completely unaffected by refluxing in concentrated nitric acid, aniline, n-butylamine or dimethylformamide. The infrared spectrum (KBr) had strong CF_2 absorption bands at 1185, 1275 and 1310, a CH_2 band at 1480(m) and bands at 855(m) and 770(m) cm.$^{-1}$. There was only a trace of absorption in the double bond region (1640 cm.$^{-1}$).

Anal. Calcd. for $(C_6H_4F_8)_x$: C, 37.90; H, 2.12; F, 59.98. Found: C, 37.29; H, 2.41; F, 59.19.

# Small-Ring Compounds. XXX. Reactions of Phenylcyclobutadienoquinone with Methanol[1,2]

BY FRANK B. MALLORY[3] AND JOHN D. ROBERTS

The thermal reaction of phenylcyclobutadienoquinone (I) with methanol gives 2,4-dimethoxy-3-phenylcyclobutenone (II), 3-phenyl-4-hydroxy-4-methoxy-2-butenoic acid lactone (III) and dimethyl phenylsuccinate (IV). The relative yields of the three products depend on the reaction temperature. A possible mechanism is presented that accounts for the formation of the three products by way of a common intermediate: 2-hydroxy-2-methoxy-3-phenylcyclobutenone (V). The structures of II and III were established by spectroscopic analysis, chemical degradation and independent synthesis.

Phenylcyclobutadienoquinone[4,5] (I) reacts thermally with methanol to give three principal products. Evidence will be presented for the formulation of these products as 2,4-dimethoxy-3-phenylcyclobutenone (II), 3-phenyl-4-hydroxy-4-methoxy-2-butenoic acid lactone (III) and dimethyl phenylsuccinate (IV).
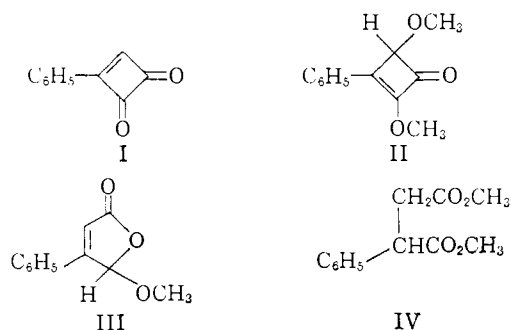
The relative yields of II-IV were found to depend mainly on the reaction temperature. Thus, when a solution of the quinone I in methanol was maintained at reflux for 12 days in the dark,[6] the product was the dimethoxyketone II, slightly contaminated with the diester IV. Substantially the same result was obtained when the reaction was carried out in a stainless-steel bomb heated[7] to 90–135° for 5–12

hours with or without acid catalyst. The highest yield (49%) of the dimethoxyketone II was obtained from the acid-catalyzed reaction.

A series of reactions in a stainless-steel bomb at somewhat higher temperatures (*ca.* 150°[7]) for 1 to 2 days gave equimolar mixtures of the methoxylactone III (*ca.* 40% yield) and the diester IV (*ca.* 40% yield); no dimethoxyketone II was detected.

When the bomb was heated in an electric furnace[8] at 150°, which constitutes the highest reaction temperature used, the predominant product was the diester IV (64% yield), along with a small amount of the methoxylactone III (8% yield). That the diester IV was not formed from the methoxylactone III as precursor was demonstrated by recovering III unchanged after heating with methanol at 145–170° for 24 hours.

The absence of excess pressure in the bomb when it was opened at the completion of each reaction indicated that no appreciable amounts of gaseous products were formed.[9]

Although no detailed mechanistic study was undertaken, it is possible to formulate reasonable reaction paths for the formation of the several products by way of a common intermediate, the hemiketal of I, 2-hydroxy-2-methoxy-3-phenylcyclobutenone (V), which could exist in equilibrium with methanol and the quinone I.
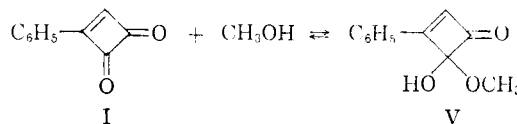
The dimethoxyketone II could arise from V by reaction with methanol of the carbonium ion resulting from the acid-induced loss as water of the hemiketal hydroxyl.

