Carbon-13 Labelling Study of the Coenzyme B_{12}-dependent Methylitaconate ⇌ α-Methyleneglutarate Model Rearrangement Reaction and Examination of Potential Cyclopropane Intermediates

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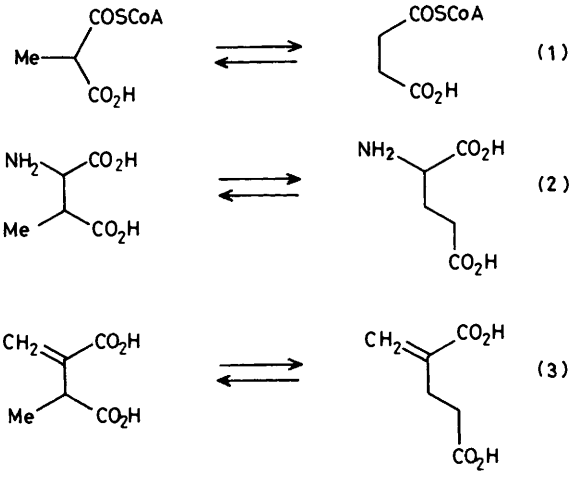
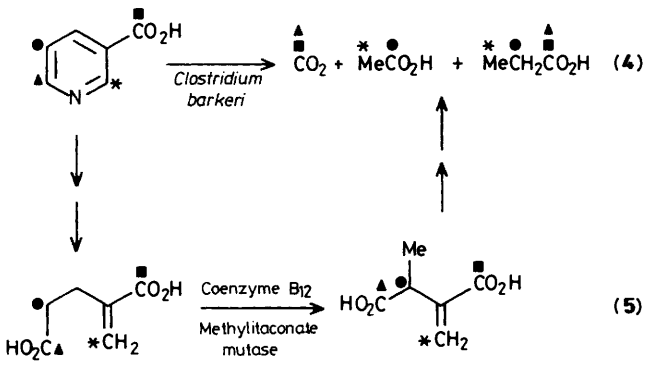
The model rearrangement mimicking the coenzyme B_{12}-dependent, enzyme-catalysed interconversion of α-methyleneglutaric acid with methylitaconic acid has been carried out with a carbon-13 label. This experiment demonstrates beyond doubt that the acrylate group is the migrating group in the model, as it is in the enzyme-catalysed rearrangement. Experiments designed to probe the possible occurrence of cyclopropylmethyl intermediates in the model rearrangement are also described. To this end the *cis*- and *trans*-bromomethylcyclopropanediacids (**16a**) and (**20a**) were prepared starting from the common precursor cyclopropane-1,1,2-tricarboxylic acid (**13**). Thus, (**13**) was converted into the anhydride (**14**) which was, in turn, reduced to the lactone (**15**). Opening of the lactone with HBr in acetic acid gave the desired *trans*-diacid (**16a**). For the *cis*-diacid (**20a**), the anhydride (**14**) was hydrolysed, esterified with diazomethane, then reduced with lithium triethylborohydride. Conversion of the resulting alcohol (**19**) into the bromide (**20a**) was effected with phosphorus tribromide. An extensive series of experiments involving treatment of the acids and their methyl and tetrahydropyranyl esters with vitamin B_{12s} was carried out. No methylitaconic acid (**3a**) could be detected in any of the reaction mixtures. However, α-methyleneglutaric acid (**2a**) and methylglutaconic acid (**21**) were observed as the reaction products. The methyl toluene-*p*-sulphonate (**22**) and iodide (**23**) were also examined and yielded results analogous to those obtained with the bromides.

Vitamin B_{12}, in the form of its coenzyme, is an obligatory cofactor in eleven enzyme-catalysed rearrangement reactions.[1] Of these, three are carbon-skeleton rearrangement reactions: methylmalonyl-CoA ⇌ succinyl-CoA [equation (1)], β-methylaspartic acid ⇌ glutamic acid [equation (2)] and methylitaconic acid ⇌ α-methyleneglutaric acid [equation (3)].[1]



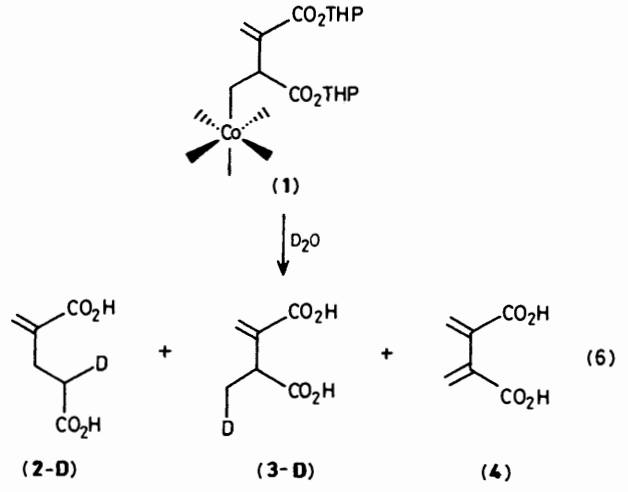
The methylitaconate ⇌ α-methyleneglutarate rearrangement [equation (3)] is one of a number of steps in the energy-yielding catabolism of nicotinic acid by the bacterium *C. barkeri*. During an investigation of the degradation pathway using [5-^{14}C]nicotinic acid,[2] it was observed that the products, propionate, acetate, and carbon dioxide, were labelled as indicated in Scheme 1 below [equation (4)]. The labelling pattern indicates that the acrylate group is the migrating group in the coenzyme B_{12}-dependent step [equation (5)] of the



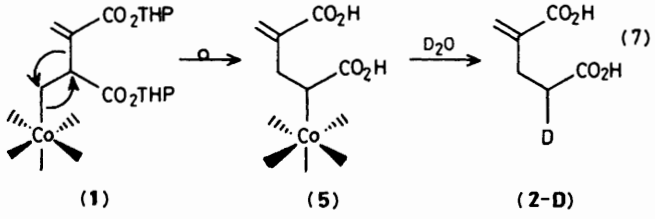
Scheme 1.

degradation sequence. Involvement of the carboxy group as the migrating function would produce a markedly different labelling pattern in the products.

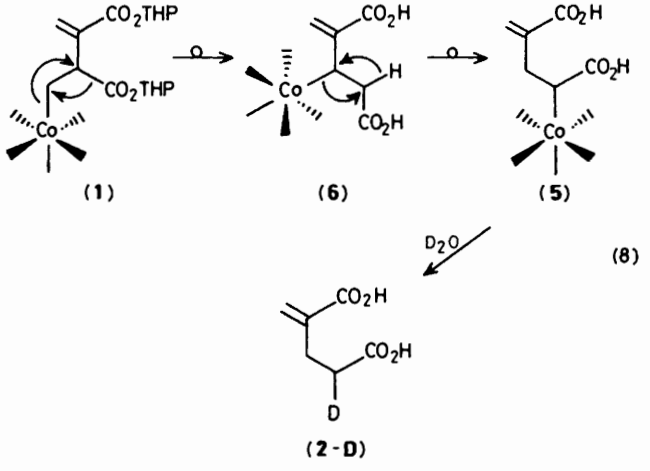
We discovered[3] a model reaction which mimics the rearrangement reaction of equation (3). In the model, bis-(tetrahydropyranyl)methylitaconate [bis(tetrahydropyranyl)-but-1-ene-2,3-dicarboxylate] was attached through the 4-position to the cobalt atom of vitamin B_{12} yielding the model intermediate (**1**). Carbon-skeleton rearrangement [equation (6)] occurred spontaneously at ambient temperature, in aqueous solution yielding α-methyleneglutaric acid (**2**) (but-1-ene-2,4-dicarboxylic acid), methylitaconic acid (**3**) (but-1-ene-2,3-dicarboxylic acid), and buta-1,3-diene-2,3-dicarboxylic acid (**4**).[4] When the model reaction was carried out in D_2O [equation (6)], the products α-methyleneglutaric acid (**2-D**) and methylitaconic acid (**3-D**) were labelled on the γ-carbon [equation (6)].[4] If it is assumed that the position of deuterium indicates the place once occupied by cobalt [as indicated in formula (**5**)] and that there have been no hidden rearrangements of hydrogen and cobalt, this result can be interpreted as



indicating that the acrylate is the migrating group. This straightforward scheme is shown in equation (7). If the carboxyl

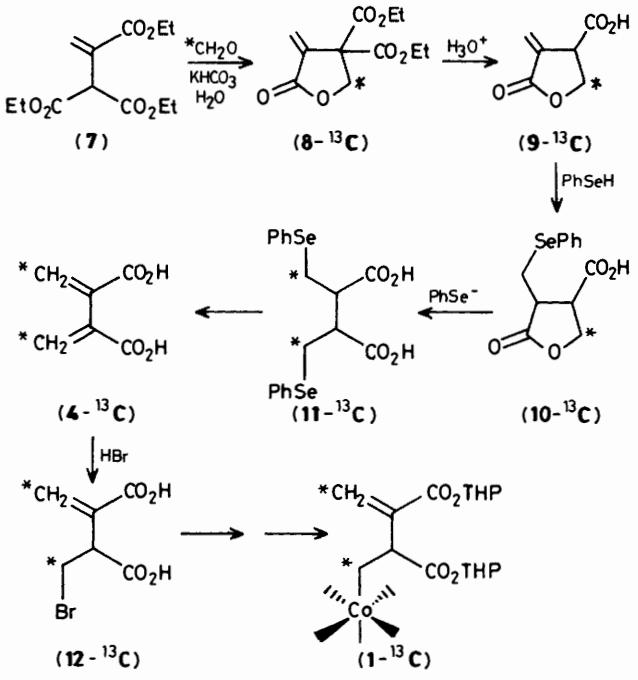


group were the migrating group, and if that migration were followed by a double shift of cobalt and hydrogen [formula (**6**)], then the same labelling result would obtain, as shown below in equation (8). No β-deuteriated α-methyleneglutarate



was detected among the products (*ca.* 2% would easily have been observed) from the reaction in D_2O. However, the putative double migration [shown in formula (**6**)] could be a high-yield reaction. If the model reaction [equation (6)] is to be a faithful representation of the enzymic rearrangement, it is important to establish beyond question that the acrylate is the exclusive migrating group, as it is in the enzymic reaction.

