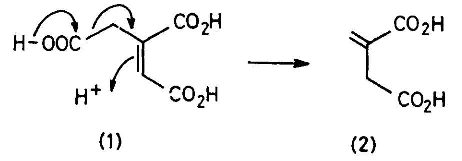
Stereochemistry of the Introduction of the Hydrogen Atom at C-2 of *cis*-Aconitic Acid during its Transformation into Itaconic Acid in *Aspergillus terreus*: a ^{2}H N.M.R. Approach†

By Bianca M. Ranzi, Fiamma Ronchetti, Giovanni Russo,* and Lucio Toma

(*Istituto di Chimica Organica dell'Università di Milano, Centro di Studio per le Sostanze Organiche Naturali del C.N.R., Via Venezian* 21, 20133 *Milano, Italy*)

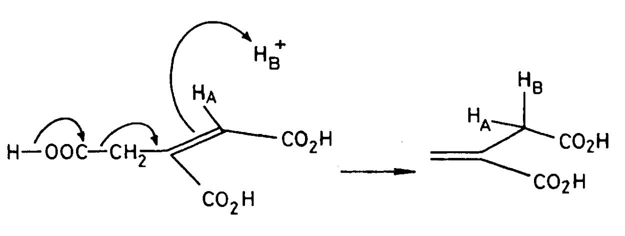
Summary By ^{2}H n.m.r. spectroscopy it is shown that, during the conversion of *cis*-aconitic acid (**1**) into itaconic acid (**2**), the proton coming from the medium is introduced at C-2 of (**1**) on the 2-*re*,3-*si* face.

Bentley and Thiessen[1] have reported the transformation of *cis*-aconitic acid (**1**) into itaconic acid (**2**) by *Aspergillus terreus* and have proposed the mechanism shown in Scheme 1, which involves a decarboxylation and a 1,3-allylic rearrangement, with the introduction of a proton from the medium.



Scheme 1

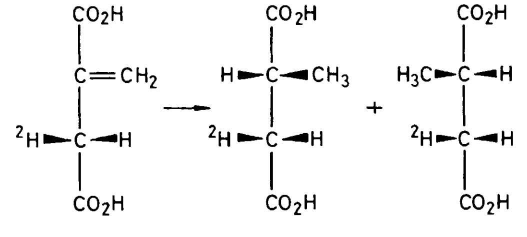
We now report the results of a ^{2}H n.m.r. study which establishes that the introduction of this proton occurs on the 2-*re*,3-*si* face‡ (Scheme 2) of *cis*-aconitic acid.



Scheme 2

An enzyme preparation of *cis*-aconitic acid decarboxylase (CAD) in ^2H_2O (99·95% ^{2}H) obtained[1] from *Aspergillus terreus* ATCC 10020 was incubated with *cis*-aconitic acid to yield [3-^{2}H]itaconic acid (**3**) (0·6 ^{2}H/molecule by n.m.r. and m.s.).

To determine the configuration at C-3 of [3-^{2}H]itaconic acid (**3**), it was submitted to catalytic hydrogenation (Pd/C in MeOH) to yield racemic [3-^{2}H]methylsuccinic acid (**4**) and (**5**), the ^{2}H n.m.r. spectrum of which showed two fairly well resolved singlets, at 2·74 and 3·18 p.p.m., in a 1:1 ratio.§



(**3**) 3*R* (**4**) 2*R*,3*R* (**5**) 2*S*,3*R*

The (2*RS*)-[3-^{2}H]methylsuccinic acid was diluted with unlabelled material to give a sample containing 0·1 ^{2}H/molecule. This was partially resolved with quinine[2] to afford (2*R*)-[3-^{2}H]methylsuccinic acid (**4**) containing 30% (by polarimetry) of the (2*S*)-isomer (**5**).

The ^{2}H n.m.r. spectrum of the resolved sample displayed the same peaks at 2·74 and 3·18 p.p.m., the intensity ratio of which changed to 72:28. ^{1}H N.m.r. analysis[3] of methylsuccinic acid allows the assignment of the signal at 2·74

---

† Presented at the 2nd European Symposium on Organic Chemistry, Stresa (Italy), June 1—5, 1981, and at the Meeting of the Italian Biochemical Society (Liguria-Lombardia-Piemonte Section), Pavia (Italy), June 6, 1981.

‡ The same results were obtained, in an independent way: D. Arigoni and B. Marinoni, unpublished results; B. Marinoni, Ph.D. Thesis, E.T.H. Zürich, 1973.

§ The spectra were recorded in pyridine at 30·7 MHz on a Varian XL-200 spectrometer using C^2HCl_3 as internal reference.

p.p.m. to the 3-*pro-R* hydrogen of (2*R*)-methylsuccinic acid (**4**), whereas the signal to 3·18 p.p.m. can be attributed to the same hydrogen in the (2*S*)-isomer (**5**).¶ Therefore, it follows that the deuteriated resolved methylsuccinic acid (**4**) has the (2*R*,3*R*)-configuration, the 2·74 p.p.m. peak (72%) in the ^{2}H n.m.r. spectrum being due to the 3-deuteron in the (2*R*)-isomer, while the peak at 3·18 p.p.m. (28%) is due to the 3-deuteron of the (2*S*)-isomer (**5**) still present in the mixture owing to the non-complete resolution of the racemate.

The above data indicate that the deuteriated itaconic acid has the (3*R*)-configuration; this means that during the formation of itaconic acid from *cis*-aconitic acid the hydrogen atom coming from the medium assumes the 3-*pro-R* position, being introduced on the 2-*re*,3-*si* face of *cis*-aconitic acid, as shown in Scheme 2.

We thank Dr. G. Severini Ricca for the n.m.r. spectra.

(*Received, 7th July* 1981; *Com.* 785.)

¶ The opposite is true for the 3-*pro-S* hydrogen.

[1] R. Bentley and C. P. Thiessen, *J. Biol. Chem.*, 1957, **226**, 703, and preceding papers.
[2] J. F. Tocanne and C. Asselineau, *Bull. Soc. Chim. Fr.*, 1965, 3346.
[3] L. Zetta and G. Gatti, *Tetrahedron*, 1972, 3773.