Animals (all female) were sacrificed 2 weeks after inoculation. Homogenized left kidneys were examined bacteriologically. The infection rate in several experiments is demonstrated in Table I.

From these results, it is concluded that kidney infecting dose[6] (KID_{100}) in whole the animals of our *E. coli* strain to be 2... 3×10^5 bacteria in the conditions described. The reproducibility of the results at various times over the year was very good. There was no lethality in about 400 rats infected in the same manner.

Further experiments were performed to get information about duration of the infection by the use of greater amounts of bacteria than the KID_{100}. We wanted to be sure that a great part of the animals should get a longer-lasting infectious process in their kidneys. Rats of either sex were under examination. At various intervals different groups of animals were sacrificed and their kidneys checked for infection. Bacterial strains were identified by haemolysis, fermentation pattern, and serologically. In all the bacteriologically positive cases we found pure cultures of our *E. coli* strain. Saline injected, and suspensions of ground sand in saline intrarenally inoculated, male and female rats in groups up to 15 animals served as controls. They were sacrificed within a range from 2 weeks up to 8 months. In these animals we never observed kidney infection.

Table II shows that there are no great differences between male and female rats up to 4 or 5 weeks. 5 months after infection, nearly half the female animals had positive bacteriological cultures from their infected kidneys, whereas the kidney of only 1 male rat out of 13 exhibited bacterial growth.

Because of the relatively small number of animals available for statistical evaluation, we are not able to decide whether the observed sex difference was possibly present at an earlier stage of the infectious process in the kidneys. Nevertheless, it must be stated from our results that planning and interpretation of similar experiments have to consider the sex of the animals used.

Zusammenfassung. Lebende *E. coli*-Bakterien wurden bei Ratten direkt in die Niere injiziert. Für die Infektion war eine bestimmte Mindest-Keimmenge erforderlich. Bei Injektion höherer Dosen (Chronizität) überstanden die männlichen Tiere die Infektion offenbar schneller und leichter als die weiblichen.

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Sensitivity of some Plant Pathogenic Fungi Towards Plant Metabolites: Antifungal Activity of some Chalcones, Dihydrochalcones and Flavanones

As a part of our programme on the chemical constituents occurring in some plants bearing antifungal properties[1,2], we were able to isolate and characterize 2 new compounds, flemichapparin-B (I) and flemichapparin-C (II)[3] from the roots of *Flemingia chappar* (Fam: Leguminosae) which were found to show antifungal activity[4] against 3 plant pathogens, *Helminthosporium oryzae*, *Curvularia lunata* and *Alternaria solani*. Besides these 2 modified isoflavonoids, 2 new naturally occurring chalcones of unusual aromatic substitution pattern, flemichapparin- (III)[5] and flemichapparin-A (VII)[6], along with 2′,4′-dihydroxychalcone-(IV)[7], were isolated from the aerial parts of *F. chappar*. The antifungal activity of these chalcones and their corresponding derivatives (dihydrochalcones and flavanones) have now been assayed by studying their effects on 4 growth stages of fungi, viz., spore germination, germ tube growth, vegetative growth in colony, and sporulation. The compounds (III–X) have also been studied with a number of fungi representing different classes, and their minimum inhibitory concentration has been determined against a selected test organism.

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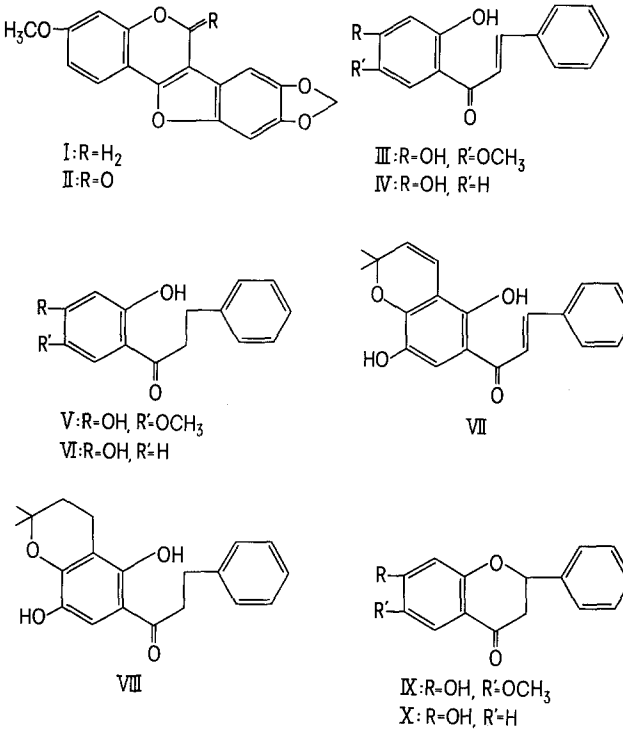
Table I. Effect of chalcones and their derivatives at 105 µg/ml concentration on spore germination of 3 plant pathogenic fungi

