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Raoulic acid: A Novel Bioactive C_{25} Terpene Acid from *Raoulia australis*

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Key Words: *Raoulia australis;* raoulic acid; C_{25} terpene acid.

Abstract: Examination of the P388 active extracts of the New Zealand plant, ***Raoulia australis***, has resulted in the isolation of a novel bicyclic C_{25} terpene acid. The structure was solved by NMR studies on an ozonolysis-type derivative.

As part of a search for new bioactive compounds from New Zealand plants, *Raoulia australis* has been examined due to the *in vitro* anti-leukemic (P388) and antibacterial activity[1] exhibited by non-polar extracts of this unusual Asteraceae species. *R. australis* is a species endemic to New Zealand and forms dense cushion-like mats which can be up to 1 m in diameter. This study reports the structure of the major bioactive constituent of this plant, a bicyclic C_{25} terpene acid which has been named raoulic acid (1).

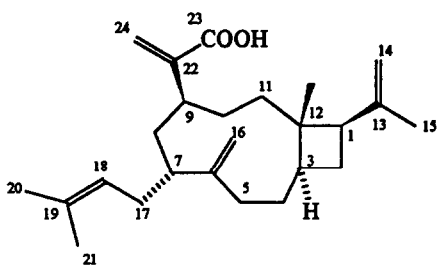

Fig. 1. Structure of raoulic acid (1)

Extraction of dried whole plant material with hexane and a two-step fractionation by SiO_2 column chromatography and rp hplc readily yielded 1 as a colourless oil.[2] Raoulic acid is present in unusually high concentration comprising ca. 70% of the hexane extract which in turn comprises approx. 8% of the dry weight of plant material. The molecular formula of 1 was established as $C_{25}H_{38}O_2$ from hrcims and the ^{13}C nmr spectrum. A methyl ester was readily formed with diazomethane thereby establishing an acid group as the only oxygen-containing functionality present in the molecule. One trisubstituted and three exo-methylene type double bonds were evident from the ^{13}C nmr spectra, and the uv and nmr data suggested that one of these double bonds was conjugated with the acid group. The remaining unsaturations could then be assigned to a bicyclic carbon skeleton.

Apart from the methyl and olefinic signals most of the remaining signals in the ^1H nmr spectrum were part of a broad overlapping region of the spectrum with limited coupling detail apparent. A C-H

COSY experiment established the position of all of the ^1H nmr signals for each of the carbons however the lack of resolution of the signals in the ^1H nmr spectrum and the near or complete coincidence of several of the signals in the ^{13}C nmr spectrum did not allow a complete structural determination from subsequent COLOC and H-H COSY experiments.[3] However, several partial structures, shown below, were deduced from these latter experiments. Some of the C-C linkages, denoted in bold, were confirmed by an INADEQUATE experiment.

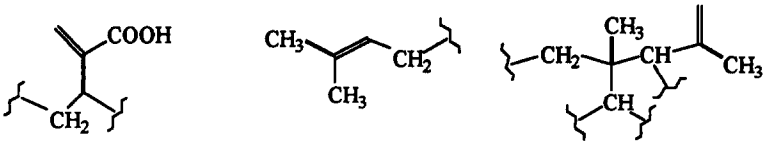

Fig. 2. Partial structures of **1** obtained from nmr experiments

The absence of further unambiguous connectivities between these partial structures presented a difficult structure determination problem and prompted an examination of further derivative or cleavage products. The abundance of double bonds suggested that oxidative cleavage might afford a suitable product. Ozonolysis was unsuccessful, however reaction of the methyl ester of **1** with the Lemieux-Johnson reagent (OsO_4-$NaIO_4$)[4] yielded small amounts of a product, **2**, which had spectra consistent with oxidative cleavage of all of the double bonds in **1** and with the retention of the ester group.[5] The retention of the bicyclic skeleton of **1** was confirmed by the molecular formula of **2**, $C_{20}H_{28}O_6$ (5 C=O). The ^1H nmr spectrum showed **2** was clearly quite amenable to structure solution in that many of the protons, particularly those adjacent to carbonyl groups, had signals which were free of overlap and had distinctive coupling detail. Also, many of the other signals showed enough chemical shift variation in spectra run in C_6D_6, $CDCl_3$ or a mixture of both solvents to enable the important connectivities to be deduced from HMQC, HMBC and H-H COSY nmr experiments.

