Labilization of C—N Linkages by Dibenzocyclohepten-5-yl Ring System

H. V. MAULDING ˣ, A. F. MICHAELIS, and J. P. NAZARENO

Abstract □ The hydrolysis of several 5-aminodibenzo[*a,d*]cycloheptenes is discussed. The relative reactivity of the various compounds was closely related to the amino substituents at position 5 of the tricyclic nucleus. Both secondary and tertiary amines were prepared. They showed instability in aqueous solution below the pKa with invariant velocity constants at pH 0.15–7.0. This is indicative of spontaneous decomposition of the molecule *via* a carbonium ion or through the kinetically equivalent solvent attack on C_5 of the dibenzocycloheptene ring system. The fully aromatic ring system leads to a two magnitude enhancement of the velocity constants as compared to those of the corresponding 10,11-dihydrodibenzo[*a,d*]cycloheptenes. The cycloheptene nucleus is unstable when the adjacent nitrogen is cationic. When positively charged, the compounds undergo decomposition by nucleophilic attack of hydroxylic oxygen either on a carbonium-ion-like substrate or the protonated parent compound. The rate law may be written as $k_{obs} = k_0\ [H^+]/([H^+] + K_a)$. These results indicate cleavage of the C—N bond to be S_N1 in character. Degradation was primarily to the bis-ether by way of the 5-hydroxy intermediate, with evidence of traces of the ketone. Kinetic analyses were by the acid–dye procedure, utilizing methyl orange as the anionic component. This method is specific for the reactions involved and yields first-order plots showing linearity through several half-lives.

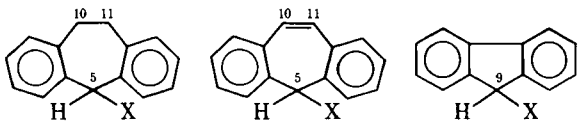
Keyphrases □ 5-Aminodibenzo[*a,d*]cycloheptenes—hydrolysis rates, compared to cycloheptanes, effect of 5-amino substituents □ Dibenzo[*a,d*]cycloheptenes—hydrolysis rates compared to cycloheptanes, effect of 5-amino substituents □ Hydrolysis—dibenzo[*a,d*]cycloheptenes, effect of 5-amino substituents

Dibenzo[*a,d*]cycloheptenes and their 10,11-dihydro analogs have served as the nucleus for numerous medicinal agents and possess an isosteric relationship with the phenothiazines (1). Dibenzo[*a,d*]cycloheptanes (I) exhibit antihistaminic activity (2, 3) while the analogous dibenzocycloheptenes (II) are active as anticonvulsants (4–6).

It was previously reported (7) that 5-aminodibenzo[*a,d*]cycloheptanes (I) hydrolyze in aqueous solutions at pH values below the pKa, *i.e.*, where an appreciable fraction of the amine is protonated. The intriguing feature of a highly labile carbon–nitrogen bond at C_5 (Structures I and II) was further investigated in this work. The effect of unsaturation in the seven-membered ring was examined quantitatively.

Several substituted 9-aminofluorenes (III) were studied with no indication of instability under the conditions utilized (8).

Molecules of Structure II show approximately a 100-fold increase in reactivity in aqueous acid over the 10,11-dihydro analogs (I). The products of the transformation are primarily the bis-ether and some alcoholic intermediate, with small amounts of ketone probably formed by air oxidation of the alcohol.



X = mono- or disubstituted aliphatic or cyclic amine

I II III

EXPERIMENTAL

Preparation of Compounds—The compounds prepared are listed in Tables I and II. The general procedures are illustrated with specific examples.

*5-Chloro-5*H*-dibenzo[*a,d*]cycloheptene*—A solution of 5-hydroxy-5*H*-dibenzo[*a,d*]cycloheptene[1] (5 g, 0.024 mole) was prepared in toluene (44 ml, dried over phosphorus pentoxide), with stirring, in a 100-ml round-bottom flask. The red material accumulating in the flask was removed by decantation of the solution into a second flask. Then thionyl chloride (2.08 ml) was added dropwise to the solution and the solution was cooled in an ice bath. The contents were allowed to come to room temperature for 30 min. The solvent was removed *in vacuo*, with the chloride being utilized without further purification.

*5-(1-Morpholino)-5*H*-dibenzo[*a,d*]cycloheptene (Compound 7, Table I)*—Solid 5-chloro-5*H*-dibenzo[*a,d*]cycloheptene (3.4 g) was added with stirring to a solution of morpholine (30 ml). After 15 min, the solution was heated on a steam bath for 30 min, followed by removal of solvent *in vacuo*. The semisolid residue was treated with 50 ml of 0.1 *N* NaOH. The basic solution was taken up in a separator and extracted with 2 × 50 ml of ether.

The ether extract was shaken out with 2 × 50 ml of distilled water and then extracted with 3 × 30-ml portions of 0.1 *N* HCl. The acid solution was neutralized with concentrated ammonia, with the resultant solid being extracted into ether and dried over anhydrous sodium sulfate. The ether was evaporated and the material was crystallized from methanol, yielding 1.9 g, 45%, mp 116–118° [lit. (9) mp 116–118°].

Hydrochlorides—These salts were prepared by dropwise addition of ethereal hydrochloric acid to an ethereal solution of amine. The white precipitate was collected and then recrystallized from methanol–ether (Table I).

9-Pyrrolidinofluorene (Compound 3, Table II)—A mixture of 2-chlorofluorene[1] (10 g, 0.05 mole) and pyrrolidine (3.5 g, 0.05 mole) was refluxed in 50 ml of benzene overnight. The solvent was removed under reduced pressure. The resultant mass was treated with 50 ml of 0.1 *N* NaOH followed by three 50-ml portions of distilled water. The solid was dissolved in methanol with heating and crystallized once from hot methanol, yielding 5.2 g, mp 86–90°.

9-Pyrrolidinofluorene Methiodide (Compound 4, Table II)—Two hundred milligrams of 9-pyrrolidinofluorene, 10 ml of methyl iodide, and 25 ml of acetonitrile were heated overnight at 50° (oil bath). The solvents were removed and the resultant material was crystallized from methanol–ether, yielding 112 mg, mp 191–192° dec.

