Tetrahedron Letters, Vol. 34, No. 28, pp. 4461-4464, 1993
Printed in Great Britain

0040-4039/93 $6.00 + .00
Pergamon Press Ltd

Bershacolone, an Unprecedented Diterpene Cyclobutene from *Maprounea africana*

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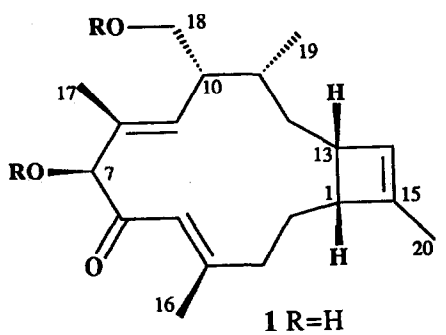
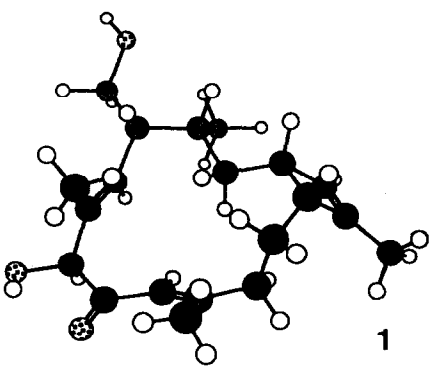
Abstract: The organic extract of *Maprounea africana* was found to contain bershacolone (1), which was defined by spectral methods as a unique diterpene containing a cyclobutene ring within a novel carbon skeleton.

The CH_2Cl_2/MeOH extract of roots of *Maprounea africana*, collected in the Central African Republic, showed activity in the NCI's primary anti-HIV screen.[1] In the course of fractionating and purifying the anti-HIV constituents of the *M. africana*, we isolated what proved to be a novel diterpene as an inactive side fraction. Our growing interest in unusual diterpenes from the Euphorbiaceae[2,3] prompted us to investigate this compound further.

Bershacolone (1) was isolated[4] as a colorless, optically active glass from the CCl_4 soluble fraction of the organic extract. CIMS (NH_3) provided *m/z* 319 $[MH]^+$ for a molecular formula of $C_{20}H_{30}O_3$, which was confirmed by HREIMS, *m/z* 300.2046 ($[M-H_2O]^+$, 4.3 mmu dev). The IR spectrum showed, in addition to a broad OH stretch (3340 cm^{-1}), a characteristic absorption for a conjugated carbonyl moiety at 1688 cm^{-1}. This was substantiated by the UV absorption (λ_{max}^{EtOH} 246 nm, ε=10,000), as well as the ^{13}C NMR spectrum (Table 1), which displayed resonances at δ199.0, 120.6 and 160.6. The presence of four additional olefinic carbons in the ^{13}C spectrum revealed that the molecule was bicyclic. Analysis of the ^1H NMR data (Table 1) showed one hydroxymethyl and four methyl groups, suggesting that bershacolone was of diterpenoid biogenesis. However, close examination of known diterpene carbon skeletons uncovered none which accommodated these structural features.



1 R=H
2 R=Ac



1

The ^1H-^1H COSY analysis of bershacolone (**1**) began at the C-18 hydroxymethyl group (δ3.83, 3.49). This geminal pair was coupled to the H-10 methine (δ2.25), which was bordered on one side by a trisubstituted olefin (H-9, δ5.46) and on the other by a methine (H-11, δ1.55) bearing a methyl group (H_3-19, δ0.99). The H-11 multiplet was further coupled to the H-12 methylene (δ1.90, 0.65), which in turn was coupled to a methine at δ2.62. The COSY spectrum also disclosed a pair of vicinal methylenes (H-2, H-3); a TOCSY experiment showed correlations between each of these methylenes and the H-1 methine resonance at δ2.64. Allylic correlations (COSY) were detected between the olefinic protons at δ6.20, 5.82, 5.46 and the vinyl methyls at δ2.09, 1.59, 1.38, respectively. The proton at δ4.61 showed no coupling by COSY analysis; however, the HMBC spectrum exposed a correlation to the carbonyl, which also showed a correlation to the α proton (δ6.20) of the α,β-unsaturated ketone. HMBC spectra also linked this α proton to a vinyl methyl (δ17.6) and the methylene at δ39.4, thus placing both these groups as substituents of the fully substituted β-carbon (δ160.6). This deduction was affirmed by the correlations of both protons on this methylene (δ2.44, 1.81) with both the α and β carbons of the unsaturated ketone. The secondary carbinol methine at δ4.61 (H-7) showed HMBC correlations to δ137.2 (C-8), 133.7 (C-9) and 10.9 (C-17), thus allowing closure of the macrocycle between C-7 and C-8.

