Synthesis and Quantitative Structure–Activity Relationships of Antibacterial 1-(Substituted Benzhydryl)-4-(5-nitro-2-furfurylideneamino)piperazines

D. K. YUNG *x, M. L. GILROY *, and D. E. MAHONY ‡

Received August 8, 1977, from the * *College of Pharmacy, Faculty of Health Professions, and the* ‡ *Department of Microbiology, Faculty of Medicine, Dalhousie University, Halifax, N.S., Canada.* Accepted for publication October 20, 1977.

Abstract □ 1-Benzhydryl -4- (5-nitro-2-furfurylideneamino)piperazine and 11 substituted analogs were prepared and examined for *in vitro* antimicrobial activity. The compounds were active against *Bacillus cereus* 7, *Bacillus megaterium* 122, *Bacillus subtilis* 104, *Clostridium perfringens* 13, and the tetracycline-resistant *Clostridium perfringens* 37. Regression analyses on the antibacterial activity data based on the Hansch approach, using π, π^2, and σ parameters, yielded several statistically significant correlation equations. 1-Benzhydryl-4-(5-nitro-2-furfurylideneamino)piperazine stopped the protein and DNA syntheses in *C. perfringens* 13, as indicated by precipitable radioactivity. The compound, however, showed no effect on the cell wall synthesis in the bacteria.

Keyphrases □ Piperazines, substituted—synthesized, antibacterial and antifungal activity evaluated □ Antibacterial activity—evaluated in various substituted piperazines □ Antifungal activity—evaluated in various substituted piperazines □ Structure–activity relationships—various substituted piperazines evaluated for antibacterial and antifungal activity

Previously (1), 1-benzhydryl-4-(5-nitro-2-furfurylideneamino)piperazine (I*a*) prevented the growth of *Clostridium perfringens* 28 and *Bacillus subtilis* 104. The present study was undertaken to investigate the antibacterial activity of 11 substituted analogs of I*a* (I*b*–I*l*) (Table I) and to correlate the activity with the hydrophobic (π and π^2) and electronic (σ) properties of the substituents. In addition, the effect of the compounds, as represented by I*a*, on the macromolecular and cell wall syntheses of *Clostridium perfringens* 13 was studied.

RESULTS AND DISCUSSION

Chemistry—The synthetic routes to I*a*–I*l* are outlined in Scheme I. The physical properties of I*a*–I*l* and the intermediates II*a*–II*l*, III*a*–III*l*, and IV*a*–IV*l* are summarized in Tables I–IV, respectively.

With the exception of II*a*, II*b*, and II*d*, which were obtained commercially, the substituted benzhydryl chlorides were prepared by treatment of the appropriate benzhydrols with anhydrous hydrogen chloride (2). Those required benzhydrols that were not readily available (Table V) were synthesized by either Grignard reaction of aldehydes or zinc–sodium hydroxide reduction of the corresponding benzophenones (3). 4-*tert*-Butylbenzophenone was prepared by the Friedel–Crafts ketone synthesis (4). The other benzophenones were obtained from commercial sources.

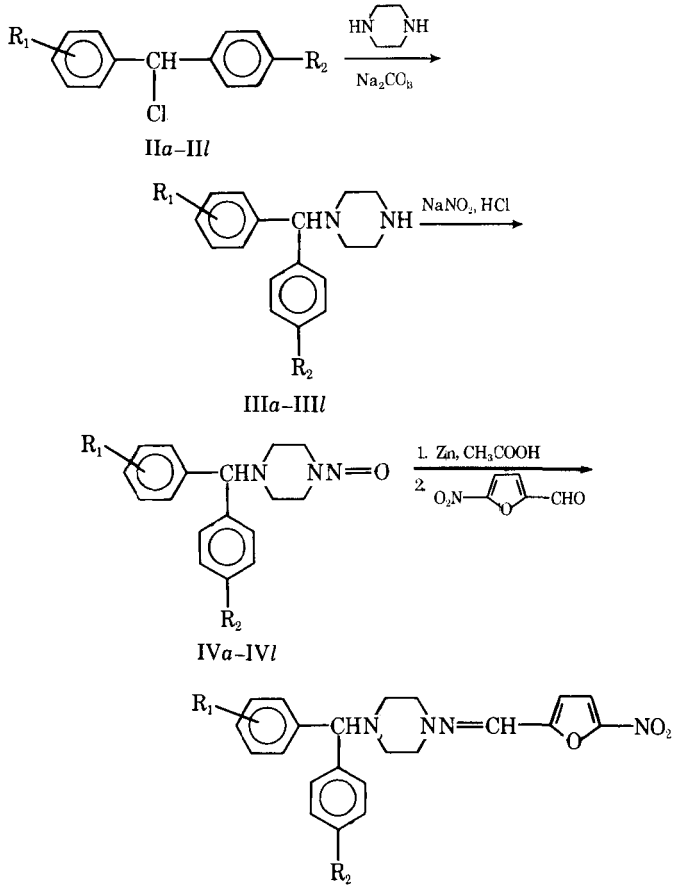
When absolute ethanol was used as the reaction solvent in early attempts to prepare III*f* and III*i*–III*l*, the desired monosubstituted piperazines were obtained in poor yields. Instead, the corresponding benzhydryl ethyl ethers (Table VI) were isolated as the major products in these reactions. In subsequent reactions with chloroform as the reaction solvent, the formation of the undesirable benzhydryl ethyl ethers was not detected. The purity of I*a*–I*l* was established by TLC (silica gel) with benzene–ethyl acetate (3:7) or benzene–acetone (4:1) as the eluant before the microbial evaluations.

