생 약 학 회 지
Kor. J. Pharmacogn.
17(1) : 85~90 (1986)

Antimicrobial Activities of Hydroxybiphenyl Derivatives

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Abstract—It has been elucidated that magnolol and honokiol, isolated from the stem bark of *Magnolia obovata*, had potent antibacterial activity against a cariogenic bacterium *Streptococcus mutans.*[1] They also show a significant antibacterial activity against *Bacillus anthracis*, which causes malignant pustule and woolsorter disease in human.[2] Some hydroxybiphenyl derivatives are synthesized from starting materials, phenylphenols and biphenols by means of Claisen's rearrangement and Elb's method to develop more potent antibacterial chemicals and to investigate the structure-activity relationships. The introduction of allyl groups to the aromatic rings of starting materials shows increase of antibacterial activities, but the number and positions of them do not effect their activities. Furthermore, the introduction of hydroxy group to aromatic rings also increases the activity.

Keywords—Magnolol · honokiol · *Streptococcus mutans* · *Bacillus anthracis*

It has been reported that *Streptococcus mutans* OMZ176 is a particularly strong cariogenic bacterium in animals and human.[3,4] For the purpose of developing antibacterial agents, sixty kinds of crude drugs were tested for their activity. Among them, the methanol extract of Magnoliae Cortex (the stem bark of *Magnolia obovata*) had potent antibacterial action against the strain.[1] To isolate and identify the active principles, the methanol extract of the stem bark of *M. obovata* was fractionated into gross chemical classes. The distribution and purification of the active principles were monitored by the paper disk method.[5,6] In this procedure, two active principles, magnolol (I) and honokiol (II) were isolated.

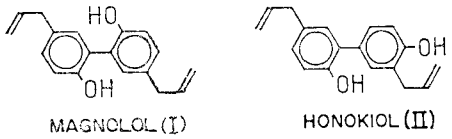

Fig. 1. Structure of magnolol (I) and honokiol (II).

Both compounds (Fig. 1) were identified by rf values, mp, ir and nmr in comparison with

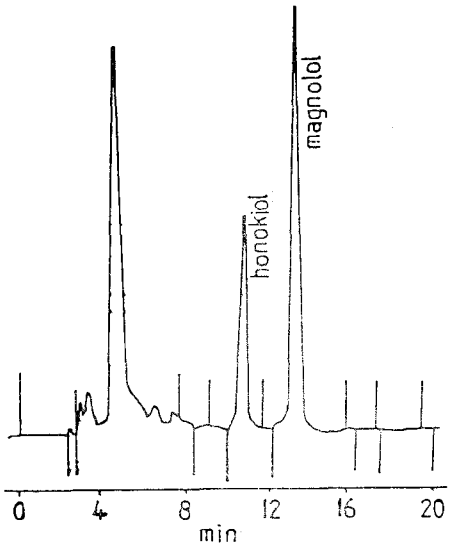

Fig. 2. HPLC chromatogram of MeOH extract of stem bark of *Magnolia obovata.* Analytical conditions: column, μBondaPak C18, 30×7.8 mm; eluent, MeOH-H_2O (4 : 1, v/v); flow rate, 2 ml/min; detector, UV 254 nm.

authentic samples. For the quantitative analysis of I and II in the dried stem of *M. obovata*, the methanol extract of it was applied to HPLC (Fig. 2). The amounts of I and II were identified as 1.94% and 0.44%, respectively, from the standard addition method.

Antibacterial Activity of I and II

According to the paper disk method,[5,6] the antibacterial activity of I and II was tested against *S. mutans* (Table I). The active principles, I and II were potent antibacterial agents. The inhibitory zones of I and II were greater in diameter than that of a typical antibacterial alkaloid, berberine, under comparable conditions. The diameter of inhibitory zones was a linear function of logarithmic concentration in a range of 5 μg to 40 μg/disk. The minimal inhibitory concentration (MIC) of the antibacterial agents were determined by the broth dilution method. Both I and II completely inhibited the bacterial growth of *S. mutans* in 6.25 μg/ml. The minimal time for bactericidal action of I and II were determined. To determine the minimal time for the bactericidal action, which is one of the most important criteria required for an oral bactericide, the cariogenic bacterium was exposed to a solution of I and II for indicated periods of pre-

