monoamyl derivative. The main fraction (VI) distilled initially at 130–155° (12 mm.) and on redistillation 4.24 g. (42% of theor.) was obtained at 134–136° (13 mm.)

Method D: The Eschweiler-Clarke methylation¹⁴ was used adding the diamine slowly to the mixture of formaldehyde and formic acid. Reaction was then completed by refluxing for 3.5 hr. The mixture was acidified with concentrated HCl and the solvent removed under reduced pressure. The residue was recrystallized from ethanol as indicated in the Table to give XXI.

Method E: Acetylation.—The addition of N,N'-diisopropylethylenediamine to excess acetic anhydride was accompanied by heat evolution. The mixture was refluxed for 30 min. and concentrated under reduced pressure to a solid. Two recrystallizations from acetone gave pure XXIV.

Acknowledgment.—We wish to thank Mr. L. Brancone and associates for microanalyses, Mr. W. Fulmor and associates for spectral data, Mr. E. Ruso for high pressure catalytic reductions and Mr. L. Binovi for assistance on certain preparations.

(14) M. L. Moore, "Organic Reactions," Vol. V, 307 (1949).

Antituberculous Agents. III. (+)-2,2 -(Ethylenediimino)-di-l-butanol^{1,2} and Some Analogs

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N-Hydroxyalkyl ethylenediamines have been synthesized by various methods as part of the further study of the antimycobacterial activity of analogs of N,N'diisopropylethylenediamine. In these hydroxylated compounds an even higher structural selectivity has been observed along with a remarkable stereospecificity. Correlation of biological activity with the postulated ability to form a certain type of chelate is discussed. (+)-2,2'-(Ethylenediimino)-di-1-butanol is two to four times as active as streptomycin against human mycobacteria in mice.

In a series of diamines related to N,N'-diisopropylethylenediamine³ high antituberculous activity *in vivo* was remarkably specific with

⁽¹⁾ A preliminary communication (Paper I) on this compound has been published by: R.G. Wilkinson, R. G. Shepherd, J. P. Thomas and C. Baughn, J. Am. Chem. Soc., 83, 2212 (1961).

 ⁽²⁾ Biological data have been published by J. P. Thomas, C. Baughn, R. G. Wilkinson and R. G. Shepherd, Am. Rev. Resp. Dis., 83, 891 (1961).

⁽³⁾ The study of these related compounds is reported (in Paper II) by R. G. Shepherd and R. G. Wilkinson, J. Med. Pharm. Chem., 5, 823 (1962).

respect to the type of N-alkyl substituent and the length of, or substitution on, the alkylene chain. These structure-activity relationships led us to speculate that the steric factors involved might correlate with the ability to form a metal chelate of type I. With copper, sterically-hindered ethylenediamines are known^{4,5} to form chelates of type I rather than the more commonly formed⁵ chelates of type II. The sharp decrease in activity which occurs when R is a secondary alkyl group larger than *sec*-butyl may be due to the increased hindrance toward formation of either type of chelate or, as Basolo⁵ suggested, long alkyl groups such as *n*-butyl may rotate in such a way as to stabilize a chelate of type II with respect to attack by solvent at the metal.



Following this line of thought, it was postulated that a tetradentate chelate of type III incorporating the principal features of type I would not require two molecules of the diamine for the completion of the chelate structure, thus increasing the possibility of formation in dilute solution. Therefore, a series of such N,N'-bis(hydroxy-alkyl)alkylenediamines was prepared with particular emphasis on the hydroxylated derivatives of N,N'-diisopropyl-, di-sec-butyl- and di-t-butyl-ethylenediamines.³

The simplest preparation of these diamines was the condensation of an amino alcohol with an alkylene dihalide or, for unsymmetrical diamines, with an alkylaminoalkyl halide. Certain amino alcohols were prepared by reduction of amino acid esters with lithium aluminum hydride. Alternatively, the amino acid ester was first condensed with oxalyl chloride and the desired diamine was then obtained on reduction of all four carboxylic functions with lithium aluminum hydride. Among other methods the reductive alkylation of ethylene-

⁽⁴⁾ M. Calvin and A. B. Martell, "Chemistry of the Metal Chelate Compounds," Prentice Hall, Inc., New York, N. Y. 1952, p. 179.

