Stereochemistry of β-Carboxy- and β-Hydroxymethyl-muconic Derivatives

By **A. T. Ainsworth** and **G. W. Kirby,***† Department of Chemistry, Imperial College, London S.W.7

N.m.r. spectroscopy has been used to determine the stereochemistry of various β-carboxy- and β-hydroxymethyl-muconic derivatives. Long-range spin coupling between the olefinic protons is discussed. Isomerisation of the αβ- and γδ-double bonds has been shown to involve participation of the δ- and α-carboxy-groups, respectively. Treatment of both β-carboxy-*cis-cis*- and *cis-trans*-muconic acid with hot aqueous alkali gave, in contrast with an earlier report, *trans*-glutaconic acid rather than β-carboxy-*trans-trans*-muconic acid. A convenient preparation of δ-deuteriated β-carboxymuconic derivatives is described.

THE structures of the β-methylmuconic acids (I and II; X = Me, R = H) have been unambiguously determined by chemical and n.m.r. spectroscopic investigations.[1] The *cis-trans*-isomer ‡ (I; X = Me, R = H) was obtained from β-methylmuconic anhydride by hydrolysis; *cis* ⟶ *trans* isomerisation of the γδ-double bond occurred under even the mildest acidic conditions. Isomerisation of the αβ-double bond to give the *trans-trans*-acid (II; X = Me, R = H) required vigorous treatment with concentrated acid or alkali. The stereochemistry and interconversions of the β-carboxymuconic acids are less well understood. We now report the n.m.r. spectra and deduce the stereochemistry of various β-carboxy- and β-hydroxymethyl-muconic derivatives.

Oxidation of vanillin with sodium chlorite in sulphuric acid gives[2] a crystalline product, $C_8H_8O_6$. Sarkanen *et al.*[3] showed this to be a β-carboxymuconic derivative and, from its method of preparation, concluded that it was probably the *cis-cis*-ester (III; X = CO_2H, R^1 = Me, R^2 = H). However, the stability of this compound in acidic solution (cf. the method of preparation) and the contrasting instability of β-methyl-*cis-cis*-muconic acid (presumably a transient intermediate in the formation of β-methyl-*cis-trans*-muconic acid from β-methylmuconic anhydride) made further investigation desirable. The n.m.r. spectra of the monoester and derived di- and tri-esters were very similar to each other (see Table). The bands for the γ- and δ-protons were unambiguously assigned by examination of the spectra of the corresponding methyl β-carboxy[δ-^{2}H]muconate, prepared by chlorite oxidation of the deuterated vanillin

† *Present address:* Chemistry Department, University of Technology, Loughborough, Leicestershire.

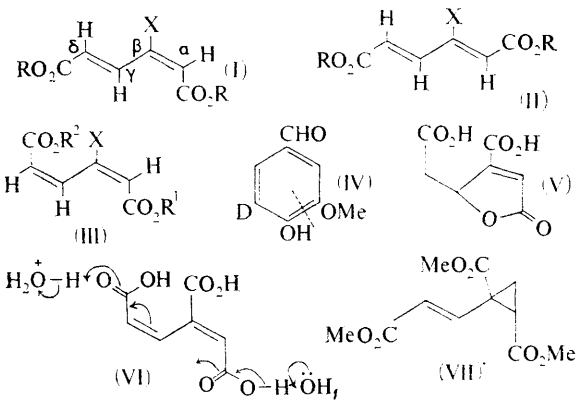
‡ In agreement with earlier usage (ref. 1), the stereochemistry of the αβ- and γδ-double bonds is designated in this order.

[1] J. A. Elvidge, R. P. Linstead, and P. Sims, *J. Chem. Soc.*, 1951, 3386 and 3398; J. A. Elvidge, *ibid.*, 1959, 474.

[2] R. M. Husband, C. D. Logan, and C. B. Purves, *Canad. J. Chem.*, 1955, **33**, 68.

[3] K. V. Sarkanen, K. Kakehi, R. A. Murphy, and H. White, *Tappi*, 1962, **45**, 24.

