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STUDY OF THE LIPOPHILIC CHARACTER OF A SERIES OF β-CARBOLINES

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SUMMARY

The lipophilic character of a series of β-carbolines has been studied. The R_M values were measured by means of a reversed-phase thin-layer chromatographic (TLC) technique and compared with the R_M values obtained by high-performance TLC (HPTLC), the log k' obtained by high-performance liquid chromatography (HPLC), and the log P values. The best equation shows a very good linear relationship between our R_M values and the classical log P values obtained using an octanol–water system. The choice of a pH of 13.0 for the TLC system allowed the measurement of the R_M values of molecules in their non-ionized form. The deviations from the linear relationship shown by the R_M(HPTLC) and log k' values of two compounds were due to the fact that both compounds were at least partially ionized at the pH of 7.0 at which the HPTLC and HPLC determinations were carried out.

INTRODUCTION

β-Carboline derivatives are new drugs which exert their pharmacological action by interacting with the benzodiazepine receptor in the mammalian central nervous system (CNS)[1]. Chemically unrelated to the benzodiazepines, they have been classified according to their spectrum of biological activity as (a) agonists (anxiolytic), (b) inverse agonists (anxiogenic) or (c) antagonists (without any biological effect but preventing the interaction of agonists and inverse agonists with the receptor)[2]. Qualitative studies have recently been devoted to structure–activity relationships for this

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class of drugs[3–8]. On the other hand, there have been few reports of studies of the quantitative structure-activity relationships (QSAR) for the β-carboline series[9,10]. However, in a previous QSAR study we showed the significant influence of the lipophilic character, as expressed by means of R_M(HPTLC) (high-performance thin-layer chromatography), log k' [high-performance liquid chromatography (HPLC)] or log P values, on the receptor binding affinity of a series of β-carbolines[11].

For several years our laboratory has been using a reversed-phase TLC technique for measuring R_M values as an expression of the lipophilic character of molecules. The purpose of this work was to measure the R_M values for the series of β-carbolines as a further contribution to the study of their lipophilic character.

EXPERIMENTAL

Chemicals

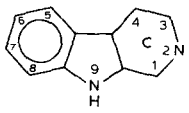
β-Carboline derivatives 5–10 and 15 were a generous gift from Dr. R. Schmiechen (Schering, Berlin, F.R.G.); the other derivatives were obtained from Sigma (St. Louis, MO, U.S.A.) (Table I). All other chemicals and solvents were of analytical-reagent or HPLC grade.

Determination of R_M values

The details of the reversed-phase TLC technique have been described previously[12]. A non-polar stationary phase was obtained by impregnating the silica gel G

TABLE I

STRUCTURES OF β-CARBOLINES



No.	*Compound*	R_1	R_3	R_4	R_5	R_6	R_7	*Ring C*
1	Harman	CH_3	H	H	H	H	H	Aromatic
2	Harmine	CH_3	H	H	H	H	OCH_3	Aromatic
3	Harmol	CH_3	H	H	H	H	OH	Aromatic
4	–	H	CH_2OH	H	H	H	H	Aromatic
5	β-CCM	H	$COOCH_3$	H	H	H	H	Aromatic
6	β-CCE	H	$COOC_2H_5$	H	H	H	H	Aromatic
7	Pr-CC	H	$COOC_3H_7$	H	H	H	H	Aromatic
8	DMCM	H	$COOCH_3$	C_2H_5	H	OCH_3	OCH_3	Aromatic
9	ZK91296	H	$COOC_2H_5$	CH_2OCH_3	$OCH_2C_6H_5$	H	H	Aromatic
10	ZK93426	H	$COOC_2H_5$	CH_3	$O_iC_3H_7$	H	H	Aromatic
11	–	H	$COONHCH_3$	H	H	H	H	Aromatic
12	Harmaline	CH_3	H_2	H_2	H	H	OCH_3	Non-aromatic
13	Harmalol	CH_3	H_2	H_2	H	H	OH	Non-aromatic
14	ZK93423	H	$COOC_2H_5$	CH_2OCH_3	H	$OCH_2C_6H_5$	H	Aromatic
15	Nor-harman	H	H	H	H	H	H	Aromatic

layer with silicone DC 200 (350 cSt) (Applied Science Labs., State College, PA, U.S.A.). The impregnation was carried out by developing the plates in a 5% silicone solution in diethyl ether. The mobile phase, saturated with silicone, was an aqueous buffer (glycine at pH 13.0), alone or mixed with various amounts of acetone.

Two plates were developed simultaneously in a chromatographic chamber containing 200 ml of mobile phase. The β-carbolines were dissolved in methanol (1–2 mg/ml) and 1 μl of solution was spotted randomly on the plates in order to avoid any systematic error. The developed plates were dried and the spots detected under UV light (254 nm). The R_M values were calculated by means of equation $R_M = \log[(1/R_F) - 1]$.

