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Anticariogenic β-Carboline Alkaloids from *Commelina communis*

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Abstract □ The methanolic extract of *Commelina communis* (aerial part) showed antibacterial activity aganist a cariogenic bacterium, *Streptococcus mutans* OMZ 176. The active principles were identified to be β-carboline alkaloids, 1-carbomethoxy-β-carboline, norharman and harman, which were bactericidal in the minimal inhibitory concentration (MIC) of 100 μg/m*l* against the strain.

Keywords □ *Commelina communis 1-carbomethoxy*-β-carboline, norharman, harman, anticariogenic activity, *Streptococcus mutans* OMZ 176.

Commelina communis (Commelinaceae) is widely distributed in Korea and has been used in traditional medicine for hepatitis, jaundice, hypertention, etc[1]. The pigments of flower have been reported by Tamura *et al.*, [2] the alkalidal components and nonalkaloidal components of aerial part by us[3,4].

In the course of extended study to develop anticariogenic agents[5], we found that the alkaloidal fraction extracted from this plant had antibacterial activity against a cariogenic bacterium, *Streptococcus mutans* OMZ 176. The present paper deals with the isolation and identification of anticariogenic components.

EXPERIMENTAL METHODS

Melting points were determined on Yanaco melting point apparatus and uncorrected. IR spectra were recorded in KBr disc by Perkin Elmer 1310 Specctrometer. ^{1}H-NMR spectra were taken with Bruker AM-300 NMR Spectrometer with TMS as an internal standard. Column chromatography was carried out on silica gel 60. TLC and preparative TLC were performed on precoated silica gel 60GF_{254} plates. The plant material was collected in Kyungsan on Sep. to Aug., 1979.

Isolation of active components

The aerial part of *Commelina communis* (32 kg) was extracted with methanol twice and concentrated. The methanolic extract (4 kg) was extracted twice with 2 *l* of 3% aqueous HCl. The resulting acidic solution was adjusted to pH 10 with NH_4OH, extracted with 500 m*l* of ethyl acetate and concentrated. The ethyl acetate extract (6g) was applied on a column of silica gel and eluted with *n*-hexane-ethyl acetate (2:1) and chloroform-methanol (50:1) systems. The fractions were monitored by TLC and positive color reaction with Dragendorff reagent. The fractions showing Rf 0.47 (*n*-hexane-EtOAc-MeOH=6:4:1) were pooled and concentrated *in vacuo* to give a white needle crystal, which was recrystallized in ethanol (component I, 18 mg). The fractions eluted by chloroform-MeOH (50:1) systems were also monitored by TLC and positive color reaction with Dragendorff reagent. The fractions

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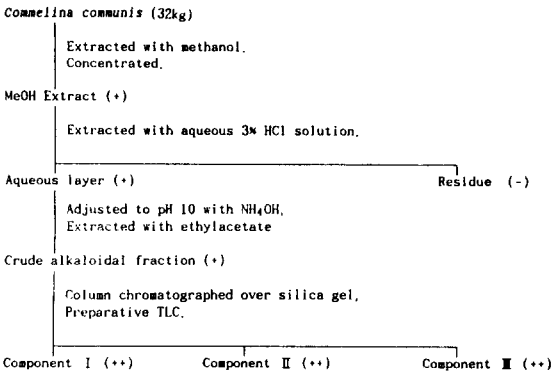

Scheme 1. Systematic fractionation and isolation procedures monitored with antibacterial activity against *S. mutans* OMZ 176.

The antibacterial activity was examined with paper disc method and each sample was prepared in 100 μg per disc. The activity was represented as follows;

− : no inhibitory zone was formed by adding 100 μg/disc.

+ : inhibitory zone of below 10 mm in diameter was formed.

++ : 10~13 mm in diameter.

showing Rf 0.34 were pooled, concentrated, and applied on preparative TLC (EtOAc-AcOH-H_2O=25:1:8, upper layer) to give component II (8 mg) and III (4 mg), respectively.

Component I

Recrystallization from *n*-hexane yielded white needles. mp 160-161℃. IR (cm^{-1}): 1678 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 9.91 (1H, br.s, NH), 8.58 (1H, d, *J*=5 Hz, 3-H), 8.14 (1H, d, *J*=8 Hz, 5-H), 8.13 (1H, d, *J*=5 Hz, 4-H), 7.67-7.55 (2H, m, 6.8-H), 7.36-7.27 (1H, m, 7-H), 4.12 (3H, s, -OMe).

