

On the Biosynthesis of Ethylene

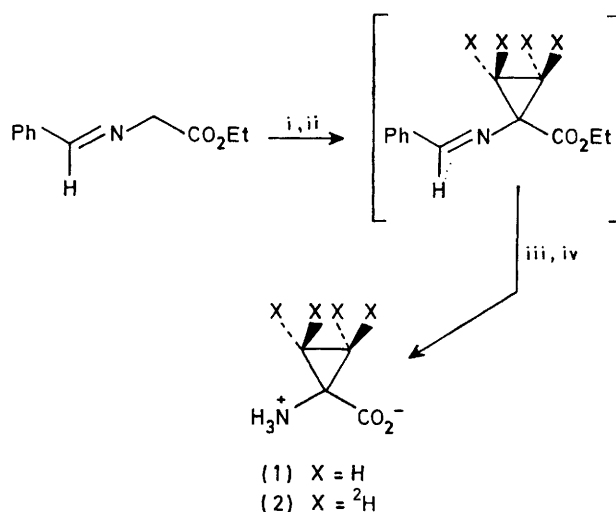
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The conversion of 2,2,3,3-tetradeuterioaminocyclopropanecarboxylate (ACC) into tetradeuterioethylene was observed in apple slices; this result proves that the biosynthesis of ethylene, in this tissue, occurs without exchange of the cyclopropane hydrogen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

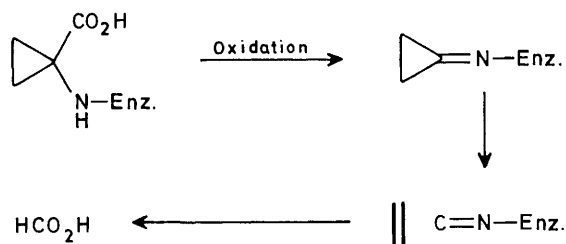
The role of ethylene as a plant growth regulator is established,¹ as is its biosynthesis from methionine,² via 1-aminocyclopropane-1-carboxylate (ACC) (1).^{3,4} However rigorous proof of the biosynthetic pathway is lacking. Thus Adams and Yang³ trapped a radioactive gas, assumed to be ethylene, from the feeding of uniformly ¹⁴C-labelled ACC to apple tissue, and Lürssen⁴ 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 [2,2,3,3-²H₄]-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



[†] The [²H₄]-ACC, (2) [$\delta(^2\text{H}, \text{D}_2\text{O})$ 0.95 and 1.10 p.p.m. (4 ²H, 2 br. s) only] was shown to contain <1% of residual unlabelled trideuterio-monoprotio-ACC *etc.* by ¹H 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 1. Reagents and conditions: i, Pr₂NLi (2.6 equiv.), tetrahydrofuran, hexamethylphosphoramide, -65 °C; ii, C₂D₄Br₂; iii, 6 M HCl, heat; iv, Dowex 50W - X 8(H), recrystallisation (H₂O-EtOH).



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 dibromide⁹ [$\delta(^2H, CH_2Cl_2)$ 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 ($M^{+} - Br$) of relative intensities m/e 110:111:112:113:114 = 2:100:3:

§ [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 \times 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.

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 nature⁹ and also possibly involves pyridoxal⁴ then a chemically feasible route for this conversion is shown in Scheme 2.

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References

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- 3 D. O. Adams and S. F. Yang, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 170.
- 4 K. Lürssen, K. Naumann, and R. Schroder, *Z. Pflanzenphysiol. Bd.*, 1979, **92**, s. 285.

** Authentic [2H_4]ethylene dibromide (P. & S. Biochemicals Limited) [$\delta(^2H, CH_2Cl_2)$ 3.645 p.p.m. (4 2H , s)] was shown to contain >99% deuterium by mass spectroscopy [intensities of base $M^{+} - Br$ 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].