STRUCTURAL STUDIES ON PYRROLOMYCINS C, D AND E

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Pyrrolomycins C, D and E are new members of the pyrrolomycin group of antibiotics produced by an *Actinosporangium* sp. The structures of pyrrolomycins C and D were determined to be 2,3-dichloro-5-(3′,5′-dichloro-2′-hydroxybenzoyl)pyrrole and 2,3,4-trichloro-5-(3′,5′-dichloro-2′-hydroxybenzoyl)pyrrole, respectively, by means of spectroscopic and synthetic approaches while that of pyrrolomycin E was shown to be 5-chloro-2-(3′,5′-dichloro-2′-hydroxyphenyl)-3-nitropyrrole by spectroscopic data.

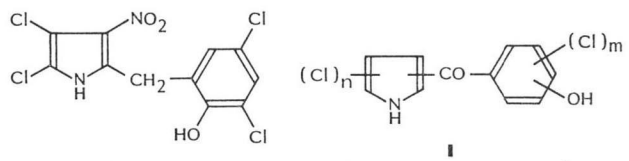
In the preceding paper[1], the isolation and characterization of the new antibiotics, pyrrolomycins (PM) C, D and E produced by *Actinosporangium vitaminophilum* SF-2080 were reported. This paper describes structural studies on PM-C, D and E which were carried out by spectroscopic analysis and synthetic correlation.

Spectroscopic Analysis of PM-C and D

PM-C isolated as yellow needles showed mp 220~221°C and PM-D isolated as yellow needles showed mp 195~198°C. They are insoluble in water but become soluble on addition of sodium carbonate, forming a light yellow solution. Elemental analysis and mass analysis established the molecular formulae of PM-C as $C_{11}H_5Cl_4NO_2$ and of PM-D as $C_{11}H_4Cl_5NO_2$. The UV absorption maximum of PM-C is 327 nm ($E^{1\%}_{1cm}$ 455) and of PM-D is 336 nm ($E^{1\%}_{1cm}$ 380) in neutral methanol solution. The ^1H NMR spectrum of PM-C in acetone-d_6 showed two singlets at 11.12 ppm and 11.75 ppm (broad) and of PM-D in the same solvent at 10.3 ppm (2H, broad). These signals disappeared on addition of D_2O, suggesting NH or OH groups. The presence of NH or OH groups was also shown by IR absorption bands at 3270 cm^{-1} (PM-C) and 3260 cm^{-1} (PM-D). The ^{13}C NMR spectra summarized in Table 1 revealed the close relationship between the two antibiotics, *i.e.*, they showed one carbonyl carbon and ten aromatic carbons of analogous chemical shifts and similar hydrogen-carbon coupling. A comparison of these results with those for PM-B[2] suggested a polychlorinated aroylpyrrole as the common skeleton (I) of PM-C and D.

The location of the aroyl group on the pyrrole ring was determined by UV spectroscopy. As shown in Chart 2, the absorption maximum of PM-C and D resembled that of a 2-aroylpyrrole such as

Chart 1.



Pyrrolomycin B

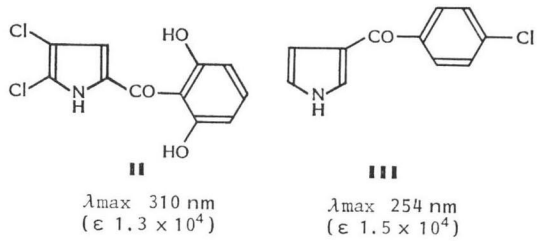
I

Possible structure of pyrrolomycins C and D

Table 1. ^{13}C NMR spectra of PM-C and D in acetone-d_6.

PM-C	PM-D
183.2 (s)	183.0 (s)
154.5 (s)	153.7 (s)
133.8 (d)	134.0 (d)
129.2 (d)	130.1 (s)
128.8 (s)	129.9 (d)
125.1 (s)	126.1 (s)
124.2 (s)	125.9 (s)
124.0 (s)	124.8 (s)
123.1 (s)	123.6 (s)
119.6 (d)	121.1 (s)
111.8 (s)	118.5 (s)

Chart 2.



