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Antifeedant Activity of Dihydro-β-Agarofuran Sesquiterpenes from Celastraceae against *Spodoptera littoralis*

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Key Word Index—*Maytenus canariensis*; *M. magellanica*; *M. chubutensis*; *Orthosphenia mexicana*; *Rzedowskia tolantonguensis*; Celastraceae; dihydro-β-agarofuran sesquiterpenes; *Spodoptera littoralis*; Lepidoptera; Noctuidae; antifeedant activity.

Abstract—Fifteen dihydro-β-agarofuran sesquiterpenes from different species of Celastraceae were assayed for possible antifeedant activity against Egyptian cotton leafworm *Spodoptera littoralis* (Boisduval) larvae, using the leaf disk method. The activity was evaluated by calculating the feeding ratio (FR) from the relationship between the consumed areas of treated disks (CTD) and control disks (CCD) at regular intervals; for comparison purposes a FR_{50} (50% of CCD) was established. In general, the compounds exhibited moderate-to-low antifeedant activity. Compound **4** (9α-benzoyloxy-8α,2-methylbutyroyloxy-1α,6β,15-triacetoxy-4β-hydroxydihydro-β-agarofuran) was the most active of the series. At a dose of 0.1 μg cm^{-2}, this compound gave a $FR_{50} = 0.23$ and in non-choice test conditions, nearly 100% leaf disk protection.

Introduction

The powdered roots of a Chinese plant, *Trypterigium wilfordii* Hook (Celastraceae), have been used since time immemorial to kill leaf-eating insects. Tests made in 1935 (Swingle *et al.*, 1941) demonstrated that *Bombyx mori* and *Malacosoma americana* larvae were deterred from feeding on leaves dusted with the processed plant. Later, the alkaloid wilfordine (Beroza, 1951; 1952; 1953) and the triterpene celastrol (Acree and Haller, 1950) were isolated from this plant and were considered responsible for its insecticidal activity. Wilfordine has also been obtained from *Euonymous alatus* (Yamada *et al.*, 1978). The extract of another member of the Celastraceae, *Austroplenckia populnea*, is active against the larvae of the nematode worm *Strongyloides stercolaris*. This extract contains polyester sesquiterpenes (Herz *et al.*, 1984) with dihydro-β-agarofuran skeletons. A paper (Redfern *et al.*, 1988) on *Celastrus angulatus* Max. (Misc. Publ. Agric. Res. Bur. China, 1935) from China reported that powder from its roots was used to protect other plants from *Colaphellus bowringi* (Misc. Publ. Agric. Res. Bur. China, 1935), *Hymenia recurvalis* (Bottger and Jacobsen, 1950) and *Locusta migratoria migratorioides* (Misc. Publ. Agric. Res. Bur., 1935). The same article (Redfern *et al.*, 1988) reported the isolation and structure determination of celangulin, a sesquiterpene which showed antifeedant activity in the fall armyworm (*Spodoptera frugiperda*) diet bioassay (Redfern, 1985).

This paper describes the antifeedant activity of 15 polyester dihydro-β-agarofuran sesquiterpenes from various Celastraceae which were selected for their variety of origins and range of stereochemistries and esterifying groups. All the sesquiterpenes had been obtained in the course of our research programme into secondary metabolites from Celastraceae used in folk medicine which has to date yielded antibiotic (González *et al.*, 1988a) and cytostatic compounds (González *et al.*, 1988b).

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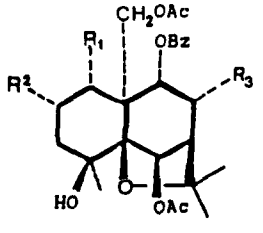
The antifeedant activity was evaluated by applying the vegetable disk method using fifth-instar *Spodoptera littoralis* (Lepidoptera: Noctuidae) larvae, as described elsewhere (Bellés *et al.*, 1985; Bellés and Piulachs, 1983).

