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Synthesis and biological activity of spirobenzopyranone derivative as analogs of thelepin, isolated from the marine annelid *Thelepus setosus*

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Abstrat : For the further development of thelepin analog as antibiotic agents, we undertook the synthesis of spirobenzopyranone derivative 5 as thelepin analog by oxidative phenol coupling. The spirobenzopyranone analog 5 showed high activity against *Bacillus subtilis* (IFO 3108) in 5 μg/disc(Received September 20, 1992, accepted November 11, 1992).

Many bromophenols and their related compounds have been isolated from marine organisms.[1] From the marine annelid *Thelepus setosus* Higa *et al.*[2] have isolated five bromophenol compounds including thelephenol 1 and thelepin 2 . Thelepin 2 exhibits antifungal activity at a level comparable with griseofulvin 3 , which was isolated from the microorganism *Penicillium griseofulvum*, and is very interesting because of sharing structural similarity with griseofulvin 3[3] (Fig. 1).

Tsuge *et al.*[4] synthesized of thelepin 2 and its analogs they have also reported that spiro-p-quinol ethers 4 showed significant *in vitro* activity against *Trichophyton mentagraphytes*. Thus, spiro-p-quinol ester 4 (X=Cl) applied at 0.5 μg/m*l*, inhibited *T. mentagraphytes in vitro*.

For the further development of thelepin analogs as antibiotic agents, we were interested in determining the influence of ring B of thelepin 2 on the biological activity, when a coumaran form i of thelepin 2 was replaced with chroman-4-one form ii.

For this purpose, we undertook the synthesis of the spiro-benzopyranone analog 5 as thelepin 2 analog by oxidative phenol coupling. Our strategy for the synthesis of the spirobenzopyranone derivative 5 *via* intermediate deoxybenzoin 9 is summarized in Fig. 2.

The biological activity of synthesized analog was tested against fungi (*Botrytis allii* and *B. cinerea*) and bacteria (*Bacillus subtilis* and *Escherichia coli*) by a paper disc method.

Materials and Methods

Chemicals and instruments

Melting points (m.p.) were determined on micro-melting point apparatus (Yanagimoto No. 1593). All melting points were uncorrected. IR spectra were measured on a JASCO IR-810 infrared spectrometer, and ^{1}H-NMR was recorded on a JEOL JNM FX (270 MHz) spectrometer. The mass spectrum was recorded on JEOL HX-105 spectrometer. Microanalyses were performed at the Analytical Laboratory of the Faculty of Science at Tohoku University. Preparative TLC was carried out on Merck Kieselgel 60PF$_{254}$ of 0.7 mm thickness.

Key words : Marine annelid, telephenol, thelepin, spiro-p-qinol ester, chroman-4-one, coumaran, deoxybenzoin, Frider-Crafts coupling, Fries rearrangment, spirobenzopyranone, antigram-positive bacteria activity
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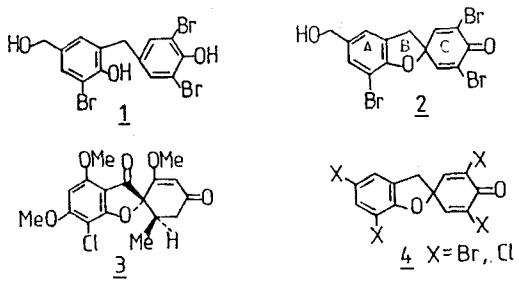

Fig. 1. Thelephenol, thelepin and griseofulvin.

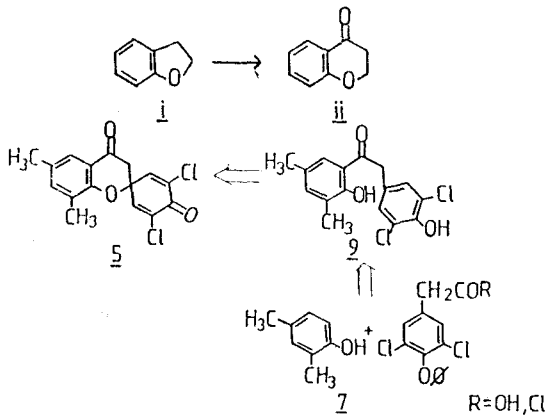

Fig. 2. Strategy for the synthesis of spirobenzopyranone analog 5.

