On the Stereochemistry of Ethylene Biosynthesis

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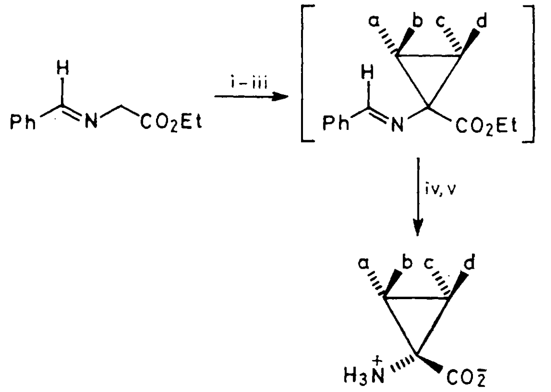
The conversion of (i) an equal mixture of the two isomers of *cis*-2,3-dideuterio-1-aminocyclopropanecarboxylate or (ii) (±)-*trans*-2,3-dideuterio-1-aminocyclopropanecarboxylate by apple slices gave in both cases a 1:1 mixture of *cis*- and *trans*-1,2-dideuterioethylene; in contrast the chemical oxidation (NaOCl) of these substances proceeded with complete retention of configuration to *cis*-dideuterioethylene and *trans*-dideuterioethylene, respectively.

The plant hormone ethylene is derived from methionine *via* 1-aminocyclopropanecarboxylic acid (ACC) (**1**).[1,2] Recently we demonstrated that [2,2,3,3-$^2H_4$]ACC (**2**) was converted by apple slices into [$^2H_4$]ethylene without loss of deuterium.[3] Stereospecifically labelled (**1**) was prepared by bromination (aqueous KBr_3, 0 °C, dark) of both *trans*- and *cis*-dideuterioethylenes[4] to *meso*- and (±)-dibromoethylenes, respectively. These were then allowed to react with the lithio-derivative of benzylidene glycine ethyl ester, as shown in Scheme 1, to yield *cis*-[$^2H_2$]ACC† (**3**) and (**4**) {as a 1 : 1 mixture of 1-amino-[*c*-2,*c*-3-$^2H_2$]-*r*-1-cyclopropanecarboxylic acid (**3**) and 1-amino-[*t*-2,*t*-3-$^2H_2$]-*r*-1-cyclopropanecarboxylic acid (**4**)} and (±)-*trans*-[$^2H_2$]ACC‡ (**5**) {as 1-amino[2*S*,3*S*-$^2H_2$]cyclopropanecarboxylic acid (**5**) and its enantiomer}. The stereochemistry of these substances was confirmed by comparison with the computer simulated ^1H n.m.r. spectra.§ These materials were used in the feeding experiments as follows: *cis*-[$^2H_2$]ACC (**3**) and (**4**) (10 mg, 22 mol % glycine) in water (1.5 ml) was fed to apple slices¶ (25 g) at 15 °C in the dark and the flask stoppered for 12—18 h. Direct i.r. analysis** of the headspace showed a 1 : 1 mixture of *cis*[6]- (ν_{max} 842 cm^{-1}) and *trans*[6]-(ν_{max} 988 and 727 cm^{-1}) dideuterioethylenes [Figure 1(A)]. The same result was obtained when (±)-*trans*-[$^2H_2$]ACC (**5**) (5 mg, 49 mol % glycine) in water (1.5 ml) was fed to the apple tissues (25 g) [Figure 1(B)]. This *cis*: *trans*



(**1**) a = b = c = d = ^1H
(**2**) a = b = c = d = ^2H
(**3**) a = c = ^1H; b = d = ^2H
(**4**) a = c = ^2H; b = d = ^1H
(**5**) a = d = ^1H; b = c = ^2H

Scheme 1. *Reagents and conditions:* i, Pr^i_2NLi (1.25 equiv.), tetrahydrofuran, −78 → −60 °C; ii, deuteriated ethylene dibromide, −60 → 15 °C; iii, Pr^i_2NLi (1.25 equiv.), −60 → 15 °C; iv, 6 M HCl, reflux; v, Dowex 50W — X8(H) and Dowex 1 × 8 — 400 (Acetate form), recrystallisation (H_2O–MeCOMe).

