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GAS CHROMATOGRAPHIC QUANTITATION OF UNDERIVATIZED AMINES IN THE DETERMINATION OF THEIR OCTANOL–0.1 *M* SODIUM HYDROXIDE PARTITION COEFFICIENTS BY THE SHAKE-FLASK METHOD*.

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SUMMARY

The use of gas chromatography (GC) for the determination of 0.1 *M* sodium hydroxide–octanol partition coefficients (log *P*) for a wide variety of ethylamines is demonstrated. The conventional shake-flask procedure (SFP) is utilized, with the addition of an internal reference, which is cleanly separated from the desired solute and solvents on a 10% Apiezon L, 2% potassium hydroxide on 80–100 mesh Chromosorb W AW column. The partitioned solute is extracted from the aqueous phase with chloroform and analyzed by GC. The method provides an accurate and highly reproducible means of determining log *P* values, as demonstrated by the low relative standard errors. The technique is both rapid and extremely versatile. The use of the internal standard method of analysis introduces consistency, since variables like the exact weight of solute are not necessary (unlike the traditional SFP) and the volume of sample injected is not critical. The technique is readily accessible to microgram quantities of solutes, making it ideal for a wide range of volatile, amine-bearing compounds.

INTRODUCTION

Partition coefficients in the octanol–water system (log *P*) have been widely utilized in rational drug design for the characterization of hydrophobic properties of drugs. Numerous methods have been devised for the determination of this important physiochemical parameter. The shake-flask procedure (SFP) described by Leo *et al.*[1] has been widely applied and criticized[2–14]. Recent efforts have produced many methods which utilize high-performance liquid chromatography (HPLC) for the mea-

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surement of lipophilicity[2,5–9,12–15,*]. The present study is concerned with the determination of the log *P* values of substituted ethylamines, including a number of non-aromatic ethylamines which were prepared as part of a series of novel inhibitors of norepinephrine N-methyltransferase (NMT), the enzyme which catalyzes the N-methylation of norepinephrine to yield epinephrine[16,17]. Determination of the log *P* values of the non-aromatic ethylamines is complicated by the lack of a detectable UV chromophore which is normally the method of choice for quantitation in partition experiments. Several alternative methods for the quantitation of solutes lacking a suitable chromophore have been described. In the case of the SFP, Church and Hansch[18] have suggested the use of either gas chromatography (GC), Nessler's analysis (a colorimetric procedure for compounds such as amides, ureas, and carbamates, which yield ammonia upon hydrolysis), or liquid scintillation counting of a labeled, radioactive molecule. Of these, GC has the greatest potential for general use owing to its sensitivity, widespread use and applicability to a variety of compounds. Alternative methods to the SFP for the determination of log *P* values have also been described which would be useful in the case of non-aromatic amines, such as potentiometric titration[19,*], and reversed-phase thin-layer chromatography (RP-TLC)[15,20,21,*]. However, Kubinyi[15] and Martin[21] have recently reported on the limitations encountered with these latter two methods. It is also possible to calculate the log *P* values utilizing the additive nature of either the hydrophobic substituent constant (π)[22] or the fragment constants of either Rekker[23] or Leo and co-workers[24,25]. However, such calculations do not adequately account for the effects of conformation on partitioning, which is an important limitation in the case of several of the solutes in this study[26].

An extensive data base of log *P* values has been compiled and is updated semiannually[27]. Surprisingly, only a few log *P* values for aliphatic amines have been reported. Of these, several are questionable[**] and the other reported log *P* values either are listed as unpublished results[***], or are measured in solvent systems other than octanol–water[28], in the compilation cited above[27]. Thus, there exists a need for a simple, reliable and reproducible procedure for determining the log *P* values of this important class of compounds suitable for quantitative structure activity relationship (QuSAR) studies. We describe herein a simple and reproducible method which meets the above requirements and utilizes GC.

EXPERIMENTAL

Materials

Structures for the compounds used in this study are shown in Table I. The hydrochloride salts of all the compounds in this study were synthesized in our laboratory according to published procedures and purified by crystallization[16,29–42] with

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* For a complete list of references, see the Ph.D. dissertation by M.A.P.