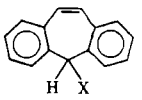
Solubility Analysis—*Method A*—Fifty milligrams of 5-pyrrolidinodibenzocycloheptene (Compound 5, Table I) was placed in a stoppered glass vial with the addition of 10 ml of buffer solution. Samples were turned at 20 rpm in a constant-temperature water bath at 23.6 ± 0.1°. Five-milliliter samples were withdrawn by filter pipet and monitored in the UV at 290 nm.

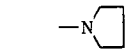
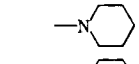
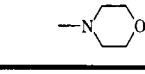
Method B—Ten milligrams of the propyl compound (Compound 3, Table I) was dissolved in 1 ml of water. Nine milliliters of buffer was added followed after 1 hr by filtration. The filtrate was monitored at 290 nm, and the pH was measured[2].

TLC of 5-Aminodibenzocycloheptenes and 9-Aminofluo-

[1] Aldrich Chemicals, Milwaukee, Wis.
[2] Metrohm pH meter.

Table I—Physical Constants of 5-Aminodibenzocycloheptenes



Compound	X	Melting Point	Yield, %	Molecular Weight	Molar Absorptivity, $\epsilon \times 10^{3}$[a]
1	$-N(C_2H_5)_2 \cdot HCl$	180–184°	32[b]	299.9	25.2
2	$-NH(CH_2CH{=}CH_2) \cdot HCl$	192–194°	20[b]	283.8	20.5
3	$-NH(CH_2CH_2CH_3) \cdot HCl$	115–117°	22[b]	285.8	26.7
4	$-NCH_3(CH_2CH_2OH)$	105–106°	18[c]	263.3	24.7
5		130–131°	54[d]	261.4	26.8
6		115–117°	39[d]	273.4	26.9
7		116–118°	45[d]	275.4	—[e]

[a] Absorptivity measured by employing a 4-ml sample (0.25–0.05 mg/ml), buffering at pH 3.44, and adding 25 ml of methyl orange solution, followed by extraction into 100 ml of chloroform. Plots of absorbance *versus* concentration then were prepared from these data. The ϵ values were calculated from moles of amine and absorbance of colored complex indicative of methyl orange. [b] Recrystallized from methanol–ether, Ref. 9. [c] Recrystallized from aqueous methanol. [d] Recrystallized from methanol, Ref. 9. [e] No satisfactory formation of colored complex.

renes (Tables I and II)—The compounds in Table I were chromatographed, using 10 μl of a 1–2% solution. Several solvent systems on silica gel GF[3] plates were used with a development distance of 15 cm. These are listed as follows (system, table, compound, and R_f): chloroform–methanol (98:2), I, 1, 0.15; chloroform–methanol (98:2), I, 5, 0.4; chloroform–methanol (98:2), I, 4, 0.2; chloroform–methanol (98:2), I, 6, 0.9; chloroform, I, 6, 0.45; chloroform–methanol (90:10), I, 1, 0.65; chloroform–methanol (90:10), I, 2, 0.95; chloroform–methanol (90:10), I, 4, 0.75; chloroform–methanol (90:10), I, 5, 0.85; chloroform, II, 1, 0.5; chloroform, II, 2, 0.4; and chloroform, II, 3, 0.2. The amines could be visualized by short-wave UV light (255 nm) as well as by means of Dragendorff reagents or bromcresol purple (15 mg/100 ml of ethanol).

Solution Preparation and Solvents—The pH 3.44 citrate buffer and methyl orange solution were prepared as previously stated (7).

Other solvents and chemicals were of reagent quality and were used without further treatment. Buffers were made to ionic strength of 0.2 except where specified.

Kinetic Procedures—A solution containing 25 mg of each compound was placed in 100 ml of 0.1 *N* HCl in volumetric flasks previously equilibrated at the specified temperatures, giving about 10^{-3} *M* solutions. Four-milliliter aliquots of the acidic solution were periodically withdrawn and neutralized with 0.1 *N* NaOH (4 ml) followed by 4 ml of pH 3.44 citrate buffer and 25 ml of methyl orange solution.

The aqueous layer was extracted three times with 30-ml portions of chloroform in a separator, and the chloroform solutions were collected and diluted to 100 ml in a volumetric flask. The absorption spectra of the yellow-colored complexes were recorded on a spectrophotometer over the 350–500-nm range.

Other kinetic procedures were carried out by the same analytical method after adjusting the pH of the aliquot drawn from the reaction flask to 3.44 prior to addition of methyl orange indicator.

The reaction products gave no color attributable to ion-pair formation with methyl orange when extracted using 10^{-3} *M* concentrations. Additionally, the intensity of the colorimetric analysis was not altered when the by-products were mixed at 10^{-3} *M* with solutions of the intact molecules.

Preparation and Properties of Reaction Products—*5-Hydroxydibenzo[a,d]cycloheptene (IV)*—One hundred milligrams of 5-morpholinodibenzo[*a,d*]cycloheptene (Compound 6, Table I) was heated for 24 hr at 23.6° in 100 ml of 0.1 *N* HCl. The white solid was filtered, dried, and streaked (in 0.5 ml of chloroform) on preparative silica gel GF plates[3] with development in chloroform. The spot corresponding to alcohol (R_f 0.3) was scraped off and eluted with chloroform, followed by filtration and evaporation, yielding 10 mg of a white powder, mp 121–123°, corresponding to the authentic alcohol[1], mp 122.5–124°. Mixed melting points of the two compounds resulted in no depression.

The UV spectrum was the same as that of the authentic alcohol. TLC of both the authentic alcohol and the product isolated in: (*a*) chloroform–diethylamine (99:1), (*b*) alcohol USP, and (*c*) ether–petroleum ether–methanol (20:80:1) gave identical R_f values of 0.3, 0.5, and 0.85, respectively.

Anal.—Calc. for $C_{30}H_{22}O$: C, 86.5; H, 5.8. Found: C, 86.4; H, 5.7.