The reason for the melting-point discrepancy between the previously prepared I*a* and that prepared in this study is not clear. Satisfactory spectral and analytical data were obtained for both compounds. It was postulated that geometric isomerism might have occurred. However, since the NMR spectra of the two compounds were exactly the same, the likelihood of the two compounds being a pair of *anti*- and *syn*-isomers was small.

Antimicrobial Testing—Compounds I*a*–I*l*, at a concentration of 50 μg/ml, were initially screened for antimicrobial activities against three Gram-positive bacteria, *B. subtilis* 104, *Staphylococcus pyogenes* 11, and *Streptococcus faecalis* 107; six Gram-negative bacteria, *Alcaligenes faecalis* 144, *Escherichia coli* 8, *Proteus vulgaris* 74, *Pseudomonas aeruginosa* 10145; *Salmonella thompson* 140, and *Serratia marcescens* 25; one acid fast bacterium, *Mycobacterium phlei* 111; one anaerobic Gram-positive bacterium, *C. perfringens* 13; and two fungi, *Candida albicans* 48 and *Saccharomyces cerevisiae* 53. The compounds showed activities only against *B. subtilis* 104 and *C. perfringens* 13.

The compounds were studied further to determine their minimum inhibitory concentrations (MIC) against *B. subtilis* 104 and *C. perfringens* 13 as well as *Bacillus cereus* 7, *Bacillus megaterium* 122, and the tetracycline-resistant *Clostridium perfringens* 37. The MIC values are expressed in molar concentrations as log (1/*C*) (Table VII). As a group, the compounds were more inhibitory against the *Clostridium* species than the *Bacillus* species. Compound I*g* was the least active in inhibiting the growth of all five microorganisms tested.

Dimethylformamide was used as the solvent. The effect of this solvent on the bacterial growth was studied prior to the microbiological testings, using *B. subtilis* 104, *Clostridium perfringens* 2, *E. coli* 8, and *Staphylococcus albus* 20 as the representative bacteria. Results indicated that the minimum inhibitory dilution of dimethylformamide in brain heart



Scheme I

0022-3549/78/0700/0900$01.00/0

Table I—1-(Substituted Benzhydryl)-4-(5-nitro-2-furfurylideneamino)piperazines

Compound	R_1	R_2	Yield, %	Melting Point	Recrystallization Solvent	Formula		Analysis, % Calc.	Found
I*a*	4-H	H	74	164.5–165.5°[a]	Ethanol	$C_{22}H_{22}N_4O_3$	C	67.67	67.78
							H	5.68	5.64
							N	14.35	14.02
I*b*	4-Cl	H	37	129–130°	Ether–petroleum ether	$C_{22}H_{21}ClN_4O_3$	C	62.19	62.15
							H	4.98	5.15
							Cl	8.34	8.35
							N	13.19	13.20
I*c*	3,4-Cl_2	H	42	120–122°	Ether–petroleum ether	$C_{22}H_{20}Cl_2N_4O_3$	C	57.52	57.79
							H	4.39	4.43
							Cl	15.44	15.44
							N	12.20	11.90
I*d*	4-Br	H	36	132–134°	Ether–petroleum ether	$C_{22}H_{21}BrN_4O_3$	C	56.30	56.20
							H	4.51	4.62
							Br	17.03	17.37
							N	11.94	11.62
I*e*	4-Cl	Cl	32	106–108°	Ether–petroleum ether	$C_{22}H_{20}Cl_2N_4O_3$	C	57.52	57.21
							H	4.39	4.89
							Cl	15.44	15.15
							N	12.20	11.77
I*f*	4-CH_3	H	30	129–131°	Petroleum ether	$C_{23}H_{24}N_4O_3$	C	68.30	68.43
							H	5.98	6.14
							N	13.85	13.50
I*g*	4-$C(CH_3)_3$	H	69	163–164°	Ethanol	$C_{26}H_{30}N_4O_3$	C	69.93	70.10
							H	6.77	6.93
							N	12.55	12.23
I*h*	3-CH_3	H	20	80–81°	Ether–petroleum ether	$C_{23}H_{24}N_4O_3$	C	68.30	67.85
							H	5.98	6.39
							N	13.85	13.58
I*i*	4-$CH(CH_3)_2$	H	25	163–163.5°	Ethanol	$C_{25}H_{28}N_4O_3$	C	69.42	69.35
							H	6.52	6.61
							N	12.95	12.83
I*j*	4-CH_3	CH_3	42	150–151°	Ethanol	$C_{24}H_{23}N_4O_3$	C	69.38	69.11
							H	5.58	5.59
							N	13.49	13.19
I*k*	2-OCH_3	H	28	130–131°	Ether–petroleum ether	$C_{23}H_{24}N_4O_4$	C	65.70	65.74
							H	5.75	6.13
							N	13.33	13.20
I*l*	4-OCH_3	H	29	129–131°	Ether–petroleum ether	$C_{23}H_{24}N_4O_4$	C	65.70	65.91
							H	5.75	5.89
							N	13.33	13.16

[a] Lit. (1) mp 147–148°.

infusion broth was 1:32. The solvent content in the subsequent test solutions was much lower than this dilution, usually below 1:256.

