Koumbalones A and B, New Casbane Diterpenes from Maprounea africana

Yoel Kashman, Matthew W. Bernart, Mark Tischler, John H. Cardellina II., and Michael R. Boyd

J. Nat. Prod., **1994**, 57 (3), 426-430• DOI: 10.1021/np50105a020 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 3, pp. 426-430, March 1994

KOUMBALONES A AND B, NEW CASBANE DITERPENES FROM *MAPROUNEA AFRICANA*

YOEL KASHMAN,[1] MATTHEW W. BERNART,[2] MARK TISCHLER,[3] JOHN H. CARDELLINA II, and MICHAEL R. BOYD*

Laboratory of Drug Discovery Research & Development, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Frederick, MD 21702

ABSTRACT.—The organic extract of *Maprounea africana* yielded koumbalones A [**1**] and B [**2**], defined by spectral methods as new variations on the casbane ring system. The gross structures, relative configurations, and solution conformations were determined by a combination of spectral analyses and molecular modeling. Because koumbalone A spontaneously converts to koumbalone B at room temperature, koumbalone B may be an artifact of isolation.

We recently reported the novel diterpene bershacolone (1), discovered during our investigation of the HIV-inhibitory activity in the organic extracts obtained from roots of *Maprounea africana* Muell. Arg. (Euphorbiaceae). Access to the AIDS-antiviral constituents was complicated by a complex array of triterpenes (2) and two new diterpenes of the casbane family, the subject of this report.

Koumbalones A and B [**1** and **2**] were isolated in the same sequence of steps that yielded bershacolone (1). Koumbalone A, an optically active solid, analyzed for $C_{20}H_{28}O_3$ by hreims; the ir indicated a hydroxyl group (3470 cm^{-1}) and suggested a conjugated ketone (1667 cm^{-1}). Two such ketones were revealed in the ^{13}C-nmr spectrum (see Table 1—δ 198.9, 122.1, 163.2 and 199.7, 136.5, 145.4), as well as by the uv spectrum (λ max 254 nm, ε 18000). The presence of a third olefin (^{13}C nmr) left two unassigned sites of unsaturation, meaning that koumbalone A was bicyclic.

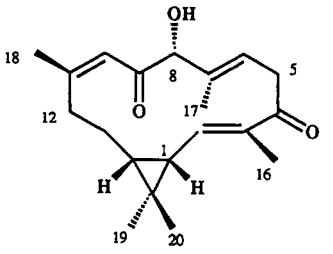
The well-dispersed ^1H-nmr spectrum (Table 1) displayed five distinct methyl groups, thus indicating a probable terpenoid biogenesis for koumbalone A. Conventional ^1H-^1H COSY nmr analysis disclosed a rather lengthy spin-system beginning with a vinyl methyl (δ 1.84, H_3-16) with allylic coupling to an olefinic proton (δ 6.07, H-2). A correlation between this olefinic proton and a methine (δ 1.53, H-1) led to a cross-peak between H-1 and a second methine (δ 1.10, H-14). This latter methine was vicinal to a diastereotopic methylene (δ 2.15 and 0.92, H-13), which was further coupled to another diastereotopic center (δ 3.86 and 1.76, H-12). These latter protons showed allylic coupling to an olefinic proton singlet (δ 5.78, H-10). Another spin-system starting with an olefinic proton (δ 5.51, H-6), which was correlated with a deshielded methylene (δ 3.82 and 3.00, H-5), as well as a vinyl methyl (δ 1.60, H_3-17), could be discerned.

An HMQC experiment provided the ^{13}C-nmr assignments for koumbalone A; the ^{13}C-nmr shifts suggested the *E* geometry for the olefins bearing H_3-16 and H_3-17 (δ 11.5, 11.1, respectively). The HMBC data established connectivities within the molecule. The secondary carbinol proton (δ 4.60, H-8) was bordered on one side by a carbonyl (δ 198.9, C-9) and on the other by the unconjugated olefin. A correlation between H-10 (δ 5.78) and C-9 placed H-10 as a substituent of the α-carbon (δ 122.1) of an α,β-unsaturated ketone. H-10 was also correlated to the C-18 vinyl methyl (δ 27.0, indicating a *Z* olefin), which was a substituent of C-11 (δ 163.2), the β-position

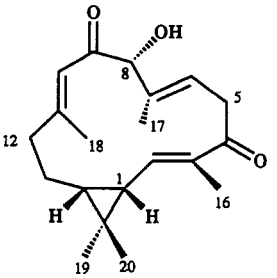
[1]On leave from Tel Aviv University.

[2]Current address: Hauser Chemical Research, Boulder, Colorado.

[3]Current address: American Cyanamid/ Lederle Laboratories, Pearl River, New York.