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† Analysis by ^1H n.m.r. (300 MHz) showed that this material contained residual glycine, δ[D_2O, sodium 3-(trimethylsilyl)tetradeuteriopropionate (TSP) = 0.00] 3.56(2H, s), (22 mol %), and the *cis* : *trans* ratio was 99 : 1. For (**3**) and (**4**), δ(D_2O, ^2H decoupled, TSP 0.00) 1.19(2H, s) and 1.34(2H, s).

‡ ^1H N.m.r. analysis as before showed glycine (49 mol %) and a *trans*: *cis* ratio of 93 : 7. For (**5**), δ(D_2O, ^2H decoupled) 1.19(1H, d, *J* 7.7 Hz), and 1.34(1H, d, *J* 7.7 Hz). We have shown that the presence of glycine does not affect ethylene production from apples.

§ The AA′BB′ system of ACC was analysed using the iterative program DAVINS.[5]

¶ Chivers Delight apples (Broadlands Fruit Farm, Bath, U.K.) were harvested and stored for 3 months at 15 °C prior to use. Individual apples were peeled, the cores removed, and cut into slices. All biological experiments were performed upon randomised slices from the same intact apples.

** Carbon dioxide (with KOH solution) and water vapour (by cooling to −78 °C) were removed from the gas sample prior to i.r. analysis.

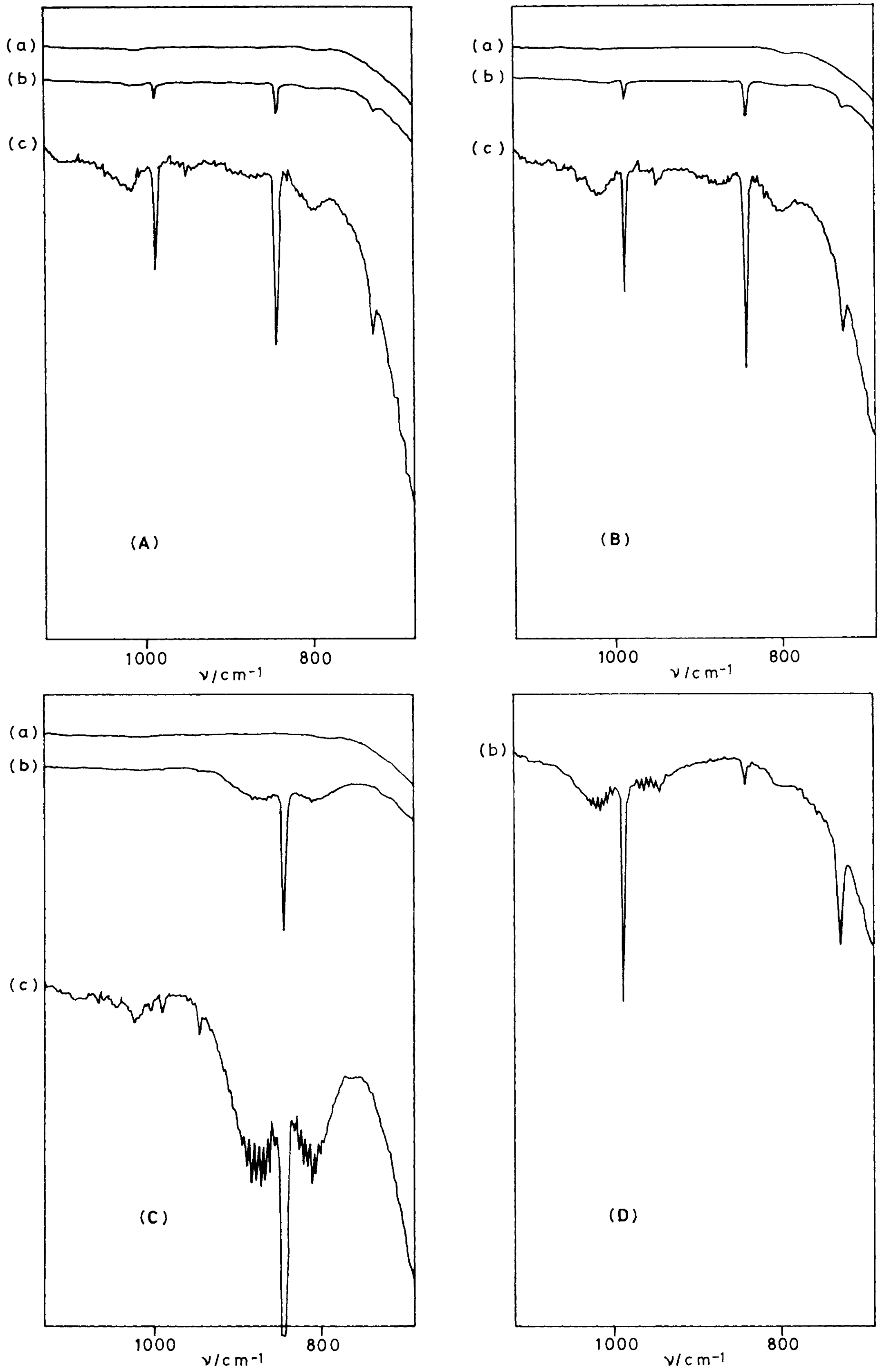
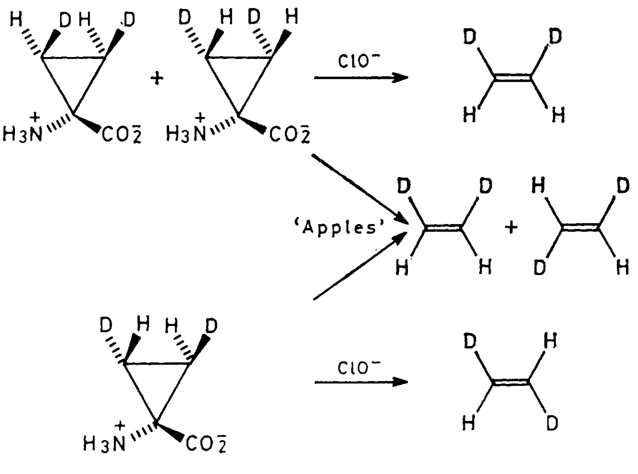


