amino acid standards and extracts were analyzed by GLC, using trimethylsilylalanine as an internal standard.

It is apparent, based on collective TLC and GLC data, that the free amino acid contents of higher marine fungi grown under uniform conditions are qualitatively similar. The amino acids common to all species examined in this study included alanine, glycine, valine, proline, leucine, isoleucine, serine, threonine, hydroxyproline, aspartic acid, methionine, glutamic acid, phenylalanine, ornithine, lysine, tyrosine, tryptophan, cystine, cysteine, and histidine.

To what extent these compounds were synthesized by the fungi or merely taken up from the culture medium remains to be determined because the medium, which contained yeast extract, cannot be considered as being totally chemically defined. However, an assay of yeast extract[5] indicated that alanine, proline, serine, ornithine, tryptophan, cystine, cysteine, and hydroxyproline are absent from this product. Also, arginine, a component of yeast extract, was not detected by us in any of the fungal mycelium examined.

Consequently, we feel that the array of amino acids detected in the mycelium represents metabolites synthesized by the organisms, at least in part. In addition, because natural sea water contains numerous amino acids, the cultivation procedures are not unrealistically artificial and this investigation allows better understanding of the chemical–ecological role of fungi in the marine biosphere as well as the overall metabolic activities of these organisms.

The occurrence of hydroxyproline in all species examined is noteworthy, because this amino acid is believed to occur only rarely in fungi (9). Whether hydroxyproline actually occurs less commonly in terrestrial than in marine fungi or has been overlooked in analyses of the former requires further clarification. The compound is a prime component of collagen in animals including marine species (10). Degens *et al.* (11) reported that hydroxyproline is not ubiquitous in sea water, whereas Pocklington (6) found it readily detectable. Owing to their abundance in salt marshes (12), the higher marine fungi could be an important source of this and other amino acids in detritus feeding animals of coastal and estuarine environments.

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5-[3-(2-Chloroethyl)-1-triazenyl]imidazole-4-carboxamide and a Possible Mechanism of Action of 5-[3,3-Bis(2-chloroethyl)-1-triazenyl]imidazole-4-carboxamide

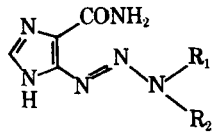
Keyphrases □ Chloroethyltriazene—synthesis, antileukemic activity □ Imidazoles, triazenyl—preparation, antileukemic activity □ (Chloroethyltriazenyl)imidazole—explosive decomposition, hydrolysis to aminoimidazolecarboxamide □ Antileukemic activity—chloroethyltriazene □ Bis(2-chloroethyl)triazenes—possible mechanism of action

To the Editor:

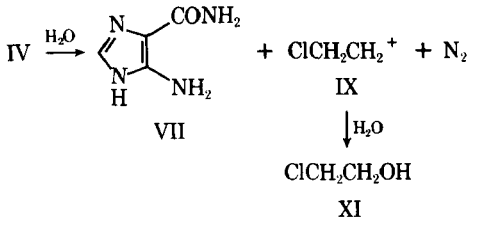
Of a variety of imidazole, pyrazole, benzenoid, and other triazenes evaluated against lymphoid leukemia L-1210 in mice, 5-[3,3-bis(2-chloroethyl)-1-triazenyl]imidazole-4-carboxamide[1] (I) was, by far, the most effective. This compound effected some cures in standard L-1210 tests (1, 2), and combinations of I with nitrosoureas cured the majority of animals with advanced leukemia L-1210 (3). The greater effectiveness of this compound and the presence of the $(ClCH_2CH_2)_2N$— group might suggest that the mechanism of action of I differs from that of other dialkyltriazenes.

Skibba and coworkers (4–6) showed that ^{14}C-labeled dacarbazine[1] [5-(3,3-dimethyl-1-triazenyl)imidazole-4-carboxamide, II] is converted by microsomal preparations from rat liver to 5-aminoimidazole-4-carboxamide[1] (VII), formaldehyde, and nucleic

[1] The following abbreviations of Compounds I–III and VII have been employed in the literature: Compound I (NSC-82196), BIC; Compound II (NSC-45388), DIC and DTIC; Compound III (NSC-407347), MIC; and Compound VII, AIC.