(IV).[4] The γδ-coupling constant, *ca.* 12 c./sec., for all three esters supports[5] a *cis*-configuration for the γδ-double bond. However, the γ-proton absorbed at higher field, τ 2·77—2·79, than the corresponding protons, τ 2·13, in dimethyl *cis-cis*-muconate (III; X = H,



$R^1 = R^2 = Me$).[5] Two explanations of this high-field absorption were considered. Interaction between the β- and δ-carboxy-functions in β-carboxy-*cis-cis*-muconic derivatives might cause twisting about the βγ-single bond, and thereby reduce the deshielding effect[6] of the

N.m.r. spectra of β-substituted muconic derivatives

	Compound				τ values			J (c./sec.)		
	X	R^1	R^2	Solvent	H_α	H_γ	H_δ	αγ	αδ	γδ
(III)	CO_2H	Me	H	D_2O	3·21	2·79	3·79	2·1	0·9	11·7
	CO_2H	Me	Me	$CDCl_3$	3·12	2·77	3·81	2	1	12
	or									
	CO_2Me	Me	H							
	CO_2Me	Me	Me	$CDCl_3$	3·30	2·79	3·92	2	1	12
	CO_2H	H	H	D_2O	3·20	2·78	3·79	2·1	0·9	11·7
	X	R								
(I)	CO_2Me	Me		$CDCl_3$	3·23	1·70	3·32			16·5
	CO_2H	H		D_2O	3·29	2·05	3·55	0·9	0·7	16·5
	CH_2OH	Me		$CDCl_3$	3·73	1·51	3·88			16
(VIII)	X = OH			Me_2CO	3·70	1·84	3·78			9·5
	X = H			$CDCl_3$	4·07	1·68	3·84	1·0	1·8	10
(X)				DMSO *	3·87	2·65	3·82			10
(X)	Methyl ester			$CDCl_3$	4·03	2·98	3·82			10
(IX)				DMSO *	3·59	3·15	3·83			12·7
(IX)	Methyl ester			$CDCl_3$	3·69	3·20	3·87			12·7

* Dimethyl sulphoxide.

α-methoxycarbonyl group on the γ-proton. Alternatively, the product from the chlorite oxidation of vanillin might have the *trans-cis*-configuration. Stanier and his colleagues have shown[7] that microbiological oxidation of 3,4-dihydroxybenzoic (protocatechuic) acid gives β-carboxy-*cis-cis*-muconic acid, isolated as the trisodium salt. The *cis-cis*-configuration assigned to this compound follows reasonably from its formation by cleavage of a six-membered ring and from the fact that it was prepared and isolated under essentially neutral conditions. The monoester from the oxidation of vanillin was therefore treated with aqueous sodium hydroxide (3 equiv.) at room temperature to give the corresponding trisodium salt, the n.m.r. and u.v.[8] spectra of which were identical with those of a sample of the biosynthetic trisodium salt provided by Professor R. Y. Stanier (University of California). Moreover, an enzymatic analysis, carried out in Professor Stanier's laboratory, confirmed the identity of the two materials.

The reluctance of the methyl β-carboxy-*cis-cis*-muconate (III; X = CO_2H, R^1 = Me, R^2 = H) to isomerise in acid was surprising. In contrast, the corresponding triacid, prepared by cautious acidification of the trisodium salt at 0°, isomerised rapidly to give β-carboxy-*cis-trans*-muconic acid (I; X = CO_2H, R = H).[3,8] The configuration of this acid followed from the n.m.r. spectrum; the chemical shift, τ 2·05, of the γ-proton showed the expected deshielding by the α-carboxy-group and the γδ-coupling constant, 16·5 c./sec., was typical of a *trans*-double bond. The ready isomerisation of β-carboxy-*cis-cis*-muconic acid and the contrasting stability of the α-monoester suggest that isomerisation of the γδ-double bond requires participation by a free α-carboxy-group. The lactone (V),[8] however, is not an intermediate, since it accumulates slowly in acidic solution after isomerisation is complete and since no deuterium is incorporated into the *cis-trans*-acid when the isomerisation is performed in deuterium oxide solution. The cyclisation depicted in (VI) and its reversal after rotation about the newly formed γδ-single bond accommodate these results. Loss of a proton from the α-carboxy-group, either during a concerted cyclisation or by a preliminary ionisation, is required, and protonation occurs on oxygen but not on carbon. Pure β-carboxy-*cis-trans*-muconic acid was best obtained by treatment of the lactone (V) with sodium methoxide in methanol. Methylation of the acid with diazomethane (3 equiv.) gave the corresponding triester. An excess of diazomethane produced the cyclopropane derivative (VII).

