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Rep. Fac. Sci. Kagoshima Univ. (Math., Phys. & Chem.),
No. 27, 45-52, 1994.

Limonoid Antifeedants from the Root Bark of Chinese *Melia Azedarach* L.

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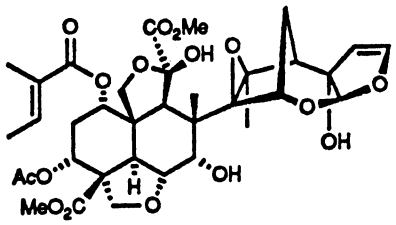
(Received Sept. 12, 1994)

Abstract

Nine new limonoids have been isolated as insect antifeedants along with six known limonoids from the root bark of Chinese *Melia azedarach* Linn (Meliaceae).

Introduction

Meliaceae plants are a rich source of limonoids. The neem tree *Melia Azadirachta indica* Juss and the related tree *Melia azedarach* Linn are attracting considerable interest, particularly because of their insect antifeedant properties. The most active constituents are classified to the azadirachtin-type C-seco limonoids [1] and the second appear to be intact apo-euphol limonoids, e.g. trichilins [2] with a 14, 15-epoxide and a C-19/C-29 bridged acyl acetal.



azadirachtin

Melia azedarach has been reported to be a native of Persia, India and China [3], but naturalized in a number of continents including Africa, Australia and the Americas. It has been used medicinally in many of these places for the treatment of a variety of human disorders [4]. In China, an extract of the bark is used as an anthelminthic [5]. Biologically

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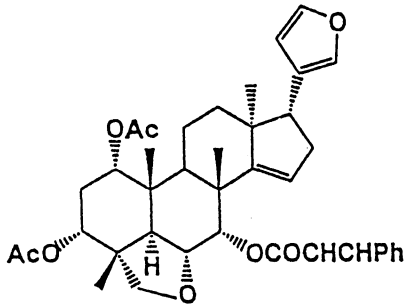
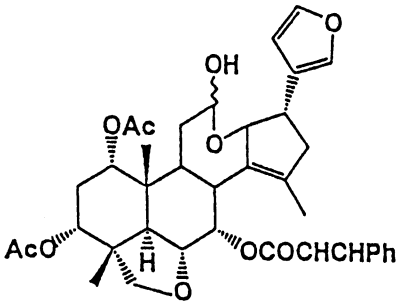
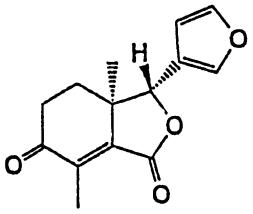
from Nigerian



nimbolin A

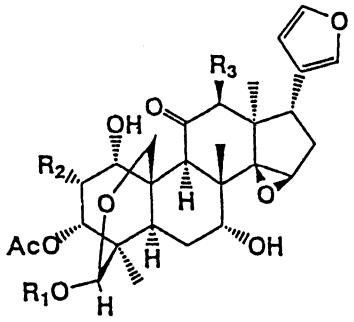


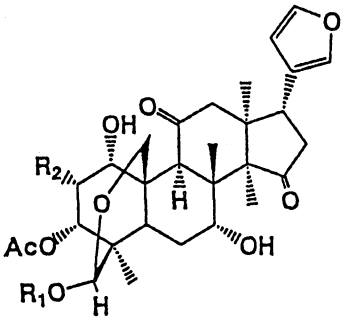
nimbolin B



fraxinellonone

from Australian

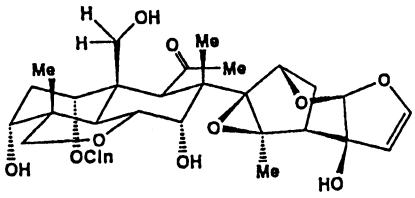




1 R_1 = $MeCH_2CHMeCO$, R_2 = AcO, R_3 = H
2 R_1 = Me_2CHCO, R_2 = AcO, R_3 = H
3 R_1 = $MeCH_2CHMeCO$, R_2 = AcO
4 R_1 = Me_2CHCO, R_2 = AcO

