A NEW ANTIBIOTIC, BACIPHELACIN

Sir:

A new antibiotic, named baciphelacin, has been isolated from the culture filtrate of *Bacillus thiaminolyticus* IFO 3967/B-1-7. Baciphelacin is a fat-soluble, substituted benzene antibiotic, mainly effective against Gram-positive bacteria (Table 1) and Newcastle Disease virus in cultured chick embryo fibroblasts (minimal inhibitory concentration measured by the method of E. C. HERRMANN Jr.[1], 2 μg/ml). Baciphelacin is also effective against P388 lymphatic leukemia in CDF_1 mice. Treatment with baciphelacin (10 mg/kg/day for 9 days, i.p.) showed about a 50 % increase in mean survival time for P388 implanted mice (10^6 cells/head, i.p.).

The culture medium was composed of 1.0 % glucose, 0.5 % ammonium acetate, 0.7 % meat extract, 1.0 % Polypeptone and 0.3 % sodium chloride (pH 7.0). Cultures were grown at 30°C for 3 days.

The active substance was extracted from the culture filtrate with ethyl acetate at pH 9.5 and the extract was concentrated *in vacuo* to a syrup. Ether was added to the syrup and the mixture was kept overnight at 5°C. The active syrupy precipitate was collected and purified further by column chromatography on silica gel and silicic acid using a solvent system of chloroform - methanol (the concentration of methanol was linearly increased from 0 to 30 %). About 50 mg of white powder of baciphelacin was obtained from 100 liters of the culture broth.

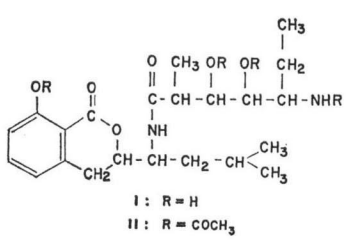
Baciphelacin (**I**) gave positive color reactions with ninhydrin, GREIG-LEABACK[2] and $FeCl_3$ reagents. The ultraviolet spectrum, λ_{max}^{MeOH} nm(ε): 216 (29,000), 245 (6,050), 314 (4,900) and the infrared spectrum, λ_{max}^{KBr}: 1695, 1680, 810, 697 cm^{-1}, indicated the presence of a benzoic acid moiety. The molecular formula $C_{22}H_{34}N_2O_6$ was estimated from that of the tetraacetate (**II**). Acetylation of **I** with acetic anhydride in pyridine gave the tetraacetate of **I** (**II**), m.p. 219°C, $C_{30}H_{42}N_2O_{10}$, UV λ_{max}^{MeOH} nm(ε): 207 (37,000), 246 (7,300), 314 (5,050), Mass M^+ *m/e* 590.

Table 1. Antimicrobial spectrum of baciphelacin.

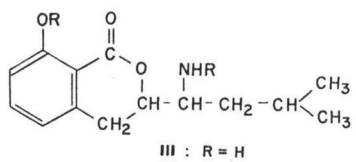
Test organism	MIC (μg/ml)
Staphylococcus aureus FDA 209P	5
Staphylococcus aureus No. 87*	5
Bacillus subtilis PCI 219	5
Sarcina lutea PCI 1001	5
Escherichia coli NIHJ	50
Klebsiella pneumoniae IFO 3512	10
Proteus vulgaris IFO 3045	>100
Proteus morganii IFO 3848	>100
Proteus mirabilis IFO 12255	>100
Pseudomonas aeruginosa IFO 3080	100
Pseudomonas aeruginosa NCTC 10490	100

* Clinical isolate, resistant against penicillin, streptomycin and macrolide group antibiotics.

