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Studies on Pillaromycin A. I.[1] The Properties of Pillaromycin A and Pillaromycinone

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A molecular formula, $C_{28}H_{28}O_{11}$, has been given for pillaromycin A. Acid hydrolysis of pillaromycin A afforded an aglycone, pillaromycinone, $C_{20}H_{18}O_7$ and a new sugar, pillarose, $C_8H_{12}O_5$. From the results of spectrometrical and chemical studies on pillaromycinone and its derivatives the presence of the following functional groups was elucidated in pillaromycinone; two *sec*-hydroxyls, a *tert*-hydroxyl, two phenolic hydroxyls, a chelated carbonyl and an acetyl conjugated with α,β-unsaturated double bond.

Pillaromycin A (I, PMA) is a yellow antibiotic obtained from the culture of *Streptomyces flavovirens* No. 65786. This antibiotic has a colchicine-like antitumor activity and an ultraviolet and visible (UV) spectra similar to those of chromomycin A_3[3] and A_2,[4] but it is less toxic than above antibiotics. These facts have stimulated the author's interest on its chemical structure.

PMA (I) was extracted with ethyl acetate (EtOAc) from the culture filtrate and crystallized from solvents such as ethanol, acetone, ether and EtOAc. The solvent free crystals of I exhibited mp 208°, $C_{28}H_{28}O_{11}$,[5] and were soluble in aq. sodium bicarbonate ($NaHCO_3$) solution. I reduced Fehling's reagent and gave a blue color with the aq. $FeCl_3$:$K_3Fe(CN)_6$ solution (Barton, *et al.*[6]), but showed negative $Mg(OAc)_2$ reaction. Its characteristic UV spectrum (Fig. 1) suggested the presence of carbonyls and aromatic rings, and its infrared absorption (IR) spectrum (Fig. 1) also showed the carbonyl bands at 1720 (isolated), 1678 (conjugated) and 1635 (chelated) cm^{-1}. The nuclear magnetic resonance (NMR)[7] spectra (Fig. 2 in CD_3OD,

1) M. Shibata, E. Higashide, K. Wada, K. Mizuno, M. Asai, M. Imanishi and A. Miyake, Japan. Patent S. 41-7839 (1966); M. Shibata, M. Asai, K. Mizuno, A. Miyake and S. Tatsuoka, *Proc. Japan Acad.*, **40**, 296 (1964).
2) Location: *Juso, Higashiyodogawa-ku, Osaka.*
3) K. Mizuno, *J. Antibiotics* (Tokyo), *Ser. A*, **16**, 22 (1963).
4) K. Mizuno, M. Imanishi, M. Asai, A. Miyake and M. Shibata, Japan. Patent S. 40-14591 (1965).
5) The molecular weight of crystalline Pillaromycin A (I) containing one molar diethylether was 619.7±5 from X-ray analysis.
6) G.M. Barton, R.S. Evans and J.A.F. Gardner, *Nature*, **170**, 249 (1952).
7) 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).

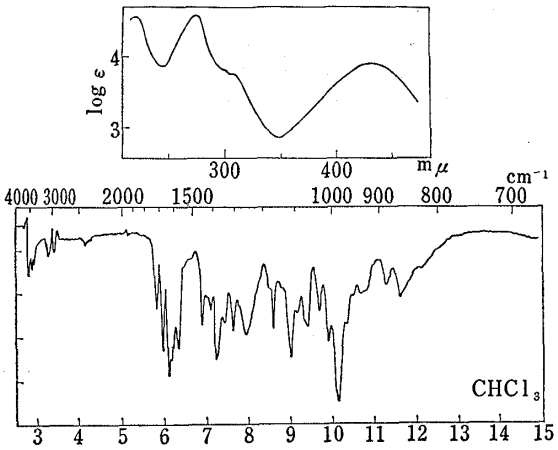


Fig. 1. UV and IR spectra of Pillaromycin A (I)

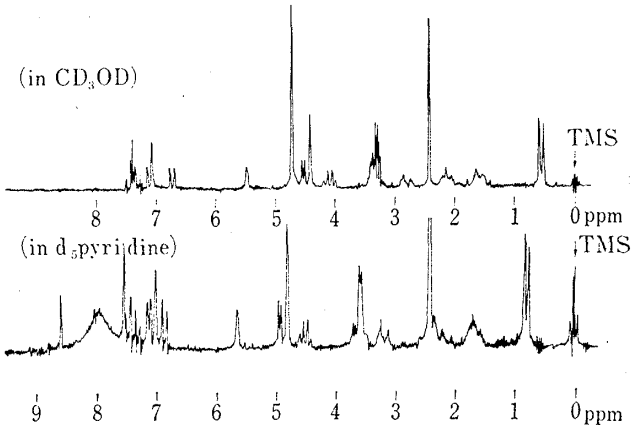


Fig. 2. NMR Spectra of Pillaromycin A (I) (100 Mc)

d_5-pyridine, 100 Mc) presented the signals which were assigned to CH–CH_3 (one), CH_3 (one, singlet), aromatic and vinyl protons (five), and alicyclic methine or methylene protons (six).

