

Chemistry of *Toona ciliata* and *Cedrela odorata* graft (Meliaceae): chemosystematic and ecological significance*

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Abstract: *Toona* differs from other genera of the Swietenioideae, notably by the absence of limonoids of the mexicanolide group. Our earlier phytochemical studies on *T. ciliata*, showed the presence of limonoids with intact carbon skeleton, B-*seco*-limonoids with rings A, C and D intact and five new pregnanes. The latter group of compounds has so far been recorded in four genera of the Melioideae. Thus, the difference in meliacin composition and the finding of pregnanes do not seem to favour the affiliation of *Toona* to the Swietenioideae. From the stem of *Cedrela odorata* grafted on *T. ciliata* var. *australis* were isolated sesquiterpenes, cycloartanes, stigmasterol, campesterol, sitostenona, limonoids, apotirucallane, tirucallanes and catechin. The limonoids were of little value to clarify the basis of the induced resistance in the graft against *Hypsipyla grandella*. The cycloartanes and catechin could have been translocated from *Toona* stock to the *Cedrela* graft.

INTRODUCTION

The family Meliaceae provides the most valuable timbers, such as mahogany (*Swietenia*) and cedar (*Cedrela*), which have been illegally exported from Brazil. At present they are scarce and efforts to establish large scale homogeneous plantations have almost invariably failed due to larval attacks by the shoot borer *Hypsipyla*. Main damage is caused by the larvae, which destroy the succulent terminal shoots by boring into the tip and tunnelling in the juvenile stems of saplings and seedlings. Re-sprouting of the plants, followed by repeated attacks of the insect, generally results in the development of numerous side branches and consequently in badly formed trees, unsuitable for timber production. *Hypsipyla grandella* is considered to be the most harmful species in Latin America. *Toona ciliata*, the Australian red cedar, introduced to Brazil shows excellent growth and an absence of attacks by *H. grandella*, in contrast to the native *Cedrela odorata* [1].

Toona was originally described by Endlicher (1840) as a section of *Cedrela*. Roemer later (1846) recognized that it could be separated by a number of sound morphological characters, raising *Toona* to generic rank. Thus, the old world species of *Cedrela* were transferred to *Toona* (Endlicher) M. J. Roemer. The two genera were placed by Harms (1940) in the tribe Cedreleae under Cedreloideae. Pennington and Styles (1975), in their more recent monograph, include Cedreleae into the Swietenioideae [1].

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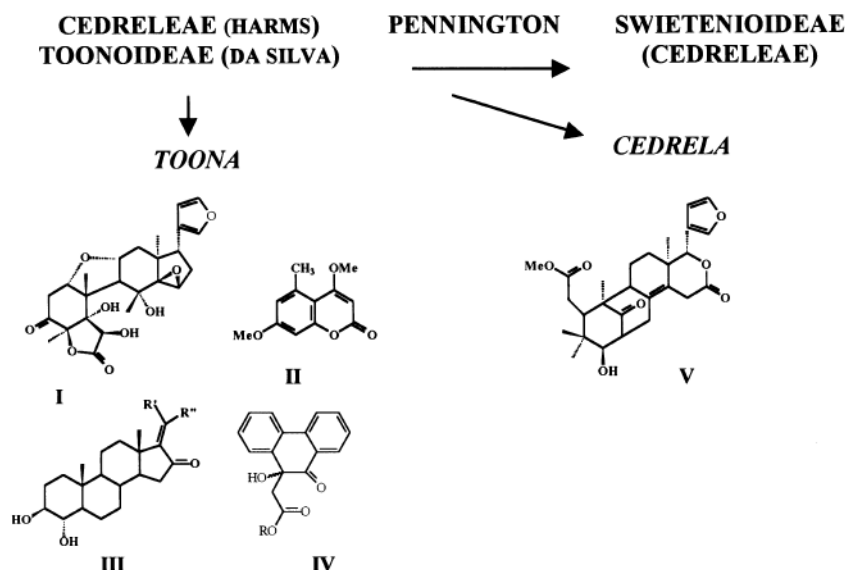
RESULTS AND DISCUSSION

