Biosynthesis of α-Cyclopiazonic Acid. Steric Course of Proton Removal during the Cyclisation of β-Cyclopiazonic Acid in *Penicillium griseofulvum* †,[1]

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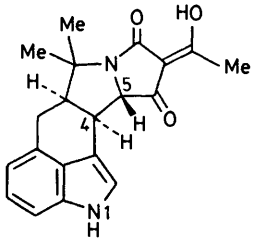
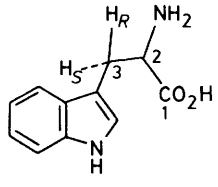
Feeding experiments with (3*R*)- and (3*S*)-[3-^{3}H]tryptophan have shown that, in *Penicillium griseofulvum* Dierckx, incorporation of tryptophan into α-cyclopiazonic acid (1) proceeds with loss of the 3-*pro-S* hydrogen atom. [3-$^2H_2$]Tryptophan was converted by *P. griseofulvum* into α-cyclopiazonic acid which was shown by ^{2}H n.m.r. spectroscopy to contain deuterium only at the expected C-4 position. (2*RS*)-[2-^{3}H,3-^{14}C]Tryptophan was incorporated into α-cyclopiazonic acid (1) with loss of *ca.* 50% of the tritium. These findings are consistent with cyclisation of β-cyclopiazonic acid (4) to α-cyclopiazonic acid (1) *via* a 1,4-didehydro-derivative, with C–C bond formation at C-4 occurring from the side of the molecule opposite to that from which proton removal takes place.

INVESTIGATIONS on the biosynthesis of α-cyclopiazonic acid (1),[2] a mycotoxin produced by cultures of *Penicillium griseofulvum* Dierckx, have shown that mevalonic acid, acetic acid, and tryptophan are precursors of this metabolite.[3] An intermediate involved in the biosynthesis from tryptophan (2) is now known to be the tetramic acid (3),[4] which in turn is converted into β-cyclopiazonic acid (4).[5] In this paper we report studies on the stereochemistry of the final oxidative cyclisation of β-cyclopiazonic acid to α-cyclopiazonic acid.[6]



(1)



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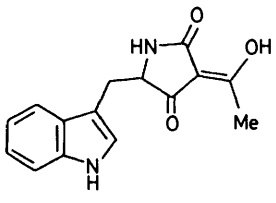

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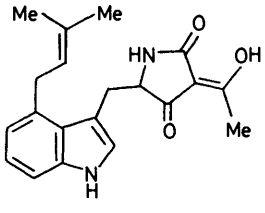


(4)

Samples of stereospecifically labelled [3-^{3}H]tryptophan[7] (see Table), mixed with [3-^{14}C]tryptophan to provide a reference label, were fed to cultures of *Penicillium griseofulvum* Dierckx, CSIR 1082. The configurational purity of the precursors at C-3 was established as *ca.* 94% through biosynthetic incorporation into indolmycin.[8] ‡ Good incorporations (18—25%) of the labelled tryptophans into α- (1) and β-cyclopiazonic acid (4) were observed. The results indicate that retention of tritium from (3*R*)-[3-^{3}H]tryptophan (2; H_R = T, H_S = H) during conversion into α-cyclopiazonic acid (1) was essentially complete for both the (2*S*)- and (2*RS*)-forms of the amino acid. Conversely, (3*S*)-[3-^{3}H]tryptophan (2; H_R = H, H_S = T) lost almost all its tritium. As expected the racemic mixture of (3*RS*)-tritiated tryptophans was converted into (1) with loss of half the tritium. All the tritiated tryptophans were incorporated into β-cyclopiazonic acid (4) with high retention of tritium, thus confirming the integrity of the methylene group during the early stages of biosynthesis.

Incorporation of labelled tryptophan into the cyclopiazonic acids (4) and (1) in *Penicillium griseofulvum* Dierckx

Configuration of precursor	^{3}H : ^{14}C ratio	^{3}H : ^{14}C ratios and retention of ^{3}H (%) in (4)	(1)
(2*S*,3*R*)-[3-^{3}H,3-^{14}C]	4.53 : 1	4.44 (98.0)	4.32 (95.4)
	1.67 : 1	1.61 (96.4)	1.60 (95.8)
(2*RS*,3*SS*)-[3-^{3}H,3-^{14}C]	4.60 : 1	4.52 (98.3)	0.21 (4.6)
	3.99 : 1	—	0.20 (5.0)
	2.41 : 1	—	0.14 (5.8)
(2*RS*,3*RR*)-[3-^{3}H,3-^{14}C]	5.69 : 1	5.60 (98.4)	5.37 (94.4)
	2.16 : 1	—	2.09 (96.7)
(2*S*,3*RS*)- + (2*R*,3*RS*)-[3-^{3}H,3-^{14}C]	4.16 : 1	3.92 (94.2)	2.09 (50.2)
(2*RS*)-[2-^{3}H,3-^{14}C]	7.32 : 1	—	3.57 (48.8)