Full acetylation of I gave the pentaacetate (IV), mp 195°, $C_{38}H_{38}O_{16}$, showing that the chelated carbonyl in I had disappeared. The NMR spectrum (in $CDCl_3$) of IV showed new signals assigned to alcoholic O-Ac (three) and phenolic O-Ac (two). This fact indicated that I had three acetylatable alcoholic hydroxyls and two phenolic hydroxyls.

Hydrolysis of I with aqueous acetic acid gave an aglycone named pillaromycinone (II, PMN) and a new sugar named pillarose (III). From the hydrolysate II was extracted with EtOAc, and crystallized from the same solvent, mp 202°, $C_{20}H_{18}O_7$. II gave a blue color with the reagent of Barton, *et al.*[6] The UV spectrum of II (Fig. 3) was closely similar to that of I. In IR spectrum of II the presence of the characteristic bands at 1678 (conjugated CO) and 1640 cm^{-1} (chelated CO) and the absence of an isolated carbonyl were observed. In its NMR spectra (Table I, Fig. 4 in CD_3OD, d_5-pyridine) the signals were assigned to CH_3 (one, singlet), alicyclic protons (three), O–$\overset{|}{\underset{|}{C}}$–H (two) and aromatic and vinyl protons (five). II was insoluble in $CDCl_3$, therefore its isopropylidene derivatives (soluble in $CDCl_3$, see in Part II) were used for the NMR study of II to show the presence of two chelated hydroxyls in the lower magnetic field.

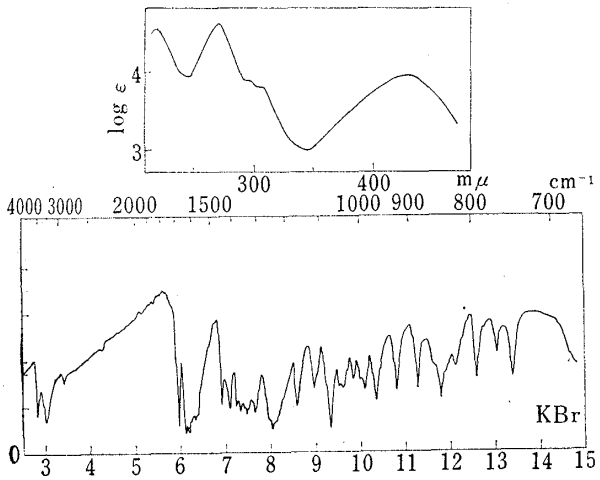


Fig. 3. UV and IR Spectra of Pillaromycinone (II)

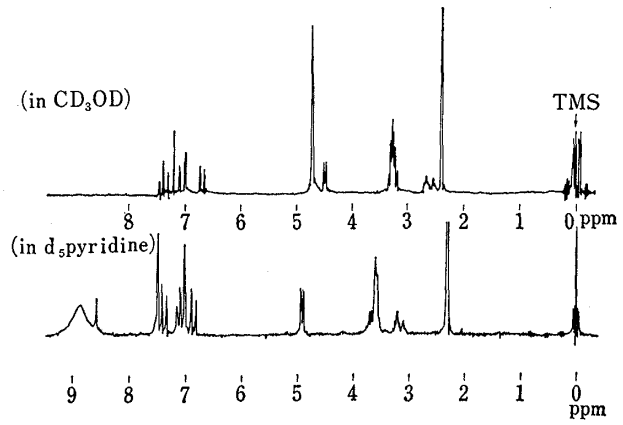


Fig. 4. NMR Spectra of Pillaromycinone (II) (100 Mc)

Table I. NMR Spectra of Pillaromycinone (II) and Pillaromycinone Tetraacetate (V) (100 Mc in CD_3OD & d_5-Pyridine)

