Circular Dichroism Studies of Aryl Diastereoisomers. 3.[1] Cupra A Spectra of Chloramphenicol Derivatives

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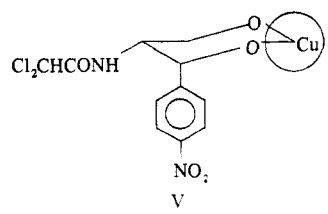
The use of Cupra A solutions for the measurement of CD spectra of aryl diastereosiomers in the chloramphenicol series is shown to be an especially valuable tool for determining absolute stereochemistry because alterations in the nature and position of electron-attracting and electron-releasing aryl substituents do not influence the spectra obtained sufficiently to confuse interpretation. The situation contrasts markedly with that resulting from the customary use of methanolic solutions. Cupra A spectra are presented for 24 chloramphenicol analogs, all of whose spectra can be interpreted satisfactorily in light of the absolute configuration of the solute.

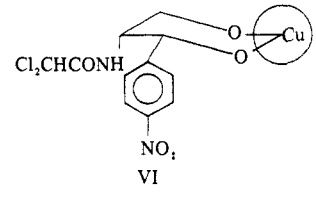
Measurement of optical rotations at the Na D line of cuprammonium solutions of glycols is well known to be useful for the assignment of the absolute configuration of carbohydrates.[2] Recently this technique has been applied effectively to the solution of stereochemical problems involving alicyclic 1,2-diols and 1,2-amino alcohols through the use of circular dichroism (CD) measurements.[3-5] Because of difficulties in finding a straightforward relationship using ordinary solvents between the sign of the ^1L_b transition and conformation-absolute configuration in an extended series of chloramphenicol-ephedrine analogs when electron-releasing and electron-attracting substituents are introduced into various positions in the aromatic ring,[1] we have applied the cuprammonium technique to these substances. Even though the chloramphenicols are quite flexible compared with the substances previously measured, it was anticipated that chelation would solve the rotamer distribution problem and that alteration of the chromophore to the copper d → d orbital transitions would reduce the electronic influence of the aromatic ring. It is now found that this device does provide a clear and unambiguous solution to the stereochemical problem. In these spectra the dominant influence is the spatial arrangement of side-chain atoms in the vicinity of the complexed ion and only the orientation, but not the electronic nature, of the aromatic ring affects the spectra.

Figure 1 records the CD spectra of the four diastereoisomeric chloramphenicols I-IV in Cupra A solution. (1*R*,2*R*)-Chloramphenicol (I) shows a positive band at about 650 nm, a negative band at about 520 nm, and a positive Cotton effect at 290 nm. (1*S*,2*S*)-Chloramphenicol (II), of course, has an enantiomeric spectrum. None of these bands are observable in the methanol spectra of these substances and none are seen in the Cupra A solvent blank. Thus, the bands must be due to chelate complex formation. The two enantiomeric erythro derivatives III and IV give spectra dramatically different in intensity from the threo analogs I and II in that they have essentially only a single high-intensity end absorption in the 350-nm region. It is quite possible that visible peaks are present but of intensities too low for accurate measurement. It is apparent from the spectra that 1*R* stereochemistry determines a positive Cotton effect in the 350-nm region regardless of the stereochemistry at C_2. The stereochemistry at C_2 can be assigned confidently from the intensity of the visible bands, with the 2*R* analogs of I having much more intense bands than the 2*S*. The opposite relationship holds for the enantiomeric series.

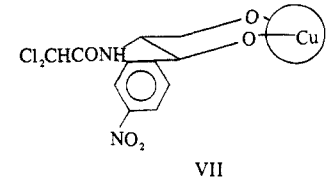
In contrast to the 1,2-glycols and amino alcohols whose curves have been published,[3-5] complexation with the chloramphenicols must be 1,3 and involve six-membered rings. The expected conformational equilibrium for the threo analogs I and II is illustrated in formulas V and VI where it will be seen that either the aryl ring or the dichloroacetamido function must be axial. No clear preference for V or VI is intuitively reasonable so an equilibrium involving substantial populations of both forms is anticipated. The erythro analogs III and IV are less ambiguous from a conformational standpoint (VII and VIII). Conformer VII has both bulky



