# PRADIMICINS M, N, O AND P, NEW DIHYDROBENZO[*a*]NAPHTHACENEQUINONES PRODUCED BY BLOCKED MUTANTS OF *ACTINOMADURA HIBISCA* P157-2

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Four blocked mutants which accumulated new dihydrobenzo[*a*]naphthacenequinone metabolites, designated pradimicins M, N, O and P, have been isolated from cultures of mutants of *Actinomadura hibisca* P157-2 resulting from treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. The structures of the four compounds were determined by spectral analysis. Pradimicins N, O and P contain D-alanine, while pradimicin M does not. The conformations at C-5 and C-6 of these compounds are different from those of the original pradimicins.

Several biologically active dihydrobenzo[*a*]naphthacenequinone derivatives have recently been isolated from various microorganisms. The potent antifungal antibiotics pradimicins A (**1**) and C (**2**) were isolated from the culture filtrate of *Actinomadura hibisca* P157-2 (ATCC 53557)[1~4]. Pradimicinone (**4**), the aglycone of pradimicins and its dealanyl derivative (**5**) were obtained by acid hydrolysis of pradimicins A and C. In addition, benanomicins A (**3**) and B[5], SF2446[6], KS-619-1[7], G-2A and G-2N[8] all possess the 5,6-dihydrobenzo[*a*]naphthacenequinone nucleus.

In the course of improving the pradimicin producing strain, we have generated four mutants blocked in pradimicin biosynthesis. The mutants produced 5,6-dihydrobenzo[*a*]naphthacenequinone metabolites designated pradimicins N (**6**), P (**7**), O (**8**) and M (**9**). In this report, we describe the isolation of the producing mutants and isolation, physico-chemical properties and structural determination of the four new metabolites.

## Materials and Methods

### Strain Maintenance

*A. hibisca* P157-2 and its mutant strains were grown on a solid medium which contained soluble starch 0.5%, glucose 0.5%, fish meat extract 0.1%, yeast extract 0.1%, NZ-case 0.2%, NaCl 0.2%, $CaCO_3$ 0.1% and agar 1.6%, pH 7.2, and were maintained at 4°C.

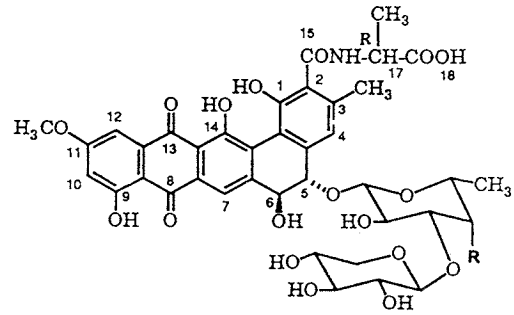
### Isolation of Mutants

*N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) was used as a mutagen with strain P157-2 as described in the previous paper[9].

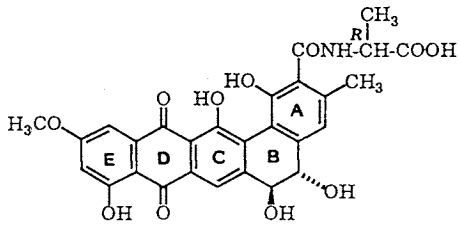
Each mutant was transferred from the solid medium into a vegetative medium (medium A: 10 ml in 50-ml Erlenmeyer flask) consisting of glucose 3%, soybean meal 3%, Pharmamedia 0.5%, yeast extract 0.1% and $CaCO_3$ 0.3%, pH 7.0, and cultivated at 28°C for 7 days at 200 rpm on a rotary shaker.

First, antifungal activity of the whole fermentation broths against *Cryptococcus neoformans* IAM 4514 inoculated in Sabouraud dextrose agar (Neopeptone 1%, glucose 2%, and agar 1.5%, pH 9.0) was determined. The broth was diluted by serial 2-fold dilution. The sample (80 $\mu$l) was put into a well (8 mm

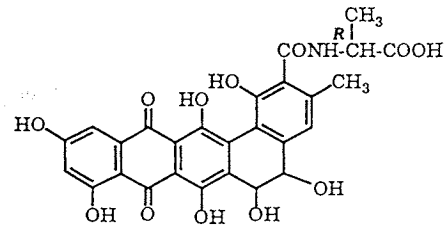
Fig. 1. Structures of several benzo[α]naphthacenequinone metabolites.



