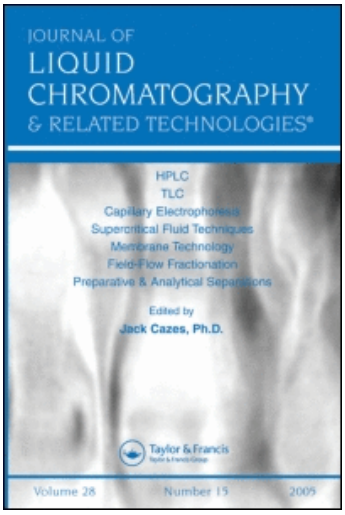

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EVALUATION OF LIQUID-LIQUID PARTITION COEFFICIENTS OF PRECOCENES AND RELATED ANALOGUES BY HPLC

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ABSTRACT

The relative hydrophobicities of natural precocenes (Ie and Ig) and of twenty-five synthetic analogues have been studied in a reversed-phase HPLC system. Using methanol-water as the mobile phase, a linear relationship of the capacity factor (log k') over a limited range of methanol fraction volumes was established for every solute and log P was related to the extrapolated k'_w value with pure water as eluent. The resulting correlation accomodates two sets of data ($r = 0.992$). For comparison purposes, results on determination of log P of selected models by using the conventional "shaking flask" method are also reported. Apparently, from our results there is not a straightforward relationship between log P values and precocene AJH activity.

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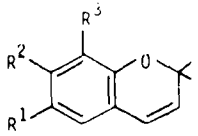
INTRODUCTION

Precocenes I and II (1e and 1g, Fig. 1), natural products isolated from Ageratum houstonianum, were the first compounds reported to elicit anti-juvenile hormone (AJH) activity when administered to a restricted number of hemimetabolous insect species (1). Studies on metabolism and mode of action on these insects have shown that precocenes selectively destroy corpora allata (CA), the glands where juvenile hormones are biosynthesized, probably by a mechanism involving a bioactivation within the gland, leading to a highly reactive epoxy intermediate which exerts extensive cytotoxic action (2). Analogously, this type of effect has also been observed on in vitro cultures of CA of some holometabolous insects (3).

Since the discovery of these compounds, many analogues have been prepared in this and other laboratories to unveil the specific structural features that confer this kind of activity (4). However, an examination of the literature revealed that only qualitative estimation of structure-activity relationships have been so far reported for precocenes and related analogues. In this context, different efforts to correlate the AJH activity of precocene analogues with ^{13}C NMR chemical shifts of significant carbon atoms of the benzopyranic framework (C-3 and C-4), either from the parent compounds (5) or from the corresponding 3,4-epoxy derivatives (6), have failed to render satisfactory results.

On the other hand, the development of quantitative structure-activity relationships (QSAR) by Hansch and co-workers (7,8), has revealed that the biological activity of a particular set of related compounds is predominantly a function of their lipophilic nature. In this sense, since precocenes are rather lipophilic molecules, it can be assumed that important events occurring prior to the postulated bioactivation within the corpora allata, such as penetration through cuticle, transport by lipoproteins and crossing cellular membranes, must be strongly



1a : $R^1 = R^2 = R^3 = H$

1b : $R^1 = R^3 = H$; $R^2 = CH_3$

1c : $R^1 = R^3 = H$; $R^2 = Cl$

1d : $R^1 = R^2 = H$; $R^3 = Cl$

1e : $R^1 = R^3 = H$; $R^2 = OCH_3$

1f : $R^1 = R^3 = H$; $R^2 = OCH_2CF_3$

1g : $R^1 = R^2 = OCH_3$; $R^3 = H$

1h : $R^1 = OCH_3$; $R^2 = OCH_2CH_3$; $R^3 = H$

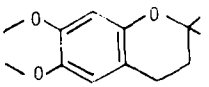
1i : $R^1 = OCH_3$; $R^2 = OCH_2CF_3$; $R^3 = H$

