UDC 576.852.2.1095.18:547.728

23

93. Shinsaku Natori: Antibacterial Effect of Lichen Substances and Related Compounds. V.[1] Dibenzofuran Derivatives. (3).

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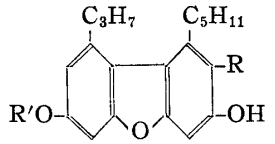
Starting from the antibacterial action of dibenzofuran group of lichen substances, i.e. didymic acid derivatives (I) and usnic acid (II), the author and his associates have been examining the relationship between chemical structure and antibacterial activity of dibenzofuran derivatives. In the previous papers[1,2] of this series, syntheses of about forty kinds of dibenzofuran derivatives, especially having a substituent at the 3-position of the ring, with or without a further substituent at the 8-position, were described and the compounds possessing amino, guanidino, or hydroxyl group at the 3-position showed a considerably strong inhibitory action against *Mycobacterium tuberculosis* A. T. C. C. No. 607 and $H_{37}Rv$ in synthetic media.

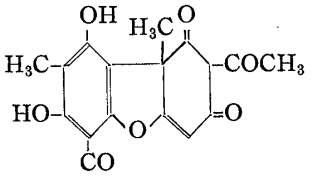
In the present paper, further syntheses and antibacterial activity of about sixty dibenzofuran derivatives, which consist of 3-, 2-, 4-, or 1-monosubstituted compounds, 2,3-, 1,4-, or 2,8-disubstituted compounds, and some tetrahydrodibenzofuran derivatives, are reported in elucidating the structure-activity relationship.

(I)

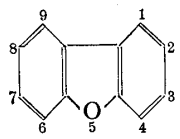


R=COOH, R'=CH_3: Didymic acid

R=R'=H: Decarboxynordidymic acid



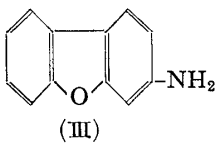
(II)



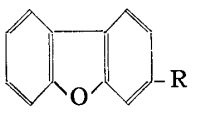
Syntheses of Dibenzofuran Derivatives

I. 3-Substituted Derivatives

3-Aminodibenzofuran (III), which was the most effective of the compounds reported in the previous paper,[2] was converted into amidino derivative (VI) as an analog of the effective guanidino compound.[2] 3-Amino compound (III) was also derived to *sec*-, *tert*-, and *quat*-amines (VII, VIII, and XI) in order to observe the effect of stepwise methylation. Sodium 3-dibenzofurylaminomethanesulfonate (XII) was prepared to gain a more water-soluble compound. The syntheses were carried out as shown in Chart 1.



(III)



(IV) R=Br

(V) R=CN ⟶ (VI) R=$-C(=NH)-NH_2$

(VII) R=$N(CH_3)_3I$

(VIII) R=$N(CH_3)_2$

(IX) R=$NHSO_2 \cdot C_6H_4 \cdot CH_3$ ⟶

(X) R=$N(CH_3)SO_2 \cdot C_6H_4 \cdot CH_3$ ⟶

(XI) R=$NHCH_3$

(XII) R=$NHCH_2SO_3Na$

Chart 1.

Of these, 3-bromo- (IV),[3] 3-cyano- (V),[4] 3-dimethylamino- (VIII),[5] and 3-methylamino-dibenzofuran (XI)[5] are known compounds, in which (XI) showed somewhat different m.p. from that described in

* Hongo, Tokyo (名取信策).

1) Part IV: S. Shibata, S. Natori, T. Kawakami, M. Okano, Y. Tsuchimoto: This Bulletin, **2**, 45(1954).
2) S. Shibata, S. Natori, Y. Sumi: J. Pharm. Soc. Japan, **72**, 1333(1952).
3) M. N. Cullinane, H. J. H. Padfield: J. Chem. Soc., **1935**, 1131.
4) W. Borsche, W. Bothe: Ber., **41**, 1941(1908).
5) W. H. Kirkpatrick, P. T. Parker: J. Am. Chem. Soc., **57**, 1123(1935).

the literature.

3-Dibenzofuranamidine Hydrochloride (VI)—To a suspension of 3-cyanodibenzofuran (V) (0.6 g.) in EtOH (dehydrated, 12 cc.), dry HCl gas was passed through till red-violet solution was formed. After 10 days' standing at a room temperature, the deposited colorless crystals (imidoether hydrochloride) were collected and washed thoroughly with dehyd. Et_2O. The dried crystals were again dissolved in EtOH saturated with NH_3 (20 cc.), warmed at around 50° for 4 hrs., and kept standing at a room temperature for several days. After evaporation of the solvent, the residue was extracted with 10% HCl, and the hydrochloride separated from the extracts after cooling was recrystallized from water to colorless needles, m.p. 296~298°. *Anal.* Calcd. for $C_{13}H_{10}ON_2 \cdot HCl \cdot H_2O$: C, 58.79; H, 4.95; N, 10.58. Found : C, 58.85; H, 4.98; N, 10.16.

3-Dimethylaminodibenzofuran Methiodide (VII)—3-Aminodibenzofuran (III) was exhaustively methylated with MeI in the presence of Na_2CO_3. Recrystallization from MeOH afforded colorless scales, m.p. 189~190°(with efferv.), in a quantitative yield. *Anal.* Calcd. for $C_{15}H_{16}ONI$: C, 51.01; H, 4.56; N, 3.96. Found : C, 51.23; H, 4.91; N, 4.21.

