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Dithyreanitrile: an unusual insect antifeedant from *Dithyrea wislizenii*

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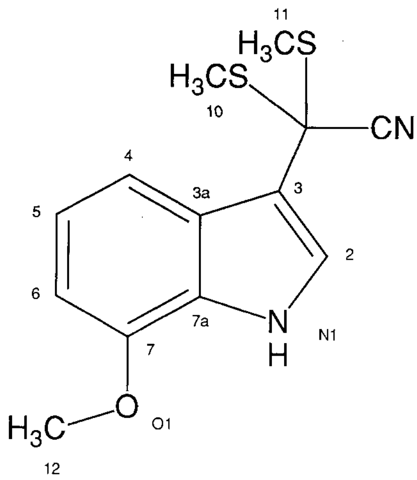
Summary. Dithyreanitrile, a novel sulfur-containing indole alkaloid, was isolated from the seeds of *Dithyrea wislizenii* (Cruciferae). Dithyreanitrile inhibits feeding of fall armyworm *(Spodoptera frugiperda)* and European corn borer *(Ostrinia nubilalis)* larvae. Dithyreanitrile, the first natural product with two sulfur atoms and a nitrile attached to the same carbon, was characterized by X-ray diffraction, spectroscopy, and chemical synthesis.
Key words. Insect antifeedant; indole alkaloid; X-ray diffraction.

Over thirty years have passed since Fraenkel's classic paper directed attention to the deterrent effects of plant metabolites towards insects[1]. Our knowledge of the chemistry and biology of plant-insect interactions has increased dramatically in the intervening years, and a large number of compounds representing virtually every structural type has been implicated in plant defense[2]. Even with all of the results accumulated by the systematic study of plant-insect interactions, the search for insect antifeedants can still produce surprising new compounds. We wish to report the isolation, structural characterization, and biological activity of dithyreanitrile, a most unusual insect antifeedant from *Dithyrea wislizenii*, a member of the Cruciferae native to the southwestern U.S. and northern Mexico[3]. Dithyreanitrile is a sulfur-containing indole alkaloid with a novel functional group – a carbon bonded to two S-methyls and a nitrile.

Seeds of *D. wislizenii* Engelm. were collected and authenticated by USDA botanists. The ground seeds (4.8 kg) were steeped for 24 h in water/ethanol (1:4), the solvent was removed, and the process repeated two more times. The collected solvent was partitioned three times with hexane (200 ml of hexane per 800 ml of solvent). The hexane layers were combined and evaporated to yield 78 g of oil, and the aqueous ethanol layer was evaporated to dryness yielding 263 g of residue. The ethanolic residue was partitioned between ether and water, yielding 44 g of ether-soluble material. The ether-soluble material was chromatographed on silica gel with chloroform and increasing amounts of methanol to yield 570 mg of active material that was further purified by HPLC using a Whatman partisil 10 PAC 20 Magnum column and chloroform elution. Final purification was accomplished on a Rainin Dynamax Macro 12″ silica column with chloroform as the eluting solvent. This procedure yielded 44 mg of dithyreanitrile (1×10^{-3}% yield).

The isolation of dithyreanitrile was monitored by a two-choice insect antifeedant bioassay[4]. Disposable Petri dishes with a moistened filter paper on the bottom had 12 1-cm diameter green bean leaf disks, half treated and half control, placed around the circumference. The disks had been dipped for 5 s either in a 1% or a 10% w/v solution of the extract being tested, or in solvent (control). The solvents used for the controls – hexane, chloroform, methanol or 95% ethanol – were selected to correspond to the solubility characteristics of the material being tested. After allowing 5 min for the solvent to evaporate the disks were arranged alternately in each Petri dish. Six fall armyworm *(Spodoptera frugiperda)* larvae, of roughly equal size, that had been reared on pinto bean artificial diet[5] for 9 days posteclosion and starved overnight, were placed in the center of each Petri dish and allowed to feed for 3 h. Assays were conducted in darkness at 27 °C and 60% relative humidity in three simulta-



Chemical line drawing of dithyreanitrile (**1**).

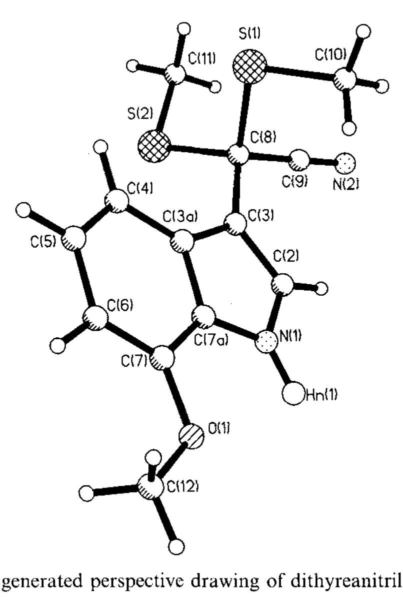
neous replicates. The percentage of each disk eaten was estimated visually, and feeding ratios, defined as the percentage of extract-treated disks consumed divided by the percentage of control disks consumed, were calculated. A feeding ratio of 1.0 means that equal quantities of treated and control leaf tissue were eaten (no deterrence). A ratio of 0.5 or less was arbitrarily set as showing some deterrence and 0.2 or less as being indicative of strong deterrence. Dithyreanitrile gave a feeding ratio of 0.11 at the 1% concentration level. Dithyreanitrile was also a feeding deterrent to European corn borer *(Ostrinia nubilalis)* larvae.

