Activity of Triterpenoids from *Cedrela fissilis* (Meliaceae) against *Spodoptera frugiperda*

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ABSTRACT The activity of five triterpenoids, oleanolic acid, oleanonic acid, hispidol A, piscidinol A and odoratol, isolated from stems and fruits of *Cedrela fissilis* (Meliaceae), was evaluated against the fall armyworm *Spodoptera frugiperda*. Toosendanin was used as a positive control. A prolongation of the larval phase was observed for insects treated with oleanolic acid, oleanonic acid and odoratol at 1.0, 10.0 and 50.0 mg/kg when compared with the control. All the tested compounds reduced pupal weight. The best results were obtained with odoratol that showed 90% of larval mortality. Correlation between insecticidal activity and chemical structure has been discussed**.**

KEY WORDS : *Cedrela fissilis*, Meliaceae, *Spodoptera frugiperda*, Triterpenoids

INTRODUCTION

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is a major pest of many crops in the Americas. In Brazil, it is one of the most important pests of maize plantations, with production losses reaching 34% (Beserra *et al.*, 2002).

These insects are usually controlled using agrochemicals, when the defoliation is noticed in maize crops. However, due to the problems associated with the use of pesticides, especially the possibility of insecticidal resistance and reduction of predator and parasitoid insects, emphasis is being shifted to natural control (Batista-Pereira *et al.*, 2002).

The increasing interest in the possible application of secondary metabolites in pest management has directed the investigation toward search for new sources of biologically active natural products with low mammalian toxicity, lack of neurotoxic mode of action, low persistence in the environment and biodegradability, as well as to avoid the development of resistance by the insect pest (Cespedes *et al.*, 2000).

Members of Meliaceae are known to produce limonoids, a group of secondary metabolites of variable structure and diversity of biological properties. Azadirachtin is the best known example of limonoids, isolated from *Azadirachta indica* (Meliaceae). This compound and its analogues are potent insect antifeedants and ecdysis inhibitors (Champagne *et al.*, 1992). *Cedrela fissilis* (Meliaceae) known as tropical cedar, is a valuable source of timber. In Brazil, it can be found from the Amazon forest as far south as Espírito Santo State. Previous investigation of fruits and seeds from this species have shown the presence of the limonoids fissinolide, mexicanolide and 3β-hydroxyisomexicanolide occurring in this plant species (Zelnik and Rosito, 1966, 1971).

In this paper we describe the evaluation of the activity of five triterpenoids, oleanolic acid (**1**),

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oleanonic acid (**2**), hispidol A (**3**), piscidinol A (**4**) and odoratol (**5**) (Fig. 1) isolated from stems and fruits of *C. fissilis* against *S. frugiperda*.

MATERIALS AND METHODS

Plant Material

The fruits and stems of *Cedrela fissilis* Vell. were collected in São Carlos-SP, Brazil in 2001 and identified by Dr. Maria Inês Salgueiro Lima, Department of Botany, Universidade Federal de São Carlos, where a voucher specimen (6701) was deposited.

Isolation of Compounds

The powdered air-dried fruits and stems (400 g) from *C. fissilis* were extracted by maceration three times (72 h) with 1000 ml hexane, CH_2Cl_2 and/or methanol at room temperature. The solvent was removed under reduced pressure by rotary evaporation. The CH_2Cl_2 extract (5.0 g) from fruits and the methanolic extract from stems of *C. fissilis* were subjected to vacuum liquid chromatography (VLC) on silica gel (70–230 mesh) using a hexane-CH_2Cl_2-EtOAc-MeOH gradient (100:0 → 0:100**;** 1.5 l of each solvent).

The EtOAc soluble fraction of CH_2Cl_2 extract from fruits of *C. fissilis* (3.0 g) was chromatographed on silica gel (70-230 mesh) and eluted with hexane-CH_2Cl_2-acetone gradient, giving 7 fractions (A→G). Fraction B was chromatographed as above to give 8 fractions. Fraction B-3 was twice chromatographed with hexane-CH_2Cl_2-acetone (6:3:1) to afford compounds **1** (25.9 mg) (Maillard *et al.*, 1992) and **2** (36.5 mg) (Fatope *et al.*, 2002).

