were obtained for all compounds. In some instances, additional proof of structure was provided by NMR spectroscopy on a Bruker WH-90 instrument in Me_2SO-d_6 and Me_2SO-d_6 plus D_2O. 2,4-Diamino-6-quinazolinecarbonitrile and the corresponding 5-chloro- and 5-methyl-6-quinazolinecarbonitriles were prepared according to a published procedure.[14]

6-[[(Substituted-phenyl)amino]methyl]-2,4-quinazolinediamines (IVa; 1–17, Table I). Procedure I. A mixture of 5.6 g (0.03 mol) of 2,4-diamino-6-quinazolinecarbonitrile, 5.8 g (0.03 mol) of 3,4-dichlorobenzenamine, and 1 g of Raney nickel in 135 mL of 67% aqueous HOAc at an initial pressure of 50 psig of hydrogen was shaken at 28 °C for 22 h. The reaction mixture was filtered, and the filter cake was washed with HOAc. The filtrate and wash were combined and evaporated to dryness under vacuum. The residue was triturated with hot H_2O, recrystallized from 20% aqueous HOAc, dried, and equilibrated in air to afford 7.3 g (57%) of 6-[[(3,4-dichlorophenyl)amino]methyl]-2,4-quinazolinediamine acetate dihydrate (1), mp 204–208 °C.

Compounds 2–17 were prepared analogously.

6-[[(Substituted-phenyl)nitrosoamino]methyl]-2,4-quinazolinediamines (IVb; 18–22, Table II). Procedure II. A solution of 0.43 g (0.0062 mol) of $NaNO_2$ in 4 mL of H_2O was added in portions over a 3-h period to a chilled solution of 1.5 g (0.003 mol) of 6-[[[4-chloro-3-(trifluoromethyl)phenyl]-amino]methyl]-2,4-quinazolinediamine (10) in 50 mL of DMF and 30 mL of 60% aqueous HOAc. The mixture was stirred at 0–5 °C for an additional 2 h and then poured into iced dilute NH_4OH. The resulting precipitate was collected, washed with H_2O, and recrystallized from 80% EtOH (charcoal) to afford 0.9 g (70%) of 6-[[[4-chloro-3-(trifluoromethyl)phenyl]nitrosoamino]-methyl]-2,4-quinazolinediamine (20), mp 216–217 °C.

Compounds 18, 19, 21, and 22 were prepared similarly.

***N*-(Substituted-phenyl)-*N*-[(2,4-diamino-6-quinazolinyl)methyl]formamides (IVc; 23–25 and 27, Table II). Procedure III.** A suspension of 3.5 g (0.009 mol) of 6-[[(3,4-dichlorophenyl)amino]methyl]-5-methyl-2,4-quinazolinediamine acetate (11) in 30 mL of 90% HCO_2H was heated under reflux for 2 h, cooled, and concentrated to dryness under vacuum. A solution of the residue in 10% aqueous EtOH was made basic with NH_4OH. The resulting solid was collected, recrystallized from 80% aqueous EtOH, dried, and equilibrated in air to afford 1.4 g (43%) of *N*-[(2,4-diamino-5-methyl-6-quinazolinyl)-methyl]-*N*-(3,4-dichlorophenyl)formamide (25), which foams at 130–133 °C, resolidifies, and melts at 233–234 °C.

The double melting point of this material suggested the possibility of structural alteration upon heating. However, IR and NMR spectra of a sample that had been heated at 160 °C for 0.5 h indicated that the material had lost water but had not changed structurally.

***N*-[(2,4-Diamino-6-quinazolinyl)methyl]-*N*-(3,4-dichlorophenyl)acetamide (IVd; 26, Table II). Procedure IV.** A mixture of 3.3 g (0.01 mol) of 6-[[(3,4-dichlorophenyl)-amino]methyl]-2,4-quinazolinediamine (4) and 1.1 g (0.01 mol) of Ac_2O in 80 mL of HOAc was stirred on the steam bath for 5 h, allowed to cool overnight, and concentrated to dryness under vacuum. A solution of the residue in hot water was made basic with NH_4OH. The resulting precipitate was collected, washed with H_2O, dried, and recrystallized from EtOH–H_2O to afford 2.6 g (65%) of 26, which foams at 108–110 °C, resolidifies, and melts at 224–225 °C.

