119 (33), 103 (45), 91 (43), 81 (27), 73 (55), 69 (33), 55 (41), 43 (23), 43 (31), 41 (41). – FAB-MS: *m/z* 515 (M^++Na), 433 (M^++H-AcOH), 391, 373, 301, 283.

Antimicrobial activity of herical

The antifungal and antibacterial activities in the conventional paper disc/agar diffusion and the se-

rial dilution assay were measured as described previously (Anke *et al*., 1977).

Cell culture and cytotoxicity testing of herical

Ehrlich ascites tumor (ECA) cells and L-1210 cells ATCC CCL 219 were grown in suspension culture in F-12 medium containing 20% respectively 15% of horse serum and 0.12% $NaHCO_3$. Balb 3T3 cells clone A31 ATCC CCL 163 and Maloney murine sarcoma virus transformed Balb 3T3 cells ATCC CCL 163.2 were grown in Dulbecco's modified Eagle's medium containing 10% of fetal calf serum and 0.1 mM glutamine. KB cells ATCC CCL 17 were grown in MEM-Earle medium containing 10% of fetal calf serum and HeLa S3 cells ATCC CCL 2.2 were grown in F-12 medium containing 10% of fetal calf serum and 0.12% $NaHCO_3$. All media contained 65 μg/ml of penicillin and 100 μg/ml of streptomycin. Except for the ECA cells all cultures were incubated in a humidified atmosphere containing 5% of CO_2. The incubation temperature for all cultures was 37 °C. Cytotoxicity was tested in microtiter plates (Falcon) with 200 μl of medium containing 10^6 cells/ml. At suitable intervals, usually 24 and 48 h, the cells were examined under the microscope either directly or after staining with trypan blue.

Transport studies in cells of the ascitic form of Ehrlich Carcinoma (ECA)

The uptake and the incorporation of ^{14}C-leucine, ^{14}C-uridine, and ^{14}C-thymidine into trichloroacetic acid (TCA)-precipitable material (protein, RNA, and DNA) of ECA cells grown was measured as described previously (Anke *et al.* 1981). After preincubation (30 min) with the antibiotic, 1 ml of the cell suspension was incubated in Eppendorf cups with 3.7×10^3 Bq ^{14}C-leucine (2.01×10^9 Bq/mmol), 3.7×10^3 Bq ^{14}C-uridine (2.22×10^9 Bq/mmol), or 3.7×10^3 Bq ^{14}C-thymidine (1.92×10^9 Bq/mmol) for 10 min at 37 °C. The cells were immediately centrifuged (10 000×*g*) and the pellet either suspended in 5% TCA (incorporation) or directly added to the liquid scintillation fluid (uptake).

Hemolytic effect of herical on porcine erythrocytes

The hemolytic effect of **3** on porcine erythrocytes was tested according to (Kupka *et al*. 1979). 0.5% Brij 58 [polyoxyethylene(20) oleyl ether] was used as standard for the induction of complete hemolysis.

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