Relative ratios* of the components identified in the cyanogenetic secretion of 3 polydesmoid millipeds

	I C_6H_5COCN	II $C_6H_5CH(OH)CN$	III C_6H_5CHO	IV $C_6H_5CH(CN)CO_2C_6H_5$	V $C_6H_5CO_2H$	VI $CH(CH_3)_2CH_2CO_2H$	VII $C_{13}H_{27}CO_2H$	VIII $C_{17}H_{35}CO_2H$
Pseudopolydesmus serratus	33 ± 4	+	100	11 ± 2	< 5	< 2	< 2	< 2
Apheloria trimaculata	18 ± 3	+	100	ND	ND	ND	ND	ND
A. corrugata	1 ± 1	+	100	ND	ND	ND	ND	ND

*Relative ratios of the components are based on gas chromatographic peak area comparisons. The mean area of the benzaldehyde peak was arbitrarily assigned a value of 100 and all other values are relative to that assigned value. Calculations are based on secretion samples from 10 individual millipeds per species. I = benzoyl cyanide; II = mandelonitrile; III = benzaldehyde; IV = mandelonitrile benzoate; V = benzoic acid; VI = isovaleric acid; VII = myristic acid; VIII = stearic acid. +, detected by thin layer chromatography only. ND, not detected.

layer chromatography (Silica gel 6060 plates, developed in 5:1 benzene/chloroform, and in petroleum ether, with 2,4-dinitrophenylhydrazine or I_2 as detection agents).

The gas chromatographs had demonstrated the presence of one major component beside benzaldehyde, of longer retention time than the latter. This component proved to have gas chromatographic characteristics and a mass spectrum [m/e 132 (6), 131 (67), 105 (100), 77 (90), 56 (63), 55 (41)] identical to those of an independently prepared sample of benzoyl cyanide (m.p. 30–31 °C)[14].

The secretion of P. serratus contained 9 additional minor components, of which 5 were present in sufficient quantity for identification. One of these showed a retention time and mass spectrum [m/e 237 (10), 116 (77), 105 (100), 89 (27), 77 (65), 51 (29)] identical to those of an authentic sample of mandelonitrile benzoate (m.p. 57–59 °C), prepared as previously described[15]. The other 4 proved to be carboxylic acids. They were converted to methyl esters by treatment with ethereal diazomethane, and identified on the basis of gc/ms data as benzoic[16], isovaleric[17], myristic[18] and stearic[19] acid.

The results are summarized in the accompanying table, which also gives quantitative data on those compounds whose relative ratios could be meaningfully calculated by gas-chromatographic peak comparisons (Varian 1200 gas chromatograph; 3 m stainless steel column, 10% OV-17 on Gaschrom Q).

Benzoyl cyanide has not been previously isolated from either animals or plants. However, as we are reporting elsewhere[20], the compound occurs also in the defensive secretion of geophilid centipedes, which have most probably evolved the ability to produce this substance independently from millipeds. Benzoic acid and mandelonitrile benzoate have been previously reported from polydesmoid millipeds[5,10,21], but the latter compound had been thought to be an artifact arising during chemical analysis of the secretion[21]. In Pseudopolydesmus, at least, both these compounds are real components of the secretion, as evidenced by our finding that they were detectable in secretion samples gas-chromatographed within seconds after discharge. Isovaleric acid has also been reported previously from a polydesmoid milliped[5], but stearic and myristic acids have not[22].

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Fungitoxic properties of Rosa chinensis Jacq.

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Summary. During a systematic survey of higher plants for their fungitoxicity, the flowers of Rosa chinensis Jacq. were found to exhibit strong antifungal properties. On chemical investigation the antifungal principle was isolated as a shining, needle-shaped crystalline substance. It was identified as gallic acid. It exhibited fungistatic action against as many as 17 fungi at 3% concentration.

Plants are known to contain various antimicrobial substances[2,3]. Surprisingly, the antifungal principles of higher plants have received relatively little attention. During our systematic survey of higher plants for their fungitoxic activity, the flowers of Rosa chinensis Jacq. were found to exhibit strong antifungal activity. In the present communication, various antifungal properties of the methanolic extract of the rose flowers and isolation of the active principle as well as its antifungal properties have been reported.

The inhibitory properties of the flowers were determined by the modified paper disc technique[4]. The flowers (20 g fresh weight) were extracted with 100 ml methanol. 2 ml of the methanolic extract was impregnated gradually in a filter paper disc (15 mm diameter) by evaporating the solvent after each addition. Discs impregnated with the same volume of pure methanol served as control. The assay discs were aseptically transferred to petri plates containing Czapek's agar medium. A mycelial disc (5 mm diameter) cut from a 7-day-old culture of the test fungi viz., Cephalosporium sacchari Butler, Curvularia pallescens Boedijn and Fusarium nivale Cesati. was aseptically inoculated, upside downwards, in the centre of each disc. The plates were incubated at 28 °C ($\pm$ 1) for 6 days and observations recorded on the seventh day. Experiments were repeated twice and each contained 5 replicates.

