Antineoplastic Agents LXIV: 1,4-Bis(2′-chloroethyl)-1,4-diazabicyclo[2.2.1]heptane Dihydrogen Dimaleate

GEORGE R. PETTIT [x], DONALD P. GIESCHEN, and WILLIAM E. PETTIT

Received May 9, 1979, from the *Cancer Research Institute and Department of Chemistry, Arizona State University, Tempe, AZ 85281.* Accepted for publication June 12, 1979.

Abstract □ The 1,4-bis(2′-chloroethyl)-1,4-diazabicyclo[2.2.1]heptane dication (II) exhibits remarkable antineoplastic activity. Detailed evaluation of several dianion derivatives showed a curative response level against the murine P-388 lymphocytic leukemia, colon 26, CD8F$_1$ mammary, and the Walker 256 carcinosarcoma (rat) tumor systems. In addition, significant cancer chemotherapeutic activity was found against the murine L-1210 lymphoid leukemia, colon 38, and B16 melanocarcinoma tumor systems. The bicyclo dication (II) first was isolated, evaluated, and stored as the diperchlorate derivative (II*a*). Because of the promising anticancer activity of II*a*, procedures were developed for obtaining other anion derivatives for comparative biological purposes. Several naturally occurring substances were evaluated, and the dihydrogen dimaleate derivative (II*i*) obtained by an ion-exchange technique was the most suitable.

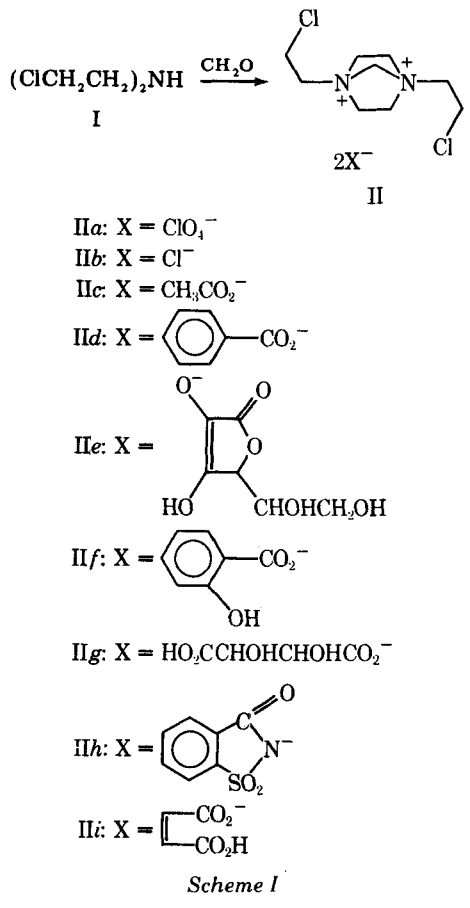
Keyphrases □ Antineoplastic agents—1,4-bis(2′ - chloroethyl) - 1,4-diazabicyclo[2.2.1]heptane cation, synthesis, diperchlorate and dihydrogen dimaleate salts □ 1,4-Bis(2′-chloroethyl)-1,4-diazabicyclo-[2.2.1]heptane cation—antineoplastic activity, synthesis, diperchlorate and dihydrogen dimaleate salts □ Heterocyclic anticancer agents—1,4-bis(2′-chloroethyl)-1,4-diazabicyclo[2.2.1]heptane cation, synthesis, diperchlorate and dihydrogen dimaleate salts

An early exploration of potential anticancer drugs in these laboratories led to the first 1,4-diazabicyclo[2.2.1]-heptane heterocyclic system. The facile preparation of 1,4-bis(2′-chloroethyl)-1,4 - diazabicyclo[2.2.1]heptane diperchlorate (II*a*, NSC 57198) from bis(2-chloroethyl)-amine (I) and formaldehyde has been reported (Scheme I) (1). Later, the structural elucidation (2), preparation of related substances (3), and confirmation of the structural assignment of II*a* by X-ray crystallography (4) were described. Meanwhile, II*a* has been evaluated against the National Cancer Institute's most important experimental tumor systems (Table I). Its promising antineoplastic activity encouraged the investigation of other presumably more suitable (7, 8) anions.

