from where an overflow of material begins to CA via NCA I from the 7th day onwards. Consequently the neurosecretory material becomes again more abundant in the CA on the 7th day. This overflow of neurosecretory material renders the glands inactive (figure 3). The moulting of 2nd stage nymphs occurs from 12th to 14th day. The CA of 2nd instar nymphs ready to moult, and also those of freshly moulted 3rd stage nymphs, present histological characteristics of an inactive gland and contain abundant neurosecretory material. This pattern is repeated in subsequent nymphal instars too.

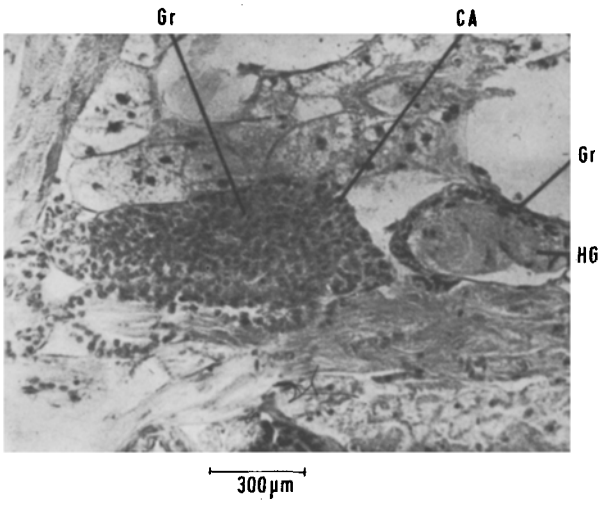

Fig. 5. Photomicrograph of the frontal section of the Corpora allata of an adult female killed 4 h after the final moult showing sparse neurosecretory granules (Gr). HG, hypocerebral ganglion. Note the scattered nuclei and relatively large size of the gland.

The CA of freshly moulted adult females (1 h after final moult) are loaded with neurosecretory material and are much smaller than those of 4 h old females. The CA of 1 h old females have a low volume of cytoplasm and highly crowded nuclei and can therefore be regarded as inactive (figure 4). But only 4 h after the final moult, the CA of adult females show a notable increase in size, have more cytoplasm and less crowded nuclei, and contain only a few neurosecretory granules (figure 5). It seems that, as soon as most of the neurosecretory material has gone out from the CA, the restraint or inhibition is over and the CA resume their activity.

However, the present histological findings can be interpreted in another way also. It may well be that the small amount of brain hormone reaching the CA perhaps stimulates the glands for a certain time; then, as the time for moulting approaches, the activity of the CA which has to be restrained is checked by the overflow and consequent presence of abundant neurosecretory material in these glands. The other possibility is that the presence of small amount of brain hormone does not stimulate the glands but just allows them to do a certain degree of their function. The secretory cells of the CA are allowed to produce a certain amount of juvenile hormone under the supervision of brain NSC (which may not be working completely independently of the CA) for a certain period after which the overflow and high concentration of brain neurosecretory material restrains their activity. In any case, the involvement of brain neurosecretory material in the control of the activity of CA seems to be fairly certain.

A fungitoxic principle from the leaves of *Lawsonia inermis* Lam.

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Summary. During antifungal screening of higher plants, the leaves of *Lawsonia inermis* were found to exhibit strong fungitoxicity. On chemical investigation, the antifungal factor was found to be 2-hydroxy-1,4-naphthoquinone (Lawsone). The minimum effective dose against test organism was found to be 1000 ppm. Lawsone was found to exhibit fungicidal activity, wide fungitoxic spectrum and nonphytotoxicity.

Plants are known to contain various antimicrobial substances[2,3]. Although the antifungal activity of extracts of higher plants is well demonstrated[4–7], the characterization of the fungitoxic principle has received little attention. The leaves of *Lawsonia inermis* Lam. were found to exhibit strong toxicity during antifungal screening of higher plants. The present communication deals with the isolation, identification and fungitoxic properties of the active principle.

Experimental procedures and results. 20 g of leaves as well as stem, root and bark were extracted separately with methanol and screened for antifungal activity by a modified paper disc method[8] against the test organism *Helminthosporium oryzae* Breda de Haan. Of various parts tested, only the leaves of *Lawsonia inermis* exhibited fungitoxicity. For the isolation of the active principle, 500 g of fresh leaves were extracted with methanol several times until the last eluate became colourless. The solvent from this extract was evaporated under reduced pressure. The residue was dissolved in distilled water and extracted with n-butanol and chloroform separately. The solvents from the n-butanol and the chloroform fractions and water from the aqueous fraction were removed by evaporation under reduced

