it may be necessary to add a small amount of formic acid to the tablet material to release salicylic acid into the solution (3, 11).

The average result for 10 individual weighings from a prepared 325-mg commercial sample composite was 0.048% salicylic acid with a relative standard deviation of 3.9%.

Table I shows a comparison of the results of the fluorometric HPLC procedure with the official USP XIX procedure. The samples are the same as those reported in Table II in Ref. 8. The results were obtained on composites prepared from commercial tablet samples.

Table II shows a comparison of the fluorometric HPLC procedure with the USP XIX method and with a semiautomated colorimetric procedure (8) for samples collected during the aspirin survey. The results listed for each manufacturer were not determined on the same composite.

Baum and Cantwell (12) recently reported the simultaneous determination of salicylic acid in aspirin formulations by HPLC with UV detection. The Baum and Cantwell method was noted only after the HPLC procedure was being used routinely on manufactured formulations in this laboratory.

The hydrolysis of aspirin to salicylic acid (12) is well recognized. For this reason, a time limit was placed on the sample preparation in the procedure. Sample preparation times exceeding 10 min were unsatisfactory for accurate and precise recoveries.

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Aspirin—A National Survey III: Determination of Impurities in Bulk Aspirin and Aspirin Formulations by High-Pressure Liquid Chromatography and Spectrophotometry

ROSS D. KIRCHHOEFER ˣ, JOHN C. REEPMEYER, and WILLIAM E. JUHL

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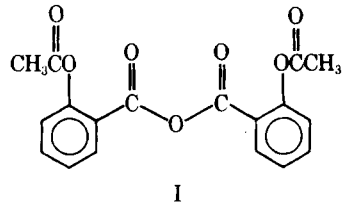
Abstract □ A quantitative high-pressure liquid chromatographic method, using a reversed-phase column and an aqueous acetic acid–methanol solution as the mobile phase, was employed for the determination of *O*-acetyl-*O*-salicylsalicylic acid and *O*-salicylsalicylic acid in pharmaceutical aspirin preparations. The aspirin was dissolved, filtered, and injected into the chromatograph. The absorbance of the impurities was measured at 254 nm. Acetylsalicylic anhydride was determined by a spectrophotometric method. The aspirin was dissolved in pH 11.3 buffer and extracted with benzene. An aliquot of the benzene was evaporated, and the residue was dissolved in α-benzamidocinnamate–pyridine reagent. The acetylsalicylic anhydride was measured using the difference between the absorbance at 362 and 372 nm. Possible interference of aspirin with the procedure is discussed. Thirty-four bulk aspirin and 172 tablet formulations were examined. Results for *O*-acetyl-*O*-salicylsalicylic acid, *O*-salicylsalicylic acid, and acetylsalicylic anhydride are given.

Keyphrases □ *O*-Acetyl-*O*-salicylsalicylic acid—high-pressure liquid chromatographic determination □ Analgesics—determination of impurities in aspirin □ Aspirin—determination of impurities □ *O*-Salicylsalicylic acid—high-pressure liquid chromatographic determination □ Acetylsalicylic anhydride—spectrophotometric determination

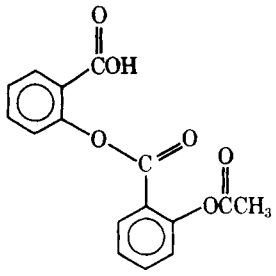
Acetylsalicylic anhydride (I), *O*-acetyl-*O*-salicylsalicylic acid (II), and salicylic acid have been determined in aspirin tablets by high-pressure liquid chromatography (HPLC) (1–5), GLC (1, 3, 6–9), TLC (10, 11), and spectrophotometry (12, 13).

The HPLC procedure of Ali (1) was used in these laboratories to determine impurities in aspirin products. However, certain reversed-phase HPLC columns did not separate I from another impurity, which was isolated and identified as *O*-salicylsalicylic acid (III) (14). Compound I also is decomposed rapidly while in contact with the mobile phase. Therefore, this procedure was used only for the measurement of II and III. Additional impurities were found in commercial samples but have not been identified.

