PERGAMON

Phytochemistry 60 (2002) 419–424

PHYTOCHEMISTRY

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Degraded limonoids from *Melia azedarach* and biogenetic implications

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Received 14 November 2001; received in revised form 6 February 2002

Abstract

The roots of *Melia azedarach* yielded three new degraded limonoids: teracrylmelazolide A, melazolide A and teracrylmelazolide B, possessing a previously undescribed skeleton. The known pyroangolensolide, fraxinellone and its three derivatives, 30-hydroxyfraxinellone, 9α- and 9β-hydroxyfraxinellone, were also isolated. Their stereochemisty was elucidated on the basis of spectral data and chemical reactions. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Melia azedarach; Meliaceae; Chinaberry; Degraded limonoids; Teracrylmelazolide A; Teracrylmelazolide B; Melazolide A; Pyroangolensolide; 30-Hydroxyfraxinellone, 9α- and 9β-Hydroxyfraxinellone

1. Introduction

Limonoids represent a large group of natural products mainly found in plants belonging to the families Rutaceae and Meliaceae; they have attracted considerable attention because of their various and significant biological properties. The chemistry and bioactivity of limonoids have been reviewed (Akhila and Rani, 1999; Dreyer, 1968).

In connection with the characterization of these terpenoids, a small group of compounds has also been isolated and classified as "degraded limonoids". They account for a total of nine molecules, all of them possessing the furan ring and the C,D-ring portion (Nakatani et al., 1998; Okamura et al., 1997) of the original tetranortriterpenoid skeleton which can be exemplified by the 7-deacetyl-7-oxogedunin **1** and methyl angolensate **2** structures.

In this paper, we report the isolation and the structure elucidation of six new,[1] along with two known, degraded limonoids from the roots of a *Melia azedarach* (chinaberry) tree grown in the alpine region of Italy. Based on spectroscopic data, three new compounds were designated as melazolide A, 3-teracrylmelazolide A and 3-teracrylmelazolide B; they are uniquely characterized by the correspondence to the A-ring portion of an original limonoid skeleton. Related to the C,D-ring portion, the known pyroangolensolide was obtained for the first time from a natural source; we also identified the known fraxinellone and three new derivatives: 30-hydroxyfraxinellone, 9α- and 9β-hydroxyfraxinellone. Compounds **1** and **2** occurred in the root extract as well.

2. Results and discussion

3-Teracrylmelazolide A **3** exhibited M^+ at *m/z* 322.17760 corresponding to a molecular formula $C_{18}H_{26}O_5$ that implies six unsaturations. The ^{13}C NMR spectrum accounted for two double bonds and two estereal carbonyls, thus requiring two rings. The ^1H NMR spectrum showed three vinylic methyls as well as two methyls and one hydroxymethylene group located on quaternary carbons. COSY data revealed a –CH=CH–CH–O– and a –CH–CH$_2$– spin systems whose connectivity could be extended from HMBC correlations: key interactions were observed from carbons with protons H_β-6 (C-5, C-7, C-10); H_a-19 (C-5, C-10); H-3 (C-1′, C-29); H-2 (C-4, C-10); H-28 (C-5, C-4, C-3); H-1 (C-3, C-5); H-2′ (C-1′, C-7′); H-7′ (C-3′, C-4′) thus establishing a benzofuran skeleton and suggesting the

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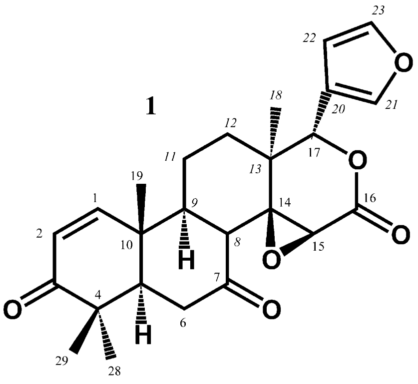
[1] We adopt the limonoid numbering for the structural formulae and spectroscopic data; IUPAC nomenclature and numbering are given in the Experimental part.