Acknowledgment. The authors are indebted to Drs. M. W. Fisher and C. L. Heifetz of Warner-Lambert Co. for the antibacterial studies and Dr. Joan Shillis and Co-workers for the L1210 tissue culture studies. We also thank William Pearlman for conducting the hydrogenations, C. E. Childs and associates for the microanalyses, and Dr. J. M. Vandenbelt and co-workers for the determination of spectral data.

Registry No. 1·$C_2H_4O_2$, 52128-44-6; 2·$C_2H_4O_2$, 87183-25-3; 3·$xC_2H_4O_2$, 52128-40-2; 4·$C_2H_4O_2$, 52128-16-2; 5·$C_2H_4O_2$, 52128-08-2; 6·$C_2H_4O_2$, 52128-18-4; 7·$C_2H_4O_2$, 87174-61-6; 8·$C_2H_4O_2$, 52128-04-8; 9·$C_2H_4O_2$, 52128-06-0; 10·$C_2H_4O_2$, 52128-20-8; 11·$C_2H_4O_2$, 52128-34-4; 12·$C_2H_4O_2$, 52128-32-2; 13·$C_2H_4O_2$, 52128-30-0; 14·$C_2H_4O_2$, 52128-10-6; 15·$C_2H_4O_2$, 52128-36-6; 16·$C_2H_4O_2$, 52128-12-8; 17·$^3/_2C_2H_4O_2$, 52128-14-0; 18, 52128-45-7; 19·$C_2H_4O_2$, 52128-22-0; 20, 52128-23-1; 21, 52128-38-8; 22·$C_2H_4O_2$, 52128-25-3; 23, 52128-46-8; 24, 52128-27-5; 25, 52128-37-7; 26, 52128-26-4; 27, 52128-28-6; V (Z = H), 18917-68-5; V (Z = Cl), 18917-75-4; V (Z = Me), 18917-72-1.

Notes

An Extension of the *f*-Fragment Method for the Calculation of Hydrophobic Constants (Log *P*) of Conformationally Defined Systems[1]

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Received March 14, 1983

An extension of the popular fragment methods for the calculation of octanol–water partition coefficient (log *P*) values of conformationally defined compounds is presented. Correction factors for both trans-antiperiplanar and gauche conformational isomers have been developed for both the Rekker and Leo fragment methods and successfully applied to a large, diverse group of conformationally defined phenethylamines. This approach is easy to use and only requires one additional correction factor per isomer. This method thus allows, for the first time, conformation to be taken into account for the fragment calculation of log *P* values.

The partition coefficient in the octanol–water system (log *P*) has been widely employed in quantitative structure–activity relationship (QSAR) studies as a measure of hydrophobicity. Since the experimental determination of log *P* values can be impractical and time consuming, accurate and straightforward methods for theoretical determination of this important property are desired. The initial work toward this aim was that of Hansch and Fujita.[2] It resulted in the hydrophobic substituent param-

(1) Portions of this paper were presented at the 184th National Meeting of the American Chemical Society, Kansas City, MO, Sept 12–17, 1982; see "Abstracts of Papers"; American Chemical Society: Washington, DC, 1982; Abstr MEDI 048.

0022-2623/83/1826-1760$01.50/0

Chart I. Example Log P Calculations of Compounds 17-20

Trans-Antiperiplanar Conformation[a]

Rekker:

$$\log P_{calcd} = 11\,C + 11\,H + NH_2\ (aliphatic) + C_{trans}$$
$$11\,(0.155) + 11\,(0.182) - 1.420 - 0.289 = 2.00$$

Leo:

$$\log P_{calcd} = f_{C_6H_4} + 5f_C + 7f_H + f_{NH_2} + F_{GBr} + (7-1)F_b + F_{trans}$$
$$1.67 + 5\,(0.2) + 7\,(0.23) - 1.54 - 0.22 + 6\,(-0.09) + 0.17 = 2.15$$

Gauche Conformation[b]

Rekker:

$$\log P_{calcd} = 11\,C + 11\,H + NH_2\ (aliphatic) + C_{gauche}$$
$$11\,(0.155) + 11\,(0.182) - 1.420 - 0.578 = 1.71$$

Leo:

$$\log P_{calcd} = f_{C_6H_4} + 5f_C + 7f_H + f_{NH_2} + F_{GBr} + (7-1)F_b + F_{gauche}$$
$$1.67 + 5\,(0.2) + 7\,(0.23) - 1.54 - 0.22 + 6\,(-0.09) + 0.00 = 1.98$$

[a] Compounds 17 and 18. [b] Compounds 19 and 20.