1j : $R^1 = OCH_2CF_3$; $R^2 = OCH_3$; $R^3 = H$

1k : $R^1 = Cl$; $R^2 = OCH_2CH_3$; $R^3 = H$

1l : $R^1 = Br$; $R^2 = OCH_2CH_3$; $R^3 = H$

1m : $R^1 = {}^tBu$; $R^2 = OCH_2CH_3$; $R^3 = H$



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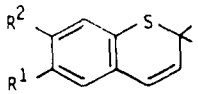
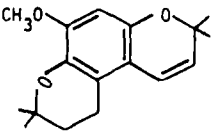


3a : $R^1 = H$; $R^2 = OCH_3$

3b : $R^1 = R^2 = OCH_3$



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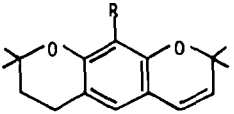
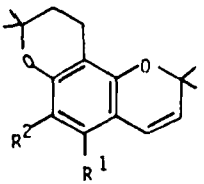


5a : R = CH_3

5b : R = CH_2CH_3

5c : R = OCH_3



6a : $R^1 = H$; $R^2 = CH_2CH_3$

6b : $R^1 = H$; $R^2 = Cl$

6c : $R^1 = H$; $R^2 = Br$

6d : $R^1 = H$; $R^2 = {}^tBu$

6e : $R^1 = H$; $R^2 = +OCH_2CH_2+_3OCH_3$

6f : R^1, R^2: $OC(CH_3)_2CH_2CH_2-$

6g : R^1, R^2: $CH_2CH_2C(CH_3)_2O-$

Figure 1- Natural precocenes (1e and 1g) and related synthetic analogues.

dependent on this physicochemical property. Therefore, it might also be anticipated that an evaluation of appropriate hydrophobic parameters for natural precocenes and representative synthetic analogues could provide valuable data to develop reliable QSAR studies in this field.

The use of partition coefficients, P, derived from n-octanol-water system has become a standard method (7) for quantifying the hydrophobicity of a given compound. These coefficients defined as : $\log P = \log C_{OCT} - \log C_{WATER}$, are usually determined experimentally.

For that purpose, the conventional "shaking flask" method has been widely used (9), although this is a tediuos procedure with a limited range up to log P = 4, which represents an important restriction for sets of highly lipophilic derivatives.

Conversely, application of modern chromatographic techniques, such as TLC (10) and reversed-phase HPLC (11,12), have overcome most of the drawbacks exhibited by the conventional partitioning method. In particular, HPLC has become very attractive since highly precise data with respect to retention times can be obtained and it is assumed that this parameter can be used as a measure of the partition between non-polar bonded stationary phase and the more polar eluent. Additionally, Braumann et al. (12) have also pointed out that due to the compartmentalized structures of biomembranes where lipids and proteins are asymmetrically arranged and cannot move freely, the dynamic chromatographic process is a better model for the behavior of drugs passing through membranes.

Studies on partition coefficients by HPLC so far reported mainly deal with the correlation between log P and the logarithm of the capacity factor,k', obtained with octadecylsilica as the bonded stationary phase and given by $k' = (t_R - t_0)/ t_0$, where t_R and t_0 are the retention times of a retained and an unretained solute in a given system, respectively.

In this context, there have been notable improvements on correlation between log P and log k' by increasing the similarities between the n-octanol-water and reversed-phase HPLC systems (11).

In the present report, a study on the determination of liquid-liquid partition coefficients of natural precocenes and related synthetic analogues (see Fig. 1), by using reversed-phase retention behavior is described. For comparison purposes, results on determination of log P of selected models by using the conventional "shaking flask" method are also reported.

For the reversed-phase HPLC correlations, we have adopted the approach developed by Bràumann and Grimme (13). Accordingly, by using methanol-water as the mobile phase, a linear relationship between the volume fraction of the organic modifier and the logarithm of the capacity factor (log k') over a limited range has been established for each compound and the relationship between log P and the extrapolated k' value with pure water as eluent (k_w) has been determined.

MATERIALS AND METHODS

Precocene Analogues

Most of the precocene analogues used in this study have been described elsewhere: 1a (14), 1b (15), 1c,d (16), 1e,1g,1h (17), 1f,1i,1j (18), 2 (19), 3a,b (20), 5a-c,6a,6g (21) and 6b (22). Compounds 1k-m, 4 and 6c-f have not been reported previously. They were prepared by using general procedures developed in this laboratory (21) and satisfactorily characterized by analytical and spectroscopic means.

