BIPHENYLS, A NEW CLASS OF COMPOUND THAT INHIBITS PLATELET-ACTIVATING FACTORS

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Honokiol (1), a constituent of *Magnoliae* cortex, and related synthetic biphenyls inhibited the platelet aggregation induced by a few aggregating agents. In particular, compounds 3, 5, and 10 had an inhibitory effect on platelet activating factor (PAF), which provides a new class of PAF inhibitor.

KEYWORDS neolignan; biphenyl; honokiol; platelet aggregation; inhibitor; platelet-activating-factor; arachidonic acid; collagen; structure-activity relationship

The biphenyl neolignans honokiol (1) and magnolol (2) isolated from *Magnoliae* cortex have a few biological activities, including central depressant effects,¹⁾ anti-microbial activity against Gram-positive and acid-fast bacteria and fungi,²⁾ and Ca²⁺-blocking activity.³⁾ Here we report that 1 and related synthetic biphenyls have, in addition to their announced activities, inhibitory effects in the platelet aggregation induced by a few aggregation agents, including platelet-activating factor (PAF), sodium arachidonate (AA), and collagen (COL). In particular, the inhibitory effect of the synthetic biphenyls 3, 5, and 10 on the PAF-induced platelet aggregation provides a new class of inhibitor. We also analyzed the structure-activity relationship of the biphenyl compounds and their activities.

MATERIALS AND METHODS

Materials: The biphenyl compounds were synthesized as follows: 1 and 3 from corresponding quinol-acetates and the Grignard reagents according to our previous report⁴⁾; 4 and 5 from 3 by methylation and acetylation; 6⁵⁾ from 2-iodo-4-propylanisole by the Ullmann reaction; 7 from 6 by acetylation; 8 from 1,1'-dieugenol⁶⁾ which was prepared by oxidative coupling reaction of eugenol, followed by hydrogenation; 9 from 8 by acetylation; 10⁷⁾ from 2-allyl-6-methoxyphenol by oxidative coupling reaction.

Platelet aggregation: Mature male albino rabbits weighing 2-3 kg were used. Blood was collected from the carotide artery by cannulation and placed in centrifuge tubes containing 1/10 volume of a 3.8% sodium citrate solution to prevent coagulation. The blood samples were centrifuged at 1,000 rpm for 10 min to obtain platelet-rich plasma (PRP). The remaining blood samples were centrifuged at 3,000 rpm for 10 min to obtain platelet-poor plasma (PPP). Inhibition of platelet aggregation was determined by a Type Aggrecoorder II (Kyoto Daiichi Kagaku) using the method by Born.⁸⁾ PRP (222.5 μ l) was put in a cuvette and warmed at 37°C with stirring (1200 rpm),

- 1: $R^1 = R^3 = \text{OH}$, $R^2 = R^4 = \text{H}$
 2: $R^1 = R^4 = \text{H}$, $R^2 = R^3 = \text{OH}$
 3: $R^1 = R^3 = \text{OH}$, $R^2 = \text{H}$, $R^4 = \text{OMe}$
 4: $R^1 = R^3 = R^4 = \text{OMe}$, $R^2 = \text{H}$
 5: $R^1 = R^3 = \text{OAc}$, $R^2 = \text{H}$, $R^4 = \text{OMe}$

- 6: $R^1 = R^2 = R^5 = R^6 = \text{H}$, $R^3 = R^4 = \text{OH}$
 7: $R^1 = R^2 = R^5 = R^6 = \text{H}$, $R^3 = R^4 = \text{OAc}$
 8: $R^1 = R^6 = \text{H}$, $R^2 = R^5 = \text{OMe}$, $R^3 = R^4 = \text{OH}$
 9: $R^1 = R^6 = \text{H}$, $R^2 = R^5 = \text{OMe}$, $R^3 = R^4 = \text{OAc}$
 10: $R^1 = R^6 = \text{OAc}$, $R^2 = R^5 = \text{OMe}$, $R^3 = R^4 = \text{H}$

Table I. Inhibitory Effect of the Biphenyl Compound, Honokiol and Compounds, on Platelet Aggregation in PRP from Rabbits

Compound	IC_{50} (mM)			
	PAF	AA	COL	ADP
1	—*	0.076	—	—
3	0.52	0.105	0.24	—
4	—	—	—	—
5	0.21	0.044	0.27	—
6	—	0.42	0.70	—
7	—	—	—	—
8	—	—	—	—
9	—	—	—	—
10	0.06	0.07	0.07	—

* — are significantly shown non-inhibitory effect.

