UDC 576.852.2.095.18:547.728

95. Shinsaku Natori : Antibacterial Effect of Lichen Substances and Related Compounds. VII.[1] The Structure-Activity Relationship observed in Compounds related to Dibenzofuran and an Approach to the Elucidation of the Mode of Action.

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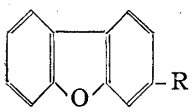
On the basis of bacteriostasis of lichen dibenzofuran derivatives[2] the author and his associates synthesized about 140 compounds related to dibenzofuran and examined their antibacterial activities.[1,3~5] Summarizing the structure-activity relationship observed in these series of compounds, some approaches to the mode of action were made, on which the present paper chiefly concerns.

I. Relationship between the Chemical Structure and Antibacterial Action in Compounds related to Dibenzofuran

In these compounds examined so far, none of them showed significant bacteriostatic activity against *Staph. aureus* and *E. coli*, but some of them exhibited fairly remarkable inhibitory effect on *Mycobac. tuberculosis* A.T.C.C. No. 607 and $H_{37}Rv$ so far as synthetic medium without serum was employed. In the previous papers, the author had elucidated the relationship between the chemical structure and antibacterial action, especially against *Mycobac. tuberc.* A.T.C.C. No. 607. These results may be summarized as follows :

a) The kind of the substituents at the 3-position of dibenzofuran : Twenty-four kinds of 3-monosubstituted dibenzofuran derivatives (I) can be classified into the following three groups on the basis of their activity[3~5].

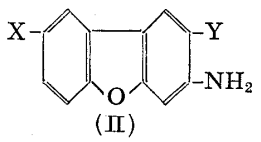

(I)

Ineffective : R=NO_2, NHAc, NAc_2, $NHSO_2C_6H_4CH_3$, $N(CH_3)SO_2C_6H_4$-CH_3, OCH_3, $OCOCH_3$, Cl, CN, COOH, $CONH_2$, $COOC_2H_5$, $CONHNH_2$.
Slightly effective : R=NHMe, NMe_2, NMe_3I, $NHNH_2$, $C(=NH)NH_2$, $NHCONH_2$.
Effective : R=NH_2, $NHC(=NH)NH_2$, $NHCH_2SO_3Na$, OH.

These classification of the substituent was generally applicable to other monosubstituted derivatives belonging to these series of compounds.[1,4,5]

b) The position of the substituents : As for amino and hydroxyl derivatives, the compounds substituted at the 3-position were the most effective, 2-amino or 2-hydroxyl compound followed, and 4- or 1-substituted derivatives were far less inhibitory.[5]

c) Influence of further introduction of a subitituent to the effective monoamino or hydroxyl derivatives : When the effective 3-aminodibenzofuran was further substituted at 8-[4] or 2-position,[5] the antitubercular activity was reduced in the following order :


(II)

(II) Y=H; X=H, Cl $\gg$ $CONHNH_2$ > NO_2, COOH, $COOC_2H_5$, NH_2.

(II) X=H; Y=H $\gg$ OCH_3, C_2H_5 > NH_2, OH > NO_2.

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1) Part VI : S. Natori, M. Ito, T. Nakagome : This Bulletin, **5**, 548(1957).
2) S. Shibata, Y. Miura, H. Sugimura, Y. Toyoizumi : J. Pharm. Soc. Japan, **68**, 303(1948); Japan. Med. J., **2**, 22(1949); S. Shibata, T. Ukita, Y. Miura, T. Tamura : J. Pharm. Soc. Japan, **68**, 298(1948); Japan. Med. J., **1**, 152(1948).
3) S. Shibata, S. Natori, Y. Sumi : J. Pharm. Soc. Japan, **72**, 1333(1952).
4) S. Shibata, S. Natori, T. Kawakami, M. Okano, Y. Tsuchimoto : This Bulletin, **2**, 45(1954).
5) S. Natori : This Bulletin, **5**, 539(1957).

