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Studies on Pillaromycin A. II.[1] The Structure of Pillaromycinone

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The structure of pillaromycinone (II) was elucidated. The ultraviolet (UV) spectrum of PMN tetraacetate (VI) was similar to that of 1,2,3,4-tetrahydro-1-oxophenanthrene. On oxidative degradation pillaromycinone dimethyl ether (IX) gave 3-methoxy-1,2,4,5-benzenetetracarboxylic acid (X) and 3-methoxyphthalic acid (XI). Zinc dust distillation of II gave anthracene and naphthacene. The UV spectrum of II resembles to that of anhydrodemethyltetracycline.

From the chemical and spectrometric data the structure of β-substance (IV) was suggested to be 1,2,3,4,4a,5,12,12a-octahydro-12-oxo-4,10,11,12a-tetrahydroxynaphthacene.

The nuclear magnetic resonance (NMR) and the spin decouplings of isopropylidene PMN (XVI) and PMN (II) gave the structure (3(*S*), 4(*R*), 4a(*R*), 12a(*R*)) 2-acetyl-3,4,4a,5,-12,12a-hexahydro-12-oxo-3,4,10,11,12a-pentahydroxynaphthacene to PMN (II).

The ultraviolet and visible absorption (UV) spectra of the pentaacetate (V) of pillaromycin A (I, PMA), tetraacetate (VI) of pillaromycinone (II, PMN), diacetate (VII) of pillaronone (III) and tetraacetate (VIII) of dihydroanhydroxypillaromycinone (IV, β-substance) (Fig. 1) were similar to that of 1,2,3,4-tetrahydro-1-oxophenanthrene.[3] II was subjected to zinc dust distillation, to give anthracene and naphthacene in good yield. Reaction of II with dimethyl sulfate gave the PMN dimethyl ether (IX), mp 171°, $C_{22}H_{22}O_7$. This derivative

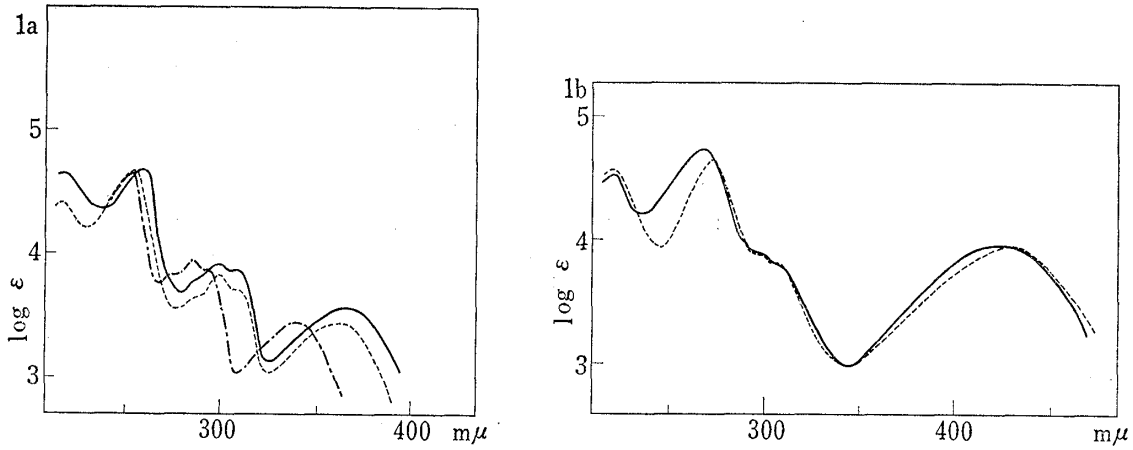
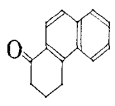


Fig. 1a. UV Spectra of Pillaromycinone Tetraacetate (VI), Dihydroanhydroxypillaromycinone Tetraacetate (VIII) and 1,2,3,4-Tetrahydro-1-oxophenanthrene

1b. UV Spectra of Anhydrodemethyltetracycline (XII) and Pillaromycinone (II)

