Wallach[36] which in our hands gave a mixture of carvomenthone, carvotanacetone and carvacrol that had to be resolved by extraction with alkali followed by gas chromatography. Pure carvotanacetone showed $\lambda_{max}^{alc}$ 236 mμ (ε 8650), 317 mμ (ε 45), in accord with the reported values,[16] and $\lambda_{max}^{film}$ 5.98μ(C=O). The semicarbazone had m.p. 176–179°, reported[37] 177–178°. The semicarbazones from the two sources had identical infrared absorption spectra (KBr disks) and the mixture had m.p. 176–178°.

(36) O. Wallach, *Ann.*, **414**, 353 (1918).

(37) I. Heilbron and H. M. Bunbury, "Dictionary of Organic Compounds," Oxford University Press, New York, N. Y., 1953.

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[CONTRIBUTION FROM MERCK, SHARPE AND DOHME RESEARCH LABORATORIES, A DIVISION OF MERCK AND CO., INC., RAHWAY, N. J.]

# Coenzyme Q. XIX. Alkoxy Homologs of Coenzyme $Q_{10}$ from Methoxy Group Exchange

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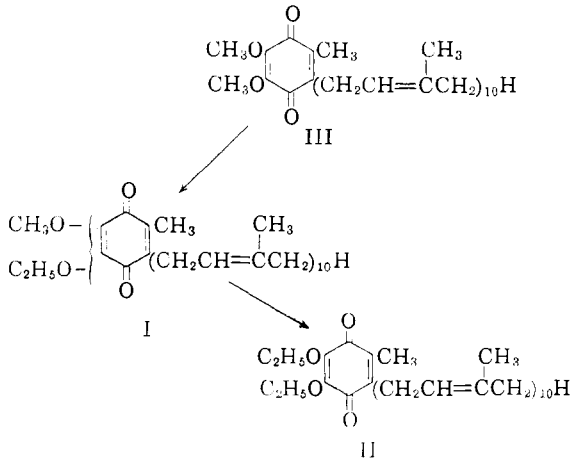
The reactivity of the methoxy groups of coenzyme $Q_{10}$ has been utilized to prepare the diisoamoxy- and diisopropoxy homologs of coenzyme $Q_{10}$. The monoethoxy homolog of $Q_{10}$ obtained during the isolation of $Q_{10}$ from beef heart has been shown by degradation and synthetic studies to be a mixture of the two monoethoxy oriented derivatives, indicating comparable reactivity of the two methoxy groups of $Q_{10}$. The syntheses have made available the new 4-ethoxy-2-methyl-3,5,6-trimethoxy- and 5-ethoxy-2-methyl-3,4,6-trimethoxyphenylacetic acids.

Our initial experience on the isolation and structural elucidation of ethoxy homologs of coenzyme $Q_{10}$ was reported,[1] and the extended study of the reactions of coenzyme $Q_{10}$ with ethanol has been described.[2] It was found that during the hot alkaline saponification in ethanolic solution, which is a step in the isolation of coenzyme $Q_{10}$ from beef heart tissue, the monoethoxy- (I) and diethoxy- (II) homologs of coenzyme $Q_{10}$ (III) were formed. The extent of the conversion of coenzyme $Q_{10}$ to these ethoxy homologs depends upon several aspects of the reaction conditions. These ethoxy homologs were not obtained when methanol was substituted for ethanol in the procedure. It was shown that the ethoxy derivatives are artifacts of isolation. The presence of pyrogallol and nitrogen atmosphere for the hot saponification step minimizes the exchange reaction, but does not always

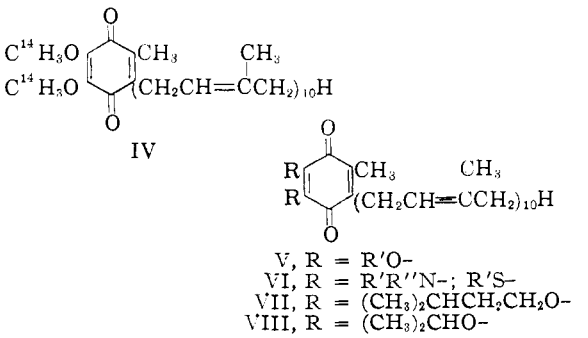


completely suppress this reaction, particularly when the exposure of coenzyme $Q_{10}$ to these conditions is prolonged.