Table I. Antimicrobial activity of magnolol, honokiol and berberine against *Streptococcus mutans* OMZ176

Concentration (μg/disk)[2]	Diameter of inhibitory zones (mm)[1]		
	magnolol	honokiol	berberine
5	—[3]	—	—
10	9.5	10.5	—
20	13.1	15.1	—
40	15.5	18.3	—
60	19.4	20.8	9.6

1) Mean values from three observations
2) Added amounts of compounds per disk
3) No inhibitory zone was formed

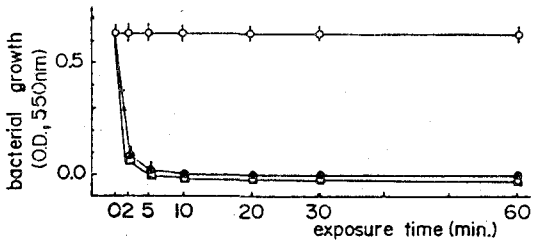

Fig. 3. Bacterial growth *vs.* time exposed to active principles I and II. Bacterial cells were exposed to 100 μg/ml of I and II.
○: Control, ●: I, □: II

incubation. Thereafter, the surviving bacteria were measured as usual by cultivation at 37° for 48 hours. The strain was almost sterilized in 2~5 minutes by exposing to the solution (Fig. 3).

The active principles, I and II were tested against another Gram-positive bacteria, *Bacillus anthracis* ATCC11949, *B. anthracis* ATCC14186 and *B. anthracis* ATCC14578 to survey their

Table II. Antibacterial activity of magnolol, honokiol, berberine and erythromycin against *Bacillus anthracis* ATCC11949, ATCC 14186, and ATCC14578

Compounds	μg/disk[2]	Diameter of inhibitory zone(mm)[1]		
		11949	14186	14578
Magnolol	5	17.1	13.1	11.9
	10	18.2	16.7	15.7
	20	20.4	18.1	17.1
	40	21.3	20.2	19.1
Honokiol	5	12.2	9.4	10.5
	10	16.8	12.3	12.3
	20	20.0	14.9	14.1
	40	24.4	17.1	15.9
Berberine	5	—[3]	—	—
	10	—	—	—
	20	8.9	—	—
	40	11.2	—	—
Erythromycin	5	28.9	20.1	15.1
	10	31.2	22.4	17.8
	20	32.6	24.1	20.0
	40	36.4	26.4	23.0

The legends are same as shown in Table I

antibacterial activity. Both I and II were also potent antibacterial activity as shown in Table II and reported paper.[2)]

Structure-activity Relationships

In order to obtain some information about structure-activity relationships in the hydroxybiphenyl compounds, and structural requirements for more potent antibacterial agents, some derivatives of I, *o*-phenylphenol (VII), *p*-phenylphenol (VIII), *o*, *o'*-biphenol (IX) and *p*, *p'*-biphenol (X) were synthesized and tested for their antibacterial activity against *S. mutans*.

(a) Synthetic I derivatives modified OH group.

Three I derivatives, O, O'-dimethylmagnolol (III), O, O'-diacetylmagnolol (IV) and O, O'-dibenzoylmagnolol (V), were synthesized from I by methylation, acetylation and benzoylation,

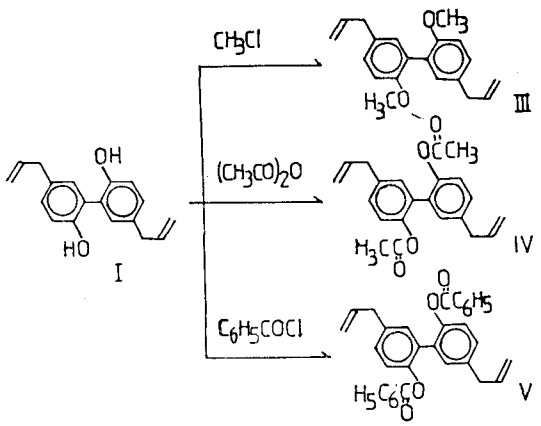

Fig. 4. Synthetic magnolol derivatives modified OH group O, O'-dimethylmagnolol(III) O, O'-diacetylmagnolol(IV) and O, O'-dibenzoylmagnolol(V).