HPLC Analyses

All chemicals used as calibration standards were commercially available (mostly from Fluka A.G., Buchs, Switzerland) and they

were utilized without further purification.

The HPLC system consisted of two Waters pumps model 510 (Waters Assoc., Milford, USA), a Waters U6K injector and an octadecylsilane column (25 X 0.46 cm i.d.. 10 μm Spherisorb particle size, repacked by Tracer Analytical, Barcelona, Spain). The system was fitted with a Waters model 481 absorbance detector operated at a fixed wavelength of 275 nm (benzene, toluene and bromobenzene were monitored at 254 nm). Chromatographic data were recorded and processed on a Waters M 730 data module system.

Throughout the study, the mobile phase consisted of different volume fractions of methanol (HPLC grade, Carlo Erba) in water, prepared with a Waters automated gradient controller (for mixtures of 85% and 90% in methanol, solvent mixture was previously prepared to minimize baseline problems). The flow rate of the mobile phase was set at a constant 1 mL/min .

A volume of 1 μL of a 2-4 X 10^{-4} M solution of the sample in methanol was injected in each experiment and two independent runs of each sample were carried out. By this procedure a reproducibility of retention times better than 0.5% was achieved. Column dead time was systematically determined by injection of a methanolic solution of acetone. The retention times were the same at volume fractions of 0.70 and 0.90, which confirmed that retention due to interactions with the stationary phase could be disregarded.

The standard error for log k' determinations was lower than 0.002.

"Shaking flask" Assays

According to a procedure described by Nahum and Horvath (9), the solute(s) and two internal references (Ri) having a known log P value were partitioned, in duplicate experiments, between n-octanol (Fluka A.G.) and water, in 50 mL round bottomed-flasks. Solute was dissolved in n-octanol (2 mL) to achieve a

concentration of ca. 10^{-4} M and 1 mL of the solution was added to water (20 mL). The flask was vigorously shaken (Vibromatic 384 Selecta, Barcelona, Spain) for 40 min at room temperature. Then the mixture was separated by centrifugation (10 min at 1500 g, Beckmann TJ-6R, Palo Alto, USA). Finally, 0.5 μL of the organic phase and 100 μL in the case of aqueous phase were injected into the HPLC system in two independent runs. The partition coefficient of the solute of interest P_S was calculated from the formula:

$$P_S = P_r \frac{A_{s,o}/A_{r,o}}{A_{s,w}/A_{r,w}}$$

where P_r is the n-octanol-water partition coefficient of the internal reference R; $A_{s,o}$ and $A_{s,w}$ are the respective peak areas of the solute from n-octanol and water phases, and $A_{r,o}$ and $A_{r,w}$ are the respective peak areas of the internal reference from both phases.

RESULTS AND DISCUSSION

Determination of liquid-liquid partition coefficients by HPLC.

An octadecylsilane column was chosen to perform the HPLC analyses on the basis of good correlations between log P and log k' so far reported in related studies with this type of column (12).

Inasmuch as the use of a totally aqueous mobile phase would lead to excessive long retention times for compounds like precocenes, an organic modifier was added to the mobile phase. Methanol was chosen as modifier since this solvent has exhibited the least interference with hydrophobic partition mechanisms within most common reversed-phase HPLC solvents (12).

The application of a HPLC system for the determination of partition coefficients by correlation requires previous calibra-

TABLE 1

Dispersion Analysis for Retention Times (R_T) and log k' Values of Four Calibration Standards. Mobile Phase: Methanol-Water 75-25 (v/v).

Standards	n	R (min)[a]	log k' [a]
Anisole	7	5.36 ± 0.005	-0.158 ± 0.002
Naphthalene	7	9.50 ± 0.005	0.302 ± 0.001
Phenantrene	7	19.98 ± 0.05	0.726 ± 0.001
Pyrene	7	34.03 ± 0.11	0.990 ± 0.002

[a]Values corresponding to mean ± S.D.

tion of the system by using standards with known log P values. In our case, we selected eleven compounds, for which both log P and standard deviation in their evaluation were known from bibliographic sources. They also exhibited intense UV absorption, either at 254 nm or at 275 nm and, finally, the set of standards chosen covered a log P range from 1.56 (benzonitrile) to 4.88 (pyrene), thus giving rise to a sufficiently wide range where most of the log P values for precocene derivatives could be included.