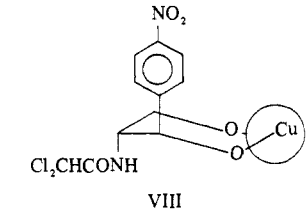
V



VI



VII



VIII

groups equatorially disposed and should produce a dominant effect on the spectrum. The higher intensity visible CD maxima in derivatives I and II (V and VI) suggest that conformer V, having an axial aryl group in close proximity to the metal atom, plays a very significant role in the spectrum regardless of the absolute population of V at equilibrium. The low intensity of the peaks in derivatives III and IV (VII and VIII) is probably a consequence of the equatorial disposition of both bulky groups and their corresponding distance from the central metal atom.

With the type spectra (I-IV) in hand, next examined were the spectra of the long series of differently substituted chloramphenicols which had proven so troublesome to interpret in earlier studies when methanol solvent was used.[1] The results are summarized in Table I where it will be seen that all derivatives having the 1*R*,2*R* stereochemistry give strong positive CD bands in the ultraviolet region. When the visible band is resolved into two oppositely signed peaks, the signs are the same as the *p*-NO_2 derivative. When only a single peak is found, it is always negative. Thus, the spectroscopic problem associated with assignment of absolute configuration in this series is solved.

The reason why some derivatives have their visible bands

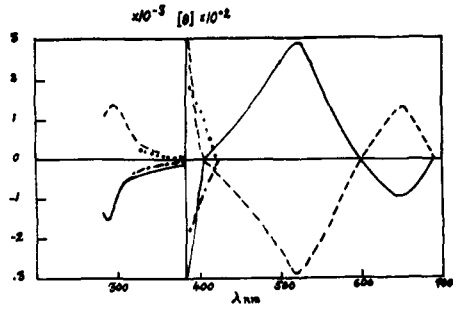


Figure 1. CD spectra of (1*R*,2*R*)-chloramphenicol (I) (- - -), (1*S*,2*S*)-chloramphenicol (II) (—), (1*S*,2*R*)-chloramphenicol (III) (- · - · -), and (1*R*,2*S*)-chloramphenicol (IV) (· · · ·) in Cupra A solution.

Table I. Circular Dichroism Prominances of Variously Substituted Chloramphenicol Derivatives in Cupra A Solution

Substance	Visible region peaks (400–700 nm)		Uv region peaks (200–400 nm)
I (1*R*,2*R*)	+ (600–700 nm)	– (400–600 nm)	+
II (1*S*,2*S*)	–	+	–
III (1*S*,2*R*)			–
IV (1*R*,2*S*)			+
IX (1*R*,2*R*)	+	–	+
X (1*R*,2*R*)	+	–	+
XI (1*R*,2*R*)	+	–	+
XII (1*S*,2*S*)	–	+	–
XIII (1*R*,2*R*)	–	–	+
XIV (1*R*,2*R*)	–		+
XV (1*R*,2*R*)	–		+
XVI (1*R*,2*R*)	–		+
XVII (1*S*,2*S*)	+		–
XVIII (1*R*,2*R*)	–		+
XIX (1*R*,2*R*)	+	–	+
XX (1*R*,2*R*)	–		+
XXI (1*R*,2*R*)	–		+
XXII (1*R*,2*R*)	–		+
XXIII (1*R*,2*R*)	–		+
XXIV (1*R*,2*R*)	–		+
XXV (1*R*,2*R*)	–		+
XXVI (1*R*,2*R*)	–		+
XXVIII (1*R*,2*R*)	–		+
XXVII (1*R*,2*R*)	+		–

split into two oppositely signed Cotton effects is not readily apparent. The side chain has the same constitution in each case so conformational inhomogeneity or asymmetric solvation seems unlikely explanations. If exciton coupling is responsible, it must be proceeding over a considerable distance. Fortunately, the effect produces constant signs when it appears and does not confuse interpretation.

