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Some Structure-Activity Relationships for Bactobolin Analogs in the Treatment of Mouse Leukemia P388[1)]

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Bactobolin, (3*S*,4*R*,4a*R*,5*R*,6*R*)-4-(L-alanylamino)-3-(dichloromethyl)-3,4,4a,5,6,7-hexahydro-5,6,8-trihydroxy-3-methyl-1H-2-oxa-1-naphthalenone, is an antitumor antibiotic produced by *Pseudomonas*. In the present studies, 14 bactobolin analogs with or without substituents on the amino group attached to the 4 position of the bactobolin nucleus were synthesized and evaluated for activity against mouse leukemia P388. These compounds were given intraperitoneally to mice bearing ascitic leukemia P388. Compounds with the L-seryl group and some L-alanine derivatives on the amino group were found to possess antileukemic activity, but at the dose levels tested, they were neither more effective nor more potent than bactobolin which increased the lifespan of leukemic mice by 110% over the control at an optimal dose of 2.5 mg/kg/day. The other analogs examined were found to be ineffective and nonlethal for mice at doses of 10 mg/kg/day or less. The structure–activity relationships for bactobolin analogs are discussed.

Keywords——bactobolin; bactobolin analogs; antitumor antibiotic; antileukemic activity; mouse leukemia P388; structure–activity relationship

Bactobolin is an antibiotic produced by *Pseudomonas*.[2–4)] Its chemical structure is 3-dichloromethylactinobolin, (3*S*, 4*R*, 4a*R*, 5*R*, 6*R*)-4-(L-alanylamino)-3-(dichloromethyl)-3,4,4a,-5,6,7-hexahydro-5,6,8-trihydroxy-3-methyl-1H-2-oxa-1-naphthalenone.[2,5)]

This chlorine-containing antibiotic has been shown to possess antitumor activity against mouse leukemias L1210 and P388, and several lines of rat hepatomas at low doses, *e.g.*, 1 mg/

kg,[2,3,6] although actinobolin has been reported to have antitumor activity only at high doses such as 1 g/kg.[7-9] We also found that N′-alanyl- or 5-deoxybactobolin, a minor product isolated from broth cultures of *Pseudomonas yoshitomiensis* Y-12278, a bactobolin-producing strain, possessed antitumor activity against leukemias L1210 and P388[6] (N or N′ refers to the amino group attached to the 4 position or that of the L-alanine moiety of bactobolin, respectively, in this paper). These minor substances were, however, less effective in increasing the lifespan of leukemic mice than bactobolin. Evaluation of the antitumor activity of additional bactobolin analogs may provide further information concerning the structure–activity relationships of compounds of this type. For this purpose, compounds with or without substituents at the N position were synthesized and evaluated for activity against mouse leukemia P388.

Materials and Methods

Compounds——Bactobolin was isolated from broth cultures of *Pseudomonas yoshitomiensis* Y-12278 in our laboratories. Bactobolin analogs were synthesized by Munakata.[10] The methods of synthesis and the chemical properties of these analogs were reported previously.[10] These compounds were dissolved in 0.9% NaCl solution just prior to use. Drug solutions were administered intraperitoneally (*i.p.*) to mice in a volume of 10 ml/kg body weight.

Animals and Tumor Cells——Female DBA/2 and CDF_1 (BALB/c × DBA/2)F_1 mice were obtained from Charles River Japan, Inc., Kanagawa. P388 leukemic cells were passaged at weekly intervals by *i.p.* implantation into 8-week-old DBA/2 mice. For evaluating the antileukemic activity of test compounds, leukemic cells harvested from DBA/2 mice bearing 6-day-old tumor were inoculated *i.p.* into 8-week-old CDF_1 mice (10^6 cells/mouse) on day 0. Drug solutions were administered daily on days 1—4. The survival times of leukemic mice were recorded, and results are expressed as percent increase in median survival time of treated mice over controls (ILS%).

