Table I—Hypolipidemic Effect of 4-(*n*-Alkylamino)benzoic Acids and Clofibrate in Normal Rats

n-Alkyl Group	Oral Dose in Diet[a], mg/kg/day	Percent Lowering[b] of Serum	
		Sterol	Triglyceride
C_{12}	86	11[c]	68[d]
	47	20[e]	60[d]
	25	14[f]	49[d]
C_{14}	82	9	47[d]
	46	9	49[d]
	23	9	36[d]
C_{16} (cetaben)	89	44[d]	69[d]
	64	30[d]	48[f]
	42	26[d]	40[f]
	22	27[d]	38[f]
	10	27[d]	29[d]
C_{18}	91	18[g]	40[e]
	48	6	28[c]
	23	14[f]	29[c]
Clofibrate	375	26[d]	43[c]
	106	5	30
	33	0	32

[a] The method used was described in detail in Ref. 2. Oral dosing was by normal rat chow containing drug; 0.05% in the diet corresponds to a measured average daily dosage of 46 mg/kg, *etc.* [b] Percent lowering of the mean serum concentrations relative to the mean values in control animals in the same test; control groups averaged 75 ± 3 mg % sterol and 85 ± 6 mg % triglyceride. The significant level (p), determined by the Student t test, is denoted by the appropriate footnote. Numbers without a footnote are not statistically significant lowerings. [c] $p < 0.05$. [d] $p < 0.001$. [e] $p < 0.005$. [f] $p < 0.02$. [g] $p < 0.01$.

Most important was the presence of only half as much luminal narrowing in four major coronary arteries (5). Accompanying this effect on lumen size were increases in myocardial blood flow in the nonstressed and stressed heart. In the stressed heart, regional blood flow toward the endocardium was improved as well. Reductions in luminal narrowing and incidence of atherosclerotic lesions also were seen in several major peripheral arteries. In the aortas of treated monkeys, average 48% reductions in cholesterol accumulation resulted predominantly from decreases in esterified cholesterol. Additionally, there was complete prevention of a 2.5-fold calcium increase in the aortic wall and a 70% inhibition of the increase in aortic fibrous connective tissue. Up to now, such pronounced therapeutic effects on this atherosclerosis model (5, 8, 9) have not been reported with any other drug.

The reduced cholesterol ester content in the rabbit and monkey aortas may result from cetaben inhibition of the cholesterol-esterifying enzyme in the aortic wall. With a cell-free preparation of fatty acyl-CoA: cholesterol acyl transferase (ACAT) from rabbit aortas, cetaben sodium substantially inhibited the enzyme at therapeutically obtainable concentrations[1] (~5 μg/ml). This effect is especially significant in view of the greater proportion of esterified to free cholesterol in atheromatous lesions in humans (10) as well as the lower experimental reversibility of ester deposition in rabbits (11).

In a series of papers, we shall fully report on the biological activity described here and elaborate the structure–activity relationships of alkyl size; branching, cyclization, substitution, and unsaturation of the alkyl group; further substitution of the amino nitrogen and its position isomers; additional substitution on the benzene ring and its replacement with other rings; and carboxyl group derivatization, modification, and extension.

Effective atherosclerosis therapy probably will require drugs that have several modes of action such as those displayed by cetaben sodium. Cetaben sodium is now undergoing clinical trials as an antiatherosclerotic agent in humans.

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pKa of 2-Methylamino-5-chlorobenzophenone, a Diazepam Hydrolysis Product

Keyphrases □ Methylaminochlorobenzophenone—pKa, diazepam hydrolysis product, structure–activity relationships □ Diazepam—hydrolysis products, methylaminochlorobenzophenone, pKa, structure–activity relationships □ Structure–activity relationships—diazepam hydrolysis products, pKa

To the Editor:

The compound 2-methylamino-5-chlorobenzophenone (I) is the aromatic product of diazepam (II) hydrolysis in acidic aqueous solution (1). It was of interest to estimate the pKa of I because of its importance in kinetic stability and analytical studies of prazepam (2) and II (1, 3–8).

The pKa of I[1] was determined spectrophotometrically[2] at 25° at 415 nm in a concentration of 4.07×10^{-5} *M* (10 μg/ml). All samples contained 7% (v/v) ethanol. The pH was varied by preparing samples in 0.02–0.40 *N* HCl. The spectrum of the conjugate acid was obtained in 60% (w/w) H_2SO_4 ($H_0 = -4.4$), and that of the nonionized base was obtained in buffers of pH 7.20 and 9.23. The conjugate acid species showed no absorbance at 415 nm, and the base had an absorbance of 0.23. The other spectral characteristics included decreasing absorbance at 410–420 nm and a hypsochromic shift from ~268 to 238 nm with increasing

[1] Ro 5-4365, lot PP-4 Hoffmann-La Roche, Nutley, N.J.
[2] Beckman DB-GT grating spectrophotometer, Beckman Instruments, Fullerton, Calif.

