Anal. Calcd for $C_{10}H_{11}N_5O$: C, 55.3; H, 5.06; N, 32.25. Found: C, 55.51; H, 5.12; N, 32.02.

2',3'-Dideoxyadenosine (III). 2',3'-Dideoxy-2',3'-didehydroadenosine (VI) (0.28 g, 0.0012 mole) analytically pure, was dissolved in 60 ml of ethanol and the solution was hydrogenated at atmospheric pressure for 1 hr, at room temperature, in the presence of 0.15 g of 5% palladium on carbon. The catalyst was removed by filtration and washed with 50 ml of boiling ethanol. The colorless solution was evaporated to dryness *in vacuo* (oil pump) at 30° (bath temperature). The resulting white solid was recrystallized from ethanol to yield 2',3'-dideoxyadenosine (III), 0.19 g, 67.2%, mp 183–185°, $[\alpha]^{23}D$ −26.8° (*c* 1.00, H_2O) (lit[13] $[\alpha]^{25}D$ −25.2° (*c* 1.01, H_2O)), λ_{max}^{MeOH} 259.5 mμ (ε 14,600).

Anal. Calcd for $C_{10}H_{13}N_5O_2$: C, 51.1; H, 5.5; N, 29.8. Found: C, 51.3; H, 5.7; N, 30.1.

2',3',5'-Trideoxyadenosine (VIII). Method 1. 6-Amino-9-(5'-S-ethyl-5'-thio-2',3',5'-trideoxy-2',3'-didehydro-β-D-*glycero*-pentofuranosyl)purine (VII, 0.900 g, 0.00325 mole) was dissolved in 80 ml of ethanol and W-7 Raney nickel (14 g) was added. The mixture was refluxed for 6.5 hr. The catalyst was removed by filtration and washed with 500 ml of boiling ethanol. Evaporation of the combined filtrate *in vacuo* gave a solid which was extracted with three 15-ml portions of boiling benzene. The combined benzene extracts were concentrated to 10 ml and cooled to room temperature overnight to yield 0.071 g of 2',3',5'-trideoxyadenosine (VIII), mp 156–159°. A mixture melting point with an authentic sample of 2',3',5'-trideoxyadenosine[14] was undepressed.

Anal. Calcd for $C_{10}H_{13}N_5O$: C, 54.8; H, 5.9; N, 32.0. Found: C, 54.5; H, 5.8; N, 32.2.

Method 2. 2',3',5'-Trideoxy-2',3'-didehydroadenosine (VI, 0.035 g) was dissolved in 10 ml of ethanol and the solution was hydrogenated at atmospheric pressure for 30 min in the presence of 0.02 g of 5% Pd–C. The filtrate was chromatogramed in four solvent systems (Table I) against an authentic sample of 2',3',5'-trideoxyadenosine.[14] Both spots were found to run identical in the four solvent systems. The colorless filtrate was evaporated to dryness *in vacuo* at 30° (bath temperature) and the resulting solid was recrystallized from benzene to yield pure VI, mp 155–159°. A mixture melting point with an authentic sample[14] was undepressed. The infrared spectrum was identical with that of 2',3',5'-trideoxyadenosine.[14]

Evidence for the Biological Incorporation of Radioactivity from Benzoate-1-C^{14} into the Quinone Carbons of Coenzyme Q_9

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Abstract: 3',6'-Diacetoxy-4',5'-dimethoxy-2'-methylphenylacetic acid (obtained by ozonolysis of the diacetate of coenzyme Q_9 hydroquinone) was converted to the lactone of 3',6'-dihydroxy-4',5'-dimethoxy-2'-methylphenylacetic acid, by acid hydrolysis. On treatment of the latter with hydrogen peroxide in alkaline solution, the major acidic products were malonic acid and the epoxide of α-methyl-α,β-dihydroxytricarballylic acid. When coenzyme Q_9 biosynthesized from benzoate-1-C^{14} in the rat was subjected to the above degradation, it was found to contain radioactivity only in one or both of its quinone carbon atoms.