PII: S0031-9422(02)00107-3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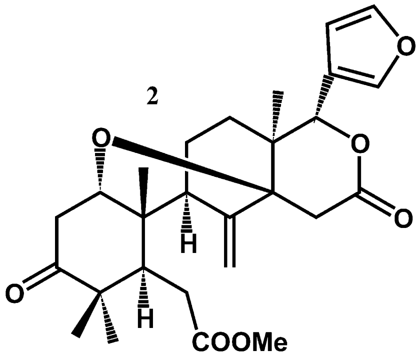

2

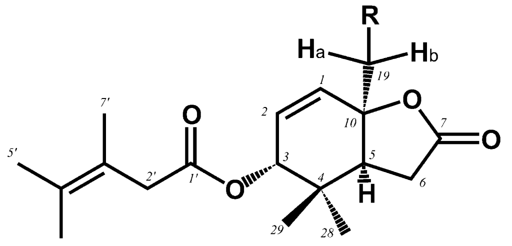
presence of a 3,4-dimethyl-3-pentenoate moiety linked to C-3. This estereal side chain was also confirmed by intense fragments at *m/z* 128 (acid residue) and 195 (cyclic part).

The relative configuration was deduced by differential NOE and NOESY experiments that showed intense spatial enhancement between signals for H-3 and H_{β}-6 protons that is only possible for a *cis* ring junction and an α-oriented ester side chain. Besides, the response of signals of H-3 and H_b-19 after irradiation respectively at Me-29 and Me-28, allowed the assignment of the chemical shifts of the geminal methyls. Inspection of a Dreiding model revealed the existence of several conformations: the one with C-19, Me-28 and H-3 in a pseudo-axial positions with respect to the six membered ring and carboxyl oxygen external (28ax), accounted well for the experimentally observed NOEs. Here the H-5–C-5–C-6–H_{α}-6 and H-5–C-5–C-6–H_{β}-6 dihedral angles measured respectively −30° and −152°; application of the Karplus rule furnished $J_{5,6\alpha}=8.0$ Hz and $J_{5,6\beta}=10.1$ Hz in contrast to the observed value (9.6 Hz and 5.6 Hz resp.). MM calculations suggested two low-energy, nearly equally populated conformers originating from simultaneous rotation around the C-2–C-3–C-4–C-5 and H-5–C-5–C-6–H_{β}-6 dihedral angles. Thus the 28ax conformer is in fast equilibrium with a conformer possessing Me-29 in the pseudo-axial and C-19 in the pseudo-equatorial positions and carboxyl oxygen internal (29ax). Here the H-5–C-5–C-6–H_{α}-6 and H-5–C-5–C-6–H_{β}-6 dihedral angles measured 21° and −99°; application of the Karplus rule furnished $J_{5,6\alpha}=9.0$ Hz and $J_{5,6\beta}=1.5$ Hz. All this fits with the averaged values of $J_{5,6\alpha}$ and $J_{5,6\beta}$ observed in the proton spectrum of **3**.

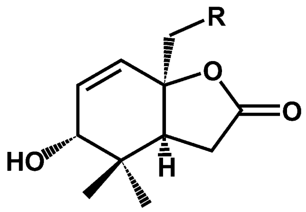
The spectral features of melazolide A **4** and 3-teracrylmelazolide B **5** revealed their close structural relationship with 3-teracrylmelazolide A. In compound **4**, the NMR signals for the acyl substituent were absent and the resonances of C-3 ($\Delta\delta$ −1.6), H-3 ($\Delta\delta$ −1.1), C-2 ($\Delta\delta$ +3.7) and C-4 ($\Delta\delta$ +1.0) exhibited the expected highfield or lowfield shifts. EIMS and HREIMS fully substantiated the formula $C_{11}H_{16}O_4$ and hence **4** as melazolide A. The only difference between 3-teracrylmelazolide B and **3** was the presence of a singlet methyl instead of the hydroxymethylene group; HREIMS on the molecular ion confirmed the composition $C_{18}H_{26}O_4$, therefore the new compound must be depicted as **5**. Its saponification furnished melazolide B **6**.

