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Bispolides, Novel 20-Membered Ring Macrodiolide Antibiotics from *Microbispora*

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Abstract Seven new related compounds named bispolides A1, A2, A3, B1, B2a, B2b and B3, have been found in a culture of *Microbispora* sp. A34030, isolated from a Malaysian soil sample. The planar structures were elucidated to be new 20-membered ring macrodiolide antibiotics on the basis of MS and NMR spectroscopic analyses. These antibiotics showed a good anti-MRSA activity *in vitro*.

Keywords bispolide, macrodiolide antibiotic, 20-menbered ring, symmetric cyclic dimmer, *Microbispora* sp.

In our screening program of new microbes and their useful metabolites from the tropical resources in Malaysia, seven new related compounds, bispolides A1, A2, A3, B1, B2a, B2b and B3, have been found in a culture of *Microbispora* sp. A34030. The strain was isolated from a soil sample collected at a forest station in the Forest Research Institute (FRIM), Selangor, Malaysia and was deposited in a Japanese International depository, the International Patent Organism Depositary (IPOD) of the National Institute of Advanced Industrial Science and Technology, under the deposit number of FERM BP-10505.

The liquid seed culture (1 ml) of *Microbispora* sp. A34030 was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of a culture medium composed of glucose 1.0%, cotton seed meal 1.0%, powdered yeast extract 1.0% and calcium carbonate 0.2% (pH 7.0). Forty flasks (4 liters) were cultured at 28°C for 12 days on a rotary shaker at 225 rpm. The mycelium separated by centrifugation was extracted with 100 ml of methanol (twice) and concentrated to 50 ml. The concentrate was extracted with 150 ml of ethyl acetate and evaporated to dryness, which was applied to a column of silica gel (Merck, Art.1.07734.1000, 21 g). The column was eluted with $CHCl_3$ (200 ml), $CHCl_3$ - MeOH (50 : 1, 200 ml), $CHCl_3$ - MeOH (25 : 1, 156 ml) and $CHCl_3$ - MeOH (25 : 4, 120 ml) in this order. The active eluate in the 25 : 4 ratio was concentrated to give 243 mg of the crude product which was further purified by HPLC (Agilent, ZORBAX XDB-C18 Prep HT 5 μm, 21.2×150 mm) with CH_3CN - H_2O (4 : 1) at 12.5 ml/minutes. Thus, seven highly purified components of bispolides were obtained; B1 (15.6 mg) at Rt 4.8 minutes, A1 (28.8 mg) at 6.9 minutes, B2a (19.8 mg) at 9.7 minutes, B2b (10.8 mg) at 11.2 minutes, A2 (45.8 mg) at 15.5 minutes, B3 (20.6 mg) at 23.6 minutes, and A3 (50.0 mg) at 32.7 minutes. The physicochemical properties are shown in Table 1.

Bispolide A3 showed *m/z* 1183.7019 [$(M+Na)^+$, calcd for $C_{64}H_{104}O_{18}Na$, 1183.7120] in the HRFABMS. As shown in Table 2, only 31 and 32 signals were observed in the ^{13}C NMR of bispolides A1 and A3, respectively, and their

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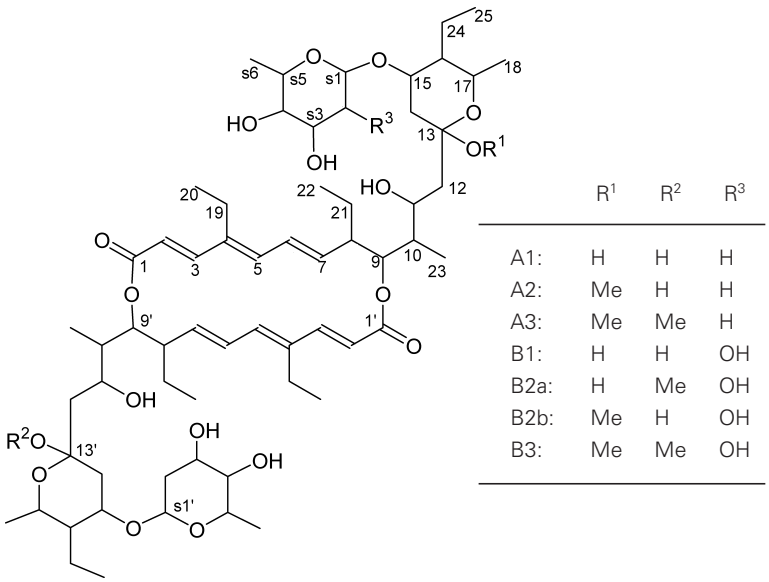
Table 1 Physicochemical properties of bispolides