Two separate spin systems were evident from the H-H COSY data. One is the system linking C-1 through to C-5. Only four of the proton signals in this system were free of overlap in the ^1H nmr spectrum, however the connections through the methylene groups at C-2 and C-4 are clearly defined from COSY cross peaks. The second spin system links C-7 through to C-11 and includes the H-7 to H-15 linkage. The two methine protons, H-7 and H-9, are separated by the single methylene group at C-8. H-9 shows further coupling to an ethylene unit (C-10,11).

When these partial structures were compared with the two- and three-bond C-H connectivity data from HMBC experiments (Table 1) the structure could be unambiguously assembled as **2**. The important linkages which defined the structure of the ring system were (i) those about the angular methyl (C-19) linking C-11, -3 and -1 to C-19 via C-12, therefore defining a four membered ring, and (ii) those from H-5 and H-7 to C-6 to form a ten-membered ring. This data also enabled the side chains at C-1 and -7 to be determined from coupling of H-1 and H-14 to C-13 and H-7 to C-16. The side chain at C-9 must be an α-oxo methyl ester group since there is a C-H coupling between C-17 and H-9 and hence the only possible placement of the ester group is adjoining C-17.

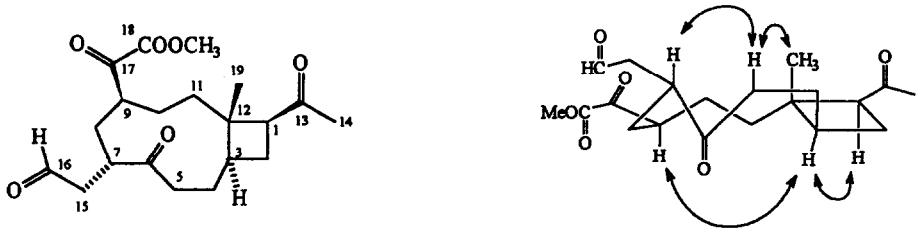

Fig. 3. Structure of **2** and observed nOe enhancements

Table 1. ^{1}H and ^{13}C NMR Data for **2** ($CDCl_3$)

Assignment	^{13}C	^{1}H	HMQC correlations to C #	NOE
1	53.00	2.71 dd 9.7, 8.2	2, 11, 12, 13, 19	11α, 3
2	24.34	~ 1.90 (2H)		
3	37.06	2.12 m	11, 12, 19	1, 9, 11α
4	25.44	~ 1.78 ~ 1.50		
5	39.43	2.90 dt 16.2, 3.3 (Hβ) 2.52 m (Hα)	3, 4, 6	
6	214.08			
7	42.61	3.28 m	6, 8, 9, 15, 16	5β, 8β, 15
8	30.00	~ 2.02 1.82 m	6, 7, 9, 10, 15, 17	
9	46.09	3.20 m	7, 8, 10, 11, 17	3, 11α
10	22.72	~ 1.78 ~ 1.50		
11	41.28	~ 1.92 (Hβ) 1.47 m (Hα)	3, 9, 10, 19	1, 3, 9
12	46.97			
13	207.36			
14	30.19	1.99 s	13	
15	47.18	2.92 dd 19, 9 2.48 dd 19, 4	6, 16	
16	199.55	9.66 s		
17	195.95			
18	161.87			
19	14.77	0.94 s	1, 3, 11, 12	5β, 11β
OMe	53.00			

The relative stereochemistry of 2 was obtained from phase-sensitive NOESY spectra. The important positive NOE's are shown above and in Table 1. The angular methyl, arbitrarily assigned as β, exhibits a positive NOE with one of the H-5 protons, which in turn shows a further NOE with H-7, placing H-7 on the same face as the angular methyl. These are the only important correlations on the upper side of the molecule. Two crucial NOE's are observed between H-1 and H-3 and between H-9 and H-3 placing these protons on the underside of the molecule. From an examination of a Dreiding model of **2** it is evident that for these interactions to occur, H-1, H-3 and H-9 must be α as represented in **2** and H-7 must be β. Thus, the ring junction is trans, and the side chains at C-1, C-7 and C-9 are cis, trans and cis, respectively, relative to the angular methyl.

