**Zusammenfassung**

Zur Charakterisierung des Anthocyanins in den feuerroten Blüten von *Canna generalis* Bailey haben wir sowohl auf papierchromatographischem Wege als auch durch präparative Darstellung und chemische Analyse bewiesen, dass der genannte Farbstoff nichts anderes als das Keracyanin (3-O-Rhamnoglucosidyl-cyanidin) ist.

(Eingegangen am 15. Dezember 1953)

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## 13. Shoji Shibata, Shinsaku Natori, Tomokichi Kawakami, Minoru Okano, and Yasuyoshi Tsuchimoto: Antibacterial Effect of Lichen Substances and Related Compounds. IV[1]. Dibenzofuran Derivatives. (2).

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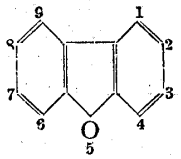
In the course of studies on the relationship between the antibacterial action and the chemical structure of lichen substances[2], the behavior of some dibenzofuran compounds, such as didymic acid derivatives, seemed to be of interest and synthesis of dibenzofuran derivatives was undertaken in order to examine their antibacterial action. In the previous paper,[1] the synthesis and antibacterial effects of 20 kinds of dibenzofurans substituted in the 3-position and their 8-chloro derivatives were reported by Shibata, Natori, and Sumi.

The present paper describes further studies on the dibenzofuran derivatives containing substituents in 3-, 3,8-, or 2,3-positions.




### Experimental

**I. Syntheses of Dibenzofuran Derivatives**—Following 22 compounds were synthesized:

**Dibenzofurans Substituted in 3-Position and their 2- or 8-Chloro Derivatives**—3-Dibenzofuranurea (I), 3-hydrazino-(II), 3-tosylamino-(III), 2-chloro-3-acetylamino-(IV), 2-chloro-3-amino-(V), 3-diacetylamino-8-chloro-(VI), 3-cyano-8-chloro-dibenzofuran (VII), 8-chloro-3-dibenzofurancarboxylic acid (VIII), ethyl 8-chloro-3-dibenzofurancarboxylate (IX), 8-chloro-3-dibenzofurancarboxylic acid amide (X), and hydrazide (XI).

**Dibenzofurans Substituted in 3,8-Positions**—3,8-Dinitro-(XII), 3,8-diamino-(XIII), 3-amino-8-nitro-(XIV), 3-chloro-8-nitro-(XV), 3-chloro-8-amino-(XVI), 3-nitro-8-acetyl-dibenzofuran (XVII), 3-nitro-8-dibenzofurancarboxylic acid (XVIII), ethyl 3-nitro-8-dibenzofurancarboxylate (XIX), 3-amino-8-dibenzofurancarboxylic acid (XX), ethyl 3-amino-8-dibenzofurancarboxylate (XXI), and 3-amino-8-dibenzofurancarboxylic acid hydrazide (XXII).

Of these, (I)[3], (II)[4], (III)[5], (XII)[6], (XIII)[6], (XIV)[6], (XVII)[3], and (XVIII)[3] are known com-

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1) Part III: S. Shibata, S. Natori, Y. Sumi: J. Pharm. Soc. Japan, 72, 1333 (1952).
2) S. Shibata, Y. Miura, H. Sugimura, Y. Toyoizumi: J. Pharm. Soc. Japan, 68, 300, 303 (1948); Japan Med. J., 1, 518 (1948), 2, 22 (1949).
3) H. Gilman, P. T. Parker, J. C. Bailie, G. E. Brown: J. Am. Chem. Soc., 61, 2836 (1939).
4) W. Borsche, W. Bothe: Ber., 41, 1949 (1908).
5) W. H. Kirkpatrick, P. T. Parker: *Ibid.*, 57, 1123 (1935); colorless needles, m.p. 142.5°.
6) N. M. Cullinane: J. Chem. Soc., 1932, 2365.

pounds and were synthesized in accordance with directions given in respective literatures.

