Polyene Acids. Part 11.[1] Preparation of α-Tritiated (or Deuteriated) Conjugated Enoic and Dienoic Acids and their Examination by Triton Magnetic Resonance Spectroscopy

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Decarboxylation in boiling pyridine of conjugated ene[*OO*′-^{3}H]dioic acids (from aryl or alkyl aldehydes and malonic acid, followed by exchange tritiation with labelled water) efficiently provided α-tritiated *trans*-αβ-unsaturated acids. Extended to croton- and cinnam-aldehydes, the method yielded conjugated dienoic acids labelled at both α- and γ-positions as shown by ^{3}H n.m.r. Labelling at only the α-position was achieved by thermal monodecarboxylation of the intermediate from cinnamaldehyde. Mechanisms are discussed. Similar reactions can be used for the preparation of deuteriated αβ-unsaturated acids.

αβ-UNSATURATED acids, including conjugated dienoic acids, bearing a tritium label on the α-carbon, were required for other studies. We therefore examined possible methods for specific incorporation of the isotope, trying deuterium labelling in initial experiments.

Atkinson and his collaborators [2] labelled the α-position of various acetic acids by heating the alkali metal salts in alkaline deuterium oxide. They extended this convenient method to the labelling of allylic positions in αβ-unsaturated acids and indicated that senecioic acid underwent exchange labelling also at the α-position. Far more strongly basic conditions than are feasible in water might have been considered necessary for this last exchange. Nevertheless, the observed slow loss of tritium from [α-^{3}H]cinnamic acid in sodium hydroxide at 160° [3] confirms that α-exchange in αβ-unsaturated acids can indeed occur in aqueous medium. We did not, however, have success with concentrated alkali in deuterium oxide for the exchange labelling of cinnamic acid in the α-position. We therefore resorted to a route [3] employing the Doebner condensation [4] of aryl aldehydes with malonic acid in boiling pyridine: in the presence of isotopically labelled water, the monodecarboxylation of the enedioic acid leads to an α-labelled product. We obtained improved incorporation by separating the stages and in particular by use of preformed malonic [*OO*′-$^2H_2$ or -^{3}H] acid. In this way, [α-^{2}H and -^{3}H]cinnamic and furyl[α-^{2}H]acrylic acid were obtained. Extension of the procedure to the aliphatic aldehydes, butyraldehyde and acetaldehyde, readily afforded [α-^{2}H]hex-2-enoic and [α-^{2}H]crotonic acid, respectively. Label remaining in the carboxy group was of course replaced by ^{1}H by exchange, before examination of the products. In each case, the ^{1}H n.m.r. spectrum confirmed the regiospecificity of the deuteriation through the marked reduction in intensity of the α-proton signal only. The spectra also confirmed, by the magnitude of $J_{\alpha\beta}$ in each case, that the acids had been isolated in the *trans*-form.

The αβ-unsaturated acid product may arise [5] through loss of carbon dioxide from the anion of the unsaturated dicarboxylic acid (in pyridine) and rapid transfer, to the resulting α-carbanion, of hydrogen ion from the medium. An alternative possibility [6] involves addition of pyridine to the β-position of the unsaturated dicarboxylic acid, accompanied by protonation (or deuteriation, *etc.*) at the α-position, followed by concerted loss of carbon dioxide and pyridine to yield the αβ-unsaturated acid.

When the unsaturated aliphatic aldehyde, crotonaldehyde, was condensed similarly with malonic [*OO*′-$^2H_2$]acid and the crotonylidenemalonic [*OO*′-$^2H_2$]acid heated in pyridine, sorbic acid resulted which bore a label in both the α- and the γ-position. Indeed, the γ-site carried more label (76%) than the α-site (67%). The two-site labelling also occurred when a mixture of crotonaldehyde, pyridine, malonic acid, and a trace of tritiated water was kept and later heated. In this case the labelling of the sorbic acid was demonstrated unambiguously by the two-line ^{3}H n.m.r. spectrum obtained with ^{1}H decoupling.[7] Nearly twice as much tritium was present in the γ-position as in the desired α-position.

Comparative data for the present analyses of labelled sorbic acid were obtained by measuring the ^{1}H n.m.r. spectrum of all-*trans*-sorbic acid in deuteriochloroform and in perdeuteriodimethyl sulphoxide (see Experimental section). This was necessary because published spectra were incompletely [8] or incorrectly [9] assigned, or were for the methyl ester in carbon tetrachloride.[1] In the last case, a transposition error had resulted in the γ- and δ-proton shifts being listed in reverse order.