** The calculated log *P* values (via Leo's *f* fragment constants[24,25]) deviated ($\geq \pm 2$ S.D.) from the observed values.

*** Professor Toshio Fujita of Kyoto University (Kyoto, Japan) has measured the log *P* values of a number of aliphatic amines and has submitted these values to the Pomona College Medicinal Chemistry Data Base[27]. Though the method used for their determination has not been reported, these log *P* values show an excellent agreement between calculated (via Leo's *f* fragment constants[24,25]) and observed values.

the exception of fenfluramine hydrochloride (2) and methamphetamine hydrochloride (24), which were gifts from the A. H. Robins Company (Richmond, VA, U.S.A.), and 4-phenylbutylamine (16), 2-aminoheptane (20) and *d*-amphetamine sulfate (33), which were purchased from Aldrich (Milwaukee, WI, U.S.A.). All of the compounds used in this study were fully characterized by spectroscopic methods and gave satisfactory combustion analyses. The purity of the compounds was checked prior to partitioning by GC. Doubly distilled water was prepared with a Corning Mega-Pure distilling unit. Reagent grade octanol (Fischer Scientific, Fair Lawn, NJ, U.S.A.) was purified according to the procedure of Church and Hansch[18] and saturated with 0.1 *M* sodium hydroxide prior to partitioning. Pre-saturation of solvents and partitionings of the solute were conducted at ambient temperature (25 ± 3°C). Chloroform (Fischer Scientific), cyclohexylamine (CHA) and di-*n*-butylamine (DBA) (the latter two compounds both 99% pure and Gold Label) were purchased from Aldrich and used without further purification. Transfer of small volumes was accomplished with an Eppendorf pipette (50, 100 and 200 μl). Partitioning samples were prepared in 50-ml glass centrifuge tubes (Corning No. 8064, 148 mm × 28 mm with a standard taper 16 glass stopper) (Fischer Scientific, Pittsburgh, PA, U.S.A.). Samples for GC analysis were transferred into 2-ml vials (HP No. 5080-8712) and sealed with a crimp-on cap with a silicon rubber septum and PTFE coating on the inside surface (HP No. 5080-8713).

Instrumentation

A Hewlett-Packard (HP) Model 5880A gas chromatograph (Avondale, PA, U.S.A.) equipped with a level four terminal, HP 7672 automatic sampler and flame-ionization detector was used. The coiled glass column (1.8 m × 2 mm I.D.) was packed with 10% Apiezon L, 2% potassium hydroxide on 80–100 mesh Chromosorb W AW (HP).

The gas flow-rates were: hydrogen, 30 ml/min; compressed air, 300 ml/min; and helium (carrier gas), 30 ml/min. The temperatures were: 300°C (detectors) and 250°C (injection port). The standard oven temperature profile listed in Table II was used, unless otherwise stated (see the *Gas chromatography* section and Table II for changes).

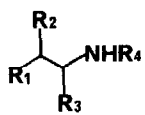
Partition samples were shaken on a Kraft Model S-500 shaker-in-the-round (Kraft Apparatus, Minneola, NY, U.S.A.) at a rate of 120 shakes per minute. Tubes were centrifuged at 24°C with a Lourdes Beta-Fuge Model A-2 (Vernitron Medical Products, Calstadt, NJ, U.S.A.).

Methods

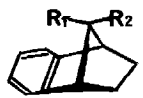
Shake-flask partitioning experiments. The following general procedure represents a typical partitioning experiment, in which each compound was partitioned at two different concentrations. Approximately 0.020 g of the solute was placed in a 100-ml volumetric flask and dissolved in 0.1 *M* sodium hydroxide (octanol-saturated; referred to as standard solution I). An aliquot of this solution was removed and diluted with an equal volume of 0.1 *M* sodium hydroxide (octanol-saturated), to yield standard solution II. A 10.0-ml aliquot of standard solution I was transferred to a 50-ml centrifuge tube and 100 μl of octanol (0.1 *M* sodium hydroxide saturated) were added for a 1:100 ratio. Similarly, a 10.0-ml aliquot of standard solution II was