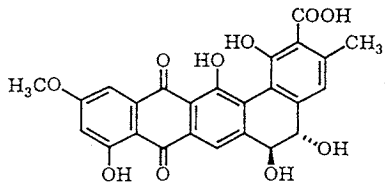
Pradimicin A (**1**) R=NHCH$_3$
Pradimicin C (**2**) R=NH$_2$
Benanomicin A (**3**) R=OH



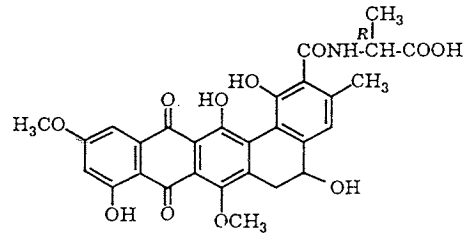
Pradimicinone (**4**)



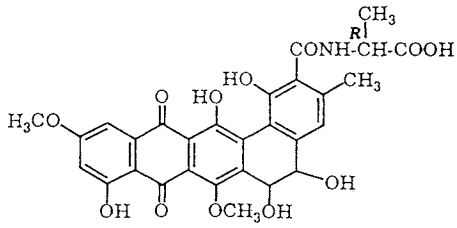
Pradimicin P (**7**)



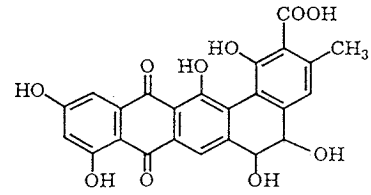
Dealanyl pradimicinone (**5**)



Pradimicin O (**8**)



Pradimicin N (**6**)



Pradimicin M (**9**)

i.d.) in the agar medium and incubated at 37°C for 2 days. The diameter of the inhibitory zone was measured and broths with weaker activity than the parent strain were selected. Next, we looked for new products in these selected broths using silica gel TLC. The broth was extracted with an equal volume of butanol at pH about 2.0. The butanol extract (20 μl) was loaded on a silica gel plate (60 $F_{254}$, 0.25 mm thick, E. Merck) and developed with methyl acetate - 1-propanol - 28% ammonia (9 : 21 : 12). New products were identified based on the color and Rf value as compared to the known components on the plate. Rf values: 0.47 for pradimicin M, 0.46 for pradimicin O, 0.44 for pradimicin N, 0.22 for pradimicin P, 0.28 for pradimicin A, 0.42 for pradimicin B and 0.23 for pradimicin C.

Fermentation

An agar plug of the mutant was transferred into 100 ml of medium A in a 500-ml Erlenmeyer flask and cultivated at 28°C for 6 days at 200 rpm. This culture (5 ml) was transferred into 500-ml flasks containing 100 ml of fresh medium A. The fermentation was carried out at 28°C for 7 days.

Isolation of New Metabolites

The fermentation broth of strain A1077 (1.5 liters) was centrifuged at 5,000 rpm for 10 minutes at room temperature. The supernate was adjusted to pH 2.0 with 3 N HCl and mixed with an equal volume of ethyl acetate. The ethyl acetate layer was separated and extracted with an equal volume of water at pH 8.5 (adjusted with 3 N NaOH). The aqueous layer was adjusted to pH 2.0 and extracted with a half volume of ethyl acetate. The ethyl acetate layer was concentrated *in vacuo* to give 5.0 g of crude solid. The solid was subjected to silica gel column chromatography (Wakogel C-200, column size: i.d. 3 × 80 cm) eluted with a linear gradient of methylene chloride - methyl acetate (9 : 1 to 1 : 1). The eluted fractions (10 ml) were tested by silica gel TLC as described above. The fractions containing the desired product were pooled, concentrated to dryness and then applied to a reversed-phase silica gel column (ODS-A60, Yamamura Chemical Lab., column size: i.d. 2.5 × 50 cm) which was developed with acetonitrile - 0.15% (11 mM) $KH_2PO_4$ buffer, pH 3.5 (1 : 1). The eluate was analyzed by HPLC (Waters M600, YMC A-301-3, ODS, i.d. 4.6 mm × 100 mm, 3 $\mu$m, Yamamura Chemical Lab.) eluted with acetonitrile - 0.15% $KH_2PO_4$ buffer, pH 3.5 (3 : 7) and detected at 254 nm. The fractions containing a single product were pooled and concentrated to dryness. The residue was dissolved in a mixture of methanol - water (1 : 1, pH 8.0) and passed through a column of Sephadex LH-20 eluted with the same solvent mixture. The fractions containing single products were concentrated to dryness, redissolved in acetonitrile, and 0.1 N hydrochloric acid was added. The resulting red precipitate was filtered, washed once with chilled water and dried *in vacuo*, yielding 30 mg of pradimicin N. By a similar procedure, 134 mg of pradimicin P (strain C2248, 480 ml), 40 mg of pradimicin M (strain B2162, 9 liters) and 18 mg of pradimicin O (strain C2099, 4 liters) were obtained.

