On the Mechanism of Action of Vitamin B_{12}. Model Studies Directed toward the Hydrogen Abstraction Reaction

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A model rearrangement reaction to explore the hydrogen abstraction step in the coenzyme B_{12} dependent rearrangement of methylmalonyl-SCoA to succinyl-SCoA has been developed. The model was prepared according to the following scheme. Vinyllithium reacted with 5-chloropent-2-one (XIII) yielding 2-vinyl-2-methyltetrahydrofuran (XIV). Hydroboration followed by oxidative workup gave tetrahydro-2-methyl-2-furanethanol (XV). Treatment of XV with *p*-toluenesulfonyl chloride yielded (2-methyltetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate (XVI). Condensation of XVI with *O,S*-diethyl thiomalonate anion gave the adduct ethyl α-[(ethylthio)carbonyl]tetrahydro-2-methyl-2-furanbutanoate (XVII). The latter was alkylated with dibromomethane yielding ethyl α-(bromomethyl)-α-[(ethylthio)carbonyl]tetrahydro-2-methyl-2-furanbutanoate (X). Treatment of the bromide X with vitamin B_{12s} resulted in rapid rearrangement to ethyl α-[2-(ethylthio)-2-oxoethyl]tetrahydro-2-methyl-2-furanbutanoate (XVIII). Some unrearranged product, ethyl α-methyl-α-[(ethylthio)carbonyl]tetrahydro-2-methyl-2-furanbutanoate (XIX), was also isolated together with a small amount of unreacted bromide X. An authentic sample of XVIII was prepared as follows. Condensation of ethyl *tert*-butyl malonate with *tert*-butyl bromoacetate with sodium hydride yielded 1,2-di-*tert*-butyl 1-ethyl 1,1,2-ethanetricarboxylate (XX). The latter was condensed with XVI yielding 1,2-di-*tert*-butyl 2-ethyl 4-(tetrahydro-2-methyl-2-furanyl)-1,2,2-butanetricarboxylate (XXI). Stirring XXI with trifluoroacetic acid yielded the diacid, 2-ethyl dihydrogen 4-(tetrahydro-2-methyl-2-furanyl)-1,2,2-butanetricarboxylate (XXII). Decarboxylation to 1-ethyl hydrogen [1-(tetrahydro-2-methyl-2-furanyl)ethyl]butanedioate (XXIII) was effected by heating. Authentic rearrangement product (XVIII) was then prepared by condensation of ethanethiol with the acid XXIII by using *N,N'*-dicyclohexylcarbodiimide. An authentic sample of the unrearranged product XIX was prepared by reaction of XVII with sodium hydride in HMPA followed by treatment with methyl iodide. An authentic sample of the regioisomer ethyl β-[(ethylthio)carbonyl]tetrahydro-2-methyl-2-furanpentanoate (XXVIII) was prepared as follows. 1,1-Di-*tert*-butyl 2-ethyl 1,1,2-ethanetricarboxylate (XXIV) was treated with sodium hydride and then the resulting anion was condensed with tosylate XVI yielding 2,2-di-*tert*-butyl 1-ethyl 4-(tetrahydro-2-methyl-2-furanyl)-1,2,2-butanetricarboxylate (XXV). Hydrolysis of XXV with trifluoroacetic acid–water yielded 1-ethyl dihydrogen 4-(tetrahydro-2-methyl-2-furanyl)-1,2,2-butanetricarboxylate (XXVI), which was decarboxylated to 1-ethyl hydrogen [2-(tetrahydro-2-methyl-2-furanyl)ethyl]butanedioate (XXVII) by heating at 80 °C. The desired thioester XXVIII was obtained by condensing XXVII with ethanethiol in the presence of dicyclohexylcarbodiimide. The thioester XXVIII was shown to be readily distinguished from the product of rearrangement XVIII with high-field NMR. When the B_{12}-based rearrangement reaction was carried out in D_2O, one deuterium was found at the 2-position in XVIII. A small amount of deuterium was found in the 5-position on the tetrahydrofuran ring of XVIII. The unrearranged product XIX showed one deuerium on the methyl carbon and a small amount of deuterium on the ring. Inclusion of potassium carbonate in the D_2O reaction raised the ring deuteration of both XVIII and XIX to approximately 30%. In this reaction deuterium was also found to be incorporated in the 5-position of the tetrahydrofuran ring of the starting bromide X.

Introduction

Vitamin B_{12} is a biologically potent substance of broad metabolic significance for both mammalian and bacterial organisms. Following the isolation of vitamin B_{12} by Folkers and Smith,[1] the discovery of coenzyme B_{12} by Barker,[2] and the synthesis of vitamin B_{12} by Woodward[3] and Eschenmoser,[4,5] the most important remaining questions concern the biosynthesis of vitamin B_{12}[6] and the mechanism of action of coenzyme B_{12}. The latter problem will be considered here from the point of view of nonenzymic chemical models.[7]

Coenzyme B_{12} is an obligatory cofactor in 11 enzyme-catalyzed rearrangement reactions.[8] Of these, three are reversible carbon-skeleton rearrangements: β-methylaspartic acid $\rightleftarrows$ glutamic acid (eq 1),[9] methylmalonyl-SCoA $\rightleftarrows$ succinyl-SCoA (eq 2),[10] and β-methylitaconic acid

(1) Rickes, E. L.; Brink, N. G.; Konivszy, F. R.; Wood, T. R.; Folkers, K. *Science* **1948**, *107*, 396. Smith, E. L.; Parker, L. F. J. *Biochem. J.* **1948**, *43*, viii.

(2) Barker, H. A.; Weissbach, H.; Smyth, R. D. *Proc. Natl. Acad. Sci. U.S.A.* **1958**, *44*, 1093. Hogenkamp, H. P. C.; Barker, H. A. *J. Biol. Chem.* **1960**, *236*, 3097.

(3) Woodward, R. B. *Pure Appl. Chem.* **1968**, *17*, 519; **1971**, *25*, 283; **1973**, *33*, 145.

(4) Eschenmoser, A. *Q. Rev. Chem. Soc.* **1970**, *24*, 366; *Pure Appl. Chem. Suppl.* **1971**, *2*, 69.

(5) See also: Stevens, R. V. *Tetrahedron* **1976**, *32*, 1599.

(6) For a cogent summary of recent progress and for leading references see: Battersby, A. R.; Fookes, C. J. R.; Matcham, G. W. J.; McDonald, E. *Nature (London)* **1980**, *285*, 17.

(7) (a) Dowd, P.; Shapiro, M.; Kang, K. *J. Am. Chem. Soc.* **1975**, *97*, 4754. (b) Dowd, P.; Trivedi, B. K.; Shapiro, M.; Marwaha, L. K. *J. Am. Chem. Soc.* **1976**, *98*, 7875. (c) Dowd, P.; Shapiro, M. *J. Am. Chem. Soc.* **1978**, *98*, 3724. (d) Bidlingmaier, G.; Flohr, H.; Kempe, U. M.; Krebs, T.; Retey, J. *Angew. Chem.* **1975**, *87*, 877. (e) Flohr, H.; Pannhorst, W.; Retey, J. *Angew. Chem.* **1976**, *88*, 613. (f) Scott, A. I.; Kang, K. *J. Am. Chem. Soc.* **1977**, *99*, 1977. (g) Scott, A. I.; Kang, J.; Dalton, D.; Chung, S. K. *J. Am. Chem. Soc.* **1978**, *100*, 3603. (h) Chemaly, S.; Pratt, J. M. *J. Chem. Soc., Chem. Commun.* **1976**, 988. (i) Scott, A. I.; Kang, J.; Dowd, P.; Trivedi, B. K. *Bioorganic Chem.* **1980**, *9*, 227. (j) See, however: Scott, A. I.; Hansen, J. B.; Chung, S.-K. *J. Chem. Soc., Chem. Commun.* **1980**, 388. These workers have reported multiple deuterium incorporation.

(8) *Reviews*: Smith, E. L. "Vitamin B_{12}", 3rd ed.; Methuen: London, 1965. Weissbach, H. Peterkofsky, A.; Barker, H. A. In "Comprehensive Biochemistry"; Florkin, M., Stotz, E. H., Eds.; Elsevier: Amsterdam, 1965; Vol. 16, pp 180–208. Stadtman, T. C. *Science* **1965**, *171*, 859. Bernhauer, K. *Angew. Chem., Int. Ed. Engl.* **1964**, *3*, 200. Brown, D. G. *Prog. Inorg. Chem.* **1973**, *18*, 187–286. Abeles, R. H. *Adv. Chem. Ser.* **1971**, No. 100, 346. Babior, B. M. "Cobalamin"; Wiley-Interscience: New York, 1975. Abeles, R. H. In "Biological Aspects of Inorganic Chemistry"; Addison, A. W., W. R. Cullen, W. R., Dolphin, D., James, B. R., Eds.; Wiley: New York, 1977; p 245. Babior, B. M. *Acc. Chem. Res.* **1975**, *8*, 376. Abeles, R. H.; Dolphin, D. *Ibid.* **1976**, *9*, 114.

(9) Barker, H. A.; Weissbach, H.; Smyth, R. D. *Proc. Natl. Acad. Sci., U.S.A.* **1958**, *44*, 1093.

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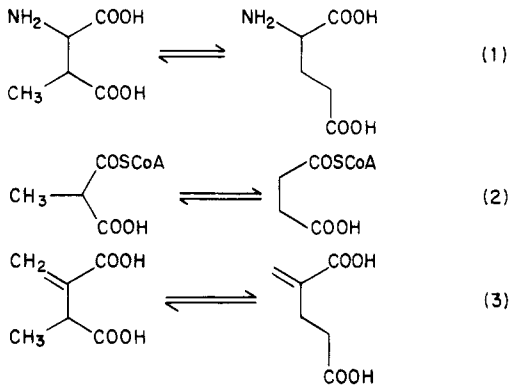
$\rightleftarrows$ α-methyleneglutaric acid (eq 3).[11]



(1)

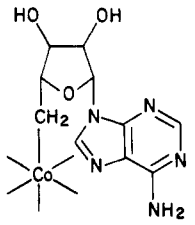
(2)

(3)

Carbon labeling experiments have been employed to show that in the β-methylaspartate rearrangement (eq 1) the glycyl fragment migrates to the methyl carbon, in the methylmalonyl-SCoA reaction (eq 2) the carbonyl-SCoA fragment migrates to methyl, and in the methylitaconate reaction (eq 3) the acrylic acid fragment is the migratory group.

The unusual feature of these reactions is the reversible conversion of an apparently unactivated methyl group into a methylene group followed by incorporation of the latter into the backbone chain of the product (eq 1–3). This transformation has had no analogues in organic chemistry; thus there have been no suitable nonenzymatic model reactions. In addition, there is, at the present time, no experimental knowledge of any reactive intermediates in these carbon-skeleton rearrangement reactions; the only acceptable substrates for the enzymes which govern the rearrangements are the substances shown in eq 1–3 above.

Hydrogen transfer accompanies group migration. Following the observation that no exchange occurs with the protons of water, it was hypothesized, for a brief period, that the hydrogen transfer might be an intramolecular reaction. In fact, the apparent hydrogen migration is an intermolecular hydrogen abstraction and transfer reaction mediated by the 5′-methylene of the deoxyadenosine I of the coenzyme. This was demonstrated by Abeles and his



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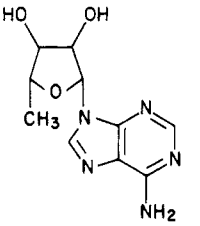
colleagues[12] in their study of the conversion of ethylene and propylene glycols to acetaldehyde and propionaldehyde, respectively, and has subsequently been shown to apply[13] to the other members of the coenzyme B_{12} dependent rearrangement series. These experiments form the cornerstone upon which mechanistic speculation in this area is founded.

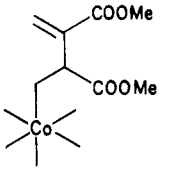
The hydrogen labeling experiments demonstrate the involvement of the deoxyadenosine group I in the hydrogen transfer reaction. However, the mechanism by which hydrogen abstraction occurs is still obscure. There are no nonenzymic models which mirror the hydrogen abstraction in the context of the enzymic transformation, but Breslow and Khanna[14] have observed hydrogen abstraction in a cyclodecane model.

The problem is 2-fold. Not only must there exist a path by which hydrogen is abstracted from the unactivated methyl group of the substrate but, in order to complete the catalytic cycle, hydrogen must be removed at a later stage from the unactivated methyl group of the intermediate 5′-deoxyadenosine II.[15]



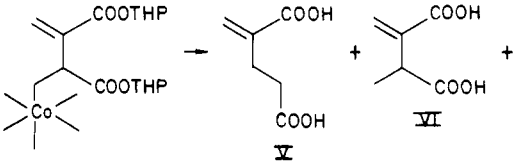
II

Model Studies. A study of vitamin B_{12} derivatives, distinguished from previously known alkyl cobalamins by having substrate bonded to the central cobalt atom, was initiated.[7a] Since from a structural standpoint, methylitaconic acid and α-methyleneglutaric acid of the rearrangement (eq 3) discovered by the Stadtmans[11] are the simplest of the carbon-skeleton rearrangement substrates, the methyl carbon of the methylitaconate dimethyl and bis(tetrahydropyranyl) esters was attached to cobalt as shown in III and IV.