3-Methylaminodibenzofuran (XI)—3-Aminodibenzofuran (III) was tosylated by the usual method to give tosylamino compound (IX) of m.p. 142° after recrystallization from MeOH. *Anal.* Calcd. for $C_{19}H_{15}O_3NS$: N, 4.15. Found : N, 4.10.

The tosylamino derivative was methylated with Me_2SO_4 and KOH, giving N-methyltosylamino derivative (X) as colorless needles, m.p. 114~115°, after recrystallization from MeOH. *Anal.* Calcd. for $C_{20}H_{17}O_3NS$: N, 3.99. Found : N, 4.14.

Hydrolysis of the N-methyltosylamino compound with $AcOH-H_2SO_4$ gave the objective compound (XI), which was separated as the hydrochloride, and the free base liberated by the action of NH_4OH was recrystallized from $MeOH-H_2O$ to colorless needles, m.p. 73~74°(literature[5]), m.p. 48~49°). *Anal.* Calcd. for $C_{13}H_{11}ON$: C, 79.17; H, 5.62; N, 7.10. Found : C, 78.79; H, 5.34; N, 7.00.

Sodium 3-Dibenzofurylaminomethanesulfonate (XII)—A mixture of EtOH solution (20 cc.) of 3-aminodibenzofuran (III) (1.8 g.) and aqueous solution (10 cc.) of sodium hydroxymethanesulfonate (monohydrate, 1.5 g.) was refluxed for 30 mins., the crystals that separated at the end of the period were collected after cooling, and recrystallized from BuOH saturated with H_2O to colorless minute needles, m.p. 263~266°(decomp.). The yield was almost quantitative. *Anal.* Calcd. for $C_{13}H_{10}O_4NSNa$: C, 52.16; H, 3.36; N, 4.68. Found : C, 52.36; H, 3.18; N, 4.44.

II. 2-Substituted Derivatives

In order to confirm the effect of the substituted position on antibacterial activity, attempts were made on the syntheses of monosubstituted dibenzofurans having NH_2 or OH in positions other than 3, viz. 2-, 4-, or 1-position.

As for 2-substituted derivatives, nitro (XIII),[6~8] bromo (XIV),[9] amino (XV),[6,9~11] acetamido (XVI),[6,11] hydroxy (XVII),[11,12] methoxy (XVIII),[12] carboxy (XX),[13] and ethyl (XXI)[14,15] derivatives were prepared by

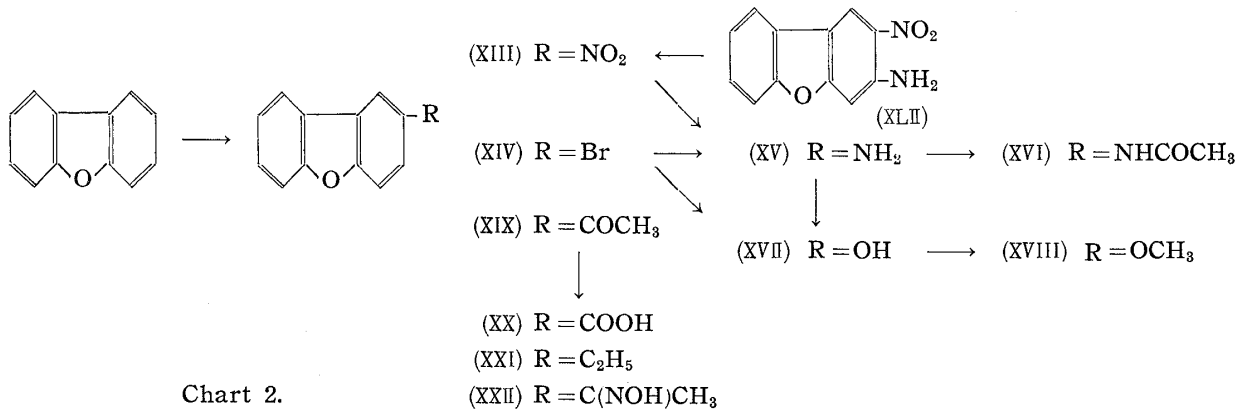


Chart 2.

6) H. Gilman, G.E. Brown, W.G. Bywater, W.H. Kirkpatrick : J. Am. Chem. Soc., **56**, 2473(1934).
7) S. Yamashiro : J. Chem. Soc. Japan, **57**, 715(1936).
8) H. Gilman, R.K. Ingham : J. Am. Chem. Soc., **75**, 4843(1953).
9) F. Mayer, W. Krieger : Ber., **55**, 1659(1922).
10) I.G. Farbenindustrie : D.R.P. 591,213 (Frdl., Fortschr. Teerfarb.-Fabrikat., **20**, 436(1933)).
11) H. Gilman, B.G. Bywater, P. Parker : J. Am. Chem. Soc., **57**, 885(1935).
12) H. Gilman, P.R. Van Ess : *Ibid.*, **61**, 1365(1939).
13) H. Gilman, P.T. Parker, J.C. Bailie, G.E. Brown : *Ibid.*, **61**, 2836(1939).
14) W. Borsche, B. Schacke : Ber., **56**, 2498(1923).
15) Brit. Pat. 633,151 (C.A., **44**, 5914(1950)).

the routes shown in Chart 2 following the directions shown in the respective literatures. 2-Hydroxy derivative (XVII) was obtained in a better yield by the decomposition of the Grignard compound of 2-bromo derivative (XIV) with air than by potash fusion of the bromo derivative in a sealed tube with $CuSO_4$ or by diazotization and decomposition of 2-aminodibenzofuran (XV).

