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LIMONOIDS FROM THE FIJIAN MEDICINAL PLANT DABI (*XYLOCARPUS*)

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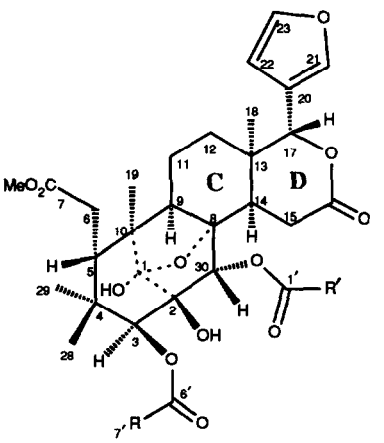
ABSTRACT

Two new limonoids, xyloccensin I (**1**) and xyloccensin J (**2**), have been isolated from the Fijian medicinal plants *Xylocarpus granatum* and *X. moluccensis*. The structures of these were proposed after extensive 2D NMR analyses. All proton and carbon resonances of **1** and **2** have been assigned. Corrections are reported for the NMR data of important known compounds related to **1** and **2** which include xyloccensin F (**3**) and swietenine (**4**). Unfortunately, compounds **1** and **2** failed to show positive bioactivity in a variety of primary screens.

INTRODUCTION

Our search for bioactive substances from Fijian marine organisms has yielded a variety of unique anti-parasitic active natural products.[1,2] We reasoned that phytochemical studies on Fijian medicinal plants ought to be similarly rewarding as they are now a subject of interest in several laboratories.[3,4,5,6] Accordingly, a chemical investigation was begun on a mangrove tree with the native name of 'dabi' (or 'legilegi') of the botanical genus *Xylocarpus*. Historically, local people refer to this as "the puzzle-nut tree"[7] and its bark pressings are used to treat fevers including those caused by malaria.[8]

Fruit kernels of *Xylocarpus granatum* Koenig and *X. moluccensis (Lam.) M. Roem*, of the family Meliaceae, were collected from Nananu-i-Ra island, Viti Levu, Fiji. The nonpolar extracts of these fruits yielded two terpenoids, xyloccensin I (**1**) and xyloccensin J (**2**), which are new examples of the mexicanolide (= xyloccensin) family of limonoid chemomarkers typically isolated from the Meliaceae. These compounds are closely related to a known limonoid, xyloccensin F (**3**).[9] In spite of this relationship, our structure elucidation work was prolonged because the NMR data in the literature of **3** (see Table 1) was incomplete, and the specific details of its structure elucidation have never been published.[10] Furthermore, the NMR data for other close derivatives such as swietenine (**4**),[11] with structures established



(**1**) R = CH_3, R'

(**2**) R = CH_3, R'

(**3**) R = R' = (xyloccensin F)

by X-ray analysis,[12] contain many unassigned or misassigned ^{13}C NMR resonances. A 2D NMR study of **1** & **2** was required to unambiguously elucidate the final overall structures as well as the ester regiochemistry and relative stereochemistry at the chiral centers.

Table 1. ^{13}C NMR Data[+]

	1°	2°	3°	4°
C#	($CDCl_3$)	($CDCl_3$)	($CDCl_3$)	($CDCl_3$)
1	107.1	107.1	107.0	216.5
2	82.2	82.3		57.6
3	80.1	80.5	80.1	78.5
4	38.7	38.7		39.0
5	40.2	40.3		49.0
6	32.3	32.3		72.8
7	173.9	173.9		175.9
8	81.0	80.9	82.1	138.3
9	63.1	63.2	63.2[#]	45.5
10	42.6	42.7		50.4
11	19.6	19.7		21.2
12	35.8	35.8	36.1[#]	34.6[#]
13	36.1	36.1		36.7
14	46.3	46.3		45.1
15	28.9	28.9		29.6[#]
16	169.9	169.8	169.8	168.5
17	77.0	77.1	76.9	76.7
18	22.1	22.2		22.8[#]
19	14.8	17.9		14.6[#]
20	120.8	120.8		121.4
21	143.0	143.0		143.1[#]
22	109.9	109.9		109.2
23	141.6	141.6		140.5[#]
28	23.7	23.8		23.0
29	21.9	21.9		21.2
30	75.6	75.6	75.5	123.6
OCH_3	51.9	51.9		
1′	174.4	174.5		
2′	40.6	33.9		
3′	26.9	19.5		
4′	11.8	21.0		
5′	21.0			
6′	171.3	171.4		
7′	21.3	21.3		