The mixture of hexane and CH_2Cl_2 soluble fractions of methanolic extract from stems of *C. fissilis* (11.8 g) was eluted on Sephadex LH-20 with CH_2Cl_2/MeOH (1:1) to give 12 fractions (A→L). Fraction B was chromatographed on silica gel (230-400 mesh) using hexane-CH_2Cl_2-MeOH (6:3.5:0.5). Fractions B-3 and B-5 were twice eluted on silica gel (230-400 mesh) with CH_2Cl_2-MeOH gradient to give compounds **3** (35.6 mg) (Jolad *et al.*, 1981) and **4** (40.2 mg) (McChesney *et al.*, 1997). Fraction D was chromatographed as fraction B. Fraction D-3 was eluted three times using the same conditions applied to fraction B-5 to give compound **5** (23.2 mg) (Chan *et al.*, 1968).

All compounds were rigorously characterized by spectroscopic methods (UV, IR, ^{1}H NMR, ^{13}C NMR, HMBC, HSQC, MS) and chemical data which corresponded to the data already available in literature.

Biological Activities

Larvae of *S. frugiperda* were obtained from the Insect Bioassay Laboratory of Universidade Federal de São Carlos, Brazil, and reared on artificial diets (Kasten *et al.*, 1978). They were maintained in an incubation chamber with a photo phase of 12:12h (L:D), 70 ± 5 % relative humidity and 25 ± 1°C.

For each treatment and control, 30 neonate larvae of *S. frugiperda* were used. A solution of triterpenoid was added to ascorbic acid (1.56 g; an ingredient of the diet), after evaporation, the mixture was incorporated to the artificial diet in which bean and wheat germ are the basic ingredients (Kasten *et al.*, 1978) at final concentrations of 1.0, 10.0, 50.0 and 100.0 mg/kg (triterpenoids **3** and **5**). For oleanolic acid (**1**), oleanonic acid (**2**) and piscidinol A (**4**) final concentrations used were 1.0, 10.0 and 50.0 mg/kg. Diet for the control was prepared similarly but without any treatment. Toosendanin (**6**) was used as standard. The diets were placed in previously sterilized glass tubes (8.5 ´ 2.5 cm), into which larvae of *S. frugiperda* were introduced individually. The obtained pupae were weighed one day after pupation and were transferred into plastic cups and maintained until the emergence of adults. Daily observations were made and the following parameters were evaluated: (i) duration of larval and pupal phases; (ii) weight of pupae and (iii) percentage of dead insects (mortality) at the end of each phase.

Statistical Analysis

Data were submitted to an analysis of variance ANOVA (Zar, 1984) and the averages were compared applying the Tukey test ($P \leq 0.05$) for pupal weight. Data of duration of larval and pupal phases were submitted to Kruskal Wallis test. Each tube containing one insect, independent of the

development phase was considered as one replicate, therefore, the number of replicates was different for each treatment. For evaluation of the mortality of the larval and pupal phases, the experimental unit was constituted by the mean of five tubes with one larva each, with six replicates per treatment.

RESULTS AND DISCUSSION

A prolongation of the larval phase was observed for insects treated with oleanolic acid (**1**), oleanonic acid (**2**) and odoratol (**5**) at 1.0, 10.0 and 50.0 mg/kg when compared with the control. The toosendanin (**6**) also showed prolongation of larval phase at 50.0 and 100.0 mg/kg treatment. Only the larvae fed with odoratol (**5**) at 1.0, 10.0 and 50.0 mg/kg showed alterations in the pupal phase when compared with the control. All the tested compounds reduced pupal weight.

A prolongation of larval duration by 6.5 days as compared to controls (22.7 days) was observed for larvae treated with odoratol (**5**) at 1.0 mg/kg in addition to a significant reduction (42.04 mg) of pupal weight, when compared with controls (272.74 mg) (Table 1). However, odoratol (**5**) at 10.0 mg/kg, there was further delay in development and larval duration was prolonged by 10.6 days and the pupal weight was reduced to 50.64 mg. Thus with the increase in treatment of odoratol (**5**), there was continued

Table 1. Mean duration of larval stage and pupal weight with triterpenoids (1- 5) and limonoid (6) administered in the artificial diet of *Spodoptera frugiperda*