The results described so far do not rigorously define the configuration of the αβ-double bond in β-carboxy-*cis-cis*-muconic acid. The n.m.r. spectrum is abnormal (see also below) and the conversion into β-carboxy-*cis-trans*-muconic acid might conceivably involve isomerisation of both double bonds. Further supporting evidence was, however, readily obtained. Husband *et al.*[2] found that formation of the compound, $C_8H_8O_6$, during the oxidation of vanillin, involved an intermediate 'aldehyde', $C_8H_8O_5$. The n.m.r. spectrum of the 'aldehyde', in acetone, shows that it exists entirely in the lactol form (VIII; X = OH). Also, the low-field absorption, τ 1·84, of the γ-proton must result from deshielding by the α-methoxycarbonyl group attached to a *cis*-αβ-double bond. Reduction of the lactol with sodium borohydride gave the corresponding δ-lactone ester (VIII; X = H), obtained earlier by Sarkanen *et al.*[9] from the oxidation of vanillyl alcohol with sodium chlor-

[4] G. W. Kirby and L. Ogunkoya, *J. Chem. Soc.*, 1965, 6914.
[5] J. A. Elvidge and P. D. Ralph, *J. Chem. Soc.* (*C*), 1966, 387.
[6] L. M. Jackman and R. H. Wiley, *Proc. Chem. Soc.*, 1958, 196.
[7] L. N. Ornston and R. Y. Stanier, *J. Biol. Chem.*, 1966, **241**, 3776, and earlier papers.
[8] D. L. MacDonald, R. Y. Stanier, and J. L. Ingraham, *J. Biol. Chem.*, 1954, **210**, 809.
[9] C. W. Dence, M. K. Gupta, and K. V. Sarkanen, *Tappi*, 1962, **45**, 29.

ite. These workers showed that treatment of the lactone ester (VIII; X = H) with sodium hydroxide (1 mol.), followed by acidification, gave a lactone acid, $C_7H_6O_4$. However, treatment with sodium hydroxide (2 mol.) gave an isomeric lactone acid. No firm stereochemical assignments were made for these three compounds. This can now be done by consideration of the n.m.r. spectra (see Table). The first lactone acid is the γ-lactone (IX) and the second the δ-lactone (X). The structure (IX) follows from the coupling constant, $J_{\gamma\delta}$ 12·7 c./sec., associated with an open-chain double bond. In the spectrum of compound (X) $J_{\gamma\delta}$ is 10 c./sec., and the γ-proton absorbs at τ 2·65 [cf. τ 1·68 in lactone (VIII; X = H)]. The rapid interconversion (VIII; X = H) ⟶ (IX), in the presence of alkali (1 mol.) is surprising, and provides a second example of neighbouring carboxy-group participation. The lactone ester (VIII; X = H), in deuterium oxide, was treated with potassium deuteroxide (1 mol.) at room temperature, and the solution was examined immediately by n.m.r. spectroscopy. The lactone ring had already opened, since a band at τ 5·70, attributable to a hydroxymethylene group, was present. The band at τ 5·70 slowly disappeared and was replaced by a lactonic methylene absorption at τ 4·85. Growth of this new band was accompanied by the appearance of a methanol peak, τ 6·67. Thus the γ-lactone (IX) is formed directly in solution and not after acidification of the reaction mixture. The *cis*-γδ-double bond survives acidification since there is no suitably orientated α-carboxy-group to assist isomerisation (see above). Isomerisation of the αβ-double bond must involve the cyclic addition (XI) followed by rotation about the αβ-bond and ring-opening. The resulting salt (XII; R = Me) can then cyclise to give the γ-lactone (IX). A second molar proportion of alkali produces the double salt (XII; R = Na) which, when the reaction mixture is acidified, can cyclise in the alternative sense to yield the second δ-lactone (X). The proposed mechanism was tested as follows. Isomerisation of the αβ-double bond requires an α-methoxycarbonyl function to assist internal Michael addition by the δ-carboxylate anion [see (XI)]. In the presence of a large excess of alkali, bimolecular hydrolysis of the ester might compete with isomerisation. This was in fact observed. The lactone ester (VIII; X = H) was treated with an excess of 5N-sodium hydroxide. The reaction mixture was acidified, and the liberated acids were methylated with diazomethane. The major reaction product (I; X = CH_2OH, R = Me) had, as predicted, a *cis*-αβ-double bond. The relevant portions of the n.m.r. spectrum of this diester (see Table) closely resembled those reported [1] for dimethyl β-methyl-*cis-trans*-muconate (I; X = Me, R = Me). Isomerisation of the γδ-double bond during isolation of the product is consistent with the presence of a *cis*-oriented α-carboxy-group (see above). To summarise: the stereochemistry of the lactol (VIII; X = OH) and of the derived lactones follows unambiguously from their n.m.r. spectra. Since the lactol and the monoester (III; X = CO_2H, R^1 = Me, R^2 = H) are formed from vanillin under the same conditions, we conclude that the latter is (α)-methyl β-carboxy-*cis-cis*-muconate.

