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# (−)-LOLIOLIDE, AN ANT-REPELLENT COMPOUND FROM *XANTHOXYLLUM SETULOSUM*

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As part of our on-going studies on chemical defenses against insect herbivory (1-5), we report here the isolation of a potent ant-repellent compound from *Xanthoxyllum setulosum* P. Wilson (Rutaceae). This species is one of the many native plants found to escape attack of the highly polyphagous leafcutter ants *Atta cephalotes* (Hymenoptera, Formicidae, Attini) in Santa Rosa National Park, Costa Rica (6). The isolation sequence was guided by a laboratory bioassay that has been previously described (7,8). Investigation of the polar fraction of the $CHCl_3$ extract led to the isolation of a potent ant-repellent, (−)-loliolide. This represents the first report of loliolide's occurrence in this species and the family Rutaceae, and the first report of its biological activity as well. This compound has been reported from *Lolium perenne* (Poaceae) (9), *Digitalis purpurea, D. lanata* (Scrophulariaceae) (10) and *Canscora decusata* (11).

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were recorded on a Thomas-Hoover melting point apparatus and are uncorrected. The ir spectra were obtained on FTIR IBM instruments IR/98. The $^1$H-nmr spectra were obtained on a Bruker WM-360 spectrometer, while the $^{13}$C-nmr spectra were recorded on a JEOL FX-90Q instrument. $CDCl_3$ was used as solvent with TMS as internal standard. Mass spectra (70 ev) were recorded with a Hewlett Packard 5985-B instrument.

EXTRACTION AND ISOLATION OF (−)-LOLIOLIDE.—Collections were made from plants previously authenticated as *X. setulosum* by R. Liesner, Missouri Botanical Gardens, St. Louis, Mo. Dried leaves of *X. setulosum* (1 kg, collected from Santa Rosa, Costa Rica, in July 1983) were successively extracted with $CHCl_3$ and 95% EtOH. Both extracts were concentrated in vacuo and bioassayed. The $CHCl_3$ extract (29 g), in which the ant-repellent activity was found, was partitioned between hexane and 50% aqueous MeOH (1-5). Only the polar fraction (1.1 g) showed significant activity ($p<0.001$), and it was further purified by flash chromatography over silica gel (4:1, hexane-$Me_2CO$). Material from the fourth pooled fraction, which had crystallized out as needles from the solvent mixture, was found to possess ant-repellent activity. This compound was identified as (−)-loliolide by comparison of its physical and spectra data (mp, $[\alpha]_D$, ir, $^1$H and $^{13}$C nmr, and ms) with published data (9).

BIOLOGICAL ASSAYS.—The laboratory bioassay, using several hundred ants from a captive colony of *A. cephalotes*, involves a forced-choice test between pressed rye flakes (60) treated with this compound and control flakes (60) treated only with solvent. This technique and the modified binomial test we use to assess statistical significance have been described in detail elsewhere (7,8). When the test flakes are treated with (−)-loliolide at a concentration of 6.8 mg/g of rye flakes, there is a significant preference for the control flakes $p<0.05$). This assay concentration is more than tenfold higher than that in the leaves, as indicated by the amount of material we recovered from the plant sample, but loliolide is the only repellent compound we have been able to isolate from this plant species. Thus, our experiments have not established that loliolide functions as a natural plant defense against leafcutter ants, but they do clearly show that this compound is a potent leafcutter repellent.

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