Two unsuccessful attempts were made at assigning the absolute configuration at C-3 in compound **6**. The *R*- and *S*-MTPA ester derivatives of melazolide B, furnished negative $\Delta\delta$ ($=\delta_S-\delta_R$) values for Me-28 (−0.06) and Me-29 (−0.01) and positive values for H-1 (+0.08), H-3 (+0.13), H-19 (+0.16), H-5 (+0.05) and H_{β}-6 (+0.02); this implies a conformational deviation of the MTPA moiety from the ideal position and consequently unreliable results (Ohtani et al., 1991). Moreover the alcohol **6** did not react with *p*-anisoyl chloride thus preventing the use of the exciton chirality method applied to allylic alcohols (Harada et al., 1981). Eventually we turned to the CD technique that establishes a relationship between the chirality of non planar, α,β-unsaturated ketones and the sign of the R-band Cotton effect (Legrand and Rougier, 1977). Melazolide B underwent clean oxidation by PCC to the enone functionality. MM calculations for the conjugated ketone suggested the presence of one major conformer owing to the 28ax:29ax population ratio as 93/7; a long-range W coupling between H-1 and H-5 in the proton spectrum, supported experimentally the computational result. The negative Cotton effect at 340 nm proved the 5*S*,10*R* absolute stereochemistry. The stereochemistry of melazolides is comparable to that of limonoids **1** and **2** at C-5 but needs inversion of chirality at C-10.

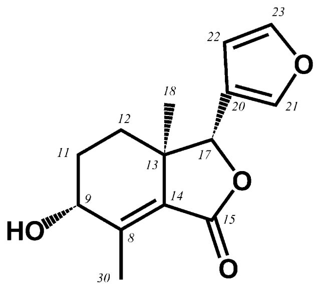
The EIMS spectra of compounds **7** and **8** had molecular ions at $m/z=248$ and the high resolution established their molecular formula as $C_{14}H_{16}O_4$ and hence seven unsaturations. The ^{13}C NMR spectra revealed an identical set of signals: one lactone carbonyl, six olefinic carbons and, in the saturated carbon region, 2*d* deshielded, 2*t*, 2*q* and 1*s*. The ^{1}H NMR spectra showed signals consistent with a β-substituted furan, a quaternary methyl, an olefinic methyl carbon and a singlet oxymethine; however they differed substantially in the coupling pattern of the proton at the secondary alcohol function: in fact, in the more abundant isomer it appeared as a broad doublet whilst in the minor isomer it had a



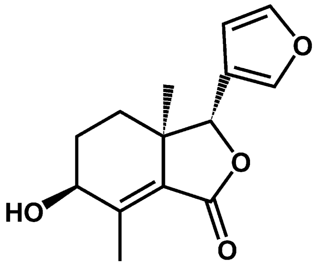
3 3-teracryl-melazolide A R=OH
5 3-teracryl-melazolide B R=H



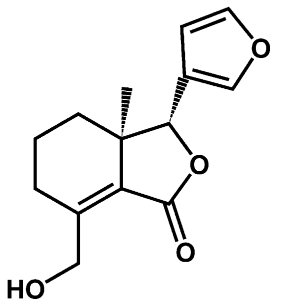
4 melazolide A R=OH
6 melazolide B R=H



7 9α-hydroxyfraxinellone



8 9β-hydroxyfraxinellone



9 30-hydroxyfraxinellone

triplet multiplicity. Extensive homonuclear decoupling allowed the assignment of all the resonances and identified their structures as 9α- and 9β-hydroxyfraxinellone respectively. Proton signals for compound **7** are compatible with the chemical shifts reported for the alcohol obtained from $NaBH_4$ reduction of fraxinellonone (Boustie et al., 1990).

Repeated elutions on silica or cyanopropyl stationary phases, could not eliminate a roughly 1:4 impurity accompanying **8**, so that they had to be examined as an inseparable mixture. Clues to the structure of **9** emerged from differences with **8** in the ^1H and ^{13}C NMR spectra, here signals attributable to hydroxymethyl and methylene groups replaced those corresponding to methyl and hydroxymethine groups respectively.