With all heteroatoms accounted for, the remaining site of unsaturation and carbons (two olefinic and a vinyl methyl) could only be accommodated by a cyclobutene ring. Because of overlap of key resonances in NMR spectra of **1**, we prepared the acetate **2**[5], in which the pairs H-1 and H-13, H-2 and H_3-16, C-1 and C-10, and C-9 and C-14 were all resolved at 500 and 125 MHz, respectively. The chemical shifts and coupling constants assigned to the cyclobutene in **1** and **2** were consistent with literature precedents.[6-9] A 169 Hz $^1J_{C,H}$ at C-14, obtained from a fully coupled HMQC experiment, provided further support of the cyclobutene moiety.[6-8] Orientation of the cyclobutene trisubstituted olefin within a hypothetical head-to-tail isoprene array was supported by an HMBC correlation between H-20 and C-1 in **2** and by an NOE observed between H-13 and H-14 in **2**. A *cis* ring juncture was evident from the NOE observed between H-1 and H-13 in **2**.

The relative stereochemistry of the remaining three chiral centers and the solution conformation of the molecule were probed by NOEDS. An NOE between H-1 and H_3-16 in **2** implied that both of these entities lie on the same upper "face" of the molecule as drawn. For this to occur, the $\Delta^{4,5}$ olefin must be oriented so that H-5 is on the opposite "face". In both **1** and **2**, we detected NOEs between H-5 and both H-7 and H-9, thus placing H-7 and H-9 on the lower "face." An NOE in **1** between H-9 and H-18 placed the hydroxymethyl on the lower "face" as well. In **2**, we observed an NOE between H-13 and the downfield H-12 diastereotopic proton, thus placing H-12 in the upper "face." NOEs observed between H_3-19 and both the hydroxymethyl protons and H-12' place all these groups on the lower "face". Molecular modeling[10], using the NOE data as constraints, provided the three dimensional conformation shown for **1**.

Bershacolone represents an unprecedented diterpene skeleton (bershacolane) with a bicyclo [11.2.0] pentadecane ring system. While there have been several reports on the triterpene constituents of *Maprounea*,[11-13] the only diterpene previously described from this genus was a resiniferol analog.[14]

Table 1. NMR data for Bershacolone, 1[a]

H#	$\delta^{13}C$[b]	$\delta^{1}H$	m	J (Hz)	COSY	TOCSY	HMBC	nOe (%)
1	48.7	2.64	m		2'	2,2',3,3',20	14,15	
2	25.6	2.05	dddd	13, 12, 8.4, 2.5	2',3,3',14(w)	1,2',3,3'		
2'	----	0.92	dt	8.3, 12	1, 2, 3	1,2,3,3',20		2(13),3'(0.3),12(0.3),12'(1)
3	39.4	2.44	dt	8.4, 12	2, 2',3'	1,2,2',3'	2,4,5	2(3),3'(10)
3'	----	1.81	dd	12, 8.3	2, 3	1,2,2',3	1,2,4,5	3(18),5(8.6)
4	160.6	----	-					
5	120.6	6.20	br s		16	3',16	3,6,16	3'(7),7(6.5),9(1),12(3)
6	199.0	----	-					
7	83.6	4.61	s			5(w)	6,8,9,17	5(4),9(7)
8	137.2	----	-					
9	133.7	5.46	d	10.7	10,17	11,12,17,19	18	5(2),7(8),12(0.2),18'(2)
10	48.8	2.25	tdd	8, 3.7, 10.7	9,11,18,18'	11,12,12',18,18',19	9,11,18,19	12'(2),17(3),18(2),18'(1.3)
11	37.0	1.55	m		10,12',19	9,10,12,12',13,18,19	12,19	
12	36.0	1.90	dd	13, 3.7	12',20	9,11,12',13,19	11	5(2),9(1),12'(2)
12'	----	0.65	dt	13, 11.5	11,12,13	10,11,12,13,19,20	1,11,13,14	2'(1.5),7(2.5),10(5),12(21),14(2),17(2)
13	42.9	2.62	m		12',20	11,12,12',19,20	3	
14	133.7	5.82	s		20			19(1)
15	148.0	----	-					
16	17.6	2.09	s		5	3',5	3,4,5	
17	10.9	1.38	q	1.0	9	9	8	10(4)
18	64.2	3.83	dd	10.7, 4	10,18'	10,11,19	8	10(2),18'(10),19(2)
18'	----	3.49	dd	10.7, 8.2	10,18	10,11,19		9(1.5),18(10)
19	17.4	0.99	d	6.3	11	9,10,11,12,12',13,18	11,12(w)	10(3),12'(1.3),13(2),14(10),18(3),18'(1)
20	13.9	1.59	d	1.0	12	1,2',12',13,14	1,14,15	