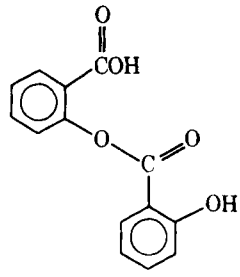
Bundgaard (5) postulated the presence of III and *p*-acetoxybenzoic acid in aspirin formulations based on their HPLC retention times. Bundgaard indicated that these



I



II



III

0022-3549/80/0500-0550$01.00/0

Table I—Amounts of I–III as Percent of Declared Aspirin in Aspirin Tablets

Tablet[a]	Tablet Dosage	I	II	III
A	324 mg	0.004	0.148	0.02
	324 mg	0.005	0.175	0.01
	324 mg	0.004	0.193	0.02
	324 mg	0.007	0.170	0.02
B	421 mg	0.075	0.210	0.02
	421 mg	0.067	0.506	0.03
	421 mg	—[b]	0.358	0.04
	421 mg	—[b]	0.247	0.02
C	324 mg	0.006	0.131	Trace[c]
	324 mg	0.036	0.065	Trace
	81 mg	0.036	0.037	0.02
	324 mg	0.009	1.057	0.02
	324 mg	0.002	0.046	Trace
	324 mg	0.003	0.269	—[d]
	324 mg	0.007	0.041	0.01
	160 mg	—[b]	0.036	Trace
D	486 mg[e]	0.006	0.141	0.01
	486 mg[e]	0.043	0.415	0.02
	324 mg	0.011	0.346	0.01
	324 mg	—[b]	0.301	0.01
	324 mg	0.018	0.237	0.01
	324 mg	0.026	0.223	0.02
	486 mg[e]	0.004	0.142	—[d]
	486 mg[e]	0.006	0.128	0.01
	486 mg[e]	0.003	0.173	0.01
E	324 mg[e]	—[b]	0.610	0.06
F	324 mg[e]	0.015	0.241	0.06
	324 mg[e]	—[b]	0.506	0.06
	324 mg[e]	—[b]	0.247	0.03
G	324 mg	0.018	0.099	—[d]
	324 mg	0.011	0.169	Trace
	324 mg	0.012	0.029	—[d]
H	81 mg	0.033	0.051	—[d]
	81 mg	0.050	0.068	—[d]
	81 mg	0.004	0.018	—[d]
	324 mg	0.005	0.022	—[d]
	324 mg	0.006	0.020	—[d]
	324 mg	0.008	0.029	—[d]
	324 mg	0.005	0.089	—[d]
	81 mg	—[b]	0.054	Trace
I	324 mg	0.002	0.092	0.01
	81 mg	—[b]	0.097	0.03
	324 mg	0.003	0.147	0.02
	324 mg	0.001	0.095	0.01
	324 mg	0.003	0.991	0.02
J	324 mg	0.022	0.109	0.01
K	324 mg	0.012	0.097	0.02
	81 mg	0.034	0.418	0.04
	81 mg	0.011	0.083	0.01
	324 mg	0.001	0.065	0.01
L	324 mg[e]	0.038	1.040	0.03
	324 mg[e]	0.039	0.540	0.03
	324 mg[e]	0.040	0.371	0.02
M	324 mg	0.006	0.230	0.01
N	324 mg	0.023	0.173	—[d]
	324 mg	0.014	0.165	—[d]
	324 mg	0.022	0.079	—[d]
	324 mg	0.003	0.094	0.01
O	324 mg[e]	0.002	0.155	0.01
	324 mg	0.023	0.199	0.02
P	324 mg	0.001	0.056	Trace
Q	324 mg	—[b]	0.013	—[d]
	324 mg	0.014	0.033	Trace
	324 mg	0.024	0.088	0.01
R	324 mg	0.007	0.016	—[d]
	324 mg	0.004	0.027	—[d]
	324 mg	0.030	0.055	Trace
	324 mg	0.006	0.060	Trace
	324 mg	0.005	0.091	—[d]
S	324 mg	0.003	0.419	0.08
	324 mg	—[b]	0.094	0.01
	324 mg	0.007	0.872	0.16
	324 mg	0.004	0.548	0.09
	324 mg	0.004	0.490	0.08