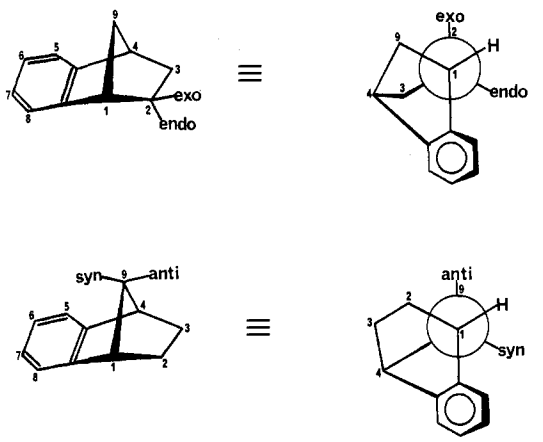


Figure 1. Newman projections of four conformationally defined benzonorbornene isomers.

eter, π_X, for the functional group X. More recently, the f_X-fragment values of Rekker[3] and Leo[4,5] have become available. While it has been recognized[6] that molecular conformation can affect the partition coefficient, little attention has been paid to the development of suitable π or f-fragment parameters for molecules with frozen conformations.[7] For example, the calculated log P by either fragment method would give an identical result for the four conformational isomers 2-*endo*-, 2-*exo*-, 9-*syn*-, and 9-*anti*-aminobenzonorbornene (**17**–**20**).

As part of our study to map out the active-site binding requirements for the enzyme norepinephrine *N*-methyltransferase (NMT, EC 2.1.1.18; also known as phenylethanolamine *N*-methyltransferase, PNMT), we had available a number of conformationally defined ("rigid") NMT substrates and inhibitors of the phenethylamine type.[8-10] We have recently determined[11,12] the log P values of these substrates and inhibitors in order to conduct a QSAR analysis. Since many of the log P values obtained were on compounds with structural types that have not been previously reported, we had within our means the potential of extending the f-fragment methods of Rekker[3] and Leo.[4,5] In this paper we report the development of

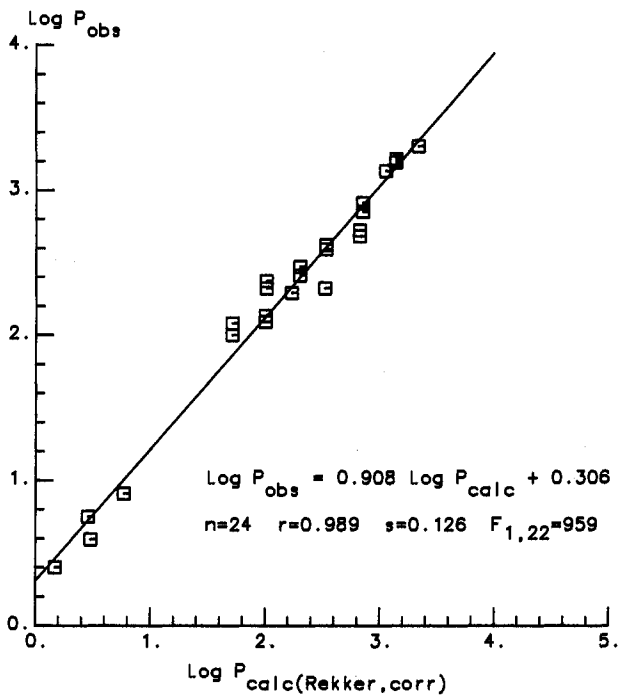
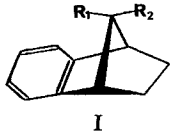


Figure 2. Plot of log P_{obsd} vs. log $P_{calcd(Rekker,corr)}$.