Bis(5H-dibenzo[a,d]cyclohepten-5-yl) Ether (V)—One hundred milligrams of the morpholinodibenzocycloheptene (Compound 6, Table I) was heated at 80° in 100 ml of 0.1 *N* HCl for 24 hr. The white precipitate was filtered and dried, yielding 41 mg (39%), mp 202–208°. Washing with cold petroleum ether removed contaminating ketone and alcohol as ascertained by TLC in chloroform–diethylamine (99:1), with the resultant product melting at 208–211° [lit. (10) mp 210–212°].

TLC in chloroform showed traces of alcohol and ketone. Mass spectroscopy showed a molecular ion at 406, giving two fragments at $M^+ = 211$ and 195.

Anal.—Calc. for $C_{30}H_{22}O$: C, 90.4; H, 5.6. Found: C, 90.1; H, 5.5.

5H-Dibenzo[a,d]cyclohepten-5-one (VI)—Degraded solutions contained a spot on TLC attributable to the ketone. This compound was not identified *per se* but may be present as a result of air oxidation of the alcohol. Solutions containing the decomposition products of the amine exhibited fluorescent spots of identical R_f to the authentic ketone[1] when subjected to TLC in the solvent systems previously listed as applicable to the alcohol (IV).

RESULTS AND DISCUSSION

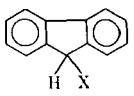
Several 5-aminodibenzo[*a,d*]cycloheptanes were recently reported to undergo cleavage at the C—N bond in aqueous acid by a route that may be visualized as proceeding *via* a carbonium ion or a kinetically equivalent mechanism (7). From the standpoint of resonance-stabilized structures, these 5-aminodibenzocycloheptanes (I) allow spreading of the positive charge over seven carbon atoms, presumably leading to the observed high reactivity. The 5-aminodibenzocycloheptenes (II) should be expected to be even more unstable in aqueous media due to the possibility of charge distribution over nine carbon atoms—the entire ring system—of the tricyclic skeleton, the center of which is composed of a potential tropylium ion.

Tropylium ions are among the most stable carbonium ions yet prepared. This longevity is partially due to distribution of six π-electrons evenly over the π-orbitals comprising the heptagonal ring, thus fulfilling the criteria for aromaticity (11, 12). More stable cations are generated when a number of resonance structures can be drawn indicative of distribution of the positive charge over several atoms rather than its confinement to a single atom.

Preparation of Compounds—Amines involved in this investigation were prepared from literature methods (6). The sequence

[3] Analtech, Newark, Del.

Table II—Physical Constants of 9-Aminofluorenes



Compound	X	Melting Point	Yield, %	Molecular Weight	Molar Absorptivity, $\epsilon \times 10^{3a}$	Analysis, % Calc.	Found
1		86–90°	44[b]	235.3	30.0	C 86.8 H 7.3 N 6.0	86.4 7.7 5.9
2		92–96°	21[b]	247.3	30.2	C 87.4 H 6.9 N 5.7	87.1 7.0 5.5
3		145–149°	37[b]	251.3	—[c]	C 81.2 H 6.8 N 5.6	81.7 6.8 5.6
4		191–192°	47[d]	377.3	27.3	C 57.3 H 5.4 N 3.7 I 33.6	57.4 5.5 3.5 33.0

[a] Absorptivity was measured by employing a 4-ml sample (0.25–0.05 mg/ml), buffering at pH 3.44, and adding 25 ml of methyl orange solution, followed by extraction into 100 ml of chloroform. Plots of absorbance *versus* concentration were then prepared from these data. The absorbance of the colored complex (indicative of methyl orange) was plotted *versus* the concentration of the amine (moles per liter) to determine ϵ. [b] Recrystallized from methanol. [c] No complex formation. [d] Recrystallized from methanol–ether.

consisted of conversion of the 5-hydroxy analog[1] into the chloride on treatment with equimolar thionyl chloride in toluene followed by amination with the appropriate primary or secondary amine. The 5-aminodibenzocycloheptenes (II) were then crystallized or converted to the hydrochlorides for further utilization. The compounds studied along with pertinent physical data are listed in Table I.

Table II gives some 9-aminofluorenes prepared for solvolytic investigation.

Evaluation of Rate Constants—Decomposition of the various 5-aminodibenzocycloheptenes (II) in aqueous solution (pH 7 and below) was monitored by colorimetry using the acid–dye method with methyl orange as the anionic component (7, 13). Complexes were of the amines (Tables I and II) and methyl orange (1:1). The molecularity was determined by extraction of a fixed quantity of amine in pH 3.44 buffer with increasing amounts of methyl orange indicator (7). Small discrepancies from the equimolar ratio were the result of volatile impurities—likely water—in the methyl orange indicator. Figure 1 represents a typical plot for ascertaining the molecularity of the complex.

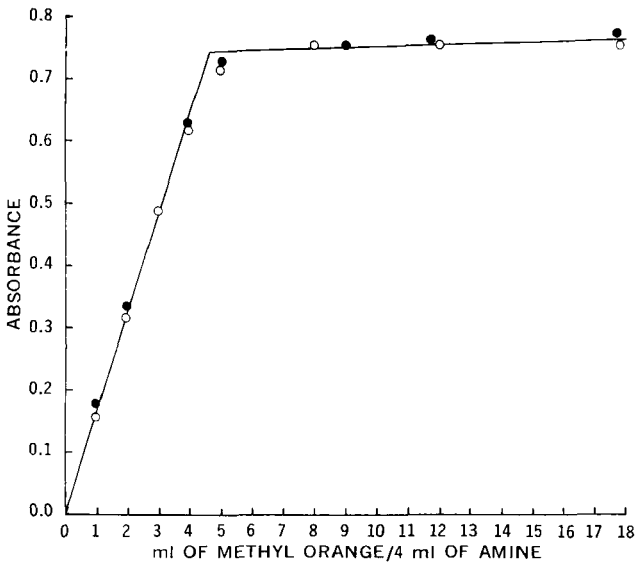
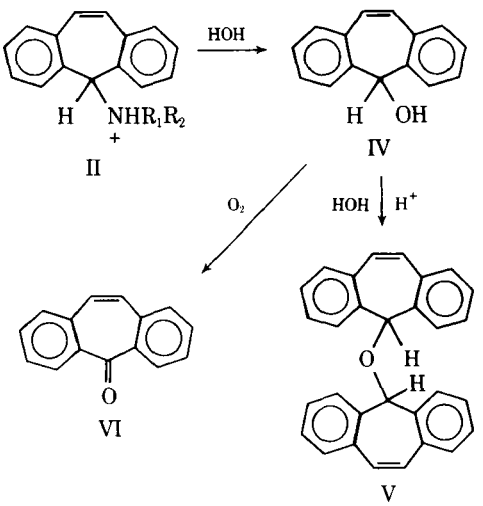