BACKGROUND

Initial isolation and structural investigations (1, 2) of diperchlorate II*a* emphasized that this heterocyclic system was very susceptible to nucleophilic attack, especially by hydroxyl ion, and this observation was confirmed by other investigators (9). As predicted, the very poor nucleophilicity of perchlorate is ideally suited to stabilizing the novel bridged heterocyclic system. Compound II*a* specimens prepared 17 years ago show no evidence of decomposition; II*a* has proven to be stable at temperatures up to 210–222° but burns violently when deliberately ignited. The corresponding dichloride derivative (II*b*), formed in the reaction of I with formaldehyde, was described previously (9). Attempts to improve its isolation (4% yield) and characterization, *e.g.*, by ion-exchange techniques, were not successful.

The objective of this investigation was to locate an anion such as that derived from ascorbic acid [vitamin C (10)] or another naturally occurring substance compatible with human use. The ascorbic acid derivative (II*e*) seemed attractive due to the possibility of increasing the immune response involved with interferon enhancement (11). Derivatives of the readily available maleic acid, a constituent of citrus and grape juices (12, 13), and D-tartaric acid also were considered. These three possibilities were inspected as part of a large series of anions of decreasing nucleophilicity derived from the following acids: acetic, pKa 4.74, II*c*; benzoic, 4.20, II*d*; ascorbic, 4.17, II*e*; salicylic, 2.97, II*f*; D-tartaric, 2.93, II*g*; saccharin, 1.60, II*h*; and maleic, 2.0, II*i*.



Scheme I

Two methods were found for displacing the perchlorate anions. The first procedure was based on the minimal solubility of potassium perchlorate in ethanol–acetonitrile mixtures and was workable except with maleic and D-tartaric acids since their corresponding potassium salts were not sufficiently soluble in this solvent. However, the nucleophilicity of the acetate, benzoate, and ascorbate anions was sufficient to cause decomposition of II*c*–II*e* during isolation. While disalicylate II*f* was stable only in the cold, disaccharin salt II*h* was obtained conveniently by this method and only slowly decomposed at ambient temperatures over 6 months. At this point, the potential bladder carcinogenic properties of saccharin became known; except for preliminary antineoplastic studies (Table I), II*h* was not considered further.

Ion-exchange chromatography was the alternative method utilized for displacing the perchlorate anions. Both ditartarate II*g* and dihydrogen dimaleate II*i* were synthesized by this method. However, II*g* was unstable at room temperature and could be maintained only at approximately −10°. Fortunately, II*i* has been maintained under normal conditions without any detectable change for over 3 years[1]. The well-established

[1] One important criterion for stabilizing cation II is an anion derived from an acid with a dissociation constant corresponding to pKa 2 or less.

0022-3549/79/1200-1539$01.00/0

Table I—Tumor System (5, 6)

Compound	PS[a] Dose, mg/kg	PS[a] T/C, %[b]	PS[a] Cures, %	LE[c] Dose, mg/kg	LE[c] T/C, %	C8[d] Dose, mg/kg	C8[d] T/C, %	C6[e] Dose, mg/kg	C6[e] T/C, %	C6[e] Cures, %
II*a*	400	268	50	150	190	400	47	400	271	60
	200	190		100	189	200	62	200	272	89
	100	154		66.0	154			100	175	30
	50	145		44.0	140			50.0	129	
								25.0	128	
								12.5	120	
II*i*	200 (toxic)	192		200	180			200	186	20
	100	146		100	148	100	45	100	155	10
	50.0	123		50.0	120	50.0	44	50.0	133	10
	25.0	113		25.0	90	25.0	78	25.0	114	10
						12.5	84	12.5	102	10
II*h*	400	296	50					400		20
	200	240						200	171	30
	100	198						100	134	
	50.0	171						50.0	116	
								25.0	106	
III*a*	2.0 (toxic)			2.0	97					
	1.0	167		1.0	154					
	0.50	145		0.50						
	0.25	120		0.25	125					
	0.10	110		0.12	110					
III*b*	> 0.30 (toxic)									
	0.20	111		0.20	125					
	0.10	116		0.10	110					
	0.05	102		0.05	102					