		OH	H_8	H_6	H_7	H_1	H_9	OH	H_3	$H_{4,5}$	H_{4a}	Me_{14}
	δ (ppm)		7.42 t	7.24 s	7.07 q	7.03 s	6.73 q	4.75 s	4.52 d	3.34—3.18 m	2.63 oct.	2.41 s
II	(CD_3OD)		1H	1H	1H	1H	1H		1H	3H	1H	3H
	J (cps)		8		8 1.5		8 1.5		4		12 4 2	
	δ	8.0 b	7.42 t	7.48 s	7.05 q	7.00 s	6.84 q		4.98 d	3.63—3.43 m	3.09 oct.	2.31 s
II	(d_5-Py.)		1H	1H	1H	1H	1H		1H	3H	1H	3H
	J		8		8 1.5		8 1.5		4		12 4 2	

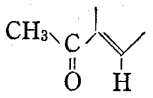
		H_8	H_6	H_7	H_1	H_9	H_3	H_4	H_5	$H_{5,4a}$	OH	OAc_{11}	Me_{14}	OAc_{10}	$OAc_{3,4}$
	δ	7.6 t	7.6 s	7.5 q	7.16 s	7.2 q	6.37 d	5.05 q	3.79 q	3.21 m	3.3	2.52 s	2.43 s	2.19 s	2.01 s
V	(d_5-Py.)	1H	1H	1H	1H	1H	1H	1H	1H	2H	1H	3H	3H	3H	6H
	J	6		6 1.5		6 1.5	4	4 12	12 2						

Stepwise acetylation of II gave PMN tetraacetate (V), needles, mp 241°, $C_{28}H_{26}O_{11}$. In its IR spectrum the presence of OH band (3400 cm^{-1}) was observed. The NMR spectrum of V (Table I) in d_5-pyridine showed the signals assigned to alcoholic O–Ac (two), phenolic O–Ac (two), CH_3 (one, s[8]), alicyclic protons (three), *tert*-OH (one, substituted with D_2O), Ac–O–C–H (two), vinyl proton (one, s.) and aromatic protons (four), showing that the two chelated hydroxyls in II had disappeared. These data indicated that two *sec*-hydroxyls and two phenolic hydroxyls in II were acetylated. Prolonged acetylation of V yielded PMN pentaacetate (VI). In the IR and NMR spectra of VI no signal of the *tert*-hydroxyl was found, but gave a new signal of an alcoholic O-Ac (1.97 ppm).

PMN (II) gave PMN monothiosemicarbazone (VII), and PMN mono-2,4-dinitrophenylhydrazone (VIII). The absorptions at 303 mμ in VII and 376 mμ in VIII indicated that VII and VIII had been derived from an α,β-unsaturated carbonyl compound.[9] The CH_3 signal (clean singlet) in the NMR spectrum (in d_5-pyridine) of II seen at 2.31 ppm was therefore ascribed to an acetyl conjugated with α,β-unsaturated bond. This was also supported by dihydropillaromycinone (IX), mp 196°, $C_{20}H_{20}O_7$, a catalytic reduction product of II in methanol over Adam's Pt. The IR spectrum of IX showed that the conjugated carbonyl had been altered into an isolated carbonyl.

Catalytic reduction of II in methanol over Pd–C gave three reduction products. The main product was named pillaronone (X, α-substance),[10] mp 235°, $C_{20}H_{18}O_5$, and the minor components were dihydroanhydroxypillaromycinone (XI, β-substance), mp 246°, $C_{20}H_{20}O_6$, and tetrahydrodianhydroxypillaromycinone (XII, γ-substance), mp 260°, $C_{20}H_{22}O_5$. The ε values of IX and XI at 233 mμ were smaller than those of I and II by *ca.* 8000. The IR spectrum of XI showed the band of an isolated carbonyl. These changes in absorptions were interpreted that the double bonds conjugated with an acetyl in I and II were hydrogenated to give IX and XI.

8) s=singlet, d=doublet, t=triplet, q=quartette, m=multiplet.
9) F.A. Braude and E.R.H. Jones, *J. Chem. Soc.*, **1945**, 498.
10) X was also obtained in good yield by reacting IX with $C_2H_5SH \cdot HCl$ in methanol followed by the desulfulization of the given monomercaptal (XIII) with Raney-Ni in ethanol.



From the above observations seven oxygen atoms in II are characterized as follows: two *sec*-hydroxyls, one *tert*-hydroxyl, two chelated phenolic hydroxyls, one acetyl and one chelated conjugated carbonyl.

Stepwise acetylation of XI gave the triacetate (XIV) and the tetraacetate (XV). The IR spectrum of XIV showed the characteristic bands of OH (3400 cm^{-1}), phenolic O–CO (1785), alcoholic O–CO (1755), isolated CO (1720) and conjugated CO (1690). In the NMR spectrum (in $CDCl_3$) of XIV there appeared signals of alcoholic OAc (one) and phenolic OAc (two). The spectrum also showed that the methyl signal of CO–CH_3 (singlet) moved to 2.08 ppm, the methine signal of O–$\overset{|}{C}$–H (one) shifted lowerfield and two signals of chelated phenolic hydroxyls had disappeared. XV showed no band of hydroxyl in the IR spectrum and showed two signals of alcoholic OAc and no signal of methine proton shifted lowerfield in the NMR spectrum.

