(11) G. Redl, R. D. Cramer, and C. E. Berkoff, *Chem. Soc. Rev.*, **3**, 273 (1974).
(12) T. Hilditch, *J. Chem. Soc.*, 1828 (1926).
(13) D. Swern, T. W. Findley, and J. T. Scanlan, *J. Am. Chem. Soc.*, **66**, 1925 (1944).
(14) C. Overberger and A. Drucker, *J. Org. Chem.*, **29**, 360 (1964).
(15) J. Pritchard and P. Lauterbur, *J. Am. Chem. Soc.*, **83**, 2105(1961).
(16) Professor M. Anteunis, personal communication.
(17) T. Cohen and T. Tsuji, *J. Org. Chem.*, **26**, 1681 (1961).
(18) J. Fried, T. S. Santhanakrishnan, J. Himizu, C. H. Lin, S. H. Ford, B. Rubin, and E. O. Grigas, *Nature (London)*, **223**, 208 (1969).
(19) Rohm and Haas Co., U.S. Patent 2,826,591 (1958); *Chem. Abstr.*, **52**, 12456*b* (1958).
(20) J. Coleman, C. Ricciuti, and D. Swern, *J. Am. Chem. Soc.*, **78**, 5342 (1956).
(21) J. C. Castillo and E. J. deBeer, *J. Pharmacol. Exp. Ther.*, **90**, 104 (1947).
(22) J. R. Wardell, D. F. Colella, A. Shetzline, and P. J. Fowler, *J. Pharmacol. Exp. Ther.*, **189**, 167 (1974).
(23) C. Takeguchi and C. J. Sih, *Prostaglandins*, **2**, 169 (1972).

Synthesis and Antitumor Activity of 2-Deamino- and N^2-(γ-Hydroxypropyl)actinomycin D[1]

Stanley Moore, Michio Kondo, Michelle Copeland, Johannes Meienhofer,*

Chemical Research Department, Hoffmann-La Roche Inc., Nutley, New Jersey 07110, and the Children's Cancer Research Foundation† and Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02115

and Randall K. Johnson

Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland 20014.
Received March 3, 1975

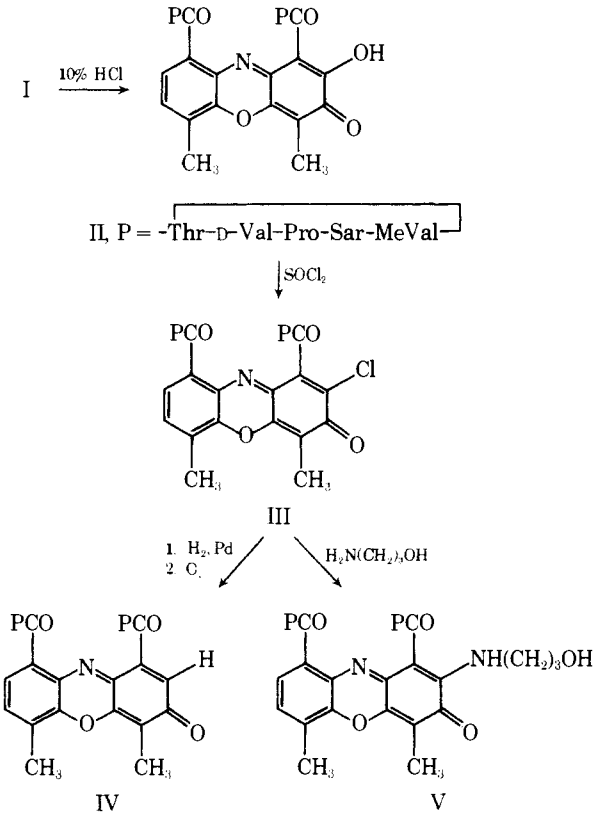
2-Deamino- and N^2-(γ-hydroxypropyl)actinomycin D were synthesized by modification of the parent actinomycin D molecule at the 2 position of the phenoxazinone moiety. The common intermediate was 2-deamino-2-chloroactinomycin D. Catalytic hydrogenation of this material afforded the 2-deamino derivative while treatment with γ-hydroxypropylamine yielded the N^2-(γ-hydroxypropyl) derivative. These 2-substituted actinomycin D derivatives were less potent in microbiological assays than the parent compound. Evaluation of activity in vivo against three murine tumor systems indicated that optimal dose levels of 2-deaminoactinomycin D were 50 times greater than toxic dose levels of actinomycin D. N^2-(γ-Hydroxypropyl)actinomycin D exhibited antitumor activity similar to the parent compound.

