Communications to the Editor

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THE ANTAGONIST EFFECTS OF COMPOUNDS DERIVED FROM KHELLACTONE ON PLATELET-ACTIVATING FACTOR

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Crude coumarin constituents of Peucedanum praeruptorum Duun. including praeruptorin A (= Pd-Ia) (2) and B (= Pd-II) (11) had an antagonistic effect on a specific platelet activating factor (PAF) in the platelet aggregation induced by several aggregating agents. This provides a new class of PAF antagonists. Studies of the structure-activity relationship with khellactone (1) derivatives and PAF antagonistic activity indicate that the cis isomers of 1 at the C-3' and C-4' positions are more favorable than trans isomers, and the acyl moiety at the C-3' position of 1 requires an appropriate molecular size.

KEYWORDS — Peucedanum praeruptorum; praeruptorin A (= Pd-Ia); praeruptorin B (= Pd-II); khellactone; PAF antagonist; platelet aggregation; structure-activity relationship

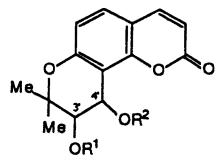
Among the naturally occuring coumarins, various biological activities of the compounds derived from khellactone (1), namely, piscicidal,[1] in vitro uterine relaxation,[1] vasodilation,[1,2] hypotension,[1] calcium antagonism,[3,4] platelet aggregation,[5] inhibition of histamine release,[3,4] and acetylcholine-, barium chloride- and serotonin-induced spasms of isolated rat and rabbit intestine,[1,2,6] have been reported. We had reported that natural (the constituent of Peucedanum japonicum Thunb.) and synthetic prenyl coumarins have calcium antagonistic, histamine antagonistic, and cerebral blood flow-increasing effects.[7] Here we report that crude coumarin (the mixture 2 and 11 derived from Peucedanum praeruptorum Duun.; corresponding to praeruptorin A (= Pd-Ia) and B (= Pd-II)[8,9]) has a specific antagonistic effect on the platelet activating factor (PAF)[10] in the platelet aggregation induced by several aggregating agents. And we analyzed the structure-activity relationship of compounds derived from 1 and their activities.

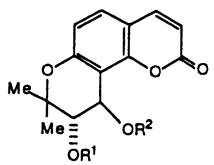
MATERIALS AND METHODS

Materials: Coumarin (a mixture of 2 and 11) was isolated from the hexane extracts of the root of Peucedanum praeruptorum Duun. The khellactone derivatives were synthesized as follows: 1,[11,12] 4,[11,12,14] 12,[15] 17,[12] and 24,[15] from

seselin[16]; 3[11,12,13] and 10[17] from 1; 5,[18] 6,[18] 7,[18] 8,[18] and 9[18] from 4; 13[17] and 16[19] from 12; 18,[13] 19,[17] 20,[17] 21,[17] 22,[17] and 23[17] from 17; 25[17] from 24.

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 Aggrecorder II (Kyoto Daiichi Kagaku) using the method by Born.[20] PRP (222.5 μl) was put in a cuvette and warmed at 37°C with stirring (1,200 rpm), then 2.5 μl of a test solution was added. Exactly 1 min later, 25 μl of aggregating agents were added. The light transmissions of PRP and PPP were taken as 0% and 100% aggregation, respectively. Aggregation agents such as adenosine diphosphate (ADP), PAF, sodium arachidonate (AA), and collagen (COL) were prepared as follows: ADP and AA were dissolved in 0.9% saline at a final concentration of 10 μM and 300 μM, respectively; PAF was dissolved in 0.2% bovine serum albumin at a final concentration of 0.01 μg/ml, and collagen was diluted with SKH Horm buffer to a final concentration of 30 μg/ml. 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 determined graphically from the resulting dose-response curves. Statistical significance was determined by Student's or Cochran's t-test after an F-test.





