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Biosynthesis of Hibarimicins

III. Structures of New Hibarimicin-related Metabolites Produced by Blocked Mutants

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Structures of metabolites produced by blocked mutants of *Microbispora rosea* subsp. *hibaria* TP-A0121, hibarimicin-producer, were determined by spectroscopic analysis. HMP-Y6 is the dimer of the west half of hibarimicin B, the aglycon of which is the genuine biosynthetic intermediate. HMP-P1 is the shunt product arising from the release of a methanol molecule from hibarimicinone. HMP-P4, the glycoside of HMP-P1, is glycosylated with two amicetoses and two digitoxoses same as hibarimicin B. HMP-M1, M2, M3 and M4 are shunt products derived from the monomeric undecaketide intermediates.

In the preceding paper, biosynthesis of hibarimicin was proposed on the basis of analysis of the metabolites from the blocked mutants and cosynthesis experiments[1]. This paper describes the isolation and characterization of seven new metabolites produced by blocked mutants of *Microbispora rosea* subsp. *hibaria* TP-A0121.

Results and Discussion

The structures of the metabolites from the blocked mutants were determined by a combination of DEPT, DQF-COSY, TOCSY, HMQC, HMBC and ROESY NMR experiments. The ^{1}H and ^{13}C NMR data of these metabolites are summarized in Tables 1 and 2, and Experimental section. Other physico-chemical properties are given in Experimental section.

HMP-M1

Molecular formula of HMP-M1 was determined as $C_{22}H_{20}O_8$ based on the FAB-MS (*m/z* 413 $[M+H]^+$) and NMR data. In the ^{1}H NMR spectrum, one methyl, three methylene, two methine groups and four aromatic protons were observed. In addition, ^{13}C NMR spectrum demonstrated twelve quaternary carbon signals due to one sp^3, five sp^2 and six oxygenated sp^2 carbons. DQF-COSY experiment revealed three spin systems: H-4/H-4a/H-5, H-7/H-8/H-9 and H-14/H-15/H-16. UV-vis spectrum showed the absorption maxima at 220, 266 and 414 nm, suggesting the presence of chromomycin-like chromophore[2]. Taking these observations into account, HMBC experiments established the structure of HMP-M1 (Fig. 2). The presence of 1,8-dihydroxynaphthalene substructure was confirmed by the HMBC correlation from H-7 and H-9 to C-10a, that

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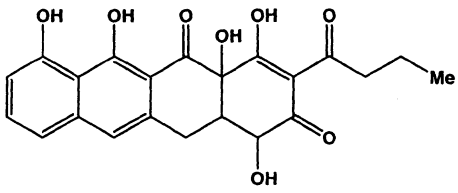
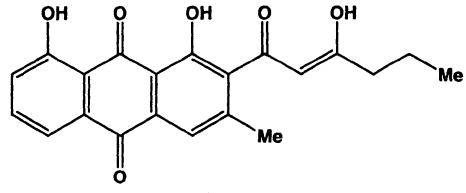
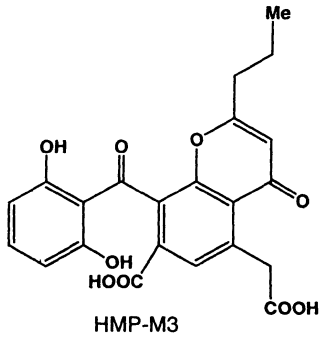
Fig. 1. Metabolites produced by the blocked mutants of *Microbispora rosea* subsp. *hibaria* TP-A0121.



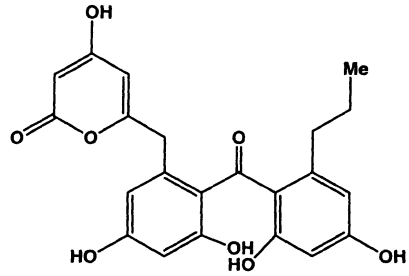
HMP-M1



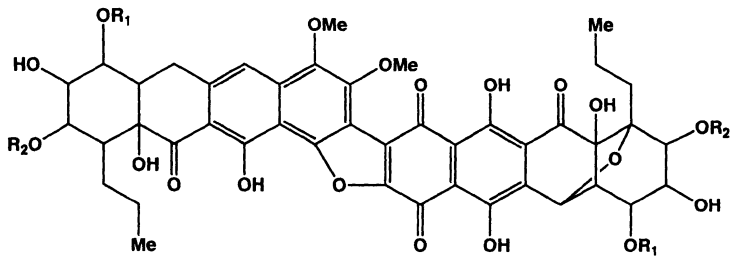
HMP-M2



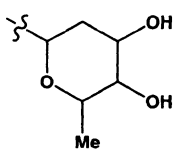
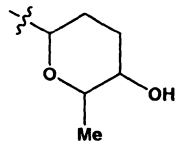
HMP-M3