Materials and Methods

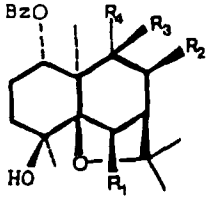
The compounds used were isolated from five species of Celastraceae; *Maytenus canariensis* (Loes.) Kunk et Sund. (Sunding, 1971) (compounds **1–4**); *Maytenus magellanica* Lam. (Lourteig and O'Donnell, 1955b) (compounds **14** and **15**); *Maytenus chubutensis* Speg. (Lourteig and O'Donnell, 1955a) (compounds **9–12** and **13**); *Orthosphenia mexicana* Standley (Rzedowsky, 1957) (compounds **5** and **6**); *Rzedowskia tolantonguensis* González-Medrano (González-Medrano, 1981) (compounds **7** and **8**).

They were grouped as accurately as possible by their chemical similarities within the following types of basic polyhydroxy skeleton: **1**: 4β-hydroxyalatol (Sugiura *et al.*, 1972); **2–4**: isoalatol (Sugiura *et al.*, 1972; González *et al.*, 1989a); **5–7**: isopentahydroxydihydro-β-agarofuran (Baxter *et al.*, 1979; González *et al.*, 1985; González *et al.*, 1988c); **8**: 4β-hydroxycelarbicol (Smith *et al.*, 1976; González *et al.*, 1985); **9** and **10**: 6β-hydroxypentahydroxy-β-agarofuran (Baxter *et al.*, 1979; González *et al.*, 1989b; González *et al.*, 1990); **11**: alatol (Sugiura *et al.*, 1972; González *et al.*, 1989b; González *et al.*, 1990; **12** and **13**: 3,4-dideoxymaytol (Kupchan and Smith, 1977; González *et al.*, 1989b; González *et al.*, 1990); **14**: 2β,3β,4β-trihydroxycelapanol (Wagner *et al.*, 1975; González, unpublished results); **15**: 2β,3β,4β,trihydroxycelarbicol (Smith *et al.*, 1976; González *et al.*, unpublished results).

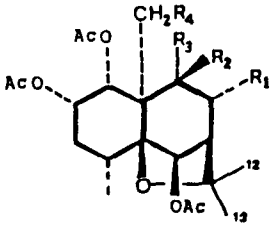
The insects used for the assay were *S. littoralis* larvae which had recently moulted to the fifth instar, the breeding conditions of the colony in the laboratory were 25±2°C, 60–70 r.h. and 18 h in photophase, and a semi-artificial diet (Poitout and Bues, 1974) was given.



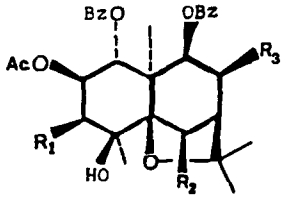
	R_1	R_2	R_3
1	OBz	OAc	OAc
2	OBz	H	OAc
3	OCinn	H	OAc
4	OAc	H	OMeBut



	R_1	R_2	R_3	R_4
5	ONic	OH	H	OAc
6	OAc	OAc	H	OAc
7	ONic	OAc	H	OAc
8	ONic	H	OAc	H



	R_1	R_2	R_3	R_4
9	OAc	OBz	H	OH
10	OAc	OBz	H	OAc
11	OAc	H	OBz	OH
12	H	OBz	H	OH
13	H	OBz	H	OAc



	R_1	R_2	R_3
14	OAc	H	OAc
15	OH	OAc	H

The antifeedant assays were made with a choice test using lettuce disks, *Lactuca sativa*, 1.0 cm^2 in area, weighing approx. 33.65±0.07 mg (Bellés and Piulachs, 1983; Bellés *et al.*, 1985). The compounds were uniformly distributed over the surface of the disk by application of 10 µl of acetone solution with a microsyringe and the solvent was evaporated off. The control disk (CD) was also treated with acetone. In each bioassay, four treated disks (TD) and four CD were placed alternatively in an 85-mm-diameter Petri dish in the presence of five larvae weighing between 40 and 50 mg and kept in constant conditions of 30 (±0.5)°C, 70% r.h. and total darkness in a culture oven. Each experiment was repeated five times.