The 'abnormal' n.m.r. spectra of the β-carboxy-*cis-cis*-muconic derivatives require further comment. The long-range coupling constants for the olefinic protons of planar butadiene derivatives are well documented.[5,10] Typical values [5] (signs are omitted throughout) for *cis-trans*-derivatives [as (I)] are, $J_{\gamma\alpha}$ 0·9 and $J_{\alpha\delta}$ 0·7 c./sec., and for *cis-cis*-derivatives [as (III)], $J_{\alpha\gamma}$ 1·2 and $J_{\alpha\delta}$ 1·6 c./sec. β-Carboxy-*cis-trans*-muconic acid (I; X = CO_2H, R = H) showed normal long-range coupling (see Table). However the *cis-cis*-ester (III; X = CO_2H, R^1 = Me, R^2 = H) gave the abnormal values, $J_{\alpha\gamma}$ 2·1 and $J_{\alpha\delta}$ 0·9 c./sec. Reduction in the magnitude of $J_{\alpha\delta}$ can be attributed to non-planarity of the diene system (see above). This is supported by comparison of the u.v. absorption [8] of the *cis-cis*- ($\lambda_{max.}$ 255 mμ) and *cis-trans*- ($\lambda_{max.}$ 265 mμ) acids. However, $J_{\alpha\gamma}$ is greater than that observed for coplanar dienes, which suggests that coupling between the α- and γ-protons in the twisted conformation is more akin to allylic coupling in monoolefins. The lactone ring in the ester (VIII; X = H) imposes planarity upon the *cis-cis*-diene system; the observed coupling constants, $J_{\alpha\gamma}$ 1·0 and $J_{\gamma\delta}$ 1·8 c./sec., are unexceptional.

Attempts were made to prepare β-carboxy-*trans-trans*-muconic acid (II; X = CO_2H, R = H). The β-carboxy-*cis-cis*- and *cis-trans*-muconic acids were each heated with 20% aqueous sodium hydroxide (the procedure successfully used for the β-methylmuconic acids). *trans*-Glutaconic acid (XIII) was the major product from both reactions. Examination of the total reaction mixture by n.m.r. spectroscopy revealed none of the expected β-carboxy-*trans-trans*-muconic acid. Presumably, the presence of a β-carboxy-group induces addition of hydroxide to the αβ-double bond followed by a retro-aldol cleavage. Again, attempted isomerisation of the monoester (III; X = CO_2H, R^1 = Me, R^2 = H) with 48% hydrobromic acid led to laevulinic acid (XIV) rather than to β-carboxy-*trans-trans*-muconic acid.

[10] R. T. Hobgood and J. H. Goldstein, *J. Mol. Spectroscopy*, 1964, **12**, 76; A. A. Bothner-By and R. K. Harris, *J. Amer. Chem. Soc.*, 1965, **87**, 3451.

