Isotopic Enrichment by Asymmetric Deuteriation. An Investigation of the Synthesis of Deuteriated (*S*)-(−)-Methylsuccinic Acids from Itaconic Acid

David J. Hardick, Ian S. Blagbrough,*,† and Barry V. L. Potter

Contribution from the Department of Medicinal Chemistry, School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, U.K.

Received February 21, 1995[⊗]

Abstract: Two different asymmetric hydrogenation methodologies based on gaseous hydrogen at 1 atm pressure and transfer hydrogenation from the decomposition of formic acid, in the presence of a rhodium catalyst, have been used to prepare (*S*)-(−)-methylsuccinic acid enriched with the deuterium isotope. NMR and mass spectroscopic evidence indicates that complex labeling occurs. We interpret this labeling pattern as the result of an equilibrium which exists between the olefin and a catalyst-alkyl intermediate in a Wilkinson-type mechanism. This phenomenon has not been reported in similar experiments with (*E*)-phenylitaconic acid where the expected *cis*-addition of deuterium from either deuterium gas at 1 atm, or deuteriated formic acid under transfer hydrogenation conditions, was observed. In our studies reducing itaconic acid (methylenebutanedioic acid), there was no loss of asymmetric induction (above 90% ee), approximately 2.4 deuterons were incorporated into (*S*)-(−)-methylsuccinic acid, a ratio of 1.8:1 methyl: methine deuteriation was observed, and there was no evidence for olefin isomerization into conjugation with both carboxylic acid groups. Following these isotopic enrichment studies, we present the first evidence for reversible transfer addition of deuterium to methylsuccinic acid, either by gaseous or transfer hydrogenation at atmospheric pressure. Such a mechanism has recently been eliminated for reduction at elevated pressures. These results have general applicability to the synthesis of isotopically labeled homochiral substituted carboxylic acids and also in interpreting the ^{13}C NMR data which are generated by the simultaneous presence of several deuterium containing isotopomers.

Introduction

Asymmetric hydrogenation of a prochiral olefin to introduce one or more chiral centers is important and topical, due mainly to the mild reaction conditions involved and the high optical purities which can be achieved in the product.[1−7] In particular, rhodium and ruthenium catalysts incorporating various optically active phosphine ligands have proved highly successful for the synthesis of a range of enantiopure compounds,[1−7] including *N*-acylated amino acids, e.g., *N*-acetylphenylalanine,[1] and (*R*,*R*)-4-propyl-9-hydroxynaphthoxazine, a novel dopamine agonist.[2] Noyori,[1,3] Halpern,[4] and their co-workers have extensively studied the mechanisms of asymmetric hydrogenation using such ruthenium based catalysts. Furthermore, by replacing hydrogen for deuterium or tritium,[8−10] the technique is also a powerful tool in the synthesis of homochiral, isotopically labeled substrates for ligand affinity, biosynthetic, or kinetic studies.

The importance of asymmetric hydrogenation has generated detailed mechanistic information on the kinetics of the reaction and the origin of stereocontrol, often from studies involving isotopic labels.[11−13] The chiral reduction of itaconic acid (methylenebutanedioic acid) (**1**) and related olefins to homochiral diacids has been well documented using both transfer hydrogenation (formic acid in the presence of a tertiary amine) or gaseous hydrogen.[14−20] Brunner and co-workers,[14] for instance, have examined the synthesis of (*S*)-(−)-methylsuccinic acid by transfer hydrogenation, employing a range of rhodium catalysts and tertiary amines in the presence of formic acid. They have consistently achieved high ee and efficient chemical conversions.

Although hydrogenation of itaconic acid (**1**) has been reported recently,[14−20] deuteriation of (**1**) has rarely been addressed. Deuteriation of (*E*)-phenylitaconic acid by Leitner, Brown, and

† FAX: 011-44-1225-826114; Phone: 011-44-1225-826795.

⊗ Abstract published in *Advance ACS Abstracts,* November 1, 1995.

(1) Miyashita, A.; Yasuda, A.; Takaya, H.; Toriumi, K.; Ito, T.; Souchi, T.; Noyori, R. *J. Am. Chem. Soc.* **1980**, *102*, 7932−7934.

(2) Melillo, D. G.; Larsen, R. D.; Mathre, D. J.; Shukis, W. F.; Wood, A. W.; Colleluori, J. R. *J. Org. Chem.* **1987**, *52*, 5143−5150.

(3) (a) Noyori, R. *Science* **1990**, *248*, 1194−1199. (b) Noyori, R.; Takaya, H. *Acc. Chem. Res.* **1990**, *23*, 345−350. (c) Takaya, H.; Ohta, T.; Mashima, K.; Noyori, R. *Pure Appl. Chem.* **1990**, *62*, 1135−1138. (d) Ohta, T.; Takaya, H.; Noyori, R. *Tetrahedron Lett.* **1990**, *31*, 7189−7192. (e) Noyori, R. *Tetrahedron* **1994**, *50*, 4259−4292.

(4) (a) Ashby, M. T.; Halpern, J. *J. Am. Chem. Soc.* **1991**, *113*, 589−594. (b) Ashby, M. T.; Khan, M. A.; Halpern, J. *Organometallics* **1991**, *10*, 2011−2015.

(5) ApSimon, J. W.; Collier, T. L. *Tetrahedron* **1986**, *42*, 5157−5254.

(6) (a) Brown, J. M. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 190−203. (b) Brunner, H. *Synthesis* **1988**, 645−654.

(7) Kawano, H.; Ikariya, T.; Ishii, Y.; Saburi, M.; Yoshikawa, S.; Uchida, Y.; Kumobayashi, H. *J. Chem. Soc., Perkin Trans. I* **1989**, 1571−1575.

(8) Lockley, W. J. S. *J. Labelled Cmpds. Radiopharm.* **1984**, *21*, 45−57.

(9) Parnes, H.; Shelton, E. J. *Synth. Appl. Isot. Labelled Compd. Proc. Int. Symp. (II)*; Elsevier: Amsterdam, 1985 (published 1986); pp 159−160.

(10) Taudien, S.; Schinkowski, K.; Krause, H. *Ger. Offen*. DE 4,108,572 [*Chem. Abs*. **1993**, *118*, 39411q].

(11) Landis, C. R.; Halpern, J. *J. Am. Chem. Soc.* **1987**, *109*, 1746−1754.

(12) Brown, J. M.; Chaloner, P. A.; Morris, G. A. *J. Chem. Soc., Perkin Trans. II* **1987**, 1583−1588.

(13) Leitner, W.; Brown, J. M.; Brunner, H. *J. Am. Chem. Soc.* **1993**, *115*, 152−159.

(14) Brunner, H.; Graf, E.; Leitner, W. *Synthesis* **1989**, 743−745.

(15) Yamashita, M.; Kobayashi, M.; Sugiura, M.; Tsunekawa, K.; Oshikawa, T.; Inokawa, S.; Yamamoto, H. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 175−178.

