

Collected Papers on **Antibiotics**

Section V

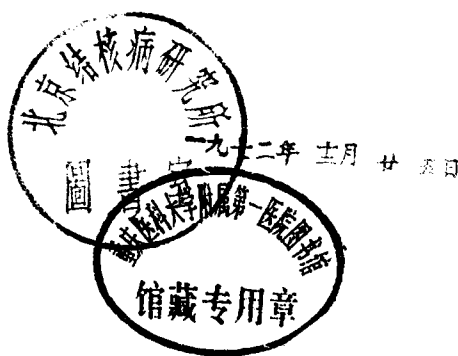
(New Antibiotics in 1971)

September 1972

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Section V

《New Antibiotics in 1971》



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AN ANTIBIOTIC, ALBIMYCIN

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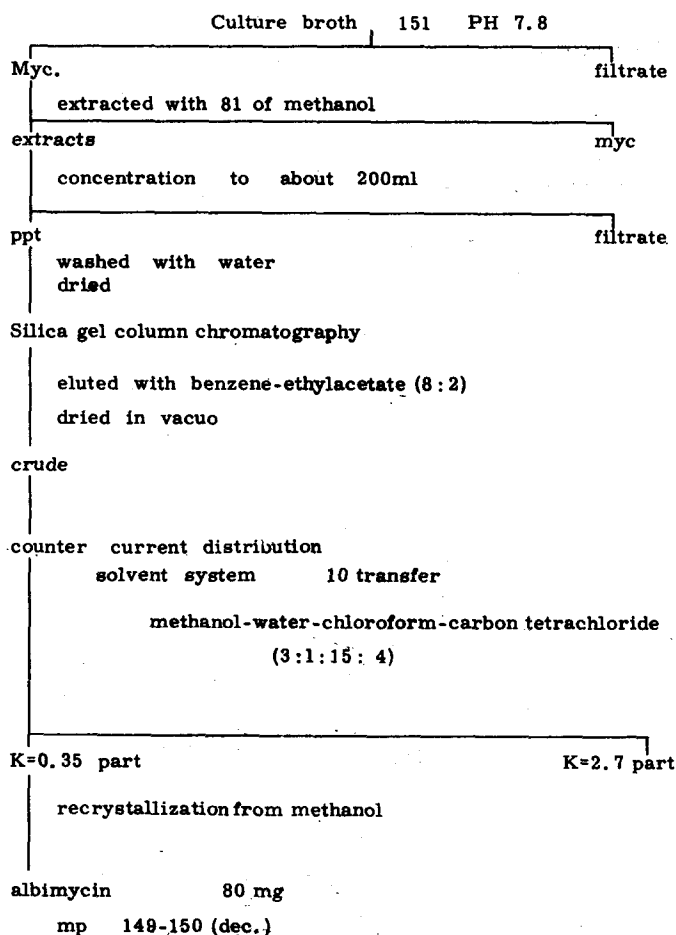
In 1965, at the 147th meeting of the Japan Antibiotics Research Association, we reported about albimycin. This antibiotic is similar in some properties to oligomycin groups, so we could not exactly distinguish it from oligomycins at that time. To recognize the differences in each, we again studied albimycin's properties. From the results, I think the differences between the albimycin and oligomycin groups could be determined; the study of chemical structure followed. So that at this time we want to explain these results.

To produce albimycin we selected an effective culture medium for promoting growth of mycelia, as shown in Table 1. After about 90 hrs, albimycin activity against *Piricularia oryzae* reached a maxi-

Table 1 Medium for jar fermentation

dextrine	1%	soybean meal	1%	NaCl	0.5%
K ₂ HPO ₄	0.2%	CaCO ₃	0.2%	PH	7.0

Isolation



mum and in that time the pH of broth was 7.8. From both broth and mycelia, we obtained the albimycin by a solvent extraction procedure. The wet mycelial cake contained more than 80% albimycin activity. The purification of this antibiotic was done by silica gel column chromatography, and its active fractions were eluted with mixed solvents of 8 parts benzene and 2 parts ethylacetate. From these active fractions, we obtained the crude albimycin. To this crude albimycin, the counter current distribution method was applied. The solvent system was the mixture methanol-water-chloroform-carbon tetrachloride (3:1:1.5:4). At the $K=0.35$ part of its transfer, we recognized the pure albimycin, which was then recrystallized from methanol.