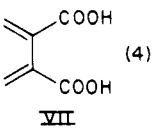


III

Spontaneous rearrangement of the bis(tetrahydropyranyl) methylitaconate ester–cobalamin IV (eq 4) provided the first model in the B_{12} rearrangement series.[7a]



IV V VI



(4)

VII

The model reaction occurs in the absence of enzyme, at

(10) Stadtman, E. R.; Overath, P.; Eggerer, H.; Lynen, F. *Biochem. Biophys. Res. Commun.* **1960**, *2*, 1. Eggerer, H.; Overath, P.; Lynen, F.; Stadtman, E. R. *J. Am. Chem. Soc.* **1960**, *82*, 2643. Stjernholm, R.; Wood, H. G. *Proc. Natl. Acad. Sci., U.S.A.* **1961**, *47*, 303. Flavin, M.; Ochoa, S. *J. Biol. Chem.* **1957**, *229*, 965.

(11) Kung, H. F.; Cederbaum, S.; Tsai, L.; Stadtman, T. C. *Proc. Natl. Acad. Sci., U.S.A.* **1970**, *65*, 978. Tsai, L.; Pastan, I.; Stadtman, E. R. *J. Biol. Chem.* **1966**, *241*, 1807. Kung, H. F.; Stadtman, T. C. *Ibid.* **1971**, *246*, 3378.

(12) Abeles, R. H.; Zagalak, B. *J. Biol. Chem.* **1966**, *241*, 1245. Frey, P. A.; Abeles, R. H. *Ibid.* **1966**, *241*, 2732. Frey, P. A.; Essenberg, M. K.; Abeles, R. H. *Ibid.* **1967**, *242*, 5369.

(13) Retey, J.; Arigoni, D. *Experientia* **1966**, *22*, 783.

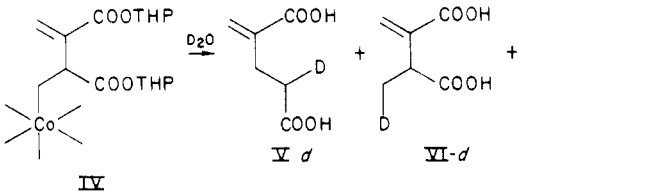
(14) Breslow, R.; Khanna, P. L. *J. Am. Chem. Soc.* **1976**, *98*, 1297, 6765.

(15) For a discussion see: Kerwar, S. S.; Smith, T. A.; Abeles, R. H. *J. Biol. Chem.* **1970**, *245*, 1169.

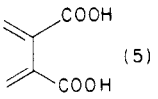
(16) That the methylitaconate contains no deuterium at the methine carbon indicates that formation of the carbon–cobalt bond, is, in this instance, not an elimination–addition sequence as it might, a priori, have been supposed to be.

ambient temperature, near physiological pH, in aqueous solution and in the dark. Study of this model and those subsequently developed from it[7] promises to afford new insight into the mechanism of action of vitamin B_{12}.

In order to learn more about the model reaction, the rearrangement of eq 4 was carried out in D_2O.[7b] The products α-methyleneglutaric-γ-d_1 acid (V-*d*) and methylitaconic-d_1 acid (VI-*d*) each were found to contain one atom of deuterium (eq 5).



IV V-*d* VI-*d* (5)

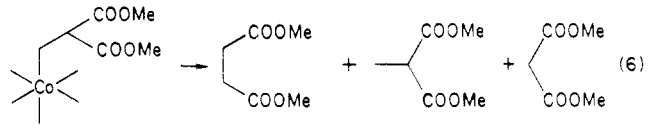


VIII

The rearranged product α-methyleneglutaric acid V-*d* was deuterated exclusively on the γ-carbon. Since the site of deuteration is indicative of the position of the cobalt prior to hydrolysis, the acrylic acid group must be the exclusive migrating group, as it is in the enzyme-catalyzed transformation. To this extent, the nonenzymic rearrangement is a faithful reflection of the enzymic transformation.[7b]

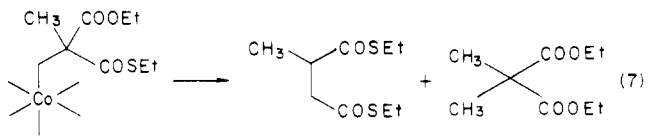
Following the development of the methylitaconate model,[7a] analogous models have been devised for the methylmalonyl-SCoA ⇄ succinyl-SCoA transformation (eq 2). Retey and his colleagues[7d] prepared a cobaloxime-based model in which a methylmalonate is attached to cobalt and rearrangement is brought about by photolysis with a high-pressure mercury lamp. They introduced the device of substituting the acidic malonate proton by a methyl group, thereby avoiding the complications which can arise from ionization at that position.

A second methylmalonate model IX[7c] was constructed by attachment of dimethyl methylmalonate through its methyl group to the full cobalamin nucleus. The adduct showed excellent characteristic visible spectral properties, consistent with the presence of the carbon–cobalt bond, and underwent spontaneous decomposition to succinate, methylmalonate, and malonate (eq 6).



IX (6)

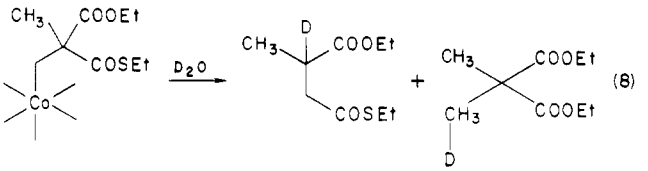
A further model based on the cobalamin nucleus was developed[7f] (eq 7) by using the blocking methyl group of Retey.[7d] In this model, a thioester is the migrating group (eq 7). This rearrangement (eq 7) can be carried out so



(7)

that it is catalytic in vitamin B_{12}.[7i] When the reaction was carrried out in EtOD, deuterium was incorporated into the product at the methine carbon[7g,j] (eq 8). This is analogous to the result from the methylitaconate model IV.[7b]

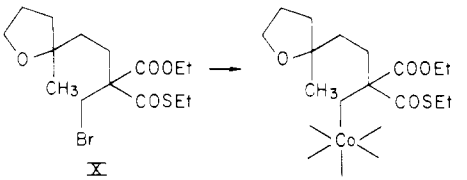
When the enzyme-catalyzed rearrangements (eq 1–3) are carried out in deuterated or tritiated water, no isotopic



(8)

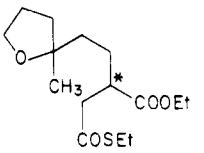
label is incorporated into the substrates of any of the carbon-skeleton rearrangement reactions (eq 1–3). Thus, there exists a discontinuity between the model reaction and the enzymic reaction. This is not surprising; there is no provision in the model for hydrogen transfer.

The blocking methyl group does not interfere with the model malonate carbon-skeleton rearrangement. Accordingly, a more elaborate group might be attached to the malonate carbon to probe for the occurrence of hydrogen abstraction in the context of the carbon-skeleton rearrangement reaction. The plan was developed to prepare the precursor bromide X and to study the rearrangement of its adduct XI with vitamin B_{12} in order to learn whether hydrogen abstraction can occur from proximate unactivated centers. The methyl group is intended to represent



X XI

the methyl group of 5′-deoxyadenosine. If the adduct XI rearranges in the expected sense, with migration of the thioester group, a reactive center (starred) or transient carbon metal bond will be generated adjacent to the carbethoxy group and six atoms removed from the hydrogens of the methyl group on the tetrahydrofuran ring shown in XII. If hydrogen abstraction occurs, then the hydrogen

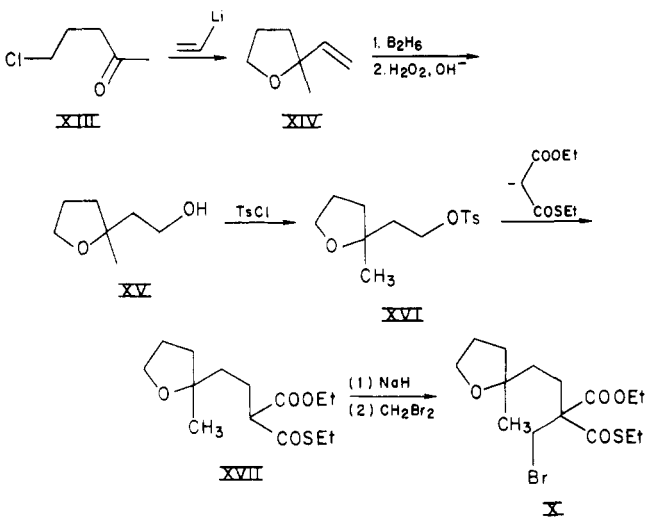


at the methine carbon of the succinate will be derived from the tetrahydrofuran ring or its methyl group. Such a hydrogen-transfer reaction might be detected either by labeling the position under siege on the tetrahydrofuran ring or by carrying out the rearrangement reaction in D_2O. In the latter instance, hydrogen instead of deuterium will be incorporated at the methine position of the succinate group if internal hydrogen abstraction takes place. The driving force for such a transformation may be provided by exchange of a tertiary carbon–cobalt bond for a more stable primary one. However this would leave unexplained the apparent spontaneity of the carbon-skeleton rearrangement step.

Results

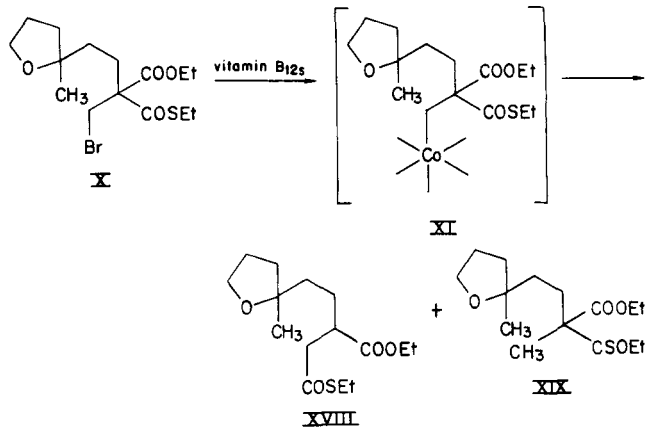
The desired model cobalamin XI was synthesized by the route outlined in Scheme I. Treatment of 5-chloropentan-2-one (XIII) with vinyllithium yielded the vinyltetrahydrofuran XIV. Addition of borane to XIV followed by oxidative workup yielded the alcohol XV, which was converted to the tosylate XVI. Displacement of the tosylate by *O,S*-diethyl thiomalonate was not a smooth reaction; it was difficult to drive the reaction to completion without causing decomposition of the product thio-

Scheme I



malonate XVII. Accordingly, the reaction was carried to partial completion and the desired thiomalonate XVII was separated from unreacted tosylate XVI, suitable for reuse in subsequent reactions, by chromatography. Synthesis of the desired bromide X was completed by reaction of the thiomalonate XVII with methylene dibromide with sodium hydride as the condensing agent.

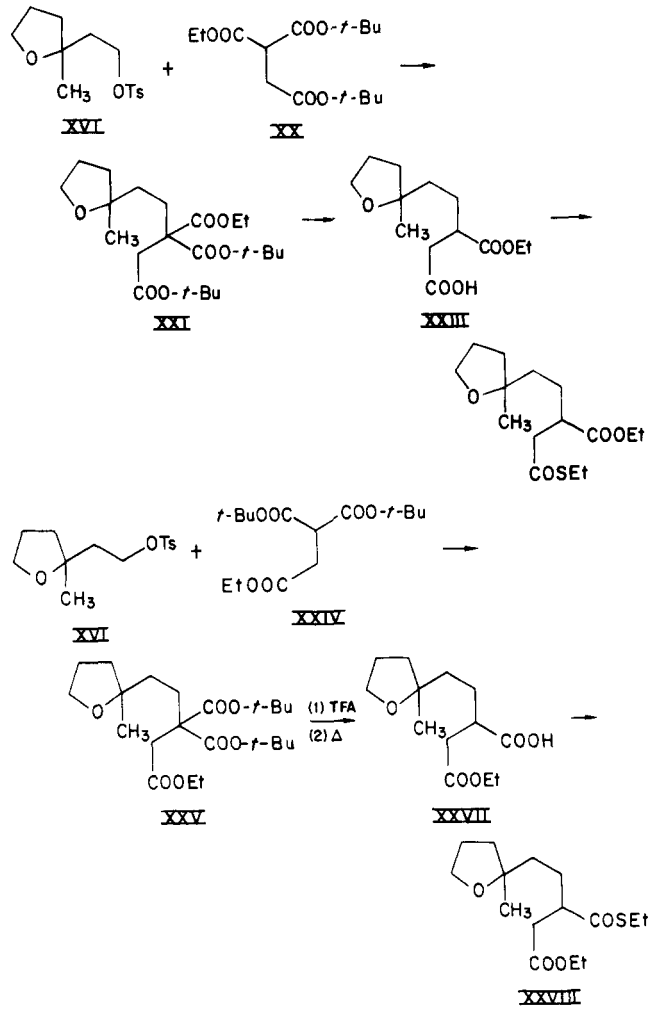
The bromide X was reacted with vitamin B_{12s}. The reaction product failed to yield visible spectra indicative of carbon–cobalt bond formation; however, the rearrangement product XVIII was formed rapidly and in good yield.



It was important that the structure of the rearranged product XVIII be established conclusively and unambiguously. This was done by synthesis of authentic samples of the two possible regioisomers XVIII and XXVIII.