The methanolic extract of flowers of Rosa chinensis Jacq. completely inhibited the growth of all the three test fungi. Besides, the growth of Alternaria solani Jones and Grout, A. humicola Oudem., Curvularia lunata Boedijn, Fusarium oxysporum Schlechte, Helminthosporium oryzae Breda de Haan, H. sativum Pammel, King and Bakke, Pythium aphanidermatum Fitz. and Rhizopus nigricans Ehrenb. was also inhibited. However, the methanolic extracts obtained from roots, leaves and stems failed to show any fungitoxicity.

Rose flowers
↓ Ext. $\bar{c}$ MeOH
MeOH extract (A. F. A. +)
↓ Conc.
Conc. MeOH ext. → Needles (150 mg)
↓ ReConc.
ReConc. MeOH ext. (A. F. A. at 5% conc.) → Needles (50 mg)

200 mg
↓
+ R_f = 0.33 (circular paper chro.)
+ blue colour with $FeCl_3$
+ m. p. = 253 °C (d)
+ m. p. of acetate = 174 °C
+ A. F. A. at 3% Conc. against test fungi

Hydrolyzed $\bar{c}$ 7% HCl
Ext. $\bar{c}$ ether

Etherial ext. (A F. A. at 4% Conc.)
Aqueous ext. (A. F. A. –)

Circular paper chromatography
a) Solvent: phenol saturated $\bar{c}$ H_2O
b) Developer $FeCl_3$
↓
Zones
Rf. 1: 0.8 yellow – Kaempferol No. A. F. A.
2: 0.6 yellow – Quercetin No. A. F. A.
3: 0.33 blue – Gallic acid – A. F. A. at 3% Conc. against test fungi

Flow sheet

Mycelial inhibition (per cent) of various fungi at different concentrations of gallic acid

Fungi tested	% concentration of gallic acid in assay plates			
	2%	2.5%	3%	3.5%
Aspergillus variecolor Thom and Raper	13.04	30.43	57.97	100
A. terreus Thom	9.67	38.70	70.96	100
A. nidulans Wint.	17.91	40.29	65.67	100
A. niger Van Tiegh.	2.81	1.40	8.45	5.63
A. flavus Link	7.35	1.47	4.41	2.94
Alternaria humicola Oudem.	13.46	38.46	100	100
A. solani Jones and Grout	8.92	48.21	100	100
Cephalosporium sacchari Butler.	11.11	27.27	100	100
Curvularia lunata Boedijn	22.44	67.34	100	100
C. pallescens Boedijn	4.76	50.00	100	100
Cladosporium herbarum Link	28.20	64.10	100	100
Chaetosphaeronema herbarum Moesz.	5.88	9.80	21.56	5.88
Chaetomium indicum Corda	27.27	47.27	100	100
Fusarium nivale Ces.	10.00	66.00	100	100
F. oxysporum Schlechte.	23.07	40.30	100	100
Helminthosporium oryzae Breda de Haan	33.33	66.66	100	100
H. sativum Pammel, King and Bakke	21.73	54.34	100	100
Leptospherulina trifolii Petr.	20.00	48.57	68.57	100
Memnoniella echinata Gall.	11.11	59.25	100	100
Nigrospora sphaerica Mason	22.50	57.50	100	100
Penicillium funiculosum Thom.	6.25	15.62	3.12	3.12
P. oxalicum Currie and Thom.	10.52	2.63	13.15	5.26
Paecilomyces fusisporus Saksena	38.43	65.38	100	100
Pythium aphanidermatum Fitz.	23.07	38.46	100	100
Rhizopus nigricans Ehrenb.	10.00	55.00	100	100

The methanolic extract obtained either from shade dried flowers or autoclaved flowers (15 lb for 20 min) did not lose the fungitoxic property. The extract allowed to stand at room temperature (28 °C $\pm$ 1) for 112 days also inhibited the growth of the test fungi. The methanolic extract obtained from flowers exposed beyond 150 °C were also effective against the test fungi.

The chemical investigation of flowers coupled with the fungitoxic assay of various fractions resulted in the isolation of a shining needle-shaped crystalline substance which exhibited fungitoxicity at 3% concentration against all the test fungi (see flow sheet). The crystals were identified as Gallic acid by colour reaction (deep blue colour with Ferric chloride), melting point determination (253 °C decomp.), spectral studies (UV, IR and NMR spectra) and melting point of their acetate derivative (m.p. of acetate 154 °C). Their further identity was established by co-chromatography (R_f 0.33) and mixed melting point determination (mixed m.p. 253 °C decomp.).