Compound	CD[f] Dose, mg/kg	CD[f] T/C, %	B1[g] Dose, mg/kg	B1[g] T/C, %	LL[h] Dose, mg/kg	LL[h] T/C, %	RO[i] Dose, mg/kg	RO[i] T/C, %	WA[j] Dose, mg/kg	WA[j] T/C, %	WA[j] Cures, %
II*a*	400	6	100	146					80	562	100
	200	24	50.0	116					40	561	66
	100	44	25.0	136	100	136			20	237	33
	50.0	57							10	128	33
	25.0	104							5	128	
	12.5	65									
II*i*	400	0	100	138			200	121			
	200	4	50.0	130			100	124			
	100	19	25.0	129			50.0	110			
	50.0	78	12.5	128			25.0	94			
	25.0	85	6.25	117							
	12.5	16									
II*h*	600	12	200	143							
	400	43	100	120							
	200	43	50.0	117							
	100	93	25.0	108							
	50.0	67									
	25.0	75									
III*a*											
III*b*									0.15	39	
									0.075	85	
									0.037	78	

[a] P-388 lymphocytic leukemia. [b] Test/control. [c] L-1210 lymphoid leukemia. [d] Colon 38. [e] Colon 26. [f] $CD8F_1$ mammary tumor. [g] B16 melanocarcinoma. [h] Lewis lung carcinoma. [i] Ridgway osteogenic sarcoma. [j] Walker carcinosarcoma 256.

stability of II*i* seems consistent with the relatively poor nucleophilic properties expected for the hydrogen maleate anion. The relative instability observed with the saccharin anion may have a less obvious explanation and will be explored.

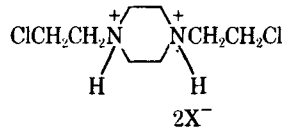
The stable diperchlorate (II*a*) and dihydrogen dimaleate (II*i*) salts showed substantial promise in key experimental tumor systems (Table I) and are being considered for clinical trial; II*a* had the more favorable therapeutic index. Biological data obtained with the disaccharin salt (II*h*) (prior to significant decomposition) also have been included. Comparison of these data with those obtained for piperazine III precludes the possibility that the high antineoplastic activity displayed by the 1,4-bis(2′-chloroethyl)-1,4-diazabicyclo[2.2.1]heptane dication is due significantly to production of this 2-chloroethylamine. The methylene-bridged diquaternary system II definitely is not a nitrogen mustard, and its mechanism of action appears to depend on *in vivo* formation and latent release of a substance(s) other than piperazine III. The *in vivo* production of a very reactive carbon–carbon or carbon–nitrogen bond-forming species (IV or V) derived from formaldehyde is an attractive prospect.

EXPERIMENTAL

Glass-distilled water was employed in the ion-exchange procedures. All other solvents were redistilled. Melting points were determined by the capillary method (oil bath) and are uncorrected. IR spectra[2] (potassium bromide) and PMR (^{1}H-NMR) spectra[3] (δ in parts per million with respect to tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate) were recorded. ^{13}C-NMR spectra (in parts per million downfield from the internal standard tetramethylsilane or *p*-dioxane, 67.4 ppm) also were measured[4] (at 22.6 MHz). NMR spectra were determined in deuterium oxide solution unless otherwise noted. Elemental microanalyses were performed[5].

