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An insect growth inhibitor from *Trichilia roka* (Meliaceae)

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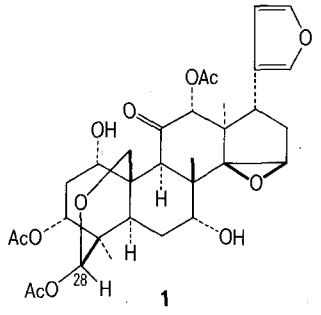
Summary. A limonoid 'sendanin' has been identified by spectroscopic data in the fresh fruit of a tropical tree, *Trichilia roka* (Meliaceae). It has an insect growth inhibitory activity against 4 important cotton insect pests. The absolute configuration of sendanin has been proposed based on CD-data.

On the basis of insect resistance of *Trichilia roka* (Meliaceae), information provided by Bwana Mganga, the local medicine man in East Africa[2], seeds, barks and leaves of this tropical tree were collected at Diani Beach near Mombasa, Kenya. The root bark has recently yielded a series of new limonoids 'trichilins' as insect antifeedants against the Southern armyworm, *Spodoptera eridonia* and the Mexican bean beetle, *Epilachna varivestis*[3] with leaf assay[4].

The seed oil of *T. roka* has long been known in East Africa to prevent jigger infection[5]. The crude aqueous methanol extract of the fresh fruits was found to possess strong insect growth inhibitory activity against the important North American cotton insect pests, pink bollworm, *Pectinophora gossypiella;* fall armyworm, *S. frugiperda;* tobacco budworm, *Heliothis virescens* and cotton bollworm, *H. zea.* Separation of this crude extract into hexane, ether, ethyl acetate and water-soluble portions indicated the ether extract to be the active fraction. Biological activity was monitored with an artificial diet feeding medium[6] into which the fractions were incorporated, and observations were made with the above mentioned insects.

The active principle was isolated as colorless prisms, $C_{32}H_{40}O_{12}$ (desorption chemical ionization mass spectrometry in NH_3 and elemental analysis), m.p. 254–255 °C, from this ether extract by careful silica gel chromatography. The spectroscopic data (UV, FTIR, MS, ^{1}H- and ^{13}C-NMR) suggest that this insect growth inhibitor to be the known 'sendanin' (**1**) previously isolated from another Meliaceae tree, *Melia azedarach* var. *japonica*[7]. This identification was confirmed by direct comparison with the authentic sample.



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Since sendanin was first isolated as an artifact, the C-28 acetate of the natural product[8], this is the 1st report of the isolation of sendanin from a natural source. Although the structure of sendanin was elucidated by X-ray crystallography, the absolute configuration was not established. The CD-spectrum of sendanin (MeOH), $\Delta\varepsilon_{307} - 1.6$ and $\Delta\varepsilon_{216} + 6.3$ is almost identical with those of trichilins[3] and aphanastantin[9]. Since the absolute configuration of aphanastantin was suggested based on triterpenoid biosynthesis[10], the absolute configuration of sendanin is that shown in 1. It should be noted from biosynthetic point of view that trichilins isolated from the root bark of *T. roka* are all oxidized at the C-2 position, while sendanin isolated from the fruit of the same source is not oxidized at that position.

The artificial diet feeding assay mentioned above was employed to study the effects of ingested sendanin in a 'no choice' situation. Various concentrations of sendanin were dissolved in acetone, applied to α-cellulose, evaporated to

Table 1. Activity of sendanin on survival and growth rate of newly-hatched larvae of 4 species of agricultural pest insects in a 10-day artificial diet feeding assay

Species	LD_{95} (ppm)[a]	ED_{50} (ppm)[b]
Pectinophora gossypiella	200	9
Heliothis zea	c	55
H. virescens	c	60
Spodoptera frugiperda	c	11

[a]LD_{95}-value is the lethal dose for 95% death. [b]ED_{50}-values are the effective doses for 50% growth inhibition. [c]Less than 10% deaths resulted from concentrations of sendanin as high as 1000 ppm.

Table 2. 48-h leaf disk 'choice' assay with 3rd instar larvae of fall armyworm and cotton bollworm against sendanin

Species	PC_{95} (µg/disk)[a]
Spodoptera frugiperda	6.2
Heliothis zea	37.9

[a]PC_{95}-values are concentrations of sendanin resulting in 95% protection of treated leaf disks when compared to untreated leaf disks.

dryness, and added to solid nutrients, vitamins, and agar components of a meridic artificial diet[6]. Newly-molted larvae of the 4 species of aforementioned insects were placed singly on portions of the diet in plastic vials. Daily observations on growth and molting were made; larval weights were determined after 10 days (equivalent in time to 4th instar control larvae). These feeding experiments showed that sendanin inhibits growth in the 4 tested species. Thus, ED_{50}-values for growth inhibition ranged from 9 to 60 ppm, with pink bollworm being the most sensitive and *Heliothis* complex the least (table 1).