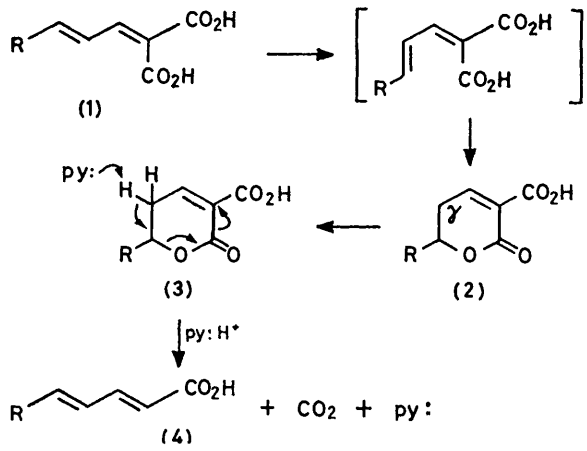
Extension of the labelling method to the phenyl analogue of sorbic acid, 5-phenylpenta-2,4-dienoic acid, made it necessary to obtain more ^{1}H n.m.r. data for this compound than was easily available.[10] The acid with

[1] Part 10, J. A. Elvidge and P. D. Ralph, *J. Chem. Soc.* (*B*), 1966, 243.
[2] J. G. Atkinson, J. J. Csakvary, G. T. Herbert, and R. S. Stuart, *J. Amer. Chem. Soc.*, 1968, **90**, 498.
[3] A. Latif, T. M. Saleh, and K. V. Sarkanen, *Holzforschung*, 1967, **21**, 46.
[4] O. Doebner, *Ber.*, 1902, **35**, 2129.
[5] E. J. Corey, *J. Amer. Chem. Soc.*, 1952, **74**, 5897; 1953, **75**, 1163; J. Klein and A. Y. Meyer, *J. Org. Chem.*, 1964, **29**, 1038.
[6] E. J. Corey and G. Fraenkel, *J. Amer. Chem. Soc.*, 1953, **75**, 1168.
[7] J. P. Bloxsidge, J. A. Elvidge, J. R. Jones, R. B. Mane, and E. A. Evans, *J. Chem. Research* (*S*), 1977, 258.
[8] N. S. Bhacca, D. P. Hollis, L. F. Johnson, and E. A. Pier, 'NMR Spectra Catolog,' Varian Associates, Palo Alto, 1963, vol. 2, No. 462.
[9] Sadtler Standard Spectra, No. 11295M.
[10] Sadtler Standard Spectra, No. 18848M.

m.p. 165° has long been considered to be the all-*trans* compound:[11] it undoubtedly has the *trans*-αβ configuration, since $J_{\alpha\beta}$ is 15 Hz. The more recent suggestion that this acid has a *cis*-γδ configuration appears to be based on an erroneous interpretation of the ^{1}H n.m.r. spectrum:[12] the multiplet near to δ 7 is not first order and arises not just from H_δ as indicated[12] but also from H_γ. Because the signal from H_β is hidden by the phenyl resonance, full analysis of the ^{1}H n.m.r. spectrum was not immediately practicable. Nevertheless, the form of the unobscured second-order pattern from the γ- and δ-protons is such that the approximate shifts can be deduced and assigned (Experimental section), though not the associated coupling constants.

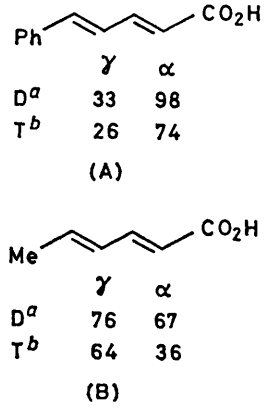
The unexpected two-site labelling of sorbic acid proved not to be unique in that labelling in the same two (α,γ) positions of 5-phenylpenta-2,4-dienoic acid occurred when fully labelled cinnamylidenemalonic [*OO*′-$^2H_2$]acid was decarboxylated in boiling pyridine, but the extent of the deuterium incorporation was now 98 and 33%, respectively. Similar *relative* labelling with tritium in the α- and γ-positions of 5-phenylpenta-2,4-dienoic acid resulted from decarboxylation of cinnamylidenemalonic acid in boiling pyridine in the presence of tritiated water, as shown by the ^{3}H n.m.r. spectrum of the product. Regiospecific α-labelling was, however, achieved by thermal decarboxylation of cinnamylidenemalonic [*OO*′-^{3}H]acid under nitrogen at 210° for 5 min, although the chemical yield was poor. The ^{3}H n.m.r. spectrum of the isolated 5-phenylpenta-2,4-dienoic acid, observed with ^{1}H decoupling, comprised only one singlet from the α-position.