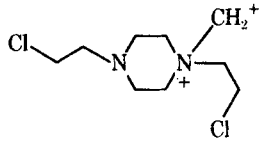
Bis(2-chloroethyl)amine Hydrochloride (I-HCl)—Purification

[2] Perkin-Elmer 299 spectrophotometer.
[3] Varian Associates T-60A or XL-100.
[4] By Dr. J. Witschel, Jr., employing a Bruker WH-90 NMR spectrometer.
[5] Spang Microanalytical Laboratory, Eagle Harbor, Mich.

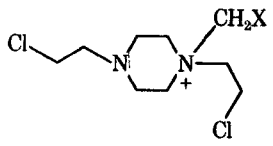


III

IIIa: X = Cl^-
IIIb: X = $HO_2CCH{=}CHCO_2^-$



IV



V

X = —NR_2, —OR, or —SR
R = H or alkyl

of currently available commercial bis(2-chloroethyl)amine hydrochloride, nitrogen mustard hydrochloride, is less efficient than the following improved (14, 15) synthesis. A solution of thionyl chloride (520 ml or 860 g) in chloroform (460 ml) was added slowly through a dropping funnel to a solution of 2,2′-iminodiethanol[6] (212 g) in 600 ml of chloroform. The reaction mixture was contained in a 5-liter, three-necked round-bottom flask equipped for efficient refluxing and rapid mechanical stirring and suspended in a cold water bath (for maintaining the reaction temperature at ~20°).

When the viscous material that formed on the stirring blade reached a constant mass, the remaining thionyl chloride was added rapidly. The water bath was heated, and the mixture was brought to reflux temperature slowly (to minimize foaming). After ~30 min, the yellow solution became clear and was allowed to cool. The crystalline product was collected and washed successively with chloroform (3 × 125 ml) and ether (200 ml) to yield I-HCl (250 g) as colorless crystals decomposing at 212–214°. Two recrystallizations from acetone–absolute ethanol (4:1) provided I-HCl (140 g) as needles decomposing at the same temperature; IR: 2975, 2760, 2440, 1595, 1460, 1445, 1355, 1305, 1050, 1000, 960, 885, 860, 840, 785, 755, 700, and 675 cm^{-1}. The ^{1}H-NMR data were reported previously (16).

Recrystallization of I-HCl obtained by this method was unnecessary for the preparation of II*a*.

1,4-Bis(2′-chloroethyl)-1,4-diazabicyclo[2.2.1]heptane Diperchlorate (II*a*)—The following procedure based on earlier studies (1, 2) is convenient and reliable for preparation of II*a*. A 10% NaOH solution (140 ml) was added in portions to I-HCl (50.0 g) in 100 ml of water. The mixture was extracted with ether (100 ml and 2 × 50 ml), and the combined extract was washed with saturated sodium chloride solution and dried (ice bath) over anhydrous magnesium sulfate. Solvent was removed by rotary evaporation (ice bath temperature) to afford the thermally unstable bis(2-chloroethyl)amine (I*a*) as a colorless oil.

Upon dissolution of the amine in absolute ethanol (150 ml), 37% formaldehyde solution (50 ml) was added quickly. After 1 day at room temperature, the mixture was cooled (ice bath) and 27 ml of 70% perchloric acid was added slowly. Crystallization of diperchlorate II*a* began within a few minutes; when completed, 32.2 g of II*a* was collected and recrystallized from water–ethanol to afford colorless crystals (24.2 g) decomposing at 222–223° [as found for the original elemental analytical sample (1)]; ^{13}C-NMR (deuterium oxide): 37.43 (-CH_2CH_2Cl), 59.73 (ring CH_2CH_2–), 60.05 (-CH_2CH_2Cl), and 83.39 (ring CH_2–). Other spectral and analytical data were reported earlier (2).