**2-Chloro-3-acetylaminodibenzofuran (IV)**—3-Acetylaminodibenzofuran[7] dissolved in glacial acetic acid was treated with an equivalent amount of chlorine in the same medium at a room temperature. The crystalline pricipitate settled out at the end of a day and was recrystallized from diluted alcohol to colorless needles of m.p. 187.5°. Yield, quantitative. *Anal.* Calcd. for $C_{14}H_{10}O_2NCl$: C, 64.74; H, 3.88; N, 5.39. Found: C, 64.54; H, 3,74; N, 5.68.

**2-Chloro-3-aminodibenzofuran (V)**—Hydrolysis of 3-acetylamino compound (IV) with 2% alcoholic potassium hydroxide and subsequent recrystallization from hydrated alcohol gave colorless leaflets, melting at 136.5°. *Anal.* Calcd. for $C_{12}H_8ONCl$: C, 66.37; H, 3.69; N, 6.45. Found: C. 66.51; H, 4.13; N, 6.70.

**3-Diacetylamino-8-chlorodibenzofuran (VI)**—Acetylation of 3-amino-8-chlorodibenzofuran[1] with acetic anydride and anhydrous sodium acetate yielded 3-monoacetamino compound[1] of m.p. 206~208° after recrystallization from benzene. From the mother liquor benzene was evaporated to dryness and the residue was repeatedly recrystallized from methanol. Colorless prisms, m.p. 142.5~143.5°, were obtained. *Anal.* Calcd. for $C_{16}H_{12}O_3NCl$: C, 63.69; H, 4.01; N, 4.64. Found: C, 63.67; H, 4.14; N, 5.01.

**3-Cyano-8-chlorodibenzofuran (VII)**—3-Amino-8-chlorodibenzofuran[1] was diazotized and decomposed with cuprous cyanide following the procedure for preparation of 3-cyanodibenzofuran[4]. The precipitate was recrystallized from ethyl acetate or butanol with addition of activated carbon and then from a mixture of alcohol and ethyl acetate to colorless needles, m.p. 208~209°. *Anal.* Calcd. for $C_{13}H_6ONCl$: C, 68.59; H, 2.66; N, 6.15. Found: C, 68.45; H, 2.96; N, 6.22.

**8-Chloro-3-dibenzofurancarboxylic Acid (VIII)**—3-cyano compound (VII) (0.8 g.) was hydrolyzed by boiling with 10% butanolic potassium hydroxide solution (30 cc.) for 3 hrs. After cooling, potassium salt of the product that separated out was filtered off, suspended in water, acidified with diluted hydrochloric acid, and again filtered. After recrystallization from glacial acetic acid or xylene, pale yellow fine needles of m.p. 289~290° were obtained. Yield, 0.4 g. *Anal.* Calcd. for $C_{13}H_7O_3Cl$: C, 63.31; H, 2.86. Found: C, 63.57; H, 3.16.

**Ethyl 8-Chloro-3-dibenzofurancarboxylate (IX)**—(VIII) (0.2 g.) was suspended in dehydrated alcohol saturated with hydrogen chloride (30 cc.) and boiled for 2.5 hrs., when the mixture formed a clear solution. After evaporation, fine needles were separated, washed with diluted alcohol, and recrystallized from methanol to needle crystals of m. p. 108~110°. *Anal.* Calcd. for $C_{15}H_{11}O_3Cl$: C, 65.58; H, 4.03. Found: C, 65.34; H, 4.29.

**8-Chloro-3-dibenzofurancarboxylic Acid Amide (X)**—3-Cyano compound (VII) (0.4 g.) was dissolved in alcoholic potassium hydroxide solution, 30% hydrogen peroxide added, and the mixture was warmed on a boiling water bath for 20 mins. After concentration of the reaction mixture, the separated crystals were collected by filtration and recrystallized from methanol to colorless needles, m.p. 271~272°. *Anal.* Calcd. for $C_{13}H_8O_2NCl$: C, 63.54; H, 3.28; N, 5.70. Found: C, 63.68; H, 3.43; N, 5,24.