Synthesis

The phenylacetic acid 6 was prepared by starting from p-hydroxybenzoic acid in seven steps according to the general synthetic method.

(1) 2,4-Dimethylphenyl m-dichloro-p-benzyloxyphenylacetate 8

To a stirred of 3,5-dichloro-4-benzyloxyphenylacetic acid (6, 0.9 g) in trifluoroacetic anhydride (15 m*l*) at 0℃ under Ar atmosphere was added 2,4-dimethylphenol (7, 0.48 g). The mixture was stirred overnight at room temperature. After evaporating *in vacuo*, the residual solid was treated with water (20 m*l*) and extracted with AcOEt. The AcOEt layer was washed successively with 5% aq. $NaHCO_3$ and sat. NaCl, dried over $MgSO_4$, and concentrated. Chromatography of the residue on silica gel with AcOEt-benzene (1 : 10) gave 8 (0.19 g, 76%) as yellow oil.

R_f 0.61 (AcOEt : benzene=1 : 10). IR ν_{max} (film) cm^{-1} : 1756, 1600, 1558, 1498, 1262. ^{1}H-NMR ($CDCl_3$) δ : 2.02 (3H, s), 2.27 (3H, s), 3.75 (2H, s), 5.03 (2H, s), 6.84~6.99 (3H, m), 7.30~7.42 (5H, m), 7.55 (2H, br d, J=5.86 Hz).

(2) 3′,5′-Dichloro-2,4′-dihydroxy-deoxybensoin 9

A mixture of phenyl ester 8 (0.83 g, 2 mmol) and finely powdered aluminium chloride (0.8 g, 6 mmol) was heated for 1.5 hr under N_2 atomosphere at 140~150℃. The reaction mixture was poured into ice-water, made acidic by the addition of 1 N HCl, and extracted with AcOEt. The AcOEt layer was washed successively with H_2O, aq. 5% $NaHCO_3$, and sat. NaCl, dried over $MgSO_4$, and concentrated. Chromatography of the residue on silica gel with AcOEt-benzene (1 : 10) gave deoxybenzoin 9 (0.53 g, 64%), m.p. 142℃ (from benzene).

R_f 0.35 (AcOEt : benzene=1 : 10). IR ν_{max} (KBr) cm^{-1} : 3340, 1628, 1600, 1564, 1496, 1150. ^{1}H-NMR ($CDCl_3$) δ : 2.23 (3H, s), 2.30 (3H, s), 4.19 (2H, s), 5.81 (1H, s), 7.11~7.21 (4H, m), 12.19 (1H, s).

(3) (±)-3′,5′-Dichloro-6,8-dimethylspiro[benzopyran-2(3H), 1′-(2′,5′-cyclohexadiene)]-4,4′-dione 5

The deoxybenzoin 9 (420 mg, 1 mmol) was suspended in 60 m*l* of water and treated with 7.74 g of K_2CO_3, and added dropwise to the stirred solution of 2.01 g of potassium ferricyanide in 30 m*l* of water. The reaction mixture was allowed to stir for 20 hr, filtered, and extracted with AcOEt. The AcOEt layer was washed with sat. NaCl, dried over $MgSO_4$, and concentrated. Chromatography of the residue on silica gel with AcOEt-benzene (1 : 5) gave 291 mg of a crude product. The crude product was purified on TLC to afford 155 mg (48%) of the spirobenzopyranone 5 as yellow needles, m.p. 218℃ (from AcOEt-hexane).

R_f 0.26 (AcOEt : benzene=1 : 5). IR ν_{max} (KBr) cm^{-1} : 1672, 1630, 1600, 1502, 1278. ^{1}H-NMR (acetone-d) δ : 2.17 (3H, s), 2.26 (2H, s), 2.58 (3H, s), 7.13 (1H, m), 7.22 (1H, m), 7.74 (2H, s). MS m/z : 323 (M^+). Anal. Found : C, 59.38; H, 3.61; Cl, 22.08. Calcd. for $C_{18}H_{12}O_3C_2$: C, 59.46; H, 3.74; Cl,21.94%.

Biological assays

The antimicrobial activity was determined by the paper disc method. A solution containing the test compound at the defined concentration (5, 25, 50,

and 100 μg/disc) was poured on to paper layer in Petri dish.

