*STRUCTURAL BASIS FOR INHIBITION OF PROTEIN SYNTHESIS BY EMETINE AND CYCLOHEXIMIDE BASED ON AN ANALOGY BETWEEN IPECAC ALKALOIDS AND GLUTARIMIDE ANTIBIOTICS**

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Communicated by Alfred Gilman, September 22, 1966

The therapeutic properties of the ipecac alkaloids in the treatment of certain dysenteries and their effectiveness as emetics and expectorants was recognized as early as the 17th century.[1] (−)-Emetine, an active principle of ipecac, was reported to be an amebicidal agent by Vedder[2] and recently has proved beneficial in the therapy of nonspecific granulomas.[3] The present communication will show that emetine is a potent inhibitor of protein synthesis in mammalian and other cells, a mode of action which may account for its therapeutic and toxic properties.

The glutarimide antibiotics, of which (−)-cycloheximide is the best known member, are derived from various species of *Streptomyces*. These compounds also inhibit protein synthesis and exert amebicidal,[4] antitumor,[5, 6] and fungicidal[7] actions. An analogy between the glutarimide antibiotics and the ipecac alkaloids will be demonstrated in this paper based on (*a*) similarities in their mode of action, (*b*) their configurational resemblances, and (*c*) their structure-activity relationships. The analogy provides a structural basis for the inhibition of protein synthesis by the ipecac and glutarimide compounds and suggests new pharmaceutical preparations of potential value.

Methods and Materials.—Protein and polyphenylalanine synthesis by extracts of different species was measured by the procedure of Lamfrom,[8] for rabbit reticulocytes; of Downey *et al.*,[9] for *Saccharomyces cerevesiae;* of Nirenberg and Matthaei,[10] as modified by Nathans *et al.*,[11] for *E. coli;* and of Penman and Summers,[12] for HeLa cells. Components of the reaction mixtures are described in the legend to Table 1. Following the period of incubation, reactions were terminated by addition of 5% TCA (trichloroacetic acid) and the acid-insoluble material was washed by the procedure of Siekievitz.[14] The precipitates were collected on Millipore membrane filters, and the radioactivity was determined in a Nuclear-Chicago low-background counter with an efficiency of 21% for C^{14}. The transfer reaction was assayed in extracts of rat liver as described by Gasior and Moldave.[13] (−)-Emetine and (−)-N-methylemetine were kindly provided by Dr. N. Whittaker of the Wellcome Laboratories, Kent, England; (−)-cephaeline and (+)-O-methylpsychotrine by Sandoz, Ltd., Basle; (−)-2,3-dehydroemetine and (±)-1,2,3,4,5,11b-trisdehydroemetine by Dr. A. Brossi of Hoffmann-La Roche, Nutley, N.J.; and (±)-isoemetine by Dr. J. Elks of the Glaxo Laboratories, Middlesex, England. *E. coli* B sRNA was obtained from Nutritional Biochemicals, poly U (polyuridylic acid) from the Miles Laboratories, and radioisotopes from New England Nuclear. C^{14}-phenylalanyl-sRNA and C^{14}-leucyl-sRNA were prepared enzymatically by the procedure of von Ehrenstein and Lipmann.[15] Coliphage MS2 RNA was a gift from Dr. D. Nathans.

Inhibition of Protein Synthesis by Emetine.—Species specificity: The effect of emetine on protein synthesis in certain mammalian cells, yeast, and plants is shown in Table 1. Emetine can effect a 50 per cent inhibition of protein synthesis in HeLa cells and gametophytes of *Anemia phylitidis* at media concentrations of less than 10^{-6} *M*. Under these *in vivo* conditions, emetine is concentrated 10- to 20-fold by the cells. Protein synthesis in cell-free extracts prepared from HeLa cells and reticulocytes was inhibited by 50 per cent at concentrations of 8×10^{-5} *M* and 2×10^{-5} *M*, respectively.