(16) Chiba, T.; Miyashita, A.; Nohira, H. *Tetrahedron Lett.* **1991**, *32*, 4745−4748.

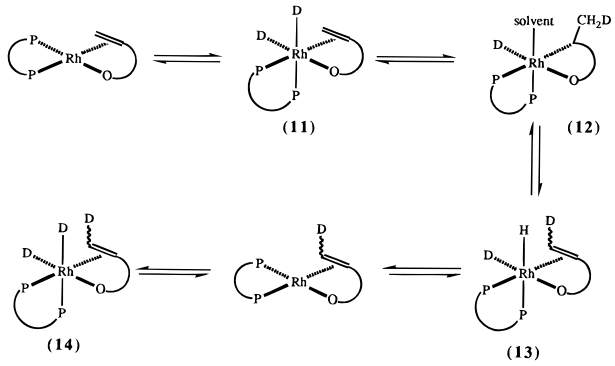
S0002-7863(95)00597-X CCC: $12.00

Brunner[13] using either transfer hydrogenation or gaseous deuterium at atmospheric pressure gave exclusively dideuteriated (phenylmethyl)butanedioic acid as expected. The production of one diastereoisotopomer, in this reduction, is consistent with the addition of deuterium across one face of the carbon−carbon double bond. The differences between these results and those of our studies are also consistent with the observed difference in kinetics for the reduction of (*E*)-phenylitaconic acid and itaconic acid (**1**) under similar conditions. These authors also report the 1,2-addition of two deuterium atoms exclusively in the transfer deuteriation of itaconic acid (**1**) with a cationic rhodium(I) catalyst, as analyzed by ^{1}H and ^{13}C-DEPT NMR spectroscopy.[13]

Our interest in the deuteriation of (*S*)-(−)-methylsuccinic acid using itaconic acid (**1**) was based on the demand for isotopically labeled norditerpenoid alkaloids which contain a 2-methylsuccinimido moiety, e.g., methyllycaconitine[21,22] or nudicauline.[23] We were convinced, in accordance with literature precedent,[13] that transfer or gaseous hydrogenation of **1** at 1 atm, catalyzed by rhodium and in the presence of a chiral phosphine ligand, would afford *cis*-addition of deuterium to the prochiral alkene. We are not aware of any precedent for deuteriation of methylsuccinic acid in excess of two deuterons. Ojima and co-workers proposed such a scheme for the rhodium mediated reduction of 2-acetamidoacrylic acid (*N*-acetyl dehydroalanine) to afford the 3,3-dideuterio product, together with further deuteriated acids.[24] However, in methanol at 20 atm of deuterium gas, there was no detectable scrambling of deuterium and the 2,3-addition product was obtained exclusively, with only two atoms of deuterium incorporated. Kagan,[25] Koenig,[26] and co-workers, in this Journal, have also ruled out similar schemes leading to the incorporation of three or more deuterons into α-acylaminocinnamic acids and α-benzamidocinnamic acids, by gaseous hydrogenation, employing various rhodium(I) catalysts. However, when we repeated the literature procedures, utilizing either deuteriated formic acid or deuterium gas, we observed complex labeling patterns and a higher enrichment of deuterium than could be explained from simple addition across the carbon−carbon double bond of (**1**).

We now report new results on the deuteriation of itaconic acid (**1**) to give a complex mixture of polydeuteriated (*S*)-(−)-methylsuccinic acids (**2**). We believe that, for certain substrates, an equilibrium with *both ^{2}H and ^{1}H* bound to the rhodium catalyst does occur (see Scheme 1) and that this ^{1}H originates by elimination from the deuteriated methyl group formed *in situ*. These findings have wider implications for enantiospecific isotopic labeling of other substituted carboxylic acids with deuterium or tritium by asymmetric hydrogenation of a double bond.

Scheme 1



Results

^{1}H NMR Studies. Following the procedure of Brunner, Brown, and co-workers,[13,14] we converted itaconic acid-d_2 (COOD-d^2) into deuteriated (*S*)-methylsuccinic acid, in 64% yield after recrystallization, by transfer hydrogenation using formic acid-d_2/α-methylbenzylamine-d_2 in DMSO-d_6 with a rhodium(III) catalyst ($RhCl_3 \cdot nH_2O$) and (2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-diphenylphosphino-2-(diphenylphosphinomethyl)-pyrrolidine (BPPM). The ^{1}H NMR spectrum of the isolated and recrystallized methylsuccinic acid, in comparison to that of unlabeled (*S*)-(−)-methylsuccinic acid [$CDCl_3$, δ 11.50-9.00 (2H, br s, COOH), 2.99−2.87 (1H, m, H2), 2.72 (1H, dd, H3, 16.9, 8.8 Hz), 2.56 (1H, dd, H3, 16.9, 4.8 Hz), 1.28 (3H, d, H5, 7.3 Hz)], indicated that several isotopomers were present. In the deuteriated (*S*)-(−)-methylsuccinic acid ^{1}H NMR spectrum, the H3 methylene protons, for instance, resonated as doublets at δ 2.56 ppm and δ 2.71 ppm (AB system) with a geminal coupling constant of 17.2 Hz, indicative of significant incorporation of deuterium at H2. However, a small, but reproducible incorporation of protium was also detected at H2 (δ 3.00−2.86 ppm, multiplet). From the integrals of the H3 methylene proton signals, which do not contain deuterium (*vide infra*), the percentage of compounds with protium at H2 compared to all isotopomers was typically 32−37%. Further evidence for this came from an inspection of the H3 methylene signals. For instance, about each peak of the doublet at δ 2.71 ppm (*vide supra*), there were two further signals of lower intensity corresponding to the coupling of H3 to protium at H2 (ABM system, 17.2 Hz, 8.8 Hz). A possible reason for this mixture of protium and deuterium containing products is exchange of the labile acid proton of the formic acid for protium, thereby giving rhodium hydride intermediates, rather than rhodium deuteride, as the catalyst for the hydrogenation. Replacement of DMSO-d_6 by DMSO-h_6 did not alter the percentage of protium found at H2. Replacement of formic acid-d_2 with deuterium gas (1 atm) resulted in only starting material being recovered, which is not unexpected given that rhodium(III) catalysts are slower than rhodium(I) catalysts, in this reaction and also that transfer hydrogenation effects a faster rate of reduction than that with gaseous hydrogen.

