On the Biosynthesis of Ethylene: Further Evidence for Stepwise Enzymatic Cyclopropane Ring Cleavage

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The conversion of a series of 2,3-dimethylated 1-aminocyclopropanecarboxylates by apple tissues into mixtures of *cis*- and *trans*-butenes is reported; the results are in accord with a stepwise enzymatic mechanism of cyclopropane ring opening.

Recently we reported that the conversion of deuterated monoalkylated 1-aminocyclopropanecarboxylates(ACCs) by apple tissue into alkenes occurs with a net stereochemical bias.[1] These results were consistent with the hypothesis that the biosynthesis of ethylene from ACC occurs by a stepwise mechanism in an active site whose intrinsic topology determines the stereochemical bias observed from substrates other than ACC.[2] In order to probe further the specificity of this enzyme, we have prepared a series of 2,3-dimethylated ACCs and challenged them with fresh apple tissue. In these cases the sterically more demanding methyl group replaces the deuterium used in the previous study.[1]

The first of these substrates, (1*R*,2*S*,3*R*)-(**1**)† {Scheme 1; ^{1}H n.m.r., δ_H‡ (D_2O, 300 MHz) 0.91—0.94 (6H, m, Me), 1.50—1.56 (2H, m, CH); *m/z* [NH_3 direct chemical ionisation (d.c.i.)] 130 (MH^+, 100%)} in which the methyl groups are *cisoid* to the amino function gave good conversion (4%) with apple tissues§ into a 5:1 mixture of *cis*-:*trans*-butenes. The second substrate, (1*R*,2*R*,3*S*)-(**2**) [Scheme 1; ^{1}H n.m.r., δ_H‡ (D_2O, 300 MHz) 1.05—1.10 (6H, m, Me), 1.40—1.50 (2H, m, CH); *m/z* (NH_3 d.c.i.) 130 (MH^+, 100%)] was found to be a

Table 1. Conversion of 2,3-dimethylated ACCs (**1**), (**2**), (**3**), and (**5**) into *cis*-/*trans*-butenes by apple tissues.

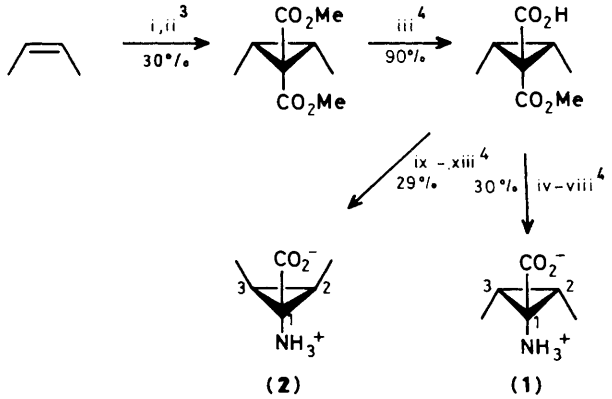
Substrate	Product *cis*-: *trans*-Butene G.c.	^{1}H N.m.r.[b]	Conversion (%)[a]
(**1**)	5:1	5:1	4
(**2**)	—	—	<0.02
(±)-(**3**)	4:1	4:1	0.4
(−)-(**5**)	4:1	4:1	0.4

[a] Calibrated against a *cis*-butene standard by g.c. analysis. [b] N.m.r. ratios were determined by integration of the olefinic and methyl resonances.

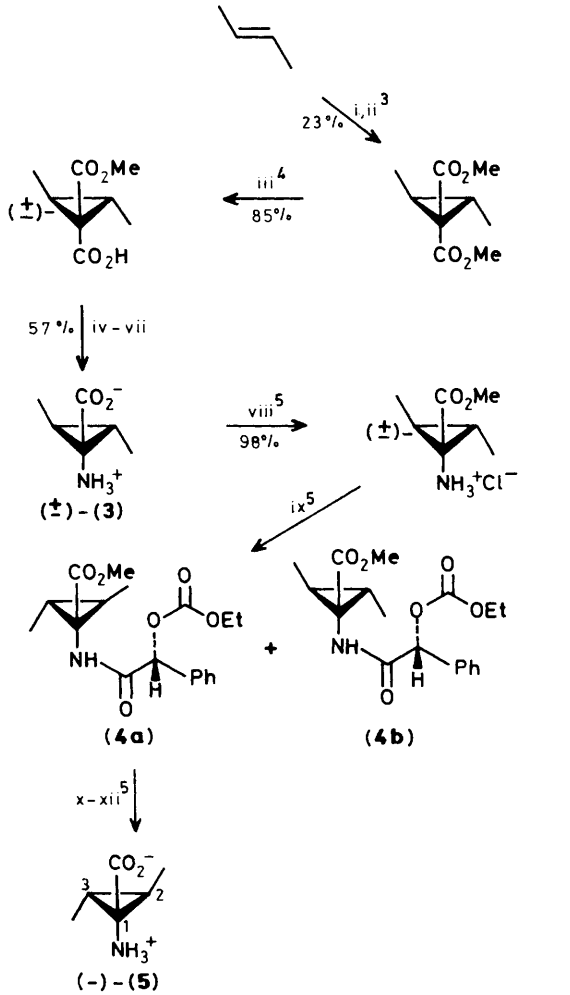
† The numbering systems adopted are indicated on structures.

