ml. of water. The resulting precipitate was collected by filtration. The product was recrystallized from ethanol to afford 1.1 g. of 4-methylaminobenzonitrile, m.p. 86°. In the case of ethyl-, propyl-, and butylaminobenzonitrile, method B was better than method A.

4-Methylaminobenzothioamide (Table III).—4-Methylaminobenzonitrile (2 g.) was dissolved in a mixture of 10 ml. of pyridine and 5 ml. of triethylamine, and the solution was treated with H_2S for 4 hr. The reaction mixture was evaporated under reduced pressure and the residual product was triturated with water. The precipitated product was collected by filtration and purified by recrystallization from ethanol to yield 2 g. of pure product, m.p. 170°. Other alkylaminobenzothioamides were prepared by the same method.

TABLE III

4-ALKYLAMINOBENZOTHIOAMIDES: p-$RNHC_6H_4CSNH_2$

R	M.p., °C.	% calcd. C	% calcd. H	% found C	% found H
CH_3	170	57.80	6.06	58.14	6.06
C_2H_5	165	59.96	6.71	60.46	6.69
n-C_3H_7	164	61.81	7.26	61.84	7.43
i-C_3H_7	172	61.81	7.26	61.68	7.32
n-C_4H_9	131	63.42	7.74	63.42	7.96
i-C_4H_9·H_2O	183	58.37	8.02	58.45	7.96
n-C_5H_{11}	142	64.82	8.16	64.06	8.07
i-C_5H_{11}	154	64.82	8.16	64.61	8.31
$C_6H_5CH_2$	178	69.38	5.82	69.06	6.05
C_6H_5	174	68.39	5.30	68.77	5.71

4-Phenylaminobenzothioamide.—A solution of 10 g. of NaCN in 25 ml. of water was added to a solution of 8 g. of $CuSO_4$ in 50 ml. of water. A diazo solution was prepared from 9.5 g. of p-aminodiphenylamine, 45 ml. of 6% HCl, and 4 g. of $NaNO_2$. The diazo solution was added to the warm well-stirred CuCN solution in 10 min. After 15 min., the reaction mixture was extracted with ether. The ether was distilled, the resulting syrup (1.4 g.) was treated with H_2S as usual to afford 1.1 g. of the crude 4-phenylaminobenzothioamide. Recrystallization from ethanol gave 0.6 g. of pure substance, m.p. 174°.

Extinction Coefficient, E_{max}, in Infrared Absorption Spectra.—The CN stretching band (2215–2230 cm.$^{-1}$) was measured in KBr disks (10 μmoles in 1 g.).

In Vitro **Antituberculous Activity.**—The *in vitro* test against human-type tubercule bacilli, strain H37Rv, using Kirchner's medium was conducted according to the method described in a previous paper.[6] The minimum inhibitory concentrations (MIC) are shown in Table I.

(6) S. Kakimoto and K. Yamamoto, *Japan. J. Tuberc.*, **6**, 27 (1958).

Antituberculous Compounds. XXIII.[1] Alkyl- and Acylisonicotinic Acid Hydrazides

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Among acyl derivatives of isonicotinic acid hydrazide (INH) Rieche, *et al.*,[2] reported that in the series having unbranched carbon chains from C_6 to C_{18}, the undecanoyl derivative was the most active and showed approximately the same activity as INH against tubercle bacilli.

Acetyl, propionoyl, and butyryl derivatives of INH have almost no activity. In the literature,[3] these substances are all described as anhydrous compounds, but we have found that they crystallize with water of crystallization from water or aqueous solvent. As alkyl derivatives of INH, N-isopropyl-N′-isonicotinoylhydrazine has been reported as a good antituberculous compound, but only a few other alkyl derivatives have been described with chemical data and biological activities. Fox and Gibas[4] reported the synthesis of monoalkyl derivatives of INH, and McMillan, *et al.*,[5] prepared some higher homologs.

We have prepared the ethyl, propyl, and butyl derivatives and have shown that these compounds are more active than the acyl derivative containing the same number of carbon atoms, as shown in Table I.

TABLE I

MINIMUM INHIBITORY CONCENTRATION OF ALKYL DERIVATIVES OF INH AGAINST H37Rv IN KIRCHNER'S MEDIUM (28 DAYS, 38°)

$C_5H_4NCONHNHR$

R	MIC, μmole/l.
H (INH)	1
$COCH_3$	400
C_2H_5	40
COC_2H_5	400
C_3H_7	40
COC_3H_9	400
C_5H_{11}	5

Experimental Section

N-Acyl-N′-isonicotinoylhydrazine.—The crude crystalline material obtained by the literature[3] methods was recrystallized from water or aqueous ethanol and acetone. The pure crystalline material contained solvate water as shown in Table II. Anhydrous substances were obtained by recrystallization from absolute ethanol or acetone and by drying under reduced pressure.