TABLE 1

EFFECT OF EMETINE ON PROTEIN AND POLYPHENYLALANINE SYNTHESIS IN DIFFERENT SPECIES

Emetine conc. (*M*)	HeLa Cells[a, d] Incorp. (cpm)	Inhib. (%)	*A. phylitidis*[a, d] Incorp. (cpm)	Inhib. (%)	Reticulocytes[b, d] Incorp. (cpm)	Inhib. (%)	*S. cerevisiae*[b, c] Incorp. (cpm)	Inhib. (%)
0	2262		25,700		7585		1435	
10^{-3}	22	99	1,640	94	76	99	85	94
10^{-4}	35	98	—	—	384	95	787	45
10^{-5}	41	97	5,830	77	6056	20	1148	20
10^{-6}	120	95	6,790	74	7410	2	1392	3

HeLa cells: The reaction mixture, composed of 3 ml of leucine-depleted Eagle's medium containing 1.2 × 10^6 cells and 0.05 μc leucine-C^{14} (specific activity 1 μc/μM), was incubated at 37° for 1 hr. Emetine concentrations represent concentration in the media. *Reticulocytes*: The reaction mixture contained, in a final volume of 0.4 ml, 16 μmoles Tris HCl, pH 7.4; 1.6 μmole Mg acetate, 8 μmoles KCl; 1 μmole mercaptoethanol; 0.33 μmole ATP; 0.083 μmole GTP; 3 μmoles phosphoenolpyruvate; 25 μg pyruvate kinase; 0.02 ml of the amino acid mixture, omitting leucine; 6.0 mμmoles leucine-C^{14}, specific activity 252 μc/μmole; 1.0 mg ribosomes; and 1.0 mg "pH 5 enzymes." Incubation was at 37° for 2 min. *S. cerevisiae:* The reaction mixture contained, in a final volume of 0.5 ml, 50 μmoles imidazole, pH 7.0; 3 μmoles Mg acetate; 50 μmoles NH_4Cl; 0.4 μmole spermine; 1 μmole GTP; 50 μg poly U; 0.07 mg C^{14}-phenylalanyl-sRNA, 65,000 cpm/mg; 2 μmoles phenylalanine-C^{12}; and 0.3 mg *S. cerevisiae* ribosomes. Incubation was at 25° for 10 min. *Anemia phylitidis:* Assay performed using intact gametophytes of this fern by measuring leucine-C^{14} incorporation into TCA-insoluble material.[28]

a Whole cells.
b Cell-free preparation.
c Polyphenylalanine synthesis. See text for inhibition of endogenous protein synthesis in this species.
d Protein synthesis.

In extracts of *S. cerevisiae*, the synthesis of polyphenylalanine from phenylalanyl-sRNA[16] was selected as a suitable model for the study of protein synthesis *in vitro* (Table 1). Emetine also inhibits the low levels of endogenous protein synthesis observed when poly U is omitted from the reaction mixture by 66 per cent at 10^{-3} *M*, 46 per cent at 10^{-4} *M*, and 22 per cent at 10^{-5} *M*. Polyphenylalanine and endogenous protein synthesis, catalyzed by similar extracts from *S. pastorianus*, were inhibited by emetine. The lack of toxicity *in vivo* against these two yeasts can be attributed to the demonstrated failure of the alkaloid to penetrate the cell wall.

The only organism tested whose extracts were resistant to the effect of emetine was the bacterium *E. coli*. Phage MS2 RNA, polyuridylic acid, and endogenous "messenger" RNA stimulated the incorporation of 4.4, 8.7, and 0.6 mμmoles of C^{14}-phenylalanine, respectively, into material insoluble in TCA. These reactions were unaffected by the presence of the alkaloid at a concentration of 10^{-3} *M*. In *E. coli*, as in *S. cerevisiae*, emetine is not concentrated from the media by the cells to any appreciable degree, and no *in vivo* toxicity of the inhibitor is observed.

TABLE 2

STEREOSPECIFICITY OF IPECAC ALKALOIDS AS AMEBICIDES AND AS INHIBITORS OF PROTEIN SYNTHESIS

Inhibitor	Relative amebicidal activity*	Concentration Required for 50% Inhibition of Protein Synthesis *In vivo*† (μM)	*In vitro*‡ (μM)
Active			
(—)-Emetine	1000	0.05	25
(—)-Dehydroemetine	1000	0.05	25
(—)-Cephaeline	>1000	0.04	17
Inactive			
(±)-Trisdehydroemetine	<10	>50	>2500
(±)-Isoemetine	<10	>50	>2500
(−)-N-methylemetine	<10	>50	>2500
(+)-O-methylpsychotrine	<10	>50	>2500

* Adapted in part from reported values in literature[17, 18] as determined relative to emetine in growing cultures of *E. histolytica*. Certain assays were performed by Dr. N. Entner using published techniques.[19]
† Determined in suspension cultures of HeLa cells as described in Table 1.
‡ Determined in cell-free preparation of reticulocyte ribosomes as described in Table 1.