In addition to the compounds included in the earlier methanol studies,[1] several additional analogs were measured in Cupra A solution to explore further the range of utility of this technique. For example, moving the *p*-NO_2 function to the meta or ortho positions alters the point symmetry group of the chromophore and spectroscopic differences in ordinary solvents are to be expected. Figure 2 records the methanol spectra of *m*-(1*R*,2*R*)- (IX) and *p*-(1*R*,2*R*)-chloramphenicol (X). The spectrum of IX correlates reasonably well with that of *p*-(1*R*,2*R*)-chloramphenicol (I) and assignment of stereochemistry would not be difficult using the ^1L_b band. The ^1L_a band is of such low intensity that it could not be used safely. The spectrum of X is rather more complex. The ^1L_b band is resolved into two bands of opposite sign. This effect can be ascribed either to conformational inhomogeneity, asymmetric solvation, or exciton coupling. Recent studies on similar compounds suggest

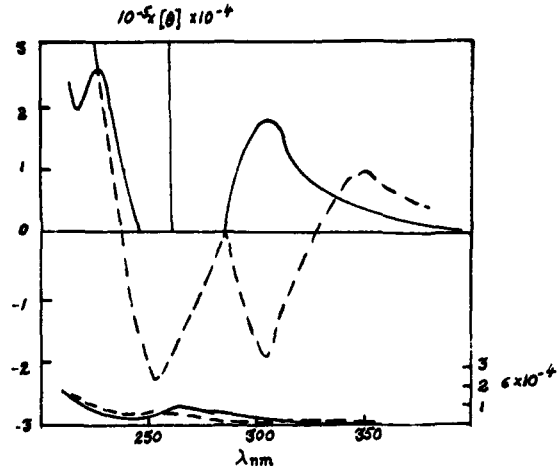


Figure 2. CD spectra in methanol of the meta (IX, —) and ortho (X, - - -) analogs of (1*R*,2*R*)-chloramphenicol.

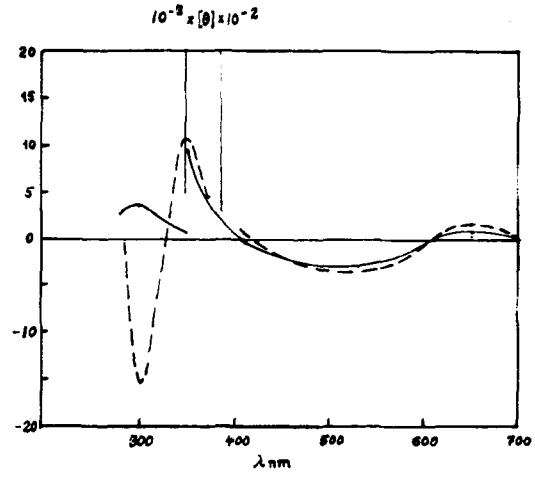
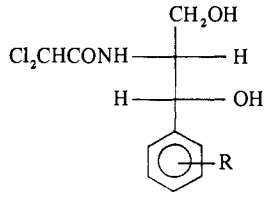


Figure 3. CD spectra of the meta (IX, —) and ortho (X, - - -) analogs of (1*R*,2*R*)-chloramphenicol in Cupra A solution.

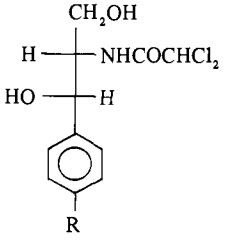
strongly that exciton coupling, due to twisting of the chromophore, is likely responsible. One cannot compare CD curves of compounds belonging to different spectroscopic classes, so noncorrelation of this curve with the others is not unexpected. There are not sufficient model substances to treat the methanol spectrum of X confidently on an empirical basis. Most gratifyingly, Figure 3 shows the Cupra A spectra of IX and X and shows conclusively that the spectroscopic complexities introduced into the methanol spectra by altering the positioning of the aryl substituent are not paralleled in the Cupra A spectra which are now closely similar to those of the para derivative.

Another derivative where the contrast between methanol and Cupra A is especially instructive is the *p*-phenylthioureido analog XXVIII. The methanol spectrum contains a number of peaks due to the complex transitions of the aryl substituent and it is difficult *a priori* to separate out the ^1L_b transition. The Cupra A spectrum, on the other hand, presents a perfectly normal appearance demonstrating very convincingly the simplifying effect of Cupra A in canceling out the influence of substituent changes in the now distant phenyl ring.