Carbon-13 *Labelling Experiments.*—Resolution of the ambiguity presented by equation (8) can be achieved by means of a carbon label. Accordingly, we prepared the carbon-13 labelled model (**1**-^{13}**C**) by the path outlined in Scheme 2.



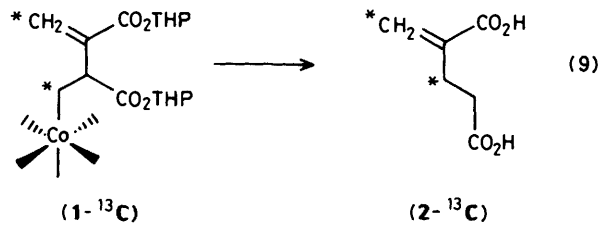
Scheme 2.

Thus, treatment of the triester (**7**) with 90% ^{13}C-enriched formaldehyde yielded the labelled diester lactone (**8**-^{13}**C**). The latter was hydrolysed and decarboxylated to the methylene lactone (**9**-^{13}**C**). After extensive investigation, we found successive phenylselenylation and oxidative elimination proceeding by way of (**10**-^{13}**C**) and (**11**-^{13}**C**) produced buta-1,3-diene-2,3-dicarboxylic acid (**4**-^{13}**C**), enriched with carbon-13 at the vinyl methylene carbons. Treatment of (**4**-^{13}**C**) with hydrogen bromide in acetic acid gave the bromide (**12**-^{13}**C**). Esterification of (**12**-^{13}**C**) with dihydropyran was followed by alkylation of the cobalt atom of vitamin B_{12} yielding the model (**1**-^{13}**C**).

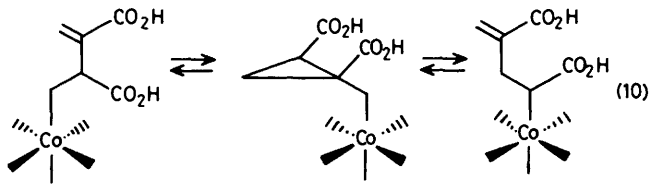
The n.m.r. spectrum of the labelled model (**1**-^{13}**C**) showed a triplet (*J* 159 Hz) for the enriched vinyl carbon at δ 121.5 and two closely spaced triplets (*J* 142 Hz) at δ 27.4 and 27.0 p.p.m. corresponding to the cobalt-bonded methylene carbon. The presence of two triplets is due to the diastereoisomerism caused by the asymmetric centre of the methylitaconate group. The asymmetric tetrahydropyranyl groups are apparently too remote to cause additional resolvable splitting of the ^{13}C n.m.r. spectrum. The chemical shift of the cobalt-bonded methylene group is in good agreement with that (δ 24.3 p.p.m.) observed for the 5′-methylene group in coenzyme B_{12}.[5]

The model (**1**-^{13}**C**) was allowed to stand at room temperature, in the dark, in aqueous solution, for thirty days while slow decomposition of the carbon-cobalt bond took place. After isolation and purification, the α-methyleneglutarate product (**2**-^{13}**C**) was examined using ^{13}C n.m.r. spectroscopy. The spectrum showed two lines at δ 129 and 28 p.p.m. which coincided with those of the vinyl carbon and β-carbon of authentic α-methyleneglutaric acid. No trace (2% would easily have been observed) of enriched carbon-13 was observed at δ 33 p.p.m. corresponding to the γ-carbon. We conclude that the acrylate group is the exclusive migrating group in the model rearrangement [equation (9)] as it is in the enzyme-catalysed reaction.

Studies of Potential Cyclopropane Intermediates.—A cyclopropylmethylcobalt complex was suggested[6a,7] as a logical



intermediate for the rearrangement of equation (6); one such possibility is shown in equation (10).



We wished to learn whether a start from the cyclopropyl intermediate would yield the same products observed in the model rearrangement [equation (6)]. Although there now has been a substantial amount of work exploring cyclopropane models in the vitamin B_{12} model series,[7] we intended to examine the cyclopropylmethyl model directly related to the substrates with both carboxyl groups intact. This had not been done previously.

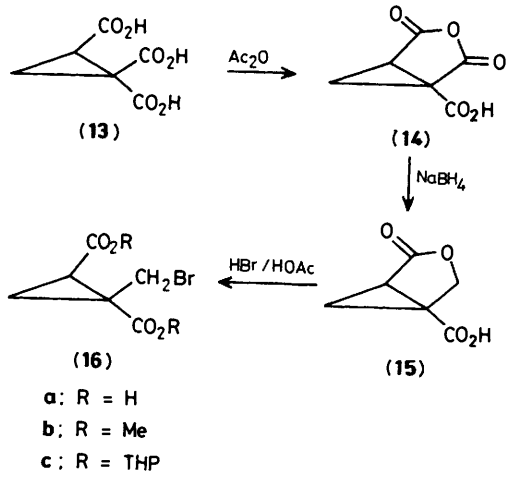
Neither *cis*- nor *trans*-1-methylcyclopropane-1,2-dicarboxylic acid is a substrate for the enzyme α-methyleneglutarate mutase.[2g] However, it is easy to envisage pathways involving cyclopropylmethyl intermediates that do not pass through the 1-methylcyclopropane-1,2-dicarboxylic acids at any intermediate stage.

In considering such a model, it is important to be aware that, if the carbon-cobalt bond were to break yielding a radical or carbanion, ring-opening would lead to the resonance-stabilized α-methyleneglutarate intermediate with radical or carbanion adjacent to the carboxyl group as opposed to the methylitaconate reactive intermediate with the reactive centre on a primary carbon. Accordingly, the rationale for undertaking this series of experiments was the following. If equilibration among the carbon-cobalt-bonded species in equation (10) is rapid, then the methylitaconate isomer will be strongly populated. It is undoubtedly the energy minimum among the three structures because the primary (methylitaconate) carbon-cobalt bond is more stable than the neopentyl (cyclopropylmethyl) or the secondary (methyleneglutarate) carbon-cobalt bonds. Equilibration among the three isomers of equation (10) must occur by a process of dissociation, rearrangement, and recombination. If rearrangement to the methylitaconate form is not sluggish, then methylitaconate should appear among the products of rearrangement.

Consideration of the three coenzyme B_{12}-dependent carbon-skeleton rearrangements raises another question. The cyclopropylmethyl intermediate hypothesis is applicable to the α-methyleneglutarate $\rightleftharpoons$ methylitaconate[2] and succinyl-CoA $\rightleftharpoons$ methylmalonyl-CoA rearrangements,[8] because in both instances the migrating group, thioester or acrylate, is unsaturated. The anomalous rearrangement in this context is the glutamate $\rightleftharpoons$ β-methylasparate[9] transformation in which there is no opening for formation of a cyclopropylmethyl intermediate involving the migrating glycine, because the migrating centre is saturated. Attempts[10] to find evidence for the introduction of an unsaturated intermediate through the involvement of pyridoxal phosphate or α-ketoglutarate in this rearrangement have yielded negative results.

Because, in the latter rearrangement, the case is still open to the infusion of new evidence and because the reservation expressed above assumes that the mechanism of the glutamate rearrangement must be common to the other two rearrangements in this series, it seemed worthwhile to explore the cyclopropylmethyl reaction in the model series where it was possible to do so. Accordingly, we prepared the *cis*- and *trans*-1-bromomethylcyclopropanecarboxylic acids (**16a**) and (**20a**) and explored their reactivities and that of their esters with vitamin B_{12s}.

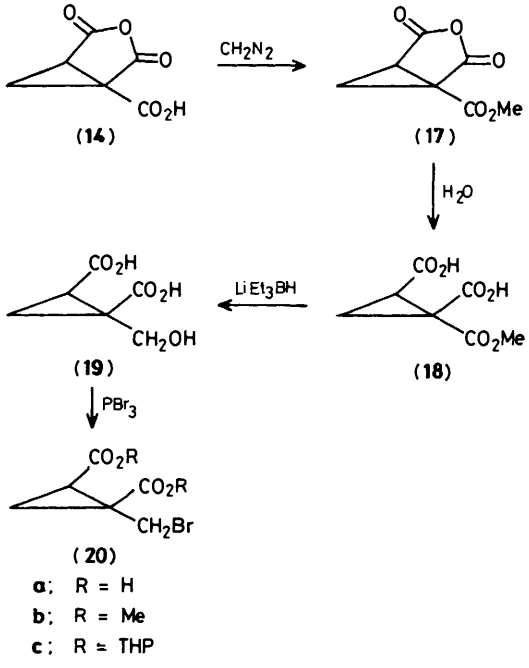
Synthesis of both the *trans*-diacid (**16a**) and the *cis*-diacid (**20a**) begins with cyclopropane-1,1,2-tricarboxylic acid (**13**)[11] and takes advantage of the selectivity associated with modern reducing agents. Thus, in the formation of the *trans*-diacid (**16a**) (Scheme 3) the anhydride (**14**) was reduced with sodium



Scheme 3.

borohydride exclusively to the lactone (**15**).[12] The latter was opened to *trans*-1-bromomethylcyclopropane-1,2-dicarboxylic acid (**16a**) with 32% hydrogen bromide in acetic acid.