(2) Fujita, T.; Iwasa, J.; Hansch, C. *J. Am. Chem. Soc.* **1964**, *86*, 5175.
(3) Rekker, R. F.; de Kort, H. M. *Eur. J. Med. Chem.* **1979**, *14*, 479.
(4) Leo, A.; Jow, P. Y. C.; Silipo, C.; Hansch, C. *J. Med. Chem.* **1975**, *18*, 865.
(5) Hansch, C.; Leo, A. J. "Substituent Constants for Correlation Analysis in Chemistry and Biology", Wiley: New York, 1979, Chapter IV.
(6) Hopfinger, A. J.; Battershell, R. D. *J. Med. Chem.* **1976**, *19*, 569.
(7) The calculation of some rigid condensed ring aromatic compounds and some aromatic heterocycles is presented in Rekker, R. F. "The Hydrophobic Fragmental Constant. Its Derivation and Application. A Means of Characterizing Membrane Systems", Elsevier: New York, 1977; pp 82–92. In addition, a few conformationally restricted molecules such as tetralin and indan are calculated; however, no substituents are present at centers with frozen conformation where more than one conformational possibility would exist (e.g., no examples of frozen gauche or trans-antiperiplanar conformations are given).
(8) Grunewald, G. L.; Borchardt, R. T.; Rafferty, M. F.; Krass, P. *Mol. Pharmacol.* **1981**, *20*, 377.
(9) Rafferty, M. F.; Grunewald, G. L. *Mol. Pharmacol.* **1982**, *22*, 127.
(10) Grunewald, G. L.; Pleiss, M. A.; Rafferty, M. F. *Life Sci.* **1982**, *31*, 993.
(11) Grunewald, G. L.; Pleiss, M. A.; Gatchell, C. L.; Pazhenchevsky, R.; Rafferty, M. F., unpublished work using the method briefly described in ref 12 (submitted to *J. Chromatogr.*).
(12) The log P values of the amines were determined on the neutral species by partitioning in the traditional shake-flask method between 1-octanol and 0.1 N NaOH. The phases were analyzed by gas chromatography (10% Apiezon L, 2% KOH on 80–100 mesh Chromosorb WAW).

Table I. Observed and Calculated Log P Values Determined by the Rekker and Leo f-Fragment Methods[a]



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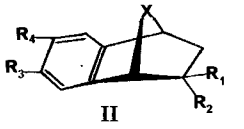

II

no.	code name	type	R_1	R_2	R_3	R_4	X	log P_{obsd}[b]	log $P_{calcd(Rekker,corr)}$[c]	Δ[d]	log $P_{calcd(Leo,corr)}$[e]	Δ[f]
1	2PX	II	$NHCH_2CH_2CH_3$	H	H	H	CH_2	3.30	3.34	−0.04	3.33	−0.03[g]
2	6-CF-2HX	II	NH_2	H	H	CF_3	CH_2	3.21	3.14	0.07[g]	2.98	0.23
3	7-CF-2HX	II	NH_2	H	CF_3	H	CH_2	3.19	3.14	0.05[g]	2.98	0.21
4	2PN	II	H	$NHCH_2CH_2CH_3$	H	H	CH_2	3.13	3.05	0.08	3.16	−0.03[g]
5	6-CF-2HN	II	H	NH_2	H	CF_3	CH_2	2.91	2.85	0.06[g]	2.81	0.10
6	7-CF-2HN	II	H	NH_2	CF_3	H	CH_2	2.85	2.85	0[g]	2.81	0.04
7	2EX	II	$NHCH_2CH_3$	H	H	H	CH_2	2.72	2.82	−0.10	2.79	−0.07[g]
8	NMX	II	$NHCH_3$	H	H	H	CH_2CH_2	2.68	2.82	−0.14[h]	2.82	−0.14[h]
9	2EN	II	H	$NHCH_2CH_3$	H	H	CH_2	2.62	2.53	0.09	2.62	0.0[g]
10	NMN	II	H	$NHCH_3$	H	H	CH_2CH_2	2.59	2.53	0.06[h]	2.65	−0.06[h]
11	9MA	I	H	$NHCH_3$				2.47	2.30	0.17[g]	2.25	0.22
12	2MX	II	$NHCH_3$	H	H	H	CH_2	2.41	2.30	0.11[g]	2.25	0.16
13	9MS	I	$NHCH_3$	H				2.37	2.01	0.36	2.08	0.29[g]
14	NHX	II	NH_2	H	H	H	CH_2CH_2	2.32	2.52	−0.20[g]	2.66	−0.34
15	2MN	II	H	$NHCH_3$	H	H	CH_2	2.32	2.01	0.31	2.08	0.24[g]
16	NHN	II	H	NH_2	H	H	CH_2CH_2	2.29	2.23	0.06[g]	2.49	−0.20
17	9HA	I	H	NH_2				2.13	2.00	0.13	2.09	0.04[g]
18	2HX	II	NH_2	H	H	H	CH_2	2.09	2.00	0.09	2.09	0.0[g]
19	9HS	I	NH_2	H				2.08	1.71	0.37	1.92	0.16[g]
20	2HN	II	H	NH_2	H	H	CH_2	2.00	1.71	0.29	1.92	0.08[g]
21	OMX	II	$NHCH_3$	H	H	H	O	0.91	0.77	0.14	0.79	0.12[g]
22	OHX	II	NH_2	H	H	H	O	0.75	0.46	0.29	0.51	0.24[g]
23	OMN	II	H	$NHCH_3$	H	H	O	0.59	0.48	0.11	0.62	−0.03[g]
24	OHN	II	H	NH_2	H	H	O	0.40	0.17	0.23	0.34	0.06[g]