We have previously shown that benzoate-1-C^{14} (*i.e.*, benzoate with the label on the ring carbon atom which carries the carboxyl group) is incorporated into coenzyme Q_9 (CoQ_9, **1**) in the rat.[1] When the radioactive CoQ_9 was degraded by ozonolysis of the diacetate of CoQ_9 hydroquinone, as previously described,[2] all of the radioactivity was in the benzoquinone moiety obtained as 3',6'-diacetoxy-4',5'-dimethoxy-2'-methylphenylacetic acid (**2**). To gain further information about the conversion of benzoate to the benzoquinone nucleus, it was necessary to locate the labeled atoms in the substituted phenylacetic acid (**2**). We have therefore investigated the hydrolysis and alkaline peroxide oxidation of **2**. From these reactions, two products have been characterized. The first is a novel, epoxytricarboxylic acid (**4**) which contains virtually all of the radioactivity found in CoQ_9 biosynthesized from benzoate-1-C^{14}. The second is malonic acid, which is devoid of radioactivity. These findings, together with other relevant data, lead to the conclusion that the label from benzoate-1-C^{14} is incorporated biologically into one or both of the quinone carbons of CoQ_9.

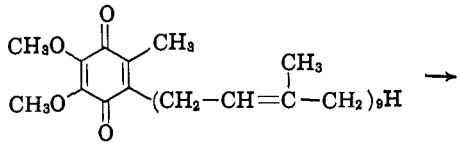
(1) R. E. Olson, R. Bentley, A. S. Aiyar, G. H. Dialameh, P. H. Gold, V. G. Ramsey, and C. M. Springer, *J. Biol. Chem.*, **238**, 3146 (1963). For a review of recent work, see R. E. Olson, *Federation Proc.*, **24**, 85 (1965).

(2) R. Bentley, V. G. Ramsey, C. M. Springer, G. H. Dialameh, and R. E. Olson, *Biochemistry*, **4**, 166 (1965).

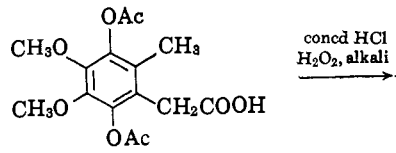
Results and Discussion

In this work, sufficient quantities of the substituted phenylacetic acid **2** were obtained by ozonolysis of the diacetate of 2,3-dimethoxy-5-methyl-6-phytylbenzohydroquinone. When **2** was allowed to stand at room temperature with concentrated hydrochloric acid, the acetyl groups were removed and the initial product lost water to form the lactone of 3',6'-dihydroxy-4',5'-dimethoxy-2'-methylphenylacetic acid (**3**). This compound was insoluble in sodium bicarbonate and was not oxidized to a quinone with silver oxide in ether.

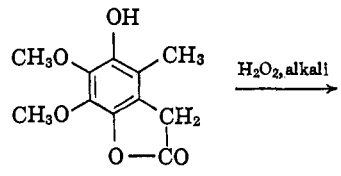
In oxidation experiments, the lactone **3** was treated with 30% hydrogen peroxide solutions in the presence of KOH. The temperature of the reaction was initially 0° and was raised slowly to 60–65° until all of the peroxide was consumed. On acidification of the mixture, CO_2 was released in amounts averaging 2 moles/mole of lactone. In examinations for neutral materials, small amounts of methanol could be identified by the chromotropic acid test; a small amount of a neutral material obtained by ether extraction was shown not to



1



2



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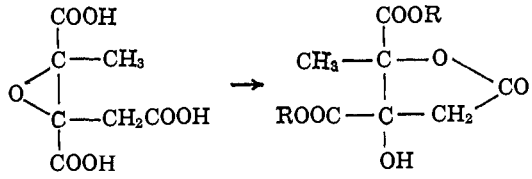


4 5

be dimethyl oxalate, a possible oxidation product, on the basis of gas chromatography.

The acidic products obtained from these oxidations were of great interest. The steam-volatile fraction contained traces of formic acid and larger amounts of acetic acid. The major products were obtained by continuous ether extraction of the mixture and were separated by partition chromatography on Celite columns,[3] using 1-butanol–chloroform (35:65). Early 10-ml fractions (16–21) from these columns contained malonic acid, but from the later fractions (27–35) a new compound was obtained. This material, which crystallized from ether–pentane (1:3), had mp 155–156°; on paper chromatography in acidic (ethyl acetate–5 *M* formic acid, 1:1)[4] and basic (ethanol–NH_4OH–water, 8:1:1)[5] solvent systems it behaved as a tricarboxylic acid with an R_f value slightly different from that of citric acid. Its nature as the tricarboxylic acid **4** was confirmed by the preparation of a trisodium salt and a trimethyl ester. In the infrared spectra of **4** and its sodium salt, bands characteristic of the COOH and COO^- functions, respectively, were observed, but no bands were seen for a γ-lactone in the 5.5-μ region. Similarly, in the trimethyl ester, the infrared spectrum showed an ester C=O band at 5.7 μ, and no bands characteristic of OH or γ-lactone C=O. The proposed structure **4** was also confirmed by the nmr spectrum of the trimethyl ester (see the Experimental Section). In addition, the mass spectrum of the ester showed a parent peak at M = 246 ($C_{10}H_{14}O_7$) and two prominent peaks at M = 31 (OCH_3) and M = 59 ($OCOCH_3$).