Compounds	Concentration (mg/kg)	Duration of larval phase (days) (± SD)[a]	Weight of pupae (mg)[b]
Oleanolic acid (**1**)	1.0	28.2 ± 4.8) a	242.33 b
	10.0	28.4 ± 1.8) a	226.18 b
	50.0	28.4 ± 4.7) a	225.29 b
Control		22.7 ± 3.0) b	272.74 a
Oleanonic acid (**2**)	1.0	26.0 ± 2.9) a	242.29 b
	10.0	28.3 ± 3.1) a	238.25 b
	50.0	29.2 ± 5.7) a	224.57 b
Control		22.7 ± 3.0) b	272.74 a
Hispidol A (**3**)	1.0	21.2 ± 3.0) b	252.89 a
	10.0	21.5 ± 5.1) b	252.75 a
	50.0	19.9 ± 1.7) ab	251.68 a
	100.0	21.0 ± 2.3) ab	252.38 a
Control		22.7 ± 3.0) a	272.74 a
Piscidinol A (**4**)	1.0	22.9 ± 3.5) a	242.85 b
	10.0	22.5 ± 2.9) a	238.95 b
	50.0	22.7 ± 2.3) a	231.89 b
Control		22.7 ± 3.0) a	272.74 a
Odoratol (**5**)	1.0	29.2 ± 5.3) ab	230.80 bc
	10.0	33.3 ± 4.9) ab	222.20 bc
	50.0	53.3 ± 3.8) a	179.37 c
Control		22.7 ± 3.0) b	272.74 a
Toosendanin (**6**)	1.0	19.1 ± 2.7) b	259.35 a
	10.0	19.9 ± 3.4) b	258.96 a
	50.0	24.4 ± 3.3) ab	216.22 b
	100.0	31.0 ± 5.2) a	203.14 b
Control		22.7 ± 3.0) ab	272.74 a

[a]Means followed by the same letters within columns indicates no significant difference ($P \leq 0.05$) in the Kruskal Wallis test.

[b]Means followed by the same letters within columns indicates no significant difference ($P \leq 0.05$) in the Tukey test.

Table 2. Mean mortality (%) of the larval phase of *Spodoptera frugiperda* with triterpenoids (1–5) and limonoid (6) administered in the artificial diet

	Concentration (mg/kg)[a]				
Compound	Control	1.0	10.0	50.0	100.0
Oleanolic acid (**1**)	10.0 b	40.0 a	43.3 a	56.7 a	
Oleanonic acid (**2**)	10.0 b	43.3 a	46.7 a	53.3 a	
Hispidol A (**3**)	10.0 b	30.0 ab	33.3 ab	33.3 ab	56.7 a
Piscidinol A (**4**)	10.0 b	30.0 a	33.3 a	33.3 a	
Odoratol (**5**)	10.0 b	46.7 b	63.3 ab	66.7 ab	90.0 a
Toosendanin (**6**)	10.0 a	10.0 a	10.0 a	23.3 a	26.7 a

[a]Means followed by the same letters within a row indicates no significant difference ($P \leq 0.05$) in the Tukey test.

prolongation of larval period and further reduction in pupal weight (At 50.0 mg/kg treatment prolongation of the larval phase of 30.6 days and reduction of pupal weight to 93.47 mg was recorded, Table 1). The larvae treated with odoratol (**5**) also reduced pupal phase when compared to the controls. These data suggest that odoratol (**5**) acts as a larval growth inhibitor and antifeedant. As a consequence, the insect could be more vulnerable to the action of entomopathogens, entomophagous agents and environmental variations (Tanzubil and McCaferry, 1990). Adults emerging from low-weight pupae could be more debilitated and would have lower capacity of competition for vital activities than individuals from healthy pupae (Batista-Pereira *et al.*, 2002).

Oleanolic acid (**1**) and oleanonic acid (**2**) also demonstrated prolongation of larval phase followed by reduction of pupal weight. The larvae treated with oleanolic acid (**1**) showed prolongation of larval phase by 5.5 (1.0 and 10.0 mg/kg) and 5.7 days (50.0 mg/kg), respectively when compared with controls (22.7 days) (Table 1). The reduction of pupal weight was of 30.41; 46.46 and 47.25 mg at 1.0, 10.0 and 50.0 mg/kg treatments, respectively, when compared with the controls (272.74 mg) (Table 1). For the larvae treated with oleanonic acid (**2**) at similar level of treatments prolongation of larval phase was by 4.3; 5.6 and 6.5 days followed by a reduction of pupal weight by 30.45; 34.49 and 48.17 mg, respectively (Table 1). Thus Oleanolic acid (**1**) and oleanonic acid (**2**) were also the larval growth inhibitors.