The same tendency was also observed in 3-hydroxydibenzofuran derivatives; 3-hydroxy- and 3-hydroxy-8-chlorodibenzofuran showing some activity,[3] while 1,9-dimethyl-3,7-dihydroxy-, 1-butyl-3,7-dihydroxy-9-propyl-(decarboxynordidymic acid), and 1-butyl-2-carboxy-3-hydroxy-7-methoxy-9-propyl-dibenzofuran (didymic acid) were slightly effective[4] against the tubercle bacillus, and 2-amino- and 2-nitro-3-hydroxydibenzofuran were not effective.[5]

In the case of 2-amino- and 4-amino derivatives, almost the same phenomena had been already pointed out in a previous paper.[5]

d) Replacement of the ring oxygen with isosteric atoms: Although isosterism was assumed to be applicable in these compounds, the replacement generally caused reduction of the activity.[1]

e) Hydrogenation of the ring: Hydrogenation also reduced the activity.[5]

Based on these results, the requirements in the chemical structure for exhibiting antitubercular activity may be summarized to: i) The size, planarity, and, presumably, aromaticity of the ring, ii) the ring oxygen atom with a lone electron pair, and iii) an appropriate substituent at the 3-position capable of forming some kind of a bond, e.g. hydrogen bond.

II. Some Observations on the Mode of Action of Compounds related to Dibenzofuran

Since the structure-activity relationship was clarified as mentioned above, it was thought worth while searching for means of elucidating the mode of action of these compounds.

First, influence of pH and coexistance of metal ions or some structurally related essential metabolites on the antibacterial action against *Mycobac. tuberc.* A.T.C.C. No. 607 was examined. The activity was also reëxamined in the coexistance of the two related compounds in order to obtain confirmation of the identity of the active site of those compounds. As an important physicochemical property influencing the antibacterial activity, surface tension of the solution of effective compounds was measured.

Further, the respiration of *Mycobac. tuberc.* A.T.C.C. No. 607 in the presence of sodium 3-dibenzofurylaminomethanesulfonate was investigated.

It was reported in the previous papers[1,4,5] that the antibacterial activity of dibenzofuran derivatives against *Mycobac. tuberc.* was remarkably reduced by the addition of serum as was observed in Kirchner's medium. In order to obtain some information on the problem, the inhibitory effect was comparatively examined in the presence of serum, serum albumin, and egg albumin. The inhibitory effect was also examined in the presence of some nonionic surface active agents.

Materials and Methods

Antibacterial Agents—Dibenzofuran derivatives reported in the preceding papers,[1~5] were submitted to the examination. 3-Aminodibenzofuran was usually used as a standard effective compound of the series, while sodium 3-dibenzofurylaminomethanesulfonate was selected as a comparatively water-soluble derivative.

Other Agents—Other agents used in these experiments were mainly commercial products, which were purified by appropriate methods, if necessary.

Antibacterial Activity in the Presence of other Agents—Antibacterial activity was examined by almost the same method as described in the previous paper.[5] *Mycobacterium tuberculosis* A.T.C.C. No. 607 was chiefly employed as the test organism, using Kirchner's medium devoid of serum as a basal medium. Other agents, of which the interaction with the dibenzofuran derivatives was examined, were added beforehand to the medium or afterwards to the series. A 3-day culture of *Mycobac. tuberc.* A.T.C.C. No. 607 in the modified Dubos medium (asparagine-glycerol-Polysorbate 80 (Tween 80) medium) was diluted with 10 volumes of physiological saline, and the suspension (0.1 cc.) was inoculated to each tube containing 3 cc. of the solution (inoculum, ca. 10^5/3 cc.). The resutls were read after 3 days' incubation at 37°. Each experiment was duplicated more than twice.

Measurement of Surface Tension—Measured by du Noüy's tensiometer at 20~22°. As 3-amino- and 3-hydroxy-dibenzofurans are insoluble in water, they were measured in dilute Me_2CO solutions.

Respiratory Inhibition for *Mycobac. tuberc.* A. T. C. C. No. 607—Conventional manometric method was employed. The organism was incubated in a Tween 80-asparagine-glucose medium using Monod's shake culture apparatus for 24 hrs. (A) or incubated in Sauton's medium without shaking for 4 days (B). From these cultures, the bacterial cell suspension was prepared by the usual method. The contents of the vessels are shown in Table I. Measurements were made at 37°.

Table I.