In the preparation of the *cis*-diacid (**20a**) (Scheme 4) the

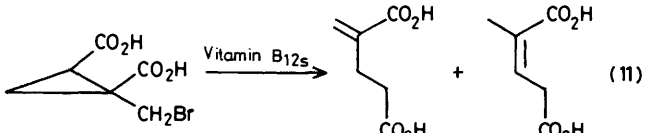


Scheme 4.

anhydride (**14**) was esterified with diazomethane. The ester anhydride (**17**) was opened to the diacid ester (**18**) upon treatment with water. Reduction of the ester group in (**18**) was effected selectively with lithium triethylborohydride[13] (superhydride) yielding the alcohol (**19**), which was converted into *cis*-1-bromomethylcyclopropane-1,2-dicarboxylic acid (**20a**) by treatment with PBr_3.

The *cis*- and *trans*-dimethyl and bis(tetrahydropyranyl) esters (**16b**), (**16c**), (**20b**), and (**20c**) were also prepared by straightforward procedures described in the Experimental section.

Using these compounds, we began to explore the reactions with vitamin B_{12s}. At the outset we found that treatment of the *cis*-bromo diacid (**20a**) with vitamin B_{12s} did not result in stable carbon-cobalt bond formation under a variety of reaction conditions. When the reaction was examined for products, no trace of methylitaconic acid (**3**) or buta-1,3-diene-2,3-dicarboxylic acid (**4a**) was observed. An analogous result was obtained with the *cis*- and *trans*-methyl and tetrahydropyranyl esters (**16b**), (**16c**), (**20b**), and (**20c**) where the only product formed was α-methyleneglutarate (**2**). The products from reaction of the *cis*-diacid (**20a**) [equation (11)] included not only α-methyl-

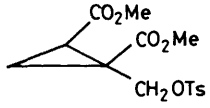


(20a) (2a) (21) (11)

eneglutaric acid (**2a**) but also 2-methylglutaconic acid (**21**). Control reactions in which α-methyleneglutaric acid (**2a**) was subjected to the reaction conditions yielded no evidence of the formation of 2-methylglutaconic acid (**21**).

There are two possibilities for the ring-opening reaction. If the carbon-cobalt bond is formed, it may be unstable, perhaps because the cobalt is attached to a neopentyl carbon, and lead directly to the ring-opening reaction. If so, then the cyclopropylmethyl cobalamin [*e.g.* (**10**)] cannot be the sole intermediate in the model rearrangement, since the latter reaction also yields methylitaconic acid (**3**) and butadienedicarboxylic acid (**4a**) as products. In this model rearrangement, equilibria are biased by the carboxyl group so that ring-opening occurs only toward the stabilized α-methyleneglutarate intermediate. This is consistent with the recent finding of Golding[7c] in which ethoxycarbonyl-substituted but-3-enyl and cyclopropylmethyl cobaloximes yielded a rearrangement product with ring-opening proceeding in one direction—toward the ester-bearing carbon. This is to be contrasted with the result obtained with the 1- and 2-methylbut-3-enyl(pyridine)cobaloximes which equilibrate rapidly and in which the 2-methyl isomer is favoured.[14]

Since the carbon-cobalt bond was not observed in this series of experiments, it is possible that the reaction proceeds by an electron-transfer mechanism. If an electron is transferred from vitamin B_{12s} to the bromomethylcyclopropyls, cleavage of the carbon-bromine bond will lead to the corresponding carbanion or free radical which will spring open to the α-methyleneglutarate anion or radical. In this scheme, formation of the carbon-cobalt bond is never consummated. To test this idea, the methyl toluene-*p*-sulphonate (**22**) of the *cis*-dimethyl ester



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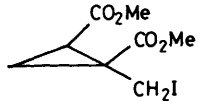

(23)

was prepared to suppress the electron-transfer process and encourage displacement by the nucleophilic cobalt of vitamin B_{12s}. Although consumption of the methyl toluene-*p*-sulphonate (**22**) was much slower than reaction of the bromides, the product composition was unchanged. Only α-methyleneglutaric acid (**2a**) was observed. Neither methylitaconic acid (**3a**) nor butadienediacid (**4**) was detected among the products. A similar result was observed starting with dimethyl 1-iodomethylcyclopropane-1,2-dicarboxylate (**23**).

We feel that the key to testing the ideas outlined above lies with the formation of the carbon-cobalt bond. If it is not formed, electron-transfer reactions can take place at the expense of the desired rearrangement processes. In the case at hand and in an earlier attempt to form a model cobalamin from γ-bromo-α-methyleneglutaric acid, it seems quite likely that electron-transfer processes have intervened to circumvent the rearrangement reactions.

Experimental

Carbon-13 *Labelling Experiment.*—*3,3-Bis(ethoxycarbonyl)-2-methylene*[4-^{13}C]-γ-*butyrolactone*[15] (**8**-^{13}**C**). In a 100 ml three-neck flask triethyl prop-3-ene-1,1,2-tricarboxylate (**7**) (8.74 g, 33 mmol) was treated with H_2O (5 ml) and $KHCO_3$ (200 mg). Under reduced pressure ^{13}C-labelled formaldehyde (1.0 g, 32 mmol) (Merck, 90% enriched) was sublimed with a heat gun into the mixture. The mixture was stirred at 45 °C for 48 h under nitrogen. The mixture was filtered through dry silica gel (50 g) with ethyl acetate (500 ml) to give the product (**8**-^{13}**C**) (9.3 g) and a small amount of starting triester (**7**). The crude product was absorbed onto silica gel (10 g), then placed on a column of silica gel (1 kg) packed in 3:1 hexane–ethyl acetate. The column was eluted with hexane–ethyl acetate (4 750 ml; 3:1) to remove the starting triester. Further elution with additional hexane–ethyl acetate (600 ml; 3:1) followed by hexane–ethyl acetate (2 400 ml; 1:1) gave a colourless oil (6.21 g, 75%).

The proton n.m.r. spectrum ($CDCl_3$) of (**8**-^{13}**C**) showed two one-proton vinyl methylene singlets at δ 6.62 and 6.3, a two-proton methylene singlet at δ 4.77 (unlabelled lactone) and methylene doublet ($J_{^{13}C-H}$ 159 Hz) at δ 4.77 (^{13}C-enriched lactone), a four-proton quartet (*J* 6 Hz) at δ 4.25, and a six-proton triplet at δ 1.28. The i.r. spectrum (neat) showed bands at 2 950m, 1 770s, 1 730s, 1 440m, 1 260s, 1 200s, 1 090m, and 1 010s cm^{-1}. The mass spectrum (15 eV) showed peaks at *m*/*z* (relative intensity): 243 (M^+, 0.5%), 198 (M^+ − OEt, 9), 171 (M^+ − CO_2Et, 66), and 143 (100). The ^{13}C-enrichment was established to be 90.6% by the 198:197 mass ratio. The proton-coupled ^{13}C n.m.r. spectrum ($CDCl_3$) showed a lactone carbonyl singlet at δ 167.5, an ester carbonyl singlet at δ 166, a quaternary unsaturated carbon singlet at δ 131, a methylene triplet (*J* 163 Hz) at δ 129, a ^{13}C-enriched lactone methylene triplet (*J* 159 Hz) at δ 69.5, an ester methylene triplet (*J* 144 Hz) at δ 63.0, a quaternary lactone carbon singlet at δ 59.0, and an ester methyl quartet (*J* 127 Hz) at δ 13.9 p.p.m.

3-Carboxy-2-methylene[4-^{13}C]-γ-*butyrolactone* (**9**-^{13}**C**). In a 250 ml round-bottom flask were placed 3,3-bis(ethoxycarbonyl)-2-methylene[4-^{13}C]-γ-butyrolactone (**8**-^{13}**C**) (6.20 g, 25 mmol) and 20% HCl (60 ml). The mixture was vigorously stirred at 45 °C for 16 h. The HCl solution was evaporated under reduced pressure and the resulting oily crystals were washed with carbon tetrachloride to recover unchanged starting material. The carbon tetrachloride was evaporated and the recovered starting material was treated again with 20% HCl (60 ml). After 16 h of vigorous stirring at 45 °C the HCl solution was evaporated to give white crystals. Two reactions gave white crystals of (**9**-^{13}**C**) (5.52 g, 86%), m.p. 107—109 °C (lit.,[6b] 105—107 °C).

The proton n.m.r. spectrum ([$^2H_6$]acetone) of (**9**-^{13}**C**) showed two one-proton, vinyl methylene doublets (*J* 2 Hz) at δ 6.28 and 6.02, a two-proton methylene multiplet at δ 4.6 (unlabelled lactone) and methylene doublet ($J_{^{13}C-H}$ 157 Hz) of multiplets at δ 4.6 (^{13}C-enriched lactone), and a one-proton methine multiplet at δ 4.2. The i.r. spectrum (KBr) showed bands at: 3 400s, 1 770s, 1 710m, and 950m cm^{-1}. The mass spectrum (70 eV) showed peaks at *m/z* (relative intensity) 143 (M^+, 9%), 142, 113 ($M^+ - {}^{13}CHO$, 100), 98 ($M^+ - CO_2H$, 36). The *m/z* 143:142 mass ratio indicated a 92% ^{13}C-enrichment. The proton-coupled ^{13}C n.m.r. spectrum ([$^2H_6$]-acetone) of (**9**-^{13}**C**) showed a lactone carbonyl singlet at δ 171, a carboxylic acid singlet at δ 169, a quaternary methylene singlet at δ 134, a methylene triplet (*J* 165 Hz) at δ 124, a ^{13}C-enriched methylene triplet (*J* 157 Hz) at δ 67.5, and a methine doublet (*J* 141 Hz) at δ 44.0 p.p.m.