Fraxinellone and pyroangolensolide were identified by spectral comparison (EIMS, ^1H and ^{13}C NMR data) with previously reported values (Nakatani et al., 1998; Okamura et al., 1997; Fernandez-Mateos et al., 1995). Fraxinellone has been known for a long time as a degraded limonoid, isolated from either Rutaceae and Meliaceae plants. It is to be pointed out in this context, that this is the first report on the isolation from a natural source of pyroangolensolide, which has in precedence been prepared through pyrolysis of methyl angolensate or synthesized as enantiomeric mixture. Optical rotations and CD spectrum of our natural fraxinellone and pyroangolensolide agreed with literature data and this denotes 17*R* chirality for compounds **7**, **8** and **9**.

Degraded limonoids are thought to be derive from a tetranortriterpenoid whose structure remains highly uncertain; our findings allow us to speculate about their biogenesis (Table 1). The formation of the allylic carbocations **10** or **11**, that later lose H^+ or are quenched by water, can rationalize the various natural metabolites derived from the C,D-rings. A possible intermediate on the route towards melazolides may be the carbocation **12** which is followed by lactonization; this could explain the inverted stereochemistry at C-10. It is appealing to imagine that the degraded limonoids of both melazolide and fraxinellone types originate from the same molecule: to this end the carboxylic acid derivatives of B-seco limonoids (e.g. **2**) are suitable precursors. However, isolation of further new compounds similar to melazolides and/or chemical investigation on the alleged precursors, need to be performed in order to clarify the biogenetic mechanism.

3. Experimental

3.1. General experimental procedures

Flash chromatography (FC): Merck-Kieselgel 60 (70–230 mesh), Merck RP-18 LiChroprep (40–65 μm). TLC: Merck-Kieselgel 60 PF_{254}. HPLC: Reinin Dynamax 60A CN (8 μm); 25×1 cm column, solvent flux 3 ml/min. UV: Perkin-Elmer Lambda3 (λ_{max} in nm, ε in mol^{-1} l cm^{-1}). Optical rotation: JASCO-DIP-181 polarimeter, $[\alpha]_D$ in deg ml dm^{-1} g^{-1}. NMR: Varian XL-300 (^1H at 299.94 MHz, ^{13}C at 75.4 MHz), δ in ppm using residual solvent signals as internal standard ($CDCl_3 = 77.0$, $CHCl_3 = 7.25$), *J* values in Hz, multiplicities and peak assignments from DEPT, ^{1}H,^{1}H-COSY, $^1J_{CH}$- and $^nJ_{CH}$-COSY. MS: Kratos MS80 with home-built acquisition system. Molecular mechanics (MM) calculations were carried out with the PCMODEL V7.5 computer program, searching for the global energy minima.

3.2. Plant material

Roots of *M. azedarach* were obtained from one plant from those growing in the garden of ISAFA (Villazzano-Trento) where a voucher specimen is deposited.

3.3. Extraction and isolation

The dried roots of *M. azedarach* (423 g) were immersed in AcOEt at r.t. for 1 week; the extract (12.1 g) was subjected to FC on silica and eluted with gradient of AcOEt in hexane to afford 15 fractions. Fractions I (0.43 g) and N (0.29 g), collected with hexane/AcOEt 65:35 and 40:60 resp., were chromatographed on RP-18 with a gradient of MeOH in H_2O to yield 12 and 18 fractions resp. The initial eluates from both FC were evaporated and the residues were submitted to HPLC on CN-column with a gradient of EtOH in hexane thus furnishing fraxinellone (75 mg), 3-teracrylmelazolide B (3 mg), pyroangolensolide (5 mg) on one hand; on the other hand they gave the mixture 9β-hydroxyfraxinellone and 30-hydroxyfraxinellone (4 mg), 9α-hydroxyfraxinellone (8 mg), melazolide A (1 mg) or 3-teracrylmelazolide A (5 mg), methyl angolensate (10 mg), 7-deacetyl-7-oxogedunin (13 mg).