Reaction of itaconic acid-d_2 with deuterium gas (1 atm) catalyzed by a rhodium(I) catalyst, Rh(COD)Cl(BPPM),[24] (COD is 1,5-cyclooctadiene), in CH_3OD/toluene, gave (*S*)-(−)-methylsuccinic acid, in 77% yield after recrystallization, with protium once more at H2, but with a higher enrichment of deuterium at H2 than had been observed in the formic acid-d_2 experiments. Typically, the percentage of isotopomers with protium at H2, compared to all deuterium labeled methylsuccinic acid species, ranged from 12% to 24%, despite rigorously excluding all sources of protium. We believe that multiple deuteriation of

(17) Yoshikawa, K.; Yamamoto, N.; Murata, M.; Awano, K.; Morimoto, T.; Achiwa, K. *Tetrahedron: Asymmetry* **1992**, *3*, 13−16.
(18) Shao, L.; Takeuchi, K.; Ikemoto, M.; Kawai, T.; Ogasawara, M.; Takeuchi, H.; Kawano, H. *J. Organomet. Chem.* **1992**, *435*, 133−147.
(19) Marinetti, A.; Mathey, F.; Ricard, L. *Organometallics* **1993**, *12*, 1207−1212.
(20) Morimoto, T.; Chiba, M.; Achiwa, K. *Chem. Pharm. Bull.* **1993**, *41*, 1149−1156.
(21) Nambi Aiyar, V.; Benn, M. H.; Hanna, T.; Jacyno, J.; Roth, S. H.; Wilkens, J. L. *Experientia* **1979**, *35*, 1367−1368.
(22) Blagbrough, I. S.; Coates, P. A.; Hardick, D. J.; Lewis, T.; Rowan, M. G.; Wonnacott, S.; Potter, B. V. L. *Tetrahedron Lett.* **1994**, *35*, 8705−8708.
(23) Rollins, Y. D.; Hall, M.; Heman, K. L.; Dobelis, P.; Walrond, J. P.; Rose, G. M.; Leonard, S. *Society Neurosci. Abstr.* **1994**, *20*, 466.33.
(24) Ojima, I.; Kogure, T.; Yoda, N. *J. Org. Chem.* **1980**, *45*, 4728−4739.
(25) Detellier, C.; Gelbard, G.; Kagan, H. B. *J. Am. Chem. Soc.* **1978**, *100*, 7556−7560.
(26) Koenig, K. E.; Knowles, W. S. *J. Am. Chem. Soc.* **1978**, *100*, 7561−7564.

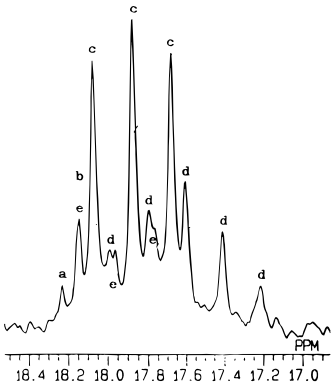
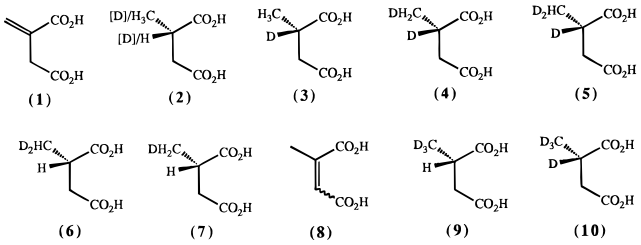


Figure 1. Part of the ^{13}C NMR spectrum (methyl region) for deuteriated (*S*)-(−)-methylsuccinic acid after gaseous hydrogenation: (a) protiomethylsuccinic acid, (b) **3**, (c) **4**, (d) **5**, and (e) **7**.

Chart 1



methylsuccinic acid occurs because of a dynamic equilibrium between the olefin, in this case itaconic acid (**1**), and the metal alkyl complex. Evidence for this is manifest from several sources.

^{13}C NMR Studies. The ^{13}C NMR spectrum of unlabeled (*S*)-(−)-methylsuccinic acid shows five resonances and interpretation follows from an inspection of the DEPT spectra, [CD_3OD δ 180.03 (CO, α to C2), 176.43 (CO, α to C3), 39.16 (CH_2), 37.75 (CH), 18.23 (CH_3)]. The carboxylic acid assignments are based on tabulated data for succinic acid, butanoic acid, and 2-methylpropionic acid in $CDCl_3$.[27] Following deuteriation of itaconic acid, in the manner described above using deuterium gas, a multiplet in the region δ 18.3−17.1 ppm was observed for this product as shown in Figure 1. Dilution of this sample with 20% by weight of unlabeled methylsuccinic acid indicated that a trace of unlabeled methylsuccinic acid at δ 18.23 ppm, [identified as (a) in Figure 1], was present following the deuteriation. The signal upfield of this unlabeled acid at δ 18.14 ppm (b) is consistent with a β-upfield shift (0.09 ppm, typically 0.1 ppm)[28] by virtue of deuterium at H2 (**3**). The prominent triplet (1:1:1) centered at δ 17.87 ppm (c) is for the dideuteriated acid (**4**), whilst the pentet (1:2:3:2:1) centered at δ 17.60 ppm (d) is for the trideuteriated species (**5**). Each resonance for (**4**) and (**5**) is shifted upfield from δ 18.14 ppm by 0.27 and 0.54 ppm, respectively, because of consecutive α-substitution with deuterium atoms (typically 0.25 ppm per α-deuterium atom).[28] It is likely that both these multiplets are from the dideuteriated (**4**) and trideuteriated (**5**) acids, respectively, i.e., with a deuteriated methine, and not just from successive incorporation of one and then two deuterium atoms into the methyl group. The evidence of a dideuteriated species (**6**) (protium methine) is less clear *from this region* of the ^{13}C NMR spectrum. Such a diacid (**6**) would resonate as a pentet shifted 0.09 ppm downfield from the one centered at δ 17.60 ppm (d). There are other deuteriated species as well, from the peaks appearing as a shoulder at approximately δ 17.99 and δ 17.79 ppm (e). These are possibly attributable to the monodeuteriated methylsuccinic acid (**7**) which would resonate as a 1:1:1 triplet centered at approximately δ 17.96 ppm, upfield from the unlabeled methylsuccinic acid by 0.27 ppm. From the C3 methylene resonance at δ 39.06 ppm in the ^{13}C NMR spectrum, no incorporation of deuterium could be detected at this carbon atom, although this signal is shifted upfield by 0.10 ppm from that found in unlabeled methylsuccinic acid (δ 39.16 ppm) because of a β-isotope effect from deuterium at H2. In our deuteriation studies, using both gaseous and transfer hydrogenation, there was always evidence for two ^{13}C methylene signals, arising from either protium at H2 (methine) or deuterium at H2. The ^{13}C methylene signal with protium at H2, at lower field, was weak in comparison to that for the ^{13}C signal arising from deuterium at H2. This lack of deuteriation at H3 is a significant measure of no olefin isomerization to the corresponding (*Z*)-citraconic or (*E*)-mesaconic acid isomers (**8**), although rhodium(I) and -(III) species are well-known as olefin isomerization catalysts.[29−34]

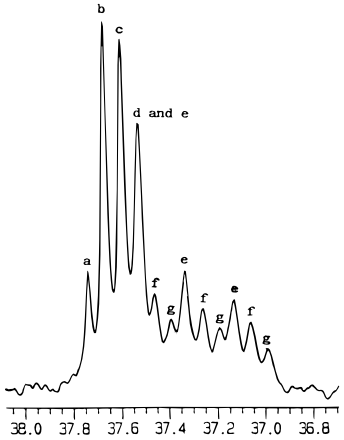


Figure 2. Part of the ^{13}C NMR spectrum (methine region) for deuteriated (*S*)-(−)-methylsuccinic acid after transfer hydrogenation: (a) protiomethylsuccinic acid (b) **7**, (c) **6**, (d) **9**, (e) **3** or **4**, (f) **4** or **5**, and (g) **5** or **10**.