Ring opening of the hemiketal V could occur at higher temperatures to form the stereoisomeric vinylketenes VI and VII.

(1) Supported in part by the National Science Foundation.

(2) Presented before the Organic Section at the Second Delaware Valley Regional Meeting of the American Chemical Society, February 5, 1958, Philadelphia, Penna.

(3) General Electric Co. Predoctoral Fellow, 1956–1957.

(4) E. J. Smutny and J. D. Roberts, THIS JOURNAL, 77, 3420 (1955).

(5) E. J. Smutny, M. C. Caserio and J. D. Roberts, *ibid.*, 82, 1793 (1960).

(6) A photochemical reaction of I with methanol was discovered that gave 15–25% of dimethyl phenylsuccinate (IV) and 10–30% of a rather unstable white crystalline solid of m.p. 219° dec., which had the elemental composition $C_{11}H_{10}O_5$ and which contained one methoxyl group per molecule. The reaction was typically carried out at reflux temperature with irradiation for 15 min. in a Pyrex flask with a General Electric A-H6 mercury arc. Considerable work was done toward the elucidation of the structure of the compound of composition $C_{11}H_{10}O_5$, but the results are not now correlatable in terms of any single structural formula; t *cf.* F. B. Mallory, Ph.D. Thesis, Calif. Inst. of Technology, 1958.

(7) The temperature of the oil-bath used to heat the bomb is specified; the effective reaction temperatures in this arrangement were al-

ways somewhat lower since only the lower part of the bomb was immersed in the bath.

(8) In this case the effective reaction temperature was undoubtedly the same as the furnace temperature.

(9) It has been shown previously[15] that carbon monoxide is evolved when pure I is heated to its melting point.
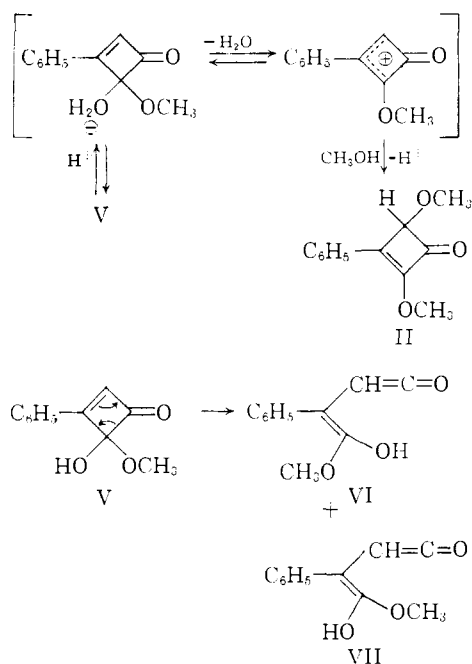
Both the methoxylactone III and the diester IV could be formed from VI.
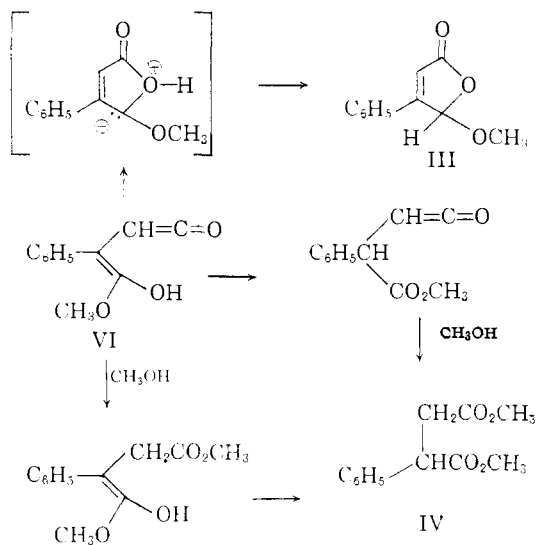
The ketene VII, in which internal cyclization is not sterically possible, would lead only to the diester IV.

