Table I—Distribution and Elimination Rate Constants

Case	k_{12}	k_{21}	k_{el}
1	1.0	1.5	0.01 → 4.0
2	1.0	0.1	0.01 → 4.0
3	0.1	1.0	0.01 → 4.0
4	0.1	0.15	0.01 → 4.0

half-lives are increased between 1.4 and 3.3 times the minimum possible value. For most drugs, however, biological half-lives in conditions of normal renal function may be considerably longer than the minimum value and a linear relationship might be expected after a much smaller increase in half-life.

Even when the extreme case applies, it is evident from these data that dose adjustment assuming a linear relationship between ln $2/t_{1/2}$ and C_{cr} is a realistic procedure regardless of the pharmacokinetic model. The errors in the assumption would be greatest in conditions of relatively mild renal insufficiency, where dose adjustment might not be considered necessary.

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Free Amino Acids in Higher Marine Fungi

Keyphrases □ Marine fungi (higher)—determination of free amino acids from Ascomycetes and Fungi Imperfecti □ Fungi, marine—determination of free amino acids □ Amino acids, free—content of Ascomycetes and Fungi Imperfecti determined

To the Editor:

Attention has been directed to the potential importance of higher marine fungi as contributors of metabolites to marine ecosystems and producers of bioactive compounds (1). Continuing our investigations on the overall metabolic capabilities of these organisms, we now report on the free amino acids of selected marine Ascomycetes and Fungi Imperfecti. Related published reports are limited to that by Schafer and Lane (2), who identified 12 amino acids from hydrolyzed peptides of the Ascomycete *Lulworthia floridana* Meyers, and Kirk's (3) cytochemical investigations of marine pyrenomycete ascospores.

Shake cultures of 10 isolates (Table I) were grown on a yeast extract–glucose–inorganic salts[1] medium and harvested after 7 days as described previously (4). Mycelia were washed thoroughly in cold water,

[1] Rila Marine Mix, Rila Products, Teaneck, N.J.

Table I—Organisms Investigated

Ascomycetes	Isolate[a]
Lignincola laevis Höhnk	R-2
Nais inornata Kohlm.	R-4
Leptosphaeria oraemaris Linder	R-13
Corollospora maritima Werdermann	R-19
Halosphaeria mediosetigera Cribb *et* Cribb	R-524
Halosphaeria hamata (Höhnk) Kohlm.	R-577
Haligena elaterophora Kohlm.	R-601
Halosphaeria appendiculata Linder	R-605
Fungi Imperfecti	
Culcitalna achraspora Meyers *et* Moore	F-1
Zalerion maritimum (Linder) Anastasiou	R-6

[a] Refers to isolate number of particular species as cataloged in the mycological collection of P. W. Kirk, Jr., Old Dominion University, Norfolk, VA 23508

dried in forced air at 40° for 48 hr, and defatted with petroleum ether (bp 30–60°) for 12 hr in a soxhlet apparatus. Extracts of free amino acids were then prepared according to the method of Heathcote *et al.* (5), modified by extending the aqueous extraction phase to 48 hr. These extracts were desalted and purified according to Pocklington (6). The extracts, free from interfering ions and peptides, were analyzed by TLC and GLC.

Aqueous extracts were spotted on thin-layer cellulose plates and developed in a two-dimensional system: isopropanol–butanone–1 *N* HCl (12:3:5) in the first direction and butanol–acetic acid–water (2:1:1) in the second. Identification of the amino acids was achieved by comparing chromatograms of reference amino acids with those of extracts and mixtures of the two. The amino acid spots were revealed by heating plates sprayed with ninhydrin solution.

GLC analyses were based on the method described by Zumwalt *et al.* (7). The instrument[2] used was equipped with a flame-ionization detector. Instrument conditions included: silanized[3] borosilicate glass column, 1.8-m × 0.3-cm (6-ft × 0.125-in.) o.d., packed with 3% methyl silicone on calcined diatomaceous earth[4]; carrier gas, nitrogen at 45 ml/min; oven, 75° increased by 7.5°/min to 300°; detector, 250°; flash heater, 200°.

Reference trimethylsilyl amino acid derivatives were prepared as follows (8). One milligram of amino acid was dissolved in 50 ml of methanol. One milliliter of this stock solution was placed in a 0.6 × 5.1-cm (0.25 × 2-in.) screw-capped vial containing 1 ml of anhydrous methylene chloride. This was heated to 70° in a sand bath to remove the solvent and azeotropically remove all moisture. While the vial remained in the sand bath, its contents were further dried under a stream of nitrogen for 30 min.

To the dried residue was added 0.25 ml of bis(trimethylsilyl)trifluoroacetamide, and this mixture was heated for 1 hr in a sand bath at 135°. After refluxing, the vial was allowed to cool spontaneously. Derivatives of the dried free amino acid extracts were prepared in an identical manner. Freshly prepared

[2] Hewlett-Packard model 402.
[3] Sylon, Supelco, Inc.
[4] 3% OV-1 on Gas Chrom Q, Applied Sciences Labs., Inc.

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] Difco Laboratories, Inc., Detroit, Mich.

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.