New Limonoids from *Melia toosendan*

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The root bark of *Melia toosendan* afforded four new limonoids with a C-19/C-29 bridged acetal structure, together with two known limonoids, 12α-hydroxyamoorastatone and its 12-acetate. The new compounds were elucidated as 1-O-acetyltrichilin H and 29-O-substituted amoorastatone derivatives, named neoazedarachins A, B and D, by spectroscopic and chemical means. Their antifeedant activity was also studied.

**Key words:** limonoid; antifeedant; *Melia toosendan*; 1-O-acetyltrichilin H; neoazedarachins

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release from the effect of the 1α-hydroxyl group in the 1,3-diaxial relationship in 7. The Δ configuration at C-29 was established from the chemical shifts of the 3β- and 6β-H signals at δ 5.70 and 2.04, which were largely influenced by the orientation of the 29-O function. \(^{\text{8}}\) W-shaped long-range couplings between the 5-H and one (δ 4.45) of the 19-methylene signals and the 9-H and 30-H signals, and NOE connectivities between the 18-H signal and the 9, 21 and 22-H signals supported the stereochemistry of 1.

Compound 2 was found by accurate mass measurement \([\text{HRFAB-MS}] m/z: 639.2799 (\text{MNa}^+) (\text{Δmmu} + 1.7)]\) to have the molecular formula C\(_{32}\)H\(_{42}\)O\(_{11}\). The CD spectrum (\(\Delta e_{295} = -24.5, \Delta e_{354} = -23.1\) and \(\Delta e_{194} = -14.2\), \(n - \pi^*\) of keto groups) suggested the presence of plural carbonyl groups. The \(^1\)H-(Table) and \(^1\)C-NMR spectra showed the presence of a C-19/C-29 bridged acyl acetal structure [19-H₂, δ 3.97 and 4.14 (each 1H, d, \(J = 12.0\) Hz); and 29-H, δ 5.77 (1H, s), δ 63.91 (C-19) and δ 94.4 (C-29)] together with three tertiary Me \([\delta 0.82 (28\text{-H}), 0.88 (18\text{-H})\) and 1.21 (30\text{-H})], one acetyl (δ 2.10) and a \(\beta\)-substituted furan moiety \([\delta 7.38, 7.30\) and 6.42 (each 1\text{H})]. The acyloxy side chain attached to C-29 was 2-methylbutanoyl in 2, being different from 2-methylpropanoyl in 1. The spectral data for 2 were closely related to those of azedarachin A (8), which had been isolated from \(M.\) \textit{azedarach}, \(^{\text{9}}\) having the same molecular formula. However, the \(^1\)H- and \(^13\)C-NMR spectra of 2 showed the presence of an additional carbonyl group at δ 219.0 instead of the epoxide at δ 71.4 and δ 59.5 in 8 and a signal at δ 3.77 (1H, s) that was assigned to the 15-H in 8 disappeared while a new signal was observed at δ 3.09 (br. s) in 2. These signals strongly suggested the presence of a 15-keto group, which is consistent with the CD data.

The structure of 2 was finally elucidated from the transformation of 8 to 2 by treating with a catalytic amount of TsOH. This acid-catalyzed rearrangement of the D ring is known in limonoids. \(^{\text{9}}\) The stereochemistry at C-14 was confirmed from the chemical shifts of 14α-H at δ 3.09 and of 7β-H at δ 4.24, the downfield shifts of both signals being attributable to the effect of 7-OH in a 1,3-diaxial relationship and to the anisotropic effect of the 15-keto group, respectively.

The structure of 3, having the molecular formula C\(_{31}\)H\(_{39}\)O\(_{11}\), assigned from the (M\(\text{Na}^+)\) ion at \(m/z \)625.2648 in the HRFAB-MS spectrum, was readily established by analogy with 2 since similar Cotton effects (\(\Delta e_{294} = -24.3, \Delta e_{354} = -22.7\) and \(\Delta e_{194} = -16.2\)) were observed in the CD spectrum and since the proton shifts of rings A, B, C and D of both compounds corresponded to each other. The only difference was the acyl side chain at C-29, which was 2-methylpropanoyl in 3 and 2-methylbutanoyl in 2. Compound 3 being the D-ring keto isomer of azedarachin B (9), \(^{\text{9}}\) which had earlier been isolated from \(M.\) \textit{toosendan}, was also confirmed from the formation of 3 by an acid treatment of 9.

