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# Isolation and Structural Characterisation of Kamahine C: An Unusual Spiroketal found in a Native New Zealand Honey.

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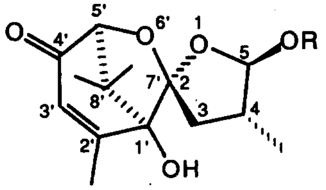
***Abstract:*** *The diethyl ether extract of honey from the flowers of the New Zealand native tree Kamahi (Weinmannia racemosa) has been found to contain three diastereoisomers of an unusual degraded carotenoid with a 14 carbon skeleton. After acetylation, one of the isomers,* ***1b****, was fully characterised by multidimensional* $^{1}H$ *and* $^{13}C$ *NMR spectroscopy and single crystal X-ray crystallography, showing that the parent alcohol (Kamahine C,* ***1a****) was 4,5-dihydro-1',5-dihydroxy-2',4,8',8'-tetramethylspiro[furan-2(3)H,7'-[6]oxabicyclo-[3.2.1]oct[2]ene]-4'-one.*

The non-carbohydrate extractives of New Zealand honeys have been found to contain a diverse range of *nor*-carotenoids[1] which have been shown to be of significant interest as floral source markers. Analysis of these extractives by gas chromatography is proving to be a viable replacement for pollen microscopy.[2] Additionally, some honeys show strong anti-bacterial activity,[3] although the relationship of this activity to the non-carbohydrate extractives is not yet clear. The diethyl ether extract of honey from Kamahi (*Weinmannia racemosa*) yielded a fraction containing three novel diastereoisomeric Kamahines A-C. Herein we report the structure elucidation of Kamahine C **1a** on the basis of NMR and X-Ray crystallographic analysis of its acetate **1b**.



**1a** R = H
**1b** R = Ac

A concentrated aqueous honey solution (600 g in 800 ml) was subject to continuous liquid-liquid extraction with diethyl ether. The extract (600 mg) was subject to further purification by flash chromatography and preparative tlc ($SiO_2$, diethyl ether/petroleum spirit), yielding a fraction (15 mg) containing Kamahines A-C. This fraction was just separable on a non-polar capillary GC column, but inseparable by preparative techniques, including reverse-phase hplc. High resolution electron-impact GC/MS showed a molecular ion at m/z 268.1307 (calulated m/z for $C_{14}H_{20}O_5$ = 268.1311). Low-resolution EIMS showed a peak at ($M^{+\cdot}$ - 17), suggesting loss of a tertiary hydroxyl radical, and a base peak at m/z 152, consistent with a 2,6,6-trimethylcyclohex-2-en-1,4-dione fragment.

It was apparent from the $^{1}$H and $^{13}$C NMR spectra that the three compounds were diastereoisomeric, and spectrum editing using the DEPT[4] sequence showed that each isomer possessed 4 methyl, 1 methylene, 4 methine and 5 quaternary carbons. Kamahines A and C interconverted in deuterochloroform solution, while Kamahine B appeared to be stable.[5] Acetylation of the mixture (pyridine/acetic anhydride) followed by further preparative thin-layer chromatography afforded two fractions, one was shown to be an inseparable mixture of monoacetylated Kamahine A and B whereas the other fraction was essentially pure monoacetoxy-Kamahine C (**1b**). From the mass spectral fragmentation pattern and 1D $^{1}$H and $^{13}$C spectra, the presence of a 1,5-dioxysubstituted 2,6,6-trimethylcyclohex-2-en-4-one fragment was suspected, as were the presence of ketal and acetal groups. The 2D$^{1}$H-$^{1}$H COSY and HOHAHA[6,7] spectra showed **1b** to have two independent spin systems, and combination of this information with long-range $^{2}J$ and $^{3}J$ $^{13}$C-$^{1}$H correlations (obtained by both heteronuclear chemical shift correlation[8] and inverse-mode HMBC[9] experiments), suggested the presence of C9 and C5 fragments. Furthermore, the connectivity between the fragments was unambiguously shown from the key long-range C-H correlations H5' - C7', H5 - C7' and OH1' - C7' (Figure 1).

