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Antimicrobial Activities of Hydroxybiphenyl Derivatives (II)
Synthesis and Antibacterial Activities of Allylhydroxybiphenyl Compounds against a Cariogenic Bacterium *Streptococcus mutans* ATCC OMZ176

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Abstract □ Naturally occurring diallyldihydroxybiphenyl compounds, magnolol and honokiol were reported to have potent antibacterial activities against most of Gram positive bacteria (1-4). To developmore potent antibacterial agents, some allylhydroxybiphenyl derivatives were synthesized from the starting compounds, o-phenyl-phenol (I), *p*-phenylphenol (IV), *o,o'*-biphenol (VII) and *p,p'*-biphenol (XII). Among the newly synthesized compounds (III, VI, IX, XI, XIV and XVI), the antibacterial activities of 2-allyl-*p*-phenylphenol (VI), 6-allyl-*o,o'*-biphenol (IX), 2, 2'-diallyl-*o,o'*-biphenol (XIV) and 2, 2', 6-triallyl-*p,p'*-biphenol (XVI) were more potent than those of magnolol and honokiol against a cariogenic bacterium, *Streptococcus mutans* ATCC OMZ176.

Keywords □ Allylhydroxybiphenyl derivatives, *Streptococcus mutans* ATCC OMZ176

Magnolol and honokiol are naturally occurring diallyldihydroxybiphenyl compounds. Magnolol was isolated from the stem bark of Magnolia obovata Thunberg and its structure was elucidated by Sug (5). Honokiol was isolated and characterized by Fujita *et al.* (6).The two components were reported to have potnet antibacterial activities against most of Gram positive bacteria including a cariogenic bacterium, *Streptococcus mutans* ATCC OMZ176. (1-4). For developing more potent antibacterial agents than these two components, some allylhydroxybiphenyl compounds were synthesized by Claisen's rearrangement (7-8) and tested for their activities against a cariogenic bacterium, *S. mutans* ATCC OMZ176.

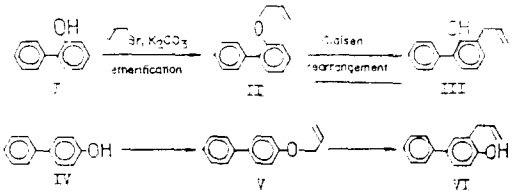
EXPERIMENTAL METHODS

All the melting points and boiling point were uncorrected. The IR spectra were taken with Perkin-Elmer 783, NMR with Varian EM 360. The turbidity of the bacterial cell suspensions was checked with Cecil 393. The chemicals of *o*-phenylphenol (I) and *p-phenylphenol (IV) were the products of Wako, o,o'*-biphenol (VII) and *p, p'*-biphenol (XII) were Aldrich allylbromide was Tokyokasei and Bacto Brain Heart Infusion (BHI) was Difco.

Synthesis of allylmonohydroxybiphenyl derivatives (Scheme 1, *comp. III and VI)*

Two steps, etherification and Claisen rearrangementiiii were applied to the synthesis of comp. III and VI. In the first step, allylbiphenylethers (2-allyloxybiphenyl, comp. II and 4-allyloxybiphenyl, comp. V) were synthesized from 0.01mol comp.I (1.7g) or 0.01 mol comp. IV (1.7g) and 0.01 mole allylbromide (1.21g) in 0.01mol pot. carbonate (2g) acetone solution. The reaction was carried out at room temperature for 4 hours. After the reaction, the reaction mixture was cooled, filtrated, and concentrated. The concentrated product was extracted with ehter and washed with N-hydrochloric acid. The ethereal layer was then dried over anhydrous sulfate and purified by silica gel column chromatography with pertroleum ether and benzene (2 : 1, v/v). In the second step, comp. II and V were refluxed in N,N-diethylaniline (20ml) for 30 minutes. By the procedures of two steps, 6-allyl-*o*-phenylphenol (comp. III, yield 18%) and 2-allyl-*p*-phenylphenol (comp. VI, yield 20%) were synthesized from comp. I and IV, respectively.

