be much stronger on both silkworms than that of original strain, *sotto*. No change of toxic activity was found in the all 3 mutants after the heat treatment.

Scanning electron micrographs of sporeless mutants showed only regularly shaped bipyramidal crystalline bodies laid free on a background of lysed cell envelopes which sometimes form cell ghosts or networks (Figures 1–3). In contrast, many ellipsoidal spores were observed besides octahedral crystalline bodies and cell envelopes in the original wild strains (Figure 4). In general, crystalline bodies of mutant S162 (Figure 3) were much smaller than those of the mutants M1 (Figure 1) and M67 (Figure 2), a condition which also occurs in the original *sotto* strain. All mutants had a size and shape of crystalline bodies similar to those of their parent strains.

The combination of biological inviability, and complete retention of toxic activity in preparations derived from sporeless mutants of *Bacillus thuringiensis* would provide a safer source of microbial insecticides as well as being a valuable source of starting material for the purification and characterization of the δ-endotoxin.

Summary. Three sporeless mutants of *Bacillus thuringiensis*, 2 derived from var. *thuringiensis* and 1 from var. *sotto* were selected after mutagenic treatment. They were completely lacking in ability to form spores, yet maintained intact insecticidal activity.

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[4] The authors are indebted to Dr. Takeda and Mr. Y. Kawamura for the scanning electron micrography and microscopic inspection, respectively. We wish to acknowledge Miss A. Ohsawa's skillful technical assistance.

[5] Daiwa Kasei, K. K. Hieda, Kosei-cho, Koga, Shiga, 520–30 (Japan).

Isatin-3-Anils as Excystment and Cysticidal Agents Against *Schizopyrenus russelli*

Generally relapses have been encountered in treated cases of chronic amoebiasis. This has been attributed to the fact that the drugs discovered so far have little or no effect on the cystic stage of amoeba.

Isatins have generally been associated with antiviral activity[1]. Other biological responses exhibited by isatins include antibacterial[2], anthelmintic[3] and hypotensive actions[4]. In this communication we wish to report for the first time their cysticidal action and their ability to cause excystment. A series of isatin-3-anils(I) have been screened for their cysticidal activity and their role as excystment agents.

It is interesting to note that certain compounds of this series have shown cysticidal activity and also caused excystment simultaneously. This type of behaviour in a single substance has perhaps not been reported earlier.

Materials and methods. Isatin-3-anils(I) were obtained by condensing isatin with aromatic amines in ethanolic medium containing 2 drops of glacial acetic acid. The resulting condensed products were then subjected to Mannich reaction conditions[5].

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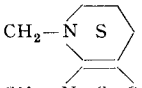
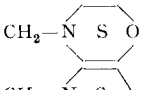
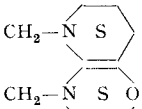
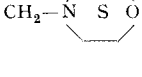
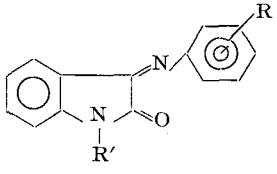
Isatin-3-anils (I) as excystment and cysticidal agents

Specimen No.	R	R′	Excystment (%)	Mortality (%)
1	4—OCH_3	H	50	20
2	2—OCH_3	H	28	—
3	4—CH_3	H	15	—
4	3—CH_3	H	30	40
5	4—Cl	H	nil	37
6	4—Ph	H	10	40
7	4—OCH_3	CH_2— 	nil	28
8	3—CH_3	CH_2— 	nil	20
9	4—Ph	CH_2— 	5	—
10	4—Ph	CH_2— 	10	10
11 Control (cysts + *E. coli* extract)			96	—

Cysts from a pure line culture of *Schizopyrenus russelli* were used in this work. The amoebae were grown on non-nutrient agar (2.5%; w/v), 0.5% (w/v) NaCl; pH 6.8–7), plates supplied with a 3-day-old culture of *Escherichia coli*, grown on nutrient agar slopes, as food. 3- to 7-day-old cysts were harvested and viable cysts, free from living or dead bacteria, were obtained[6].

