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Inhibition of Platelet Aggregation by Colchicine, Thiocolchicine, and Their Phenolic and Glucosidic Congeners.

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Abstract □ Inhibition of platelet aggregation by colchicine, thiocolchicine, O-demethylated and 3-glucosidic congeners was measured in vitro. 2- And 3-demethylated analogs of colchicine and thiocolchicine were found to be as potent as colchicine and thiocolchicine, respectively, whereas 1-demethylated compounds were found to be considerably less active. Both glucosides, colchicoside and thiocolchicoside, were not very active in this assay. 2,3-Didemethylcolchicine, which is much less toxic than colchicine, showed similar inhibitory effects on platelet aggregation as colchicine.

Keywords □ Inhibition of platelet aggregation, Colchicinoids, Thiocolchicinoids.

Colchicine (**1**), the best known of the alkaloids of *Colchicum autumnale*, has antitumor and anti-inflammatory activities[1,2], and is a valuable drug against acute gout attacks[3]. Studies of phenolic congeners, present in plants and available by partial synthesis, showed that cleavage of the O-CH_3 groups in ring A afforded compounds which bound less to tubulin and had lower antitumor activity[1,4,5]. Ether cleavage of thiocolchicine (**7**), a partially synthetic analog of colchicine with a SMe-group at C-10 instead of an OMe-group, similarly afforded phenols[6]. The biological evaluation of the thiophenols paralleled findings made with the 10-OMe analogs[7]. The sugar alkaloid colchicoside (**6**) and its thio analog thiocolchicoside (**11**) are also of interest since **6**, unlike **1** and other nonsugar alkaloids, does not bind to tubulin[8].

An assay measuring inhibition of platelet aggregation, a crucial factor in ischemic disorders[9,10], was used to evaluate crude plant extracts[11]. This assay has been used by us to evaluate compounds derived from colchicine (**1**) and thiocolchicine (**7**), hoping that it would give additional information on these compounds and also allow an assessment for its use as a routine screening method to find compounds inhibiting platelet aggregation.

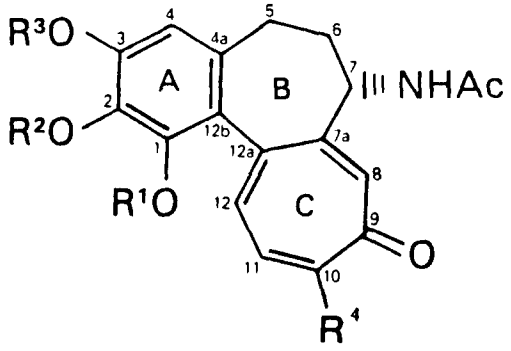
EXPERIMENTAL METHODS

Materials

The sugar alkaloid colchicoside was obtained from Dr. V. Simanek, Institute of Chemistry of the Medical Faculty, Palachy University, Olomouc, Czechoslovakia. Thiocolchicine and thiocolchicoside were generously supplied by Dr. J. Gaignault, Roussel-Uclaf, Romainville, France. O-Demethylated congeners of colchicine and thiocolchicine were prepared by the published procedures[5,6,7]. ADP (adenosine 5′-diphosphate dicyclohexylammonium salt), AA (arachidonic acid sodium salt) and collagen (acid soluble preparation from calf skin), were purchased from Sigma, Chemical Co., St. Louis, MO.

Methods

Preparation of platelet rich plasma (PRP), from citrated rat blood, and determination of platelet aggregation, by the modified smear method of Yun-Choi et al.[11], was as follows: Blood was drawn from hearts of $CHCl_3$-anesthetized Sprague-Dawley rats (males, 220 ± 30g) into a plastic syringe containing 1/10 vol. of 2.2% trisodium citrate solution. The citrated blood was centrifuged



1 $R_1 = R_2 = R_3 = CH_3$, $R_4 = OCH_3$
2 $R_1 = H$, $R_2 = R_3 = CH_3$, $R_4 = OCH_3$
3 $R_1 = R_3 = CH_3$, $R_2 = H$, $R_4 = OCH_3$
4 $R_1 = R_2 = CH_3$, $R_3 = H$, $R_4 = OCH_3$
5 $R_1 = CH_3$, $R_2 = R_3 = H$, $R_4 = OCH_3$
6 $R_1 = R_2 = CH_3$, $R_3 = Gluc.$, $R_4 = OCH_3$
7 $R_1 = R_2 = R_3 = CH_3$, $R_4 = SCH_3$
8 $R_1 = H$, $R_2 = R_3 = CH_3$, $R_4 = SCH_3$
9 $R_1 = R_3 = CH_3$, $R_2 = H$, $R_4 = SCH_3$
10 $R_1 = R_2 = CH_3$, $R_3 = H$, $R_4 = SCH_3$
11 $R_1 = R_2 = CH_3$, $R_3 = Gluc.$, $R_4 = SCH_3$