It is evident that the reactivity of the methoxy group of 1,4-benzoquinone is much more similar to that of a carboxylic methyl ester than to that of typical aliphatic or aromatic methyl ethers. The reaction of methoxy-1,4-benzoquinone with various nucleophilic reagents to cause displacement of the methoxy group has been studied previously.[3-5]

Nucleophilic displacement of a methoxy group of a benzoquinone derivative by $RO^-$, such as $C_2H_5O^-$, has not been seen in the literature, but the ethoxy homologs of coenzyme $Q_{10}$ appear to be examples of such a reaction.

This reactivity of the methoxy groups of coenzyme $Q_{10}$ offers an approach to the preparation of radioactive coenzyme $Q_{10}$ (IV), higher alkoxy homologs (V), and nitrogen- and sulfur-containing derivatives (VI) by reaction with appropriate nucleophiles. We have utilized this approach



V, R = R'O-
VI, R = R'R''N-; R'S-
VII, R = $(CH_3)_2CHCH_2CH_2O$-
VIII, R = $(CH_3)_2CHO$-

and prepared the diisoamoxy (VII) and diisopropoxy- (VIII) homologs of coenzyme $Q_{10}$. These homologs are of interest to study for possible inhibition of coenzyme $Q_{10}$ in various succino- and cytochrome enzyme systems.[6]

(1) B. O. Linn, N. R. Trenner, C. H. Shunk and K. Folkers, THIS JOURNAL, **81**, 1263 (1959).

(2) B. O. Linn, N. R. Trenner, B. A. Arison, R. G. Weston, C. H. Shunk and K. Folkers, *ibid.*, **82**, 1647 (1960).

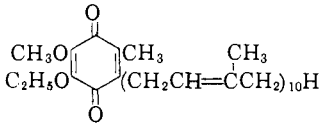
(3) D. Buckley, H. B. Henbest and P. Slade, *J. Chem. Soc.*, 4891 (1957).

(4) D. Buckley, S. Dunstan and H. B. Henbest, *ibid.*, 4901 (1957).

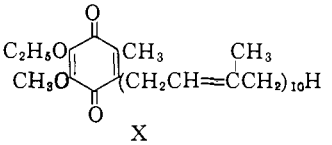
(5) J. A. D. Jeffreys, *ibid.*, 2153 (1959).

(6) D. Hendlin and T. Cook, *J. Biol. Chem.*, **235**, 1187 (1960).

Since there are two possible monoethoxy homologs (IX and X) of coenzyme $Q_{10}$, it was of interest to reduce, methylate and oxidize the monoethoxy

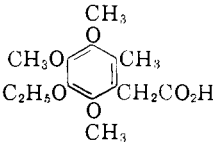


IX

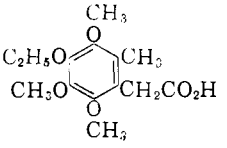


X

product[1,2] obtained from the isolation process to a substituted phenylacetic acid, and compare the product of degradation with the two corresponding synthetic acids XI and XII.



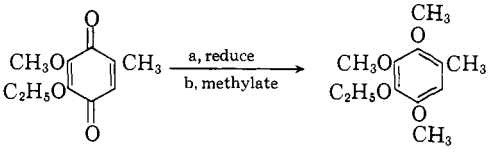
XI



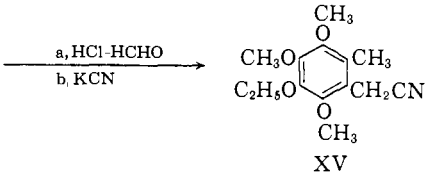
XII

The monoethoxy product[1,2] was degraded by the same three-step sequence which was used[7] for coenzyme $Q_{10}$ itself. The product of the degradation behaved as a mixture of the two substituted phenylacetic acids XI and XII, according to $R_f$ and infrared data. The monoethoxy homolog of coenzyme $Q_{10}$ must be a mixture (I) of IX and X, although paper chromatography did not distinguish between the two components. However, a one-carbon difference, *i.e.*, the monoethoxy homologs (IX and X) *versus* $Q_{10}$ is sufficient to make possible separation on papergrams.[2]

5 - Ethoxy - 2 - methyl - 3,4,6 - trimethoxyphenylacetic acid (XI) was obtained by the alkaline hydrolysis of the nitrile XV which in turn was synthesized from 5-ethoxy-6-methoxy-2-methylbenzoquinone[8] (XIII) by the reaction sequence including XIV.



XIII XIV



XV

4 - Ethoxy - 2 - methyl - 3,5,6 - trimethoxylphenylacetic acid (XII) was synthesized from commercially available 3-ethoxy-4-hydroxybenzaldehyde (XVI) by the reaction sequence XVII through XX and completed by hydrolysis of the nitrile XX.