Figure 1—*Determination of the molecularity of the 5-pyrrolidinodibenzocycloheptene complex (Compound 5, Table I) by means of adding increasing volumes of 7.6 × 10⁻⁴ M methyl orange indicator solution to 4-ml portions of 7.6 × 10⁻⁴ M amine in pH 3.44 buffer. Point of intersection of the lines is indicative of the ml of methyl orange/4 ml of equimolar amine. The value is a little greater than 4 ml of methyl orange, which may be attributed to volatile impurities (possibly water) found in the reagent methyl orange. Black and white circles represent two different experiments.*

The principal products of the solvolysis in water (Scheme I) were the alcohol (IV), the bis-ether (V), and the ketone (VI). The allyl and propyl derivatives (Compounds 2 and 3, Table I) gave a fourth substance, which was separated by TLC but not identified. The transformations shown in Scheme I are analogous to those previously reported for the 10,11-dihydro analogs (7). At lower temperatures the alcohol predominates, while at 70° and above the ether is the primary product.

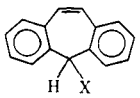
Scheme I illustrates the general reaction sequence, although the formation of the ether (V) has not been proven to go entirely through the alcohol. Some ether could conceivably result from reaction of the amine (II) with the alcohol (7).

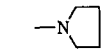
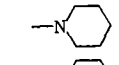
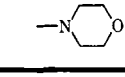
The ketone (VI) was present in trace amounts, possibly due to air oxidation of the alcohol (IV), while the latter serves as an intermediate. None of the materials formed on hydrolysis (IV, V, and VI) interferes with the analytical procedure when mixed with reactant amines in equimolar concentrations.



Scheme I

Table III—Substituent Effect of Substituted Amino Group on Rate Constants for Hydrolysis of 5-Aminodibenzo[*a*,*d*]cycloheptenes, about 8 × 10^{-4} *M*[a], in 0.1 *N* HCl



Compound	X	k_{obs}, hr^{-1}[b] 80°	70°	60°	50°
1	$-N(C_2H_5)_2 \cdot HCl$	(45.0)[c]	(10.3)[c]	2.5[d]	0.53
2	$-NH(CH_2CH{=}CH_2) \cdot HCl$	3.1	0.84	0.24	0.043
3	$-NH(CH_2CH_2CH_3) \cdot HCl$	0.89	0.21	0.065	0.011
4	$-NCH_3(CH_2CH_2OH)$[e]	(120.0)[c]	(32.0)[c]	7.6	2.1
5		0.27	0.054	0.013	0.0023
6		1.7	0.46	0.12	0.020
7		—[f]	—	—	—

[a] Initial concentration of 25 mg/100 ml with 4-ml samples withdrawn and analyzed as specified in the *Experimental* section. [b] Rate constants were determined in triplicate. Constants listed are the average of three runs with no deviations greater than ±15% of the arithmetic mean. [c] Values were determined by extrapolation of log k_{obs} = constant − $Ea/2.303RT$. [d] Other values for the diethylamino compound were: 40°, 0.11; and 23.6°, 0.059. [e] Other value: 0.58, 40°. [f] Reaction extremely rapid with $t_{1/3}$ of about 30 min at 23.6° as estimated from TLC plates.

First-order disappearance of starting material was followed by:

$$\log(A - A_\infty) = \log(A_0 - A_\infty) - k_{obs}t/2.303 \quad \text{(Eq. 1)}$$

where A_0, A, and A_∞ are absorbances of the ion-pair in chloroform at times zero, t, and infinity, respectively, and k_{obs} is the apparent or observed first-order rate constant in $hours^{-1}$.

Figure 2 illustrates typical plots of log absorbance *versus* time for the decomposition of the 5-aminodibenzocycloheptenes (Table I). Residual absorbances, A_∞, of less than 2.5% of the initial A_0 were observed. These were primarily blank extractions of slight amounts of indicator into the chloroform. Graphs of the first-order process were linear through up to six half-lives. Readings were taken at 340 ± 2 nm, with blank determinations being subtracted from the absorbances found.

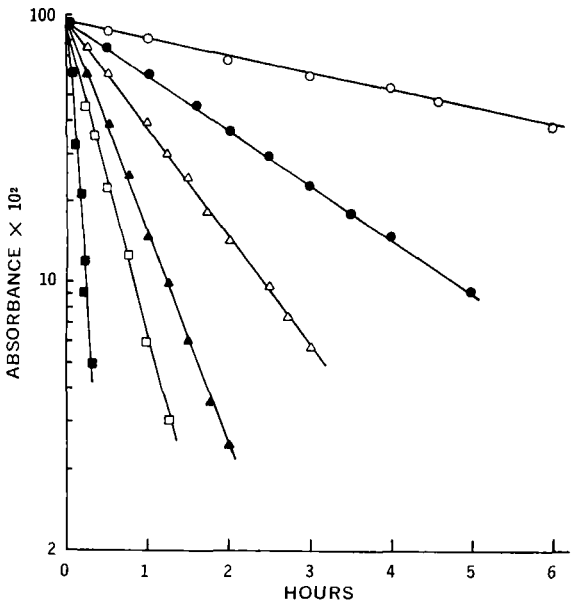


Figure 2—*First-order plots for hydrolysis of several 5-aminodibenzocycloheptenes in 0.1 N HCl at 80° unless otherwise indicated. Twenty-five milligrams of substrate was dissolved in 100 ml of 0.1 N HCl followed by taking a 4-ml aliquot, adjusting the pH to 3.44, and adding 25 ml of methyl orange solution. The complex was then extracted into 100 ml of chloroform and monitored as a function of time. Compounds studied were: ■, —$NCH_3CH_2CH_2OH$, 50°; □, —$NHCH_2CH{=}CH_2$; ▲, piperidino; △, —$NHCH_2CH_2CH_3$; ●, —$N(CH_2CH_3)_2$, 50°; and ○, pyrrolidino. Values for velocity constants are listed in Table III.*

Concentrations at 2.5 mg/100 ml solution were monitored, employing longer path length cells and/or less chloroform volume for extraction. Values for the rate constants were the same within experimental error as those obtained with 25 mg of amine/100 ml of solution. Centrifugation was circumvented by allowing the chloroform solutions to clarify for 5–24 hr prior to analysis.