II

λmax 310 nm
(ε 1.3 × 10⁴)

III

λmax 254 nm
(ε 1.5 × 10⁴)

II[3,4], but differed from the maximum of the 3-aroylpyrrole **III**[5], suggesting the 2-substituted structure.

The location of hydroxyl group and chlorine atoms were determined by EI mass spectra. As shown in Fig. 1, both antibiotics exhibited an abundant fragment ion at *m/z* 189 attributable to the dichlorohydroxybenzoyl ion. A difference was observed regarding the number of chlorine atoms attached to the pyrrole ring: PM-C showed a fragment ion at *m/z* 135 attributed to a dichloropyrrole and PM-D at *m/z* 169 due to a trichloropyrrole. Based on this finding, the gross structures **IV** and **V** were proposed for PM-C and D, respectively. However, the positions of chlorine atoms and the hydroxyl group in the pyrrole ring and benzoyl moiety could not be determined unambiguously by spectroscopic analysis. Consequently, a synthetic approach was chosen to solve this problem.

Synthesis PM-C and D

There were reported several routes[6~9] for the synthesis of pyoluteorin (**II**), but none showed good

Fig. 1. Mass spectra of PM-C and D (EI).

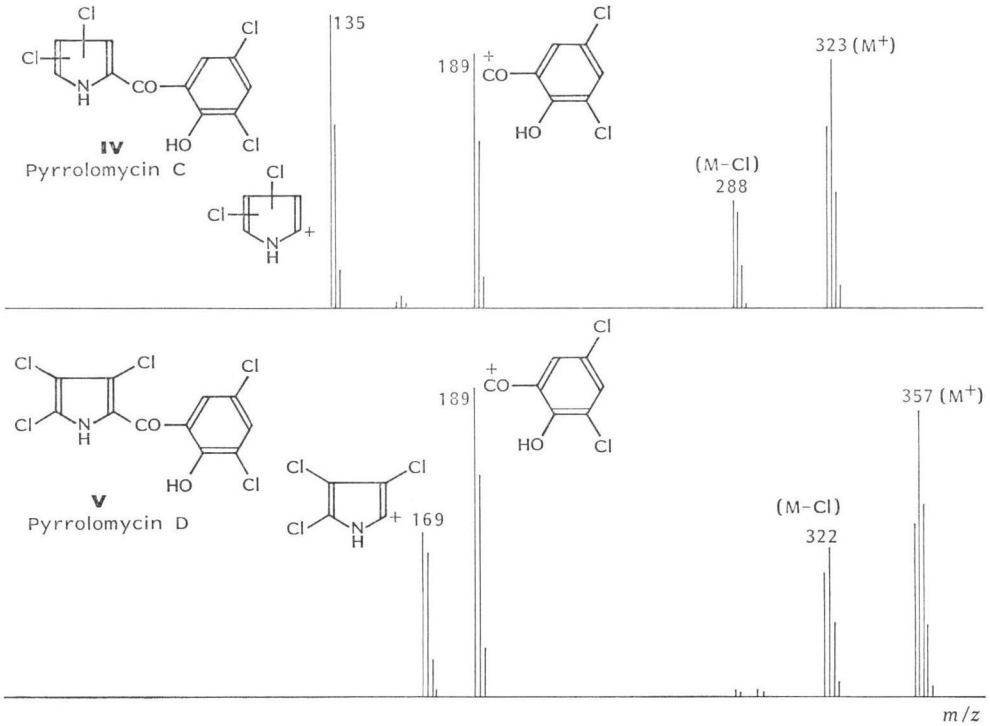
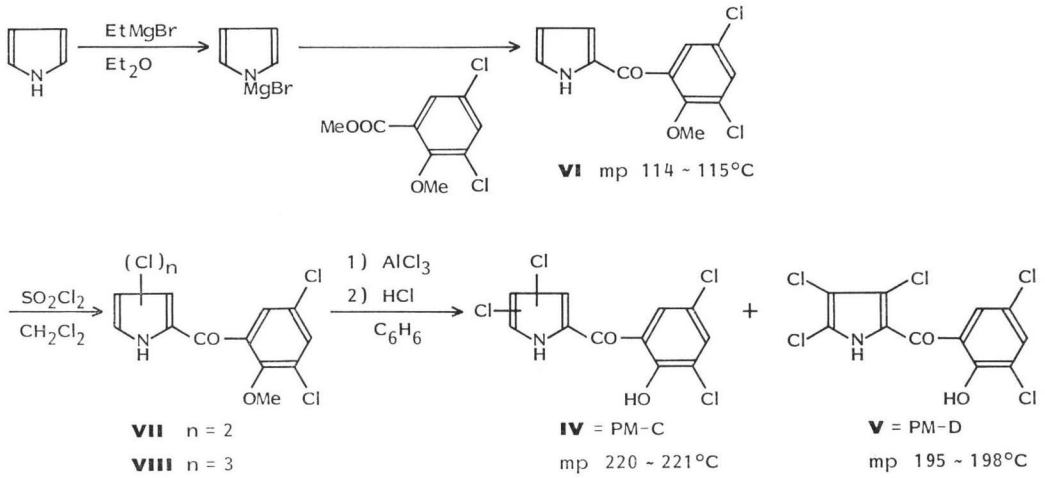