Among clinically used antitumor agents actinomycin D (I) (Figure 1) is one of the few possessing curative effects against two different tumors,[2,3] i.e., Wilms' tumor[4] and gestational choriocarcinoma.[5] Unfortunately, its spectrum of antitumor activity in man is relatively narrow and its administration difficult due to its high toxicity. The development of modified actinomycins possessing a broader range of activity and/or lower toxicity is thus highly desirable.[6]

We wish to report syntheses of 2-deaminoactinomycin D (IV) and N^2-(γ-hydroxypropyl)actinomycin D (V) (Figure 1). The 2-amino function seems to play a role in the biological activity of actinomycins.[7,8] Consequently, many 2-substituted derivatives have been prepared and evaluated.[9-12] Although a few N^2-alkyl derivatives exhibited low antibacterial activity (ca. 10% that of actinomycin D), most other 2-substituted derivatives were completely inactive, including 2-deamino-2-hydroxyactinomycin[13] and 2-deamino-2-chloroactinomycin.[13,14] These results suggested that the 2-amino group is essential for actinomycin activity. Conclusive proof, however, required synthesis and evaluation of actual 2-deaminoactinomycin in which the 2-amino group would be replaced by hydrogen. Rationale for the preparation of V was twofold: (a) the lower homolog N^2-(β-hydroxyethyl)actinomycin[11] exhibited some antibacterial activity and appears to interact with double-stranded DNA,[8] and (b) the cytotoxic agent acrolein, which may contribute to the antitumor activity of cyclophosphamide,[15] could perhaps be liberated from V along with actinomycin D by enzymatic processes in vivo.

The synthetic route to these derivatives is outlined in Scheme I. 2-Deamino-2-hydroxyactinomycin D (II) was produced by treatment of actinomycin D (I) with 10% HCl for 4.5 hr at 60°.[13] This derivative was converted to 2-deamino-2-chloroactinomycin D (III) by reaction of I with

Scheme I



† The synthetic part of this project was started at the Children's Cancer Research Foundation in collaboration with M.K. and M.C. and completed at Hoffmann-La Roche Inc. in collaboration with S.M.

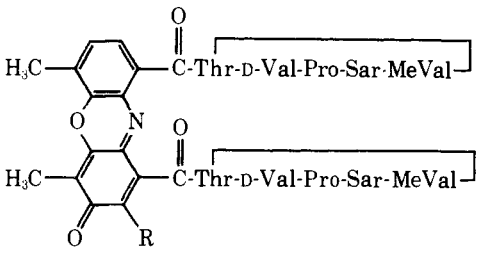


Figure 1. Structures of actinomycin D (I), 2-deaminoactinomycin D (IV), and N^2-(γ-hydroxypropyl)actinomycin D (V).

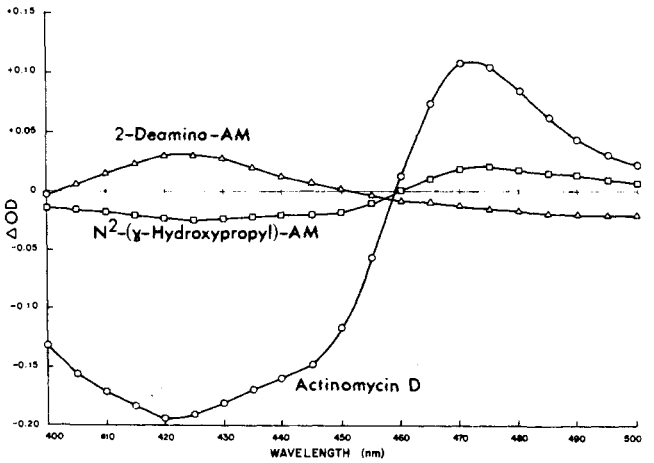


Figure 2. Difference spectra were obtained manually with a Gilford Model 2000 spectrophotometer by recording the spectrum of a solution containing an actinomycin (derivative) and DNA against a reference solution containing only the same concentration of the actinomycin. All solutions were 0.001 *M* Tris–HCl and 0.005 *M* NaCl buffer (pH 7.4). The concentration of *Micrococcus lysodeikticus* DNA was 38 μg/ml, of actinomycin D 38 μg/ml, and of both 2-deaminoactinomycin (IV) and N^2-(γ-hydroxypropyl)actinomycin (V) 38 and 76 μg/ml.

thionyl chloride in benzene.[13,14] Catalytic hydrogenation of III afforded 2-deaminoactinomycin D (IV). Treatment of III with 3-hydroxypropylamine yielded N^2-(γ-hydroxypropyl)actinomycin D (V). Careful intermediate and final purification was conducted by Sephadex LH-20 column chromatography using ethanol as an eluent. Heavily loaded thin-layer chromatograms employing two different solvent systems indicated the absence of actinomycin D (<0.05%) in any of the intermediates and the final products IV and V.