From examination of the C2 methine carbon resonance (Figure 2), isotopomers **6**, **9**, and possibly **10** can also be distinguished. The unlabeled (perprotio) methylsuccinic acid methine resonates at δ 37.73 ppm [identified as (a) in Figure 2] (from a sample spiked with authentic methylsuccinic acid), and the three signals immediately upfield from this signal arise from successive β-isotope shifts (0.06−0.08 ppm) concomitant with compounds **7**, **6**, and **9** (37.66 (b), 37.58 (c), and 37.52 ppm (d), respectively). Signals (e) (centered at 37.32 ppm), (f) (centered at 37.25 ppm), and (g) (centered at 37.19 ppm), are all triplets by virtue of deuterium at C2. Each one is shifted upfield through a β-isotope effect in substituting more atoms of deuterium into the methyl group, as in compounds **3**, **4**, and

(27) Pretsch, E.; Clerc, T.; Seibl, J.; Simon, W. *Tables of Spectral Data for Structure Determination of Organic Compounds*; Springer-Verlag: Berlin Heidelberg, 1989; p C191.

(28) Wehrli, F. W.; Wirthlin, T. *Interpretation of ^{13}C NMR Spectra*; John Wiley and Sons: England, 1983; p 108.

(29) Rinehart, R. E.; Lasky, S. *J. Am. Chem. Soc.* **1964**, *86*, 2516−2518.

(30) Schrock, R. R.; Osborn, J. A. *J. Am. Chem. Soc.* **1976**, *98*, 2134−2143.

(31) (a) Grieco, P. A.; Nishizawa, M.; Marinovic, N.; Ehmann, W. J. *J. Am. Chem. Soc*. **1976**, *98*, 7102−7104. (b) Grieco, P. A.; Marinovic, N. *Tetrahedron Lett*. **1978**, 2545−2548. (c) Blagbrough, I. S.; Pattenden, G.; Raphael, R. A. *Tetrahedron Lett*. **1982**, *23*, 4843−4846.

(32) (a) Corey, E. J.; Suggs, J. W. *J. Org. Chem*. **1973**, *38*, 3224. (b) Corey, E. J.; Suggs, J. W. *Tetrahedron Lett*. **1975**, 3775−3778.

(33) (a) Biellmann, J. F.; Jung, M. J. *J. Am. Chem. Soc*. **1968**, *90*, 1673−1674. (b) Genet, J. P.; Ficini, J. *Tetrahedron Lett*. **1979**, 1499−1502.

(34) (a) Cramer, R. *J. Am. Chem. Soc*. **1966**, *88*, 2272−2282. (b) Cramer, R. *Acc. Chem. Res*. **1968**, *1*, 186−191.

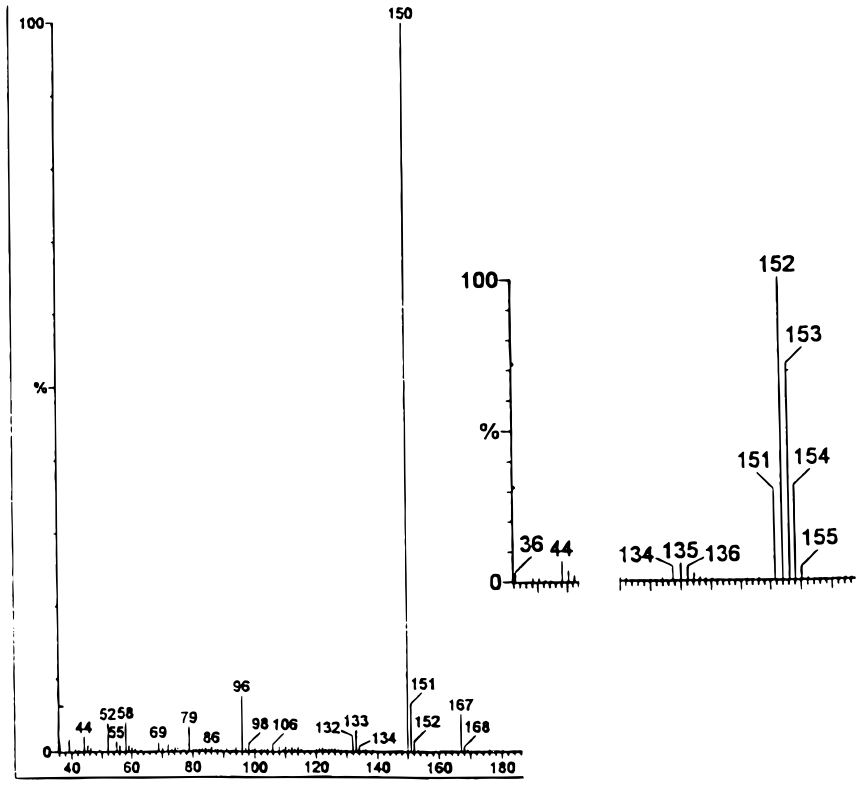


Figure 3. The mass spectrum (ammonia CI) of unlabeled methylsuccinic acid with (inset) deuteriated (*S*)-(−)-methylsuccinic acid.

5, overlapped with **4**, **5**, and **10**. The presence of tetradeuteriated **10** was confirmed by high resolution mass spectrometry. These results for the C2 methine carbon were observed after transfer hydrogenation of itaconic acid with formic acid-d_2 in the presence of $[Rh(COD)Cl]_2$ and BPPM, and comparable resonances were also obtained in the ^{13}C NMR spectrum following deuteriation of **1** with deuterium gas.

^{2}H NMR Studies. ^{2}H NMR spectroscopy also indicated that there was an 1.8:1 ratio for deuterium in the methyl group (δ 1.16 ppm, in MeOH) compared to the methine (δ 2.78 ppm), consistent with a higher enrichment of deuterium in the methyl group than could be expected from simple *cis*-addition of the deuterium across the carbon−carbon double bond of itaconic acid (**1**). The efficient incorporation of poly-deuterium in the methyl position means that addition−elimination followed by further addition of deuterium (D_2) must have occurred. From our studies with gaseous deuterium, there was an average of 2.4 deuterons per molecule of methylsuccinic acid after an aqueous acidic workup.