General

UV and IR spectra were recorded on a Shimadzu UV-260 spectrophotometer and an Analect JIR-fx6160 FT spectrophotometer, respectively. FAB-MS were obtained on a Jeol JMS-AX505H with a 5 KeV beam of energetic xenon atoms and 3 KV as an acceleration voltage using *meta*-nitrobenzyl alcohol as a matrix. HRFAB-MS spectra were obtained by the same equipment using double target probe and polyethylene glycol 600 as an external mass standard. $^1H$ and $^{13}C$ NMR spectra were recorded on Jeol JNM-GX400 spectrometer with DMSO-$d_6$ as an internal standard. $^{13}C$ NMR spectra were obtained by measuring the samples prepared at pH 2 in DMSO-$d_6$ at 60°C.

## Results

### Isolation of Mutants

Three sets of MNNG mutation experiments were carried out starting from *A. hibisca* P157-2, the parent pradimicin-producing strain (Table 1). The blocked mutant A1077 produced pradimicin N as the dominant metabolite. In the first set of experiments, strain A1017 was also isolated as the pradimicin A producer. As this strain showed favorably abundant and uniform spores upon growth, it was subjected to the next set of experiments. Two blocked mutants C2099 and C2248 were isolated from the mutation of strain A1017. The former co-produced pradimicins A and O, while the latter yielded pradimicins A and P. Mutant B2162 was isolated from the third set of mutations of strain A1077, and found to co-produce

Table 1. Isolation of mutants of *Actinomadura hibisca* P157-2.

| Experiment | Strain treated | No. of mutants tested | Mutants isolated | Major product |
|---|---|---|---|---|
| | | | | Pradimicin |
| 1 | P157-2 | 1,627 | A1017 | A |
| | | | A1077 | N |
| 2 | A1017 | 1,412 | C2099 | O |
| | | | C2248 | P |
| 3 | A1077 | 780 | B2162 | M |
| Total | | 3,819 | | |

pradimicins M and N.

Morphological observations and cultural characteristics of the four blocked mutants were almost identical with those of the parent strain P157-2 (data not shown).

## Fermentation and Isolation

Production of the new metabolites was achieved on the flask fermentation scale. The metabolites were purified by silica gel and reversed-phase silica gel column chromatographies to analytically pure material. All the new metabolites showed no anticandidal activity at 100 $\mu$g/ml in Sabouraud dextrose agar medium (pH 7.0).

## Physico-chemical Properties

Physico-chemical properties of the compounds **6**, **7**, **8** and **9** are summarized in Table 2 in comparison with **4** and **5**[1,2]. The molecular formulae of **6**, **7**, **8** and **9** were determined by HRFAB-MS as $C_{29}H_{25}NO_{12}$, $C_{27}H_{21}NO_{12}$, $C_{29}H_{25}NO_{11}$ and $C_{24}H_{16}O_{10}$, respectively. The UV spectra of **6** and **8** are similar to those of **1**, **2**, **4**, and **5**, indicating that they have similar hydroxyl substitutions on the anthracenequinone core. The visible absorption spectra of **7** and **9** in alkaline medium showed a more pronounced bathochromic shift than those of **6** and **8**, suggesting a polyhydroxyanthraquinone structure such as G-2A, G-2N[8]. In the IR spectra of these compounds, strong absorptions were obtained around 1622~1603 $cm^{-1}$ due to the C=O stretching of the hydrogen-bonded quinone carbonyl groups with the *peri* hydroxyl[10]. The absorption band at around 1720 $cm^{-1}$ in **6**~**8** was assigned to a carboxyl group since it disappeared in the spectra of their sodium salts. An amido carbonyl band at around 1660 $cm^{-1}$ was detected in **4**, **6**, **7** and **8**, but not in **5** and **9**. In the FAB-MS, **4**, **6**, **7** and **8** displayed a characteristic $(M-88)^+$ fragment ion due to the cleavage of alanine.