3-*Carboxy*-2-*phenylselenomethyl*[4-^{13}C]-γ-*butyrolactone* (**10**-^{13}**C**). In a 500 ml three-neck flask were placed diphenyl diselenide (12.12 g, 38.9 mmol) and NaH (5.6 g, 116 mmol; 50% oil dispersion). The contents of the flask were placed under nitrogen and dry tetrahydrofuran (THF) (75 ml) was added. The mixture was stirred for 1 h at 45 °C. The white reaction mixture was cooled in an ice-bath and cold 10% HCl was added slowly. To this freshly generated solution of benzeneselenol was added 3-carboxy-2-methylene[4-^{13}C]-γ-butyrolactone (**9**-^{13}**C**) (5.52 g, 38.9 mmol). After stirring for 24 h at 45 °C, the mixture was evaporated to dryness and allowed to stand at room temperature for another 24 h. The mixture was triturated with hexanes to remove the excess of selenium reagent. The resulting crude product was dried in acetone over $MgSO_4$, filtered, and evaporated to give a white powder (9.2 g, 85%).

The proton n.m.r. spectrum ([$^2H_6$]acetone) of (**10**-^{13}**C**) showed two aromatic multiplets at δ 7.6 and 7.3, a two-proton broad multiplet at δ 4.45, and a three-proton broad multiplet at δ 3.4. The i.r. spectrum (KBr) showed bands at 3 100—2 800s (CO_2H) and 1 760s (C=O) cm^{-1}. The mass spectrum (15 eV) showed peaks at *m/z* (relative intensity): 301, 299 (M^+, 100%, 51), 158, 156 ($PhSeH^+$, 81, 37), and 143 (M^+ − PhSe, 19). Exact mass: calc. for $^{13}C_1C_{11}H_{12}O_4{}^{78}Se$, 298.9942; found 298.9942. The proton-coupled ^{13}C n.m.r. spectrum ([$^2H_6$]-acetone) of (**10**-^{13}**C**) showed a lactone carbonyl singlet at δ 176, a carboxylic acid singlet at δ 172, three aromatic doublets (*J* 158 Hz) at δ 133, 130, and 128, an aromatic singlet at δ 131, a ^{13}C-enriched methylene triplet (*J* 154 Hz) at δ 67.5 for the major diastereoisomer, a ^{13}C-enriched methylene triplet (*J* 154 Hz) at δ 69.0 for the minor diastereoisomer, a doublet (*J* 140 Hz) at δ 44, and a triplet (*J* 140 Hz) at δ 27.6 p.p.m.

1,2-*Bis*(*phenylseleno*[^{13}C]*methyl*)*ethane*-1,2-*dicarboxylic acid* (**11**-^{13}**C**). In a 500 ml three-necked, round-bottom flask were placed diphenyl diselenide (15.5 g, 49.8 mmol) and NaH (7.17 g, 149 mmol; 50% oil dispersion). Under a nitrogen atmosphere dry THF (300 ml) and hexamethylphosphoramide (1.0 ml) were added and the mixture was stirred at 60 °C for 1 h. The reaction was cooled in ice and glacial acetic acid (10 ml) was added slowly followed by powdered sodium hydroxide (2.25 g). The mixture showed a pH in the range of 8—10. To this solution 2-phenylselenomethyl-3-carboxy[4-^{13}C]-γ-butyrolactone (**10**-^{13}**C**) (5.0 g, 16.6 mmol) was added and the mixture was stirred at 60 °C for 13.5 h. The reaction mixture was cooled in ice and acidified with cold concentrated HCl (18 ml). The acidic mixture was treated with silica gel (20 g), evaporated to dryness, and placed on a column of dry silica gel 60 (30 g). The column was eluted with hexanes (500 ml), followed by 20% ethyl acetate–hexane (400 ml) to remove the excess of selenium reagent. The product was eluted with ethyl acetate (500 ml) and gave a powder (6.72 g, 88%), m.p. 151—161 °C.

The proton n.m.r. spectrum ([$^2H_6$]acetone) of (**11**-^{13}**C**) showed a four-proton aromatic multiplet at δ 7.5, a six-proton aromatic multiplet at δ 7.3, and a six-proton broad multiplet at δ 3.2. The i.r. spectrum (KBr) showed bands at 3 200—2 600s (CO_2H), 1 660s (C=O), 1 390m, 1 360m, and 720m cm^{-1}. The mass spectrum (15 eV) showed peaks at *m/z* (relative intensity): 441, 439, 437 (M^+ − OH, 30%, 30, 9), 283, 281 (M^+ − OH − PhSeH, 43, 40), and 158, 156 (PhSeH, 100, 36). Exact mass: calc. for $^{13}C_1C_{17}H_{16}O_4{}^{80}Se_2$, 440.9464. Found: 440.9465. The proton-coupled ^{13}C n.m.r. spectrum ([$^2H_6$]acetone) of (**11**-^{13}**C**) showed three aromatic doublets (*J* 160 Hz) at δ 133.5, 130, and 128, an aromatic singlet at δ 130.5, a doublet (*J* 130 Hz) at δ 49.5, and a ^{13}C-enriched methylene triplet (*J* 144 Hz) at δ 27.0 p.p.m.

[1-^{13}C]*Buta*-1,3-*diene*-2,3-*dicarboxylic acid* (**4**-^{13}**C**). A solution of 1,2-bis(phenylseleno[^{13}C]methyl)ethane-1,2-dicarboxylic acid (**11**-^{13}**C**) (6.72 g, 14.6 mmol) in ethyl acetate (400 ml) was cooled to −78 °C and treated with dry ozone for 50 min, then the excess of ozone was displaced with nitrogen at −78 °C. The mixture was placed in an ice-bath and allowed to warm to room temperature. After 12 h the solvent was evaporated leaving a yellow solid which, after trituration with hexanes, gave a yellow powder (4.17 g). The powder was dissolved in hot diethyl ether (30 ml) then precipitated with chloroform (30 ml). After cooling, the product was filtered off and washed with chloroform to give white, powdery buta-1,3-diene-2,3-dicarboxylic acid (**4**-^{13}**C**) (1.59 g, 76%), m.p. 182—185 °C (lit.,[15] m.p. 185—187 °C).

The proton n.m.r. spectrum ([$^2H_6$]acetone) of (**4**-^{13}**C**) showed a pair of vinyl methylene singlets at δ 6.2 and 5.88 corresponding to the unlabelled butadiene (**4**-^{13}**C**) and a pair of vinyl methylene doublets ($J_{^{13}C-H}$ 161 Hz) at δ 6.2 and 5.88 for the ^{13}C-enriched butadiene (**4**-^{13}**C**). The i.r. spectrum (KBr) showed bands at 3 600—2 500s (CO_2H), 1 665s (C=O), 1 430m, 1 280m, 1 120s, and 900s cm^{-1}. The mass spectrum (15 eV) showed peaks at *m/z* (relative intensity) 143 (M^+, 65%), 125 ($M^+ - H_2O$, 80), 99 ($M^+ - CO_2H$, 100). The *m/z* 143:142 mass ratio indicated a 90% ^{13}C-enrichment. The proton-coupled ^{13}C n.m.r. spectrum ([$^2H_6$]acetone) of (**4**-^{13}**C**) showed a carboxylic acid singlet at δ 167, a quaternary carbon singlet at δ 141, and a ^{13}C-enriched methylene triplet (*J* 161 Hz) at δ 127.4 p.p.m.

4-*Bromo*[1,4-$^{13}C_2$]*but*-1-*ene*-2,3-*dicarboxylic acid* (**12**-^{13}**C**). A solution of [1-^{13}C]butadiene-2,3-dicarboxylic acid (**4**-^{13}**C**) (500 mg) in dioxane (50 ml) (freshly distilled from Na benzophenone ketyl) was treated with 32% HBr (0.7 ml). After 16 h at 25 °C, the solution was treated with decolourizing carbon and filtered. Evaporation gave an oil which crystallized following addition and evaporation of carbon tetrachloride (5 × 10 ml). The weight of the crude product (m.p. 100—105 °C) was 690 mg. The proton n.m.r. spectrum ([$^2H_6$]-acetone) showed the desired (**12**-^{13}**C**), contaminated with 23—25% of unchanged butadienedicarboxylic acid (**4**-^{13}**C**). Because the bromobutenedicarboxylic acids tends to lactonize very readily, the mixture was esterified with dihydropyran and reacted directly with vitamin B_{12s} (*vide infra*).

The proton n.m.r. spectrum ([$^2H_6$]acetone) of bromobutenedicarboxylic acid (**12**-^{13}**C**) [m.p. 123—125 °C (lit.,[3] 118—119 °C)] showed a pair of vinyl doublets ($J_{^{13}C-H}$ 162 Hz) at δ 6.44 and 5.95 for the bromodiacid enriched with ^{13}C at the olefinic carbon, two one-proton vinyl singlets at δ 6.44 and 5.95 for the bromodiacid unlabelled at the vinyl methylene, and a broad three-proton multiplet at δ 3.8 p.p.m. The i.r. spectrum (KBr) showed bands at: 3 500—2 500s, 1 710s, 1 440m, 1 230s, and 870m cm^{-1}. The ^{13}C n.m.r. spectrum ([$^2H_6$]acetone) of (**12**-^{13}**C**) showed a triplet (*J* 162 Hz) at δ 129 for the ^{13}C-enriched vinyl carbon, and a triplet (*J* 150 Hz) at δ 31.9 p.p.m. for the ^{13}C-enriched bromomethyl carbon.

Bis(*tetrahydropyranyl*) 4-*bromo*[1,4-$^{13}C_2$]*but*-1-*ene*-2,3-*dicarboxylate*. Dihydropyran (5 ml, freshly distilled from sodium) was added to 4-bromo[1,4-$^{13}C_2$]but-1-ene-2,3-dicar-

boxylic acid (**12-^{13}C**) (650 mg) and the mixture was stirred at room temperature for 5 h. Unchanged dihydropyran was removed under high vacuum to leave the product as an oil (1.3 g).