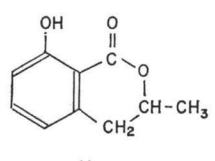
Acid hydrolysis of **I** (6 N HCl, 105°C, 24 hours) gave the chromophore moiety (**III**) as colorless needles, m.p. $\geq$204°C (decomp.), $C_{14}H_{19}NO_3 \cdot HCl$, Mass M^+ *m/e* 249, UV λ_{max}^{MeOH} nm(ε): 210 (23,600), 246 (6,950), 314 (4,530), IR λ_{max}^{KBr}: 1685 (C=O), 805, 697 cm^{-1} (aromatic), showing positive color reactions with ninhydrin ($-NH_2$) and $FeCl_3$ (phenolic OH). Diacetate of **III** (**IV**), $C_{18}H_{23}NO_5$, Mass M^+ *m/e* 333, was obtained by acetylation of **III**. The UV spectrum of **III** agreed very closely with that of mellein[3] (**V**), λ_{max}^{MeOH} nm(ε): 212 (20,000), 246 (6,500), 314 (4,100), indicating the presence of a similar basic skeleton. The NMR spectrum of **IV** showed the following functional groups: $\underline{CH_3}\!>\!CH\!-\!\underline{CH_3}$ (δ 0.91, 6H, d), $\underline{CH_3}$-CO-NH- (δ 1.94, 3H, s), $\underline{CH_3}$-CO-O-C_6H_5- (δ 2.28, 3H, s), CH_3-CO-$\underline{NH}$-$\underline{CH}$- (δ 6.31, 1H, d) and aromatic *p*, *m*-three protons (δ 6.97, 1H, d; δ 7.08,



I : R = H
II : R = COCH$_3$



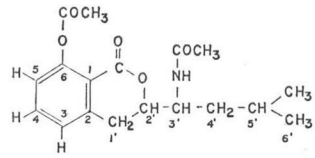
III : R = H
IV : R = COCH$_3$



V

1H, d; δ 7.47, 1H, t). The high resolution mass spectrum of **III** indicated the following fragment ion peaks: $C_{14}H_{19}NO_3$ (M^+), $C_{10}H_{10}NO_3$ ($M^+ - C_4H_9$), $C_{10}H_7O_3$ ($M^+ - C_4H_9 - NH_3$), $C_9H_7O_3$ ($M^+ - C_5H_{12}N$) and $C_5H_{12}N$ (B^+). The fragment $C_5H_{12}N$ was assumed to be the side chain $\left(\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\!CH\text{-}CH_2\text{-}CH(NH_2)-\right)$, this was supported by the fragment ion peak *m/e* 192 $\left[M^+ - C_4H_9\left(\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\!CH\text{-}CH_2-\right)\right]$. All protons

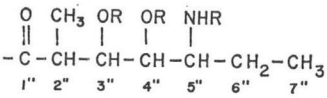
Table 2. NMR Spectrum of **IV** (**III**-diacetate)



Chemical shift		Results of spin-decoupling study						
		Following proton (δ, ppm) was irradiated						Addition of D_2O
Proton	δ(ppm)	3.00	4.50	4.30	1.40	1.65	0.93	
3-H	6.97 (1H, d)							
4-H	7.47 (1H, t)							
5-H	7.08 (1H, d)							
1′-CH_2	3.00 (2H, dd)	*	dd→d					
2′-CH	4.46 (1H, m)	m→dd	*					
3′-CH	4.28 (1H, m)			*	m→d-like			col.
4′-CH_2	1.34 (2H, m)			col.	*			
5′-CH	1.65 (1H, m)					*	m→t-like	
6′-CH_3	0.91 (6H, d)					d→s	*	
N-$COCH_3$	1.94 (3H, s)							
O-$COCH_3$	2.28 (3H, s)							
NH	6.31 (1H, d)			d→s				dis.

s=singlet, d=doublet, t=triplet, m=multiplet, col.=collapsed, dis.=disappeared

Table 3. NMR Spectrum of the side chain of **II**.

