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BIOLOGICALLY ACTIVE DITERPENOIDS FROM SOLIDAGO SPECIES - PLANT-INSECT INTERACTIONS

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ABSTRACT

Solidago (Asteraceae) is a widespread clonal genus important in the native prairie grassland of North America. The insect predators of Solidago, which are well known, respond to interspecific and clonal differences between and within Solidago species. As part of a study of insect predation as influenced by the taxonomic relationship of environmentally adjacent plants, we have investigated the role of Solidago secondary metabolites in mediating insect predation. Several ent-kaurane derivatives have been obtained from the leaves of Solidago species and shown to influence feeding behavior of larvae and adults of Trirhabda canadensis (Coleoptera, Chrysomelidae). Data obtained thus far suggest a multichemical effect.

INTRODUCTION

Recent advances in ecology and in our understanding of animal behaviour have revealed the wide-ranging importance of secondary metabolites--"natural products" (allelochemicals)--in regulating the interactions between plants and animals in nature. Indeed, the role of such compounds is now known to be their raison d'etre in the evolutionary sense. Those who seek to understand the role of allelochemicals in the environment are assisted by discoveries in the more classical areas of natural products chemistry devoted to molecular structure determination, chemical reactivity, and structure-activity relationships.

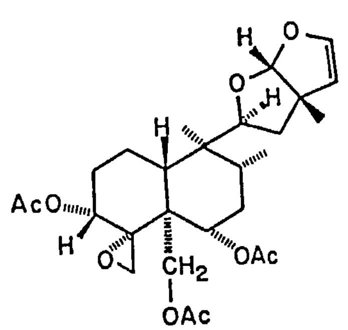
Any study seeking to comprehend the role allelochemicals play in the environment--that is, the way in which they may influence or control many of the complex interactions that occur between living organisms in natural communities (refs.1,2), is intrinsically difficult since plants, even a single plant, may possess a large number of different classes of allelochemical and at the same time may interact with many and varied organisms in widely different ways.

Thus the complexity of the potential chemical interactions would appear to be enormous (refs.3,4). Nevertheless over recent years great progress has been made towards understanding the role of natural products in insect-plant interactions (refs.5,6,7,8).

The genus Solidago provides an outstanding opportunity for North American investigators to explore the role of allelochemicals in the native prairie. Species of the genus are widespread in North America and are characteristic of the original flora of the Great Plains. Solidago belongs to the Asteraceae (Compositae), a family with a rich chemistry in terms of its secondary metabolites (refs.9,10). In the research to be described we have investigated the role of Solidago secondary metabolites in mediating the resistance of four related Solidago species to feeding by the beetle Trirhabda canadensis (Coleoptera, Chrysomelidae). This study forms part of an on-going collaborative investigation of the role of allelochemicals in associational resistance in the North American native prairie.

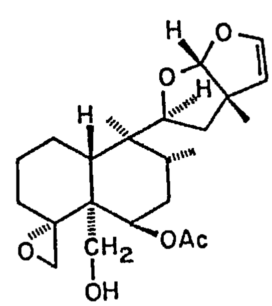
Biological Activity of Diterpenoids Against Insects

Several groups of diterpenoids are known to be active either as larval growth inhibitors or as feeding deterrents against a number of agricultural and forest pests (refs.11-13). A number of compounds have been studied in some detail in these respects. Particularly well studied are the antifeedant activities of the highly oxygenated clerodane diterpenoids caryoptin 1 and clerodin 2 from Clerodendron and Caryopteris spp. (Verbenaceae) against the tobacco cutworm Spodoptera litura L. (refs.14-16).



