On the Biosynthesis of Ethylene. Determination of the Stereochemical Course using Modified Substrates

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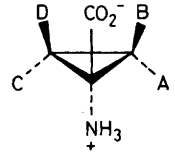
The conversion of a series of specifically 2-alkylated-3-deuteriated-1-aminocyclopropanecarboxylates by apple tissue into mixtures of *cis*- and *trans*-1-deuterioalk-1-enes is reported; the results are in accord with a stepwise enzymatic mechanism of cyclopropane ring opening in which stereochemical equilibration is faster than the subsequent bond breaking process.

Previously[1] we demonstrated that the conversion of 1-aminocyclopropanecarboxylate (ACC) (**1a**) into ethylene by apple tissue occurs without exchange of the cyclopropane hydrogen atoms of ACC and also this process unexpectedly proceeded with complete loss of stereochemistry, since both forms of *cis*-[2,3-$^2H_2$]ACC (**1b**), (**1c**) or (±)-*trans*-[2,3-$^2H_2$]ACC (**1d**), (**1e**) gave an equal mixture of *cis*-(**2a**) and *trans*-[1,2-$^2H_2$]-ethylene (**2b**),[2] a result subsequently confirmed in mung beans by others.[3] This stereochemical outcome may result from an enzyme bound ring-opened cyclopropane intermediate *e.g.* the radical (**3**), which through rapid rotation about C(3)–C(4) could lose its stereochemical integrity and subsequently collapse to an equal mixture of *cis*- and *trans*-dideuterioethylenes (Scheme 1). A similar stereochemical course was observed in chemical oxidations with transition metal oxidants such as copper(II), permanganate, and ferrate ions,[4] whereas hypochlorite oxidation gave complete retention.[2]

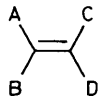
Of the four stereoisomers of 2-ethyl-ACC only the 1*R*,2*S*-enantiomer (**1f**) proved an effective† substrate with postclimacteric apples or mung bean hypocotyls to give but-1-ene (**2c**).[5] From these observations, Yang proposed that the site for alkene formation was enzymatic in nature and presumably the topology of the enzyme site could only accommodate the ethyl group when it occupies the *pro-S* position of the *pro-R* methylene group.

In order to probe the stereochemical course of ethylene biosynthesis we prepared specifically alkylated and deuteriated ACC's, in racemic and enantiomerically pure form[6,7] and tested these as substrates with apple tissue. The results of incubation of racemic and enantiomerically pure substrates are given in Tables 1 and 2 respectively.

These data show that, whereas for the 2-ethyl-ACC enantiomers only the 1*R*,2*S*-form (**1f**) is an effective substrate, as Yang found,[5] in the 2-methyl-ACC series, the 1*R*,2*S*-(**1p**), 1*R*,2*R*-(**1r**), and the 1*S*,2*R*-(**1q**) enantiomers all behave as substrates. Doubtless this reflects the relative sizes of ethyl *vs.* methyl substituents. The alkene stereochemistries from these



(1)

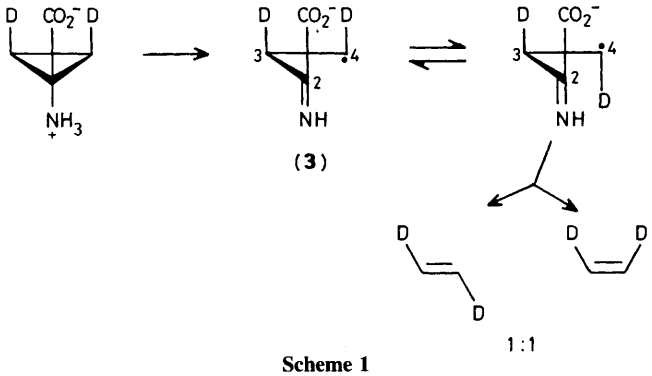


(2)

	A	B	C	D
a	H	H	H	H
b	H	D	H	D
c	D	H	D	H
d	D	H	H	D
e	H	D	D	H
f	Et	H	H	H
g	H	Et	H	H
h	H	H	Et	H
i	H	H	H	Et
j	Et	H	D	H
k	D	H	Et	H
l	Et	H	H	D
m	H	D	Et	H
n	Me	H	D	H
o	D	H	Me	H
p	Me	H	H	D
q	H	D	Me	H
r	H	Me	D	H
s	D	H	H	Me
t	H	Me	H	D
u	H	D	H	Me

	A	B	C	D
a	H	D	H	D
b	H	D	D	H
c	Et	H	H	H
d	Et	H	D	H
e	Et	H	H	D
f	Me	H	D	H
g	Me	H	H	D

† The relative conversions of (**1a**):(**1f**):(**1g**):(**1h**):(**1i**) with mung bean hypocotyls was reported as 350:100:0.5:1.2:0.5.