Comp: III: Yellowish liq., bp. 248°, IR ν_{max}^{KBr} cm^{-1}: 3280 (OH), 756 (1, 2, 3-substituted benzene), 1640, 1410, 990 and 915 (-CH=CH_2), NMR($CDCl_3$, TMS)δ :3.5 (2H, d, aryl-$C\underline{H}_2$



Scheme 1. Synthetic Procedure of Allylmonohydroxybiphenyl Derivatives.

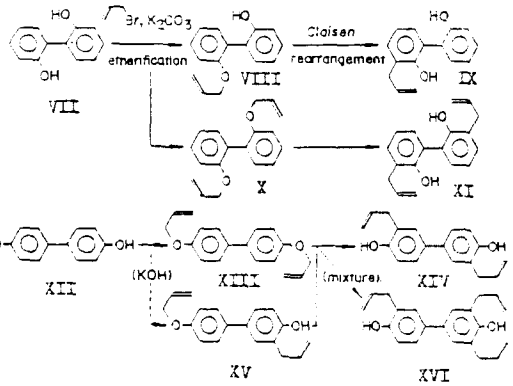
-CH=), 5.1 (1H, bs, -OH), 5.4 (2H, m, -CH=C$\underline{H}_2$), 6.0 (1H, m, -C$\underline{H}$=CH$_2$), 6.8-7.6 (8H, m, aromatic H).

Comp. VI: Whitish crystal, mp. 72-73℃, IR ν_{max}^{KBr} cm^{-1}: 3300(OH), 825 (1,2,4-substituted benzene), 1640, 1410, 990 and 915 (-CH=CH$_2$), NMR(CDCl$_3$, TMS) δ:3.5 (2H, d, aryl-C$\underline{H}_2$-CH=), 5.1 (2H, m, -CH=C$\underline{H}_2$), 5.4 (1H, bs, OH), 5.9 (1H, m, -C$\underline{H}$=CH$_2$), 6.8-7.6 (8H, m, aromatic H).

Synthesis of allyldihydroxybiphenyl derivatives (Scheme 2, comp. IX, XI XIV and XVI)

The Claisen rearrangement(7-8) was also applied to the synthesis of allyldihydroxybiphenyl derivatives. 6-Allyl-*o,o'*-biphenol (comp. IX) and 6,6'-diallyl-o,o'-biphenol (comp. XI) were synthesized from 0.04 mol comp. VII, 0.1 mol allylbromide and 0.1 mol pot. carbonate in acetone and methanol (1 : 2, v/v) solution. The etherification was carried out at room temperature for 4 hours. In the procedure, two compounds, 2-allyloxy-2'-hydroxybiphenyl (comp. VIII) and 2,2'-diallyloxybiphenyl (comp. X) were produced. Comp. VIII and comp. X were separated by silica gel column chromatography with the solvents of chloroform and methanol (9 : 1, v/v) and purified by recrystallization. With the Claisen rearrangement(7-8), comp. IX (yield 43%) and comp. XI (yield 12%) were prepared from comp. VIII and comp. X, respectively.

2,2'-Diallyl-*p,p'*-biphenol (comp. XIV) and 2,2'6-triallyl-*p,p'*-biphenol (comp. XIV) were synthesized form the mixture of 4,4'-diallyloxy-biphenyl (comp. XIII) and 3-allyl-4'-allyloxy-4-hydroxybiphenyl (comp. XV) through the Claisen rearrangement(7-8). Comp. XIII, one of the intermediates of comp. XIV and XVI, was prepared from 0.04 mol comp. XII, 0.1 mol allylbromide and 0.1 mol pot. carbonate in acetone and methanol (1 : 2, v/v). Comp. XV, the other intermediate of comp. XIV and XVI, was prepared from 0.04 mol comp. XII, 0.1 mol allylbromide and 0.1 mol pot. hydroxode(9) in actetone and methanol (1 : 2, v/v). The yields of comp. XIV and XVI were 36% and 8%, respectively.