A concentration of 2 mg/ml of the test compounds (in 1% agar suspension) was employed for excystment experiments[7]. 50 to 75 cysts were placed as a hanging drop suspension in a cavity slide (25 ± 1 °C), housed in a moist chamber. The percentage excystment was calculated from the count of amoebae and the unexcysted cysts. A cyst was considered excysted only when an amoeba escaped from it and was found moving in the surrounding medium.

For cysticidal activity[7], the test compound was freed from the cysts, after initial treatment, by repeated centrifugation. The cysts were then treated with an excystment agent (*E. coli* extract), after getting rid of trophozoites in cases where excystment had taken place by treating the sample for 2 h with 2% HCl, and the number of cysts excysted were counted. The cysts that did not excyst were presumed to be dead. The number of cysts taken in each experiment varied from 200 to 350. The results are described in the Table.



I

Acute toxicity. The acute toxicity tests were done by feeding orally the test compounds (900 mg/kg) to albino rats weighing 20–25 g of either sex and fed on a diet of milk and bread). No mortality was recorded during 7 days of observation.

Summary. A series of isatin-3-anils (with or without a N-piperidino/morpholinomethyl substituent) have been screened for their cysticidal activity against *Schizopyrenus russelli*. Their ability to cause excystment has also been studied.

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[8] Division of Microbiology, Central Drug Research Institute, Lucknow, India.

Action of *Bacillus thuringiensis* Preparation Against Larch Bud Moth, *Zeiraphera diniana* (Gn.), Enhanced by β-Exotoxin and DDT[1]

The larch bud moth *Zeiraphera diniana* (Guénée), a monovoltine tortricide moth of the subfamily Olethreutinae, is a primary pest of larch in subalpine forests of Austria, France, Italy, and Switzerland[2]. In larch forests, situated at 1400 to 2100 m above sea level, the density of the insect fluctuates in cycles, with gradations of 8–10 years duration[3,4]. Tree defoliation in the phase of culmination impairs the touristic attraction of the forests attacked, reduces wood production considerably[5], and leads to noticeable tree mortality when defoliation takes place in 2 successive years, as for instance in 1973/74. Research is therefore being conducted at our Department to find suitable means to control of this pest.

Large scale applications of insecticides in the culmination phase of a gradation showed that a single application of DDT could reduce larval populations by 97% and thus prevent defoliation for the whole gradation cycle, whereas a single treatment with the organophosphate phosphamidone, causing 93% mortality, could prevent defoliation only in the year of application but not in the succeeding year[6]. The latter treatment caused heavy mortality of birds in the forests treated[7], and since meanwhile DDT has been banned, chemical control offers no solution of the problem of *Z. diniana*.

More than 10 years ago, the author tried selective microbial control of *Z. diniana* by means of biopreparations of *Bacillus thuringiensis* Berliner[8]. Unpublished experiments in 1963 on 3 and 4 ha plots, treated by helicopter[9] at the rate of 40 l per ha, resulted in population reduction of 60–75%. These values, after correction for natural mortality, corresponded to 41% induced mortality on plots treated with Thuricide 90T (7.5 l/ha) or Bactospeine (3.3 kg/ha), and 64% mortality on plots treated with Biospor Hoechst (3.3 kg/ha). Repetition of the treatment with Biospor on the same plot with a dose of 4 kg/ha in 1964 resulted in a 95% reduction of the larval population, corresponding to 90% induced larval mortality. Unfortunately, the producer of Biospor stopped the production of this most promizing preparation in 1965.

Large scale applications of Bactospeine against *Z. diniana* in France have been reported by GRISON et al.[10,11]

[1] Contribution No. 83 of the research team for the investigation of the population dynamics of the larch bud moth. The research was aided by a grant of the Swiss National Funds for the Advancement of Scientific Research.
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