The morpholino derivative (Compound 7, Table I) decomposed quickly under ambient conditions but failed to form an extractable complex with methyl orange. It was more readily extractable from pH 3.44 buffer into chloroform than the piperidino congener (Compound 6, Table I). The morpholino was completely removed on one extraction, while the piperidino showed measurable absorbance in the aqueous layer after three extractions (15 ml of chloroform–15 ml of pH 3.44 buffer).

When equimolar amounts of amine and methyl orange were prepared (7 × 10^{-4} *M*) followed by shake-out with equal volumes of chloroform, the morpholino passed into the organic layer while the dye remained in the aqueous layer; however, the piperidino formed a readily extractable complex. This extractability of noncomplexed amine into the organic layer and subsequent failure of acid–dye interaction has been described elsewhere (14).

The oxygen atom *per se* is not the cause of this phenomenon because the —$NCH_3(CH_2CH_2OH)$ analog responds in the normal manner. The pKa values of all compounds in Table I should be similar enough to eliminate this as the factor causing the spurious result.

The 9-aminofluorenes (Table II) exhibited no tendency to degrade on prolonged treatment at 80°, with the morpholino again failing to complex with the dye.

pKa Estimation—Equilibrium solubility analysis of 5-propylaminodibenzo[*a*,*d*]cycloheptene (Compound 3, Table I) and 5-pyrrolidinodibenzo[*a*,*d*]cycloheptene (Compound 5, Table I) was carried out for approximation of the pKa's (15). An excess of the pyrrolidino analog was allowed to equilibrate with various buffers

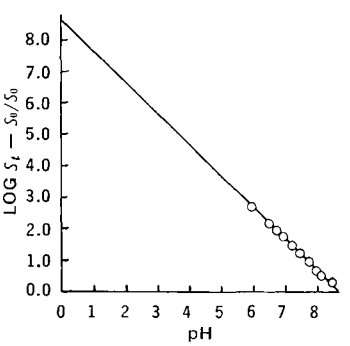
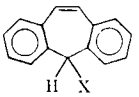


Figure 3—*Plot of log* $S_t - S_0/S_0$ *versus pH from Eq. 5. This figure represents data obtained from solubility analysis of the 5-pyrrolidinodibenzo*[a,d]*cycloheptene (Compound 5, Table I) at 23.6°. Point of intersection with the* y*-axis is the pKa value, slope* = −1. S_t *is solubility of protonated and neutral species, with* S_0 *being solubility of neutral or uncharged base.*

Table IV—Observed First-Order Rate Constants (k_{obs}, hr^{-1}) for Decomposition of 5-Aminodibenzo[*a*,*d*]cycloheptenes in Aqueous Solution[a,b]



Compound	X	Temperature	pH[c,d] 0.1	1.1	2	3.4	5	6	7	Water
1	$-N(C_2H_5)_2 \cdot HCl$	60°	0.52	0.53	—	—	0.49	—	0.48	0.44
2	$-NH(CH_2CH{=}CH_2) \cdot HCl$	70°	—	0.84	—	0.89	0.78	—	0.80	0.82
3	$-NH(CH_2CH_2CH_3) \cdot HCl$	80°	0.89	0.88	0.82	0.91	—	—	0.71	0.77
4	$-NCH_3(CH_2CH_2OH)$	50°	—	2.1	—	—	—	2.3	—	2.0
5		80°	0.28	0.29	—	0.31	—	—	0.27	0.22
6		80°	1.82	1.89	1.71	1.74	1.68	1.76	1.79	1.61
7		—	—[e]	—	—	—	—	—	—	—

[a] Followed by acid–dye method, initial concentration of 25 mg/100 ml except at pH 7 where 10 mg/100 ml was used with extraction of complex into 50 ml of chloroform. See *Experimental* section. [b] Most kinetic runs were carried out in duplicate, with results reported as averages of total number of runs. [c] The pH values of buffers were measured on a Metrohm pH meter. The pH values of hydrochloric acid solutions were determined from activity coefficients employing Ref. 19. The slight variation with temperature of the solutions was not considered. [d] The pH 1.1 and 0.1 buffers were made from 0.1 and 1.0 *N* HCl, respectively. The pH 2 buffer was made from 0.05 *M* $NaH_2PO_4 \cdot H_2O$ with H_3PO_4 added to give the proper pH. The pH 5 buffer was made to ionic strength 0.1 from sodium hydroxide on addition of acetic acid. The pH 3.44 buffer was made as stated in Ref. 7. The pH 6 and 7 buffers were prepared from 0.05 *M* $Na_2HPO_4 \cdot 7H_2O$ by addition of hydrochloric acid. [e] Could not be assayed by acid–dye method. [f] Compounds 4, 5, and 6 were dissolved by addition of equimolar hydrochloric acid.

in a constant-temperature bath, 23.6°, overnight followed by UV analysis of the filtrate. The morpholino and propylamino were dissolved in 0.1 *N* HCl and water prior to the addition of buffer. Instability prevented their long equilibration.

Amines and their salts may be treated in a manner similar to that recently discussed for acids and their anions (16). The ionization constant, K_a, for a protonated amine may be expressed as:

$$K_a = \frac{[B][H^+]}{[BH^+]} \qquad \text{(Eq. 2)}$$

or, if certain assumptions are made, can be stated in terms of solubilities (15):

$$K_a = \frac{[H^+]S_0}{[S_{BH^+}]} \qquad \text{(Eq. 3)}$$

when:

$$S_t = S_0 + S_{BH^+} \qquad \text{(Eq. 4)}$$

where S_t, S_0, and S_{BH^+} represent total solubility, solubility of the neutral molecule, and solubility of the protonated amine, respectively, in moles per liter.