Structural specificity: The capacity of various ipecac alkaloids to inhibit protein synthesis in HeLa cells and in cell-free preparations from rabbit reticulocytes has been compared to their reported amebicidal activity in Table 2. A detailed report of the inhibitory activity of all isomers and analogues will be published elsewhere. The relative efficacy of the alkaloids tested as inhibitors of *in vivo* protein synthesis in HeLa cells corresponds closely to their comparative ED_{50} for inhibition of protein synthesis in cell-free preparations and to the reported *in vitro* toxicity of these compounds against cultures of *Entamoeba histolytica*. Referring to the structure of emetine shown in Figure 1, the data indicate that the (R) configuration at C-1′ and the secondary nitrogen atom at the 2′ position are essential requirements for activity. These conclusions are based in part on the inactivity of the epimer with the (S) configuration at C-1′ (isoemetine) and the loss of activity with unsaturation at the 1′-2′ position (O-methylpsychotrine) or by substitution of the secondary nitrogen (N-methylemetine). Unsaturation at the 2-3 position (dehydroemetine) destroys the asymmetry at carbons 2 and 3 without the loss of biological activity, but further oxidation to 1,2,3,4,5,11b-trisdehydroemetine creates a positive charge at the tertiary nitrogen atom and results in loss of activity (Table 2).

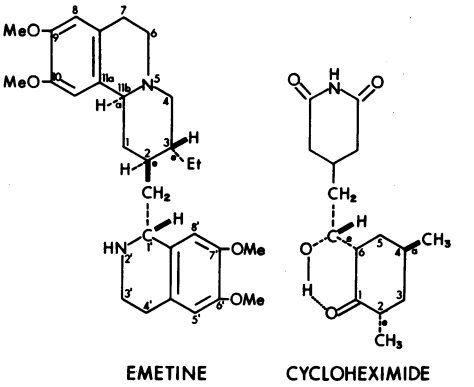

FIG. 1.—Structural formulae of emetine and cycloheximide. Hydrogen-bonding between the ketone and hydroxyl groups of cycloheximide is indicated by dotted line.

Site of action: Emetine inhibits the aminoacyl-sRNA transfer reaction in protein biosynthesis (Table 3). This reaction represents a series of steps resulting in the incorporation of the aminoacyl moiety into a polypeptide-bound form. Activation of amino acids, synthesis of aminoacyl-sRNA, and chain initiation or release of completed polypeptides do not appear to be affected by emetine. If the alkaloid is added after protein synthesis has been initiated, the nascent peptide remains firmly attached to the intact polyribosome structure.[20] This response differs from that induced by puromycin, which results in the breakdown of polyribosomes and the release of polypeptides.[21] Inhibition of protein synthesis is as rapid and effective in preformed ribosome-messenger RNA-transfer enzyme complexes, which exist in reticulocyte preparations and in rat liver microsomes, as it is in partially purified systems from rat liver in which transfer enzymes are added to desoxycholate-treated ribosomes. In the latter case, higher concentrations of emetine are required to achieve the same degree of partial inhibition although the stereospecificity of the alkaloid is retained (Table 3). The exact site of action of emetine remains to be determined but appears to be in the

TABLE 3

EFFECT OF EMETINE ON THE TRANSFER REACTION OF PROTEIN SYNTHESIS

Conditions	Leucine incorporation (cpm)	Inhibition (%)
Complete system	1180	—
− Transfer enzymes	34	—
+ Emetine, 10^{-2} *M*	212	82
+ Emetine, 10^{-3} *M*	531	55
+ Emetine, 10^{-4} *M*	932	21
+ Isoemetine, 10^{-2} *M*	1073	9
+ Isoemetine, 10^{-3} *M*	1189	0

The reaction mixture contained, in a volume of 0.5 ml, 25 μmoles Tris HCl, pH 7.4; 0.1 μmole GTP; 3 μmoles $MgCl_2$; 40 μmoles NH_4Cl; 2 μmoles glutathione; 1 mg transfer enzyme fraction; 2 mg ribosomes; 0.05 mg C^{14}-leucyl-sRNA, 87,000 cpm/mg; and 2 μmoles leucine-C^{12}.

enzymatic formation of the peptide bond on the polyribosome. Attachment of emetine to its site of action may be affected by the structure of the ribosome.