To ascertain that dimethylformamide does not increase the susceptibility of the bacteria, the MIC of penicillin G sodium against *C. perfringens* 2 was determined using water alone or water–dimethylformamide as the solvent. When the drug was dissolved in water, the MIC was 0.020 ± 0.007 μg/ml; in water–dimethylformamide, the MIC value was 0.016 ± 0.006 μg/ml. There was no statistically significant difference ($p < 0.05$) between the two values. On the basis of this observation, dimethylformamide was considered to be a suitable solvent and the MIC values obtained in the testings were considered a true indication of the antibacterial activities.

Regression Analysis—The relationships between the physicochemical properties and the antibacterial potency of I*a*–I*l* were analyzed by the Hansch approach. The physicochemical parameters were π, π^2, and σ. The π and σ parameter values are given in Table VII. No collinearity was found between π and σ for the compounds studied ($r^2 = 0.16$). Only those regression equations that were shown to be statistically significant ($p < 0.05$) by the F test are listed in Table VIII. The regression coefficients in these equations were found to be significant at the $p < 0.05$ level by the t-test.

In the *B. cereus* 7 system, Eq. 1 best correlated the antibacterial activity data and accounted for 60% ($r^2 = 0.60$) of the variance. For *B. megaterium* 122, the best correlation was obtained with both the π and σ

Table II—Substituted Benzhydryl Chlorides[a]

Compound	R_1	R_2	Yield, %	Melting Point or Boiling Point/mm	Formula		Analysis, % Calc.	Found
II*c*	3,4-Cl_2	H	69	134–136°/0.1	$C_{13}H_9Cl_3$	C	57.49	57.68
						H	3.34	3.59
						Cl	39.17	39.40
II*e*	4-Cl	Cl	63	160–166°/1.5[b]	$C_{13}H_9Cl_3$	—	—	—
II*f*	4-CH_3	H	81	128–132°/1.0[c]	$C_{14}H_{13}Cl$	—	—	—
II*g*	4-$C(CH_3)_3$	H	79	130–135°/0.2[d]	$C_{17}H_{19}Cl$	—	—	—
II*h*	3-CH_3	H	97	100–102°/0.1	$C_{14}H_{13}Cl$	C	77.59	77.89
						H	6.05	6.11
						Cl	16.36	16.16
II*i*	4-$CH(CH_3)_2$	H	71	118–124°/0.1	$C_{16}H_{17}Cl$	C	78.51	78.51
						H	7.00	7.03
						Cl	14.49	14.19
II*j*	4-CH_3	CH_3	94	43.5–44.5°[e]	$C_{15}H_{15}Cl$	—	—	—
II*k*	2-OCH_3	H	68	110–119°/0.1	$C_{14}H_{13}ClO$	C	72.26	72.51
						H	5.63	5.87
						Cl	15.24	15.12
II*l*	4-OCH_3	H	90	61–62°[f]	$C_{14}H_{13}ClO$	—	—	—

[a] 4-Chloro-, 4-bromo-, and benzhydryl chlorides (II*b*, II*d*, and II*a*, respectively) were obtained commercially. [b] Lit. (9) bp 189–193.5°/5 mm. [c] Lit. (4) bp 136°/0.4 mm. [d] Lit. (4) bp 158–160°/1.5 mm. [e] Lit. (3) mp 45–46°. [f] Lit. (10) mp 61°.

Table III—1-(Substituted Benzhydryl)piperazines

Compound	R_1	R_2	Yield, %	Melting Point or Boiling Point/mm	Formula		Analysis, % Calc.	Found
III*a*	4-H	H	52	160–162°	$C_{17}H_{20}N_2$	C	80.91	81.95
						H	7.99	8.05
						N	11.10	10.87
III*b*	4-Cl	H	47	190–192°/1.8	$C_{17}H_{19}ClN_2$	C	71.19	71.08
						H	6.68	6.72
						Cl	12.36	12.15
						N	9.77	9.08
III*c*	3,4-Cl_2	H	37	178–183°/0.6	$C_{17}H_{18}Cl_2N_2$	C	63.56	63.71
						H	5.65	5.80
						Cl	22.07	21.97
						N	8.72	7.95
III*d*	4-Br	H	9	174–176°/0.3	$C_{17}H_{19}BrN_2$	C	61.63	62.11
						H	5.78	5.84
						Br	24.13	23.92
						N	8.46	7.99
III*e*	4-Cl	Cl	51	110.5–111°[a,b]	$C_{17}H_{18}Cl_2N_2$	—	—	—
III*f*	4-CH_3	H	57	168–172°/1.0[c]	$C_{18}H_{22}N_2$	C	81.15	81.43
						H	8.33	8.50
						N	10.52	10.28
III*g*	4-$C(CH_3)_3$	H	74	126–128°[d]	$C_{21}H_{28}N_2$	C	81.77	81.74
						H	9.15	9.05
						N	9.08	8.85
III*h*	3-CH_3	H	53	134–136°/0.3[e]	$C_{18}H_{22}N_2$	—	—	—
III*i*	4-$CH(CH_3)_2$	H	35	186–190°/0.2	$C_{20}H_{26}N_2$	C	81.54	80.80
						H	8.90	8.63
						N	9.52	9.75
III*j*	4-CH_3	CH_3	42	92–94°[f]	$C_{19}H_{24}N_2$	C	81.38	81.69
						H	8.63	8.38
						N	9.99	9.64
III*k*	2-OCH_3	H	71	91–92°[f]	$C_{18}H_{22}N_2O$	C	76.56	76.54
						H	7.85	7.70
						N	9.92	9.28
III*l*	4-OCH_3	H	66	170–175°/0.4[g]	$C_{18}H_{22}N_2O$	—	—	—