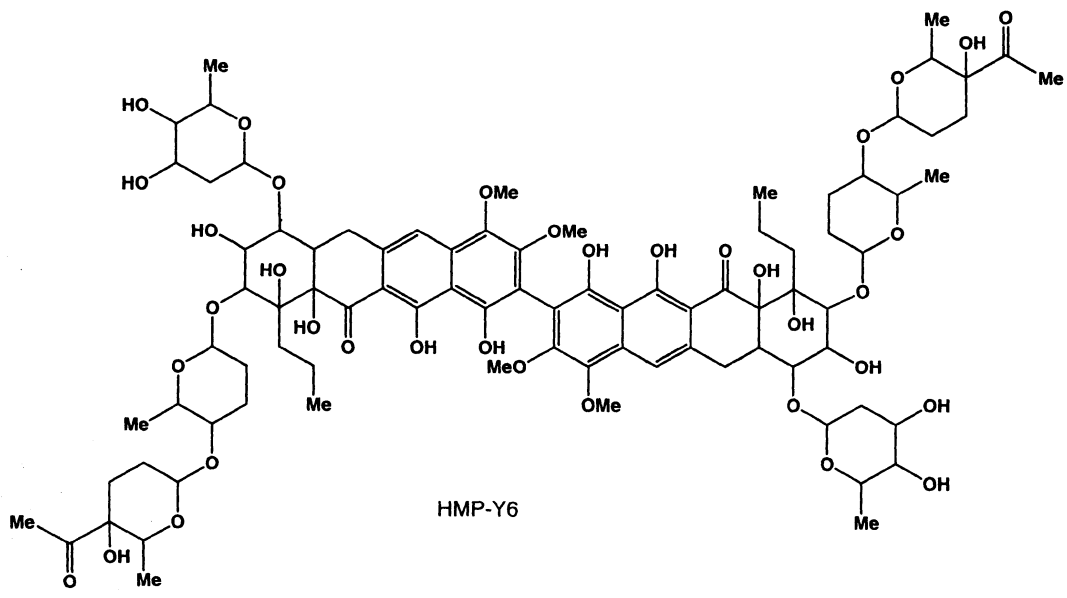


HMP-M4



HMP-P1 R_1=R_2=H

HMP-P4 R_1=  R_2= 



HMP-Y6

Table 1. ^{1}H and ^{13}C NMR data for HMP-P4 (acetone-d_6).

Position	^{13}C	^{1}H	Position	^{13}C	^{1}H
1	152.51		1'	176.98[f]	
2	116.93[a]		2'	126.12[g]	
3	148.29		3'	152.70	
4	146.36		4'	183.82[f]	
5	136.64		5'	117.17[a]	
6	113.01	7.44 (s)	6'	152.41	
7	145.12		7'	150.54	
8	29.60	3.18 (dd, 6.4, 17.4), 3.75 (dd, 13.2, 17.4)	8'	68.77	5.69 (s)
9	45.41	2.73 (m)	9'	56.16	3.24 (br.s)
10	76.97	4.15 (dd, 5.5, 9.6)	10'	77.76	4.30 (dd, 3.5, 7.9)
11	71.63	4.03 (m)	11'	75.67	3.95 (m)
12	87.22	3.65 (d, 8.8)	12'	86.60	3.85 (d, 7.1)
13	80.40		13'	83.89	
14	78.81		14'	87.00	
15	206.48		15'	195.63	
16	114.25[b]		16'	126.40[g]	
17	161.69		17'	159.34	
18	107.98		18'	114.52[b]	
19	37.29	1.40 (m)	19'	35.07	0.86 (m), 1.65 (m)
20	18.25	1.02 (m), 1.35 (m)	20'	17.06	0.87 (m), 1.16 (m)
21	15.43	0.68 (t, 7.1)	21'	15.13	0.57 (t, 6.8)
3-OMe	62.55	4.05 (s)			
4-OMe	62.00	4.00 (s)			
DG1	99.44	5.32 (d, 3.2)	DG1'	98.98	5.41 (br.s)
DG2	36.08[c]	1.85 (m), 2.22 (m)	DG2'	36.36[c]	1.91 (m), 2.21 (m)
DG3	68.11	3.85 (m)	DG3'	68.11	3.85 (m)
DG4	74.00	3.04 (m)	DG4'	73.77	2.99 (m)
DG5	65.83	4.00 (m)	DG5'	65.55	4.03 (m)
DG6	18.70[d]	1.24 (d, 6.4)	DG6'	18.42[d]	1.11 (d, 6.1)
AM1	104.29	4.53 (d, 6.6)	AM1'	104.29	4.47 (d, 8.0)
AM2	31.87[e]	1.45 (m), 1.88 (m)	AM2'	31.86[e]	1.45 (m), 1.73 (m)
AM3	31.45	1.40 (m), 1.86 (m)	AM3'	31.45	1.40 (m), 1.86 (m)
AM4	71.25	3.04 (m)	AM4'	71.29	3.04 (m)
AM5	77.44	3.37 (m)	AM5'	77.36	3.34 (m)
AM6	18.52[d]	1.20 (d, 6.1)	AM6'	18.42[d]	1.12 (d, 5.9)

a-g: exchangeable

from H-8 to C-6a, that from H-6 to C-6a, C-7, C-10a and C11a, that from H-8 and H-9 to C-10, and that from H-6 and H-9 to C-11. Long-range couplings of H-5 to C-4, C-4a, C-5a, C-6, C-11a and C12a and that of H-6 to C-12 (δ 201.14) established the conjugated six-membered ketone substructure. The HMBC correlation from H-4 to C-3, C-4a and C-5, H-4a to C-1, C-3 and C12a and that from H-14 to C-2 and C-13 confirmed the connectivity of the remaining atoms. Another six-membered ring fused at the position of C-4a and C-12a to make the enol-form of 1,3-diketone with a substitution of *n*-butanoyl group at C-2.

HMP-M2

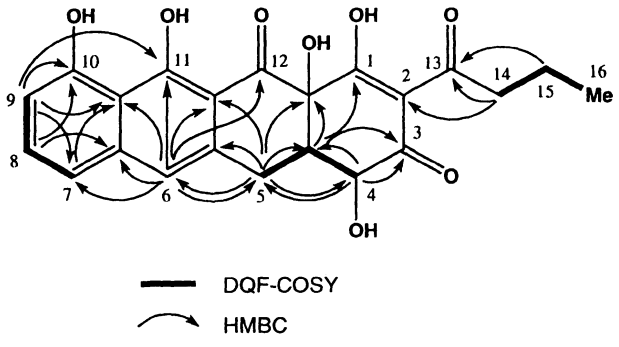
The ^{13}C NMR spectrum in combination with DEPT and HMQC spectral data revealed signals due to three carbonyl carbons, nine sp^2 quaternary carbons, five aromatic and olefinic protons, two methylenes and two methyl groups. These NMR and the FAB-MS (*m/z* 367 $[M+H]^+$) data allowed to determine the molecular formula as $C_{21}H_{18}O_6$.