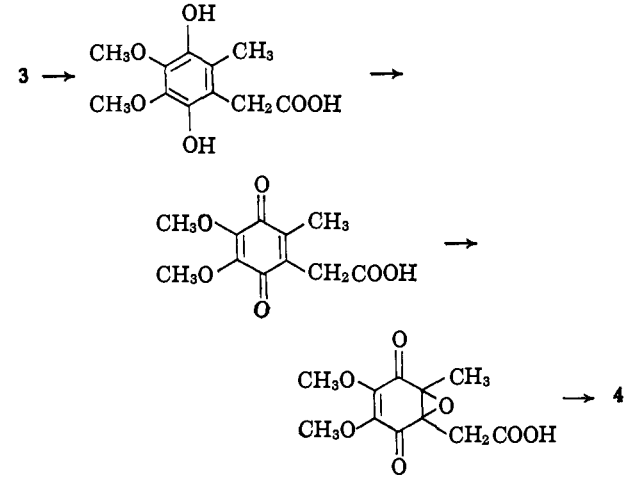
(3) E. F. Phares, E. H. Mosbach, F. W. Denison, and S. F. Carson, *Anal. Chem.*, **24**, 660 (1952).
(4) M. L. Buch, R. Montgomery, and W. L. Porter, *ibid.*, **24**, 489 (1952).
(5) F. Brown, *Nature*, **167**, 441 (1951).

Synthetic proof for the structure **4** was obtained by preparation of the corresponding glycol by treatment of γ-methyl-*cis*-aconitic anhydride with osmium tetroxide. The purified product obtained from this reaction was the γ-lactone of α-methyl-α,β-dihydroxytricarballylic acid (**5**, R = H); it showed expected bands in the infrared spectrum for OH, γ-lactone C=O, and acid C=O. The dimethyl ester (**5**, R = CH_3) was prepared and showed an nmr spectrum in agreement with the assigned structure.

On hydrolysis of the epoxy acid **4** with perchloric acid, a lactone acid with the same R_f in the acidic solvent system[4] as compound **5** (R = H) was obtained. Esterification of the acid gave a product which was identical with the synthetic ester **5** (R = CH_3) by mixture melting point, paper chromatography,[4] gas–liquid chromatography (15% adipate polyester on Chromosorb W at 190°), and infrared and nmr spectra.

The formation of the epoxide structure by degradation of the hydroquinone derivative **3** is apparently similar to the formation of alkylnaphthoquinone epoxides by treatment of the alkylnaphthoquinones with hydrogen peroxide and sodium carbonate.[6]

Presumably the lactone ring is first opened in the alkaline solution, and, after oxidation to the quinone, an unstable benzoquinone epoxide is formed as an



intermediate. If this mechanism is generally correct, there will be a *cis* relationship between the tertiary carboxyl groups of **3**.

In tracer experiments, CoQ_9 was biosynthesized from benzoate-1-C^{14}, either in intact rats, or in a rat liver slice system.[7] The purified, radioactive, CoQ_9 samples were degraded first to the substituted phenylacetic acid (**2**) and then to the epoxytricarboxylic acid **4**; in the degradation with alkaline peroxide, malonic acid was also isolated. In addition, a portion of the substituted phenylacetic acid was subjected to the Kuhn–Roth oxidation, and the acetic acid was isolated for radioactivity determination. The results of these degradations are summarized in Table I. With three samples of CoQ_9, there was no radioactivity in the acetic and malonic acids, while the epoxytricarboxylic acid contained from 94 to 98% of the original activity. As shown below, the epoxy acid contains those carbons assayed in