## Structure Elucidation

The structures of the newly isolated pradimicins **6**~**9** were determined on the basis of $^1H$ and $^{13}C$ NMR spectral analyses (Tables 3 and 4). The complete NMR assignment of pradimicinone (**4**) has been carried out previously using 2D NMR[2] and biosynthetic experiments[11]. When the NMR spectrum of **6** was compared to that of **4**, one singlet aromatic proton signal at $\delta$ 8.11 (7-H) of **4** was missing, whereas an additional methoxy methyl signal was found at $\delta$ 3.90. The *O*-methyl proton exhibited a correlation peak to 6-H ($\delta$ 5.03) in the 2D homonuclear NOE (NOESY) experiment. In the long range $^1H$-$^{13}C$ COSY spectrum of **6**, the *O*-methyl proton ($\delta$ 3.90) coupled with the new quarternary carbon at $\delta$ 153.7 ($^3J_{CH}$), which also coupled with the methine proton at 6-H ($\delta$ 5.03). These results indicated that **6** was the 7-methoxy analog of **4**.

The $^1H$ NMR spectra of **7** closely resembled **6** except for the absence of two methoxy methyl signals observed in **6**. In the NOESY experiment, two hydroxy protons at $\delta$ 12.18 and 12.76 showed cross peaks with 12-H ($\delta$ 7.29) and 6-H ($\delta$ 5.10), respectively. Therefore, the structure of **7** was determined to be 7,11-*O*-demethyl analog of **6**.

The $^1H$ and $^{13}C$ NMR spectra of **8** showed that **8** differed from **6** only at the C-6 position. A methine carbon signal at $\delta$ 63.8 in the $^{13}C$ NMR spectrum of **6** appeared as a methylene carbon signal at $\delta$ 31.5 in that of **8**. The $^3J_{HH}$ coupling constants between C-5 methine and C-6 methylene of **8** were 9.4 and 3.9 Hz, and NOE's of 5-H to 4-H and of 6-H to C-7 methoxy were observed. Furthermore, the pseudomolecular ion $(M+H)^+$ of **8** in FAB-MS spectrum was 16 mass units lower than that of **6**. From

Table 2. Physico-chemical properties of pradimicinone and the dealanyl pradimicinone, pradimicins N, P, O and M.

| | Pradimicinone (**4**) | Dealanyl pradimicinone (**5**) | Pradimicin N (**6**) | Pradimicin P (**7**) | Pradimicin O (**8**) | Pradimicin M (**9**) |
|---|---|---|---|---|---|---|
| Molecular formula | $C_{28}H_{23}NO_{11}$ | $C_{25}H_{18}O_{10}$ | $C_{29}H_{25}NO_{12}$ | $C_{27}H_{21}NO_{12}$ | $C_{29}H_{25}NO_{11}$ | $C_{24}H_{16}O_{10}$ |
| HRFAB-MS ($(M+H)^+$ $m/z$) | | | | | | |
| Calcd: | 550.1349 | 478.0900[a] | 580.1455 | 552.1142 | 564.1506 | 465.0822 |
| Found: | 550.1337 | 478.0889[a] | 580.1449 | 552.1169 | 564.1537 | 465.0803 |
| UV $\lambda_{max}$ nm ($\varepsilon$) | | | | | | |
| in MeOH | 232 (31,200), 290 (25,700), 468 (10,200) | 240 (28,300), 274 (18,200), 322 (12,900), 476 ( 9,300) | 236 (26,800), 287 (19,300), 479 (10,100) | 237 (30,700), 287 (26,400), 356 ( 5,100), 512 (16,600) | 236 (31,000), 291 (27,000), 472 (13,200) | 240 (36,300), 301 (24,600), 466 (15,400) |
| in 0.01 N HCl-90% MeOH | 233 (32,100), 300 (28,000), 458 (10,900) | 239 (28,100), 287 (20,600), 461 (10,200) | 236 (26,300), 287 (19,000), 475 (10,300) | 237 (30,700), 287 (26,800), 511 (17,200) | 235 (31,400), 290 (26,800), 490 (13,200) | 241 (35,600), 300 (24,600), 464 (14,900) |
| in 0.01 N NaOH-90% MeOH | 216 (32,000), 244 (30,700), 319 (13,300), 502 (13,700) | 225 (23,800), 241 (22,800), 272 (20,900), 320 (10,000), 510 (11,300) | 223 (28,600), 264 (sh, 20,400), 320 (sh, 8,600), 509 (11,100) | 245 (29,300), 304 (24,000), 336 (10,700), 556 (22,400) | 223 (34,100), 240 (sh, 29,300), 265 (sh, 24,600), 320 (sh, 11,900), 503 (14,200) | 239 (34,800), 311 (20,600), 330 (21,400), 530 (12,300) |
| IR $\nu_{max}$ (KBr) $cm^{-1}$ | 3392, 1728, 1662, 1622, 1607 | 3381, 1718, 1603 | 3418, 1728, 1660, 1617 | 3397, 1718, 1666, 1606 | 3430, 3248, 1733, 1669, 1617, 1602 | 3374, 1606 |