⁽⁵⁾ F. Basolo and K. R. Murmann, J. Am. Chem. Soc., 76, 211 (1954).

diamine with hydroxy ketones was moderately satisfactory, and the reaction of ethylenediamine with expoxides was suitable where the hydroxyl group was not desired on a terminal carbon.

In many of these preparations, stereoisomers were encountered and in some cases the racemic and *meso* forms could be separated. In each case the higher-melting form has been arbitrarily assigned the *meso* configuration.⁶ Only with 2,2'-(ethylenediimino)-di-1-butanol was the preparation of all the stereoisomers attempted. Resolution of its racemic base (compound 9) with (+)-tartaric acid was incomplete whereas the intermediate racemic 2-aminobutanol was readily resolved⁷ with either (+)- or (-)-tartaric acid. Synthesis of the highly active (+)-diamine (Compound 11) and of the (-)-isomer (Compound 10) was carried out through the amino alcohol enantiomers.

As can be seen from Table I, (+)-2,2'-(ethylenediimino)-di-1butanol (Compound 11) is four times as active as N,N'-diisopropylethylenediamine (Compound 1). It is also at least four times as active as its parent N,N'-di-sec-butylethylenediamine³ (mixture of meso and racemic forms) and was found to be considerably less toxic than either of these diamines. By parenteral administration to mice infected with a human strain of Mycobacterium tuberculosis this (+)hydroxylated diamine was two to four times as active as streptomycin and had an efficacy index (ratio of maximum tolerated dose to median effective dose) about four times as great.^{1,2} A similar comparison by oral administration showed it to have an efficacy index at least equal to that of isoniazid. It has no appreciable activity in vitro or in vivo against other bacteria, fungi or viruses. The stereoisomers of 2.2'-(ethylenediimino)-di-1-butanol were strikingly different in antituberculous activity: the meso form (Compound 8) was only onetwelfth as active as the (+)-isomer (Compound 11) and the (-)isomer (Compound 10) was only $1/_{500}$ th as active. In contrast, the texicities in mice were the same.^{1,2} Structural analogs (Compounds, 6, 7, 12, 13 and 16) of this highly active compound were for the most part considerably less active (Table I).

The following comparisons of the structure-activity relationships in the hydroxylated series with those in the parent series are of interest (see Table I). It is obvious that compounds containing hydroxylated primary alkyl groups (Compounds 2, 3, 6, 7) are no more

⁽⁶⁾ R. Stern, J. English and H. Cassidy, J. Am. Chem. Soc., 79, 5798 (1957), reported that in 90% of 130 pairs of diastereomers the meso isomer was the higher-melting.

⁽⁷⁾ F. H. Radke, R. B. Fearing and S. W. Fox, J. Am. Chem. Soc., 76, 2802 (1954). Dr. H. G. Arlt, Jr., and associates of these Laboratories have found the L-glutamic acid procedure therein to be suitable for large scale preparations.