[+] Multiplicity of carbon resonances are not shown but were determined from an APT data and reaffirmed by ^1H-^{13}C COSY (J = 140 Hz) data.
° at 62.5 MHz
* Ref. 9
# Ref. 11
# Our assignments or reassignments

RESULTS AND DISCUSSION

The petroleum extracts of each *Xylocarpus* collection yielded a mixture of **1** and **2**, with the former as the dominant component. An APT ^{13}C NMR spectrum of xyloccensin I revealed a partial formula of $C_{34}H_{44}$ (Table 1) which was comparable to the molecular formula of $C_{34}H_{46}O_{12}$ determined from the HREIMS data *m/z* = 646.2993. Two OH groups were indicated by the differing H count in these two formulae as no carboxylic acid carbons were evident in the ^{13}C NMR spectrum. Four low-field ^{13}C NMR signals between δ 109 - 143 were suggestive of a β-substituted furan. Additional unsaturation, as four ester groups, was denoted by ^{13}C NMR signals at δ 174.4, 173.9, 171.3 and 169.9, with one as a methyl ester (δC = 51.9 (q), δH = 3.67 (s)). Collectively these moieties accounted for eleven of the twelve oxygens. In addition to the OMe there were six sp^3 oxygen substituted carbons indicated by their ^{13}C NMR resonances between δ 75 -107. Three of these must have OC(=O)R groups attached and the remaining three could be accounted for by C-OH and C-O-C-OH moieties; the hemiacetal carbon being assigned to the ^{13}C resonance at δ107.1 (s). A total of six rings were required to account for the remaining units of unsaturation.

The phytochemical literature of the Meliaceae contains xyloccensin F (**3**),[9,10] a hexacyclic limonoid with a hemiacetal residue, whose spectroscopic properties are close to those noted above for **1**. Actually only eight carbon and three proton NMR resonances were reported for **3** by Taylor,[9] and resonances analagous to all of these could be observed (see Table 1) in spectra we obtained for **1**. Subsequently, we determined that several of the assignments and associated peak multiplicities originally reported for **3** were in error. Nonetheless a composite structure **1**, without R and R′ regiochemistry and relative stereochemistry, could be proposed. This overall framework was also independently assembled by analysis of 2D COSY, ^1H-^1H and ^1H-^{13}C (J = 140 Hz) NMR spectra. For example, the ^1H NMR data

revealed, in addition to the β-furan ring, four separate coupled proton spin systems: H-5··H-6; H-9··H-11··H-12; H-14··H-15; and H-4′··H-3′··H-2′··H-5′. The unequivocal chemical shift assignments in hand for these protons were then applied to assign carbon signals in the ^{1}H-^{13}C (J = 140 Hz) NMR spectra (see Table 1). Remaining ambi-guous assignments in the ^{13}C NMR of **1** included the CH's at δ 80.1/4.88, 77.0/5.6 and 75.6/6.20, along with the nonprotonated carbons at δ 42.6, 38.7, and 36.1. These resonances were deciphered by obtaining a ^{1}H-^{13}C COSY (J = 9 Hz) spectrum, and the most important correlations are summarized in Table 2. At this point, the position of all of the functional groups within each of the fused *cis* rings, as well as the position of all ring substituents, could be verified from the long range $^{2}J_{C\text{-}H}$ and $^{3}J_{C\text{-}H}$ correlations shown in Table 2. Assignment of the ester regiochemistry at C-3/30 provides an illustration of this process. The CH's of positions 3 and 30 were distinguished by correlations to C-30 shown in Table 2. The C=O attached to C-3 showed a $^{3}J_{C\text{-}H}$ correlation to δ 4.88 (s, H-3) and a $^{2}J_{C\text{-}H}$ correlation to δ 2.02 (s, H_3-7′), while the C=O attached to C-30 showed $^{3}J_{C\text{-}H}$ correlations to δ 6.20 (s, H-30) and δ 1.05 (d, H_3-H-5′). With all of the ^{13}C and ^{1}H resonances firmly assigned in **1**, revisions could then be made to literature data for **3**[9] and **4**,[11] as noted by the # symbol in Table 1.