	324 mg	0.004	0.493	0.08
	324 mg	0.008	0.418	0.09
	324 mg	0.012	0.492	0.08
	324 mg	0.007	0.518	0.10
	324 mg	—[b]	0.513	0.09
	324 mg	0.018	0.470	0.11[f]
T	81 mg	0.044	0.166	Trace
	81 mg	0.006	0.106	0.01
	324 mg[e]	0.007	0.383	0.25
	324 mg[e]	—[b]	0.513	0.04
	324 mg[e]	0.001	0.355	0.04
	324 mg[e]	0.004	0.202	0.03
U	81 mg	0.015	0.124	0.02
	81 mg	0.008	0.099	0.02
	81 mg	0.012	0.107	0.02
	324 mg[e]	0.005	0.231	0.04
	324 mg[e]	0.005	0.199	—[d]
	324 mg[e]	0.004	0.193	0.01
	324 mg[e]	0.012	0.137	0.02
	81 mg	0.010	0.140	0.01
	324 mg[e]	—[b]	0.219	0.03
V	81 mg	0.008	0.090	0.01
	81 mg	0.003	0.125	0.01
	81 mg	0.003	0.092	0.01
	81 mg	0.003	0.147	0.02
	324 mg[e]	0.008	0.197	0.02
	324 mg[e]	0.005	0.171	0.02
	324 mg[e]	0.007	0.202	0.02
	324 mg[e]	0.004	0.215	0.02
	324 mg[e]	0.004	0.188	0.02
W	324 mg	0.004	0.026	—[d]
	324 mg	0.012	0.152	0.09
	324 mg	0.002	0.067	0.01
X	81 mg	0.013	0.140	Trace
	81 mg	0.012	0.116	0.01
	81 mg	0.028	0.117	Trace
	324 mg	0.012	0.142	0.001
	324 mg	0.004	0.094	—[d]
	324 mg	0.001	0.071	0.01
	81 mg	0.021	0.207	0.03
	81 mg	0.022	0.207	0.02
	324 mg	0.002	0.054	Trace
Y	324 mg	0.006	0.012	Trace
	648 mg	0.007	0.094	Trace
	324 mg	0.009	0.060	0.01
	486 mg[e]	0.014	0.286	0.02
	81 mg	0.008	0.041	—[d]
	648 mg	0.001	0.108	0.01
	324 mg	0.001	0.077	0.01
Z	324 mg	0.001	0.031	—[d]
	486 mg	0.001	0.077	0.01
	486 mg	0.001	0.129	0.01
AA	81 mg	0.015	0.308	0.03
	300 mg	0.288	0.177	0.02
BB	324 mg	0.001	0.028	—[d]
CC	324 mg[e]	0.010	0.598	0.06
	324 mg[e]	0.019	0.480	0.07
DD	324 mg[e]	—[b]	0.285	Trace
	324 mg[e]	—[b]	0.682	0.05
	324 mg[e]	—[b]	0.276	0.02
EE	325 mg	0.003	0.331	—[d]
	81 mg	—[b]	0.027	0.01
	325 mg	0.002	0.281	0.01
	81 mg	—[b]	0.194	0.01
	81 mg	—[b]	0.229	Trace
	81 mg	0.022	0.212	—[d]
	325 mg	0.003	0.296	—[d]
	325 mg	0.002	0.319	—[d]
	325 mg	0.002	0.336	—[d]
FF	324 mg	0.013	0.276	0.03
	324 mg	0.010	0.231	0.01
	324 mg	0.024	0.190	0.02
	324 mg	0.012	0.195	0.02
GG	81 mg	0.010	0.120	Trace
	324 mg	0.244	0.028	—[d]
	324 mg[e]	0.019	1.358	0.05
	324 mg	0.012	0.026	—[d]
	324 mg[e]	—[b]	0.833	0.04
	324 mg[e]	0.027	0.499	0.02
HH	65 mg	—[b]	0.081	0.02
	320 mg	0.003	0.036	Trace
	320 mg	0.002	0.027	—[d]
II	324 mg[e]	—[b]	0.253	0.01
	324 mg[e]	—[b]	0.340	0.02
JJ	81 mg	0.010	0.120	Trace
	81 mg	0.005	0.175	Trace
	324 mg[e]	0.014	0.241	0.02