The fact that the products III and IV were isolated in equivalent amounts in the intermediate-temperature reactions could be interpreted to indicate that the stereochemical course of the ring opening of V is random, leading to equivalent amounts of the vinylketenes VI and VII, and that the ring closure of VI to give the methoxylactone III is very much faster than the competing reactions of VI, which lead to the diester IV. Alternatively, to avoid the necessity of assuming that VI leads exclusively to III and essentially not at all to IV, it might be considered that the ring opening of the hemiketal V gives mostly VI, and that VI gives predominantly cyclization to III, but some reaction

with methanol to give IV. In this interpretation, the amount of the diester IV arising from both VI and VII is viewed as being fortuitously equivalent to the amount of the methoxylactone III arising from VI.

Predominant formation of the diester IV at still higher temperatures may be due to pronounced temperature dependencies on the stereochemistry of the ring-opening reactions of V and/or the ratio of the rates of reaction of VI to form III and IV. Another possibility is a sharply increasing importance with temperature of direct conversion of the quinone I to the diketene VIII, which would react with methanol to afford only the diester IV.
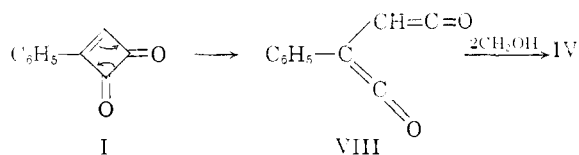
Ring openings of cyclobutenones by way of vinyl-ketenes, such as those postulated above, have been demonstrated in other cases.[10]

Establishment of the Structure of 2,4-Dimethoxy-3-phenylcyclobutenone (II).—The low-melting dimethoxyketone II was found to have the molecular formula $C_{12}H_{12}O_3$ with two methoxyl groups per molecule. Regeneration of the quinone I in good yield from the hydrolysis of the dimethoxyketone II in refluxing, dilute hydrochloric acid indicated that no skeletal change had occurred in the formation of II from I. The proton n.m.r. spectrum of a pure molten sample of II showed four peaks with relative areas of 5:1:3:3, corresponding to five phenyl hydrogens, one tertiary hydrogen and two nonequivalent methoxyl groups. The infrared absorption spectrum of II showed strong absorption bands at 1755 and 1635 cm.$^{-1}$ characteristic of carbonyl and double-bond stretching frequencies, respectively, in the 3-phenylcyclobutenaoe series. The dimethoxyketone II was found to be identical with a substance synthesized from the quinone I by successive treatments with sodium methoxide and dimethyl sulfate.[11]
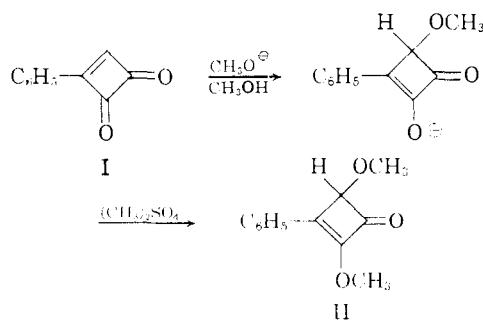
Establishment of the Structure of 3-Phenyl-4-hydroxy-4-methoxy-2-butenoic Acid Lactone (III). —The crystalline methoxylactone III was found to have the molecular formula $C_{11}H_{10}O_3$ with one methoxyl group per molecule. The proton n.m.r.

(10) (a) E. F. Jenny and J. D. Roberts, THIS JOURNAL, **78**, 2005 (1956); (b) E. F. Silversmith, Y. Kitahara, M. C. Caserio and J. D. Roberts, *ibid.*, **80**, 5840 (1958).

(11) L. Skatteb∅l and J. D. Roberts, *ibid.*, **80**, 4085 (1958).

spectrum of the pure melted material showed four peaks with relative areas 5:1:1:3, corresponding to five phenyl hydrogens, one vinyl hydrogen, one tertiary hydrogen and one methoxyl group. The infrared spectrum of III showed double-bond absorption at 1637 cm.$^{-1}$ and a strong carbonyl absorption at 1770 cm.$^{-1}$. The high frequency of this carbonyl absorption supports the methoxylactone structure for III, as opposed to the isomeric structures methyl β-formylcinnamate (IX) or methyl 2-keto-3-phenyl-3-butenoate (X), by analogy with similar molecules.[12] The aldehyde structure IX was further eliminated by the absence of the characteristic aldehyde hydrogen peak in both the proton n.m.r. spectrum and the infrared spectrum of the methoxylactone III, and by the failure of III to form a dimedone derivative. However, under the alkaline conditions of the Tollens test, hydrolysis of III to the open-chain aldehyde, β-formylcinnamic acid (XI), apparently occurred since a silver mirror was obtained.

