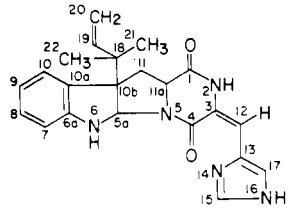
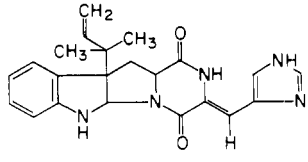
Configuration of the 3,12 Double Bond of Roquefortine

The *E* configuration of the 3,12 double bond of roquefortine, a metabolite of the commercially important fungus *Penicillium roqueforti*, was established by comparison of its spectral properties with those of a photoisomer. The structure (*E*)-3-(1*H*-imidazol-4-ylmethylene)-6-(1*H*-indol-3-ylmethyl)-2,5-piperazinedione is proposed for the major product of an acid-catalyzed de-isoprenylation reaction of roquefortine.

Roquefortine is a metabolite of *Penicillium roqueforti* strains isolated from blue cheese (Scott et al., 1977) and has itself been found in blue cheese at estimated concentrations of up to 6.8 ppm (Scott and Kennedy, 1976). The same alkaloid has been isolated in Japan under the name roquefortine C (Ohmomo et al., 1977). Neurotoxic properties have been attributed to roquefortine (Scott et al., 1976) but these were not observed in recent experiments by Arnold et al. (1978). Roquefortine was assigned either structure I



I



II

or II (10b-(1,1-dimethyl-2-propenyl)-6,10b,11,11a-tetrahydro-3-(1*H*-imidazol-4-ylmethylene)-2*H*-pyrazino[1′,2′:1,5]pyrrolo[2,3-*b*]indole-1,4(3*H*,5a*H*)-dione) without determination of the configuration of the 3,12 double bond (Scott et al., 1976). This has now been shown to be *E* by comparison of spectral properties of roquefortine with those of a photoisomer.

EXPERIMENTAL SECTION

Photoisomerization of Roquefortine. Ninety milligrams of roquefortine in 100 mL of 95% ethanol was irradiated under nitrogen for 6 h at a wavelength of 330 nm. After evaporation of solvent, the residue was chromatographed on a column of 9 g of Florisil. Elution with chloroform–methanol (99:1) yielded 78 mg of photoproduct, which was further purified by preparative thin-layer chromatography (TLC) (Scott and Kennedy, 1976) to give a pale buff, amorphous solid.

Acid Fragmentation of Roquefortine. A solution of 160 mg of roquefortine in 5 mL of methanol–1 N hydrochloric acid (4:1) was refluxed for 3 h, cooled, and passed through a column of 3 g of Amberlite IR-45 (OH), previously washed with 5 mL of methanol–1 N hydrochloric acid (4:1). The column was washed with 40 mL of methanol and the combined eluates were evaporated and dissolved in chloroform–methanol (1:1). The reaction mixture was separated by preparative TLC on E. Merck silica gel 60 F-254 using chloroform–methanol (4:1) as solvent system and chloroform–methanol (1:1) to elute the products from the silica gel. The major product (53 mg) was further purified by preparative TLC, then added to a small column (1 g) of silica gel 60 and eluted with chloroform methanol (3:1). Evaporation of a methanol solution yielded 17 mg of crystalline solid, mp 252–253 °C (dec).

***cis*-Urocanic Acid.** Photoisomerization of *trans*-urocanic acid (Edlbacher and Heitz, 1943) afforded *cis*-urocanic acid, mp 178–179 °C.

Nuclear Magnetic Resonance (NMR) Spectroscopy. ^1H and ^{13}C NMR spectra of irradiated roquefortine and the ^{13}C NMR spectrum of the fragmentation product were recorded on a Bruker HX-90E F.T. NMR spectrometer operating at 90 MHz or 22.63 MHz. ^{13}C NMR spectra of *cis*- and *trans*-urocanic acid were measured on a Bruker WP-80 F. T. NMR spectrometer at 20.1 MHz. Broad band and off-resonance decoupling were used for ^{13}C NMR spectra. All signals were measured in ppm (δ) downfield from tetramethylsilane internal standard.

High-Resolution Mass Spectroscopy. Mass spectra were recorded on Varian MAT 311 and AEI MS 50 mass spectrometers.