*3.4. (−)-(3aS,5R,7aR)-4,4-Dimethyl-7a-hydroxymethyl-2-oxo-2,3,3a,4,5,7a-hexahydrobenzo[b]furan-5-yl 3,4-dimethyl-3-pentenoate (**3**)*

$[\alpha]_D$ −226° (EtOH, *c* 0.26). ^{1}H NMR: δ 6.03 (1H, *dd*, $J_{2,1}=10.0$ Hz, $J_{2,3}=4.1$ Hz, H-2), 5.86 (1H, *dd*, $J_{1,2}=10.0$ Hz, $J_{1,3}=0.9$ Hz, H-1), 5.10 (1H, *dd*, $J_{3,2}=4.1$ Hz, $J_{3,1}=0.9$ Hz, H-3), 3.77 (1H, *dd*, $J_{gem}=12.2$ Hz, $J_{19a,OH}=5.8$ Hz, H_a-19), 3.52 (1H, *dd*, $J_{gem}=12.2$ Hz, $J_{19b,OH}=7.3$ Hz, H_b-19), 3.05 (2H, *br.s*, H-2′), 2.82 (1H, *dd*, $J_{gem}=17.0$ Hz, $J_{6\alpha,5}=9.6$ Hz, H_α-6), 2.73 (1H, *dd*, $J_{5,6\alpha}=9.6$ Hz, $J_{5,6\beta}=5.6$ Hz, H-5), 2.50 (1H, *dd*, $J_{gem}=17.0$ Hz, $J_{6\beta,5}=5.6$ Hz, H_β-6), 2.18 (1H, *br.t*, $J_{OH,19b}=7.3$ Hz, $J_{OH,19a}=5.8$ Hz, 19-OH), 1.70 (3H, *br.s*, H-7′), 1.68 (6H, *br.s*, H-5′ and H-6′), 0.99 (3H, *s*, H-28), 0.92 (3H, *s*, H-29). ^{13}C NMR: δ 175.5 (*s*, C-7), 171.6 (*s*, C-1′), 131.0 (*d*, C-2), 128.9 (*s*, C-4′), 127.3 (*d*, C-1), 120.4 (*s*, C-3′), 85.0 (*s*, C-10), 72.8 (*d*, C-3), 67.6 (*t*, C-19), 42.3 (*d*, C-5), 40.1 (*t*, C-2′), 35.6 (*s*, C-4), 31.7 (*t*, C-6), 23.1 and 23.2 (2*q*, C-28 and C-29), 20.6 and 20.7 (2*q*, C-5′ and C-6′), 19.2 (*q*, C-7′). EIMS (70 eV) *m*/*z* (rel. int.): 248 [M] (4), 181 (5), 152 (100), 124 (13). HREIMS 248.1048±0.05 [$C_{14}H_{16}O_4$], calc. 248.1049.

*3.5. (−)-(3aS,5R,7aR)-4,4-Dimethyl-7a-hydroxymethyl-5-hydroxy-2,3,3a,4,5,7a-hexahydrobenzo[b]furan-2-one (**4**)*

$[\alpha]_D$ −94° (EtOH, *c* 0.033). ^{1}H NMR: δ 6.10 (1H, *dd*, $J_{2,1}=10.2$ Hz, $J_{2,3}=3.8$ Hz, H-2), 5.78 (1H, *dd*, $J_{1,2}=10.2$ Hz, $J_{1,3}=1.2$ Hz, H-1), 4.00 (1H, *dd*, $J_{3,2}=3.8$ Hz, $J_{3,1}=1.2$ Hz, H-3), 3.75 (1H, *d*, $J_{gem}=11.9$ Hz, H_a-19), 3.60 (1H, *d*, $J_{gem}=11.9$ Hz, H_b-19), 2.75 (2H, *m*, H-5 and H_α-6), 2.49 (1H, *m*, H_β-6), 1.02 (3H, *s*, H-28), 0.98 (3H, *s*, H-29). ^{13}C NMR: δ 175.5 (*s*, C-7), 134.7 (*d*, C-2), 126.1 (*d*, C-1), 85.2 (*s*, C-10), 71.2 (*d*, C-3), 66.7 (*t*, C-19), 42.0 (*d*, C-5), 36.6 (*s*, C-4), 31.3 (*t*, C-6), 22.7 and 23.6 (2*q*, C-28 and C-29). EIMS (70 eV) *m*/*z* (rel. int.): 212 [M] (1), 194 (4), 181 (59), 163 (23), 135 (100). HREIMS 212.1043 0.05 [$C_{11}H_{16}O_4$], calc. 212.1048.