Expt. No.	Main chamber: 0.02*M* phosphate buffer (cc.)	Main chamber: 0.1*M* glucose (cc.)	Main chamber: H_2O (cc.)	Main chamber: Na 3-dibenzofuryl-aminomethane-sulfonate (*M*)	Center well: 20% KOH (cc.)	Side arm: Cultural method	Side arm: Volume of suspension (cc.)	Side arm: Dry wt. of the organism (m.g.)
1	1.0	1.0	0.5	10^{-3}~10^{-6}	0.2	A	0.5	5.0
2	1.0	0	1.5	10^{-3}~10^{-5}	0.2	A	0.5	5.0
3	1.0	1.0	0.5	10^{-3}~10^{-6}	0.2	B	0.5	2.6

Results and Discussions

Influence of pH on the Antibacterial Activity—There has been many examples in which the minimum inhibitory concentrations of antibacterial agents vary with the change of pH of the media.[6] From these results it has been discussed whether ionic form or neutral molecule of the agent exhibits the activity.[7] As the effective compounds of dibenzofuran series also belong to amines or phenols and slightly ionized at physiological pH, the antibacterial activity of 3-aminodibenzofuran, sodium 3-dibenzofurylaminomethanesulfonate, and 3-hydroxydibenzofuran was comparatively examined at pH 6, 7, and 8, using *Mycobac. tuberc.* A. T. C. C. No. 607 as the test organism.

Table II.

Compound	pH	log reciprocal molar concn. 4	4.5	5	5.5	6	6.5	7
3-Aminodibenzofuran	6	−	−	−	−	±	+	++
	7	−	−	−	−	±	++	++
	8	−	−	−	−	±	+	++
Sodium 3-dibenzofuryl-aminomethanesulfonate	6	−	−	−	−	−	++	++
	7	−	−	−	−	+	++	++
	8	−	−	−	−	±	+	++
3-Hydroxydibenzofuran	6	−	−	−	+	++	++	++
	7	−	−	±	+	++	++	++
	8	−	−	±	+	+	++	++

The results, shown in Table II, indicated that the activity was scarcely influenced by the pH. Since the pKa value of 3-aminodibenzofuran was reported as 3.3[8] or 3.54,[9] the molar concentration of the ammonium cation existing at the minimum inhibitory concentration of the amine was calculated approximately as $10^{-8}M$ for pH 6, $10^{-9}M$ for pH 7, and $10^{-10}M$ for pH 8, while the neutral molecule retained concentration of ca. $10^{-5.5}M$ at each pH. Although it could not be excluded that the ammonium cation competes with hydrogen cation to exhibit the activity, it seemed more likely that the neutral molecule plays a chief role in the activity. The assumption will become more certain, if the active site of the amine and the phenol is proved to be the same. The fact that the further introduction of a substituent to 3-aminodibenzofuran always reduced the activity also seemed to support the assumption.

Influence of Metal Ion on the Antibacterial Activity—The activities of antibacterial agents have been often influenced extensively by the coexisting metal ions. In some

6) e.g. C.L. Fox, Jr., H.M. Rose: Proc. Soc. Exptl. Biol. Med., **50**, 142(1942); F.C. Schmelkes, *et al.*: *Ibid.*, **50**, 145(1942); P.B. Cowles, I.M. Klotz: J. Bacteriol., **56**, 277(1948).
7) c.f. A. Albert: "Selective Toxicity," Methuen & Co., London(1951).
8) E. Sawicki, F.E. Ray: J. Am. Chem. Soc., **75**, 2519(1953).
9) K.A. Allem, J. Cymerman-Craig, A.A. Diamantis: J. Chem. Soc., **1954**, 234.

cases, the mode of action of antibacterial agents has been well explained by their metal complex formation.[10] The correlation between antibacterial activity and copper-complex formation was also reported with diphenyl ether and usnic acid derivatives.[11] In this respect, antibacterial activity of 3-aminodibenzofuran against *Mycobac. tuberc.* A. T. C. C. No. 607 was examined with the addition of Fe^{2+}, Fe^{3+}, Cu^{2+}, Co^{2+}, Zn^{2+}, and Mn^{2+} as their sulfate in $10^{-4}M$ concentration. The influence of Cu^{2+} was also examined on 3-hydroxy-, 4-amino-, and 4-hydroxy-dibenzofurans and on sodium 3-dibenzofurylaminomethanesulfonate. In any of these cases, no increase or decrease of the inhibitory effect was observed.