(7) D. E. Wolf, C. H. Hoffman, N. R. Trenner, B. H. Arison, C. H. Shunk, B. O. Linn, J. F. McPherson and K. Folkers, THIS JOURNAL, **80**, 4752 (1958).

(8) W. K. Anslow and H. Raistrick, *Biochem. J.*, **32**, 694 (1938).

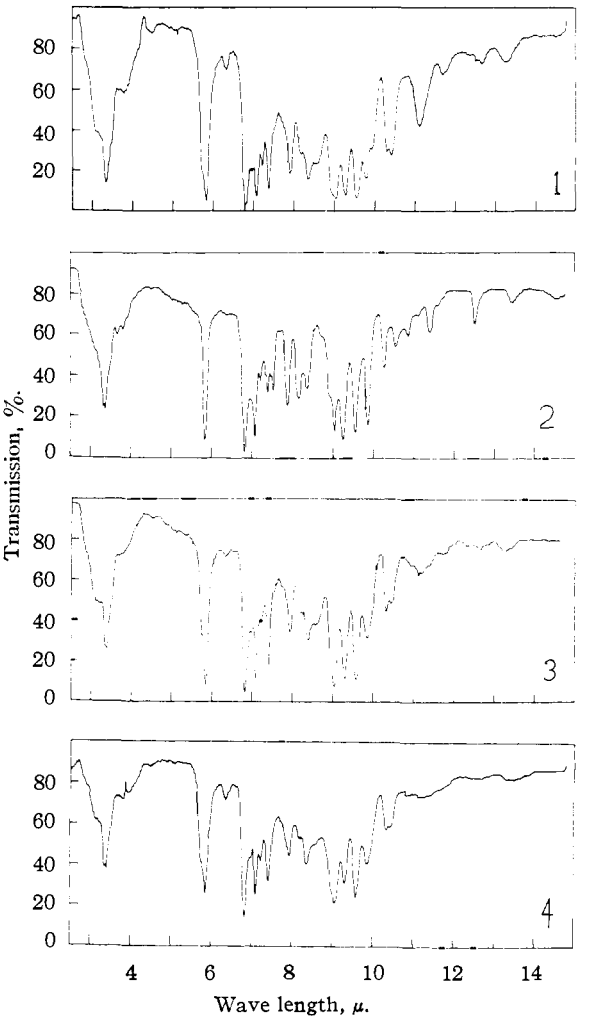
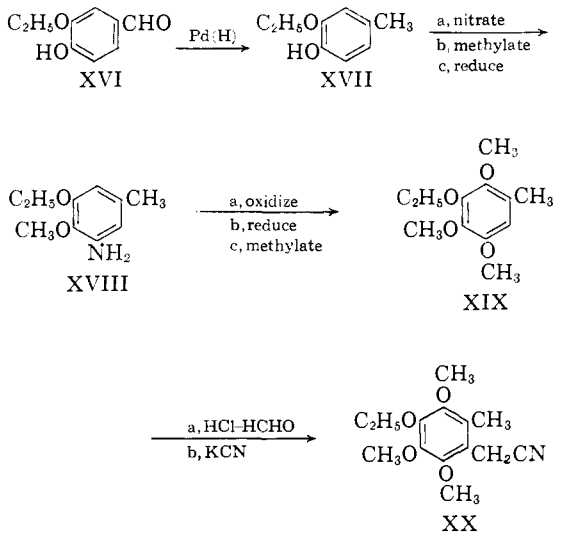



Fig. 1.—Comparison of infrared spectra: curve 1, XI, 5-ethoxy-2-methyl-3,4,6-trimethoxyphenylacetic acid; curve 2, XII, 4-ethoxy-2-methyl-3,5,6-trimethoxyphenylacetic acid; curve 3, mixture (50:50) of XI and XII; curve 4, acid(s) obtained by oxidation of the ethoxy homolog of dimethyldihydrocoenzyme $Q_{10}$.

By comparison of the infrared curves it can be seen that the oxidation product obtained from the monoethoxy derivative of coenzyme $Q_{10}$ has a spectrum most similar to the 50:50 mixture of the two isomeric alkoxyphenylacetic acids XI and XII.