The albimycin thus obtained had a melting point at from 149° to 150°C with decomposition. Its optical rotation was -48.1° , the electrometric titration indicated no pKa, and it did not contain nitrogen, phosphorus, sulfur or halogen. The molecular weight was measured by the various methods; the results are shown in Table 2. The ion peak of albimycin triacetate by high mass spectrography was found at m/e 958. The experimental results indicated the molecular formula of albimycin to be

Table 2 Properties of albimycin

mp	149-150° (dec.)			
$[\alpha]_D^{13.5}$	-48.1 (C 1.93, MeOH)			
pKa	none			
C ₄₇ H ₇₈ O ₁₃	Calc. C 66.31 H 9.24 O 24.45			
M. W. 851.09	Found 66.38 9.25 (24.37)			
	66.28 9.42 (24.30)			
MW	794.6 (osm. CHCl ₃)			
	820 (Berger acetone)			
	845.8 (x-ray)			
	821 (thermoelectr. acetone)			
O-Me	0%	C-Me	173% (C-Me x 10 17.67%)	
UV	219 mμ (log 4.54 sh.)			
	225 (" 4.58)			
	232.5 (" 4.56)			
	240 (" 4.35 sh.)			
	270-295 (" 1.97 sh.)			

Color reaction

+ : Tollen, Fehling, Iodoform test,
KMnO₄, Br₂, Hydroxamic acid test,
conc. H₂SO₄, (dark red)

- : FeCl Legal

2 mol. of periodate consumed

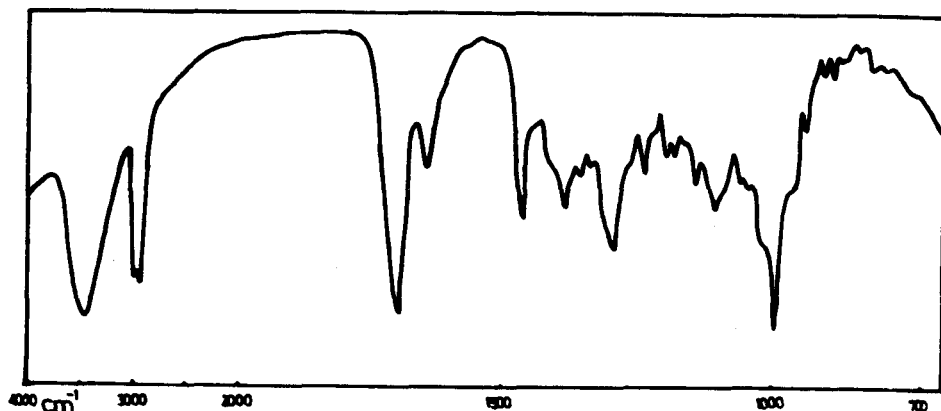


Fig. 1. Infrared spectrum of albimycin (KBr).

$C_{47}H_{78}O_{13}$; it contains no O-Me but contains 10 C-Me. In the ultraviolet region, albimycin has an absorption maxima at 225 m μ and 232.5 m μ and shoulders at 219 m μ , 240 m μ and 270-295 m μ in methanol. It shows positive reaction as follows: Tollen, Fehling reaction, Iodoform test, $KMnO_4$, Br_2 , Hydroxamic acid test, and with conc. H_2SO_4 it changes to dark red. In regard to $FeCl_3$ and Legal reaction, it shows negative.

The infrared absorption spectrum of albimycin is shown in Fig. 1.

The NMR spectrum of albimycin in the 100 Mc condition in $CDCl_3$, is shown in Fig. 2.