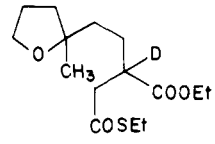
In the first instance the tosylate XVI was reacted with the protected succinate XX. Hydrolysis and decarboxylation of the resulting triester XXI yielded the acid ester XXIII, which was converted to the thioester XVIII, identical in every respect with that obtained from the B_{12} rearrangement reaction.

The alternative regioisomer XXVIII was prepared by condensation of the tosylate XVI with the protected succinate XXIV. The resulting triester XXV was hydrolyzed, decarboxylated, and converted to the regioisomeric thioester XXVIII. This substance is clearly distinguished in its NMR spectrum from the B_{12} rearrangement product XVIII. The ABX patterns (described in the Experimental Section) associated with the succinate parts of the two thioesters XVIII and XXVIII are quite distinct in the 250-MHz proton NMR spectra.



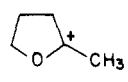
With the demonstration that the rearrangement does occur in this more elaborate model and the establishment of the structure of the rearrangement product XVIII, the possible occurrence of internal hydrogen abstraction was examined.

When the model reaction was carried out in D_2O the rearranged product XVIII-*d* contained one atom of deuterium at the succinate methine carbon. The adjacent methylene group of the succinate was observed to be a clean AB quartet. Splitting of the latter by the neighboring methine proton, readily observed in the parent XVIII, was absent in XVIII-*d* indicating full deuterium incorporation at the methine carbon. Thus, within the limits of detection of the NMR method little internal hydrogen abstraction was indicated. However, when the mass spectrum of XVIII-*d* was examined it was interesting.



XVIII-*d*

The mass spectrum of XVIII shows the base peak at *m/z* 85 arising from the methyltetrahydrofuran fragment ion XXIX. The identity of fragment XXIX is supported by the value of 85.0651 found for its exact mass. This is

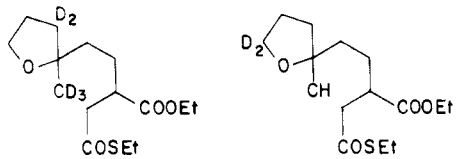


in good agreement with the value 85.0653 expected for an

ion of composition C_5H_9O. In the mass spectrum of the parent compound XVIII the peak at m/z is 6% as intense as the base peak at m/z 85. This is readily accounted for by natural abundance carbon-13, uncomplicated by the superposition of other fragments which might make deuterium analysis difficult.

The mass spectrum of the rearrangement product XVIII-*d* showed the peak at m/z 85 as the base peak in the spectrum. The peak at m/z 86 was 14% as intense as the peak at m/z 85, indicating the presence of deuterium in the methyltetrahydrofuran part of XVIII-*d*. The result was reproducible, but the significance of the relatively small increment in the intensity of the m/z 86 peak was unclear. Further experiments revealed that inclusion of potassium carbonate in the reaction mixture, to the extent of 12% of saturation, yielded rearranged product XVIII-*d* in which the m/z 86 peak was 50% as intense as the m/z 85 base peak. This could no longer be ignored or dismissed as an instrumental artifact. The methyltetrahydrofuryl unit contains deuterium. Two questions arise. Where is the deuterium located? How did it gain entry?

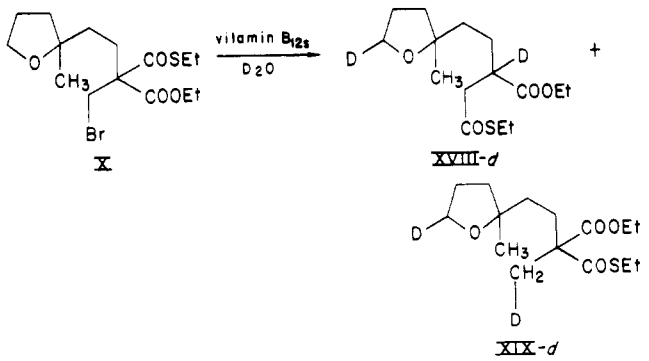
To answer the first question, we had recourse to deuterium NMR spectroscopy. Although the chemical shifts in the deuterium NMR spectrum closely approximate those observed in the proton NMR spectrum, the lines are



XVIII-d_5 XVIII-d_2

broad and it was helpful to have a model compound for direct comparison of the chemical shifts. Accordingly, the deuterated rearrangement products XVIII-d_5 and XVIII-d_2 were prepared and used for spectral comparison with the product XVIII-*d* obtained from the model reaction carried out in D_2O.

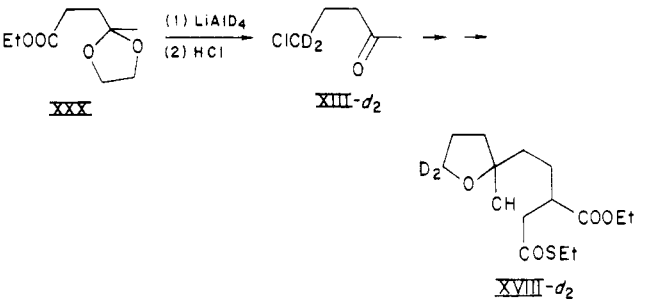
Comparison of the deuterium NMR spectra of XVIII-d_1 and XVIII-d_5 showed that no deuterium was incorporated into the methyl group or the position β to the oxygen on the tetrahydrofuran ring. These are the two positions with



X XVIII-*d* XIX-*d*

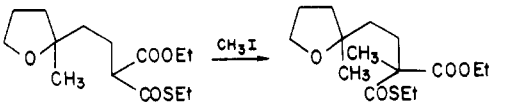
protons six atoms removed from the active center generated by migration of the thioester group. It is also clear from comparison of the spectra of XVIII-*d* and that of the rearrangement product XVIII-d_2, specifically deuterated in the position α to oxygen on the tetrahydrofuran ring, that the deuterium in XVIII-*d* is incorporated at the position α to the oxygen on the tetrahydrofuran ring.

The rearrangement product XVIII-d_2 was prepared by $LiAlD_4$ reduction of ethyl levulinate ethylene ketal XXX followed by ketal hydrolysis and conversion to the chloro ketone XIII-d_2 using hydrochloric acid. The remainder of the synthesis, including vitamin B_{12} mediated rearrangement to XVIII-d_2, followed lines outlined above.



XXX XIII-d_2 XVIII-d_2

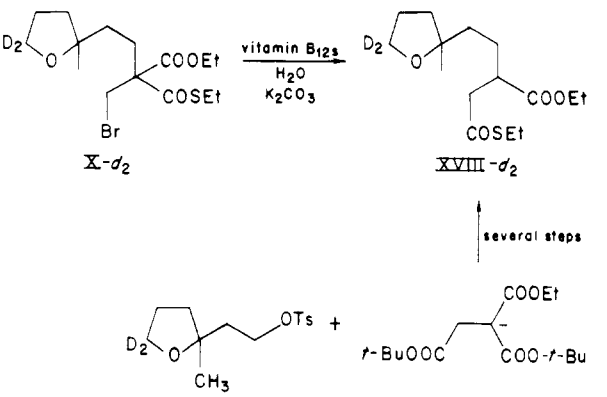
A small amount of unreacted bromide X was usually observed together with unrearranged reduced product XIX. The structure of the latter was established by synthesis of an authentic sample by methylation of the thiomalonate XVII. When XIX-*d* was recovered from the



XVII XIX

rearrangement carried out in D_2O, it was also found to be deuterated in the α-position on the tetrahydrofuran ring to the extent of approximately 30%. When the bromide X was recovered from the rearrangement conducted in D_2O, it too was found to carry deuterium in the α-position—to the extent of 7%—less than that found in XVIII-*d* and XIX-*d*.

The labeled product XVIII-d_2 was prepared by rearrangement of the bromide X-d_2 and by synthesis. The deuterium NMR spectra of the two samples were identical showing that in the B_{12} mediated rearrangement deuterium is not translocated to the methine position in the succinate part of the molecule. When the reaction is run in water,



X-d_2 XVIII-d_2

less exchange of deuterium (ca. 15%) was observed at the α-position on the tetrahydrofuran ring. We ascribe this to a deuterium isotope effect. Of course, such an isotope effect might also compromise the other part of the experiment in which hydrogen transfer between the succinate and tetrahydrofuran parts of the molecule is being probed.

A control reaction in which the rearrangement product XVIII was carried through the reaction conditions showed no exchange of deuterium into either the succinate or tetrahydrofuran part of the molecule. Neither rearrangement nor exchange occurs in the absence of hydroxocobalamin.

Conclusions

Examination of the deuterated models in H_2O and the undeuterated model in D_2O has revealed no evidence which might be construed to favor the view that hydrogen ab-

straction occurs from unactivated carbon as an adjunct to or consequence of the B_{12} dependent model rearrangement reaction described above. This is of course but a single model and steric or other effects may intervene to prevent hydrogen abstraction from taking place. If attachment or association is to the massive B_{12} nucleus, close approach of the reactive center to the tetrahydrofuran nucleus may be difficult. Alternatively, there may be other factors at work in the enzymic hydrogen abstraction cum rearrangement reactions of which we have no appreciation at the present time.

The exchange of hydrogen at the α-position of the tetrahydrofuran ring is highly unusual; such exchange has no precedent among previous reactions in the B_{12} model series. There does not *appear* to be a direct link between the succinate–methylmalonate rearrangement and the exchange which occurs on the tetrahydrofuran ring. There may be an indirect or associative connection between the two reactions. Products which appear to enjoy the most contact with the corrin nucleus—the rearranged product XVIII and the unrearranged reduced product XIX—show the greatest extent of exchange on the tetrahydrofuran ring, while the recovered starting bromide X shows some exchange but less than the other two products. At first, the exchange occurring on the tetrahydrofuran ring seemed to be a side issue of some interest but not central to the dual problems of hydrogen abstraction from unactivated carbon and subsequent rearrangement. However, in the reactions of some members of the B_{12} rearrangement series, the ethylene glycol and propylene glycol dehydrase and the ribonucleotide reductase reactions, it is carbon-bound hydrogen adjacent to oxygen which is removed. It remains to be seen whether there is any relationship between the exchange observed in the model reaction above and these enzyme-catalyzed rearrangements. It must be borne in mind that in the first two instances the enzymatic conversion of ethylene and propylene glycols to their respective aldehydes, there is no exchange with solvent water. Exchange does occur in the ribonucleotide reductase reaction. The observation of facile exchange in the tetrahydrofuran ring in the model reaction described here warrants further pursuit and definition.

Experimental Section

Melting points were determined on a Fisher-Johns hot stage melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Varian T-60A 60-MHz spectrometer or on the 250- and 600-MHz spectrometer at the Mellon Institute NIH-NMR Facility for Biomedical Studies. Chemial shifts are recorded in parts per million (δ units) relative to tetramethylsilane as internal standard. Carbon-13 NMR spectra were recorded on a Jeol JNM-FX 60 Fourier-Transform 60-MHz spectrometer. Infrared spectra were recorded on a Perkin-Elmer Model 247 Grating Infrared Spectrophotometer as thin films between sodium chloride plates, as dilute solutions in chloroform or carbon tetrachloride, or as KBr pellets. Absorption bands are reported in wave numbers (cm^{-1}) and are uncalibrated. Low-resolution mass spectra were recorded on an LKB 9000 Gas Chromatograph-Mass Spectrometer by direct insertion at ionizing voltages of 70 and 15 eV. High-resolution mass spectra were recorded on a Varian MAT CH-5 spectrometer. Ultraviolet spectra were recorded on a Cary 14 or a Varian Super Scan-3 spectrometer.

Analytical thin-layer chromatography was carried out on Merck PF_{254} 0.25-mm silica gel plates. Compounds were visualized by UV light at 254 nm, by iodine vapor, and by charring after spraying with a 10% solution of cobalt chloride in sulfuric acid. For preparative thick-layer chromatography, commercially available 20 × 20 cm Merck PF_{254} 2-mm silica gel plates were used. Compounds were visualized by UV light at 254 mm. Column chromatographic separation was carried out with Baker 60-200 mesh or E. Merck 70–230 mesh silica gel.

Common reagents were purchased from the Fischer Scientific Company, J. T. Baker Company, and Aldrich Chemical Company and were used without further purification unless otherwise specified. Hydroxocobalamin was purchased from the Merck Company. Vinyllithium was purchased as a 2 M solution in THF from the Research Organic/Inorganic Chemical Corporation and borane was purchased as a 0.94 M solution in THF from Alfa Products.

Distilled reagent grade solvents were used for all chromatographic separations. The following solvents were purified, dried, and stored as indicated: tetrahydrofuran, benzene, and diethyl ether were freshly distilled from the sodium–benzophenone ketyl. Pyridine, hexamethylphosphoramide (HMPA), and triethylamine were distilled from calcium hydride and stored over 4A molecular sieves.

All reactions involving oxygen-sensitive or moisture-sensitive compounds were performed under a nitrogen atmosphere. All model reactions were performed in a flask deaerated by evacuating and flushing with nitrogen 10 times with a nitrogen bubbler. Preparations of alkylcobalamins were performed in a darkroom by addition of the appropriate alkyl bromide to vitamin B_{12}s.