caryoptin

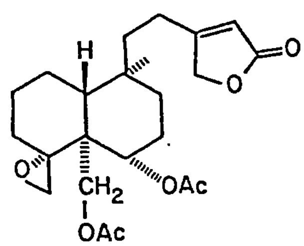
1



clerodin

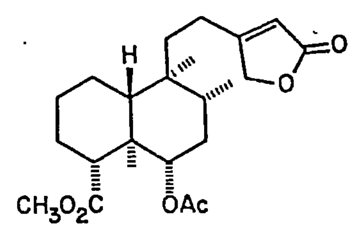
2

The bitter-tasting leaves of Ajuga remota, an East African medicinal plant, known locally to be resistant to insects, led to the isolation of another group of clerodanes, the ajugarins (e.g. ajugarin I 3 and ajugarin IV 4), which are



ajugarin I

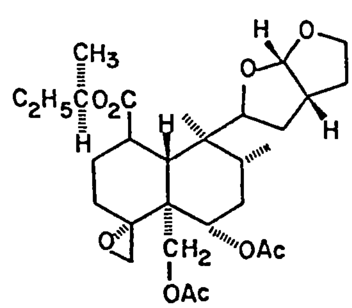
3



ajugarin IV

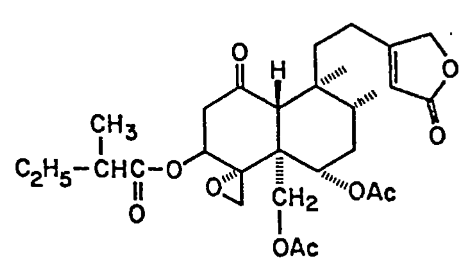
4

active against both the monophagous African armyworm, Spodoptera exempta, and the polyphagous S. littoralis (refs.11,17). When added to artificial diets ajugarins are also insecticidal to the silkworm, Bombyx mori, but merely inhibit the growth of the pink bollworm, Pectinophora gossypiella (ref.17). A sufficient number of highly oxygenated clerodanes having insect antifeedant properties has now been isolated that Belles et al. (ref.18) have been able to discuss structure-activity relationships in this area; for example ajugareptansin and ajugareptansone A (5 and 6) respectively from A. reptans (refs.19,20) and the ivains (e.g. ivain I 7 (ref.21)) from A. iva also exhibit high antifeedant



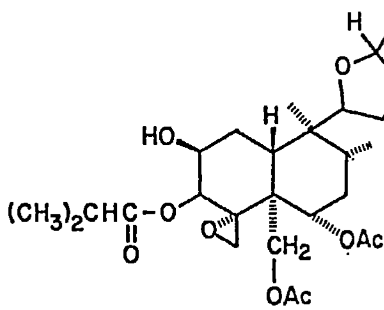
ajugareptansin

5



ajugareptansone A

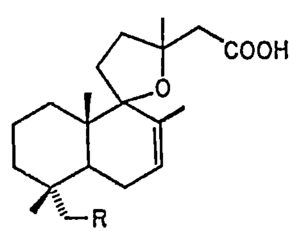
6



ivain
7

activity against the African armyworm and other lepidopterous species. It appears that "the presence in the clerodane structure of one spiroepoxide substituent at C-4 and two acetate groups at C-6 and C-18, eventually, together with the hexahydrofurofuran moiety at C-9" (ref.21), are necessary for biological activity, the ivains being considerably less active than members of the ajugareptansin and ajugapitin(14,15-dehydro-)series. Such findings are reminiscent of structure-activity correlations for cytotoxicity among sesquiterpene lactones, and suggest that some commonality may exist between the modes of action of these groups of compounds.

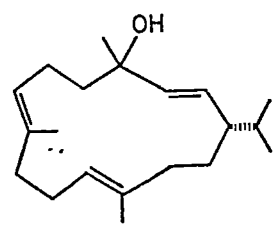
The labdane-derived grindelanes have representatives showing significant insect antifeedant activity. Two grindelane diterpenoids, 18-hydroxy- and 18-succinoyloxy-grindelic acids (**8** and **9**, respectively) present in the bicarbonate-



8 *R = OH: 18-hydroxygrindelic acid*
9 *R = OCOCH$_2$CH$_2$COOH: 18-succinoyloxygrindelic acid*

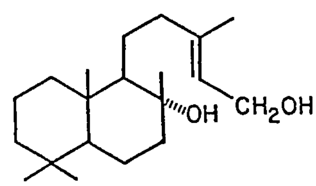
soluble fraction of extractives of Chrysothammus nauseosus (Pall.) Britt, the rabbit bush, were found by Rose (ref.22) to inhibit feeding of third instar Colorado beetles. Other grindelic acid derivatives also inhibited feeding by the aphid Schizapis graminum (ref.23).