Table 2. Selected 2D NMR Correlations of 1

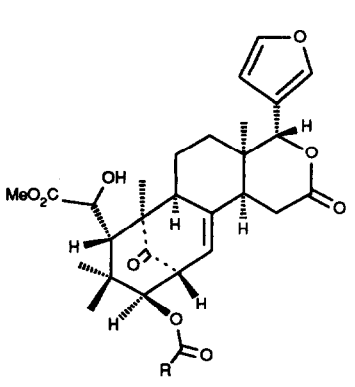
^{1}H-^{13}C COSY (J = 9)

δC	C#	Long Range Correlations to H#	
		$^{2}J_{C\text{-}x}$	$^{3}J_{C\text{-}x}$
174.4	CO-1′	-	H-5′,H-30
173.9	CO-7	H-6	OCH_3,H-5
171.3	CO-6′	-	H-3,H_3-7′
169.9	CO-16	H-15	H-14
120.8	C-20	H-17,H-21,H-22	H-23
81.0	C-8	H-9,H-14	H-11
77.0	C-17	-	H-14,H_3-18
75.6	C-30	-	H-3,H-9,H-14
40.2	C-5	-	H-3,H_3-19,H_3-28,H_3-29
38.7	C-4	H-3,H-5	H-6,H_3-28,H_3-29

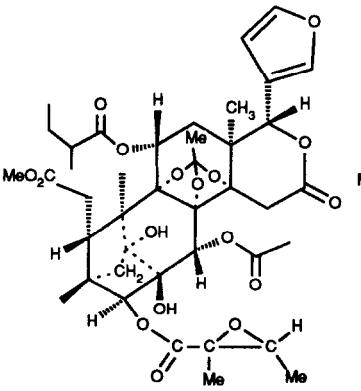
^{1}H - ^{1}H NOESY

δH	H#	NOE Correlations to H#
6.37	H-22	H_3-18,H-23
6.20	H-30	H-5,H-17
5.16	H-17	H-30,H-21
2.19	H-14	H-9,H-15a,H_3-18
1.22	H_3-29	H-3

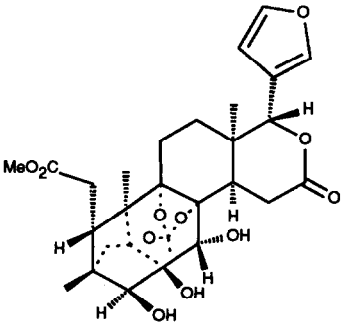
One approach to deducing the relative stereochemistry at the 11 chiral ring centers of **1** was to assume a biogenetic relationship between this compound and related limonoids whose structures had been secured by X-ray analysis. Such basis compounds consist of swietenine (**4**),[12] utilin (**5**),[13] and trideacetyl xyloccensin E (**6**) (=phragmalin).[14] This trio have parallel stereochemistry at nine centers including: C-2, C-3, C-5, C-9, C-10, C-13, C-14, C-17 and C-30. An almost identical substitution pattern exists within the CD rings between **4**, **6** and **1**; correspondingly, respective differences in ^{13}C



swietenine (**4**)



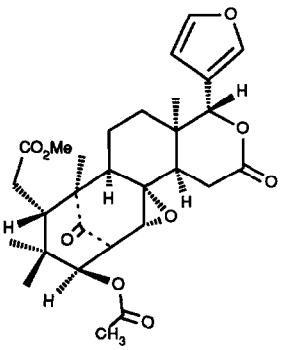
utilin (**5**)