The assignment of the structure and relative stereochemistry of raoulic acid then follows by comparison of **2** with those partial structures previously elucidated for **1**. Raoulic acid represents a new terpene skeleton of uncertain biogenesis, but possibly arises from diprenylation of a sesquiterpenoid precursor of the germacrane type which are common metabolites in other Asteraceae species[6]. The relatively rare 4-membered ring appears to play some part in the biological activity of **1** as an isomeric co-metabolite with this ring opened, also isolated during this work, is considerably less active in the P388 assay. Studies on the biological activity of **1** and related metabolites in the extract are continuing.

Acknowledgements: To A P Druce and B P J Molloy (Landcare NZ Ltd) for assistance in the collection of plant material and to H Wong (Industrial Research NZ Ltd) for nmr spectra.

References and Notes

1. Bioassay-guided fractionation identified raoulic acid as the major active constituent. Activity data: P388 murine leukemia LC_{50} 5μg/mL and min. inhib. conc. (MIC) vs. methcillin-resistant *S. aureus* using an agar dilution technique 25 μg/mL.
2. Spectral data for **raoulic acid** (**1**): $[\alpha]_D^{20}$ = -3.7° (c = 2.2, $CHCl_3$): ^1H NMR ($CDCl_3$) 6.35 s and 5.65 s (H-24); 5.12 t 7 (H-18); 5.10 s and 4.97 s (H-16); 4.76 s and 4.55 s (H-14); 2.92 t (br) 10 (H-9), 2.25 t (br) 6 (H-1), 1.68 s (3H) (H-20), 1.57 s (6H) (H-21,15), 0.80 s (3H) (H-25); ^{13}C NMR ($CDCl_3$) 173.7 (C-23), 151.4 (C-6), 145.5 (C-13), 144.9 (C-22), 132.2 (C-19), 126.2 (C-24), 123.2 (C-18), 112.2 (C-16), 109.0 (C-14), 51.1 (C-1), 46.5 (C-12), 42.2 (C-11), 41.4 (C-9), 39.7 (C-8), 39.2 (C-7), 37.4 (C-17), 37.3 (C-5) 34.1 (C-3) 29.0 (C-10), 28.8 (C-2), 25.8 (C-20), 23.2 (C-15,4), 17.8 (C-21), 14.5 (C-25); CIMS m/z (rel. intensity) 371 (30), 301 (25), 259 (20), 233 (100), 187 (40); HRCIMS calcd. for $C_{25}H_{39}O_2$ 371.2950, found 371.2940; IR (cm^{-1}) 3000 br, 2955, 2910, 1690; UV (hexane) λ_{max} (E) 232 (9 200) and 192 (4 000).
3. Nmr experiments were run at 500 MHz on a Varian Unity instrument (phase sensitive NOESY, Double quantum filtered COSY) or at 300 MHz on a Bruker AC 300 instrument (All other experiments) using manufacturer supplied software.
4. Pappo, R., Allen, Jr.; D.S., Lemieux, R.U.; and Johnson, W.S. *J. Org. Chem.* **1956**, *21*, 478.
5. Compound **2** was prepared from 500 mg raoulic acid methyl ester by stirring with 100 mg OsO_4 in 50 ml THF at r.t. with the addition of 15 g $NaIO_4$ and 30 ml H_2O over 5 hrs. After overnight stirring the mixture yielded, on workup, 20 mg **2** as a colourless oil: $[\alpha]_D^{25}$ = -74.3° (c = 0.9, $CHCl_3$); ^1H and ^{13}C NMR, Table 1; EIMS m/z (rel. intensity) 364 (2), 321 (5), 259 (10), 217 (10), 111 (45); HREIMS calcd. for $C_{20}H_{28}O_6$ 364.1886, found 364.1868; IR (cm^{-1}) 1723, 1702.
6. Mabry, T.J.; Bohlmann, F.J. Summary of the chemistry of the Compositae. In *Biol. Chem. Compos., [Symp.]*; Heywood, V.H.; Harborne, J.B.; Turner, B.L. Eds.; Academic: London, 1975; pp. 1097-1104.

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