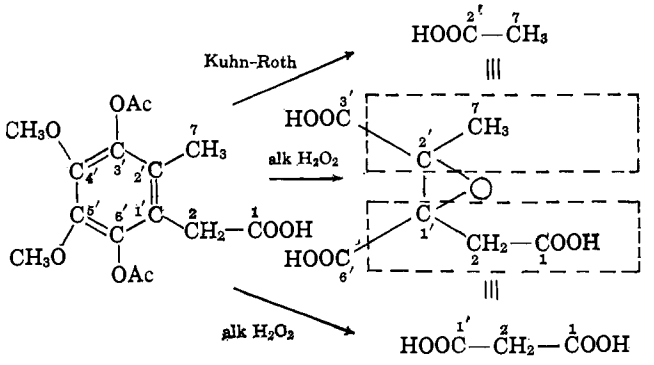
(6) L. F. Fieser, W. P. Campbell, E. M. Fry, and M. D. Gates, *J. Am. Chem. Soc.*, **61**, 3216 (1939).
(7) P. H. Gold, G. H. Dialameh, and R. E. Olson, *Federation Proc.*, **20**, 228 (1961); A. S. Aiyar and R. E. Olson, *ibid.*, **23**, 425 (1964).

Table I. Degradation of Labeled CoQ Samples Biosynthesized from Benzoate-1-C^{14}

Expt no.[a]	CoQ_9 hydroquinone acetate[b]	Phenylacetic acid[c]	Kuhn–Roth acetic acid	Malonic acid	Epoxy acid[d]	Distribution of C^{14}, %: C-7 + C-2′[e]	C-1 + C-2 + C-1′	C-3′ + C-6′[f]
1 (*in vivo*)	1,181	1,154	15	...	...	1.5	...	...
2 (*in vivo*)	500	452	..	0	488	...	0	98
3 (*in vitro*)	34,913	32,714	49	474	32,730	0.2	1.3	92.5

[a] In experiment 1, 0.5 mcurie of benzoate-1-C^{14} (2.0 mcuries/mmole) was injected intraperitoneally into ten rats and the animals were sacrificed after 3 hr. Total body CoQ_9 was isolated as previously described: R. E. Olson, G. H. Dialameh, R. Bentley, C. M. Springer, and V. G. Ramsey, *J. Biol. Chem.*, **240**, 514 (1965). In experiment 2, 1.0 mcurie of benzoate-1-C^{14} was injected into 20 rats receiving 1% sodium benzoate in the diet. The rats were sacrificed in 3 hr and CoQ_9 was isolated from the livers. In experiment 3, 0.1 mcurie of benzoate-1-C^{14} (8.9 mcuries/mmole) was incubated with 40 g of rat liver slices in 200 ml of Krebs–Ringer bicarbonate buffer, pH 7.4; the gas phase was 95% O_2–5% CO_2, and incubation was continued for 4 hr. [b] The values recorded in these columns are specific activities, expressed as dpm/μmole. Corrections have been made for the dilution of the originally isolated CoQ_9 with carrier. [c] Compound **2**. [d] Compound **4**. [e] For the numbering used, see text diagram. [f] This value is derived by subtraction of the per cent C^{14} in (C-7 + C-2′) + (C-1 + C-2 + C-1′) from that in the epoxytricarboxylic acid (C-1 + C-2 + C-7 + C-1′ + C-2′ + C-3′ + C-6′).

malonic (C-1, C-2, C-1′) and acetic acids (C-7, C-2′), together with the two quinone carbon atoms (C-3′, C-6′). The lack of activity in acetic and malonic acids, therefore, indicates that benzoate-1-C^{14} labels either one or both of the quinone carbons. This conclusion is in harmony with the fact that Olsen, *et al.*,[8] have



isolated radioactive 2-decaprenylphenol from *Rhodospirillum rubrum* cultures in the presence of *p*-hydroxybenzoate-U-C^{14}. It is clear from this experiment that the decaprenyl side chain does not replace the carboxyl group of the *p*-hydroxybenzoate since in this case the product would have been the 4-decaprenylphenol. A similar relationship is seen in the structure of the antibiotic grifolin (2-farnesyl-5-methylresorcinol), for which orsellinic acid is a possible precursor.[9] The other alkylation reaction involved in CoQ biosynthesis is a methylation. In microorganisms, such methylations take place directly on the aromatic nucleus and do not require replacement of carboxyl groups. A similar type of methylation in general CoQ biosynthesis would be in harmony with the present observations in the rat.