There has been many reports[13~16] on the acetylation of dibenzofuran and the chief reaction product was well established to be 2-acetyldibenzofuran (XIX), which was recorded as an oily substance, accompanying crystalline 2,8-diacetyl compound (XXXVII). Further purification and separation were performed in this study as follows :

Acetylated Dibenzofurans—Dibenzofuran (50.4 g.) in CS_2(150 cc.) was acetylated with AcCl (24 g.) in the presence of $AlCl_3$ (60 g.) in a usual manner, and the reaction products, after working as usual, were distilled *in vacuo* to yield the following fractions : i) b.p$_4$ 104~107°, recovery of the starting material (20.1 g.); ii) b.p$_4$ 150~160°, colorless oil which solidified after cooling (22.8 g.); iii) b.p$_4$ 175~185°, colorless mass(5.1 g.); and iv) residue (ca. 2 g.). The 2nd and 3rd fractions were purified further through alumina column using benzene as the developer. The 2nd fraction was revealed to be composed chiefly of the main reaction product, 2-acetyldibenzofuran (XIX), which was isolated as colorless needles of m.p. 71~72° after recrystallization from MeOH. Yield, 17.7 g. *Anal.* Calcd. for $C_{14}H_{10}O_2$ (2-Acetyldibenzofuran) : C, 79.98; H, 4.79. Found : C, 79.53; H, 4.82. Oxime (XXII) : Colorless prisms (from EtOH), m.p. 132~133°. *Anal.* Calcd. for $C_{14}H_{11}O_2N$: N, 6.22. Found : N, 6.25.

The 2nd fraction was contaminated with a small amount of colorless scales of m.p. 126~129°, more sparingly soluble in MeOH, analytical data of which agreed with monoacetyl compound. *Anal.* Calcd. for $C_{14}H_{10}O_2$: C, 79.78; H, 4.79. Found : C, 80.07; H, 5.01.

The chromatographic separation of the 3rd fraction chiefly gave 2,8-diacetyldibenzofuran (XXXVII) as colorless needles, m.p. 162~163°, showing agreement with the literature.[13] *Anal.* Calcd. for $C_{16}H_{12}O_3$: C, 76.18; H, 4.80. Found : C, 76.36; H, 4.86.

III. 4-Substituted Derivatives

Since Gilman and his collaborators[17] found that the metallation of dibenzofuran occurred preferentially at the 4-position, many 4-substituted derivatives[8,12] were synthesized through 4-metallated dibenzofuran. However, it remained obscure why 4-substituted derivatives prepared by Yamashiro[18] through different methods showed discrepancy in melting points with those of Gilman's compound. In order to get confirmation of this point, 4-dibenzofurancarboxylic acid (XXIII) was synthesized by ring closure as shown in Chart 3. As the product showed identity with the compound prepared by the decomposition of dibenzofuryllithium with carbon dioxide,[5,17,19] other 4-substituted derivatives, i.e. bromo- (XXV),[13] hydroxy- (XXVI),[20,21] amino- (XXVII),[8] and acetamido-dibenzofuran (XXVIII),[5] were prepared by the respective treatment of 4-dibenzofuryllithium.

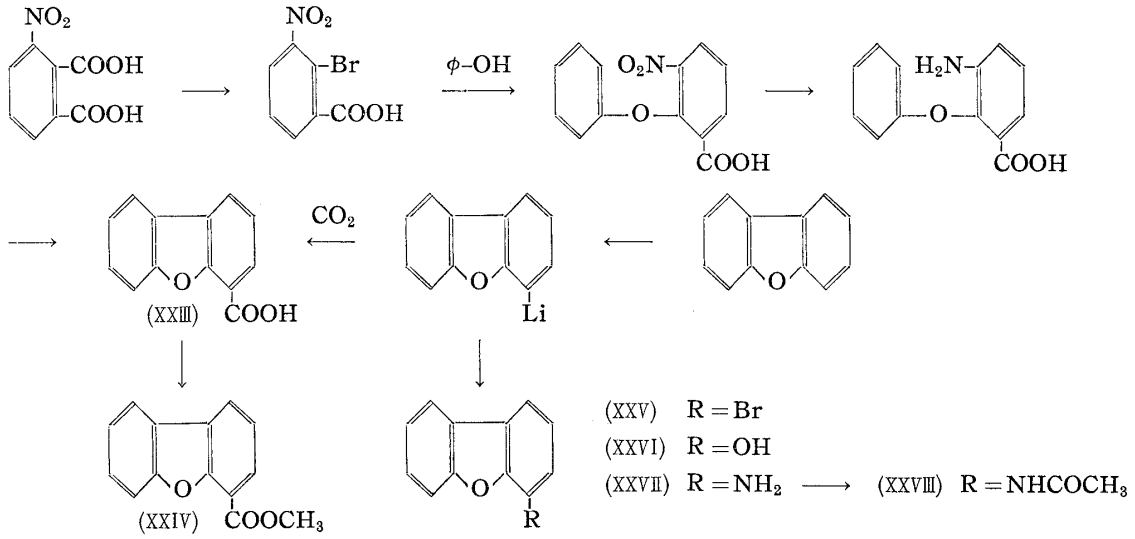


Chart 3.

16) P. Galewsky : Ann., **264**, 187(1891).
17) H. Gilman, R.V. Young : J. Am. Chem. Soc., **56**, 1415(1934).
18) S. Yamashiro : J. Chem. Soc. Japan, **57**, 715(1936); **59**, 186, 443, 945(1938); Bull. Chem. Soc. Japan, **16**, 61(1941); **17**, 10, 76, 172(1942).
19) H. Gilman, M.W. Van Ess, D.M. Hayes : J. Am. Chem. Soc., **61**, 643(1939).
20) H. Gilman, R.V. Young : *Ibid.*, **57**, 1121(1935).
21) H. Gilman, L.C. Cheney, H.B. Willis : *Ibid.*, **61**, 951(1939).

