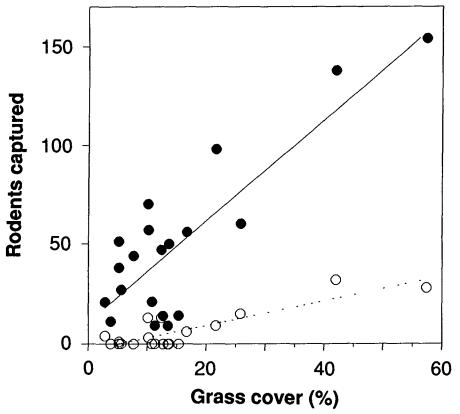

Fig. 3. Number captured per plot since 1983 of rodent species typical of grassland habitat as a function of mean percentage of cover of all tall perennial grasses plus *Aristida adscensionis*. Unshaded symbols represent only the most specialized grassland species, *Sigmodon hispidus, S. fulviventer,* and *Baiomys taylori;* shaded symbols represent the above species plus *Reithrodontomys* spp. Least-squared regression lines are fitted, and both are statistically significant ($P < 0.0001$).

may facilitate decomposition of litter, establishment of many annuals, and foraging of birds (*8*). Conversely, the reduction in soil disturbance following exclusion of kangaroo rats promoted the establishment and persistence of tall grasses, and this in turn favored colonization by specialized grassland rodent species.

Although kangaroo rats presumably cause size-selective seed predation and extensive soil disturbance wherever they are abundant, the kind and magnitude of changes reported here would probably not be duplicated if kangaroo rats were removed from different kinds of desert habitats. Our experimental site is near the zone of natural transition from desert to grassland, so that certain abiotic conditions or the presence or absence of keystone species can cause a shift between alternative vegetation types. Elsewhere in the southwestern United States grazing by domestic livestock is known to cause degradation of arid grassland to desert shrubland (*10*). At our site, however, the effects on vegetation of the exclusion of kangaroo rats in combination with livestock exclusion were much greater than those produced by the exclusion of cattle and horses alone. Grazing livestock were excluded from our entire 20-ha site since 1977, but no significant change in vegetation has yet been detected across the fenceline (*11*).

In the present case the "keystone" organism whose removal caused large changes in ecosystem structure and dynamics was not a single species, but a guild of three taxonomically related and ecologically similar kangaroo rat species. Removal of the largest and behaviorally dominant of these (*D. spectabilis*) had significant effects on the abundance and distribution of other desert rodents (*4, 12*), but it required the removal of all three species to cause wholesale changes in vegetation. On the other hand, the eight common and several rare species of desert rodents that remained after kangaroo rats had been removed clearly did not play the same keystone role and were not able to prevent the conversion of desert to grassland.

Twenty-five years after the concept of "keystone species" was first introduced, examples have been found in a number of taxonomic groups and habitat types (*1, 3*). It remains, however, to develop a general conceptual framework that will predict which kinds of organisms play key roles in different kinds of ecosystems. Native species are increasingly being eliminated from local habitats and larger regions as a result of human activities. It is critical to develop a theoretical basis for assessing the effects of these species on ecosystems so that, if extirpation of keystone organisms cannot be avoided, their roles can be replaced by other native or exotic species or by active ecosystem management.

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Characterization of "Peak E," a Novel Amino Acid Associated with Eosinophilia-Myalgia Syndrome

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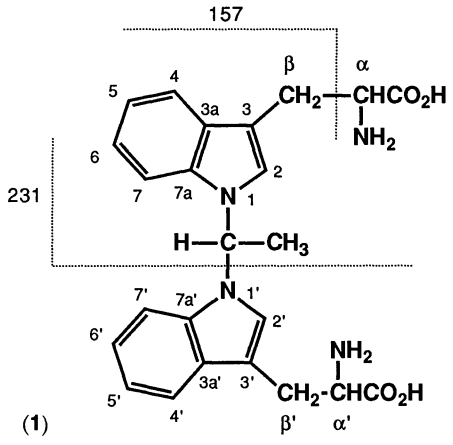
Epidemiologic studies strongly associate eosinophilia-myalgia syndrome (EMS) with ingestion of tryptophan containing a contaminant ("peak E"). Prior reports have suggested that peak E is the di-tryptophan Nα-aminal of acetaldehyde. Spectral and chemical studies now demonstrate that peak E is 1,1′-ethylidenebis[tryptophan]. This novel amino acid may be the etiological agent responsible for EMS, or it may be a marker of a still unidentified causal agent.