Chemical systematics within the Cedreloideae

Chemically, the family Meliaceae is distinguished by the frequent occurrence of limonoids. The mexicanolide group occurs widely in the genera of the Swietenioideae. The Melioideae, which has shown absence of attacks of *H. grandella*, appears to be the most prolific in production of A,B-*seco* limonoids but relatively poor in mexicanolide types. The C-*seco* limonoids have so far been recorded in four genera of the Melioideae (*Turrea*, *Trichilia*, *Melia*, and *Azadirachta*) [1]. As reported in previous papers [1] the known limonoids from *Cedrela* are typical of the Swietenioideae. On the other hand, *Toona* differs from other genera of this subfamily, notably by the absence of limonoids of the mexicanolide group. Our earlier phytochemical studies on *T. ciliata*, showed the presence of limonoids with intact carbon skeleton 21-hydroxycedrelonolide, 23-hydroxycedrelonolide, 6 α -acetoxo-14 β ,15 β -epoxyazadirone, rings-A,C,D-intact-ring-B-*seco*-limonoids (**I**) 12-deacetoxystoonacilin, 5 α ,6 β ,8 α -trihydroxy-28-norisotoonafolin and 5 α ,6 β ,8 α ,12 α -tetrahydroxy-28-norisotoonafolin, the coumarin siderin (**II**) [1–3] and five new pregnanes (**III**) 3 β ,4 α -dihydroxy-5 α -pregnan-17(20)-(Z)-en-16-one, 3 β ,4 α -dihydroxy-5 α -pregnan-17(20)-(E)-en-16-one, 2 α ,3 α -dihydroxy-5 α -pregnan-17(20)-(Z)-en-16-one, 2 α ,3 α -dihydroxy-5 α -pregnan-17(20)-(E)-en-16-one and 3 β ,4 α -dihydroxy-5 α -pregnan-16-one. The latter group of compounds has so far been recorded in four genera of the Melioideae, *Melia*, *Turrea*, *Trichilia* and *Aglaia* and B-*seco*-limonoids with rings A, C and D intact are feature that are largely confined to this subfamily. Thus, the difference in meliacin composition and the finding of pregnanes do not seem to favour the affiliation of *Toona* to the Swietenioideae. Moreover, we have examined the roots of *T. ciliata* since they have never been investigated before and in order to determine if the above differences still remain in this organ. The dichloromethane extract afforded four new 9,10-dihydrophenanthrenes (**IV**) which were identified on the basis of spectroscopic analysis as 9-hydroxy-9-(*tert*-butoxycarbonylmethyl)-10-oxophenanthrene, 9-hydroxy-9-(ethoxycarbonylmethyl)-10-oxophenanthrene, 9-hydroxy-9-(*n*-butoxycarbonylmethyl)-10-oxophenanthrene and 9-hydroxy-9-(benzyloxycarbonylmethyl)-10-oxophenanthrene. This appears to be the first record of phenanthrenes from Meliaceae or from the allied families of the order Rutales (Rutaceae, Meliaceae, Simaroubaceae and Cneoraceae). In addition, these compounds represent a novel group of unsubstituted phenanthrenes. Oxidation involving phenyl rings, as hydroxyl and methoxyl substituents, has been found in Orchidaceae. It can of course be argued that the roots were collected with a trace of other roots which do not belong to *T. ciliata*. However, subsequent to this work we ourselves have found these compounds in stock of an 5-year-old tree of *C. odorata* grafted on *T. ciliata* (part to be published and [4]). Therefore, all these phytochemical findings are in favour to include *Toona* into Melioideae or, even better, to treat it as a separate subfamily, Cedreloideae, as did Harms. However, this latter name is based on genus *Cedrela* and eventually will have to be replaced by Toonoideae. *Cedrela*, as discussed before, with their mexicanolide limonoids (**V**) has been added into Swietenioideae (Scheme 1).

Chemistry of *Cedrela odorata* graft

Cedrela odorata has been grafted to stems of *T. ciliata* and the resistance has been translocated from the *Toona* stock to the *Cedrela* graft [4]. The chemistry of these grafts have not been previously studied. Thus, we have now examined the stem of *C. odorata* grafted on *T. ciliata* var. *australis*, in order to determine if secondary metabolites present in the latter could be translocated into the former. The stem of *C. odorata* graft, afforded (Fig. 1) the sesquiterpenes calamenene (**VI**), 7-hydroxycalamene and 5-hydroxycalamene, the cycloartanes (**VII**) cycloartenol (**1**), 24-methylenecycloartan-3 β -ol (**2**), cycloartenone (**3**), 24-methylenecycloartan-3-one (**4**), 30-nor-24-methylenecycloartan-3-one (**5**), cycloeucalenol (**6**) and 3 β -O- β -D-glucopyranosylcycloeucalenol (**7**), the ring-D-lactone-limonoids (**VIII**) gedunin (**1**), 7-deacetylgedunin (**2**), 7-deacetoxy-7-oxogedunin (**3**), photogedunin (**4**), 1 α -methoxy-1,2-dihydrogedunin (**5**), the B,D-*seco* limonoids (**IX**) methylangolensate (**6**), mexicanolide limonoids (**X**) 3 β -deacetylfiissinolide (**7**), febrifugin (**8**) and intact limonoids (**XI**) azadiradione (**9**), the apotirucallane 20,21,22,23-tetrahydro-23-oxoazadirone (**10**), the tirucallanes (**XII**) tirucallol (**1**), 22 ξ -hydroxytirucalla-7,24-dien-3,23-dione (**2**), 22 ξ ,3 α -dihydroxytirucalla-7,24-dien-23-one (**3**), 22 ξ ,3 β -dihydroxytirucalla-7,24-dien-23-one (**4**), odoratone (**5**), odoratol (**6**), *iso*-odoratol (**7**) and pentaol (**8**) and the flavonoid (**XIII**) catechin [4]. Thus, *C. odorata* graft produces a considerable number of limonoids which are