*3.6. (3aS,5R,7aR)-4,4,7a-Trimethyl-2-oxo-2,3,3a,4,5,7a-hexahydrobenzo[b]furan-5-yl 3,4-dimethyl-3-pentenoate (**5**)*

^{1}H NMR: δ 5.85 (1H, *s*, H-1), 5.84 (1H, *d*, $J_{2,3}=2.4$ Hz, H-2), 5.09 (1H, *d*, $J_{3,2}=2.4$ Hz, H-3), 3.05 (2H, *br.s*, H-2′), 2.72 (1H, *dd*, $J_{gem}=17.8$ Hz, $J_{6\alpha,5}=9.2$ Hz, H_α-6), 2.51 (1H, *dd*, $J_{gem}=17.8$ Hz, $J_{6\beta,5}=6.9$ Hz, H_β-6), 2.39 (1H, *dd*, $J_{5,6\alpha}=9.2$ Hz, $J_{5,6\beta}=6.9$ Hz, H-5), 1.70 (3H, *br.s*, H-7′), 1.68 (6H, *br.s*, H-5′ and H-6′), 1.50 (3H, *s*, H-19), 0.99 (3H, *s*, H-28), 0.92 (3H, *s*, H-29). ^{13}C NMR: δ 175.0 (*s*, C-7), 171.6 (*s*, C-1′), 131.3 (*d*, C-2), 128.9 (*s*, C-4′), 127.6 (*d*, C-1), 120.4 (*s*, C-3′), 82.8 (*s*, C-10), 72.6 (*d*, C-3), 48.2 (*d*, C-5), 40.1 (*t*, C-2′), 35.7 (*s*, C-4), 31.4 (*t*, C-6), 27.5 (*q*, C-19) 23.3 and 23.4 (2*q*, C-28 and C-29), 20.6 and 20.7 (2*q*, C-5′ and C-6′), 19.2 (*q*, C-7′). EIMS (70 eV) *m*/*z* (rel. int.): 248 [M] (4), 181 (5), 152 (100), 124 (13). HREIMS 248.1048 0.05 [$C_{14}H_{16}O_4$], calc. 248.1049.

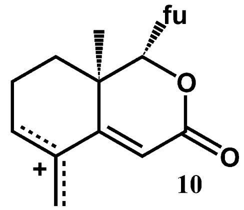
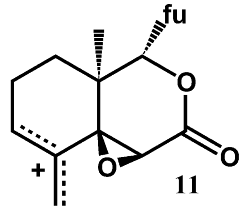
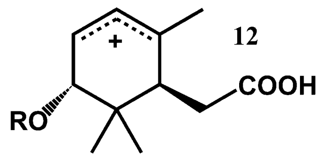
*3.7. (−)-(3aS,5R,7aR)-4,4,7a-Trimethyl-5-hydroxy-2,3,3a,4,5,7a-hexahydrobenzo[b]furan-2-one (**6**)*

$[\alpha]_D$ −75° (EtOH, *c* 0.027). ^{1}H NMR: δ 5.85 (1H, *dd*, $J_{2,1}=10.0$ Hz, $J_{2,3}=3.2$ Hz, H-2), 5.77 (1H, *dd*, $J_{1,2}=10.0$ Hz, $J_{1,3}=1.2$ Hz, H-1), 4.04 (1H, *dd*, $J_{3,2}=3.2$ Hz, $J_{3,1}=1.2$ Hz, H-3), 2.65 (1H, *m*, H_α-6), 2.45 (2H, *m*, H-5 and H_β-6), 1.53 (3H, *s*, H-19), 1.02 (3H, *s*, H-28), 1.00 (3H, *s*, H-29).