Dithyreanitrile (**1**) was obtained as an off-white crystalline solid, m.p. 135 °C (sharp), having a molecular formula $C_{13}H_{14}N_2OS_2$ as indicated by mass measurement and by an off-resonance decoupled ^{13}C NMR spectrum. The chemical ionization mass spectrum of **1** exhibited an ion at *m/z* 281 ($MH^+ + 2$), consistent with two sulfur atoms, and had major ions at *m/z* 252 ($MH^+ - HCN$) and 231 ($MH^+ - CH_3SH$). The structure of **1** was initially elucidated by single crystal X-ray diffraction techniques. Dithyreanitrile crystallized in the monoclinic space group $P2_1/n$ with $a = 10.747(2)$, $b = 9.864(2)$, $c = 13.513(2)$ Å and $\beta = 107.87(7)°$. A calculated and approximately measured density of 1.36 g/cc indicated that one molecule of composition $C_{13}H_{14}N_2OS_2$ formed the asymmetric unit. The space group also indicated that dithyreanitrile was either achiral or occurred as a racemate. All unique diffraction maxima with $2\theta \leq 114°$ were collected at room temperature using graphite-monochromated Cu $K\alpha$ radiation, 1° ω-scans, and 50% backgrounds on either side of the scan. Of the 1833 reflections collected in this fashion, 1207 (66%) were judged observed ($|F_0| \geq 3\sigma(|F_0|)$) after correction for Lorentz, polarization and background effects. An initial phasing model was found by a direct methods approach, and this model was extended through successive cycles of tangent formula refinement and electron density syntheses. When all nonhydrogen atoms had been located, least-squares refinements were used to optimize atomic coordinates. Hydrogens were included at ideal geometries. The final discrepancy index was 0.052, and a computer-generated perspective drawing is given in the figure[6].

Spectroscopic data were completely consistent with the X-ray defined structure[7]. Proton NMR indicated the presence of two equivalent S-methyl groups (δ 2.28), one O-methyl group (δ 3.95), three adjacent aromatic protons, and one additional aromatic proton coupled only to an exchangeable proton (NH, δ 8.44). A strong signal at δ 15.7 in the ^{13}C NMR spectrum of **1** also indicated the presence of two identical S-methyl groups.

To check on the possibility that the activity we observed was a contaminant, not dithyreanitrile, a synthetic sample was prepared[8]. The synthetic material not only had identical chemical and spectral properties with those of the natural product, it gave identical results in the bioassay.

The crucifers are well known as major sources of nitrile and sulfur-containing secondary metabolites[9]. These compounds are responsible for the characteristic odors and flavors of common spices and vegetables such as mustard, cabbage, radish, and broccoli. Many of these same compounds are also known to produce a broad variety of physiological responses in, or inhibit the growth of, plants, animals, insects and microorganisms[9–12]. However, dithyreanitrile (**1**) is the first example of a natural product having two sulfur atoms and a nitrile group bonded to the same carbon atom. A literature search revealed only a few synthetic compounds with this general type of functionality[13]. An obvious possibility for the mode of action of dithyreanitrile is hydrolysis, possibly enzyme catalyzed, of the native compound to hydrogen cyanide, methane thiol, and the corresponding carboxylic acid. This hypothetical mechanism shares some similarities to the mechanism of action



A computer-generated perspective drawing of dithyreanitrile (**1**).

of cyanogenic glycosides, and work in our laboratory is exploring its feasibility.

Dithyreanitrile is one of the simplest compounds with insect antifeedant properties to have been reported. Possibly this simplicity will allow it or an analog to be exploited on a broad scale.

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Drimane sesquiterpenoids in Mediterranean *Dendrodoris* nudibranchs: Anatomical distribution and biological role

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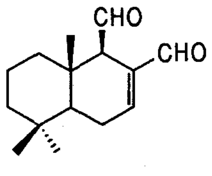
Summary. Two Mediterranean species of *Dendrodoris* nudibranchs have elaborated a very sophisticated defensive strategy against predators, involving the denovo biosynthesis of a series of drimane sesquiterpenoids, some of which are strongly ichthyodeterrent. Anatomical distribution of the drimane terpenoids in different sections and egg masses of the mollusc is reported, together with further studies aimed at finding out how the animals are protected against the noxious effects of their own allomones.

Key words. Chemical defense; *Dendrodoris* species; polygodial; drimane sesquiterpenoids; nudibranch.

Naked nudibranch molluscs exhibit a series of defensive strategies[2,3] against potential predators, which include the use of chemicals obtained either from the diet or by de novo biosynthesis. Polygodial (**1**), already known as plant metabolite[4] strongly anorectic for insects[5], is the defensive allomone isolated from two Mediterranean *Dendrodoris* species[6,7], *D. limbata* and *D. grandiflora*, and from some other Pacific porostome nudibranchs[8]. *Dendrodoris* molluscs are able to biosynthesize de novo[7,9,10] both the active polygodial, which can be isolated by chromatographic procedures from their mantles, and some inactive structurally related drimane esters (**2**), which are stored in the viscera.



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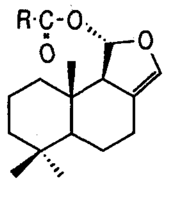

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The biological properties of **1** (antifeedant to fish, anorectic to insects, hot-tasting to humans) are most probably due to the simultaneous interaction of both the aldehydic groups with primary amine moieties[11]. Analogously, other terpenoidic dialdehydes display the same activities when particular structure-activity parameters, such as the distance between the two aldehydic groups and the molecular bulkiness, are taken into consideration[12]. Owing to the toxicity of **1** to *D. limbata*[10], the related sesquiterpenoid olepupuane (**3**) was strongly suspected[13] to be the masked form of the allomone present in the animal.

We now report the results we obtained when studying two questions which remained to be clarified:

1) Is polygodial completely absent in *Dendrodoris* species?
2) Where are the drimane sesquiterpenoids localized in *Dendrodoris* nudibranchs?