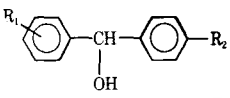
[a] Lit. (11) mp 106°. [b] Recrystallized from ether–petroleum ether. [c] Solidified on standing, mp 75.5–77.5°. [d] Recrystallized from *n*-hexane. [e] Lit. (12) bp 150°/0.05 mm. [f] Recrystallized from petroleum ether. [g] Solidified on standing, mp 80–81°. Lit. (13) bp 185–190°/0.5 mm.

Table IV—1-(Substituted Benzhydryl)-4-nitrosopiperazines

Compound	R_1	R_2	Yield, %	Melting Point	Recrystallization Solvent	Formula		Analysis, % Calc.	Found
IV*a*	4-H	H	81	109–110°[a]	Ethanol–water	$C_{12}H_{19}N_3O$	—	—	—
IV*b*	4-Cl	H	92	120–122°	Ethanol	$C_{17}H_{18}ClN_3O$	C	64.65	64.83
							H	5.74	5.87
							Cl	11.22	11.49
							N	13.31	13.10
IV*c*	3,4-Cl_2	H	68	135–136.5°	Ethanol	$C_{12}H_{17}Cl_2N_3O$	C	58.29	57.91
							H	4.89	5.08
							Cl	20.25	20.06
							N	12.00	11.83
IV*d*	4-Br	H	73	128–129.5°	Ethanol	$C_{17}H_{18}BrN_3O$	C	56.68	56.86
							H	5.04	5.18
							Br	22.18	22.32
							N	11.66	11.64
IV*e*	4-Cl	Cl	86	157.5–158.5°	Ethanol–acetone	$C_{17}H_{17}Cl_2N_3O$	C	58.29	58.34
							H	4.89	5.22
							Cl	20.25	19.89
							N	12.00	11.58
IV*f*	4-CH_3	H	94	86–88°	Ethanol	$C_{18}H_{21}N_3O$	C	73.19	73.16
							H	7.17	7.29
							N	14.23	13.84
IV*g*	4-$C(CH_3)_3$	H	77	138–139°	Ethanol–water	$C_{21}H_{27}N_3O$	C	74.74	74.71
							H	8.07	8.05
							N	12.45	12.17
IV*h*	3-CH_3	H	76	88.5–91°	Ethanol–ether	$C_{18}H_{21}N_3O$	C	73.19	73.49
							H	7.17	7.43
							N	14.23	14.21
IV*i*	4-$CH(CH_3)_2$	H	70	88–89.5°	Ethanol–ether	$C_{20}H_{25}N_3O$	C	74.27	73.97
							H	7.79	7.78
							N	12.99	12.86
IV*j*	4-CH_3	CH_3	90	139.5–140.5°	Ethanol–water	$C_{19}H_{23}N_3O$	C	73.75	73.85
							H	7.49	7.39
							N	13.58	13.59
IV*k*	2-OCH_3	H	86	143.5–144.5°	Ethanol	$C_{18}H_{21}N_3O_2$	C	69.43	69.70
							H	6.80	6.74
							N	13.50	13.36
IV*l*	4-OCH_3	H	67	80–81°	Ether–petroleum ether	$C_{18}H_{21}N_3O_2$	C	69.43	69.07
							H	6.80	6.72
							N	13.50	13.18

[a] Lit. (14) mp 110–111°.

Table V—Substituted Benzhydrols [a]



R_1	R_2	Synthetic Method [b]	Yield, %	Melting Point or Boiling Point/mm	Formula
3,4-Cl_2	H	A	65	164–166°/0.3	$C_{13}H_{10}Cl_2O$ [c]
4-Cl	Cl	B	96	91.5–93° [d]	$C_{13}H_{10}Cl_2O$
4-CH_3	H	B	91	53–54° [e]	$C_{14}H_{14}O$
4-$C(CH_3)_3$	H	B	97	79–81° [f]	$C_{17}H_{20}O$
3-CH_3	H	A	81	51–53° [g]	$C_{14}H_{14}O$
4-$CH(CH_3)_2$	H	A	62	53–56° [h]	$C_{16}H_{18}O$
4-CH_3	CH_3	B	96	69–70° [i]	$C_{15}H_{16}O$
2-OCH_3	H	A	53	134.5–135.5° [j]	$C_{14}H_{14}O_2$
4-OCH_3	H	B	97	63–65° [k]	$C_{14}H_{14}O_2$

[a] 4-Chloro, 4-bromo-, and benzhydrols were obtained commercially. [b] A = Grignard reaction; B = zinc–sodium hydroxide reduction. [c] *Anal.*—Calc. for $C_{13}H_{10}Cl_2O$: C, 61.68; H, 3.98; Cl, 28.01. Found: C, 62.02; H, 4.06; Cl, 28.16. [d] Lit. (15) mp 93–93.5°. [e] Lit. (16) mp 53.4–54°. [f] Lit. (4) mp 82°. [g] Lit. (16) mp 54–55°. [h] Lit. (17) mp 59.5–60.5°. [i] Lit. (3) mp 71°. [j] Lit. (3) mp 139°. [k] Lit. (4) mp 59–60°.