Morgan [11] has reported the preparation of β-carboxy-*cis-trans*-muconic acid by oxidation of protocatechuic acid with silver oxide, to give the corresponding *o*-quinone, followed by oxidation with peracetic acid. The m.p. of the derived trimethyl ester was given as 91—92°. Our trimethyl β-carboxy-*cis-trans*-muconate had m.p. 75—76°. More significantly, the isomerisation of β-carboxy-*cis-trans*- to β-carboxy-*trans-trans*-muconic acid by 20% aqueous sodium hydroxide was stated to proceed in 55% yield. We conclude that Morgan's acid, and the compounds derived from it, cannot have the structures he assigned to them. Unfortunately, we have been unable to repeat his preparation of the supposed β-carboxy-*cis-trans*-muconic acid, and samples of the original compounds are no longer available for direct comparison with ours.

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. Mass spectra were measured with an A.E.I. MS9 double-focusing spectrometer with an ionising potential of 70 ev. N.m.r. spectra were run with a Varian A-60 spectrometer on permanent loan to Professor D. H. R. Barton, F.R.S. from the Wellcome Trust; deuteriochloroform was used as solvent except where otherwise stated.

Oxidation of Vanillin and [5-^{2}H]*Vanillin*.—Oxidation [2] of vanillin with sodium chlorite at pH 0·5 gave (α)-methyl β-carboxy-*cis-cis*-muconate, m.p. 142—144° (lit.,[2] 143—144°). Similarly, oxidation of [5-^{2}H]vanillin (IV) [4] gave the corresponding δ-deuteriomuconic derivative. The n.m.r. spectrum (D_2O; t-butyl alcohol, τ 8·77, as internal standard) showed bands at τ 6·19 (s, OMe), and 2·75 and 3·19 (both doublets, J 2·1 c./sec., γ- and α-protons).

Dimethyl β-*Carboxy*-cis-cis-*muconate*.—Methylation [2] of the monoester (above) with methanol–sulphuric acid gave a dimethyl ester, m.p. 124—125° (lit.,[2] 125—126°). The stereochemistry followed from the n.m.r. spectrum (see Table) but the position of the new methyl ester group was not determined.

Trimethyl β-*Carboxy*-cis-cis-*muconate*.—The monoester (above) (0·5 g.) in methanol (10 ml.) was treated with methyl iodide (5 ml.) and silver oxide (2 g.) with shaking at room temperature for 24 hr. Filtration and evaporation of the filtrate gave the oily trimethyl ester (0·48 g.) (cf. ref. 2).

Trisodium β-*Carboxy*-cis-cis-*muconate*.—The corresponding monomethyl ester (1 g.) in water (42 ml.) containing sodium hydroxide (0·65 g.) was kept at room temperature for 15 min. The solution was then diluted with methanol (210 ml.) at 0°. Propan-2-ol (250 ml.) was added in portions (10 ml.) at 0° during 4 hr. with stirring, and the precipitated trisodium salt (1·2 g.) was collected. This salt had u.v. and n.m.r. spectra identical with those of biosynthetic material [7,8] provided by Professor R. Y. Stanier (University of California). An enzymatic analysis, carried out in Professor Stanier's laboratory, confirmed the identity of the two trisodium salts.

β-*Carboxy*-cis-cis-*muconic Acid*.—The trisodium salt (1 g.) in water (10 ml.) was treated at 0° with N-hydrochloric acid (11·8 ml.). The solution was rapidly extracted with ice-cold ether (7 × 50 ml.) and the extracts were dried (Na_2SO_4) and evaporated at 0—10°. The product was crystallised from ethyl acetate–light petroleum (b.p. 40—60°) at room temperature to give β-carboxy-*cis-cis*-muconic acid, m.p. 144—145° (lit.,[3] 144—145°).

β-*Carboxy*-cis-trans-*muconic Acid*.—(α)-Methyl β-carboxy-*cis-cis*-muconate (1 g.) was treated with 5% aqueous sodium hydroxide (20 ml.) at room temperature for 3 hr. The solution was acidified with N-hydrochloric acid (Congo Red) and extracted with ethyl acetate (5 × 50 ml.). The extract was dried (Na_2SO_4) and evaporated. Crystallisation of the residue from ethyl acetate–methylene dichloride gave β-carboxy-*cis-trans*-muconic acid (0·79 g.), m.p. 155—159° (varied with rate of heating). Satisfactory analyses on this material were not obtained (see also refs. 3 and 8) but the n.m.r. spectrum (see Table) agreed with that of the analytically pure material (see below).