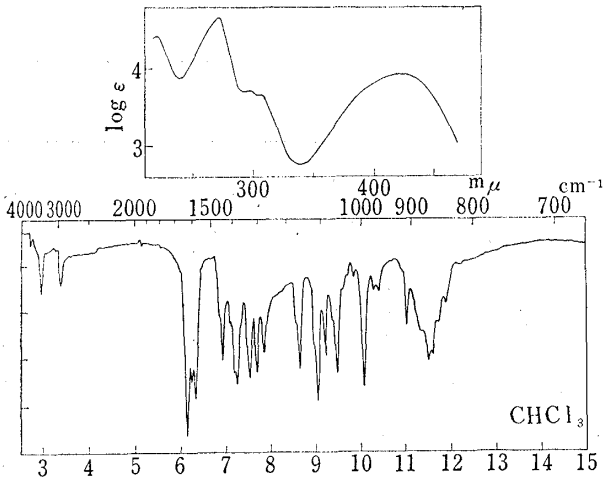


Fig. 5. UV and IR Spectra of Pillaronone (X)

Thus, the six oxygen atoms in XI were assigned as follows:

Readily acetylatable OH (*sec*-hydroxyl)	one
Hardly acetylatable OH (*tert*-hydroxyl)	one
Acetylatable phenolic OH	two
Isolated CO	one
Chelated conjugated CO	one

The UV and IR spectra of X are shown in Fig. 5. The value of ε at 233 mμ was lower than those of I and II, andin the IR spectrum the band of isolated carbonyl recognized in XI had disappeared.

Acetylation of X gave the monoacetate (XVI), $C_{22}H_{20}O_6$ and the diacetate (XVII), mp 248°, $C_{24}H_{22}O_7$. In the IR spectrum of XVII, the bands of phenolic O–CO were recognized, but neither band of hydroxyl nor alcoholic O–CO was recognized. This indicated the absence of alcoholic hydroxyl in X. The NMR spectrum of XVII (Table II) showed the presence of phenolic O–Ac (two), CH_3 (one, 1.69 ppm, s, shifted higher field), O–$\overset{|}{C}$–H (one), aromatic protons (four), new alicyclic protons (five) and the absence of the vinyl proton. The fact that no acetyl was recognized in X may be interpreted that the CH_3CO recognized in IX and XI had been converted into a CH_3–$\overset{|}{C}$– in X.

Reaction of X with 2,4-dinitrophenylhydrazine in HCl solution yielded the 2,4-dinitrophenylhydrazone (XVIII). The absorption at 360 mμ is specific to the 2,4-dinitrophenylhydrazone of isolated carbonyl.[9] XVIII was found to be identical with the 2,4-dinitrophenylhydrazone of XI, and this finding was explained that in HCl solution X was converted into XI.

Thus, the five oxygen atoms in X were ascribed to:

Acetylatable phenolic OH	two
Chelated conjugated CO	one
Ether oxygen	two

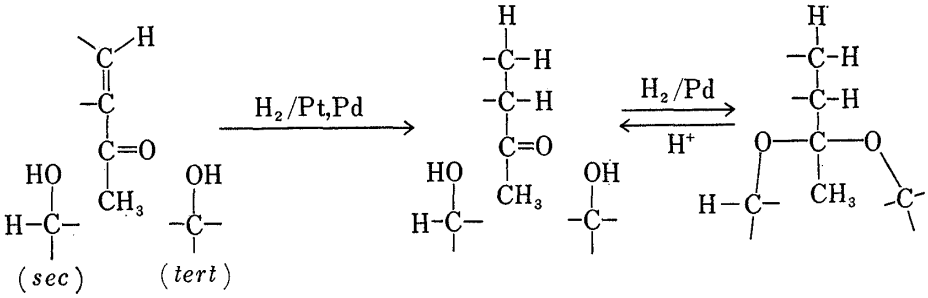
Table II. NMR Spectra of Pillaronone (X) and Pillaronone Diacetate (XVII) (100 Mc in $CDCl_3$ & d_5-Pyridine)