[a] $(M)^-$.

Table 3. $^1$H NMR chemical shifts of pradimicins N, P, O and M.

| Proton | Pradimicin N (6) | Pradimicin P (7) | Pradimicin O (8) | Pradimicin M (9) |
|---|---|---|---|---|
| 3-$CH_3$ | 2.31 (s) | 2.34 (s) | 2.33 (s) | 2.56 (s) |
| 4-H | 6.79 (s) | 6.84 (s) | 6.95 (s) | 7.11 (s) |
| 5-H | 4.50 (d, $J$=3.4) | 4.53 (d, $J$=3.4) | 4.57 (dd, $J$=9.4, 3.9) | 4.58 (d, $J$=3.4) |
| 6-H | 5.03 (dd, $J$=3.4, 4.0) | 5.10 (d, $J$=3.4) | 2.82 (dd, $J$=9.4, 15.6)<br>3.10 (m) | 5.03 (d, $J$=3.4) |
| 7-H | — | — | — | 8.61 (s) |
| 7-$OCH_3$ | 3.90 (s) | — | 3.80 (s) | — |
| 10-H | 6.91 (d, $J$=2.4) | 6.67 (d, $J$=2.4) | 6.91 (d, $J$=2.6) | 6.59 (d, $J$=2.6) |
| 11-$OCH_3$ | 3.94 (s) | — | 3.94 (s) | — |
| 12-H | 7.26 (d, $J$=2.4) | 7.29 (d, $J$=2.4) | 7.26 (d, $J$=2.6) | 7.14 (d, $J$=2.6) |
| 16-NH | 8.60 (d, $J$=7.3) | 8.37 (d, $J$=7.0) | 8.57 (d, $J$=7.3) | — |
| 17-H | 4.39 (qui, $J$=7.3) | 4.46 (dq, $J$=7.0, 7.3) | 4.39 (qui, $J$=7.3) | — |
| 17-$CH_3$ | 1.36 (d, $J$=7.3) | 1.36 (d, $J$=7.3) | 1.34 (d, $J$=7.3) | — |

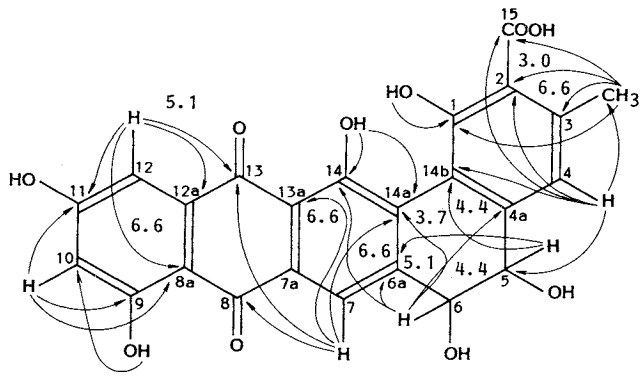
Multiplicity and coupling constants (Hz) are in parentheses.