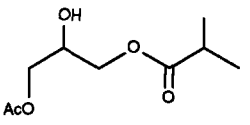
deactylxyloccensin E (**6**)

shifts at C-13, C-14, C-17 and C-18 in **4** versus **1** were each less than 1.2 ppm (Table 1) and documents their parallel geometries in this portion of the molecule. Also, the δ = 22.1 shift of Me-18 of **1** (δ 22.8 for **4**) was consistent with a *cis*-CD ring junction.[15] The stereochemical assignments of the CD rings made it possible to use Me-18, H-17 and H-14 as stereo anchor points. Thus, a NOESY correlation (see Table 2) from H-17 to H-30 and from H-30 to H-5 indicated a *cis* orientation between these respective protons. Similarly, a NOESY correlation from H-14, to Me-18 and to H-9 also indicated their mutual *cis* relationship. A significant NOESY correlation was observed from H-3 to Me-29, but no correlations were observed from H-3 to H-5, or from H-3 to H-30. This provided circumstantial support for the *trans* relationship shown for these proton sets in **1**. We have found rigid six-membered ring Me ^{13}C chemical shifts to be very sensitive to changes in stereochemistry.[16] The difference in shifts at Me-19, Me-29, and Me-28 between **4** and **1** was less than 0.8 ppm at each carbon. Therefore, the stereochemistry of substituents vicinal to these methyls must be identical in these two compounds. This, along with arguments cited above, substantiates the relative configurations proposed in **1** at C-3, C-5, and C-10. Inspection of molecular models which incorporate all the stereochemical constraints established at this point revealed that the fused rings could only be constructed if the remaining undefined centers have the geometry shown in **1**.

Xyloccensin J (**2**) was obtained as colorless small needles, m.p. 213-215 °C. The molecular formula of $C_{33}H_{44}O_{12}$ was deduced by LREIMS, and the M^+ of *m/z* = 632 is 14 amu less than that of **1**. The ^1H and ^{13}C NMR spectra of compound **2** were almost identical as compared to **1**, except that the triplet at δ 0.87 due to an *sec*butyrate methyl was absent. The presence of a new isopropyl group was noted by methyl proton doublets at δ 1.08 (J = 7.2 Hz) and 1.07 (J = 6.7 Hz), each of which was coupled to a methine proton at δ 2.61. The remaining NMR assignments for **2**, shown in Table 1, were based on 2D COSY, ^1H-^1H and ^1H-^{13}C (*J* = 140 & 9 Hz) NMR spectra. That **1** and **2** were also similar in overall stereochemistry could be seen from the closeness of the ^{13}C NMR shifts of the related carbons.



xylocarpin (**7**)



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Xyloccensins I & J were also isolated from *X. moluccensis*. In addition, the same glyceride ester **8** was isolated from both organisms. The presence of such identical mixtures of these different metabolites from the different *Xylocarpus* species presents an interesting situation. This may be consistent with the speculation that both plants are actually an identical species and differ only at the varietal level.[9] Fijian specimens of these two species have similar fruits, but their leaves and habitats are different. Also, it has been noted that East African specimens of *X. mekongensis* are a small fruited variety of *X. granatum*.[9] The seeds from African collections of *X. moluccensis* and *X. mekongensis* are both a source of xylocarpin (**7**), but this compound was not observed from our Fijian material.

Both compounds **1** and **2** were subjected to extensive *in vitro* bioassays. Neither compound was active in a broad screening effort which included assays for antimicrobial, antiviral, anthelmintic, and antikinase responses. These negative results are in contrast to bioassay evaluations which have shown that some limonoids are active as insect antifeedants[17] and possess cytotoxic activity.[18]

EXPERIMENTAL SECTION

The NMR spectra were recorded at 300 MHz for ^1H, and 75.0 MHz for ^{13}C. Multiplicities of ^{13}C NMR resonances were determined from APT data, and from COSY experiments. Low resolution electron impact mass spectrometry data were obtained at UCSC, while high resolution mass spectral data were obtained from the UC Berkeley MS laboratory.