[a] See ref 11 for the source of the compounds. [b] From ref 11. [c] Calculated using the fragment values of Rekker[3] and the correction factors for conformationally defined systems derived from this study. [d] Residual value [log P_{obsd} − log $P_{calcd(Rekker)}$]. [e] Calculated using the fragment values of Leo[4,5] and the correction factors for conformationally defined systems derived from this study. [f] Residual value [log P_{obsd} − log $P_{calcd(Leo)}$]. [g] Smallest absolute residual obtained with this method. [h] Absolute residual the same for both methods.

Table II. Comparison of Observed and Calculated Log P Values without Correction for Conformation

no.	log P_{obsd}	log $P_{calcd(Rekker,uncorr)}$ [a]	Δ [b]	log $P_{calcd(Leo,uncorr)}$ [c]	Δ [d]
			Trans-Antiperiplanar Conformation		
1	3.30	3.63	−0.33	3.22	0.08
2	3.21	3.43	−0.22	2.86	0.35
3	3.19	3.43	−0.24	2.86	0.33
7	2.72	3.11	−0.39	2.68	0.04
8	2.68	3.11	−0.43	2.71	−0.03
11 [e]	2.47	2.59	−0.12	2.14	0.33
12	2.41	2.59	−0.18	2.14	0.27
14	2.32	2.81	−0.49	2.55	−0.23
17 [e]	2.13	2.29	−0.16	1.98	0.15
18	2.09	2.29	−0.20	1.98	0.11
21	0.91	1.06	−0.15	0.68 [j]	0.23
22	0.75	0.75	0	0.40 [j]	0.35
			−0.24 ± 0.14 [f]		0.17 ± 0.18 [f]
			exo only: −0.26 ± 0.15 [g]		exo only: 0.15 ± 0.19 [g]
			Gauche Conformation		
4	3.13	3.63	−0.50	3.22	−0.09
5	2.91	3.43	−0.52	2.86	0.05
6	2.85	3.43	−0.58	2.86	−0.01
9	2.62	3.11	−0.49	2.68	−0.06
10	2.59	3.11	−0.52	2.71	−0.12
13 [h]	2.37	2.59	−0.22	2.14	0.23
15	2.32	2.59	−0.27	2.14	0.18
16	2.29	2.81	−0.52	2.55	−0.26
19 [h]	2.08	2.29	−0.21	1.98	0.10
20	2.00	2.29	−0.29	1.98	0.02
23	0.59	1.06	−0.47	0.68 [j]	−0.09
24	0.40	0.75	−0.35	0.40 [j]	0.00
			−0.41 ± 0.13 [f]		0.00 ± 0.13 [f]
			endo only: −0.45 ± 0.11 [i]		endo only: −0.03 ± 0.12 [i]

[a] Calculated using the fragment values of Rekker without correction for conformation. [b] Residual [log P_{obsd} − log $P_{calcd(Rekker,uncorr)}$]. [c] Calculated using the fragment method of Leo without correction for conformation. [d] Residual [log P_{obsd} − $P_{calcd(Leo,uncorr)}$]. [e] Compound with anti conformation, see Results and Discussion. [f] Mean plus or minus standard deviation for all compounds (n = 12). [g] Mean plus or minus standard deviation for exo compounds only (n = 10). [h] Compound with syn conformation, see Results and Discussion. [i] Mean plus or minus standard deviation for endo compounds only (n = 10). [j] Calculated using an estimated f_O^{1R} of −1.54 (private communication with A. Leo) and F_{P2}.