	OH	H_8	H_7	H_6	H_9	H_4	$H_{2,5}$	H_{4a}	H_1	H_3	OAc_{11}	OAc_{10}	Me_{14}
δ	10.19	7.42	7.09	6.89	6.88	4.36	2.85—2.68	2.58	2.3—1.96				1.68
X (ppm)	b.s	t	q	s	q	m	m	m	m				s
(d_5-Py.)		1H	1H	1H	1H	1H	3H	1H	4H				3H
J (cps)		8	8 1.5		8 1.5								

	H_8	H_7	H_6	H_9	H_4	H_2	H_5	$H_{5,4a}$	$H_{1,3}$	OAc_{11}	OAc_{10}	Me_{14}
δ	7.44	7.6	7.52	7.03	4.36	3.08	2.92	2.8 —2.6	2.2 —1.9	2.48	2.36	1.69
XVII	t	q	s	q	m	m	(q)	m	m	s	s	s
($CDCl_3$)	1H	1H	1H	1H	1H	1H	1H	2H	4H	3H	3H	3H
J	7	7 1.5		7 1.5								

The two ether oxygens in X were seemed to be derived from the isolated carbonyl, *sec*-hydroxyl and *tert*-hydroxyl of XI.

In short, the above facts may be interpreted as follows: A) II has the $>C=\underset{|}{C}-COCH_3$ structure (CH_3 2.31 ppm), B) the double bond is saturated when catalytically hydrogenated over Pt or Pd–C to form an isolated $COCH_3$ in IX and XI (CH_3 2.09 ppm), and C) when Pd–C is used, the reduction proceeds further to form a ketal in X (CH_3, 1.69 ppm) which is cleaved with a strong acid.



(A) pillaromycinone II — (B) β—substance XI — (C) pillaronone X

Chart 1

Experimental[11]

Pillaromycin A (I, PMA)——Culture filtrate (500 liter) of *Streptomyces flavovirens* No. 65786 was extracted at pH 2 with EtOAc (75 liter × 2). The crude yellow precipitate (200 g) obtained from the extract was dissolved in EtOAc (3 liter) and the solution extracted with aq. 2% $NaHCO_3$ (0.5 liter × 2). The aqueous layer was adjusted to pH 3 with HCl and extracted with EtOAc (0.75 liter × 2). The EtOAc layer was passed through a charcoal column (3.7 × 38 cm), then the active component was eluted with the same solvent. The active eluate was concentrated and ethanol (*ca.* 300 ml) was added to the concentrate to give crystalline pillaromycin C.[1] After filtration the filtrate was mixed with ether (*ca.* 1.2 liter) and placed in cooled place to yield yellow prisms (*ca.* 20 g) of I containing one mole of ether. The crystalline recrystallized from ethanol–ether lost the ether at 120° and melted at 208°, $[\alpha]_D^{22}$ −37° (c=1.0, MeOH). Crystallization from acetone, ethanol and EtOAc gave crystals having one mole of crystallization solvent, respectively. The crystalline having one mole of EtOAc lost the EtOAc when dried at 55°. *Anal.* Calcd. for $C_{28}H_{28}O_{11}$: C, 62.21; H, 5.22. Found: C, 61.92; H, 5.15. *Anal.* Calcd. for $C_{28}H_{28}O_{11}\cdot C_4H_{10}O$: C, 62.53; H, 6.23. Found: C, 62.56; H, 6.40. UV

11) All melting points were uncorrected.

λ_{max}^{EtOH} mμ ($\varepsilon \times 10^{-3}$): 220 (37.6), 275 (41.4), 298 (8.0), 309 (6.5), 431 (8.75) (Fig. 1 shows IR and UV spectra, Fig. 2 shows NMR spectra).

Pillaromycinone (II, PMN)——I (50 g) in 50% acetic acid (1.5 liter) was hydrolyzed at 75° for 3 hr. The hydrolyzate was extracted with EtOAc (0.4 liter × 3). The EtOAc extract was treated with water (1.5 liter) containing $NaHCO_3$ (400 g), washed with water, dried and concentrated. The resulting yellow precipitate (30 g) was recrystallized from EtOAc (300 ml) to provide yellow needles, mp 202° molecular wt. 366.2(osmotic method) $[\alpha]_D^{23}$ +550° (c=1.0, MeOH). *Anal.* Calcd. for $C_{20}H_{18}O_7$: C, 64.86; H, 4.89. Found: C, 64.80; H, 4.82. UV λ_{max}^{EtOH} mμ ($\varepsilon \times 10^{-3}$): 221 (36.9), 274 (43.8), 296 (7.8), 308 (6.2), 428 (8.83) (Fig. 3 shows IR and UV spectra, Fig. 4 and Table I show NMR spectra, respectively).