Microbiological assays (Table I) followed literature procedures.[16-18] N^2-(γ-Hydroxypropyl)actinomycin D (V) was eight times less active than actinomycin D against *Bacillus subtilis* and *Sarcina subflora;* however, against *Staphylococcus aureus* V had only 1% the activity of actinomycin D. 2-Deaminoactinomycin (IV) showed ca. 1 and 0.05%, respectively, the activity of the parent compound in these systems. The inhibition of an exponentially growing culture of *B. subtilis* (Marburg) plotted against time[18] provided ID_{50} values of 0.04 μg/ml for actinomycin D compared with 38.0 μg/ml for 2-deaminoactinomycin (IV) and 0.6 μg/ml for N^2-(γ-hydroxypropyl)actinomycin (V). The effect of *Micrococcus lysodeikticus* DNA on the absorption spectra was studied by difference spectroscopy between 400 and 500 nm (Figure 2). Depression in optical density in the visible wavelength region 400–450 nm and bathochromic shift, indicative of actinomycin binding to DNA, showed the N^2-(γ-hydroxypropyl) derivative V to undergo considerably

Table I. Antibacterial Activities of 2-Position Derivatives of Antinomycin D

Organism	Minimal inhibitory concentration, μg/ml: Actinomycin D	2-Deaminoactinomycin D	N^2-(γ-Hydroxypropyl)-actinomycin D
B. subtilis	0.09	10.0	0.7
S. subflora	0.05	4.0	0.4
Staph. aureus	0.03	>50	3.0

weaker interaction with DNA than actinomycin D. The spectra for the 2-deamino derivative IV, however, were entirely atypical for actinomycin and uninterpretable as to DNA binding. No acrolein liberation was observed by a fluorometric test[19] when the N^2-(γ-hydroxypropyl) derivative V was incubated with modified Fenton reagent[20] at 37° under conditions where both cyclophosphamide[15] and isophosphamide[20] give rise to acrolein formation. Thus, metabolic acrolein does not seem to contribute to the antitumor activity of V. The in vivo antitumor activities of 2-deaminoactinomycin D (IV) and N^2-(γ-hydroxypropyl)actinomycin D (V) were compared with the activity of actinomycin D (I) in three experimental murine tumor systems. The derivatives were tested over broad dosage ranges on three schedules of treatment employing standard screening methodology.[21]

In leukemia L1210 (Table II), actinomycin D, at optimal doses, produced a modest increase in life span (ILS) ranging from 31 to 78% on the various treatment schedules. 2-Deaminoactinomycin D was inactive (ILS <25%) at the highest dose levels tested. N^2-(γ-Hydroxypropyl)actinomycin D had antitumor activity at optimal doses which was approximately equivalent to that of actinomycin D.

In the more sensitive P388 leukemia (Table III), treatment with actinomycin D resulted in extensive increases in life span (110–262%) and a number of long-term survivors. At the highest doses tested, 2-deaminoactinomycin D had relatively low activity on two schedules of administration but was inactive when administered daily. N^2-(γ-Hydroxypropyl)actinomycin D had therapeutic activity comparable to actinomycin D.

In a solid tumor system, B16 melanoma (Table IV), optimal dose levels of actinomycin D increased life span 38, 48, and 61% on the three schedules of administration. 2-Deaminoactinomycin D had borderline activity (27% ILS) at the highest dose tested on a daily treatment schedule. Activity equivalent to actinomycin D was observed for the N^2-(γ-hydroxypropyl)-substituted derivative.

These studies indicate that the 2-amino function does play an important, though not completely essential role in the antitumor activity of the actinomycins. Deletion of the 2-amino group does not result in a complete loss of antitumor activity as indicated by the moderate activity of 2-deaminoactinomycin D against the highly sensitive P388 leukemia. However, the 2-deamino derivative was inactive against leukemia L1210 at doses up to 50 times the LD_{10} of the parent compound. Toxicity was not observed at the highest dose levels of 2-deaminoactinomycin D and the possibility exists that, at extremely high doses, this derivative could possess antitumor efficacy equal to actinomycin D.