Table 2. ^{1}H and ^{13}C NMR data for HMP-Y6 ($CDCl_3$).

Position	^{13}C	^{1}H	Position	^{13}C	^{1}H
1	152.62		DG1	98.95	5.42 (d, 2.7)
1-OH		9.62 (s)	DG2	35.10	1.95 (m), 2.42 (dd, 3.0, 13.6)
2	112.21		DG3	67.57	4.03 (d, 2.2)
3	155.07		DG4	73.13	3.16 (dd, 2.2, 9.8)
4	138.10		DG5	65.39	3.83 (m)
5	134.59		DG6	18.15	1.30 (d, 6.3)
6	112.21	7.39 (s)	AM1	103.55	4.53 (d, 8.5)
7	139.52		AM2	30.78	1.65 (m), 1.93 (m)
8	27.98*	3.04 (dd, 6.3, 17.4), 3.76 (dd, 11.2, 17.4)	AM3	29.72	1.70 (m), 2.20 (m)
9	44.85	2.70 (ddd, 5.8, 6.6, 12.2)	AM4	78.88	3.26 (dt, 5.1, 10.0)
10	76.69	4.23 (dd, 5.4, 9.8)	AM5	75.65	3.54 (dq, 8.8, 6.1)
11	71.04	4.17 (dd, 8.3, 9.8)	AM6	18.41	1.33 (d, 6.1)
11-OH		4.66 (s)	AT1	99.25	4.94 (d, 2.9)
12	86.91	3.76 (d, 8.0)	AT2	25.07	1.68 (m), 2.03 (m)
13	79.70		AT3	28.06*	1.50 (m), 2.22 (m)
14	77.40		AT4	79.10	
14-OH		3.98 (s)	AT5	67.04	4.28 (q, 6.6)
15	203.19		AT6	14.94	0.95 (d, 6.4)
16	110.40		AT7	211.05	
17	165.00		AT8	25.38	2.24 (s)
17-OH		15.04 (s)			
18	109.00				
19	37.37	1.52 (m), 1.70 (m)			
20	18.30	1.14 (m)			
21	15.42	0.87 (t, 7.1)			
3-OMe	61.01	3.84 (s)			
4-OMe	61.45	3.92 (s)			

* exchangeable

Fig. 2. NMR analysis of HMP-M1.



DQF-COSY experiment showed two spin systems, H-6/H-7/H-8 due to the aromatic portion and H-14/H-15/H-16 due to the *n*-propyl group. The presence of 1,9-dihydroxyanthraquinone moiety was confirmed by the HMBC correlation of 1-OH (δ 12.44) to C-1, C-2 and C-10a, that of 9-OH (δ 12.00) to C-8, C-9 and C-9a, that of H-4 to C-2 and C-10a, and that of H-6 to C-5, C-5a and C-9a. Long-range couplings from 17-Me to C-2, C-3 and C-4 established the substitution of the methyl group at C-3. The olefinic proton H-12 showed the HMBC correlations to C-11 and C-13 and the methylene H-14 of the *n*-propyl group to C-12 and C-13. Since the phenolic hydrogen of 1-OH is likely hydrogen-bonded to the carbonyl oxygen, 3-oxo-*n*-hexanoyl side chain was determined to be linked at C-2 (Fig. 3).

Fig. 3. NMR analysis of HMP-M2.

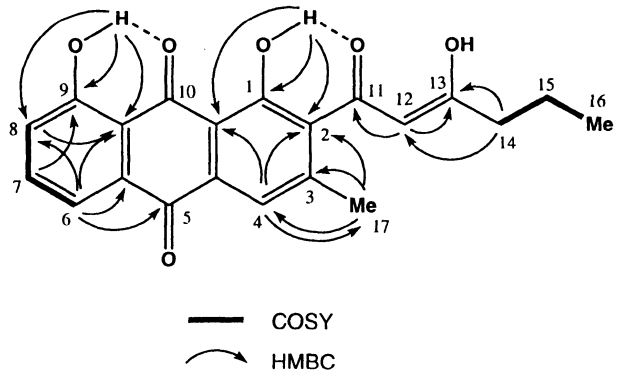


Fig. 4. NMR analysis of HMP-M3.

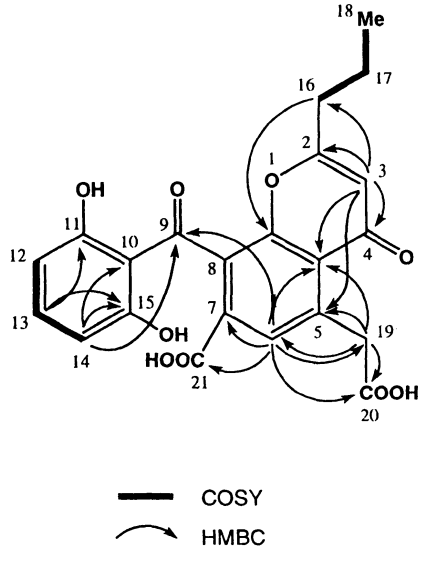
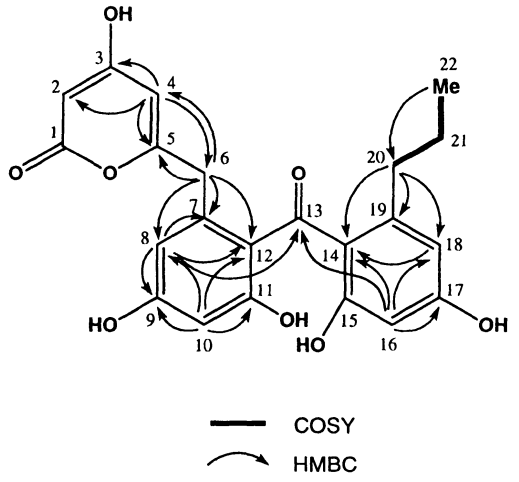