Mass Spectrometry Studies. Mass spectrometry of the deuteriated methylsuccinic acid (which readily indicates the ratio of each isotopomer, unlike ^{13}C NMR spectroscopy), indicated that the dideuteriated acid (*m*/*z* 152) was the most abundant (base peak), with trideuteriated (*m*/*z* 153) and then monodeuteriated acids (*m*/*z* 151) being next at 72% and 30% of the base peak, respectively (Figure 3, inset). The unlabeled methylsuccinic acid ($M_r = 132$) showed an *m*/*z* value (ammonia CI MS) of 150 (base peak) with an $M + 1$ isotope peak (6%) (Figure 3). The peak at *m*/*z* 154, in the deuteriated acid (>30% of the base peak), can be attributed to methylsuccinic acid with four atoms of deuterium, as the intensity of this peak cannot be accounted for by the isotope peaks of *m*/*z* 152 and *m*/*z* 153 alone, although it will incorporate the signals for these isotopes. In these deuteriation studies, there was only ever evidence for trace quantities of unlabeled methylsuccinic acid at *m*/*z* 150 (not shown). Analysis of the accurate mass data indicated that 1 to 4 atoms of deuterium per molecule of methylsuccinic acid were present and confirmed, for instance, that *m*/*z* 154 was attributable to the tetradeuteriated methylsuccinic acid (**10**) and not to other (e.g., ^{13}C) isotopomers. The calculated mass for tetradeuteriated acid (**10**) is 154.1017, and the mass found was 154.102. If one more protium atom was present in the molecule (replacing one deuteron) together with a ^{13}C isotope (for ^{12}C), a mass of 154.0985 would be required, using ^{13}C 13.00335, ^1H 1.0078, and ^2H 2.0141 mass isotopes, respectively. This is 3 millimass units lower than the found value. A mechanism consistent with these results is shown in Scheme 1.

Discussion

In a recent paper in this Journal, Leitner and co-workers have reported kinetic studies for the transfer hydrogenation of **1** using either DCO_2H or HCO_2D.[13] They observed a primary kinetic isotope effect when the formyl hydrogen atom of formic acid was replaced with deuterium, but no such effect was observed when the carboxyl hydrogen was exchanged for deuterium. In the same study, they also observed that transfer deuteriation of **1** led exclusively to 1,2-addition of two deuterium atoms, whilst a similar procedure with the dimethyl ester of **1** led to the 1,3-addition product only.[13] This change in mechanism, in hydrogen transfer, probably accounts for the significantly lower optical induction observed in the enantioselective hydrogenation of esters in comparison with their corresponding carboxylic acids. For this reason, we elected to reduce diacid **1**.

Several authors have proposed a Wilkinson-type mechanism for gaseous or transfer hydrogenation of itaconates, 2-acetamidoacrylic acid, and α-acylaminocinnamic acids.[13,14,26] This mechanism involves coordination of the alkene to the rhodium which is also chelated by the chiral phosphine. Rhodium hydride catalyst intermediates are formed from either the decomposition of formic acid or from gaseous hydrogen, and then transfer of hydrogen can take place between the metal center and the alkene to form a rhodium−alkyl bond. Transfer of another hydrogen atom from the metal then releases the

hydrogenated substrate so that the process can repeat in a catalytic cycle. From our deuteriation studies, we believe that an equilibrium exists between the olefin and the rhodium−alkyl intermediate, so that when hydrogen is replaced with deuterium, multiple incorporation of the deuterium isotope occurs, as in Scheme 1.

An intermediate can be formed which possesses bound itaconic acid and chiral phosphine groups, and this will undergo reversible addition of deuterium to give a species such as **11** (see Scheme 1). Transfer of deuterium to the coordinated double bond of the itaconic acid will give a rhodium alkyl species (**12**), and if we assume that the metal alkyl bond is formed between the rhodium and the α-carbon of the diacid, then the newly formed methyl group in **12** is free to rotate. If this transfer step of deuterium is considered to be reversible, then on re-forming the double bond of itaconic acid it is possible to remove a hydrogen atom from this freely rotating methyl group, forming a rhodium *hydride* species (**13**) and a deuteriated alkene.[35−37] Presumably, exchange occurs because the deuterium delivered to the terminal methylene group of the alkene is interchanged with one of the original prochiral hydrogens on the newly formed methyl group. This hydrogen atom is transferred to the rhodium metal. If there is a substituent (e.g., methyl or phenylmethyl) on the alkene, the ^{2}H and ^{1}H cannot interconvert without rotation to effect the necessary *E*-to-*Z* isomerization. Hence, our observation with itaconic acid (**1**) may be a restricted one. Ojima and co-workers found that *N*-acetyl dehydroalanine was reduced, in methanol at 20 atm of deuterium gas, to afford the 2,3-dideuterio addition product only, with no polydeuteriation or scrambling of isotopic label.[24] As the hydrogen and deuterium atoms on the metal center will interchange with one another, or with noncoordinated deuterium, there is the possibility of transferring another deuterium atom from **14** to the methyl group of the methylsuccinic acid. Further evidence for this mechanism accounting for the observed deuterium scrambling and polydeuteriation comes from the rhodium(I) catalyzed deuteriation of cycloalkenes, in which typically two or three deuterons (and even up to seven deuterons) were incorporated in cyclooctene when reduced at subatmospheric pressure.[36,37]

Protium found at the α-position (H2) in methylsuccinic acid is impossible to eliminate because there is always the chance that on final cleavage of the rhodium alkyl intermediate, some protium will be transferred to the α-carbon atom of the diacid. This is a significant result because, in kinetic studies on asymmetric hydrogenation, no equilibrium was invoked for this olefin/metal-alkyl intermediate. In the reduction of (*E*)-phenylitaconic acid with deuterium gas at 1 atm, and also using formic acid-d_2, both catalyzed by [Rh(NBD)(DPPB)]CF_3SO_3, [NBD is norbornadiene and DPPB is 1,4-bis(diphenylphosphino)butane], the 2*R*,1*S* isotopomer was produced exclusively, with no evidence for further deuterium incorporation.[13] This is perhaps due to restricted rotation of the phenylmethyl group once bound to the rhodium intermediate so that any equilibration that might occur results in transfer of the same hydrogen between the metal center and the substrate. Alternatively, the equilibration may be sensitive to nature of the surrounding ligands. From our studies, and also from those of previous workers,[1−7,14] there was no loss of asymmetric induction despite extensive equilibration between the olefin and the rhodium alkyl complex,[25] as in Scheme 1. This was observed with both labeled and unlabeled methylsuccinic acid giving optical purities in excess of 90% ee (by optical rotation) following recrystallization from toluene. Although the deuteriation of itaconic acid (**1**) has been discussed primarily from the results of experiments with deuterium gas, a similar multiple deuteriation occurred via transfer hydrogenation using formic acid-d_2 and Rh(COD)Cl-(BPPM) in DMSO-h_6, despite different kinetics between the two reactions. Brown, Kemball, and Sadler have described the deuteriation of 2-methylpropene, with deuterium gas, over various supported metal catalysts.[38] With rhodium, in particular, they found up to three atoms of deuterium in the isolated product, 2-methylpropane, but mainly with the isotope in only one of the three possible methyl groups. If a mechanism as in Scheme 1 is operative, then deuterium should be scrambled to all three methyl groups. These workers proposed that a metal vinyl intermediate is formed which can contain hydrogen or deuterium at the terminal alkenyl carbon atom. Further reduction of the alkylidene rhodium−carbon double bond, with deuterium or protium, will give a metal alkyl species which will finally eliminate the saturated product with enhanced deuterium exchange in only one methyl group. This mechanism possibly accounts for the absence of deuterium observed at H3 of methylsuccinic acid, analogous to the high enrichment of deuterium observed in only one of the three methyl groups of 2-methylpropane.