TABLE	

SYNTHESIS, PROPRETIES AND ANTIMICOBACTERIAL ACTIVITY OF HYDROXY N,N'-DISUBSTITUTED ETHYLENEDIAMINES

						-Analy	/ses	(Relative ^d	In vitro ^e
			$M.p. \circ C.^{b}$	Yield, ^c	Cal	led. ovi	er Four	ы	activity	activity
	Compound	Isomer and formula	B.p., $^{\circ}$ (mm.) ^{<i>a</i>}	%	ບ	Н	z	ប	in vivo	meg./ml.
17	(i-PrNHCH2)2	$C_8H_{20}N_2$	b. 169–169.5	60	66.6	14.0	19.4			
					66.7	13.8	19.6			
		C8H20N2 · 2 HCl	m. 258-259	58°	44.3	10.2	12.9	32.6	1.0	×
					44.6	10.4	12.8	32.3		
24	(HOCH2CH2NHCH2)2	C6H16N2O2							<0.03i	
30	(CH _a CHOHCH ₂ NHCH ₂) ₂	$C_8H_{20}N_2O_2$							<0.03i	
$4^{\mathbf{h}}$	(CH3CHNHCH2)2	meso-C8H20N2O2	b. 150-170 (0.1)	12'						60
			m. 137.5–141.5							
	CH2OH	meso-C8H20N2O2 · 2 HCl	m. 201.5-204.5	10"	38.6	8.9	11.2	28.5	<0.06i	s
					38.4	9.1	11.6	28.3		
54		(\pm) -C ₈ H ₂₀ N ₂ O ₂ ·2 HCl	m. 184–186.5	"6	38.6	8.9	11.2	28.5	<0.06i	15
•					38.4	8.9	11.3	28.1		
(0,1)	(C ₂ H ₅ CHCH ₂ NHCH ₂) ₂	meso-C10H24N2O2	m. 149–150		58.8	11.8	13.7		<0.06i	<250i
	– HO				58.4	11.9	13.3			
11		(\pm) -C ₁₀ H ₂₄ N ₂ O ₂	m. 94.0-95.5		58.8	11.8	13.7		<0.06i	< 1000
					58.7	11.9	13.5			
8^{h}	(C ₂ H ₆ CHNHCH ₂) ₂	me80-C10H24N2O2	b. 167-170 (0.6)	371	58.8	11.8	13.7			
			m. 135.8–136.5		58.7	11.6	14.0			
	LU2UI		1 100 1 000	~ * *	6 G T	1 0	101	0 20	06 V	9
		meso-Ci01241N2O2+2 11C4	6, #02~6, 602, 111	F.C.	43.6	0.0 2.0	10.3	25.6		c
46		(\pm) -C ₁₀ H ₂₄ N ₂ O ₂	b. 167-170 (0.6)	42^{r}	58.8	11.8	13.7			
			m. 75–76		59.0	12.1	14.1			
		(土)-CteH24N2O2・2 HCl	m. 179–180	38'	43.3 6	9.5 7.7	10.1	25.6 25.3	¢I	Ċ)
-					40.4		4.0T			

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10 ^{4,4})	(C ₂ H ₆ CHNHCH ₂) ₂	$(-) \cdot C_{10} H_{24} N_2 O_2$	b. 163170 (0.1) m. 8880	53 <i>°</i>	58.8	11.8	13.7 13.9			
	CH ₂ OH			-						
		(-)-C ₁₀ H ₃₄ N ₂ O ₂ ·2 E CI	m. 201.5-202.5	50'	43.3 43.3	9.9 9.9	10.1 10.3	25.6 25.3	<0.004i	200
114.4		(+)-Cl ₀ Hz ₁ N ₂ O ₂	b. 160–170 (0.15) m. 87.5–88.8	40 ^a	58.8 58.8	11.8 11.8	13.7 13.7			
		(+)-C10H24N2O2·2 HCl	m. 201.8-202.6	674	43.3 43.5	9.5	10.1	25.6 25.6	al t	1
12^{f}	(CH ₂ CHNHCH ₂) ₂	Mixed CutH24N2O2	b. $135-140(0.3)$ m. $128-144$	"б	58.8 58.7	11.8 11.5	13.7 13.6			
	CH ₃ CHOH	isomers								
		C10H24N2O2.2 HCl	m. 196–218	16	43.3 43.4	9.6 9.6	10.1 10.3	25.6 25.3	<0.03i	>250i
13/	(CH3CHNHCH2)2	Mixed C10H24N2O2	b. 158-165 (0.3)	27	58.8	11.8	13.7		<0.03i	250
	 CH2CH2OH	opt. isomers			58.8	11.7	13.8			
14^{i}		(\pm) -C ₁₁ H ₂₆ N ₂ O ₂	b. 150–190 (0.4)							
		(土)-C ₁₂ H26N2O2・2 HCl	m. 238–239	1	47.5	9.3	9.2	23.4	< 0.015i	>250i
15	CH2				47.6	9.4	9.5	23.5		
		meso-C12H26N202.2 HCl	m. 246.5–247.5	0.5^{*}	47.5	9.3	9.2	23.4	<0.015i	>250i
	CH ₂ OH				47.7	9.2	9.6	23.4		
16^{h}	(CH ₃ CNHCH ₂) ¹	C ₁₀ H24N2O2·2HCI	m. 260.5–261.5 (gas evol.)	52^x	43.3 43.4	9.4 9.5	10.1 9.8	25.6 25.6	<0.03i	09
	CH*OH									
17 ^t	сн _а он 1	CloHMN204.2 HCl	m. 226.5–227.5 dec.	55 ^u	38.8 38.8	8.5 8.9	9.1	22.9 22.5	<0.06i	250
	(CH ₃ CNHCH ₂) ₂									
	I CH2OH									
18 ^{m,n}	[(HOCH2)2CNHCH2]2	CloH34N2O6.2 HCI.H2O	m. 214–215 (gas evol.)	46°	33.5 33.9	7.9 7.9	7.8 8.0	19.8 19.8	<0.03i	>250i