The i.r. spectrum (neat) showed bands at: 2 950s, 1 720s, 1 120m, and 1 020m cm^{-1}. The ^{13}C n.m.r. spectrum ($CDCl_3$) showed a vinyl methylene triplet (*J* 164 Hz, with further slight splitting of the peaks of the triplet due to diastereoisomerism) at δ 130 for the ^{13}C-enriched vinyl carbon, and a triplet (*J* 148 Hz) at δ 31.3 p.p.m. for the ^{13}C-enriched bromomethyl carbon.

Reaction of Vitamin B_{12s} *with Bis(tetrahydropyranyl) 4-Bromo*[1,4-$^{13}C_2$]*but-1-ene-2,3-dicarboxylate* (**1-^{13}C**).—A special apparatus [3b] with two 250 ml round-bottom flasks joined together with a tube containing a fritted disc was used for the reaction. The labelled tetrahydropyranyl ester prepared from bromobutenedicarboxylic acid (**12-^{13}C**) (500 mg) was placed in flask B. A solution of vitamin B_{12a} (2.3 g, 17 mmol) in water (100 ml) was placed in flask A. The solid addition tube was charged with $NaBH_4$ (0.462 g, 12 mmol). The system was deoxygenated by evacuating under high vacuum (0.01 mmHg) for 40 min followed by filling with nitrogen, then evacuating and filling with nitrogen ten times. Finally, the vessel was capped with a nitrogen balloon. The vitamin B_{12a} solution was cooled in an ice–salt bath. One third of the $NaBH_4$ was added. The ice–salt bath was removed to allow the mixture to warm to room temperature, then the final portion of $NaBH_4$ was added. After stirring for 1.5 h the grey-green colour of vitamin B_{12s} indicated the completion of the reduction. At this stage the apparatus was moved to a dark-room that was lit with a dim red light. The double-flask apparatus was tilted allowing the solution of vitamin B_{12s} to react with the bis(tetrahydropyranyl) 4-bromo[1,4-$^{13}C_2$]but-1-ene-2,3-dicarboxylate. The mixture was stirred vigorously for 15 min at 25 °C. A u.v. spectrum at this point indicated [3b] formation of the carbon-cobalt bond. The reaction mixture was treated with cold acetone (3 l) and allowed to stand in an ice–salt bath for 10 min. The acetone was decanted and the crystals were filtered off and washed with cold acetone (3 × 100 ml). A second crop crystallized from the original solution and was washed with cold acetone. The red crystals from the first and second crops were combined and air-dried (2.76 g). The u.v.–visible spectrum of the product (**1-^{13}C**) showed bands at 315 and 515 nm. Upon irradiation of the cuvette with a 150 W sun lamp, the spectrum was converted to that of hydroxocobalamin with its characteristic intense band at 352 nm. The proton-coupled ^{13}C n.m.r. spectrum (D_2O solvent, dioxane reference) of (**1-^{13}C**) showed a ^{13}C-enriched vinyl methylene triplet (*J* 159 Hz) at δ 126 and two ^{13}C-enriched methylene triplets (–CH_2–Co) (*J* 142 Hz) at δ 27.39 and 27.0 p.p.m.

The crystals of the vitamin B_{12} butenedicarboxylic acid adduct (**1-^{13}C**) were dissolved in water (75 ml). The pH of the solution was 9.4 and was lowered to 8.3 by the addition of 10% acetic acid. The mixture was allowed to stand in the dark at 22 °C. The u.v. spectrum of the reaction mixture showed complete carbon-cobalt bond cleavage after 30 days. The basic reaction mixture was extracted continuously with ether for 24 h. The basic ether extract contained no product and was discarded. Concentrated HCl (1 ml) was added slowly to lower the pH below 1.0 and the acidified mixture was extracted continuously with ether for 60 h. The ether from the acid extract was evaporated to dryness to give the crude product (480 mg). The ^{13}C n.m.r. spectrum showed the vinyl methylene of ^{13}C-labelled α-methyleneglutaric acid (**2-^{13}C**) at δ 126 and the β-methylene ^{13}C-enriched carbon at δ 28 p.p.m.

The crude product was placed on silica gel (2 g) and eluted with ethyl acetate (70 ml) to give the product (430 mg). This was dissolved in ethyl acetate (1 ml) and chloroform (3 ml) was added precipitating butadienedicarboxylic acid (36 mg), m.p. 173—175 °C (lit.,[16] 185—187 °C). The mother liquor was adsorbed on silanized silica gel (200 mg), placed on a 21 × 8.5 cm column of silanized silica gel (20 g) and eluted with hexanes (110 ml) followed by 2% glacial acetic acid in hexanes. Fractions (40 ml each) were collected. Fraction 5 containing crude α-methyleneglutaric acid (**2-^{13}C**) (14 mg) was dissolved in ethyl acetate (0.2 ml) and treated with chloroform (0.2 ml) causing some impurities to precipitate. Filtration and evaporation gave crude α-methyleneglutaric acid (**2-^{13}C**) (11 mg). Fraction 6 from the column weighed 11 mg and contained substantial amounts of α-methyleneglutaric acid (**2-^{13}C**). It was rechromatographed on silanized silica gel (20 g) to give crude α-methyleneglutaric acid (**2-^{13}C**) (5 mg). The 11 mg and 5 mg samples of α-methyleneglutaric acid (**2-^{13}C**) were combined and chromatographed on a 30 × 1 cm column of silanized silica gel (20 g) eluting with 2% glacial acetic acid in hexanes while fractions (40 ml each) were collected. Fraction 4 containing 8 mg of product (**2-^{13}C**) was rechromatographed on a silanized silica gel column (20 g; 30 × 1 cm) eluting with 2% glacial acetic acid in hexanes collecting 10 ml fractions. Fractions 15—18 were combined yielding 3.5 mg of product. The product was washed with carbon tetrachloride (1 ml) and gave pure α-methyleneglutaric acid (**2-^{13}C**) (3 mg), m.p. 125—128 °C (lit.,[3b] 128—130 °C). The proton n.m.r. spectrum ([$^2H_6$]acetone) of the ^{13}C-enriched α-methyleneglutaric acid (**2-^{13}C**) showed two doublets ($J_{^{13}C-H}$ 159 Hz) at δ 6.2 and 5.77 corresponding to the enriched olefinic carbon, an olefinic doublet (allylic $^3J_{^{13}C,H}$ 8 Hz) at δ 6.2, and an olefinic doublet (allylic $^3J_{^{13}C,H}$ 6 Hz) at δ 5.77 for the α-methyleneglutaric acid enriched at the β-carbon. The ^{13}C n.m.r. spectrum ([$^2H_6$]acetone) showed the enriched β-carbon at δ 28.0. The γ-carbon at δ 33.0 p.p.m. showed no ^{13}C-enrichment; 2% would easily have been detected.

Cyclopropylmethyl Model Experiments.—*Cyclopropane-1,1,2-tricarboxylic acid* (**13**). A mixture of ethyl 2,3-dibromopropionate (100 g, 380 mmol) and diethyl malonate (67 g, 420 mmol) was placed in a 3 l flask equipped with a mechanical stirrer. Benzyltriethylammonium chloride (33 g, 140 mmol) was added with stirring. The reaction mixture was cooled in an ice-bath while 50% sodium hydroxide (500 ml) was added with vigorous stirring over a period of 1 h. When the addition was complete, the ice-bath was removed and the reaction mixture was stirred for 12 h at room temperature. At this point, the flask was cooled in an ice-bath and concentrated hydrochloric acid (600 ml) was added slowly with stirring. The aqueous layer, still basic, was extracted once with ethyl acetate (200 ml). The organic layer was discarded. The aqueous layer was concentrated to 100 ml at 45 °C with a rotary evaporator. The concentrate was cooled in ice and acidified to pH 1 with concentrated hydrochloric acid (150 ml), then it was extracted with ethyl acetate (4 × 250 ml). The extract was dried (anhydrous $MgSO_4$ and evaporated to give a crude, yellow, oily solid (57.10 g). The crude products from three such runs were combined, recrystallized twice from acetone–chloroform to give a white powder (51.2 g, 26%), m.p. 198.5—199.5 °C (lit.,[11] 184 °C).

The proton n.m.r. spectrum ([$^2H_6$]acetone) of (**13**) showed three cyclopropyl protons, each a doublet-of-doublets, at δ 2.54 (*J* 8.7 and 6.9 Hz), 1.88 (*J* 6.9 and 4.5 Hz), and 1.66 (*J* 8.7 and 4.5 Hz). The i.r. spectrum (KBr) showed bands at 3 200—2 900s (CO_2H), 1 665s (C=O), 1 395m, 1 260s, 1 200m, and 830w cm^{-1}. The mass spectrum (15 eV) showed peaks at *m/z* (relative intensity): 157 (M^+ − OH, 4%), 156 (M^+ − H_2O, 3), 139 (5), 138 (M^+ − $2H_2O$, 23), 129 (M^+ − CO_2 − H_2O, 16), 128 (7), 114 (37), 113 (5), 112 (61), 111 (25), 110 (7), and 84 (100). Exact mass: calc. for $C_6H_5O_5$: 157.0137. Found: 157.0134. The proton-coupled ^{13}C n.m.r. spectrum ([$^2H_6$]acetone) showed three carboxylic acid singlets at δ 171.7, 171.4, and 168.7, a cyclopropyl quaternary carbon singlet at δ 37.6, a cyclopropyl

methine doublet (*J* 171 Hz) at δ 29.3, and a cyclopropyl methylene triplet (*J* 168 Hz) at δ 20.9 p.p.m.

Cyclopropane-1,1,2-*tricarboxylic acid* 1,2-*anhydride* (**14**). Acetyl chloride (10 ml) was added to cyclopropane-1,1,2-tricarboxylic acid (**13**) (2g, 11 mmol), and the mixture was stirred for 5 h at 40 °C. The excess of acetyl chloride was evaporated using a rotary evaporator while the product was protected with a calcium sulphate drying tube. The product consisted of a white powder (1.73 g, 96.5%), m.p. 153—155 °C.