Compounds	pH of the solutions	Germination (%) of spores of		
		H. oryzae	*A. solani*	*C. lunata*
Flemichapparin (III)	8.5	80	20	0
2′,4′-Dihydroxychalcone (IV)	8.5	100	59	60
Dihydroflemichapparin (V)	10.0	0	90	0
2′,4′-Dihydroxy-dihydrochalcone (VI)	10.0	0	0	0
Flemichapparin-A (VII)	9.8	0	0	0
Tetrahydroflemichapparin-A (VIII)	10.0	0	0	0
6-Methoxy-7-hydroxyflavanone (IX)	9.5	100	0	0
7-Hydroxyflavanone (X)	8.5	100	0	0
Control	7.0	100	95	70

Table II. Effect of different chalcones and their derivatives at 105 µg/ml on germ tube growth of 1 plant pathogenic fungus, *H. oryzae*

Compounds	Effect on germ tube growth		
	Length after 24 h (µm)	Morphology after 24 h	Spore germination (%)
Flemichapparin (III)	48–80	Very short, swelling and excessive branching type	80
2′,4′-Dihydroxychalcone (IV)	96–160	Short and swelling type of germ tube	100
Dihydroflemichapparin (V)	Nil	Nil	0
2′,4′-Dihydroxydihydrochalcone (VI)	Nil	Nil	0
Flemichapparin-A (VII)	Nil	Nil	0
Tetrahydroflemichapparin-A (VIII)	Nil	Nil	0
6-Methoxy-7-hydroxyflavanone (IX)	480–800	Smooth slightly short germ tube	100
7-Hydroxyflavanone (X)	160–320	Smooth but some what short germ tube	100
Control	800–1000	Smooth walled long germ tube	100



Materials and methods. The following 5 test organisms were used in this work: *Helminthosporium oryzae, Alternaria solani, Curvularia lunata, Aspergillus niger* and *Rhizopus nigricans*. Spore suspensions from 12-day-old cultures on PDA with cold (5 °C) sterile distilled water were used in the germination tests by cavity-slide method[8], making observations after 4–12 h incubation at 28 °C. The compounds, (III), (VII) and (IV), as well as their corresponding dihydro and flavanone derivatives (V, VI, VIII–X), were assayed. The solutions of these compounds in 1% aqueous NaOH were prepared at double the required strength. Effects on growth and sporulation were studied by adding requisite quantity of solutions of these compounds to PDA medium in petri dishes so as to get 50 µg/ml concentration of the compounds in the medium; growth was estimated by diameter of colony; sporulation was measured from the spore suspensions from the plates. The minimum inhibitory concentration (MIC) of the compounds (VI–VIII) against *H. oryzae* was determined.

Results and discussion. The compounds (VII), (VIII) and (VI) were found to be highly successful inhibitors of all the three test fungi. The spore germination of the fungi, *H. oryzae, A. solani* and *C. lunata* were inhibited

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Table III. Effect of compounds (50 µg/ml) on growth and sporulation of some plant pathogenic fungi

Compounds	*H. oryzae*		*A. niger*		*Rh. nigricans*	
	Growth	Sporulation	Growth	Sporulation	Growth	Sporulation
Flemichapparin (III)	1.5	0	0.9	4	0.0	NG
2′,4′-Dihydroxy chalcone (IV)	1.2	4	3.0	4	2.0	2
Dihydroflemichapparin (V)	3.0	0	1.0	4	5.0	2
2′,4′-Dihydroxy dihydrochalcone (VI)	2.0	2	2.5	4	3.8	0
Flemichapparin-A (VII)	0.6	2	0.4	4	0.0	NG
Tetrahydroflemichapparin-A (VIII)	3.0	0	0.0	0	0.0	NG
6-Methoxy-7-hydroxy flavanone (IX)	0.5	1	1.5	4	5.0	3
7-Hydroxy flavanone (X)	0.5	3	3.6	4	5.0	0
Control	6.0	5	3.6	5	5.0	5

Growth measurement: Diameter of colony in cm; NG, no growth; Sporulation grade: 0, no; 1, very scanty; 2, slight; 3, low; 4, medium; 5, high.