Similarities between Ipecac Alkaloids and Glutarimide Antibiotics.—Species specificity: Cycloheximide has previously been demonstrated to inhibit protein synthesis in mammalian cells,[22, 23] certain species of yeast,[24, 25] protozoa,[26] and plants[27, 28] but, like emetine, is inactive against extracts prepared from *E. coli.*[29]

Structural specificity: Siegel, Sisler, and Johnson have tested various isomers of the glutarimide antibiotics and related compounds for their capacity to inhibit protein synthesis.[30] Replacement of the imide hydrogen of cycloheximide by a methyl group, esterification of the hydroxyl with acetate, or conversion of the ketone to an oxime results in great diminution or complete loss of activity. These results suggested that the keto, hydroxyl, and imide N groups (cf. Fig. 1) were involved in a three-point attachment of the glutarimide antibiotics to their receptor. Since epicycloheximide was not available, the probable importance of the correct configuration of the carbon bearing the hydroxyl was not demonstrated directly. However, any modification of the spatial position of this hydroxyl group in relation to the cyclohexanone ring as in neocycloheximide, which has an axially oriented side chain, or inactone, in which C_{5-6} is unsaturated, results in loss of biological activity. Existing data may also be interpreted as evidence for a hydrogen-bonded conformation in the biologically active forms. β-Dihydrocycloheximide, in which intramolecular hydrogen-bonding is sterically prevented, is inactive, while α-dihydrocycloheximide, in which stronger hydrogen-bonding is demonstrable,[31] has some activity.[30] The active compound, streptimidone, which has an open chain in place of the cyclohexanone ring, would still assume the conformation of cycloheximide if stabilized by intramolecular hydrogen-bonding.

Site of action: Cycloheximide inhibits the transfer reaction in protein synthesis and has no effect on amino acid activation or the synthesis of aminoacyl-sRNA.[25] In the presence of this inhibitor, nascent polypeptide is not released from the ribosome,[32] there is no breakdown of the polyribosome structure,[32] and chain initiation or release of completed polypeptides is not prevented. All of the active ipecac alkaloids resemble the glutarimide antibiotics in these respects, but cycloheximide differs from emetine in that it acts reversibly; that is, activity can be restored to cycloheximide-treated HeLa cells by resuspension in fresh media while the action of emetine under similar conditions is irreversible. This apparent difference is reconciled by comparing the activities of streptovitacin A and acetoxycycloheximide to that of emetine. The action of these glutarimide antibiotics, which differ from cycloheximide only in having an equatorial hydroxyl or acetoxy substitution at the C-4 position (cf. Fig. 1), closely resembles that of emetine in being partially or totally irreversible.[33, 34] It appears that the property of irreversibility is conferred by a secondary binding site which is not essential for inhibition of protein synthesis.

Structural Basis of Activity.—Configurational, conformational, and electrostatic considerations: The established structures of emetine and cycloheximide are compared in Figure 1, and their stereochemical similarities can be appreciated by examination of the Dreiding models portrayed in Figure 2. Cycloheximide itself lacks methoxy groups, but streptovitacin A and acetoxycycloheximide, both active isomers, have hydroxyl and acetoxy substitutions, respectively, at the position which

corresponds to the C-7′ methoxy group of emetine. Infrared data on cycloheximide[31, 37] provide evidence for hydrogen-bonding, stabilizing a conformation in