Component II

Recrystallization from ether yielded pale yellow needles. mp 193-195℃. IR (cm^{-1}): 1626 (aromatic C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 10.46 (1H, br.s, NH), 8.93 (1H, s, H-1), 8.43 (1H, d, *J*= Hz, H-3), 8.12 (1H, d, *J*=8 Hz, H-5), 7.97 (1H, d, *J*= Hz, H-4), 7.57-7.45 (2H, m, H-6, 8), 7.35-7.18 (1H, m, H-7).

Component III

Recrystallization from ether yielded pale yellow needles. mp 230-233℃. IR (cm^{-1}): 1625 (aromatic C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 10.56 (1H, br.s, NH), 8.31 (1H, d, *J*= Hz, H-3), 8.11 (1H, d, *J*=8 Hz, H-5), 7.83 (1H, d, *J*= Hz, H-4), 7.55-7.46 (2H, m, H-6, 8), 7.25-7.20 (1H, m, H-7), 2.82 (3H, s, -CH_3).

The antibacterial activity of each fraction and the components was monitored with the procedure as shown in Scheme 1.

Antibacterial activity

Antibacterial activity was examined with paper disk method on BHI agar plate and the minimum inhibitory concentration was determined in BHI broth as described in previous reports[5-7], using a cariogenic bacterium, *Streptococcus mutans* OMZ 176.

To examine the antibacterial activity of each fraction or components with the paper disc method, *S. mutans* was cultivated in brain heart infusion (BHI, Difco Lab.) at 37° overnight. The turbidity of bacterial suspension was adjusted with the same broth to the optical density of 0.07 absorbance at 550 nm. The bacterial cell suspension (0.6 m*l*) was poured uniformly on the agar plates made of BHI as medium. Discs were carefully placed on the seeded plates. Culture was carried out at 37° for 24 hrs. Antibacterial activity in paper disc method was measured as inhibitory zones around paper discs (6 mm in diameter) for each fraction (100 μg/disc) and purified compounds.

The minimum inhibitory concentration was determined with two-fold dilution method[5-7]. Test compounds were dissolved in a minimum volume of ethanol and prepared for two-fold step dilution series of the solution. The solution (0.1 m*l*) was then added to the inoculated BHI broth (4.9 m*l*) which had ca. 0.01 unit at 550 nm. The value of MIC was determined with visual judging from the results of bacterial growth in the series of test tubes.

RESULTS AND DISCUSSION

Anticariogenic fraction and components

As described in Scheme 1, the distribution and purification of the active components were monitored by the paper disc assay method[5-7]. For the isolation and identification of antibacterial components, the methanolic extract of *C. communis* was treated with HCl, and fractionated acidic soluble fraction

Table I. Anticariogenic activities of 1-carbomethoxy-β-carboline, norharman and harman against *S. mutans* OMZ 176

Compounds	Diameter of inhibitory zone (mm)[a]				MIC[c] (μg/ml)
	10[b]	20	40	80	
I	7.3± 0.4[d]	9.0± 0.4	10.8± 0.3	12.5± 0.3	100
II	7.1± 0.1	8.4± 0.4	9.7± 0.3	11.3± 0.	100
III	6.3[e]	7.8± 0.3	9.2± 0.2	10.8± 0.2	100

[a]Mean values from four observations.
[b]Added amounts (μg) per disc.
[c]Minimum inhibitory concentration.
[d]Mean± standard deviation.
[e]Calculated by least square method.

and residue. Acidic soluble fraction showed antibacterial action, but no activity was found in the residue. The acidic solution was adjusted to pH 10 with NH_4OH and extracted with ethyl acetate, concentrated to give crude alkaloid fraction. The alkaloidal fraction showed antibacterial activity but no activity in the mother liquour, these results showed that the active principles were in alkaloidal fraction.

Therefore, the alkaloidal fraction was column chromatographed on silica gel column. The major three components (component I, II and III) were isolated from the fraction. Component I, showed antibacterial activity, was easily obtained from silica gel column, and identified as 1-carbomethoxy-β-carboline from the comparision of spectral data[8]. Component II and III were not separated completely each other on the column chromatography. They could be obtained from preparative TLC. The physicochemical data of component II were identical with those of norharman[9,10], and component III was identified as harman[8,10].

Component I, II and III are all β-carboline alkaloids and have been known as a widespread chemotaxonomic distribution[11].

Antibacterial activity of component I, II and III

The β-carboline alkaloids have been known to act principally on the central nerve system and muscle[11], but have not been reported on the antibacterial activity. The antibacterial activity of component I (1-carbomethoxy-β-carboline), II (norharman) and III (harman) are as shown in Table I. Component I, II and III showed slightly different potency in antibacterial activity with paper disc method[5-7], but in MIC test, they showed same values of 100 μg/ml. These results showed that the isolated β-carbolines had almost same anticariogenic activity. The antimicrobial activity of 1-alkalated β-carbolines was reported by Kosuge *et al.*[12] against *Trichophyton interdigitale*. The paper reported that the introduction of low alkyl group such as methyl, ethyl and propyl at C-1 position in β-carboline structure decreased the activity. The fact that norharman had slightly superior activity than that of harman in paper disc method (Table. I), though the difference of activity values was meaningless, may agree with the result of early report[12].

