Stereochemistry of the Dehydrogenation of (2*S*)-Histidine in the Biosynthesis of Roquefortine and Oxaline

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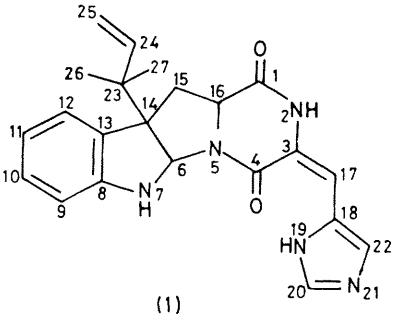
Summary A comparison of spectral data indicates the *E* configuration for the dehydrohistidine unit in both roquefortine and oxaline; incorporation of (2*S*,3*S*)- and (2*S*,3*R*)-[3-^{3}H]histidine into roquefortine by *Penicillium roqueforti* and into oxaline by *Penicillium oxalicum* proceeded in each case with removal of the *pro-S* hydrogen atom from C(3).

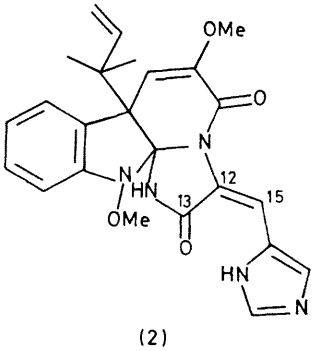
NUMEROUS $\alpha\beta$-dehydroamino acids have in recent years been recognised as constituents of metabolites derived from micro-organisms[1] and one, dehydroalanine, has been identified at the active site of the enzyme histidine ammonia-lyase[2,3] and (2*S*)-phenylalanine ammonia-lyase.[4] Only a few studies, however, have been reported on the stereochemical course of the *in vivo* formation of $\alpha\beta$-dehydroamino acids.[5–7]

The recent isolation and structure elucidation of the neurotoxin roquefortine (**1**) from cultures of *Penicillium roqueforti*[8] and our work on the structure of oxaline (**2**), a metabolite isolated from *Penicillium oxalicum*,[9,10] prompted us to investigate the stereochemistry of the dehydrogenation of (2*S*)-histidine in these two metabolites.

X-Ray crystallography[9] and ^{13}C n.m.r. spectroscopy[10] established the *E* configuration for the 12,15 double bond in oxaline (**2**). The signal due to C(13) (166·1 p.p.m.) in the

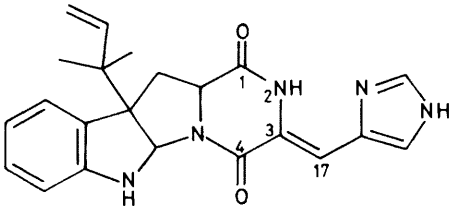


(1)

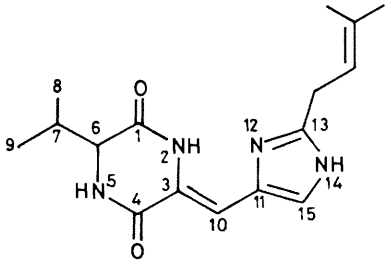


(2)

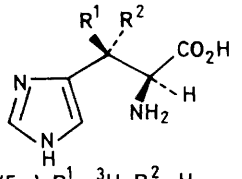
coupled nuclear Overhauser enhanced ^{13}C n.m.r. spectrum of (**2**) appears as a doublet with $^{3}J(CH)$ 10·0 Hz. The magnitude of $^{3}J(CH)$ is indicative of the *E* configuration.[6,7,11] The assignment of the natural abundance ^{13}C n.m.r. spectrum of roquefortine (**1**) derived from coupled, proton noise decoupled, and selective proton decoupled spectra and selective population inversion experiments[12] is given in Table 1 and enabled us to determine the hitherto unknown 3,17 double bond configuration. The value of 8·6 Hz for $^{3}J(CH)$ between C(4) and H(17) favours the *E* configuration for roquefortine. This result was verified as follows. The ^{13}C n.m.r. assignments of the reported[13] photoproduct of roquefortine, for which the trivial name 'isoroquefortine' (**3**) is suggested, are given in Table 1. The magnitude of $^{3}J(CH)$ between C(4) and H(17), 4·7 Hz is indicative of the *Z* configuration for the 3,17 double bond as shown in (**3**). This result is in agreement with the 4·9 Hz for $^{3}J(CH)$ observed between C(4) and H(10) in viridamine (**4**)[14] which has the *Z*-configuration.[15]