	A1	A2	A3
Appearance	Colorless powder	Colorless powder	Colorless powder
Molecular formula	$C_{62}H_{100}O_{18}$	$C_{63}H_{102}O_{18}$	$C_{64}H_{104}O_{18}$
FAB-MS *m/z* (M^-)	1132.1	1146.1	1160.0
(MNa^+)	1155.9	1169.9	1184.0
HRFABMS *m/z* (MNa^+)			1183.7019
Calcd. exact MS	1132.69	1146.71	1160.72
Calcd.			1183.7120
$[\alpha]_D$ in MeOH	+80.0° (*c* 0.84)	+176.1° (*c* 0.72)	+418.7° (*c* 0.74)
Temp. at	25°C	25°C	25°C
UV λ_{max}^{MeOH} (nm)	298	298	298

	B1	B2a	B2b	B3
Appearance	Colorless powder	Colorless powder	Colorless powder	Colorless powder
Molecular formula	$C_{62}H_{100}O_{19}$	$C_{63}H_{102}O_{19}$	$C_{63}H_{102}O_{19}$	$C_{64}H_{104}O_{19}$
FAB-MS *m/z* (M^-)	1148.9	1162.1	1162.1	1176.1
(MNa^+)	1171.7	1185.9	1185.9	1199.9
Calcd. exact MS	1148.69	1162.7	1162.7	1176.72
$[\alpha]_D$ in MeOH	+115.0° (*c* 0.13)	+103.7° (*c* 0.15)	+126.3° (*c* 0.12)	+91.7° (*c* 0.13)
Temp. at	23°C	23°C	26°C	23°C
UV λ_{max}^{MeOH} (nm)	298	298	298	298



	R^1	R^2	R^3
A1:	H	H	H
A2:	Me	H	H
A3:	Me	Me	H
B1:	H	H	OH
B2a:	H	Me	OH
B2b:	Me	H	OH
B3:	Me	Me	OH

Fig. 1 Structures of bispolides.

structures were shown to be symmetric cyclic dimmers (Fig. 1) by the 2D NMR analyses. Bispolide A2 was suggested to be a 13-*O*- or 13′-*O*-monomethyl derivative on one of two hemiacetal OH groups.

The planar structures of four B group antibiotics, bispolides B1, B2a, B2b and B3, were elucidated to have a different glycoside in the side chain from A group by the extensive MS (FAB-MS, MS/MS-B/E linked scan) and NMR (2D such as HMQC) studies (data not shown). Bispolide A group antibiotics have two 2,6-dideoxyhexopy-