1,4-Bis(2′-chloroethyl)-1,4-diazabicyclo[2.2.1]heptane Disaccharin (II*h*)—Freshly prepared (from saccharin and potassium hydroxide in water) potassium saccharin (29.2 g) was dissolved in 95% ethanol (900 ml), and the hot solution was poured rapidly into a solution of diperchlorate II*a* (28.1 g) in acetonitrile (200 ml). After 2.25 hr, the potassium perchlorate precipitate was collected, the filtrate was concentrated to 150 ml, and potassium perchlorate was collected again. The remaining acetonitrile solution was concentrated to a yellow oil, which was dissolved in water and lyophilized to yield 37.4 g (96%) of disaccharin II*h* as pale-yellow flakes; IR: 3460, 3100, 1725, 1610, 1405, 1360, 1315, 1125, 1065, 1025, 880, 835, 770, 735, 705, 660, 610, and 585 cm^{-1}; PMR: δ 4.16–4.41 (m, 8H), 4.48 (s, 8H), 5.66 (s, 2H), and 7.85 (s, 4H).

Attempts to prepare a specimen for elemental analyses led to decomposition, precluding this characterization step. However, the IR and, especially, the ^{1}H-NMR spectra showing the methylene bridge signal at δ 5.66 were in complete accord with the assigned structure. When stored at ambient temperatures in a sealed container, II*h* underwent slow transformation. In approximately 6 months, the original substance remained in only a minor amount. The products are presently under investigation.

By employing the general procedure used to obtain disaccharin II*h*, the following reactions were attempted in hot ethanol–acetonitrile solutions. In each case, freshly prepared potassium acetate, benzoate, ascorbate, and salicylate were allowed to react separately with II*a*. The minimal solubilities of dipotassium D-tartarate and maleate in ethanol–acetonitrile mixtures precluded their use in these syntheses. The diacetate II*c* and dibenzoate II*d* were destroyed rapidly during the isolation. The diascorbate II*e* was transformed partially during isolation, and further characterization proved impractical.

Reaction between potassium salicylate (12.4 g in 250 ml of 95% ethanol) and diperchlorate II*a* (15.0 g) in acetonitrile (200 ml) provided nearly the theoretical amount of potassium perchlorate. Removal of solvent afforded disalicylate II*f* as a pale-yellow oil; PMR (acetonitrile-d_3): δ 4.0–4.8 (complex, 16H), 5.25 (s, H_2O), 5.95 (s, 2H), 6.7–7.1, 7.2–7.5, and 7.8–8.1 (complex, aromatic). Approaches to purifying II*f* led to its rapid destruction, which was monitered easily by observing the disappearance of the methylene bridge protons at δ 5.9 in the PMR spectra. Application of an ion-exchange technique (*cf.*, II*i*) to preparation of the acetate, benzoate, and salicylate derivatives also was unsuccessful[7].

1,4-Bis(2′-chloroethyl)-1,4-diazabicyclo[2.2.1]heptane Dihydrogen Dimaleate (II*i*)—In a typical experiment, a 100-ml volume of ion-exchange resin[8] (chloride form) in a chromatography column was treated with 40 volumes of 1 *N* NaOH/volume of resin. The resulting resin (hydroxide form) was washed with water until the eluate was neutral to pH paper[9]. The resin bimaleate was prepared by elution with 600 ml of 1 *N* maleic acid. The large maleic acid excess allowed conversion to II*i* rather than to the monomaleate derivative. Excess acid was removed by a final elution with 200 ml of water.

A solution of diperchlorate II*a* (20.0 g) in 1 liter of water was passed through the resin. Elution with an additional 0.5 liter of water and lyophilization of the combined fractions afforded 18.2 g (85%) of dihydrogen dimaleate II*i*. Three recrystallizations from ethanol provided an analytical specimen melting at 118–119° (slight decomposition); IR: ν_{max} 3440, 3010, 2920, 2850, 1580, 1495, 1385, 1365, 1195, 1110, 1070, 995, 875, 865, 750, and 695 cm^{-1}; PMR: δ 4.16–4.41 (m, 8H), 4.47 (s, 8H), 5.68 (s, 2H), and 6.32 (s, 2H).