Scheme 2. Synthetic Procedure of Allyldihydroxybiphenyl Derivatives.

Comp. IX: Reddish white crystal, mp. 51.9℃, IR ν_{max}^{KBr} cm^{-1}: 750 (1,2-substituted benzene), 784 (1,2,3,-substituted benzene), NMR (CDCl$_3$, TMS) δ: 3.4 (2H, d, aryl-C$\underline{H}_2$-CH=), 5.1 (2H, m, -CH=C$\underline{H}_2$), 6.0(1H, m, -C$\underline{H}$=CH$_2$), 6.2 (2H, bs, OH), 6.7-7.3 (7H, m, aromatic H).

Comp. XI: Brownish white crystal, mp. 37.5℃, IR ν_{max}^{KBr} : 772 (1,2,3-substituted benzene), NMR (CDCl$_3$, TMS) δ: 3.4 (4H, d, aryl-C$\underline{H}_2$-CH=), 5.1 (4H, m, -CH=C$\underline{H}_2$), 5.2 (2H, bs, OH), 6.0 (2H, m, -C$\underline{H}$=CH$_2$), 6.7-7.2 (6H, m, aromatic H).

Comp. XIV: Yellowish white crystal, mp. 75.2℃, IR ν_{max}^{KBr} cm^{-1}: 813 (1,2,4-substituted benzene), NMR (CDCl$_3$, TMS) δ : 3.4 (4H, d, aryl-CH$_2$-CH=), 5.2 (6H, m, -CH=CHi), 5.3 (3H, bs, OH), 6.0 (3H, m, -CH=CH$_2$), 6.7-7.4 (5H, m, aromatic H).

Comp. XVI: Brownish grey crystal, mp. 84℃, IR ν_{max}^{KBr} : 813 (1,2,4-substituted benzene), 869 (1,2,3,5-substituted benzene), NMR (CDCl$_3$, TMS) δ : 3.4 (6H, d, aryl-C$\underline{H}_2$-CH=), 5.2 (6H, m, -CH=C$\underline{H}_2$), 5.3 (3H, bs, OH), 6.0 (3H, m, -C$\underline{H}$=CH$_2$), 6.7-7.4 (5H, m, aromatic H).

Antibacterial test

For the test, the strain of *B. anthracis* was cultured in liquid BHI broth at 37℃ overnight and subcultured again for 6 hours. The bacterial cell suspension was adjusted with the same sterile broth to 0.07 optical density unit at 550nm and then used for the test. For the paper disk method, 0.6ml of the bacterial cell suspension was poured uniformly into the plates made of BHI as medium. Five paper diskes containing 5, 10, 20, 40, 80 and 120mg of the compounds and one control were carefuly placed on the seeded plates. The culture was carried out at 37℃ for 24 hours.

Minimal time for bactericidal action of comp. III and VI

The precultured bacterial suspension (0.1ml) which had about 0.07 optical density unit at 550 nm was mixed with BHI broth (4.9ml) containing comp. III or comp. VI (100μg/ml). The suspension (0.1ml) was transferred to BHI medium (5 ml) at an indicated time. The bacterial growth was cultured for 48 hours and measured turbidimetrically at 550 nm.

RESULTS AND DISCUSSION

1. For the purpose of more potent antibacterial agents than naturally occurring compounds, magnolol and honokiol, 6-allylhydroxybiphenyl derivatives are synthesized by Claisen etherification and rearrangement (7-8). In the synthetic procedure, comp. XV and XVI are newly occurred. These compounds may be occurred from the results of the mechanisms of C-alkylation (10-11) and intermolecular migration of allyl groups in strong base medium like pot. hydroxide (9) rather than partial rearrangement in the allylether of diallylresorcinol (12).