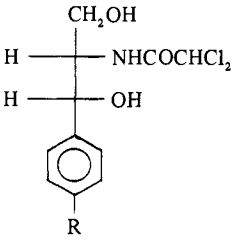
It is apparent by inspection that the Cupra A spectra of 1,3-diols present a different appearance from those of the 1,2-glycols previously published.[3-5] It was of some special interest, therefore, to examine the Cupra A spectrum of desdichloroacetyl-(1*R*,2*R*)-chloramphenicol (XXVII) in which



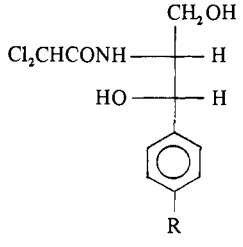
I, R = *p*-NO_2
IX, R = *m*-NO_2
X, R = *o*-NO_2
XI, R = H
XIII, R = *p*-CH_3CO
XIV, R = *p*-NH_2CO
XV, R = *p*-NH_2
XVI, R = *p*-C_6H_5
XVIII, R = *p*-(c-Pr)CONH
XIX, R = *p*-Br
XX, R = *p*-I
XXI, R = *p*-CN
XXII, R = *p*-CH_3SO_2
XXIII, R = *p*-NH_2CONH
XXIV, R = *p*-$C_6H_5NHCONH$
XXV, R = *p*-CH_3O_2C
XXVI, R = *p*-CH_3OCONH
XXVIII, R = *p*-C_6H_5-NHCSNH



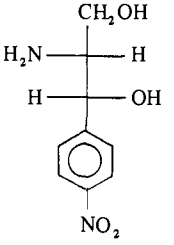
II, R = NO_2
XII, R = H
XVII, R = C_6H_5



III, R = NO_2

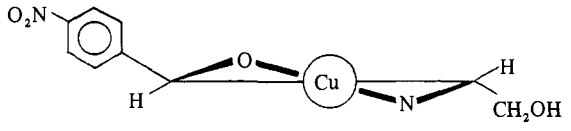


IV, R = NO_2



XXVII

derivative it is now possible to have 1,2-chelation. Indeed, Figure 4 shows the typical pattern of a δ chelate with a single visible maximum as might be expected, for, now, both bulky substituents should be equatorial XXIX. This



XXIX

finding serves to correlate the present study with earlier work[2-4] and to confirm the inference that 1,3-chelation occurs in the chloramphenicol series and accounts for the difference in spectra.

It will be noted, too, that the signs of the transitions are opposite to those of the chelated chloramphenicols which show only a single intense visible maximum. This precludes the possibility that the spectra of the latter are due to hydrolysis before chelation.

These findings allow one to assign stereochemistry with confidence in the aryl series when either 1,2- or 1,3-chelation is possible. This renders somewhat less pressing from a practical standpoint the necessity of rationalizing the aromatic transitions in more traditional solvents but does not in any way reduce the challenge and value of doing so. Our current work is devoted to this objective.

Experimental Section

Circular dichroism measurements (in deg cm^2/dmole) were performed at ambient temperature (cell compartment = 29°) on a

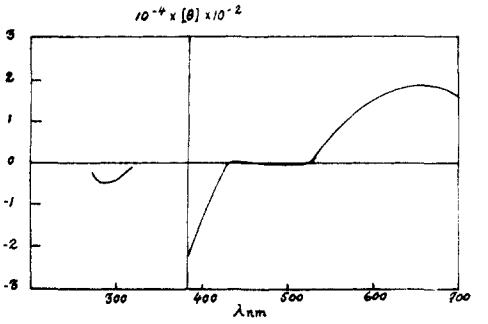


Figure 4. CD spectrum of desdichloroacetyl-(1*R*,2*R*)-chloramphenicol (XXVII) in Cupra A solution.

JASCO UV/ORD/5 instrument fitted with a CD attachment. Solutions were made in Cupra A[2] at the concentrations specified in Table I. The figures listed for intensity are approximate as the reaction between glycols and Cupra A is an equilibrium process.

D-*threo*-(1*R*,2*R*)-1-*p*-**Nitrophenyl-2-dichloroacetamido-1,3-propanediol (I)**: CD (*c* 1.042, Cupra A) $[\theta]_{690}$ 0, $[\theta]_{675}$ 72, $[\theta]_{660}$ 60, $[\theta]_{645}$ 128, $[\theta]_{602}$ 0, $[\theta]_{530}$ −285, $[\theta]_{410}$ 0, $[\theta]_{350}$ 750; CD (*c* 0.2084, Cupra A) $[\theta]_{350}$ 820, $[\theta]_{320}$ 1740; CD (*c* 0.042, Cupra A) $[\theta]_{340}$ 1535, $[\theta]_{290}$ 5120.