## Experimental

**Diisoamoxy Homolog of Coenzyme$Q_{10}$ (VII).**—To 50 ml. of anhydrous ethyl ether was added 500 mg. (580 μmoles) of coenzyme $Q_{10}$ and 5.80 ml. (5.80 mmoles) of 1.00 *N* sodium isoamoxide in isoamyl alcohol. The reaction solution was protected from moisture and from direct light and was kept under a nitrogen atmosphere while being stirred and heated at reflux for 2.5 hours. The mixture was cooled, and the reaction was stopped by the addition of 9 ml. of 1 *N* hydrochloric acid. Ether and water were added. The ether layer was separated, washed free of acid with water, and then dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure leaving a red viscous residue. This residue was dissolved in 25 ml. of hexane (Skellysolve B) and passed onto a column, 1.8 × 56 cm., of 60–100 mesh Florisil, 50 g., packed in hexane. The column was washed with 500 ml. of hexane and developed with 1.0 l.




of 2% ethyl ether in hexane. An orange band was eluted in the next 1.0 l. of 2% ether solution. Concentration of this fraction under reduced pressure yielded 142 mg. of residue. This material was dissolved in hexane and further purified on a column, i.d. 1.1 cm., containing 8 g. of 50–100 mesh Decalso. The resulting orange band was washed with 300 ml. of hexane and then eluted with 100 ml. of 2% ethyl ether in hexane. The latter fraction was concentrated under reduced pressure, giving 93 mg. of the diisoamoxy homolog of coenzyme $Q_{10}$ (VII): in isoöctane ultraviolet absorption at $\lambda_{max}$ 271 m$\mu$ ($E^{1\%}_{1cm}$ 134), $\lambda_{min}$ 236 m$\mu$ ($E^{1\%}_{1cm}$ 39); in carbon disulfide infrared bands characteristic for 2,3-dialkoxy-1,4-benzoquinones[2] at 6.03 (C=O), 6.18 (C=C) and 7.92 $\mu$ (=C—O); n.m.r. analysis was consistent with structure VII.

*Anal.* Calcd. for $C_{67}H_{106}O_4$ (975.52): C, 82.48; H, 10.95; alkoxy, 2.00 moles per mole of sample. Found: C, 82.28; H, 11.10; alkoxy, 2.09 moles.

**Diisopropoxy Homolog of Coenzyme $Q_{10}$ (VIII).**—A solution containing 2.9 mmoles of sodium isopropoxide was prepared by the reaction of 67 mg. of sodium in 250 ml. of redistilled isopropyl alcohol, which had been dried by refluxing over magnesium methoxide. The solution was diluted with 250 ml. of sodium-dried ethyl ether and was freed from oxygen by bubbling high purity, anhydrous nitrogen through the solution. The system was protected from moisture with a Drierite tube, from air by a slightly positive pressure of nitrogen, and from light by a covering. To the solution was added 5.00 g. (5.8 mmoles) of coenzyme $Q_{10}$, and the resulting reaction mixture was stirred slowly for 1.0 hour at 25°. The mixture was acidified with 30 ml. of 1.0 *N* hydrochloric acid and diluted with 150 ml. of water and 250 ml. of ether. The ether layer was separated, extracted with three 100-ml. portions of water, and dried over anhydrous magnesium sulfate. The ether was evaporated under reduced pressure leaving 5.4 g. of a red. viscous residue which was dissolved in isoöctane and evaporated under reduced pressure. The residue was redissolved in isoöctane and evaporated several times in order to remove the last traces of isopropyl alcohol.

A solution of the residue in 50 ml. of *n*-hexane was passed onto a column, 2.7 × 97 cm., of Davison activated silica gel, 300 g. of 100–200 mesh, packed in *n*-hexane. Using a flow rate of 700 ml./hr., the column was washed with 840 ml. of *n*-hexane and was then developed with the following ethyl ether–hexane solutions: 730 ml. of 1% ether, 200 ml. of 3% ether and 910 ml. of 6% ether. An orange band was eluted with the next 330 ml. of 6% ether solution. Evaporation of this eluate gave 2.85 g. of a red, viscous residue which contained three components by papergram analysis.[2]