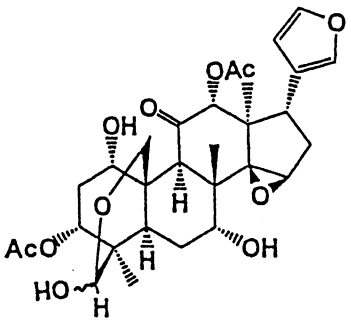
meliatoxin A_1 (1)
meliatoxin A_2 (2)
meliatoxin B_1 (3)
meliatoxin B_2 (4)

from American

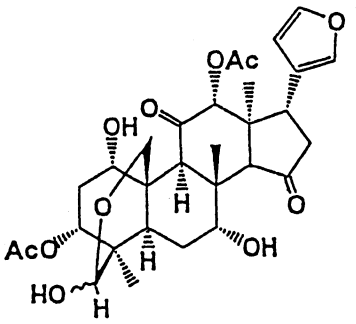


1-cinnamoylmelianolone

from Chinese



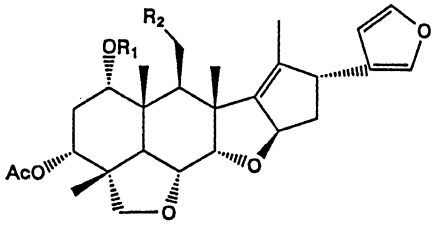
chuanliansu



iso-chuanliansu

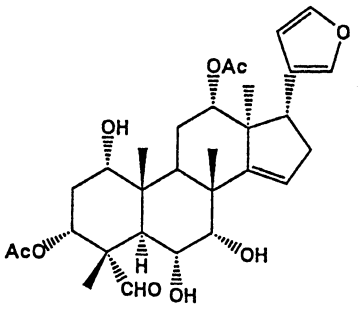
Fig. 1. Isolated limonoids from *M. azedarach* L.

from Japanese (*M. azedarach* L. var. *japonica* Makino)

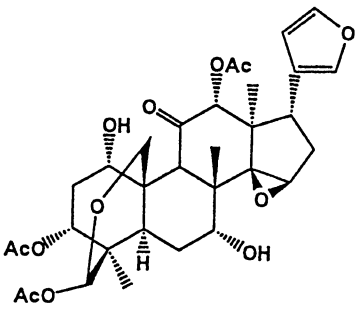


1 R_1 = COPh, R_2 = CHO
2 R_2 = COCH = CHPh, R_2 = CO_2Me

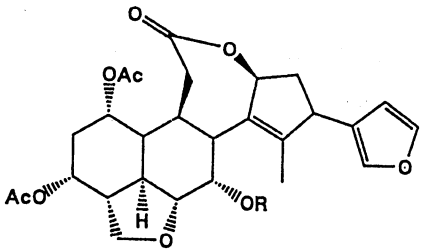
ohchinal (1)
ohchinin acetate (2)



sendanal

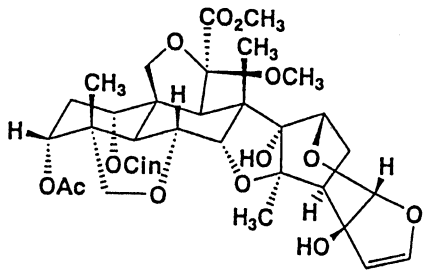


sendanin



3 R = COPh ohchinolide A
4 R = Tig ohchinolide B

from Okinawan



1-cinnamoyl-3-acetyl-11-methoxymeliacarpinin

Fig. 2. Isolated limonoids from *M. azedarach* L. (continued).

active substances from the plant have been extensively studied in Nigeria, Australia, China, the U.S.A. and Japan, and the isolated limonoids have been extended to several types; i.e., degraded limonoids [6], azadirachtin [7] and related compounds [8], toosendanin (chuanliansu) [9] and a related compound [5], and salannin-type C-seco limonoids [10], etc.

Recentry, we isolated a new meliacarpinin as insect antifeedant from Okinawan *M. azedarach* L. [11]. In the continuous study of antifeedants from Meliaceae plants, we have isolated nine new three types limonoids along with six known limonoids from the root bark of Chinese *M. azedarach* L. collected at Guangzhou. They are antifeedants active against the larvae of the Japanese pest insect *Spodoptera exigua* Hübner (Boisduval).