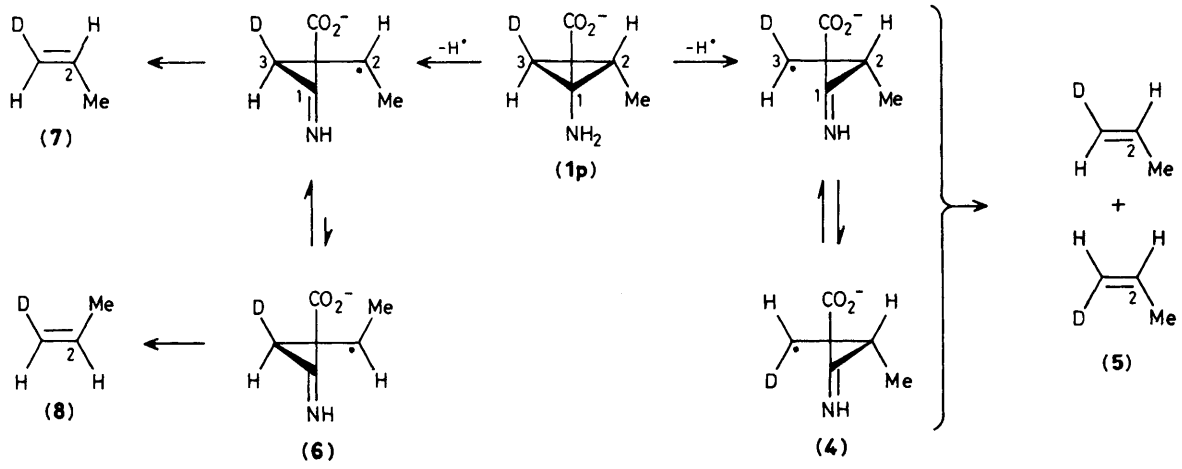


Scheme 1

Table 1.

Entry	Racemate	Product	Alkene geometry[a]	Net stereochemical bias
1	(**1f**),(**1h**)	(**2c**)	—	—
2	(**1g**),(**1i**)	—	—	—
3	(**1j**),(**1k**)	(**2d**):(**2e**)	53:47	Retention[b]
4	(**1l**),(**1m**)	(**2d**):(**2e**)	47:53	Retention[b]
5	(**1n**),(**1o**)	(**2f**):(**2g**)	55:45	Retention
6	(**1p**),(**1q**)	(**2f**):(**2g**)	42:58	Retention
7	(**1r**),(**1s**)	(**2f**):(**2g**)	65:35	Inversion
8	(**1t**),(**1u**)	(**2f**):(**2g**)	36:64	Inversion

[a] Ratios (±5%) of *cis*- and *trans*-[1-^{2}H]alkene determined by ^{1}H and ^{2}H n.m.r. spectroscopy of the products of *trans* bromination by comparison with authentic standards. The ratios were consistent with direct gas i.r. analysis of the 1-deuteriopropene products from entries 4—8 prior to bromination. [b] See footnote‡.

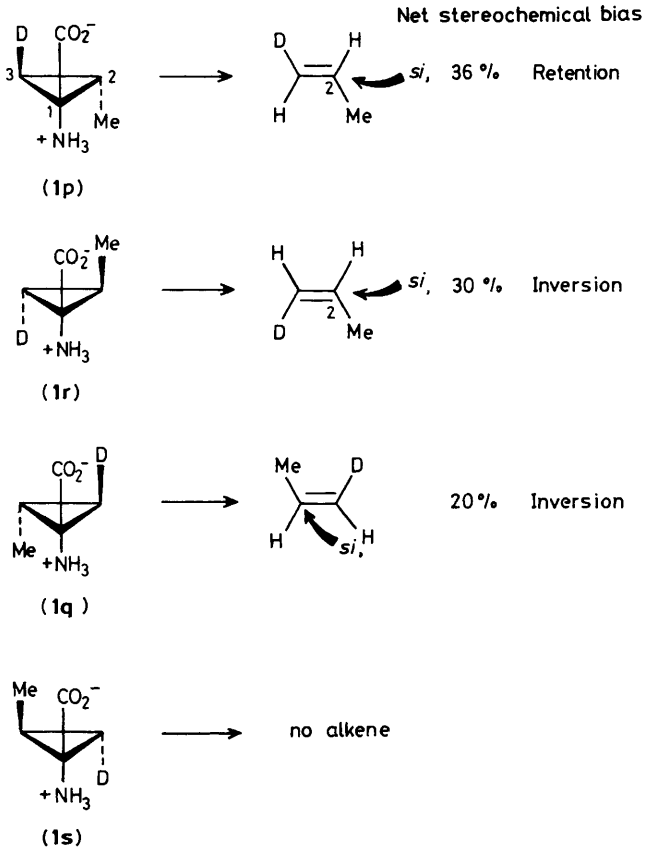


Scheme 2

Table 2.

