A NEW ANTIBIOTIC, HONDAMYCIN.* I
ISOLATION AND CHARACTERIZATION

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A new antibiotic, hondamycin, was isolated from the mycelium of *Streptomyces griseochromogenes* var. *albicus*[1], as colorless crystals. The antibiotic is active against phytopathogenic fungi and *Trichophyton* spp. Structural studies showed that the antibiotic has a molecular formula of $C_{47}H_{78}O_{13}$ and a molecular weight of about 850. This result was confirmed by high resolution mass spectrography of hondamycin triacetate.

In the course of a systematic screening program for new antibiotics, a strain, *Streptomyces griseochromogenes* var. *albicus*, has been found to produce an antifungal substance which shows inhibitory effects against phytopathogenic fungi and *Trichophyton* spp. This antibiotic has been isolated as colorless hexagonal crystals and found to be a new antibiotic which we have named hondamycin.

The present paper deals with the isolation, physical and chemical properties of hondamycin.

Isolation

Hondamycin was isolated from both the broth and mycelia by organic solvent extraction procedures. The broth was filtered at pH 7.8 and more than 80 % of the activity was found in the wet mycelial cake which was extracted twice with methanol. The combined extracts were concentrated *in vacuo* to remove the solvent and the precipitate was extracted with ethylacetate or butylacetate at pH 7.0. The solvent extract was washed with water, followed by the removal of the solvent by concentration of the extract *in vacuo*, whereupon the activity was obtained in the dark oily material. Ten volumes of *n*-hexane were added to the concentrate and the precipitate was then dried *in vacuo*.

In the case of the broth filtrate one third volume of butylacetate was added and after washing the butylacetate layer with water to remove impurities, it was concentrated *in vacuo*. Ten volumes of *n*-hexane were added to the concentrate and the precipitate which contains the active substance was dried *in vacuo*.

Purification of the antibiotic could be achieved by column chromatography on silica gel. The crude material was dissolved in a solvent mixture of benzene and ethylacetate (9 : 1) and applied to a column of silica gel in the same solvent mixture. The active substance was adsorbed completely and the column was washed with

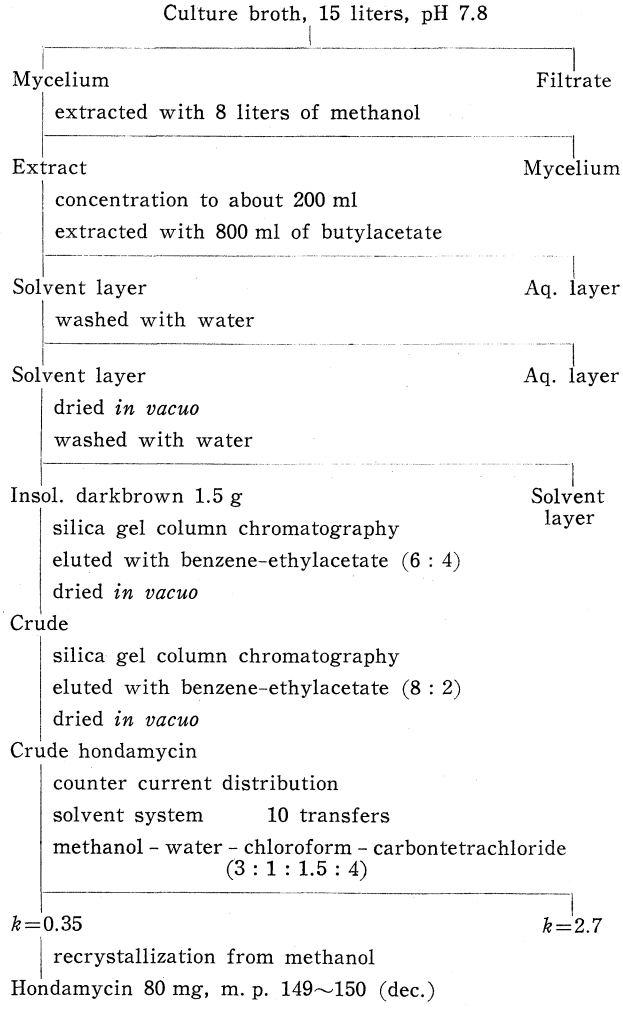
* Hondamycin was reported in the name of albimycin at the 147th meeting of the Japan Antibiotics Research Association, Nov. 26, 1965.

large volumes of the solvent (benzene – ethylacetate, 9 : 1). No active substance was eluted. The application of another solvent system (benzene – ethylacetate, 6 : 4) resulted in the elution of the active portion.

