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Short Communication

R_M values of some colchicines and colchiceinamides determined by reversed-phase thin-layer chromatography

Damjan Glavač

Faculty of Science and Technology, University of Ljubljana, Ljubljana (Yugoslavia)

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ABSTRACT

The R_M values of twelve colchicines and eight colchiceinamides were measured using reversed-phase thin-layer chromatography. The R_M values were calculated by extrapolation from the linear range of a plot of R_M values *versus* the composition of the mobile phase. The results showed that in the colchicine series substitution at the nitrogen in position C_7 decreases the lipophilicity, whereas in the colchiceinamide series substitution at the nitrogen in position C_{10} increases lipophilicity. The influence of other substituent groups on the R_M values are considered.

INTRODUCTION

The usefulness of partition coefficients and R_M values [$R_M = \log(1/R_F - 1)$] as an expression of the lipophilic character of the molecule in quantitative structure–activity relationship studies has been shown for several series of biologically active compounds such as penicillins [1], steroids [2], benzodiazepins [3] and nucleosides [4]. Colchicine and many of its derivatives are powerful mitotic poisons, anti-inflammatory agents and inhibitors of tubulin, but their effects were only observed at near toxic levels [5]. The relationship between the physicochemical properties and biological activity of some colchicinoids was studied by Quinn and co-workers [6,7], who showed that there is a parabolic dependence of the antitumour potency on the partition coefficient [6].

The purpose of the work reported here was to study a larger number of natural colchicines and their synthetic analogues to compare their lipophilic characters. The colchicinoids given in Table I were either isolated from natural sources [8,9] or synthesized in this laboratory [10].

EXPERIMENTAL

The R_M values were measured using a reversed-phase thin-layer chromatography technique which allowed the partitioning of colchicinoids between the polar mobile phase and the non-polar stationary phase. The mobile phase consisted of a phosphate buffer (pH 7.4) in various mixtures with acetone. The stationary phase was obtained by impregnating precoated silica gel 60 F_{254} plates (0.25 mm, Merck) with 5% (v/v) liquid paraffin (Merck) in diethyl ether. The method of impregnating the plates and other details of the chromatographic technique have been described elsewhere [1–3]. The plates were left in the chamber for 12 h, that is, for several hours after the paraffin solution had reached the top of the plates.

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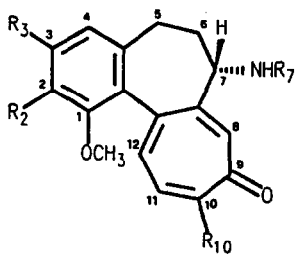
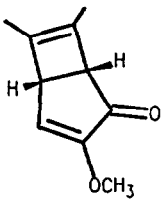
TABLE I