**8-Chloro-3-dibenzofurancarboxylic Acid Hydrazide (XI)**—A mixture of the ester (IX) (0.1 g.) in alcohol (10 cc.) and 61% hydrazine hydrate (6 cc.) was warmed on a water bath for 2 hrs. After cooling, the separated fine needles were filterd and washed thoroughly with warm alcohol; the hydrazide, m.p. 274~277° (decomp.), is sparingly soluble or insoluble in almost all the organic solvents. *Anal.* Calcd. for $C_{13}H_9O_2N_2Cl$: C, 59.89; H, 3.48; N, 10.75. Found: C, 60.14; H, 3.28; N, 11.12.

**3-Chloro-8-nitrodibenzofuran (XV)**—To a suspension of 3-amino-8-nitrodibenzofuran[6] (0.6 g.) in diluted hydrochloric acid (200 cc.), sodium nitrite (0.12 g.) was gradually added under ice cooling and stirring to form a clear diazonium solution, which was dropped into a freshly prepared cuprous chloride solution (from 0.6 g. of $CuSO_4 \cdot 5H_2O$) and warmed on a water bath for 2 hrs. After cooling the precipitate was filterd off and recrystallized from alcohol to pale yellow, long needles of m.p. 207°. Yield, 0.15 g. *Anal.* Calcd. for $C_{12}H_8O_3NCl$: C, 58.18; H, 2.42. Found: C, 58.11; H, 2.34.

**3-Chloro-8-aminodibenzofuran (XVI)**—8-Nitro compound (XV) (0.2 g.) was reduced with tin and concd. hydrochloric acid for 8 hrs. on a water bath, when the pale yellow-colored needles of the material disappeared gradually, and colorless prisms separated out. The reaction mixture was made alkaline with ammonia and extracted with ether, to which dry hydrogen chloride gas was introduced to precipitate the hydrochloride of the amine (XVI). It melted at 302~304° after darkening around 180°, when recrystallized from 50% alcohol. *Anal.* Caled. for $C_{12}H_8ONCl \cdot HCl$: C 56.72; H, 3.57; N, 5.51. Found: C, 56.09; H, 3.88; N, 5.59.

The free base liberated by the action of ammonia was recrystallized from hydrated alcohol to colorless needles, m.p. 138~141°.

**Ethyl 3-Nitro-8-dibenzofurancarboxylate (XIX)**—3-Nitro-8-dibenzofurancarboxylic acid[3]

7) K. Tatematsu, B. Kubota: Bull. Chem. Soc. Japan, 9, 448 (1934).

(0.5 g.) in alcohol was refluxed on a water bath with the addition of concd. sulfuric acid for 4 hrs., when a clear solution was obtained. The crystals that precipitated after cooling were filtered and recrystallized from acetone to pale yellow, fine needles, m. p. 180~181.5°. Yield, 0.4 g. *Anal.* Calcd. for $C_{15}H_{11}O_5N$: C, 63.16; H, 3.88; N, 4.91. Found: C, 62.74; H, 3.74; N, 5.00.

**3-Amino-8-dibenzofurancarboxylic Acid (XX)**—3-Nitro-8-dibenzofurancarboxylic acid[3] (0.3 g.), suspended in alcohol, was hydrogenated using palladium-carbon as a catalyst. After the theoretical amount of hydrogen was absorbed, the catalyst was filtered off, and the filtrate was evaporated. The residue was recrystallized from methanol to colorless leaflets of m.p. 273~278° (decomp). Yield, 0.2 g. *Anal.* Calcd. for $C_{13}H_9O_3N$: C, 68.72; H, 3.99; N, 6.18. Found: C, 68.98; H, 4.39; N, 6.68.