As of August 1990, EMS was linked to 27 deaths and over 1500 cases (*1*). Epidemiological studies (*2, 3*) have associated EMS with the ingestion of L-tryptophan (Trp) produced by a single manufacturer, suggesting that a contaminant is responsible for EMS. Recently we reported the discovery of a contaminant (peak E) in the Trp samples consumed by EMS patients that was absent in the Trp consumed by asymptomatic controls (*3*). A significant association exists between the presence of peak E and EMS-associated Trp (*3, 4*). Two groups have previously reported that peak E is the di-tryptophan Nα-aminal of acetaldehyde (*4, 5*). We present chemical and spectral data which show that peak E is actually the isomeric 1,1′-ethylidenebis[tryptophan] (**1**).

Peak E was isolated by high-performance liquid chromatography (HPLC) (*3*). Fast atom bombardment–mass spectrometry (FAB-MS) revealed peaks at mass-to-charge ratios (*m/z*) of 157, 231 (base peak), and 435 ($[M + H]^+$). High-resolution FAB-MS gave a mass of 435.2041, consistent with the molecular formula $C_{24}H_{27}N_4O_4$.

The proton nuclear magnetic resonance (^1H NMR) spectrum of peak E in D_2O (*6*) was similar to that of Trp in terms of



chemical shifts and coupling patterns, but with two significant differences. First, several of the signals were doubled; ^{1}H-^{1}H correlation spectroscopy (COSY) showed that the doubling was not due to coupling but to two slightly nonequivalent Trp units. The nonequivalence was more apparent in deuterated dimethyl sulfoxide (DMSO-d_6). Second, a quartet at 7.03 ppm and doublet at 2.06 (both of which were not present in Trp) were observed for peak E; these signals were shown to be coupled (J = 6.7 Hz) by a COSY experiment and attributed to the presence of an ethylidene (>CHCH$_3$) unit.

Hydrolysis of peak E (0.1% aqueous trifluoroacetic acid, pH 2, 25°C) slowly produced Trp with concomitant disappearance of peak E, as observed by HPLC. Acetaldehyde and its hydrate were detected by ^{1}H NMR during hydrolysis in D_2O. The ultraviolet (UV) spectrum of peak E in H_2O was similar to that of Trp, showing a broad band at 281.5 nm but with less pronounced shoulders. More significantly, the band was broader and at a longer wavelength than observed in Trp. Peak E lacked the characteristic band at ~3400 cm^{-1} of the indole N–H stretch by Fourier transform infrared spectroscopy. Because a bathochromic shift in the UV spectrum is observed with 1-alkyltryptophans and NMR indicated no additional substituents on the indole carbons, attachment of the ethylidene at the 1-position was suspected. Infrared data also suggested that the bridging occurred at the indole nitrogens rather than at the α-amino groups.

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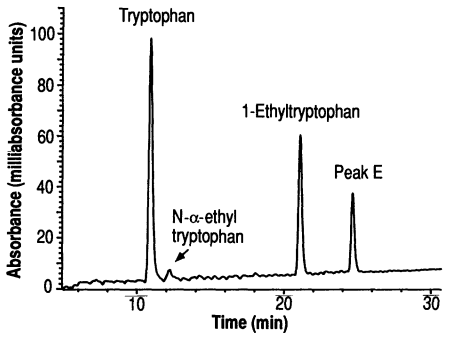
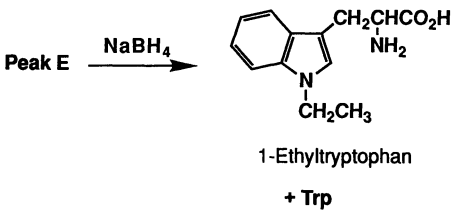