Figure 1. (a) Evacuated i.r. cell; (b) liberated ethylene in air; (c) scale enlargement of (b).

ratio was confirmed by chemical reduction[7] ($Na-NH_3$) of a 1 : 1 mixture of *meso*- and (±)-dideuterioethylene dibromides, prepared by equilibrating *meso*-dideuterioethylene dibromide with lithium bromide (hexamethylphosphoramide, tetrahydrofuran, 100 °C, 15 h), which gave an identical mixture of dideuterioethylenes. Addition of glycine in the absence of ACC gave only endogenous ethylene (ν_{max} 951 cm^{-1}), unchanged from the glycine free control. When the apple slices were preheated to 50 °C for 6 h prior to addition of *cis*-[$^2H_2$]-ACC (**3**) and (**4**) no deuteriated ethylene could be detected. A similar result was obtained if the apple slices were boiled in water for 2 h prior to the addition of *cis*-[$^2H_2$]ACC (**3**) and (**4**). That apple slices do not interconvert the dideuterioethylene was shown by incubating *trans*-[$^2H_2$]ethylene with apple slices (15 °C, 18 h, dark) followed by i.r. analysis, a result in accord with previous findings in etiolated pea seedlings.[8]



Scheme 2

In complete contrast to the biosynthetic results, the chemical oxidation with sodium hypochlorite in the presence or absence of mercury(II) chloride[9] proceeded with complete retention of configuration, *i.e.*, *cis*-[$^2H_2$]ACC (**3**) and (**4**) yielded only *cis*-[$^2H_2$]ethylene [Figure 1(C)] and *trans*-[$^2H_2$]-ACC (**5**) gave *trans*-[$^2H_2$]ethylene [Figure 1(D)].

In summary, the complete lack of stereospecificity seen in the biosynthetic production of ethylene from ACC implies a quite different mechanism for this process as compared to the stereospecific chemical pathway, Scheme 2.[10]

We thank the S.E.R.C. for financial assistance to B. J. R., and Dr. A. E. Derome and Dr. L. D. Field for assistance with the computer simulations of ACC.

Received, 10th January 1983; Com. 051

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