In Vitro **Antituberculous Activity.**—The *in vitro* test against human tubercule bacilli, strain H37Rv, using Kirchner's medium was conducted according to the method described in a previous paper.[6] The minimum inhibitory concentration (MIC) is shown in Table I.

TABLE II

$C_5H_4NCONHNHCOR$

R	Water of crystn., moles	M.p., °C.	Anhydrous m.p., °C.	% calcd. C	% calcd. H	% calcd. H_2O	% found C	% found H	% found H_2O
CH_3	2	76	158	44.64	6.09	16.75	44.48	6.38	16.61
C_2H_5	2	95	131.5	47.15	6.60	15.72	47.13	6.41	15.82
C_3H_7	1	84	139	53.32	6.71	8.00	53.32	6.81	7.76

(1) Part XXII: S. Kakimoto and I. Tone, *J. Med. Chem.*, **8**, 867 (1965).

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The Use of Substituent Constants in the Correlation of Demethylation Rates

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In continuing our study[1-4] of substituent effects on the biological activity of congeneric drugs we have in

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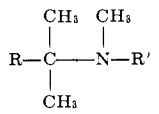
TABLE I

DATA USED IN DERIVING EQUATIONS ON THE DEMETHYLATION OF AMINES BY RAT MICROSOMES

No.	R	R′	Log *BR* Obsd. at 1 hr.	Log *BR* Calcd.[a]	pK_a − 9.5	log *P*
1	Am	Am	0.712	0.59	0.90	4.55
2	Am	Bu	0.532	0.35	0.90	4.05
3	Bu	Bu	0.167	0.09	1.00	3.55
4	Am	Pr	0.090	0.12	0.90	3.55
5	Pr	Pr	−0.770	−0.35	0.90	2.55
6	*i*-Bu	*i*-Bu	−0.032	0.03	0.60	3.19
7	*sec*-Bu	*sec*-Bu	−0.377	−0.24	1.60	3.19
8	*t*-Am	*i*-Pr	−0.347	−0.32	1.70	3.08
9	*t*-Am	*t*-Bu	−0.523	−0.32	2.40	3.47
						
10	HC≡C	CH_3	−0.022	−0.05	−1.60	1.77
11	HC≡C	Et	0.041	0.11	−1.30	2.27
12	HC≡C	Pr	0.352	0.34	−1.30	2.77
13	HC≡C	*i*-Pr	0.362	0.12	−0.80	2.59
14	HC≡C	Bu	0.586	0.58	−1.30	3.27
15	HC≡C	*sec*-Bu	0.407	0.33	−0.70	3.09
16	HC≡C	*t*-Bu	0.608	0.15	−0.20	2.98
17	HC≡C	$C_6H_5CH_2$	0.813	1.20	−2.40	3.96
18	CH_2=CH	*i*-Pr	−0.301	−0.43	1.80	2.89
19	$COCH_3$	*i*-Pr	0.782	−0.33	−0.20	0.93
20	$CHOHCH_3$	*i*-Pr	−0.700	−1.16	1.90	1.39
21	A		0.726	0.84	−0.60	5.53
22	B		0.896	0.72	−0.10	4.17
23	C		0.650	0.65	0.10	3.44
24	D		0.625	0.58	00	2.73
25	E		0.458	0.53	−0.20	2.21
26	F		0.461	0.49	−0.60	1.75

[a] The aliphatic calculated values were obtained with eq. 4. The values for **21–26** were obtained with eq. 5.

this report analyzed the results from the elegant demethylation studies of McMahon.[5,6] McMahon has presented excellent evidence for the suggestion of Brodie and co-workers that demethylation of drugs is brought about by enzymes located in fatty portions of the cell. The reaction studied *in vivo* and *in vitro* by McMahon was

$$R_1R_2NCH_3 \longrightarrow R_1R_2\ddot{N}H + CH_2O \quad (1)$$

Using the data for the first 18 compounds in Table I, we have derived eq. 2–4 by the method of least squares.

$$\log BR = -0.200(pK_a - 9.5) + 0.162 \quad n = 18,\ r = 0.611,\ s = 0.373 \quad (2)$$