IX   X

XI

Hydrogenation of the methoxylactone III over palladium-on-carbon gave a crystalline solid of composition $C_{11}H_{12}O_3$. This solid is assigned the structure 3 - phenyl - 4 - hydroxy - 4 - methoxybutanoic acid lactone (XII). The infrared absorption spectrum of XII showed no double-bond absorption but strong carbonyl absorption at 1795 cm.$^{-1}$. Such high-frequency carbonyl absorption is characteristic of lactones; and the shift of 25 cm.$^{-1}$ to higher frequencies compared with the carbonyl peak of III is the expected consequence of the destruction of the conjugation of the carbonyl group.

The saturated methoxylactone XII was subjected to Clemmensen reduction and gave β-phenylbutyric acid (XIII) as identified by comparison of the amide with an authentic sample. Phenylsuccinic acid (XIV) was obtained from XII by acid hydrolysis followed by treatment with silver oxide. The aldehyde XV was presumably an intermediate in these reactions.

XII   XV

XIII   XIV

Hydrolysis of the methoxylactone III with refluxing 2 N hydrochloric acid gave a solid believed to be 3 - phenyl - 4,4 - dihydroxy - 2 - butenoic acid lactone (XVI). The structure is supported by elemental analysis, spectral data and synthesis. The infrared spectrum of XVI in a potassium bromide disk showed a sharp hydroxyl absorption peak at 3240 cm.$^{-1}$, strong carbonyl absorption at 1735 cm.$^{-1}$ and double-bond absorption at 1635 cm.$^{-1}$. These values support the hydroxylactone structure for XVI by analogy with similar molecules[12] and help to rule out the isomeric open-chain structure, β-formylcinnamic acid (XI), which would be expected[12] to show infrared absorption at 1708 and 1690 cm.$^{-1}$ due to the aldehyde and acid carbonyl groups, respectively. Furthermore, XVI dissolved in aqueous sodium bicarbonate only after standing overnight as expected for a hydroxylactone but not for a carboxylic acid.

XVI

The structures assigned to the various lactones mentioned above have been further supported by synthesis. It has been shown previously[13] that mild alkaline treatment of 3-phenyl-4,4-dichlorocyclobutenone (XVII) gives 3-phenyl-4,4-dichloro-2-butenoic acid (XVIII). In the present work, treatment of the dichloro acid XVIII with silver nitrate in aqueous ethanol gave the hydroxylactone XVI.

XVII   XVIII

XVI

The hydroxylactone XVI was converted to the methoxylactone III by a treatment with methanol in the presence of a catalytic amount of sulfuric acid.

Experimental

Microanalyses are by Dr. A. Elek, Los Angeles, Calif. Melting points and boiling points are uncorrected.

Reaction of Phenylcyclobutadienoquinone (I) with Methanol. A. At 112–115° with Acid Catalyst.—A 1-l. stainless-steel bomb containing a mixture of 15 g. (0.095 mole) of the quinone I, 5 drops of concentrated sulfuric acid and 700 ml. of absolute methanol was heated in an oil-bath[7] at 112–115° for 12 hr. The reaction mixture was cooled to 0°, shaken with anhydrous sodium carbonate, and filtered. No solid quinone I was obtained by concentration of the filtrate. The residue after concentration was flash distilled to give 10.4 g. of distillate and 5.9 g. of residual black tar. The distillate was redistilled through a semi-

(12) J. F. Grove and H. A. Willis, *J. Chem. Soc.*, 877 (1951).

(13) J. D. Roberts, G. B. Kline and H. E. Simmons, Jr., THIS JOURNAL, **75**, 4765 (1953).

micro column[14] and afforded 9.5 g. (49%) of the dimethoxy-ketone II, b.p. 115° (1 mm.), contaminated with the quinone I, and possibly with dimethyl phenylsuccinate (IV), as indicated by infrared spectra.