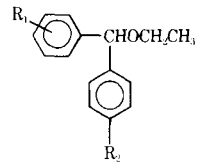


Table VI—Substituted Benzhydryl Ethyl Ethers

R_1	R_2	Yield, %	Boiling Point/mm	n_D^{25}	Formula		Analysis, % Calc.	Found
4-CH_3	H	62	106–112°/0.4	1.5484	$C_{16}H_{18}O$	C	84.91	85.21
						H	8.02	7.99
4-$CH(CH_3)_2$	H	33	123–126°/0.2	1.5414	$C_{18}H_{22}O$	C	84.99	84.81
						H	8.72	8.50
4-CH_3	CH_3	48	120°/0.5	1.5468	$C_{17}H_{20}O$	C	84.95	85.56
						H	8.39	8.48
2-OCH_3	H	96	124°/0.5	1.5559	$C_{16}H_{18}O_2$	C	79.31	79.34
						H	7.49	7.32
4-OCH_3	H	39	146–148°/1.0	1.5565	$C_{16}H_{18}O_2$	C	79.31	78.99
						H	7.49	7.81

terms as indicated by Eq. 4. This equation explained almost 80% (r^2 = 0.790) of the variation in the data. Although the π or σ term in the single-parameter equations (Eqs. 2 and 3) was significant, the r value in each of these equations was inferior to that in Eq. 4. Inclusion of π^2 in Eq. 4 did not improve the correlation coefficient.

In the *B. subtilis* 104 bacterial system, Eq. 5 gave the best correlation, although it explained only 44% (r^2 = 0.438) of the variance. Linear regression with σ was not statistically significant, the r value being only 0.183. Addition of a π^2 term to Eq. 5 did increase the r value to 0.787, but the t-test showed that both terms were not statistically justified. Thus, the electronic characteristic of the substituents, as measured by the σ term, apparently was not important for activity against *B. subtilis* 104. This result is in contrast with the other two *Bacillus* bacterial systems in which the electronic effect of the substituents played a role in the activity of the compounds.

The only equation containing a π^2 term in Table VIII is Eq. 7. This highly significant equation ($F_{2,9}$ = 18.01; $F_{2,9;\alpha=0.001}$ = 16.39) indicated that the activity of the compounds against *C. perfringens* 13 was a parabolic function of π and predicted an ideal lipophilic value (π_0) of 0.456 for this bacterial system. The fact that the single-parameter equation (Eq. 6) with a π term showed a much smaller correlation coefficient than Eq. 7 supported the existence of a parabolic relationship between the hydrophobic property and activity. Equation 8 explained slightly below 70% (r^2 = 0.693) of the variance in the antibacterial activity against *C. perfringens* 37. It can be seen from Eq. 8 that the hydrophobic and electronic natures of the substituents were significant in determining the level of activity of the compounds against this tetracycline-resistant strain of *C. perfringens*.

Effect of Ia on Macromolecular Synthesis in *C. perfringens* 13—The effect of Ia on protein and DNA syntheses in *C. perfringens* 13 is shown in Fig. 1. Based on the total incorporation of radioactive precursors of these macromolecules, as indicated by precipitable radioactivity, both protein and DNA syntheses were stopped by the compound. This cessation of syntheses occurred within 20–30 min after the addition of Ia and was probably immediate.

Effect of Ia on Cell Wall Synthesis in *C. perfringens* 13—To examine any abnormal cellular morphology that might indicate interaction of Ia with cell wall synthesis, *C. perfringens* 13 was grown in brain heart infusion broth containing 10% (w/v) sucrose. Such a medium allowed the development of spheroplasts when the bacteria were treated with penicillin (5) and bacteriocin 28 (6). No spheroplasts or abnormal cell shapes were apparent in experiments with Ia; in conjunction with isotope data

Table VII—Aromatic Substituent Constants and Antibacterial Activities [a] **(Log 1/*C*) of Ia–Il**

Compound	π [b]	σ [c]	*B. cereus* 7	*B. megaterium* 122	*B. subtilis* 104	*C. perfringens* 13	*C. perfringens* 37
Ia	0.00	0.00	6.61	5.10	5.40	6.38	6.38
Ib	0.70	0.23	6.94	4.53	5.44	6.52	6.16
Ic	1.46	0.60	6.68	4.26	4.57	6.07	6.75
Id	0.86	0.23	7.29	4.27	4.88	6.46	6.40
Ie	1.40	0.46	6.37	4.26	4.57	6.28	6.20
If	0.60	−0.17	6.62	4.81	4.81	6.62	6.32
Ig	1.98	−0.20	5.16	4.25	4.25	5.69	4.93
Ih	0.51	−0.07	6.02	4.81	5.41	6.62	6.49
Ii	1.40	−0.15	5.14	4.54	4.84	5.92	5.32
Ij	1.20	−0.34	6.33	4.82	5.12	6.15	5.73
Ik	−0.33	−0.27	6.64	4.83	5.13	6.33	5.81
Il	−0.04	−0.27	6.64	5.13	4.83	6.21	6.33

[a] These values are the average of at least three MIC determinations. [b] Reference 18. [c] Reference 19.