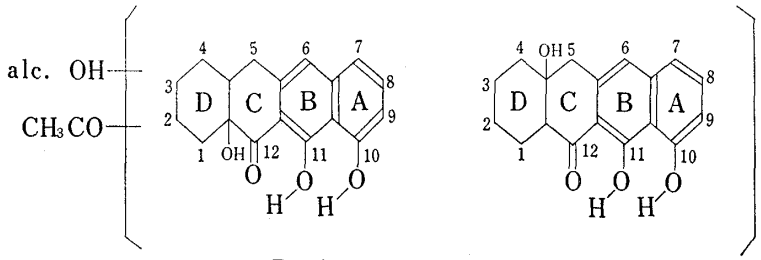
——: VI
------: VIII
—•—: 1,2,3,4-tetrahydro-1-oxophenanthrene
——: XII
------: II

1) Part I: M. Asai, *Chem. Pharm. Bull.* (Tokyo), **18**, 1699 (1970).
2) Location: *Juso, Higashiyodogawa-ku, Osaka.*
3) R.A. Friedel and M. Orchin, *J. Am. Chem. Soc.*, **69**, 1985 (1947).

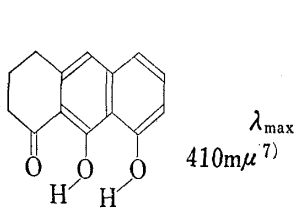


afforded, when oxidatively degradated, 3-methoxy-1,2,4,5-benzenetetracarboxylic acid (X), and 3-methoxyphthalic acid (XI), both being identified with the authentic samples. The NMR of a half-and-half mixture[4] of pillaronone (III) and its diacetate (VII) in $CDCl_3$ showed two signals assigned to chelated hydroxyls (each 1/2 proton) at 9 and 15 ppm.

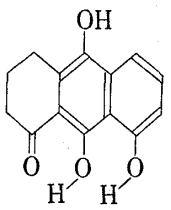
From these findings the parent skeletons (models (1), (2)) may be given for I, II, III and IV.[5] The possibility of model (2) was eliminated since only two phenolic hydroxyls had been recognized in I, II, III and IV and they had four aromatic ring protons. Thus, the structures of I, II, III and IV were found analogous to model (1). This finding together with the facts



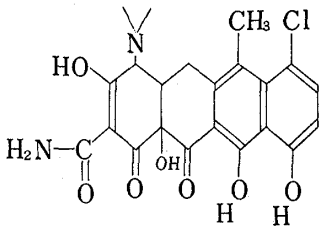
that the unsaturation number of IV was eleven and that zinc-dust distillation of IV gave naphthacene has further suggested that the parent structure of IV would be either D-1 or D-2. The λ_{max}^{EtOH} of I, II, III and IV in visible region are 431, 428, 420 and 420 mμ, respectively and those of the model compounds ((1)—(6)) structurally related to I, II, III and IV are as follows:



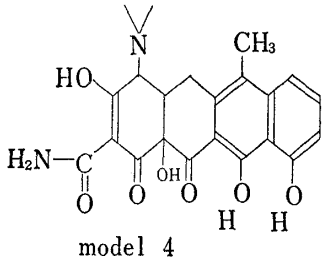
λ_{max} 410mμ [7]



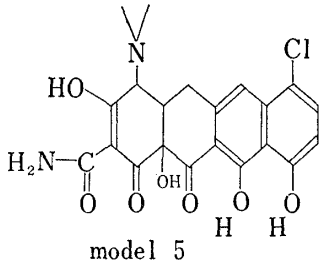
428mμ [8]



445mμ [9]



428mμ [8]



430mμ [10]

4) Pillaronone diacetate (VII) was utilized as the solubilizing agent and had no contribution to the absorption in the lower magnetic field (9 and 15 ppm).

5) The quinone configurations[6] which had been introduced mainly from the oxidative degradation result must be ruled out, since acetates of I and II were obtained without passing through reductive acetylation.