Table 2 ^{13}C NMR data for bispolides

Position	A1	A2	A3	B1	B2a	B2b	B3
1, 1′	168.5	168.0, 168.6	168.0	169.2	168.6, 169.2	168.4, 168.9	168.6
2, 2′	116.3	116.3, 116.4	116.4	116.8	116.8, 117.0	116.5, 116.6	116.9
3, 3′	148.5	148.3, 148.4	148.3	149.1	148.9, 149.0	148.6, 148.7	149.0
4, 4′	138.5	138.3, 138.4	138.3	139.0	138.9, 139.0	138.6, 138.7	138.9
5, 5′	138.0	137.9, 138.0	137.9	138.6	138.5, 138.6	138.2, 138.3	138.5
6, 6′	129.6	129.3, 129.5	129.5	130.2	129.9, 130.1	129.8, 129.8	129.9
7, 7′	141.3	141.2, 141.5	141.5	141.9	141.8, 142.1	141.5, 141.8	142.0
8, 8′	49.5	49.6, 49.8	49.8	50.1	50.2, 50.3	49.9, 50.0	50.4
9, 9′	74.9	74.7, 74.9	74.7	75.5	75.3, 75.5	75.0, 75.2	75.3
10, 10′	40.1	40.1, 40.1	40.1	40.7	40.6, 40.7	40.4, 40.4	40.7
11, 11′	70.1	69.4, 70.1	69.5	70.6	70.0, 70.6	69.6, 70.3	70.0
12, 12′	44.8	40.3, 44.8	40.4	45.4	41.0, 45.3	40.8, 45.0	41.0
13, 13′	97.5	97.5, 101.4	101.4	98.1	98.1, 101.9	97.8, 101.6	102.0
14, 14′	40.6	38.3, 40.6	38.3	41.1	38.9, 41.1	38.7, 40.8	38.9
15, 15′	69.1	69.0, 69.0	69.0	69.6, 72.4	69.5, 72.4	69.2, 72.2	69.8, 72.5
16, 16′	48.9	48.0, 48.9	48.0	49.4	48.6, 49.3	48.2, 49.1	48.6
17, 17′	66.9	66.9, 68.1	68.2	67.5	67.5, 68.7	67.0, 68.3	68.7
18, 18′	19.0	18.8, 19.0	18.8	19.6	19.3, 19.6	19.0, 19.3	19.4
19, 19′	19.7	19.7, 19.7	19.7	20.3	20.3, 20.3	20.0, 20.0	20.3
20, 20′	13.5	13.4, 13.5	13.5	14.1	14.0, 14.0	13.8, 13.8	14.1
21, 21′	23.2	23.2, 23.3	23.4	23.7	23.8, 24.0	23.5, 23.7	24.0
22, 22′	11.7	11.7, 11.7	11.7	12.3	12.3, 12.3	12.0, 12.0	12.3
23, 23′	9.3	9.3, 9.3	9.3	9.9	9.9, 9.9	9.6, 9.7	9.9, 10.0
24, 24′	19.4	19.3, 19.4	19.3	19.9, 19.9	19.8, 19.8	19.4, 19.6	19.9
25, 25′	8.8	8.8, 8.8	8.8	9.4, 9.4	9.3, 9.3	9.0, 9.0	9.4
s1, s1′	93.5	93.0, 93.5	93.5	94.0, 97.1	94.1, 97.1	93.7, 96.8	94.1, 97.1
s2, s2′	33.2	33.2, 33.2	33.2	33.7, *73.5	33.7, *73.5	33.5, *73.2	33.7, *73.5
s3, s3′	65.7	65.7, 65.7	65.7	66.3, *72.5	66.2, *72.5	66.0, *72.2	66.3, *72.5
s4, s4′	71.3	71.2, 71.2	71.2	71.8, *70.2	71.7, *70.1	71.5, *69.7	71.8, *70.0
s5, s5′	66.7	66.7, 66.7	66.7	67.3, *69.6	67.3, *69.7	67.1, *69.4	67.3, *69.8
s6, s6′	16.8	16.8, 16.8	16.8	17.4, 18.2	17.4, 18.1	17.1, 17.8	17.4, 18.2
13, 13′-OMe		46.8	46.8		47.3	47.0	47.4

^{13}C NMR data (δ_C, ppm) were measured in acetone-d_6 at 100 or 125 MHz.
* Assignments are exchangeable in the same column.

ranoses in the side chain, while B group antibiotics have each one of 6-deoxyhexopyranose and 2,6-dideoxyhexopyranose. Different cleavages of glycosylated side chains in bispolides B2a and B2b were observed in the MS/MS measurements (data not shown).

Therefore, all seven bispolide antibiotics were proposed to have a novel 20-membered symmetric cyclic structure consisting of two conjugated trienes, as shown in Fig. 1. These structures of bispolide antibiotics suggest to be the relation to 16-menbered macrodiolide antibiotics, such as azalomycin B [1, 2] and elaiophylin [3~6], but bispolides are quite different from 16-membered macrodiolides consisting of two conjugated dienes.

Bispolide antibiotics exhibited the inhibiting activities against Gram-positive bacteria, especially a good activity against clinically-isolated strains of methicillin-resistant *Staphylococcus aureus* (MRSA) at 1~4 μg/ml (MRSA isolate 1, A1: 2 μg/ml, B1: 2; isolate 2, A1: 2, B1: 1; isolate 3, A1: 4, B1: 2; MSSA ATCC29213, A1: 4, B1: 2). Further chemical and biological studies on bispolides will be reported in due course.

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