2-Vinyl-2-methyltetrahydrofuran (XIV). A solution of 60 g (0.498 mol) of 5-chloro-2-pentanone (XIII) in 100 mL of tetrahydrofuran was added at −40 °C under a nitrogen atmosphere over a period of 1.5 h to a solution of 274 mL of vinyllithium (2 M solution in THF, 0.548 mol). The reaction mixture was brought to 0 °C over a 2-h period and kept in an ice bath for 2 h. The reaction was then brought to room temperature and stirred overnight. The reaction mixture was taken up in 1000 mL of ether, washed with four 75-mL portions of brine, and dried over $MgSO_4$. The ether was removed by distillation at 60 °C (oil bath temperature). The residual oil was distilled bulb-to-bulb at room temperature under vacuum (1 mm), the product being collected in a liquid nitrogen cooled trap. The distilled liquid weighed 49.6 g and was a 1:1.4 mixture of product XIV and tetrahydrofuran. The yield of the product XIV was 36%.

The proton NMR spectrum ($CDCl_3$) showed a one-proton vinyl doublet of doublets at δ 5.82 (J = 10 and 16 Hz), a one-proton vinyl doublet of doublets at δ 5.13 (J = 2 and 16 Hz), a one-proton vinyl doublet of doublets at δ 4.92 (J = 2 and 8 Hz), a two-proton methylene multiplet at δ 3.72 (CH_2O), a four-proton ring methylene multiplet at δ 1.8, and a three-proton methyl singlet at δ 1.32. The infrared spectrum (neat) showed carbon–carbon double bond absorption at 1640 cm^{-1}. The mass spectrum (15 eV) showed peaks at m/e (relative intensity) 112 (1.6, M^+), 97 (14, $M^+ - CH_3$), 85 (100, methyltetrahydrofuryl).

2-Methyl-2-(2-hydroxyethyl)tetrahydrofuran (XV). Borane (230 mL, 0.94 M solution in THF, 0.214 mol) was added over a period of 1.5 h to a solution of 40 g of 2-vinyl-2-methyltetrahydrofuran (XIII) (0.357 mol) in 80 mL of THF at 0 °C under nitrogen. The reaction was stirred at room temperature for 2.5 h and then cooled in an ice bath. Water (19 mL) was added slowly, followed by 80 mL of 3 N NaOH and 70 mL of 30% H_2O_2. The reaction was then stirred at room temperature for 2 h. The THF solution was decanted from the solid borane complex which was washed with three 30-mL portions of THF. The solvent was evaporated and the residue was distilled under vacuum yielding 20.2 g (100%) of the alcohol XV, bp 47–48 °C (0.4 mm). The proton NMR spectrum ($CDCl_3$) showed a four-proton multiplet at δ 3.8 (CH_2OH and CH_2O), a one-proton hydroxyl singlet at δ 3.4, a six-proton multiplet at δ 1.8, and a three-proton methyl singlet at δ 1.4. The infrared spectrum (neat) showed broad hydroxyl absorption at 3400 cm^{-1}. The mass spectrum (15 eV) showed peaks at m/e (relative intensity) 115 (11, $M^+ - CH_3$), 85 (100, methyltetrahydrofuryl); exact mass calcd for $C_6H_{11}O_2$ ($M^+ - CH_3$) 115.0759, found 115.0766.

(2-Methyltetrahydrofuran-2-yl)ethyl Tosylate (XVI). To a solution of 15 g (0.115 mol) of 2-methyl-2-(2-hydroxyethyl)-tetrahydrofuran (XV) in 36.5 g (0.461 mol) of dry pyridine at 0 °C was added slowly 30.8 g (0.161 mol) of *p*-toluenesulfonyl chloride. The reaction mixture was stirred at 0 °C for 1.5 h and kept at 0 °C for 48 h. The reaction was diluted with 750 mL of ether and the ether suspension was extracted with five 30-mL portions of 10% HCl followed by five 25-mL portions of brine. The ethereal solution was dried over $MgSO_4$, filtered, and

evaporated. The crude product was purified by column chromatography on 600 g of silica gel. The polarity of the eluant was increased from hexane (100 mL) to 10% ether–hexane (150 mL), 25% ether–hexane (250 mL), 40% ether–hexane (200 mL), and 60% ether–hexane (400 mL). The tosylate XVI, R_f 0.48 (60:40, ether–hexane), was eluted in 60% ether–hexane. The yield was 28.14 g (86%). The proton NMR spectrum ($CDCl_3$) showed a four-proton aromatic AB quartet at δ 7.73 and 7.24 (J = 8 Hz), a two-proton methylene triplet at δ 4.1 (J = 7 Hz, CH_2OTs), a two-proton methylene multiplet at δ 3.73 (CH_2O), a three-proton aromatic methyl singlet at δ 2.43, a six-proton multiplet at δ 1.86, and a three-proton methyl singlet at δ 1.13. The mass spectrum (70 eV) showed peaks at m/e (relative intensity) 172 (3.5, TsOH), 112 (7.0, methylvinyltetrahydrofuryl), 97 (22, vinyltetrahydrofuryl), 85 (100, methyltetrahydrofuryl); exact mass calcd for $C_7H_{12}O$ (M^+ – TsOH) 112.0888, found 112.0889.

Ethyl α-[(Ethylthio)carbonyl]tetrahydro-2-methyl-2-furanbutanoate (XVII). A solution of 8.056 g (0.0457 mol) of *O,S*-diethyl thiomalonate in 30 mL of dry benzene was added over a period of 45 min to a suspension of 2.7 g (0.056 mol, 50% suspension in mineral oil) of NaH in 60 mL of dry benzene under nitrogen at room temperature. The reaction was stirred at room temperature for 1.5 h and then treated with a solution of 10 g (0.035 mol) of the tosylate XVI in 10 mL of dry benzene. The reaction mixture was heated at 50 °C for 24 h. It was taken into 600 mL of ether and the ether solution was washed with five 30-mL portions of 5% HCl followed by three 25-mL portions of brine. The ether extract was dried over $MgSO_4$, filtered, and evaporated. The resulting oil was purified by column chromatography on 600 g of silica gel. The column was eluted with hexane (100 mL), 10% ether–hexane (250 mL), 20% ether–hexane (300 mL), 30% ether–hexane (300 mL), and 60% ether–hexane (300 mL). The desired malonate XVII (2.17 g, 21%) was eluted in 40% ether–hexane. The unreacted tosylae XVI (4.086 g) was eluted in 60% ether–hexane. The proton NMR spectrum ($CDCl_3$) of the thiomalonate XVII showed a two-proton quartet at δ 4.14 (J = 7 Hz, $COOCH_2CH_3$), a two-proton multiplet δ 3.8 (CH_2O), a one-proton methine triplet at δ 3.5 (J = 7 Hz), a two-proton quartet at δ 2.9 (J = 7 Hz, $COSCH_2CH_3$), an eight-proton multiplet at δ 2.0–1.8, a six-proton triplet at δ 1.23 (J = 7 Hz, $COOCH_2CH_3$ $COOCH_2CH_3$ and $COSCH_2CH_3$) and a three-proton methyl singlet at δ 1.16. The infrared spectrum (neat) showed ester and thioester carbonyl absorption at 1730 and 1670 cm^{-1}, respectively. The mass spectrum (70 eV) showed peaks at m/e (relative intensity) 227 (12, M^+ – SEt), 209 (7, M^+ – SEt – H_2O), 181 (M^+ – COSEt – H_2O), 85 (100, methyltetrahydrofuryl); exact mass calcd for $C_{12}H_{19}O_4$ (M – SEt) 227.1283, found 227.1282.

Ethyl α-(Bromomethyl)-α-[(ethylthio)carbonyl]tetrahydro-2-methyl-2-furanbutanoate (X). A solution of 2.0 g (0.007 mol) of ethyl α-[(ethylthio)carbonyl]tetrahydro-2-methyl-2-furanbutanoate (XVII) in 6 mL of dry tetrahydrofuran was added slowly to a suspension of 0.499 g (0.0105 mol, 50% suspension in mineral oil) of NaH in 6 mL of tetrahydrofuran containing 1.86 g (0.0105 mol) of hexamethylphosphoramide at room temperature under nitrogen. The reaction mixture was stirred at room temperature for 1 h and then treated with 1.81 g (0.01 mol) of dibromomethane. The reaction mixture was refluxed at 80 °C for 20 h, then diluted with 150 mL of anhydrous ether, and filtered and the solvent evaporated under vacuum. In order to remove the hexamethylphosphoramide, the residue was passed through 50 g of silica gel with 50% ether–hexane. The crude product was then purified by column chromatography on 350 g of silica gel. Fractions of 25-mL volume were collected after elution with hexane (200 mL), 5% ether–hexane (400 mL), 10% ether–hexane (400 mL), and 20% ether–hexane (500 mL). The bromide X was eluted in 20% ether–hexane as 2.072 g (78%) of an oil, R_f 0.46 (12:88 ether–hexane, four developments).

The 250-MHz proton NMR spectrum ($CDCl_3$) showed a two-proton quartet at δ 4.2 (J = 7.2 Hz, $COOCH_2CH_3$), a four-proton multiplet at δ 3.8 (CH_2O and CH_2Br), a two-proton quartet at δ 2.93 (J = 7.2 Hz, $COSCH_2CH_3$), an eight-proton multiplet at δ 2.4–1.8, a three-proton triplet at δ 1.26 (J = 7 Hz, $COOCH_2CH_3$), a three-proton triplet at δ 1.25 (J = 7 Hz, $COSCH_2CH_3$), and a three-proton methyl singlet at δ 1.23. The infrared spectrum (neat) showed absorption at 1730 and 1670 cm^{-1} corresponding to the ester and thioester carbonyls, respectively. The mass spectrum (15 eV) showed peaks at m/e (relative intensity) 321, 319 (5.7, M^+ – SEt), 303, 301 (1.6, M^+ – SEt – H_2O), 85 (100, methyltetrahydrofuryl); exact mass calcd for $C_{13}H_{20}O_4Br$ – (M^+ – SEt) 319.0545, found 319.0531.

Reaction of the Bromide X with Vitamin B_{12} in H_2O. A solution of 0.060 g (0.0016 mol) of $NaBH_4$ in 1 mL of water was added to a solution of 0.0909 g (0.000067 mol) of hydroxocobalamin in 2 mL of water under nitrogen. The reaction mixture was stirred at room temperature for 30–45 min until it became grey-green. The reaction mixture was taken into the dark room and a solution of 0.080 g (0.00021 mol) of the bromide X in 0.25 mL of absolute methanol was added. The reaction mixture was stirred at room temperature at pH 11 in the dark for 24 h. The reaction was extracted with three 25-mL portions of ether, dried over $MgSO_4$, filtered, and evaporated. The resulting oil, weighing 0.052 g, was spotted on a 2-mm silica gel preparative plate and developed four times in 24:76 ether–hexane. Two UV- active bands were separated.

Band 1 (R_f 0.39) was the rearranged product XVIII weighing 0.035 g (54.4%). The 250-MHz NMR spectrum ($CDCl_3$) of the rearranged product XVIII showed a two-proton quartet at δ 4.13 (J = 7.2 Hz, $COOCH_2CH_3$ and a two-proton multiplet at δ 3.8 (CH_2O). In the succinate region (δ 2.5–3.1) there was a quartet (J_{AB} = 16.2 Hz, $J_{A,X}$ = 4.6 Hz) assigned to one of the methylene protons at δ 2.67, a thioester methylene quartet at δ 2.88 which masks the succinate methine proton. The presence of the latter causes a broadening at the base of the thioester quartet. This broadening is absent when the methine position is deuterated. A second quartet (J_{AB} = 16.2 Hz, J_{AX} = 8.3 Hz) assigned to the second succinate methylene proton appears at δ 2.97. In this instance, three of the four peaks of the quartet are visible, the fourth peak at δ 2.92 is masked by one of the peaks of the thioester quartet. In addition, there was an eight-proton multiplet at δ 2.0–1.8, a three-proton triplet at δ 1.25 (J = 7.2 Hz, $COOCH_2CH_3$), a three-proton triplet at δ 1.24 (J = 7.2 Hz, $COSCH_2CH_3$), and a three-proton methyl singlet at δ 1.16. The infrared spectrum (neat) showed carbonyl absorption at 1730 and 1680 cm^{-1} corresponding to the ester and thioester groups. The mass spectrum (70 eV) showed peaks at m/e (relative intensity) 241 (13.4, M – SEt), 195 (17, M^+ – COSEt – H_2O), 85 (100, methyltetrahydrofuryl); exact mass calcd for $C_{13}H_{21}O_4$ (M^+ – SEt) 241.1439, found 241.1427.