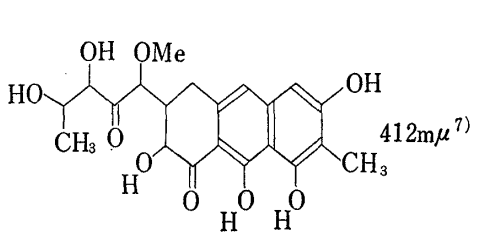
6) M. Shibata, M. Asai, K. Mizuno, A. Miyake and S. Tatsuoka, *Proc. Japan Acad.*, **40**, 296 (1964).

7) M. Miyamoto, K. Morita, Y. Kawamatsu, S. Noguchi, R. Marumoto, K. Tanaka, S. Tatsuoka, K. Nakanishi, Y. Nakadaira and N.S. Bhacca, *Tetrahedron Letters*, **34**, 2355 (1964).

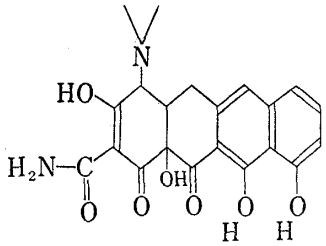
8) F.A. Hochstein, C.R. Stephens, L.H. Conover, P.P. Regna, R. Pasternack, P.N. Gordon, F.J. Pilgrim, K.J. Brunings and R.B. Woodward, *J. Am. Chem. Soc.*, **75**, 5455 (1953).

9) C.W. Waller, B.L. Hutchings, R.W. Broschard, A.A. Goldman, W.J. Stein, C.R. Stephens, L.H. Conover, R. Pasternack, F.A. Hochstein, W.T. Moreland, P.P. Regna, F.J. Pilglim, K.J. Brunings and R.B. Woodward, *J. Am. Chem. Soc.*, **76**, 3568 (1954).

10) J.S. Webb, R.W. Broschard, D.B. Cosulich, W.J. Stein and C.F. Wolf, *J. Am. Chem. Soc.*, **79**, 4563 (1957).



model 6



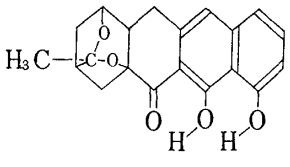
model 7

Chart 1

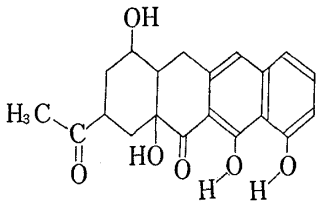
Compared with model (1) and (6), other models ((2)—(5)) have the absorption maxima which shifted by 15—35 mμ to longer wave region. The reasons of these shifts were ascribed[8,10] to oxygen atom on C_{10} in model (2), or to methyl radical on C_6 in models (3), (4), and to chlorine atom on C_7 in models (3), (5), respectively. It must be noted that the contribution of the 12a-OH (*tert*-hydroxyl) to those shifts has not been referred to.

To confirm the effect of the 12a-OH, ledermycin® (demethylchlorotetracycline) was converted to anhydrodemethyltetracycline (XII) model (7). XII had the absorption maxinum at 423.5 mμ in visible region, (Fig. 1), showing the longer wave length shift compared with those of models (1) and(6). This fact supported the possibility that the phenomenon would be due to the effect of the 12a-OH. Thus, the parent structure of I, II and IV was proposed as D-1. And two compounds (X and XI) may be given by a different demethylation during the oxidative degradation of IX, the dimethylether of II.

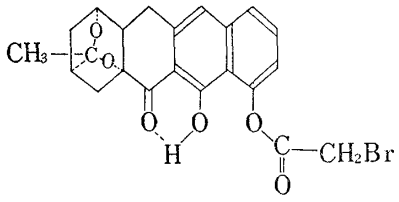
Beside the 12a-OH in formula D-1 the *sec*-hydroxyl and the acetyl should be present additionally in such position that they could formed ketal combination (see formula C in Part I) in IV. In II, the vinyl proton showed a clean singlet and the acetyl was conjugated to the vinyl bond, these findings indicated that the acetyl was on C_2 of D ring, and the *sec*-hydroxyl was on C_4 of D ring. Thus, the structure of β-substance was presumed to be formula IV and the structure of pillaronone was elucidated to be formula III.