Table III. Antimicrobial activity of magnolol derivatives modified "OH" group against S. mutans OMZ176

Concentration (μg/disk)[2)]	Diameter of inhibitory zones(mm)[1)]		
	O, O'-dimethyl-magnolol	O, O'-diacetyl-magnolol	O, O'-dibenzoyl-magnolol
5	—[3)]	—	—
10	—	—	—
20	—	—	—
40	—	—	—

The legends are same as shown in Table I

Table IV. Antibacterial acitivities of related compounds of I and II

Compounds	Diameter of inhibitory zone (mm)[1)]					
	5 μg[2)]	10 μg	20 μg	40 μg	80 μg	120 μg
biphenyl	—[3)]	—	—	—	—	—
o-phenylphenol	—	—	—	—	8.4	10.1
p-phenylphenol	—	—	—	—	—	9.4
o, *o'*-biphenol	—	—	—	—	—	8.5
p, *p'*-biphenol	—	—	—	—	—	8.9
chavicol	—	—	—	—	—	—

The legends are same as shown in Table I

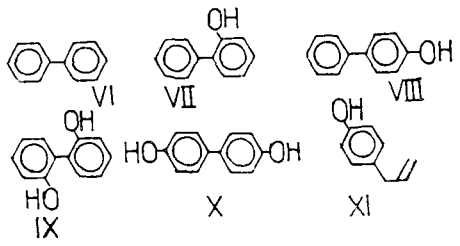

Fig. 5. Related compounds of I and II, biphenyl (VI), *o*-phenylphenol (VII), *p*-phenylphenol (VIII), *o*, *o'*-biphenol (IX), *p*. *p'*-biphenol (X) and chavicol (XI)

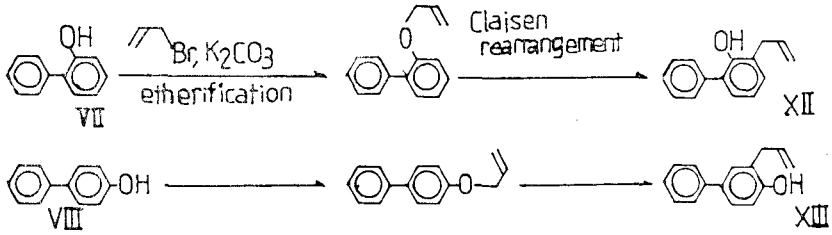

Fig. 6. Synthetic procedure of allylphenylphenol derivatives 6-allyl-*o*-phenylphenol (XII), 2-allyl-*p*-phenylphenol (XIII).