I: $R_1 = R_2 = CH_2CH_2Cl$
II: $R_1 = R_2 = CH_3$
III: $R_1 = H$, $R_2 = CH_3$
IV: $R_1 = H$, $R_2 = CH_2CH_2Cl$

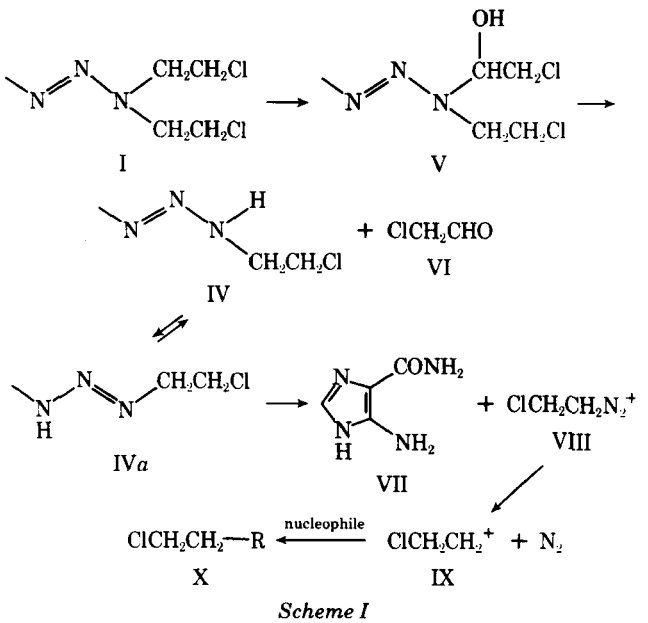


Scheme II

acids containing radioactive 7-methylguanine. Analogous results were obtained by incubating labeled II with microsomal preparations from mouse tumor tissues (7). These studies showed that II is demethylated by microsomal oxidases and that a methylating agent is generated by this process.

Similarly, studies of the metabolism of ^{14}C-labeled II (labeled at the methyl groups or at position 2 of the imidazole ring) in rats (4, 5), dogs (8, 9), mice (9), and patients (4, 5, 8–10) identified the aminoimidazole (VII) and expired carbon dioxide as metabolic products *in vivo*. Utilization of VII and of formate (both derived from labeled II) in the biosynthesis *de novo* of purine nucleotides may explain the observed labeling of nonmethylated purines (6, 9) and may account in part for the radioactivity of nucleic acids (6, 11); however, the identification of 7-methylguanine as a constituent of nucleic acids and as a urinary excretion product after administration of II confirms the metabolic generation of a methylating agent *in vivo* (6).

5-(3-Methyl-1-triazenyl)imidazole-4-carboxamide[1] (III) was shown earlier to have activity against L-1210 leukemia and to dissociate spontaneously in solution to the aminoimidazole (VII) and, presumably, to the methyldiazonium ion and then to the methylcarbonium ion (12). Taken together, the enzymatic, metabolic, biological, and chemical studies indicated that the dimethyltriazene (II) is activated by dealkylation to the monomethyltriazene (III) and that the methylcarbonium ion is the reactive species responsible for activity. Presumably, other active dialkyltriazenes are metabolized similarly, and III or an analogous monoalkyltriazene is the immediate progenitor of a methyl or other alkyl carbonium ion.



Scheme I

It has been suggested (13, 14) that I may act by dissociation to bis(2-chloroethyl)amine (nor-nitrogen mustard) and 5-diazoimidazole-4-carboxamide. However, if I is metabolized by microsomal oxidases in a manner similar to II and to phenyltriazenes (15), two highly reactive species should be formed (Scheme I). The presumed intermediate (V) should decompose spontaneously to 5-[3-(2-chloroethyl)-1-triazenyl]imidazole-4-carboxamide (IV) and to chloroacetaldehyde (VI).

By analogy to other monoalkyltriazenes (12, 16), IV should dissociate to VII and to the chloroethyldiazonium ion (VIII), which should, in turn, decompose to nitrogen and a chloroethylcarbonium ion (IX). Either the chloroethylcarbonium ion or chloroacetaldehyde (or both) may be responsible for the activity of I. One of the presumed intermediates, IV, has been prepared, studied for stability, and evaluated against leukemia L-1210.

The mono(chloroethyl)triazene (IV) was prepared by treating 2-chloroethylamine free base in dry ethyl acetate solution with 5-diazoimidazole-4-carboxamide (added in one portion) by a procedure similar to previously described procedures (12). A white precipitate was washed thoroughly with ethyl acetate, slurried with absolute ethanol in the dark, and dried *in vacuo* at room temperature[2], yielding 83%; violent explosive decomposition[3] at 114° (inserted at 80°, 3°/min); strong IR bands at 3475, 3250, 3070, 1635, 1585, 1420, and 1380 cm^{-1}.