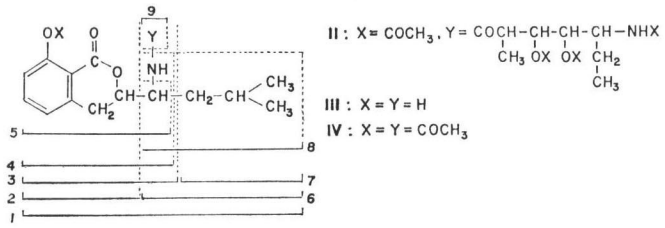


R = $COCH_3$ (**VI**)

Chemical shift		Results of spin-decoupling study					
		Following proton (δ, ppm) was irradiated					
Proton	δ(ppm)	0.97	5.39	5.11	4.51	1.46	6.68
2″-CH_3	0.97 (3H, d)	*					
2″-CH	1.55 (1H, m)	col.	col.				
3″-CH	5.37 (1H, d)		*	d→s			
4″-CH	5.11 (1H, dd)		dd→d	*	dd→s-like		
5″-CH	4.45 (1H, m)			col.	*	m→d-like	col.
5″-NH	6.68 (1H, d)				d→s-like		*
6″-CH_2	1.45 (2H, m)	col.			col.	*	
7″-CH	0.94 (3H, t)	*					

s=singlet, d=doublet, t=triplet, m=multiplet, col.=collapsed

Table 4. Mass fragmentation patterns of **II**, **III** and **IV**.



	(1) M^+	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
II	590	205	533	234	216	385	57	85	300
III	249	163	192	—	175	86	57	—	—
IV	333	205	276	234	216	128	57	86	43

	M^+-15	M^+-42	$M^+-15-42$	M^+-60	$M^+-60-42$	$M^+-42\times3$	$M^+-42\times4$	(2)-42	(3)-42	(3)-42×2	(3)-42×3	(4)-42	(4)-85	(5)-42
II	575	548	533	530	488	464	422	163	491	349	307	192	149	175
IV	318	291	276	273	231	—	—	163	234	192	—	192	149	175

	(6)-42	(6)-60	(6)-60-42	(6)-42-42	(6)-42×3	(9)-42	(9)-60	(9)-60-42	(9)-42-42	(9)-42×3
II	343	325	283	301	258	258	240	198	216	174

of **IV** were assigned as shown in Table 2 and confirmed by NMR spin-decoupling studies of **IV**. From all of the above evidence, structure **III** was proposed for the chromophore of baciphelacin.

When the molecular formula of the chromophore moiety $C_{14}H_{19}NO_3$ (**III**) is substracted from that of baciphelacin $C_{22}H_{34}N_2O_6$ (**I**), $C_8H_{15}NO_3$ remains as the side chain moiety. Comparing the NMR spectrum of **II** with that of **IV**, one additional secondary methyl, two O-acetyl, one imino, one ethyl, two methines attached to O-acetyl and two methines were observed. The NMR spin-decoupling studies on **II** clarified the presence of partial structure (**VI**) as shown in Table 3. The UV spectrum of baciphelacin (**I**) is almost identical with that of **III**, indicating that the chromophore moiety of **III** is also present in baciphelacin. Mass fragmentation patterns of **II**, **III** and **IV** indicated that they were split according to the same fragmentation scheme as shown in Table 4.

From these results, structure **I** was proposed for baciphelacin.

Acknowledgments

We thank Drs. R. TAKEDA and M. ISONO for their continued interest and encouragement throughout this work.

HISAYOSHI OKAZAKI
TOYOKAZU KISHI

Central Research Laboratories,
Takeda Chemical Industries, Ltd.,
Osaka, Japan

TERUHIKO BEPPU
KEI ARIMA

Department of Agricultural Chemistry,
Faculty of Agriculture,
The University of Tokyo,
Tokyo, Japan

(Received June 5, 1975)

References

1) HERRMANN, Jr., E. C.; J. CABLIKS, C. ENGLE & P. L. PERLMAN: Agar diffusion method for detection and bioassay of antiviral antibiotics. Proc. Soc. Expt. Biol. Med. 103: 625~628, 1960
2) GREIG, C. G. & D. H. LEABACK: Use of chlorine in the detection of compounds on paper chromatograms. Nature 188: 310~311, 1960
3) BLAIR, J. & G. T. NEWBOLD: Lactones. II. The structure of mellein. J. Chem. Soc. 1955: 2871~2875, 1955