Results and Discussion

The ether extract of the root bark contained a variety of limonoids which were detected by the characteristic color with Ehrlich's reagent on TLC. The antifeeding limonoids from *M. azedarach* were also very sensitive to traces of acid and gradually decomposed on a silica column [12]. It was, therefore, necessary to use flash chromatography and HPLC separation techniques, and the isolation of the various congeners, **1—15**, was a tedious process requiring careful combined use of normal and reversed phase HPLC.

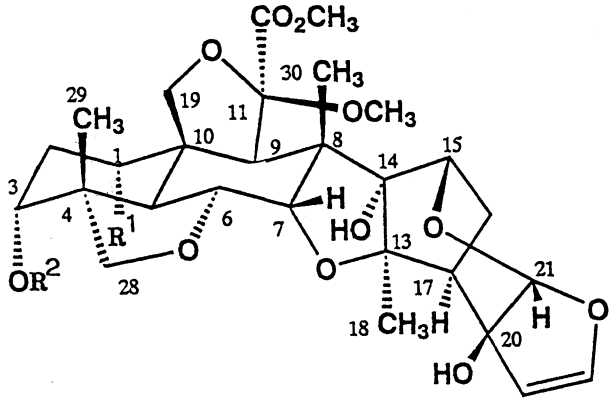
A powder, insoluble in 50% hexane/ether, from the ether extract of the root bark (375g) was flash-chromatographed with 1—3% MeOH/CH_2Cl_2 and each of the limonoid fractions was rechromatographed on a flash column with hexane/ether solvent system. Finally, very careful combined use of normal- and reversed-phase HPLC gave three new meliacarpinins (**1—3**), three new trichilin-type limonoids (**4—6**) along with four known trichilins (**7—10**), three new sendanin-type limonoids (**11—13**) designated as azedarachins, and two known ring-C seco limonoids (**14** and **15**). These compounds showed antifeedant activities at 50—600 ppm, corresponding to the concentration of 1—12 $\mu g\ cm^{-2}$, by a conventional leaf disk method [13] against the larvae of the voracious pest insect *Spodoptera exigua* Hübner (Boisduval).

Meliacarpinins (1—3) [14] and [15]

The structures of compounds **1** (0.8 mg; $C_{33}H_{44}O_{12}$, $[\alpha]_D$ −6.7°), **2** (6.8 mg; $C_{35}H_{46}O_{14}$, $[\alpha]_D$ −8.3°) and **3** (2.2 mg; $C_{35}H_{46}O_{14}$, $[\alpha]_D$ +9.1°) were elucidated as 1-deoxy-3-tigloyl-11-methoxymeliacarpinin (**1**), 1-tigloyl-3-acetyl-11-methoxymeliacarpinin (**2**) and 1-acetyl-3-tigloyl-11-methoxymeliacarpinin (**3**), respectively, by extensive ^1H and ^{13}C NMR studies including COSY, DEPT spectra and NOE experiments. Their NMR data were almost superimposable on each other except for some changes of substituent groups and also showed strong resemblances to that of 1-tigloyl-3-acetyl-11-methoxyazadirachtinin (**16**) [16] except for some changes of substituent groups and the presence of an additional methyl group instead of the lack of one methoxycarbonyl group.

Their ^1H NMR data and NOE enhancements (for example, Figure 3) revealed the stereochemistries of the B, C and D rings to be same with that of **16** and that the 4β-methoxy-

carbonyl group in **16** changed to methyl group, which was deduced from the high shifts of 3β-, 6β- and 28β-H due to the removal of the anisotropic effect of the 29-carbonyl group. The substitution patterns and stereochemistries around the A-ring were also elucidated by the ^{1}H NMR studies, in which W-type long range couplings were observed between 1β-H and 3β-H singnals. In **1**, an additional W-type long range coupling was observed between 1α-H and 19-H_a signals and an irradiation of 2'-Me singnal of tigloyl at C-3 enhanced the signal due to 3β-H. On the other hand, a NOE was observed between 3'-methyl of tigloyl at C-1 and 9-H signals in **2**, but it was not observed in **3**.