(1) Against *Botrytis allii* IFO 9430 and *B. cinerea* AHU 9573

The used medium was potato sucrose medium (pH was not corrected. potato 300 g/*l*, sucrose 20 g, and agar powder 15 g). The treated culture was incubated at 26~28℃ for 4~5 days, and the growth-inhibited zone around the disc was measured.

(2) Against *Bacillus subtilis* IFO 3108 and *Escherichia coli* IFO 6036

The used medium was agar malt medium (pH 7.0, meat juice 1.5 g/0.5 *l*, pepton 5 g, NaCl 2.5 g, agar powder 7.5 g). The treated culture was incubated at 35℃ for 16 hr, and the growth-inhibited zone around the disc was measured.

Results and Discussion

Our first objective was focused on the preparation of the deoxybenzoin 9. It was hoped that reaction of this compound might occur C-O oxidative ring-coupling to give spirobenzopyranone analog 5 *via* the biradical 10. The oxidation of phenolic intermediates is an important step in the biogenesis of diverse type of natural product.[5-7]

Initial attempt to effect Friedel-Crafts coupling between commercial 2,4-dimethylphenol 7 and the acid chloride 11 in the presence of Lewis acid was unsuccessful, providing many side products. The C-acylation in trifluoroacetic anhydride was reported by Taub *et al.*[8]. Several attempts to prepare 9 by direct C-acylation of the phenol 7 with the phenylacetic acid 6 in trifluoroacetic anhydride gave, the phenyl ester 8 as an O-acylation product. We had achieved the synthesis of the deoxybenzoin 9 by a Fries rearrangement on the phenyl ester 8. The direct Fries rearrangement of the phenyl ester 8 with aluminium chloride in the absence of solvent occurred deprotonation of the benzyl protecting group, afforded only the deoxybenzon 9 (Fig. 3). The structure of 8 and 9 were assigned by their ^{1}H-NMR or IR spectra. The IR spectrum of 8 showed the C=O bond in the 1756 cm^{-1}. But, the IR spectrum of 9 showed the O-H bond in the 3340 cm^{-1} and the unsaturated C=O bond in the 1628 cm^{-1}. The ^{1}H-NMR spectrum of 9 in $CDCl_3$ showed chemical shift of O-H proton was removed by D_2O exchange.

The generation of a radical from a phenol takes place under the influence of an oxidizing agent of the required potential for the removal of the phenolic hydrogen atoms, or an electron from the corresponding phenoxide anion. As the reagent of intramolecular oxidative ring-coupling of 9 to give spirobenzopyranone analog 5 was used potassium fer-

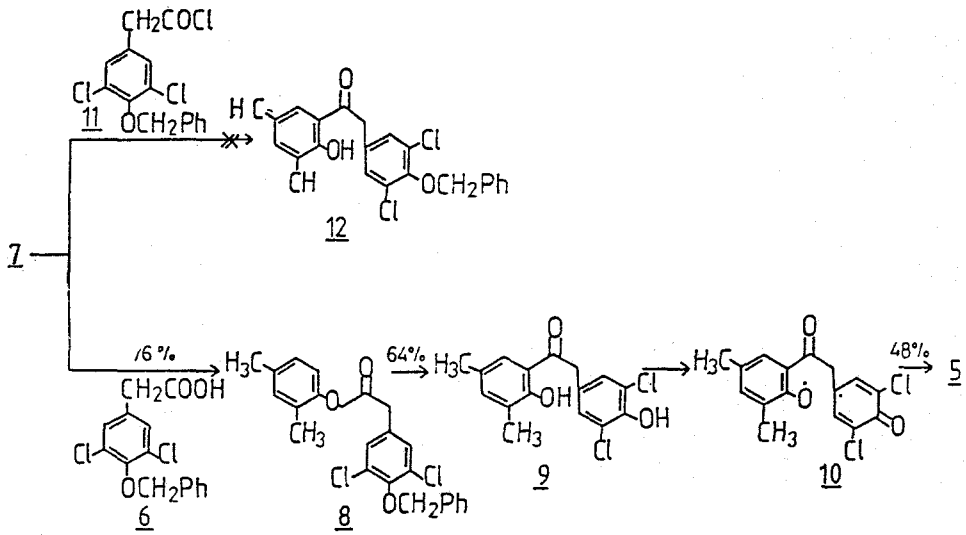

Fig. 3. Synthetic scheme of spirobenzopyranone analog 5.

ricyanide. The structure of 5 was supported by its IR spectrum which showed the C=O bond absorption at 1672 and 1630 cm^{-1}. Its ^{1}H-NMR spectrum in CD_3COCD_3 showed chemical shift of the vinylic protons at δ 7.74 (2H, s).

