Evaluation of the Steric Course of Enoyl Reduction in Higher Plants and Insects *via* Coupling to 1-Alkene Biosynthesis. A Model Study with *Carthamus tinctorius* (Asteraceae) and *Tribolium castaneum* (Coleoptera; Tenebrionidae)

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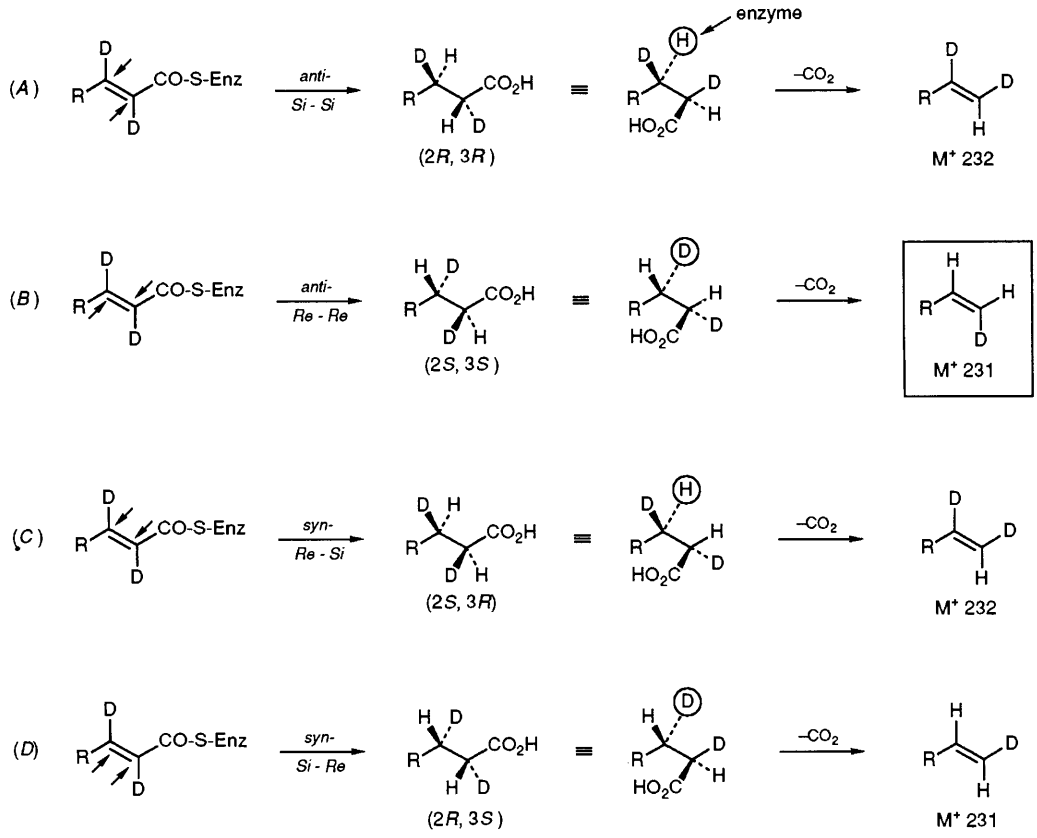
(*E*)-12-Phenyl[2-$^2H_2$]dodec-2-enoic acid is *in vivo* reduced and oxidatively decarboxylated by plant (*Carthamus tinctorius*) and insect (*Tribolium castaneum*) model systems to (*Z*)-11-phenyl[1-^2H]undec-1-ene; the known *anti*-elimination of the carboxy group and the C(3)-H_S hydrogen atom in conjunction with the (*Z*)-configuration of the alkene demands an *anti*-2*Re*,3*Re* addition of two hydrogen atoms across the double bond of the precursor acid.