Band 2 (R_f 0.49) weighing 0.014 g is a mixture of starting bromide X and unrearranged product XIX. This mixture was spotted on a 0.25-mm silica gel plate and developed five times in 18:82 ether–hexane. The band at R_f 0.42 containing unrearranged product XIX was removed from the plate. Elution of the product from the silica gel with ether yielded 0.003 g (4.5%) of an oil. The 250-MHz NMR spectrum ($CDCl_3$) of XIX showed a two proton quartet at δ 4.16 (J = 7.2 Hz, $COOCH_2CH_3$), a two-proton multiplet at δ 3.8 (CH_2O), a two-proton quartet at δ 2.9 (J = 7 Hz, $COSCH_2CH_3$), an eight-proton multiplet at δ 2.0–1.6, a three-proton methyl singlet at δ 1.46, a three-proton triplet at δ 1.26 (J = 7 Hz, $COOCH_2CH_2$), a three-proton triplet at δ 1.25 (J = 7 Hz, $COSCH_2CH_3$), and a three-proton methyl singlet at δ 1.18. The infrared spectrum (neat) showed carbonyl absorption at 1730 and 1680 cm^{-1} corresponding to ester and thioester groups, respectively. The mass spectrum (70 eV) showed peaks at m/e (relative intensity) 241 (35, M^+ – SEt), 223 (15.4, M^+ – SEt – H_2O), 195 (10.3, M^+ – COSEt – H_2O), 85 (100, methyltetrahydrofuryl); exact mass calcd for $C_{13}H_{21}O_4$ (M^+ – SEt) 241.14398, found 241.14393. A second band at R_f 0.5 containing starting bromide X was removed from the plate and eluted from the silica gel with ether. An oil weighing 0.009 g (11%) was obtained which had spectral properties identical with those of the starting bromide X.

Reaction of the Bromide X with Vitamin B_{12s} in D_2O. A solution of 0.060 g (0.0016 mol) of $NaBH_4$ in 1 mL of D_2O was added to a solution of 0.090 g (0.000067 mol) of hydroxocobalamin in 2 mL of D_2O under nitrogen. The reaction mixture was taken into the dark room and a solution of 0.080 g (0.00021 mol) of the bromide X in 0.25 mL of MeOD was added. The reaction mixture was stirred at room temperature in the dark at pH 11 for 24 h. The reaction was extracted with three 25-mL portions of ether, dried over $MgSO_4$, filtered, and evaporated. The residual oil, weighing 0.050 g, was spotted on a 2-mm silica gel plate and

developed four times in 24:76 ether–hexane. Two UV active bands were separated. Band 1 (R_f 0.37) was the rearranged product XVIII-*d* weighing 0.038 g (58%). The 250-MHz NMR spectrum of XVIII-*d* was identical with that of XVIII with the exception of the succinate region (δ 3.1–2.5) where the deuterated product XVIII-*d* showed a doublet (J_{AB} = 16.2) at δ 2.68, a thioethyl quartet (J = 7.4 Hz) at δ 2.88 (the latter now appears as a clean quartet since the methine proton, now a deuterium, is no longer observed) and a doublet (J_{AB} = 16.2 Hz) at δ 2.94, one component of which overlaps with the lowest field peak of the thioethyl quartet. The mass spectrum (70 eV) showed peaks at *m/e* (relative intensity) 242 (17, M^+ – SEt), 196 (22.9, M^+ – COSEt – H_2O), 86 (14), 85 (100, methyltetrahydrofuryl). The peaks at *m/e* 241 and 242 had an intensity ratio of 3.5:39 corresponding to 92% deuterium incorporation in the rearranged product XVIII-*d*.

Band 2 at R_f 0.46, weighing 0.018 g, was a mixture of starting bromide X and unrearranged product XIX-*d*. This mixture was spotted on 0.25-mm silica gel plate and developed five times in 18:82 ether–hexane. The band at R_f 0.42 containing unrearranged product XIX-*d* was removed from the plate. Elution of the product from the silica gel with ether yielded 0.002 g (3%) of an oil. The 250-MHz proton NMR spectrum ($CDCl_3$) showed a two-proton quartet at δ 4.14 (J = 7 Hz, $COOCH_2CH_3$), a two-proton multiplet at δ 3.8 (CH_2O), a two-proton quartet at δ 2.9 (J = 7.2 Hz, $COSCH_2CH_3$), an eight-proton multiplet at δ 2.0–16, a two-proton methyl multiplet at δ 1.46 (CH_2D), a three-proton triplet at δ 1.26 (J = 7 Hz, $COOCH_2CH_3$), a three-proton triplet at δ 1.25 (J = 7 Hz, $COSCH_2CH_3$), and a three-proton methyl singlet at δ 1.18. The mass spectrum (70 eV) shows peaks at *m/e* (relative intensity) 242 (32, M^+ – SEt), 224 (12.8, M^+ – SEt – H_2O), 196 (8.5, M^+ – COSEt – H_2O), 85 (100, methyltetrahydrofuryl). The peaks at *m/e* 241 and 242 had an intensity ratio of 11:52 corresponding to 82% deuterium incorporation in the unrearranged product XVI-*d*.

Reaction of the Bromide X with Vitamin B_{12s} in D_2O with Potassium Carbonate. Hydroxocobalamin (0.090 g, 0.000067 mol) was added to a solution of 0.362 g (0.0026 mol) of anhydrous K_2CO_3 in 2 mL of D_2O. The hydroxocobalamin is not soluble in this solution. The suspension was placed under nitrogen and a solution of 0.060 g (0.0016 mol) of $NaBH_4$ in 1 mL of D_2O was added. The reaction mixture was stirred at room temperature for 0.5 h. The reduced vitamin B_{12} was not dissolved but remained as a dark grey precipitate in a brown colored solution. The reaction mixture was removed to the darkroom and a solution of 0.080 g (0.00021 mol) of bromide X in 0.25 mL of MeOD was added. The reaction mixture was stirred at room temperature and pH 12.6 for 24 h. At the end of the reaction, the homogeneous red solution was extracted with four 25-mL portions of ether, dried over $MgSO_4$, filtered, and evaporated. The resulting oil was spotted on a 2-mm silica gel prep plate and developed four times in 24:76 ether–hexane. Two UV active bands were separated. Band 1 at R_f 0.38 was rearranged product XVIII-*d* weighing 0.030 g (45.3%). The infrared and 250-MHz NMR spectral data were not distinguishable from those of the rearranged product XVIII-*d* previously isolated from the reaction in D_2O. However, in the mass spectrum of the rearranged product XVIII-*d* the peak at *m/e* 86 is 50% of that at *m/e* 85, indicating incorporation of deuterium in the methyltetrahydrofuran ring. The position of incorporation of deuterium was established by deuterium NMR spectroscopy. The deuterium NMR spectrum ($CHCl_3$) showed resonances at δ 3.77 corresponding to the deuterium on carbon next to oxygen in the tetrahydrofuran ring and at δ 2.83 corresponding to the deuterium on the succinate methine carbon ($CDCH_2$).

Band 2 at R_f 0.48 was a mixture of starting bromide X and unrearranged product XIX-*d* weighing 0.022 g. The mixture was spotted on an 0.25-mm silica gel plate and developed five times in 18:82 ether–hexane. Unrearranged product XIX-*d*, R_f 0.41, weighing 0.011 g (17%) was isolated. The infrared and proton NMR spectra were very similar to those of the unrearranged product XIX-*d* obtained from the D_2O reaction. The mass spectrum (15 eV) showed peaks at *m/e* (relative intensity) 242 (19, M^+ – SEt), 224 (10, M^+ – SEt – H_2O), 196 (8, M^+ – COSEt – H_2O), 86 (48, methyltetrahydrofuryl-d_1), 85 (110, methyltetrahydrofuryl). The presence of the strong peak at *m/e* 86 suggested that deuterium was incorporated also within the methyltetrahydrofuran nucleus of the unrearranged product XIX-*d*. The deuterium NMR spectrum ($CHCl_3$) showed resonances at δ 3.77 corresponding to the deuterium on carbon adjacent to the oxygen on the methyltetrahydrofuran ring and δ 1.43 corresponding to the deuterium on the methyl group of the methylmalonate portion of the molecule. The recovered starting bromide, R_f 0.48, weighed 0.007 g. The infrared and proton NMR spectra were very similar to those of an authentic sample of the bromide X. The mass spectrum showed peaks at *m/e* 85 and 86 in the ratio 100:13 indicating 7–8% deuterium incorporation on the methyltetrahydrofuran ring. The deuterium NMR spectrum ($CHCl_3$) showed a peak at δ 3.78 corresponding to the presence of deuterium on the carbon adjacent to oxygen in the tetrahydrofuran ring.

Control Reaction. Hydroxocobalamin (0.045 g, 0.0335 mol) was added to a solution of 0.181 g (0.00013 mol) of anhydrous K_2CO_3 in 1 mL of D_2O. The hydroxocobalamin is not soluble in this solution. The suspension was placed under nitrogen and a solution of 0.030 g (0.00079 mol) of $NaBH_4$ in 0.5 mL of D_2O was added. The reaction mixture was stirred at room temperature for 0.5 h. The reduced vitamin B_{12s} was not dissolved but remained as a dark grey precipitate in a brown colored solution. The reaction mixture was taken into the darkroom and a solution of 0.030 g (0.0001 mol) of rearranged product XVIII in 0.125 mL of MeOD was added. The reaction mixture was stirred at room temperature for 24 h. At the end of this time, the pH of the reaction mixture was approximately 12.5 and it was a homogeneous red solution. The reaction was extracted with three 25-mL portions of ether, dried over $MgSO_4$, filtered, and evaporated. The residue was spotted on a 2-mm silica gel plate and developed four times in 24:76 ether–hexane. The UV active band at R_f 0.38 was removed from the plate and eluted with ether. The recovered rearranged product XVIII thus isolated weighed 0.019 g (64%) and had identical infrared, NMR, and mass spectral properties with those of the rearranged product XVIII obtained from the B_{12} rearrangement reaction. In particular, in the mass spectrum the peak at *m/e* 86 was 5% as intense as the base peak at *m/e* 85.

The strongest peak in the spectrum if the M^+ – SEt fragment at *m/e* 241. In the rearranged product XVIII from rearrangement in H_2O the peak at *m/z* 242 is 17.6% as intense as the 241 peak, a value slightly larger than that (13%) which might be accounted for by natural abundance carbon-13. In the rearranged product XVIII from the control reaction the peak at *m/e* 242 is 41% as intense as the peak at *m/e* 241 indicating the probability of base-catalyzed exchange of deuterium into the succinate portion of the molecule.

1,2-Di-*tert*-butyl 1-Ethyl 1,1,2-Ethanetricarboxylate (XX). A solution of 2.0 g (0.0106 mol) of ethyl *tert*-butyl malonate in 15 mL of dry benzene was added over a period of 30 min to a suspension of 0.560 g (50% suspension in mineral oil, 0.0117 mol) of sodium hydride in 10 mL of benzene at room temperature under a nitrogen atmosphere. After the addition was complete, the reaction mixture was stirred at room temperature for 25 min. *tert*-Butyl bromoacetate (2.289 g, 0.0111 mol) was added and the reaction mixture was heated at 50 °C for 4 h. After dilution with 50 mL of anhydrous ether, the solid material was removed by filtration and the filtrate was evaporated. The crude product was distilled under vacuum yielding 2.065 g (64%) of the triester XX, bp 115–118 °C (0.4 mm). The proton NMR spectrum ($CDCl_3$) showed a two-proton quartet at δ 4.14 (J = 7 Hz, $COOCH_2CH_3$), a one-proton methine triplet at δ 3.6 (J = 7 Hz, $CHCH_2$), a two-proton doublet at δ 2.72 (J = 7 Hz, $CHCH_2$), an eighteen-proton *tert*-butyl singlet at δ 1.45, and a three-proton methyl triplet at δ 1.22 (J = 7 Hz, $COOCH_2CH_3$). The infrared spectrum (neat) showed ester carbonyl absorption at 1740 cm^{-1}.

1,2-Di-*tert*-butyl 2-Ethyl 4-(Tetrahydro-2-methyl-2-furanyl)-1,2,2-butanetricarboxylate (XXI). A solution of 0.994 g (0.0033 mol) of 1,2-di-*tert*-butyl 1-ethyl 1,1,2-ethanetricarboxylate (XX) in 5 mL of dry tetrahydrofuran was added slowly to a suspension of 0.2 g (0.042 mol, 50% suspension in mineral oil) of NaH in 4 mL of tetrahydrofuran containing 0.670 g (0.0037 mol) of HMPA at room temperature under nitrogen. The reaction mixture was stirred at room temperature for 45 min and then treated with the 0.85 g (0.003 mol) of tosylate XVI. The reaction

mixture was refluxed at 75 °C for 24 h. It was then diluted with 300 mL of anhydrous ether and filtered and the solvent evaporated under vacuum. The residue was purified by column chromatography on 200 g of silica gel. The column was eluted with hexane (100 mL), 10% ether–hexane (150 mL), 20% ether–hexane (200 mL), 30% ether–hexane (250 mL), 40% ether–hexane (250 mL), 50% ether–hexane (200 mL), and 60% ether–hexane (200 mL). The desired ester XXI weighing 0.755 g (72%), R_f 0.41 (40:60 ether–hexane) was eluted with 40% ether–hexane. The tosylate XVI (0.130 g) was recovered in 60% ether–hexane.

The proton NMR spectrum ($CDCl_3$) of the ester XXI showed a two-proton quartet at δ 4.2 (J = 7 Hz, $COOCH_2CH_3$), a two-proton multiplet at δ 3.8 (CH_2O), a two-proton methylene singlet at δ 2.83, an eight-proton multiplet at δ 2.0–1.8, an eighteen-proton *tert*-butyl singlet at δ 1 43, a three-proton triplet at δ 1.26 (J = 7Hz, $COOCH_2CH_3$), and a three-proton methyl singlet at δ 1.16. The infrared spectrum (neat) showed a broad ethyl ester and *tert*-butyl ester carbonyl absorption at 1740 cm^{-1}. The mass spectrum (15 eV) showed peaks at m/e (relative intensity) 358 (1, $M^+ - (CH_3)_2C{=}CH_2$), 303 (1.6, $M^+ - [(CH_3)_2C{=}CH_2]_2$), 285 (11, $M^+ - (CH_3)_2C{=}CH_2 - OC_4H_9$), 257 (6.7, $M^+ - COOC_4H_9 - (CH_3)_2C{=}CH_2$); exact mass calcd for $C_{18}H_{30}O_7$ ($M^+ - C_4H_8$) 358.1992, found 358.1997.

