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The Zaragozic Acids: Structure Elucidation of a New Class of Squalene Synthase Inhibitors

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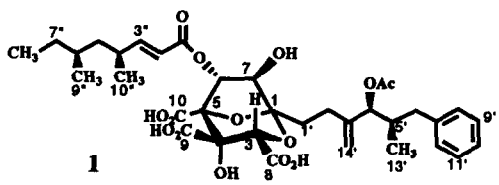
Abstract: *Structures of two novel fungal metabolites, zaragozic acids A (1) and B (2), characterized by a novel 2,8-dioxobicyclo[3.2.1]octane-4,6,7-trihydroxy-3,4,5-tricarboxylic acid core, are proposed predominantly on the basis of 2D NMR.*

It has been shown that clinical intervention with drugs such as lovastatin, belonging to the general class of HMG-CoA reductase inhibitors, offers new promise for the treatment of atherosclerosis.[1] Recent evidence shows that cholesterol-lowering drugs can retard and cause regression of coronary artery disease.[2] The search therefore continues unabated for other promising inhibitors of enzymes in the *de novo* cholesterol biosynthetic pathway. We report here on the structure elucidation of two novel fungal metabolites, zaragozic acids A (**1**) and B (**2**), which belong to a new class of potent picomolar competitive inhibitors of squalene synthase, isolated from two different fungal cultures *Sporormiella intermedia* and *Leptodontiium elatius* respectively.[3]



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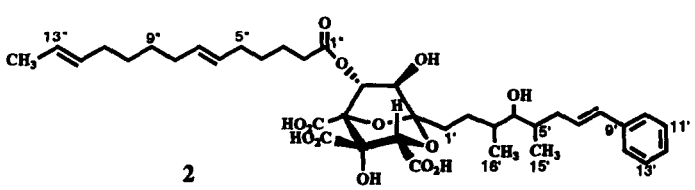

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Zaragozic acid A (**1**) is a tricarboxylic acid[4] having the empirical formula $C_{35}H_{46}O_{14}$, deduced from HR-EIMS of the penta-TMS derivative on silylation (found m/z 1050.4829; calcd for $C_{35}H_{46}O_{14}$ + $(SiC_3H_8)_5$ m/z 1050.4864) and ^{13}C NMR analysis. This was supported by the formation of a trimethyl ester (**3**) with diazomethane in ethyl acetate (HR-EIMS: found m/z 732.3329 [M^+]; calcd for $C_{38}H_{52}O_{14}$ m/z 732.3357).

Table 1. Full NMR assignments for Zaragozic acid A (1) and B (2) in CD_3OD[a]

C #	1 (400 MHz, 40° C) ^{13}C	^{1}H	mult.	C #	2 (500 MHz, RT) ^{13}C	^{1}H	mult.
core							
1	106.9			1	107.5		
3	76.8 (149)	5.26	s	3	76.6	5.24	s
4	75.7			4	75.7		
5	91.3			5	91.1		
6	81.3 (160)	6.30	d, 2.0	6	81.0	6.23	d, 2.0
7	82.8 (147)	4.03	d, 2.0	7	82.0	4.03	d, 2.0
8	170.3			8	170.3		
9	172.7			9	172.6		
10	168.7			10	168.6		
C1-alkyl side chain							
1'	35.2 (128)	2.02	m, 2H	1'	33.9	1.89	m, 2H
2'a	26.7 (127)	2.34	m	2'a	27.5	1.76	m
2'b		2.45†	m	2'b		1.40	m
3'	147.9			3'	36.7	1.73	m, 2H
4'	80.4 (144)	5.08	d, 4.8	4'	78.4	3.28	t, 5.5
5'	37.9 (127)	2.23	m	5'	36.9	1.85	m
6'a	41.0 (125)	2.68	dd, 6.2, 13.4	6'a	38.6	2.33	m
6'b		2.43	dd, 8.6, 13.4	6'b		2.08	dt, 14.5, 8.0
7'	141.7			7'	130.2	6.25	dt, 16.0, 7.5
8',12'	130.3 (157)	7.17	m, 2H	8'	132.6	6.40	d, 16.0
9',11'	129.4 (159)	7.24	m, 2H	9'	139.3		
10'	127.0 (160)	7.13	m	10',14'	127.0	7.35	br d, 8.0, 2
13'	14.3 (126)	0.86*	d, 3H	11',13'	129.5	7.25	t, 8.0, 2H
14'a	111.7 (157)	4.96	br s	12'	127.9	7.15	tt, 7.5, 1.2
14'b		5.02	br s	15'	15.2	0.98	d, 7.0, 3H
15'	172.2			16'	14.8	0.97	d, 6.5, 3H
16'	20.9 (129)	2.08	s, 3H				
C6-acyloxy side chain							
1"	166.7			1"	173.6		
2"	120.0 (162)	5.78	dd, 1.2, 15.8	2"	34.8	2.29	m
3"	157.6 (153)	6.85	dd, 8.4, 15.8	3"	25.3	1.58	qt, 7.5, 2H
4"	35.6 (128)	2.45†	m	4"	30.1	1.32‡	m
5"a	44.5 (124)	1.13^	m	5"	33.5^	1.92†	m
5"b		1.38	m	6"	131.9*	5.35*	m
6"	33.2 (124)	1.32‡	m	7"	131.0*	5.38*	m
7"a	30.9 (124)	1.13^	m	8"	33.5^	1.92†	m
7"b		1.32‡	m	9"	30.3	1.32‡	m
8"	11.5 (124)	0.86*	t, 3H	10"	30.3	1.32‡	m
9"	19.3	0.86*	d, 3H	11"	33.2^	1.92†	m
10"	20.6 (126)	1.02	d, 6.6, 3H	12"	132.6	5.35*	m
				13"	125.7	5.38*	m
				14"	18.1	1.62	m, 3H