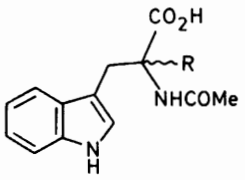
The location of the tritium at C-4 of α-cyclopiazonic acid was supported from the observation that treatment of biosynthetically tritiated α-cyclopiazonic acid (1) with alkali, under conditions known [2] to effect exchange of the C-5 proton, caused no loss of tritium. Unambiguous proof of the location of the proton derived from C-3 of tryptophan in α-cyclopiazonic acid was provided by a feeding experiment with (2*RS*)-[3-$^2H_2$]tryptophan. The ^{2}H n.m.r. spectrum of the deuteriated α-cyclopiazonic acid exhibited a signal only at δ 3.65, the resonance position of the C-4 proton.

It has been suggested by Schabort *et al.*[6] that 4,5-didehydro-β-cyclopiazonic acid is an intermediate in the

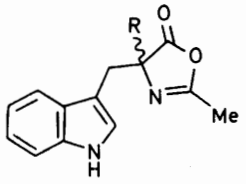
† This strain (CSIR 1082) of *Penicillium griseofulvum* Dierckx was identified as such by Dr. J. I. Pitt, CSIRO, Australia and was known previously as *Penicillium cyclopium* Westling.

‡ We thank Professor H. G. Floss (Purdue University) for this determination.

oxidative cyclization of β- (4) to α-cyclopiazonic acid (1). This mechanism would result in the loss of a hydrogen atom from both C-5 of β-cyclopiazonic acid and C-2 of tryptophan. However, Holzapfel and Schabort have shown that (5*RS*)-[5-^{3}H]-β-cyclopiazonic acid is incorporated into α-cyclopiazonic acid (1) with complete retention of tritium.[9] Similarly, we have observed retention of tritium on feeding (2*RS*)-[2-^{3}H]tryptophan to cultures of *P. griseofulvum*. The labelled tryptophan was prepared [10] *via* ring closure of *N*-acetyltryptophan (5) by dicyclohexylcarbodi-imide in pyridine to give the corresponding oxazolone (6). Addition of tritiated water then effected exchange at the chiral centre in a rapid step to produce (7) which without isolation underwent slow hydrolytic opening of the oxazolone ring. Basic hydrolysis of the *N*-acetyl derivative (8) afforded (2*RS*)-[2-^{3}H]tryptophan. When deuterium oxide was used this method gave (2*RS*)-[2-^{2}H]tryptophan with *ca.* 80% deuterium at C-2 as determined by ^{1}H n.m.r.



(5) R = H
(8) R = T
(9) R = D



(6) R = H
(7) R = T

spectroscopy and mass spectrometry. A sample of tritiated tryptophan was mixed with (2*RS*)-[3-^{14}C]tryptophan, as an internal standard, to give a ^{3}H : ^{14}C ratio of 7.32 : 1. This precursor was fed to cultures of *P. griseofulvum* to give α-cyclopiazonic acid (6.0% incorporation of ^{14}C) with a ^{3}H : ^{14}C ratio of 3.57 : 1, which corresponds to a 48.8% retention of tritium. This result is readily explained on the assumption that the (2*R*)-[2-^{3}H]-tryptophan is converted into (2*S*)-tryptophan *via* indolylpyruvic acid by a transaminase reaction.[11] The resultant (2*S*)-[2-^{3}H,3-^{14}C]tryptophan (^{3}H : ^{14}C 3.66 : 1) is then incorporated into α-cyclopiazonic acid with essentially complete retention of tritium (97.5%).

The above results are consistent with cyclisation *via* a 1,4- but not a 4,5-didehydro-derivative of (4) with C–C bond formation at C-4 occurring from the side of the molecule opposite to that from which proton removal takes place.