Results

The effects of bactobolin and its analogs on the lifespan of mice bearing ascitic leukemia P388 are shown in Table I. The lifespan of mice was increased by 110% over the control following administration of 2.5 mg/kg/day of compound **1**, bactobolin. The dose of 5 mg/kg/day bactobolin was lethal to mice. Among bactobolin derivatives with substituents at the N′ position (compounds **2**—**8**), compounds **2**—**5** were found to be effective (ILS≧25%) with maximum ILS values of 45 to 100% at the dose levels tested. The optimal and toxic doses of compound **2** or **3** were 5 and 10 mg/kg/day or 2.5 and 5 mg/kg/day, respectively. The optimal doses of compounds **4** and **5** were not less than 10 mg/kg/day. The bactobolin derivatives shown to be inactive (compounds **6**—**8**) were nonlethal to mice at doses of 10 mg/kg/day or less.

Among the bactobolin analogs with amino acids other than L-alanine, *i.e.*, with D-alanyl (**9**), L-phenylalanyl (**10**), L-seryl (**11**) and L-2-aminobutyryl groups (**12**), at the N position, only compound **11** was found to be effective with a maximum ILS of 62% at a dose of 25 mg/kg/day. The lethal doses of these compounds for mice were higher than 25 mg/kg/day.

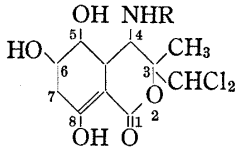
Compounds with no substituent (**13**) and acetyl (**14**) and phenylacetyl groups (**15**) at the N position were found to be ineffective. No mice died of toxicity at a dose of 50 mg/kg/day of these compounds.

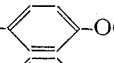
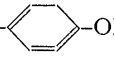
Discussion

The antibiotic bactobolin is structurally a 3-dichloromethyl analog of actinobolin.[2,5]

In the present series of bactobolin analogs, compounds with or without substituents at the N position (an amino group on the 4 position of the bactobolin nucleus) were evaluated for activity against mouse leukemia P388. Bactobolin (**1**) and N′-O-*t*-butyl-L-tyrosyl- (**2**) and N′-L-tyrosylbactobolins (**3**) showed the most potent activities. Compared to bactobolin,

TABLE I. Effect of Bactobolin and Its Analogs on the Lifespan of Mice Bearing Ascitic Leukemia P388.



Compd. No.	Structure R	ILS (%) at daily doses (mg/kg) of						
		0.5	1	2.5	5	10	25	50
1	(L)-$COCH(CH_3)NH_2$	60	70	110	Toxic			
2	(L,L)-$COCH(CH_3)NHCOCH(NH_2)CH_2$-  -$OC(CH_3)_3$	50	60	90	100	Toxic		
3	(L,L)-$COCH(CH_3)NHCOCH(NH_2)CH_2$-  -OH	33	50	58	Toxic			
4	(L)-$COCH(CH_3)NHCOCH_2CH_2NH_2$					9	18	45
5	(L,DL)-$COCH(CH_3)NHCH(COOH)CH_2COOH$	0	8	25	33	50		
6	(L)-$COCH(CH_3)NHCOCH_3$						0	9
7	(L)-$COCH(CH_3)NHCOCH_2N(CH_3)_2$		0	0	0	9		
8	(L)-$COCH(CH_3)NHCSNH$- 							10
9	(D)-$COCH(CH_3)NH_2$	0	10	0	0	0	0	0
10	(L)-$COCH(NH_2)CH_2$- 		0	0	0	0	0	
11	(L)-$COCH(NH_2)CH_2OH$		5	14	14	33	62	
12	(L)-$COCH(NH_2)CH_2CH_3$		5	5	5	5	5	
13	-H						20	20
14	$-COCH_3$					11	0	11
15	$-COCH_2$- 					11	11	11