(200 × g) for 10 min at room temperature to give supernatant platelet rich plasma (PRP). Saline (10 μl) and 10 μl of the solution of the test sample prepared in 20% EtOH-saline (or 10 μl of 20% EtOH-saline as control) were added to 160 μl of PRP in a polyethylene tube. The mixture was incubated for 2 min at 37 °C and 20 ul of either one of the solutions of ADP, AA or collagen was added, to give final concentrations of 7.5×10^{-7} g/ml, 6×10^{-5} g/ml and 1×10^{-5} g/ml respectively. After vigorous agitation for 10 sec, and incubation at 37 °C for 4 min, thin smears were prepared on glass slides and quickly dried in the air. The smears were stained with a Wright-Giemsa stain, washed and dried. The stained smears were subjected to an examination under an ordinary light microscope using an oil immersion objective lens (1000 ×). The degrees of aggregation of platelets were judged as described by Yun-Choi et al.[11].

RESULTS AND DISCUSSION

Inhibition of platelet aggregation induced by adenosine 5'-diphosphate (ADP), arachidonic acid (AA) and collagen, used to detect biologically active compounds in crude plant extracts[11], was measured with colchicinoids **1 – 6**, and thiocolchicinoids **7 – 11**. Compounds **3, 8** and **10**, not soluble in 10% EtOH-saline were used as suspensions, a factor making an assessment of their inhibitory activity difficult. From the results listed in the Table, the following conclusions can be made: Colchicine (**1**) and thiocolchicine (**7**), both binding well to tubulin and having antitumor pro-

Table I. Effects of colchicinoids and thiocolchicinoids against ADP, arachidonic acid (AA) and collagen induced platelet aggregation

comp.	conc. of comp. / aggregating agent	1×10^{-3} M			5×10^{-4} M	
		ADP[a]	AA[b]	collagen[c]	AA[a]	collagen[b]
1	colchicine	+	±	±	+	+
2	1-O-demethylcolchicine	+ +	+	+ +		
3	2-O-demethylcolchicine[d]	+ +	±	±	±	+
4	3-O-demethylcolchicine	+	−	−	±	±
5	2,3-O-didemethylchochicine	+	±	±	+	+
6	chochicoside	+ +	+ +	+		
7	thiocolchicine	±	±	−	±	±
8	1-O-demethylthiocolchicine[d]	+ +	+	+		
9	2-O-demethylthiocolchicine	+	±	±	±	±
10	3-O-demethylthiocolchicine[d]	+	+	±	+	+
11	thiocolchicoside	+ +	+	+		

[a] ADP: 7.5×10^{-7} g/ml, [b] AA: 6×10^{-5} g/ml, [c] collagen: 1×10^{-5} g/ml,
[d] tested as suspensions
Degrees of platelet aggregation induced: −, no aggregation; ±, slight aggregation; +, intermediate aggregation, + +, as much aggregation as PRP plus aggregating agent alone.
The data represents the average of a minimum of five tests.

perties[1,7], clearly inhibited induced platelet aggregation. From O-demethylated congeners, the 3-demethylated compounds **4** and **10**, and the 2-demethylated compounds **3** and **9**, similarly inhibited aggregation as **1** and **7**, whereas the 1-demethylated compounds **2** and **8** were found considerably less potent. This finding parallels results obtained with these compounds in assays measuring inhibition of tubulin and P388 induced lymphocytic leukemia[1,5,7]. 2,3-Didemethylcolchicine (**5**), a natural alkaloid[1], which does not bind well to tubulin and has low antitumor activity[12], but excellent anti-inflammatory activity in an assay measuring suppression of edema in rat-pad[2,13], inhibited induced platelet aggregation similarly as colchicine (**1**). Both sugar alkaloids, colchicoside (**6**) and thiocolchicoside (**11**), did not suppress induced platelet aggregation.

It can be concluded that inhibition of platelet aggregation, induced by ADP, AA and collagen, of compounds structurally related to colchicine[1], were marked with the same compounds which were already shown to be biologically active in different screenings[1,5,7,12].

The finding that 2,3-didemethylcolchicine (**5**), which does not bind well to tubulin, had low antitumor activity[12] and markedly inhibited the rat pad edema[13], also inhibits induced platelet aggregation, suggests that this assay, requiring only mg's of material, may be of value to detect anti-inflammatory agents.

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13. Compound **5**, measured as described in (2) showed anti-inflammatory activity equal to that of colchicine. (Personal communication by K. Sugio, Faculty of Pharmaceutical Science, Tohoku University, Sendai, Japan). This compound, however, was much less active than colchicine when given by the oral route in an assay measuring uric acid induced edema (personal communication by Dr. K. Nakamura, Nippon Roche Research Center, Kamakura, Japan).