The purification of II was effected by two additional distillations through a semi-micro column[14] to give a slightly yellow liquid, b.p. 105° (0.5 mm.), $n^{23.5}D$ 1.5910. Subsequent crystallization from hexane gave nearly white crystals of m.p. 34–37°. The ultraviolet absorption spectrum of II in cyclohexane solution showed λ_{max} 302 mμ with a shoulder at 314 mμ. The infrared and ultraviolet spectra of this material were identical with the spectra of the compound whose synthesis has been reported earlier,[11] which had $n^{23.5}D$ 1.5922, m.p. 37–39°. The melting point on admixture of these substances was not depressed.

Reconversion of II to I.—A mixture of 0.85 g. (0.0042 mole) of 2,4-dimethoxy-3-phenylcyclobutenone (II) and 10 ml. of 1 N hydrochloric acid was heated under reflux for 15 min.; then 1 ml. of concentrated hydrochloric acid was added and the mixture was maintained at reflux for an additional 8 min. Phenylcyclobutadienoquinone (I) (0.5 g., 75%) was obtained by crystallization from the cooled reaction mixture and identified by its infrared spectrum.

Reaction of I with Methanol. B. At 150°.—In a typical reaction a 1–1. stainless-steel bomb that contained a mixture of 20.0 g. (0.127 mole) of the quinone I and 500 ml. of absolute methanol was heated in an oil-bath[7] at about 150° for 40 hr. The bomb was allowed to cool and the solvent removed by distillation. The residue was distilled through a semi-micro column.[14]

Fraction	Temp., °C.	Press., mm.	Wt., g.
1	114–120	1.1	10.0
2	120–132	1.1	2.0
3	132–135	1.1	8.1
Residue (black tar)			5.5

It was shown by means of infrared spectra that fraction 1 was dimethyl phenylsuccinate (IV), fraction 3 was 3-phenyl-4-hydroxy-4-methoxy-2-butenoic acid lactone (III) and fraction 2 was a mixture (approximately 1:1) of III and IV. Thus, the total yields were 11.0 g. (39%) of the diester IV and 9.1 g. (38%) of the methoxylactone III.

The identity of a sample of the dimethyl phenylsuccinate (IV) that had been recrystallized from hexane to give m.p. 57.9–58.5° was established by its failure to depress the melting point of an authentic sample, which was prepared by methods previously described[15] and had m.p. 58.5–59.5° (lit.[15b] m.p. 57.5–58.5°).

The methoxylactone III was purified by redistillation through a semi-micro column[14] to give a nearly colorless liquid, b.p. 124° (0.5 mm.), which solidified on standing; three recrystallizations from hexane gave a white solid, m.p. 52.5–53.0°. The ultraviolet absorption spectrum in ethanol solution showed λ_{max} 276.5 mμ, ϵ 20,000.

Anal. Calcd. for $C_{11}H_{10}O_3$: C, 69.46; H, 5.30; CH₃O, 16.32; mol. wt., 190. Found: C, 69.59; H, 5.38; CH₃O, 16.22; mol. wt. (Rast), 186.

Reaction of I with Methanol. C. At 150° in An Electric Furnace.—A 1–1. stainless-steel bomb containing a mixture of 20.0 g. (0.127 mole) of the quinone I and 500 ml. of absolute methanol was shaken and maintained at 150° in an electric furnace for 31 hr. The isolation procedure was as in B above, and there was obtained 18 g. (64%) of dimethyl phenylsuccinate (IV), 2 g. (8%) of the methoxylactone III and 5 g. of tarry residue.

Treatment of 3-Phenyl-4-hydroxy-4-methoxy-2-butenoic Acid Lactone (III) with Methanol.—A sealed tube containing a solution of 0.6 g. (0.0032 mole) of the methoxylactone III in 30 ml. of absolute methanol was heated in an oil-bath at 145° for 22 hr., and then at 170° for an additional 2 hr. The solvent was removed by distillation and the residue distilled to give 0.4 g. (67% recovery) of the methoxylactone III, b.p. 135° (1 mm.), identified by its infrared spectrum. No dimethyl phenylsuccinate (IV) was detected.