Mice were given *i.p.* implants of 10^6 leukemic cells. Five mice were used in each group. The median survival times of control groups were 9—12 days. Some compounds were prepared as salts: HCl salts for compounds **1**, **9**, **12** and **13**; HBr salt for compound **11**; HCl·1/2iso-C_3H_7OH salt for compound **4**; HCl·iso-$C_3H_7OH \cdot 3H_2O$ salts for compounds **2** and **3**.

these analogs (except for compound **3**) showed low toxicity to mice as reflected in an increase in effective dose or a loss of antileukemic effectiveness. Although most compounds were not evaluated for antileukemic activity up to toxic dose levels, the following tentative conclusions on the structure–activity relationships for bactobolin analogs can be made.

1. Bactobolin Derivatives

Bactobolin derivatives with the L-alanyl (data reported previously[6]), O-*tert*-butyl-L-tyrosyl (**2**) and L-tyrosyl groups (**3**) at the N′ position were found to be effective. Their optimal doses were 2.5 or 5 mg/kg/day, which is equal to or only twice that of bactobolin. The bactobolin derivatives with the β-alanyl (**4**), (1,2-dicarboxy)ethyl (**5**), acetyl (**6**), N,N-dimethylglycyl (**7**) and thiocarbamoyl groups (**8**) at the N′ position were found to be ineffective or less potent than compounds **2** and **3**. These results suggest that the preferred substituents at the N′ position for potent antileukemic activity are α-aminoacyl groups in which the amino group is primary. Loss of the basic character of derivatives (compounds **6** and **8**) may result in a loss of effectiveness.

2. Other Analogs and Bactobolin

Among 5 compounds with α-amino acids at the N position, bactobolin (**1**) and a compound containing the L-seryl group (**11**) were found to be effective, but compounds with the D-alanyl (**9**), L-phenylalanyl (**10**) and L-2-aminobutyryl groups (**12**) showed neither toxicity nor antileukemic effect. Compounds with no substituent (**13**) and with acetyl (**14**) and phenylacetyl groups (**15**) at the N position were without effect. These results suggest that the N position

should be substituted with α-aminoacyl groups to obtain antileukemic activity. The amino hydrogen may form a hydrogen bond with certain cell components. The configuration and bulk of substituents on the α-carbon atom are also critical factors.

On the other hand, bactobolin and actinobolin have the same skeleton, hexahydroisocoumarin. As cited above, the former was found to possess antitumor activity at 2.5 mg/kg/day or less, while the latter has been reported to have antitumor activity at high doses such as 1 g/kg/day.[7-9] Since the structural difference is the presence or absence of the 3-dichloromethyl group, it is apparent that the high antitumor potency of bactobolin is due to the presence of this group. Ueda *et al.*[5] reported that the L-alanylamino moiety at the 4 position of bactobolin is linked to the isocoumarin ring by a hydrogen bond, and that a hydrophilic hole is formed by this moiety and the dichloromethyl group. This hydrophilic hole may play some role in the antitumor action. It is also likely that the L-alanine moiety at the N position of these two antibiotics is essential for their antitumor actions.

Although no improvement in antileukemic effectiveness over bactobolin was observed among compounds in the series of bactobolin analogs used in this study, additional structural modifications of this antibiotic to obtain analogs with improved therapeutic effectiveness are being attempted on the basis of the present findings.

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Photochemistry of Flavonoids. III.[1] Photorearrangement of Flavonols

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Irradiation of flavonols (**1**) in methanol gave 3-arylphthalides (**3**) which were formed *via* the diketones (**4**). Metal ions (Cu^{2+}, Ni^{2+}, Fe^{3+}, Co^{2+} and Be^{2+}) inhibited this rearrangement.

Keywords——flavonols; photochemical rearrangement; 3-arylphthalides; methyl phenylglyoxylates; metal ions; 3-aryl-3-hydroxy-1,2-indandiones