Results and discussion. After DAB incubation in the presence of H_2O_2, mitochondria appeared heavily stained (figure 1); maximal staining occurred when the reaction was carried out at pH near 8. The staining was localized both in the intercristal spaces and between the outer and inner membranes, while no other organelle was stained. After the incubation with KCN, aminotriazole or antimycin A, the staining was less pronounced and more irregular, but on the whole DAB deposition seemed little affected (figures 2, 3 and 4). In the presence of methanol-nitroferricyanide, no staining was observed in mitochondria (figure 5).

Our results indicate that, in Prototheca moriformis, it is possible to detect a peroxidase activity in the presence of H_2O_2 which is nearly insensitive to KCN, aminotriazole and antimycin A, and is completely inhibited by methanol-nitroferricyanide.

We have at present no biochemical information on the enzyme(s) responsable of this peroxidase activity, but a peroxidative activity of mitochondria, demonstrated by DAB method, has been described in other unicellular organisms[3-5].

In Prototheca, this peroxidase activity seems neither due to catalase, because DAB deposition is not inhibited by aminotriazole, nor to cytochrome oxidase, because DAB deposition occurs only in the presence of H_2O_2 and is insensitive to KCN.

We think that DAB deposition could not be dependent on a peroxidase reaction of cytochrome c[6], as the peroxidase activity is not inhibited by antimycin A; but we cannot exclude a participation of a haemoprotein. At present we favour the hypothesis that the peroxidase activity described in this paper could be due to a mitochondrial peroxidase similar to those described in other unicellular organisms[7-9].

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Benzoyl cyanide in the defensive secretion of polydesmoid millipeds[1]

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Summary. A novel cyanogenetic compound, benzoyl cyanide, was isolated from the defensive secretion of 3 polydesmoid millipeds (Pseudopolydesmus serratus, Apheloria corrugata and A. trimaculata). The secretion of the 3 species also contains mandelonitrile and benzaldehyde, and that of P. serratus contains mandelonitrile benzoate, benzoic acid, isovaleric acid, myristic acid and stearic acid.

One of the more striking chemical defenses of animals, remarkable in part because of its similarity to the defenses of certain plants, is the cyanogenetic glandular apparatus of millipeds of the order Polydesmida. When disturbed, these animals discharge droplets of an odorous fluid from a series of glands that open along the sides of the body, and they depend on this response for protection against predaceous enemies[2]. The 2 primary known components of these secretions are hydrogen cyanide and benzaldehyde[3-7], which are not secreted as such by the glands, but are derived, as they are in cyanogenetic plants that produce these compounds[8], from the cyanohydrin mandelonitrile[9]. The mechanism whereby the mandelonitrile stored in the glands is exposed to catalyst and forced to dissociate into benzaldehyde and hydrogen cyanide at the moment of glandular discharge has been described[9]. Polydesmoid millipeds are a diversified lot, and recent work has indicated that their secretions might be chemically more complex and variable than generally suspected[5,10,11]. We have now reinvestigated 3 polydesmoid species that had previously been shown to be cyanogenetic[6,7,12] – Pseudopolydesmus serratus, Apheloria corrugata and A. trimaculata – and found them to produce, besides mandelonitrile, a novel additional cyanogenetic compound, benzoyl cyanide. Moreover, the secretion of P. serratus was found to contain several ancillary components, which were also identified.

The millipeds stemmed from the environs of Ithaca, N.Y. Secretion was obtained by manipulating and gently tapping the animals, taking up the discharged fluid in capillary tubing, and transferring it to carbon disulfide or ether. Apparatus used in the analyses included a gas chromatograph (Varian 2100 with flame ionization detector; 2.4 m glass column, 5% OV-1 on Gaschrom Q) and a gas chromatograph/mass spectrometer (Finnigan 3300) coupled to a computer (Systems Industries 150).

Liberation of hydrogen cyanide from the discharged secretion of all 3 species was clearly indicated by the blue coloration that developed on strips of filter paper impregnated with copper acetate/benzidine acetate reagent[13] held beside the secretion. Presence of benzaldehyde was confirmed by gas chromatographic and mass spectral comparison with an authentic sample. Mandelonitrile as such could not be demonstrated by gas chromatography since it dissociated at the high instrument temperatures. However, its presence in fresh samples of secretion from all 3 species was demonstrated by thin

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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 im-