(continued)

Table I—*Continued*

Tablet[a]	Tablet Dosage	I	II	III
	324 mg	0.002	0.052	—[d]
	324 mg	0.001	0.093	—[d]
KK	486 mg[e]	0.010	0.231	0.02
	486 mg[e]	0.038	0.337	0.01
	486 mg[e]	—[b]	0.339	0.02
	486 mg[e]	0.008	0.346	0.02
	486 mg	0.044	0.306	0.02
LL	325 mg[e]	—[b]	0.140	0.02
	325 mg[e]	—[b]	0.217	0.04
	325 mg[e]	—[b]	0.185	0.02

[a] A = Bell Pharmacal, Greenville, S.C.; B = Block Drug Co., Memphis, Tenn.; C = Bowman Pharmaceuticals, Canton, Ohio; D = Bristol-Myers Co., New York, N.Y.; E = Chromalloy American Corp., Culver City, Calif.; F = Otis Clapp & Sons, Boston, Mass.; G = Cord Laboratories, Detroit, Mich.; H = Davis Manufacturing Co., Knoxville, Tenn.; I = Dewey Products Co., Grand Rapids, Mich.; J = Ferndale Laboratories, Ferndale, Mich.; K = Freeda Vitamins, New York, N.Y.; L = ICN Pharmaceuticals, Cincinnati, Ohio; M = Lannett Co., Philadelphia, Pa; N = Eli Lilly & Co., Indianapolis, Ind.; O = Mallard, Detroit, Mich.; P = Manhattan Drug Co., Hillside, N.J.; Q = Marshall Pharmacal Corp., Hackensack, N.J.; R = McKesson Laboratories, Fairfield, Conn.; S = Norwich-Eaton Pharmaceuticals, Norwich, N.Y.; T = Oak Park Pharmaceuticals, Fredonia, Wis.; U = Pennex Products Co., Pittsburgh, Pa; V = L. Perrigo Co., Allegan, Mich.; W = Pill Mill, Grand Rapids, Mich.; X = Plough, Memphis, Tenn.; Y = Rexall Drug Co., St. Louis, Mo; Z = Richlyn Laboratories, Philadelphia, Pa; AA = Sein-Mendez Labs, Rio Piedas, Puerto Rico; BB = Stanback Co., Salisbury, N.C.; CC = Standard Pharmacal Co., Chicago, Ill.; DD = Stanley Laboratories, Portland, Ore.; EE = Sterling Drug, New York, N.Y.; FF = E. R. Squibb & Sons, New York, N.Y.; GG = Sun Laboratories, Portland, Ore.; HH = Vale Chemical Co., Allentown, Pa.; II = Walgreen Co., Chicago, Ill.; JJ = West-Ward, Eatontown, N.J.; KK = Whitehall Laboratories, New York, N.Y.; and LL = Zenith Laboratories, Hoboken, N.J. [b] No valid reading was obtained. [c] Trace = <0.003% of II or <0.01% of III. [d] Not detected. [e] Buffered tablet formulation. [f] Sample from which *O*-salicylsalicylic acid was isolated and identified.

impurities were present in salicylic acid, the starting material for aspirin. The presence of III in aspirin formulations was confirmed in this laboratory and was determined to be a commonly occurring impurity in commercial aspirin preparations. This paper presents the first procedure for the quantitation of III in aspirin and its formulations.

The spectrophotometric procedure of Bundgaard and Bundgaard (13) was modified and used to determine I in bulk aspirin and in plain and buffered tablets, and an HPLC method was used to determine II and III in these products. Thirty-four bulk aspirin products, representing 12 foreign and domestic bulk drug suppliers, and 172 tablet formulations, representing 38 manufacturers, were analyzed for I–III.

EXPERIMENTAL

Chromatographic Method—*Apparatus*—A high-pressure liquid chromatograph[1] with a single-wavelength 254-nm detector was used. The operating conditions, column, and reagents were as described previously (15), except that the flow rate was 1.5 ml/min and the amount of acetic acid was increased to 2.5%.

Standard Stock Solution—Approximately 10 mg of *O*-acetyl-*O*-salicylsalicylic acid, synthesized according to the general method of Chattaway (16), and 10 mg of *O*-salicylsalicylic acid[2] were weighed accurately, transferred to a 10-ml volumetric flask, and dissolved in and diluted to volume with methanol.

Standard Working Solution—A 1.0-ml aliquot of the stock solution was transferred to a 10-ml volumetric flask and diluted to volume with the mobile phase. This solution was transferred to the HPLC vial, the vial was capped, and 40 μl of the solution was injected.

Sample Preparation—An amount of powder equivalent to 324 mg of aspirin for tablet formulations or 700 mg for bulk aspirin was transferred to a 25-ml erlenmeyer flask. The mobile phase (10.0 ml) was added, and the solution was shaken or treated ultrasonically for 2–3 min. The solution was filtered, if needed, through a 0.6-μm filter[3]. After the first milliliter was discarded, the HPLC vial was filled and capped, and 40 μl of the solution was injected.

[1] Model 1084A, Hewlett-Packard Co., Avondale, PA 19311.
[2] K&K Laboratories, Plainview, NY 11803.
[3] BDWP01300, Millipore Corp., Bedford, MA 01730.