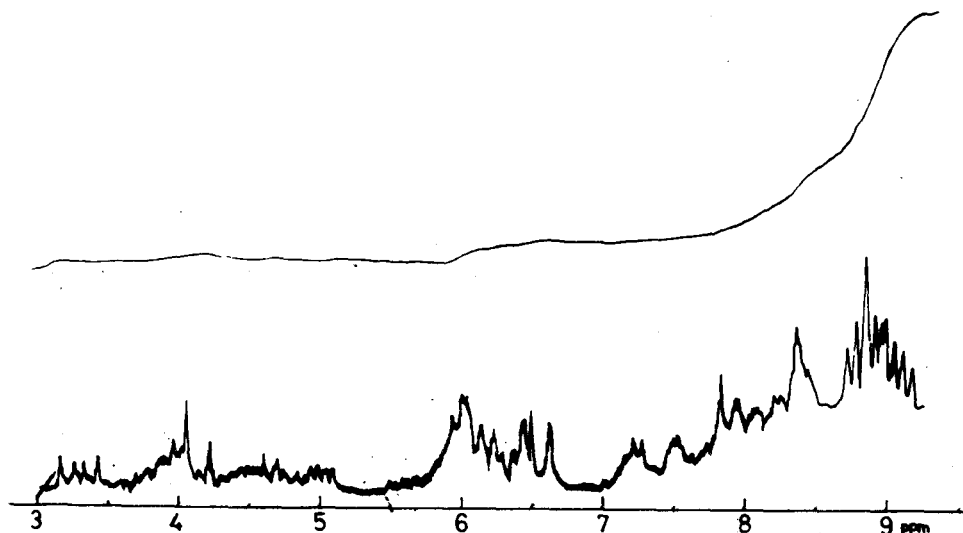


Fig. 2. Nmr spectrum of albimycin (100 MC in $CDCl_3$).

Albimycin is soluble in most organic solvents but insoluble in water. The R_f values of albimycin in ascending paper chromatography and thin layer chromatography are shown in this Table 3.

The antimicrobial spectra of albimycin is shown in Table 4.

Albimycin shows inhibitory effect strongly against phytopathogenic fungi and Trichophyton, but it has no effect against bacteria and yeasts. As regards the toxicity of albimycin in mice, the results are shown in Table 4. Many of the known antibiotics produced by streptomyces such as oligomycin A, B and C, phycomycin, ormycin, A-272 substance, and botrycidin have their antimicrobial activity limited to fungi. These compounds contain only carbon, hydrogen and oxygen atom in their empirical formula, as in albimycin. Furthermore, the UV absorption spectrums are almost the same. So as shown in Table 5 their antibiotics are similar to each other.

As compared with these compounds, albimycin is different from oligomycin A in elemental analysis,

Table 3

TLC of albimycin (silica gel G)		
Solvent system		R_f
Benzene-ethylacetate	(1:1)	0.42
Benzene-methanol	(5:1)	0.3
Hexane-acetone	(3:7)	0.38
Hexane-ethylacetate	(1:1)	0.24

PPC of albimycin (Toyo Roshi No. 51)		
Solvent system		R_f
16% aqueous n-propanol		0.19
18% aqueous n-propanol		0.27

Table 4 Antimicrobial spectra of albigmycin

Test organisms	MIC (μ g/ml)
<i>Trichophyton asteroides</i>	1
" <i>rubrum</i> F-86	0.1
" <i>gypseum</i>	1
" <i>mentagrophytes</i>	0.5
<i>Piricularia oryzae</i>	0.1
<i>Botrtis cinerea</i>	1
<i>Alternaria citri</i>	0.01
<i>Glomerella cingulata</i> G-24	0.5
<i>Penicillium chrysogenum</i> Q176b	0.1
<i>Rhodotorula glutinis</i>	5
<i>Aspergillus niger</i>	5
<i>Blastomyces dermatidis</i>	1
<i>Hystoplasma capsulatum</i>	1
<i>Englena gracilis</i>	25

Toxicity (LD₅₀)

Oral > 500 mg/kg

Intraperitoneal 2.5 mg/kg

Table 5

	albigmycin	oligomycin A	oligomycin B	oligomycin C	phycomycin	orymycin	A-272	botrycidin
m. p. (°C)	149-150 (dec.)	140-141 150-151 (dec.)	160-161	198-200	133.5-135	157-158 (dec.)	127-128	—
(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 found C 66.38 H 9.26	C ₄₅ H ₇₄ O ₁₁ found C 67.76 H 9.53	C ₄₅ H ₇₂ O ₁₂ found C 66.88 H 9.04	 found C 69.9 H 9.8	C ₂₄ H ₄₀ O ₇ or C ₂₀ H ₃₄ O ₈	C ₂₅ H ₄₂ O ₇ found C 66.02 H 9.47	 found C 66.25 H 9.53	C ₃₉ H ₆₄ O ₁₀ or C ₃₉ H ₆₄ O ₁₁ found C 66.35 H 9.18
M. W. Calcd. found	851 845.8 (x-ray)	790 790 (mass)	804 804 (mass)	742 (mass)			776 740 (mass)	701 (osm.)