Pillaromycin A Pentaacetate (IV, PMA Pentaacetate)——I (1 g) was acetylated with acetic anhydride (3 ml) in pyridine (6.6 ml) for 20 hr. A crude acetate (1.3 g) obtained from the reaction mixture was dissolved in benzene–EtOAc (1:1) containing 1% oxalic acid and passed through a silica gel column (50 g) (0.05—0.20 mm, Merck) and developed with the same solvent. The blue green fluorescent eluate was concentrated (*ca.* 10 ml) and added cyclohexane to yield a crystalline powder, mp 195°, $[\alpha]_D^{22}$ +27° (c=1.0, MeOH). *Anal.* Calcd. for $C_{38}H_{38}O_{16}$: C, 60.79; H, 5.10. Found: C, 60.69; H, 5.39. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1785 (CO), 1755 (CO), 1725 (CO), 1685 (CO). UV λ_{max}^{EtOH} mμ ($\varepsilon \times 10^{-3}$): 220 (32.6), 261.5 (45.08), 288 (5.62), 301 (8.21), 311 (7.28), 368 (3.25). NMR (100 Mc in $CDCl_3$): 2.43 (3H, s, $COCH_3$), 2.34 (6H, s, $COCH_3$), 2.23 (3H, s, $COCH_3$), 2.05 (3H, s, $COCH_3$), 1.97 (3H, s, $COCH_3$). Mass Spectrum[12] *m/e*: 708 (M^+-42).

Pillaromycinone Tetraacetate (V. PMN Tetraacetate)——II (1 g) in pyridine (6.6 ml) was acetylated with acetic anhydride (3 ml) for 2 hr. From the resulting mixture a crude powder (1.3 g) was obtained. The product was recrystallized from EtOAc, mp 241°, $[\alpha]_D^{22}$ +230° (c=1.0, MeOH). *Anal.* Calcd. for $C_{28}H_{26}O_{11}$: C, 62.45; H, 4.86. Found: C, 62.57; H, 4.80. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 1770 (CO), 1750 (CO), 1695—1690 (CO). UV λ_{max}^{EtOH} mμ ($\varepsilon \times 10^{-3}$): 220 (33.2), 261 (45.18), 288 (5.28), 300 (8.02), 310 (7.10), 366 (3.345). NMR (100 Mc in $CDCl_3$) shows in Table I. Mass Spectrum *m/e*: 538 (M^+).

Pillaromycinone Pentaacetate (VI, PMN Pentaacetate)——II (100 mg) in pyridine (0.7 ml) was acetylated with acetic anhydride (0.3 ml) for 24 hr. The reaction mixture was treated with ice–water to yield a precipitate (80 mg). Silica gel G (Merck) (30 g) in EtOAc (68 ml) containing 1% oxalic acid was spread on a large glass plate (20 × 20 cm) so as to prepare a thin-layer. A solution of the above crude product in EtOAc was applied to the thin-layer and developed with benzene–EtOAc (1:1) containing 0.5% oxalic acid. The blue fluorescent band was extracted with EtOAc, the extract was concentrated and treated with petroleum ether. The resulting white powder was recrystallized with EtOAc–ether–petroleum ether. *Anal.* Calcd. for $C_{30}H_{28}O_{12}$: C, 62.08; H, 4.83. Found: C, 61.88; H, 4.75. IR $\nu_{max}^{CHCl_3}$: No. OH band. UV λ_{max}^{EtOH} mμ ($\varepsilon \times 10^{-3}$): 215 (35.35), 260 (42.70), 288 (sh), 300 (7.38), 310 (7.00), 360 (3.02). NMR (60 Mc in $CDCl_3$): 2.02 (3H, s), 2.08 (3H, s), 2.11 (3H, s), 2.38 (6H, s), 2.44 (3H, s, $COCH_3$). Mass Spectrum *m/e*: 580 (M^+), 538 (M^+-42).

Pillaromycinone Monothiosemicarbazone (VII)——The mixture of II in ethanol (10 ml) and thiosemicarbazide (100 mg) in water (10 ml) was left standing overnight. The resulting crystals were recrystallized from 50% ethanol, mp 250° (decomp.). *Anal.* Calcd. for $C_{21}H_{21}O_6N_3S \cdot H_2O$: C, 54.71; H, 4.99; S, 6.94. Found: C, 54.67; H, 4.98; S, 6.46. UV λ_{max}^{EtOH} mμ ($\varepsilon \times 10^{-3}$): 223 (44.8), 273 (55.38), 303 (36.05), 428 (12.22).