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						-Anal	yses	($\operatorname{Relative}^{d}$	In vitro ^c
			$M.p. \circ C.^{b}$	${ m Yield},^{c}$	Cal	led. ove	er Four	pu	activity	activity
	Compound	Isomer and formula	B.p., ° (mm.)"	%	c	Н	z	Ũ	oaia ui	meg./ml.
10^{I}	$(n-PrCHNHCH_2)_2$	meso-C12H28N2O2	m. 133.5-135	20^{z}						
		meso-C ₁₂ H ₂₈ N ₂ O ₂ ·2 HCl	m. 194.5-196	196	47.2	9.9	9.2	23.2	< 0.25i	60
	CH ₂ OII				47.1	9.8	9.2	23.3		
20^{l}		$(\pm)-C_{12}H_{28}N_2O_2$	m. 69–72	12^{w}						
		(±)-C ₁₂ H ₂₈ N ₂ O ₂ ·2 HC1	m. 182–184.5	11,	47.2	9.9	9.2	23.2	0.25	
					47.1	10.1	9.2	22.9		
21°	(i-PrCHNHCH ₂) ₂	Mixed C ₁₂ H ₂₈ N ₂ O ₂ -2 HCl	m. 231.5-233.5	43*	47.2	9.9	9.2	23.2	<0.25i	250
	 CH20H	opt. isomers			47.0	9.8	9.3	23.1		
22^{p}	(NHCH ₂) ₂	$meso-C_{14}\Pi_{28}N_2O_2$	m. 205–210	394	65.6	11.0	10.9		<0.03i	250
~	HUY				65.8	11.0	11.1			
23^{p}		(\pm) - $C_{14}H_{28}N_2O_2$	m. 126–128	42^{w}	65.6	11.0	10.9		< 0.03i	250
					65.6	11.0	10.8			
2400	$(\langle NHCH_2)_2$	Ch4 H16 N2O2 - 0.125	m. 228-232	5100	68.1	6.7	11.4		<0.03i	125
	HO HO				68.2	6.9	11.5			
259	(EtCHNCH ₂) ₂	$(+)-(.12H_{38}N_{3}O_{2}\cdot 0.25H_{2}O_{3})$	b. 132-134 (0.1)	67	60.9	12.1	11.8		2.0	>250i
	 				61.0	12.2	11.8			
96	(EtCII-NCII)	Mixed CidH20N00.	h. 135–138 (0.07)	37	64.6	12.4	10.8		0.12	ũ
; 1		opt.			64.2	12.4	10.8			
	HOCH ² Et	isomers								
27^{c}	(EtCH-NCH ₂) ₂	$(\pm) - C_{18} \Pi_{32} N_2 O_6$	m. 76–79	52^{bh}	58.0	8.7	7.5		< 0.06i	
					58.0	9.0	7.6			
~	AcOCH2 Ac			10000		t	1			
		meso-Ci8H32N2Oa	m. 112-113.5	87	08.0 57 8	x x	9 L 2		< 0.121	>2501
~~							-			

TABLE I (continued)