After the active fractions were combined, they were concentrated *in vacuo* and dried.

Further purification of the antibiotic was accomplished using chromatography on silica gel with the same solvent system. The antibiotic was partially eluted by a mixture of benzene – ethylacetate (8 : 2). The eluate containing only hondamycin was concentrated to a syrupy state under vacuum and crude crystals of hondamycin were obtained by treatment of the oily syrup with a small volume of methanol. The crude crystalline fraction obtained from methanol was further purified by counter-current distribution with a mixture of methanol – water – chloroform – carbon tetrachloride (3 : 1 : 1.5 : 4). The active fractions (K=0.35) were collected and gave colorless hexagonal crystals after crystallization from methanol or ether. Recrystallization was repeated from methanol with no change in its properties.

Fig. 1.



Physical and Chemical Properties

Crystalline hondamycin forms a colorless hexagonal plate crystal, melting at 149~150°C with decomposition. Its optical rotation is $[\alpha]_D^{13.5} = -48.1$ ($c=1.93$ in methanol). Electrometric titration indicates no pKa.

Hondamycin does not contain nitrogen, phosphorus, sulphur or halogen. The molecular weight determined by various techniques was as follows:

the osmotic method	794.6 ($CHCl_3$)
the Signer and Berger method	820 (acetone)
the single crystal X-ray diffraction method	
a=10.35 Å, b=17.4 Å, c=27.4 Å, $\alpha=\beta=\gamma=90°$	
d=1.138, 4 mol. in unit cell	845.8
the thermoelectric method	821 (acetone)

The results of elementary analysis of hondamycin were as follows:

Anal. Calcd. for $C_{47}H_{78}O_{13}$: C 66.31,
H 9.24, O 24.45, $10\times$C-CH_3 17.67
Found: C 66.38,
H 9.25, O 24.37, C-CH_3 17.3

The ion peak of the hondamycin triacetate by the high resolution mass spectrography was found at m/e 958 (958.5957; calcd. for $M^+-H_2O=C_{53}H_{82}O_{15}$: 958.5653).

These experimental results indicated the molecular formula of hondamycin to be $C_{47}H_{78}O_{13}$ with no O-Me group.

In the ultraviolet region hondamycin has absorption maxima at 225 mμ ($\log\varepsilon=4.58$) and 232.5 mμ ($\log\varepsilon=4.56$), and shoulders at 219 mμ ($\log\varepsilon=4.54$), 240 mμ ($\log\varepsilon=4.35$) and 270~295 mμ ($\log\varepsilon=1.97$) in methanol as shown in Fig. 2.

Fig. 2. Ultraviolet absorption spectrum of hondamycin in methanol.

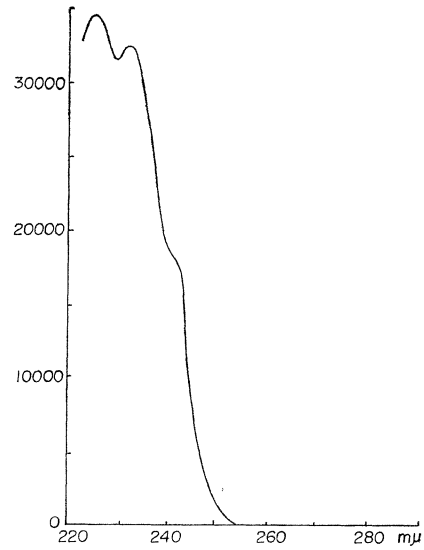
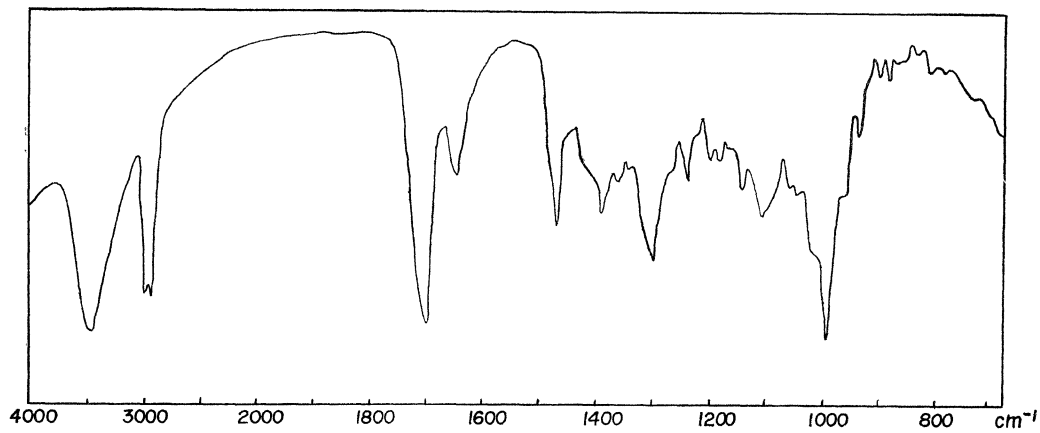