1



2

of the enone moiety. The HMBC experiment also yielded correlations between H-8 and both ends of the C-6–C-7 olefin. However, the large $J_{H5\text{-}H6}$ value (11.5 Hz), together with the HMQC assignments, placed the carbon (δ 125.3) bearing H-6 vicinal to C-5. By a process of elimination, the quaternary carbon (δ 135.1) correlated to H-8 (HMBC) had to be assigned as C-7. Another carbonyl (δ 199.7, C-4), which accounted for the last oxygen in the molecular formula, showed correlations with both H-2 and H-5. Because the olefinic proton H-2 is bordered on one side by H-1 ($J_{H1\text{-}H2}$=11.2 Hz), then the final sp^2 carbon (δ 136.5) in koumbalone A must be C-3.

This combination of homo- and hetero-nuclear 2D nmr experiments gave a 14-membered carbocycle with three vinyl methyls. The remaining unassigned components in the diterpene were two methyls which appeared as singlets in the ^{1}H-nmr spectrum and a shielded quaternary carbon (δ 30.2), all of which had to be incorporated into a second ring in

TABLE 1. Nmr Data for Koumbalone A [**1**].[a]

H#	δ^{13}C	δ^{1}H	m	*J* (Hz)	COSY *TOCSY*	HMBC	nOe (%)
1 . . .	29.4	1.53	dd	11.2, 8.5	*2*,5,13′,*14*		2(1),14(6),16(2.5),20(0.8)
2 . . .	145.4	6.07	dd	11.2, 1.5	1,*5*,*13*,*13*′,16	4,16	5(7),6(2),13′(5),17(0.7), 19(1.3)
3 . . .	136.5	—					
4 . . .	199.7	—					
5 . . .	33.6	3.82	dd	14, 11.5	5′,6,8,17	4,6,7,8	2(3.8),5′(14)
5′. . .		3.00	br d	14	5,6,17		5(8.7),6(3.6)
6 . . .	125.3	5.51	br d	11.5	5,5′,17		2(1.2),5′(1.6),8(5.5), 10(2.1)
7 . . .	135.1	—					
8 . . .	82.7	4.60	d	3	5,6,10,12	6,7,8,17	6(2.2),10(6)
9 . . .	198.9	—					
10 . .	122.1	5.78	s		*8*,17,*18*	9,11,13,18	6(3),8(7),18(5)
11 . .	163.2	—					
12 . .	40.7	3.86	m		10,12′,13,13′,14		12′(8)
12′. .		1.76	dt	2.2, 12	*2*,10,12,13,13′		2(1),12(9),14(5),18(1.2)
13 . .	26.9	2.15	br d	14	12,12′,13′		12(2.5),13′(15),14(2)
13′. .		0.92	br q	12	12,12′,13,14,19		2(6),13(8),17(0.5)
14 . .	36.6	1.10	m		1,2,12,13′		1(2),12′(2),13(1),18(0.4)
15 . .	30.2	—					
16 . .	11.5	1.84	d	1.5	2		1(2)
17 . .	11.1	1.60	t	3	5,5′,6,8	6,7,8	2(2.4),5(2.5),19(0.4)
18 . .	27.0	1.90	d	1.5	*10*	10,11	1(1),10(5),12′(1),14(1)
19 . .	15.8	1.03	s			1,15	2(4.8),13(1),20(0.5)
20 . .	28.6	1.18	s			1,13,19	1(2),19(1.3)

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

order to satisfy the remaining unsaturation specified by the molecular formula. One of these methyls (δ 1.03, H_3-19) was in fact correlated by HMBC to the shielded quaternary carbon (C-15), thus establishing the methyl as a substituent of C-15. The only sites available to place C-15 on the macrocycle were the C-1 and C-14 methines, which gave a cyclopropyl moiety in conjugation with the C-2–C-4 enone. Evidence for the geminal placement of C-19 and C-20 was seen in nOe enhancements between H_3-19 and H_3-20 (δ 1.18). Moreover, both these signals showed HMBC correlations to the C-1 cyclopropyl methine (δ 29.4).

With the planar structure of koumbalone A in hand, we uncovered an isomer in the literature, 14-dehydroagrostistachin (3), which differs from koumbalone A only in the position of the hydroxyl moiety. Comparison of spectral data for the two molecules showed some similarities, but numerous significant differences, especially in the chemical shifts of the carbinol carbons (δ 82.7 for koumbalone A and 69.1 for 14-dehydroagrostistachin).