III



IV



XIII

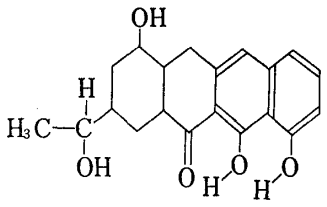
The X-ray analysis[11] of the crystalline pillaronone monobromoacetate (XIII) also supported the above deduction.

The γ-substance (XIV) showed the absorption maximum at 407 mμ in visible region and had a new signal of a *sec*-methyl in the NMR spectrum. In the NMR spectrum of tetraacetate (XV) of XIV, the presence of two phenolic O–Ac and two alcoholic O–Ac, a new methine and a *sec*-methyl (d.) was recognized. Therefore, the structure of γ-substance could be presented as below:

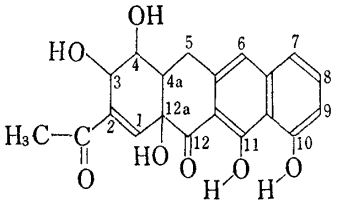
On catalytic reduction of II the loss of a *sec*-hydroxyl and the vinyl bond[12] was observed to give IV, therefore II was presumed to have either formula E or F.

11) K. Kamiya, M. Asai, M. Nishikawa, K. Mizuno, Y. Tomiie and I. Nitta, *Chem. Pharm. Bull.* (Tokyo), **18**, 1724 (1970).

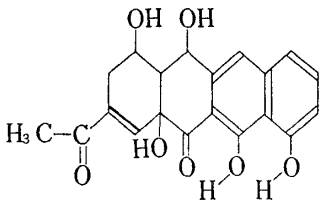
12) The vinyl bond should be on C_1–C_2, because the vinyl proton was observed as a clean singlet.



XIV



E



F

To investigate the propriety of the formulae E and F, II was derived to isopropylidene PMN (XVI), mp 145°, $C_{23}H_{22}O_7$. Acetylation of XVI gave isopropylidene PMN diacetate (XVII), and isopropylidene PMN triacetate (XVIII). No methine proton adjacent to oxygen atom shifted to lower field was observed in the NMR[13] spectra of XVI, XVII and XVIII (Fig. 2, Table I). This finding suggested that in XVII two phenolic hydroxyls of XVI were acetylated, in XVIII a *tert*-hydroxyl of XVII was further acetylated and the *tert*-hydroxyl (12a-OH) of XVI was not involved in the isopropylidene configuration. When H_4[14] (4.37 ppm) of XVI was irradiated, the doublet at 5.03 ppm collapsed to a clean singlet and was assigned to H_3. This finding indicated that PMN had the formula E.

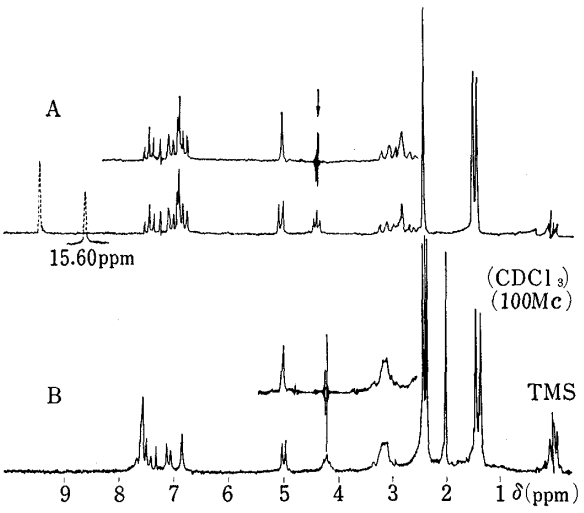


Fig. 2. NMR Spectra of Isopropylidene PMN (XVI) (A) and Isopropylidene PMN Triacetate (XVIII) (B) (100 Mc in $CDCl_3$)