Fig. 5. NMR analysis of HMP-M4.



revealed the 2-substituted resorcinol substructure (Fig. 4). The presence of substituted coumarin substructure was confirmed by the long-range coupling from H-3 to C-2, C-4, C-4a and C-5, that from H-6 to C-4a, C-7, C-19 and C-21 and that from H-19 to C-4a, C-5 and C-6. The HMBC correlation of H-3 to C-16 and that of H-16 to C-8a revealed the substitution of *n*-propyl side chain at C-2. In addition, the long-range couplings from H-6 and H-19 to C-20 and that from H-6 to C-21 demonstrated the substitutions of carboxymethyl at C-5 and carboxyl at C-7 respectively. Finally, the $^4J_{C,H}$ couplings from H-6 and H-12 (H-14) to the carbonyl carbon C-9 completed the connection of two aromatic substructures at C-9. Thus, the gross structure of HMP-M3 was determined as shown in Fig. 4.

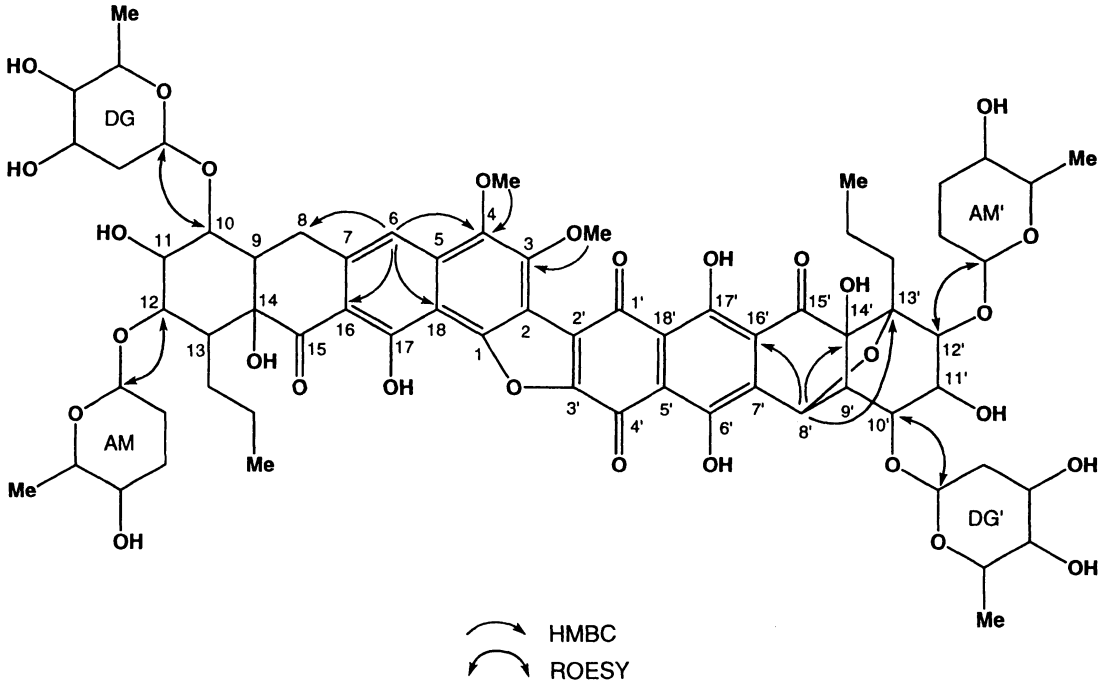
HMP-M3

^{13}C NMR spectrum of HMP-M3 demonstrated twenty carbon signals, two of which were overlapped due to the C_2-symmetry of benzene ring as described below. In the ^1H NMR spectrum, one methyl and three methylene groups and five aromatic protons were observed. Based on these spectral data and FAB-MS (*m/z* 427 $[M+H]^+$), molecular formula was determined as $C_{22}H_{18}O_9$. DQF-COSY showed the presense of two spin systems: H-16/H-17/H-18 and H-12/H-13/H-14 corresponding to the *n*-propyl group and 1,2,3-trisubstituted benzene ring respectively. HMBC correlation from H-13 to the oxygenated sp^2 carbon, C-11 (C-15), and that from H-14 to C-11 (C-15) and C-10

HMP-M4

The ^{13}C NMR spectrum in combination with DEPT, HMQC and COSY spectral data revealed signals due to eight oxygenated sp^2 quaternary carbons, four sp^2 quaternary carbons, six aromatic and olefinic protons, one methylene and one *n*-propyl group. The FAB-MS gave the molecular ion peak $[M+H]^+$ at *m/z* 413, which allowed to identify the molecular formula as $C_{22}H_{18}O_9$ combining with the NMR data. The four-bond coupling between H-16 and H-18, the HMBC correlation of H-16 and H-18 to C-14, that of H-16 to C-17, and that of H-20 to C-14, C-18 and C-19 formed the *n*-propyl resorcinol substructure (Fig. 5). Another resorcinol moiety was deduced by the observation

Fig. 6. NMR analysis of HMP-P4.