Evaluation procedure. The consumed area of treated disks (CTD) and those of the controls (CCD) were measured simultaneously at regular 30-min intervals for 4 to 5 h to calculate the Feeding Ratio (FR) (Antonius and Sarto, 1981); FR = CTD/CCD. We used FR_{50}, the ratio when the CD had been 50% consumed; these values could be obtained by the extrapolation of the closest empirical data.

Results and Discussion

The results of the antifeedant tests are given in Table 1 and it can be concluded therefrom that the most active products were **7** and **4**, with a $FR_{50} < 0.5$ at a dose of 0.1 µg cm^{-2}. When the dose used was 1.0 µg cm^{-2}, compound **15** also showed a $FR_{50} < 0.5$ and at a dose of 10.0 µg cm^{-2}, all the compounds were active in this order of magnitude except for **5** and **8** which gave a $FR_{50} > 0.5$.

Compounds **4** (at doses from 10 to 0.1 µg cm^{-2}) and **7** (at doses of 10 and 1 µg cm^{-2}) maintained nearly 100% leaf disk protection after all the control disks had been consumed, i.e. $FR_{50} = FR_{100}$, and this continued to be observed even when the assay was prolonged for 2 h in these non-choice test conditions.

It is difficult to compare our findings with those reported in the literature for other compounds as different tests have been used by other authors working on the same lines. For example, the antifeedant effects of celangulin against *Spodoptera frugiperda* (Redfern, 1985; Redfern *et al.*, 1988) were not suitable for comparison as the activity

TABLE 1. FEEDING RATIOS OF TEST COMPOUNDS

Compound	Dose (µg cm^{-2})	FR_{50}±S.E.M.
1	10	0.35±0.19
2	10	0.24±0.11
	1	0.58±0.06
3	10	0.27±0.17
4*	10	0
	1	0
	0.1	0.23±0.07
	0.01	0.69±0.08
5	10	0.74±0.06
6	10	0.12±0.07
	1	0.40±0.02
7*	10	0.04±0.03
	1	0.15±0.15
	0.1	0.45±0.28
8	10	0.68±0.14
9	10	0.07±0.06
	1	0.53±0.32
10	10	0.13±0.05
	1	0.71±0.09
11	10	0.16±0.15
	1	0.65±0.25
12	10	0.36±0.09
13	10	0.19±0.06
	1	0.54±0.09
14	10	0.38±0.05
15	10	0.24±0.19
	1	0.44±0.12

All assays were performed in quintuplicate.

***4**, doses of 10, 1 and 0.1 µg cm^{-2}; **7**, doses of 10 and 1 µg cm^{-2}; in both cases part of the T.D. was eaten and then left for good.

had been studied by adding the compound to the diet. Conversely, some comparisons could be made with the synthetic compound, triphenyl tin acetate which was studied under identical conditions as those described below, giving a reported FR_{50} of 0.37 at a dose of 10.0 μg cm^{-1} (Bellés and Piulachs, 1983). Thus, 12 of the products studied here, i.e. **1–4**, **6**, **7**, **9–13** and **15** (see Table 1), are more active than triphenyl tin acetate, which is often used as a standard for antifeedant studies (Chapman, 1974). The antifeedant activity of a series of natural or semi-synthetic clerodane diterpenes (Bellés *et al.*, 1985) subjected to the same assay could also be used for comparison purposes. The more active compounds of this series, 14,15-dihydroajugapitin, ajugapitin and 2-acetyl-14,15-dihydroajugapitin, gave $FR_{50} = 0.32$, 0.41 and 0.62, respectively, at a dose of 0.1 μg cm^{-2}. Only compound **4** showed comparable activity and it, unlike the three clerodane diterpenoids, maintained antifeedant capability even in non-choice test conditions which is obviously of interest for practical application.

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