Beneficial effects of this type of labeling pattern occur when a receptor ligand or biosynthetic precursor, for example, needs to be isotopically enriched as much as possible. In our research, multiple isotopic labeling of (*S*)-(−)-methylsuccinic acid is important for bioorganic studies involving different norditerpenoid alkaloids, e.g. methyllycaconitine and nudicauline, which typically have binding constants at nicotinic acetylcholine receptors in the nM range.[39]

Summary

The analysis of the ^{13}C NMR and mass spectroscopic data presented here should also prove useful in other studies of deuterium labeling by asymmetric hydrogenation. One of the characteristics of this multiple labeling, prominent in the ^{1}H NMR spectra, is that protium is consistently found at the methine carbon atom, and this can give the misleading impression that a low enrichment of isotope has been incorporated into the substrate. We have shown that a high enrichment of deuterium in methylsuccinic is possible (on average 2.4 equiv are incorporated, but species with up to four deuterons are present) by virtue of multiple labeling of the methyl group, even though typically 13% of H2 methine is protium rather than deuterium. These deuterium isotope labeling experiments highlight the need to consider carefully the mechanism for gaseous and transfer hydrogenation, as this can directly influence deuterium (and presumably tritium) isotope enrichment.

Experimental Section

General Remarks. NMR spectra were recorded using Jeol GX 270 (operating at 270 MHz for ^{1}H) or Jeol EX 400 (operating at 400 MHz for ^{1}H or 100 MHz for ^{13}C) spectrometers. Chemical shift values are in parts per million on the δ scale and are referenced either to tetramethylsilane, δ 0.00 ppm in $CDCl_3$, or deuteriated methanol, δ 49.80, ppm in CD_3OD. High resolution mass spectra were recorded with a ZAB-E VG analytical mass spectrometer (reverse geometry)

(35) Atkinson, J. D.; Luke, M. O. *Can. J. Chem.* **1970**, *48*, 3580−3582.
(36) Güngör, M.; Jardine, F. H.; Wheatley, J. D. *J. Chem. Soc., Dalton Trans.* **1988**, 1653−1656.
(37) Güngör, M.; Jardine, F. H.; Wheatley, J. D. *Polyhedron* **1988**, *7*, 1827−1829.
(38) Brown, R.; Kemball, C.; Sadler, I. H. *Proc. R. Soc. London A* **1989**, *424*, 39−56.
(39) Coates, P. A.; Blagbrough, I. S.; Hardick, D. J.; Rowan, M. G.; Wonnacott, S.; Potter, B. V. L. *Tetrahedron Lett.* **1994**, *35*, 8701−8704.
(40) Eisenbraun, E. J.; McElvain, S. M. *J. Am. Chem. Soc.* **1955**, *77*, 3383−3384.

using chemical ionisation with ammonia gas at an optimum pressure (source voltage 8 kV). The masses of the ions of interest were measured using a standard peak-matching procedure employing perfluorokerosene (PFK) as a reference material. PFK was simultaneously admitted to the source with the sample. Low resolution mass spectra were recorded with either a VG Biotech Quattro II quadrupole mass spectrometer or a VG Analytical Autospec mass spectrometer using chemical ionisation with ammonia or isobutane gas respectively. Optical rotations were recorded in absolute ethanol with an Optical Activity AA10 polarimeter. Melting points were determined using a Kofler hot-stage apparatus (Cambridge Instruments) and are uncorrected.

Transfer hydrogenation of itaconic acid from the decomposition of formic acid was performed according to the procedure of Brunner and co-workers.[14] Gaseous hydrogenations were carried out at 1 atm, in an all glass apparatus, taking care to purge the system thoroughly with deuterium gas (99.8 atom % D) by successive evacuation/purge cycles (typically 3−4 times).

Reagents. Toluene was freshly distilled from sodium/benzophenone ketyl before use. DMSO-h_6 was distilled *in vacuo* with the heart-cut fraction being retained. This was then stored over 4 Å activated molecular sieves before use. CH_3OD and DMSO-d_6 were purchased from Aldrich and were not purified further. Formic acid-d_2 (99 + atom % D, 95% in D_2O) was purchased from Janssen and was not purified further. Deuterium gas (99.8 atom % D) was purchased from Aldrich in lecture bottles. (*S*)-α-Methylbenzylamine-d_2 was prepared by dissolving (*S*)-α-methylbenzylamine-h_2 (6.8 g) in dichloromethane (20 mL) followed by washing with D_2O (3 × 5 mL). The organic phase was dried ($MgSO_4$) and then concentrated at 20 mmHg pressure before distillation *in vacuo* (0.5 mmHg). Triethylamine was distilled from calcium hydride and stored under nitrogen. Itaconic acid-d_2 acid was prepared by dissolving, with warming, itaconic acid-h_2 (99+%, Aldrich) in D_2O followed by lyophilization. This procedure was repeated twice more. BPPM was purchased from Fluka, and $RhCl_3 \cdot nH_2O$ and $[Rh(COD)Cl]_2$ were obtained from Johnson Matthey Chemicals.