Enantiomer	Product	Alkene geometry[a]	Net stereochemical bias
(**1f**)	(**2c**)	—	—
(**1h**)	—[b]	—	—
(**1p**)	(**2f**) : (**2g**)	32 : 68	36% Retention
(**1q**)	(**2f**) : (**2g**)	60 : 40	20% Inversion
(**1r**)	(**2f**) : (**2g**)	65 : 35	30% Inversion
(**1s**)	—	—	—

[a] As footnote a, Table 1. [b] Ratio of conversion of (**1f**) : (**1h**) *ca.* 25 : 1.



Scheme 3

2-methyl-ACC enantiomers (Table 2) show that, unlike the complete scrambling observed with [2,3-$^2H_2$]ACC's,[2] there is now a *net stereochemical bias*. Our interpretation of these results may be seen by consideration of the case of the 1*R*,2*S*-enantiomer (**1p**) (Scheme 2). We suggest that initial oxidative cleavage of the cyclopropane may occur both at C(1)–C(2) and C(1)–C(3). Since the C(1)–C(3) cleavage will result in complete scrambling of the deuterium stereochemistry by rotation around C(2)–C(3) in (**4**), if this process is faster than the subsequent C(2)–C(1) cleavage the alkene product from this manifold will be an equal mixture of *cis*- and *trans*-[1-^{2}H]propene, (**5**). Thus the net stereochemical bias derives only from initial cleavage of C(1)–C(2) to an equilibrating pair of radicals (**6**). If the rates of collapse of each of these to alkenes (**7**) and (**8**) are similar then the stereochemical outcome will reflect the conformational populations of (**6**), presumably determined by the topology of the active site, as well as the partitioning between (**4**) and (**6**). By reference to the *si* face at C(2) of alkenes (**7**) and (**8**), which specifies the stereochemical relationship between the alkene fragment, C(2)–C(3), and C(1) in the precursor cyclopropane[8] (Scheme 3), then (**1p**) and (**1r**) give, within our experimental error, the same stereochemical outcome, as expected from a pair of equilibrating radicals, (**6**), differing only in the stereochemistry of one deuterium atom. However the other pair (**1q**) and (**1s**), cannot be compared in this way since only (**1q**) is a substrate, giving 20% net inversion, whereas (**1s**) does not convert. From this it appears, assuming equal access to the active site, that the steric restraints imposed on the ring-opened intermediate from (**1q**) are different from those controlling binding of the pair (**1q**) and (**1s**). Interestingly, the ethyl series (**1j**) and (**1l**), with the same absolute configuration of the ethyl group as that in the methyl analogue (**1p**), gave only 6% net retention, Table 1. Although these were incubated as racemates their enantiomers, (**1k**) and (**1m**) respectively, were not substrates,‡ *vide supra*. The origin of this lower level of net retention, 6% *vs.* 36%, is unclear

‡ As only the 1*R*,2*S*-enantiomer (**1f**) of 2-ethyl-ACC gives an effective conversion into but-1-ene, it is assumed that it is only this entantiomer that contributes from the incubation of the racemates (**1j**), (**1k**) and (**1l**), (**1m**).

although it may arise from a better enzymatic 'fit' of the smaller methyl series.

The results obtained in this study cannot be explained§ by the operation of any non-asymmetric processes since chemical oxidations with one electron reagents, *e.g.* K_2FeO_4[4] of the racemates (**1n**), (**1o**) or (**1t**), (**1u**) gave cleanly a 1 : 1 mixture of *E*- and *Z*-1-deuteriopropenes, while two electron oxidation with hypochlorite[4] gave complete retention of stereochemistry in the alkenes from (**1n**), (**1o**) and (**1t**), (**1u**).

In summary, the formation of substituted alkenes from methylated ACC's with net stereochemical bias supports the contention that the biosynthesis of ethylene occurs by a stepwise mechanism in an enzyme active site, whose intrinsic topology may result in other than the complete scrambling of the stereochemistry originally observed.[2,3]

§ Although a non specific chemically based one electron or two electron process may contribute to the total unsaturated product obtained from incubation of these alkylated ACC's with apples, they cannot totally explain the different alkene ratios obtained from enantiomeric substrates, *e.g.* (**1p**) *vs.* (**1q**).

Received, 5th July 1985; Com. 955

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