Steric Course of Proton Removal During the Cyclisation of β-Cyclopiazonic Acid in *Penicillium cyclopium*

By PIETER S. STEYN* and ROBERT VLEGGAAR
(*National Chemical Research Laboratory, C.S.I.R., Pretoria, Republic of South Africa*)
NICHOLAAS P. FERREIRA
(*Microbiological Research Group, C.S.I.R., Pretoria*)
GORDON W. KIRBY*
(*Department of Chemistry, University of Glasgow, Glasgow* G12 8QQ)
and MICHAEL J. VARLEY
(*Chemistry Department, University of Technology, Loughborough, Leicestershire* LE11 3TU)

Summary Incorporation of (3*R*)- and (3*S*)-[3-^{3}H,3-^{14}C]tryptophan into α-cyclopiazonic acid in *Pencillium cyclopium* Westling proceeds with loss of *pro*-*S*-tritium showing that, in the final cyclisation step, formation of the new C–C bond occurs from the opposite side of the molecule to proton removal.

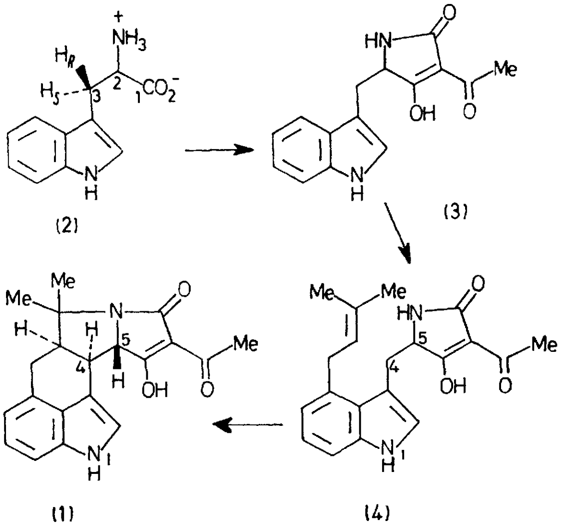
α-CYCLOPIAZONIC acid (**1**) is formed biosynthetically from tryptophan (**2**)[1] *via* the tetramic acid (**3**)[2] and β-cyclopiazonic acid (**4**).[3] We now report studies on the stereochemistry of the final, oxidative cyclisation reaction, (**4**) → (**1**).

TABLE

Incorporation of [3-^{3}H, 3-^{14}C]tryptophan (**2**) into the cyclopiazonic acids, (**4**) and (**1**), in *Penicillium cyclopium* Westling.

Tryptophan		^{3}H/^{14}C Ratios and retention of ^{3}H (%) in	
Configuration	^{3}H/^{14}C ratio	(4)	(1)
L-(3*R*)	4·53 ± 0·11	4·44(98)	4·32(95)
DL-(3*S*)	4·60 ± 0·10	4·52(98)	0·21(4·6)
DL-(3*R*)	5·69 ± 0·07	5·60(98)	5·37(94)
DL-(3*RS*)	4·16 ± 0·08	3·92(94)	2·09(50)

Samples of stereoselectively labelled [3-^{3}H]tryptophan[4] (see Table), mixed with [3-^{14}C]tryptophan to provide a reference label, were fed to cultures of *Penicillium cyclopium* Westling. Good incorporations (18—25%) into the cyclopiazonic acids, (**1**) and (**4**), were observed. 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 L- and DL-forms of the amino-acid. Conversely, (3*S*)-[3-^{3}H]tryptophan (**2**; H_R = H,



---

† The configurational purity of the precursors[4] at C-3 was *ca.* 94%.

$H_S = T$) lost almost all of its tritium. As expected, the (3*RS*) mixture of 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. Treatment of biosynthetically tritiated (**1**) with alkali, under conditions known[5] to effect exchange of the C-5 proton, caused no loss of tritium.

Holzapfel has shown[6] that tritiated β-cyclopiazonic acid (**4**) derived from [2-^{3}H]tryptophan is incorporated into α-cyclopiazonic (**1**) with complete retention of tritium. This finding, taken with our results, is consistent with cyclisation[3] *via* a 1,4- but not a 4,5-didehydro-derivative of (**4**) with C–C bond formation at C-4 occurring from the opposite side of the molecule to proton removal.

We thank the S.R.C. for financial support.

(*Received*, 21*st April* 1975; *Com.* 459.)

---

[1] C. W. Holzapfel and D. C. Wilkins, *Phytochemistry*, 1971, **10**, 351.

[2] R. M. McGrath, P. S. Steyn, and N. P. Ferreira, *J.C.S. Chem. Comm.*, 1973, 812.

[3] J. C. Schabort, D. C. Wilkins, C. W. Holzapfel, D. J. J. Potgieter, and A. W. Neitz, *Biochim. Biophys. Acta*, 1971, **250**, 311; J. C. Schabort and D. J. J. Potgieter, *ibid.*, p. 329.

[4] G. W. Kirby and M. J. Varley, *J.C.S. Chem. Comm.*, 1974, 833.

[5] C. W. Holzapfel, *Tetrahedron*, 1968, **24**, 2101.

[6] C. W. Holzapfel, personal communication.