2-Ethyl Dihydrogen 4-(Tetrahydro-2-methyl-2-furanyl)-1,2,2-butanetricarboxylate (XXII). A reaction mixture consisting of 0.216 g (0.00063 mol) of the ester XXI, 1.1 g (0.0096 mol) of trifluoroacetic acid, and 0.148 g (0.0082 mol) of H_2O was stirred at room temperature for 20 h. The solvent was evaporated under vacuum and the residue was purified by column chromatography on silica gel. The column was eluted with 50% ethyl acetate–hexane (100 mL), ethyl acetate (100 mL), and 1% acetic acid in ethyl acetate (70 mL). The diacid XXII weighing 0.170 g (90%), R_f 0.13 (80:20:0.2 ethyl acetate–hexane–acetic acid), was eluted in 1% acetic acid–ethyl acetate. The proton NMR spectrum ($CDCl_3$) of the diacid XXII showed a two-proton carboxylic acid singlet at δ 11.1, a two-proton quartet at δ 4.2 (J = 7 Hz, $COOCH_2CH_3$), a two-proton quartet at δ 3.82 (CH_2O), a two-proton methylene singlet at δ 3.06, an eight-proton multiplet at δ 2.0–1.8, a three-proton triplet at δ 1.26 (J = 7 Hz, $COOCH_2CH_3$), and a three-proton methyl singlet at δ 1.16. The infrared spectrum ($CHCl_3$) showed broad carboxylic acid absorption between 3500 and 2800 cm^{-1} and absorption at 1740 and 1700 for ethyl ester and carboxylic acid carbonyls, respectively. The mass spectrum (15 eV) showed peaks at m/e (relative intensity) 269 (0.2, $M^+ - CH_3 - H_2O$), 243 (1.5, $M^+ - CO_2 - CH_3$), 213 (0.8, $M^+ - CO_2 - OEt$), 195 (3, $M^+ - CO_2 - OEt - H_2O$), 85 (100, methyltetrahydrofuryl); exact mass calcd for $C_{12}H_{19}O_5$ ($M - CO_2 - CH_3$) 243.1232, found 243.1232.

1-Ethyl Hydrogen 2-[2-(Tetrahydro-2-methyl-2-furanyl)ethyl]butanedioate (XXIII). The neat diacid XXII (0.11 g, 0.00036 mol) was heated under vacuum (0.4 mm) at 80 °C for 20 h and then at 95 °C for 3 h. The resulting crude acid mixture was purified by column chromatography on 12 g of silica gel. The column was eluted with 20% ethyl acetate–hexane (100 mL), 40% ethyl acetate–hexane (50 mL), 50% ethyl acetate–hexane (50 mL), and 1% acetic acid–ethyl acetate (40 mL). The desired product XXIII, R_f 0.43 (80:20:0.2 ethyl acetate–hexane–acetic acid), weighing 0.040 g (43%) was eluted in 50% ethyl acetate–hexane. Some of the diacid XXII (0.045 g) was recovered in 1% acetic acid–ethyl acetate.

The proton NMR spectrum ($CDCl_3$) of the acid XXIII showed a one-proton carboxylic acid singlet at δ 10.4, a two-proton quartet at δ 4.13 (J = 7.2 Hz, $COOCH_2CH_3$), a two-proton multiplet at δ 3.8 (OCH_2), a three-proton multiplet at δ 2.8 ($CHCH_2$), an eight-proton multiplet at δ 2.0–1.8, a three-proton triplet at δ 1.26 (J = 7.2 Hz, $COOCH_2CH_3$), and a three-proton methyl singlet at δ 1.16. The infrared spectrum ($CHCl_3$) showed broad absorption between 3300 and 2800 cm^{-1} for the carboxylic acid and absorption at 1730 cm^{-1} corresponding to the ester and carboxylic acid carbonyls. The mass spectrum (70 eV) showed peaks at m/e (relative intensity) 243 (0.5, $M^+ - CH_3$), 213 (0.5, $M^+ - OEt$), 195 (1.4, $M^+ - OEt - H_2O$), 180 (1.0, $M^+ - OEt - CH_3 - H_2O$), 85 (100, methyltetrahydrofuranyl); exact mass calcd for $C_{12}H_{19}O_5$ ($M^+ - CH_3$) 243.1232, found 243.1234.

Authentic Ethyl α-[2-(Ethylthio)-2-oxoethyl]tetrahydro-2-methyl-2-furanbutanoate (XVIII). *N,N'*-Dicyclohexylcarbodiimide (0.043 g, 0.00021 mol) was added to a solution of 0.045 g (0.00017 mol) of the acid XXIII and 0.0216 g (0.00035 mol) ethanethiol in 0.5 mL of dry tetrahydrofuran under nitrogen at room temperature. The reaction mixture was stirred at room temperature for 20 h. The dicyclohexylurea was removed by suction filtration and washed with three 10-mL portions of anhydrous ether. The solvent was removed from the filtrate under vacuum. The crude product was then spotted on a 2-mm silica gel plate and developed three times in 30:70 ether–hexane. The UV active band at R_f 0.35 was removed from the plate and the product was eluted from the silica gel with ether. Evaporation of the ether yielded 0.019 g (36%) of the desired thioester (XVIII). The infrared, 250-MHz NMR, and mass spectra were identical with those of the rearranged product XVIII isolated from the vitamin B_{12} rearrangement reaction described above.

Authentic Sample of Ethyl α-(Methyl)-α-[(ethylthio)-carbonyl]tetrahydro-2-methyl-2-furanbutanoate (XIX). A solution of 0.1 g (0.00035 mol) of ethyl α-[(ethylthio)carbonyl]-tetrahydro-2-methyl-2-furanbutanoate (XIII) in 0.5 mL of dry tetrahydrofuran was added to a suspension of 0.025 g (50% suspension in mineral oil, 0.00052 mol) of NaH in 1.5 mL of tetrahydrofuran containing 0.093 g (0.00052 mol) of HMPA at room temperature under nitrogen. The reaction mixture was stirred at room temperature for 1 h and then treated with 0.074 g (0.00052 mol) of methyl iodide. The reaction mixture was heated at 65 °C for 20 h, then diluted with 30 mL of anhydrous ether, and filtered and the solvent evaporated under vacuum. The residue was passed through 5 g of silica gel with 50% ether–hexane to remove hexamethylphosphoramide. The solvent was then removed under vacuum yielding 0.092 g (88%) of the desired product XIX, R_f 0.43 (18:82 ether–hexane five developments). The infrared, 250-MHz NMR, and mass spectra were identical with those of the unrearranged product XIX isolated from the vitamin B_{12} rearrangement reaction described above.

2,2-Di-*tert*-butyl 1-Ethyl 4-(Tetrahydro-2-methyl-2-furanyl)-1,2,2-butanetricarboxylate (XXV). A solution of 0.994 g (0.0033 mol) of 1,1-di-*tert*-butyl 2-ethyl 1,1,2-ethanetricarboxylate (XXIV) in 5 mL of tetrahydrofuran was added to a suspension of 0.2 g (0.0042 mol, 50% suspension in mineral oil) of NaH in 4 mL of tetrahydrofuran containing 0.67 g (0.0037 mol) of hexamethylphosphoramide under nitrogen at room temperature. The reaction mixture was stirred at room temperature for 45 min and then treated with 0.85 g (0.003 mol) of tosylate XVI. The reaction mixture was refluxed at 75 °C for 24 h. It was then diluted with 300 mL of anhydrous ether and filtered and solvent was evaporated under vacuum. The residue was purified by column chromatography on 200 g of silica gel. The column was eluted with hexane (100 mL), 10% ether–hexane (150 mL), 20% ether–hexane (200 mL), 30% ether–hexane (250 mL), 40% ether–hexane (250 mL), 50% ether–hexane (200 mL), and 60% ether–hexane (200 mL). The desired product, R_f 0.41 (40:60 ether–hexane), was eluted as 0.698 g (51%) of an oil in 40% ether–hexane. Some of the tosylate XVI (0.161 g) was recovered in 60% ether–hexane.

The proton NMR spectrum ($CDCl_3$) of XXV showed a two-proton quartet at δ 4.16 (J = 7 Hz, $COOCH_2CH_3$), a two-proton multiplet at δ 3.8 (CH_2O), a two-proton methylene singlet at δ 2.86, an eight-proton multiplet at δ 2.0–1.8, an eighteen-proton *tert*-butyl singlet at δ 1.46, a three-proton methyl triplet at δ 1.24 (J = 7 Hz, $COOCH_2CH_3$), and a three-proton methyl singlet at δ 1.18. The infrared spectrum ($CHCl_3$) showed a broad ethyl ester and *tert*-butyl ester carbonyl absorption at 1740 cm^{-1}. The mass spectrum (15 eV) showed peaks at m/e (relative intensity) 358 (0.5, $[M^+ - (CH_3)_2C{=}CH_2]$), 303 (1.5, $M^+ - H - [(CH_3)_2C{=}CH_2]_2$), 285 [10.9, $M^+ - OC_4H_9 - (CH_3)_2C{=}CH_2$], 85 (100, methyltetrahydrofuranyl); exact mass calcd for $C_{14}H_{21}O_6$ [$M^+ - OC_4H_9 - (CH_3)_2C{=}CH_2$] 285.1338, found 285.1337.

1-Ethyl Dihydrogen 4-(Tetrahydro-2-methyl-2-furanyl)-1,2,2-butanetricarboxylate (XXVI). A reaction mixture consisting of 0.38 g (0.00092 mol) of the triester XXV, 1.6 g (0.014 mol) of trifluoroacetic acid, and 0.215 g (0.0119 mol) of H_2O was stirred at room temperature for 20 h. The solvent was removed under vacuum and the residue was purified by column chromatography on 18 g of silica gel. The column was eluted with 50% ethyl acetate–hexane (150 mL), ethyl acetate (150 mL), and 1% acetic acid–ethyl acetate (100 mL). The

product XXVI, R_f 0.12 (80:20:02 ethyl acetate–hexane–acetic acid), was eluted in 1% acetic acid–ethyl acetate yielding 0.234 g (84%) of an oil.

The proton NMR spectrum ($CDCl_3$) of XXVI showed a two-proton carboxylic acid singlet at δ 12.0, a two-proton quartet at δ 4.1 (J = 7 Hz, $COOCH_2CH_3$), a two-proton multiplet at δ 3.82 (CH_2O), a two-proton methylene singlet at δ 3.06, an eight-proton multiplet at δ 2.0–1.8, a three-proton triplet at δ 1.24 (J = 7 Hz, $COOCH_2CH_3$), and a three-proton methyl singlet at δ 1.18. The infrared spectrum ($CHCl_3$) showed broad absorption between 3500 and 2800 cm^{-1} for the carboxylic acid group and absorption at 1740 and 1710 for the ethyl ester and carboxylic acid carbonyls, respectively. The mass spectrum (70 eV) showed peaks at m/e (relative intensity) 243 (2.5, $M^+ - CO_2 - CH_3$), 213 (1.8, $M^+ - CO_2 - OEt$), 195 (5, $M^+ - CO_2 - OEt - H_2O$), 85 (100, methyltetrahydrofuranyl); exact mass calcd for $C_{12}H_{19}O_5$ ($M^+ - CO_2CH_3$) 243.1232, found 243.1228.

4-Ethyl 1-Hydrogen 2-[2-(Tetrahydro-2-methyl-2-furanyl)ethyl]butanedioate (XXVII). The neat diacid XXVI (0.18 g, 0.0006 mol) was heated at 80 °C under vacuum (0.4 mm) for 20 h. The crude product was then purified by column chromatography on 10 g of silica gel. The column was eluted with 20% ethyl acetate–hexane (100 mL), 40% ethyl acetate–hexane (50 mL), and 50% ethyl acetate–hexane (50 mL). The mono acid XXVII, R_f 0.43 (80:20:02 acetate–hexane–acetic acid), was eluted in 50% ethyl acetate–hexane yielding 0.124 g (81%) on an oil.

The proton NMR spectrum ($CDCl_3$) showed a one-proton acid singlet at δ 11, a two-proton quartet at δ 4.1 (J = 7 Hz, $COOCH_2CH_3$), a three-proton multiplet at δ 2.7 ($CHCH_2$), an eight-proton multiplet at δ 2.0–1.8, a three-proton multiplet at δ 1.24 (J = 7 Hz, $COOCH_2CH_3$), and a three-proton methyl singlet at δ 1.18. The infrared spectrum ($CHCl_3$) showed broad absorption between 3300 and 2800 cm^{-1} for the carboxylic acid and absorption at 1730 cm^{-1} for the ester and carboxylic acid carbonyls. The mass spectrum (15 eV) showed peaks at m/e (relative inensity) 243 (2.4, $M^+ - CH_3$), 225 (1.2, $M^+ - CH_3 - H_2O$), 213 (1.7, $M^+ - OEt$), 195 (2.8, $M^+ - OEt - H_2O$), 85 (100, methyltetrahydrofuranyl); exact mass calcd for $C_{12}H_{19}O_5$ ($M^+ - CH_3$) 243.1232, found 243.1229.