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29"	EtechNhchchi CHaoh Cha eachan	Mixed CuH2sN5O201460 opt. isomers	h. 149–1	150 (0.01)	69	59.3 59.6	12.0 11.8	12.6 12.6		<0.12i	250
307	CH20H CH20H (EtCHNHCH3)2CH2	meso-C ₁₁ H ₃₆ N ₂ O ₂	b. 130–1	170 (0.08)							
	CH2OH	meso-C11Hz6NzO2 · 2 HCl · Hz0	m. 139–1	142	28²	42.7 42.9	9.8 10.0	9.1 9.0	22.9 22.7	<0.06i	250
31 {]		(土)-C ₁₁ H ₂₀ N ₂ O ₂	b. 150-	170 (0.3)	×	60.5 60.5	12.0 11.8	12.8 12.6		<0.06i	09
32 ¹	EtCHNHCH1CH1NH-I-Pr CH1OH	(\pm) -C ₈ H ₂ N ₂ O·H ₂ O	b. 85–8(§ (0.01)	63	62.0 61.8	12.7 12.5	16.1 15.7		2.0	15
33 <i>m</i>	EtCH-NCH2CH2NH-i-Pr HOCH2 Et	(土)-C ₁₁ HzsN2O	b. 82-8	3 (0.03)	50	65.3 65.0	12.9 12.8	13.8 13.5		0.12	250
" 'T' points isomer	the boiling points (designat are corrected; designat s are isolated, the theor diamine base ^d Relativo	ed by b.) are uncorrected and ar ed by m. ^c Yields are based etical yield of each would be e antinvechacterial activity ² is	e subjec on the 50%.	t to the us total pos Yields of	ual ina sible d hydro	ccurae iamine chloric he dru	aies in ; thu les ar o diet	vacuu 18, wł e bas	um distil nere bot ed on t signific	lation. ^b All a h racemic an he alkylene o sant (4 days o	melting d <i>meso</i> lihalide

tested is indicated by < 0.03i, etc. which are calculated by dividing this dosage into the minimal effective dose of the standard. Testing² prolongation of survival time, taking N,N'-diisopropylethylenediamine dihydrochloride as unity. Inactivity at the highest dosage was carried out by Drs. J. P. Thomas and C. Baughn of these Laboratories using mice infected with H37R, strain of Mycobacterium tuber-^e The activity in vitro is the minimum concentration of the drug in mcg./ml. in agar which will cause 100% inhibition of a streak culture of avirulent Mycobacterium smeamatis (ATCC 607). In those cases (Compounds 1, 9 and 11) where Dr. Thomas used H37R_a strain for in vitro studies the inhibitory concentrations for each compound were approximately half those for M. smegmatis. Inactivity at highest concentration tested is designated by >250i. Testing was done by Miss M. Hauck and Mr. A. C. Dornbush of these Laboratories. ⁷ Prepared by method B. For compound 1 see ref. 3. ⁹ These compounds were purchased from Visco Chemical Co. ^h Prepared by method A with reaction time 0.3 to 1 hr. ⁴ The piperazine stereoisomers, compounds 14 and 15, were isolated as by-products in the preparation of compounds 8 and 9. They were contained in a high-boiling fraction from which the meso and racemic ethylenediamine ulosis.

TABLE I NOTES (Continued)