Fig. 3. Infrared absorption spectrum of hondamycin (KBr).



The infrared absorption spectrum of hondamycin is shown in Fig. 3 and the nmr spectrum in Fig. 4.

Hondamycin was slightly soluble in benzene and carbon tetrachloride and insoluble in *n*-hexane, petroleum ether and water but freely soluble in various organic solvents such as methanol, ethanol, butanol, amyl alcohol, acetone, methylisobutylketone, ethyl acetate, butyl acetate, chloroform, carbon disulfide, pyridine and acetic acid.

Hondamycin gave positive reactions to BAEYER, TOLLENS, FEHLING, hydroxamic acid tests and iodoform test but negative reactions to ferric chloride and 2,4-dinitrophenylhydrazine. With sulfuric acid, hondamycin shows a color change to red and decolorizes a bromine-chloroform solution.

The Rf values of hondamycin in ascending paper chromatography were as

Fig. 4. N.M.R. spectrum of hondamycin (100 MC, in $CDCl_3$, TMS as a reference)

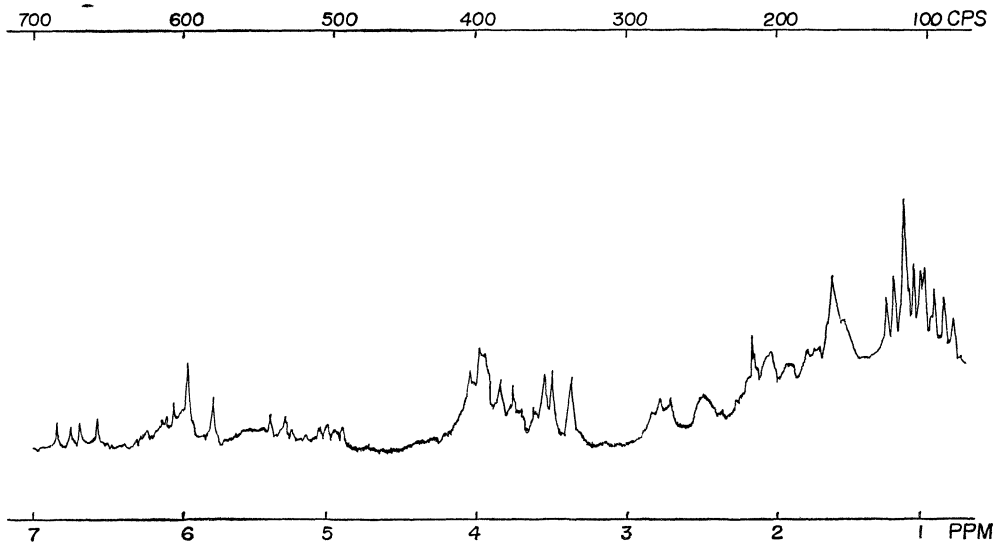
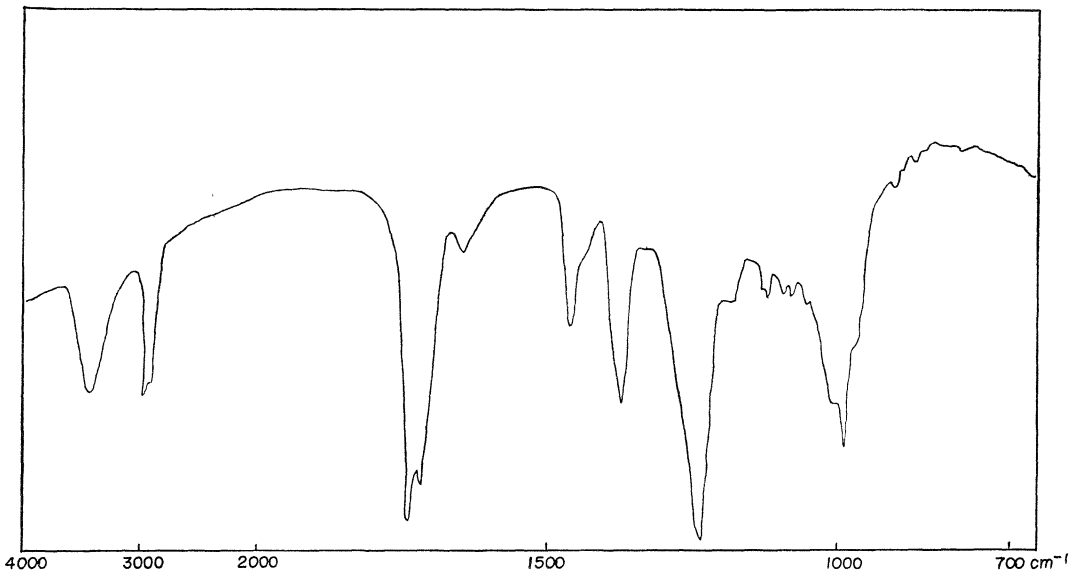