Hispidol A (**3**) and piscidinol A (**4**) did not show any significant variation in the larval and pupal stages though some reduction in pupal weight due to piscidinol A (**4**) treatment was recorded (Table 1).

Toosendanin (**6**) used as standard showed comparable activity with the compounds evaluated (Table 1), however, odoratol (**5**) was apparently better than toosendanin (**6**) in its activity against *S. frugiperda*. In fact, odoratol (**5**) was the most active compound as a larval growth inhibitor (Tables 1 and 2). Oleanolic (**1**) and oleanonic acids (**2**) possessed similar activity, which can be attributed to their similar chemical structure with only difference of a hydroxyl in **1** replaced by a carbonyl in **2** (Figure 1). Both compounds showed significant effect on larval and pupal growth (Table 1). Hispidol A (**3**) and piscidinol A (**4**) though similar in their activity yet they were slightly more active than Oleanolic type of compounds. In terms of the mortality hispidol A (**3**) and piscidinol A (**4**) induced moderate mortalities (< 40 %) (Table 2). Oleanolic acid (**1**) and oleanonic acid (**2**) induced > 50% mortality at 50 mg/kg level compared to hispidol, which was required at 100 mg/kg level to induce similar level of mortality. Odoratol (**5**), however, was most effective compound, inducing 66.7% mortality at 50.0 mg/kg treatment and 90% at 100 mg/kg treatment. It was obvious that the compounds evaluated in present study were significantly more active than the standard toosendanin (**6**) (Table 2). However, earlier studies have shown that oleanolic acid (**1**) was a moderate feeding attractant and induced significant post-ingestive toxicity to *Sitophilus oryzae* (Pungitore *et al.*, 2005) besides being antifeedant against *Heliothis*