Anal.—Calc. for $C_{17}H_{24}Cl_2N_2O_8$: C, 44.85; H, 5.31; Cl, 15.57; N, 6.15; O, 28.11. Found: C, 44.95; H, 5.36; Cl, 15.56; N, 6.14; O, 27.99.

The ion-exchange technique for obtaining II*i* was applied analogously to the preparation of the dihydrogen ditartarate II*g* from 20 g of II*a*. The 1.5-liter water eluate led to 20.2 g of glassy residue on lyophilization. The product was precipitated three times with ether from ethanol–water, and a water solution was relyophilized to yield II*g* as a pale-yellow powder; PMR: δ 4.16–4.41 (m, 8H), 4.47 (s, 8H), 4.83 (s, 2H), and 5.68 (s, 2H). Because of the persistent decomposition of this salt on further attempts at purification and relatively short storage at ambient temperatures, satisfactory elemental analyses were not obtained.

REFERENCES

(1) G. R. Pettit and J. A. Settepani, *Chem. Ind. (London)*, **1964**, 1805.

(2) G. R. Pettit, D. C. Fessler, and J. A. Settepani, *J. Org. Chem.*, **34**, 2978 (1969).

(3) D. C. Fessler, G. R. Pettit, and J. A. Settepani, *J. Med. Chem.*, **12**, 542 (1969).

(4) D. J. Abraham, R. D. Rosenstein, and G. R. Pettit, *ibid.*, **14**, 1141 (1971).

(5) G. R. Pettit, and G. C. Cragg, "Biosynthetic Products for Cancer Chemotherapy," vol. 2, Plenum, New York, N.Y., 1978.

(6) R. I. Geran, N. H. Greenbert, M. M. Macdonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep. (part 3)*, **3**, 2 (1972).

[6] Aldrich Chemical Co.

[7] Experiments were performed by Mr. Scott D. Sucher.

[8] Mallinckrodt Amberlite IRA-400.

[9] Hydrion.

(7) J. C. Schumacher, "Perchlorates," Reinhold, New York, N.Y., 1960, pp. 65, 171–179.
(8) B. Blank, N. W. Di Tullio, A. J. Krog, and H. L. Saunders, *J. Med. Chem.*, **21**, 489 (1978).
(9) H. Böhme and H. Orth, *Arch. Pharm.*, **300**, 148 (1967).
(10) I. M. Sharman, *Endeavour*, **1**, 97 (1977).
(11) B. V. Siegel and J. I. Morton, *Experientia*, **33**, 393 (1977).
(12) E. P. Yufera, J. Sanchez, and J. Alberola, *Rev. Agroquim. Tecnol. Aliment.*, **5**, 121 (1965); through *Chem. Abstr.*, **63**, 12235e (1965).
(13) O. Colagrande, *Ann. Microbiol. Enzimol.*, **9**, 62 (1959); through *Chem. Abstr.*, **54**, 18681a (1960).
(14) F. G. Mann, *J. Chem. Soc.*, **1934**, 461.
(15) K. Ward, *J. Am Chem. Soc.*, **57**, 914 (1935).
(16) G. R. Pettit, J. A. Settepani, and R. A. Hill, *Can. J. Chem.*, **43**, 1792 (1965).

ACKNOWLEDGMENTS

Supported by Public Health Service Research Grant CA-16049-02-05 from the National Cancer Institute, the Fannie E. Ripple Foundation, Mrs. Mary Dell Pritzlaff, the Olin (Spencer T. and Ann W.) Foundation, Mr. Robert B. Dalton, the Phoenix Coca Cola Bottling Co., and the ARCS Foundation Phoenix Chapter.

The authors thank Dr. M. I. Suffness and Miss B. J. Abbott for undertaking these biological studies. Appreciation also is extended to Dr. J. D. Douros, Dr. R. Engle, Dr. K. Paull, and Dr. H. B. Wood for encouragement and assistance.