2. The antibacterial activities of synthetic allylhydroxybiphenyl derivatives (comp. III, VI, IX, XI, XIV and XVI) and starting chemicals (comp. I, IV, VII and XII) against *S. mutans* are as shown in Table I. Among the newly synthesized compounds, the antibacterial activities of 2-allyl-p-phenylphenol (comp. VI), 6-allyl-*o,o'*-biphenol (comp. IX), 2,2'-diallyl-*o,o'*-biphenol (comp. XIV) and 2,2',6-triallyl-*p,p'*-biphenol (XVI) are more potent against *S. mutans* than those of

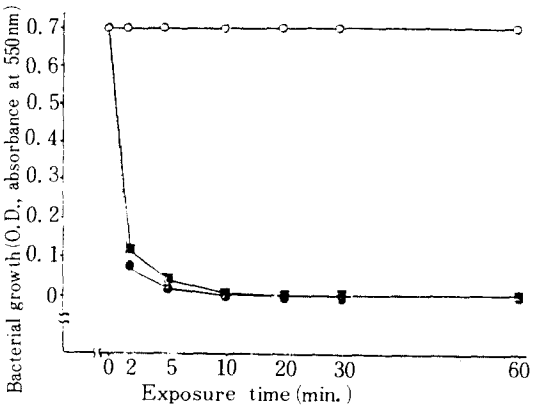


Fig.1. Bacterial grow *vs.* time exposed to 2-allyl-*p*-phenylphenol (III) and 2,2'-diallyl-*p,p'*-biphenol (VI).

: Control; bacterial culture without sample ■: Bacterial culture containing 100mg/ml of 2-ally-*p*-phenylphenol ●: Bacterial culture containing 100mg/ml of 2,2'-diallyl-*p,p'*-biphenol.

Table I. Antibacterial Activities of Starting, Synthesized and Related Compounds against *Streptococcus mutans* ATCC OMZ176.

Compounds	Diameter of inhibitory zone (mm)[1]					
	5[2]	10	20	40	80	120
o-phenylphenol (I)	–[3]	–	–	–	8.4	10.1
p-phenylphenol (IV)	–	–	–	–	–	9.4
o, o'-biophenol (VII)	–	–	–	–	–	8.5
p, p'-biophenol (XII)	–	–	–	–	–	8.9
6-allyl-*o*-phenylphenol (III)	9.3	10.6	13.8	15.8	17.8	19.0
2-allyl-*p*-phenylphenol (VI)	12.5	14.4	17.2	19.9	22.1	24.1
6-allyl-*o, o'*-biphenol (IX)	12.5	14.8	17.5	20.1	23.0	24.8
6,6'-diallyl-*o, o'*-biophenol (XI)	11.5	13.3	15.9	18.2	19.4	21.8
2,2'-diallyl-*p, p'*-biophenol (XIV)	13.0	17.0	20.9	24.5	29.0	30.9
2,2',6-triallyl-*p, p'*-biophenol (XVI)	12.7	16.5	20.0	22.9	27.7	29.4
magnolol[4]	–	9.5	13.1	15.5	18.6	21.6
honokiol[4]	–	10.5	15.1	18.3	21.5	23.3

1) Added amounts (μg) per a disk
2) Mean values from 3 observations
3) No inhibitory zone was formed
4) Reported values in reference 3.

magnolol and honokiol as reported in the previous paper(3). From the results, the number and positions of allyl groups in the hydroxybiphenyl rings may influence to the antibacterial activities. It has also been clarified that the introduction of allyl groups to the hydroxybiphenyl compounds to enhance the activities in comparison with those of starting materials. We will study the structure -activity relationships in hydroxybiphenyl compounds.
relationships in hydroxybiphenyl compounds.

3. The minimal time for the bactericidal action of comp. III and VI are determined as shown in Fig. 1. This is one of the most important criteria required for an oral bactericide. The cells of *S. mutans* are sterilized in 2-5 minutes by exposing to 100 μg/ml of comp. III or comp. VI. The synthesized allylhydroxybiphenyl derivatives may be used as an oral bactericide or general antibacterial agents after testing of toxicity.

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