The proton n.m.r. spectrum ([$^2H_6$]acetone) of (**14**) showed the three cyclopropyl protons as a doublet-of-doublets (*J* 8.9 and 4.9 Hz) at δ 3.34, a triplet (*J* 4.9 Hz) at 2.42, and a doublet-of-doublets (*J* 8.7 and 4.9 Hz) at 2.35. The i.r. spectrum (KBr) showed bands at 3 100s (CO_2H), 1 860s, 1 760s, 1 725s, 1 420s, 1 295m, 1 240m, 1 220m, 1 150m, and 1 000w cm^{-1}. The mass spectrum (15 eV) showed peaks *m*/*z* (relative intensity): 157 ($M^+ + H$, 2%), 156 (M^+, 2), 139 (3), 138 ($M^+ - H_2O$, 6), 129 (4), 112 ($M^+ - CO_2$, 27), 111 (10), and 84 ($M^+ - CO_2 - CO$, 100). Exact mass: calc. for $C_6H_4O_5$: 156.0059. Found: 156.0059. The proton-coupled ^{13}C n.m.r. spectrum ([$^2H_6$]acetone) showed three carboxylic acid singlets at δ 166.9, 165.3, and 164.7, a cyclopropyl quaternary carbon singlet at δ 34.2, a cyclopropyl methine doublet (*J* 171 Hz) at δ 29.3, and a cyclopropyl methylene triplet (*J* 168 Hz) at δ 20.9 p.p.m.

trans-1-*Hydroxymethylcyclopropane*-1,2-*dicarboxylic acid* γ-*lactone* (**15**). A solution of cyclopropane-1,1,2-tricarboxylic acid 1,2-anhydride (**14**) (8.57 g, 55 mmol) in dry THF (100 ml) (distilled from the sodium benzophenone ketyl) was placed in a 250 ml flask equipped with a balloon adapter and a solids addition tube charged with $NaBH_4$ (1.70 g, 45 mmol). The system was placed under N_2 and cooled to 0 °C with an ice-bath. The $NaBH_4$ was added gradually and the mixture was stirred for 6 h in ice then for 12 h at room temperature. The mixture was cooled in an ice-bath and 10% hydrochloric acid (3 ml) was added to destroy the excess of hydride, then the mixture was acidified to pH 1 with concentrated hydrochloric acid (10 ml). The product was obtained by extracting with ether (3 × 150 ml). The ether was dried (anhydrous $MgSO_4$) and evaporated to leave an oily solid (8.41 g). The solid was recrystallized twice from ether–chloroform to give a white powder (3.76 g, 48%), m.p. 158.5—159.5 °C.

The proton n.m.r. spectrum ([$^2H_6$]acetone) of (**15**) showed a two-proton lactone AB quartet (*J* 9.3 Hz) at δ 4.67 and 4.30, a one-proton doublet-of-doublets (*J* 9.5 and 4.5 Hz) at δ 2.5, a doublet-of-doublets (*J* 9.5 and 4.5 Hz) at δ 1.99, and a one-proton triplet (*J* 4.5 Hz) at δ 1.39. The i.r. spectrum (KBr) showed bands at 3 100—2 900s (OH, CO_2H), 1 695s (C=O), 1 410m, 1 290w, 1 195m, 1 000m, 940w, and 810w cm^{-1}. The mass spectrum (15 eV) showed peaks at *m*/*z* (relative intensity): 142 (M^+, 6%), 141 ($M^+ - H$, 1), 125 ($M^+ - OH$, 4), 124 ($M^+ - H_2O$, 18), 115 (2), 114 ($M^+ - CO$, 38), 113 (5), 97 (19), 96 ($M^+ - CO - H_2O$, 100), 86 (11), 85 (8), and 84 (6). Exact mass: calc. for $C_6H_6O_4$: 142.0266. Found 142.0269. The proton-coupled ^{13}C n.m.r. spectrum ([$^2H_6$]acetone) showed two carboxylic acid singlets at δ 173.7 and 170.6, a lactone methylene triplet at δ 68.9 (*J* 154 Hz), a cyclopropyl quaternary carbon singlet at δ 30.7, a cyclopropyl methine doublet (*J* 186 Hz) at 27.3, and a cyclopropyl methylene triplet (*J* 168 Hz) at δ 19.4 p.p.m.

trans-1-*Bromomethylcyclopropane*-1,2-*dicarboxylic acid* (**16a**). *trans*-1-Hydroxymethylcyclopropane-1,1,2-tricarboxylic acid-γ-lactone (**15**) (2.12 g, 15 mmol) was placed under nitrogen and treated with 82 g of a 32% solution of hydrogen bromide in acetic acid. The mixture was stirred for 40 h at 45 °C. The hydrogen bromide–acetic acid solution was evaporated and the residue was flushed by the addition and evaporation of benzene (50 ml). The crude product was crystallized from acetone–$CHCl_3$ (10 ml; 50:50), then washed repeatedly with small portions of $CHCl_3$ to give a white powder (1.90 g, 57%), m.p. 197—201 °C.

The proton n.m.r. spectrum of (**16a**) ([$^2H_6$]acetone) showed a two-proton bromomethyl AB quartet (*J* 10.5 Hz) at δ 4.16 and 3.86, and three cyclopropyl protons, each a doublet-of-doublets, at δ 2.6 (*J* 8.5 and 4.5 Hz), 1.78 (*J* 8.5 and 4.5 Hz), and 1.58 (*J* 6.7 and 4.5 Hz). The i.r. spectrum (KBr) showed bands at 3 010—2 850s (CO_2H), 2 600w, 1 680s (C=O), 1 440s, 1 420s, 1 300s, 1 250s, 1 200s, 1 000w, and 940m cm^{-1}. The mass spectrum (15 eV) showed peaks at *m*/*z* (relative intensity): 206, 204 ($M^+ - H_2O$, 7%), 178, 176 ($M^+ - H_2O - CO$, 23), 143 (14), 142 (4), 126 (8), 125 ($M^+ - H_2O - Br$, 100), 124 (4), 114 (10), 99 (7), 98 (19), 97 (20), 82 (12), and 80 (12). Exact mass: calc. for $C_6H_5{}^{79}BrO_3$: 203.9422. Found: 203.9421. The proton-coupled ^{13}C n.m.r. spectrum ([$^2H_6$]acetone) of (**16a**) showed two carboxylic acid singlets at δ 171.8 and 171.3, a cyclopropyl quaternary carbon singlet at δ 34.2, a bromomethyl triplet (*J* 161 Hz) at δ 31.97, a cyclopropyl methine doublet at δ 29.3, and a cyclopropyl methylene triplet (*J* 168 Hz) at δ 23.5 p.p.m.

cis-1-*Methoxycarbonylcyclopropane*-1,2-*dicarboxylic acid* (**18**). A slight excess of cold ethereal diazomethane was added to a cold solution of cyclopropane-1,1,2-tricarboxylic acid-1,2-anhydride (**14**) (9.42 g, 60 mmol) in dry THF (50 ml) (freshly distilled from sodium benzophenone ketyl). The yellow colour was allowed to persist for 10 min, then acetic acid was added to destroy the excess of diazomethane. Water (8 ml) was added and the solution was stirred 12 h to hydrolyse the anhydride. The mixture was evaporated to dryness. The residue was dissolved in ether (100 ml) and dried (anhydrous $MgSO_4$). Evaporation and crystallization from ether–hexane gave white powder (7.69 g, 68%), m.p. 132.5—134 °C.

The proton n.m.r. spectrum ([$^2H_6$]acetone) of (**18**) showed a three-proton methyl ester singlet at δ 3.75, and three cyclopropane ring protons, each a doublet-of-doublets at δ 2.5 (*J* 8.7 and 6.9 Hz), 1.84 (*J* 6.9 and 4.5 Hz), and 1.61 (*J* 8.7 and 4.5 Hz). The i.r. spectrum (KBr) showed bands at 2 950s, 1 700s (C=O), 1 425m, 1 280s, 1 210m, and 1 145m cm^{-1}. The mass spectrum (15 eV) showed peaks at *m*/*z* (relative intensity): 171 ($M^+ - OH$, 4%), 170 ($M^+ - H_2O$, 2), 157 ($M^+ - OMe$, 13), 156 (6), 143 ($M^+ - CO_2H$, 32), 142 (40), 139 (9), 138 (50), 128 (11), 127 (7), 126 (90), 113 (45), 112 ($M^+ - CO_2Me - OH$, 100), 111 (18), 110 (17), 98 (70), and 84 (32). Exact mass: calc. for $C_7H_7O_5$: 171.0294. Found: 171.0294. The proton-coupled ^{13}C n.m.r. spectrum ([$^2H_6$]acetone) showed three carboxylic acid singlets at δ 170.5, 169.9, and 166.8, a methyl ester quartet (*J* 149 Hz) at δ 53.0, a cyclopropyl quaternary carbon singlet at δ 37.3, a cyclopropyl methine doublet (*J* 171 Hz) at δ 28.1, and a cyclopropyl methylene triplet (*J* 168 Hz) at δ 19.9.

cis-1-*Hydroxymethylcyclopropane*-1,2-*dicarboxylic acid* (**19**). A solution of *cis*-1-methoxycarbonylcyclopropane-1,2-dicarboxylic acid (**18**) (2.97 g, 15 mmol) in dry THF (20 ml) (distilled from sodium benzophenone ketyl) was placed under an atmosphere of nitrogen and cooled to 0 °C, when a 1M solution of lithium triethylborohydride[13] (80 ml) in THF was added. After stirring for 30 h at 0 °C the mixture was quenched with water (1 ml). The alkylboranes were oxidized by first adding 3M-sodium hydroxide (20 ml) followed by slow addition of 30% hydrogen peroxide (30 ml). The internal temperature at this stage was kept below 20 °C by cooling in an ice–salt bath. After the addition was complete, the solution was stirred for 30 h at room temperature. The mixture was extracted with ether (2 × 100 ml), and the ether extract was discarded. The aqueous layer was acidified to pH 1 with concentrated hydrochloric acid (35 ml), then continuously extracted with ether for four days. The ether extract was dried (anhydrous $MgSO_4$) and evaporated to give a dark oil (2.95 g). The crude product was placed on silica gel (6 g). The column was washed with hexane (50 ml) then the product was eluted with ethyl acetate (200 ml) to leave an oil

(2.81 g), which crystallized from ethyl acetate to give a white powder (1.57 g, 65%), m.p. 78—81 °C.