3.8. (3aS,7aR)-4,4,7a-Trimethyl-2,3,3a,4,5,7a-hexahydrobenzo[b]furan-2,5-dione

UV λ_{max}^{EtOH} nm (logε): 220 (4.26). CD $\Delta\varepsilon_{340}$ -0.4 (EtOH, *c* 0.00037). ^{1}H NMR: δ 6.55 (1H, *dd*, $J_{1,2}=10.3$ Hz, $J_{1,5}=1.9$ Hz, H-1), 6.01 (1H, *d*, $J_{2,1}=10.3$ Hz, H-2), 2.68 (1H, *m*, H_α-6), 2.63 (1H, *m*, H-5), 2.27 (1H, *m*, H_β-6), 1.73 (3H, *s*, H-19), 1.28 (3H, *s*, H-28), 1.14 (3H, *s*, H-29).

Table 1

Degraded limonoid	Hypothesized intermediate	Proposed precursors
Azedaralide Pyroangolensolide	 **10**	Deoxyandirobin Methyl angolensate
Calodendrolide Isofraxinellone Fraxinellone 9α-, 9β-Hydroxyfraxinellone 30-Hydroxyfraxinellone Fraxinellonone	 **11**	Andirobin Limonin
3-Teracrylmelazolide A and B	 **12**	Andirobin Deoxyandirobin

3.9. (−)-(3R,3aR,6R)-3-(3′-Furanyl)-3a,7-dimethyl-6-hydroxy-1,3,3a,4,5,6-hexahydrobenzo[c]furan-1-one (7)

$[\alpha]_D$ −11.8° (EtOH, *c* 0.187). ^{1}H NMR: δ 7.46 (1H, *m*, H-21), 7.44 (1H, *m*, H-23), 6.31 (1H, *m*, H-22), 4.90 (1H, *br.s*, H-17), 4.25 (1H, *br.dd*, H-9), 2.20 (3H, *d*, $J_{30,9}=0.8$ Hz, H-30), 2.20 (1H, *m*, H_β-11), 1.84–1.69 (3H, H_α-11 and H-12), 0.95 (3H, *s*, H-18). ^{13}C NMR: δ 169.3 (*s*, C-15), 148.1 (*s*, C-8), 143.6 (*d*, C-23), 139.8 (*d*, C-21), 130.1 (*s*, C-14), 120.0 (*s*, C-20), 108.4 (*d*, C-22), 83.1 (*d*, C-17), 71.7 (*d*, C-9), 43.6 (*s*, C-13), 31.4 (*t*, C-12), 28.6 (*t*, C-11), 20.4 (*q*, C-18), 14.2 (*q*, C-30). EIMS (70 eV) *m*/*z* (rel. int.): 248 [M] (5), 181 (12), 152 (100), 124 (18). HREIMS 248.1048±0.05 [$C_{14}H_{16}O_4$], calc. 248.1049.

3.10. (−)-(3R,3aR,6S)-3-(3′-Furanyl)-3a,7-dimethyl-6-hydroxy-1,3,3a,4,5,6-hexahydrobenzo[c]furan-1-one (8)

$[\alpha]_D$ −86° (EtOH, *c* 0.175). ^{1}H NMR: δ 7.46 (1H, *m*, H-21), 7.43 (1H, *m*, H-23), 6.34 (1H, *m*, H-22), 4.93 (1H, *br.s*, H-17), 4.12 (1H, *br.t*, $J_{9,11}=3$ Hz, H-9), 2.24 (3H, *d*, $J_{30,9}$=small, H-30), 1.95 (2H, *m*, H-11), 1.63 (2H, *m*, H-12), 0.84 (3H, *s*, H-18). ^{13}C NMR: δ 169.7 (*s*, C-15), 145.9 (*s*, C-8), 143.5 (*d*, C-23), 139.9 (*d*, C-21), 130.5 (*s*, C-14), 120.1 (*s*, C-20), 108.4 (*d*, C-22), 83.1 (*d*, C-17), 67.3 (*d*, C-9), 43.6 (*s*, C-13), 27.8 (*t*, C-11), 26.7 (*t*, C-12), 19.0 (*q*, C-18), 15.7 (*q*, C-30). EIMS (70 eV) *m*/*z* (rel. int.): 248 [M] (4), 181 (5), 152 (100), 124 (13). HREIMS 248.1048±0.05 [$C_{14}H_{16}O_4$], calc. 248.1049.