It followed that thermal monodecarboxylation of cinnamylidenemalonic acid proceeds straightforwardly, albeit in low yield, with the expected transfer of the carboxy hydrogen to the α-position. In boiling pyridine, however, the monodecarboxylation in good yield of both cinnamylidene- and crotonylidene-malonic acid is presumably accompanied by lactonisation[13] and ring opening: otherwise it is difficult to account for the transfer of carboxy hydrogen (the source of the label) to both α- and γ-positions. Analogy with the condensation between citral and malonic acid in pyridine, which leads to a δ-lactone product,[14] also suggests that the heating of the 2,4-dienedioic acids (1) in pyridine effects lactonisation by addition of the *Z*-carboxy to the γδ-double bond in the s-*cis*-form of the molecules. Label would thus be introduced into the γ-position, but would be lost again through elimination ring opening, because both the addition, (1) ⟶ (2), and the ring opening, as (3), would be expected to proceed by *trans*-mechanisms. However, the γ-position of the lactones (2) is allylic so that under the reaction conditions exchange labelling there would be expected and the 2,4-dienoic acid (4) resulting from the ring opening and decarboxylation stages (3) would then necessarily be αγ-labelled. The observed relative extents of labelling of the sites, as shown in (A) and (B), have further implications. The high α-incorporation in the 5-phenylpenta-2,4-dienoic



acid (A) may mean a preponderance of straight monodecarboxylation of (1; R = Ph) in boiling pyridine. Alternatively the steps (3) may follow formation of the lactone sufficiently rapidly for there to have been only a little allylic exchange in lactone (2).

The isotopic incorporation observed for sorbic acid [see (B)] suggests that there is little or no straight monodecarboxylation of the dienedioic acid (1; R = Me) in boiling pyridine. Evidently the lactone (2; R = Me)



[a] Actual incorporation % per site. [b] Relative incorporation, % of total.

is formed and persists long enough for extensive exchange to occur at its γ-position. As a result of this allylic exchange, protons in addition to isotopic hydrogen ions would be available from the medium for combination with the incipient carbanion arising at the decarboxylation step at (3).

Consistent with these various suggestions is the fact that the dienoic acid products (4) have been isolated in their highest m.p. forms, long regarded as the all-*trans* geometrical isomerides, so that the processes depicted predominate. There are indications however that

[11] Beilstein, 'Handbuch der Organischen Chemie,' 4th edn., vol. E II 9, p. 440.
[12] O. S. Stepanova, A. I. Galatina, and Ng. V. Tong, *Vop. Stereokhim.*, 1971, **1**, 76.
[13] *Cf.* A. Riedel, *Annalen*, 1908, **361**, 96.
[14] C. E. Berkoff and L. Crombie, *Proc. Chem. Soc.*, 1959, 400.

parallel reactions occur to a minor extent. That the αβ-double bond in the products (4) is undoubtedly *trans*, as shown by the ^{1}H n.m.r. spectra, agrees with the concerted mechanism at (3) although sequential ring opening and decarboxylation cannot be excluded. That decarboxylation does not precede the elimination ring opening of the lactonic intermediate (2) appears certain, because otherwise the *cis*-αβ product would be formed.[1] Aspects of these findings warrant kinetic and other investigations, which we intend to pursue.

EXPERIMENTAL

Proton and triton n.m.r. spectra were obtained, the latter with proton-spin decoupling,[7] as previously described [15,16] by means of a Bruker WH 90 pulse spectrometer operating at 90 and 96 MHz (nominal), respectively, with deuteriated solvents, and tetramethylsilane as internal standard. Only relevant n.m.r. observations are reported. Tritiated samples were counted with a Beckman LS 100 liquid scintillation counter by using NE-250 liquid scintillator (Nuclear Enterprises Ltd.). Molecular ions (of deuteriated products) were measured with an A.E.I. MS 12 mass spectrometer.