Table III. New Correction Factors for the Rekker and Leo Fragment Methods for Calculating Log P Values of Conformationally Defined Systems [a]

Rekker method: correction factor [b]	Leo method: correction factor
$C_{trans} = -0.289 \equiv -1C_m$	$F_{trans} = +0.17$
$C_{gauche} = -0.578 \equiv -2C_m$	$F_{gauche} = +0.00$

[a] See Chart I for an example of log P calculations utilizing these factors. [b] C_m is the magic constant of Rekker that is employed for proximity effects.

f-fragment correction values that take conformation into consideration.

Results and Discussion

The structures of the 24 amines included in this study and the measured partition coefficients (log P_{obsd}) are shown in Table I. In these conformationally defined ring systems, the exo and anti isomers correspond to trans-antiperiplanar arrangements of the aromatic ring and amino group about the phenethylamine portion of each compound, whereas the endo and syn isomers correspond to gauche arrangements of the phenethylamine portion as shown in Figure 1.[13] For the Rekker method, calculated log P values used the following factors: C = 0.155; H = 0.182; NH_2 (aliphatic) = −1.420; F (aliphatic) = −0.476; NH (aliphatic) = −1.814; O (aliphatic) = −1.595, and C_M = 0.289.[3] For the Leo method, the values were determined by using the standard f-fragment and correction factors.[4,5]

When we compared the log P_{obsd} with the calculated log P values by both the Rekker [log $P_{calcd(Rekker,uncorr)}$] and Leo [log $P_{calcd(Leo,uncorr)}$] methods, it was apparent that a rela-

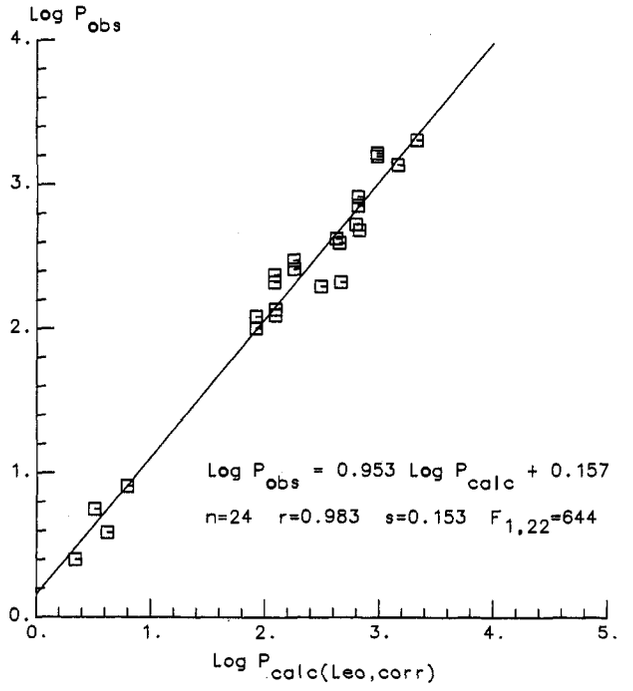

Figure 3. Plot of log P_{obsd} vs. log $P_{calcd(Leo,corr)}$.

(13) For a review of the properties of these and other conformationally defined amines of the current study, see: Grunewald, G. L.; Creese, M. W.; Walters, D. E. *ACS Symp. Ser.* 1979, *no. 112*, 439.

tively constant deviation occurred for similar conformational differences (e.g., the values calculated by the Rekker method for all the *exo*-amines differed by -0.26 ± 0.15 from the log P value observed experimentally for each *exo*-amine). These deviations are summarized in Table II. We have utilized these average deviations for each conformational type (e.g., gauche) to derive the appropriate correction factor to be used to include conformation in the calculated log P value. These correction factors are shown in Table III. Inclusion of the correction factors for conformation then allowed a calculation of the Rekker [log $P_{\text{calcd(Rekker,corr)}}$] and Leo [log $P_{\text{calcd(Leo,corr)}}$] values listed in Table I. In the modification of both the Rekker or Leo procedure, the appropriate additional factor from Table III was added after the normal fragment calculation was completed to compensate for the effect of conformation on hydrophobicity. An example set of calculated log P values is shown in Chart I. The corrected log P values calculated from both the Rekker and Leo methods are shown graphically in Figures 2 and 3, respectively. As can be seen from the plots in Figures 2 and 3, the calculated (corrected) log P for this diverse set of amines is in excellent agreement with the observed values. The regression equations for both plots are also shown in Figures 2 and 3; the correlation coefficients, r, are 0.989 for the Rekker method and 0.983 for the Leo method. Thus, when our new conformational correction factors are applied to the compounds of this data set, excellent agreement between calculated and measured values arises. It is significant to note that the correlation applies over a wide log P range (0.4–3.3).