To estimate reproducibility of retention times and, consequently, of log k' parameters, four of the above standards, covering a wide range of retention values, were tested. As depicted in Table 1, the results showed excellent reproducibility which allowed us to perform the whole HPLC analysis with two independent injection runs for every solute.

The values of log k' obtained from analysis of retention behavior of each standard at five different methanol compositions (90, 85, 80, 75 and 70%), were fitted to a linear function; the

TABLE 2

Regression Analysis of the Relationship between the Volume Fraction of Methanol Φ_M and log k' for Standards.

$\log k' = \log k'_w - S_{\Phi_M}$

Standard	log k'_w [a]	r	log P [b]
Benzonitrile	1.938 ± 0.068	0.999	1.56
Nitrobenzene	2.005 ± 0.031	0.999	1.84 ± 0.05
Anisole	2.069 ± 0.017	0.999	2.08 ± 0.03
Benzene	2.159 ± 0.017	0.999	2.12 ± 0.05
Toluene	2.642 ± 0.020	0.999	2.74 ± 0.06
Bromobenzene	2.814 ± 0.027	0.999	2.99
Benzophenone	2.850 ± 0.041	0.999	3.18
Naphthalene	3.064 ± 0.037	0.999	3.38 ± 0.13
Diphenyl ether	3.642 ± 0.050	0.999	4.20 ± 0.09
Phenantrene	3.967 ± 0.067	0.999	4.53 ± 0.10
Pyrene	4.440 ± 0.131	0.998	4.88

[a] Values corresponding to mean ± S.D.

[b] Partition coefficients were taken from Hansch and Leo (7).

intercept of the correlation line with the ordinate was taken as the corresponding log k'_w value of the standard (see Table 2). As shown, excellent correlation coefficients were obtained for all standards assayed ($\geq$0.998) and the deviations calculated for log k'_w values were lower than those reported for the respective log P values (7), thus confirming the high accuracy of the HPLC technique.

The relationship between log k'_w and log P for the set of standards was fitted into the following linear equation:

$$\log P = 1.324 \log k'_w - 0.757 \quad \text{(Equation 1)}$$

with n = 11, r = 0.992 and test F $F > F^{1}_{9}(99.9\%)$. This correlation can be considered as very satisfactory when compared with previous reports for related studies (13,23).

At this point, additional correlations were carried out to compare those obtained from the relationship between log k'_w and log P (equation 1) with those derived from log k' data at only one organic modifier percentage, which have been determined by application of the procedure recently reported by Haky and Young (23). In all cases, correlation coefficients obtained using the latter approach were significantly lower than those derived from log k'_w . Moreover, since it was also observed that values of r increased with decreasing methanol percentages (0.871 for 90% to 0.974 for 70%), in our opinion, this constituted an additional confirmation that better results would be obtained when the method of extrapolation to 0% of organic modifier is applied.

The above HPLC analytical treatment was then applied to natural precocenes I and II (1e and 1g) and twenty-five synthetic analogues prepared in our laboratory, covering a wide range of structures (Fig. 1). Thus, respective log k' values were obtained from analysis of retention behavior using the same methanol volume fractions as in standards (exceptional, extreme hydrophobicity exhibited by compound 7d led us to discard data derived for this compound at 70% methanol volume fraction). Then, log k'_w values for each precocene analogue were calculated by extrapolation of the corresponding linear correlation as described above for standards and results obtained are depicted in Table 3. As shown, correlation coefficients were also excellent (>0.998) for all cases.

Finally, interpolation and extrapolation (for cases of strong hydrophobic derivatives with log k'_w values > 4.25) of respective log k'_w values in equation 1 permitted calculation of corresponding partition coefficients of natural precocenes and

TABLE 3

Regression Analysis of the Relationship between the Volume Fraction of Methanol and log k' for Natural Precocenes and Synthetic Analogues[a]. Calculated Values for log P Using Standards Calibration Correlation (Equation 1).