The \(^1\)H-NMR spectrum of compound 4 (Table) having the molecular formula C\(_{31}\)H\(_{39}\)O\(_{11}\) (\((-\) HRFAB-MS \(m/z: 545.2387 [\text{M-H}]^- (\Delta\text{mmu} 0.0)])\), showed a three-proton singlet at δ 3.32 assignable to a methoxy group, together with three tertiary methyl signals. A compar-
son of the spectral properties of 4 with those of 2 and the CD Cotton effect \((\Delta \varepsilon_{255} + 4.4, \Delta \varepsilon_{295} - 19.7, \Delta \varepsilon_{365} - 16.4\) and \(\Delta \varepsilon_{316} - 9.9\)) due to the keto functions in 4 indicated that they were also closely related, including the C-19/C-29 bridged acetel and C-15 keto structures. However, the \(^1H\)-NMR spectrum of 4 lacked the 2-methylbutanoyl signals and showed the presence of a methoxy signal, with an upfield shift of the 29-H signal to \(\delta 4.19\) in 4 from \(\delta 5.77\) in 2. These data showed that compound 4 was a 29-O-methyl derivative of amoorastatone (5).

The endo configuration of the methoxy group was elucidated from the W-shape long-range coupling between the 29- and 5-H signals and the chemical shift change of the 3β- and 6β-H signals at \(\delta 5.35\) and \(\delta 2.15\) in 2 to \(\delta 4.90\) and \(\delta 2.49\) in 4, respectively. Acetylation of 4 gave a 7,12-diacetate (10), in which the 14-H signal was shifted upfield to \(\delta 2.81\) from \(\delta 3.10\) in 4 to confirm its configuration to be \(\alpha\).

On the other hand, the \(\alpha\)-configuration of the 12-hydroxy group was unambiguously assigned from the chemical shifts of the furan protons and from the results of a CD study of 12-p-bromobenzoate 11. The furan signals were observed at higher fields in the benzoate. Thus, the chemical shifts for compound 4/its 12-benzoate were as follows: 21-H, 7.30/7.21; 22-H 6.41/6.20; and 23-H 7.38/7.28. The upfield shifts of the furan ring protons can be accounted for by the ring current of the benzoate aromatic ring which is located on the same side as the furan ring. On the other hand, the CD spectrum of 11 showed negatively split interaction bands between the benzoate and furan chromophores at 246 \((\Delta \varepsilon - 3.5, \pi \rightarrow \pi^*\) transition of the benzoate) and 210.5 nm \((\Delta \varepsilon + 2.1, \pi \rightarrow \pi^*\) transition of the furan) and negative carbonyl bands at 306 \((\Delta \varepsilon - 3.9)\) and 320 nm \((\Delta \varepsilon - 2.7)\) due to \(n \rightarrow \pi^*\) transitions of the 11- and 15-keto groups (Fig. 2). This negatively split Cotton effect would be in accordance with the negative chirality between the benzoate and furan chromophores.\(^{10}\)

Finally, the structure of 4 was confirmed from O-methylation of 12-hydroxyamoorastatone (5) with a catalytic amount of TsOH in dried MeOH, which afforded 4 as the main product. Compound 4 is the first natural 29-endo derivative to be found in C-19/C-29 bridged acetel limonoids.

The antifeedant activity of the isolated compounds was tested against third-instar larvae of Spodoptera littoralis (Boisdoual) by the conventional leaf disk method.\(^{11}\) The most potent were meliacarpins, which were active at 50 ppm,\(^{12}\) corresponding to a concentration of ca. 1 \(\mu g/cm^2\). Azedarachin B (9) was the next most active at 200 ppm, followed by 12-hydroxyamoorastatone (5, 250 ppm), iso-chuanliansu (6, 300 ppm), and 1-O-acetyltrichilin H (1, 400 ppm) and neoazedarachins (2-4, 400 ppm). These results show that isomerization of the D-ring epoxide to a 15-keto and acylation of the 12- and 29-OH groups reduced the activity, but that the side-chain change at C-29 did not influence their activity.

**Experimental**

Instrumental analyses. \(^1H\)- and \(^13C\)-NMR spectra were measured with a JEOL FX-400 spectrometer. IR and UV spectra were recorded with JASCO FT/IR 5300 and Shimadzu UV-210A spectrometers, respectively. Optical rotation was measured with a JASCO J-20A apparatus, and CD spectra with a JASCO J-720 spectropolarimeter. HPLC was performed on Waters \(\mu\) Bondapshere 5 \(\mu\) C18 100 A in a 19×150 mm column and TLC on Kieselgel 60 (Merck).