Cutler et al. have found that the cuticular diterpenoids of green tobacco have both allelopathic and insect deterrent activity (ref.24). Present in the cuticle are duvane and/or labdane diterpenoids such as the duvatriene 10 and the labdadienol 11. The levels of these specific cuticular compounds are be-



α & β-4,8,13-duvatrien-1-ol

10

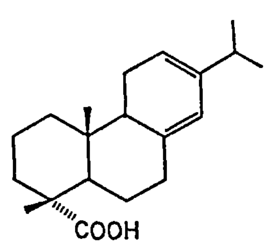


Labda-13en-8α,15-diol

11

lieved to be responsible for the observed resistance of some types of tobacco to green peach aphids (Myzus persicae Sulzer), tobacco budworm (Heliothis vivescens F.), and tobacco hornworm (Manduca sexta L.) (ref.25).

The diterpene resin acids such as levopimaric acid 12 and the numerous

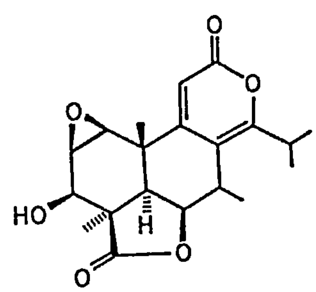


levopimaric acid

12

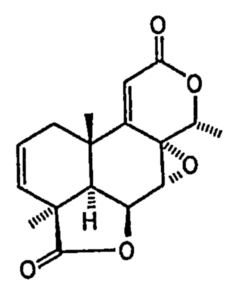
chemically related C_{20}-acids have also been implicated in pest resistance. Among the conifer-associated saw flies many prefer to feed on mature needles rather than on the current season's needles of their respective hosts. Bioassay studies with needle extracts indicate that diterpene resin acids are important. Ali and Benjamin (ref.26) observed that the monophagous saw fly species *Neodiprion swainei* Midd. and *N. rugifrons* Midd. did not feed on juvenile foliage of their host jack pine, *Pinus banksiana* Lamb. The antifeedants were found by Ikeda *et al*. to be 13-keto-8(14)-podocarpen-18-oic acid and dehydroabietic acid. A number of other examples of such bioactivity for diterpenoid acids are known, for instance from other studies by Benjamin *et al*. (refs.27-30).

In the biosynthetic sense, the norditerpene lactones and related compounds, such as nagilactone C **13** and podolide **14**, may be regarded as products of oxida-



nagilactone C

13

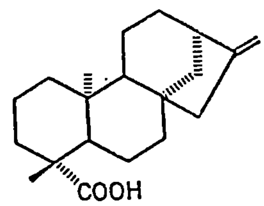


podolide

14

tive metabolism and chemical rearrangement of substances derived from the initial cyclizations of geranylgeraniol; that is, compounds such as **13** and **14** are derived from simpler, less oxygenated precursors. It is interesting that the resistance of *Podocarpus nivalis* and *P. hallii* to insect attack is attributed to the high concentration of **13** in the foliage (refs.31,32). As part of an apparently multichemical defense mechanism, three of the nagilactones and podolide show insecticidal activity against *Heliothis zea*, *Spodoptera frugiperda*, and *Pectinophora gossypiella* (ref.33).

Among the tetracyclic diterpenoids, several kauranoid and related carboxylic acids, e.g. *ent*-kaur-16-en-19-oic acid **15**, have been shown to inhibit insect growth (ref.34). Kauranes, trachyloban-19-oic acid, and *ent*-kaur-16-en-19-oic acid found in the florets of *Helianthus annuus*, inhibit larval development of the sunflower moth, *Homeosoma electellum* L. (ref.35) and of several other lep-



ent-kaur-16-en-19-oic acid

15

idopterous species (ref.36). These acids are widely distributed in other Helianthus species and may be important in the resistance of sunflowers to insects. Herz et al. (ref.37) have recently shown that two other kauranes from the sunflower, namely ciliaric acid and angeloylgrandifloric acid, when fed to the sunflower moth result in both higher mortality and retardation of growth.