Table 4. $^{13}$C NMR chemical shifts of pradimicinone, dealanyl pradimicinone, pradimicins N, P, O and M.

| Carbon | Pradimicinone (4) | Dealanyl paradimicinone (5) | Pradimicin N (6) | Pradimicin P (7) | Pradimicin O (8) | Pradimicin M (9) |
|---|---|---|---|---|---|---|
| C-1 | 150.9 (s) | 155.3 (s) | 151.8 (s) | 152.0 (s) | 151.9 (s) | 159.8 (s) |
| C-2 | 126.8 (s) | 118.2 (s) | 127.7 (s) | 127.7 (s) | 126.5 (s) | 112.0 (s) |
| C-3 | 137.3 (s) | 139.5 (s) | 137.4 (s) | 137.5 (s) | 137.3 (s) | 142.8 (s) |
| 3-$CH_3$ | 19.0 (q) | 21.5 (q) | 18.7 (q) | 18.6 (q) | 19.0 (q) | 23.7 (q) |
| C-4 | 117.6 (d) | 117.9 (d) | 123.8 (d) | 123.7 (d) | 117.6 (d) | 119.8 (d) |
| C-4a | 140.8 (s) | 143.5 (s) | 139.6 (s) | 139.7 (s) | 142.7 (s) | 138.9 (s) |
| C-5 | 71.4 (d) | 71.5 (d) | 71.2 (d) | 71.0 (d) | 66.1 (d) | 69.9 (d) |
| C-6 | 72.3 (d) | 72.2 (d) | 63.8 (d) | 62.8 (d) | 31.5 (t) | 61.8 (d) |
| C-6a | 149.7 (s) | 149.4 (s) | 144.1 (s) | 137.8 (s) | 143.0 (s) | 145.7 (s) |
| C-7 | 115.7 (d) | 115.2 (d) | 153.7 (s) | 155.3 (s) | 151.5 (s) | 119.6 (d) |
| 7-$OCH_3$ | — | — | 62.5 (q) | — | 60.8 (q) | — |
| C-7a | 131.1 (s) | 131.2 (s) | 121.7 (s) | 112.4 (s) | 121.6 (s) | 131.5 (s) |
| C-8 | 184.9 (s) | 185.1 (s) | 185.2 (s) | 187.6 (s) | 185.3 (s) | 189.0 (s) |
| C-8a | 110.0 (s) | 110.0 (s) | 111.0 (s) | 109.2 (s) | 110.9 (s) | 108.9 (s) |
| C-9 | 164.6 (s) | 164.6 (s) | 164.6 (s) | 165.4 (s) | 164.3 (s) | 164.4 (s) |
| C-10 | 106.8 (d) | 106.6 (d) | 107.4 (d) | 108.4 (d) | 107.0 (d) | 107.7 (d) |
| C-11 | 165.8 (s) | 165.8 (s) | 165.4 (s) | 164.4 (s) | 165.2 (s) | 165.5 (s) |
| 11-$OCH_3$ | 56.2 (q) | 56.2 (q) | 56.3 (q) | — | 56.1 (q) | — |
| C-12 | 107.4 (d) | 107.3 (d) | 106.4 (d) | 108.6 (d) | 106.0 (d) | 108.8 (d) |
| C-12a | 134.2 (s) | 134.4 (s) | 133.7 (s) | 134.7 (s) | 133.9 (s) | 135.0 (s) |
| C-13 | 187.3 (s) | 187.1 (s) | 188.0 (s) | 186.4 (s) | 186.9 (s) | 181.0 (s) |
| C-13a | 115.2 (s) | 115.2 (s) | 115.4 (s) | 110.8 (s) | 115.1 (s) | 113.3 (s) |
| C-14 | 156.5 (s) | 157.5 (s) | 155.2 (s) | 154.0 (s) | 155.7 (s) | 158.5 (s) |
| C-14a | 125.8 (s) | 125.5 (s) | 130.8 (s) | 132.4 (s) | 130.6 (s) | 130.5 (s) |
| C-14b | 113.7 (s) | 114.2 (s) | 115.4 (s) | 115.2 (s) | 114.5 (s) | 116.0 (s) |
| C-15 | 167.0 (s) | 171.3 (s) | 167.2 (s) | 167.0 (s) | 167.0 (s) | 173.7 (s) |
| C-17 | 47.6 (d) | — | 47.8 (d) | 47.6 (d) | 47.5 (d) | — |
| 17-$CH_3$ | 16.8 (q) | — | 16.9 (q) | 16.8 (q) | 16.8 (q) | — |
| C-18 | 173.7 (s) | — | 173.9 (s) | 173.7 (s) | 173.7 (s) | — |

these results **8** was determined to be the 6-deoxy-derivative of compound **6**. Upon acid hydrolysis, **6**, **7** and **8** gave alanine together with the corresponding aglycones. The alanine was determined to have the D-configuration based on HPLC analysis as previously reported[2].