Compound	log k'_w [b]	log P_{calc} [c]
2	2.644 ± 0.044	2.74 ± 0.058
1g	2.666 ± 0.069	2.77 ± 0.091
1h	3.110 ± 0.060	3.36 ± 0.079
1e	3.186 ± 0.038	3.46 ± 0.090
3b	3.234 ± 0.036	3.52 ± 0.048
1a	3.155 ± 0.087	3.42 ± 0.115
4	3.600 ± 0.031	4.01 ± 0.041
1b	3.616 ± 0.047	4.03 ± 0.062
1d	3.639 ± 0.040	4.06 ± 0.053
3a	3.837 ± 0.031	4.32 ± 0.041
5c	3.950 ± 0.040	4.47 ± 0.053
1i	3.962 ± 0.059	4.49 ± 0.078
1c	4.006 ± 0.037	4.55 ± 0.049
1j	4.194 ± 0.060	4.79 ± 0.079
6e	4.242 ± 0.061	4.86 ± 0.081
1f	4.293 ± 0.063	4.93 ± 0.083
1k	4.266 ± 0.052	4.89 ± 0.069
1l	4.459 ± 0.054	5.15 ± 0.072
6g	4.953 ± 0.069	5.80 ± 0.091
6b	5.210 ± 0.050	6.14 ± 0.066
5a	5.258 ± 0.048	6.20 ± 0.064
6c	5.355 ± 0.044	6.33 ± 0.058
1m	5.685 ± 0.074	6.77 ± 0.098
5b	5.776 ± 0.053	6.89 ± 0.070
6a	5.834 ± 0.042	6.97 ± 0.056
6f	6.364 ± 0.056	7.67 ± 0.074
6d	6.744 ± 0.129	8.17 ± 0.171

[a] Correlation coefficient was in all cases > 0.999.

[b] Values corresponding to mean ± S.D.

[c] Considering the value of correlation coefficient derived from Equation 1 ($r=0.992$; test $F \geq {}^{1}_{9}F(99.9\%)$), it has been assumed that the correlation is confident enough to be directly applied for inferring log P solute values from the corresponding log k'_w determinations.

TABLE 4

Partition Coefficients (log P) Calculated by the Conventional "Shaking Flask" Method and Using HPLC with Internal Reference (Nahum and Horvath, 1980).

Compound	Reference	log P_r	$(A_{s,o}/A_{s,w})/(A_{r,o}/A_{r,w})$	log P_s [a]
Anisole	Naphthalene	3.38	0.0513	2.09 ± 0.08
Naphthalene	Phenantrene	4.53	0.0776	3.42 ± 0.04
Phenantrene	Anisole	2.10	281.84	4.55 ± 0.03
1a	Phenantrene	4.53	0.0603	3.31 ± 0.03
1e	Anisole	2.10	36.308	3.66 ± 0.04
1e	Phenantrene	4.53	0.661	3.35 ± 0.03

[a] Values corresponding to mean ± S.D.

synthetic analogues so far assayed. Results of these calculations are also depicted in Table 3.

"Shaking flask" Assays.

For comparison purposes, log P values for chromene 1a and precocene I (1e) were calculated according to the procedure developed by Nahum and Horwath (9), where partition is carried out using conventional "shaking flask" techniques and analysis of distribution of solutes is performed by reversed-phase HPLC (Table 4). The method, usually tedious, could not be used for most compounds due to the high lipophilicity exhibited by precocene derivatives. Thus, for log P values over 4, compounds were not soluble enough in water to be analyzed by HPLC. Moreover, in cases as 1a and 1e, a 20:1 water: n-octanol volume

ratio should be used, which also led to additional practical disadvantages.

In summary, our results have shown that evaluation of partition coefficients of hydrophobic compounds, such as precocene analogues, by use of extrapolated log k'_W values from reversed-phase HPLC retention parameters, constitute a convenient and valuable tenchique which offers high accuracy, and good reproducibility.