‡ Referenced to internal *H*OD, δ = 4.63.

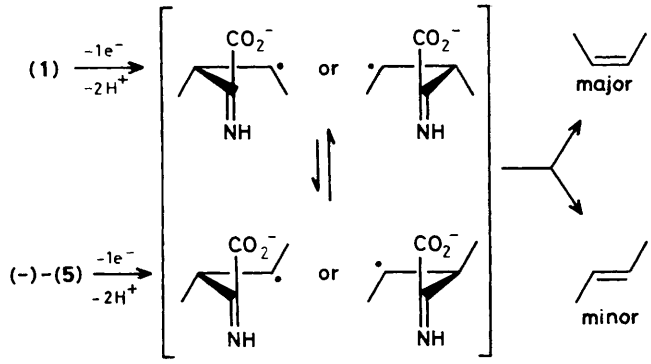
§ Feeding experiments to apple tissues were performed as previously described, see ref. 2.



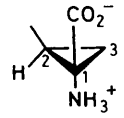
Scheme 1. *Reagents*: i, $(MeS)_2$, SO_2Cl_2; ii, $NaCH(CO_2Me)_2$, MeOH, heat; iii, KOH, MeOH, then H_3O^+; iv, $N_2H_4{\cdot}H_2O$, 80 °C, 3 days; v, $NaNO_2$ (1.5 equiv.), HCl, 0 °C, 1 h; vi, toluene, 90 °C, 1 h; vii, 6 M HCl, 60 °C, 18 h; viii, Dowex 50W-X8 (H) ion exchange; ix, $NEtPr^i_2$ (1.2 equiv.), EtOCOCl (1.5 equiv.), tetrahydrofuran (THF), −10 °C, 1 h; x, NaN_3, (3 equiv.) in water (5 ml), 0 °C, 45 min; xi, toluene, 85 °C, 1 h; xii, 6 M HCl, 100 °C, 6 h; xiii, Dowex 50W-X8 (H) ion exchange.



Scheme 2. *Reagents:* i, $(MeS)_2$, SO_2Cl_2; ii, $NaCH(CO_2Me)_2$, MeOH, heat; iii, KOH, MeOH, then H_3O^+; iv, $(PhO)_2PON_3$, THF, 15 °C, 3 h; v, toluene, 90 °C, 1 h; vi, 6 M HCl, 100 °C, 6 h; vii, Dowex 50W-X8 (H) ion exchange; viii, MeOH, HCl; ix, ethyl 1,2-dihydro-2-ethoxy-1-quinoline carboxylate (EEDQ) (2 equiv.), (*R*)-(−)-2-hydroxy-2-phenylacetic acid, NEt_3 (1 equiv.), CH_2Cl_2, 18 h, 65%; x, recrystallization (EtOAc–petroleum); xi, 6 M HCl, 100 °C, 8 h; xii, Dowex 50W-X8 (H) ion exchange.



Scheme 3



(1*S*,2*S*)-(**6**)

very poor substrate with apple tissues (yield <0.02%). The racemate (**3**) [Scheme 2; ^1H n.m.r., δ_H‡ (D_2O, 300 MHz) 0.98—1.09 (1H, m, CH), 1.025 (6H, 2 × d, *J* 8 Hz, Me), 1.39—1.48 (1H, m, CH); *m/z* (d.c.i.) 130 (MH^+, 100%)] gave conversion (0.4%) into a 4 : 1 mixture of *cis*-:*trans*-butenes, for which the (1*R*,2*R*,3*R*)-(**5**) enantiomer¶ {$[\alpha]_D^{20}$ −49° (*c* 0.225, H_2O)} was found to be the only significant biological precursor to butene production (Table 1).

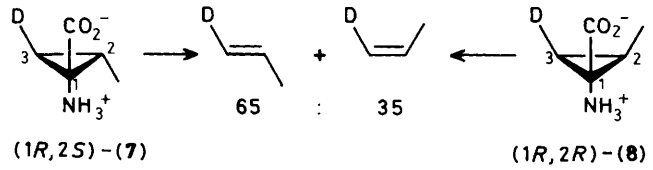
In a separate study the chemical oxidation of substrates (**1**) and (**2**) with ferrate (FeO_4^{2-}) ions was examined.[6] In contrast to the biological system, both substrates were efficiently converted into a 1 : 2 mixture of *cis*- and *trans*-butenes.