The HPTLC determinations were previously carried out on Whatman KC 18F plates[11]. A Camag Nanomat (Camag, Berlin, F.R.G.) was used to spot the compounds on the plates (about 100 nl of each β-carboline solution in methanol). The solutes were detected under UV light (254 nm). Solvent mixtures of methanol–phosphate buffer (pH 7.0) in the concentration range 45–80% were used as mobile phases.

Determination of HPLC retention times

The HPLC measurements were previously performed on a Spectra-Physics chromatograph consisting of an SP 87000 solvent delivery system and an SP 8750 organizer module. A Varian Aerograph UV detector was operated at 254 nm. A μBondapak C_{18} column (30 cm × 3.9 mm I.D.) from Waters (Milford, MA, U.S.A.) was used. The mobile phase was methanol in various mixtures with phosphate buffer (pH 7.0; ionic strength = 0.05 *M*) at a flow-rate of 1 ml/min. The β-carboline solutions in methanol were injected into the column by a 10-μl loop.

The experiments were performed at room temperature. The retention time of potassium nitrate was taken as t_0. The capacity factor, k', was evaluated from the t_0 value and the retention time of the solute, t_R, by the equation $k' = (t_R - t_0)/t_0$. For each compound the retention data were measured at different methanol concentrations (20–80%) in the mobile phase.

Measurement and calculation of log P values

The log *P* values for three compounds (harman, harmine and nor-harman) were determined at pH 13.0 in octanol–water. The log *P* values of the other β-carboline derivatives were calculated from the experimental log *P* value for nor-harman, by taking advantage of the additive property of the Hansch π values[13].

RESULTS

The reversed-phase TLC of the β-carboline derivatives showed that increasing acetone concentrations in the mobile phase resulted in decreasing R_M values. The equations describing the linear relationship between R_M values and acetone concentrations allowed the calculation of extrapolated R_M values at 0% acetone in the mobile phase (Table II). The R_M values, as measured in the chromatographic system at pH 13.0, should be an expression of the lipophilic character of the β-carbolines in their non-ionized form. The R_M(HPTLC) and log k' values reported in Table II were obtained in a similar way by extrapolation from the linear relationship between partition data and organic solvent concentrations in the mobile phase. However, the

TABLE II

LIPOPHILIC PARAMETERS OF β-CARBOLINES

Compound	R_M	R_M(*HPTLC*)	*log k'*	*log P*
1	2.06	3.35	2.54	3.50
2	2.02	3.10	2.73	3.56
3	1.75	2.82	2.10	3.06
4	1.42	2.62	2.01	2.55
5	1.97	2.92	2.83	3.16
6	2.08	3.50	2.65	3.68
7	2.36	3.54	3.15	4.24
8	2.16	3.70	2.98	4.14
9	2.74	4.60	3.86	4.56
10	2.83	4.76	3.89	5.18
11	1.24	2.71	2.11	2.75
12	2.18	1.92	1.71	3.71
13	1.44	2.04	1.10	3.06
14	2.56	3.93	3.98	4.56
15	1.82	3.12	2.51	3.17

R_M (HPTLC) and log k' values of harmaline and harmalol in those systems should reflect the partial ionization of both compounds at pH 7.0 (Table II). Finally, the log P values reported in Table II were calculated from the experimental log P value for nor-harman at pH 13.0 and therefore they should refer to their non-ionized form.

The relationship between R_M and log P values is described by eqn. 1, which shows a very good correlation coefficient.

$$R_M = -0.133(\pm\ 0.198) + 0.594(\pm\ 0.053)\log P \quad (1)$$
$$(n = 15;\ r = 0.952;\ s = 0.148;\ F = 125.9;\ P < 0.005)$$

The log P values explain 90% of the variance in the R_M values ($R^2 = 0.906$). The equation holds over a range of R_M values, showing a 38.9-fold difference in lipohilicity. However, the slope of eqn. 1 is lower than 1 and indicates the wider range of the log P values. An intercept different from zero indicates a systematic difference between the two systems.

On the other hand, eqns. 2 and 3, describing the relationship between the R_M values and the R_M(HPTLC) and log k' values, respectively, are not as good as eqn. 1.

$$R_M = 0.561(\pm\ 0.324) + 0.457(\pm\ 0.097)R_M(\text{HPTLC}) \quad (2)$$
$$(n = 15;\ r = 0.793;\ s = 0.295;\ F = 22.03;\ P < 0.005)$$

$$R_M = 0.766(\pm\ 0.237) + 0.477(\pm\ 0.085)\log k' \quad (3)$$
$$(n = 15;\ r = 0.841;\ s = 0.262;\ F = 31.40;\ P < 0.005)$$

This is mainly due to the R_M(HPTLC) and log k' values of harmaline and harmalol, which had been measured at pH 7.0. In fact, eqns. 4 and 5, calculated without these two compounds, were found to be much better. In particular, their slopes are very close to that of eqn. 1.