then 2.5 μl of a test solution was added. Exactly 1 min later, 25 μl of aggregating agents were added. The light transmission of PRP and PPP was taken as 0% and 100% aggregation, respectively. Aggregation agents such as PAF, AA, COL, and adenosine diphosphate (ADP) were prepared as follows: the PAF was dissolved in 0.2% bovine serum albumin at a final concentration of 0.01 $\mu\text{g/ml}$; the AA and ADP were dissolved in 0.9% saline at a final concentration of 10 μM and 300 μM , respectively; and the COL was diluted to a final concentration of 30 $\mu\text{g/ml}$ with SKF Horm buffer (Collagen Horm Co., Ltd., West Germany) to sustain its activity. Test samples were dissolved in dimethyl sulfoxide. The inhibition rates of various test compounds on platelet aggregation were expressed as the IC_{50} value which were determined by measuring the inhibitory effects of each agent at four different inhibitor concentrations in more than five experiments and analyzed graphically from the resulting dose-response curves. Statistical significance was determined by Student's or Cochran's t-test after an F-test.

RESULTS AND DISCUSSION

Preliminary investigations of the inhibition of platelet aggregation in PRP from rabbits showed that **1** dose-dependently inhibited AA-induced platelet aggregation (IC_{50} potency: 0.076 mM; Table I). According to such information, we synthesized several related biphenyl compounds and determined their inhibitory effects in platelet aggregation induced by several aggregating agents (Table I), by studying their structure-activity relationship.

Experimental results indicate that **10** is more active than other compounds in all respects except ADP-induced aggregation. Compound **9**, a position isomer of **10**, had no potency. Compounds **3** and **5** were potent but **4** had no effect. These results indicate that the hydroxyl or acetoxyl group at the C-4 position is required to inhibit platelet aggregation induced by PAF, AA, and COL. Further, it may be necessary to have, in addition to the AA and COL antagonistic activities, a methoxy substituent at the C-3' position to inhibit PAF, as the compounds **3**, **5**, and **10** have.

The present results are of interest in regard to the medical uses of *Magnoliae* cortex, a Chinese herbal medicine and a parent plant of honokiol **1**, and may provide an additional new series of antagonist compounds for PAF-induced platelet aggregations from the natural sources, kadsurenone from *Piper futokadsura*,⁹⁾ gingolide B from *Gingo biloba*,⁹⁾ and praeruptorin A (= Pd-Ia) and B (= Pd-II) from *Peucedanum praeruptorum*.¹⁰⁾

REFERENCES AND NOTES

- 1) a) K. Watanabe, H. Watanabe, Y. Goto, M. Yamaguchi, N. Yamamoto, and K. Hagino, *Journal of Medicinal Plant Research*, **49**, 103 (1983); b) H. Watanabe, K. Watanabe, and K. Hagino, *J. Pharmacobio-Dyn.*, **6**, 184 (1983); c) K. Watanabe, H. Watanabe, Y. Goto, N. Yamamoto, and M. Yoshizaki, *Jpn. J. Pharmacol.*, **25**, 605 (1975).
- 2) a) A. M. Clark, F. S. El-Feraly, and W.-S. Li, *J. Pharm. Sci.*, **70**, 951 (1981); b) K. Ito, T. Iida, K. Ichino, M. Tsunozuka, M. Hattori, and T. Namba, *Chem. Pharm. Bull.*, **30**, 3347 (1982); c) T. Namba, M. Tsunozuka, M. Hattori, S. Kadota, and T. Kikuchi, *Proc. Symp. Wakan-Yaku*, **15**, 179 (1982); d) T. Namba, M. Tsunozuka, and M. Hattori, *Journal of Medicinal Plant Research*, **44**, 100 (1982).
- 3) J. Yamahara, S. Miki, H. Matsuda, and H. Fujimura, *Yakugaku Zasshi*, **106**, 888 (1986).
- 4) T. Takeya, T. Okubo, and S. Tobinaga, *Chem. Pharm. Bull.*, **34**, 2066 (1986).
- 5) M. Fujita, H. Itokawa, and Y. Sashida, *Yakugaku Zasshi*, **93**, 422 (1973).
- 6) A. Nishiyama, H. Eto, Y. Terada, M. Iguchi, and S. Yamamura, *Chem. Pharm. Bull.*, **31**, 2820 (1983).
- 7) Compound **10** was synthesized from 2-allyl-6-methoxyphenol by oxidation with $Mn(ClO_4)_2 \cdot 9H_2O$ and $KMnO_4$ in Ac_2O .
- 8) G. V. R. Born, *J. Physiol.*, **162**, 67 (1962).
- 9) "Biological Active Ether Lipids", Ed., by P. Braquet, H. K. Mangold, and B. B. Vargaftig (Progress in Biochemical Pharmacology, Vol. **22**, Ed., by Paoletti Kargel, 1988).
- 10) N. Takeuchi, T. Kasama, K. Mayuzumi, K. Tamaru, H. Fukaya, T. Ohno, and S. Tobinaga, *Chem. Pharm. Bull.*, **36**, 4221 (1988).

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