The biological results obtained in respect of the relative effectiveness of (**1**) and (**5**) but not (**2**) are consistent with the previous study of mono-methylated ACCs,[1] in which all isomers except the (1*S*,2*S*)-isomer (**6**) were effective substrates, *i.e.* all isomers bearing an alkyl substituent in the same absolute stereochemical disposition as in (**6**) are not effective substrates for the apple enzyme. The predominance of the

¶ Resolution of the enantiomers of (**3**) followed from coupling to the diastereoisomeric (*R*)-mandelate derivatives (**4**) [δ_H (partial) (300 MHz, $CDCl_3$) 3.61, 3.64 (2 × 3H, 2 × s, 2 × CO_2Me)] from which (**4a**) was separated by recrystallization (ethyl acetate–petroleum) {m.p. 142—144 °C; *m/z* (NH_3 d.c.i.) 367 (MNH_4^+, 60%), 350 (MH^+, 100%); δ_H (partial) (300 MHz, $CDCl_3$) 3.6 (3H, s) 4; $[\alpha]_D^{20}$ −66° (*c* 0.35, $CHCl_3$)} and hydrolysed to (**5**) (Scheme 2).[5] The absolute configuration of (**4a**) follows from *X*-ray crystallographic studies (Figure 1).‖

‖ *Crystal data* for (**4a**): $C_{18}H_{23}O_6N_1$; *M* = 349.4, monoclinic, space group $P2_1$, *a* = 19.643(2), *b* = 9.439(2), *c* = 10.419(2) Å, β = 94.02(2)°, *U* = 1927.1 Å^3, *Z* = 4, D_c = 1.20 g cm^{-3}. 4202 Independent reflections (1 < 2θ < 150°) gave 2578 observed reflections [$I \geq 3\sigma(I)$]. Data were collected on an Enraf-Nonius CAD4 diffractometer using graphite monochromated Cu-K_α radiation (λ = 1.5418 Å). The structure was solved using MITHRIL.[7] All refinement was carried out on a VAX11/750 computer using CRYSTALS.[8] The final *R* value is 0.061, R_w 0.069. †† Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

†† The two molecules in the asymmetric unit are related by a pseudo-operator which could not be accommodated by a change of space group. The two molecules differ mainly in the orientation of the phenyl group which appears to be able to librate fairly freely.



Scheme 4

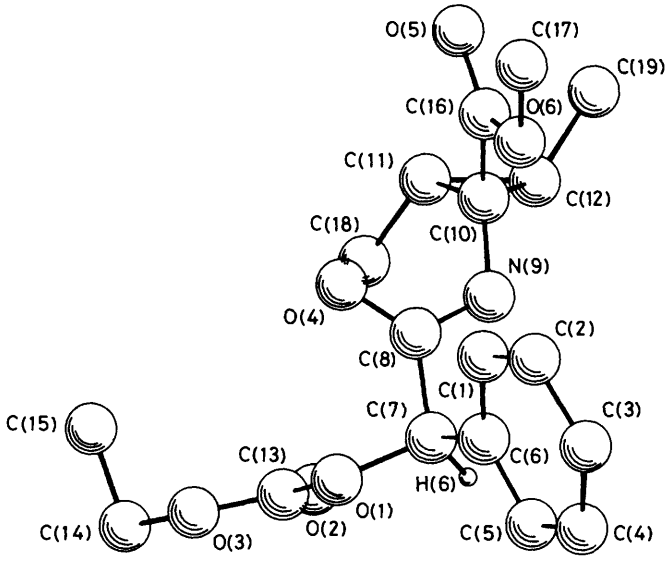


Figure 1. Molecular structure of (**4a**) showing the atom numbering scheme.

thermodynamically less stable *cis*-butene from both (**1**) and (**5**) is more striking than the previous results with deuterated mono-methylated ACCs[1] since in this case the stereochemical bias afforded by the active site is clearly contra-thermodynamic. This result may arise from a common radical intermediate whose conformation, prior to olefin formation, is determined by the active site topology, Scheme 3, as was previously suggested[1] for the (1*R*,2*S*)-(**7**) and (1*R*,2*R*)-(**8**) mono-methylated ACCs, Scheme 4.

In conclusion, the results reported here provide further support for the view that ethylene synthetase operates *via* a stepwise and homolytic mechanism in which active site topology directs the stereochemical course of the process.

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References

1 J. E. Baldwin, R. M. Adlington, G. A. Lajoie, and B. J. Rawlings, *J. Chem. Soc., Chem. Commun.*, 1985, 1496.
2 R. M. Adlington, J. E. Baldwin, and B. J. Rawlings, *J. Chem. Soc., Chem. Commun.*, 1983, 290.
3 Prepared by analogous methods to the literature procedure, see J. Ohishi, *Synthesis*, 1980, 690.
4 Prepared by analogous methods to the literature procedures, J. E. Baldwin, R. M. Adlington, and B. J. Rawlings, *Tetrahedron Lett.*, 1985, 481.
5 Prepared by analogous methods to the literature procedures, J. E. Baldwin, R. M. Adlington, B. J. Rawlings, and R. H. Jones, *Tetrahedron Lett.*, 1985, 485.
6 J. E. Baldwin, D. A. Jackson, R. M. Adlington, and B. J. Rawlings, *J. Chem. Soc., Chem. Commun.*, 1985, 206.
7 G. J. Gilmore, 'MITHRIL, A computer program for the automatic solution of crystal structures from *X*-ray data,' Department of Chemistry, University of Glasgow, 1983.
8 D. J. Watkin, J. R. Carruthers, and P. W. Betteridge, 'CRYSTALS User Guide,' Chemical Crystallography Laboratory, Oxford, 1985.