of the four-bond coupling between H-8 and H-10, the HMBC correlation of H-10 to C-8, C-9, C-11 and C-12, and that of H-8 to C-7, C-9 and C-12. In addition, the HMBC correlation from H-6 to C-7, C-8 and C-12 confirmed the substitution of the methylene group at C-7. Long-range $^4J_{C,H}$ couplings from H-8 and H-16 to C-13 established that these two resorcinol substructures were linked at C-13 carbonyl carbon. The remaining atoms, three oxygenated sp^2 carbons, two aromatic protons and three oxygens, were deduced to form the hydroxy-α-pyrone ring. H-4 exhibited correlations to C-2, C-3, C-5 and C-6. Long-range coupling of H-6 to C-4 and C-5 established the connectivity between the pyrone ring and the remaining substructure. The H-2 proton was not detected in CD_3OD because of the chemical exchange with deuterium and the carbon signal at C-2 was broad and the intensity was very weak.

and both acetylhexoses on amicetoses. The NMR assignment deduced from the 2D-NMR experiments is summarized in Table 1. Significant HMBC and ROESY correlations are shown in Fig. 6. HMBC correlations were observed from H-6 to C-4, C-8, C-16 and C-18, from 3-OMe to C-3 and from 4-OMe to C-4. The presence of the ether bridge at C-8′ was confirmed by the long-range coupling from the angular proton H-8′ to C-13′. The ^{13}C NMR assignments around the furan ring connecting the naphthalene and naphthoquinone moieties were tentative because the contiguous quaternary carbons did not allow the detection of long-range couplings. The attachment of digitoxoses (DG) at C-10 and C-10′ and of amicetoses (AM) at C-12 and C-12′ was confirmed by ROESY correlations. Anomeric configurations of digitoxose and amicetose were determined to be α and β respectively by the coupling constants $J_{1,2}$.

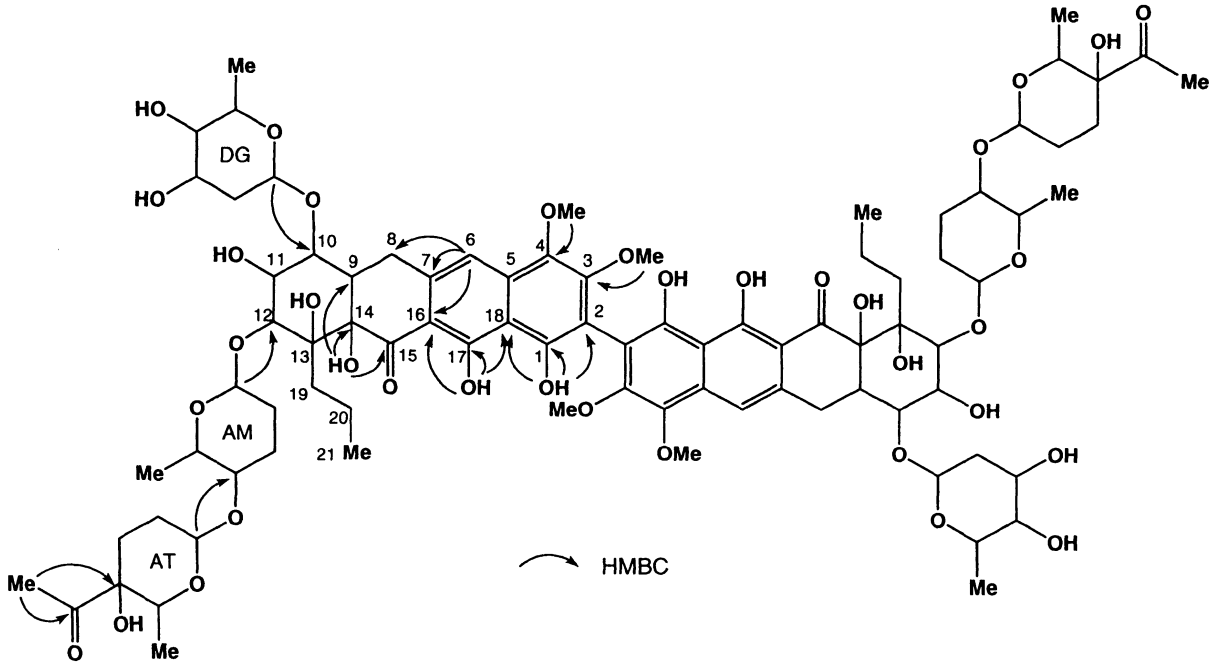
HMP-P4

Molecular formula was determined as $C_{68}H_{84}O_{29}$ based on the FAB-MS, which gave the parent ion peak $[M+H]^+$ at *m/z* 1381, and NMR data. By comparing the ^1H and ^{13}C NMR spectra of HMP-P4 with those of hibarimicin B[3], two major differences were observed: losses of 3′-OMe group

HMP-P1

The FAB-MS gave the parent ion peaks $[M]^+$ at *m/z* 893 and $[M+H]^+$ at *m/z* 894. The acid-catalyzed methanolysis of HMP-P4 afforded the aglycon identical with HMP-P1. The complete NMR assignment of HMP-P1 will be reported elsewhere along with that of hibarimicinone.

Fig. 7. NMR analysis of HMP-Y6.



HMP-Y6

The FAB-MS gave the parent ion peak $[M+Na]^+$ at *m/z* 1749, indicating the molecular formula $C_{86}H_{118}O_{36}$, whereas the ^{13}C NMR spectrum showed only 43 carbon signals. These spectral data suggested that HMP-Y6 had a symmetric structure. Comparison of the ^1H and ^{13}C NMR spectra of HMP-Y6 with those of hibarimicin B[3] led to the confirmation of the structure as shown in Fig. 1. The one-bond C–H bondings were determined by HMQC and proton spin systems by DQF-COSY and TOCSY experiments (Table 2). Significant C–H long-range couplings detected in the HMBC spectrum are summarized in Fig. 7. HMBC correlation from 17-OH to C-16, C-17 and C-18, that from 1-OH to C-1, C-2 and C-18, that from 3-OMe to C-3, that from 4-OMe to C-4, and that from 14-OH to C-9, C-14 and C-15 established the substitution pattern on the aglycon moiety. Long-range coupling of DG-1 to C-10, that of AM-1 to C-12, and that of AT-1 to AM-4 confirmed the positions of glycosidic linkage. The anomeric configurations of digitoxose (DG), amicetose (AM) and acetylhexose (AT) were determined to be α, β and α respectively on the basis of the $J_{1,2}$ values. The gross structure of HMP-Y6 was determined as the dimer of the west half of hibarimicin B.