For Part LXIII of this series, see J. Polonsky, Z. Varon, C. Marazano, B. Arnoux, G. R. Pettit, J. M. Schmidt, and M. Ochi, *Experientia*, in press.

Tablet Position and Basket Type Effects in Spin-Filter Dissolution Device

STEPHEN A. HOWARD ˣ, JOHN W. MAUGER, AURWAN KHWANGSOPHA, and DEBORAH A. PASQUERELLI

Received December 18, 1978, from the *School of Pharmacy, West Virginia University, Morgantown, WV 26506.* Accepted for publication June 26, 1979.

Abstract □ The effects of stirring and basket placement on tablet dissolution using the previously developed Shah spin-filter device were investigated. Visualization of flow and dissolution patterns was possible by testing nondisintegrating colored tablets. Dissolution experiments were conducted on nondisintegrating double-layered tablets containing salicylic acid as the dissolving layer and ethylcellulose as an inert nondissolving layer. Visual observations revealed that color was drawn more rapidly from the tablet face resting on the bottom of the basket. Dissolution data from multilayered tablets revealed that when the salicylic acid face was resting on the bottom of the basket, the dissolution was appreciably more rapid than when it was facing up in the basket. This phenomenon was found for several stirring speeds.

Keyphrases □ Dissolution devices—spin filter, effects of tablet position and basket type, comparison to USP basket method □ Dissolution testing systems—spin filter compared to USP basket method, effects of tablet position and basket type □ Dosage forms—tablets, dissolution testing, spin filter compared to USP basket method, effects of tablet position and basket type

The dissolution profile of oral solid dosage forms is useful for controlling formulation and process variables that influence the bioavailability of the active ingredient.

An apparatus receiving acceptance by the scientific community is the spin-filter dissolution device developed by Shah *et al.* (1, 2). This apparatus provides a dynamic *in situ* microporous nonclogging rotating filter, which permits continuous and efficient filtration of the dissolution fluid. Furthermore, its relatively large filter area extends over most of the dissolution fluid, which permits representative sampling of the bulk dissolution medium. The smooth cylindrical surface of the rotating filter assembly without impeller blades and its agitation over a long vertical axis provide uniform, mild, laminar, nonturbulent, and reproducible stirring even at relatively high speeds.

In addition to a change in the stirring method from the USP apparatus (3), the basket mesh size was changed from the 40 mesh used in the USP basket to 12 mesh (1, 2). This change was made to prevent clogging of the basket pores by disintegrating tablets.

BACKGROUND

A study was undertaken to examine the spin-filter dissolution device hydrodynamically. Acceptance will probably increase since the spin filter was announced as acceptable for industrial dissolution testing by the Food and Drug Administration (4).

Several studies have been undertaken to examine dissolution devices hydrodynamically and to compare the spin-filter device to other dissolution devices (5–11). Skelly (12) and Grostic (13) highlighted the need for dissolution device standardization and suggested the use of nondisintegrating salicylic acid tablets as a calibrator to overcome device-to-device and laboratory-to-laboratory variations.

A carefully controlled study of the diffusion and hydrodynamic variables in the spin device seemed necessary. Nondisintegrating tablets were used so that hydrodynamic flow patterns rather than basket clogging or particle buildup would be the chief measurement parameter. Dye studies were initiated to measure visibly the large-scale flow patterns that would affect the dissolution of disintegrating as well as of nondisintegrating tablets. Nondisintegrating salicylic acid tablets were used (5) in this study, although special double-layered tablets were valuable for measuring position face as a variable. Direct correlation with nondisintegrating tablets may not be warranted, but such a study would provide valuable information in quantitating hydrodynamic effects and would apply directly to nondisintegrating dosage forms such as sustained-release products.

EXPERIMENTAL

All reported dissolution data were obtained using a device[1] reported by Shah *et al.* (1, 2) with the sample basket removed. The basic features

[1] Rotating filter dissolution device, courtesy of The Upjohn Co., Kalamazoo, Mich.

0022-3549/79/1200-1542$01.00/0