Ethyl β-[(Ethylthio)carbonyl]tetrahydro-2-methyl-2-furanpentanoate (XXVIII). *N,N'*-Dicyclohexylcarbodiimide (0.043 g, 0.00021 mol) was added to a solution of 0.045 g (0.00017 mol) of the acid XXVII and 0.0216 g (0.00035 mol) of ethanethiol in 0.5 mL of dry tetrahydrofuran under nitrogen at room temperature. The reaction mixture was stirred at room temperature for 20 h. Precipitated dicyclohexylurea was removed by filtration and washed with three 10-mL portions of anhydrous ether. The solvent was removed from the filtrate under vacuum. The crude product was then spotted on a 2-mm silica gel plate and developed three times in 30:70 ether–hexane. The UV active band at R_f 0.34 was removed from the plate and the product was eluted with ether. Evaporation of the solvent yielded 0.018 g (34%) of the desired product XVIII. The 250-MHz proton NMR spectrum ($CDCl_3$) showed a two-proton quartet at δ 4.12 (J = 7.2 Hz, $COOCH_2CH_3$), a two-proton multiplet at δ 3.8 (CH_2O), a one-proton methine multiplet at δ 3.03, a two-proton quartet at δ 2.88 (J = 7.4 Hz, $COSCH_2CH_3$), a one-proton doublet of doublets at δ 2.74 (J = 8.5 and J_{AB} = 15.4 Hz, H_A), a one-proton doublet of doublets at δ 2.42 (J = 5.7 and J_AB = 15.4, H_B), an eight-proton multiplet at δ 2.0–1.6, a six-proton triplet at δ 1.25 (J = 7.2 Hz, $COOCH_2CH_3$ and $COSCH_2CH_3$), and a three-proton methyl singlet at δ 1.15. The infrared spectrum ($CHCl_3$) showed absorption at 1730 and 1680 cm^{-1} corresponding to ester and thioester carbonyls, respectively. The mass spectrum (15 eV) showed peaks at m/e (relative intensity) 241 (41, $M^+ - SEt$), 223 (10.7, $M^+ - SEt - H_2O$), 195 (35, $M^+ - COSEt - H_2O$), 85 (100, methyltetrahydrofuryl); exact mass calcd for $C_{13}H_{21}O_4$ ($M^+ - SEt$) 241.1440, found 241.1444.

5-Chloro-2-pentanone-*1,1,1,3,3-d*$_5$ (XIII-*d*$_5$). A mixture of 100 g (0.833 mol) of 5-chloro-2-pentanone (XIII), 8 g (0.0578 mol) of anhydrous potassium carbonate, and 100 g of D_2O was refluxed at 105 °C (oil bath temperature) for 30 min under a nitrogen atmosphere. The upper organic layer weighing 81 g was separated from the aqueous layer, treated with 80 g of fresh D_2O and 6.48 g (0.0468 mol) of anhydrous potassium carbonate, and refluxed again at 105 °C for 30 min. The organic layer weighing 43 g was separated and passed through a 200-g column of silica gel to remove colored impurities. The product weighing 35.4 g was eluted with 15% ether–hexane then further purified by distillation under vacuum yielding 32.9 g (32%) of the chloro ketone XIII-d_5, bp 72–74 °C (19 mm). The proton NMR spectrum ($CDCl_3$) showed a two-proton triplet at δ 3.56 (J = 6 Hz, $ClCH_2CH_2$) and a two-proton multiplet at δ 2.13 ($CH_2CH_2CD_2$). The infrared spectrum (neat) showed carbon–deuterium absorption at 2200 and 2100 cm^{-1} and carbonyl absorption corresponding to the ketone at 1700 cm^{-1}.

Ethyl α-(Bromomethyl)-α-[(ethylthio)carbonyl]tetrahydro-2-(methyl-*d*$_3$)-2-furan-3,3-*d*$_2$-butanoate (X-*d*$_5$). The sequence of reactions leading to the deuterated bromo thioester X-d_5 from the deuterated chloro ketone was the same as that for the nondeuterated bromo thioester X described above. The proton NMR spectrum ($CDCl_3$) of X-d_5 showed a two-proton quartet at δ 4.16 (J = 7 Hz, $COOCH_2CH_3$), a four-proton multiplet at δ 3.86 (OCH_2, CH_2Br), a two-proton quartet at δ 2.9 (J = 7 Hz, $COSCH_2CH_3$), a six-proton multiplet at δ 2.4–1.8, a three-proton triplet at δ 1.26 (J = 7 Hz, $COOCH_2CH_3$), and a three-proton triplet at δ 1.24 (J = 7 Hz, $COSCH_2CH_3$). The infrared spectrum (neat) showed carbon–deuterium absorption at 2200 cm^{-1} and carbonyl absorptions at 1730 and 1670 cm^{-1} corresponding to the ester and thioester groups. The mass spectrum of the bromide X-d_5 showed peaks corresponding to the methyltetrahydrofuryl fragment at m/e 86 (d_1, 1.5%), 87 (d_2, 9%), 88 (d_3, 23%), 89 (d_4, 37%), and 90 (d_5, 27%). The level of deuteration was thus approximately 74%. In other respects the fragmentation pattern was similar to that of the bromide X.

Reaction of the Bromide X-*d*$_5$ with Vitamin B$_{12s}$ in H$_2$O. A solution of 0.060 g of $NaBH_4$ (0.0016 mol) in 1 mL of water was added under nitrogen to a solution of 0.090 g (0.000067 mol) of hydroxocobalamin in 2 mL of water. The reaction mixture was stirred at room temperature for 30–45 min until it became grey-green. The reaction mixture was taken into the darkroom and a solution of 0.080 g (0.00021 mol) of the bromide X-d_5 in 0.25 mL of absolute methanol was added. The resulting resolution, pH 11, was stirred at room temperature in the dark for 24 h. At the end of this time, the reaction was extracted with three 25-mL portions of ether, dried over $MgSO_4$, filtered, and evaporated. The crude product was spotted on a 2-mm silica gel preparative plate and developed four times in 24:76 ether–hexane. Band 1 at R_f 0.4 was found to be rearranged product XVIII-d_5 weighing 0.038 g (59%). The 250-MHz proton NMR spectrum ($CDCl_3$) showed a two-proton quartet at δ 4.14 (J = 7.2 Hz, $COOCH_2CH_3$), a two-proton multiplet at δ 3.8 (OCH_2), a one-proton doublet of doublets at δ 2.96 (J = 8.1 and J_{AB} = 14.6 Hz, H_A), a methine proton resonance at the same chemical shift as that of the thioester methylene quartet at δ 2.87 (J = 7.2 Hz), a one-proton doublet of doublets at δ 2.67 (J = 4.6 and J_AB = 14.6 Hz, H_B), a six-proton multiplet at δ 2.0–1.8, a three-proton triplet at δ 1.25 (J = 7.2 Hz, $COOCH_2CH_3$), and a three-triplet at δ 1.24 (J = 7.2 Hz, $COOCH_2CH_3$). The deuterium NMR spectrum showed resonance at δ 0.93 and at δ 1.5 corresponding to the deuteriums on the methyl and C-3 methylene carbons of the methyltetrahydrofuran ring, respectively. No absorption could be observed in the region of δ 2.9 which would correspond to transfer of deuterium from the methyltetrahydrofuran ring to the methine carbon of the succinate part of the molecule. It was estimated that 5% of such deuterium incorporation could easily have been detected. The infrared spectrum ($CHCl_3$) showed carbon–deuterium absorption at 2200 cm^{-1} and carbonyl absorptions at 1730 and 1680 cm^{-1} corresponding to ester and thioester groups, respectively. The mass spectrum showed peaks corresponding to the methyltetrahydrofuran fragment at m/e 86 (d_1, 1.8%), 87 (d_2, 7.5%), 88 (d_3, 22.4%), 89 (d_4, 37.7%), and 90 (d_5, 30.5%). The fragmentation pattern was, in all other respects, similar to that of the rearranged product XVIII.

Band 2 at R_f 0.48 was found to be the unrearranged product XIX-d_5 weighing 0.012 g (18%). The proton NMR spectrum ($CDCl_3$) showed a two-proton quartet at δ 4.18 (J = 7.2 Hz, $COOCH_2CH_3$), a two-proton triplet at δ 3.8 (J = 6.5 Hz, OCH_2CH_2), a two-proton quartet at δ 2.9 (J = 7 Hz, $COSCH_2CH_3$), a six-proton multiplet at δ 2.0–1.8, a three-proton methyl singlet at δ 1.46, and a six-proton triplet at δ 1.26 (J = 7.2 Hz, $COOCH_2CH_3$ and $COSCH_2CH_3$). The deuterium NMR spectrum showed peaks at δ 1.18 and 1.5 corresponding to the methyl and methylene groups on the tetrahydrofuran ring. No resonance

could be seen corresponding to the methyl group of the methylmalonate unit at δ 1.46, which would correspond to an intramolecular hydrogen-transfer reaction. The infrared spectrum ($CHCl_3$) showed carbon–deuterium absorption at 2200 cm^{-1} and carbonyl absorptions at 1730 and 1680 cm^{-1} corresponding to ester and thioester groups, respectively. The mass spectrum showed fragmentation similar to that of the unrearranged product XIX with peaks for the methyltetrahydrofuryl fragment at m/e 86 (d, 2%), 87 (d_2, 7.2%), 88 (d_3, 20%), 89 (d_4, 38.6%), and 90 (d_5, 32%).

Reaction of the Bromide X-d_5 with Vitamin B_{12s} in H_2O with Potassium Carbonate. Hydroxocobalamin (0.090 g, 0.000067 mol) was added to a solution of 0.362 g (0.0026 mol) of anhydrous K_2CO_3 in 2 mL of H_2O. The hydroxocobalamin is not soluble in this solution. The suspension was placed under nitrogen and a solution of 0.060 g (0.0016 mol) of $NaBH_4$ in 1 mL of H_2O was added. The reaction mixture was stirred at room temperature for 30 min. The reduced vitamin B_{12s} was not dissolved but remained as a dark grey precipitate in a brown colored solution. The reaction mixture was taken into the darkroom and a solution of 0.080 g (0.00021 mol) of the bromide X-d_5 in 0.25 mL of absolute methanol was added. The reaction mixture, pH 12.5, was stirred at room temperature for 24 h at which time it was a homogeneous red solution. The reaction was extracted with four 25-mL portions of ether, dried over $MgSO_4$, filtered, and evaporated. The resulting oil was spotted on a 2-mm silica gel plate and developed four times in 24:76 ether–hexane.

Band 1 at R_f 0.38 was the rearranged product XVIII-d_5 weighing 0.032 g (50%). The spectral data obtained on this product was identical with that obtained for the rearranged product isolated from reaction in water without added potassium carbonate described above. Band 2 at R_f 0.47 was a mixture of unrearranged product XIX-d_5 and starting material X-d_5 weighing 0.018 g. This mixture was spotted on 0.25-mm silica gel plate and developed five times in 18:82 ether–hexane. The unrearranged product XIX-d_5, R_f 0.39, weighed 0.009 g (14%) and had very similar spectral data to that of the unrearranged product XIX-d_5 isolated from the reaction in water. The recovered bromide X-d_5, R_f 0.47, weighed 0.005 g.

Ethyl Levulineate Ethylene Acetal (XXX). A mixture of 72 g (0.5 mol) of ethyl levulineate, 500 mL of dry benzene, 34.1 g (0.55 mol) of ethylene glycol, and 0.250 g (0.0013 mol) of p-toluenesulfonic acid was refluxed for 18 h, using a Dean–Stark apparatus. The reaction was allowed to cool and washed with three 30-mL portions of 10% $NaHCO_3$, followed by three 25-mL portions of brine. The organic extract was dried over $MgSO_4$, filtered, and evaporated. The crude product was distilled under high vacuum yielding 92.68 g (98%) of the ketal XXX, bp 55–56 °C (0.1 mm). The proton NMR spectrum ($CDCl_3$) of XXX showed a two-proton quartet at δ 4.13 (J = 7 Hz, $COOCH_2CH_3$), a four-proton singlet at δ 3.93 (OCH_2CH_2O), a four-proton multiplet at δ 2.4–1.8, a three-proton methyl singlet at δ 1.33, and a three-proton triplet at δ 1.26 (J = 7 Hz, $COOCH_2CH_3$). The infrared spectrum (neat) showed ester carbonyl absorption at 1740 cm^{-1}.

2,2-(Ethylenedioxy)pentan-5-ol-5,5-d_2 (XXXI). A solution of 22.39 g (0.119 mol) of the ester ketal XXX in 200 mL of anhydrous ether was added over a period of 45 min to a suspension of 5.0 g (0.119 mole) of $LiAlD_4$ in 200 mL of anhydrous ether. The reaction refluxed during the addition. It was stirred at room temperature for 2 h and then decomposed by the addition of 10 mL of water. The mixture was diluted with 400 mL of ether and the salts were removed by filtration. The ether solution was dried over $MgSO_4$, filtered, and evaporated yielding 17.12 g (97%) of the desired alcohol. The proton NMR spectrum ($CDCl_3$) showed a four-proton singlet at δ 3.96 (OCH_2CH_2O), a one-proton hydroxyl singlet at δ 2.06, a four-proton singlet at δ 1.73, and a three-proton methyl singlet at δ 1.36. The infrared spectrum (neat) showed hydroxyl absorption at 3400 cm^{-1} and C–D absorption at 2200 and 2100 cm^{-1}. The mass spectrum (70 eV) showed peaks at m/e (relative intensity) 133 (21, $M^+ - CH_3$), 115 (8, $M^+ - CH_3 - H_2O$), 87 (100, $M^+ - C_3H_5D_2O$).