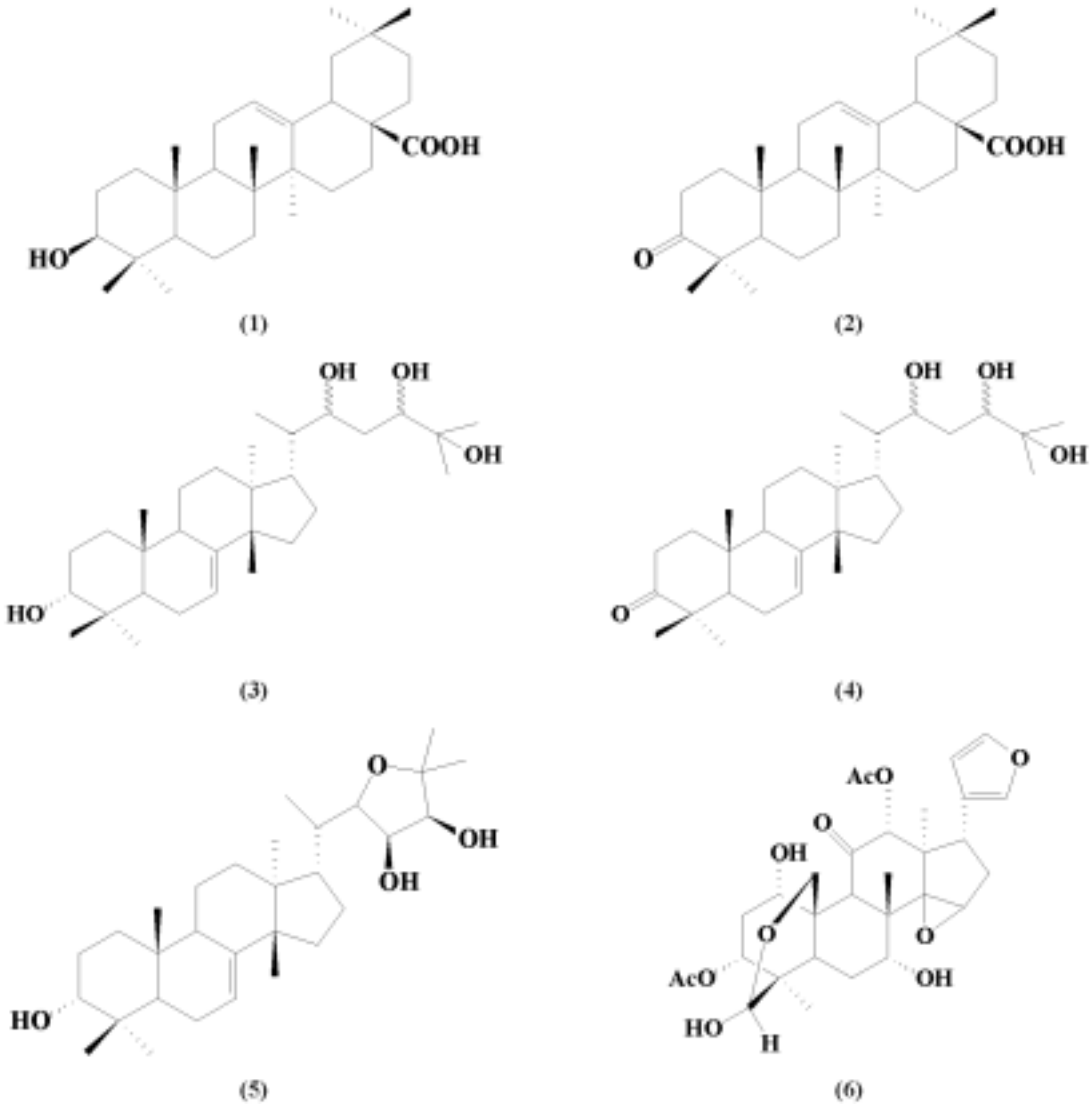

Fig. 1. Triterpenoids (1–5) isolated from *Cedrela fissilis* and toosendanin (6).

zea (Argandoña and Faini, 1993). The other compounds, though known, have not been evaluated against any other insect species. The high insecticidal activity of odoratol (**5**) can probably be related with the presence of two hydroxyl groups at C-23 and C-21, as shown in other tetranortriterpenoids (Suresh *et al.*, 2002). Céspedes *et al*. (2000) suggest that the presence of an oxygenated function at C-23 was necessary for the activity of photogedunin epimeric acetate mixture and photogedunin epimeric mixture against *S. frugiperda*, both isolated from *Cedrela salvadorensis* and *Cedrela dugessi* (Meliaceae). Odoratol (**5**) showed higher larval mortality than cedrenolide, isolated from *C. salvadorensis* against European corn borer, *Ostrinia nubilalis* Hübner (Jimenez *et al*., 1997; Arnason *et al*., 1987).

Therefore triterpenoids oleanolic acid (**1**), oleanonic acid (**2**) and odoratol (**5**), seem to be good insect growth inhibitors and antifeedants, which are either comparable or even more active than the recent Chinese commercial insecticide toosendanin (**6**). Odoratol (**5**) showed significant activity, which suggests its potential for further exploitation as insect control agent in an IPM strategy.

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ABREVIATIONS USED

CH_2Cl_2, dichloromethane; VLC, vacuum liquid chromatography; EtOAc, ethyl acetate; MeOH, methanol; UV, ultra violet; IR, infra red; ^{1}H NMR, hydrogen resonance magnetic nuclear; ^{13}C NMR, carbon resonance magnetic nuclear; HMBC, heteronuclear multiple quantum correlation; HSQC, heteronuclear single quantum correlation; MS, mass spectrometry.

REFERENCES

Argandoña, V.H. and Faini, F.A. (1993) Oleanolic acids content in *Baccharis linearis* and its effects on *Heliothis zea* larvae. *Phytochemistry*, **33**, 1377–1379.

Arnason, J.T., Philogène, B.J.R., Donskov, N. and Kubo, I. (1987) Limonoids from the Meliaceae and Rutaceae reduce feeding, growth and development of *Ostrinia nubilalis*. *Entomol. Exp. Appl*., **43**, 221–226.