Fig. 1. An HPLC trace showing the formation of 1-ethyltryptophan from peak E by reduction with sodium borohydride ($NaBH_4$); $NaBH_4$ was added to peak E in water, allowed to react for 10 hours, acidified to pH ~2 with HCl, and analyzed. The peak labeled 1-ethyltryptophan co-eluted with an authentic sample.

Conclusive evidence for bridging through the indole nitrogens was obtained from the following experiments. First, ^{1}H NMR showed that peak E in DMSO-d_6 lacked the N-1 proton signal at δ ~ 11 ppm; this signal was present in all model compounds that were unsubstituted at the 1-position. Second, addition of NaOD to peak E in DMSO-d_6 caused no significant change (0.1-ppm shift observed) in the chemical shift of the bridging C–H, although a major upfield shift (1.3 ppm) was recorded for the α-protons. If the ethylidene were attached to the α-amino groups, a similar upfield shift would be expected for the methine signal because of deprotonation of the attached amines. Furthermore, comparison of the chemical shift (7.03 ppm) of the bridging methine to that of models clearly showed that the linkage was between the two indole nitrogens instead of the α-amino groups (7). Finally, the position of the bridging in peak E was confirmed by $NaBH_4$ reduction, which cleaves the labile aminal linkage. If the bridging were between the α-amino groups, *N*α-ethyltryptophan and Trp would be the expected products. Instead, reduction of peak E afforded 1-ethyltryptophan, Trp, and only a very minor amount of *N*α-ethyltryptophan (*8*) (Fig. 1). Each product co-eluted with authentic materials and displayed the correct UV spectrum.



Structure **1** is consistent with the FAB-MS data described above: fragmentation at the aminal linkage would give the peak at *m/z* 231; a second cleavage between the α and β-carbons would give the *m/z* 157 peak. 1,1′-Alkylidenebisindole derivatives are unusual; **1** is an isomer of that previously proposed (*4*, *5*), in which the Trp units are linked by the α-amino groups. Although **1** hydrolyzes under acidic conditions (half-life ~12 hours, pH 2, 25°C), the half-life of gastric emptying following ingestion of water is ~10 min (*9*). Thus, significant quantities of peak E would likely reach the small intestine and be available for absorption.

At present, the biological significance of **1** is unknown. This novel amino acid may be the substance responsible for EMS or a marker of the manufacturing conditions leading to the formation of an as yet unidentified causal agent.

Note added in proof: Subsequent to submission of this manuscript, a revised structure identical to **1** was reported (*10*).

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6. ^{1}H NMR for peak E (500 MHz, D_2O) δ 7.563 (d, 1H, 4 or 4′), 7.556 (d, 1H, 4 or 4′), 7.38 (d, 1H, 7 or 7′), 7.37 (d, 1H, 7 or 7′), 7.324 (s, 1H, 2 or 2′), 7.318 (s, 1H, 2 or 2′), 7.12 [t, 2H, (two of 5, 6, 5′, 6′)], 7.06 [t, 2H, (two of 5, 6, 5′, 6′)], 7.03 [q, 1H, J = 6.7 Hz, C*H* (bridging)], 3.89 [dd, 2H, CH and CH (α, α′-protons)], 3.30 (dd, 1H, CH*H* or CH′*H*′), 3.27 (dd, 1H, CH*H* or CH′*H*′), 3.16 (dd, 1H, CH*H* or CH′*H*′), 3.13 (dd, 1H, CH*H* or CH′*H*′), 2.06 [d, 3H, J = 6.7 Hz, C*H*$_3$ (bridging)]. Abbreviations = d, doublet; s, singlet; t, triplet, q, quartet; and dd, doublet of doublets.
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