Many plants and insects are able to synthesize terminally unsaturated hydrocarbons from saturated fatty acids by oxidative decarboxylation. The biocatalysts and the exact mechanism of the reaction are as yet unknown, but the site-specificity of the enzymes and the overall stereochemical course of the transformation have been thoroughly investigated with plant and insect model systems. In fact, the steric course was found to be identical in both cases and proceeds *via* an *anti*-elimination of the carboxy group and the C(3)-H_S hydrogen atom of the precursor acid [Scheme 1; right half, *e.g.* path (*A*)]. The data were obtained from *in vivo* feeding studies with the dyers thistle *Carthamus tinctorius*[1] (Asteraceae) and the flour beetle *Tribolium confusum*[2] (Coleoptera; Tenebrionidae). Unnatural, deuterium labelled 12-phenyl-



Scheme 1 Left half: Compilation of the four stereochemical alternatives of hydrogen transfer reactions to the two trigonal centres of an α,β-unsaturated fatty acid. Right half: Transformation of the resulting chiral 12-phenyl[2,3-$^2H_2$]dodecanoic acids into labelled 1-alkenes *via anti*-elimination of C(1) and the C(3)-H_S hydrogen atom (encircled).

dodecanoic acids were used as surrogates for the genuine families of (un)saturated C_{16}- and C_{18}-fatty acid precursors.[3] The artificial probes are readily available,[4] chemically stable, and smoothly converted into 11-phenylundec-1-enes by the above plant and insect species. Owing to the unique mass spectral fragmentation pattern of the resulting unnatural 11-phenylundec-1-enes the metabolic fate of the administered acid can be precisely followed, and superposition of the product(s) even by very complex hydrocarbon blends from plants or insects does not occur.[3] Interestingly, the immediate degradation product, namely 11-phenylundec-1-ene (*ca.* 80%) is often accompanied by a smaller amount of 13-phenyltridec-1-ene (*ca.* 20%). Hence, it follows that the 12-phenyldodecanoic acid can not only serve as a substrate for the oxidative decarboxylation, but may also suffer chain elongation (at least one cycle) prior to the oxidative degradation. Owing to this channelling into the fatty acid anabolism (14-phenyltetradecanoic acid) followed by oxidative decarboxylation (13-phenyltridec-1-ene), it should be possible in principle to evaluate the stereochemical course of at least some reactions of the anabolic sequence (condensation–reduction–dehydration–reduction) using the known stereochemical course of the oxidative decarboxylation as an analytical method. For example, administration of (*E*)-12-phenyl[2,3-$^2H_2$]dodec-2-enoic acid to the above plant or insect model systems should give reliable information on the steric course of their enoyl reductases, since it is only the saturated acid which is converted into the deuterium labelled 11-phenylundec-1-ene. The strategy and the principal stereochemical alternatives of such reductions are compiled in Scheme 1.

Each of the four possible modes of hydrogen transfer [Scheme 1, left half, path (*A*) → (*D*)] should result in a certain, well defined diastereoisomer of the labelled 12-phenyl[2,3-$^2H_2$]dodecanoic acid. After release from the enzyme, the free acids will become the substrates of the oxidative decarboxylation and yield 1-alkenes *via anti*-elimination of C(1) and the C(3)-H_{Si} hydrogen atom.[1] The resulting deuterium labelled 11-phenylundec-1-enes can be detected either by mass spectrometry owing to their different deuterium content [Scheme 1, path (*A*) and (*C*) *versus* path (*B*) and (*D*)] or by IR spectroscopy (GC–FTIR) owing to their different (*E*)- or (*Z*)-configuration. The synthesis of the model substrates and the administration techniques are described elsewhere.[1,2,4]