respectively by the compounds [(V–VIII)], [(III), (VI–X)] and [(III), (V–X)]. All these compounds save (IV) showed some inhibitory activity against any of the 3 test fungi. However, the compounds (IX), (X) and (V) showed specificity in their inhibitory action (Table I). The compounds (III), (IV) and (X) inhibited or disturbed the growth of germ tubes of the same fungus, allowing 80–100% germination of spores of *H. oryzae*. Germ tube growth was significantly reduced by these 3 compounds. Moreover, (III) and (IV) resulted in swellings of germ tubes (Table II). The compounds (VII), (III) and (VIII) were found to be highly potent growth inhibitors of all the fungi tested (Table III). Two of them (VII and VIII) appeared to be good germination inhibitors (Table I). Further, the compound (X) was found to be a specific inhibitor for *H. oryzae* alone. Differential growth inhibitory activity was shown by (V) and (IX), being inhibitory to all except *Rh. nigricans*, and by (IV), being inhibitory to all except *A. niger*. Sporulation inhibitory activity towards *H. oryzae* and *A. niger* was observed in only (VIII). The compounds (III) and (V) showed antisporulant activity for *H. oryzae* while (VI) and (X) exhibited the same property for *R. nigricans* (Table III). This is a very useful observation, since the importance of sporulation in secondary spread of such pathogens is very well-known[9]. The MIC for (VI) and (VIII) was found to be 25 µg/ml and that for (VII) was 30 µg/ml. The high antifungal activity of these 3 compounds at such a low concentration is very promising. The compounds (III–X) appear to be somewhat broad spectrum in their activity towards different types of plant pathogenic fungi. Further, these types of chalcones and their corresponding dihydro and flavanone derivatives show fungitoxic property since most of them inhibited the fungi in different growth phases. Further work in this area is now underway[10].

Résumé. Action antifongique et antisporulante de dérivés chalcones et flavones.

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H^+ Secretion and Na^+-K^+-Dependent ATPase System in the Human Gastric Mucosa

H^+ secretion by the human stomach is one of the active cation transport processes[1,2]. During secretion the H^+ is concentrated 10^6 times against its concentration gradient in the stomach. It is well known that the H^+ secretion is an ATP-dependent process[2]. Two enzymatic ways are present for the hydrolysis of ATP at the level of the cell membrane: membrane ATPase system[3,4] and adenyl cyclase system[5]. Although both of the 2 systems were obtained in the human gastric mucosa[6–12], the importance of these systems is unknown in the H^+ secretion.

In this paper, a positive and mathematically significant correlation has been proved to be present between the H^+ secretion and the Na^+-K^+-dependent ATPase activity from human fundic gastric mucosa.

Material and methods. In 45 patients with peptic ulcer (20 patients with gastric and 25 patients with duodenal ulcers), the H^+ secretion by the stomach was measured without application of any drug (basal acid output). The H^+ secretion was expressed in mEq/h. These patients underwent resection of stomach because of peptic ulceration. During operation a piece was cut out from the fundic part of the stomach. The gastric mucosa and the muscular layer were separated from each other and the membrane ATPase was prepared from fundic gastric mucosa with differential centrifugation ($20{,}000 \times g$ and $40{,}000 \times g$) and treatment with 2.0 *M* NaI solution according to the method previously described[6]. The membrane ATPase activity was measured in an incubation system at 37 °C, by liberation of inorganic phosphorus[7].

ATPase activity of membrane fraction from human fundic gastric mucosa

Mg^{2+}-dependent ATPase	0.81 ± 0.13
Total/Mg^{2+}-dependent and Na^+-K^+-dependent/ATPase	2.95 ± 0.10
Na^+-K^+-dependent ATPase	2.14 ± 0.11
Total + ouabain (10^{-4} *M*)	0.77 ± 0.13
Ouabain (10^{-4} *M*) inhibition	2.18 ± 0.12

The ATPase activity was determined in presence of 2 m*M* Mg^{2+} (Mg^{2+}-dependent part) and of 2 m*M* Mg^{2+}, 80 m*M* Na^+ and 33 m*M* K^+ (total ATPase). Na^+-K^+dependent ATPase was calculated as the difference between the total and Mg^{2+}-dependent part alone. Ouabain was dissolved in the same salt solution (2 m*M* Mg^{2+}, 80 m*M* Na^+ and 33 m*M* K^+). ATPase activity was expressed as means ± SEM of 10 patients, in µmoles of inorganic phosphorus (P_i) liberated by the transformation of ATP into ADP/mg membrane protein/h.

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