HMP-M1, M2, M3 and M4 were identified as the shunt metabolites arising from the monomeric precursors. The structure of HMP-M1 dictates the mode of cyclization from an undecaketide to a tetracyclic intermediate in the early stage of biosynthesis. The D ring (The ring was conventionally denoted A, B, C and D from the left to the right.) was not formed in HMP-M2 due to the decarboxylation. The B ring of HMP-M3 was oxidaively cleaved and the pyranone ring was formed instead of the D ring. The cyclase gene is considered to be disrupted in the producer of HMP-M4: the cyclization pattern is definitely different from hibarimicin. Structures of HMP-P1 and P4 are generated by the intramolecular Michael addition of phenolic hydroxyl group to the quinone in the biaryl moiety and the release of methanol molecule to make the furan ring. These HMP-P type metabolites were produced both in the culture broth of the parent strain of hibarimicin and during the purification of hibarimicins. HMP-6, in which the aglycon is not oxidized to quinone, is the dimer of the west half of hibarimicin B. Although other components showing the similar UV-vis spectra to that of HMP-Y6 were detected in the fermentation extract of the strain, isolation was not successful because of the instability. The biological activity of these metabolites from the blocked mutants is under evaluation in comparison with hibarimicins.

Experimental

Instrumental Analysis

Melting points were determined on a Yanagimoto apparatus and are uncorrected. All NMR experiments were performed on a JEOL JNM-LA400 NMR spectrometer in the solvents specified. The FAB-MS spectra were measured on a JEOL JMS-HX110A spectrometer. UV-visible spectra were recorded on a Beckman DU640 spectrophotometer. IR spectra were recorded on a Shimadzu FTIR-300 spectrophotometer. TLC analysis was performed on a silica gel RP-18F254S (Merck, Art 15389) with the solvent of acetonitrile - water (55 : 45).

Isolation of Mutant Metabolites

HMP-M1

The fermented broth (3 liters) was extracted with ethyl acetate (3 liters) and the extract was evaporated to dryness (4.06 g). The crude material was dissolved in methanol (50 ml) and applied on a column of Diaion HP-20 (300×40 mm, i.d., Mitsubishi Chemical Industries Ltd.) and the column was washed with 50% aqueous methanol (1 liter) and eluted with 80% aqueous acetone (1 liter). Evaporation of the eluate gave crude HMP-M1 (630 mg). Portion of this material (400 mg) was applied onto a column of YMC GEL ODS (450×40 mm, i.d., ODS-AM 120-S50), and developed with a mixture of acetonitrile - 0.15% KH_2PO_4 (pH 3.5) (50 : 50). Evaporation of the eluate yielded a semi-pure HMP-M1 (100 mg), which was further purified by Sephadex LH-20 gel filtration (450×20 mm, i.d.) with the eluent of methanol to give pure HMP-M1 (84.0 mg) as a pale yellow amorphous: m.p. 191~193°C; TLC Rf 0.30; ^{1}H NMR (DMSO-d_6): δ 0.91 (3H, t, 7 Hz, H-16), 1.57 (2H, m, H-15), 2.73 (H, dd, 13 and 17 Hz, H-5), 2.86 (2H, t, 7 Hz, H-14), 3.00 (1H, ddd, 4, 5 and 13 Hz, H-4a), 3.32 (1H, dd, 4 and 17 Hz, H-5), 4.92 (1H, s, H-4), 6.84 (1H, d, 8 Hz, H-9), 7.14 (1H, d, 8 Hz, H-6), 7.21 (1H, d, 8 Hz, H-7), 7.51 (1H, t, 8 Hz, H-8); ^{13}C NMR (DMSO-d_6): δ 13.62 (C-16), 17.72 (C-15), 26.14 (C-5), 40.56 (C-4), 43.63 (C-4a), 67.15 (C-4), 79.96 (C-12a), 109.41 (C-11a), 110.22 (C-9), 110.66 (C-2), 112.33 (C-10a), 117.56 (C-6), 118.03 (C-7), 132.50 (C-8), 135.12 (C-5a), 139.21 (C-6a), 157.17 (C-10), 164.08 (C-11), 191.04 (C-1), 197.16 (C-3), 201.14 (C-12), 204.39 (C-13); UV λ_{max}^{MeOH} nm (log ε): 220 (4.32), 266 (4.60), 414 (3.88) $\lambda_{max}^{MeOH+HCl}$ 221 (4.48), 269 (4.71), 415 (3.98), $\lambda_{max}^{MeOH+NaOH}$ 222 (4.41), 269 (4.70), 330 (3.86), 425 (4.23); IR ν_{max} (KBr): 3430, 2960, 1680, 1660, 1450, 1435, 1225, 1120, 1075 cm^{-1}; FAB-MS *m/z* 413 $[M+H]^+$, 435 $[M+Na]^+$.

HMP-M2 and M3

The fermented whole broth (5 liters) was extracted with acetone (10 liters) and the acetone was removed *in vacuo*. The aqueous layer (6 liters) was charged on a column of Diaion HP-20 (500×80 mm, i.d.). The column was washed with water (2 liters) and eluted with 80% aqueous acetone (2 liters). The eluate was evaporated and extracted with ethyl acetate and the extract was evaporated to give crude material (512 mg), which was then applied onto a column of YMC GEL ODS (450×40 mm, i.d.). The column was eluted with a mixture of acetonitrile - 0.15% KH_2PO_4 (pH 3.5) (40 : 60~70 : 30). Fractions containing HMP-M2 and HMP-M3 were pooled, evaporated and extracted with ethyl acetate and the extracts was evaporated respectively. The residues were further chromatographed on a column of Sephadex LH-20 (450×22 mm, i.d.) and eluted with CH_2Cl_2 - methanol (1 : 1) to give HMP-M2 (7.0 mg) and M3 (50.0 mg).