Since there is some controversy[14] as to the choice of the Rekker[3] or Leo[4,5] fragment approach, we have determined new correction factors for both methods. Although the theoretical foundation of both methods is different, the two methods appear to predict the log P values of these conformationally defined systems well. The Leo method predicts 14 compounds better than the Rekker method, while the latter predicts eight compounds better than the former. The remaining two compounds are predicted equally well by both methods.

In summary, a valuable extension of the popular fragment method for calculating log P values has been presented for conformationally defined compounds. With our new correction factor, both the Rekker and Leo fragment procedures give excellent agreement with measured log P values for a wide range of pharmacologically important, conformationally defined amines. This approach is easy to use and only requires one additional correction factor per isomer. With these new correction factors, a beginning has been made toward the inclusion of conformation into the calculation of log P values.

Acknowledgment. This work was supported by Grants HL 21887, GM 22988, and DA 01990 from the U.S. Public Health Service. Support was also received from the University of Kansas General Research Fund. We gratefully acknowledge Professors E. A. Coats (College of Pharmacy, University of Cincinnati, Cincinnati, OH), A. J. Leo (Pomona College, Claremont, CA), and T. J. Reitz (Bennington College, Bennington, VT) for many helpful discussions about this study.

Registry No. 1, 86943-77-3; 2, 83118-50-7; 3, 83118-51-8; 4, 86992-67-8; 5, 83118-48-3; 6, 86022-72-2; 7, 86943-78-4; 8, 18883-05-1; 9, 86992-68-9; 10, 18883-06-2; 11, 86943-79-5; 12, 62624-27-5; 13, 86992-69-0; 14, 15537-20-9; 15, 58742-05-5; 16, 14342-36-0; 17, 14098-20-5; 18, 62624-26-4; 19, 72597-35-4; 20, 58742-04-4; 21, 86943-80-8; 22, 73159-84-9; 23, 86992-70-3; 24, 73208-84-1; NMT, 9037-68-7.

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Synthesis of 3-Hydroxy-3-cyclohexylbutyric Acid Derivatives. 1. Cyclic Homologues of 3-Hydroxy-3-methylglutaric Acid

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Z and *E* isomers of 3-methyl-3-(carboxymethyl)hexahydro-1(3*H*)-isobenzofuranones (I), lactones of 3-hydroxy-3-(2-carboxycyclohexyl)butyric acids (II), were prepared and tested on cholesterol biosynthesis in vitro. Compound I of the *Z* series was prepared through its ethyl ester by hydrogenation, over Rh/Al_2O_3 catalyst, of the phenyl ring of 3-methyl-3-[(ethoxycarbonyl)methyl]-1(3*H*)-isobenzofuranone. Compound I of the *E* series was prepared, through its ethyl ester, by Reformatsky reaction from ethyl (*E*)-2-acetylcyclohexanecarboxylate. 3-Methyl-3-(carboxymethyl)-5,6,7,8-tetrahydro-1(3*H*)-isobenzofuranone, 3-methyl-3-ethyl-5,6,7,8-tetrahydro-1(3*H*)-isobenzofuranone, and 3-methyl-3-(carboxymethyl)-1(3*H*)-isobenzofuranone were also prepared and tested. The above compounds inhibited acetate incorporation in cholesterol and fatty acids in rat liver slices at 5×10^{-3} M but lack specific inhibitory activity on HMG-CoA reductase.

3-Hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) is the metabolic precursor of mevalonic acid in cholesterol biosynthesis, and its reduction by HMG-CoA reductase is considered to be the rate-limiting step in the biosynthetic pathway from acetate to cholesterol.[1] Moreover, 3-hydroxy-3-methylglutaric acid (HMG) reportedly has regulatory effects on cholesterol biosynthesis both in vitro and in vivo[2] and blood cholesterol lowering activity in rats and humans.[3]

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