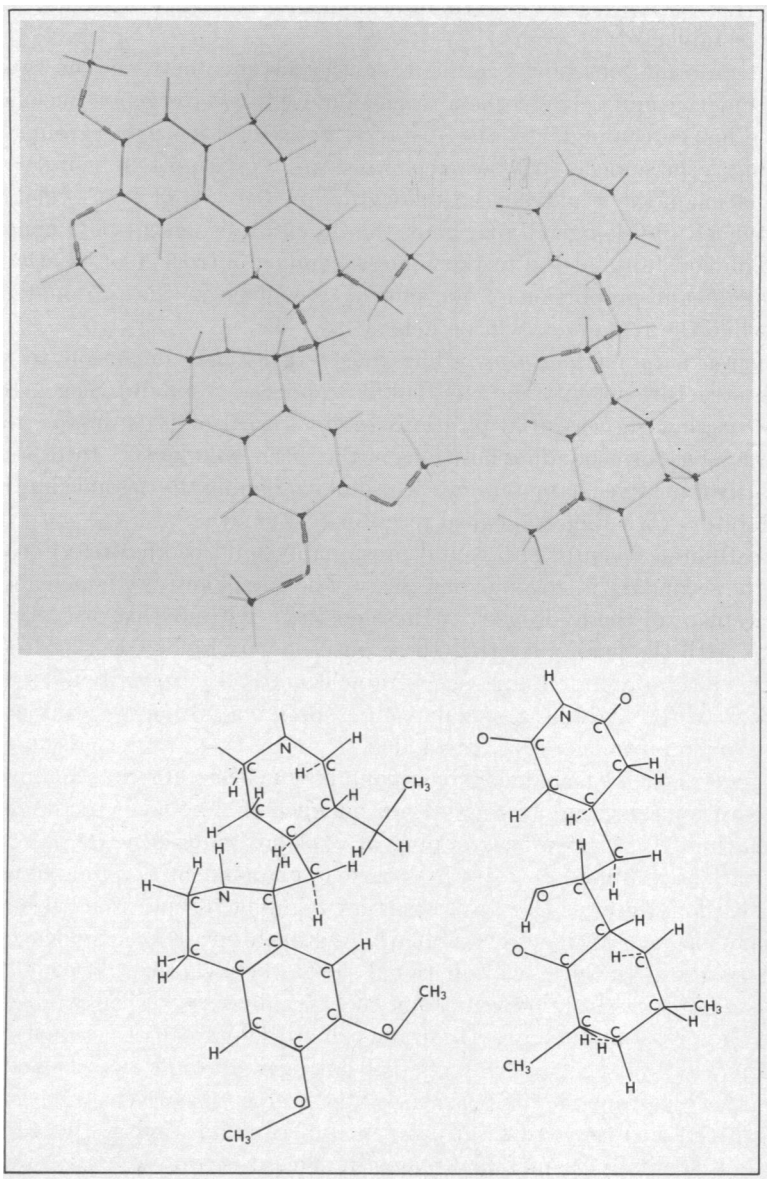

FIG. 2.—Photograph of Dreiding stereomodels of emetine (*left*) and cycloheximide (*right*) with atoms designated in the illustration below. The axial methyl group at C-4 of cycloheximide is the only substituent larger than a hydrogen atom in either compound which is not equatorial to its respective ring. Although both cycloheximide and emetine can be rotated 180° around the methylene groups from the position shown, such a conformation for emetine has been rejected because of steric hindrance by the bulky ethyl group. The absolute configuration (R) at the 1′ position of emetine has been related by Battersby *et al.*[35] to D-alanine. The configuration of the corresponding asymmetric carbon in the side chain of cycloheximide has been assigned by Johnson *et al.*[36] to the (R) series, correcting a previous assignment by Okuda.[31] In the conformation shown, cycloheximide is superimposable over the portion of the emetine molecule illustrated in the lower half of the figure.

which the quasi ring, shown in Figures 1 and 2, would correspond to the nitrogen-containing ring of the tetrahydroisoquinoline nucleus of emetine. The actual extent to which this hydrogen-bonded form of cycloheximide exists in aqueous solution has not been established.

In the conformation shown in Figure 2, the distance between the two nitrogen atoms of emetine and between the nitrogen and hydroxyl oxygen of cycloheximide is 5.5 Å. This corresponds to the distance between functional groups of many drugs[38] and is the same as that between two turns of an α-protein helix.[39]

Dissociation of the weakly acidic imide grouping ($pK_a = 11.1$) of cycloheximide is suppressed at physiological pH, and the function is essentially neutral. The dissociation constants of the tertiary and secondary nitrogens of emetine are such that a significant proportion of the molecules will be in the protonated form at pH 7.5 while the remainder will be neutral.

Structure-activity relationships: The most significant conclusion to be drawn from the structure-activity data in Table 3 and the results of Siegel, Sisler, and Johnson[30] is that the essential positions for biological activity in the glutarimide antibiotics have corresponding positions in the ipecac alkaloids. Interpretations of a necessarily tentative character may be useful in defining the possible interactions of these inhibitors with their biological receptor (enzyme).

One postulated receptor site would presumably bind the hydroxyl of cycloheximide or the secondary nitrogen of emetine. The loss of activity which accompanies the replacement of the hydrogens at these positions with methyl or acetyl groups is consistent with the possibility that these positions are involved in hydrogen-bonding to the receptor. Although an intramolecularly hydrogen-bonded conformation appears to be favored in cycloheximide, preferential bonding of the hydroxyl hydrogen to a receptor site is not precluded.