	albigmycin triacetate	oligomycin A tetraacetate	oligomycin B tetraacetate				A-272 tetraacetate	
Formula and Analysis	C ₅₃ H ₈₄ O ₁₆ found C 65.41 H 8.19	C ₅₃ H ₈₂ O ₁₅ found C 65.93 H 8.71	C ₅₃ H ₈₀ O ₁₆ found C 65.29 H 8.45					
M. W. Calcd. found (mass)	976 958 (M-H ₂ O)	958 958	972 972				944	

molecular weight and biological properties. Albigmycin gave triacetate under routine conditions. This was confirmed by high resolution mass spectrum and elemental analysis, and its triacetate value was calculated from the NMR spectrum of albigmycin triacetate. On the other hand, oligomycin A, B and A-272 substance gave tetraacetate under the same conditions. The highest peak of tetraacetate for oligomycin B in mass spectrum was observed at m/e 972 by W. F. Prouty, et al. But albigmycin triacetate showed its highest peak at m/e 958. And then the molecular weight of A-272 substance was 776 by mass spectrum.

Recently in a communication from an editor of the Journal of Antibiotics (an international journal), we learned that no free OH band remained in the infrared spectrum of oligomycin A tetraacetate. But, as shown in Fig. 3, we could recognize an OH band, which indicates tertiary OH in the infra-red

spectrum of albimycin triacetate.

By paper chromatography, albimycin was compared simultaneously with oligomycins and ormymycin to use propanol aq. solution for the developing solvents on the same paper. As shown in Table 6, to use the 16% and 18% n-propanol aq. solution, we could see some difference of the R_f between albimycin, ormymycin and oligomycins. And then, as shown in Table 6, we could recognize some different activity

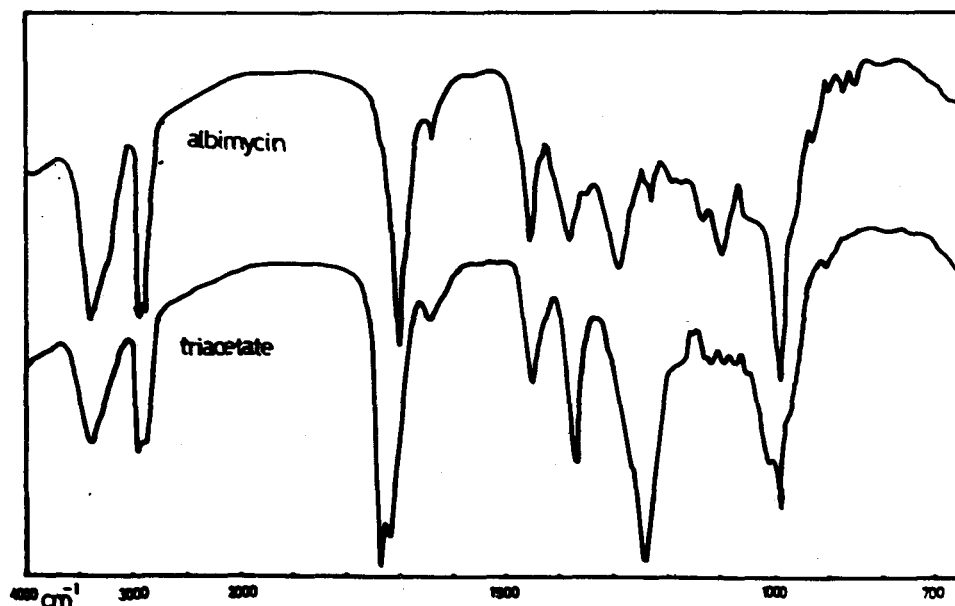


Fig. 3. Infrared spectrum of albimycin and albimycin triacetate (KBr).