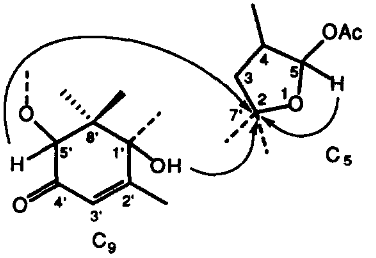


Fig. 1. Long-range C-H correlations connecting C$_9$ and C$_5$ fragments

These data showed that **1b** possessed a novel tricyclic skeleton, and this structure represents the first reported spiro[furan-2,7'-oxabicyclo[3.2.1]octane]. The orientation of the furan ring with respect to the enone system was determined by NOE difference experiments.[10] The key interactions are shown in Figure 2. In particular, the NOE enhancement observed between H3a and 2'-Me showed that the furan ring was oriented such that C3 was *cis* to the enone system. Additional NOE experiments showed that H4 and H5 were *trans* with respect to each other and the strong NOE enhancement observed between 4-Me and H5 showed that 4-Me was *cis* to H5. Full NMR data, with COSY, NOE and long-range C-H correlations, are given in Table 1.

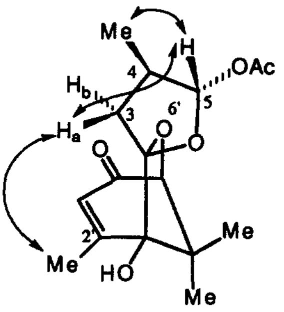


Fig. 2. Key NOE enhancements

Table 1. NMR data for **1b**

| Carbon | $\delta^{13}C$ | $\delta^{1}H$ (J Hz) | COSY | HMBC (H→C) | NOE |
|---|---|---|---|---|---|
| 4-Me | 16.1(q) | 1.10 (d, $^{3}J$=6.9Hz) | H4 | 3, 4, 5 | 4, 3a, 5 |
| 8'-Me$_{ax}$ | 18.1(q) | 1.00 (s) | 8'-Me$_{eq}$ | 8'-Me$_{eq}$ 8', 1', 5' | 8'-Me$_{eq}$, 5' |
| 2'-Me | 20.3(q) | 2.06 (d, $^{4}J$=1.5Hz) | H3' | 1', 2', 3' | 3', 3a, 8'-Me$_{ax}$ |
| MeCO | 21.3(q) | 2.12 (s) | | | |
| 8'-Me$_{eq}$ | 21.8(q) | 1.27 (s) | 8'-Me$_{ax}$ | 1', 5', 8', 8'-Me$_{ax}$ | 8-Me$_{ax}$, 5' |
| 4 | 36.8(d) | 2.51 (m) | H5, H3a, H3b,4-Me | 5 | 4-Me, 3b, 5 |
| 3 | 40.5(t) | H3a: 1.66 (dd, $^{2}J$=13.0, $^{3}J$=9.9Hz), H3b: 2.09 (dd, $^{2}J$=13.0, $^{3}J$=7.3Hz) | H4 | 3a→1', 7', 4, 4-Me, 5<br>3b→ 7', 4, 4-Me, 5 | 3b, 4-Me<br>3a, 4 |
| 8' | 52.0(s) | | | | |
| 1' | 84.9(s) | 2.90 (s(broad), OH) | | 2', 7' | |
| 5' | 89.9(d) | 3.98 (d, $^{4}J$=2.2Hz) | H3' | 1', 3', 4', 7', 8'-Me$_{eq}$ | 8'-Me$_{eq}$, 8'-Me$_{ax}$ |
| 5 | 103.6(d) | 5.85 (d, $^{3}J$=4.8Hz) | H4 | 4, 7' | 4-Me, 4, 3a |
| 7' | 114.6(s) | | | | |
| 3' | 125.4(d) | 5.91 (dq, $^{4}J$=2.2, $^{4}J$=1.5Hz) | 2'-Me, H5' | 1', 2'-Me | 2'-Me, 8'-Me$_{ax}$ |
| 2' | 164.8(s) | | | | |
| MeCO | 170.8(s) | | | | |
| 4' | 194.6(s) | | | | |

During the course of the NMR study, crystallisation of **1b** was induced, and a single-crystal X-ray diffraction study was carried out.[11] The structure and relative stereochemistry (Figure 3) was identical to the NMR derived structure. Although **1b** crystallised in an asymmetric space group, it was not possible to determine the absolute configuration from the crystallographic data.