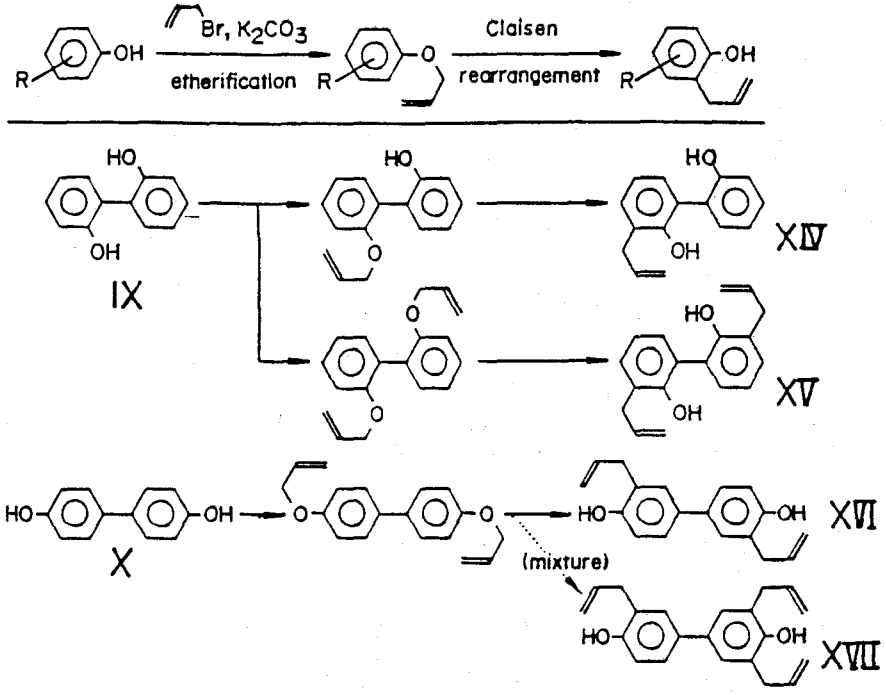

Fig. 7. Synthetic procedure of allylbiphenol derivatives. 6-Allyl-*o*, *o*′-biphenol (XIV), 6, 6′-diallyl-*o*, *o*-biphenol (XV), 2, 2′-diallyl-*p*, *p*′-biphenol (XVI), 2, 2′, 6-triallyl-*p*, *p*′-biphenol(XVII).

respectively (Fig. 4). The derivatives of I, modified OH group, III, IV and V showed no antibacterial activity against *S. mutans*,(Table III) from which OH group in the hydroxybiphenyl compounds is thought to be an essential element for the antibacterial activity.

(b) The antibacterial activity of related compounds with I and II.

For the comparison of antibacterial activity with I and II, the related compounds (Fig. 5), biphenyl (VI), *o*-phenylphenol (VII), *p*-phenylphenol (VIII), *o*, *o*′-biphenol (IX), *p*, *p*′-biphenol (X) and chavicol (XI) were tested (Table IV). The compounds VI and XI had no antibacterial activity, and the others were very weak in activity comparing with that of the compounds I and II. Therefore, it has been clarified that the introduction of allyl group to the hydroxybiphenyl ring to increase the antibacterial activity. It is interesting that chavicol (XI), the monomer of magnolol (I), has no antibacterial activity even though it has allyl group and hydroxy

Table V. Antibacterial activities of synthetic allylphenylphenols and allylbiphenols

Compounds	Diameter of inhibitory zone (mm)[1]					
	5μg[2]	10μg	20μg	40μg	80μg	120μg
6-allyl-*o*-phenylphenol	9.3	10.6	13.8	15.8	17.8	19.0
2-allyl-*p*-phenylphenol	12.5	14.4	17.2	19.9	22.1	24.1
6-allyl-*o*, *o*′-biphenol	12.5	14.8	17.5	20.1	23.0	24.8
6, 6′-diallyl-*o*, *o*′-biphenol	11.5	13.3	15.9	18.2	19.4	21.8
2, 2′-diallyl-*p*, *p*′-biphenol	13.0	17.0	20.9	24.5	29.0	30.9
2, 2′, 6-triallyl-*p*, *p*′-biphenol	12.7	16.5	20.0	22.9	27.7	29.4

The legends are same as shown in Table I

group in the benzene ring. These results allow to conclude that the basic chemical moiety having antibacterial activity of the activie principles, I and II, is hydroxybiphenyl.

(c) The antibacterial activity of synthetic derivatives of phenylphenols and biphenols

To develop more potent antibacterial compounds, some allylhydroxybiphenyl compounds (Fig. 6, XII and XIII; Fig. 7, XIV~XVII) were synthesized by Claisen's rearrangement[7] from starting materials, *o*-phenylphenol (VII), *p*-phenylphenol (VIII), *o*,*o'*-biphenol (IX) and *p*,*p'*-biphenol (X). In the synthesis of allylphenylphenols (Fig. 6, XII and XIII) and allylbiphenols (Fig. 7, XIV~XVII), two steps, etherification and rearrangement were occurred. In the first step, allylphenylether derivatives were prepared from starting materials and allylbromide in acetone, and in the second step, the allylphenylphenols or the allylbiphenols were synthesized in N, N-diethylaniline from the prepared allylphenylethers. As shown in Table V, the antibacterial activities of synthetic compounds XII, XIII, XIV and XV are similar to those of I and II, but the synthetic compounds XVI and XVII show more potent antibacterial activities in comparison with those of I and II. The introduction of allyl groups to the aromatic rings of phenylphenol or biphenol show the increase of antibacterial activities, but the number and