Trimethyl β-*Carboxy*-cis-trans-*muconate*.—The *cis-trans*-acid (above) (100 mg.) in methanol (5 ml.) and ether (5 ml.) was treated with ethereal diazomethane (3 mol.; standardised by titration with benzoic acid). The solution was evaporated immediately at room temperature to give the required trimethyl ester (120 mg.) as needles, m.p. 65—70°. Sublimation (50°; $1·5 \times 10^{-4}$ mm.) gave *trimethyl* β-*carboxy*-cis-trans-*muconate*, m.p. 75—76° (Found: C, 52·3; H, 5·4. $C_{10}H_{12}O_6$ requires C, 52·6; H, 5·3%).

In one experiment a slight excess of diazomethane was used. The oily product was chromatographed on Merck silica gel GF_{254} plates with benzene as solvent. A component R_F *ca.* 0·5 was eluted and examined by n.m.r. and mass spectrometry: τ 2·89 (H_γ) and 3·96 (H_δ), $J_{\gamma\delta}$ 16 c./sec.; 6·22 and 6·32 (3 OMe), 7·34 (H_α, t, J 7·5 c./sec.) and 8·14 (CH_2 of cyclopropane, d, J 7·5 c./sec.); M^+ at *m/e* 242·078 ($C_{11}H_{14}O_6^+$ requires *m/e* 242·079) and fragment ion peaks at *m/e* 211, 183, and 156·044 ($C_7H_8O_4^+$ requires *m/e* 156·042). After distillation, the *cyclopropane ester* (VII) was obtained as an oil (Found: C, 54·5; H, 6·1. $C_{11}H_{14}O_6$ requires C, 54·5; H, 5·8%).

Preparation of β-*Carboxy*-cis-trans-*muconic Acid from the Lactone* (V).—Impure β-carboxy-*cis-trans*-muconic acid was heated in 90% formic acid to give the lactone (V),[8] m.p. 187—188° (lit.,[8] 187—189°). This lactone (100 mg.) in methanol (5 ml.) and ether (5 ml.) was treated with ethereal diazomethane (2 mol.). The solvent was evaporated off to leave the corresponding dimethyl ester as an oil (110 mg.), purified by chromatography on neutral alumina (grade III) with benzene as eluant, followed by distillation (50°; 2×10^{-5} mm.). The *lactone dimethyl ester* was obtained as a viscous oil (Found: C, 50·25; H, 4·6. $C_9H_{10}O_6$ requires C, 50·5; H, 4·7%). The structure was confirmed by the n.m.r. spectrum: τ 3·27 (H_α, d.), 4·43 (H_γ, octet), 6·10 and 6·28 (2OMe), 6·76 (H_δ, q), and 7·28 ($H_{\delta'}$, q); J values: αγ 2·1, γδ 3·6, γδ′ 7·6, and δδ′ 16·4 c./sec. The spectrum of the parent lactone (V) was similar but lacked the methoxy-signals.

The lactone (V) (400 mg.) in dry methanol (10 ml.) was treated dropwise with sodium methoxide (200 mg.) in dry methanol (5 ml.). After 1 hr. at room temperature, water (30 ml.) was added, and the solution was acidified (Congo Red) with 0·1N-hydrochloric acid. The product was extracted with ether (5 × 50 ml.). Evaporation of the extract and crystallisation of the residue from ethyl acetate–methylene dichloride gave β-carboxy-*cis-trans-muconic acid* (78 mg.), m.p. 160—161° (Found: C, 45·05; H, 3·3. $C_7H_6O_6$ requires C, 45·2; H, 3·25%).

The Lactol (VIII; X = OH).—Prepared [2] by oxidation of vanillin with sodium chlorite at pH 4·0, the lactol (VIII; X = OH), m.p. 103—105° (lit.,[2] 104—105°), gave n.m.r.

[11] L. R. Morgan, *J. Org. Chem.*, 1962, **27**, 1208.

signals (in acetone) at τ 6·21 (MeO), 3·70 [*HC*(OH)·O], and 2·8 (HO). The olefinic signals are listed in the Table. No aldehydic absorption was observed.