4-Dibenzofurancarboxylic Acid (XXIII)—3-Amino-2-phenoxybenzoic acid[22] (2.5 g.), prepared by the condensation of 2-bromo-3-nitrobenzoic acid[23] and phenol and subsequent reduction, was suspended in 10% HCl (70 cc.) and diazotized with $NaNO_2$ (0.75 g.) at about 0° for 3 hrs. The diazonium salt suspension was dropped into boiling 50% H_2SO_4 (70 cc.), the decomposition was completed in 3 hrs., and the deposited crystals were collected after cooling. Recrystallization from xylene or AcOH gave colorless needles, m.p. 203~205°; yield, 0.9 g. *Anal.* Calcd. for $C_{13}H_8O_3$: C, 73.58; H, 3.80. Found: C, 73.86; H, 3.89.

The identity of the sample with that prepared from dibenzofuryllithium by Gilman's method[5] was confirmed by mixed fusion. Admixture of the respective methyl esters,[19] m.p. 88~90°, also showed no depression of the melting point.

IV. 1,4-Disubstituted and 1-Substituted Derivatives

Nitration of 4-acetamidodibenzofuran (XXVIII) gave a mixture of 3-nitro and 1-nitro derivatives irrespective of the reaction temperature.[24] On the contrary, bromination took place chiefly at the 1-position to give 4-acetamido-1-bromodibenzofuran (XXXI),[12] from which 1-hydroxy (XXXIV),[12] 1-amino (XXXV),[12] and 1-acetamido (XXXVI)[12] derivatives were synthesized through 4-amino-1-bromo (XXXII)[12] and 1-bromo (XXXIII)[12] compounds as intermediates (Chart 4).

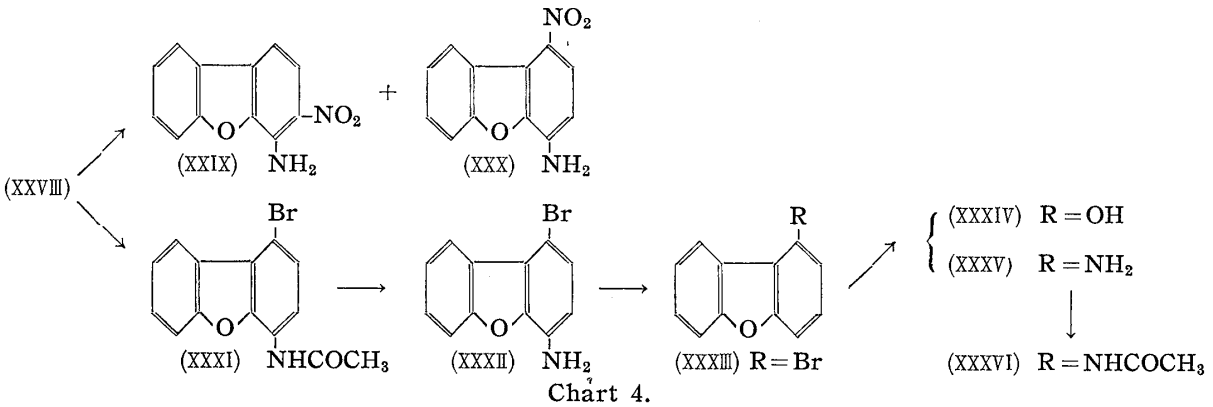
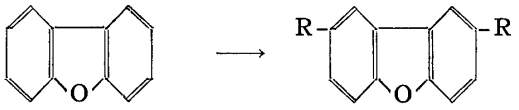


Chart 4.

V. 2,8-Disubstituted Derivatives

Following four compounds were synthesized by the known methods: 2,8-Diacetyl- (XXXVII),[13] -dibromo- (XXXVIII),[25,26] -diamino- (XXXIX),[27] and -diacetamido-dibenzofurans (XL)[27] (Chart 5); (XXXVII) was mentioned above.



(XXXVII) R=$COCH_3$
(XXXVIII) R=Br ⟶ (XXXIX) R=NH_2
⟶ (XL) R=$NHCOCH_3$

Chart 5.

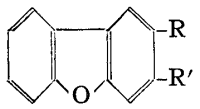
VI. 2,3-Disubstituted Derivatives

Antibacterial actions of amines and phenols are often much regulated by their basic or acidic strength. To make this point clear, 2- or 3-amino- and -hydroxy-dibenzofurans were substituted further at the 3- or 2-position by the synthetic procedure shown in Chart 6.

Of these, (XLI),[6,14] (XLII),[6,14] (XLIII),[28] (XLIV),[6,14] (XLVI),[29] (XLVII),[29] (LI),[28] and (LII)[28] are known compounds and do not require further mention. Nitration of 2-hydroxy- (XVII) and 2-ethyl-dibenzofuran (XXI) occurred at the 3-position in either case. Position of the nitro group was established in the former by the fact that the same hydroxynitro compound (XLVIII) was obtained from 2-methoxy-3-nitrodibenzofuran (LI) and in the latter by its derivation to 3-nitrodibenzofuran.

2,3-Diacetamidodibenzofuran (XLV)—2,3-Diaminodibenzofuran (XLIV) was acetylated by the usual manner to form colorless needles, m.p. 267~271°, after recrystallization from AcOH. *Anal.* Calcd. for $C_{16}H_{14}O_3N_2$: C, 68.07; H, 5.00; N, 9.92. Found: C, 68.20; H, 4.95; N, 10.21.