3.11. (3R,3aR)-3-(3′-Furanyl)-3a-methyl-7-hydroxymethyl-1,3,3a,4,5,6-hexahydrobenzo[c]furan-1-one (9)

^{1}H NMR: δ 7.47 (1H, *m*, H-21), 7.44 (1H, *m*, H-23), 6.35 (1H, *m*, H-22), 5.00 (1H, *br.s*, H-17), 4.50 and 4.25 (2H, *d*, $J_{\text{gem}}=14$ Hz, H-30), 2.45 (1H, *br.dd*, $J=20$ and 7 Hz, H_β-9), 2.26 (1H, *m*, H_α-9), 1.7–1.9 (3H, H-11 and H_α-12), 1.45 (1H, *m*, H_β-12), 0.90 (3H, *s*, H-18). ^{13}C NMR: δ 170.9 (*s*, C-15), 152.4 (*s*, C-8), 143.6 (*d*, C-23), 139.9 (*d*, C-21), 130.0 (*s*, C-14), 120.1 (*s*, C-20), 108.4 (*d*, C-22), 84.6 (*d*, C-17), 62.5 (*d*, C-30), 43.2 (*s*, C-13), 31.1 (*t*, C-12), 28.5 (*t*, C-9), 20.4 (*q*, C-18), 17.8 (*t*, C-11).

Acknowledgements

We are grateful to Dr. Carla Vender and the ISAFA (Istituto Sperimentale per l'Assestamento Forestale e per l'Alpicoltura, Villazzano-Trento) for providing the plant material. This work was supported by the Provincia Autonoma di Trento (Project on insect pest control) and by M.U.R.S.T. (Cofin2000).

References

Akhyla, A., Rani, K., 1999. Chemistry of the neem tree. Fortschr. Chem. Organ. Naturstoffe 78, 47–149.

Boustie, J., Moulis, C., Gleye, J., Fouraste, I., Servin, P., Bon, M., 1990. A degraded limonoid from *Fagaropsis glabra*. Phytochemistry 29, 1699–1701.

Dreyer, D.L., 1968. Limonoid bitter principles. Fortschr. Chem. Organ. Naturstoffe 26, 190–244.

Fernandez-Mateos, A., Benito, M.G., Coca, G.P., Gonzalez, R.R., Hernandez, C.T., 1995. Synthesis of DL-pyroangolensolide. Tetrahedron 51, 7521–7526.

Harada, N., Iwabuchi, J., Yokota, Y., Uda, H., Nakanishi, K., 1981. A chiroptical method for determining the absolute configuration of allylic alcohols. J. Am. Chem. Soc. 103, 5590–5591.

Legrand, M., Rougier, M.J., 1977. Application of the optical activity to stereochemical determinations. In: Kagan, H.B. (Ed.), Stereochemistry, Vol. 2. George Thieme, Stuttgart, pp. 123–126.

Nakatani, M., Huang, R.C., Okamura, H., Iwagawa, T., Tadera, K., 1998. Degraded limonoids from *Melia azedarach*. Phytochemistry 49, 1773–1776.

Ohtani, I., Kusumi, T., Kashman, Y., Kakisawa, H., 1991. High-field FT NMR application of Mosher's method. The absolute configuration of marine terpenoids. J. Am. Chem. Soc. 113, 4092–4096.

Okamura, H., Yamauchi, K., Miyawaki, K., Iwagawa, T., Nakatani, M., 1997. Synthesis and biological activities of degraded limonoids, fraxinellonone and its related compounds. Tetrahedron Lett. 38, 263–266.