bases were removed by crystallization from a 1:1 chloroform, petroleum ether (90-100°) mixture. On acidification of the concentrated filtrate with ethanolic HCl the piperazine salts deposited and were separated due to the low solubility of the higher-melting meso isomer in aqueous ethanol. ^j Prepared by Mr. R. Zambrano and Dr. P. J. Kohlbrenner of these Laboratories by reaction of ethylenediamine with 1,2-butylene oxide. ^k The specific rotations of the optically active isomers in water (c = 2) are: (+)-base $[\alpha]^{25}D + 13.7^{\circ}$, (+)-dihydrochloride $[\alpha]^{25}D$ + 7.6; (-)-base $[\alpha]^{25}D$ - 16.1°, (-)-dihydrochloride $[\alpha]^{25}D$ - 7.5°. The m.p. of the (+)-monohydrochloride was 91.2-91.6°. The principal infrared bands, identical in the (+) and (-) bases, are: 3,310, 3,180, 2,990, 2,900, 1,475, 1,385, 1,374, 1,360, 1,228, 1,148, 1,095, 1,068, 1,055, 1,015, 990, 885, 846, 839, 816 and 768 cm⁻¹. The differences from the spectra of the meso and racemic isomers will be discussed in a later publication. ¹ Prepared by method A with reaction time of 2 to 4 hr. ^m Prepared by method A with reaction time of 18 to 20 hr. ⁿ The dihydrobromide salt of this compound was reported by J. S. Pierce and J. Wotiz, J. Am. Chem. Soc., 66, 879 (1944), m.p. 205-206°. ^o Prepared by method C. ^p Prepared by method D. ^q Prepared by method E. ^r Prepared by method F. ⁸ Recrystallized from EtOH-H₂O. ¹ Recrystallized from EtOH. ^u Recrystallized from MeOH. "Recrystallized from acetone. ^w Recrystallized from $^{\nu}$ Recrystallized from benzene. ^x Recrystallized from aqueous 1-propanol. MeOH-Me₂CO. ² Recrystallized from EtOH-Me₂CO. ^{aa} Compound 24 was best prepared by heating to reflux for 20 hr. a mixture of ethylene dibromide with two equivalents of o-aminophenol and two equivalents of sodium bicarbonate in 90% 1-butanol. The product crystallized on cooling and was recrystallized by dissolving in ethanol with dilute HCl, decolorizing and neutralizing with dilute ammonium hydroxide. ^{bb} Recrystallized from Et₂O-Me₂CO.

active than those with primary alkyl groups in the parent series. Of the hydroxylated secondary alkyl groups (Compounds 4, 5, 8, 9, 10, 11, 12, 13, 19, 20, 21, 22, 23, 29, 32, 33), only the (+)-1-hydroxy-2butyl (Compound 11) substituent gives appreciable activity, in clear contrast with its enantiomer (Compound 10), its higher or lower homologs (Compounds 4, 5, 19, 20, 21) or the isomeric 3- or 4-hydroxy-2-butyl (Compounds 12, 13) substituents. In the hydroxylated t-butyl compounds (Compounds 16, 17, 18) no detectable activity was encountered as opposed to the relatively high activity present in the *t*-butyl and *t*-amyl derivatives of the parent series. The introduction (by the Eschweiler-Clark modification of the Leuckart reaction) of two N-methyl groups into (\pm) -2,2'-(ethylenediimino)di-1-butanol (Compound 25) caused virtually no loss of activity in vivo while N-ethyl groups (Compound 26) cut activity 16-fold. In contrast, two N-methyl groups introduced into N,N'-diisopropylethylenediamine cut activity³ at least eightfold. Significantly, methylation of both compounds drastically reduced the activity in vitro (M. smeqmatis) which may indicate that the N-methyl groups of the hydroxylated diamine are being removed in vivo to generate activity (*M. tuberculosis*) due to the parent (Compound 9). The in vitro activity of the analogs, conveniently using a non-pathogenic organism, provided a means of sorting out analogs with high in vitro activity and poor in vivo activity. In such instances, a parenteral (daily subcutaneous) test² in mice served to differentiate between pharmacology unfavorable to oral administration and intrinsically poor activity.

Other substitution on the nitrogens of 2,2'-(ethylenediimino)-di-1butanol was detrimental to activity. The *meso* and racemic forms of the piperazine (Compounds 15, 14) and tetraacetyl (Compounds 27, 28) analogs of the parent compound were inactive. Lengthening of the alkylene chain to give 2,2'-(trimethylenediimino)-di-1-butanol (racemic and *meso* isomers, Compounds 31, 30) caused a loss of activity as also occurred in the N,N'-dialkyl series. On the other hand, N,N'-diisopropyl-1,2-propanediamine³ showed good activity whereas 2,2'-(propylenediimino)-di-1-butanol (Compound 29) did not. A possible reason for this contrast, indicated from molecular models⁸ of type IIIa chelate, may be the destabilizing interaction, in certain conformations, of the methyl (R') group with the ethyl (R) group in the 1-hydroxy-2-butyl substituent. No interaction occurs with the isopropyl substituent having the smaller methyl groups and the possibility of rotation around the C–N bond.