The proton n.m.r. spectrum ([$^2H_6$]acetone) of (**19**) showed a hydroxymethyl AB quartet (*J* 11.5 Hz) at δ 3.9 and 3.6, and three cyclopropane protons, each a doublet-of-doublets, at δ 2.03 (*J* 8.5 and 6.1 Hz), 1.66 (*J* 6.1 and 4.4 Hz), and 1.30 (*J* 8.5 and 4.4 Hz). The i.r. spectrum (KBr) showed bands at 3 200s (OH, CO_2H), 1 700s (C=O), 1 400m, 1 230m, and 1 030w cm^{-1}. The mass spectrum (15 eV) showed peaks at *m/z* (relative intensity): 143 (M^+ − OH, 4%), 142 (M^+ − H_2O, 21), 141 (7), 125 (4), 124 (13), 115 (M^+ − CO_2H, 14), 114 (M^+ − CO − H_2O), 113 (7), 112 (12), 98 (M^+ − CO_2H − OH, 39), 97 (M^+ − CO_2H − H_2O, 100), 96 (79), 84 (26), 86 (33), 88 (17), 73 (99), 70 (42), 69 (29), and 68 (14). Exact mass calc. for $C_6H_6O_4$: 142.0266. Found: 142.0267. The proton-coupled ^{13}C n.m.r. spectrum ([$^2H_6$]acetone) showed two carboxylic acid singlets at δ 172.1 and 171.9, a hydroxymethyl triplet (*J* 144 Hz) at δ 64.5, a cyclopropyl quaternary carbon at δ 30.32, a cyclopropyl methine doublet (*J* 166 Hz) at δ 25.0, and a cyclopropyl methylene triplet (*J* 166 Hz) at δ 16.3 p.p.m.

cis-1-*Bromomethylcyclopropane*-1,2-*dicarboxylic acid* (**20a**). A suspension of *cis*-1-hydroxymethylcyclopropane-1,2-dicarboxylic acid (**19**) (1.425 g, 8.91 mmol) in ether (9 ml) was warmed to 40 °C in a flask fitted with a reflux condenser and a calcium sulphate drying tube. Phosphorus tribromide (3.36 g, 12.5 mmol) was added and the mixture was stirred for 12 h at 40 °C. The mixture was cooled in an ice-bath, water (1 ml) was added, and the mixture was stirred for 0.5 h. The solvent was removed and the resulting brown oil was chromatographed on silica gel (125 g) with ethyl acetate–hexanes (3:1). Fractions 3 and 4 (20 ml each) were combined and recrystallized from ether yielding a white powder (618 mg, 28%), m.p. 158—159 °C. The product bromide showed R_F 0.15 on thin layer chromatography (silica gel; 1:1 hexane–ethyl acetate, 1% HOAc).

The proton n.m.r. spectrum ([$^2H_6$]acetone) of (**20a**) showed a two-proton bromomethyl AB quartet (*J* 10.3 Hz) at δ 4.12 and 3.45, and three cyclopropane ring protons, each a doublet-of-doublets, at δ 2.3 (*J* 10.7 and 8.6 Hz), 1.9 (*J* 8.6 and 7.8 Hz), and 1.35 (*J* 10.7 and 7.8 Hz). The i.r. spectrum (KBr) showed bands at 3 450s (CO_2H), 2 850s, 1 680s (C=O), 1 420s, 1 325m, 1 202m, and 910m cm^{-1}. The mass spectrum (70 eV) showed *m/z* (relative intensity): 207, 205 (M^+ − H_2O, 3%), 178, 176 (M^+ − CO − H_2O, 5), 143 (M^+ − Br, 8), 142 (4), 126 (11), 125 (M^+ − Br − H_2O, 100), 99 (11), 98 (15), 97 (46), 82 (16), 81 (37), 80 (15), 69 (29), 67 (7), 55 (26), and 53 (9). Exact mass calc. for $C_6H_5{}^{81}BrO_3$: 206.9480. Found: 206.9479. The proton-coupled ^{13}C n.m.r. spectrum ([$^2H_6$]acetone) showed two carboxylic acid singlets at δ 172.5 and 172.3, a bromomethyl triplet (*J* 156 Hz) at δ 37.3, a cyclopropyl quaternary carbon singlet at δ 36.1, a cyclopropyl methine doublet (*J* 164 Hz) at δ 32.6, and a cyclopropyl methylene triplet (*J* 162 Hz) at δ 20.2 p.p.m.

Dimethyl cis-1-*bromomethylcyclopropane*-1,2-*dicarboxylate* (**20b**). Ice-cold diazomethane in ether was added to an ice-cold suspension of *cis*-1-bromomethylcyclopropane-1,2-dicarboxylic acid (**20a**) (310 mg, 1.39 mmol) in ether (3 ml) until the yellow colour of diazomethane persisted for 10 min. The excess of diazomethane was destroyed with glacial acetic acid and the solution was evaporated. The crude product was filtered through silica gel (3 g) using ethyl acetate (25 ml). Evaporation of the solvent gave a colourless oil (350 mg, 100%).

The proton n.m.r spectrum ([$^2H_1$]chloroform) of (**16b**) showed two three-proton methyl ester singlets at δ 3.76 and 3.69, a bromomethyl AB quartet (*J* 10.5 Hz) at δ 4.02 and 3.19, a one-proton cyclopropyl doublet-of-doublets (*J* 8.22 and 4.93 Hz) at δ 2.4, a one-proton cyclopropyl triplet (*J* 4.93 Hz) at δ 1.9, and a one-proton cyclopropyl doublet-of-doublets (*J* 8.22 and 4.93 Hz) at δ 1.45. The i.r. spectrum (neat) showed bands at 2 960m (CH), 1 740s (C=O), 1 445s, 1 400m, 1 360s, 1 210s, 1 180s, and 1 000w cm^{-1}. The mass spectrum (15 eV) showed *m/z* (relative intensity): 252, 251 (M^+, 2%), 221, 219 (M^+ − OMe, 6), 220, 218 (8), 193, 191 (1), 192, 190 (M^+ − CO_2Me − H, 2), 171 (2), 170 (M^+ − HBr, 17), 169 (3), 139 (M^+ − OMe − HBr, 100), 129 (3), 127 (2), 116 (2), 112 (3), and 111 (10). Exact mass calc. for $C_7H_8{}^{81}BrO_3$: 220.9636. Found: 220.9636.

Bis(*tetrahydropyranyl*) cis-1-*bromomethylcyclopropane*-1,2-*dicarboxylate* (**20c**). Under a nitrogen atmosphere *cis*-1-bromomethylcyclopropane-1,2-dicarboxylic acid (**20a**) (50 mg, 0.24 mmol) was treated with dihydropyran (200 mg) and toluene-*p*-sulphonic acid (1 mg). After stirring for 18 h the mixture was dissolved in ether (2 ml) and washed with saturated, aqueous sodium hydrogen carbonate (8 × 2 ml). The ether solution was dried (anhydrous Na_2SO_4) and evaporated to give an oil which was filtered through basic aluminium oxide (3 g) with hexane–ethyl acetate (4:1) containing 1% ethyldi-isopropylamine. The product consisted of an oil (167 mg) containing some dihydropyran. The product is quite sensitive so purification was not attempted.

The proton n.m.r. spectrum ([$^2H_1$]chloroform) showed two methine singlets at δ 6.05 and 5.97, a six-proton multiplet at δ 3.6 and a fifteen-hydrogen multiplet at δ 1.6 in addition to resonances due to dihydropyran. The i.r. spectrum (neat) showed bands at 2 940s (CH), 2 850s, 1 730s (C=O), 1 440w, 1 350w, 1 250w, 1 020m, and 900m cm^{-1}. The mass spectrum showed *m/z* (relative intensity): 257 (M^+ − X, 11%), 252 (6), 201 (M^+ − THP − HBr − CO, 18), 200 (6), 199 (6), 197 (6), 186 (11), 185 (M^+ − THP − HBr − CO_2, 45), 184 (11), 183 (31), 182 (27), 181 (11), 169 (22), 168 (16), 167 (21), 1.55 (21), 139 (18), 133 (25), 113 (50), 101 (OTHP, 100), 100 (30), 99 (75), 98 (30), and 97 (56).

Dimethyl trans-1-*bromomethylcyclopropane*-1,2-*dicarboxylate* (**16b**). Ice-cold diazomethane in ether was added to an ice-cold suspension of *trans*-1-bromomethylcyclopropane-1,2-dicarboxylic acid (**16a**) (212 mg, 0.95 mmol) in anhydrous ether (5 ml) until the yellow colour of diazomethane persisted for 10 min. The excess of diazomethane was destroyed with glacial acetic acid and the solution was evaporated. The product was filtered through silica gel (3 g) using ethyl acetate (25 ml). Evaporation of the solvent gave a colourless oil (235 mg, 98%).