Table 6 Paper chromatographic behaviour of albimycin, ormymycin, and oligomycins with an aqueous n-propanol solution

Solvent system	R _f values		
	albimycin	ormymycin	oligomycins
16% aq. n-propanol	0.19	0.21	0.30, 0.23 0.15
18% aq. n-propanol	0.27	0.34	0.44, 0.35 0.24

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

Antimicrobial spectrum of albimycin and oligomycins

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

of albimycin and oligomycins in the antimicrobial spectrum. Especially against *Aspergillus fumigatus*, *Trichophyton mentagrophytes* and *Trichophyton rubrum* there showed the difference on the inhibitory activity of albimycin and oligomycins.

So we think albimycin is a different antibiotic from oryomycin, A-272 substance and oligomycin A, B and C, from these results. Albimycin has a different pattern of N.M.R. spectrum at 60 Mc in CDCl_3 , as shown in Fig. 4, and then albimycin gave different derivatives (octahydroalbimycin)

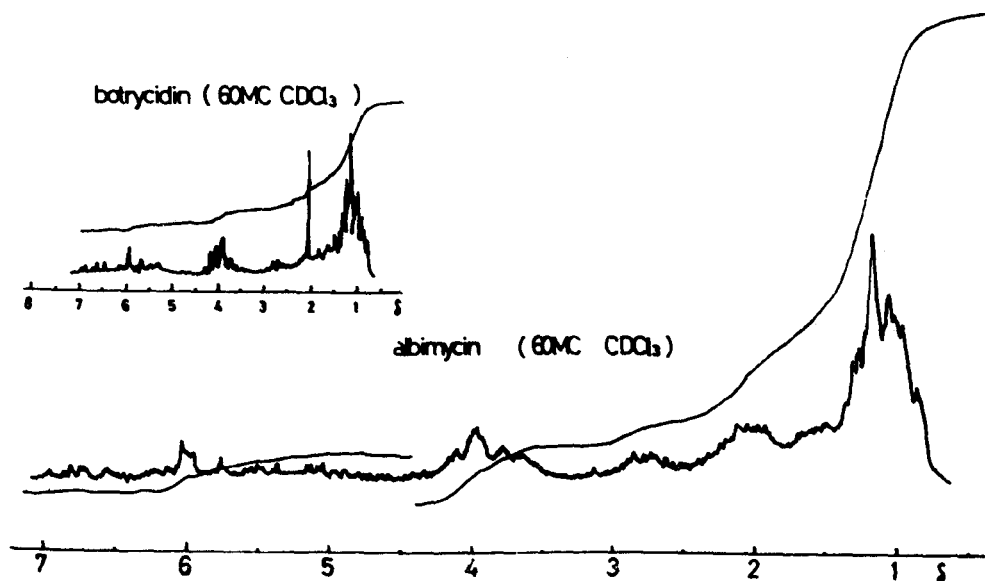
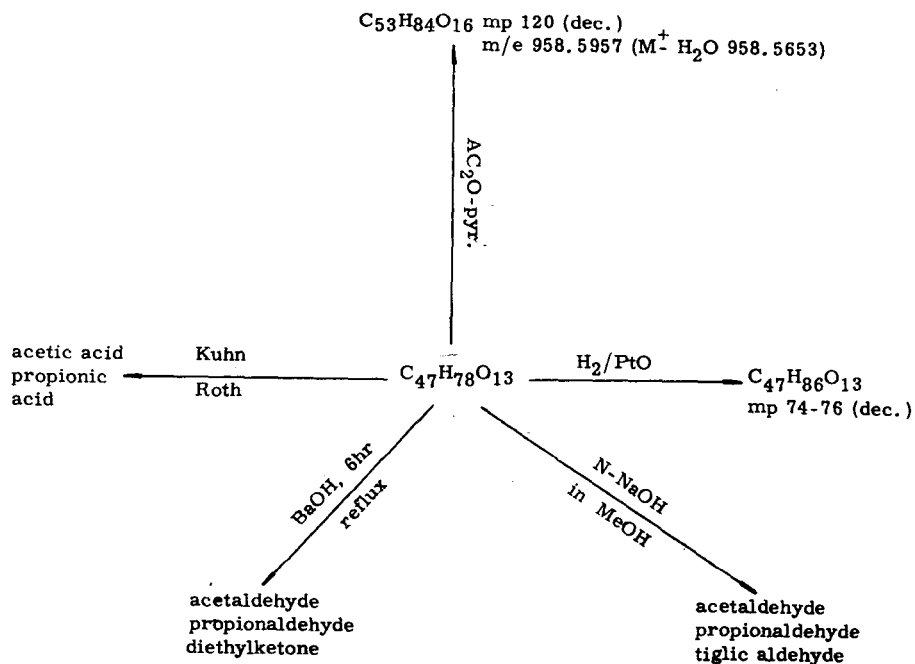


Fig. 4.