Chart 3. Synthesis of PM-C and D.



results for the synthesis of PM-C and D. For example, FRIEDEL-CRAFTS acylation of pyrrole[9] using 3,5-dichloro-2-methoxybenzoyl chloride as an acylating agent caused considerable degradation. It was found that methyl 3,5-dichloro-2-methoxybenzoate was stable to the synthetic conditions on reaction with pyrrylmagnesium bromide. From this new method, the desired ketone **VI** was obtained in moderate yield. Chlorination of compound **VI** with 3 mol of sulfuryl chloride afforded a mixture of dichloropyrrole (**VII**) and trichloropyrrole (**VIII**). The crude mixture of chlorinated compounds **VII** and **VIII** was demethylated on treatment with anhydrous aluminium chloride in benzene. The mixture of demethylated compounds was separated into two active fractions by preparative TLC over silica gel developed with a mixed solvent system such as hexane - ethyl acetate - acetic acid (100: 20: 1). After recrystallization of each fraction from hot benzene, pure antibiotics **IV** and **V** were obtained as yellow needles. Compound **IV** was identical with natural PM-C, and **V** was identical with PM-D in their physico-chemical properties. Therefore, PM-D was identified as 2,3,4-trichloro-5-(3′,5′-dichloro-2′-hydroxybenzoyl)pyrrole (**V**) and PM-C was dichloro-2-(3′,5′-dichloro-2′-hydroxybenzoyl)pyrrole (**IV**).

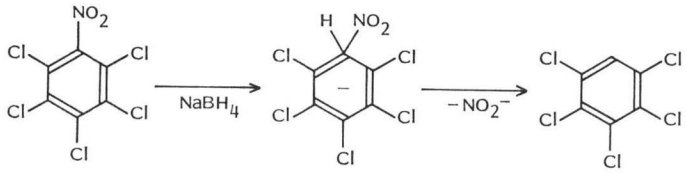
However, the structure of PM-C was not fully determined by this synthetic approach, because there remained three possibilities for the position of chlorine atoms on the pyrrole nucleus. Accordingly, an another synthetic approach was examined.

Chemical Correlation of PM-C with PM-B

The structure of PM-B[2] was established as a 2,3-dichloro derivative by X-ray analysis. If the chemical correlation of PM-C with PM-B succeeded, the position of chlorine atoms in PM-C could be assigned unambiguously.