L-*threo*-(1*S*,2*S*)-1-*p*-**Nitrophenyl-2-dichloroacetamido-1,3-propanediol (II)**: CD (*c* 0.941, Cupra A) $[\theta]_{690}$ 0, $[\theta]_{640}$ −105, $[\theta]_{597}$ 0, $[\theta]_{530}$ 300, $[\theta]_{410}$ 0, $[\theta]_{350}$ −985*; CD (*c* 0.188, Cupra A) $[\theta]_{370}$ −540, $[\theta]_{320}$ −1925*; CD (*c* 0.188, Cupra A) $[\theta]_{340}$ −1130, $[\theta]_{290}$ −6230.

D-*erythro*-(1*S*,2*R*)-1-*p*-**Nitrophenyl-2-dichloroacetamido-1,3-propanediol (III)**: CD (*c* 1.028, Cupra A) $[\theta]_{420}$ 0, $[\theta]_{344}$ −790, $[\theta]_{335}$ −620*.

L-*erythro*-(1*R*,2*S*)-1-*p*-**Nitrophenyl-2-dichloroacetamido-1,3-propanediol (IV)**: CD (*c* 1.009, Cupra A) $[\theta]_{420}$ 0, $[\theta]_{340}$ 690, $[\theta]_{335}$ 655*.

D-*threo*-(1*R*,2*R*)-1-*m*-**Nitrophenyl-2-dichloroacetamido-1,3-propanediol (IX)**: uv ϵ_{263} 8110, ϵ_{219} 18,350* (MeOH); CD (*c* 0.213, MeOH) $[\theta]_{390}$ 0, $[\theta]_{330}$ 700, $[\theta]_{303}$ 1600, $[\theta]_{285}$ 0, $[\theta]_{250}$ 0*; CD (*c* 0.0213, MeOH) $[\theta]_{245}$ 0, $[\theta]_{224}$ 26,030, $[\theta]_{217}$ 19,525, $[\theta]_{214}$ 24,530; CD (*c* 1.040, Cupra A) $[\theta]_{700}$ 0, $[\theta]_{650}$ 82, $[\theta]_{608-600}$ 0, $[\theta]_{525}$ −300, $[\theta]_{410}$ −25, $[\theta]_{400}$ 0, $[\theta]_{350}$ 950*; CD (*c* 0.104, Cupra A) $[\theta]_{350}$ 925, $[\theta]_{300}$ 3640, $[\theta]_{290}$ 2975*.

D-*threo*-(1*R*,2*R*)-1-*o*-**Nitrophenyl-2-dichloroacetamido-1,3-propanediol (X)**: uv ϵ_{350} 1860 (br), ϵ_{250} 5330, ϵ_{210} 18,030* (MeOH); CD (*c* 0.209, MeOH) $[\theta]_{415}$ 0, $[\theta]_{346}$ 8550, $[\theta]_{325}$ 0, $[\theta]_{305}$ −18,620, $[\theta]_{284}$ 0; CD (*c* 0.0209, MeOH) $[\theta]_{326}$ 0, $[\theta]_{305}$ −18,880, $[\theta]_{283}$ 0, $[\theta]_{254}$ −22,960, $[\theta]_{237}$ 0, $[\theta]_{215}$ 62,760*; CD (*c* 1.116, Cupra A) $[\theta]_{700}$ 0, $[\theta]_{650}$ 172, $[\theta]_{596}$ 0, $[\theta]_{525}$ −372, $[\theta]_{428}$ 0, $[\theta]_{400}$ 43*; CD (*c* 0.045, Cupra A) $[\theta]_{375}$ 4780, $[\theta]_{350}$ 10,760, $[\theta]_{325}$ 0, $[\theta]_{305}$ −15,300, $[\theta]_{286}$ 0.

D-*threo*-(1*R*,2*R*)-1-**Phenyl-2-dichloroacetamido-1,3-propanediol (XI)**: CD (*c* 0.974, Cupra A) $[\theta]_{700}$ 0, $[\theta]_{650}$ 40, $[\theta]_{600}$ 0, $[\theta]_{540}$ −40, $[\theta]_{480-420}$ 0, $[\theta]_{285}$ 1525, $[\theta]_{282}$ 1410*.