The saponification of the ester (XXI) also yields the same compound.

**Ethyl 3-Amino-8-dibenzofurancarboxylate (XXI)**—Catalytic hydrogenation of 3-nitro compound (XIX) in the same way as above and subsequent recrystallization from aqueous alcohol gave colorless needles of m.p. 144~145°. Yield, quantitative. *Anal.* Calcd. for $C_{15}H_{13}O_3N$: C, 70.56; H, 5.13; N, 5.48. Found: C, 71.05; H, 5.21; N, 5.40.

**3-Amino-8-dibenzofurancarboxylic Acid Hydrazide (XXII)**—A mixture of the ethyl ester (XXI) (0.1 g.), 61% hydrazine hydrate (6 cc.), and alcohol (5 cc.) was warmed on a water bath for an hour and the reaction mixture was concentrated to half its volume. Colorless leaflets precipitated after cooling and melted at 233~238°, sparingly soluble or insoluble in all the organic solvents tested. *Anal.* Calcd. for $C_{13}H_{11}O_2N_3$: N, 17.42. Found: N, 17.58.

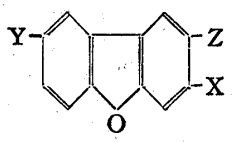
**II. Antibacterial Activity**—The antibacterial activity of these synthesized compounds and some other related dibenzofurans, whose effect has not been reported as yet, was examined by the same method as described in the previous reports[1,2,8]. Test organisms and conditions are as follow:

| Test organism | Medium | Incubation time |
|---|---|---|
| *Staphylococcus aureus*, Terashima strain | Nutrient broth | 24 hrs. |
| *Escherichia coli communior* | Nutrient broth | 24 hrs. |
| *Mycobacterium tuberculosis*, A.T.C.C. No. 607 | Lockemann-Bloch's | 3, 7, and 14 days |
| *Mycobacterium tuberculosis*, $H_{37}$ Rv | Kirchner's | 3 weeks |
| | Sauton's | 4 weeks |

The results are shown in Tables I~III.

TABLE I.

Antibacterial Activity of Dibenzofuran Derivatives against *Staph. aureus* and *E. coli*
(Medium: Nutrient broth. Incubation time: 24 hrs.)



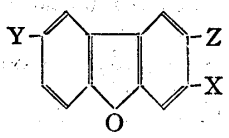

| Compound | | | | Highest dilution for complete inhibition | |
|---|---|---|---|---|---|
| X | Y | Z | No. | *Staph. aureus* | *E. coli* |
| $NHCONH_2$ | H | H | I | 40,000 | 5,000 |
| $NHNH_2$ | H | H | II | 10,000 | 5,000 |
| *p*-$CH_3$-$C_6H_4$-$SO_2NH$ | H | H | III | < 5,000 | < 5,000 |
| $CH_3CONH$ | H | Cl | IV | 5,000 | < 5,000 |
| $NH_2$ | H | Cl | V | 40,000 | 5,000 |
| $(CH_3CO)_2N$ | Cl | H | VI | 5,000 | < 5,000 |
| CN | Cl | H | VII | < 5,000 | < 5,000 |
| COOH | Cl | H | VIII | < 5,000 | < 5,000 |
| $COOC_2H_5$ | Cl | H | IX | < 5,000 | < 5,000 |
| $CONH_2$ | Cl | H | X | < 5,000 | < 5,000 |
| $CONHNH_2$ | Cl | H | XI | 10,000 | 10,000 |
| $NO_2$ | $NO_2$ | H | XII | < 5,000 | < 5,000 |
| $NH_2$ | $NH_2$ | H | XIII | < 5,000 | 5,000 |
| $NH_2$ | $NO_2$ | H | XIV | < 5,000 | < 5,000 |
| Cl | $NO_2$ | H | XV | < 5,000 | < 5,000 |
| Cl | $NH_2$ | H | XVI | < 5,000 | < 5,000 |
| $NO_2$ | $CH_3CO$ | H | XVII | < 5,000 | < 5,000 |
| $NO_2$ | COOH | H | XVIII | 10,000 | 20,000 |