Scheme 1 The chemical evidence do not seem to favour the affiliation of *Toona* to the Swietenioideae.

common in *Cedrela*, but **2**, **5**, **7**, **9**, and the apotirucallane **10** were not previously found from the latter. Azadiradione (**9**) and 20,21,22,23-tetrahydro-23-oxoazadirone (**10**) are typical of the *Toona* [2], but they might be the biosynthetic intermediates to *Cedrela* limonoids. Studies of the structure/anti-insect activity relationships for several limonoids have established that aside from the *C-seco* limonoids, the most active compounds appear to be intact apo-euphol limonoids with a 14,15-epoxide and either a 3-oxo-1-ene A ring. Absence of the 14,15 β -epoxide results in reduced activity, as with azadiradione (**9**). Thus, the finding of both limonoids **9** and **10** is of little value to clarify the basis of induced resistance in the graft. Representatives of cadinene series have been reported from stem of *C. odorata* and *T. ciliata*, but calamenene is known only from the former, 7-hydroxycalamenene was not previously found from Meliaceae and 5-hydroxycalamenene appear to be new. The tirucallanes are so far restricted to *Cedrela*, however, it seem that this is the first time that tirucallane **3** and **4** has been described in the literature. Flavonols and 3-glycoside derivatives were found in leaves of *C. sinensis*, but not so far in any other *Cedrela*. Proanthocyanidins have otherwise been reported from only stem of *T. ciliata*. The genus *Cedrela* has been widely investigated, but, to date, there are no records of cycloartanes. Our own investigations of the stem and leaves of *T. ciliata* revealed the presence of cycloeucalenol (**6**) and 3 β -O- β -D-glucopyranosylcycloeucalenol (**7**). Since the latter types and proanthocyanidins have not been found in *C. odorata*, the occurrence of both cycloartanes (**1**–**7**) and catechin (in great amounts) in the graft, suggests that these constituents could have been translocated from the *Toona* stock to the *Cedrela* graft. However, both species should be re-examined for condensed tannins and their precursors (as catechin), and *C. odorata* for cycloartanes (Fig. 1).

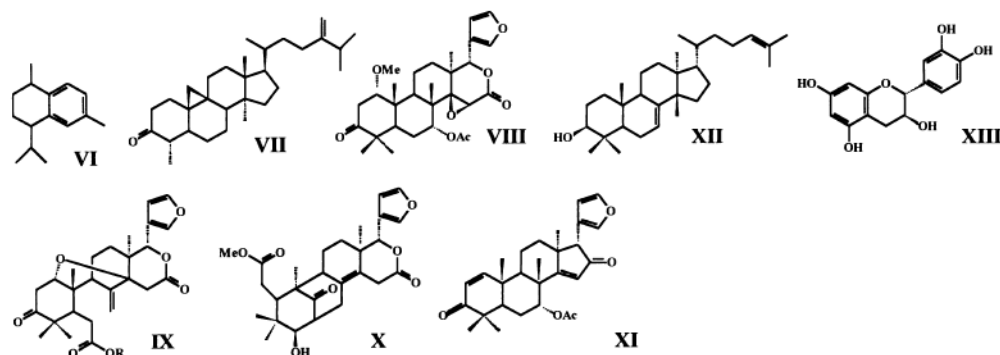


Fig. 1 Compound types of *Cedrela odorata* graft.