The antimicrobial activity of the spirobenzopyranone analog 5 is listed Table 1. The spirobenzopyranone analog 5 showed quite weak activity against *Botrytis cinerea* in 100 μg/disc. Thus, it was not active against some strain of *Botrytis allii, B. cinerea*, and *Escherichia coli*. However, it showed high activity against *Bacillus subtilis* in 5 μg/disc (Fig. 4). Also, from the structure of thelepin 2, it became obvious that the carbonyl group on the B ring of synthesized analog was not necessary to have the biological activity. Thelepin 2 has antifungal activity at a level comparable with griseofulvin 3, and although the test compound 5 had no activity against fungi. But the spirobenzopyranone analog 5 showed very interesting activity in view of having antigram-positive bacteria activity (against *B. subtilis*).

Table 1. Antimicrobial activities of the spirobenzopyranone analog (5)

Conc. (μg/disc)	Inhibited zone (mm)			
	B. cinerea AHU 9573	*B. allii* IFO 9430	*B. subtilis* IFO 3108	*E. coli* IFO 6036
5	−	−	17	−
25	−	−	22	−
50	−	−	29	−
100	+	−	36	−

+: Inhibited zone <10 mm, −: Inactive.

From these results and previous data[2,3,4,8] in the structure of thelepin 2, the coumaran form i seems to be an more effective structure than the chroman-4-one form ii for the biological activity againtst fungi. This structural necessity for biological activity could be used for finding out a leading compound for new antibiotic agents.

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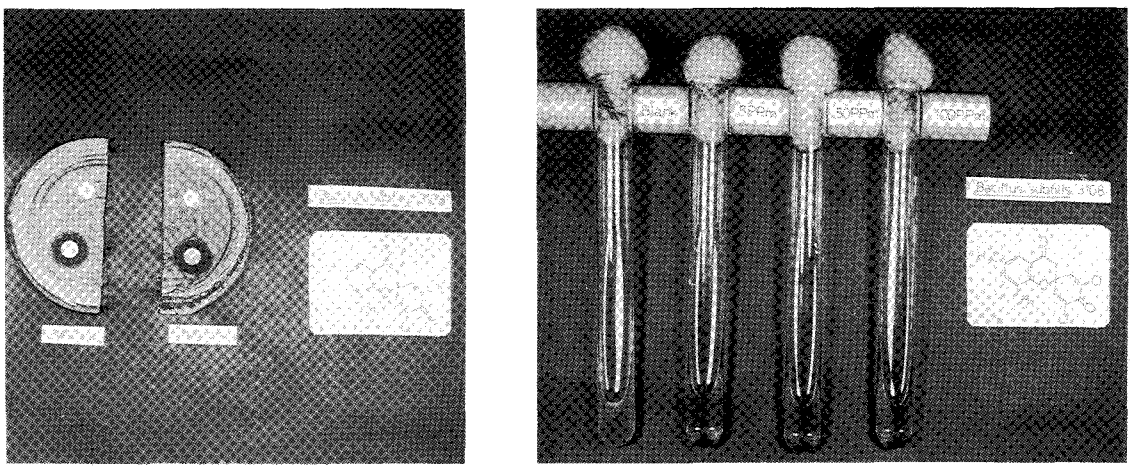

A B

Fig. 4. Growth inhibition of *Bacillus subtilis* IFO 3108 by the paper disc method (A) and the dilution method (B).

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항균성물질 thelepin의 spirobenzopyranone 유도체의 합성과 생물활성

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초록 : 해양환형동물 thelepus setosus로부터 분리된 thelepin 2의 항균활성에 착안하여 새로운 항균성 선도구조를 찾아내고자, B 환구조를 coumaran형에서 chroman-4-one형으로 전환하고 생물활성을 조사하였다. Thelepin의 spirobenzopyranone 유도체를 산화적분자내에 환반응을 이용하여 합성하였으며 진균 2종과 박테리아 2종을 대상으로 paper disc법으로 생물활성을 조사한 결과 gram-positive 박테리아인 *Bacillus subtilis*에 대하여 5 μg/disc 수준에서 억제활성을 보였다.