Table II—Amounts of I–III (Percent of Assay) in Bulk Aspirin

Tablet[a]	Bulk Drug[b]	I	II	III
A	ZZ	0.026	0.906	0.05
B	MA	0.014	0.123	Trace[c]
C	MA	0.003	0.042	—[d]
C	MA	0.024	0.148	—[d]
D	ZZ	0.044	0.210	Trace
F	MA	0.005	0.730	0.02
H	MA	0.004	0.065	—[d]
H	XX	0.007	Trace	—[d]
J	MA	0.007	0.088	Trace
K	MA	0.003	0.050	—[d]
N	ZZ	0.009	0.063	Trace
O	MA	0.020	0.058	Trace
O	MA	0.011	0.088	Trace
Q	WW	0.012	0.029	—[d]
R	VV	0.010	0.022	—[d]
S	S	0.016	0.229	0.03
T	MA	0.010	0.065	—[d]
T	MA	0.028	0.130	—[d]
V	ZZ	0.026	0.236	0.02
V	MA	0.002	0.032	—[d]
W	MA	0.005	0.023	—[d]
X	MA	0.012	0.049	—[d]
X	ZZ	0.015	0.107	Trace
Y	ZZ	0.020	0.097	Trace
Z	UU	0.320	0.073	Trace
AA	MA	0.014	0.122	0.12[e]
BB	MA	0.009	0.049	Trace
CC	TT	—[f]	0.188	Trace
EE	EE	0.068	0.345	0.02
FF	RR	0.043	0.122	0.02
GG	QQ	0.019	0.035	—[d]
HH	MA	0.019	0.455	—[d]
II	MA	0.012	0.040	0.01
LL	SS	—[f]	Trace	—[d]

[a] See Table I, footnote *a*. [b] MA = Monsanto Chemical Co., St. Louis, Mo.; QQ = Polfa-Starogard, Poland; RR = Schering AG, West Germany; SS = Kim, Romania; TT = McKesson and Robbins Co., Oak Lawn, Ill.; UU = Flavine International, Northvale, N.J.; VV = Polfa Pharmaceutical Works, Warsaw, Poland; WW = unknown, Romania; XX = Graynor Chemical Co., Pine Brook, N.J., supplier Poland; and ZZ = Dow Chemical Co., Midland, Mich. [c] Trace = <0.003% of II or <0.01% of III. [d] Not detected. [e] Salicylic acid content of 0.30%. [f] No valid reading was obtained.

Quantitation—The concentration of each impurity was determined by comparing its peak area, as measured by the data system integrator, with that of its standard.

Spectrophotometric Method—The method of Bundgaard and Bundgaard (13) was modified for determining I. Compound I was ex-

Table III—Amounts of I–III (Percent of Declared Aspirin) and Correlation of Bulk Aspirin with Manufactured Tablets

Tablet[a]	Bulk Drug[b]	Type of Sample	I	II	III
H	XX	Bulk	0.007	Trace[c]	—[d]
		324-mg tablet	0.007	Trace	—[d]
		324-mg tablet	0.005	0.089	—[d]
		324-mg tablet	0.006	0.020	—[d]
		324-mg tablet	0.004	0.018	—[d]
		324-mg tablet	0.005	0.022	—[d]
H	MA	Bulk	0.004	0.065	—[d]
		81-mg tablet	0.050	0.068	—[d]
		81-mg tablet	0.033	0.051	—[d]
T	MA	Bulk	0.028	0.130	—[d]
		324-mg tablet[e]	0.007	0.383	0.25[f]
		324-mg tablet[e]	—[g]	0.513	0.04
		324-mg tablet[e]	0.001	0.355	0.04
		Bulk	0.010	0.065	—[d]
		81-mg tablet	0.044	0.539	Trace
W	MA	Bulk	0.005	0.023	—[d]
		324-mg tablet	0.004	0.026	—[d]
O	MA	Bulk	0.020	0.058	Trace
		324-mg tablet[e]	—[g]	0.155	0.01
J	MA	Bulk	0.07	0.088	Trace
		324-mg tablet	0.022	0.109	0.01

[a] See Table I, footnote *a*. [b] MA = Monsanto Chemical Co., St. Louis, Mo.; and XX = Graynor Chemical Co., Pine Brook, N.J., supplier Poland. [c] Trace = <0.003% of II or <0.01% of III. [d] Not detected. [e] Buffered formulation. [f] Salicylic acid content of 2.1%, limit 3.0%. [g] No valid reading was obtained.

tracted from a powdered sample containing 500 mg of aspirin. To an evaporated portion of the benzene extract was added 3 ml of α-benzamidocinnamate–pyridine reagent, producing an azlactone. The solution was read in the UV region after 25 and 60 min.