By combining Eqs. 3 and 4 and writing the result in terms of negative logarithms, the relationship:

$$\log\frac{S_t - S_0}{S_0} = \text{pKa} - \text{pH} \qquad \text{(Eq. 5)}$$

is derived for the solubility of the undissociated amine in combination with its protonated form, where S_0 is constant regardless of pH.

Plotting $\log S_t - S_0/S_0$ on the ordinate against pH on the abscissa, one obtains a straight line with slope −1. The *y*-intercept is the pKa. Figure 3 illustrates an example utilizing the 5-pyrrolidino analog.

The other compounds (Table I) could not be studied by this equilibration procedure due to their tendency to degrade under ambient conditions. However, assuming rapid equilibration, the propylamine hydrochloride (Compound 3, Table I) was dissolved in a minimal volume of water with addition of buffer, followed by filtration and analysis of the filtrate. The morpholine (Compound 7, Table I) was treated analogously except for dissolution in 0.1 *N* HCl preceding addition of buffer with pKa = 7.5 ± 0.3 at 21°. The pKa values for the propylamino and pyrrolidino derivatives determined at 23.6° were 8.9 ± 0.3 and 8.8 ± 0.3, respectively.

The usual methods for pKa evaluation were not applicable because of: (*a*) the extreme insolubility of the free base precluding titration, (*b*) the lack of spectral shifts in the UV region on conversion of protonated to nonprotonated species, and (*c*) the inability to make determinations kinetically due to the poor solubility of the unionized base.

Application of Eq. 5 as shown in Fig. 3 gives a pKa value of 8.9 ± 0.3. The nature of the equation allows the point of intersection with the abscissa to give the pKa value. Examination of a graph of milligrams per milliliter in solution, S_t, against pH reveals a pKa approximately the same as that obtained from Eq. 5. The pKa is the point where $S_t = 2 \times S_0$; S_0 was estimated from the asymptote at higher pH values where practically all amine is present as the neutral species. Figure 4 is an example of the data obtained from the tedious solubility experiment with the propylamine derivative.

Reaction Routes—Tables III and IV present experimental kinetic data. The expression describing the observed velocity constant for hydrolysis of the 5-aminodibenzocycloheptenes may be given as:

$$k_{obs} = k_0 f_{BH^+} = k_0 \frac{[H^+]}{[H^+] + K_a} \qquad \text{(Eq. 6)}$$

when the fraction of neutral amine is appreciable or $k_{obs} = k_0$ when the fraction of cationic amine is 1, $f_{BH^+} = 1$. Equations of this sort are indicative of water reaction with substrate (17, 18).

The compounds investigated showed an invariant rate constant at a given temperature in water. At pH 7, the value for the velocity constant was the same, within experimental error, as that in 1 *N* HCl (Table IV). Due to the low solubility of the partially protonated compounds, only a small percentage of uncharged substrate was present, leading to the apparently identical rate constants obtained at higher hydrogen-ion concentrations.

The velocity constants were a function of temperature, solvent (water), nature of substrate, and protonation of the amino group. When hydrochloride salts were dissolved in distilled water, the rate constants were the same as those in aqueous acidic or buffer solutions as long as the nitrogen was cationic (Table IV). No ionic strength or buffer effects were noted.

Decomposition of the protonated amine (Compound II, Scheme I) appears as a spontaneous process at a specified temperature in aqueous solution. No color was observed as indicative of free carbonium ions in nonaqueous media, but it is unlikely that complete ionization would occur in water. A concerted mechanism might be expected where the solvent interacts to some extent with the substrate at all phases of the hydrolysis.

Arrhenius Parameters—Figure 5 exemplifies plots from the

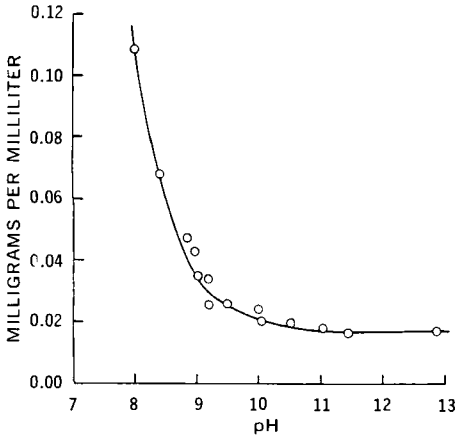


Figure 4—*Plot of solubility of 5-propylaminodibenzocycloheptene (Compound 3, Table I)* versus *pH, 23.6°. The pKa value is equivalent to point where total solubility equals twice the observed solubility at pH 12–13;* i.e., $S_t = 2S_0 = pKa$.

Arrhenius equation:

$$\ln k_{obs} = Ea/RT + \text{constant} \qquad \text{(Eq. 7)}$$

for several compounds in Table III. The apparent energies of activation are invariant for the pH ranges studied (0.1–7). The *Ea* values for the compounds under scrutiny (Fig. 5) lie between 29.8 and 32.6 kcal/mole, so it appears that the activated complexes are quite similar in all cases within the realm of experimental error.

Relative Reactivities—Removal of two hydrogen atoms and subsequent aromatization of the dibenzocycloheptane nucleus bring about almost a 100-fold enhancement of the rate constants, $k_{\text{cycloheptene}}/k_{\text{cycloheptane}}$, for the molecules with the same amino substituents at position 5 of the ring. The k_{ene}/k_{ane} values are 79, 87, and 204 for the diethylamino, pyrrolidino, and piperidino analogs, respectively, within the rate constant data for the 10,11-dihydro congeners taken from previously published results (7). That