Other oxygenated diterpenoids, e.g. the grayanotoxins and the seco-ent-kauranoids, show important activity against insects. The grayanoid kalmitoxin-I is the major antifeedant to the polyphagous gypsy moth, Lymantria dispar (ref. 38), while the isodons, isolated from Rhabdosia spp. (formerly Isodon), show growth inhibitory activity against both Spodoptera exempta and S. littoralis (refs.39,40).

The Genus Solidago

Solidago is a mostly North American genus with about 125 species. It belongs to the Asteraceae (Compositae) a family well known for the richness of its secondary chemistry (see for example ref.9). During the 1960's and 1970's Anthonsen in Finland and McCrindle and his coworkers in Canada isolated about 25 different compounds, mostly clerodanes and labdanes, from the roots of Solidago species. But during the last five years attention has turned to the aerial parts of the plant and representatives of other diterpenoid skeletal types, e.g. abietanes and kauranes, have been obtained (ref.41).

Solidago species are subject to colonization by a number of different phytophagous insects (refs.42-48), parasites, and predators (refs.49-51). Many of these insects are highly selective in their choice of food plants (refs.52-54), as are, for example, Trirhabda beetles (Coleoptera, Chrysomelidae), which will feed on certain interspersed species of goldenrods but which will avoid others. T. canadensis will readily feed on the leaves of both S. missouriensis or S. altissima but will completely reject either S. nemoralis or S. rigida, when all four species are growing in the same garden (ref.53). Messina also found (ref. 54) that T. virgata rejected S. nemoralis to a greater extent than other Solidago species under field conditions (ref.54). Thus S. nemoralis appears to be particularly well defended against Trirhabda beetles (refs.53,54), suggesting a possible role for the secondary metabolites of S. nemoralis as feeding deterrents.

The Minnesota Gardens Allelochemical Project

Our interest in Solidago species began by considering another role for plant secondary metabolites--that many herbivorous insects are thought to use the secondary chemicals present in their host plants as an aid to locating a plant suitable for feeding and/or oviposition. The ability of insects to use host chemicals as location cues is also influenced by the secondary chemicals present in neighboring non-host plant species. A key objective of the present project is to establish whether there is a systematic basis for predicting how non-host species will influence herbivore host location.

Our long term goal is to characterize the attractant, repellent, and antifeedant activity of secondary chemicals present in species that are part of a study of "associational resistance". This study is being conducted in experimental gardens in Minnesota designed by one of us (P.A. Morrow). The experimental gardens are of four types: a monoculture of Solidago altissima and three polycultures in which S. altissima is interplanted with three other species that are in the same genus (Solidago), in the same family (Asteraceae), or in different orders (Labiatae, Leguminosae, Asclepiadaceae). A variety of ecological observations exist suggesting that specialist herbivores should take longer to reject non-hosts, and make more mistakes, when non-hosts are chemically similar to the host (i.e. same genus) than when the non-hosts are distantly related species which have relatively many fewer secondary metabolites in common.

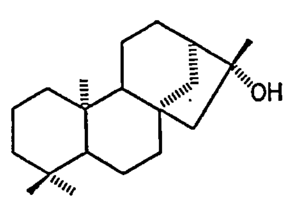
As mentioned above, the beetle Trirhabda canadensis (Chrysomelidae: Coleoptera) feeds on Solidago canadensis, the very closely similar polyploid S. altissima, and S. missouriensis. In field experiments in Minnesota with marked T. canadensis, P.A. Morrow and coworkers found that: (1) beetles placed on host and non-host species left the non-hosts much sooner than the hosts. These data sets suggest that beetles in contact with a plant use antifeedant compounds to reject host species. (2) Beetles dispersing within the different polycultures were almost never found on the non-host species. (3) Beetles released between a set of the four garden types preferentially colonized the monocultures. Among the polycultures, the beetles did not show clear preferences, although the plots containing the four Solidago species (two host species) had higher colonization percentages in two of three experiments. Work to expand these data and to perform a more detailed statistical analysis of the colonization patterns is in progress.