If the ability to form a chelate of type III or type I is a prerequisite for antituberculous activity, it is not in itself sufficient since the homologs, structural isomers and optical isomers of (+)-2,2'-(ethylenediimino)-di-1-butanol do not show appreciable activity. Also, (\pm) -C₂H₅CH(CH₂OH)NHCH₂CH₂NHCH(CH₃)₂, (Compound 32) was approximately as active as the racemic 2,2'-(ethylenediimino)di-1-butanol although it cannot form a chelate of type III, except by interaction with water. Of interest is the fact that a (+)-2-aminobutanol moiety is present in the few highly active compounds. It is possible that the ability to form a chelate of either type I or type III in combination with the ability of the compound or the chelate to fit a specific enzyme template may account for the structural and stereoisomeric selectivity in the antimycobacterial activity of these diamines. Work on analogs containing various other functional groups will be reported in additional papers in preparation.

Experimental

The following procedures were for the most part used on a 0.1 to 0.2 mole scale (8) Stewart-Briegleb models of La Pine and Co., Chicago, Ill. without attempting to obtain maximum yields. Where diastereomers were encountered, their separation was attempted both as base and hydrochloride salts, in some cases without success.

Method A. Alkylation of Amines with Alkyl Halide.—The reaction of ethylene dibromide (ethylene dichloride served equally well in case of compounds 8, 9, 10, 11, 14, 15, 18, 19 and 20, as did ethyleneglycol ditosylate with compounds 8 and 9) or trimethylene dibromide with the amino alcohols was in most cases run preferably without solvent using 4 moles of the aminoalcohol and heating to reflux for 30 min. to 2 hr. Similarly the reaction of isopropylaminoethyl chloride hydrochloride⁹ was run with 3-4 moles of (\pm) 2-aminobutanol (compound 32) or (\pm) 2-ethylamino-1-butanol¹⁰ (Cpd. 33) without solvent, refluxing for 2 and 18 hr. respectively. Isolation of the diamines by acidification of the reaction mixture with ethanolic HCl was the simplest method when it was possible to fractionally crystallize out the salt (Compounds 16 and 17). However, it usually was necessary to treat the reaction mixture with excess 10 N alkali and extract the amines for fractional distillation. Chloroform was the most desirable extraction solvent (Compounds 9, 10, 11, 14, 15, 19 and 20) but several times the extraction was complicated by the reaction of the chloroform and the amine in the presence of strong base if the temperature was slightly above 30°. Where possible, extraction was done with ethyl acetate (Compounds 30 and 31) or benzene (Compounds 26, 32 and 33). The alternate method of adding the calculated amount of sodium hydroxide or sodium methoxide, and precipitating the sodium halide with ethanol or propanol, usually was unsuccessful since substantial amounts of amine hydrohalide remained and caused decomposition during distillation. An example of this method which was applied to the preparation of the C^{14} labeled compound is given below.

(+)-2,2'-(Ethylenediimino)-di-1-butanol dihydrochloride (Compound 11).--A mixture of (+)-2-aminobutanol⁷ (9.0 g., 0.1 mole) and ethylene bromide (1.9 g., 0.01 mole) was heated at 100-115° in an oil bath for 25 min. To the cooled reaction mixture was added 1.29 g. (0.02 mole) of potassium hydroxide in 10 ml. of hot propanol. The precipitated salt was removed by filtration. Concentration of the filtrate to a gum followed by dissolving in 10 ml. of 1:1 acetone-propanol allowed an additional trace of KBr to be removed. To this filtrate was added 8.0 ml. of 7.8 N ethanolic hydrochloric acid. After cooling to -5° for 30 min., 2.12 g. of white crystals, m.p. 194.5-197.5°, were obtained. Recrystallization from 45 ml. of hot ethanol gave 1.89 g. (67%) of material, m.p. 201.8-202.6°.