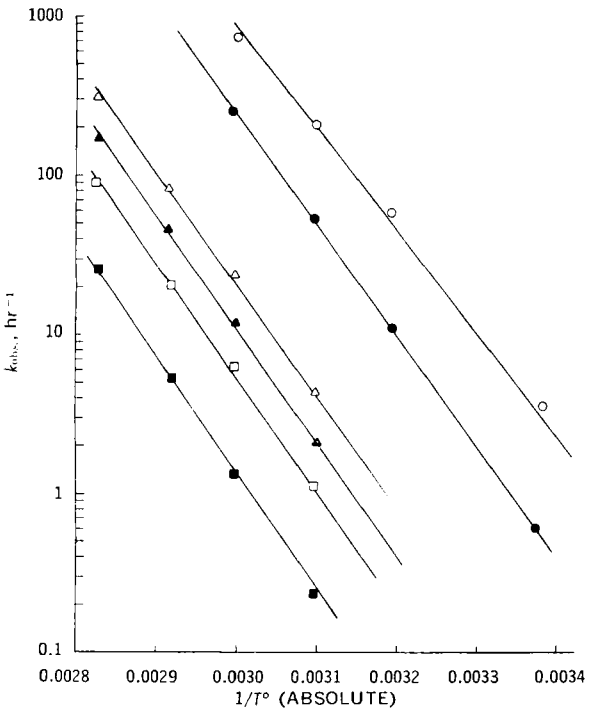


Figure 5—*Arrhenius plots for various compounds in 0.1* N *HCl. Key:* ■, $-NC_4H_8$; ▲, $-NC_5H_{10}$; □, $-NH(C_3H_8)$; △, $-NHCH_2CH{=}CH_2$; ●, $-N(CH_2CH_3)_2$; *and* ○, $-NCH_3(CH_2CH_2OH)$. *Compounds included are listed in Table III.*

the fully aromatic structures (II) react so rapidly is not as disconcerting as the fact that the hydrogenated analogs (I) react at all, because the opportunity for formation of the tropylium ion is not afforded in these molecules.

Another unexpected finding is that the k_{obs}–pH relationships (Table IV) give no evidence of an increased reaction rate as the pH scale is ascended, contrary to recently published papers dealing with tropylium ions (20–23). These data reinforce the previously stated idea that the rate-determining step is ionization or some equivalent of the 5-amino functionality, with concomitant hydroxylation by the nucleophilic solvent, water, leading to the ether and alcohol as primary products (7). In light of recent work (21, 22), it is surprising that as the pH increases the affinity of the intermediate for OH^- ions does not increase the rate constant. Other nucleophilic agents such as methanol interacted even more quickly with the protonated amines, and this will be the subject of a later report.

The initial reaction of Scheme I with water attack on the 5-amino position may be analogous to the reaction of water with alkyl iodide or other halide to give the alcohol. This series represents a unique class of transformations, probably greatly facilitated by the potential tropylium ion flanked by two phenyl rings, thus allowing dispersion of the charge over the entire framework of the ring system. The protonated amines serve as unlikely leaving groups, producing electron-deficient intermediates.

CONCLUSIONS

A series of 5-aminodibenzocycloheptenes was prepared and investigated kinetically. The velocity constants are two magnitudes larger than those for the corresponding 5-aminodibenzocycloheptanes. This can be explained on the basis of complete dispersion of the potential positive charge generated on or during cleavage of the C—N bond over the entire tricyclic skeleton.

The rate law was found to be $k_{obs} = k_0\,[H^+]/[H^+] + K_a$ and can be simplified to $k_{obs} = k_0$ at pH values below 7 where the amines are practically 100% protonated. This type of kinetic result indicates a S_N1 mechanism, although rigid proof of the proposed reaction type is difficult.

These compounds further illustrate cases where a carbon–nitrogen linkage is easily cleaved in aqueous solution, and the reaction class is rather reminiscent of hydrolysis of certain alkyl halides in aqueous solution.

REFERENCES

(1) O. Gisvold and C. O. Wilson, "Textbook of Organic Medicinal and Pharmaceutical Chemistry," 4th ed., Lippincott, Philadelphia, Pa., 1962, p. 363.

(2) N. V. Koninklijke, Pharmaceutische Fabrieken voorheen Brocades-Stheeman & Pharmacia, British pat. 969,023; through *Chem. Abstr.*, **62,** 2782h(1965).

(3) V. Mychajlzsyn and M. Protiva, *Collect. Czech. Chem. Commun.*, **24,** 3955(1959); through *Chem. Abstr.*, **54,** 8766e(1960).

(4) Belgian pat. 657,925 (July 5, 1965); through *Chem. Abstr.*, **64,** 19332h(1966).

(5) C. Van der Stelt and N. V. Koninklijke, Pharmaceutische Fabrieken voorheen Brocades-Stheeman & Pharmacia, U.S. pat. 3,357,982 (Cl. 260–268) (Dec. 12, 1967); through *Chem. Abstr.*, **69,** 10472u(1968).

(6) I. C. I. Ltd., Netherland Appl. 6,500,085 (Cl. C 07 c) (July 7, 1965); through *Chem. Abstr.*, **64,** 2036h(1966).

(7) H. V. Maulding, D. Brusco, J. Polesuk, J. Nazareno, and A. F. Michaelis, *J. Pharm. Sci.*, **61,** 1197(1972).

(8) A. H. Wragg, T. S. Stevens, and D. M. Ostic, *J. Chem. Soc.*, **1958,** 4057.

(9) Imperial Chemical Industries Ltd., Netherland Appl. 6,500,085 (July 7, 1965); through *Chem. Abstr.*, **64,** 2037b(1966).

(10) B. Giancarlo, *Gazz. Chim. Ital.*, **87,** 293(1957); through *Chem. Abstr.*, **52,** 11803d(1958).

(11) E. S. Gould, "Mechanism and Structure in Organic Chemistry," Holt, Rinehart and Winston, New York, N.Y., 1959, p. 415.

(12) W. E. Doering and L. H. Knox, *J. Amer. Chem. Soc.*, **76,** 3103(1954).

(13) T. Higuchi and E. Brochmann-Hanssen, "Pharmaceutical Analysis," Interscience, New York, N.Y., 1961, pp. 413–418.