TABLE I

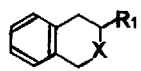
STRUCTURE OF SOLUTES



I

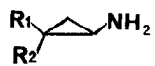


II



III

IV



V

Compound	*Type*	R_1	R_2	R_3	R_4	*X*	*Codename*	*Reference*
1	I	cyclooctyl	H	CH_3	H	–	COAM	16
2	I	*m*-$CF_3C_6H_5$	H	CH_3	CH_2CH_3	–	FEN	–
3	IV	$NHCH_2CH_2CH_3$	H	H	H	CH_2	2PX	29
4	IV	NH_2	H	H	CF_3	CH_2	6-CF-2HX	30
5	IV	NH_2	H	CF_3	H	CH_2	7-CF-2HX	30
6	IV	H	$NHCH_2CH_2CH_3$	H	H	CH_2	2PN	29
7	I	cyclohexyl	H	CH_3	H	–	CHAM	16
8	IV	H	NH_2	H	CF_3	CH_2	6-CF-2HN	30
9	IV	H	NH_2	CF_3	H	CH_2	7-CF-2HN	30
10	I	$C_6H_5CH_2CH_2$	H	H	CH_3	–	PBNM	31
11	IV	$NHCH_2CH_3$	H	H	H	CH_2	2EX	29
12	IV	$NHCH_3$	H	H	H	CH_2CH_2	NMX	32
13	IV	H	$NHCH_2CH_3$	H	H	CH_2	2EN	29
14	IV	H	$NHCH_3$	H	H	CH_2CH_2	NMN	32
15	II	H	$NHCH_3$	–	–	–	9MA	33*

16	I	$C_6H_5CH_2CH_2$	H	H	H	–	PBNH	–
17	IV	$NHCH_3$	H	H	H	CH_2	2MX	34
18	III	$NHCH_3$	–	–	–	CH_2	2MAT	35
19	II	$NHCH_3$	H	–	–	–	9MS	33*
20	I	$CH_3(CH_2)_3$	H	CH_3	H	–	2AHP	–
21	IV	NH_2	H	H	H	CH_2CH_2	NHX	36
22	IV	H	$NHCH_3$	H	H	CH_2	2MN	34
23	IV	H	NH_2	H	H	CH_2CH_2	NHN	36
24	I	C_6H_5	H	CH_3	CH_3	–	MAM	–
25	II	H	NH_2	–	–	–	9HA	37
26	IV	NH_2	H	H	H	CH_2	2HX	34
27	II	NH_2	H	–	–	–	9HS	38
28	III	NH_2	–	–	–	CH_2	2AT	35
29	I	C_6H_5	ψ-OCH_3	CH_3	CH_3	–	PME**	39
30	IV	H	NH_2	H	H	CH_2	2HN	34
31	III	CH_3	–	–	–	NH	3MTHIQ	40
32	I	C_6H_5	OCH_3	CH_3	CH_3	–	EME***	39
33	I	C_6H_5	H	CH_3	H	–	AM	–
34	V	H	C_6H_5	–	–	–	TCY	41
35	V	C_6H_5	H	–	–	–	CCY	41
36	IV	$NHCH_3$	H	H	H	O	OMX	39
37	IV	NH_2	H	H	H	O	OHX	42
38	IV	H	$NHCH_3$	H	H	O	OMN	39
39	IV	H	NH_2	H	H	O	OHN	39

* Compounds 15 and 19 were prepared in an analogous fashion to compounds 17 and 22.