Fig. 2. The summary of long range $^{1}H$-$^{13}C$ COSY of pradimicin M.




Arrows indicate the carbon coupled to the proton and the values represent the coupling constant (Hz).

Pradimicin M (**9**) showed the molecular formula of $C_{24}H_{16}O_{10}$ by HRFAB-MS which was 14 mass units smaller than that of **5**. In the $^{1}H$ NMR spectrum of **9**, a *meta*-coupling ($J=2.6$ Hz) between aromatic protons 10-H and 12-H and a vicinal coupling ($J=3.4$ Hz) between 5-H/6-H were observed along with three NOEs between methyl on C-3/4-H, 4-H/5-H and 6-H/7-H, demonstrating the same substitution as **5**. However, the lack of an *O*-methyl signal in **9** suggested that the C-11 methoxy group in **5** was replaced by a hydroxy group in **9**. The $^{1}H$-$^{13}C$ correlation of **9** was obtained based on the long range $^{1}H$-$^{13}C$ COSY spectra (Fig. 2). Two aromatic protons 7-H and 12-H at the *peri* position of the quinones coupled to quinone carbonyl carbon at $\delta$ 189.0 (C-8) and 181.0 (C-13), respectively. Furthermore, a weak W-type coupling was observed between 7-H and C-13. The C-8 quinone carbonyl carbon resonated at an 8 ppm lower field than C-13, which in turn displayed a diamagnetic shift of approximately 6 ppm compared with the C-13 of **5**. The benzylic proton 5-H coupled to two aromatic carbons C-6a ($^{3}J_{CH}=5.1$ Hz) and C-14b ($^{3}J_{CH}=4.4$ Hz), and 6-H was coupled to four carbons C-6a, C-4a ($^{3}J_{CH}=4.4$ Hz), C-14a ($^{3}J_{CH}=3.7$ Hz) and C-14 by a W-type coupling. From these data, the structure of **9** is 11-*O*-demethyl-**5** with a different stereochemistry at C-5 and C-6 from that of **5**. In all the new pradimicin components, the magnitude of vicinal coupling constants between 5-H and 6-H was around $J=3.4$ Hz (and $J=9.4$ Hz in case of **8**) which distinctly differed from those of pradimicin A and compounds **4** and **5** ($J=10.7$ Hz). Therefore, the conformations at C-5 and C-6 of these new components are different from those of the original pradimicins. The determination of the stereochemistry will be the topic of future publication.

## Discussion

The biosynthesis of 5,6-dihydrobenzo[*a*]naphthacenequinone nucleus in pradimicins and benanomicins was reported by two independent groups[11,12] where $^{13}C$-acetate-labeled antibiotics were analyzed by $^{13}C$ NMR. There have been no reports on the isolation of mutants specifically blocked in their biosynthetic pathways. Pradimicin P (**7**) was found as a major product in the fermentation of strain C2248, but was originally isolated as a minor by-product of the parent strain P157-2 (unpublished result). Pradimicin M (**9**) was isolated from the fermentation broth of a mutant B2162 which was derived from pradimicin N-producing mutant A1077 by MNNG mutation. From the structure of **9**, it is probable that **9** may be an early intermediate of pradimicin biosynthesis.

As expected, all new metabolites were devoid of antifungal activity. No glycosidated compounds of

the above characterized new aglycones have so far been isolated from fermentation broths of the mutants. However, it will be intriguing to see if glycosidated compounds with different conformations at C-5 and C-6 from those of the pradimicins have antifungal, antiviral, antibacterial, or antitumor activity.

While this publication was being written, a new α-glucosidase inhibitor, benanomicin C was published[13]. Although a direct comparison has not been carried out, benanomicin C is considered to be identical with pradimicin N from the reported data.

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