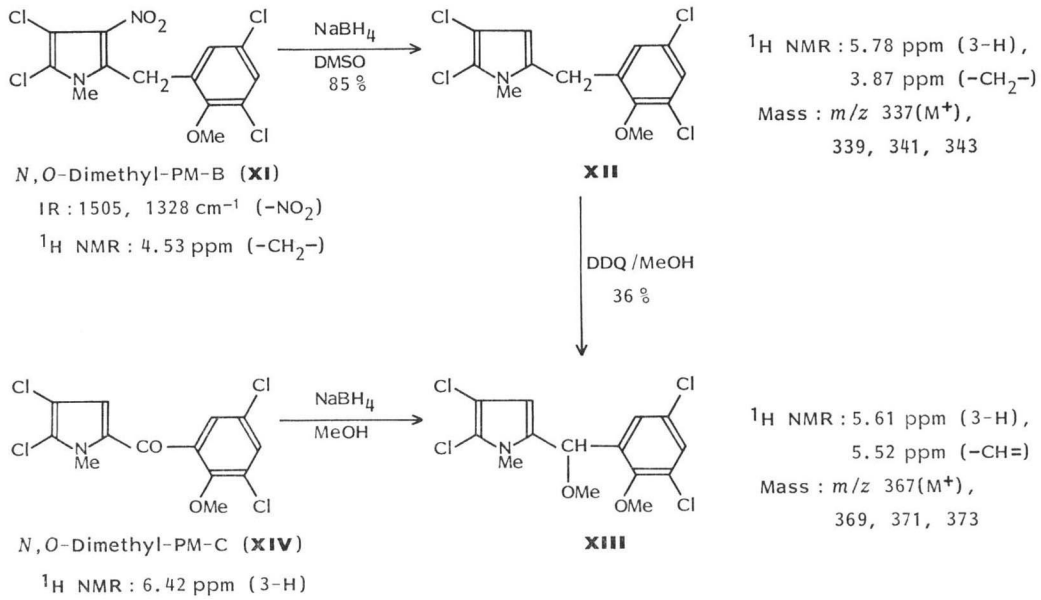
There was an interesting report which proved to be useful for this purpose. LAMSON[10] reported that, when polychlorinated nitrobenzene such as **X** was treated with sodium borohydride in dimethylsulfoxide, nucleophilic substitution of nitro group by hydride occurred. This condition could not be applied directly to the denitration of PM-B itself, but, in the case of *N*,*O*-dimethyl-PM-B (**XI**), the reaction did occur, and the denitro compound **XII** was obtained in good yield. The structure of **XII** was confirmed by ^{1}H NMR and elemental analysis. Although direct oxidation of **XII** to *N*,*O*-dimethyl-PM-C (**XIV**) failed, the new methoxy compound **XIII** was obtained from DDQ oxidation of **XII** in me-

Chart 4.



X

Chart 5. Chemical correlation of PM-B and C.



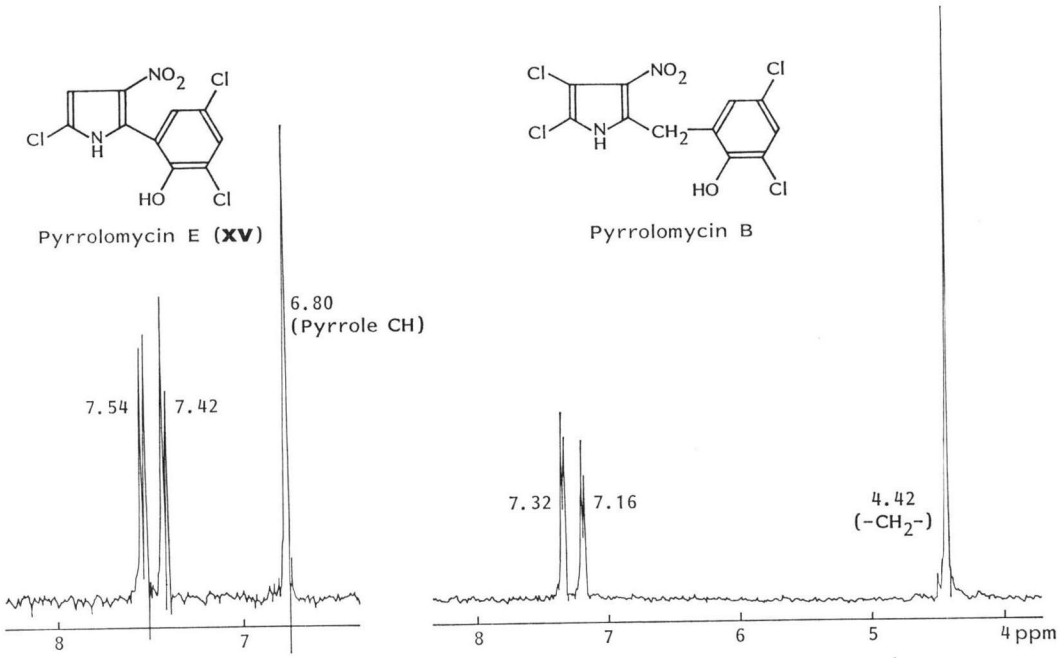
thanol. The structure of **XIII** was confirmed also by spectroscopic analyses. Simultaneously, *N,O*-dimethyl-PM-C (**XIV**) afforded the same methoxy compound **XIII** when **XIV** was hydrogenated with sodium borohydride in methanol. Thus, the chemical correlation of PM-C with PM-B was established and the structure of PM-C was assigned as 2,3-dichloro-5-(3′,5′-dichloro-2′-hydroxybenzoyl)pyrrole.