Experimental Section

Microanalyses were performed by Dr. Alfred Bernhardt, Max Planck Institut für Kohlenforschung, Mulheim, Germany. Gas chromatography was carried out with a Barber–Colman Model 10 instrument. Paper chromatography of acids was carried out with the ascending technique using the solvents of Buch, *et al.*,[4] or Brown.[5] The compounds were visualized by spraying with 0.5% aqueous $KMnO_4$.

Preparation of 3′,6′-Diacetoxy-4′,5′-dimethoxy-2′-methylphenylacetic Acid (2). 2,3-Dimethoxy-5-methyl-6-phytylbenzoquinone was prepared from 3,4-dimethoxytoluhydroquinone by the general alkylation methods of Morton, *et al.*,[10] and Rüegg, *et al.*,[11] and was acetylated as follows. A mixture of 2,3-dimethoxy-5-methyl-6-phytylbenzoquinone (4.0 g), triethylamine (8 ml), and acetic anhydride (40 ml) was held at 70–80°, and treated with portions of zinc dust until the solution became colorless; heating was then continued for a further 10 min. Ether (400 ml) was added, and after filtration through glass wool the solution was washed with portions of saturated sodium chloride solution until the washings were acid (congo red paper). The residue obtained on removal of the ether by vacuum evaporation was stirred with water (3 l.) to complete removal of acetic anhydride. The oil was extracted into chloroform and, after washing with saturated sodium chloride solution until the washings were no longer acid, the solution was dried over sodium sulfate. On evaporation, 4.8 g of a pale yellow oil was obtained. This preparation of 1,4-diacetoxy-2,3-dimethoxy-5-methyl-6-phytylbenzene was ozonized as previously described.[2] 3′,6′-Diacetoxy-4′,5′-dimethoxy-2′-methylphenylacetic acid was obtained in 34% yield, mp 135–137° alone and in admixture with material prepared by ozonolysis of the diacetate of CoQ_9 hydroquinone.

Lactone of 3′,6′-Dihydroxy-4′,5′-dimethoxy-2′-methylphenylacetic Acid (3). 3′,6′-Diacetoxy-4′,5′-dimethoxy-2′-methylphenylacetic acid (132 mg) was allowed to stand at room temperature for 21 hr with concentrated HCl (1 ml). The crystalline product was collected on a sintered glass filter and dried under vacuum over potassium hydroxide, mp 141–143°, 73 mg (74% yield). Recrystallization from ether gave **4**, mp 143–144°, λ_{max} 288 mμ (ethanol, $E_{1\,cm}^{1\%}$ 118); infrared spectrum, λ_{max}^{KBr} 2.85 (OH) and 5.55 μ (γ-lactone C=O). The lactone was not soluble in $NaHCO_3$ and was not oxidized to quinone with silver oxide in ether.

Anal. Calcd for $C_{11}H_{12}O_5$: C, 58.92; H, 5.40. Found: C, 59.16; H, 5.40.

Alkaline Peroxide Oxidations of Lactone 3. The reaction tube used for the oxidations was swept through with CO_2-free nitrogen and was connected to a series of traps. The first was surrounded by a Dry Ice–acetone mixture to condense volatile materials such as methanol; the second and third contained 0.1 *N* barium hydroxide solution to adsorb CO_2. Cold H_2O_2 (6 ml, 30% solution, not stabilized) was quickly added to the lactone (108 mg) followed by 0.45 ml of potassium hydroxide solution (50% in glass distilled water). The reaction tube was initially immersed in an ice bath, and cooling was maintained until the lactone had dissolved (2 hr). The ice bath was then removed and the temperature of the mixture was slowly raised over a period of 1 to 2 hr to 55° (the temperature was controlled so that there was a steady but not violent evolution of gas from the mixture). Finally, the temperature was brought to 60–65° until the peroxide test with starch–potassium iodide paper was negative (about 1 hr). After cooling, the mixture was acidified with 0.5 ml of concentrated H_2SO_4, and flushing with nitrogen was continued for some hours. In many experiments the yield of CO_2 was always 2 moles/mole of lactone. The Dry Ice trap contained a small amount of neutral, colorless liquid; this material did not react with 2,4-dinitrophenylhydrazine, but gave a

(8) R. K. Olsen, J. L. Smith, G. D. Daves, H. W. Moore, K. Folkers, W. W. Parson, and H. Rudney, *J. Am. Chem. Soc.*, **87**, 2298 (1965).