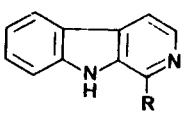

Fig. 1. Anticariogenic components isolated from *Commelina communis* against a cariogenic bacterium, *Streptococcus mutans* OMZ 176.
I, R=CO_2Me, 1-Carbomethoxy-β-carboline
II, R=H, Norharman
III, R=Me, Harman

Comparing with the activities of magnolol and honokiol[13,14] (MIC, 6.3 ug/ml) isolated from the stem bark of *Magnolia obovata* Thunb., the anticariogenecity of the β-carbolines was weak. But, the β-carbolines had almost same activities as berberine or emodin in the test of MIC (100 μg/ml) or paper disk method[5-7]. The antibacterial functional group of magnolol and honokiol is thought to be phenolic-OH[13], but it is still unknown that the mechanism of the β-carboline alkaloids on the antibacterial activity. The effect of antibacterial activity with the

introduction of substituent groups in β-carboline moiety shall be studied later.

LITERATURE CITED

1. Shanghai Science & Technological Publisher: "*The Dictionary of Chinese Drugs*" (Zhong Yao Da Ci Dian). Shougakukan, Tokyo, pp. 147 (1985).
2. Tamura, T., Kondo, T. and Goto, H.: The composition of commelinin, a highly associated metalloanthocyanin present in the blue flower petals of *Commelina communis*. *Tetrahedron Lett.*, **27**, 1801 (1986).
3. Baek, S., Seo, W., K. and Jin, K.: Studies on alkaloidal constituents of *Commelina communis*. *Yakhak Hoeji*, **34**, 34 (1990).
4. Baek, S., Seo, W., Bae, K. and Jin, K.: Studies on the iridoid, triterpenoid and steroid components of *Commelina communis*. *Yakhak Hoeji*, **34**, 64 (1990).
5. Bae, K., Kim, B., Myung, P., Chung, K. and Baek, J.: The isolation and evaluation of bioactive components from crude drugs against a cariogenic bacterium, *Streptococcus mutans* OMZ 176; an antibacterial component of Polygoni Radix and its safety. *Yakhak Hoeji*, **34**, 277 (1990).
6. Seo, W., Koo, S. and Bae, K.: Antimicrobial activities of hydroxybiphenyl derivatives. *Arch. Pharm. Res.*, **9**, 127 (1986).
7. Namba, T., Tsunezuka, M, Bae, K. and Hattori, M.: Studies on dental caries prevention by traditional Chinese medicines (part I); Screening of crude drugs for antibacterial action against *Streptococcus mutans*., *Shoyakugaku Zasshi*, **35**, 295 (1981).
8. Park, J., Kim, M., Yoo, S. and Wee, J.: Chemical studies on the alkaloidal fraction of *Panax ginseng*., Isolation of 1-carbobutoxy-β-carboline, and 1-carbomethyoxy-β-carboline., *Arch. Pharm. Res.* **10**, 197 (1987).
9. Chang, Y., Kim, S. and Han, B.: Chemical studies on the alkaloidal constituents of *Codonopsis lanceolata*, *Yakhak Hoeji*, **30**, 1 (1986).
10. Han, B., Park, J., Park, M. and Han, Y.: β-Carboline alkaloids of *Polygala tenuifolia*. *Arch. Pharm. Res.*, **8**, 243 (1985).
11. Allen, J. R. F. and Holmstedt, B. R.: The simple β-carboline alkaloids, *Phytochemistry*, **19**, 1753 (1980).
12. Wakabayashi, K., Yamamoto, T., Tsuji, K., Zenda, H. and Kosuge, T.: Studies on active principles of tar. VI. Antifungal constituents in fish meal tar., *Yakugaku Zasshi*, **98**, 898 (1978).
13. Bae, K., Seo, W. and Leem, S.: Synthesis of 4,4′-biphenol derivatives and antibacterial activities against a cariogenic bacterium, *Streptococcus mutans* OMZ 176, *Yakhak Hoeji*, **36**, 36 (1992).
14. Namba, T., Tsunezuka, M., Hattori, M., Kadota, S. and Kikuchi, T.: Studies on dental caries prevention by traditional Chinese medicines. Screening of crude drugs for inhibitory action on plaque formation, *Proc. Symp. Wakan-yaku*, **15**, 179 (1982).