**Plant materials.** The root bark was collected in December 1992 at Xiangtan in China.

Isolation of the limonoids. The air-dried root bark (1.5 kg) was defatted with hexane (20 l) and then extracted with ether (20 l) to give an extract of 12.8 g. The precipitate, which was insoluble in 50% hexane-ether, from the ethereal extract, was subjected to DCCC by using CH\(_2\)Cl\(_2\)-MeOH-H\(_2\)O (5:5:3, v/v) in an ascending mode, and the resulting limonoid fraction was flash chromatographed on SiO\(_2\) with a 50–100% ether/hexane solvents. HPLC purification of the limonoid fractions, which was eluted with ether, on a semiprep reversed-phase column with 25–50% H\(_2\)O/MeOH as the solvents, gave I (0.8 mg), 2 (3.0 mg), 3 (0.9 mg), and meliacarpinins A (2.1 mg), C (5.2 mg), and D (6.2 mg). These more-polar DCCC fractions were subjected to prep TLC with 50% benzene/acetic as the solvent, subsequent HPLC purification of the limonoid fraction in a similar manner to that just described giving 4 (16.5 mg), 5 (18.5 mg) and 6 (15.1 mg).

1-O-acetyltrichilin H (1). An amorphous powder, C\(_{18}\)H\(_{24}\)O\(_{15}\); [\(\alpha\)]\(_D\) 3.4° (c 0.08, MeOH); UV \(\lambda_{max}\) (MeOH)
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Endo-isomer of *iso-chuanlanisou* (6). NMR $\delta_H$ (CDCl$_3$ + CD$_2$OD): 0.89 (3H, s, 4a-CH$_3$), 1.02 (3H, s, 8-CH$_3$), 1.20 (3H, s, 13-CH$_3$), 1.77 (1H, m, 2a-CH), 1.77 (1H, m, 6a-CH), 2.09, 2.12 (each 3H, s, COOC$_2$H$_5$), 2.50 (1H, dt, $J=2.5$ and 13.4 Hz, 6b-CH$_2$), 2.60 (2H, d, $J=9.4$ Hz, 16-H), 2.60 (1H, m, 5-H), 2.78 (1H, dt, $J=16.4$ and 4.5 Hz, 2b-CH), 3.12 (1H, s, 14-H), 3.34 (t, $J=9.0$ Hz, 17-H), 3.77 (1H, s, 9-H), 3.95 (1H, m, 7-H), 3.98 (1H, d, $J=12.0$ Hz, 19-H$_a$), 4.12 (1H, br. d, $J=4.0$ Hz, 1-H), 4.42 (1H, d, $J=12.0$ Hz, 19-H$_b$), 4.69 (1H, s, 29-H), 4.84 (1H, br. d, $J=4.0$ Hz, 3-H), 5.06 (1H, s, 12-H), 6.29 (1H, m, 7-H), 7.32 (1H, br. s, 21-H) and 7.40 (1H, br. t, $J=1.5$ Hz, 23-H).

Treatment of azedarach A (8) with *p*-toluenesulfinic acid. Azedarach A (8, 0.7 mg) was treated with a catalytic amount of TsOH in CH$_2$Cl$_2$ (1 ml) at r.t. for 3 h. The product was purified by semiprep. HPLC with 40% H$_2$O/MeOH as the solvent to give 2 (0.4 mg).

Treatment of azedarach B (9) with *p*-toluenesulfinic acid. Azedarach B (9, 1 mg) was treated with a catalytic amount of TsOH in CH$_2$Cl$_2$ (1 ml) at r.t. for 1 h to give 3 (0.3 mg).

Acetylation of azedarach D (4). Compound 4 (2 mg) was acetylated with Ac$_2$O (0.1 ml) in Py (0.5 ml) at r.t. for 4 d. The reaction mixture was washed with H$_2$O, and the product was purified by semiprep. HPLC with 30% H$_2$O/MeOH as the solvent to give 10 (0.3 mg), C$_{29}$H$_{42}$BrO$_{14}$; FAB-MS: $m/z$ 613 (MH$^+$); selected NMR $\delta_H$ (CDCl$_3$): 0.79 (3H, s, 4a-CH$_3$), 1.03 (3H, s, 13-CH$_3$), 1.24 (3H, s, 8-CH$_3$), 2.05, 2.10, 2.12 (each 3H, s, COCH$_3$), 2.81 (1H, s, 14-H), 3.29 (1H, t, $J=9.0$ Hz, 17-H), 3.31 (3H, s, OCH$_3$), 3.73 (1H, s, 9-H), 3.95, 4.12 (each 1H, d, $J=11.3$ Hz, 19-H$_b$), 4.17 (1H, s, 29-H), 4.25 (1H, d, $J=4.4$ Hz, 1-H), 4.89 (1H, d, $J=4.4$ Hz, 2-H), 5.24 (1H, s, 12-H), 5.26 (1H, m, 7-H), 6.36, 7.30 and 7.39 (each 1H, furan-H).