Isolation and Biological Activity of Kaurane Diterpenoids from Solidago Species

When methanolic whole plant extracts of the ten species in the experimental gardens were painted on to leaves of S. canadensis and adult beetles were allowed to choose between treated and untreated shoots, the beetles rejected host plants painted with non-host extracts but accepted controls painted with solvent or with host plant extract. These field and laboratory experiments suggested that non-hosts contain antifeedant. Accordingly an activity-directed fractionation was undertaken.

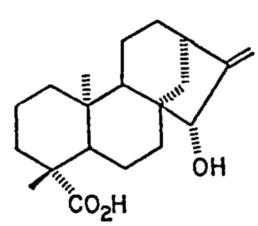
Leaves of S. nemoralis and S. altissima were collected from Professor Morrow's experimental gardens located at Cedar Creek, Minnesota. The dried, ground plant material was thoroughly extracted with petroleum ether/ether, and the secondary metabolites purified by a combination of gradient elution chromatography on silica gel, TLC, and HPLC using a Dynamax semipreparative silica column.

Four kauranoids were isolated and were identified by spectroscopic methods as ent-kaur-16-en-19-oic acid 15, ent-kauran-16α-ol 16, 15α-hydroxy-ent-kaur-16-en-19-oic acid 17, and 17-hydroxy-ent-kaur-15-en-19-oic acid 18.



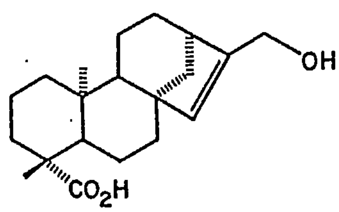
(−)-kauran-16-ol

16



15α-hydroxy-(−)-kaur-16-en-19-oic acid

17



17-hydroxy-(-)-kaur-15-en-19-oic acid

18

Antifeedant Activity of Kauranoids Isolated from S. nemoralis

A modification of the disc assay method was used to test the isolated kauranes for antifeedant activity. Freshly collected leaves of S. altissima were painted with the pure compound, dissolved in methanol, at concentrations of 1 mg/mL and 0.5 mg/mL in order to approximate the concentration present in the leaves. Control leaves were coated with methanol. Two treated and two untreated leaves were placed in a petri dish containing moistened filter paper. Eight Trirhabda larvae or four adults, collected from the same field site, were starved for several hours, introduced into the dishes and placed under standard conditions for a period of twenty-four hours. After twenty-four hours, feeding was determined in two ways, by visually estimating the total amount of damage to each leaf and by weighing the leaves before and after the experiment. There was no significant difference between the results obtained. Antifeedant activity is expressed as the ratio of the consumed area of the treated leaf/the consumed area of the control.

Most previous experiments with kauranes and closely related compounds have been concerned with their effects on insect growth and development. However in this case the deterrent effects of the isolated compounds to Trirhabda canadensis was being investigated as a first stage in experiments to understand the feeding behavior of this insect in the field.

(-)-Kaur-16-en-19-oic acid had no deterrent effect on feeding by the larvae of Trirhabda, but surprisingly was stimulatory to feeding by the adults. The three other kauranoids significantly reduced feeding in the larvae by 40 percent and by 32-49 percent in the adults. In combination the four compounds reduced feeding by 32 percent. Overall this denotes a substantial reduction in feeding by Trirhabda beetles.

However these compounds either singly or in combination do not account for the total lack of feeding activity observed either in the laboratory or in the field. Volatile compounds as yet still under investigation show powerful inhibitory action against feeding by *T. canadensis*. Resistence of *S. nemoralis* to insect attack seems to involve, as is expected, not a single class of compounds but is the result of a multichemical response.

Further work on this problem and on other aspects of the chemical components in the phenomenon of associational resistance in this biological system is in progress.

Acknowledgements

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