Fig. 5. Infrared absorption spectrum of hondamycin triacetate (KBr).



follows: 0.62 in benzene - water - acetic acid (1 : 1 : 0.2) system, 0.65 in ethanol - *n*-hexane (1 : 2), 0.41 in water - acetone (75 : 25), and 0.19 in 16 % aqueous solution of *n*-propanol.

In the case of ascending thin-layer chromatography with silica gel, the Rf values of hondamycin were 0.75 in ethanol - water (4 : 1), 0.82 in ethyl acetate - methanol (100 : 15), and 0.42 in benzene - ethyl acetate (1 : 1) respectively.

Discussion

A number of the known antibiotics produced by Streptomycetes such as oligomycins A, B, C,[2,3,4,9,10] phycomycin[7], orymycin[6], A-272 substance[5,10] and botrycidin[8] have antimicrobial activity limited to fungi. These compounds contain only carbon, hydrogen and oxygen as does hondamycin. Furthermore, oligomycins A, B, C, orymycin and botrycidin all show ultraviolet absorption maxima at approximately 225 mμ and 232 mμ with shoulders at approximately 219 mμ, 240 mμ and 270~295 mμ, as does hondamycin. As shown in Tables 1 and 4, these antibiotics are similar to each

Table 1.

	Hondamycin	Oligomycin A	Oligomycin B	Oligomycin C	Phycomycin	Orymycin	A-272	Botrycidin
m. p. (°C)	149~150 (dec.)	140~141 150~151 (dec.)	160~161	189~200	133.5~135	157~158 (dec.)	127~128	—
$[\alpha]_D$	−48.1	−54.5	−49.5	−80.7	−29.0	−54.5	−40.3	−61.5
Formula and analysis	$C_{47}H_{78}O_{13}$	$C_{45}H_{74}O_{11}$	$C_{45}H_{72}O_{12}$		$C_{24}H_{40}O_7$ or $C_{20}H_{34}O_6$	$C_{25}H_{42}O_7$		$C_{39}H_{64}O_{10}$ or $C_{39}H_{64}O_{11}$
Calcd. C	66.31	68.31	67.12			66.05		
H	9.24	9.43	9.02			9.31		
Found C	66.38	67.80	66.68	69.9		66.02	68.25	66.35
H	9.26	9.45	9.04	9.8		9.47	9.53	9.18
M. W. Calcd.	851	790	804			454	776	
Found	845.8 (X-ray)	790*	804*	742*			740*	701 (osm.)