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Table I—pKa Values of Some Aniline Derivatives

Substance	Substituent	Position	pKa[a]
Aniline	—	—	4.60[b]
Aniline	Benzoyl	4	2.2[c]
Aniline	Chloro	4	3.98[b]
N-Methylaniline	—	—	4.85[b]
N-Methylaniline	Chloro	4	3.9[c]

[a] At 25°. [b] References 10 and 11. [c] Reference 11.

absorbance, both in relation to the increasing sample acidity. The I pKa was calculated from Eq. 1 (9) to be 1.45 ± 0.04[3]:

$$\text{pKa} = \text{pH} + \log (A_b - A/A - A_i) \qquad \text{(Eq. 1)}$$

where A_b, A_i, and A = the absorbances of the nonionized base, the conjugate acid, and the various samples, respectively.

The pKa values of substituted anilines such as I are lowered more by an *ortho*- than by a *meta*- or *para*-electron-withdrawing substituent (10, 11). Thus, the inductive effect of 2-benzoyl and 4-chloro groups on *N*-methylaniline (*i.e.*, I) as regards the pKa of the anilino nitrogen atom would be predicted to exceed that of the sum of these substituents at the 4-position. From Table I, the pKa differences among anilines substituted with *N*-methyl, 4-benzoyl, and 4-chloro groups are 0.25, −2.4, and −0.62 units, respectively. The arithmetic sum of these differences is −2.77 units, thereby predicting a pKa of ~1.8 (*i.e.*, 4.6 − 2.8) for I. However, the pKa lowering caused by a 4-chloro substituent is 0.1 unit greater for *N*-methylaniline than for aniline. Therefore, the observed anilino pKa decrements (Table I), augmented by the expected superior electron-inducing capability of a 2-benzoyl (*ortho*) over that of a 4-benzoyl *N*-methylaniline substituent, appear to be in reasonable agreement with the pKa estimate of 1.4–1.5 for I.

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[3] Average of five values in the pH range of pKa ± 1 unit.

Drug Dissolution within a Cascade Barrier Bed

Keyphrases □ Dissolution—test apparatus, cascade barrier bed □ Pharmacokinetics—drug dissolution tests, apparatus, cascade barrier bed □ Cascade barrier bed—for testing drug dissolution

To the Editor:

When dissolution limits the rate of drug absorption, *in vitro* dissolution tests are useful for quality control or in formulation development only if they correlate well with *in vivo* drug levels. Where satisfactory correlations have not been found, differences in solubility behavior among the polydisperse particles produced during dosage unit disintegration may be a factor. Where the relative intrinsic solubilities of particles are size dependent and sensitive to formulation variables, a single dissolution test method cannot be relied upon. Depending on the desired degree of resolution among formulations, at least two procedures are needed that produce linearly independent functional relationships between particle size and dissolution hydrodynamics. To accomplish this result, they must control the dissolution fluid mechanics as a function of particle size.

This communication discusses a cascade barrier bed test method, which complements existing procedures by providing distinctly different dissolution conditions. It differs from the USP–NF (1, 2), stationary basket–rotating filter (3), and flowthrough cell (4, 5) methods by favoring the dissolution of small particles over large.

The device consists of a vertical cylindrical cell (Fig. 1). It is partially filled with discrete layers, B, of uniformly sized, silanized glass balls. The ball size of each layer is successively increased from the bottom to the top of the bed. The prototype tested contained five layers, each 6.25 cm in height and composed of 0.25-, 0.56-, 1-, 2-, and 4-mm balls. The cell was 50 cm in height × 1.9 cm i.d.

In preparation for a run, solvent was introduced through the efflux chamber, C, to the top of the previously loaded bed so as to preclude air entrapment. The powder was then placed on top of the bed; the solvent receiving chamber, A, was filled with solvent; and flow was initiated. Flow was controlled and maintained by hydrostatic pressure; solvent flowed from an elevated reservoir through Tygon tubing to the receiving chamber, A. Samples of effluent were collected through tubing attached to chamber C and assayed. A sintered-glass frit, D, retained and supported the bed within the cell.

In operation, drug particles are carried into the bed by viscous drag forces and by gravity to a level where they are trapped. Their location is determined by drug particle size and the pore size characteristic of each layer in the bed. Particles from polydispersed systems are thus separated into various size fractions within the bed.

Although fluid flow fields in this type of packed bed are complicated, the hydrodynamics within the various layers are simply related. With column and ball diameters in a practical range for dissolution testing, random packing fractions of uniform spheres are size independent. For a constant column cross-sectional area, the average linear velocity of the solvent also will be constant throughout the bed for incompressible fluids. Voids and pores are geo-

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