Preparation of (*S*)-(−)-Methylsuccinic Acid by Transfer Hydrogenation. Itaconic acid (3.2 g, 24.6 mmol) was added to a dry 100 mL, single-necked flask under nitrogen. BPPM (250 mg, 0.45 mmol) and $RhCl_3 \cdot nH_2O$ (100 mg, M_r = 263.3, 0.38 mmol) were added next followed by DMSO (25 mL). The reaction was stirred for 10 min before the addition of (*S*)-α-methylbenzylamine (6.25 g, 51.5 mmol). Formic acid (96%, Aldrich, 5.7 mL, 151 mmol) was then syringed slowly into the reaction with ice-bath cooling before heating the reaction to 30 °C. Gas evolution was observed at this temperature. Gradually, the color of the reaction changed from an orange/red to pale orange. After 135 h, aqueous sulfuric acid solution (2 M, 10 mL) was added to the reaction causing the precipitation of a yellow solid. This was filtered *in vacuo*, and the residue was washed with DMSO (10 mL). The clear yellow filtrate was then concentrated *in vacuo* to afford a yellow/orange oil. Aqueous HCl solution (2 M, 5 mL) was added to this oil which was then repeatedly extracted with ether. The organic phase was dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give (*S*)-(−)-methylsuccinic acid (2.81 g) as a pale yellow, waxy solid. Recrystallization from toluene gave pure (*S*)-(−)-methylsuccinic acid (2.33 g, 72%). (*S*)-(−)-Methylsuccinic acid displayed satisfactory analytical and spectroscopic data: mp 107−108.5 °C; lit. value,[40] 111−113°C; $[\alpha]_D^{20} = -14.7°$ (*c* 3.2, ethanol); lit. value[40] for the (*S*)-enantiomer $[\alpha]_D^{24} = -15.0°$ (*c* 1.89, ethanol), lit. value[40] for the (*R*)-enantiomer $[\alpha]_D^{25} = +15.5°$ (*c* 2.82, ethanol); ^{1}H NMR (D_2O, referenced to HOD at 4.75 ppm) δ 2.92−2.84 (1H, m, H2), 2.68 (1H, dd, H3, 17.5, 9.1 Hz), 2.57 (1H, dd, H3, 17.1, 5.5 Hz), 1.20 (3H, d, H5, 7.3 Hz); ^{1}H NMR ($CDCl_3$) δ 11.50−9.00 (2H, br.s, COOH), 2.99−2.87 (1H, m, H2), 2.72 (1H, dd, H3, 16.9, 8.8 Hz), 2.56 (1H, dd, H3, 16.9, 4.8 Hz), 1.28 (3H, d, H5, 7.3 Hz); ^{13}C NMR (CD_3OD, DEPT experiment) δ 180.03 (CO, α to C2), 176.43 (CO, α to C3), 39.16 (CH_2), 37.75 (CH), 18.23 (CH_3); mass spectrum (ammonia CI) *m*/*z* 150 $(M + NH_4)^+$, base, *m*/*z* 151, 6%, *m*/*z* 152 < 2% (Figure 3). M represents unlabeled acid, M_r = 132. Anal. Calcd for $C_5H_8O_4$: C, 45.4; H, 6.06. Found: C, 45.3; H, 6.15.

Preparation of Deuteriated (*S*)-(−)-Methylsuccinic Acid by Transfer Hydrogenation. Itaconic acid-d_2 (1.2 g, 9.1 mmol) was added to a dry 50 mL, single necked flask under nitrogen. BPPM (100 mg, 0.18 mmol) and $RhCl_3 \cdot nH_2O$ (40 mg, 0.15 mmol) were added next followed by DMSO-d_6 (10 mL, 99.5 + atom % D). The reaction was stirred for 10 min or until dissolution of the reactants was complete. (*S*)-α-Methylbenzylamine-d_2 (2.0 g, 16.2 mmol) was added with ice-bath cooling. A precipitate often formed at this stage, but this disappeared after the addition of formic acid-d_2. Formic acid-d_2 (2 mL, 53 mmol) was syringed slowly (typically 5 min) into the reaction, which after complete addition, was stirred at 30−35 °C. Gas evolution was observed after approximately 30 min. After 120 h, no further gas evolution was observed, and the DMSO was removed *in vacuo* to leave an orange viscous oil. This was dissolved in aqueous HCl solution (1 M, 110 mL) whereupon a precipitate appeared. This was filtered off under vacuum, and the aqueous filtrate was repeatedly extracted with ether (total volume 130 mL). The organic phase was back extracted with saturated brine (30 mL), dried ($MgSO_4$), and filtered. Concentration *in vacuo* gave deuteriated methylsuccinic acid (380 mg). Recrystallization from toluene gave pure deuteriated (*S*)-(−)-methylsuccinic acid (343 mg). Further concentration of the aqueous filtrate to ca. 20 mL (pH 1.0), and re-extraction with ether (total volume 50 mL) gave methylsuccinic acid (510 mg), (428 mg after recrystallisation from toluene, total yield, 64%). Replacement of the $RhCl_3 \cdot nH_2O$ catalyst for $[Rh(COD)Cl]_2$ (1.6 mol % of itaconic acid), in the presence of BPPM (2 mol % of itaconic acid), accelerated the reaction so that after 6 h only trace amounts of itaconic acid remained, monitored by SiO_2 TLC (solvent A, dichloromethane:methanol:glacial acetic acid, 9:0.5:0.5, visualized with a permanganate dip followed by heating). Deuteriated (*S*)-(−)-methylsuccinic acid from transfer hydrogenation (typically 32−37% protium incorporated at H2) displayed the following analytical and spectroscopic data: mp 112−113 °C; $[\alpha]_D^{20} = -14.6°$ (*c* 2.8, ethanol); ^{1}H NMR ($CDCl_3$) δ 12.50-10.00 (2H, br s, COOH), 3.00−2.86 (0.36H, m, H2), 2.71 (1H, d, H3, 17.2 Hz and dd, 17.2, 8.8 Hz), 2.56 (1H, d, H3, 17.2 Hz and dd, 17.2, 5.5 Hz), 1.30−1.27 (1.8H, br m, H5 methyl); ^{13}C NMR (CD_3OD) δ 180.06 (CO α to C2), 176.43 (CO α to C3), 39.11 (CH_2 with ^{1}Hβ at H2), 39.03 (CH_2 with ^{2}Hβ at H2); methine resonances, (Figure 2), 37.73 (protio), 37.66 (one ^{2}Hβ at H5), 37.58 (two ^{2}Hβ at H5), 37.52 (three ^{2}Hβ at H5), 37.52, 37.32, 37.13 (^{2}Hα and none or one ^{2}Hβ at H5), 37.45, 37.25, 37.05 (^{2}Hα and one or two ^{2}Hβ at H5), 37.39, 37.19, 36.99 (^{2}Hα and two or three ^{2}Hβ at H5); methyl resonances, 18.21 (protio), 18.12 (^{2}Hβ at H2), 18.04, 17.85, 17.66 (^{2}Hα and ^{2}Hβ at H2), 17.94, 17.75 (^{2}Hα and ^{1}Hβ at H2), 17.59, 17.38, 17.19 (two ^{2}Hα and ^{2}Hβ at H2), 17.48, 17.28 (two ^{2}Hα and ^{1}Hβ at H2); mass spectrum (isobutane CI) *m*/*z* 116 (67%), $(MH - H_2O)^+$, monodeuteriated; *m*/*z* 117 (100%), $(MH - H_2O)^+$, dideuteriated; 118 (66%), $(MH - H_2O)^+$, trideuteriated; 134 (8%), MH^+ monodeuteriated; 135 (15%), MH^+ dideuteriated; 136 (7.5%), MH^+ trideuteriated. Anal. Calcd for $C_5H_6O_4D_2$: C, 44.8; H, 5.97. Found: C, 44.7; H, 6.00.