STRUCTURES AND R_M VALUES OF COLCHICINES AND COLCHICEINAMIDES



β-lumicolchicine



Compound		R_2	R_3	R_7	R_{10}	R_M	Log P^c
No.	Name						
1	Colchicine	OCH_3	OCH_3	$COCH_3$	OCH_3	0.87	1.03
2	N-Deacetylcolchicine[a]	OCH_3	OCH_3	H	OCH_3	1.21	1.10
3	N-Acetoacetylcolchicine	OCH_3	OCH_3	$COCH_2COCH_3$	OCH_3	0.92	
4	Colchifoline	OCH_3	OCH_3	$COCH_2OH$	OCH_3	0.68	
5	N-Formylcolchicine	OCH_3	OCH_3	CHO	OCH_3	0.76	1.02
6	2-Demethylcolchicine	OH	OCH_3	$COCH_3$	OCH_3	0.61	
7	3-Demethylcolchicine	OCH_3	OH	$COCH_3$	OCH_3	0.57	0.93
8	Demecolcine	OCH_3	OCH_3	CH_3	OCH_3	1.29	1.53
9	2-Demethyldemecolcine	OH	OCH_3	CH_3	OCH_3	0.78	
10	3-Demethyldemecolcine	OCH_3	OH	CH_3	OCH_3	0.73	
11	β-Lumicolchicine	OCH_3	OCH_3	$COCH_3$	OCH_3	1.57	
12	Colchicoside	OCH_3	gluc.[b]	$COCH_3$	OCH_3	0.36	
13	Colchiceinamide (CA)[a]	OCH_3	OCH_3	$COCH_3$	NH_2	0.82	
14	Formyl-CA[a]	OCH_3	OCH_3	$COCH_3$	NHCHO	1.16	
15	Acetyl-CA[a]	OCH_3	OCH_3	$COCH_3$	$NHCOCH_3$	1.25	
16	Glycolyl-CA[a]	OCH_3	OCH_3	$COCH_3$	$NHCOCH_2OH$	1.07	
17	Acetoacetyl-CA[a]	OCH_3	OCH_3	$COCH_3$	$NHCOCH_2COCH_3$	1.34	
18	N-Deacetyl-CA[a]	OCH_3	OCH_3	H	NH_2	0.97	
19	2-Demethyl-CA[a]	OH	OCH_3	$COCH_3$	NH_2	0.54	
20	O-Acetyl-glycolyl-CA[a]	OCH_3	OCH_3	$COCH_3$	$NHCOCH_2OCOCH_3$	1.52	

[a] Synthesized as in Glavač *et al.* [10].
[b] β-D-glucosyl.
[c] Taken from Quinn and Beisler [6]; P = *n*-octanol–water partition coefficient.

The colchicinoids were dissolved in acetone (5 mg/ml) and 1 μl of the solution was spotted onto the plates in random allocations to avoid any systematic error. A migration of 10 cm was obtained on all plates by cutting the layer at 12 cm and spotting the compounds on a line 2 cm from the lower edge of the plate. The mobile phase, saturated with liquid paraffin, was an aqueous buffer (pH 7.4 phosphate buffer) mixed with the following amounts of acetone: 4, 6, 8, 10, 12, 14, 16, 18 and 20% (v/v). The developed plates were dried, and the compounds were detected in UV light at 250 nm, then sprayed with a saturated solution of iron(III) chloride in water and treated with gaseous hydrogen chloride. Sprayed plates were heated at 120°C. Dark green spots appeared after a few minutes on a brown background. The R_M values were calculated by the formula: $R_M = \log(1/R_F - 1)$.

RESULTS AND DISCUSSION

The chromatographic separation showed that in the system with the paraffin–phosphate buffer the test compounds did not migrate when the mobile

phase contained only phosphate buffer. The addition of acetone was necessary to obtain acceptable R_F values. The transformation of R_F into R_M values gives a series of positive and negative R_M values which were plotted *versus* the acetone concentrations. The plots are shown in Fig. 1. The test compounds could be divided into several groups on the basis of the acetone concentrations necessary to obtain a suitable range of R_M values. For each compound there was a linear relationship between the R_M values and the composition of the mobile phase. The straight lines were calculated by regression equations with the R_M values in the linearity region between 4 and 20% acetone concentration. For compound **12** (see Table I) there were few available points because with higher acetone concentrations in the mobile phase it migrated with the solvent front. The R_M values indicated in Table I are theoretical R_M values corresponding to a 0% acetone concentration in the mobile phase.