1: $R^1 = R^2 = H$

2: $R^1 = CO$(Me)$C=C$(Me)(H), $R^2 = Ac$

3: $R^1 = R^2 = Ac$

4: $R^1 = H$, $R^2 = Ac$

5: $R^1 = CO$(H)$C=C$(H)(Me), $R^2 = Ac$

6: $R^1 = COC{\equiv}CMe$, $R^2 = Ac$

7: $R^1 = CO$(Me)$C=C$(H)(Me), $R^2 = Ac$

8: $R^1 = COCHMeCH_2Me$, $R^2 = Ac$

9: $R^1 = CO$(Me)$C=C$(H)$(CH_2)_8Me$, $R^2 = Ac$

10: $R^1 = R^2 = CO$(Me)$C=C$(H)(Me)

11: $R^1 = R^2 = CO$(Me)$C=C$(Me)(H)

12: $R^1 = H$, $R^2 = Et$

13: $R^1 = CO$(Me)$C=C$(H)(Me), $R^2 = Et$

14: $R^1 = CONH(CH_2)_2Cl$, $R^2 = Et$

15: $R^1 = CONH(CH_2)_2NEt_2$, $R^2 = Et$

16: $R^1 = CONH(CH_2)_2N^+Et_3I^-$, $R^2 = Et$

17: $R^1 = H$, $R^2 = Ac$

18: $R^1 = R^2 = Ac$

19: $R^1 = CO$(H)$C=C$(Me)(H), $R^2 = Ac$

20: $R^1 = CO(CH_2)_3Me$, $R^2 = Ac$

21: $R^1 = CO\,CH_2CHMe_2$, $R^2 = Ac$

22: $R^1 = CO$(Me)$C=C$(Me)(H), $R^2 = Ac$

23: $R^1 = CO(CH_2)_8Me$, $R^2 = Ac$

24: $R^1 = H$, $R^2 = Et$

25: $R^1 = CO$(Me)$C=C$(Me)(H), $R^2 = Et$

Chart 1

Table I. Inhibitory Effects of the Crude Coumarin Component of Peucedanum praeruptorum and Etizolam on Platelet Aggregation in PRP from Rabbits

Agent	IC_{50} (mM)			
	ADP	PAF	AA	COL
Crude coumarin	0.9<	0.09	0.9<	0.74
Etizolam	0.9<	0.0016<	0.9<	0.9<

Table II. Inhibitory Effects of Khellactone Derivatives on Platelet Aggregation Induced by PAF

Compound	IC_{50} (mM)	Compound	IC_{50} (mM)	Compound	IC_{50} (mM)
1	0.9<	10	0.04	17	0.9<
2	0.09	11	0.05	18	0.54
3	0.13	13	0.50	19	0.55
5	0.09	16	0.43	20	0.46
6	0.18			21	0.61
7	0.05			22	0.36
8	0.05			23	0.9<
9	0.90			25	0.9<

RESULTS AND DISCUSSION

Preliminary investigations of the inhibition of platelet aggregation in PRP from rabbits showed crude coumarin (a mixture of 2 and 11) from the root of Peucedanum praeruptorum Duun. dose-dependently, inhibited PAF-induced platelet aggregation more strongly than ADP-, AA-, or the collagen-induced inhibitor (Table I). Etizolam,[21] a PAF antagonist, also inhibited specifically PAF-induced platelet aggregation. The IC_{50} potency of crude coumarin was 0.09 mM, approximately 1/50 of that of etizolam. The coumarin consist of 2 and 11 in a ratio of 3:2. The PAF IC_{50} antagonism of 2 and 11, the authentic samples, were 0.09 and 0.05 mM, respectively. Subsequently, the PAF antagonistic activities of synthetic khellactone derivatives, summarized in Table II, were determined by studies of structure-activity relationships.

Experimental results indicate that the cis isomers at the C-3' and C-4' position of khellactone (1) are more active than those of the corresponding trans isomers such as 3 and 18 ($p < 0.05$); 7 and 22; 5 and 19 ($p < 0.05$); 13 and 25 (no effect). On the other hand, the compounds having an acyl moiety such as the tiglic acid moiety at 3' and 4' in 1, i.e., 11 and 10, 2 and 7, were similarly potent. Similarly, the compound in which R is an unsaturated acyl group (7) exhibited activity similar to that of a saturated one (8), but increased unsaturation showed poor activity (6; $p < 0.05$). In cases of R^1 = a bulky acyl group and R^2 = an alkyl group, i.e., 9 (no effect), 13 ($p < 0.05$), and 25 (no effect), these compounds were less potent than the corresponding

compounds such as 7 and 22. Further, compound 16 with the R = $-CONH(CH_2)_2N^+Et_3$ moiety, constitutes PAF itself in part, did not show the expected activity.