8) S. Shibata, *et al.*: Japan Med. J., 1, 152 (1948); J. Pharm. Soc. Japan, 68, 298 (1948).

| | | | | | |
|---|---|---|---|---|---|
| $NO_2$ | $COOC_2H_5$ | H | XIX | < 5,000 | < 5,000 |
| $NH_2$ | COOH | H | XX | 40,000 | 10,000 |
| $NH_2$ | $COOC_2H_5$ | H | XXI | < 5,000 | 5,000 |
| $NH_2$ | $CONHNH_2$ | H | XXII | 40,000 | 20,000 |
| $NH_2$ | Cl | H | | 40,000 | 20,000 |
| Decarbonordidymic acid | | | | 640,000 | — |

TABLE II.

Antibacterial Activity of Dibenzofuran Derivatives against *M. tuber.*, A.T.C.C. No. 607
(Medium: Lockemann-Bloch's. Incubation time: 3, 7, and 14 days)

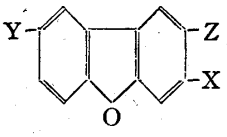



| Compound | | | | Highest dilution for complete inhibition | | |
|---|---|---|---|---|---|---|
| | | | | Incubation time | | |
| X | Y | Z | No. | 3 days | 7 days | 14 days |
| Didymic acid | | | * | 160,000 | 160,000 | 80,000 |
| Decarbonordidymic acid | | | * | 160,000 | 160,000 | 160,000 |
| 1,9-Dimethyl-3,7-dihydroxydibenzofuran | | | * | 160,000 | 160,000 | 80,000 |
| $NH_2$ | H | H | ** | 2,560,000 | 2,560,000 | 2,560,000 |
| OH | H | H | ** | 2,560,000 | 2,560,000 | 1,280,000 |
| OH | Cl | H | ** | 2,560,000 | 2,560,000 | 1,280,000 |
| $NHCONH_2$ | H | H | I | 2,560,000 | 2,560,000 | 2,560,000 |
| $NH_2$ | H | Cl | V | 1,280,000 | 1,280,000 | 1,280,000 |
| $CONHNH_2$ | Cl | H | XI | < 10,000 | < 10,000 | < 10,000 |
| $NH_2$ | $NH_2$ | H | XIII | 10,000 | < 10,000 | < 10,000 |
| Cl | $NH_2$ | H | XVI | 1,280,000 | 640,000 | 320,000 |
| $NO_2$ | COOH | H | XVIII | 10,000 | < 10,000 | < 10,000 |
| $NH_2$ | COOH | H | XX | 10,000 | < 10,000 | < 10,000 |
| $NH_2$ | $CONHNH_2$ | H | XXII | 40,000 | 20,000 | 10,000 |
| $NH_2$ | Cl | H | ** | 5,120,000 | 2,560,000 | 2,560,000 |
| Streptomycin sulfate | | | ** | 2,560,000 | 2,560,000 | 1,280,000 |
| Isonicotinic acid hydrazide | | | ** | 320,000 | 160,000 | 160,000 |

* See Part II[2]
** See Part III[1]

TABLE III.

Antibacterial Activity of Dibenzofuran Derivatives against *M. tuber.*, $H_{37}Rv$
(Medium and Incubation Time: Sauton's, 4 weeks; Kirchner's, 3 weeks)