Finally, the relationship between the partition coefficients calculated for precocene derivatives, summarized in Table 3, and the AJH activity deserve further comments. Although at present there are no definitive systematic studies on the biological activity of the whole set of compounds utilized in the present work, both qualitative and semiquantitative data on morphogenetic and antigonadotropic activities of these precocene derivatives on Oncopeltus fasciatus (24) show that high activity is elicited by compounds with very different log P values. Thus, whereas natural precocenes PI (1e) and PII (1g) define a narrow range of log P values (2.77-3.46) in which analogues of high activity such as ethoxyprecocene II (1h) are enclosed (3.36), other compounds with high or remarkable AJH activity, such as trifluoroethoxy analogue 1i or chloroderivative 6b, exhibit log P values (4.93 and 6.14, respectively) far removed from that range.

Conversely, compounds like chromene 1a or sulfur analogue 3b with values of the same magnitude as those of natural precocenes have so far not displayed any AJH activity against different insect species tested.

In spite of the need for more precise biological data to reach final conclusions, it may be anticipated that the lipophilicity of precocene analogues taken independently of other factors, such as electronic and steric requirements, cannot be directly related to AJH activity.

ACKNOWLEDGEMENT

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REFERENCES

1. Bowers, W.S., Ohta, T., Cleere, J.S. and Marsella, P.A., Science (Washington D.C.) 193, 542, 1976.

2. Hamnett, A.F. and Pratt, G.E., Life Sciences 32, 2747, 1983.

3. Bowers, W.S. and Feldlaufer, M.F., Gen. Comp. Endocrinol., 47, 120, 1982.

4. Camps, F., Coll, J., Colomina, O. and Messeguer, A., Z. Naturforsch, 39b, 1801, 1984 and references cited therein.

5. Ottridge, A.P., Jennings, R.C. and Brooks, G.T., Juvenile Hormone Biochemistry: Action, Agonism and Antagonism; Pratt, G.E Brooks, G.T., eds, Elsevier/North Holland: Amsterdam, p.381, 1981.

6. Camps, F., Colomina, O., Conchillo, A. and Messeguer, A., unpublished results.

7. Hansch, C. and Leo, A., Substituent Constants for Correlation Analysis in Chemistry and Biology; John Wiley: New York, 1979.

8. Leo, A., Hansch, C. and Elkins, D., Chem. Rev., 71, 525, 1971.

9. Nahum, A., and Horvath, C. J. Chromatogr., 192, 315, 1980.

10. Tomlinson, E., J. Chromatogr., 113, 1, 1975.

11. Kaliszan, R., J. Chromatogr., 220, 71, 1981.

12. Braumann, T., Weber, G. and Horst Grimme, L., J. Chromatogr. 261, 329, 1983.

13. Braumann, T. and Horst Grimme, L., J. Chromatogr., 206, 7, 1981.

14. Schweizer, E.E. and Meeder-Nycz, D., Chromenes, Chromanones and Chromones, Ellis, G.P., ed., John Wiley and Sons: New York, p. 11, 1977.

15. Kawase, Y., Yamaguchi, S., Horita, H., Takeno, J. and Kameyano, H. Bull. Chem. Soc. Japan, 55, 1153, 1982.

16. Hepworth, J.D., Kones, T.K. and Livingstone, R. Tetrahedron, 37, 2613, 1981.

17. Ohta, T. and Bowers, W.S., Chem. Pharm. Bull., 25, 2788, 1977.

18. Camps, F., Coll, J., Messeguer, A. and Pericás, M.A., Tetrahedron Lett., 2361, 1980.

19. Camps, F., Coll, J., Messeguer, A., Pericás, M.A., and Ricart, S. Synthesis, 126, 1979.

20. Camps, F., Coll, J., Colomina, O. and Messeguer, A., J. Heterocyclic Chem., 20, 1115, 1983.

21. Camps, F., Coll, J., Colomina, O. and Messeguer, A. Tetrahedron, 38, 2955, 1982.

22. Camps, F., Colomina, O., Conchillo, A. and Messeguer, A., J. Heterocyclic Chem., in the press.

23. Haky, J.E., and Young, A.M., J. Liq. Chromatogr., 7, 675, 1984.

24. Bowers, W.S., private communication.