** *threo*-2-(methylamino)-1-methoxy-1-phenylpropane.

*** *erythro*-2-(methylamino)-1-methoxy-1-phenylpropane.

TABLE II

OVEN TEMPERATURE PROFILES

Profile for compounds	*Initial*		*Level 1*			*Level 2*		
	Temp. (°C)	*Time (min)*	*Program rate (°C/min)*	*Final temp. (°C)*	*Final time (min)*	*Program rate (°C/min)*	*Final temp. (°C)*	*Final time (min)*
Standard	120	0.1	20	250	2	–	–	–
2, 7, 24, 33	120	0.1	20	140	2.5	20	160	2
3, 6, 10, 11, 13, 16	90	0.1	5	100	0.1	20	250	4
8	120	0.1	20	200	1	5	210	0.5
9	120	0.1	20	170	1	10	200	1
20	90	0.1	2	100	6	–	–	–
28	120	0.1	20	200	1	5	210	1.5

transferred to a 50-ml centrifuge tube and 100 μl of octanol (0.1 *M* sodium hydroxide saturated) was added for a 1:100 ratio. The two centrifuge tubes were stoppered and then shaken for 30 min at ambient temperature at a rate of 120 shakes per minute in a horizontal position. The tubes were then centrifuged two hours at 310 *g*.

For the GC analysis, two different samples were prepared for each partition experiment: a calibration sample from each of the standard solutions and a sample from each of the partition solutions. The calibration sample of the solute was prepared in the following manner: 2.0 ml of standard solution I, 1.0 ml of CHA (50 μl/100 ml doubly distilled water), and 1.0 ml chloroform were transferred into a 30-ml separatory funnel. The solute and internal reference were extracted into the chloroform, and the chloroform phase was then transferred into a 2-ml vial and sealed. A sample of the partition solutions was prepared for GC analysis in an analogous fashion. An aliquot of the aqueous phase from the partition solution (carefully removed with a volumetric pipette to avoid the removal of any octanol phase) was combined with 1 ml of the CHA solution and 1 ml chloroform in a separatory funnel. Again, the solute and internal reference were extracted into the chloroform layer, and the solution transferred to a 2-ml vial and sealed.

Gas chromatography. The internal standard method[43,44] was used in determining the relative concentrations of solute in the partition experiment. Under the most commonly employed chromatographic conditions, the internal reference (CHA) had a retention time of 1.5–1.7 min. The retention times of the solutes in this study were generally within 4–11 min, and so were well resolved from the internal standard. Compound 20 was the lone exception, in that complete resolution of the solute and CHA peaks could not be achieved. However, changing the internal reference to DBA (t_R = 4.15 min) solved the problem and allowed quantitation of 20. The octanol (t_R = 2.5 min) and chloroform (t_R = 1 min) peaks, while comparatively large, did not usually interfere. Five 1-μl injections of the calibration sample were made by an automatic sampler in order to obtain the average peak areas for both the internal reference and solute, which was then used in the calculation of the solute concentration in the partitioning sample. Six 1-μl injections of the partitioning sample were then made by the automatic sampler, and the relative amounts of solute were computed for each of the six runs. The average of these runs was used to calculate the log *P*. The entire procedure was repeated using a different concentration of solute and the log *P* values from the two determinations were compared and averaged.

Modifications in the oven temperature program were necessary for compounds 2, 7, 24 and 33 since the solute and octanol peaks had similar t_R values. A related problem arose with compounds 3, 6, 10, 11, 13, and 16 since the chloroform and internal reference peaks overlapped. Compounds 8, 9, and 28 exhibited a small shoulder on the solute peak which was attributed to the presence of trace impurities (< 1%) in the sample. All of these problems were solved by utilizing a multilevel oven temperature program in order to separate the desired peaks. The modifications to the standard profile are outlined in Table II.

Data evaluation. The partition coefficient, *P*, of the solute of interest was calculated from the relationship

$$P = \left(\frac{100 - C}{C}\right)\left(\frac{V_{aq}}{V_o}\right) \tag{1}$$

TABLE III

LOG *P* VALUES AND GC EXPERIMENTAL CONDITIONS

The ratio of octanol to 0.1 *M* sodium hydroxide in the partitioning sample (see Methods section) is given as V_o:V_{aq}. The volume of the aqueous phase of the partition solution (V_{ps}) in the partitioning sample was a whole number multiple of the volume of the standard solution (V_{ss}) in the calibration sample in order for the ratio of the area of the internal reference peak to that of the solute to be as close to unity as possible for greater accuracy during both the calibration and partitioning runs. $\sigma\mu$ = Relative standard error of the mean for the partitioning sample.