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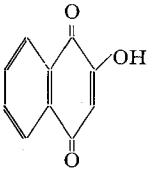
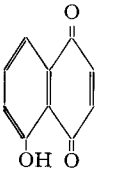
pressure. The residues obtained from the 3 fractions were subjected to antifungal testing by poisoned food technique[9]. The n-butanol fraction exhibited fungitoxicity at 1% concentration. No activity could be detected in the chloroform and aqueous fractions. The n-butanol fraction was acetylated with acetic anhydride in the presence of dry pyridine, and the acetate derivative was hydrolysed with $Ba(OH)_2$ to obtain the barium salt. This salt was then subjected to cation exchange column chromatography, using a Dowex-50 resin and 75% methanol. The eluate was treated under reduced pressure to remove the solvent, and the barium-free residue (F_1) was collected. The F_1 fraction was found to be 100% effective at 0.75% concentration in antifungal tests. It gave a deep blue colour with ferric chloride and a red brown colour with 5% sodium bicarbonate solution indicating the presence of some compound having phenolic hydroxyl group and a naphthoquinone ring. Since the presence of 2-hydroxy-1,4-naphthoquinone was reported earlier[10,11] and the F_1 fraction exhibited the presence of some hydroxy-naphthoquinone, it was considered desirable to isolate the compound from the leaves of *Lawsonia* and to test its fungitoxicity. The compound was isolated according a procedure described earlier[10]. Its identity with 2-hydroxy-1,4-naphthoquinone was confirmed by m.p. determination (m.p. 195–196 °C decomposition), mixed m.p. determination (no depression in m.p.) as well as UV, IR and NMR spectra [UV in ethanol λ_{max} 236, 272 nm, IR KBr, 3185, 1381, 874, 810, 782, 739, 1600, 1451, 1684, 1645 cm^{-1}, NMR δ 8.0, 7.6, 6.27]. The compound completely inhibited the mycelial growth of the test organism at a minimum inhibitory concentration (MIC) of 1000 ppm. To ascertain whether the fungitoxic activity of the active principle is fungistatic or fungicidal, tests were made according to Garber and Houston[12]. The compound was found to be fungicidal. It was tested for its fungitoxicity against 17 other fungi (table) and found to be active at various concentrations against all fungi tested. For testing phytotoxicity different concentrations of Lawsone viz., 1000, 2000 and 4000 ppm were sprayed on alternate days for a week on about 15-day-old plants of *Chenopodium amaranticolor* and *Zea mays* cv. Sankar Makka – Ganga – 2- and 20-day-old plants of *Solanum lycopersicum* cv. Marglove, *S. melongena* cv. Pusa purple long. The compound did not produce any visible symptoms of injury or other type of abnormality. Lawsone also had no significant effect on the percent germination and on radicle and plumule length of maize seedlings.

Discussion. Different parts of a plant have been reported to exhibit fungitoxic activity due to the distribution of the active principle in different parts[13]. However, in *Lawsonia inermis*, the activity was found to be confined to the leaves only, while other parts viz., stem, root and bark did not exhibit any activity. Chemically our fungitoxic activity was shown to be identical to 2-hydroxy-1,4-naphthoquinone (Lawsone), which in earlier observations of Karawaya et al.[14] has been shown to be absent from stem, root and bark of the plant. The role of substituted α-naphthoquinones as antifungal agents is well established. 2-methoxy-1,4-naphthoquinone[15], 5-hydroxy-1,4-naphthoquinone[16] (juglone) and 2-methal-5-hydroxy-1,4-naphthoquinone[17] have been recorded as antifungal factors from the flowers of *Impatiens balsamina*, leaves, pericarps and bark of the *Juglans regia* and leaves of *Ceratostigma willmottianum* respectively. However, the isolation of 2-hydroxy-1,4-naphthoquinone as fungitoxic principle from the leaves of *Lawsonia inermis* has been done for the first time. The lawsone has been found to be more potent than its position isomer juglone[18] which was earlier considered to be one of the strongest fungitoxic natural products from higher plants[19]. Furthermore, the present compound has been found to be nonphytotoxic, while phytotoxic activity has been reported for juglone[20]. It may be noted that the substitution of a hydroxyl-group in the second position instead of the fifth in a α-naphthoquinone system leads to more antifungal potency and nonphytotoxicity.



2-hydroxy-1,4-naphthoquinone (lawsone)



5-hydroxyl-1,4-naphthoquinone (juglone)

The strong fungitoxicity and the absence of phytotoxicity of lawsone indicates the possibility of its exploitation as an effective fungicide.

Effect of 2-hydroxy-1,4-naphthoquinone on the percent inhibition of growth of different fungal organisms

Fungi tested	Concentration of Lawsone (ppm) 1000	2000	4000
Alternaria solani	60	100	100
A. tenuis	100	100	100
Aspergillus niger	65	100	100
A. flavus	35	75	100
A. wentii	100	100	100
A. taedius	40	75	100
Absidia ramosa	100	100	100
A. corymbifera	100	100	100
Cladosporium herbarum	44	80	100
C. sphaerospermum	41	76	100
Penicillium funiculosum	5	15	100
P. oxalicum	0	19	100
P. rubrum	13	16	100
P. variabile	12	20	100
Acrophialophora fusispora	100	100	100
Circinella umbellata	84	100	100
Syncephalastrum racemosum	41	74	100

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