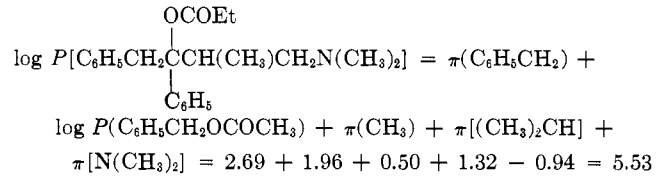
$$\log BR = 0.296 \log P - 0.805 \quad n = 18,\ r = 0.429,\ s = 0.426 \quad (3)$$

$$\log BR = 0.470 \log P - 0.268(pK_a - 9.5) - 1.305 \quad n = 18,\ r = 0.890,\ s = 0.222 \quad (4)$$

In these equations, n is the number of points used in the regression analysis, r is the multiple correlation coefficient, and s is the standard deviation. The biological response, *BR*, is the relative rate of *in vitro* demethylation by rat microsomes. *P* is the calculated partition coefficient of the amine and K_a is the ionization constant reported by McMahon. We have subtracted the constant 9.5 from pK_a for convenience in calculation. This is the pK_a value for $C_6H_5CH_2CH_2CH_2N(CH_3)_2$. We have used this molecule to obtain π for the $N(CH_3)_2$ group and for this reason have chosen it as the reference compound.

Log *P* was calculated by taking advantage of the additive nature of π and log *P*.[7,8] The following values[7,8] were used in calculating log *P*: 0.50 for CH_3 and CH_2, 0.71 for Cl, 2.69 for $C_6H_5CH_2$, −0.94 for $(CH_3)_2N$, 1.32 for $(CH_3)_2CH$, −0.76 for CH_3COO, 0.70 for CH_2=CH, 0.40 for HC≡C, −1.80 for OH, −1.26 for $COCH_3$, 1.96 for $C_6H_5CH_2OCOCH_3$, and 1.10 for $C_6H_5CH_2OH$. The appropriate aromatic or aliphatic value for π must be used.

The π values were found for the aliphatic functions by determining log *P* for compounds of the type $C_6H_5CH_2CH_2CH_2X$ and subtracting log *P* for *n*-propylbenzene. The HC≡C function is less reliable than the others since it was obtained from phenylacetylene rather than an aliphatic molecule. Resonance interaction of the two π-electron systems will cause a slightly lower $\pi_{HC\equiv C}$ value. For CH_2=CH, π was obtained by subtracting log *P* for methyl ethyl ketone from log *P* for $CH_3COCH_2CH_2CH$=CH_2.[7] The following example serves to illustrate how log *P* values have been calculated. The hydrogen atom in a C–H bond is



$$\log P[C_6H_5CH_2\overset{\overset{OCOEt}{|}}{\underset{\underset{C_6H_5}{|}}{C}}CH(CH_3)CH_2N(CH_3)_2] = \pi(C_6H_5CH_2) + \log P(C_6H_5CH_2OCOCH_3) + \pi(CH_3) + \pi[(CH_3)_2CH] + \pi[N(CH_3)_2] = 2.69 + 1.96 + 0.50 + 1.32 - 0.94 = 5.53$$

taken as zero in the calculation. Slightly different values of log *P* result for a complex compound depending on how one chooses the parts for addition, but these differences are usually small when compared with the differences in biological assays.

McMahon, in extending Brodie's idea that demethylation occurred in lipophilic tissue, pointed out that the more lipophilic amines were, the more rapidly they were demethylated. Equation 3 bears this out by showing that there is a correlation between lipophilic character and demethylation rate. McMahon also appreciated the importance of the electron density on nitrogen and for this reason measured the pK_a values of the amines. Equation 2 shows the dependence of demethylation rate on this parameter. Equation 4 then shows the combined effect of the two parameters on demethylation rates. The high correlation obtained with eq. 4 (80% of the variance in the data is "explained") is about as good as one can expect considering the difficulties in measuring *BR*. An F test indicates the additional π term in eq. 4 to be significant at >0.995. Adding a $(\log P)^2$ term[1] to eq. 4 does not result in an improved correlation.

In the first report,[5] McMahon investigated the demethylation of the amines (A–F) containing aromatic rings. Proceeding as described above, we obtain eq. 5–7.