(9) R. Bentley, "Biogenesis of Antibiotic Substances," Publishing House of the Czechoslovak Academy of Sciences, 1965, p 241.

(10) R. A. Morton, U. Gloor, O. Schindler, G. M. Wilson, L. H. Chopard-dit-Jean, F. W. Hemming, O. Isler, W. M. F. Leat, J. F. Pennock, R. Rüegg, U. Schwieter, and O. Wiss, *Helv. Chim. Acta*, **41**, 2343 (1958).

(11) R. Rüegg, U. Gloor, R. N. Goel, G. Ryser, O. Wiss, and O. Isler, *ibid.*, **42**, 2616 (1959).

positive chromotropic acid test for methanol. In preliminary experiments, the reaction mixture (after removal of CO_2) was again made alkaline and extracted continuously with ether. The neutral residue obtained on evaporation of the ether amounted to only 2.0 mg; by gas chromatography it was shown that no dimethyl oxalate was present.

When the acidified reaction mixture was steam distilled, the distillate gave a weak reaction for formic acid in the chromotropic acid test and a strong positive test for acetic acid.[12]

After removal of the steam-volatile acids, the acid solution was extracted continuously with ether for 24 hr. The dried ether extract was evaporated to a semicrystalline residue (74 mg). The residue was transferred to a Celite column (18 g) moistened with 0.5 *N* H_2SO_4,[3] which was eluted with 1-butanol–chloroform (35:65); fractions were collected every 30 min. The combination of fractions 16–21 yielded 11.7 mg of a solid, mp 125–130°, which was identified as slightly impure malonic acid on the basis of paper chromatography. On recrystallization from acetone–benzene, 5.8 mg of pure malonic acid was obtained, showing no melting point depression (135–137°) on admixture with authentic material. A second acid was obtained in larger amount (25.8 mg) from fractions 27–35; after crystallization from ether–pentane (1:3) the melting point was 155–156°. On paper chromatography, the acid, **4**, behaved as a tricarboxylic acid, and the R_f value was actually close to that of citric acid.

Anal. Calcd for $C_7H_8O_7$: C, 41.18; H, 3.95; equiv wt, 74.0. Found: C, 41.41; H, 4.00; equiv wt, 73.0.

The trisodium salt of **4** was prepared by the method of Habicht and Schneeberger.[13]

Anal. Calcd for $C_7H_5O_7Na_3$: Na, 25.5. Found: Na, 25.3.

The trimethyl ester of **4** was obtained as an oil by treatment of the acid, either with diazomethane in ether or with methanolic HCl. The infrared spectrum showed $\lambda_{max}^{CCl_4}$ 5.7 μ (ester C=O); no bands in the 3-μ region (OH) or in the 5.5-μ region (γ-lactone C=O); nmr spectrum τ 6.27, 6.30, 6.31 (OCH_3), two doublets at 6.86 (J = 17 cps) and 7.25 (J = 18 cps) (CH_2), 8.4 (CCH_3).

Anal. Calcd for $C_{10}H_{14}O_7$: C, 48.75; H, 5.73; OCH_3, 37.81. Found: C, 48.37; H, 5.88; OCH_3, 37.51.

γ-Lactone of α-Methyl-α,β-dihydroxytricarballylic Acid (5, R = H). A solution of γ-methyl-*cis*-aconitic anhydride[14] (3.0 g), $KClO_3$ (2.4 g), K_2CO_3 (0.6 g), and OsO_4 (0.09 g) in water (60 ml) was heated at 45° for 5 hr. After the addition of concentrated HCl (2.0 ml), the solution was extracted with ten 150-ml portions of ethyl acetate. The residue (1.53 g) obtained after drying the solution over Na_2SO_4 and vacuum evaporation, was dissolved in 10 ml of ethyl acetate and decolorized with Norit A. A 500-mg portion of the yellow oil obtained on evaporation was chromatographed on a column of Celite (60 g) mixed with 0.5 *N* H_2SO_4 (42 ml). The column was eluted first with 1-butanol–chloroform (10:90), then with 1-butanol–chloroform (35:65), and fractions were collected at 30-min intervals (about 8 ml initially). Fractions 48–58 were combined, and on evaporation yielded 230 mg of a colorless oil. A white, crystalline solid was obtained by dissolving the oil in ether and adding petroleum ether (bp 30–60°). The γ-lactone of α-methyl-α,β-dihydroxytricarballylic acid (**5**, R = H) had mp 185–187°; infrared spectrum λ_{max}^{KBr} 2.85 (OH), 5.63 (γ-lactone C=O), and 5.80 μ (acid C=O).