Structure of PM-E

PM-E was isolated as yellow needles, mp >250°C. Elemental analysis and mass analysis established the molecular formula $C_{10}H_5Cl_3N_2O_3$. The presence of a nitro group was shown by a mass fragment ion at *m/z* 260 ($M^+ - NO_2$) and IR absorption bands at 1555 and 1380 cm^{-1}. The ^1H NMR spectrum of PM-E in acetone-d_6 showed a broad singlet (2H) at 10.26 ppm, which disappeared on addition of D_2O, suggesting two dissociable hydrogen atoms. When treated with excess of diazomethane, PM-E was converted into a dimethyl derivative with formula $C_{12}H_9Cl_3N_2O_3$ (mp 161~162°C). The above results together with ^1H NMR analysis indicated that PM-E was a new nitropyrrole antibiotic, with structure related to PM-B.

Because of their similarity, ^1H NMR data of PM-E and B were compared as shown in Fig. 2. PM-E showed no methylene signal, but a sharp singlet at 6.80 ppm, being split into a doublet with J= 2.8 Hz on addition of trifluoroacetic acid. This indicated that the pyrrole ring and benzene moiety

Fig. 2. 100 MHz ^{1}H NMR spectra of PM-E and B (acetone-d_6).



were directly bonded in PM-E and that one β-position of the pyrrole ring was substituted by a hydrogen atom. Direct linkage of the benzene and pyrrole moieties was further supported by ^{1}H NMR spectra.

Two protons assigned to those of the benzene moiety resonated at lower field than those of PM-B. This difference could be explained in terms of the electron withdrawing effect of the nitropyrrole nucleus that was directly bound to the benzene moiety in PM-E.

It was noted that the absorption maxima due to the nitro group of PM-E in UV and IR spectra were close to those of non-conjugated nitro compounds. This indicated that the nitro group of PM-E was not substituted at the β-position of the pyrrole ring but was sterically much hindered. Direct bonding of two aromatic rings together with adjacent substitution of the nitro group were consistent with the weak nitro-aromatic conjugation in PM-E. Thus, the structure of PM-E was presumed to be 5-chloro-2-(3′,5′-dichloro-2′-hydroxyphenyl)-3-nitropyrrole (XV). The structure of PM-E was finally confirmed by X-ray crystallographic analysis of its *N*,*O*-dimethyl derivative, details of which will be reported separately.

Experimental

Melting points were determined on a Yamato MP-21 apparatus in glass capillary tubes. ^{1}H NMR spectra were recorded in a solution containing TMS as internal reference on a Varian XL-100 or Jeol FX-200 spectrometer, IR spectra in KBr disks on a Hitachi 269-10 infrared spectrophotometer, UV spectra in methanol on a Hitachi 200-20 spectrophotometer and mass spectra on a Hitachi M-80 mass spectrometer. TLC was carried out by using silica gel plates F_{254} (E. Merck) and a mixed solvent of benzene - ethyl acetate (10: 1) or hexane - ethyl acetate - acetic acid (100: 20: 1).

2-(3′,5′-Dichloro-2′-methoxybenzoyl)pyrrole (VI)

Pyrrole (0.67 g, 0.01 mol) was dissolved in 30 ml of dry benzene and 5 ml of ethyl magnesium bromide (2 mol solution in ether) was added cold and the solution was kept at 20°C for 30 minutes. To

this solution, 2.2 g (0.01 mol) of methyl-3,5-dichloro-2-methoxybenzoate was added in one portion and the mixture was stirred at 25°C for 1 hour. The reaction mixture was poured into 100 ml of ice water and acidified with 5 ml of concentrated hydrochloric acid. The organic layer was separated, dried over sodium sulfate and concentrated to dryness under reduced pressure. The residue was crystallized from hexane, and 2-(3′,5′-dichloro-2′-methoxybenzoyl)pyrrole (**VI**) was obtained as colorless crystals. Yield 1.06 g (36%), mp 114~115°C, IR (KBr) 3290 cm^{-1} (NH), 1635 (C=O).