Antibacterial Activity in the Presence of some Essential Metabolites—The minimum inhibitory concentrations of 3-aminodibenzofuran and sodium 3-dibenzofurylaminomethanesulfonate, $10^{-5.5}\sim10^{-6}$ and $10^{-5.5}M$, did not change in the presence of menadione (V. K_3, $10^{-5}M$), riboflavin (V. B_2, $10^{-4}M$), tryptophan ($10^{-4}M$), or biotin ($10^{-4}M$).

The Effect of Related Compounds to the Inhibitory Action of 3-Aminodibenzofuran—The antibacterial activity against *Mycobac. tuber.* A.T.C.C. No. 607 was examined in the coexistence of 3-aminodibenzofuran and one of the structurally related compounds, i.e. 3-hydroxy- and 2-aminodibenzofuran, sodium 3-dibenzofurylaminomethanesulfonate, 3-aminodibenzothiophene, and 2-aminofluorene. The results obtained by the couple of 3-aminodibenzofuran and 3-aminodibenzothiophene are shown in Table III as an example.

Table III.

log reciprocal molar concn. of 3-aminodibenzothiophene	log reciprocal molar concn. of 3-aminodibenzofuran: 4	4.5	5	5.5	6	6.5	7	nil
4	—	—	—	—	—	—	—	—
4.5	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—
5.5	—	—	—	—	—	±	±	+
6	—	—	—	—	±	##	##	##
6.5	—	—	—	—	+	##	##	##
7	—	—	—	—	+	##	##	##
nil	—	—	—	—	+	##	##	##

In the other four couples, similar results were obtained, showing slight additive action in some cases. Any obvious antagonistic or synergistic action was not observed in each case. From these observations the identity of the active site of these series of compounds could not be confirmed.

Surface Activity of Some Compounds—Surface activity is an important factor influencing the antibacterial activity and its correlation to the activity has been examined in many cases, especially in the compounds having aliphatic side chains.[12]

In this study, surface tension of the solution of 3-amino- and 3-hydroxy-dibenzofuran and sodium 3-dibenzofurylaminomethanesulfonate was examined by the method described above. The surface tension of the amino and the hydroxyl compounds was measured in dilute acetone solution (2, 3, and 5%) due to their slight solubility in water, showing no surface activity even in $10^{-3}M$ solution. The results with the methanesulfonate are given in Table IV.

10) e.g. H. Erlenmeyer, *et al.*: Helv. Chim. Acta, **34**, 427, 430(1951); **35**, 1763(1952); **36**, 610, 941 (1953); **37**, 95, 636, 2010(1954); **38**, 96(1955); A. Albert, S.D. Rubbo, *et al.*: Biochem. J.(London), **41**, 529, 534(1947); Brit. J. Exptl. Pathol., **26**, 160(1945); **28**, 69(1947); **31**, 425(1950); **34**, 119(1953); **35**, 478(1954); Nature, **176**, 34(1955); T. Ueno: J. Pharm. Soc. Japan, **76**, 825, 831, 839(1956).

11) F. Carl, P. Marquart: Z. Naturforsch., **46**, 280(1949)(C. A., **44**, 3157(1950)).

12) e.g. R. Adams, *et al.*: J. Am. Chem. Soc., **54**, 1548(1932); J. Pharmacol. Exptl, Therap., **45**, 121(1932); C.J. Cavallito, *et al.*: J. Am. Chem. Soc., **70**, 3724(1948); T. Noguchi: J. Pharm. Soc. Japan, **76**, 392(1956); H. Watanabe: *Ibid.*, **76**, 696(1956).

Table IV.