Solute		t_R *(min)*	V_o:V_{aq}	V_{ps}:V_{ss}	*log P observed*	$\sigma\mu$	*log* P_{calc}** *(Rekker)*	Δ***	*log* P_{calc}** *(Leo)*	Δ§
No.	*Codename*									
1	COAM	5.57	1:1000	2	3.70	0.05	3.63*,†	0.07	3.67*,†	0.03
2	FEN	4.92	1:400	4	3.36	0.01	3.34*	0.02	3.37*	−0.01
3	2PX	9.70	1:400	2	3.30	0.03	3.34	−0.04	3.33	−0.03
4	6-CF-2HX	5.44	1:400	2	3.21	0.03	3.14	0.07	2.98	0.23
5	7-CF-2HX	5.38	1:400	2	3.19	0.06	3.14	0.05	2.98	0.21
6	2PN	9.34	1:400	2	3.13	0.02	3.05	0.08	3.16	−0.03
7	CHAM	4.86	1:400	2	2.96	0.04	3.07*	−0.11	3.01*	−0.05
8	6-CF-2HN	5.30	1:400	2	2.91	0.05	2.85	0.06	2.81	0.10
9	7-CF-2HN	5.85	1:400	2	2.85	0.05	2.85	0	2.81	0.04
10	PBNM	10.89	1:200	2	2.76	0.04	2.80*	−0.04	2.69*	0.07
11	2EX	9.04	1:400	2	2.72	0.07	2.82	−0.10	2.79	−0.07
12	NMX	6.96	1:100	4	2.68	0.03	2.82	−0.14	2.82	−0.14
13	2EN	8.71	1:400	2	2.62	0.10	2.53	0.09	2.62	0
14	NMN	6.74	1:100	4	2.59	0.04	2.53	0.06	2.65	−0.06
15	9MA	5.98	1:100	4	2.47	0.02	2.30	0.17	2.25	0.22
16	PBNH	10.73	1:100	2	2.45	0.07	2.50*	−0.05	2.52*	−0.07
17	2MX	5.87	1:100	4	2.41	0.03	2.30	0.11	2.25	0.16
18	2MAT	6.13	1:100	2	2.38	0.07	2.44*	−0.06	2.21*	0.17

19	9MS	5.58	1:100	3	2.37	0.01	2.01	0.36	2.08	0.29
20	2AHP	2.87[§§]	1:200	2	2.40	0.04	2.40*	0	2.37*	0.03
21	NHX	6.74	1:100	4	2.32	0.06	2.52	−0.20	2.66	−0.34
22	2MN	5.72	1:100	3	2.32	0.05	2.01	0.31	2.08	0.24
23	NHN	6.54	1:100	4	2.29	0.10	2.23	0.06	2.49	−0.20
24	MAM	5.22	1:100	2	2.16	0.04	2.28*	−0.12	1.92*	0.24
25	9HA	5.71	1:100	2	2.13	0.04	2.00	0.13	2.09	0.04
26	2HX	5.65	1:100	2	2.09	0.06	2.00	0.09	2.09	0
27	9HS	5.39	1:100	2	2.08	0.07	1.71	0.37	1.92	0.16
28	2AT	6.22	1:100	1	2.08	0.09	2.13*	−0.05	2.05*	0.03
29	PME	4.60	1:40	2	2.05	0.04	1.79*	0.26	1.75*	0.30
30	2HN	5.47	1:100	2	2.00	0.10	1.71	0.29	1.92	0.08
31	3MTHIQ	5.20	1:100	2	1.93	0.10	1.92*	0.01	1.89*	0.04
32	EME	4.50	1:40	2	1.87	0.01	1.79*	0.08	1.75*	0.12
33	AM	4.61	1:100	1	1.81[§§§]	0.05	1.98*	−0.17	1.76*	0.05
34	TCY	4.35	1:100	2	1.58	0.10	1.61*	−0.03	1.58*	0
35	CCY	4.32	1:8.3	2	1.49	0.06	1.61*	−0.12	1.58*	−0.09
36	OMX	6.67	1:5	3	0.91	0.13	0.77	0.14	0.79	0.12
37	OHX	6.54	1:5	1	0.75	0.15	0.46	0.29	0.51	0.24
38	OMN	6.13	1:5	2	0.59	0.05	0.48	0.11	0.62	−0.03
39	OHN	5.90	1:5	1	0.40	0.14	0.17	0.23	0.34	0.06

* From this study.