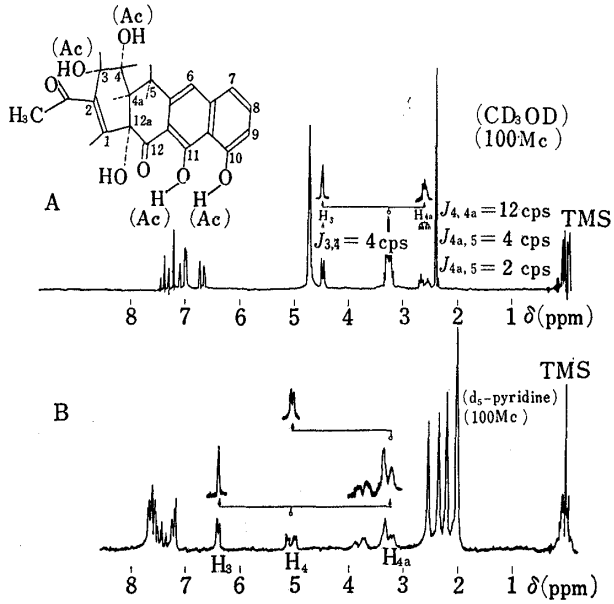


Fig. 3. NMR Spectra of Pillaromycinone (II) (A) and Pillaromycinonetetraacetate (VI) (B)

The remaining undissolved absolute configuration of II was next investigated. The result of NMR spin decoupling (in CD_3OD) of II are shown in Fig. 3. The observed coupling constants were 4 cps, 12 cps, 4 cps and 2 cps which would be allocated to J_{3-4}, J_{4-4a}, J_{4a-5a} and J_{4a-5b} respectively. Dreiding model of conformation (E′) reveals that the dihedral angles between H_3 and H_4, H_4 and H_{4a}, H_{4a} and H_{5a}, and H_{4a} and H_{5b} are approximately 45°, 180°, 45° and 60°, respectively. According to M. Karplus,[15] the coupling constants between these coupled protons were calculated and the calculated values corresponded to 4 cps for

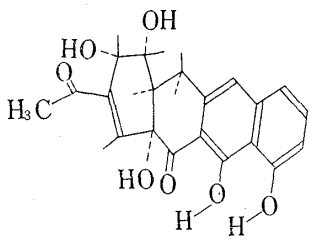
13) NMR spectra were measured on a Varian HA-100 or A-60A spectrometer. The chemical shifts were expressed in δ value from TMS (internal reference).
14) Proton at 4.37 ppm was assigned to H_4 from the NMR spectrum of β-substance.
15) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

Table I. NMR Spectra of Isopropylidene PMN (XVI) and Isopropylidene PMN Triacetate (XVIII) (100 Mc in $CDCl_3$)

	OH_{11}	OH_{10}	H_8	H_7	H_6	H_1	H_9	H_3	OH	H_4	H_5	H_{4a}	H_5	Me_{14}	Me	Me
δ (ppm)	15.60 s	9.46 s	7.46 t	7.06 q	6.93 s	6.91 s	6.71 q	5.03 d	4.43 s	4.37 t	3.10 (d)	2.86 m	2.80 m	2.39 s	1.47 s	1.40 s
XVI	1H	1H	1H	1H	1H	1H	1H	1H	1H	1H	1H	1H	1H	3H	3H	3H
J (cps)			8	8 1.5			8 1.5	6		6	12	6 12	2			

	H_8	H_7	H_6	H_1	H_9	H_3	H_4		Ac_{11}	Me_{14}	Ac_{10}	Ac_{12a}	Me	Me
δ	7.50 t	7.64 q	7.56 s	6.84 s	7.09 q	4.98 d	4.16 t	3.35—3.02 m	2.44 s	2.40 s	2.36 s	2.01 s	1.44 s	1.36 s
XVIII	1H	1H	1H	1H	1H	1H	1H	1H	3H	3H	3H	3H	3H	3H
J	7	7 1.5			7 1.5	6	6							

J_{3-4}, to 12 cps for J_{4-4a}, to 4 cps for J_{4a-5a} and to 2 cps for J_{4a-5b}, respectively, being in good agreement with the observed values. Thus II may be shown as the formula E′.