Two-dimensional NMR procedures. Literature pulse sequences[19] were used for the APT, ^1H-^1H COSY, ^1H-^{13}C COSY, and NOESY experiments.

Isolation procedures. The fruit kernels of *X. granatum* and *X. moluccensis* collected from Nananu-i-Ra were separated from their nut husks and crushed using a blender. Separately, the ground fruit kernels (seeds) of *X. granatum* (4.9 kg) and *X. moluccensis* (4.0 kg) were extracted in a soxhlet apparatus with light petroleum for 20 hours. The concentrated extract deposited an off-white solid for *X. granatum* (32 g) and a brownish white precipitate for *X moluccensis* (4.1 g).

The *X. granatum* precipitate (19.0 g) was dissolved in a minimum volume of $CHCl_3$ and chromatographed on silica gel. A gradient elution scheme involved 100 mL portions each of light petroleum containing 0, 1, 2, 5, 7.5, 10, 15, 20, 30, 50, 75 and 100% EtOAc followed by increasing concentrations of CH_3OH in EtOAc. The fractions containing compounds which produced identical R_f values when chromatographed on TLC plates were combined. A similar procedure was applied to the solid from *X. moluccensis*.

The impure mixtures of xyloccensins I & J, from the 100% EtOAc chromatographic fractions above, were subjected to repeated chromatography on silica gel columns using gradient elution technique, initially eluting with varying portions of EtOAc in light petroleum (light petroleum: EtOAc = 85:15 to 0:100) followed by increasing concentration of Et_2O in light petroleum (40:60 to 100:0). Crystals were obtained from the 100% EtOAc fraction which were then further purified on preparative thick layer or thin layer chromatography plates (~20 mg of crystals/plate) developed three times in EtOAc:Et_2O (7:93). Two bands were obtained which consisted of xyloccensin I (60 mg, R_f = 0.59, Et_2O:EtOAc, 4:1) and xyloccensin J (10 mg, R_f = 0.56, Et_2O:EtOAc, 4:1).

Xyloccensin I (1): fine white needles, m.p. 223-225 °C (from methanol); $[\alpha]_D$ -105° (c 0.06, CH_2Cl_2); IR (CH_2Cl_2) λ_{max} 3505, 3510, 2923, 1730, 1723 cm^{-1}; LREIMS (positive) *m/z* (relative intensity) 646 M^+ (30), 628 (20), 614 (5), 562 (30), 544 (32), 508 (70), 484 (69), 404 (20), 194 (50) and 110 (100); HREIMS (positive) 646.2993 [M^+ $C_{34}H_{46}O_{12}$ Δ 0.4 mmu of calcd]; ^1H NMR ($CDCl_3$, 250 MHz) δ 4.88 (s, H-3), 2.59 (bd, J = 8.4 Hz, H-5), 2.29 (bdd, J = 11.7, 9.6 Hz, H-6), 1.45 (dd, J = 2.4, 13.2 Hz, H-9), 1.62 & 1.20 (m, H-11), 1.79 & 1.26 (m, H-12), 2.19 (d, J = 9.3 Hz, H-14), 3.13 (d, J = 19.8 Hz, H-15), 2.71 (dd, J = 19.8, 9.3 Hz, H-15), 5.16 (s, H-17), 1.02 (s, Me-18), 1.08 (s, Me-19), 7.52 (s, H-21), 6.37 (bs, H-22), 7.37 (bs, H-23), 0.68 (s, Me-28), 1.22 (s, Me-29), 6.20 (s, H-30), 3.67 (s, OMe), 2.38 (m, H-2′), 1.67 & 1.20 (m, H-3′), 1.05 (d, J = 6.6 Hz, Me-5′), (t, J = 7.5 Hz, H-4′), 2.02 (s, Me-7′).