Additional testing of analogous compounds will be required to define fully the receptor site corresponding to the tertiary nitrogen of emetine or the imide nitrogen of cycloheximide. The imide grouping of cycloheximide is rendered inactive by N-methylation, implying that its hydrogen is involved in bonding since the two adjacent carbonyl groups effectively prevent bonding through the π-electrons. If the tertiary nitrogen of emetine is similarly hydrogen-bonded, the hydrogen must be supplied via the receptor. A bifunctional site in the receptor that could protonate as well as accomodate a hydrogen atom, such as a hydroxyl or an imidazole group, would satisfy the requirements. Inactivity of trisdehydroemetine may result from charge repulsion by the positively charged nitrogen atom or from the effective removal of free electrons at this position. Steric effects also seem to be involved in the area of this nitrogen atom since reversal of configuration at the adjacent 11b position [(+)-dehydroisoemetine] creates significant hindrance at the lower face of the molecule correlated with a decrease in biological activity.[17, 33]

Although C-1′ of emetine and the asymmetric carbon of the side chain of cycloheximide may be involved in binding to the receptor, it is probable that they serve instead to fix the spatial position of the secondary nitrogen atom of emetine and the hydroxyl group of cycloheximide. The asymmetric carbon at C-6 of cycloheximide would fulfill a similar function as, in order for the quasi ring of cycloheximide to correspond to the ring of emetine, the configuration at C-6 must be such that the side chain is equatorial to the cyclohexanone ring.

Structural models for the inhibition of protein synthesis: The assumption that the spatial position of two nitrogen atoms was important for biological activity led to the preparation of a number of active diamines, some of which have many times the amebicidal activity of emetine *in vitro*.[40, 41] As the stereochemistry of the carbon corresponding to C-1′ of emetine was largely ignored in the synthesis of these compounds, their inactivity as inhibitors of protein synthesis is not surprising.[33] Representative compounds are proposed (Fig. 3) which contain the minimal structural requirements for the inhibition of protein synthesis based on the foregoing analogy between the ipecac alkaloids and the glutarimide antibiotics. Although important conformation and configurational aspects contributing to total activity may have been omitted in these models, each contains the essential features for biological activity. Compound *A* is a substituted bispiperidyl methane compound with the (R) configuration at the asymmetric carbon, and compound *B* includes a polar group at the 7′ position, a substitution which could be associated with irreversible binding. The glutarimidyl moiety is illustrated in *B* to emphasize it as an optional and possibly more desirable replacement for the piperidyl group of *A*. Compound *C* is a more elaborate model with substitutions at the 3 and 6 positions, which may contribute to increased activity within the dehydroemetine series.

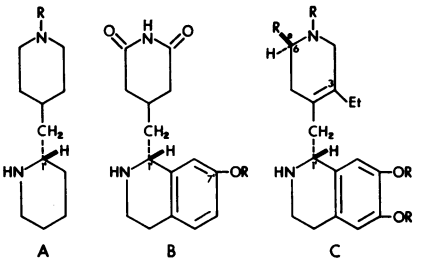

FIG. 3.—Representative model compounds containing the essential structural features for the inhibition of protein synthesis.

The flexibility provided by chemical modification of these model compounds offers a lead to new agents with an improved therapeutic index. Their potential activity against yeast and fungi and their possible application as rodent repellents[42] may prove to be of practical importance in agriculture.

Summary.—Emetine inhibits protein synthesis in certain mammalian cells, species of *Saccharomyces*, and in *Anemia phylitidis*, a higher plant, but not in cell-free extracts prepared from *E. coli*. Although distinctly different in chemical nature, (−)-emetine and the ipecac alkaloids show configurational and conformational similarities to (−)-cycloheximide and the glutarimide antibiotics and have similar effects on protein synthesis. The results of these studies define a structural basis for the inhibition of protein synthesis and suggest a synthetic approach to new compounds of potential therapeutic and agricultural importance.

The author is grateful to the many individuals whose suggestions aided the progress of this work. In particular, he is indebted to Drs. Gilbert Stork and Francis Johnson for most helpful discussions and to Dr. H. T. Openshaw for critically reviewing this manuscript. Dr. Malcolm Siegel kindly made available previously unpublished data on the biological activity of the glutarimide antibiotics, and Miss Althea Hoff rendered invaluable technical assistance.

* Supported by grants from the National Science Foundation (GB-2982) and the National Institutes of Health (AM-09310).

† Career Scientist of the Health Research Council of the City of New York.

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