Method B. Catalytic Reductive Alkylation.—Reductive alkylation of ethylenediamine with 2 moles of 4-hydroxy-2-butanone was run with platinum oxide in ethanol³ at $(2.1-3.2 \text{ kg./cm.}^2)$ and room temperature. An 80% uptake of hydrogen in 40 hr. gave Compound 13. When 3-hydroxy-2-butanone was used with the same catalyst and solvent, reduction was only 48% complete in 4 hr. at 35-110° and (63.3-91.4 kg./cm.²) to give Compound 12. In each case the products were isolated by distillation.

Method C. Lithium Aluminum Hydride Reduction.—Reduction of N,N'oxalyldivaline diethyl ester was run in ether using 5 moles of lithium aluminum

(9) Prepared from β-isopropylaminoethanol by reaction with thionyl chloride; m.p. 179.5-182°; A. C. Cope, H. R. Nace, W. R. Hatchard, W. H. Jones, M. A. Stakman, and R. B. Turner, J. Am. Chem. Soc., 71, 555 (1949).

(10) B.p. 177-180°, prepared by lithium aluminum hydride reduction of diacetyl 2aminobutanol (m.p. 77-78°); J. S. Pierce, J. M. Salsbury, W. W. Haden and L. H. Willis, J. Am. Chem. Soc., 64, 2885 (1942). hydride and refluxing for 3 hr. The mixture was treated with 10 N sodium hydroxide and the diamine extracted with ether. The crude extract gave a crystalline dihydrochloride (Compound 21).

Reduction of N-[1-(hydroxymethyl)-propyl]-2-[1-(hydroxymethyl)-propylamino]-propionamide (crude product from the reaction of excess 2-aminobutanol with α -chloropropionyl chloride) was run in tetrahydrofuran using 2 moles of lithium aluminum hydride. The mixture was refluxed for 16 hr. and then treated with aqueous sodium hydroxide according to the procedure of Amundsen and Nelson.¹¹ The solids were removed by filtration and the tetrahydrofuran filtrate distilled to give the diamine (Compound 29, a mixture of four racemates).

Method D. Alkylation With Epoxides.—The reaction of cyclohexene oxide (2.1 moles) with ethylenediamine at 140–160° for 1.5 hr. gave a solid mush which on trituration with ether left a white solid, m.p. 150–170°. This mixture of racemic and *meso* forms was heated with absolute ethanol (10 ml./g.) leaving the *meso* isomer (m.p. 196–208°) undissolved. Two additional washings with hot ethanol gave a constant melting point,¹² 205–210° (Compound 22). The alcoholic filtrates on concentration gave a solid which on recrystallization from benzene melted at 126–128°. When this base was dissolved in hydrochloric acid, and then treated with aqueous alkali, white crystals formed, m.p. 86.5–89° (lit.¹² m.p. 87–88° for the racemate). Recrystallization from benzene gave the higher-melting polymorph (Compound 23).

Method E. Leuckart Reductive Methylation.—Using the Eschweiler-Clark modification,¹³ (\pm)-2,2'-(ethylenediimino)-di-1-butanol was treated with 2.1 moles of formaldehyde and 7.5 moles of formic acid at reflux for 7 hr. The mixture was acidified with 3.6 moles of concd. hydrochloric acid, refluxed for 1.5 hr., concentrated to a gum, made alkaline with 10 N sodium hydroxide and extracted with benzene. The dried extract was distilled to yield the diamine (Compound 25).

Method F. Acetylation.—The meso and racemic forms of 2,2'-(ethylenediimino)-di-1-butanol were treated with excess (5 to 8 moles) of acetic anhydride with or without benzene. Volatiles were evaporated and the residue gave a crystalline solid on triturating with petroleum ether (90–100°). The meso product (Compound 28) was pure after filtering and washing with acetone. The racemic product (Compound 27) required two recrystallizations from a 5:1 benzene-petroleum, ether (90–100°) mixture and one from a 4:1 ether, acetone mixture to achieve analytical purity.

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⁽¹¹⁾ L. H. Amundsen and L. S. Nelson, J. Am. Chem. Soc., 73, 242 (1951).

⁽¹²⁾ M. Mousseron, R. Granger and G. Combes, Bull. Soc. Chim., 843 (1947), reported m.p. 203-204°.

⁽¹³⁾ M. L. Moore, "Org. Reactions," Vol. V, p. 302 (1949).