On the Biosynthesis of Ethylene

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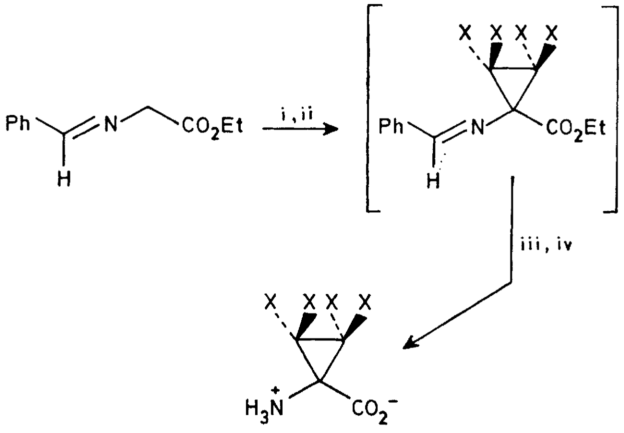
The conversion of 2,2,3,3-tetradeuterioaminocyclopropanecarboxylate (ACC) into tetradeuterioethylene was observed in apple slices; this result proves that the biosynthesis of ethylene, in this tissue, occurs without exchange of the cyclopropane hydrogen atoms of ACC.

The role of ethylene as a plant growth regulator is established,[1] as is its biosynthesis from methionine,[2] *via* 1-aminocyclopropane-1-carboxylate (ACC) (**1**).[3,4] However rigorous proof of the biosynthetic pathway is lacking. Thus Adams and Yang[3] trapped a radioactive gas, assumed to be ethylene, from the feeding of uniformly ^{14}C-labelled ACC to apple tissue, and Lürssen[4] observed an increase in ethylene production (*ca.* 100 fold) on addition of ACC to soybean leaves. In order to provide a rigorous proof of this pathway we synthesised [2,2,3,3-$^2H_4$]-ACC (**2**) as in Scheme 1, (28% overall yield). This substrate† (30 mg) in water (30 ml) was then fed to apple slices (260 g)‡ and the gaseous products were trapped in mercury perchlorate solution (0.25 M, 60 ml). Addition of lithium chloride (0.25 g) caused liberation of ethylene from its mercury complex; the evolved gas was analysed by mass spectroscopy



(**1**) X = H
(**2**) X = ^{2}H

Scheme 1. *Reagents and conditions:* i, Pr^i_2NLi (2.6 equiv.), tetrahydrofuran, hexamethylphosphoramide, −65 °C; ii, $C_2D_4Br_2$; iii, 6 M HCl, heat; iv, Dowex 50W - X 8(H), recrystallisation (H_2O–EtOH).

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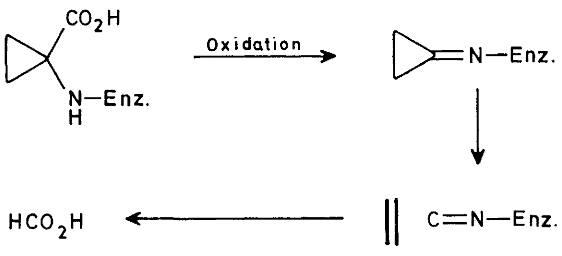
† The [$^2H_4$]-ACC, (**2**) [δ(^{2}H, D_2O) 0.95 and 1.10 p.p.m. (4 ^{2}H, 2 br. s) only] was shown to contain <1% of residual unlabelled trideuterio-monoprotio-ACC *etc.* by ^{1}H n.m.r. spectroscopy.

‡ Star Crimson apples were peeled, the core was removed, and the apple tissue was cut into slices 2—3 mm thick.



Scheme 2

and shown to be $C_2{}^2H_4$ (*m/e* found 32.0564, calculated 32.0564).§

In a separate experiment $[^2H_4]$-ACC (**2**) (20 mg) in water (20 ml) was fed to apple slices¶ (280 g) and the volatile products carried by an air stream to an aqueous KBr_3 trap. Reduction of the excess of bromine ($NaHSO_3$) and extraction with dichloromethane provided a solution which, when examined by ^2H n.m.r. spectroscopy (46.07 MHz), was shown to contain $[^2H_4]$ethylene dibromide[9] [$\delta(^2H, CH_2Cl_2)$ 3.645 p.p.m. (4 ^2H, s), >95% of ^2H content]. Analysis of this substance by g.c.–m.s. showed a fragment ion ($M^{\cdot+}$ — Br) of relative intensities *m/e* 110:111:112:113:114 = 2:100:3:98:2, identical with the ion derived from an authentic standard.** In this experiment a small amount (*ca.* 10%) of ethylene dibromide, resulting from endogenous ethylene production, was detected as fragment ions at *m/e* 107 and 109. Control experiments were performed as follows. In the absence of $[^2H_4]$-ACC only endogenous ethylene was detected, as its dibromide (by ^2H n.m.r. and g.c.–m.s. analyses). Finally when the apple slices were boiled (2 h) and the resultant purée treated as before with $[^2H_4]$ethylene dibromide, no $[^2H_4]$ethylene or ethylene was detected by the same analyses.

In conclusion, we have shown that $[^2H_4]$-ACC (**2**) is converted by apple slices into $[^2H_4]$ethylene without loss of deuterium. Since it has previously been shown that this step is oxidative in nature[3] and also possibly involves pyridoxal[4] then a chemically feasible route for this conversion is shown in Scheme 2.

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References

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§ $[^2H_4]$Ethylene was analysed on a VG Micromass ZAB 1F mass spectrometer. $[^2H_4]$Ethylene dibromide was run on a g.c.–m.s. technique. The g.c. column consisted of a 25 m × 0.3 mm ID OV 17 Quartz column heated at 95 °C, and helium at 1 ml min^{-1} was used as a carrier gas. The $[^2H_4]$ethylene dibromide appeared from the column after 75—77 s and its mass spectrum was measured directly by a VG Micromass 16F mass spectrometer.

¶ New Zealand Red Delicious apples were peeled, the core was removed, and the apple tissue cut into slices 2–3 mm thick.

** Authentic $[^2H_4]$ethylene dibromide (P. & S. Biochemicals Limited) [δ (^2H, CH_2Cl_2) 3.645 p.p.m. (4 ^2H, s)] was shown to contain >99% deuterium by mass spectroscopy [intensities of base $M^{\cdot+}$— Br fragment ion for (**2**) *m/e* 110:111:112:113:114: = 2:100:4: 98:2; intensities for (**1**) *m/e* 106:107:108:109:110: = 3:100:5:95:2].