# Biosynthesis of α-Cyclopiazonic Acid: Stereochemical Aspects of D-ring Formation

**Anthony A. Chalmers, Charles P. Gorst-Allman,* and Pieter S. Steyn**

*National Chemical Research Laboratory, Council for Scientific and Industrial Research, P.O. Box 395, Pretoria 0001, Republic of South Africa*

(3*R*)-Mevalonic acid lactone is incorporated into α-cyclopiazonic acid with retention of the 4-*pro*-*R*-hydrogen atom; [1,2-$^{13}C_2$]acetate has been used as a probe to show that the cyclization of β-cyclopiazonic acid into α-cyclopiazonic acid involves a *syn* addition to the double bond.

The biosynthesis of α-cyclopiazonic acid (**1**) has received considerable attention.[1-6] Mevalonic acid, acetic acid, and L-tryptophan have been implicated as the biogenetic precursors,[1,2] and the biogenesis has been shown to occur *via* cyclization of β-cyclopiazonic acid (**2**).[1-5] A ping-pong bi-bi mechanism has been suggested for this cyclization[4] which occurs with loss of the 3-*pro*-*S*-hydrogen atom from tryptophan.[5] In this paper we report studies on the stereochemistry

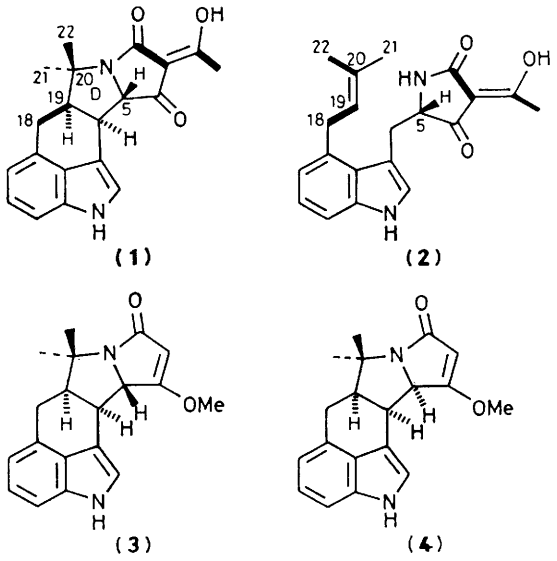


**Table 1.** Incorporation of labelled mevalonic acid lactones into α-cyclopiazonic acid (**1**).

| Mevalonic acid lactone mixture | $^3H:^{14}C$ ratio | $^3H:^{14}C$ ratio in (**1**) |
|---|---|---|
| (3*S*,4*S*)-[4-$^3$H] + (3*R*,4*R*)-[4-$^3$H] + (3*RS*)-[2-$^{14}$C] | 2.35 | 2.44 |
| (3*S*,4*S*)-[4-$^3$H] + (3*R*,4*R*)-[4-$^3$H] + (3*R*)-[3-$^{14}$C] | 1.67 | 0.83 |

of the cyclization of β-cyclopiazonic acid to α-cyclopiazonic acid with regard to the incorporation of the mevalonic acid unit and the disposition of the geminal dimethyl groups in α- and β-cyclopiazonic acids.

Samples of stereospecifically labelled [4-$^3$H]mevalonic acid lactones, mixed with (3*RS*)-[2-$^{14}$C]- and (3*R*)-[3-$^{14}$C]-mevalonic acid lactone (Table 1), were fed to cultures of *Penicillium griseofulvum* Dierckx, C.S.I.R. 1082. Analysis of the label found in the purified α-cyclopiazonic acid indicated that the (3*R*)-isomer of mevalonic acid lactone is incorporated into (**1**) with retention of the 4-*pro*-*R*-hydrogen atom.

Investigation of the stereochemical origin of the geminal methyl groups [C(21) and C(22)] in (**1**) is hampered by their equivalence to chemical reaction and the similarity of their chemical shifts in $^1$H and $^{13}$C n.m.r. spectroscopy, thus making unambiguous assignments impossible (Table 2). However, treatment of (**1**) with 0.05M sulphuric acid in methanol, followed by methylation with diazomethane, yields a mixture of *O*-methyldesacetylcyclopiazonic acid (**3**) and its 5-epimer (**4**).[1] In the $^{13}$C n.m.r. spectrum of the latter compound the two methyl groups C(21) and C(22) are assignable (Table 2). In the conformation of the normal series both methyl groups experience three γ-*gauche* interactions whereas in the 5-epimer conformation the β-methyl group experiences four γ-*gauche* and the α-methyl group two γ-*gauche* interactions. The resonance of the β-methyl group will, therefore, shift to higher field with respect to its normal series conformation resonance, whereas the resonance of the α-methyl group will shift to lower field.