Substance	Solvent	Surface tension (dynes/cm.)								
		10^{-3}	8×10^{-4}	6×10^{-4}	4×10^{-4}	2×10^{-4}	10^{-4}	10^{-5}	10^{-6}	Control
Sodium 3-dibenzofuryl-aminomethanesulfonate	H_2O	52.8	56.8	60.2	65.3	74.7	74.7	74.8	74.9	74.5

It was found that the compound showed some surface activity in a concentration higher than $4\times10^{-4}M$, but the threshold was comparatively higher than that of the inhibitory action ($10^{-5.5}M$) and the surface activity even in $10^{-3}M$ was too small to explain the effect[13] so that the surface activity could not be assumed as the major factor influencing the bacterial growth.

Influence of Sodium 3-Dibenzofurylaminomethanesulfonate on the Respiration of *Mycobac. tuberc.* A. T. C. C. No. 607—Since a decisive evidence had not been obtained to explain the mode of action from the above-mentioned experiments, the action mechanism of the series was searched in the metabolic pathways of *Mycobac. tuberc.* A. T. C. C. No. 607. As almost all the compounds of this series are sparingly soluble in water, the most water-soluble compound of the series, sodium 3-dibenzofurylaminomethanesulfonate, was submitted to test to see whether the compound inhibits the respiration of the cell suspension of the organism or not. The experimental conditions were described above. The amount of oxygen uptake measured in the presence of the agent was compared with that of the control in three experimental conditions shown in Table I. The results are shown in Table V by the ratio of oxygen consumption.

Table V.

Qo_2 ratio (with sodium 3-dibenzofurylaminomethanesulfonate : without the agent)

Expt. No.	concn. of the agent (M)			
	10^{-6}	10^{-5}	10^{-4}	10^{-3}
1	1.43	1.14	1.00	0.86
2	—	1.08	1.00	0.89
3	1.25	1.15	1.11	0.89

From these results, it was made clear that the compound inhibited the respiration of the organism in the concentration of $10^{-3}M$ but stimulated in the concentration lower than $10^{-5}M$. Comparing the concentration with that for bacteriostatic activity, i.e. $10^{-5.5}M$, the antibacterial action of the compound might not be chiefly due to respiratory inhibition.

As for usnic acid, one of the antitubercular lichen substances, it was proved that the acid inhibits the orthophosphate uptake in the oxidative phosphorylation of washed residue of the liver and kidney homogenates.[14] It may be considered that the dibenzofuran compound also exhibits inhibitory action on the growth of the organism through acting as an uncoupling agent of the oxidative phosphorylation.

Further confirmation of this point has not been obtained because of some limitation, i.e. inefficacy against bacteria other than Mycobacterium, decrease of the activity in the presence of surface active agents, and slight solubility of the compounds.

Antibacterial Action against *St. aureus* and *E. coli* in Semisynthetic Medium—The decrease of antibacterial activity of the dibenzofuran compounds against *Mycobac. tuberc.* $H_{37}Rv$ by the addition of serum was a notable phenomenon in these series. The activity against *Staph. aureus* and *E. coli* was examined previously in a nutrient broth and it was thought that the activity might heve been reduced by antagonistic action

13) A. E. Alexander, M. A. Soltys : J. Pathol. Bacteriol., **58**, 37(1946).
14) R. B. Johnson, G. Feldott, H. A. Lardy : Arch. Biochem., **28**, 317(1950); see also A. Marshak, *et al.* : J. Cellular Compt. Physiol., **31**, 321(1948) (C. A., **42**, 8976(1948)); *ibid.*, **35**, 317(1950); Y. Miura, *et al.* : Compt. rend., **232**, 1710(1951) (C. A., **45**, 9106(1951)).

of protein-like substances in the medium.

The antibacterial activity was, therefore, reëxamined in Knight's semisynthetic medium with eight dibenzofurans, which were most effective in these series. The results showed that the highest inhibitory dilutions in Knight's medium were generally higher than that in broth, but they were not more than four-fold. It was thereby found that the antibacterial activity of dibenzofuran compounds is specific against Mycobacterium.

Antibacterial Activity in the Presence of Serum—It is a well-known fact that the toxicity of some antibacterial agents is often neutralized by the serum in which albumin fraction is assumed to play a chief rôle in reversing antibacterial activity through its interaction with the agents.[15] Dibenzofuran derivatives were also proved to fall into this category.[1,4,5] In order to obtain further confirmation on this point, antibacterial activity of 3-aminodibenzofuran and sodium 3-dibenzofurylaminomethanesulfonate against *Mycobac. tuberc.* A. T. C. C. No. 607 was comparatively examined in the presence of horse serum, serum albumin, and egg albumin. The results are shown in Table VI.