The method of calculation was changed from the given procedure by subtracting the absorbance of the minimum at 372 nm from the absorbance of the maximum at 362 nm and using this net absorbance. The standard was synthesized according to Bundgaard and Bundgaard (13).

RESULTS AND DISCUSSION

Compounds II and III were stable in the chromatographic solvents and were determined by this procedure; I was unstable in the mobile phase and decomposed rapidly during analysis. Therefore, I could not be determined by HPLC. While attempting to measure I, it was found that some C_{18} reversed-phase HPLC columns would not separate I from III. However, the column (15) used in the present work separated I and III. Compound III has not previously been identified in aspirin formulations, but its presence was postulated by Bundgaard (5) based on its retention time on silica gel HPLC. Compound III has been identified in these laboratories by IR, UV, and mass spectrometry and TLC after isolation (14).

The original spectrophotometric procedure (13) gave results that indicated that aspirin did not interfere with the determination of I. However, aspirin itself will react with the reagent but at a much slower rate than I. When as little as 0.044% (0.22 mg) aspirin was extracted with I, it contributed to an artificially high value of I by 0.004% (additive with the percentage of I present) after a 30-min reaction period and by 0.008% after 60 min. During the survey, results for I averaged ~0.015%.

Aspirin interference in this analysis can be determined by following the reaction over a period of time. Absorbance readings were taken at 25 and 60 min. A continual increase in absorbance indicated the presence of aspirin, and the quantitation was considered invalid. Thorough shaking of the pH 11.3 buffer solution with benzene, followed by centrifugation, usually removed the aspirin to permit determination of I.

Table I lists the amounts of I–III found in a national survey of aspirin formulations, and Table II lists the amounts found in bulk aspirin. The levels of I–III listed for bulk aspirin from the same bulk drug suppliers sometimes were different, presumably because they were from different lots.

Linearity for II and III was established at levels down to 0.003 and 0.01% (0.01 and 0.03 mg/10 ml) of declared aspirin, respectively. A recovery (from a synthetic starch–lactose formulation) of 100% for II and III was obtained at the 0.03% level relative to aspirin.

Table III shows the only correlations available to match the same lot of bulk aspirin to the finished tablets. Bundgaard (5) stated that the levels of I and II found in tablets originate from the starting bulk aspirin. In general, the level of II was lower in bulk aspirin than in tablets manufactured from that bulk, indicating that aspirin is converted to II during or after tablet manufacture (Table III).

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Synthesis and Anticancer Activity of Asparagine Analogs

MICHAEL J. MURPHY and JAMES F. STUBBINS ˣ

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Abstract □ A series of 11 asparagines substituted on N^4 was prepared and evaluated for their ability to inhibit the growth of L5178Y leukemia cell cultures. These cells require an exogenous source of L-asparagine and should be sensitive to an asparagine antimetabolite. The compounds were prepared by reaction of phthalylaspartic anhydride with a primary or secondary amine, followed by removal of the phthalyl group with hydrazine. One compound, *N,N*-dibenzylasparagine, showed significant activity. Additional study of asparagine derivatives bearing large, lipophilic groups at N^4 is warranted.

Keyphrases □ Asparagine analogs—synthesis and anticancer activity □ Anticancer activity—asparagine analogs □ Antileukemic activity—asparagine analogs

There has been great interest in the enzyme L-asparaginase (L-asparagine amidohydrolase, E.C. 3.5.1.1) since it was shown (1) to be the active antitumor agent found in guinea pig serum. Several tumor cell types are sensitive to L-asparaginase treatment, including some human leukemias (2–4). Dramatic remissions were obtained with sensitive tumors, but resistance to the L-asparaginase treatment developed rapidly (4).

BACKGROUND

L-Asparaginase acts on sensitive tumors by removing the required exogenous supply of L-asparagine (I), whereas most normal cells and resistant tumors can synthesize L-asparagine (5). Large doses of injected L-asparaginase rapidly reduced L-asparagine below detectable levels in the plasma, but extracellular fluid concentrations were reduced by only 70% (6). Thus, leukemic cells in the bloodstream generally are killed, but some inaccessible cells have a good chance of survival on the remaining tissue levels of L-asparagine. Furthermore, those cells that survive long

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