Alkylation of the 2-amino group with a γ-hydroxypropyl

Table II. In Vivo Antitumor Activity of 2-Position Derivatives of Actinomycin D against Leukemia L1210[a]

Expt	Drug	Dosage range, mg/kg/inj	Optimal dose, mg/kg/inj	MST (range), days	ILS, %
		Daily Treatment, Days 1–9			
I	Untreated control			9.6 (8–15)	
	Actinomycin D	0.1–0.0125	0.05	13.6 (12–17)	42
	2-Deaminoactinomycin	0.4–0.0125	0.4	10.9 (9–15)	13
	N^2-(γ-Hydroxypropyl)actinomycin	0.4–0.0125	0.4	13.2 (10–17)	38
II	Untreated control			9.3 (7–14)	
	Actinomycin D	0.1–0.0125	0.1	16.6 (12–26)	78
	2-Deaminoactinomycin	4–0.25	4	9.6 (9–11)	3
	N^2-(γ-Hydroxypropyl)actinomycin	2–0.125	1	13.5 (12–15)	45
		Intermittent Treatment, Days 1, 5, and 9			
I	Untreated control			9.6 (8–15)	
	Actinomycin D	0.6–0.075	0.3	14.0 (12–17)	45
	2-Deaminoactinomycin	2.4–0.075	2.4	11.6 (9–18)	21
	N^2-(γ-Hydroxypropyl)actinomycin	2.4–0.075	2.4	12.5 (11–16)	30
II	Untreated control			9.3 (7–14)	
	Actinomycin D	0.6–0.075	0.3	14.6 (9–19)	57
	2-Deaminoactinomycin	20–1.25	20	10.8 (9–12)	15
	N^2-(γ-Hydroxypropyl)actinomycin	10–0.625	5	14.9 (12–19)	59
		Single Dose, Day 1 Only			
I	Untreated control			9.6 (8–15)	
	Actinomycin D	1.6–0.2	0.4	12.6 (9–18)	31
	2-Deaminoactinomycin	6.4–0.2	6.4	11.4 (9–15)	18
	N^2-(γ-Hydroxypropyl)actinomycin	6.4–0.2	6.4	13.8 (12–20)	43
II	Untreated control			9.3 (7–14)	
	Actinomycin D	0.8–0.1	0.4	12.1 (9–14)	30
	N^2-(γ-Hydroxypropyl)actinomycin	16–1	8	13.2 (11–15)	42

[a] 10^5 L1210 cells implanted ip on day 0 into groups of eight BDF_1 male mice. Drugs administered ip. MST (range), mean survival time in days (range of individual animal deaths); ILS, percent increase in life span.

Table III. In Vivo Antitumor Activity of 2-Position Derivatives of Actinomycin D against P388 Leukemia[a]

Drug	Dosage range, mg/kg/inj	Optimal dose, mg/kg/inj	MST (range), days	ILS (surv), %
Untreated control			10.5 (9–19)	
	Daily Treatment, Days 1–9			
Actinomycin D	0.1–0.0125	0.05	28.5 (19–39)	171 (1/8)
2-Deaminoactinomycin	1–0.25	1	11.0 (9–16)	5
N^2-(γ-Hydroxypropyl)actinomycin	0.8–0.05	0.8	31.0 (20–52)	195 (1/8)
	Intermittent Treatment, Days 1, 5, 9			
Actinomycin D	0.6–0.075	0.3	38.0 (31–38)	262 (3/8)
2-Deaminoactinomycin	7–1.68	7	18.0 (8–22)	71
N^2-(γ-Hydroxypropyl)actinomycin	4.8–0.3	4.8	26.0 (19–33)	148 (1/8)
	Single Dose, Day 1 Only			
Actinomycin D	1.6–0.2	0.4	22.0 (8–31)	110 (1/8)
2-Deaminoactinomycin	10–2.4	10	16.0 (14–19)	52
N^2-(γ-Hydroxypropyl)actinomycin	12.8–0.8	3.2	21.5 (17–30)	105

[a] 10^6 P388 cells implanted ip on day 0 into groups of eight BDF_1 male mice. MST (range), median survival time in days (range of individual animal deaths); ILS (surv), percent increase in life span (survivors/total on day 60).

substituent results in a 10- to 20-fold decrease in potency as indicated by the optimal dose levels with no change in antitumor efficacy. Toxicity was observed at twice the level of optimal antitumor efficacy. This is the same ratio of toxic to optimal doses as generally observed with actinomycin D and, therefore, no improvement in the therapeutic index was obtained. The weight changes in the test animals, at the effective and toxic dose levels of the derivative, were similar to the weight changes observed for actinomycin D.