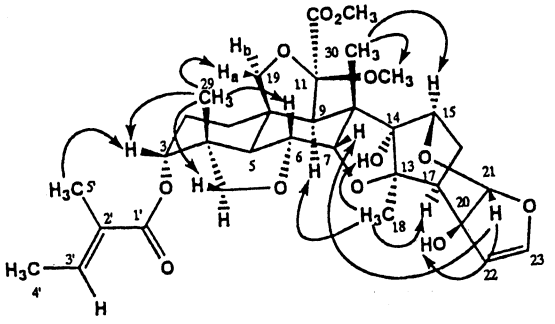


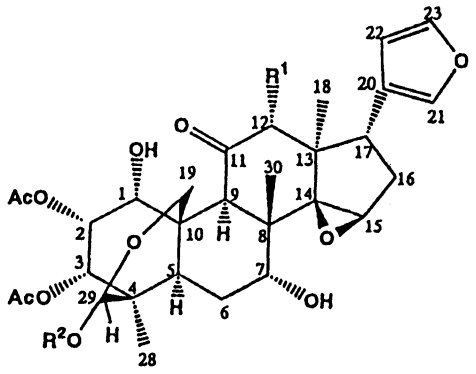
Fig. 3. Selected NOE connectivities for **1**.

1-deoxy-3-tigloyl-11-methoxymeliacarpinin (**1**)
R^1= H, R^2= Tig
1-tigloyl-3-acetyl-11-methoxymeliacarpinin (**2**)
R^1= Tig, R^2= Ac
1-acetyl-3-tigloyl-11-methoxymeliacarpinin (**3**)
R^1= Ac, R^2= Tig
1-tigloyl-3-acetyl-11-methoxyazadirachtinin (**16**)
R^1= Tig, R^2= Ac, 4β(C-29)= CO_2Me

Trichilins (4—10) [17]

Four compounds, **7—10**, were identified as trichilins B and D [2], meliatoxin A_2 [18] and aphanastatin [19], respectively, by comparison of their ^{1}H NMR spectra with those published. Insect antifeedants, **7** and **8**, were first found in an African medicinal Meliaceae plant *Trichilia roka*, and **9** was isolated as a toxin from Australian *M. azedarach*. On the other hand, **10** was reported as a cytotoxin from a Simarubaceae plant *Aphanamixis grandifolia* Bl.

NMR studies of three new limonoids, 12-*O*-acetyltrichilin B (**4**, 2.6 mg; C_{37} H_{48} O_{14}, $[\alpha]_D$ −2.5°), 1, 12-di-*O*-acetyltrichilin B (**5**, 1.7 mg; C_{39} H_{50} O_{15}, $[\alpha]_D$+0.8°) and trichilin H (**6**, 1.0 mg; C_{36} H_{46} O_{14}, $[\alpha]_D$ −20.2°), taking into account their circular dichroism (CD) data at~310 nm (n-π^* of 11-keto group), allowed us to predict their structures. Their complex ^{1}H NMR spectra were very similar to that of trichilin B (**7**), including a 14, 15-epoxide and a C-19/C-29 bridged acyl acetal system except for the differences of the 29-ester moiety and acetyl substitution. Their structures were confirmed by extensive ^{1}H and ^{13}C NMR studies including COSY, DEPT spectra and NOE experiments, and chemical transformations.

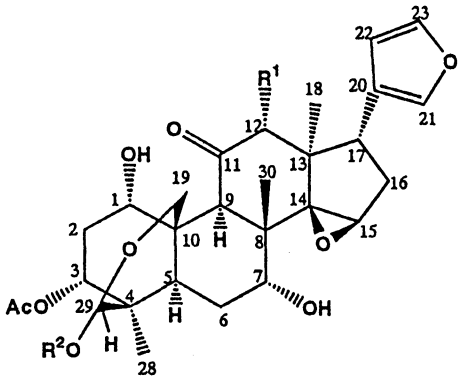


12-*O*-acetyltrichilin B (4): R^1= OAc, R^2= $COCH(CH_3)CHCH_3$
1,12-di-*O*-acetyltrichilin B (5): R^1= OAc, R^2= $COCH(CH_3)CH_2CH_3$, 1α= OAc
trichilin H (6): R^1= OAc, R^2= $CO(CH_3)_2$
trichilin B (7): R^1= OH, R^2= $COCH(CH_3)CH_2CH_3$
trichilin D (8): R^1= H, R^2= $COCH(CH_3)CH_2CH_3$
meliatoxin A_2 (9): R^1= H, R^2= $COCH(CH_3)_2$
aphanastatin (10): R^1= OH, R^2= $COCH(CH_3)CH_2CH_3$, 1α= OAc, 2α= OH