Anal. Calcd. for $C_{12}H_8Cl_2NO_2$: C 53.36, H 3.36, N 5.19, Cl 26.25.
Found: C 54.01, H 3.13, N 5.07, Cl 25.99.

2,3-Dichloro-5-(3′,5′-dichloro-2′-hydroxybenzoyl)pyrrole (**IV**) and 2,3,4-Trichloro-5-(3′,5′-dichloro-2′-hydroxybenzoyl)pyrrole (**V**)

238 mg of compound **VI** was dissolved in dry methylene chloride and cooled, to this solution was added 370 mg of sulfuryl chloride. The mixture was stirred at 20°C for 1 hour. The reaction mixture was concentrated and to the residue, 30 ml of dry benzene was added and re-concentrated. Residual solid was solved in 30 ml of dry benzene and to this solution, 0.25 g of anhydrous aluminum chloride was added and the mixture was stirred vigorously at 20°C for 2 hours. The reaction mixture was poured into 100 ml of ice water and acidified with 5 ml of concentrated hydrochloric acid. Organic layer was separated, washed with water and dried over sodium sulfate. The benzene solution was concentrated under reduced pressure to give a mixture of antibiotics (**IV**) and (**V**) as a yellow mass. This material was separated by preparative TLC on silica gel developed with hexane - ethyl acetate - acetic acid (100: 20: 1). The following fractions were obtained;

(a) 90 mg of 2,3,4-trichloro-5-(3′,5′-dichloro-2′-hydroxybenzoyl)pyrrole (**V**), which was recrystallized from 5 ml of benzene. Yield 58 mg (16%), mp 195~198°C.

Anal. Calcd. for $C_{11}H_4Cl_5NO_2$: C 36.76, H 1.12, N 3.90, Cl 49.32.
Found: C 36.41, H 1.10, N 3.78, Cl 49.58.

(b) 110 mg of 2,3-dichloro-5-(3′,5′-dichloro-2′-hydroxybenzoyl)pyrrole (**IV**), which was also recrystallized from benzene. Yield 59 mg (18%), mp 220~221°C.

Anal. Calcd. for $C_{11}H_5Cl_4NO_2$: C 40.66 H 1.55, N 4.31, Cl 43.63.
Found: C 40.92, H 1.53, N 4.24, Cl 43.26.

Compound **V** was identical with natural PM-D and **IV** with PM-C in physico-chemical properties.

N,O-Dimethylpyrrolomycin B (**XI**)

PM-B (320 mg) was treated with an excess of diazomethane in ethyl acetate. After 1 hour, excess reagent was decomposed by the addition of acetic acid and reaction mixture was concentrated to dryness. Residual solid was washed with 3 ml of methanol and filtrated. Yield 271 mg (78%), mp 172~173°C.

Anal. Calcd. for $C_{13}H_{10}Cl_4N_2O_3$: C 40.66, H 2.65, N 7.39, Cl 36.92.
Found: C 40.94, H 2.65, N 7.25, Cl 36.65.

^1H NMR ($CDCl_3$) 3.48 ppm (3H, N–CH_3), 3.82 (3H, O–CH_3), 4.53 (2H, –CH_2–), 6.85 (1H, J=2.4 Hz, 6′-H), 7.36 (1H, J=2.4 Hz, 4′-H).

2,3-Dichloro-5-(3′,5′-dichloro-2′-methoxybenzyl)-1-methylpyrrole (**XII**), Denitration of *N,O*-Dimethyl-PM-B

N,O-Dimethyl-PM-B (197 mg) was dissolved in 25 ml of dimethyl sulfoxide. To this solution, 400 mg of sodium borohydride was added in one portion. The reaction mixture was kept at 20°C for 8 hours and was extracted with 50 ml of ethyl acetate and water. Ethyl acetate layer was separated, washed with sodium chloride solution and dried over sodium sulfate. Ethyl acetate extract was concentrated and denitro compound (**XII**) was obtained as colorless foam. Yield 149 mg (85%).