Table VIII—Regression Equations Generated for Ia–Il

System	Equation[a]	r	s	F
B. cereus 7	1. $\log 1/C = -0.670\ (\pm 0.461)\ \pi + 1.287\ (\pm 1.059)\ \sigma + 6.909\ (\pm 0.475)$	0.779	0.450	0.015
B. megaterium 122	2. $\log 1/C = -0.369\ (\pm 0.199)\ \pi + 4.933\ (\pm 0.210)$	0.794	0.209	0.002
	3. $\log 1/C = -0.682\ (\pm 0.578)\ \sigma + 4.637\ (\pm 0.170)$	0.639	0.264	0.025
	4. $\log 1/C = -0.304\ (\pm 0.170)\ \pi - 0.451\ (\pm 0.390)\ \sigma + 4.882\ (\pm 0.175)$	0.889	0.166	0.001
B. subtilis 104	5. $\log 1/C = -0.352\ (\pm 0.281)\ \pi + 5.223\ (\pm 0.296)$	0.662	0.294	0.019
C. perfringens 13	6. $\log 1/C = -0.249\ (\pm 0.221)\ \pi + 6.473\ (\pm 0.233)$	0.621	0.232	0.031
	7. $\log 1/C = -0.384\ (\pm 0.201)\ \pi^2 + 0.350\ (\pm 0.341)\ \pi + 6.415\ (\pm 1.456)$	0.894	0.140	0.001
C. perfringens 37	8. $\log 1/C = -0.505\ (\pm 0.330)\ \pi + 1.300\ (\pm 0.758)\ \sigma + 6.473\ (\pm 0.340)$	0.833	0.322	0.005

[a] $n = 12$.

presented, Ia apparently had no direct action on cell wall synthesis in *C. perfringens* 13.

EXPERIMENTAL[1]

4-*tert*-Butylbenzophenone—This compound was prepared in 42% yield according to the procedure of Hughes *et al.* (4), bp 146–148.5°/0.3 mm [lit. (4) bp 205°/15 mm].

Substituted Benzhydrols (Vc–Vl)—These compounds were synthesized from aldehydes by the Grignard reactions or the by zinc–sodium hydroxide reduction of benzophenones according to Morris and Blake (3).

Substituted Benzhydryl Chlorides (IIc and IIe–IIl)—A modification of the procedure reported by Bateman *et al.* (2) was followed. A solution of the appropriate benzhydrol (0.075 mole) in anhydrous benzene (150 ml), containing suspended calcium chloride (15 g), was saturated with dry hydrogen chloride by passing the gas through the solution for 75 min. The solution was then poured onto fresh calcium chloride (15 g) and saturated again with dry hydrogen chloride for another 75 min. The solution was finally filtered, and the solvent was removed *in vacuo*. The liquid-substituted benzhydryl chlorides were vacuum distilled whereas the solid chlorides were recrystallized.

1-(Substituted Benzhydryl)piperazines (IIIa–IIIl)—These compounds were prepared according to the method of Kitchen and Pollard (7), using chloroform as the reaction solvent instead of absolute ethanol.

1-(Substituted Benzhydryl)-4-nitrosopiperazines (IIIa–IIIl)—To a cold (0–5°) suspension of the appropriate 1-(substituted benzhydryl)-piperazine (0.012 mole) in hydrochloric acid [60% (v/v), 50 ml] was added an aqueous solution (20 ml) of sodium nitrite (0.024 mole), dropwise and with stirring. The reaction mixture was strongly basified with sodium hydroxide and extracted with chloroform. Removal of the solvent *in vacuo* produced yellow crystals of the nitroso compound.

1-(Substituted Benzhydryl)-4-(5-nitro-2-furfurylideneamino)-piperazines (Ia–Il)—To a solution of the appropriate 1-(substituted benzhydryl)-4-nitrosopiperazine (0.014 mole) in acetic acid (60 ml) was added zinc dust (0.07 mole), with stirring, over 20 min at 10°. After the addition was complete, stirring was continued for 1 hr at 10° and for 2 hr at 60°. The reaction mixture was filtered hot; after cooling, the filtrate was strongly basified with sodium hydroxide.

The solution was then extracted with chloroform and, after being dried over anhydrous magnesium sulfate, the chloroform extracts were evaporated *in vacuo*. The resulting residue was redissolved in ethanol (10 ml) and then refluxed, with stirring, with an ethanolic solution (10 ml) of 5-nitro-2-furaldehyde (~0.007 mole) for 30 min. Stirring was continued at room temperature for a further 30 min. The crystals were then filtered, dried, and recrystallized.