HMP-M2: Yellow powder; m.p. 177~179°C; TLC Rf 0.31; ^{1}H NMR ($CDCl_3$): δ 1.01 (3H, t, 8 Hz, H-16), 1.72 (2H, sextet, 7 Hz, H-15), 2.40 (2H, t, 8 Hz, H-14), 2.48 (3H, s, H-17), 5.81 (1H, s, H-12), 7.31 (1H, dd, 1 and 8 Hz, H-8), 7.70 (1H, s, H-4), 7.70 (1H, t, 8 Hz, H-7), 7.84 (1H, dd, 1 and 8 Hz, H-6), 12.00 (1H, s, 9-OH), 12.44 (1H, s, 1-OH), 15.60 (1H, br, 13-OH); ^{13}C NMR ($CDCl_3$): δ 13.75 (C-16), 19.10 (C-15), 20.71 (C-17), 40.37 (C-14), 102.77 (C-12), 114.11 (C-10a), 115.83 (C-9a), 120.20 (C-6), 121.98 (C-4), 124.90 (C-8), 132.28 (C-2), 133.25 (C-5a*), 133.47 (C-4a*), 137.34 (C-7), 147.16 (C-3), 159.85 (C-1), 162.61 (C-9), 181.51 (C-5), 185.09 (C-11), 192.53 (C-10), 194.97 (C-13) (* exchangeable); UV λ_{max}^{MeOH} nm (log ε): 223 (3.99), 256 (3.93), 288 (3.85), 431 (3.75) $\lambda_{max}^{MeOH+HCl}$ 227 (3.80), 256 (3.90), 285 (3.82), 431 (3.74), $\lambda_{max}^{MeOH+NaOH}$ 236 (3.99), 264 (3.87), 299 (4.02), 514 (3.63); IR ν_{max} (KBr): 3450, 2970, 1700, 1660, 1620, 1470, 1375, 1280, 1205, 1160, 1090 cm^{-1}; FAB-MS *m/z* 367 $[M+H]^+$.

HMP-M3: Colorless powder: m.p. 162~163°C; TLC Rf 0.73; ^{1}H NMR (CD_3OD): δ 0.76 (3H, t, 8 Hz, H-18), 1.39 (2H, m, H-17), 2.44 (2H, t, 7 Hz, H-16), 4.25 (2H, s, H-19), 6.13 (1H, s, H-3), 6.31 (2H, d, 8 Hz, H-12 and H-14), 7.26 (1H, t, 8 Hz, H-13), 7.80 (1H, s, H-6); ^{13}C NMR (CD_3OD): δ 13.39 (C-18), 20.59 (C-17), 36.24 (C-16), 41.60 (C-19), 108.20 (C-12 and C-14), 111.91 (C-3), 112.48 (C-10), 124.87 (C-4a), 130.07 (C-6), 132.43 (C-8), 136.56 (C-7), 137.14 (C-5), 138.32 (C-13), 155.18 (C-8a), 163.32 (C-11 and C-15), 167.61 (C-21), 171.12 (C-2), 174.79 (C-20), 181.19 (C-4), 199.06 (C-9); UV λ_{max}^{MeOH} nm (log ε): 226 (4.66), 256 (4.41), 314 (4.17), $\lambda_{max}^{MeOH+HCl}$ 231 (4.44), 260 (4.30), 321 (4.02), $\lambda_{max}^{MeOH+NaOH}$ 238 (4.51), 302 (4.14), 403 (3.55); IR ν_{max} (KBr): 3450, 2960, 1710, 1640, 1450, 1440,

1225, 1115, 1045 cm^{-1}; FAB-MS *m/z* 427 $[M+H]^+$, 449 $[M+Na]^+$.

HMP-M4

The fermented whole broth (2 liters) was extracted with ethyl acetate (2 liters) and the extract was concentrated *in vacuo* to dryness (3.16 g). The extract was dissolved in methanol (40 ml) and charged on a column of Diaion HP-20 (300×40 mm, i.d.). The resin was washed with 30% aqueous methanol (1 liter) and eluted with 50% aqueous acetone (1 liter). Evaporation of the eluate yielded a crude HMP-M4 (360 mg). This was applied onto a column of YMC GEL ODS (450×40 mm, i.d.), and developed with a mixture of acetonitrile - 0.15% KH_2PO_4 (pH 3.5) (40 : 60). Evaporation of the eluate gave a pure HMP-M4 (200 mg) as a pale brown powder: m.p. 130~131°C; TLC Rf 0.51; ^1H NMR (CD_3OD): δ 0.74 (3H, t, 7 Hz, H-22), 1.40 (2H, br, H-21), 2.18 (2H, br, H-20), 3.56 (2H, s, H-6), 5.55 (1H, s, H-4), 6.15 (1H, d, 1 Hz, H-16), 6.15 (1H, d, 1 Hz, H-18), 6.27 (1H, d, 2 Hz, H-8), 6.32 (1H, d, 2 Hz, H-10); ^{13}C NMR (CD_3OD): δ 14.39 (C-22), 25.44 (C-21), 36.91 (C-20), 38.56 (C-6), 89.78 (C-2), 101.32 (C-16), 120.60 (C-4), 103.34 (C-10), 110.19 (C-18), 112.57 (C-8), 119.74 (C-12), 119.87 (C-14), 138.05 (C-7), 146.03 (C-19), 160.72 (C-15), 162.52 (C-17), 163.14 (C-9), 163.14 (C-11), 166.11 (C-5), 168.15 (C-1), 173.06 (C-3), 202.49 (C-13); UV λ_{max}^{MeOH} nm (log ε): 206 (4.57), 290 (4.08), 336 (3.85), $\lambda_{max}^{MeOH+HCl}$ 205 (4.33), 293 (4.07), 338 (3.84), $\lambda_{max}^{MeOH+NaOH}$ 206 (4.78), 293 (3.87), 344 (4.20); IR ν_{max} (KBr): 3450, 2960, 1675, 1620, 1455, 1310, 1260, 1175, 1020 cm^{-1}; FAB-MS *m/z* 413 $[M+H]^+$, 435 $[M+Na]^+$.