The relative stereochemistry was probed by a series of nOeds experiments. The nOe enhancements observed between H-1 and H-14 established the cyclopropyl ring protons as cis. Using Dreiding models in conjunction with the nOe data, we found it helpful to consider the koumbalone A macrocycle as having upper and lower "faces," the upper face arbitrarily containing the cyclopropyl methine protons. The nOe enhancement observed between H-1 and H_3-16 placed this vinyl methyl in the upper face as well. The *E* geometry of this vinyl group placed H-2 on the lower face. Strong nOes between H-2 and both H-5 and H-13′ put these protons on the lower face. Coupling constant analysis ($J_{H5\text{-}H6}$=11.5 Hz) located H-6 on the opposite (upper) face from H-5. Such placement was substantiated by the nOe observed between H-5′ and H-6, and by the lack of an observed nOe between H-5 and H-6. H-6 did show an nOe to the H-8 secondary carbinol, meaning that it too must reside on the upper face. These and other nOe correlations are summarized in Table 1. Molecular modeling, using Dreiding models as a guide and nOe data as constraints, provided the three-dimensional conformation illustrated in Figure 1.

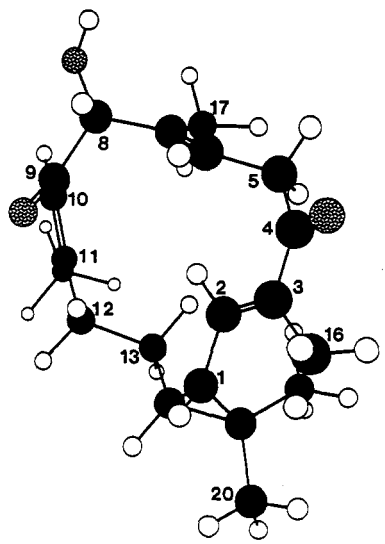


FIGURE 1. A computer generated three-dimensional perspective drawing of koumbalone A [**1**]. Only the relative configuration is implied; the enantiomer shown is an arbitrary choice.

Koumbalone B [**2**] proved to be identical in every respect to koumbalone A, except for the $\Delta^{10,11}$ olefin; nmr analyses indicated isomerization to the *E* geometry (see Table 2). An attempt to crystallize pure koumbalone A at room temperature produced significant amounts of koumbalone B. Therefore, koumbalone B is most likely an artifact resulting from photo-induced cis/trans isomerization. The positions of oxidation on the casbene skeleton are not novel; however, the combination of positions (C-4, -8, -9) is (3–7). These compounds continue the trend in oxidized casbanes in which a hydroxyl is vicinal to an enone carbonyl.

TABLE 2. Nmr Data for Koumbalone B [**2**].[a]

H#	$\delta^{13}C$	$\delta^{1}H$	m	*J* (Hz)	COSY *TOCSY*	HMBC	nOe (%)
1 ...	27.7	1.60	dd	10.8, 8.5	*13'*,14	3,16,20	16(1.5),20(1.1)
2 ...	141.5	6.26	dq	10.8, 1.8	1,5,*12,13,14*,16	3,4,16	1(0.6),5(5),6(0.4),10(0.6), 13'(4),17(0.3),19(1.1)
3 ...	138.0	—					
4 ...	199.0	—					
5 ...	38.8	3.80	dd	10, 14	5,16,17	4,6,7	2(9),5'(20),17(2,5)
5'...		2.97	br d	14, 1.6	17		5(20),6(2)
6 ...	126.3	5.57	br d	10	5,5',17	4,5,8,17	5'(1.5),8(5),10(1.3)
7 ...	135.5	—					
8 ...	83.2	4.50	s		5,17,18	6,7,9,17	6(10),10(4.4)
9 ...	198.7	—					
10 ..	119.4	6.11	q	1.7	12,12',18	9,12,18	6(1.7),8(4.1),12'(5), 13'(0.8),17(0.2)
11 ..	162.5	—					
12 ..	41.6	2.32	dt	11, 8	*1*,12',13,13'	10,11,13,18	12'(3),13'(0.4),14(0.4), 18(0.8)
12'..		1.90	dd	10, 11	*1*,13',*14*	10,11,13, 14,18	10(8),12(18)
13 ..	27.0	2.23	m		*1*,12',13',14	10,11,12, 15,18	13'(15),14(3)
13'..		0.79	br q	12		11,12,14,20	2(8),10(1),13(24), 17(0.8),19(3)
14 ..	34.4	1.19	ddd	10, 8.5, 2	13'	20	
15 ..	26.4	—					
16 ..	12.0	1.92	d	1.8	1(w)	1,2,3	1(4.5),2(0.3),5'(0.3), 6(0.4),10(0.7)
17 ..	10.7	1.40	t	1.6		6,7,8	2(0.6),5(2.5),8(0.2), 10(0.5),13'(0.4),18(0.1)
18 ..	18.3	2.13	d	1.7	12'	10,11,12	12(1.8),13(1.1),17(0.2)
19 ..	15.8	1.06	s			1,14,15,20	1(0.2),2(4.1),13'(2.6)
20 ..	29.1	1.16	s			1,14,15,19	