Anal. Calcd. for $C_{13}H_{11}Cl_4NO$: C 46.05, H 3.27, N 4.13, Cl 41.82.
Found: C 44.66, H 3.38, N 4.52, Cl 40.77.

^1H NMR ($CDCl_3$) 7.27 ppm (1H, phenyl), 6.88 (1H, phenyl), 5.78 (1H, 4-H), 3.87 (3H, O–CH_3), 3.35 (3H, N–CH_3). Mass (EI) 337, 339, 341, 343 (M^+, Cl_4).

2,3-Dichloro-1-methyl-5-[methoxy-(3′,5′-dichloro-2′-methoxyphenyl)methyl]pyrrole (**XIII**), DDQ Oxidation of Denitro Compound (**XII**)

40 mg of denitro compound (**XII**) was dissolved in 5 ml of methanol. To this solution, 40 mg of

2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) was added and reaction was maintained for 8 days at room temperature. Reaction mixture was concentrated to dryness and the residue was purified by preparative TLC over silica gel developed with benzene. New methoxy compound (**XIII**) was obtained as colorless crystals. Yield 8 mg, mp 67~74°C. ^{1}H NMR ($CDCl_3$) 7.36 (2H, phenyl), 5.61 (1H, 3-H), 5.52 (1H, =CH), 3.62 (3H, O–CH_3), 3.60 (3H, O–CH_3), 3.32 (3H, N–CH_3). Mass (EI) 367, 369 371, 373 (M^+, Cl_4). This compound was identical with the reduction product of *N,O*-dimethyl-PM-C.

2,3-Dichloro-1-methyl-5-[methoxy-(3′,5′-dichloro-2′-methoxyphenyl)methyl]pyrrole (**XIII**), Sodium Borohydride Reduction of *N,O*-Dimethyl-PM-C

N,O-Dimethyl-PM-C (95 mg) was dissolved in 10 ml of methanol. To this solution, 30 mg of sodium borohydride was added and the reaction mixture was stirred at room temperature for 1 hour. The reaction mixture was extracted with ethyl acetate and ethyl acetate layer was washed with saturated sodium chloride solution, then dried over anhydrous sodium sulfate. Ethyl acetate extract was concentrated to dryness under reduced pressure and residual foam was purified by preparative TLC over silica gel developed with benzene. 40 mg (40%) of title compound was obtained as colorless crystals. mp 68~74°C.

Anal. Calcd. for $C_{14}H_{13}Cl_4NO_2$: C 45.56, H 3.55, N 3.80, Cl 38.42.
Found: C 45.38, H 3.46, N 3.78, Cl 38.05.

N,O-Dimethyl-PM-C (**XIV**)

PM-C (325 mg) was dissolved in 20 ml of ethyl acetate and treated with excess of diazomethane at room temperature. After 10 minutes, excess diazomethane was decomposed by the addition of acetic acid and the reaction mixture was concentrated to dryness. *N,O*-Dimethyl derivative of PM-C was obtained as colorless powder. Yield 346 mg (98%), mp 85~87°C.

Anal. Calcd. for $C_{18}H_9Cl_4NO_2$: C 44.23, H 2.57, N 3.97, Cl 40.17.
Found: C 44.06, H 2.49, N 3.88, Cl 40.35.

N,O-Dimethyl-PM-E

100 mg of PM-E was suspended in ethyl acetate (15 ml) and to this mixture, excess of diazomethane in ether solution was added and the mixture was stirred at 25°C for 4 hours. The excess diazomethane was decomposed by the addition of acetic acid and the solution was concentrated under reduced pressure. The residual solid was recrystallized two times from ethanol and pure *N,O*-dimethyl-PM-E was obtained as colorless prisms. Yield 73 mg (67%), mp 161~162°C.

Anal. Calcd. for $C_{12}H_9Cl_5N_2O_3$: C 42.95, H 2.70, N 8.35, Cl 31.69.
Found: C 42.83, H 2.64, N 8.34, Cl 31.83.

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