L-*threo*-(1*S*,2*S*)-1-**Phenyl-2-dichloroacetamido-1,3-propanediol (XII)**: CD (*c* 1.150, Cupra A) $[\theta]_{700}$ 0, $[\theta]_{650}$ −50, $[\theta]_{600}$ 0, $[\theta]_{550}$ 60, $[\theta]_{470-450}$ 0, $[\theta]_{283}$ −1120, $[\theta]_{280}$ −1070*.

D-*threo*-(1*R*,2*R*)-1-*p*-**Acetophenyl-2-dichloroacetamido-1,3-propanediol (XIII)**: CD (*c* 1.06, Cupra A) $[\theta]_{700}$ −70, $[\theta]_{650}$ −33, $[\theta]_{545}$ −175, $[\theta]_{430-390}$ 0, $[\theta]_{305}$ 1930, $[\theta]_{300}$ 1495*; CD (*c* 0.106, Cupra A) $[\theta]_{350}$ 240, $[\theta]_{276}$ 5410, $[\theta]_{270}$ 2990*.

D-*threo*-(1*R*,2*R*)-1-*p*-**Carboxamidophenyl-2-dichloroacetamido-1,3-propanediol (XIV)**: CD (*c* 1.00, Cupra A) $[\theta]_{700}$ −106, $[\theta]_{490-385}$ 0, $[\theta]_{292}$ 2520, $[\theta]_{288}$ 2200*; CD (*c* 0.10, Cupra A) $[\theta]_{350}$ 50, $[\theta]_{269}$ 8550, $[\theta]_{253}$ 1160*.

D-*threo*-(1*R*,2*R*)-1-*p*-**Aminophenyl-2-dichloroacetamido-1,3-propanediol (XV)**: CD (*c* 1.12, Cupra A) $[\theta]_{700}$ −90, $[\theta]_{650}$ −115, $[\theta]_{540}$ 0, $[\theta]_{495}$ 35, $[\theta]_{460-400}$ 0, $[\theta]_{338}$ 300; CD (*c* 0.112, Cupra A) $[\theta]_{370}$ 50, $[\theta]_{300}$ 1750, $[\theta]_{290}$ 1490, $[\theta]_{270}$ 3720, $[\theta]_{252}$ 785*.

D-*threo*-(1*R*,2*R*)-1-*p*-**Phenylphenyl-2-dichloroacetamido-1,3-propanediol (XVI)**: CD (*c* 0.503, Cupra A) $[\theta]_{700}$ −45, $[\theta]_{650}$ −115, $[\theta]_{525-430}$ 0, $[\theta]_{300}$ 1900.

L-*threo*-(1*S*,2*S*)-1-*p*-**Phenylphenyl-2-dichloroacetamido-1,3-propanediol (XVII)**. The Cupra A spectrum was run qualitatively due to lack of sample. The peaks, qualitatively, occurred at the same wavelength and had the anticipated opposite sign to those of its enantiomer XVI.

D-*threo*-(1*R*,2*R*)-1-*p*-**Cyclopropylformamidophenyl-2-dichloroacetamido-1,3-propanediol (XVIII)**: CD (*c* 0.926, Cupra A) $[\theta]_{700}$

-50, $[\theta]_{650}$ -95, $[\theta]_{595-475}$ 0, $[\theta]_{290}$ 1650.

D-*threo*-(1*R*,2*R*)-1-*p*-**Bromophenyl-2-dichloroacetamido-1,3-propanediol (XIX)**: CD (*c* 1.042, Cupra A) $[\theta]_{700}$ 10, $[\theta]_{650}$ 90, $[\theta]_{595}$ 0, $[\theta]_{530}$ -100, $[\theta]_{470-450}$ 0, $[\theta]_{283}$ 1440, $[\theta]_{278}$ 1200*.