5-Nitro-2-furaldehyde—This compound was prepared by the hydrolysis of 5-nitro-2-furaldehyde diacetate (0.007 mole) in sulfuric acid [7% (v/v), 30 ml]. The solution was refluxed with stirring for 1 hr and then poured onto ice. The crude aldehyde thus obtained melted at 32–35° [lit. (8) mp 32–36°] and was used without further purification. 5-Nitro-2-furaldehyde diacetate was purchased commercially.

Antimicrobial Studies[2]—The methods of preparing the microorganisms for evaluation and determining the MIC's of the compounds were described previously (1).

Effect of Ia on Macromolecular Synthesis in *C. perfringens* 13—Protein and DNA syntheses in *C. perfringens* 13 were measured by the incorporation of radioactive precursors of the respective macromolecules, ^{14}C-protein hydrolysate and ^{3}H-thymidine[3]. Labeled protein hydrolysate and thymidine were added to brain heart infusion broth, separately, to obtain final activities of 0.3 μCi/ml. This broth was distributed in 10-ml volumes into sterile test tubes and boiled for 10 min to drive off dissolved oxygen. The cooled tubes were then inoculated with a 1:10 dilution of a 3-hr culture of *C. perfringens* 13 (0.5 ml).

After 60 min of incubation at 37°, broth containing 50 μg of Ia/ml (2 ml) was added to one-half of the culture tubes; the other half received broth only (2 ml) as control cultures. Incubation continued, and a pair of treated and control tubes was removed from the water bath at various times for assay of precipitable radioactivity.

Aliquots of 3 ml of each culture were precipitated with cold trichloroacetic acid [10% (w/v), 3 ml] for 15 min. The precipitate was collected on 2.4-cm glass fiber filter papers and washed five times with cold trichloroacetic acid (5% w/v) and once with ether. The filters were dried, placed in scintillation fluid[4] [2,5-bis[2-(5-*tert*-butylbenzoxazoyl)]thiophene in toluene, 0.4% (w/v), 15 ml], and counted for two 1-min intervals by a liquid scintillation counter[5].

Effect of Ia on Cell Wall Synthesis in *C. perfringens* 13—A 1:10 dilution of a 3-hr culture of the bacteria (0.5 ml) was added to four sterile test tubes containing brain heart infusion broth with sucrose [10% (w/v), 10 ml] that had been boiled for 10 min and cooled. After 60 min of incubation at 37°, 2 ml of Ia in the same broth was added to three of the four tubes, giving final concentrations of 8.3, 4.2, and 1.0 μg/ml. The last tube received the same volume of broth but with no Ia. Samples were

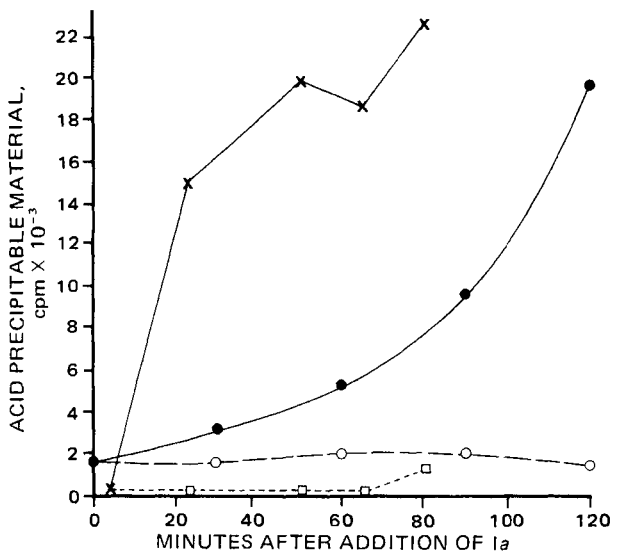


Figure 1—*Effect of Ia on protein and DNA syntheses in* C. perfringens *13 as measured by total uptake of ^{14}C-protein hydrolysate and ^{3}H-thymidine, respectively. Compound was added after 60 min of bacterial growth. Key:* ●, *protein control;* ○, *protein plus Ia;* ×, *DNA control; and* □, *DNA plus Ia.*

[1] Melting points were determined on a Thomas–Hoover capillary melting-point apparatus and are uncorrected. Boiling points are also uncorrected. Elemental analyses were performed by Robertson Laboratory, Florham Park, N.J. IR spectra were recorded on a Perkin-Elmer model 237B spectrophotometer in potassium bromide. A Varian model T-60 spectrometer was used to record the NMR spectra, with deuterated chloroform as the solvent and tetramethylsilane as the internal reference. IR and NMR spectra were in agreement with the assigned structures.

[2] The microorganisms were from the stock culture collections of the Department of Microbiology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia.

[3] New England Nuclear.

[4] Packard Instrument Co.

[5] Nuclear Chicago Mark I.

removed from each tube over 2 hr and examined by phase contrast microscopy at a magnification of 1000× for spheroplasts or abnormal cell shapes.

Regression Analysis of Antibacterial Activities—This analysis was carried out by the method of least squares[6].

REFERENCES

(1) D. K. Yung, D. E. Mahony, and L. W. Whitehouse, *J. Pharm. Sci.*, **60**, 386 (1971).

(2) L. C. Bateman, E. D. Hughes, and C. K. Ingold, *J. Chem. Soc.*, **1940**, 1014.