The assignment of the methyl resonances [C(21) and C(22)] in the $^{13}$C n.m.r. spectrum of (**2**) is straightforward as a large body of opinion places the chemical shift of the (*Z*)-methyl group at substantially higher field than that of the (*E*)-methyl group for dimethylallyl moieties in numerous model systems.[7] Moreover, the intact double labels observed here confirm this assignment if the side-chain results from a normal isoprene pathway.[8]

**Table 2.** $^{13}$C N.m.r. data[a] for compounds (**1**)—(**4**).

| | (**1**) | | (**2**) | | (**3**) | | (**4**) | |
|---|---|---|---|---|---|---|---|---|
| Carbon | δ | $^1J$(C,C)[b] | δ | $^1J$(C,C)[b] | δ | $^1J$(C,C)[b] | δ | $^1J$(C,C)[b] |
| 18 | 26.6 | 34.8 | 32.4 | 43.7 | 26.8 | 34.5 | 26.5 | 36.0 |
| 19 | 53.1 | 34.7 | 123.5 | 43.8 | 54.4 | 34.5 | 53.9 | 36.1 |
| 20 | 63.5 | 39.8 | 132.9 | 42.5 | 61.6 | 40.4 | 61.2 | 36.6 |
| 21 | 26.4 | — | 25.7 | — | 25.7 | — | 30.8 | — |
| 22 | 24.5 | 40.0 | 18.1 | 41.7 | 25.2 | 40.5 | 20.9 | 36.6 |

[a] δ in p.p.m. relative to internal $Me_4Si$ in $CDCl_3$, *J* in Hz. [b] Obtained for compounds derived from culture media supplemented with [1,2-$^{13}C_2$]acetate.

Incorporation of [1,2-$^{13}C_2$]acetate into both (**1**) and (**2**), followed by chemical modification of (**1**) into (**4**), and analysis of the $^{13}$C n.m.r. spectra of (**2**) and (**4**), allow the intact acetate units in these compounds to be identified (Table 2). As the stereochemistry of the geminal dimethyl groups is not affected in the transformation (**1**) → (**4**), the intact acetate units in (**1**) and (**2**) are as indicated in heavy print on the structural formulae. Thus [1,2-$^{13}C_2$]acetate may be used as an effective probe for the stereochemistry of the geminal dimethyl groups in the conversion of (**2**) into (**1**). As can be seen, the (*Z*)-methyl group C(22) in β-cyclopiazonic acid (**2**) becomes the β-methyl group C(22) in α-cyclopiazonic acid (**1**). Taken together with the established relative configuration at C(19) in (**1**),[9] this corresponds to a *syn* addition to the double bond in the transformation of (**2**) into (**1**).

We thank Dr. W. E. Hull, Bruker Analytische Messtechnik, Rheinstetten-FO for recording the $^{13}$C n.m.r. spectra on a Bruker WM-500 spectrometer, and Dr A. de Jesus for microbiological assistance.

*Received, 1st September 1982; Com. 1048*

## References

1 C. W. Holzapfel and D. C. Wilkins, *Phytochemistry*, 1971, **10**, 351.
2 C. W. Holzapfel and J. Schabort, *S. Afr. J. Chem.*, 1977, **30**, 233.
3 J. C. Schabort and D. J. J. Potgieter, *Biochim. Biophys. Acta*, 1971, **250**, 329; D. J. Steenkamp, J. C. Schabort, C. W. Holzapfel, and N. P. Ferreira, *ibid.*, 1974, **358**, 126; D. J. Steenkamp, J. C. Schabort, and N. P. Ferreira, *ibid.*, 1973, **309**, 440; J. C. Schabort, M. Marx, G. L. Erasmus, M. M. J. Oosthuizen, M. J. Pitout, and N. P. Ferreira, *Int. J. Biochem.*, 1978, **9**, 427; J. C. Schabort, M. Marx, and G. L. Erasmus, *ibid.*, p. 437.
4 J. C. Schabort and M. Marx, *Int. J. Biochem.*, 1978, **10**, 61.
5 A. E. de Jesus, P. S. Steyn, R. Vleggaar, G. W. Kirby, M. J. Varley, and N. P. Ferreira, *J. Chem. Soc., Perkin Trans.* 1, 1981, 3293.
6 D. C. Neethling and R. M. McGrath, *Can. J. Microbiol.*, 1977, **23**, 856.
7 A. A. Chalmers, G. J. H. Rall, and M. E. Oberholzer, *Tetrahedron*, 1977, **33**, 1735, and references cited therein.
8 D. Arigoni, *Experientia*, 1958, **14**, 153; A. J. Birch, M. Kocor, N. Sheppard, and J. Winter, *J. Chem. Soc.*, 1962, 1502.
9 C. W. Holzapfel, *Tetrahedron*, 1968, **24**, 2101.