Azedarachins (11—13) [20]

Three azedarachins, A (**11**, 1 mg; C_{33} H_{44} O_{11}, $[\alpha]_D$ −10°), 12-*O*-acetylazedarachin A (**12**, 0.7 mg; C_{35} H_{46} O_{12}, $[\alpha]_D$ +7.5°) and 12-*O*-acetylazedarachin B (**13**, 2.5 mg; C_{34} H_{44} O_{12}, $[\alpha]_D$ −55°), possessed a 2-deoxytrichilin skeleton and their structures were also elucidated by extensive ^1H and ^{13}C NMR studies including COSY, DEPT spectra and NOE experiments, taking into account the circular dichroism data. Some pertinent points related to the structural studies are follows. Their NMR spectra indicated the presence of the 14, 15-epoxide and the 19/29 bridged acyl acetal system like trichilins. The substitution pattern around the A-ring, namely, that they have a free 1α-OH and 3α-acetoxyl groups, the same as sendanin (**17**) [21], was shown by the fact that the 9-H signal was shifted to down field due to the effect of the 1-hydroxyl in a 1, 3-diaxial relationship, respectively. Their α configuration of 12-substituents were deduced by the comparison of the chemical shifts of 12β-H with those of trichilins and the NOE enhancements between the 12β-H and 8β-Me signals.

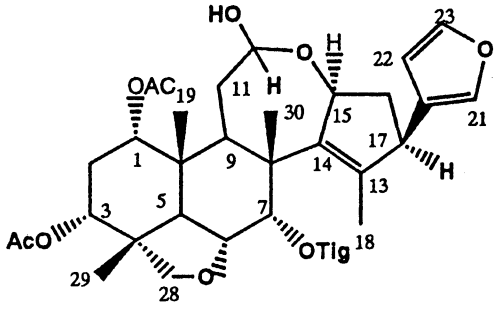
Finally, their *S*-configuration at C-29 was assigned from the chemical shifts of 3-H, which were observed at low positions, as well as sendanin (**17**) and all of trichilins, compared to that in the endo-isomer of toosendanin (29-OH) [22].



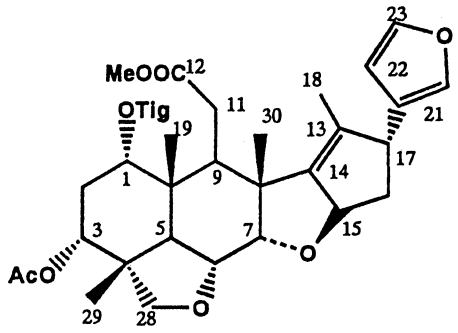
azedarachin A (11): R^1= OH, R^2= $COCH(CH_3)CH_2CH_3$
12-*O*-acetylazedarachin A (12): R^1= OAc, R^2= $COCH(CH_3)CH_2CH_3$
12-*O*-acetylazedarachin B (13): R^1= OAc, R^2= $COCH(CH_3)_2$
sendanin (17): R^1= OAc, R^2= Ac

Other limonoids (14 and 15)

Compounds **14** and **15** were identified as nimbolin E [23] and salannin [24], respectively, by comparison of their ^1H NMR spectra with their published data.



nimbolin E



salannin

Antifeedant activity

The antifeedant activities of the isolated limonoids, **1**—**15**, were tested by the conventional leaf disk method [13] against the larvae of *S. exigua*. The most potent was meliacarpinins, which active at 50—100 ppm corresponding to the concentration of 1—2 $\mu g/cm^2$. The activity is less than those of famous azadirachtin and the related compounds [25] but stronger than those of the second class limonoids [26] such as trichilins or azedarachins with a 14, 15-epoxide and a 19/29 lactol bridge, in which the 12α-OH compounds, trichilin B (**7**), aphanastatin (**10**) and azedarachin A (**11**), were most potent and active at 200 ppm. Independent of the substitution patterns in ring A and the C-28 ester moieties, the 12-acetoxy and 12-deoxy

compounds, **4—6**, **8**, **9**, **12** and **13**, were active at 400 ppm. Both nimbolin E (**14**) and salannin (**15**) showed weaker activities at 600 ppm.

Acknowledgements — We would like to thank Dr. H. Naoki, Suntory Institute for Bioorganic Research, for CI, SI and FAB mass measurements.

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