D-*threo*-(1*R*,2*R*)-1-*p*-**Iodophenyl-2-dichloroacetamido-1,3-propanediol (XX)**: CD (*c* 1.02, Cupra A) $[\theta]_{700}$ -150, $[\theta]_{640}$ -205, $[\theta]_{530-510}$ 0, $[\theta]_{480}$ 44, $[\theta]_{450-390}$ 0, $[\theta]_{297}$ 2425, $[\theta]_{288}$ 1625*; CD (*c* 0.102, Cupra A) $[\theta]_{350}$ 160, $[\theta]_{269}$ 7465, $[\theta]_{247}$ 560*.

D-*threo*-(1*R*,2*R*)-1-*p*-**Cyanophenyl-2-dichloroacetamido-1,3-propanediol (XXI)**: CD (*c* 1.09, Cupra A) $[\theta]_{700}$ -165, $[\theta]_{650}$ -200, $[\theta]_{515}$ 0, $[\theta]_{475}$ 25, $[\theta]_{430-390}$ 0, $[\theta]_{296}$ 2070, $[\theta]_{290}$ 1470*; CD (*c* 0.109, Cupra A) $[\theta]_{330}$ 240, $[\theta]_{277}$ 8065, $[\theta]_{272}$ 5700*.

D-*threo*-(1*R*,2*R*)-1-*p*-**Methylsulfonylphenyl-2-dichloroacetamido-1,3-propanediol (XXII)**: CD (*c* 1.10, Cupra A) $[\theta]_{700}$ -140, $[\theta]_{625}$ -200, $[\theta]_{515-370}$ 0, $[\theta]_{292}$ 2335, $[\theta]_{288}$ 2000*; CD (*c* 0.110, Cupra A) $[\theta]_{325}$ 500, $[\theta]_{266}$ 9550, $[\theta]_{248}$ 1290.

D-*threo*-(1*R*,2*R*)-1-*p*-**Ureidophenyl-2-dichloroacetamido-1,3-propanediol (XXIII)**: CD (*c* 1.07, Cupra A) $[\theta]_{700}$ -125, $[\theta]_{640}$ -140, $[\theta]_{500-470}$ 0, $[\theta]_{440}$ 20, $[\theta]_{420-410}$ 0, $[\theta]_{400}$ 13, $[\theta]_{305}$ 1620, $[\theta]_{298}$ 1370*; CD (*c* 0.107, Cupra A) $[\theta]_{350}$ 125, $[\theta]_{268}$ 6975, $[\theta]_{255}$ 2735.

D-*threo*-(1*R*,2*R*)-1-*p*-**Phenylureidophenyl-2-dichloroacetamido-1,3-propanediol (XXIV)**: CD (*c* 0.900, Cupra A) $[\theta]_{700}$ -215, $[\theta]_{625}$ -300, $[\theta]_{500}$ -125, $[\theta]_{400}$ -170, $[\theta]_{348}$ 0, $[\theta]_{320}$ 470, $[\theta]_{310}$ 210*; CD (*c* 0.090, Cupra A) $[\theta]_{350}$ 0, $[\theta]_{278}$ 7970, $[\theta]_{278}$ 3940.

D-*threo* (1*R*,2*R*)-1-*p*-**Carbomethoxyphenyl-2-dichloroacetamido-1,3-propanediol (XXV)**: CD (*c* 1.10, Cupra A) $[\theta]_{700}$ -120, $[\theta]_{625}$ -150, $[\theta]_{500-390}$ 0, $[\theta]_{295}$ 2245, $[\theta]_{292}$ 1925*; CD (*c* 0.11, Cupra A) $[\theta]_{350}$ 120, $[\theta]_{270}$ 7950, $[\theta]_{253}$ 2500*.

D-*threo*-(1*R*,2*R*)-1-*p*-**Methoxycarbonylaminophenyl-2-dichloroacetamido-1,3-propanediol (XXVI)**: CD (*c* 1.04, Cupra A) $[\theta]_{700}$ -120, $[\theta]_{650}$ -140, $[\theta]_{508}$ 0, $[\theta]_{485-475}$ 20, $[\theta]_{450-390}$ 0, $[\theta]_{300}$ 1925, $[\theta]_{295}$ 1550*; CD (*c* 0.104, Cupra A) $[\theta]_{300}$ 2600, $[\theta]_{269}$ 6280, $[\theta]_{254}$ 2465.