Table VI.

	Dilution ($\times 10^4$)										
	1	2	4	8	16	32	64	128	256	512	1024
3-Aminodibenzofuran	−	−	−	−	−	−	−	−	−	+	##
+1% horse serum	−	−	−	±	+	+	+	+	##	##	##
+10% horse serum	−	−	−	±	+	+	##	##	##	##	##
+0.2%[a] serum albumin[b]	−	−	±	±	+	+	+	##	##	##	##
+0.2%[c] egg albumin	−	−	−	−	−	−	−	−	−	+	##
Sodium 3-dibenzofuryl-aminomethanesulfonate	−	−	−	−	−	−	−	−	±	##	##
+1% horse serum	−	−	±	+	+	+	+	##	##	##	##
+10% horse serum	±	+	+	+	+	##	##	##	##	##	##
+0.2% serum albumin	−	−	±	+	+	+	+	##	##	##	##
+0.2% egg albumin	−	−	−	−	−	−	−	±	##	##	##

a) 0.29 mg. N/cc.
b) Fractionated by Dr. T. Katsura following Cohn's method.
c) 0.29 mg. N/cc.

From these results, the antibacterial activity of dibenzofurans was ascertained to be reversed by serum albumin but not by egg albumin.

Antibacterial Activity in the Presence of Some Surface Active Agents—Since the use of nonionic surface active agents in the cultural medium of Mycobacteria, there has been much discussion on the influence of surface active agents upon the efficacy of antibacterial agents. Although surface active agents are reported to increase the antibacterial activities in some cases, activity of many antibacterial agents have been reversed in the presence of surface active agents.[16] Usnic acid has also been reported to belong to the latter case.[17]

As the author met with the phenomenon in the course of this study, the antibacterial activity of 3-aminodibenzofuran on *Mycobac. tuberc.* A. T. C. C. No. 607 was examined in the presence of some nonionic surface active agents. Although the bacterial weight, determined by nitrogen, of submerged dispersed growth in the presence of surface active agents was about one-tenth of that of surface growth, 3-aminodibenzofuran failed to inhibit completely the growth of the organism even in a high concentration by the addition of surface active agents (Table VII). The antibacterial

15) e.g. R. J. Dubos, *et al.*: J. Exptl. Med., **83**, 409(1946), **85**, 9(1947).
16) e.g. A. S. Youmans, G. P. Youmans: J. Bacteriol., **56**, 245(1948); H. S. Forrest, P. D. Hart, J. Walker: Nature, **160**, 94(1947); C. A. Lawrence, A. L. Erlandson, Jr.: J. Am. Pharm. Assoc., **42**, 352(1953); M. Aoki, *et al.*: J. Pharm. Soc. Japan, **76**, 939(1956), **77**, 410(1957).
17) A. Stoll, *et al.*: Schweiz. Zeit. allgem. Pathol. Bacteriol., **13**, 752(1950).

actions of sodium 3-dibenzofurylaminomethanesulfonate, 3-hydroxy-, 3,7-dihydroxy-, and 1,9-dimethyl-3,7-dihydroxydibenzofurans were also reversed in the presence of a surface active agent, Tween 80 (Table Ⅷ).

Table Ⅶ.

	Dilution ($\times 10^4$)											
	1	2	4	8	16	32	64	128	256	512	1024	control
3-Aminodibenzofuran	−	−	−	−	−	−	−	−	−	++	++	++
+Tween 80(0.5%)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
+Tween 80(0.05%)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
+Tween 80(0.005%)	(±)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	± (+)	± (+)	± (+)
+Tween 20(0.05%)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	+ (+)
+Tween 40(0.05%)	(±)	(±)	(±)	(±)	(±)	(±)	(±)	(±)	(+)	(+)	++ (+)	++ (+)
+Tween 60(0.05%)	(±)	(±)	(+)	(+)	(++)	(++)	(++)	(++)	(++)	(++)	++ (+)	++ (+)
+Tween 85(0.05%)	(+)	(+)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	++ (+)	++ (+)
+Span 80(0.05%)	(+)	(+)	(+)	(+)	(+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)
+Triton N 100(0.05%)	−	−	−	(±)	(±)	(±)	(±)	(+)	(+)	++ (+)	++ (++)	++ (++)

(±), (+), (++) indicate submerged and dispersed growth.
±, +, ++, ++ indicate granular surface growth.