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

EXPERIMENTAL

COLLECTION, EXTRACTION AND CHROMATOGRAPHY.—Roots of *M. africana* Muell. Arg. (457 g dry wt) were collected near Camp Koumbala in the Central African Republic, at 600 m in sandy soil close to a water course in transitional woodlands, by J.M. Fay of the Missouri Botanical Gardens, under contract to the U.S. National Cancer Institute. A voucher specimen has been deposited in the herbarium of Missouri Botanical Garden, St. Louis, MO. The roots were air-dried, stored at −20° for three days, ground in a hammer mill, and extracted overnight at room temperature in CH_2Cl_2-MeOH (1:1), followed by MeOH. Solvents were reduced *in vacuo* to yield 25 g of crude organic extract, of which 15.7 g were 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. Hplc on Si gel [4×25 cm, i-PrOH-hexane (1:19)], followed by hplc [8 μm phenyl, 1×25 cm, MeOH-H_2O (79:21)] yielded koumbalones A (8 mg) and B (15 mg).

Koumbalone A [**1**].—A semi-crystalline solid: $[\alpha]^{24}D$ [λ (nm)] +125° (589), +130° (578), +150° (546), +360° (436) (*c*=0.1, $CHCl_3$); ir ν max 3470 (br OH), 2920, 1667, 1648, 1620, 1377, 1274, 1064, 1028, 861, 768 cm^{-1}; uv λ max (EtOH) 254 (ε 18,000) nm; eims (probe) (70 eV) *m/z* 316 $[M]^+$ (38), 298 $[M-H_2O]^+$ (13), 273 (11), 255 (9), 233 (8), 221 (10), 207 (26), 189 (39), 161 (67), 149 (34), 135 (70), 123 (100), 107 (94), 67 (58), 55 (60); hreims *m/z* 316.2021 $[M]^+$ ($C_{20}H_{28}O_3$, 1.7 mmu dev). For ^1H- and ^{13}C-nmr data, see Table 1.

Koumbalone B [**2**].—A clear glass: $[\alpha]^{24}D$ [λ (nm)] +270° (589), +280° (578), +330° (546), +790° (436) (*c*=0.7, $CHCl_3$); ir ν max 3440 (br OH), 2944, 2864, 1684, 1664, 1648, 1623, 1377, 1067, 1027, 772 cm^{-1}; uv λ max (EtOH) 247 (ε 21,000), 275 (ε 10,000) nm; cd $\Delta\epsilon_{247}=+18.7$, $\Delta\epsilon_{273}=-29.3$; eims (probe) (70 eV) *m/z* 316 $[M]^+$ (26), 298 $[M-H_2O]^+$ (21), 273 (7), 255 (5), 233 (8), 219 (7), 207 (38), 189 (34), 161 (39), 149 (35),

135 (69), 123 (90), 107 (100), 67 (59), 55 (66); hreims *m/z* 316.2019 $[M]^+$ ($C_{20}H_{28}O_3$, 1.9 mmu dev). For ^1H- and ^{13}C-nmr data, see Table 2.

Molecular Modeling.—Koumbalone A 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 nOes. The resulting structure was downloaded to a Macintosh computer and the graphic generated using Chem-3D.

ACKNOWLEDGMENTS

We thank Dr. Tom McCloud for the extractions, Dr. John Beutler for assistance with the computer modeling, and Dr. Anthony Dipple, ABL, for use of the cd spectropolarimeter.

LITERATURE CITED

1. M.W. Bernart, Y. Kashman, M. Tischler, J.H. Cardellina II, and M.R. Boyd, *Tetrahedron Lett.*, **34**, 4461 (1993).
2. M. Tischler and J.H. Cardellina II, *J. Liq. Chromatogr.*, **16**, 15 (1993).
3. Y.-H. Choi, J.M. Pezzuto, A.D. Kinghorn, and N.R. Farnsworth, *J. Nat. Prod.*, **51**, 110 (1988).
4. D. Sitton and C.A. West, *Phytochemistry*, **14**, 1921 (1975).
5. M.A. Commissiong and K.O. Pascoe, *Tetrahedron Lett.*, **25**, 711 (1984) and references cited therein.
6. Y.-H. Choi, J. Kim, J.M. Pezzuto, A.D. Kinghorn, N.R. Farnsworth, H. Lotter, and H. Wagner, *Tetrahedron Lett.*, **27**, 5795 (1986).
7. V.L.A. Moura, F.J.O. Monte, and R.B. Filho, *J. Nat. Prod.*, **53**, 1566 (1990).

Received 29 October 1993