[α-^{2}H]*Cinnamic Acid*.—(a) Benzaldehyde (2 g), malonic [*OO'*-$^2H_2$]acid (2 g; 78% incorporation; made by exchange in D_2O), and pyridine (2 ml) were heated together on the steam-bath for 4 h. Dilution with water (20 ml), acidification (HCl), and recrystallisation of the precipitate from aqueous ethanol gave [α-^{2}H]*trans*-cinnamic acid (2.6 g), m.p. 130—131 °C, M^+ 149, δ($CDCl_3$) 6.55 (d, *J* 16 Hz, α-^{1}H, 26%). Hence there is 74% incorporation of α-^{2}H.

(b) Benzylidenemalonic acid [17] (4 g) was kept with deuterium oxide (5 ml; 99.8% incorporation) for 12 h. The liquid was then removed completely under reduced pressure, and dry pyridine (15 ml) was added. After being heated on the steam-bath for 4 h, the solution was cooled, diluted with water (40 ml), and acidified (HCl). Crystallisation of the precipitate from aqueous ethanol gave [α-^{2}H]*trans*-cinnamic acid (2.5 g), m.p. 130—131 °C, M^+ 149, δ($CDCl_3$) 6.55 (d, *J* 16 Hz, α-^{1}H, 8%). Hence there is 92% incorporation of α-^{2}H.

[α-^{3}H]*Cinnamic Acid*.—To benzylidenemalonic acid (50 mg) dissolved in dry dioxan (150 μl), tritiated water (10 μl, 50 Ci ml^{-1}) was added. After 2 h, dry pyridine (150 μl) was added and the solution was heated at 80 °C for 30 min, then cooled, diluted with water (3 ml), and acidified (HCl). Recrystallisation of the precipitate from aqueous ethanol afforded [α-^{3}H]*trans*-cinnamic acid (30 mg, 162 mCi mmol^{-1}), m.p. 130—131 °C, δ($CDCl_3$) 6.55 (α-^{3}H).

2-*Furyl*[α-^{2}H]*acrylic Acid*.—Furfural (1 g), malonic [*OO'*-$^2H_2$]acid (1.3 g; 75% incorporation), and pyridine (1 ml) were heated together on a steam-bath for 10 h. Cooling, dilution of the solution with water (10 ml), acidification (HCl), and recrystallisation of the precipitate from aqueous ethanol, gave the *trans*-product (1.1 g), m.p. 140 °C, δ($CDCl_3$) 6.31 (d, *J* 16 Hz, α-^{1}H, 35%). Hence there is 65% incorporation of α-^{2}H.

[2-^{2}H]*Hex*-2-*enoic Acid*.—Butyraldehyde (1 g), malonic [*OO'*-$^2H_2$] acid (1.5 g; 75% incorporation), pyridine (0.5 ml), and piperidine (0.5 ml) were heated together on a steam-bath for 10 h. Dilution with water (10 ml), and extraction with chloroform gave the *trans*-product (500 mg), b.p. 118 °C at 20 mmHg, M^+ 115, δ($CDCl_3$) 5.82 (d, *J* 15.5 Hz, 2-^{1}H, 35%). Hence there is 65% incorporation of 2-^{2}H.

[α-^{2}H]*Crotonic Acid*.—Acetaldehyde (1 ml) was heated with malonic [*OO'*-$^2H_2$]acid (1.2 g; 75% incorporation) in pyridine (1 ml) for 10 h at 50 °C. Work-up as in the previous experiment and crystallisation from light petroleum (b.p. 40—60 °C) yielded the *trans*-product (800 mg), m.p. 68 °C, M^+ 87, δ($CHCl_3$) 5.85 (dq, *J*. 15.5, 1.75 Hz, α-^{1}H, 24%). Hence there is 76% incorporation of α-^{2}H.

[α,γ-^{2}H]*Sorbic Acid*.—Crotonaldehyde (1 g), malonic [OO'-$^2H_2$]acid (1.2 g; 78% incorporation), and pyridine (1 ml) were heated together on a steam-bath for 8 h. The solution was diluted with water and acidified (HCl). Recrystallisation of the precipitate from aqueous ethanol gave [α,γ-^{2}H]*trans,trans*-sorbic acid (800 mg), m.p. 133—134 °C, M^+ 114, δ($CDCl_3$) 5.77 (d, *J* 15.5 Hz, α-^{1}H, 33%) (hence there is 67% incorporation of α-^{2}H) and 6.25 (m, γ-^{1}H, 24%) (hence there is 76% incorporation of α-^{2}H).

trans,trans-Sorbic acid, m.p. 134°, had δ($CDCl_3$) 5.77 (d, *J* 15.5 Hz, α-H), 6.17 (q, δ-H), 6.25 (t, γ-H), and 7.33 (dq, β-H); δ[$(CD_3)_2SO$] 5.76 (d, α-H), 6.16 (q, δ-H), 6.28 (t, γ-H), and 7.16 (dq, β-H) where d, t (' 3-line '), and q (' 5-line ' multiplets) refer to apparent multiplicities at 20 Hz cm^{-1} display.