Hydrogenation of 3-Phenyl-4-hydroxy-4-methoxy-2-butenoic Acid Lactone (III).—The methoxylactone III (2.0 g., 0.01 mole) in 10 ml. of purified anhydrous tetrahydrofuran was shaken with 50 mg. of 10% palladium-on-carbon[16] at a hydrogen pressure of 50 p.s.i. for 49 hr. The catalyst was removed by filtration and the solvent evaporated at reduced pressure. The residue was distilled through a semi-micro column[14] to give 1.8 g. (89%) of 3-phenyl-4-hydroxy-4-methoxybutanoic acid lactone (XII), b.p. 117–120° (1.5 mm.), which solidified on standing. After seven recrystallizations from hexane and one sublimation, this material had m.p. 84.0–84.5°.

Anal. Calcd. for $C_{11}H_{12}O_3$: C, 68.73; H, 6.29. Found: C, 68.77; H, 6.19.

Clemmensen Reduction of 3-Phenyl-4-hydroxy-4-methoxybutanoic Acid Lactone (XII).—The general method was based on that previously described.[17] From 4.0 g. (0.021 mole) of the dihydromethoxylactone XII, 5.0 g. of amalgamated zinc, 12.5 ml. of 27% hydrochloric acid and 5 ml. of toluene there was obtained 1.6 g. of product, b.p. 76–133° (1 mm.). This material was dissolved in ether and the acidic portion extracted with 5% aqueous sodium bicarbonate. A neutral fraction, amounting to 0.5 g., was not characterized completely. Acidification of the bicarbonate extract with concentrated hydrochloric acid gave 1.0 g. (29%) of a semi-solid material (XIII). The infrared spectrum of XIII in carbon tetrachloride solution showed a broad band at about 2900 cm.$^{-1}$ characteristic of carboxylic acids; XIII was shown to be β-phenylbutyric acid by preparation of its amide derivative,[18] which had m.p. 104.5–106° after three recrystallizations from benzene–hexane and one recrystallization from ethanol–water. The melting point was not depressed on admixture with authentic β-phenylbutyramide.

Hydrolysis and Silver Oxide Oxidation of 3-Phenyl-4-hydroxy-4-methoxybutanoic Acid Lactone (XII).—A mixture of 3.8 g. of the dihydromethoxylactone XII and 35 ml. of 2 N hydrochloric acid contained in a 50-ml. flask fitted with a reflux condenser was magnetically stirred and maintained at reflux for 12 hr. The reaction mixture was extracted with ether, and the ethereal solution was separated into an acid fraction amounting to 2.2 g. and a neutral fraction amounting to 1.1 g. by treatment with 5% aqueous sodium bicarbonate. The neutral fraction was not characterized completely. A 1.9-g. portion of the acid fraction was dissolved in 10 ml. of ethanol, and a small amount of insoluble material was removed by filtration. A solution of 3.0 g. of silver nitrate in 40 ml. of water was added, and the resulting solution was cooled to 0°. A solution of 2.0 g. of potassium hydroxide in 10 ml. of water was added dropwise with swirling. The flask was stoppered, wrapped in a wet towel, and shaken mechanically for 4 hr. The solid was removed by filtration, and the filtrate was acidified with concentrated hydrochloric acid and extracted with ether. The ether was removed from the extract, and the residue was treated with carbon tetrachloride to give 0.7 g. of insoluble material, m.p. 151–158°, identified as phenylsuccinic acid (XIV) by its infrared spectrum in a potassium bromide disk. After six recrystallizations from water, XIV had m.p. 160–162.5°; the melting point was not depressed on admixture with authentic phenylsuccinic acid.[15a]

Hydrolysis of 3-Phenyl-4-hydroxy-4-methoxy-2-butenoic Acid Lactone (III).—In a typical hydrolysis, a mixture of 1.0 g. (0.0053 mole) of the methoxylactone III and 54 ml. of 2 N hydrochloric acid was heated under reflux for 1.7 hr. until no insoluble oil remained. 3-Phenyl-4,4-dihydroxy-2-butenoic acid lactone (XVI) crystallized from the solution when it was cooled to 0° and had m.p. 157–160°. Purification of XVI by five recrystallizations from benzene gave a white crystalline solid, m.p. 158.0–158.8°. The ultraviolet absorption spectrum of XVI in ethanol solution showed λ_{max} 275.5 mμ, ϵ 19,000.