The proton n.m.r. spectrum ([$^2H_1$]chloroform) showed a bromomethyl AB quartet (*J* 10.71 Hz) at δ 4.11 and 3.70, two methyl ester singlets at δ 3.78 and 3.77, and three cyclopropyl ring hydrogens, each a doublet-of-doublets, at δ 2.65 (*J* 8.63 and 6.87 Hz), 1.80 (*J* 8.63 and 4.65 Hz), and 1.65 (*J* 6.87 and 4.65 Hz). The i.r. spectrum (neat) showed bands at 2 960s (CH), 1 740s (C=O), 1 445s, 1 400s, 1 320s, 1 220s, 1 180s, 1 040m, and 900m cm^{-1}. The mass spectrum (15 eV) showed *m/z* (relative intensity): 221, 219 (M^+ − OMe, 7%), 220, 218 (14), 193, 191 (M^+ − CO_2Me, 3), 192, 190 (18), 172 (2), 171 (M^+ − HBr, 24), 170 (4), 139 (M^+ − OMe − HBr, 100), and 111 (17). Exact mass calc. for $C_7H_7{}^{79}BrO_3$: 217.9579. Found: 217.9579.

Bis(*tetrahydropyranyl*) trans-1-*bromomethylcyclopropane*-1,2-*dicarboxylate* (**16c**). Under an atmosphere of nitrogen *trans*-1-bromomethylcyclopropane-1,2-dicarboxylic acid (**16a**) (100 mg, 0.45 mmol) was treated with dihydropyran (400 mg) and toluene-*p*-sulphonic acid (1 mg). After stirring for 15 h, an i.r. spectrum showed the absence of carboxylic acid absorption. The mixture was evaporated to dryness then filtered through basic aluminium oxide (3 g) with hexane–ethyl acetate (4:1) containing 1% ethyldi-isopropylamine. The product consisted of an oil (450 mg) containing some dihydropyran.

The proton n.m.r. spectrum ([$^2H_1$]chloroform) of (**16c**) showed two methine singlets at δ 6.1 and 6.06, a six-hydrogen multiplet at δ 3.8, and a fifteen-hydrogen multiplet at δ 1.7 in addition to dihydropyran absorption. The i.r. spectrum (neat) showed bands at 2 950s (CH), 2 860m, 1 740s (C=O), 1 665w,

1 445w, and 1 100m cm^{-1}. The mass spectrum showed *m/z* (relative intensity): 201 (M^+ − THP − HBr − CO, 5%), 185 (M^+ − THP − HBr − CO_2, 5), 170 (3), 164 (2), 156 (3), 144 (3), 143 (7), 140 (5), 137 (2), 136 (2), 135 (3), 130 (5), 122 (4), 117 (5), 115 (4), 114 (3), 113 (3), 111 (3), 102 (15), 101 (OTHP, 100), 100 (15), 99 (30), 98 (30), and 97 (8).

Rearrangement of Dimethyl cis-1-*Bromomethylcyclopropane*-1,2-*dicarboxylate* (**20b**) *to Dimethyl* α-*Methyleneglutarate* (**2b**).—In side B of the special double flask [3b] was placed *cis*-dimethyl 1-bromomethylcyclopropane-1,2-dicarboxylate (**20b**) (249 mg, 1 mmol). In side A were placed vitamin B_{12a} (1.345 g, 1 mmol), ammonium iodide (2 g, 14 mmol), and methanol (25 ml). A solid addition tube was charged with zinc dust (4 g, 61 mmol) and fitted to bulb A. The system was deoxygenated by purging and filling with nitrogen ten times; then it was capped with a nitrogen balloon. The zinc was introduced by rotating the addition tube. After the red vitamin B_{12a} was reduced to green vitamin B_{12s} in flask A, it was filtered through a fritted disk into flask B to react with the cyclopropane compound. After stirring for 0.5 h an u.v. spectrum showed that only vitamin B_{12a} was present.

The MeOH was evaporated and the residue was triturated with ether (5 × 50 ml). The combined ether extracts were treated with powdered sodium thiosulphate (1 g), stirred until colourless, filtered, and evaporated to leave an oil (540 mg). The oil was chromatographed on silica gel using chloroform to separate unchanged starting bromide from the product. Dimethyl α-methyleneglutarate (**2b**) was isolated in 40% yield and identified by comparison with an authentic sample. Neither dimethyl methylitaconate (**3b**) nor dimethyl buta-1,3-diene-2,3-dicarboxylate (**4b**) was detected. The yield of recovered starting bromide (**20b**) was 30%.

Reaction of cis-1-*Bromomethylcyclopropane*-1,2-*dicarboxylic Acid* (**20a**) *with Vitamin* B_{12s}.—In side B of the special double flask [3b] was placed *cis*-bromomethylcyclopropanedicarboxylic acid (**20a**) (142 mg, 0.64 mmol). A solution of vitamin B_{12a} (895 mg, 0.66 mmol) in water (4 ml) was placed in side A and the side-arm was charged with sodium borohydride (447 mg, 11.8 mmol) dissolved in water (1 ml). The system was fitted with a balloon adapter and purged by evacuating with an aspirator and filling with argon ten times. The sodium borohydride solution was added to the red vitamin B_{12a} solution reducing it to the green vitamin B_{12s}. After stirring for 15 min, oxygen-free acetone (3.0 g, 51 mmol) was added to destroy the excess of sodium borohydride.

The apparatus was moved to a dark room, dimly lit with a red light, and the vitamin B_{12s} solution was added to the cyclopropanediacid (**20a**) by tilting the two-bulbed flask. After stirring for 15 min, a u.v. spectrum showed only vitamin B_{12a}. The aqueous layer (pH 11) was acidified with 10% hydrochloric acid and extracted with ether (4 × 50 ml). The ether extract was dried (anhydrous $MgSO_4$) and evaporated to give a crude oil (350 mg). The oil was chromatographed on silica gel (6 g) eluting with hexanes (2 × 20 ml), ethyl acetate–hexane (20 ml; 1:4), ethyl acetate–hexane (20 ml; 2:3), and ethyl acetate–hexane (20 ml; 3:2). The product was found in the 3:2 ethyl acetate–hexane fractions and yielded 46 mg of a 1.5:1 mixture of α-methyleneglutaric acid (**1a**) and methylglutaconic acid (**21**). The proton n.m.r. spectrum ([$^2H_6$]acetone) of the product showed: for methylglutaconic acid (**21**), a one-proton vinyl methylene triplet (*J* 7.4 Hz) at δ 6.97, a two-proton methylene doublet (*J* 7.4 Hz) at δ 3.3 and a methyl singlet at δ 1.85; for α-methyleneglutaric acid (**2a**), two one-proton vinyl singlets at δ 6.2 and 5.7, and a four-proton multiplet at δ 2.5 p.p.m.

Dimethyl cis-1-(p-*tolylsulphonyloxymethyl*)*cyclopropane*-1,2-*dicarboxylate* (**22**). In a dry 10 ml flask equipped with a side-arm were placed toluene-*p*-sulphonyl chloride (180 mg) and pyridine (0.2 ml). The flask was placed under nitrogen and a solution *cis*-dimethyl 1-hydroxymethylcyclopropane-1,2-dicarboxylate (**20b**) (58 mg) in pyridine (0.1 ml) was added while the mixture was cooled in ice, then additional pyridine (0.2 ml) was added. The homogeneous mixture was stirred in a cold room at 0 °C for 3 h and resulted in the precipitation of pyridinium hydrochloride as long white needles. The mixture was treated with ice (1 g) and stirred at 0 °C for 1 h. The solution was extracted with ether (4 × 3 ml). The ether extract was dried (anhydrous $MgSO_4$) and evaporated to leave the crude product (145 mg). Chromatography on silica gel (1 g) eluting with hexane–ethyl acetate (50 ml; 4:1) gave a colourless oil (**22**) (87 mg, 60%).

The proton n.m.r. spectrum ([$^2H_1$]chloroform) of (**22**) showed a four-proton aromatic AB quartet (*J* 8.3 Hz) at δ 7.8 and 7.4, a two-proton AB quartet (*J* 11 Hz) at δ 4.7 and 3.9, three three-proton methyl singlets at δ 2.46, 3.66, and 3.63, and three cyclopropyl protons, each a doublet-of-doublets, at δ 2.05 (*J* 8.3 and 5.8 Hz), 1.93 (*J* 5.8 and 5.8 Hz), and 1.32 (*J* 8.3 and 5.8 Hz). The i.r. spectrum (neat) showed bands at 2 950w, 1 730s, 1 430m, 1 350m, 1 170s, and 810m cm^{-1}. The mass spectrum (15 eV) showed peaks at *m/z* (relative intensity): 342 (M^+, 10%), 310 (100), and 283 (18). Exact mass calc. for $C_{15}H_{18}O_7S$: 342.0773. Found: 342.0777.

Dimethyl cis-1-*iodomethylcyclopropane*-1,2-*dicarboxylate* (**23**). A solution of *cis*-dimethyl 1-(*p*-tolylsulphonyloxymethyl)-cyclopropane-1,2-dicarboxylate (**22**) (8 mg) in acetone (0.6 ml) was treated with sodium iodide (10 mg). After stirring for 13 h the solvent was removed. The residue was dissolved in ether, filtered and evaporated to give a yellow oil (4 mg). The proton n.m.r. spectrum ($CDCl_3$) of (**23**) showed two three-proton methyl ester singlets at δ 3.75 and 3.65, a two-proton AB quartet (*J* 9.5 Hz) at δ 3.8 and 3.0, a one-proton cyclopropyl multiplet at δ 2.1, and a two-proton cyclopropyl multiplet at δ 1.15 p.p.m. The mass spectrum (15 eV) showed peaks at *m/z* (relative intensity): 298 (M^+, 2%), 267 (M^+ − OMe, 5), and 171 (100). Exact mass calc. for $C_8H_{11}IO_4$: 297.9702. Found: 297.9702.

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