[a] $^{1}J_{CH}$ (Hz) in parentheses; *,†, ‡, ^ overlapping ^{1}H NMR signals; *, ^ ^{13}C NMR signals can be interchanged; abbreviations: s=singlet, d=doublet, t=triplet, q=quartet, qt=quintet, m=multiplet, br=broad

The ^{13}C NMR data (Table 1) and formula indicated 5 double bonds, 5 carboxyl/ester groups and thereby 3 rings. Analysis of ^{1}H-^{1}H (COSY, ^{1}H-^{1}H decoupling), direct (HETCOR, HMQC) and long-range ^{1}H-^{13}C correlation data (LR-HETCOR, HMBC) suggested the partial structure in Fig. 1. ^{2}H-isotope induced ^{13}C shifts (see below) ruled out the presence of a hemiketal function. The assignment of the 6-acyloxy fragment as the 4,6-

dimethyl-oct-2-*E*-enoic acid was confirmed by ^{1}H NMR and MS characterization of the Na salt (^{1}H NMR (D_2O) δ0.77 (t, *J* = 7, 3H, H-8"), 0.78 (d, *J* = 6, 3H, H-10"), 0.94 (d, *J* = 7, 3H, H-9"), 5.75 (d, *J* = 17, 1H, H-2"), 6.43 (dd, *J* = 11, 17, 1H, H-3") and volatile methyl ester (GC-EIMS: m/z 184 [M^+, $C_{11}H_{20}O_2$]), produced on mild base hydrolysis. The 3 carboxyl resonances as well as the 3 undefined carbons at 75.7, 76.8 and 91.3 ppm appeared as broadened signals, which sharpen on raising the temperature and upon methylation to the trimethyl ester. It was therefore inferred that these carbons are attached to the exchange-broadened carboxyl groups.

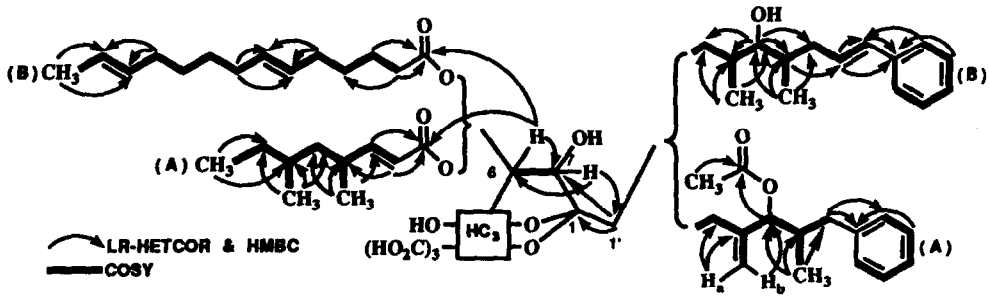
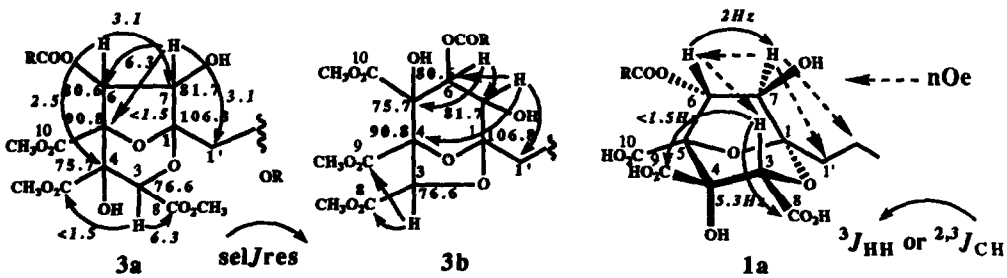


Fig. 1 Partial Structures of Zaragozic Acids A and B. Only key correlations shown.