** From ref. 26 unless otherwise indicated. Details regarding the method of calculation can be found in this reference.

*** Residual value [log $P_{obs.}$ − log $P_{calc.(Rekker)}$].

§ Residual value [log $P_{obs.}$ − log $P_{calc.(Leo)}$].

§§ See *Methods* and *Gas chromatography* section in text.

§§§ J. Schaeffer, UCLA Pharmacology Department, reports a log P = 1.76 (private communication to Pomona College Medicinal Chemistry Project[27]).

† Calculated using a correction factor of −0.48. This value is utilized for cyclooctyl and larger cycloaliphatic ring systems. A correction of −0.06 per cycloaliphatic carbon atom is applied (according to M. A. Pleiss and G. L. Grunewald, unpublished observation).

where C is the average concentration of solute in the aqueous phase of the respective partition solution and V_{aq} and V_o represent the volumes of the aqueous and organic phases, respectively.

RESULTS AND DISCUSSION

Experimental conditions as well as the partition coefficients measured by GC are listed in Table III. The log P values of several of the ring systems in Table III have not been previously reported, making this an interesting and valuable compilation. The precision of the log P measurements is quite good with this technique, as can be seen from the column of relative standard errors ($\sigma\mu$) in Table III. Also included are the calculated log P values which were derived from either the Rekker[23] or Leo[24,25] procedures. The column of residual values associated with each method is another indication of the overall accuracy of this technique.

Selection of an analytical method

The need for a practical and versatile technique for the determination of the log P of *any* amine in the neutral form exists. If the solute contains a chromophore with a suitable extinction coefficient, the existing techniques, such as UV, will generally be sufficient for the measurement of the log P value. However, in the case of UV transparent aliphatic amines, an alternative technique must be pursued. Application of derivatization is possible, where a chromophore bearing reagent is coupled to the amine-containing solute, but the need for quantitative transfer of the reagent is essential for accurate results and this tends to be a problem in the determination of the distribution ratios of amines[45]. Among the generally available analytical techniques in use in most laboratories, only GC or HPLC offered the sensitivity and reproducibility needed for solute quantitation in partition experiments. Beckett and Moffat[28] and Vree *et al.*[46] have measured the partition coefficients for a series of amines between *n*-heptane and 0.1 *M* sodium hydroxide and determined the concentrations by GC. It is possible to relate the *n*-heptane–0.1 *M* sodium hydroxide partition coefficients for compounds described in this set[28,46], that are also common with compounds in the present study (*i.e.*, 2, 24, and 33), in the manner originally described by Collander[47] and extended by Leo and Hansch[48], as shown by eqn. 2[27].

$$\log P_{oct} = 0.493 \log P_{n\text{-heptane}} + 1.272 \quad (n = 11, r = 0.954, s = 0.276) \qquad (2)$$

These calculated log P values are compared to those experimentally determined in the present study and the results are listed in Table IV. Examination of the residuals clearly indicates that the partition coefficients measured in the *n*-heptane–0.1 *M* sodium hydroxide solvent system do not satisfactorily relate to the measured log P_{oct}. Thus, the methodology discussed by both groups[28,46] was not applicable to the present study. We have modified the technique reported by Beckett and Moffat[28], utilizing the octanol–0.1 *M* sodium hydroxide solvent system, with GC determination of the solute concentration and the results are presented in Table III. These values are reported with the assumption that the recoveries of the sampled amines from water are identical and independent of the initial amine concentration. This assumption

TABLE IV

COMPARISON OF MEASURED LOG *P* VALUES TO THOSE CALCULATED FROM EQN. 2

Log P_{hep} [log *P* (*n*-heptane–0.1 *M* sodium hydroxide)] from refs. 28 and 46. Log P_{oct} calculated using eqn. 2 (see Results and discussion —Selection of an analytical method). Measured log P_{oct} from this study. Δ = measured log P_{oct} − calculated log P_{oct}.