E′

Therefore, both C_3-hydroxyl and C_4-hydroxyl are *cis* to H_{4a} and C/D ring juncture must be *cis*. The configurations of H_3, H_4, H_{4a} and 12a-OH in D ring are equatorial, axial, axial and equatorial and the configurations of H_{4a} and 12a-OH in C ring are equatorial and axial, respectively.

Conclusively the absolute configuration, (3(*S*), 4(*R*), 4a (*R*),12a(*R*))2-acetyl-3,4,4a,5,12,12a-hexahydro-12-oxo-3,4,10, 11, 12a-pentahydroxynaphthacene, was given to PMN (II).

Experimental[16]

Zinc-dust Distillation of PMN (II)——A homogeneous mixture of II (5 g) and zinc-dust (120 g) was gradually heated in a retort-type flask (200 ml) wrapped with asbestos so as to the external temperature gradually rose to 400° in 10 hr. Distillate of the naphthalene series (~250°), purple blue fluorescent distillate of the anthracene series (at 250—320°) and reddish orange fluorescent substances of the naphthacene series (at 300—350°) were fractionated. The distillate of the anthracene series (*ca.* 80 mg) were dissolved in benzene–petroleum ether (1:8) and purified by passing through a column (1.5×15 cm) of alumina (Merck). As a main product, anthracene was obtained, which was recrystallized from benzene to give white crystals (20 mg). *Anal.* Calcd. for $C_{14}H_{10}$: C, 94.34; H, 5.66. Found: C, 94.13; H, 5.87. UV $\lambda_{max}^{n\text{-hexane}}$ mμ: 222, 252, 310, 338, 355, 374. The reddish orange fluorescent fraction was recrystallized from benzene to give naphthacene (3 mg). UV $\lambda_{max}^{benzene}$ mμ: 280, 295, 355, 375, 397, 420, 446, 476.

PMN Dimethyl Ether (IX)——A solution of II (8 g) in acetone was treated with dimethylsulfate (10 g) and potassium carbonate (22 g) at 60—62° for 4 hr, and the reaction mixture was filtered. The filtrate was treated with ice–water (150 ml), sodium bicarbonate ($NaHCO_3$) (10 g) and ethylacetate (EtOAc) (100 ml). On concentrating the EtOAc extract and adding petroleum ether, a crude precipitate was obtained. The crude powder was purified by the column chromatography of bentonite–celite (Wako Pure Chemical Ind., Ltd.) (1:1) (2.7×13 cm), using chloroform–benzene–cyclohexane (1:9:1) and acetone–chloroform–benzene (2:4:2) as the developing solvent. The green fluorescent effluent was concentrated and filtered. The yellowish green products were purified by dissolving in chloroform benzene and reprecipitating with petroleum ether, mp 171°. *Anal.* Calcd. for $C_{22}H_{22}O_7$: C, 66.32; H, 5.57; OMe, 15.56. Found: C, 65.91; H, 5.55; OMe, 15.60. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (OH), 1692, 1678 (CO), 1625, 1605. UV λ_{max}^{EtOH} mμ ($\varepsilon\times10^{-3}$): 220 (36.05), 263 (35.42), 295 (sh.), 305 (4.94), 378 (5.29). NMR (60 Mc in $CDCl_3$): 2.32 (3H, s, $COCH_3$), 3.90 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), nonechelated OH.