The related zaragozic acid B (2), of molecular weight 730, also analyzed as a tricarboxylic acid.[5] The empirical formula $C_{39}H_{54}O_{13}$ was deduced from HR-EIMS data on the hexa-TMS derivative (found m/z 1147.5751; calc for $C_{39}H_{54}O_{13}$ + $(SiC_3H_8)_6$ - CH_3 m/z 1147.5701). This was corroborated by the preparation of a trimethyl ester (4) (FAB-MS: m/z 779 $[M+Li]^+$; HR-EIMS: m/z 988.5172; calcd for $C_{42}H_{60}O_{13}$ + $(SiC_3H_8)_3$ m/z 988.5220) and ^{13}C NMR analysis (Table 1), which suggested 6 double bonds, 4 carboxyl/ester groups and 3 rings. Mild base hydrolysis with LiOH in aq. MeOH, gave the novel tetradeca-6*E*, 12*E*-dienoic acid which readily yielded the corresponding methyl ester (GC-EIMS: m/z 238 [M^+, $C_{15}H_{26}O_2$]). The *E* configuration of both double bonds followed from the general observation that steric compression of the carbons flanking the double bond in a disubstituted olefin of the *Z* configuration, lead to 4-5 ppm shifts upfield of their *E* counterparts.[6] Based on analysis of all the ^{1}H-^{1}H and ^{1}H-^{13}C connectivity data on B, a similar partial structure to that for A was proposed (Fig. 1).

Of the several possible structures considered at this stage, two isomeric candidates **3a** and **3b** (depicted as their trimethyl esters), based on 1,6-anhydrohexofuranose and pyranose ring systems, were particularly difficult to distinguish. Application of the selective 2D heteronuclear *J*-resolved (sel*J*res) method[7] to 3 in



CD_3CN (+CD_3OD) indicated a small coupling between H7 and the resonance at 90.8 ppm, in favor of **3a**. Moreover, ^{13}C chemical shift arguments, based on 1,6-anhydrohexofuranose and pyranose ring systems,[8] are inconsistent with structure **3b**. ^{2}H-isotope induced ^{13}C shifts observed by comparison of spectra in CD_3OD and CD_3OH, indicated primary upfield shifts (ppm) for C4 (+0.09) and C7 (+0.10) in **3a** (C5 and C7 in **3b**) which are consistent with both structures. The secondary isotope shifts observed for C3 (+0.03), C6 (+0.03) and C9 (+0.05) in **3a**, however, are only consistent with this structure.

The configuration at C3, C6 and C7 was deduced on the basis of PS-NOESY data and the observed coupling between H6 and H7 as depicted in **1a**. The small coupling of H3 to C9 suggests a *gauche* orientation where the carboxyl at C4 is in the *endo* and hydroxyl in the *exo* configuration.

The fascinating structures for zaragozic acids A (**1**) and B (**2**) are characterized by the 2,8-dioxobicyclo [3.2.1]octane-4,6,7-trihydroxy-3,4,5-tricarboxylic acid core resembling a conformationally rigid citric acid analog. In addition to its topological similarity to presqualene pyrophosphate this moiety may prove a useful biological mimic for pyrophosphate in general. During the preparation of this manuscript the structures of the squalestatins were reported, one of which is identical to zaragozic acid A.[9] The absolute stereochemistry of the core structure of A, as depicted in (**1**) and the side chains was determined independently.[9,10]

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4. **1**: Colorless oil: FABMS: m/z 691 $[M+H]^+$; m/z 715 $([M^*Li_3 + Li]^+$ lithium adduct of a tri-lithium salt on spiking with lithium acetate; $[\alpha]_D$ = + 37° (*c* 1.29, MeOH); FTIR (ZnSe): 3471 (br), 2964, 1719, 1649, 1455, 1376, 1255, 1183, 1024, 749, 702 cm^{-1}; UV_{max} (MeOH): 214 nm (ε 19,576).
5. **2**: Colorless oil; FABMS: m/z 731 $[M+H]^+$; m/z 755 $([M^*Li_3 + Li]^+$ lithium adduct of a trilithium salt; $[\alpha]_D$ = + 5.9° (*c* 0.27, MeOH); FTIR (ZnSe): 3440 (br), 2928, 1731, 1634, 1437, 1382, 1257, 1145, 966, 744, 693 cm^{-1}; UV_{max} (MeOH) : 205 nm (ε 27,565), 251 nm (ε 20,849).
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