An F test shows that the additional electronic term in eq. 7 is not significant even at the 0.75 level. One

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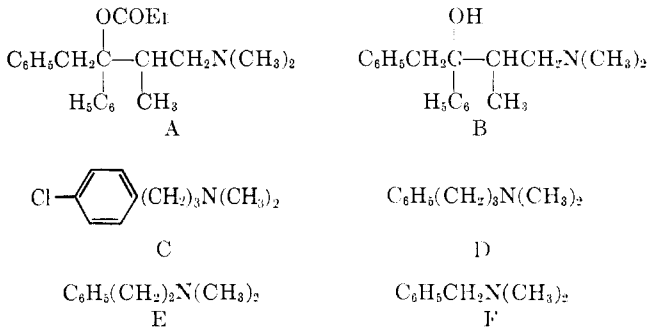


reason for this may be the relatively little variation in pK_a for these compounds compared to the variation in log P.

	n	r	s	
$\log BR = 0.094 \log P + 0.325$	6	0.782	0.116	(5)
$\log BR = 0.137(pK_a - 9.5) + 0.668$	6	0.248	0.180	(6)
$\log BR = 0.099 \log P + 0.190(pK_a - 9.5) + 0.354$	6	0.853	0.112	(7)

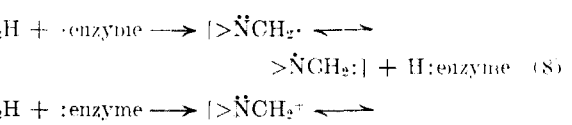
For the work in this report, P is the octanol–water partition coefficient so that the larger the value for P, the more lipophilic the compound. Thus the positive coefficients associated with log P in eq. 2–5 bear out Brodie's idea and McMahon's conclusion that the more lipophilic the compounds are, the more rapidly they are demethylated.

Where electronic terms are significant in the above equations, we note that a negative coefficient is associated with $(pK_a - 9.5)$. This means that the lower the electron density on nitrogen (as measured by $R_3N^+H \rightleftarrows R_3N + H^+$), the greater the demethylation rate. An obvious interpretation of this could be that the lower electron density reduces the chance of protonation of the nitrogen and hence the aqueous solubility of the amine. It must be kept in mind that π or log P is determined[8] so that it is independent of the degree of dissociation; hence π and pK_a are independent variables. Actually, both can play a role in the distribution of the amines between the aqueous and lipophilic phases. The role of pK_a in this process is difficult to assess accurately since it will vary with the pH of the environment and the endobio environment at the sites of action may be different from that of the external solution.

One of the most interesting aspects of McMahon's study is the minor importance of steric effects on the rate of demethylation. One might expect that the demethylation rate of a molecule with such highly branched substituents as *t*-amyl-*t*-butylmethylamine would be so different from *n*-dipropylmethylamine that an equation such as 4 without a steric parameter would give good correlations. It seems to us this would of necessity be true if any group other than a proton were involved in reaction with the nitrogen lone-pair electrons.

The work of Brown[9] has clearly demonstrated that the steric requirements for a proton reacting with highly substituted amines are uniquely low. It is also clear from Brown's work that the steric requirements of other electron-seeking substances such as BH_3, $B(CH_3)_3$, etc. do not parallel those of the proton. The fact that such good correlation is obtained using pK_a, a constant dependent on the steric and electronic requirements of protonation of the amines in question, argues strongly against the electronic term in eq. 4, reflecting action with a function larger than a proton. We interpret this to mean that an enzyme cannot be involved with the lone-pair electrons on nitrogen or those in the N–CH_3 bond in the rate-determining step of demethylation.

An attractive mechanism in which steric influence of the highly branched alkyl group would be at a minimum would be a displacement on hydrogen. Protonation of the nitrogen would inhibit either mechanism 8 or 9 by tying down the lone-pair electrons and thus preventing their stabilization of the intermediates through resonance.



Thus the role of the electron density on nitrogen in the above suggested mechanism for demethylation is complex. If it is very high, the amine is primarily in the ammonium ion form and its lipophilic character is lowered. If it is too low, then the availability of the electrons for stabilizing an intermediate is decreased. In order to test this hypothesis we have derived eq. 10 for compounds 1–18 in Table I. This gives a slightly better correlation than eq. 4. An F test indicates the squared term to be significant at >0.95. Equation 10 does indicate a slightly nonlinear dependence of log BR on the electron density, indicating a possible dual role for the lone-pair electrons. A further analysis of this point using amines having higher and lower electron densities than those studied would be worthwhile.

	r	s	
$\log BR = 0.484 \log P - 0.068(pK_a - 9.5)^2 - 0.267(pK_a - 9.5) - 1.225$	0.924	0.193	(10)

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Alkoxyalkyltetracyclines

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Modifications of tetracyclines involving the carboxamide function have previously been achieved mainly *via* the Mannich or Ritter reactions. Condensations of tetracyclines with formaldehyde and various primary or secondary amines have led to Mannich base deriva-