Preparation of (*S*)-(−)-Methylsuccinic Acid by Gaseous Hydrogenation. $[Rh(COD)Cl]_2$ (10 mg, 2 × 10^{-5} mol) was placed in a dry, single necked, 50 mL; round bottomed flask before being evacuated and purged with nitrogen gas three times. Toluene (5 mL) and BPPM (20 mg, 3.6 × 10^{-5} mol) were added to the reaction flask, and the contents were then stirred at 25 °C for 15 min. Itaconic acid (650 mg, 5 mmol) was dissolved in methanol (5 mL, HPLC grade) and added to the reaction along with triethylamine (500 mg, 4.9 mmol). The reaction, which was an orange color at this point, was then hydrogenated at atmospheric pressure for 72 h. Workup of the clear orange/yellow filtrate, as descibed for transfer hydrogenation, gave (*S*)-(−)-methylsuccinic acid as an off-white solid (575 mg, 87%) which displayed satisfactory analytical ($[\alpha]_D^{20}$) and spectroscopic (NMR, ms) data.

Preparation of Deuteriated (*S*)-(−)-Methylsuccinic Acid Using Deuterium Gas. Toluene (5 mL) was added to a dry, single necked, 50 mL, round bottomed flask under nitrogen. $[Rh(COD)Cl]_2$ (10 mg, 2 × 10^{-5} mol) was added, in one portion, and the reaction was evacuated and purged with nitrogen three times using a Schlenk line. BPPM (20 mg, 3.6 × 10^{-5} mol) was added next, and the reaction was stirred for 15 min at 25 °C. The color of the reaction changed from yellow to an orange/brown. CH_3OD (5 mL), itaconic acid-d_2 (650 mg, 4.9 mmol), and triethylamine (500 mg, 4.9 mmol) were added followed by an evacuation/purge cycle. The flask was attached to an atmospheric hydrogenation apparatus and rigorously purged with deuterium gas. After 48 h hydrogenation, TLC (solvent A) showed only traces of itaconic acid. A further 7 mg of BPPM (1.3 × 10^{-5} mol) and 7 mg of

$[Rh(COD)Cl]_2$ (1.4×10^{-5} mol) were added, and after hydrogenation for a further 69 h, no itaconic acid remained by TLC. The reaction mixture had turned an olive green color with some deposition of metal. The reaction mixture was filtered and concentrated *in vacuo*, and the residual oil was dissolved in aqueous HCl solution (2 M, 10 mL). A precipitate appeared which was filtered off under vacuum. The filtrate was extracted with diethyl ether (5×10 mL), and the organic phase was dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give an off-white solid (560 mg). A sample (270 mg) was recrystallized from toluene to give pure, deuteriated (*S*)-(−)-methylsuccinic acid as white needles (242 mg, overall 77% yield): mp 115−115.5 °C; $[\alpha]_D^{20} = -14.9°$ (*c* 2.8, ethanol); ^{13}C and ^1H NMR spectroscopy indicated that no deuterium was present at H3 (methylene) of methylsuccinic acid and that 13% of all the isotopomers present contained protium at H2 (methine); ^1H NMR ($CDCl_3$) δ 9.50−7.20 (2H, br.s, COOH), 2.90−2.80 (0.13H, m, H2), 2.64 (1H, d, H3, 17.2 Hz and dd, 17.2 Hz, 8.8 Hz), 2.48 (1H, d, H3, 17.2 Hz and dd, 17.2 Hz, 5.5 Hz), 1.23−1.20 (1.5H, m, H5); ^{13}C NMR (CD_3OD) δ 180.08 (CO α to C2), 176.44 (CO α to C3), 39.14 (CH_2 with $^1H\beta$ at H2), 39.06 (CH_2 with $^2H\beta$ at H2); methine resonances, 37.76 (protio), 37.69 (one $^2H\beta$ at H5), 37.61 (two $^2H\beta$ at H5), 37.55 (three $^2H\beta$ at H5), 37.55, 37.35, 37.15 ($^2H\alpha$ and none or one $^2H\beta$ at H5), 37.47, 37.28, 37.08 ($^2H\alpha$ and one or two $^2H\beta$ at H5), 37.41, 37.21, 37.02 ($^2H\alpha$ and two or three $^2H\beta$ at H5); methyl resonances, (Figure 1), 18.23 (protio), 18.14 ($^2H\beta$ at H2), 18.07, 17.87, 17.67 ($^2H\beta$ at H2 and $^2H\alpha$ at H5), 17.99, 17.79, 17.60, 17.41, 17.21 (two $^2H\alpha$ and $^2H\beta$ at H2); ^2H NMR (CH_3OH, referenced to methyl deuterons of CD_3OD at δ 3.30 ppm) δ 2.78 (^{2}H2) and δ 1.16 (^{2}H5) (ratio of integrals 1:1.8 respectively); mass spectrum (ammonia CI) *m*/*z* 151 $(M^\dagger + NH_4)^+$, 30%, *m*/*z* 152 $(M + NH_4)^+$, base, *m*/*z* 153 $(M^* + NH_4)^+$, 72%, *m*/*z* 154 $(M^{**} + NH_4)^+$, 31%, (Figure 3 inset). Ions are recorded as a percentage of the base peak. $M^\dagger$ represents monodeuteriated acid, $M_r = 133$; M represents dideuteriated acid, $M_r = 134$; M* represents trideuteriated acid, $M_r = 135$ and M** represents tetradeuteriated acid, $M_r = 136$; HRMS found: 151.083 (calculated for monodeuteriated acid 151.0829), 152.090 (calculated for dideuteriated acid 152.0892), 153.096 (calculated for trideuteriated acid 153.0955), 154.102 (calculated for tetradeuteriated acid 154.1017). Anal. Calcd for $C_5H_6O_4D_2$: C, 44.8; H, 5.97. Found: C, 44.6; H, 6.00.

When the deuteriation was again carried out with deuterium gas, using five times more $[Rh(COD)Cl]_2$ (50 mg, 1.01×10^{-4} mol, 2 mol % of itaconic acid) and BPPM (112 mg, 2.02×10^{-4} mol, 4 mol % of itaconic acid), but otherwise under the same conditions as above, the reduction was complete in less than 24 h with no evidence of starting material remaining by TLC (solvent A). Workup as previously described gave deuteriated (*S*)-(−)-methylsuccinic acid, (552 mg, 84% yield), which displayed a ratio of 2:1 methyl:methine deuteriation in the ^2H NMR spectrum. The ^1H NMR spectrum indicated that there was 26% of protium at C2 compared to all methylsuccinic acid isotopomers and that there was an average incorporation of two deuterons per molecule of methylsuccinic acid.

Acknowledgment. We acknowledge the financial support of The Wellcome Trust (Project Grant 036214). We thank Dr. J. A. Ballantine, Dr. C. J. Reid, and Ms. B. K. Williamson of the EPSRC Mass Spectrometry Service Centre at Swansea for isotope enrichment analysis and Dr. S. Wonnacott (University of Bath) for useful discussions. B.V.L.P. is a Lister Institute Research Professor.

JA950597W