EXPERIMENTAL

Mass spectra were determined with an A.E.I. MS9 spectrometer by direct insertion. Spectra of the relevant labelled and unlabelled materials were run consecutively and in duplicate; the deuterium content was calculated as described by Biemann.[12] ^{1}H N.m.r. spectra were recorded on a Varian EM-390 (90 MHz) spectrometer with Me_4Si as internal standard. ^{2}H N.m.r. spectra (56.3 MHz) were recorded on a Bruker WH-360 instrument for solutions in $CHCl_3$ with $CDCl_3$ (δ 7.25) as internal standard. Radioactive samples were counted on a Packard Tri-Carb 2660 instrument.

Synthesis of the (3R)- *and* (3S)-[3-^{3}H]*Tryptophans*.—The various [3-^{3}H]tryptophans listed in the Table were prepared [7] from 3-[^{3}H]formylindole *via* (*Z*)-2-acetylamino-3-(indol-3-yl)[3-^{3}H]acrylic acid.

Synthesis of (2RS)-[3-$^2H_2$]*Tryptophan*.—(2*RS*)-[3-$^2H_2$]-Tryptophan was prepared from [*methylene*-$^2H_2$]gramine * by the method of Weygand and Linden.[13]

Incorporation of Labelled Precursors and Isolation of α- *and* β-*Cyclopiazonic Acid*.—Conical flasks (10 × 500 ml) containing CSM medium [14] (100 ml) at pH 5.5 were inoculated with a spore suspension of *Penicillium griseofulvum* Dierckx, CSIR 1082, prepared as described by Neethling and McGrath,[14] but without maceration. The cultures were grown at 25 °C in shake culture (180 r.p.m.). To each of the ten flasks containing two-day old pellets was added the requisite labelled tryptophan precursor {*e.g.* (2*RS*)-[2-^{3}H,3-^{14}C]tryptophan (30 mg) $^3H_3$: ^{14}C 7.32 : 1} every 12 h from day 2 to day 5. The cultures were harvested on day 7 by filtration. The filtrates were acidified (to pH 2) with 6N-HCl and extracted with chloroform. The extracts were dried (Na_2SO_4), filtered, and evaporated to dryness. The crude cyclopiazonic acids were separated and purified by p.l.c. on silica gel using ethyl acetate–ammonium hydroxide–methanol (80 : 10 : 15) as eluant. The α- (80 mg, ^{3}H : ^{14}C 3.57 : 1) and the β-cyclopiazonic acid (not formed in this instance) were crystallised from methanol to constant ^{3}H : ^{14}C activity. Yields of α- and β-cyclopiazonic acids varied in each experiment and in some instances only α-cyclopiazonic acid was formed.

Synthesis of (2RS)-[2-^{2}H]*Tryptophan*.—A solution of (2*RS*)-*N*-acetyltryptophan (5) (1.72 g) and dicyclohexylcarbodi-imide (1.44 g) in pyridine (20 ml) was stirred at 80 °C for 5 h. Deuterium oxide (4 ml) was then added and after the mixture had been stirred for 3 h, the solvents were evaporated off. The residue was dissolved in water and after filtration the solution was evaporated; crystallization of the product from water gave compound (9) (1.32 g), δ[$(CD_3)_2SO$] 1.80 (3 H, s, NCOMe), and 3.00 and 3.23 (2 H, ABq, *J* 15.5 Hz); no discernible signal at δ 4.57 due to H-2; D content >95% by n.m.r., 94% by mass spectrometry. A solution of (9) (2.47 g) in aqueous sodium hydroxide (10%; 15 ml) was refluxed for 8 h (cellulose t.l.c. control). The mixture was diluted with water, the pH adjusted to 5.8 with acetic acid, and the solution concentrated The product was filtered off and recrystallized from aqueous EtOH to give (2*RS*)-[2-^{2}H]tryptophan (1.58 g); δ(CF_3CO_2D) 3.53 and 3.73 (2 H, ABq, *J* 15.7 Hz); D content 80% by n.m.r.

Synthesis of (2RS)-[2-^{3}H]*Tryptophan*.—The procedure as outlined for the deuterio-derivative was followed except that tritiated water (300 μl) instead of deuterium oxide was used. The (2*RS*)-[2-^{3}H]tryptophan had a specific activity of 3.74 μCi mmol^{-1}. An aliquot of this material (189 mg) was admixed with (2*RS*)-[3-^{14}C]tryptophan (50 μCi) and recrystallized (3×) from aqueous ethanol to give the doubly labelled material (120 mg), ^{3}H : ^{14}C ratio 7.32 : 1.

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