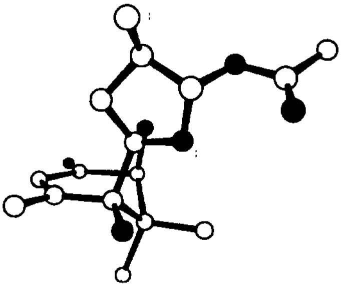


Fig. 3. Crystal structure of **1b** (hydrogens omitted for clarity, arbitrarily chosen enantiomer shown)

The Kamahines appear to be the first *nor*-carotenoids to possess a C14 skeleton.[12] From our initial investigations, it appears that the Kamahines are formed from oxidative processes occurring in the honey, rather than being components of the floral extractives, although the possibility that they exist as glucosides in the flowers cannot be ruled out. We are actively searching for likely precursors of the Kamahines, with a view to gaining some insight into the chemical transformations occurring in honeys. As part of this investigation, we expect to report the full relative and absolute stereochemistry of Kamahines A-C in the near future.

*Acknowledgements*: The authors are indebted to: Ms J. Wikaira, University of Canterbury for collection of X-ray intensity data, Assoc. Prof. B.K. Nicholson for assistance with the X-ray crystallographic analysis, and to Dr P.T. Holland for high-resolution mass spectra. Financial assistance for equipment from the University Grants Committee and New Zealand Lottery Science, and scholarships from the New Zealand Vice-Chancellors Committee for S.J.B. and Y.L. are gratefully acknowledged. Nomenclature for **1a** was provided by Dr B. Bossenbroek, Chemical Abstracts Service.

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(5) NMR data for **1a** was taken from the mixture of isomers A-C. $^1$H NMR (300MHz) δ: 0.98(3H, s, 8'-Me$_{ax}$), 1.09(3H, d, $^3$J=6.8, 4-Me), 1.31(3H, s, 8'-Me$_{eq}$), 1.60(1H, dd, $^2$J=13.1, $^3$J=8.1, H-3), 2.03(1H,dd, $^2$J=13.1, $^3$J=6.9, H-3), 2.04(3H, d, $^4$J=1.4, 2'-Me),2.25(1H,m, H-4), 2.97(1H, OH), 3.88(1H, OH), 3.97(1H, d, $^4$J=2.0, H-5'), 5.03(1H, t, J=5.9, H-5), 5.89(1H, dd, $^4$J=1.4, $^4$J=2.0, H-3'). $^{13}$C NMR (75.5MHz) δ: 15.72(q, 4-Me), 18.22(q, 8'-Me$_{ax}$), 20.40(q, 2'-Me), 21.93(q, 8'-Me$_{eq}$), 39.65(d, C-4), 41.30(t, C-3), 52.09(s, C-8'), 84.40(s, C-1'), 89.58(d, C-5'), 107.10(d, C-5), 112.93(s, C-7'), 125.28(d, C-3'), 165.52(s, C-2'), 195.17(s, C-4').

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(11) (a) clear, colourless crystals m.p. 152-154 °C: Empirical formula $C_{16}H_{22}O_6$, $M_r = 310.2$, orthorhombic, space group $P2_12_12_1$; $a = 8.660$, $b = 13.554$ and $c = 13.661$ Å. $U = 1603.5$ Å$^3$, $D_{calc} = 1.28$ g cm$^{-3}$. Of the 1790 unique intensities, 1010 were used with $I \geq 2\sigma(I)$. Intensities were measured using Mo $K_\alpha$ radiation ($\lambda = 0.71073$ Å). The structure was solved by direct methods (SHELXS86$^{11b}$). All atoms were treated isotropically, and hydrogen atoms were included in calculated positions with common isotropic temperature factors for each type of hydrogen. The refinement (SHELX76$^{11c}$) converged with $R = 0.0801$, $R_w = 0.0700$ where $w = [\sigma^2F + 0.000525F^2]^{-1}$. (b) Sheldrick, G.M. "SHELXS86, a program for solving X-ray crystal structures", University of Göttingen, Göttingen, Germany 1986. (c) Sheldrick, G.M. "SHELX76, a program for X-ray crystal structure determination", University of Cambridge, Cambridge, U.K. 1976.

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