Experimental Section

Details on materials and methods have been described before.[22] In addition, chloranil was recrystallized from benzene and thionyl chloride was distilled from linseed oil prior to use. Solvent systems for silica gel chromatography were A, *sec*-BuOH–HCOOH–H_2O (75:13.5:11.5); and B, EtOAc–acetone (2:1).

2-Deamino-2-hydroxyactinomycin D (II). A solution of actinomycin D (I) (1.2 g) in 10% HCl (300 ml) was heated for 4.5 hr at 60°. On cooling, the solution was extracted with chloroform (3 × 200 ml). The chloroform solution was washed twice with water and saline, dried, and evaporated. Investigation by TLC, at this point,

Table IV. In Vivo Antitumor Activity of 2-Position Derivatives of Actinomycin D against B16 Melanoma[a]

Drug	Dosage range, mg/kg/inj	Optimal dose, mg/kg/inj	MST (range), days	ILS (surv), %
Untreated control			28.0 (21–53)	
	Daily Treatment, Days 1–9			
Actinomycin D	0.1–0.0125	0.025	45.0 (36–53)	61 (2/10)
2-Deaminoactinomycin	2–0.125	2	35.5 (29–42)	27
N^2-(γ-Hydroxypropyl)actinomycin	1.94–0.25	0.42	43.5 (32–56)	55 (1/10)
	Intermittent Treatment, Days 1, 5, and 9			
Actinomycin D	0.6–0.075	0.3	41.5 (29–49)	48
2-Deaminoactinomycin	4–0.25	4	34 (24–45)	21
N^2-(γ-Hydroxypropyl)actinomycin	5.5–0.7	3.24	40.5 (32–42)	45 (2/10)
	Single Dose, Day 1 Only			
Actinomycin D	0.8–0.1	0.4	38.5 (33–43)	38 (1/10)
2-Deaminoactinomycin	10–0.625	10	31.5 (25–36)	12
N^2-(γ-Hydroxypropyl)actinomycin	9–1.17	9	37.0 (29–40)	32

[a] 0.2 ml of a 1:5 (weight/volume) brei of B16 melanoma implanted ip on day 0 into groups of ten BDF_1 male mice. Drugs administered ip. MST (range), median survival time in days (range of individual animal deaths); ILS (surv), percent increase in life span (survivors/total on day 60).

indicated the absence of actinomycin D. The product was chromatographed on Sephadex LH-20 using 95% ethanol as eluent: 1.0 g (83%); mp 238–240°; $[\alpha]^{25}D$ −121.7° (c 0.25, CH_3OH); TLC R_f 0.36 (A), 0 (B); uv (CH_3OH) 228 nm (ε infl 38,300), 440–444 (ε max 18,500). Anal. ($C_{62}H_{87}N_{11}O_{17}\cdot H_2O$) C, H, N [lit.[13] for the corresponding actinomycin C_3 derivative: mp 239°; $[\alpha]^{25}D$ −91° (c 0.25; CH_3OH)].

2-Deamino-2-chloroactinomycin D (III). II (1.1 g) was dissolved in dry benzene (50 ml) and chloranil (297 mg) added, followed by freshly distilled thionyl chloride (8.8 ml). The reaction mixture was refluxed under anhydrous conditions for 25 min. After cooling, the solution was evaporated to dryness and reevaporated several times from benzene. The product was precipitated from benzene with cold hexane and separated by centrifugation. This material was chromatographically homogeneous: 1.0 g (89.5%); mp 232–234°; $[\alpha]^{25}D$ −94.5° (c 0.2, acetone); TLC R_f 0.59 (A), 0.61 (B); uv max (CH_3OH) 270 nm (ε infl 18,850), 390–392 (ε max 14,250).

2-Deaminoactinomycin D (IV). III (0.3 g) was dissolved in 95% acetic acid–water (15–20 ml) and hydrogenated for 5 hr in the presence of freshly prepared[22] Pd black (0.7 g). The catalyst was removed by filtration and the filtrate evaporated. The ir spectrum of the product showed no bands in the CCl region as compared to that of III. The product was reevaporated several times from dry benzene and the residue dissolved in methanol (15 ml). A few drops of 10% triethylamine–methanol were added and oxygen was bubbled through the solution. Acetic acid (10%) was added to neutralize excess triethylamine, and the solution was evaporated to dryness and reevaporated several times from dry benzene. The product was reprecipitated from benzene–hexane. Further purification was achieved by LH-20 chromatography: 230 mg (79%); mp 242–243°; $[\alpha]^{25}D$ −7.3° (c 0.06, CH_3OH); TLC R_f 0.57 (A), 0.63 (B); uv max (CH_3OH) 260 nm (ε 15,950), 375 (11,100), 475 (5100). Anal. ($C_{62}H_{85}N_{11}O_{16}\cdot 5H_2O$) C, H, N.