[α,γ-^{3}H]*Sorbic Acid*.—To crotonaldehyde (0.1 ml) and malonic acid (0.12 g) in pyridine (0.1 ml), tritiated water (10 μl, 50 Ci ml^{-1}) was added. After 2 h, the solution was heated on a steam-bath for 2 h, and then diluted with water (3 ml) and acidified (HCl). The solid was recrystallised from aqueous ethanol to give [α,γ-^{3}H]*trans,trans*-sorbic acid (75 mg; 68 mCi mmol^{-1}), m.p. 132—133 °C, δ[$(CD_3)_2SO$] 5.79 (α-^{3}H, 36% relative incorporation) and 6.29 (γ-^{3}H, 64% relative incorporation).

5-*Phenyl*[2,4-^{2}H]*penta*-2,4-*dienoic Acid*.—Cinnamylidenemalonic [*OO'*-$^2H_2$]acid [18] (400 mg; 99% incorporation) was heated in pyridine (5 ml) under reflux for 8 h. Water (10 ml) was added, the solution was acidified (3N-HCl), and the precipitate recrystallised from benzene to afford the dienoic acid (150 mg), m.p. 164—165 °C, δ($CDCl_3$) absence of 2-^{1}H doublet (hence, *ca.* 98% incorporation of 2-^{2}H) and 6.89 (m, 4-^{1}H, 67%) (hence 33% incorporation of 4-^{2}H).

5-Phenylpenta-2,4-dienoic acid, m.p. 165 °C, had δ($CDCl_3$) 6.00 (d, *J* 15 Hz, α-H), *ca.* 6.90 (m, γ-H), *ca.* 6.96 (d, δ-H), and 7.25—7.7 (m, Ph + β-H); δ[$(CD_3)_2SO$] 6.07 (d, *J* 15 Hz, α-H), *ca.* 7.13 (m, γ-H), *ca.* 7.17 (m, δ-H), and 7.25—7.7 (m, Ph + β-H).

5-*Phenyl*[2,4-^{3}H]*penta*-2,4-*dienoic Acid*.—Cinnamylidenemalonic acid (300 mg), tritiated water (10 μl; 50 Ci ml^{-1}), and pyridine (4 ml) were heated together under reflux and the product was isolated as previously. The dienoic acid (110 mg; 180 mCi mmol^{-1}), m.p. 164—165 °C, had δ[$(CD_3)_2$-SO] 6.08 (s, 2-^{3}H, 74% relative incorporation) and 7.13 (s, 4-^{3}H, 26% relative incorporation).

5-*Phenyl*[2-^{3}H]*penta*-2,4-*dienoic Acid*.—Tritiated water (10 μl; 50 Ci ml^{-1}) was added to cinnamylidenemalonic acid (300 mg) dissolved in dry dioxan (2 ml). After 30 min, the

[15] J. M. A. Al-Rawi, J. P. Bloxsidge, C. O'Brien, D. E. Caddy, J. A. Elvidge, J. R. Jones, and E. A. Evans, *J.C.S. Perkin II*, 1974, 1635.

[16] J. M. A. Al-Rawi, J. P. Bloxsidge, J. A. Elvidge, J. R. Jones, V. M. A. Chambers, and E. A. Evans, *J. Labelled Compounds and Radiopharmaceuticals*, 1976, **12**, 293.

[17] K. C. Pandya and R. B. Pandya, *Proc. Indian Acad. Sci.*, 1941, **14A**, 112.

[18] S. E. Boxer and R. P. Linstead, *J. Chem. Soc.*, 1931, 740.

solution was evaporated under reduced pressure and the solid heated under nitrogen at 210 °C for 5 min. The residue was taken up into ether. Extraction with saturated aqueous sodium hydrogencarbonate (40 ml), separation and acidification of the aqueous layer, re-extraction of the product into ether, evaporation, and crystallisation from benzene afforded 5-phenyl[2-^{3}H]pentadienoic acid (20 mg; 60 mCi mmol^{-1}), m.p. 163—164 °C, δ[$(CD_3)_2SO$] 6.07 (s, 2-^{3}H).

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