The δ-Lactone Ester (VIII; X = H).—The lactol (VIII; X = OH) (75 mg.) in water (2 ml.) and methanol (0·5 ml.) was treated with sodium borohydride (14 mg.) at room temperature. When all the borohydride had dissolved, the solution was acidified with 0·1N-hydrochloric acid and extracted with ether (4 × 20 ml.). The extract was dried (Na_2SO_4) and evaporated. Crystallisation of the residue from ether gave the δ-lactone ester (VIII; X = H) (38 mg.), m.p. 96—97° (lit.,[9] 98—99°), τ 6·24 (MeO), 5·00 (CH_2, d, *J* 1·8 c./sec.) (see also Table).

The γ-Lactone (IX) *and the Derived Ester*.—The δ-lactone ester (above) was treated with aqueous sodium hydroxide (1 mol.) to give the γ-lactone (IX), m.p. 177—179° (lit.,[9] 179°). Recrystallisation from water raised the m.p. to 182—186° but did not cause a change in the n.m.r. spectrum (in dimethyl sulphoxide): τ 4·85 (CH_2, d, *J* 1·9 c./sec.) (see also Table). The γ-lactone was esterified in methanol with ethereal diazomethane (1 mol.). The product was crystallised from methanol and sublimed to give the *methyl ester* of the lactone (IX), m.p. 118—119° (Found: C, 57·0; H, 4·7. $C_8H_8O_4$ requires C, 57·1; H, 4·8%), τ 6·21 (MeO) and 4·77 (CH_2, d, *J* 1·5 c./sec.).

The δ-Lactone Acid (X).—The δ-lactone ester (VIII; X = H) was treated with aqueous sodium hydroxide (2 mol.) to give the δ-lactone acid (X), m.p. 194—196° (from ethyl acetate) (lit.,[9] 189°), τ 4·41 (CH_2, d, *J* 2·6 c./sec.) in dimethyl sulphoxide (see also Table).

Dimethyl β-Hydroxymethyl-cis-trans-*muconate*.—The δ-lactone ester (VIII; X = H) (200 mg.) was treated with 5N-sodium hydroxide (5 ml.) for 3 hr. at room temperature. The solution was cooled, acidified with 5N-sulphuric acid, saturated with sodium chloride, and extracted with ether (5 × 25 ml.) and ethyl acetate (5 × 25 ml.). The extracts were dried (Na_2SO_4) and evaporated. The residue, in methanol, was treated with a slight excess of ethereal diazomethane. Evaporation and chromatography on silica gel gave *dimethyl β-hydroxymethyl*-cis-trans-*muconate* (102 mg.), m.p. 103—104° (Found: C, 54·2; H, 5·8. $C_9H_{12}O_6$ requires C, 54·0; H, 6·0%).

Degradation of β-Carboxymuconates with Alkali.—(α)-Methyl β-carboxy-*cis-cis*-muconate (0·5 g.) was heated under reflux in 20% aqueous sodium hydroxide (37 ml.) for 4 hr. The solution was acidified with N-hydrochloric acid and extracted continuously with ether. Evaporation of the extract and crystallisation of the residue from ethyl acetate–methylene dichloride gave *trans*-glutaconic acid (150 mg.), m.p. 135—136°, structure established from its n.m.r. spectrum (in D_2O): τ 2·94 (H_β, doublet of triplets), 3·98 (H_α, doublet of triplets), and 6·62 (γ-CH_2, q); *J* values: αβ 15·7, αγ 1·4, and βγ 7·0 c./sec. Similar results were obtained when β-carboxy-*cis-trans*-muconic acid was treated with alkali.

Degradation of (α)-*Methyl* β-*Carboxy*-cis-cis-*muconate with Hydrobromic Acid*.—The methyl ester (1 g.) was heated at 100° in 48% hydrobromic acid (10 ml.) for 3 hr. The pH of the solution was adjusted to 5 with N-sodium hydroxide and the solution was extracted continuously with ether. Evaporation of the extract gave material (0·52 g.) identified as laevulic acid by the n.m.r. spectrum and the formation of a 2,4-dinitrophenylhydrazone, m.p. and mixed m.p. 206—207° (decomp.).

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