N^2-(γ-Hydroxypropyl)actinomycin D (V). III (300 mg) and freshly distilled 3-hydroxypropylamine (106 mg, 5 equiv) were dissolved in dry benzene (10 ml) and the reaction mixture was stirred at room temperature overnight. Acetic acid (70 mg) was added and the reaction mixture evaporated. The product was reevaporated several times from dry benzene. The derivative was reprecipitated from benzene–hexane. Further purification was achieved by LH-20 chromatography: 250 mg (81%); mp 226–228°; $[\alpha]^{25}D$ −221.8° (c 0.095, CH_3OH); TLC R_f 0.54 (A), 0.60 (B); uv max (CH_3OH) 245 nm (ε 23,500), 443 (ε max 13,300). Anal. ($C_{65}H_{92}N_{12}O_{17}\cdot 4H_2O$) C, H, N.

Acknowledgments. This work was supported in part by Research Grants C-6516 from the National Cancer Institute and FR-05526 from the Division of Research Facilities and Resources, National Institutes of Health. We thank the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute, for a supply of actinomycin D, Dr. E. Katz and Miss K. Mason, Department of Microbiology, Georgetown University, Washington, D.C., for the microbiological assays and the difference spectra, and Dr. R. A. Alarcon, Cancer Research Institute, New England Deaconess Hospital, Boston, Mass., for acrolein liberation tests.

References and Notes

(1) Synthesis of Actinomycin and Analogs. 11. For paper 10, see J. Meienhofer, *Cancer Chemother. Rep.*, **58**, 21 (1974).
(2) E. Frei, III, *Cancer Chemother. Rep.*, **58**, 49 (1974).
(3) P. A. Friedman and A. Cerami in "Cancer Medicine", J. F. Holland and E. Frei, III, Ed., Lea & Febiger, Philadelphia, Pa., 1973, pp 835–839.
(4) S. Farber, *J. Am. Med. Assoc.*, **198**, 826 (1966).
(5) J. L. Lewis, Jr., *Cancer*, **30**, 1517 (1972).
(6) S. Perry, *Cancer Chemother. Rep.*, **58**, 117 (1974).
(7) H. Brockmann, *Cancer Chemother. Rep.*, **58**, 9 (1974).
(8) E. Reich, I. H. Goldberg, and M. Rabinowitz, *Nature (London)*, **196**, 743 (1962).
(9) H. Brockmann, P. Hocks, and W. Müller, *Chem. Ber.*, **100**, 1051 (1967).
(10) H. Brockmann, W. Müller, and H. Peterssen-Borstel, *Tetrahedron Lett.*, 3531 (1966).
(11) H. Brockmann, G. Pampus, and R. Mecke, *Chem. Ber.*, **92**, 3082 (1959).
(12) H. Brockmann and B. Franck, *Angew. Chem.*, **68**, 68 (1956).
(13) H. Brockmann and B. Franck, *Chem. Ber.*, **87**, 1767 (1954).
(14) H. Brockmann, H. Gröne, and G. Pampus, *Chem. Ber.*, **91**, 1916 (1958).
(15) R. A. Alarcon and J. Meienhofer, *Nature (London), New Biol.*, **233**, 250 (1971).
(16) A. L. Demain, *J. Bacteriol.*, **75**, 517 (1958).
(17) K. Mason and E. Katz, *Arch. Biochem. Biophys.*, **160**, 402 (1974).
(18) J. V. Formica, A. J. Shatkin, and E. Katz, *J. Bacteriol.*, **95**, 2139 (1968).
(19) R. A. Alarcon, *Anal. Chem.*, **40**, 1704 (1968).
(20) R. A. Alarcon, J. Meienhofer, and E. Atherton, *Cancer Res.*, **32**, 2519 (1972).
(21) R. I. Geran, N. H. Greenberg, M. M. Macdonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**, 1 (1972).
(22) J. Meienhofer, *J. Am. Chem. Soc.*, **92**, 3771 (1970).