Benzoylation of azedarach D (4). Compound 4 (0.4 mg) was treated with 2-bromobenzoyl chloride (10 mg) in Py (1 ml) at 50°C for 4 d. After washing with H$_2$O, the reaction products were subjected to prep. TLC, using 50% Me$_2$CO/C$_6$H$_6$ as the solvent, and the limonoid fraction was purified by semiprep. HPLC with 20% H$_2$O/MeOH as the solvent to give 12-benzoate 11 (1.0 mg), C$_{29}$H$_{44}$BrO$_{14}$; FAB-MS: $m/z$ 713 and 715 (MH$^+$); UV $\lambda_{max}$ (MeOH) nm (c) 207 (9000) and 245 (8700); CD: 0.28 (3H, s, 4a-CH$_3$), 1.10 (3H, s, 8-CH$_3$), 1.69 (1H, m, 6a-CH), 1.78 (1H, dt, $J=16.1$ and 1.7 Hz, 2a-CH$_2$), 1.99 (1H, m, 6b-CH$_2$), 2.03 (3H, s, COOCH$_3$), 2.49 (1H, m, 16c-CH$_3$), 2.58 (1H, dd, $J=15.3$ and 2.0 Hz, 5-H), 2.63 (1H, m, 16b-CH$_2$), 2.71 (1H, dt, $J=16.0$ and 4.8 Hz, 2b-CH$_2$), 2.78 (1H, dt, $J=16.1$ and 1.7 Hz, 2a-CH$_2$), 3.28 (1H, br s, 12-H), 3.38 (1H, t, $J=8.5$ Hz, 17-H), 3.53 (1H, s, 9-H), 3.77 (1H, d, $J=11.7$ Hz, 19-H$_a$), 4.18 (1H, s, 12-H), 4.31 (1H, d, $J=11.7$ Hz, 19-H$_b$), 4.52 (1H, br. d, $J=4.8$ Hz, 1-H), 4.62 (1H, s, 29-H), 4.71 (1H, dt, $J=16.0$ and 4.8 Hz), 6.27 (1H, br. s, 22-H), 7.24 (1H, br. s, 21-H) and 7.31 (1H, br. s, 23-H).

Endo-isomer of 12-hydroxyamorastatone (5). NMR $\delta_H$ (CDCl$_3$ + CD$_2$OD): 0.82 (3H, s, 4a-CH$_3$), 0.83 (3H, s, 13-CH$_3$), 1.10. (3H, s, 8-CH$_3$), 1.69 (1H, m, 6a-CH), 1.78 (1H, dt, $J=16.1$ and 1.7 Hz, 2a-CH$_2$), 1.99 (1H, m, 6b-CH$_2$), 2.03 (3H, s, COOCH$_3$), 2.49 (1H, m, 16c-CH$_3$), 2.58 (1H, dd, $J=15.3$ and 2.0 Hz, 5-H), 2.63 (1H, m, 16b-CH$_2$), 2.71 (1H, dt, $J=16.0$ and 4.8 Hz, 2b-CH$_2$), 2.78 (1H, dt, $J=16.1$ and 1.7 Hz, 2a-CH$_2$), 3.28 (1H, br s, 12-H), 3.38 (1H, t, $J=8.5$ Hz, 17-H), 3.53 (1H, s, 9-H), 3.77 (1H, d, $J=11.7$ Hz, 19-H$_a$), 4.18 (1H, s, 12-H), 4.31 (1H, d, $J=11.7$ Hz, 19-H$_b$), 4.52 (1H, br. d, $J=4.8$ Hz, 1-H), 4.62 (1H, s, 29-H), 4.71 (1H, dt, $J=16.0$ and 4.8 Hz), 6.27 (1H, br. s, 22-H), 7.24 (1H, br. s, 21-H) and 7.31 (1H, br. s, 23-H),
Methylation of 12-hydroxyamoorastralactone (5). To a solution of compound 5 (4 mg) in dried MeOH (1 ml) was added a catalytic amount of TsOH. The reaction mixture was stirred at r.t. for 18 h, poured into ice-cooled water, and extracted with ether. The ethereal layer was successively washed with a 5% Na2CO3 solution and water, and concentrated. The residue was purified by semiprep. HPLC with 30-35% H2O/MeOH as the solvent to give 4 (1.0 mg).

Bioassay of the limonoid antifeedants. The antifeedant potential of the isolated compounds was tested by the conventional leaf disk method13 against third-instar larvae of S. littoralis (Boisdual). Five disks of Chinese cabbage (Brassica campestris L. var chinensis) treated with the sample solution in Me2CO were arranged with another 5 control disks immersed in Me2CO alone in a Petri dish. Ten larvae were placed in the center, and the score for the treated and untreated leaves eaten by the larvae in 2-24 h was evaluated at appropriate intervals. From these choice tests at 50, 100, 200, 250, 300 and 400 ppm, the minimum inhibitory concentration of each limonoid was determined.

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References