Anal. Calcd for $C_7H_8O_7$: C, 41.18; H, 3.95. Found: C, 41.75; H, 4.33.

The dimethyl ester (**5**, R = CH_3) was prepared with diazomethane in ether and had mp 153–155°; infrared spectrum λ_{max}^{KBr} 2.95 (OH), 5.65 (γ-lactone C=O), and 5.83 μ (ester C=O); nmr spectrum τ 6.10, 6.19 (OCH_3), 6.97, 6.99 (CH_2), 8.50 (CCH_3), 6.25 (OH).

Anal. Calcd for $C_9H_{12}O_7$: C, 46.55; H, 5.21; OCH_3, 26.73. Found: C, 46.62; H, 5.32; OCH_3, 26.48.

The same lactone (**5**, R = H) was also prepared from the epoxytricarboxylic acid (**4**, 5 mg) by heating for 1 hr at 80–85° with 1 ml of 1% aqueous $HClO_4$. After cooling in ice and saturating the solution with SO_2, the excess SO_2 was removed by a stream of nitrogen. The solution was evaporated to dryness in a stream of nitrogen at 80° and the residue was dried under vacuum. This material had the same R_f as **5** (R = H) on chromatography in the ethyl acetate–5 *M* formic acid solvent system. The residue was converted to the ester by reaction with diazomethane in ether. After removal of the ether, the residue was sublimed at 0.1 mm and 80–85°, and the sublimate was then crystallized from ether–pentane. The white crystals had mp 154–156°, and this melting point was unchanged in admixture with the synthetic ester (**5**, R = CH_3).

Acknowledgments. This research was supported in part by the National Institutes of Health through Grants No. AM-03737 and FR-5451-04. We thank Donald Lee for technical assistance and Drs. A. S. Aiyar and G. H. Dialameh for providing the biologically synthesized radioactive CoQ_9 samples for degradation. We are indebted to Mr. Ross Pitcher of Varian Associates, Pittsburgh, Pa., for the nuclear magnetic resonance and mass spectra determinations.

(12) F. Feigl, "Spot Tests in Organic Analysis," Elsevier Publishing Co., Amsterdam, 1960, pp 357, 515.

(13) E. Habicht and P. Schneeberger, *Helv. Chim. Acta*, **39**, 1316 (1956).

(14) O. Gawron and K. P. Mahajan, *Federation Proc.*, **24**, 228 (1965). We are very much indebted to Professor O. Gawron for details of this synthesis in advance of publication.

Communications to the Editor

Stereospecific 6,1 Migration of Deuterium during Rearrangement of 2-Phenylnorbornane-2,3-*cis*-*exo*-diol-5,6-d_2[1]

Sir:

In 1961 Kleinfelter and Schleyer[2] reported that diol I (without deuterium) rearranges, in sulfuric acid, to produce 3-*endo*-phenylnorbornanone-2 (II, without deuterium). We showed[3] later that this rearrangement proceeds: (1) with inversion of configuration, (2) with intramolecular migration of the original 3-*endo*-hydrogen, and (3) without migration of phenyl (the asterisks denote appropriate labels with tritium and carbon-14). These results are compatible with the presence of the bridged ion intermediates A and B. In the rearrangement A → B an intramolecular 6,1-hydride shift must take place.[3] We were curious as to whether this shift occurs (1) by a discrete shift of the *exo*-6-hydrogen, (2) through a "face-protonated" cyclopropane intermediate,[4,5] or (3) through some

(1) Research sponsored by the U. S. Atomic Energy Commission under contract with the Union Carbide Corporation.

(2) D. C. Kleinfelter and P. von R. Schleyer, *J. Am. Chem. Soc.*, **83**, 2329 (1961).

(3) C. J. Collins, Z. K. Cheema, R. G. Werth, and B. M. Benjamin, *ibid.*, **86**, 4913 (1964).

(4) J. D. Roberts and C. C. Lee, *ibid.*, **73**, 5009 (1951).

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