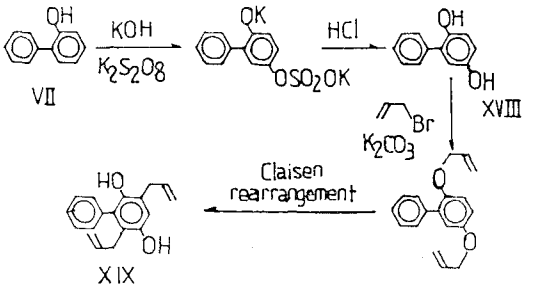

Fig. 8. Synthetic procedure of 4-hydroxy-*o*-phenylphenol (XVIII) and 3,6-diallyl-4-hydroxy-*o*-phenylphenol (XIX)

position of them in aromatic rings do not effect their activities. The antibacterial activity of para hydroxylated compounds (XIII, XVI and XVII) were more potent than that of ortho hydroxylated compounds (XII, XIV and XV).

(d) The effect with the introduction of OH group to hydroxybiphenyl ring

To survey the antibacterial activity with the introduction of OH group to the hydroxybiphenyl ring, the compound VII were oxidized by the modified Elb's persulfate oxidation method[8] (Fig. 8). The oxidized compound, 4-hydroxyphenylphenol (XVIII) were more potent antibacterial activity than that of starting material VII (Table VI) From the result, the introduction of OH group to hydroxybiphenyl ring was clarified to enhance the activity.

Table VI. Antibacterial activities of synthetic hydroxyphenylphenol and allylhydroxyphenylphenol

Compounds	Diameter or inhibitory zone (mm)[1]					
	5 μg	10μg	20μg	40μg	80μg	120μg[2]
o-phenylphenol	—	—	—	—[3]	8.4	10.1
4-hydroxy-*o*-phenylphenol	—	—	8.7	10.5	12.8	13.9
3,6-diallyl-4-hydroxy-*o*-phenylphenol	10.2	11.8	15.0	17.2	18.4	20.8

The legends are same as shown in Table I

Conclusion

It has been clarified that magnolol (I) and honokiol (II), isolated from the stem bark *Magnolia obovata*, had potent antibacterial activity against a cariogenic bacterium *Streptococcus mutans* OMZ176 and the strains of *Bacillus anthr-*

acis.[1-2] As the result of their strong bactericidal action (minimal inhibitory concentration 6.25 μg/m*l*, the cells of *S. mutans* and *B. anthracis* are sterilized in 2~5 minutes by exposing to 100 μg/m*l* of I and II, and more potent than berberine in antibacterial activity), magnolol (I) and honokiol (II) might be used as an oral bactericide or general antibacterial agents.

In order to obtain some information about structure-activity relationships in the hydroxyb iphenyl compounds, and structural requirements for more potent antibacterial agents, some synthetic procedures were carried out. The results allow to conclude that the basic chemical moiety having the antibacterial activity is hydroxybiphenyl. It has been clarified that the introduction of allyl group to the hydroxybiphenyl ring to increase the antibacterial activity. It has been also elucidated that the introduction of OH group to the hydroxyphenylphenol to enhance the activity. Among synthetic compounds, the antibacterial activities of 2,2′-dially-*p*,*p*′-biphenol (XVI) and 2,2′,6-triallyl-*p*,*p*′-biphenol (XVII) were more potent than those of magnolol (I) and honokiol (II).

Acknowledgment—The authors wish to thank Prof. T. Namba and Associate Prof. M. Hattori, Toyama Medical and Pharmaceutical Univ. for the gift of authentic samples (magnolol and honokiol) and the guidance.

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