Anal. Calcd. for $C_{10}H_8O_3$: C, 68.18; H, 4.58; mol. wt., 176. Found: C, 68.09; H, 4.52; mol. wt. (Rast), 205.

Synthesis of 3-Phenyl-4,4-dihydroxy-2-butenoic Acid Lactone (XVI).—A solution of 438 mg. (1.9 mmol.) of 3-phenyl-4,4-dichloro-2-butenoic acid (XVIII) and 703 mg.

(14) C. W. Gould, Jr., G. Holzman and C. Niemann, *Anal. Chem.*, **20**, 361 (1948).

(15) (a) "Organic Syntheses," Coll. Vol. I, J. Wiley and Sons, Inc., New York, N. Y., 1941, pp. 181, 451; (b) H. Wren and H. Williams, *J. Chem. Soc.*, **109**, 572 (1916).

(16) A. I. Vogel, "A Textbook of Practical Organic Chemistry," 2nd Ed., Longmans, Green and Co., London, 1951, p. 996.

(17) E. L. Martin, THIS JOURNAL, **59**, 1438 (1936).

(18) Procedure as used for bicyclooctanecarboxylic acids by J. D. Roberts, W. T. Moreland, Jr., and W. Frazer, *ibid.*, **75**, 637 (1953).

(4.1 mmol.) of silver nitrate in 25 ml. of ethanol and 10 ml. of water was allowed to stand at room temperature in the dark for 27 hr. Silver chloride (322 mg., 59%) was removed by filtration, an additional 0.7 g. of silver nitrate and 20 ml. of water was added to the filtrate, and the reaction was allowed to continue for 5 more days to give an additional 62 mg. of silver chloride, which brought the total to 384 mg. (71%). After removal of the silver chloride, the ethanol was evaporated from the filtrate under reduced pressure and the aqueous residue extracted with ether. The ether was removed and the residue treated with carbon tetrachloride to give a yellow solution and an insoluble white solid. This solid was recrystallized three times from water to give 20 mg. (6%) of the hydroxylactone XVI, m.p. 152–158°; the

melting point was not depressed on admixture with XVI obtained by the hydrolysis of the methoxylactone III.

Synthesis of 3-Phenyl-4-hydroxy-4-methoxy-2-butenoic Acid Lactone (III).—A solution of 0.6 g. (0.0034 mole) of the hydroxylactone XVI and 1.5 ml. of concentrated sulfuric acid in 35 ml. of absolute methanol was allowed to stand at room temperature for 24 hr. and then concentrated to 20 ml. on a steam-bath over a period of 2 hr. The solution was cooled to 0°, diluted with ether, and washed with water and 5% aqueous sodium bicarbonate. The ethereal solution was dried over anhydrous magnesium sulfate, filtered, and the ether evaporated. The residue was distilled to give 0.4 g. (62%) of the methoxylactone III, b.p. 131° (1 mm.), identified by its infrared and n.m.r. spectra.

Methylmalonyl Coenzyme A Isomerase: Investigation of a Carbon Monoxide Transfer Mechanism

BY MARTIN FLAVIN[1] AND CLARENCE SLAUGHTER

Investigation of the mechanism of the isomerization of methylmalonyl coenzyme A to succinyl coenzyme A, catalyzed by an enzyme from kidney cortex, has been hampered by the absence of any analogous model non-enzymatic "transcarboxylation" reaction. This deficiency has recently been supplied by the discovery that the cinenic acid rearrangement involves a carboxyl rather than an alkyl migration. Evidence that the non-enzymatic reaction takes place by a carbon monoxide and carbonium ion mechanism prompted an investigation of the possible intermediary formation of carbon monoxide in the reversible isomerization of methylmalonyl coenzyme A. Preliminary results do not support this mechanism for the enzymatic reaction.