22) A.A. Goldberg, H.A. Walker: J. Chem. Soc., **1953**, 1348.
23) Org. Syntheses, Coll. Vol. I, 56, 125, 408.
24) cf. H. Gilman, A.L. Jacoby, J. Swisslowsky: J. Am. Chem. Soc., **61**, 954(1939); H. Gilman, J. Swiss: *Ibid.*, **66**, 1884(1944).
25) N.M. Cullinane, H.G. Davey, H.J.H. Padfield: J. Chem. Soc., **1934**, 716.
26) W. Hoffmeister: Ann., **159**, 215(1871).
27) J. Swisslowsky: Iowa State Coll. J. Sci., **14**, 92(1939)(C.A., **34**, 6274(1940))(cf. S. Yamashiro: Footnote 18).
28) H. Gilman, S. Avakian: J. Am. Chem. Soc., **68**, 500(1946).
29) H. Gilman, M.W. Van Ess: *Ibid.*, **61**, 3146(1939).



R=H, R′=NHCOCH$_3$ ⟶ (XLI) R=NO$_2$, R′=NHCOCH$_3$ ⟶
(XLII) R=NO$_2$, R′=NH$_2$ ⟨ (XLIII) R=NO$_2$, R′=OH / (XLIV) R=R′=NH$_2$ ⟶ (XLV) R=R′=NHCOCH$_3$
R=H, R′=OH ⟶ (XLVI) R=N$_2$C$_6$H$_5$, R′=OH ⟶ (XLVII) R=NH$_2$, R′=OH
(L) R=OH, R′=NH$_2$
(XVII) R=OH, R′=H ⟶ (XLVIII) R=OH, R′=NO$_2$ + (XLIX) R=OH, x,x′-NO$_2$
(XVIII) R=OCH$_3$, R′=H ⟶ (LI) R=OCH$_3$, R′=NO$_2$ ⟶ (LII) R=OCH$_3$, R′=NH$_2$
(XXI) R=C$_2$H$_5$, R′=H ⟶ (LIII) R=C$_2$H$_5$, R′=NO$_2$ ⟶ (LIV) R=C$_2$H$_5$, R′=NH$_2$
(LV) R=COOH, R′=NO$_2$ ⟶ R=H, R′=NO$_2$

Chart 6.

2-Hydroxy-3-nitro- (XLVIII) and 2-Hydroxy-x,x'-dinitro-dibenzofuran (XLIX)—i) 2-Methoxy-3-nitrodibenzofuran (LI)[28](2 g.) in AcOH (40 cc.) and HBr(30 cc.) was boiled for 8 hrs. After cool, separated crystals were purified from Me_2CO to yellow needles, m.p. 207~209°. *Anal.* Calcd. for $C_{12}H_7O_4N$: C, 62.89; H, 3.08; N, 6.11. Found : C, 62.94; H, 3.03; N, 6.20.

ii) Nitration of 2-hydroxydibenzofuran (XVII)(1.25 g.) in AcOH(20 cc.) with HNO_3(d.=1.49, 10 cc.) under cooling proceeded in 2 hrs. The product deposited by the addition of water was separated, dried, and dissolved again in a mixture of benzene-$CHCl_3$(5:1) to remove sparingly-soluble portion (A). The solution was chromatographed through alumina column, forming two bands. From the lower red band, yellow needles, m.p. 209~210°, were separated in a yield of 0.6 g., which showed no depression of m.p. on admixture with the sample obtained from (i). The upper deep red band of the chromatogram was cut off and extracted with MeOH, treated with HCl to decompose the Al-salt, and recrystallized from AcOH to yellow scales, m.p. 242~243°. The above-mentioned (A) also gave the same product; total yield, 0.3 g. From analytical results it was assumed to be a dinitro compound. *Anal.* Calcd. for $C_{12}H_6O_6N_2$: C, 52.56; H, 2.21; N, 10.22. Found : C, 52.33; H, H, 2.35; N, 10.37.

2-Hydroxy-3-aminodibenzofuran (L)—2-Hydroxy-3-nitrodibenzofuran (XLVIII)(0.7 g.) in AcOH(5 cc.) was reduced with $SnCl_2 \cdot 2H_2O$(3.2 g.) in HCl(4 cc.). The separated hydrochloride of the objective amine was liberated by the action of NH_4OH and recrystallization from EtOH-H_2O afforded colorless scales, m.p. 204~207°(decomp., after coloring around 195°). *Anal.* Calcd. for $C_{12}H_9O_2N$: C, 72.35; H, 4.55; N, 7.03. Found : C, 72.66; H, 4.75; N, 7.11.

2-Ethyl-3-nitrodibenzofuran (LIII)—To AcOH solution (40 cc.) of 2-ethyldibenzofuran (XXI)(7.0 g.), HNO_3(d.=1.5, 4.6 cc.) was added under stirring at a room temperature and the reaction was completed by warming on a boiling water bath for 1 hr. After cool, water was added and the precipitate was taken up in Et_2O. The ethereal layer was washed thoroughly and evaporated to leave a residue, which was passed through an alumina column as a benzene solution. As a main fraction of the eluates, pale yellow needles of m.p. 102~104° was obtained by recrystallization from MeOH. Yield, 4.0 g. *Anal.* Calcd. for $C_{14}H_{11}O_3N$: C, 69.70; H, 4.59; N, 5.80. Found : C, 69.42; H, 4.70; N, 5.68.