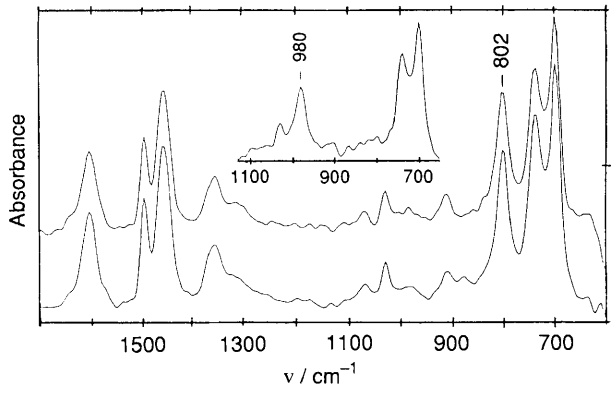


Fig. 1 Gas phase IR spectra of (*Z*)- and (*E*)-11-phenyl[1-^{2}H]undec-1-enes. Lower trace: synthetic (*Z*)-11-phenyl[1-^{2}H]undec-1-ene. Upper trace: Metabolites derived from incubation of specimens of *T. castaneum* or roots of *C. tinctorius* with 12-phenyl[2,3-$^2H_2$]dodec-2-enoic acid. Inset: (*E*)-11-phenyl[1-^{2}H]undec-1-ene.

As expected, the *in vivo* experiments with (*E*)-12-phenyl[2,3-$^2H_2$]dodec-2-enoic acid and, *e.g.*, roots of *C. tinctorius* or specimens of the flour beetle *T. castaneum* are

unequivocal and confirm the validity of the above considerations. The results can be summarized as follows: (*i*) consistent with the loss of a single deuterium atom from the α,β-unsaturated precursor acid, the 11-phenyl[1-^{2}H]undec-1-enes of both plant and insect origin display an identical molecular ion at *m/z* 231 [*cf.* Scheme 1; path (*B*) and (*D*) only], (*ii*) the 11-phenyl[1-^{2}H]undec-1-enes of plant and insect origin possess the (*Z*)-configuration [path (*B*) only]. The fingerprint region of their GC–FTIR spectra is fully congruent with that of a synthetic (*Z*)-reference[4] [*cf.* Fig. 1; vinyl wagging band at 802 cm^{-1}; inset (*E*)-isomer: 980 cm^{-1}]. Moreover, in view of both the $M^{\cdot+}$ at *m/z* 231 and the unequivocal localization of the remaining deuterium atom at C(1) of the alkene by the IR spectrum in Fig. 1, the abstracted deuterium atom must have been removed from C(3) of the administered acid [loss of the C(3)-H_S].

Owing to these findings and in agreement with the analysis of Scheme 1, the enoyl reductases of both plant and insect origin must have delivered their hydrogen atoms in an *anti-2Re,3Re* fashion to the two trigonal centres of the alkene model substrate. This is the only steric course which will result in the (2*S*,3*S*)-12-phenyl[2,3-$^2H_2$]dodecanoic acid, the known precusor of the (*Z*)-11-phenyl[1-^{2}H]undec-1-ene.[1]

The present results are remarkable in several respects. First, the steric course of the enoyl reduction in the two representatives from the plant (*C. tinctorius*) and insect (*T. castaneum*) kingdom is identical. The methodology appears to be of general applicability in other organisms, provided that there is a production of 1-alkenes from fatty acids according to a known steric course. The observed *anti-2Re,3Re* addition of the two hydrogen atoms is different from the steric courses of all previously examined enoyl reductases from fatty acid synthase complexes[5] (bacteria:[6] *syn-2Re,3Si*; rat, chicken:[7] *syn-2Si,3Re*; yeast:[8] *anti-2Si,3Si*). Thus, the stereochemical outcome of the present study represents the last of the four theoretically possible modes of enoyl reduction.[5] We are well aware that such an *in vivo* study does not answer the most important question as to whether the enoyl reductase(s) involved belong to the mitochondrial or microsomal chain elongation system or have to be considered as an integral constituent of the cytosolic fatty acid synthase. However, preliminary experiments with enantiospecifically labelled 3-hydroxy-12-phenyl[2,3-$^2H_2$]dodecanoic acids indicate already that the preceding elimination of water to give the α,β-unsaturated acid occurs *via syn*-elimination. This is in accord with the steric course of the cytosolic fatty acid synthase of all hitherto examined organisms.[5] As a consequence, the present work probably adds another example to the generally observed trend that it is only the steric course of the reduction of carbon–carbon double bonds which is a subject of divergent evolution in fatty acid synthases of the various forms of life.[5]

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