The reported R_M values allow the influence of substituent groups on the partitioning of natural

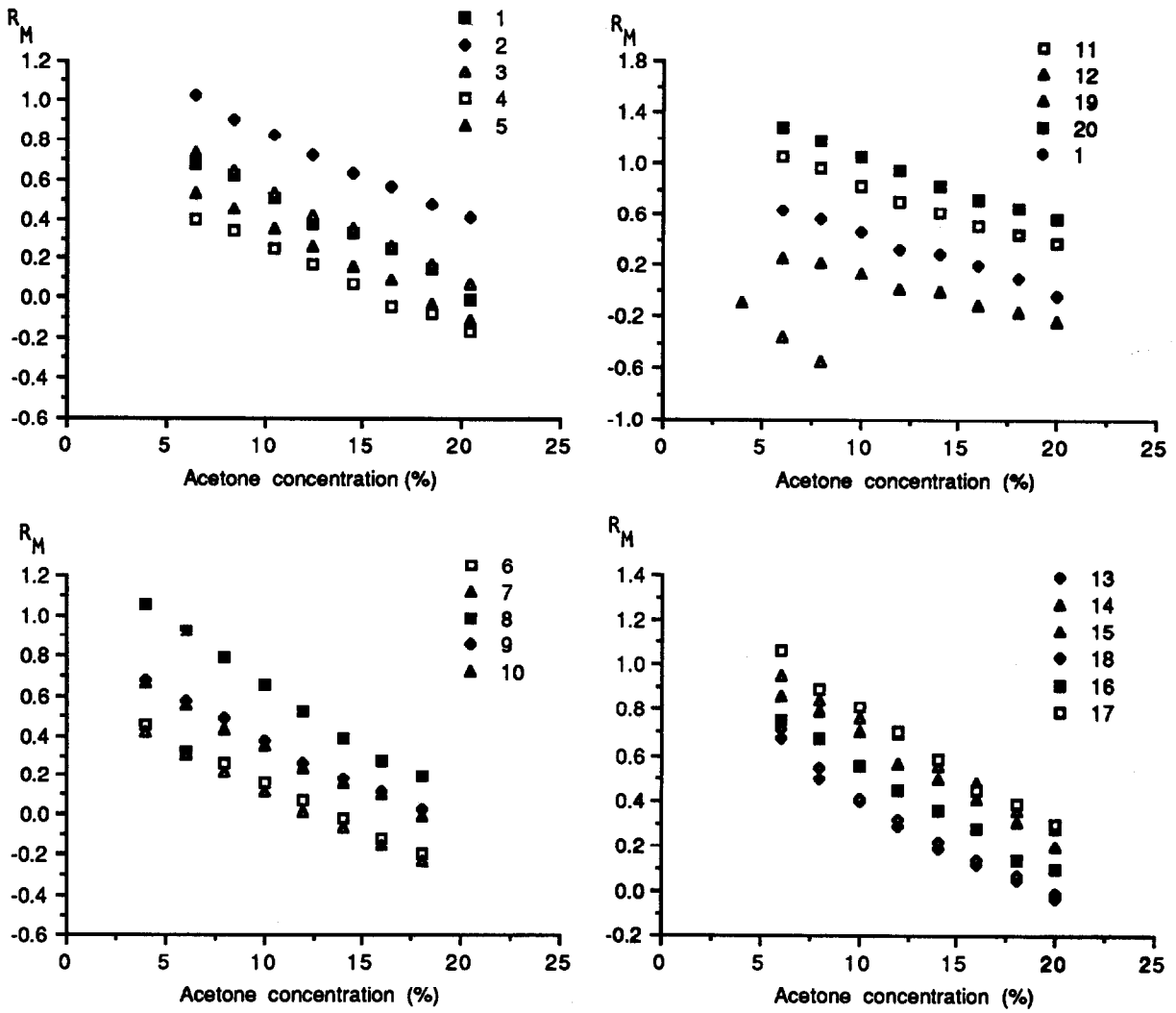


Fig. 1. Relationship between the R_M values of the studied compounds and the acetone concentration in the mobile phase. Each point represents the mean of five determinations. The compounds are numbered as in Table I.

colchicines and colchiceinamides derivatives to be studied. Higher R_M values indicate compounds more lipophilic than those represented by lower R_M values. In a series of natural colchicines the most lipophilic compounds are **2**, **8** and **11**, with $R_M > 1$, and the most hydrophilic is compound **12**, with $R_M = 0.36$.