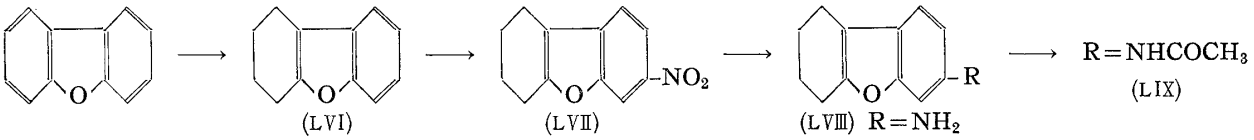
To confirm the position of the nitro group, (LIII)(0.5 g.) in pyridine (10 cc.) was oxidized with $KMnO_4$(5 g.) in H_2O(100 cc.) on a boiling water bath for 10 hrs. The precipitated MnO_2 was removed and acidification of the filtrate separated a yellow powder. Nitrodibenzofurancarboxylic acid (LV) thus formed was recrystallized from MeOH, melting at 180~182°.

The acid (0.16 g.) was heated with Cu powder (0.05 g.) in quinoline(10 cc.) at 200~210° for 1 hr. After cool, the whole was taken up in Et_2O, Et_2O layer washed thoroughly, evaporated, and the residue was again dissolved in benzene to pass through an alumina column. The eluate gave pale yellow needles, m.p. 180~182°, which showed no depression when fused with the authentic specimen of 3-nitrodibenzofuran.[2]

2-Ethyl-3-aminodibenzofuran (LIV)—2-Ethyl-3-nitrodibenzofuran (LIII) (0.6 g.) was dissolved in AcOH (6 cc.) and reduced with $SnCl_2 \cdot 2H_2O$(2 g.) in HCl(3 cc.) at around 60°. The reduction product separated out as hydrochloride, which gave the free base as colorless prisms, m.p. 68~70°, by liberation with NH_4OH and recrystallization from MeOH-H_2O. *Anal.* Calcd. for $C_{14}H_{13}ON$: C, 79.59; H, 6.20; N, 6.63. Found : C, 79.58; H, 6.39; N, 6.65.

VII. Tetrahydrodibenzofuran Derivatives

Hydrogenation of dibenzofuran ring causes the loss of planarity and a change in aromaticity, which would result in some alteration of antibacterial activity. From this respect, 7-amino-1,2,3,4-tetrahydrodibenzofuran (LVIII), corresponding to the effective 3-aminodibenzofuran (III), was prepared by the known methods[30,31] shown in Chart 7.



(LVI) (LVII) (LVIII) R=NH_2

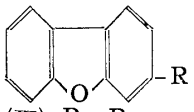
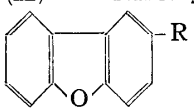
Chart 7.

Antibacterial Action of Dibenzofuran Derivatives

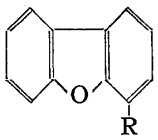
Methods—Test Organisms : *Staphylococcus aureus* Terashima strain, *Escherichia coli communior* type, *Mycobacterium tuberculosis* A.T.C.C. No. 607, and *M. tuberc.* $H_{37}Rv$.

Media : Nutrient broth was used for the former two organisms, the Lockemann-Bloch synthetic

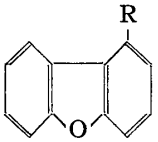
Table I. Antibacterial Activity of Dibenzofuran Derivatives

Compound	Highest dilution for complete inhibition ($\times 10^4$)				
	St. aureus Terashima	*E. coli communior*	*Mycobacterium tuberculosis*		
			A.T.C.C. No. 607	$H_{37}Rv$	
	Nutrient broth	Nutrient broth	Lockemann-Bloch	Sauton	Kirchner
Streptomycin sulfate	—	—	128~256	—	200
Isonicotinic acid hydrazide	—	—	16~32	—	1,000
p-Aminosalicylic acid	—	—	<1	—	—
p,p'-Diaminodiphenyl sulfone	—	—	32	—	—
(I) Didymic acid	8	—	16	2	<2
(I) Decarboxynordidymic acid	64	—	16	—	—
(II) Usnic acid	16	—	64	—	—
(III) 3-Aminodibenzofuran	4	2	256~512	256*	4
3-Hydroxydibenzofuran	4	2	64~128	64	2
					
(IV) R=Br	<0.5	<0.5	—	—	—
(V) CN	<0.5	<0.5	—	—	—
(VI) $C(=NH)NH_2$	1	1	1	—	—
(VII) $N(CH_3)_3I$	2	<0.5	1	<2	<2
(VIII) $N(CH_3)_2$	0.5	<0.5	8	—	—
(IX) $NHSO_2 \cdot C_6H_4 \cdot CH_3$	1	0.5	—	—	—
(X) $N(CH_3)SO_2 \cdot C_6H_4 \cdot CH_3$	<0.5	<0.5	—	—	—
(XI) $NHCH_3$	4	<0.5	32	—	—
(XII) $NHCH_2SO_3Na$	1	0.5	256~512	32*	<2
					
(XIII) R=NO_2	<0.5	<0.5	—	—	—
(XIV) Br	<0.5	<0.5	—	—	—
(XV) NH_2	1	1	64	64*	4*
(XVI) $NHCOCH_3$	<0.5	<0.5	—	—	—
(XVII) OH	2	2	32	32*	<2
(XVIII) OCH_3	<0.5	<0.5	2	—	—
(XIX) $COCH_3$	4	<0.5	<1	—	—
(XX) COOH	<0.5	<0.5	—	—	—
(XXI) C_2H_5	<0.5	<0.5	1	—	—
(XXII) $C(=NOH)CH_3$	<0.5	<0.5	—	—	—

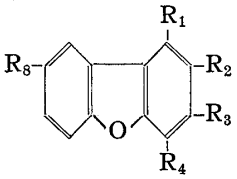
30) H. Gilman, E.W. Smith, L.C. Cheney : J. Am. Chem. Soc., **57**, 2095(1935).
31) J.v. Braun : Ber., **55**, 3761(1922).