5-Chloro-2-pentanone-5,5-d_2 (XIII-d_2). A mixture of 17.5 g (0.119 mol) of the deuterated ketal alcohol XXXI, 60 mL of concentrated hydrochloric acid, and 80 mL of water was heated in an oil bath at 125–130 °C. An azeotropic mixture of the chloro ketone XIII-d_2 and water, bp 98–103 °C, distilled from the reaction. The distillate (75 mL) was extracted with five 75-mL portions of ether. The ether extract was dried over Na_2SO_4, filtered, and evaporated. The crude product was distilled under reduced pressure yielding 11.71 g (80%) of the chloro ketone XIII-d_2, bp 50–51 °C (10 mm). The proton NMR spectrum ($CDCl_3$) showed a two-proton triplet at δ 2.63 (J = 6 Hz, $CH_2CH_2COCH_3$), a three-proton methyl ketone singlet at δ 2.16 (CH_3CO), and a two-proton multiplet at δ 2.03 ($ClCD_2CH_2CH_2$). The infrared spectrum showed carbon–deuterium absorption at 2280, 2180 cm^{-1} and a ketone carbonyl absorption at 1710 cm^{-1}. The mass spectrum (70 eV) showed peaks at m/e (relative intensity) 124 and 122 (38, 100, M^+), 109 and 107 (24, 69, $M^+ - CH_3$).

Ethyl α-(Bromomethyl)-α-[(ethylthio)carbonyl]tetrahydro-2-methyl-2-furan-5,5-d_2-butanoate (X-d_2). The 5-chloropentan-2-one-5,5-d_2 (XIII-d_2) was transformed to the bromomethylmalonyl tetrahydrofuran X-d_2 by the same sequence of reactions used to prepare the nondeuterated model substrate X.

The deuterated model X-d_2 showed in the 250-MHz proton NMR spectrum ($CDCl_3$) a two-proton quartet at δ 4.22 (J = 7.0 Hz, $COOCH_2CH_3$), a two-proton AB quartet at δ 3.8 (J = 10.7, CH_2Br), a two-proton quartet at δ 2.93 (J = 7.2, $COSCH_2CH_3$), an eight-proton multiplet at δ 2.4–1.8, a three-proton triplet at δ 1.28 (J = 7.0 Hz, $COOCH_2CH_3$), a three-proton triplet at δ 1.26 (J = 7.2 Hz, $COSCH_2CH_3$), and a three-proton methyl singlet at δ 1.21. The infrared spectrum (neat) showed carbon–deuterium absorption at 2200, 2100 cm^{-1} and strong absorption at 1730 and 1670 cm^{-1} corresponding to the ester and thioester carbonyls. The mass spectrum (15 eV) showed peaks at m/e (relative intensity) 323 and 321 (8.5, $M^+ - SEt$), 305 and 303 (4.0, $M^+ - SEt - H_2O$), 87 (100, methyltetrahydrofuryl-d_2).

Reaction of the Bromide X-d_2 with Vitamin B_{12s} in H_2O. A solution of 0.060 g (0.0016 mol) of $NaBH_4$ in 1 mL of water was added to a solution of 0.090 g (0.000067 mol) of hydroxocobalamin in 2 mL of water under nitrogen. The reaction mixture was stirred at room temperature for 45 min until it became grey-green. The reaction mixture was taken into the darkroom and a solution of 0.080 g (0.00002 mol) of the bromide X-d_2 in 0.25 mL of absolute methanol was added. The reaction mixture, pH 11, was stirred at room temperature in the dark for 24 h. It was then extracted with three 25-mL portions of ether, dried over $MgSO_4$, filtered, and evaporated. The crude material was spotted on a 2-mm silica gel preparative plate and developed four times with 24:76 ether–hexane. Two UV active bands were separated.

Band 1, R_f 0.38, was rearranged product XVIII-d_2 weighing 0.035 g (55%). The 250-MHz proton NMR spectrum ($CDCl_3$) showed a two-proton quartet at δ 4.14 (J = 7.2 Hz, $COOCH_2CH_3$), a one-proton doublet of doublets at δ 2.97 (J = 8.1 and 14.6 Hz, H_A), a methine proton resonance at the same chemical shift as that of thioester methylene quartet at δ 2.87 (J = 7.3 Hz), a one-proton doublet of doublets at δ 2.67 (J = 4.5 and 14.6 Hz, H_B), an eight-proton multiplet at δ 2.0–1.8, a three-proton triplet at δ 1.25 (J = 7.2 Hz, $COOCH_2CH_3$), a three-proton triplet at δ 1.24 (J = 7.2 Hz, $COSCH_2CH_3$), and a three-proton methyl singlet at δ 1.15. The deuterium NMR spectrum ($CDCl_3$) showed a single resonance at δ 3.78 corresponding to the presence of deuterium at the carbon adjacent to the oxygen on the tetrahydrofuran ring. The infrared spectrum ($CHCl_3$) showed carbon–deuterium absorption at 2200 and 2100 cm^{-1} and carbonyl absorption at 1730 and 1680 cm^{-1} corresponding to the ester and thioester groups. The mass spectrum (70 eV) showed peaks at m/e (relative intensity) 243 (14, $M^+ - SEt$), 197 (21, $M^+ - COSEt - H_2O$), 87 (100, methyltetrahydrofuryl-d_2). The spectrum showed a peak at m/e 86 with intensity 8.6% of that of the m/e 87 peak.

Band 2 at R_f 0.47 was a mixture of starting bromide X-d_2 and unrearranged product XIX-d_2 weighing 0.014 g. This mixture was spotted on a 0.25-mm silica gel plate and developed five times in 18:82 ether–hexane. The band containing unrearranged product XIX-d_2, R_f 0.42, was removed from the plate and eluted from the silica with ether to yield 0.004 g (6%) of an oil. The proton NMR spectrum ($CDCl_3$) showed a two-proton quartet at δ 4.16 (J = 7.2 Hz, $COOCH_2CH_3$), a two-proton quartet at δ 2.89 (J = 7.2 Hz, $COSCH_2CH_3$), an eight-proton multiplet at δ 2.0–1.8, a three-proton methyl singlet at δ 1.43, a six-proton triplet at δ 1.24 (J = 7.2 Hz, $COOCH_2CH_3$ and $COSCH_2CH_3$), and a three-proton methyl singlet at δ 1.15. The infrared spectrum ($CHCl_3$) showed

carbon–deuterium absorption at 2200 and 2100 cm^{-1} and carbonyl absorption at 1730 and 1680 cm^{-1} corresponding to the ester and thioester groups. The mass spectrum (70 eV) showed peaks at m/e (relative intensity) 243 (16.6, M^+ – SEt), 225 (10, M^+ – SEt – H_2O), 197 (8, M^+ – COSEt – H_2O), 87 (100, methyltetrahydrofuryl-d_2). The spectrum showed a peak at m/e 86 with intensity 9.3% of that at m/e 87. The second band at R_f 0.49 was removed from the plate and eluted from the silica gel with ether yielding 0.008 g of an oil. The spectral properties of the latter were identical with those of the starting bromide X-d_2.

Reaction of the Bromide X-d_2 with Vitamin B_{12s} in H_2O with Potassium Carbonate. Hydroxocobalamin (0.090 g, 0.000067 mol) was added to a solution of 0.362 g (0.0026 mol) of anhydrous K_2CO_3 in 2 mL of H_2O. The resulting suspension was placed under nitrogen and a solution of 0.060 g (0.0016 mol) of $NaBH_4$ in 1 mL of H_2O was added. The reaction mixture was stirred at room temperature for 30 min. The reduced vitamin B_{12s} was not dissolved but remained as a dark grey precipitate in a brown colored solution. The reaction mixture was taken into the darkroom and a solution of the 0.080 g (0.00021 mol) of the bromide X-d_2 in 0.25 mL of absolute methanol was added. The reaction mixture, pH 12.5, was stirred at room temperature for 24 h. The reaction was extracted with four 25-mL portions of ether, dried over $MgSO_4$, filtered, and evaporated. The residue weighing 0.049 g was spotted on a 2-mm silica gel prep plate and developed four times in 24:76 ether–hexane.

Band 1 at R_f 0.39 was the rearranged product XVIII-d_2 weighing 0.029 g (45%). The spectral data (infrared, 250-MHz NMR and deuterium NMR) were identical with those of the rearranged product XVIII-d_2 obtained in the reaction in water described above. The mass spectrum showed fragmentation (m/e 243, 197, 87) similar to that shown by the rearranged product XVIII-d_2. The spectrum showed a peak at m/e 86 with intensity 13.7% of that at m/e 87. The m/e 86 peak in the starting bromide VIII-d_2 was 8.4% of the m/e 87 peak. Band 2 at R_f 0.47 was the unrearranged product XVI-d_2 weighing 0.014 g (22%). The spectral data (infrared and nmr) were similar to those of the unrearranged product XVI-d_2. The deuterium NMR spectrum showed a single resonance at δ 3.76 corresponding to deuterium at the carbon adjacent to oxygen on the tetrahydrofuran ring. The mass spectrum showed fragmentation (m/e 243, 225, 197, 87) similar to that shown by the unreacted product (64). The spectrum showed the peak at m/e 86 with inensity 17.25% of that at m/e 87. It appears then that both rearranged and unrearranged products, XVIII-d_2 and XIX-d_2, are undergoing exchange, to a limited degree, at the position α to oxygen on the tetrahydrofuran ring.

Control Reaction without Hydroxocobalamin. A solution of 30 mg (0.8 mmol) of sodium borohydride in 0.5 mL of D_2O was added to a solution of 181 mg (1.3 mmol) of anhydrous potassium carbonate in 1 mL of D_2O. This solution was then treated with 40 mg (0.1 mmol) of bromide X in 0.1 mL of MeOD. The reaction was stirred at room temperature for 24 h and then extracted with three 20-mL portions of ether. After drying over anhydrous $MgSO_4$, evaporation yielded 39 mg of product identical in every respect with the starting bromide X. No rearrangement product was detected by TLC or NMR. The mass spectrum showed that no deuterium had been incorporated during the reaction.

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Studies Directed at a Synthesis of the Morphine Alkaloids. A Photochemical Approach

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Studies directed at a synthesis of the morphine alkaloids by photocyclization of aryl vinyl ethers (e.g., A → B) has resulted in preparation of representatives of the *trans*-morphine ring system; e.g., **49**, **51**, and **52**. Photocyclization substrates **1a–c**, **25a–e**, and **33a–d** are prepared by reaction of the appropriate substituted phenol with epoxy ketones of type **8** or by annelation of 4-oxopiperidines with (aryloxy)methyl vinyl ketones of type **30**. Photocyclization conditions have been developed to provide a generally high level of chemical efficiency; photocyclization of **33d** provides tetracyclic benzodihydrofuran **35b** in 95% yield on a 30-g reaction scale. The remaining ring in morphine is constructed by addition of cyanide ion to an immonium ion derived from enamine **40b** to give **47b**. Amino nitrile **47b** is converted to *trans*-morphine derivative **49c** by nitrile addition with methyllithium, imine hydrolysis, and cyclodehydration of the resulting methyl ketone.

The first total synthesis of morphine (R = H) and codeine (R = Me) was reported by Gates and Tschudi over 30 years ago.[1] This landmark in organic chemistry provided chemical confirmation of the structure of morphine proposed by Robinson in 1925.[2] The well-exploited analgesic properties of natural morphine and codeine have stimulated intense interest in the development of practical total syntheses of these opium-derived alkaloids. Several approaches have been developed,[3] but a common inter-

(1) Gates, M.; Tschudi, G. *J. Am. Chem. Soc.* **1952**, *74*, 1109; **1956**, *78*, 1380.

(2) Gulland, J. M.; Robinson, R. *Mem. Proc. Manchester Lit. Phil. Soc.* **1925**, *69*, 79.

(3) For some of the most recent work, see: (a) Moos, W. H.; Gless, R. D.; Rapoport, H. *J. Org. Chem.* **1983**, *48*, 227. (b) Evans, D. A.; Mitch, C. H. *Tetrahedron Lett.* **1982**, *23*, 285. (c) Rice, K. C. *J. Org. Chem.* **1980**, *45*, 3135. (d) Weller, D. D.; Weller, D. L. *Tetrahedron Lett.* **1982**, *23*, 5239. (e) Ciganek, E. *J. Am. Chem. Soc.* **1981**, *103*, 6261. (f) Szántay, C.; Blaskó, G.; Bárczai-Beke, M.; Péchy, P.; Dörnyei, G. *Tetrahedron Lett.* **1980**, *21*, 3509. (g) Schwartz, M. A.; Zoda, M. F. *J. Org. Chem.* **1981**, *46*, 4623. These papers provide references to most of the work directed at synthesis of the morphine alkaloids.

0022-3263/85/1950-0217$01.50/0