Oxidation of PMN Dimethyl Ether——To a solution of PMN di-methyl ether (IX) (6 g) in 0.5N NaOH (400 ml) was added dropwise aq. 5% $KMnO_4$ (600 ml) at 70—73° over a period of 1 hr, then methanol was

16) All melting points were uncorrected.

added to reduce the unreacted $KMnO_4$. After filtering of MnO_2 the filtrate was stirred with Amberlite IR-120 (H) (100 ml) for 15 min and passed through a column of Amberlite IR-120 (H) (200 ml), and the eluate was concentrated and shaken with EtOAc (200 ml).

a) The EtOAc layer was concentrated (*ca.* 20 ml) and chromatographed on a thick Tōyō filter paper No. 526 (40×40 cm) with pyridine–*n*-butanol–ethanol–water (1:3:1:1). The bands having *Rf* 0.35—0.39 (2b) and *Rf* 0.28—0.32 (2a) were extracted with methanol and evaporated to give crude substances (2a (100 mg) and 2b (300 mg)). The substances thus obtained were rechromatographed by the same method.

b) The aqueous layer was concentrated, paper chromatographed as above. The yellow spots colored with BCG and having *Rf* 0.03—0.10 (1a) and *Rf* 0.12—0.15 (1b) were extracted with water, and the extracts were evaporated to dryness to yield 1a (300 mg) and 1b (500 mg). They were purified by the same chromatography.

c) An aqueous solution of 2b was desalted with Amberlite IR-120 (H), and adsorbed on Amberlite IR-45 (OH) (25 ml) and eluted with aqueous ammonia (4%). The eluate was concentrated (*ca.* 20 ml), decolorized with active carbon, passed through Amberlite IR-120 (H) (15 ml) to remove ammonia and placed in an ice-box after concentration. The separated substance, 3-methoxyphthalic acid (XI), mp 164°, became the anhydride at 180°. *Anal.* Calcd. for $C_9H_6O_4$: C, 60.87; H, 3.37; OCH_3, 17.33. Found: C, 61.02; H, 3.75; OCH_3, 17.06. UV λ_{max}^{EtOH} mμ ($\varepsilon\times10^{-3}$): 210 (22.0), 298 (3.0). 3-Methoxyphthalic acid was prepared according to the method of Miyashita.[17] mp 177°. *Anal.* Calcd. for $C_9H_8O_5$: C, 55.10; H, 4.08. Found: C, 54.77; H, 4.07. UV λ_{max}^{EtOH} mμ ($\varepsilon\times10^{-3}$): 211 (18.7), 297 (2.55) (in literature),[18] 211 (22.5), 300 (3.4). The product became the anhydride at 180°.

d) The concentrate of 1b fraction was desalted with Amberlite IR-120 (H) (20 ml) and concentrated, and the Ca-salt of the resulting product was dried at 200° and analyzed. *Anal.* Calcd. for CaC_2O_4: C, 18.75; Ca, 31.29. Found: C, 19.00; Ca, 31.19.

e) The crude 1a fraction (180 mg) was mixed with water, desalted with Amberlite IR-120 (H) (20 ml) and concentrated to give crystals, recrystallized from water, 3-methoxy-1,2,4,5-benzenetetracarboxylic acid (X) mp 240°. *Anal.* Calcd. for $C_{11}H_8O_9$: C, 46.49; H, 2.84; OCH_3, 10.92. Found: C, 47.04; H, 2.94; OCH_3, 11.64. UV λ_{max}^{EtOH} mμ ($\varepsilon\times10^{-3}$): 215 (27.5), 250 (sh.), 298 (5.3). This compound was synthesized according to the method of S.R. Finn, *et al.*[19] and J.S. Robert, *et al.*[20] *Anal.* Calcd. for $C_{11}H_8O_9$: C, 46.49; H, 2.84. Found: C, 46.60; H, 2.94. UV λ_{max}^{EtOH} mμ ($\varepsilon\times10^{-3}$): 215 (27.0), 250 (sh.), 298 (5.68).