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Gallic acid exhibited fungistatic action against Alternaria humicola, A. solani, Cephalosporium sacchari, Curvularia lunata, C. pallescens, Cladosporium herbarum, Chaetomium indicum, Fusarium nivale, F. oxysporum, Helminthosporium oryzae, H. sativum, Memnoniella echinata, Nigrospora sphaerica, Paecilomyces fusisporus, Pythium aphanidermatum and Rhizopus nigricans at 3% concentration while against Aspergillus variecolor, A. terreus, A. nidulans and Leptospherulina trifolii at 3.5% concentration. However, Aspergillus niger, A. flavus, Chaetosphaeronema herbarum, Penicillium funiculosum and P. oxalicum remained unaffected (table).

The role of Phenols as fungitoxic agents is well established. Phenolic acids, viz., benzoic acid, salicylic acid and protocatechuic acid, are well known antifungal substances[5]. Benzoic acid and salicylic acids have also been recorded as antifungal factor of Populus tremuloides[6]. However, the isolation of gallic acid as an antifungal factor from Rosa chinensis in present study has been done for the first time.

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Differential effects of disuse preceding denervation on the onset and development of fibrillation in fast and slow muscles[1]

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Summary. Section of the sciatic nerve, performed after a week of muscular disuse, is followed by fibrillation earlier in the soleus (S) than in the anterior tibialis (AT) muscle of the rat. The subsequent development of fibrillation, which is different in the control denervated S as compared with the control denervated AT, tends to become similar in the disused-denervated muscles.

In a previous paper[2], it was reported that the onset of fibrillation in the denervated soleus-gastrocnemius muscles of the rat is greatly accelerated if the muscles are put into disuse for some days before denervation.

In the present work, it was investigated if disuse affects equally the fast and slow muscle fibres (both of which are present in the tested muscular group[3,4]), or preferentially affects one type of fibres. Under the same experimental conditions of disuse and denervation, the onset of fibrillation was selectively investigated in soleus (S, slow) and anterior tibialis (AT, fast) muscles. The subsequent development of fibrillation, both in control and experimental muscles, was also investigated.

Methods. Spinal cord section, or plaster cast immobilization of the limbs were performed in adult albino rats, 250–300 g in weight, as described[2]. The distal tendinous insertions of S and AT were cut on one side. In a number of cases, the whole tendo calcaneus was cut. Unilateral (cordotomized, immobilized, or control animals) or bilateral (tenotomized animals) section of the sciatic nerve was performed 6–7 days later, near the trochanter, at 3.5–4.0 cm from the point of nerve insertion into the muscles, the nerve stump to AT being 2–3 mm longer than the stump to S.

EMGraphic records were taken, under ether anaesthesia, via a pair of needle electrodes, insulated except for the tips, with an interelectrode distance of 2 mm, from the middle portions of both S and AT. Repeated insertions were performed transcutaneously in the same animal, using the fibula as a reference point for S. The records were from the superficial layers of AT, where succinic dehydrogenase activity is low[5], at a depth not greater than 2 mm, and approximately from the central layers of S. In some cases, fibrillation was acutely recorded from the exposed S, at a depth of 1–2 mm, the animal being sacrificed afterwards.

The development of fibrillation was estimated by measuring the integrated electrical activity of the muscles through a Beckman-Offner EMG integrator, at 12–24-h-intervals, over a period of a week. Fibrillation activity was also monitored on a CRO.

Results. In the previously tenotomized S, the onset of fibrillation was, on an average, as precocious as reported for the soleus-gastrocnemius group[2], occurring 25.63 ± 1.14 h after denervation (mean of 23 cases, ± S.E.); the control time in the contralateral denervated muscles was 54.31 ± 1.09 h. In the tenotomized AT, fibrillation arose consistently later: 39.86 ± 2.22 h after denervation (mean of 21 cases; control time 55.87 ± 2.13 h).

The difference between S and AT was still greater in immobilized limbs. Fibrillation began respectively 26.26 ± 2.29 h and 48.52 ± 4.32 h after denervation (mean of 10 cases).

Less markedly different were the results in the cordotomized animals. Fibrillation began 22.41 ± 1.18 h and 30.42 ± 3.52 h after denervation, respectively in S and AT (mean of 15 cases).

The development of fibrillation was first of all investigated in the simply denervated, control muscles. In S (see figure) fibrillation increased rather quickly, reaching a peak 48 h after its onset, but afterwards it fell off markedly. In AT, fibrillation developed more gradually during the first 2 days, and then leveled off, so that after 168 h the electrical activity was much about the same in AT and S.

In the animals with spinal cord transection, fibrillation development in S and AT was rather similar, increasing very slowly and very gradually throughout the whole experimental period (168 h).

In the tenotomized and in the immobilized animals, the results were less consistent. In some animals, the devel-

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