RESULTS AND DISCUSSION

The photoisomer of roquefortine had molecular formula $C_{22}H_{23}N_5O_2$ (by high-resolution mass spectrometry) with $[\alpha]_D$ −409° (*c* 0.071 in chloroform), λ_{max} (95% ethanol), 212 (log ϵ 4.236), 240 (log ϵ 4.113), and 311 nm (log ϵ 4.434) and ν_{max} (chloroform) 3464, 3197, 3093, 2982, 1676, and 1627 cm^{-1}. The ^1H NMR spectrum measured in $CDCl_3$ showed signals at δ 1.02 (s, 3 H, H-21), 1.14 (s, 3 H, H-22), 2.50 (m, 2 H, H-11), 4.09 (dd, 1 H, J = 10.5 and 6.5 Hz, H-11a), 5.00 (s, br, 1 H, NH), 5.10 (m, 2 H, H-20), 5.66 (s, 1 H, H-5a), 6.03 (m, 1 H, H-19), 6.57–7.20 (6 H, including 6.67 s, 1 H, H-12, and 7.12 s, 1 H, H-17), 7.66 (s, 1 H, H-15), 11.02 (s, br, 1 H, NH), and 11.81 (s, 1 H, NH) ppm. The peak due to H-12 shifted from δ 6.35 in a comparable spectrum of roquefortine to 6.67 for the photoisomer on account of deshielding by the carbonyl group at position 4, analogous to a shift from δ 6.45 to 7.06 for the vinylic proton of *cis*- and *trans*-3-benzylidene-1-methyl-2,5-piperazinedione (Porter and Sammes, 1970). Thus the photoisomer II has the *Z* configuration about the 3,12 double bond. The signal at δ 7.26 in the spectrum of roquefortine, assigned to H-17, shifted in the other direction to δ 7.12 in the spectrum of the isomer. The ^{13}C NMR spectrum of the photoisomer (in $CDCl_3$) closely resembled that of roquefortine (Scott et al., 1976) except for differential shifts of carbons assigned to C-3 (from δ 122.3 to 137.1), C-12 (from δ 110.9 to 105.7 or 118.0), and C-17 (from δ 134.3 to 118.0 or 105.7). Comparison of the ^{13}C NMR spectra (in Me_2SO-d_6) of *cis*- and *trans*-urocanic acid (3-(1*H*-imidazol-4-yl)-2-propenoic acid), which had signals at δ 167.1 s, 136.1 d, 134.2 s, 130.2 d, 123.0 d, and 118.5 d (cis) and δ 168.3 s, 137.9 d, 135.7 d, 134.5 s, 123.8 d, and 115.2 d (trans), showed virtually no change in the chemical shift of the singlet due to C-4 on the imidazole ring, corresponding to C-13 in roquefortine and the photoisomer. Further evidence for the 3*Z* configuration of the photoisomer of roquefortine is given by the hypsochromic shift in the longwave UV absorption band (326 nm in roquefortine), comparable to such shifts in *cis*- and *trans*-3-benzylidine-2,5-piperazinediones (Blake and Sammes, 1970; Porter and Sammes, 1970).

The 3*E* configuration in roquefortine (I) is the same as in the didehydrohistidine moiety of oxaline, an alkaloid from *P. oxalicum* (Nagel et al., 1976). Roquefortine is exceptional in that as far as is known, other microbial

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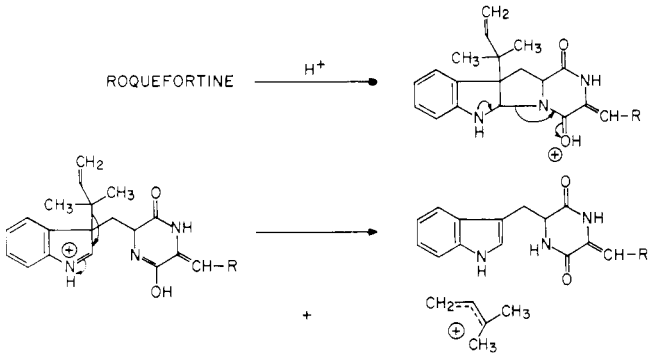
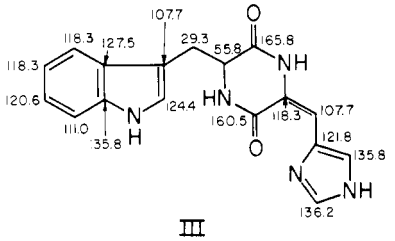


Figure 1. Probable mechanism for the acid cleavage of roquefortine; R = 1*H*-imidazol-4-yl.

piperazine-2,5-diones formally derived in part from α,β-didehydroamino acids, viridamine (didehydrohistidine) (Holzapfel and Marsh, 1977), neoechinulin A, cryptoechinuline A, and related compounds (didehydrotryptophan) (Cardillo et al., 1975; Marchelli et al., 1977), mycelianamide (didehydrotyrosine) (Kirby and Narayanaswami, 1976), and albonoursin (didehydroleucine and didehydrophenylalanine) (Shin et al., 1977), have the *Z* configuration.

Roquefortine (I) underwent an unusual facile fragmentation when refluxed in dilute methanolic hydrochloric acid. The major product, (*E*)-3-(1*H*-imidazol-4-ylmethylene)-6-(1*H*-indol-3-ylmethyl)-2,5-piperazinedione (III) (Polonsky et al., 1977), had $[\alpha]_D$ +164.5° (*c* 0.46 in pyridine), molecular formula $C_{17}H_{15}N_5O_2$ (by high-resolution mass spectrometry, with principal fragment ions of composition $C_8H_8N_4O_2$ and C_9H_8N), λ_{max} (95% ethanol) 221 (log ε 4.532), 286 sh (log ε 4.027), 291 (log ε 4.073), and 326 (log ε 4.275) nm, in agreement with the presence of two chromophores, and ν_{max} (Nujol) 3500, 3250, 1690, and 1620 cm^{-1}. The ^1H NMR spectrum (60 MHz, in pyridine) confirmed loss of the isoprene unit while signals in the ^{13}C NMR spectrum (in Me_2SO) were assigned as indicated in structure III



III

by comparison with ^{13}C NMR spectra of roquefortine (Scott et al., 1976), 3-methylindole (Fraser et al., 1976), 3-ethylidene-6-(1*H*-indol-3-ylmethyl)-1-methyl-2,5-piperazinedione (Kakinuma and Rinehart, 1974), and echinulin (Cardillo et al., 1974). The fragmentation involves breakage of a C–C bond under mild reaction conditions (Figure 1) and may be conjectured to occur in vivo during metabolism of roquefortine. Similar loss of a 1,1-dimethyl-2-propenyl side chain has been previously observed when oxaline was treated with dilute HCl but the product, which contained chlorine, was not structurally characterized (Nagel et al., 1976).

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