D-*threo*-(1*R*,2*R*)-1-*p*-**Nitrophenyl-2-amino-1,3-propanediol (XXVII)**: CD (*c* 1.13, Cupra A) $[\theta]_{700}$ +175, $[\theta]_{650}$ +190, $[\theta]_{530}$ 0, $[\theta]_{430}$ 0, $[\theta]_{360}$ -505*; CD (*c* 0.113, Cupra A) $[\theta]_{310}$ -3035*; CD (*c* 0.023, Cupra A) $[\theta]_{285}$ -4340, $[\theta]_{275}$ -2890.

D-*threo*-(1*R*,2*R*)-1-*p*-**Phenylthioureidophenyl-2-dichloroacetamido-1,3-propanediol (XXVIII)**. This material was incompletely soluble in Cupra A. It gave a broad negative band at about 650 nm and a strong positive peak at 280 nm: CD (*c* 0.479, methanol) $[\theta]_{350}$ 0, $[\theta]_{325}$ +170, $[\theta]_{322}$ +97*; CD (*c* 0.192, methanol) $[\theta]_{320}$ -304, $[\theta]_{317}$ -364, $[\theta]_{310}$ 0, $[\theta]_{305}$ +668*; CD (*c* 0.038, methanol) $[\theta]_{290}$ +607, $[\theta]_{285}$ -1820, $[\theta]_{255}$ 0, $[\theta]_{222}$ +12,135.

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Inhibition of Phenylethanolamine *N*-Methyltransferase by Benzylamines. 1. Structure–Activity Relationships

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In searching for inhibitors of phenylethanolamine *N*-methyltransferase (PNMT) as potentially useful pharmacologic agents for reducing epinephrine biosynthesis, we have found that the enzyme from rabbit adrenals is strongly inhibited by benzylamines. Among compounds with various ring substitutions, the 2,3-dichloro and 2-chloro-3-trifluoromethyl compounds were the most active inhibitors. Excellent correlation of inhibitor potency with Hansch π values and Hammett σ values associated with the aromatic substituent was obtained within single-substituent subseries. An α-methyl group in some cases reduced inhibitor activity but in other cases increased the inhibitor activity, perhaps through a steric influence. Other α-alkyl groups reduced inhibitor potency. Substitution on the nitrogen generally reduced inhibitor activity. Lengthening the alkyl chain connecting the phenyl group to the amine decreased inhibitor activity. PNMT from human or rat adrenal glands was inhibited by several of the benzylamines to a degree paralleling the inhibition of the rabbit enzyme.

Phenylethanolamine *N*-methyltransferase (PNMT) is chiefly localized in the adrenal medulla, where it has the physiological role of converting norepinephrine to epinephrine.[1] Tyrosine hydroxylase, the first and apparently rate-limiting enzyme in catecholamine biosynthesis, has often been considered an ideal target for inhibition of catecholamine production. However, inhibition of that enzyme (or of the decarboxylase or dopamine β-hydroxylase) would interfere with norepinephrine formation in the brain and in the peripheral sympathetic nervous system as well as in the chromaffin cells of the adrenal medulla. PNMT, on the other hand, represents a site for enzyme inhibition that would directly suppress formation only of the adrenal medullary hormone, epinephrine, without interfering with enzymic steps in norepinephrine formation in the adrenal gland or in the sympathetic nervous system. No information is available concerning the pharmacologic effects of inhibiting PNMT, and relatively few compounds are known to inhibit the enzyme even *in vitro*.

Previously studied inhibitors include phenylethylamines,[2-4] sulfhydryl-binding agents,[1,5,6] aminobenzimidazoles[7] and other substituted imidazoles,[8] and a few miscellaneous compounds.[3] We report here that benzylamines are potent competitive inhibitors of PNMT. Since these compounds can be viewed as structural analogs of PNMT substrates, it is not surprising that they should inhibit the enzyme competitively; however, the high degree of potency of the benzylamines as inhibitors was unexpected.

Inhibition by Benzylamines with Ring Substitutions. Table I shows the degree of PNMT inhibition by 28 compounds in the benzylamine series. Depending upon the substitution on the aromatic ring, the potency of the inhibitors covered a range of at least 10,000-fold. All but one of the compounds were better inhibitors than benzylamine itself. Seven of the compounds were more potent inhibitors than any of a group of amphetamines that we had previously reported to inhibit PNMT.[4]

We attempted to correlate structure and activity by using