Speculations on the induced resistance against *Hypsipyla grandella*

We have investigated the feeding deterrence/or growth inhibitory activities produced by extracts from stem, stem bark and leaves of *T. ciliata* against the larvae of the lepidopteran *H. grandella*. A number of compounds which were isolated from these extracts and from *C. odorata* graft were also tested. However, our *H. grandella* colony has been reared in the laboratory only for 3 generations. These insects needed more time to complete the metamorphosis from larva to adult and they oviposited less fertilized eggs than wild-types. At present we doubt if we have a good rearing system of *H. grandella* to allow us to make definitive statements. Thus, we also tested the extracts and the same compounds against other lepidopteran, *Spodoptera frugiperda*, for comparison. Despite these limitations, all extracts and compounds analyzed against *H. grandella* exhibited insecticidal activity close to those against *S. frugiperda*. However, we will present here only the effects of extracts (Table 1) and compounds (Table 2) on the growth of first instar *S. frugiperda*.

Table 1 *Spodoptera frugiperda* bioassay: results for *Toona ciliata* extracts

Extracts (2000 p.p.m.)	Larval weight (% of control)*
Control (64.02 mg)	
Hexane—stem bark	73.66 b
Methanol—stem bark	66.79 b c
Methanol—stem	62.51 b c
Dichloromethane—stem	57.61 b c d
Dichloromethane—stem bark	53.97 b c d
Methanol—leaves	47.67 c d e
Hexane—stem	40.05 d e
Hexane—leaves	33.24 e f
Dichloromethane—leaves	20.62 f

* Determined on day 8. Values followed by the same letter are not statistically significant at 5% (Newman Keuls). For each experiment (12 replicates were done), the diet blank and solvent (methanol/acetone, larval mean weight 64.02 mg) controls were first tested for consistency.

Table 2 Insect growth inhibitory activity of *T. ciliata* and *C. odorata* graft compounds. Values are the dietary concentrations for 50% growth inhibition (EC_{50})

Compounds	<i>S. frugiperda</i>
6 α -Acetoxy-14,15 β -epoxyazadirone	108.5 p.p.m.
Toonacilin	103.9 p.p.m.
12-Deacetoxytoonacilin	28.2 p.p.m.
Odoratol	21.4 p.p.m.
Gedunin	15.7 p.p.m.
Sitosterol	12.7 p.p.m.
α - and β -Amyrins—fatty acids	14.9 p.p.m.
Cycloeucalenol	7.7 p.p.m.
Cedrelone	5.2 p.p.m.

Siderin appears to be the feeding stimulant: the larval weight was 125% of the control (64.02 mg).

For a concentration of 2000 p.p.m. incorporated into the artificial diet [5] during 8 days, all extracts inhibited the growth of first instar *S. frugiperda*. The effects produced by hexane extracts of the stem and leaves and dichloromethane extract of the leaves (slower weight gain, 20.62% of the control) were more pronounced than the others. The occurrence of cedrelone in great amount in the hexane extract of the stem suggested that it could act as a growth inhibitor. It was confirmed by *H. grandella* and *S. frugiperda*.

bioassays. Cedrelone was the most potent against *S. frugiperda*, the effective concentration for 50% growth inhibition (EC_{50}) being 5.2 p.p.m. In the same test azadirachtin, one of the most efficient anti-feedants of plant origin, exhibited an EC_{50} of 0.4 p.p.m. The dichloromethane extract of leaves was the most active and it was characterized by large amounts of pregnanes, which are at present unknown to any other organs of *T. ciliata*. However, these compounds were obtained recently and they were not evaluated as a larval growth inhibitor for *S. frugiperda*. This extract also afforded cycloeucalenone and cycloeucalenol. The latter showed potent growth inhibitory activity (7.7 p.p.m.) and was also found in *C. odorata* graft.

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REFERENCES

- 1 S. M. M. Agostinho, M. F. das G. F. da Silva, J. B. Fernandes, P. C. Vieira, A. L. Pinheiro, E. F. Vilela. *Biochem. Syst. Ecol.* **22**, 323 (1994).
- 2 J. Oiano-Neto, S. M. M. Agostinho, M. F. das G. F. da Silva, J. B. Fernandes, P. C. Vieira, A. L. Pinheiro, E. F. Vilela. *Phytochemistry* **38**, 397 (1995).
- 3 J. Oiano-Neto, M. F. das G. F. da Silva, Fo E. Rodrigues, J. B. Fernandes, P. C. Vieira, A. L. Pinheiro, E. F. Vilela. *Phytochemistry* **49**, 1369 (1998).
- 4 J. R. de Paula, I. J. C. Vieira, M. F. das G. F. da Silva, Fo E. Rodrigues, J. B. Fernandes, P. C. Vieira, A. L. Pinheiro, E. F. Vilela. *Phytochemistry* **44**, 1449 (1997).
- 5 J. R. P. Parra. *Técnicas de criação de insetos*. ESALQ/USP, Piracicaba-SP-Brazil (1994).