Pillaromycinone Mono-2,4-dinitrophenylhydrazone (2,4-DNPH) (VIII)——To a solution of II (500 mg) (1.36 mmole) in methanol (*ca.* 10 ml) was added a solution of 2,4-dinitrophenylhydrazine (2,4-DNP) (270 mg) (1.36 mmole) in 4N HCl (20 ml). The separated crystalline precipitate was collected and washed with methanol, EtOAc and ether successively, mp 260° (decomp.), $[\alpha]_D^{22}$ +775° (c=1.0, pyridine). *Anal.* Calcd. for $C_{26}H_{22}O_{10}N_4 \cdot H_2O$: C, 54.93; H, 4.25; N, 9.85. Found: C, 55.40; H, 4.11; N, 9.65. UV λ_{max}^{EtOH} mμ ($\varepsilon \times 10^{-3}$): 221 (34.40), 273 (38.50), 298 (9.9), 310 (7.7), 376 (24.8), 422 (18.56).

Dihydropillaromycinone (IX)——A solution of II (1 g) in methanol (40 ml) was subjected to catalytic reduction on Adam's platinum oxide (25 mg), until 2 molar equivalents of H_2 were absorbed. From the reaction mixture a crude yellow product (0.8 g) was obtained. The product was dissolved in EtOAc (50 ml), passed through a charcoal column (1 g) and developed with EtOAc to give a yellow fraction, which yielded yellow crystals containing 1 mole of crystallization EtOAc, mp 196° $[\alpha]_D^{23}$ +174.7° (c=1.0, MeOH). *Anal.* Calcd. for $C_{20}H_{20}O_7 \cdot C_4H_8O_2$: C, 62.59; H, 6.12. Found: C, 62.61; H, 5.75. IR ν_{max}^{KBr} cm^{-1}: 1700 (CO). UV λ_{max}^{EtOH} mμ ($\varepsilon \times 10^{-3}$): 221 (26.50), 273 (44.0), 297 (5.58), 308 (4.76), 422 (8.9). NMR (100 Mc in d_5-pyridine): 2.14 (3H, s, $COCH_3$).

Pillaronone (X)——A solution of II (1 g) in methanol (40 ml) was subjected to catalytic reduction on palladium–carbon, until 3 molar equivalents of hydrogen were absorbed. The reduction product was obtained as needles, mp 235°, $[\alpha]_D^{22}$ +135° (c=1.0, acetone). *Anal.* Calcd. for $C_{20}H_{18}O_5$: C, 70.99; H, 5.36; O, 23.64. Found: C, 70.60; H, 5.39; O, 23.84. IR: Fig. 5. UV λ_{max}^{EtOH} mμ ($\varepsilon \times 10^{-3}$): 221 (26.5), 272 (46), 297 (5.2), 308 (4.5), 420 (8.9). NMR (100 Mc in d_5-pyridine): Table II.

Dihydroanhydroxy PMN (XI, β-substance), Dihydroandihydroxy PMN (XII, γ-substance)——The crude substance obtained by concentrating the mother liquor of pillaronone was treated with a little methanol.

12) Mass Spectrometry.

The dissolved part was concentrated to obtain β-substance (XI), mp 246°, which was recrystallized from MeOH. The insoluble part was recrystallized from EtOAc:glacial acetic acid to give γ-substance, mp 260° (XI). *Anal.* Calcd. for $C_{20}H_{20}O_6$: C, 67.40; H, 5.65. Found: C, 67.15; H, 5.70. $[\alpha]_D^{22}$ +197° (c=0.5, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 3200 (OH), 1700 (CO), 1635 (CO). UV λ_{max}^{EtOH} mμ ($\varepsilon \times 10^{-3}$): 220 (26.5), 270 (48), 296 (5.4), 306 (4.7), 420 (8.3). NMR (100 Mc in d_5-pyridine): 2.09 (3H, s, $COCH_3$), 1.96—2.3 (4H, m, $-CH_2-$), 2.73 (1H, m, $-\overset{|}{C}H-$), 2.97 (2H, m, $-CH_2-$), 3.48 (1H, m, $-\overset{|}{C}H-$), 4.21 (1H, m, $-\overset{|}{\underset{H}{C}}-O$) (XII). *Anal.* Calcd. for $C_{20}H_{22}O_5$: C, 70.02; H, 6.43. Found: C, 69.99; H, 6.43. IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1635 (CO), 1602, 1605 (C=C). UV λ_{max}^{EtOH} mμ ($\varepsilon \times 10^{-3}$): 220 (23.25), 267 (44.10), 295 (4.48), 305 (sh.), 407 (7.35). NMR (60 Mc in d_5-pyridine): 1.38 (dH, d, $CH-CH_3$).