Anal.—Calc. for $C_6H_9ClN_6O$: C, 33.26; H, 4.19; N, 38.79. Found: C, 33.38; H, 4.33; N, 38.62.

The thermal instability[2] of IV precluded mass spectral analysis, and the sparing solubility and solvolytic instability prevented proton magnetic resonance analysis. The IR spectrum of a specimen of IV kept at −15° for 1 year was unchanged.

In water or in 50% ethanol, IV decomposed in the dark to 5-aminoimidazole-4-carboxamide (VII). For example, a suspension of IV (200 mg) in water (50 ml) was stirred in the dark until essentially all IV had dissolved (about 8 hr). Evolution of a gas (presumably nitrogen) was observed. After filtration of the mixture to remove undissolved IV (2 mg), GLC of an

[2] Compound IV is very explosive; it is sensitive to heat and to shock. A specimen (prepared prior to the synthesis of I) decomposed explosively while being dried at 78°.

[3] The decomposition temperature is reproducible when it is determined as stated.

Table I—5-[3-(2-Chloroethyl)-1-triazenyl]imidazole-4-carboxamide[a] (IV) *versus* L-1210 Leukemia[b]

Dose, mg/kg	Mortality by Day 5	Average Weight Change Difference, g[c]	Number of 30-Day Survivors/Total	Nonsurvivors	
				Average Survival Time, days	T/C Ratio, %
Day 1 Schedule:					
100	2/6	−6.2t[d]	0/6	6.3/9.4	67t
50	0/6	−4.6t	2/6	14.0/10.0	140
37	0/6	−1.7	4/6	14.5/9.9	146
25	0/6	−2.2	6/6		
25	0/6	−2.0	5/6	15.0/9.9	151
17	0/6	−1.1	3/6	14.3/9.9	144
12.5	0/6	−1.3	3/6	18.0/10.0	180
6.25	0/6	−1.7	0/6	14.8/10.0	148
qd, Days 1–9:					
25	0/6	−4.2t	0/6	9.2/10.0	92
12.5	0/6	−3.3	0/6	16.0/10.0	160
9	0/6	−2.4	2/6	16.5/9.9	166
6.25	0/6	−2.4	3/6	20.3/10.0	203
6	0/6	−1.6	1/6	22.4/9.9	226
4	0/6	−1.0	0/6	15.5/9.9	156
3.12	0/6	−1.9	1/6	14.4/10.0	172

[a] Suspensions of this compound in saline + Tween 80 were administered intraperitoneally within 5 min of the preparation of the suspensions. [b] L-1210 cells (10^5) were implanted intraperitoneally in mice on Day 0. [c] Average weight change of treated mice minus average weight change of control mice by Day 5. [d] t = toxic.

aliquot of the reaction solution showed that it contained 2-chloroethanol (XI), which was identified by comparing its retention time with that of an authentic commercial sample. The concentration of XI in the aliquot corresponded to a yield of 70% of XI from the chloroethyltriazene (IV).

Lyophilization of the remaining reaction solution left a solid (123 mg, 93% yield as VII monohydrate) that was shown by TLC to be almost pure VII. (Trace amounts of three impurities were detected.) The identity of the residue was confirmed by its mass spectrum, which was compared with that of an authentic specimen of pure VII free base: *m/e* 126 (molecular ion, base peak), 109 (126 — NH_3), and 94.3 (metastable ion, 126 → 109). Evidence of slight alkylation of VII by the 2-chloroethylcarbonium ion (IX) during this experiment or in similar experiments was found in the mass spectra of the isolated products (all of which were predominantly VII). The following weak peaks in some spectra support this conclusion: *m/e* 188 (M^+ of product of alkyation of VII by IX), 170 (M^+ of product of alkylation of VII by IX and replacement of the chloro group by a hydroxy group), 152 (M^+ of product of alkylation of VII by IX followed by formation of a vinyl group or of a cyclic derivative of VII *via* loss of HCl), and 139 (loss of CH_2Cl or CH_2OH from an alkylated VII derivative).

As shown in Scheme II, the mode of decomposition is analogous, therefore, to that of the monomethyltriazene (III) and similar monoalkyltriazenes (12) and is in contrast to the intramolecular cyclization reaction of I (1, 17, 18).