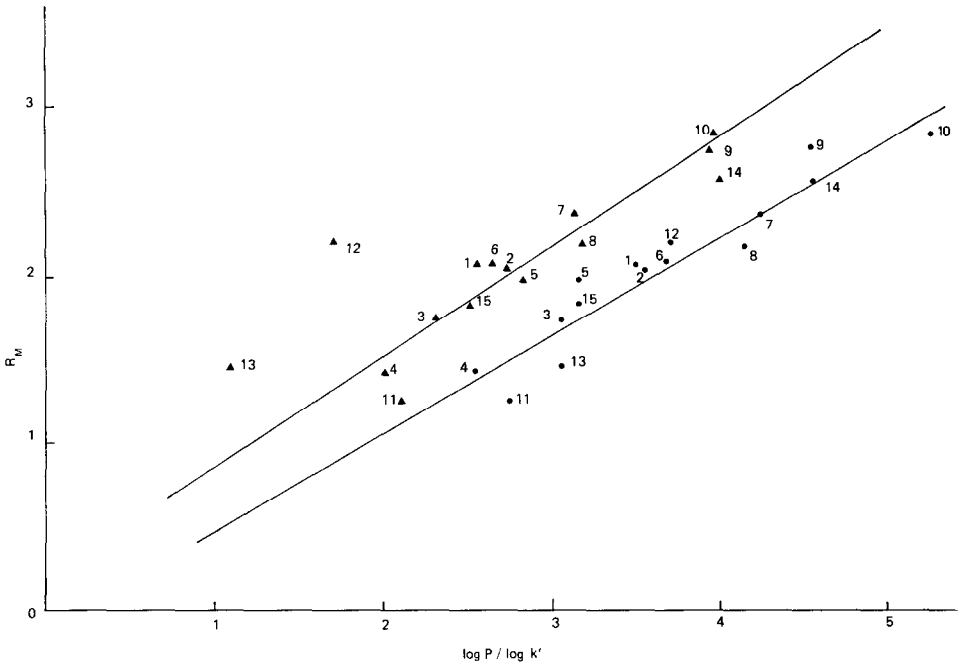


Fig. 1. Plots of R_M *vs.* log P (●) and log k' (▲) values. The straight lines were calculated from eqns. 1 and 5, respectively. For identification of compounds, see Table I.

$$R_M = -0.149(\pm 0.262) + 0.648(\pm 0.075)R_M(\text{HPTLC}) \quad (4)$$
$$(n = 13;\ r = 0.934;\ s = 0.176;\ F = 74.80;\ P < 0.005)$$

$$R_M = 0.212(\pm 0.207) + 0.649(\pm 0.070)\log k' \quad (5)$$
$$(n = 13;\ r = 0.941;\ s = 0.165;\ F = 85.54;\ P < 0.005)$$

As mentioned in the Introduction, in a previous study[11] a significant correlation had been found between R_M(HPTLC), log k' or log P values and receptor binding affinity. This was due to the fact that both lipophilicity indexes and binding assay had been measured at the same pH 7.0.

DISCUSSION

Eqn. 1 shows a very good linear relationship between R_M and log P values. The correlation coefficient is 0.954, indicating that only about 9% of the inaccuracies are not accounted for. This may be due to the fact that most of the log P values were calculated by taking advantage of the Hansch π values[13]. In any event, the R_M values, despite their narrower range as shown by the slope of eqn. 1, seem to be a reliable alternative to the log P values. The experimental determination of the octanol–water partition coefficient is likely to give more accurate and unequivocal data. However, the chromatographic method has several advantages[14]: (a) it is simple and rapid; (b) it requires little material; (c) the material does not need to be very pure because

impurities are separated during the determination; (d) the detection of spots by unspecific methods avoids the need for specific quantitative analytical methods; (e) the determination of the partition coefficient of slightly water-soluble compounds requires a long period of equilibration to achieve thorough partitioning between the phases; and (f) the range of linearity between the R_M values and the mobile phase composition allows the calculation of a theoretical R_M value at 0% acetone in the mobile phase, *i.e.*, in a standard system where all the compounds could be compared. However, this procedure has another great advantage over the determination of the R_M values at only one organic solvent concentration in the mobile phase. In fact, in this way one can avoid the error that might arise because of the different slopes of the straight lines describing the relationship between R_M values and organic solvent concentration in the mobile phase. Two compounds might have the same R_M value at a given organic solvent concentration and different extrapolated R_M values.

On the other hand the TLC technique has another important advantage over the HPLC method, which is similarly suggested as an alternative to the classical determination of the partition coefficients. All bonded-phase packing materials based on silica gel are alkali-labile. Therefore, in HPLC one has to work in the pH range 2–8 in order to avoid any alteration of the column. Particularly with basic compounds, it may be difficult to have them in the non-ionized form. In reversed-phase TLC using silicone-impregnated layers or KC 18F plates, one does not face such a problem, as it is possible to choose pH values at which any compound can be considered to be in the non-ionized form. In this way it should be possible to avoid the discrepancies resulting from eqn. 3.

In a previous paper[15] we reported $R_{Mo} = 1.82$ as the optimum lipophilic character for the activity of a series of benzodiazepines in the CNS. As the β-carbolines are assumed to interact with the same receptor, they could have a similar lipophilic character. However, as reported previously[11], the lack of a parabolic relationship between R_M values and biological activity does not allow any definite conclusion.

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