| Compound | | | | Highest dilution for complete inhibition | |
|---|---|---|---|---|---|
| | | | | Sauton's medium | Kirchner's medium |
| X | Y | Z | No. | | |
| Didymic acid | | | * | 20,000 | < 20,000 |
| $NH_2$ | H | H | ** | 5,120,000 | 80,000 |
| $NH_2$ | Cl | H | ** | 5,120,000 | 20,000 |
| $NHC(NH)NH_2$ | Cl | H | ** | 5,120,000 | 40,000 |
| $CH_3CONH$ | Cl | H | ** | 40,000 | < 20,000 |
| OH | H | H | ** | 640,000 | 20,000 |
| OH | Cl | H | ** | 1,280,000 | 40,000 |
| $NHCONH_2$ | H | H | I | 20,000 | < 20,000 |
| $NH_2$ | H | Cl | V | 2,560,000 | < 20,000 |
| Cl | $NH_2$ | H | XVI | 640,000 | 20,000 |
| $NH_2$ | COOH | H | XX | 40,000 | < 20,000 |

| | | |
|---|---|---|
| Dihydrostreptomycin sulfate | 32,000,000 | 1,000,000 |

* See Part II[2]
** See Part III[1]

## Discussion and Conclusion

Following the previous report[1], a series of dibenzofuran derivatives was synthesized in order to examine the effect of substituents on their antibacterial action. However, none of the twenty-two newly synthesized dibenzofuran derivatives showed stronger inhibitory action against *St. aureus* than decarbonordidymic acid.[2] Substitution with tosylamino, cyano, carboxyl, carboxylic ester, and carboxylic acid hydrazide groups in 3-position of dibenzofuran showed no obvious effect on the growth of staphylococci, whereas 3-dibenzofuranurea exhibited a considerable inhibitory effect. Further substitution by amino, carboxyl, and other groups at 8-position did not increase, or rather, decreased the action of 3-amino compounds[1] (Table I).

Although the same relationship between antibacterial action and chemical structure was observed with *E. coli*, the actions of these compounds against this organism were generally weaker than that against *St. aureus*; 3-nitro-8-dibenzofurancarboxylic acid (XVIII) was a rare exceptional case (Table I).

In Lockemann-Bloch's medium, 3-amino-8-chloro- and 3-guanidino-8-chloro-dibenzofurans possess a remarkable inhibitory action against *Mycobac. tuberculosis*, A.T.C.C. No. 607 as reported in the previous paper.[1] By the present experiments, it was found that 3-amino-, 3-hydroxy-, 3-hydroxy-8-chloro-, 2-chloro-3-amino-dibenzofurans, and 3-dibenzofuranurea also showed strong inhibitory action. 3-Chloro-8-aminodibenzofuran (i.e. 2-amino-7-chlorodibenzofuran) has also comparatively strong activity. When amino, carboxyl, or carboxylic acid hydrazide group was further introduced into the 8-position of 3-aminodibenzofuran, the activity diminished to almost no effect (Table II).

The active compounds were also effective against *M. tuber.*, $H_{37}Rv$, when Sauton's synthetic medium was used. On the other hand, in Kirchner's medium which contains serum, the activity of dibenzofurans tested so far was markedly diminished perhaps due to the antagoinstic substances present in serum (Table III).

It would seem worth while to study this problem and further investigations are now in progress.

The authors are indebted to Yawata Iron & Steel Co., Ltd. for supplying the material for research. Thanks are also due to Dr. K. Iwata, Department of Bacteriology, University of Tokyo, for his kind cooperation in a part of the antibacterial test for *M. tuber.*, $H_{37}Rv$. Analyses were carried out by the members of the analytical laboratory of this Institute, and the expenses of the study were supported by the Scientific Research Fund provided by the Ministry of Education for which the authors' thanks are due.

## Summary

Further syntheses of dibenzofuran derivatives were undertaken in order to examine the effect of substituents on the antibacterial activity and some relationship was observed to exist between chemical structure and antibacterial action. Although 3-amino- and 3-hydroxy-dibenzofurans and related compounds showed remarkable effect against *M. tuber.*, A.T.C.C. No. 607 in Lockemann-Bloch's medium, these effects were strongly antagonized in the presence of serum as shown by the test against *M. tuber.*, $H_{37}Rv$ in Kirchner's medium.

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