* Highest observed peak in the mass spectrum

Table 2. Antimicrobial spectrum of hondamycin and oligomycins

Test organisms	M. I. C. (mcg/ml)				
	Oligomycin				Hondamycin
	Oligs.	A	B	C	
Aspergillus parasiticus Spheare IFO 4351	4.35	1	5	50	5
Asp. niger		1	5	5	5
Asp. fumigatus	0.6	2	5	20	>100
Glomerella cingulata G-24	2.2	0.1	0.5	0.5	0.5
Botrytis cinerea		5	5	>30	0.5
Trichophyton mentagrophytes	>50				0.5
Tri. rubrum	>50				0.5
Blastomyces dermatitidis	0.04	0.1	0.1	0.1	1
Histoplasma capsulatum	1.1				1
Rhodotorula glutinis		1	>50	>50	5

Table 3. Paper chromatographic behaviour of hondamycin, orymycin, and oligomycins with an aqueous *n*-propanol solution

	Hondamycin	Orymycin	Oligomycins
16 % an aq. *n*-propanol	0.19	0.21	0.30, 0.23 0.15
18 % an aq. *n*-propanol	0.27	0.34	0.44, 0.35 0.24

Antibiotic visualized on agar plates seeded with *Piricularia oryzae*.

other. Recently revised molecular formula and molecular weight were given for oligomycins A, B and C, and A-272 by PROUTY *et al.*[9] and CHAMBERLIN *et al.*[10], and are shown in Tables 1 and 4. It was shown that oligomycins A and B had $C_{45}H_{74}O_{11}$ (M.W. 790) and $C_{45}H_{72}O_{12}$ (M.W. 804) for their molecular formula and the molecular weight of A-272 was 776 by mass spectroscopy. As compared with these compounds in Tables 1~4, however, hondamycin is different from oligomycin A in elemental analysis, molecular weight and biological properties. Hondamycin yielded a triacetate by routine condition using acetic anhydride-pyridine at room temperature. This was confirmed by high resolution mass spectrum, elemental analysis and nuclear magnetic resonance spectrum (δ 2.05 ppm, 9H, s, acetyl proton). On the other hand, oligomycins A, B and A-272 yielded a tetraacetate using the same chemical procedure. Highest peak of tetraacetate of oligomycin B in mass spectrum was observed at m/e 1014 by CHAMBERLIN, *et al.*[10] and at m/e 972 by PROUTY *et al.*[9] On the other hand, hondamycin triacetate showed highest peak at m/e 958. Furthermore no hydroxyl group can be recognized in the infrared spectrum of oligomycin A tetraacetate, but hondamycin triacetate shows additional hydroxyl groups as shown in Fig. 5. Some differences in biological properties between hondamycin and oligomycins were also observed, *i. e.* hondamycin and oligomycins showed different activity against *Aspergillus fumigatus*. The molecular weight of orymycin was shown to be 454. Using paper chromatography hondamycin was shown to be different from oligomycins and orymycin using an aqueous propanol solution as a solvent system. Hondamycin has a different pattern of nmr spectrum from that of botrycidin. Furthermore hondamycin yielded the different derivatives, *i. e.* triacetate and octahydrohondamycin, from those of botrycidin when subjected to the same chemical procedures.

Table 4.

	Hondamycin triacetate	Oligomycin-A tetraacetate	Oligomycin-B tetraacetate	A-272 tetraacetate
Analysis	$C_{53}H_{84}O_{16}$	$C_{53}H_{82}O_{15}$	$C_{53}H_{80}O_{16}$	
Calcd. C	65.16	66.35	65.40	
H	8.61	8.62	8.29	
Found C	65.41	65.93	65.29	
H	8.19	8.71	8.45	
M.W. Calcd.	976	958	972	
Found*	958 ($M-H_2O$)	958	972	944

Highest observed peak in the mass spectrum

Chemical studies show that hondamycin yields 2 moles of acetaldehyde and one mole of monocarboxylic acid, $C_{43}H_{68}O_{12}$ by the oxidation with periodate, and after ozonolysis followed by reduction with sodium borohydride, a polyol, $C_{25}H_{50}O_6$ was obtained. It is therefore concluded that hondamycin is a new antibiotic substance.

Acknowledgement

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