Introduction

The reversible rearrangement of methylmalonyl coenzyme A (I) to succinyl coenzyme A (II)

$$CoA—SCCHCO_2^- \longleftarrow CoA—SCCH_2CH_2CO_2^- \quad (1)$$

(I)   (II)

is catalyzed by an enzyme discovered 5 years ago in extracts of animal tissues, where it plays a role in the metabolism of propionic acid.[2] Interest in this reaction has recently been stimulated by the discovery that a coenzyme form of vitamin B_{12}[3] is an essential cofactor.[4] The rearrangement, which is effectively a carboxyl transfer, has been without apparent analogy in organic or biological chemistry, and the mechanism has remained obscure.

An interesting analogy has now come to light through the demonstration that the rearrangement, in concentrated sulfuric acid, of α-cinenic acid (III) to geronic acid (IV)

$$(2)$$

III   IV

involves a carboxyl-group transfer, rather than the previously assumed long range methyl migration.[5a,b] Evidence that the mechanism is a decarbonylation, followed by isomerization of the resultant carbonium ion, and recapture of carbon monoxide,[5c] has prompted the reinvestigation of the mechanism of the methylmalonyl coenzyme A (CoA) isomerase reaction reported here.

While the analogy suffers from lack of obvious potentiality for resonance stabilization of intermediate carbonium ions in the enzymatic reaction (equation 1),[6] this shortcoming was weighed against the unknown factors represented by the intervention of enzyme and coenzyme and the potentiality of the coenzyme to serve as a carbon monoxide carrier through its cobalt moiety.[7]

Experimental

Methylmalonyl CoA was prepared by a procedure yielding the monothioester unequivocally and in high yield.[8] Labeled succinyl CoA could be prepared in good yield from succinic-1,4-C^{14} acid (28 μcuries/mg.) on a 4-mg. scale, by the method previously described for methylmalonyl CoA,[2d] with the aid of microglassware. Tetrahydrofuran, which inhibits the isomerase reaction, was removed from the thioester solution by ether extraction at pH 6. The $C^{14}O$ (500 μcuries in 250 μmoles) was obtained in a break-seal bulb and freed of traces of $C^{14}O_2$ by alkali extraction before use.

(1) Established Investigator of the American Heart Association.

(2) (a) M. Flavin, Federation Proc., 14, 211 (1955); (b) M. Flavin P. J. Ortiz and S. Ochoa, Nature, 176, 823 (1955); (c) M. Flavin and S. Ochoa, J. Biol. Chem., 229, 965 (1957); (d) W. S. Beck, M. Flavin and S. Ochoa, J. Biol. Chem., 229, 997 (1957).

(3) H. Weissbach, J. Toohey and H. A. Barker, Proc. Natl. Acad. Sci., U. S., 45, 521 (1959).

(4) (a) R. M. Smith and K. J. Monty, Biochem. Biophys. Res. Comm., 1, 105 (1959); (b) E. R. Stadtman, P. Overath, H. Eggerer and F. Lynen, ibid., 2, 1 (1960); (c) J. R. Stern and D. L. Friedman, ibid., 2, 82 (1960); (d) S. Gurnani, S. P. Mistry and B. C. Johnson, Biochim. Biophys. Acta, 38, 187 (1960).

(5) (a) J. Meinwald, THIS JOURNAL, 77, 1617 (1955); (b) J. Meinwald and J. T. Ouderkirk, ibid., 82, 480 (1960); (c) J. Meinwald. H. C. Hwang, D. Christman and A. P. Wolf, ibid., 82, 483 (1960).

(6) Since, unlike the case of cinenic acid, hydrogen atoms are bonded to the relevant carbons in the enzymatic rearrangement, the latter could be alternately formulated as being mediated by a hydride-shift or an unsaturated intermediate (acrylyl CoA); M. Flavin and C. Slaughter, J. Biol. Chem., 235, 1112 (1960).

(7) Spectral evidence for a carbon monoxide derivative of vitamin B₁₂ has been reported by J. G. Heathcote; quoted in R. T. Williams (editor), "Biochemistry of Vitamin B₁₂," Biochemical Society Symposium, No. 13, Cambridge University Press, 1955, p. 15.

(8) E. G. Trams and R. O. Brady, THIS JOURNAL, 82, 2972 (1960).