At least a part of this growth inhibitory activity of sendanin can be attributed to an 'antifeedant' effect (table 2). Unlike the artificial diet bioassay, this antifeedant effect was determined in a 'choice' situation by confining either fall armyworm or cotton bollworm with both cotton leaf disks treated with sendanin dissolved in acetone and cotton leaf disks treated only with acetone[11]. Scoring of this antifeedant bioassay was done by visually estimating the amount of each leaf disk eaten after 48 h. The scores were then reported as PC_{95} (95% protective concentration) values. Thus, fall armyworm ($PC_{95} = 6.2$ µg/disk) is seen to be about 6 times more sensitive than cotton bollworm ($PC_{95} = 37.9$ µg/disk) in the antifeedant or choice bioassay. This result is similar in pattern to that found in the feeding or no-choice bioassay.

1 Insects were kindly supplied by the agencies of the USDA in Tifton, Ga, Phoenix, Az, and Brownsville, Tx. Authentic sample of sendanin was kindly provided by Prof. M. Ochi. The authors thank J.C. James for the CD measurement.
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Study of the metabolites of *Phyllosticta maydis*. I. Isolation and partial identification

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Summary. Various metabolites of the fungus *Phyllosticta maydis* have been isolated, particularly mevalonolactone. The most interesting of these compounds, which is highly toxic to corn seeds *(Zea mays)*, and which is probably a complex polyol, is the subject of the present report.

Among corn pathogens, *Phyllosticta maydis* Arny and Nelson presents, as does *Helminthosporium maydis* Nisikado and Myake race T, a relative specificity for plants with sterile male 'Texas' cytoplasm[2].

A filtrate of a *P. maydis* culture produces the same overall effects as that of a *H. maydis* culture on isolated mitochondria from Texas plants; inhibition of the coupling of respiration to ATP synthesis, inhibition of malate and α-ketoglutarate oxidations, stimulation of the oxidation of exogenous NADH, and mitochondrial swelling. Characteristic lesions are produced when the culture filtrates of these 2 fungi are applied to the foliar stalks of corn plants. The response obtained with the *H. maydis* extract, however, is the more rapid of the two.

This apparent similarity of action suggests that the toxic compounds of the 2 fungi are related. On the other hand, it is known that plant resistance to the 2 pathogenic agents is not always parallel, since mutagenesis has led to the creation of corn resistant to *H. maydis* race T[3]. Similarly, mitochondria resistant to *H. maydis* are not necessarily resistant to *P. maydis* and *vice-versa*. In parallel with ongoing genetic resistance studies, we have undertaken the characterization of metabolites produced by *P. maydis* in comparison with those produced by *H. maydis*[4].

Materials and methods. The metabolites of *P. maydis* were studied by extracting cultures grown in a modified Fries medium with glucose (30 g/l) and yeast extract (1 g/l) as sole carbon sources. Extractions of mitochondria and tests on corn leaves have been described elsewhere[3,5].

Extraction and isolation. 10 l of culture medium and the corresponding mycelium yielded 11.2 g of a water-soluble thick yellow oil, after isobutanol extraction and reduction of the aqueous phase to 0.1 its original volume. Isobutanol is preferred to the commonly used ethyl acetate, since the latter generates artifacts, regardless of its purity[4]. The resulting oil was dissolved in 30 ml of distilled water and underwent a continuous extraction by diethyl ether for 24 h. After evaporation of the ether, 4.8 g of a yellow oil was obtained, which was carefully neutralized with a cold 0.1 N solution of NaOH. A 2nd continuous extraction with ether yielded the neutral fraction (2.66 g), which was particularly active with Texas cytoplasm. Aqueous metabolite solutions which were not extractable with ether had no effect in the test system.

Spectroscopy. Proton NMR-spectra were obtained with a Varian T.60 spectrometer. The ^{13}C NMR-spectra were recorded with a Varian CFT.20 operating at 20 MHz in Fourier transformed mode. Samples were dissolved in $CDCl_3$ containing 1% TMS as internal standard. IR-spectra were obtained with a Perkin-Elmer 257 spectrometer. Samples were dissolved in CCl_4. Microanalyses were performed at the Central Microanalysis Laboratory, C.N.R.S., Gif-sur-Yvette, France. Mass-spectra were recorded with a ZAB 2.F spectrometer at the Physical Chemistry Institute of the 'Ecole Polytechnique' of Lausanne, Switzerland.

Results. Identification of metabolites. 1. Acid fraction. The last aqueous residue obtained was acidified with dilute cold 1N HCl. Organic acids were then continuously extracted with diethyl ether. A partially crystallized magma was obtained, which was then diluted in a minimum volume of