Pillaronone Monomercaptal (XIII)——The mixture of dihydro PMN (IX) (5 g) in methanol (15 ml) and ethylmercaptan (30 ml) was saturated with dry HCl gas and left standing for 4 hr. The solvent was distilled, ether was added and the separated crystals (1.8 g) were recrystallized from ethanol:ether, mp 198°. *Anal.* Calcd. for $C_{22}H_{22}O_5S$: C, 66.31; H, 5.56; S, 8.04. Found: C, 66.33; H, 5.46; S, 7.61. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2700—2800 (chelated OH), 1630 (chelated CO).

A solution of XIII (1 g) in ethanol (100 ml) was boiled with Raney-Ni for *ca.* 8 hr. After removing the Ni, the filtrate was concentrated and the resulting product was dissolved in EtOAc. The solution was treated with aqueous sodium hydrosulfite, dried and concentrated to give crystals of pillaronone (X).

Dihydroanhydroxy PMN Tetraacetate (XV, β-Substance Tetraacetate)——The method is similar to that of pillaronone diacetate (XVII). mp 248°. *Anal.* Calcd. for $C_{28}H_{28}O_{10}$: C, 64.12; H, 5.38. Found: C, 64.02; H, 5.24. IR ν_{max}^{KBr} cm^{-1}: 1775, 1735, 1690 (CO), 1620, 1600 (C=C). UV λ_{max}^{EtOH} mμ ($\varepsilon \times 10^{-3}$): 217 (26.50), 257 (48.75), 288 (5.08), 298 (7.55), 310 (5.88), 360 (2.89). NMR (60 Mc in $CDCl_3$): 2.07 (3H, s, $COCH_3$), 2.08 (3H, s, $C-COCH_3$), 2.14 (3H, s, $COCH_3$), 2.39 (3H, s, $C_6H_5-O-COCH_3$), 2.49 (3H, s, $C_6H_5-O-COCH_3$).

Pillaronone Monoacetate (XVI)——X (100 mg) in pyridine (1.2 ml) was acetylated with acetic anhydride (0.5 ml) for 18 hr. From the resulting mixture a crude product (100 mg) was obtained and crystallized with ethanol–EtOAc to yield pillaronone monoacetate. mp 237°. *Anal.* Calcd. for $C_{22}H_{20}O_6$: C, 69.46; H, 5.29. Found: C, 69.25; H, 5.15. NMR (60 Mc in $CDCl_3$): 2.36 (3H, s, $COCH_3$), 14.65 (1H, broad s, chelated OH).

Pillaronone Diacetate (XVII)——X (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 product was dissolved in hot ethanol–EtOAc, then pillaronone diacetate (XIII) separated out from the solution on standing. The crystals were recrystallized from the same solvent. mp 248°. *Anal.* Calcd. for $C_{24}H_{22}O_7$: C, 68.24; H, 5.25. Found: C, 68.17; H, 5.32. IR ν_{max}^{KBr} cm^{-1}: 1770, 1690 (CO), 1620, 1600 (C=C). UV λ_{max}^{EtOH} mμ ($\varepsilon \times 10^{-3}$): 217 (26.92), 257 (49.38), 288 (5.28), 299 (7.65), 310 (5.92), 360 (3.14). NMR (100 Mc in $CDCl_3$): Table II.

Dihydroanhydroxy PMN-2,4-DNPH (XVIII, 2,4-DNPH of β-Substance)——A solution of β-substance (XI) (100 mg) (0.29 mmole) in methanol (30 ml) was reacted with a solution of 2,4-DNP (58 mg) (0.29 mmole) in 4N HCl (15 ml) for 30 min at room temperature. The resulting precipitate was washed with water and ethanol to remove the soluble part. The insoluble yellow powder (*ca.* 50 mg) was dissolved in a mixture of EtOAc (30 ml), ethanol (40 ml) and acetic acid (4 drops) by warming and left cooling to separate crystals, mp >300°. *Anal.* Calcd. for $C_{26}H_{24}O_9N_4$: C, 58.21; H, 4.51; N, 10.44. Found: C, 58.69; H, 4.50; N, 10.25. UV λ_{max}^{EtOH} mμ ($\varepsilon \times 10^{-3}$): 221 (29.0), 272 (38.90), 296 (5.93), 310 (6.53), 360 (16.70), 415 (11.10).

This compound was also obtained from X as follows: A solution of X (100 mg) (0.296 mmole) in methanol (30 ml) was added to a solution of 2,4-DNP (58 mg) (0.295 mmole) in 4N HCl (15 ml) at 50° and the mixture was left standing for 3 hr at room temperature. The separated precipitate was collected and crystallized from ethanol.

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