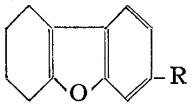
(XXIII)	R=COOH	<0.5	<0.5	—	—	—
(XXIV)	$COOCH_3$	0.5	<0.5	—	—	—
(XXV)	Br	0.5	<0.5	—	—	—
(XXVI)	OH	1	1	16	4	<2
(XXVII)	NH_2	0.5	1	16	64*	4*
(XXVIII)	$NHCOCH_3$	<0.5	<0.5	—	—	—



(XXXIII)	R=Br	<0.5	<0.5	—	—	—
(XXXIV)	OH	4	4	8	2	<2
(XXXV)	NH_2	<0.5	<0.5	<1	—	—
(XXXVI)	$NHCOCH_3$	<0.5	<0.5	—	—	—



(XXIX)	$R_3=NO_2$, $R_4=NH_2$, $R_1:R_2:R_8=H$	0.5	<0.5	—	—	—
(XXX)	$R_1=NO_2$, $R_4=NH_2$, $R_2:R_3:R_8=H$	<0.5	<0.5	<1	—	—
(XXXI)	Br, $NHCOCH_3$, 〃	<0.5	<0.5	—	—	—
(XXXII)	〃 NH_2, 〃	<0.5	<0.5	16	—	—
(XXXVII)	$R_2:R_8=COCH_3$, $R_1:R_3:R_4=H$	<0.5	<0.5	—	—	—
(XXXVIII)	Br, 〃	<0.5	<0.5	—	—	—
(XXXIX)	NH_2, 〃	<0.5	<0.5	<1	—	—
(XL)	$NHCOCH_3$, 〃	2	<0.5	—	—	—
(XLI)	$R_2=NO_2$, $R_3=NHCOCH_3$, $R_1:R_4:R_8=H$	0.5	<0.5	—	—	—
(XLII)	NO_2, NH_2, 〃	<0.5	<0.5	<1	<4	<4
(XLIII)	NO_2, OH, 〃	0.5	<0.5	<1	—	—
(XLIV)	NH_2, NH_2, 〃	4	<0.5	2	4	<4
(XLV)	$NHCOCH_3$, $NHCOCH_3$, 〃	<0.5	<0.5	—	—	—
(XLVI)	$N_2C_6H_5$, OH, 〃	<0.5	<0.5	—	—	—
(XLVII)	NH_2, OH, 〃	2	0.5	2	—	—
(XLVIII)	OH, NO_2, 〃	<0.5	<0.5	<1	—	—
(XLIX)	$R_2=OH$, x,x′-dinitro	8	0.5	4	—	—
(L)	$R_2=OH$, $R_3=NH_2$, $R_1:R_4:R_8=H$	8	0.5	2	4	<4
(LI)	OCH_3, NO_2, 〃	<0.5	<0.5	—	—	—
(LII)	OCH_3, NH_2, 〃	2	0.5	32	128*	<4
(LIII)	C_2H_5, NO_2, 〃	<0.5	<0.5	—	—	—
(LIV)	C_2H_5, NH_2, 〃	8	<0.5	16	—	—
(LV)	COOH, NO_2, 〃	<0.5	<0.5	—	—	—



(LVI)	R=H	<0.5	<0.5	<1	—	—
(LVII)	NO_2	<0.5	<0.5	<1	—	—
(LVIII)	NH_2	0.5	1	32	16	<4
(LIX)	$NHCOCH_3$	0.5	<0.5	<1	—	—

* In the case of antibacterial activity test against *Mycobac. tuberc.* $H_{37}Rv$, a minute amount of flocculent growth was occasionally observed in the bottom of the test tube in the presence of a rather high amount of the agent. In such a case it was read inhibitory unless further multiplication or surface growth was observed by extention of the incubation time and, therefore, the figures with asterisk are nearly the same as that observed by the inhibition of surface growth.

medium for *M. tuberc.* A.T.C.C. No. 607. In the case of *M. tuberc.* $H_{37}Rv$, Sauton's synthetic medium and Kirchner's medium (containing 10% horse serum) were employed and the results were compared.

Procedure : Each sample was dissolved in the medium in 1:5,000 for *St. aureus* and *E. coli*, 1:10,000 for *M. tuberc.* A.T.C.C. No. 607, and 1:20,000 for *M. tuberc.* $H_{37}Rv$, with or without the aid of Me_2CO (less than 2% for final concentration), sterilized, and serially diluted with the medium aseptically to make a dilution series, in which each tube contained 3 cc. (5 cc. for *M. tuberc.* $H_{37}Rv$) of the solution.

Inoculum : *St. aureus* and *E. coli* were incubated in nutrient broth for 24 hrs. at 37°, diluted 100-fold with physiological saline, and 0.05 cc. of this suspension was inoculated to each tube (inoculum size, ca 10^7).

In the case of *M. tuberc.* A.T.C.C. No. 607, a 4-day culture of the organisms on a glycerol-nutrient broth-agar slant was collected, minced with the 100-fold medium of the wet weight of the bacteria, and 0.03 cc. of the supernatant of the suspension was inoculated (inoculum size, ca $10^5 \sim 10^6$).