HMP-P4

The fermented whole broth (2 liters) was extracted with acetone (4 liters). After the evaporation, the aqueous layer (2.2 liters) was applied on a column of Diaion HP-20 (500×80 mm, i.d.) and the column was washed with distilled water (2 liters) and eluted with 60% aqueous acetone (2 liters). The eluate was concentrated to aqueous solution and extracted with ethyl acetate. The organic layer was concentrated to give a dark solid (400 mg). This material (400 mg) was applied onto a column of YMC GEL ODS (450×40 mm i.d.), and developed with a mixture of acetonitrile - 0.15% KH_2PO_4 (pH 3.5) (40 : 60). Fractions were evaporated, extracted with ethyl acetate and evaporated to give semi-pure HMP-P4 (62 mg). This was finally purified by Sephadex LH-20 gel filtration (450×20 mm, i.d.) with the eluent of methanol to give pure HMP-P4 (36.0 mg) as a dark purple powder: m.p. >200°C (dec.); TLC Rf 0.40; UV λ_{max}^{MeOH} nm (log ε): 260 (4.21), 291 (4.43), 399 (3.58), 548 (3.82), $\lambda_{max}^{MeOH+HCl}$ 260 (4.19), 290 (4.42), 399 (3.53), 546 (3.87), $\lambda_{max}^{MeOH+NaOH}$ 264 (4.18), 293 (4.23), 454 (3.96), 480 (4.00), 632 (3.61), 674 (3.62); IR ν_{max} (KBr): 3450, 2940, 1690, 1630, 1455, 1410, 1120, 1055 cm^{-1}; FAB-MS *m/z* 1403 $[M+Na]^+$, 1381 $[M+H]^+$, 1252 $[M-DG]^+$, 1138 $[M-DG-AM]^+$, 1007 $[M-2DG-AM]^+$, 893 $[M-2DG-2AM]^+$.

HMP-P1

The fermented broth (3 liters) was extracted with acetone (6 liters) and filtered on Celite. The filtrate was evaporated to an aqueous solution (3.5 liters), which was chromatographed on a column of HP-20 (500×80 mm, i.d.). The column was washed with water (1 liter) and eluted with 50% aqueous acetone (1 liter), and the eluent was evaporated, extracted with ethyl acetate (1 liter) and concentrated *in vacuo*. The extract was chromatographed on a column of YMC GEL ODS (450×40 mm, i.d.) with the eluent of acetonitrile - 0.15% KH_2PO_4 buffer (pH 3.5) (35 : 65). Fractions were evaporated, extracted with ethyl acetate and concentrated *in vacuo* to give HMP-P1 (14.0 mg) as black purple powder; m.p. >200°C (dec.); TLC Rf 0.66; UV λ_{max}^{MeOH} nm (log ε): 260 (4.12), 290 (4.42), 399 (3.50), 546 (3.89), $\lambda_{max}^{MeOH+HCl}$ 260 (4.07), 290 (4.37), 399 (3.49), 546 (3.87), $\lambda_{max}^{MeOH+NaOH}$ 264 (4.05), 293 (4.14), 440 (3.76), 477 (3.73), 632 (3.56), 673 (3.62); IR ν_{max} (KBr): 3450, 2935, 1690, 1630, 1455, 1410, 1220, 1055 cm^{-1}; FAB-MS *m/z* 893 $[M]^+$, 894 $[M+H]^+$.

HMP-Y6

The fermented whole broth (7 liters) was extracted with ethyl acetate (7 liters) and the extract was concentrated *in vacuo* to dryness (3.06 g). The extract was dissolved in methanol (50 ml) and applied on a column of Diaion HP-20 (300×40 mm i.d.). The column was washed with 50% aqueous methanol (1 liter) and eluted with 80% aqueous acetone (1 liter). Evaporation of the eluate yielded a crude HMP-Y6 (1.08 g). This compound was unstable under usual chromatographic conditions such as silica gel and reversed phase silica gel. The crude material (500 mg) was dissolved in methanol (20 ml) and applied on a column of Diaion HP-20SS (450×22 mm. i.d.). The column was washed with 50% aqueous methanol (0.5 liter) and eluted with 80% aqueous acetone (0.5 liter). After the evaporation, the aqueous layer was extracted with ethyl acetate and a pure HMP-Y6 (68 mg) was obtained as a yellow powder: m.p. 188~189°C; TLC Rf 0.20; UV λ_{max}^{MeOH} nm (log ε): 237 (4.88), 280 (5.11), 441 (4.68), $\lambda_{max}^{MeOH+HCl}$ 237 (4.97), 283 (5.14), 439 (4.63), $\lambda_{max}^{MeOH+NaOH}$ 237 (4.76, 278 (5.13), 444 (4.80); IR ν_{max} (KBr): 3450, 2945, 1705, 1630, 1455, 1405,

1110, 1060 cm^{-1}; FAB-MS *m/z* 1749 $[M+Na]^+$, 1187 $[M-2AT-2AM]^+$, 1053 $[M-2AT-2AM-DG]^+$, 927 $[M-2AT-2AM-2DG]^+$.

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