Xyloccensin J (**2**): small white needles, m.p. 213-215 °C (from methanol); $[\alpha]_D$ -106° (c 0.09, CH_2Cl_2); LREIMS (positive) *m/z*, (relative intensity) 632 M^+ (10), 614 (8), 572 (10), 494 (10), 406 (30), 346 (15), 238 (40) and 180 (100); ^1H NMR ($CDCl_3$, 250 MHz) δ 4.84 (s, H-3), 2.60 (bt, J = 7.5 Hz, H-5), 2.29 (bt, J = 7.5 Hz, H-6), 1.46 (dd, J = 10.4, 2.8 Hz, H-9), 1.63 & 1.20 (m, H-11), 1.79 & 1.26 (m, H-12), 2.19 (d, J = 8.8 Hz, H-14), 3.12 (d, J = 19.6 Hz, H-15), 2.71 (dd, J = 19.8, 8.8 Hz, H-15), 5.19 (s, H-17), 1.03 (s, Me-18), 1.05 (s, Me-19), 7.52 (s, H-21), 6.37 (bs, H-22), 7.37 (bs, H-23), 0.69 (s, Me-28), 1.22 (Me-29), 6.17 (s, H-30), 3.68 (s, OMe), 2.61 (m, H-2′), 1.08 (d, J = 7.2 Hz, H-3′), 1.07 (d, J = 6.7 Hz, H-4′) and 2.04 (s, Me-7′).

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REFERENCES

1. Alvi, K. A.; Tenenbaum, L.; Crews, P. *J. Nat. Prod.* **1991**, *54*, 71.
2. Jiménez, C.; Crews, P. *Tetrahedron* **1991**, *47*, 2097.
3. Cambie, R. C.; Lal, A. R.; Ahmad, F. *Phytochemistry* **1990**, *29*, 2329, and refs within.
4. (a) Jogia, M. K.; Andersen, R. J.; Mantus, E. K.; Clardy, J. *Tetrahedron Lett.* **1989**, *30*, 4919. (b) Jogia, M. K.; Andersen, R. J. *Can. J. Chem.* **1989**, *67*, 257.
5. Aalbersberg, B.; Singh, Y. *Phytochemistry* **1991**, *30*, 921.
6. Brophy, J. J.; Jogia, M. K. *J. Nat. Prod.* **1986**, *49*, 730.
7. Parham, H. B. R. *Polynesian Soc. Mem.* **1943**, *16*, 1-160.
8. Weiner, M. A. *Secrets of Fijian Medicine*, Author's self published book, Berkeley, CA. **1984**, pp. 14 & 80.
9. Connolly, J. D.; MacLellan, M.; Okorie, D.; Taylor, D. A. H. *J. Chem. Soc. Perkin Trans. I* **1976**, 1993.
10. Taylor, D. A. H. *Prog. Chem. Org. Nat. Prod.* **1984**, *45*, 21-26.
11. Taylor, D. A. H. *J. Chem. Soc. Perkin Trans. I* **1974**, 437.
12. McPhail, A. T.; Sim, G. A. *Tetrahedron Lett.* **1964**, 2599.
13. Harrison, H. R.; Hodder, O. J. R; Bevan, C. W. L.; Taylor, D. A. H.; Halsall, T. G. *J. Chem Soc., Chem. Comm.* **1970**, 1388.
14. Coetzer, J.; Baxter, W. J.; Gafner, G. *Acta. Cryst.* **1971**, *B27*, 1434.
15. Crews, P.; Jiménez, C.; O'Neil-Johnson, M. *Tetrahedron* **1991**, *47*, 3585.
16. Crews, P.; Bescansa, P. *J. Nat. Prod.* **1986**, *49*, 1041.
17. Lavie, D.; Jain, M. K.; Shpan-Gabrielith, S. R. *J. Chem. Soc., Chem. Commun.* **1967**, 910.
18. Jolad, S. D.; Wiedhopf, R. M.; Cole, J. R. *J. Pharm. Sci.* **1977**, *66*, 889.
19. For a recent review of practical aspects of 2D NMR see: Gray, G. A. In *Two-Dimensional NMR Spectroscopy*; Croasmun, W. R.; Carlson, R. M. K., Eds.; VCH: New York, **1987**; Chapter 1.