[a]$CDCl_3$, 500 MHz for ^{1}H, 125 MHz for ^{13}C
[b]Carbon resonances assigned by HMQC experiment

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4. Crude extract (15.7 g) was sequentially partitioned among hexane, CCl_4, and $CHCl_3$, and increasingly polar mixtures of MeOH/H_2O. The CCl_4 solubles (0.75 g) were applied to a Sephadex LH-20 column and eluted with CH_2Cl_2/MeOH (1:1) to yield an HIV-inhibitory fraction. Preparative silica and phenyl-bonded phase HPLC yielded 15 mg of bershacolone (**1**): $[\alpha]_D^{24}$ +440° (c 0.13, $CHCl_3$); IR: ν_{max} 3440, 2926, 1688, 1630, 1374, 1067 cm^{-1}; EIMS (probe) 70 eV *m/z* (rel. int.): 300 (3), 217 (2), 203 (16), 187 (5), 175 (18), 161 (20), 147 (22), 133 (25), 119 (39), 107 (44), 93 (71), 79 (86), 67 (58), 55 (94), 43 (100). For ^1H and ^{13}C NMR data, see Table 1.
5. Colorless glass: $[\alpha]_D^{24}$ +370 (c 0.20, $CHCl_3$); IR: ν_{max} 2927, 1740, 1701, 1627, 1371 cm^{-1}; UV: λ_{max}^{EtOH} 245 nm, ε=17,000; ^1H NMR (500 MHz, C_6D_6): δ5.77 (s, H-7, H-14), 5.64 (s, H-5), 5.24 (d, J=10 Hz, H-9), 4.14 (dd, J=11, 3.5, H-18), 4.02 (dd, J=11, 7.5, H-18'), 2.48 (m, J=13, 1.5, H-13), 2.43 (br d, J=13, H-1), 2.11 (dddd, J=11, 10, 7.5, 3.5, H-10), 1.96 (s, H_3-18Ac), 1.92 (m, H-3), 1.86 (s, H_3-7Ac), 1.70 (s, H_3-16), 1.62 (dddd, J=13, 11, 8, 2.5, H-2), 1.59 (m, H-12), 1.50 (d, J=1.5, H_3-17), 1.47 (q, J=1.5, H_3-20), 1.40 (ddd, J=13, 8, 1, H-3'), 1.28 (dtq, J=3.5, 11, 6.5, H-11), 0.86 (d, J=6.5, H_3-19), 0.66 (ddt, J=8, 1.5, 12, H-2'), 0.41 (dt, J=13, 11, H-12'); ^{13}C NMR (125 MHz, $CDCl_3$) δ193.2 (C-6), 171.0 (C-18Ac), 169.9 (C-7Ac), 159.7 (C-4), 148.0 (C-15), 135.7 (C-9), 133.4 (C-14), 130.7 (C-8), 121.0 (C-5), 85.5 (C-7), 65.5 (C-18), 48.9 (C-1), 44.8 (C-10), 43.0 (C-13), 39.5 (C-3), 37.2 (C-11), 35.8 (C-12), 25.5 (C-2), 20.9 (C-18Ac), 20.8 (C-7Ac), 17.4 (C-16, C-19), 13.9 (C-20), 11.6 (C-17).
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10. Bershacolone was modeled using Macromodel v3.0 running under VMS. The structure was input as a drawing and minimized using molecular mechanics under the MM2 force field. This structure was then constrained with a set of transannular nOe's, setting the distance restraint to 3.0 Å for each distance restrained, and minimized. The constraints were then removed and the structure reminimized. This was done to force agreement with a maximum number of nOe's. The final structure fit the nOe constraints well (all distances under 3.0 Å). The structure was downloaded to a Macintosh and the graphic generated using Chem-3D.
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15. We thank T. McCloud for extractions, J. Roman for mass spectral analyses, J.M. Fay of the Missouri Botanical Garden for plant collections, and J. Beutler for help with computer modeling.

(Received in USA 11 March 1993; accepted 18 May 1993)