For *M. tuberc.* $H_{37}Rv$., the modified Dubos medium containing Tween 80 was employed for the culture, and 0.1 cc. each of the 2-week culture was inoculated (inoculum size, 10^7).

Readings : Results were read after incubation at 37° for 24 hrs. (*St. aur.* and *E. coli*), 3 days (*M. tuberc.* A.T.C.C. No. 607), or 3 weeks and 4 weeks (*M. tuberc.* $H_{37}Rv$; Kirchner's and Sauton's medium, respetively). Each experiment was duplicated more than twice.

Results—Highest inhibitory dilution read at the above-mentioned time is recorded in Table I.

Relationship between Chemical Structure and Antibacterial Action

Following the previous papers,[1,2] about 60 kinds of dibenzofuran derivatives were prepared, and the structure-activity relationship accompanying alteration of the position and the kind of substituents on dibenzofuran ring was examined by antibacterial activity.

Although none of the present series of compounds showed stronger inhibitory action against *St. aureus* than the original lichen acids, i.e. decarboxynordidymic acid and usnic acid, some amino- and hydroxy-dibenzofuran derivatives, such as 3-methylamino- (XI), 1-hydroxy- (XXXIV), 2,3-diamino- (XLIV), 2-hydroxy-x,x'-dinitro- (XLIX), 2-hydroxy-3-amino- (L), and 2-ethyl-3-amino-dibenzofurans (LIV), showed inhibition in the same degree as 3-amino- (III) and 3-hydroxy-dibenzofurans reported previously.[2] The action of these dibenzofuran derivatives on *E. coli* was generally weaker than that on *St. aureus*, and almost all of them should be assumed to be ineffective against the test organism.

As reported in the previous papers,[1,2] inhibitory action of 3-aminodibenzofuran and its related compounds against *Mycobac. tuberc.* A.T.C.C. No. 607 was remarkable so far as Lockemann-Bloch's medium was employed. Although compounds more effective against this strain were not found in the series of compounds reported here, the existence of a structure-activity relationship was clearly observed. As for 3-monosubstituted derivatives, the following facts were noted. The gradual methylation of 3-aminodibenzofuran (III) resulted in decrease of the activity in the order of secondary, tertiary, and quaternary amines (compounds (XI), (VIII), and (VII)). Contrary to 3-guanidinodibenzofuran,[1,2] 3-amidino derivative (VI) was ineffective. Sodium 3-dibenzofurylaminomethanesulfonate (XII) was yet slightly but more soluble in water than 3-amino compound (III), and showed inhibitory effect on the organism in the same order of dilution or in somewhat higher molar concentration than the original compound.

When the amino or hydroxyl group was transferred to the positions other than 3, viz. 2, 4, or 1, the effectiveness of those groups was assumed to be retained, whereas other substituents examined so far were ineffective as in the case of the 3-position. However, the maximum dilution inhibiting the growth of the tubercle bacillus was much reduced from 3-substituted derivatives in the order of 2-, 4-, and 1-position (XV, XVII, XXVI, XXVII, XXXIV, and XXXV); 1-aminodibenzofuran (XXXV) being not inhibitory even in 1:10,000 dilution.

The active 3-amino- and 3-hydroxy-dibenzofurans were an amine and a phenol, and were ionized though weak. In some cases, it has been observed that some correlation exists in a group of compounds between their ionization at a physiological condition, viz. pKa of compounds, and their biological activity.[32] From this respect 2,3-disubstituted compounds from (XLI) to (LV) were examined. Results were the same as the other disubstituted derivatives; that is to say, further introduction of a substituent rendered decrease of the original activity, irrespective of the electron-attracting or -repulsing character of the introduced group. For example, 2-nitro- (XLII), 2-amino- (XLIV), 2-hydroxy- (L), 2-methoxy- (LII), and 2-ethyl-3-aminodibenzofuran (LIV) were all less effective than 3-aminodibenzofuran (III), and the same was true when viewed from 3-hydroxy-, 2-amino- (XV), and 2-hydroxydibenzofuran (XVII).

7-Amino-1,2,3,4-tetrahydrodibenzofuran (LVIII), which was devoid of planarity of the molecule, showed some activity, though far weaker than the corresponding fully aromatic amine (III).

These bacteriostatic compounds inhibiting the growth of *Mycobac. tuberc.* A.T.C.C. No. 607 also showed an inhibitory effect on the growth of $H_{37}Rv$ strain so far as Sauton's medium was used, and the structure-activity relationship read by the surface growth of the organism was almost similar with that observed on No. 607 strain. Next to 3-aminodibenzofuran (III), 2-methoxy-3-aminodibenzofuran (LII) was the most effective, followed by compounds (XII), (XV), (XVII), and (XXVII). If the slight flocculent growth observed in the depth of the medium was taken into account, these compounds might be regarded as less effective, and it seemed correct to say that these series of compounds including 3-amino- and 3-hydroxydibenzofuran partially inhibited the growth of the organism even in a high dilution.

When Kirchner's medium, which contained 10% horse serum, was used, the antibacterial activities of these compounds were markedly reduced. As had been pointed out in the previous paper,[1] the decrease of activity was assumed to be due to some binding components in the serum.

The structure-activity relationship and antagonistic action of serum will be discussed further in the forthcoming paper.

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Summary

About sixty kinds of dibenzofuran derivatives having one or two substituents were synthesized, and relationship between kinds and positions of substituents and antibacterial action, especially against *Mycobac. tuberc.* A. T. C. C. No. 607, was discussed.

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32) e.g. A. Albert: "Selective Toxicity," Methuen & Co., London(1951).