(14) G. Schill, *Acta Pharm. Suecica*, **2**, 13(1965).
(15) A. Albert and E. P. Sergeant, "Ionization Constants of Acids and Bases," Wiley, New York, N.Y., 1962.
(16) E. R. Garrett and C. M. Won, *J. Pharm. Sci.*, **60**, 1801(1971).
(17) E. R. Garrett, *ibid.*, **51**, 811(1962).
(18) "Advances in Pharmaceutical Science," A. H. Beckett, H. S. Bean, P. E. Carless, and E. R. Garrett, Eds., Academic Press, London, England, 1967, p. 1.
(19) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 3rd ed., Reinhold, New York, N.Y., 1958.
(20) W. von E. Doering and L. H. Knox, *J. Amer. Chem. Soc.*, **79**, 352(1957).
(21) C. D. Ritchie and H. Fleischhauer, *ibid.*, **94**, 3481(1972).
(22) C. D. Ritchie and P. O. I. Virtanen, *ibid.*, **94**, 4966(1972).
(23) *Ibid.*, **94**, 4963(1972).

ACKNOWLEDGMENTS AND ADDRESSES

Received April 12, 1974, from the *Pharmacy and Analytical Research Department, Sandoz-Wander, East Hanover, NJ 07936*
Accepted for publication July 2, 1974.
[x] To whom inquiries should be directed.

In Vitro Binding of Drugs to Colestipol Hydrochloride

HOWARD KO [x] and MAX E. ROYER

Abstract □ The *in vitro* binding of drugs by colestipol hydrochloride depended not only upon ionic strength, pH, and type of competing ion but also upon whether association could occur with other molecules. Where feasible, the initial input ratio of drug to binding agent was equivalent to that if both were administered orally in therapeutically effective amounts. The water-soluble drugs chlorpropamide-^{35}S, niacin-6-^{14}C, ascorbic acid, aspirin, salicylic acid, phenobarbital-^{14}C, sulfadiazine, penicillin G, and lincomycin hydrochloride were less than 30% bound to colestipol hydrochloride in tromethamine–sodium chloride buffer (pH 7.5, μ = 0.15), while warfarin and tetracycline hydrochloride were bound 59 and 30%, respectively. The binding of a drug determined under these conditions is an estimate of the upper limit of the percentage of the dose of orally ingested drug that would remain bound *in vivo*. The binding of tetracycline hydrochloride, sulfadiazine, benzyl penicillin, and lincomycin hydrochloride to colestipol hydrochloride was reversible, and only a small fraction of warfarin was irreversibly bound to colestipol hydrochloride. As a positive control, binding of the drugs (or bile salts) to cholestyramine was also studied. Cholestyramine bound most of the drugs investigated to a greater extent than did colestipol hydrochloride. The binding of water-soluble drugs to colestipol hydrochloride decreased in the combined presence of monoolein, oleic acid, and taurocholate. The colestipol hydrochloride binding of compounds of low aqueous solubility, vitamins A_1, D_2, and K_1, and cholesterol-^{14}C increased as the concentration of taurocholate decreased. Monoolein increased, while oleic acid decreased, the binding of taurocholate to colestipol hydrochloride. Oleic acid increased slightly the binding of taurocholate to cholestyramine; monoolein had no effect. The data are consistent with the hypotheses that: *(a)* taurocholate associates with and thus transfers compounds of low aqueous solubility to polymer binding sites, *(b)* the fraction of vitamin A_1 and cholesterol-^{14}C associated with the polymer binding sites and the fraction in solution depends upon the concentration of taurocholate, and *(c)* the binding of taurocholate depends upon the composition of micelle formed.

Keyphrases □ Colestipol hydrochloride—*in vitro* binding to various drugs, effects of ionic strength, pH, and competing ion, compared to cholestyramine □ Binding—colestipol hydrochloride and various drugs *in vitro*, effects of ionic strength, pH, and competing ion, compared to cholestyramine □ Drug binding—colestipol hydrochloride and various drugs *in vitro*, effects of ionic strength, pH, and competing ion, compared to cholestyramine □ Cholesterol-reducing agents—*in vitro* binding of various drugs to colestipol hydrochloride

Colestipol hydrochloride is a copolymer that indirectly lowers serum cholesterol in experimental animals and humans and is believed to do so through binding bile acids and their conjugates in the GI tract (1). Cholestyramine (a bile salt-sequestering agent) interferes with the absorption of thyroxine in humans (2) and other drugs in rats and dogs (3). It was conceivable that concurrently ingested drugs would be similarly bound to colestipol hydrochloride and thus be poorly absorbed.

To identify potential drug–polymer interaction problems prior to *in vivo* binding studies, the comparative *in vitro* binding to colestipol hydrochloride and cholestyramine was determined for different drugs. Initial drug–polymer ratios typical of clinical usage were employed under experimental conditions (pH and ionic strength) approximating those of the distal portion of the ileum, one of the later absorbing sites in the GI tract.

EXPERIMENTAL

Materials—Sodium taurocholate-7-^{3}H had a specific activity of 46.3 mCi/mmole[1], and sodium taurocholate-24-^{14}C had a specific activity of 6.6 μCi/mmole[2]. Unlabeled sodium taurocholate[3] was used as carrier. These compounds were homogeneous by TLC. Tetracycline-7-^{3}H hydrochloride (1200 mCi/mmole[4]), niacin-6-^{14}C (26.2 mCi/mmole[4]), D-biotin-carbonyl-^{14}C (57.5 mCi/mmole[4]), chlorpropamide-^{35}S (1.8 mCi/mmole[4]), potassium benzyl penicillin-^{14}C (20–40 mCi/mmole[4]), phenobarbital-2-^{14}C (2.97 mCi/mmole), and cholesterol-26-^{14}C (46 mCi/mmole[5]) were of 95–99% radiochemical purity as determined by paper or thin-layer chromatography.

The binding of these compounds to colestipol hydrochloride[6] and to cholestyramine[7] was studied.

[1] Supplied by Nuclear Research Chemicals Co., Orlando, Fla.
[2] Prepared by Dr. R. C. Thomas, The Upjohn Co., Kalamazoo, Mich.
[3] Grade A, Calbiochem Co., Los Angeles, Calif.
[4] Amersham/Searle Co., Chicago, Ill.
[5] New England Nuclear, Boston, Mass.
[6] Colestid, supplied by The Upjohn Co., Kalamazoo, Mich.
[7] Cuemid, Merck, Sharp and Dohme, West Point, Pa.