Table 7



from those of botrycidin (tetrahydrobotrycidin) under the same conditions.

As regards the phycomycin, Prof. T. Arai pointed out the difference of phycomycin and oligomycins in the absence of the 1175 cm band in the infrared spectrum of phycomycin which compared with these of oligomycins. But about the 1175 cm absorption band, albimycin is same with oligomycins. And then it is different from albimycin in the M. W. of phycomycin. From these results, we can think albimycin is a different antibiotic from oligomycins and other similar antibiotics.

Albimycin was found to contain no methoxyl group or acetoxyl group, and C-methyl determination gave a minimum value of 10 such groups. The presence of hydroxyl and carbonyl groups was expected. Absorption maxima in the UV spectrum indicated a conjugated diene system and other chromophores. By comparing albimycin with octahydroalbimycin on IR and NMR spectrum, the presence of trans-

Table 8

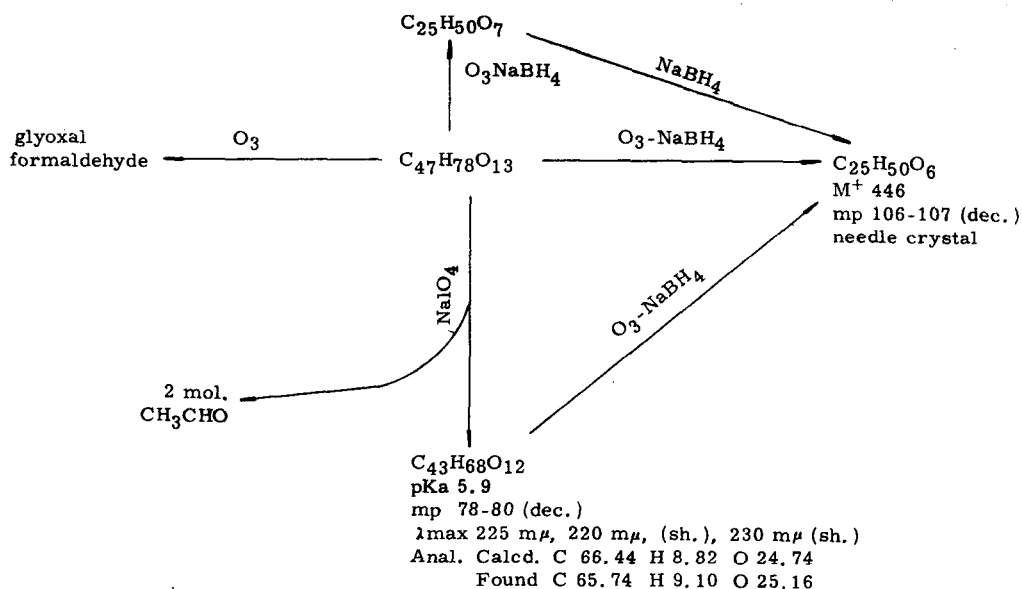


Table 9

$C_{25}H_{50}O_6$	
mp 106-107 (dec.)	$[\alpha]_D^{23} = -92.8$ (c 1.0, MeOH)
Anal. calcd. for $C_{25}H_{50}O_6 \cdot \frac{1}{3}H_2O$	C 66.33, H 11.28, O 22.39
	$C_{25}H_{50}O_6$ (MW 446.36074)
found	C 66.68, H 10.72, O 22.60
	M ⁺ m/e 446.36171
O-Me 0%, C-Me 19.3% (6 x C-Me 20.2%)	
ν_{max} 1,640 cm ⁻¹	
UV end absorption	
Color reaction	
+	: reddish yellow with conc. H ₂ SO ₄
-	: Antrone, Tollen, Fehling, Legal,
	no discoloring with KMnO ₄ ,
	Molisch, Ehrlich, Killiani, FeCl ₃ , Bial,
	Phloroglucin-HCl,
	no consumption of 10 $\bar{4}$