(3)



(4)



(5a) $R^1 = {}^3H, R^2 = H$
(5b) $R^1 = H, R^2 = {}^3H$

In order to determine which of the two diastereotopic hydrogens at C(3) of (2*S*)-histidine is removed in the dehydrogenation reaction samples of (2*S*,3*S*)- and (2*S*,3*R*)-[3-^{3}H]histidine, (**5a**) and (**5b**), respectively were prepared from 4(5)-[*formyl*-^{3}H]formylimidazole.[16] The two samples were each mixed with (2*S*)-[*ring*-2′-^{14}C]histidine as an internal standard, to give the desired $^{3}H:{}^{14}C$ ratios and crystallised to constant activity. The configurational purity of the (2*S*,3*R*)-[3-^{3}H]histidine (**5b**) sample ($^{3}H:{}^{14}C$ 6·50) was assayed using histidine ammonia-lyase (E.C. 4.3.1.3), an enzyme which stereospecifically eliminates the 3-*pro-R* hydrogen atom together with ammonia to give urocanic acid ($^{3}H:{}^{14}C$ 0·44; ^{3}H retention: 6·8%).[17]

TABLE 1. ^{13}C n.m.r. data for roquefortine (**1**) and isoroquefortine (**3**)

	(**1**) δ(c)/p.p.m.[a]	(**3**) δ(c)/p.p.m.[a]
C(1)	166·7S	165·4S
C(3)	121·9S	125·7S
C(4)	159·2S	158·2S
C(6)	78·3D	77·8D
C(8)	149·8S	150·2S
C(9)	109·1D	109·0D
C(10)	128·9D	128·8D
C(11)	119·0D	118·7D
C(12)	125·0D	125·0D
C(13)	128·5S	128·7S
C(14)	61·5S	61·6S
C(15)	36·8T	37·3T
C(16)	58·8D	59·0D
C(17)	110·9D	105·6D
C(18)	125·5S	136·8S
C(20)	136·4D	135·3D
C(22)	134·3D	117·7D
C(23)	40·9S	40·9S
C(24)	143·2D	143·4D
C(25)	114·5DD	114·4DD
C(26)	22·9Q	23·0Q
C(27)	22·5Q	22·5Q

[a] Relative to internal Me_4Si

Each of the two substrates (**5a**) and (**5b**) was fed to cultures of *P. roqueforti* HPB 061175 and *P. oxalicum* MRC 100. Good incorporations (1—4%) of the substrates into both roquefortine and oxaline were observed. The results (Table 2) indicate that the 3-*pro-S* hydrogen of (2*S*)-histidine is stereospecifically eliminated in each case whereas tritium from the 3-*pro-R* position is retained. The dehydrogenation step in the biosynthesis of both roquefortine and oxaline must involve the *syn* elimination of H(2) and the *pro-S* hydrogen at C(3) of (2*S*)-histidine.

TABLE 2. Incorporation of [3-^{3}H]histidine into roquefortine (**1**) and oxaline (**2**)

Configuration	$^{3}H:{}^{14}C$ ratio	(**1**) $^{3}H:{}^{14}C$ ratio	(**2**) $^{3}H:{}^{14}C$ ratio
2*S*,3*S* (**5a**)	6·50	0·30 (4·6)[a]	0·30 (4·6)
2*S*,3*R* (**5b**)	6·50	6·12 (94·2)	6·24 (96·0)

[a] Figures in brackets are % ^{3}H retention.

Stereospecific *syn* elimination of the 3-*pro-S* hydrogen has been observed for (2*S*)-tyrosine in the biosynthesis of mycelianamide,[5] for the incorporation of (2*S*)-tryptophan into cryptoechinuline A[6] and in the side-chain dehydrogenation of *N*-Boc-(2*S*)-tryptophan.[7] In each case the dehydroamino acid unit formed has the *Z* configuration. The hydrogen atom which is eliminated in the above quoted studies[5–7] is not the same as the one which is lost from C(3) of histidine in the biosynthesis of roquefortine and oxaline, although it is designated as 3-*pro-S* in all these instances.

The close biogenetic relationship between roquefortine and oxaline is indicated by the occurrence of roquefortine

together with oxaline in cultures of *P. oxalicum*.[18] The precursor rôle of roquefortine in oxaline biosynthesis is under investigation.

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