Anhydrodemethyl Tetracycline (XII)——A solution of $NaHCO_3$ (84 mg/17.5 ml H_2O) was added to a suspension of Ledermycin® (6-demethyl-7-chlortetracycline hydrochloride) (500 mg) in dimethylformamide (0.3 ml). The suspension once became clear, but soon 6-demethyl-7-chlortetracycline separated out. According to the method of J.H. Booth, *et al.*,[21] 6-demethyl-7-chlortetracycline (400 mg) dissolved in the mixture of methyl cellosolve (7 ml) and triethylamine (1.7 ml) was subjected to catalytic reduction on Pd-charcoal (200 mg) until *ca.* 1.5 mole of H_2 was consumed. After filtering the catalyzer the filtrate was concentrated and a small amount of water was added to give precipitate. The precipitate was dissolved in conc. HCl (1 ml) and left standing for 40 hr at room temperature. The reaction mixture was poured into water (*ca.* 20 ml), neutralized with 1N NaOH and extracted with EtOAc. The extract was washed with water and concentrated. The resulting anhydrodemethyltetracycline was recrystallized from EtOAc, mp 210°. *Anal.* Calcd. for $C_{21}H_{20}O_7N_2$: C, 61.16; H, 4.89; ,N 6.79. Found: C, 60.99; H, 4.89; N, 6.68.

γ-Substance Tetraacetate (XV)——γ-Substance (100 mg) in pyridine (1.2 ml) was acetylated with acetic anhydride (0.5 ml) at 65° for 3 hr and then at room temperature for 40 hr. The reaction mixture was treated with ice–water (20 ml) to separate a yellowish white precipitate (100 mg). The γ-substance tetraacetate (XV) was crystallized from the hot ethanol: ethyl acetate (EtOAc) and recrystallized from the same solvent to give 50 mg of XV, mp 270°. *Anal.* Calcd. for $C_{28}H_{30}O_9$: C, 65.88; H, 5.92. Found: C, 65.37; H, 5.79. NMR (60 Mc in $CDCl_3$): 1.23 (3H, d, CH–CH_3), 2.05 (3H, s), 2.12 (3H, s), 2.39 (3H, s), 2.49 (3H, s) (O–CO–CH_3).

Isopropylidene PMN (XVI)——A solution of PMN (II) (500 mg) in acetone (10 ml) was treated with conc. sulfuric acid (3 drops) and the mixture was left standing for 2.5 hr, and poured into ice–water (150 ml) to separate a yellow precipitate (0.5 g). This was purified by a charcoal column (0.5 g) chromatography. The yellow effluent was concentrated to dryness. Recrystallization of the product with ether–petroleum ether gave yellow needles, mp 145°, $[\alpha]_D^{22}+449°$ ($c=1.0$, MeOH). *Anal.* Calcd. for $C_{23}H_{22}O_7\cdot\frac{1}{2}C_4H_{10}O$: C, 67.10; H, 6.08; O, 26.90. Found: C, 67.54; H, 5.61; O, 26.76. IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 1688, 1640 (CO). UV λ_{max}^{EtOH} mμ ($\varepsilon\times10^{-3}$): 223 (36.05), 273 (42.0), 295 (sh.), 310 (sh.), 428 (8.91). NMR (100 Mc in $CDCl_3$): Table I, Fig. 2.

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Isopropylidene PMN Triacetate (XVIII)——XVI (500 mg) was acetylated with acetic anhydride (1.2 ml) in pyridine (2.4 ml) for 24 hr. From the resulting mixture a crude acetate (420 mg) was obtained. The crude acetate (100 mg) was purified by the preparative thin-layer chromatography and crystallized from EtOAc–ether–petroleum ether. mp 197°. *Anal.* Calcd. for $C_{29}H_{28}O_{10}$: C, 64.92; H, 5.26; O, 29.82. Found: C, 64.75; H, 5.16; O, 30.23. IR ν_{max}^{Nujol} cm^{-1}: 1778, 1745, 1690 (CO), 1630, 1600 (C=C). UV λ_{max}^{EtOH} $m\mu$ ($\varepsilon \times 10^{-3}$): 259 (49.30), 287 (sh.), 300 (8.17), 310 (7.26), 360 (3.32). NMR (100 Mc in $CDCl_3$): Table I, Fig. 2.

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