In standard L-1210 leukemia tests, IV was administered intraperitoneally as a suspension on Day 1 and on Days 1–9 (qd). The data in Table I indicate that there were some "cures," as evidenced by 30-day survivors. The most effective dose was 25 mg/kg on the Day 1 schedule; in two tests, 11 of 12 treated mice survived until the end of the 30-day test period. In comparable tests (1, 2) of I against L-1210 leukemia, the optimal single and daily doses were about 200–500 mg/kg and 50–75 mg/kg/day (qd 1–30 or death), respectively. These doses are about 10-fold those of the approximate optimal doses (Table I) of IV.

The results of tests *in vivo* show that IV, if it is formed as outlined in Scheme I, could account for the observed activity of I. Therefore, the results are consistent with the activation of I by microsomal oxidases. The demonstrated mode of dissociation of IV provides evidence that the chloroethylcarbonium ion is the reactive species responsible for the activity of IV (IX → X) and, after activation, of I[4]. Because of the potent activity of IV, a corollary is that the chloroethylcarbonium ion is exceptionally effective in manifesting antineoplastic activity[5].

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[4] After the initial reaction of IX with a nucleophilic center *in vivo*, the chloroethyl derivative (X) may undergo further (intermolecular or intramolecular) reaction.

[5] Although the activity of triazenes and certain other anticancer agents may be due to the generation of methyl, chloroethyl, or other carbonium ions, it does not necessarily follow that alkylation of guanine or other nucleic acid moieties is responsible for the observed activity.

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BOOKS

REVIEWS

International Aspects of Drug Evaluation and Usage. Edited by A. J. JOUHAR and M. F. GRAYSON. Longman Inc., 72 Fifth Ave., New York, NY 10011, 1973. 374 pp. 14 x 23 cm. Price $21.00.

The book records the proceedings of the International Meeting of Medical Advisors in the Pharmaceutical Industry held April 17–20, 1972 in London. The meeting was conceived as an attempt to assess the current state of the evaluation of drugs around the world. The major contributors to the book were medically qualified people working in the pharmaceutical industry. Because of the broad spectrum of contributors, the book is worthwhile reading for anyone involved in drug development in the pharmaceutical industry. Due to the international nature of the symposium, much of the material concerning, directly or indirectly, the regulatory aspect of drug development is not entirely applicable to requirements in the United States. The book does provide, however, *via* its international contributors, an interesting overview of the data required by drug regulatory agencies outside the United States. The book's most useful aspect is its exchange of experiences in drug development by people intimately involved in the work on a world-wide basis. It is always useful and consoling, if not profoundly informative, to be made aware of the approaches and frustrations of others involved in drug evaluation and development. The many chapters in this vein are quite useful in confirming one's approach to drug development, and in demonstrating that one's unanswered questions are also unanswered questions by others working in the same area.

One particularly noteworthy chapter is by C. Maxwell. Maxwell provides some extremely worthwhile comments and philosophy in his opinion and overview on the preclinical screening of new drugs, the proper philosophy for the clinical testing of drugs, and the use of statistics in clinical efficacy decision making.

Overall, the book outlines a very practical, realistic approach to drug testing and clinical evaluation. "International Aspects of Drug Evaluation and Usage" is, therefore, recommended reading by anyone involved in the clinical testing and/or supervision of clinical testing of new drugs.

Reviewed by William A. Cressman
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USAN 10 and the USP Dictionary of Drug Names, 1974 Supplement. Published for the USAN Council by the U.S. Pharmacopeial Convention, Inc., Rockville, MD 20852, 1974. vi + 100 pp. 21 × 28 cm. Price $4.75.

This recently published Supplement contains 5747 entries including all entries from the 1973 Supplement. Seventy-three new USAN names are incorporated into this Supplement and 125 new code designations are included. The USAN books also provide information on USP and NF official names, FDA established names, and brand names, abbreviations, and trivial names.

The USAN publications are a most important aspect of the effort to bring uniformity to the area of drug nomenclature. As such they should be available to and utilized by all persons involved with pharmaceuticals.

Staff Review

Everything You Wanted To Know About Drug Abuse . . . But Were Afraid To Ask. By C. L. WINEK. Dekker, New York, NY 10016, 1974. 213 pp. 15.5 × 23.6 cm. Price $12.75.

The material in this book is presented in an easy to read form of questions and answers and is divided into 14 sections generally by category of abused drug.

The book is based on the author's experience in dealing with the drug problem on a daily basis over the past 6 years. The material is based on real experiences obtained during the author's various activities concerned with the drug problem including investigating drug-related deaths and conducting toxicological analyses.

The questions included in this book are those put to the author during various speaking engagements and seminars by students, teachers, and various health-care professionals.

Staff Review