The ΔR_M values are calculated as the difference between the R_M value of the unsubstituted compound and the R_M value of a compound bearing a certain substituent. ΔR_M values allow an evaluation of the contribution of functional groups to the lipophilicity of the whole molecule. Especially interesting in this respect is the substitution at the amino side-chain in **2** in the colchicine series and **13** in colchiceinamide series. In the colchicine series a substitution of the amino group decreases the lipophilicity in the following order: $-COCH_2COCH_3$ ($\Delta R_M = -0.29$), $-COCH_3$ ($\Delta R_M = -0.34$), $-CHO$ ($\Delta R_M = -0.45$), $-COCH_2OH$ ($\Delta R_M = -0.53$). Therefore **4** is the most hydrophilic compound in this series. The introduction of a methyl group into **8** slightly increases the R_M value ($\Delta R_M = 0.08$). The cleavage of the ether groups in ring A in general decreases the R_M value. The cleavage of a methoxy group in position C_2 has slightly less influence on the lipophilicity ($\Delta R_M = -0.26$) of the whole molecule than in position C_3 ($\Delta R_M = -0.30$). The same influence was observed in the demecolcine series. The transformation of the tropolone ring in **11** increases the lipophilicity more than the introduction of a glucose moiety ($\Delta R_M = -0.51$) into ring A in **12** ($\Delta R_M = 0.70$) decreases the lipophilicity.

The log P values of five colchicinoids, determined by Quinn and Beisler [6] in an octanol–water system (for **1** and **2**) and calculated using constant π and/or fragment values for the substituent (for **5**, **7** and **8**) are reported in Table I. They are, in general, in the same decreasing order as the corresponding R_M values determined in a system.

In the series of N-acylcolchiceinamides the hydrophilic character decreases with the substitution on the nitrogen: $-COCH_2OH$ ($\Delta R_M = 0.25$), $-CHO$ ($\Delta R_M = 0.34$), $-COCH_3$ ($\Delta R_M = 0.43$), $-COCH_2COCH_3$ ($\Delta R_M = 0.52$) and $-COCH_2OCOCH_3$ ($\Delta R_M = 0.70$). Therefore, the O-acetylated compound **20** is the most lipophilic. Deacetylation on the nitrogen in position C_7 has less influence on lipophilicity of the whole molecule in **13** ($\Delta R_M = 0.15$), than in **1** ($\Delta R_M = 0.34$).

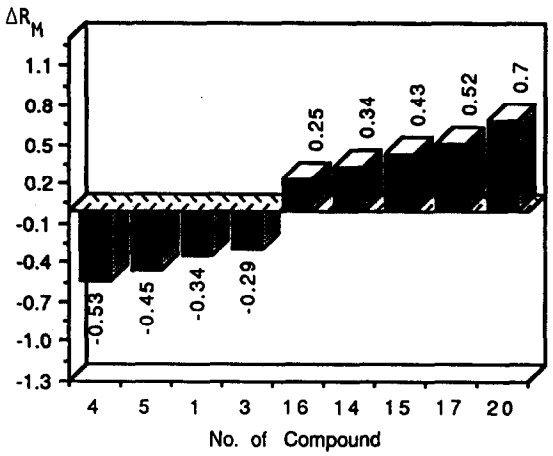


Fig. 2. Comparison of ΔR_M values of amino-substituted C_7 colchicines and amino-substituted C_{10} colchiceinamides.

In Fig. 2, ΔR_M values are given for those colchicines and colchiceinamides which are substituted by the same group in positions C_7 and C_{10}, respectively. In the colchicine series these groups decrease the lipophilicity and are on the negative scale of the plot, whereas in the colchiceinamide series the difference between the R_M value of colchiceinamide and its amino-substituted derivative give a positive ΔR_M value. This could be attributable to the ability of this group to increase the lipophilicity; colchiceinamide is the most hydrophilic in this series.

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