(3) J. F. Morris and J. T. Blake, *J. Am. Chem. Soc.*, **50**, 1811 (1928).

(4) E. D. Hughes, C. K. Ingold, and N. A. Taher, *J. Chem. Soc.*, **1940**, 949.

(5) D. E. Mahony and T. I. Moore, *Can. J. Microbiol.*, **22**, 953 (1976).

(6) D. E. Mahony, M. E. Butler, and R. G. Lewis, *ibid.*, **17**, 1435 (1971).

(7) L. J. Kitchen and C. B. Pollard, *J. Org. Chem.*, **8**, 338 (1943).

(8) P. M. Kochergin and G. A. Karpov, *Khim. Farm. Zh.*, **1967**, 9; through *Pharm. Chem. J.*, **1967**, 8.

(9) W. E. Craig and E. F. Riener, U.S. pat. 2,783,284 (1957); through *Chem. Abstr.*, **51**, 13927b (1957).

(10) J. G. Burr, Jr., and L. S. Ciereszko, *J. Am. Chem. Soc.*, **74**, 5426 (1952).

(11) H. G. Morren, British pat. 817,231 (1959); through *Chem. Abstr.*, **54**, 9970e (1960).

(12) H. G. Morren, Belgium pat. 544,751 (1956); through *Chem. Abstr.*, **54**, 12167c (1960).

(13) H. G. Morren, S. Trolin, R. Denayer, E. Grivsky, and J. Baricq, *Bull. Soc. Chim. Belg.*, **60**, 282 (1951); through *Chem. Abstr.*, **46**, 8661a (1952).

(14) J. W. Cusic and P. Yonan, U.S. pat. 3,265,687 (1966); through *Chem. Abstr.*, **65**, 13764d (1966).

(15) S. Nishida, *J. Org. Chem.*, **32**, 2692 (1967).

(16) V. J. Skiner, Jr., and C. J. Verbanic, *J. Am. Chem. Soc.*, **79**, 369 (1957).

(17) Abbott Laboratories, British pat. 752,332 (1956); through *Chem. Abstr.*, **51**, 5847a (1957).

(18) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, **71**, 525 (1971).

(19) C. Hansch, A. Leo, S. H. Unger, K. H. Kim, D. Nikaitani, and E. J. Lien, *J. Med. Chem.*, **16**, 1207 (1973).

[6] Using the Statistical Package for the Social Sciences, version 600, and the CDC 6400 computer at the Dalhousie University Computer Centre.

ACKNOWLEDGMENTS

Presented at the 37th International Congress of Pharmaceutical Sciences, The Hague, The Netherlands, September 1977.

Supported by Grant MA-5669 from the Medical Research Council of Canada.

The assistance of Miss A. R. Manzer in preparing some intermediates in the chemical synthesis is gratefully acknowledged.

Ball Milling as a Measure of Crushing Strength of Granules

ASHOK MEHTA, M. A. ZOGLIO ˣ, and J. T. CARSTENSEN *

Received April 15, 1977, from *Merrell-National Laboratories, Cincinnati, OH 45215.* Accepted for publication October 5, 1977. *Present address: School of Pharmacy, University of Wisconsin, Madison, WI 53716.

Abstract □ When granules are milled in a ball mill, the size decrease follows a modification of Kick's law. The Briggsian decay constant, here denoted as the attritional crushing strength, shows correlation with the Harwood–Pilpel crushing strength. Both crushing strengths show a correlation with the amount of granulating agent (povidone) added to the granulation, *i.e.*, the more povidone added, the harder the granule.

Keyphrases □ Granules—ball milling as a measure of crushing strength □ Hardness, granule—ball milling as a measure of crushing strength

The measurement of granule hardness is an important parameter in pharmacy research and quality control. In the simplest method, that of Harwood and Pilpel (1, 2), a granule is placed on a supported plane surface and then brought in contact with the flat bottom of a balance pan. Metal shot is placed in the pan until the granule breaks, and the weight of the shot is then determined. This weight is denoted as the crushing strength of the granule.

It was shown previously (3, 4) that the crushing strength obtained by the Harwood–Pilpel method, when used on granules, requires the averaging of many granules because the observed crushing strength is a function of the way in which the granule is positioned under the pan. The method is fully functional once this fact is realized. An average of 20 measurements suffices for good characterization but is somewhat time consuming. Another disadvantage of the Harwood–Pilpel method is that the crushing strength of granules finer than 40 mesh is difficult (impossible) to measure.

Therefore, an alternative method, equally convenient but applicable to all particle sizes, is desirable. This paper describes such a procedure and shows the correlation between it and the method of Harwood and Pilpel (1) for povidone granulations. Its applicability to other granulations (exemplified by a gelatin granulation) is also discussed.

EXPERIMENTAL

A 6.7-cm i.d. × 13.5-cm long ball mill[1] was used. Granulations were made of the compositions shown in Table I; the povidone was added in a 2-propanol solution to a lactose and corn starch mixture. The dried granules were sized, and the 14/20-mesh fraction was used for further study. Granule hardness was determined by the method of Harwood and Pilpel (1, 3, 4). The lactose used had a particle size of 10–20 μm.

In addition, the following method of determining the crushing strength

[1] Fisher Scientific, Pittsburgh, PA 15219.

0022-3549/78/0700/0905$01.00/0