It is well known that PAF has various physiological actions, not only platelet and leukocyte activation but also lowering of blood pressure, increment of vascular permeability and bronchus constriction. Elucidation of the role of PAF in a variety of pathophysiologic conditions has been facilitated considerably by the development of PAF antagonists.[10] The present results showing the specific inhibition of PAF-induced platelet aggregation of 2 and 11 are of interest in regard to the medical uses of the Peucedanum species as a herb drug for bronchial disorders, stomach pain, etc. Further, these results may provide an additional new series of PAF antagonist compounds from natural sources, kadsurenone from Piper futokadsura[10] and gingolide B from Gingo biloba.[10]

REFERENCES AND NOTES

1) E. B. Thompson, G. H. Aynilian, R. H. Dobberstein, G. A. Cordell, H. H. S. Fong, and N. R. Farnsworth, J. Natural products, 42, 120 (1979), and refs. cited therein. 2) E. Smith, N. Hosansky, W. G. Bywater, and E. E. van Tamelen, J. Am. Chem. Soc., 79, 3534 (1957). 3) T. Kozawa, K. Sakai, M. Uchida, T. Okuyama, and S. Shibata, J. Pharm. Pharmacol., 33, 317 (1981). 4) T. Suzuki, Y. Kobayashi, M. Uchida, I. Sakakibara, T. Okuyama, and S. Shibata, J. Pharmacobio-Dyn., 8, 257 (1985). 5) T. Okuyama, C. Kawasaki, S. Shibata, M. Hoson, T. Kawada, H. Osada, and T. Noguchi, Planta Medica, 1986, 132. 6) R. E. Willette and T.O. Soine, J. Pharm. Sci., 51, 149 (1962). 7) N. Takeuchi, I. Maruyama, J. Ohki, S. Tobinaga, T. Kasama, Y. Aida, K. Watanabe, M. Koizumi, and K. Mayuzumi, J. Pharmacobio-Dyn., 8, s-53 (1985). 8) T. Okuyama and S. Shibata, Planta medica, 42, 89 (1981). 9) Z. X. Chen, B. S. Huang, Q. L. She, and G. F. Zeng, Acta Pharm. Sin., 14, 486 (1979). 10) "Biologically Active Ether Lipids", Ed., by P. Braquet, H. K. Mangold, and B. B. Vargaftig (Progress in Biochemical Pharmacology, Vol 22, Ed., by R. Paoletti), Karger, Basel, 1988. 11) S. Bal-Tembe, D. N. Bhedi, N. J. de Souza, and R. H. Rupp, Heterocycles, 26, 1239 (1987); Although the synthesis of 2 from 4 was reported by them in only 0.8% yield, we synthesized 2 by acylation of 4 with angeloyl chloride in benzene in 21.1% yield. 12) H. D. Schroeder, W. Bencze, O. Halpern, and H. Schmid, Chem. Ber., 92, 2338 (1959). 13) S. N. Shanbhag, M. L. Maheshwari, and S. C. Bhattacharyya, Tetrahedron, 23, 1235 (1967). 14) F. Bohlmann and H. Franke, Chem. Ber., 104, 3229 (1971). 15) K. Hata, M. Kozawa, Y. Ikeshiro, and K. -Y. Yen, Yakugaku Zasshi, 88, 513 (1968). 16) J. Hlubucek, E. Ritchie, and W. C. Tayler, Aust. J. Chem., 24, 2347 (1971). 17) 10, 13, 19, 20, 21, 22, 23, and 25 were synthesized by the reactions with corresponding alcohols 1, 12, 17, and 24 and the acid chlorides with refluxing in benzene. 18) 5, 6, 7, 8, and 9 were synthesized by the reactions with 4 and corresponding acids in the presence of dicyclohexylcarboimide and 4-pyrrolidinopyridine in methylene chloride, with refluxing. 19) 16 was synthesized by the reaction with 12 and 2-chloroethylisocyanate in chloroform in the presence of pyridine at 50°C to give 14, followed by the reaction of 14 with dimethylamine in DMF at 65°C and the treatment of 15 with ethyliodide with refluxing. 20) G. V. R. Born, J. Physiol., 162, 67 (1962). 21) T. Tahara, H. Mikashima, M. Terasawa, and Y. Maruyama, Chem. Pharm. Bull., 35, 2119 (1987).

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