Table Ⅷ.

	Dilution ($\times 10^4$)											
	1	2	4	8	16	32	64	128	256	512	1024	control
Sodium 3-aminomethanesulfonate	−	−	−	−	−	−	−	−	±	++	++	++
+Tween 80(0.05%)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
3-Hydroxydibenzofuran	−	−	−	−	−	−	±	±	++			
+Tween 80(0.05%)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)			
3,7-Dihydroxydibenzofuran	−	−	+	++	++	++	++					
+Tween 80(0.05%)	(±)	(+)	(+)	(+)	(+)	(+)	(+)					
1,9-Dimethyl-3,7-dihydroxydibenzofuran	−	−	−	−	−	++	++					
+Tween 80(0.05%)	(±)	(±)	(+)	(+)	(+)	(+)	(+)					

The mechanism of these phenomena would be explained by the occlusion of the agents into the micelle of surface active agents, which causes decrease of concentration of the agents around the organism.[18] The activity of sodium 3-dibenzofurylaminomethanesulfonate, which is more soluble in water than in organic solvents, was also reversed by the surface active agents. The fact might suggest that the compound exhibited the activity after it was metabolised to an active form, such as free amine form.

The interaction of the dibenzofuan derivatives with serum and nonionic surface active agents was assumed to be worth while to study, on which some experiments are now in pregress.

III. Conclusion

As reported in the preceding four papers,[1,3~5] the author and his associates synthesized about 150 kinds of dibenzofuran derivatives to examine their antibacterial activities and a distinct structure-activity relationship was established as was summarized in this paper.

Although some dibenzofuran derivatives seemed to be fairly effective against Mycobacterium so far as synthetic media were employed, none of them would be

18) c.f. A.E. Alexander, A.J.H. Tomlinson : "Surface Chemistry," Interscience Publishers Inc., New York, 317(1949); M. Aoki, *et al.* : *loc. cit.*

useful as chemotherapeutic agents, because the effetiveness was markedly reversed by the serum.

(**Addendum**) About forty derivatives of this series were submitted to the tests for efficacy against Salmonella-Dysentery group bacilli and as antitumor agents. In the former test, 3-amino-, 3-amino-8-chloro-, and 3-guanidino-8-chloro-dibenzofurans inhibited the growth of following organisms at $10^{-4}M$: *Staph. aureus* Terashima, *E. coli communis*, *Shigella dysenteriae* Ewing I, *S. flexneri* 2a, *S. flexneri* sulfathiazole-resistant strain, *Salmonella typhi* S 57, *Sal. enteritidis* 5168, and *Sal. paratyphi* A 1015. In the antitumor test, 4-hydroxy-, 2-hydroxy-, 2-hydroxy-x,x'-dinitro-, 2-hydroxy-3-amino-dibenzofurans and didymic acid showed some activity by the method[19] based on the inhibition of the dehydrogenase activity of carcinoma cell suspension buried in the agar. The activity exhibited by 2-hydroxy-3-aminodibenzofuran was thought to be interesting, because 2-methoxy-3-amino-dibenzofuran had been reported as a carcinogenic substance.[20] A part of these tests has been reported[21] and the rest will be published in future.

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Summary

The relationship between the chemical structure and antibacterial activity observed in compounds related to dibenzofuran was conclusively elucidated. Some attempts were also made for studying the mode of action of these compounds.

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19) S. Yamazaki, *et al.*: J. Antibiotics, **9**, 135(1956).
20) A private communication from Prof. Ng. Ph. Buu-Hoi, The Radium Institute, The University of Paris.
21) S. Akiya: Japan. J. Exptl. Med., **26**, 91(1956).