Batista-Pereira, L.G., Petacci, F., Fernandes, J.B., Corrêa, A.G., Vieira, P.C., Da Silva, M. F.G.F. and Malaspina, O. (2002) Biological activity of astilbin from *Dimorphandra mollis* against *Anticarsia gemmatalis* and *Spodoptera frugiperda*. *Pest Manage. Sci*., **58**, 503–507.

Beserra, B.B., Dias, C.T.S. and Parra, J.R.P. (2002) Distribution and natural parasitism of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) eggs at different phenological stages of corn. *Florida Entomologist*, **85**, 588–592.

Céspedes, C.L., Calderón, J.S., Lina, L. and Aranda, E. (2000) Growth inhibitory effects on fall armyworm *Spodoptera frugiperda* of some limonoids isolated from *Cedrela* spp. (Meliaceae). *J. Agric. Food Chem*., **48**, 1903–1908.

Champagne, D.E., Koul, O., Isman, M.B., Scudder, G.G.E. and Towers, G.H.N. (1992) Biological activity of limonoids from the Rutales. *Phytochemistry*, **31**, 377–394.

Chan, W.R., Holder, N.L., Snatzke, G., Fehllaber, H.W. and Taylor, D.R. (1968) Extractives of *Cedrela odorata*. II. The structures of *Cedrela* tetracyclic triterpenes odoratol, isoodoratol and odoratone". *J. Chem. Soc. C*., **20**, 2485–2489.

Fatope, M.O., Salihu, L., Asante, S.K. and Takeda, Y. (2002) Larvicidal activity of extracts and triterpenoids from *Lantana camara*. *Pharm. Biol*., **40**, 564–567.

Jimenez, A., Mata, R., Pereda-Miranda, R., Calderón, J., Isman, M. B., Nicol, R. and Arnason, J.T. (1997) Insecticidal limonoids from *Swietenia humilis* and *Cedrela salvadorensis*. *J. Chem. Ecol*., **23**, 1225–1234.

Jolad, S.D., Hoffmann, J.J., Schram, K.H. and Cole, J.R. (1981) Constituents of *Trichilia hispida* (Meliaceae). 4. Hispidols A and B, Two New tirucallane triterpenoids. *J. Org. Chem*., **46**, 4085–5088.

Kasten, P.J.R., Precetti, A.A.C.M. and Parra, J.R.P. (1978) Dados biológicos comparativos de *Spodoptera frugiperda* (JE Smith, 1797) em duas dietas artificiais e substrato natural. *Rev. Agric.*, **53**, 68–78.

Maillard, M., Adewunmi, C.O. and Hostettamann, K. (1992) A triterpene glycoside from the fruits of *Tetrapleura tetraptera*. *Phytochemistry*, **31**, 1321–1323.

McChesney, J., Dou, J., Sindelar, R.D., Goins, D.K., Walker, L.A. and Rogers, R.D. (1997) Tirucallane-type triterpenois: nmr and X-ray diffraction analyses of 24-*epi*-piscidinol A and piscidinol A. *J. Chem. Crystallogr.*, **27**, 283–290.

Pungitore, C.R., García, M., Gianello, J.C., Sosa, M.E. and Toon, C.E. (2005) Insecticidal and antifeedant effects of *Junellia aspera* (Verbenaceae) triterpenes and derivatives on *Sitophilus oryzae* (Coleoptera: Curculionidae). *J. Stored Prod. Res.*, **41**, 433–443.

Suresh, G., Gopalakrishnan, G., Wesley, S.D., Singh, N.D.P., Malathi, R. and Rajan, S.S. (2002) Insect antifeedant activity of tetranortriterpenoids from the Rutales. A perusal of structural relations".

J. Agric. Food Chem., **50**, 4484–4490.

Tanzubil, P.B. and McCaferry, A.R. (1990) Effects of azadirachtin and aqueous neem seed extracts on survival, growth and development of the African armyworm, *Spodoptera exempta*. *Crop Prot.*, **9**, 383–386.

Zar, J.H. (1984) *Biostatistical Analysis*, Prentice-Hall Inc, Englewood Cliffs, NJ.

Zelnik, R. and Rosito, C.M. (1966) Le Fissinolide. *Tetrahedron Lett.*, **7**, 6441–6444.

Zelnik, R. and Rosito, C.M. (1971) Tetranortriterpenoids from *Cedrela fissilis*. *Phytochemistry*, **10**, 1955–1956.

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