Solute		*log* P_{hep}	*Calculated log* P_{oct}	*Measured log* P_{oct}	Δ
No.	*Codename*				
2	FEN	2.74	2.62	3.36	0.74
24	MAM	1.24	1.88	2.16	0.28
33	AM	0.53	1.53	1.81*	0.28

* See sixth footnote in Table III.

appears to be valid. In all cases two different solute concentrations were partitioned and assayed against a calibration sample. The log *P* values for both determinations show excellent agreement (see the column of relative standard errors listed in Table III). Also, a wide range of lipophilicity was studied, again with very consistent results. In all cases the observed value was in excellent agreement with the corresponding calculated values. This can be seen from an examination of the column(s) of residual values in Table III.

The internal standard method[43,44] as described in the Methods section provided a means for correcting for errors in a number of steps in the partition experiment which affect procedures employing UV quantitation. Since the same solute solution is used to prepare both the calibration standard and the partition experiment, exact weighing of solute is not necessary. The resolving power of GC also allows log *P* measurements to be made with samples which contain small amounts of impurities, which by their additional weight would bias the results obtained in normal SFP methods. Within the present study, three of the compounds examined (8, 9, and 28) were found to have an unidentified impurity present (< 1%) which was separable during GC analysis. An indication that the trace impurities did not significantly affect the partitioning of the major constituent is found in that the log *P* values calculated for these compounds using the fragment method of either Rekker[23] or Leo[24,25] are in excellent agreement with the measured values ($\Delta \leqslant |0.10|$), see Table III. Finally, by adjusting the peak heights of the internal standard *vs.* solute to an approximate ratio of one (accomplished by varying the size of the aliquot of aqueous phase removed for GC sample preparation), any correction for non-linearity in the detector response is made with concentration.

The lipophilicity of the solute determined the ratio of octanol to aqueous phase that was employed. This was another easily modified variable that led to the overall precision seen in the observed log *P* values listed in Table III. The approximate lipophilicity of the solute could generally be estimated prior to analysis by calculating the log *P* from *f* fragment constants[24,25]. This factor becomes very important as the lipophilicity of the solute increases. In the case of a very lipophilic solute (log $P \geqslant 3$) it is extremely important that small volumes of octanol be used or there will be insufficient material left in the aqueous phase for analysis[1,18].

CONCLUSIONS

The GC method for the determination of log P values is advantageous since it is one of the few existing methods that allows for the accurate determination of this important property for *any* volatile amine in the neutral form. It has been demonstrated that this technique can provide reliable log P values for a diverse set of solutes of pharmacological interest. The widespread use of GC as an everyday analytical tool, coupled with the commercial availability of the necessary columns, makes this an attractive method. This technique, as designed, has several strong points: (1) the use of the internal standard method[43,44] of analysis introduces consistency, since variables like the exact weight of the solute are not necessary (unlike the traditional SFP) and the volume of sample injected is not critical; (2) the presence of trace impurities does not interfere with the evaluation of the log P and can be dealt with by simple adjustments in the procedure; (3) the method provides an accurate and highly reproducible means of determining log P values, as demonstrated by the relative standard errors of the mean ($\sigma\mu$) found in Table III. The experimental design allows for a check on each determination, since at least two different concentrations are run; (4) the choice of CHA as the internal reference allows for a clean distinction of the t_R generally seen among the solvent, solute and internal reference peaks. This procedure is not limited to ethylamine-type solutes, since GC columns have been developed for a large array of substituted amines, especially the medicinally important class of ethanolamines[49], as well as all other volatile solutes. The only apparent limitation of this procedure occurs when either solute adsorption occurs on the walls of the flask or the lipophilicity of the solute is extremely high, causing detergent or solubility related problems[1].

In summary, a convenient, simple and readily accessible technique for the determination of log P values of any volatile amine has been demonstrated. The reproducibility of this procedure has allowed us to determine the log P values for a diverse set of lipophilic amines.

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