This residue was dissolved in 25 ml. of redistilled isooctane and passed onto a column, 2.7 × 94 cm., of 60–100 mesh Florisil, 280 g., packed in isoöctane. Using a flow rate of 200 ml./hr., the column was washed with 210 ml. of isoöctane and was then developed with the following ethyl ether–isooctane solutions: 70 ml. of 2% ether, 70 ml. of 4% ether, 90 ml. of 6% ether and 1010 ml. of 8% ether. An orange band was eluted with the next 540 ml. of 8% ether solution. The residue, 1.18 g., from this eluate appeared to contain a single component by papergram analysis.[2] Crystallization from 100 ml. of ethanol gave orange crystals, 0.98 g., of the diisopropoxy homolog of coenzyme $Q_{10}$ (VIII): m.p. 36–37° with softening at 33°; in isoöctane ultraviolet absorption at $\lambda_{max}$ 271 m$\mu$ ($E^{1\%}_{1cm}$ 135), $\lambda_{min}$ 236 m$\mu$ ($E^{1\%}_{1cm}$ 32); in carbon disulfide infrared bands characteristic of the 2,3-dialkoxy-1,4-benzoquinones[2] at 6.04 (C=O), 6.19 (C=C) and 7.92 $\mu$ (=C—O); n.m.r. analysis was consistent with structure VIII.

*Anal.* Calcd. for $C_{63}H_{98}O_4$ (914.37): C, 82.75; H, 10.25; alkoxy, 2.00 moles per mole of sample. Found: C, 82.66; H, 10.29; alkoxy, 1.83 moles.

**Dimethyl Ether of Monoethoxy Dihydro Coenzyme $Q_{10}$,4(5)-Ethoxy-2-methyl-3,5(4),6-trimethoxy-[3'-methyl-2'-butenyl-enakis-(3'-methyl-2'-butenylene)]-benzene.**—Eight hundred and twenty milligrams of the ethoxy homolog of coenzyme $Q_{10}$ (I)[1,2] was dissolved in about 100 ml. of ethanol, and an excess of a saturated aqueous solution of sodium hydrosulfite was added. The reaction mixture became colorless. It was blanketed with carbon dioxide by addition of pieces of Dry Ice and diluted with about 100 ml. of water. The hydroquinone was extracted with ether, and the ether solution was washed well with water, then with saturated salt solution, while maintaining the atmosphere of carbon dioxide.

The ether extract of the hydroquinone was evaporated *in vacuo*, and the residue was a light yellow oil.

The hydroquinone was dissolved in 40 ml. of ethanol, and the solution was placed in a flask equipped with a stirrer and nitrogen gas inlet. To the stirred solution was added 1.6 ml. of dimethyl sulfate. Aqueous sodium hydroxide (34%) was added dropwise until 2 ml. had been added. The mixture was stirred several hours. It was then diluted with about 20 ml. of water, and extracted repeatedly with ether. The ether extract was washed with 0.1 *N* hydrochloric acid, then twice with saturated salt solution. It was dried over magnesium sulfate, filtered and evaporated *in vacuo* to a light yellow oil (800 mg.).

**Oxidation of the Ethoxy Homolog of Dimethyldihydrocoenzyme $Q_{10}$ to a Mixture of 4- and 5-Ethoxy-2-methyl-3,5-(4)6-trimethoxy-phenylacetic Acids.**—Eight hundred and fifty milligrams of 4(5)ethoxy-2-methyl-3,5(4),6-trimethoxy-[3'-methyl-2'-butenyl-enakis-(3'-methyl-2'-butenylene)]-benzene was dissolved in 800 ml. of acetone and oxidized with 60 ml. of 5% (aqueous) potassium permanganate at room temperature. After standing overnight, 10 ml. of 2.5 *N* aqueous sodium hydroxide was added to the reaction mixture. Another equal volume of alkali was added after 2 hours. When the permanganate color was gone, the mixture was filtered to remove the manganese dioxide, and the acetone was evaporated *in vacuo*.

The manganese dioxide residue and the residue from acetone were both extracted with water giving a strongly alkaline solution. After filtration, this aqueous extract was acidified with hydrochloric acid and extracted with ether. Acidic material was obtained from the ethereal solution by extraction with 10% aqueous sodium carbonate. The alkaline solution was then acidified and extracted with ether. After washing with water and drying over magnesium sulfate, the ether extract yielded 152 mg. of acidic material as a yellow oil.

Purification of the crude acidic fraction was accomplished by first distilling at reduced pressure which yielded a nearly colorless distillate between 90° and 120° (1 mm.) (47 mg.). The distillate was then passed over a column of cellulose fiber using 1-butanol saturated with 5 *N* ammonium hydroxide as the developing solvent. Fractions with high absorption densities at 278 m$\mu$ were combined. These fractions yielded a nearly colorless oil after distillation in a tube at about 1 mm. (100–120°). This oil( XI + XII) failed to crystallize either as a distillate or when dissolved in chloroform and the solvent allowed to evaporate.

For comparison with synthetic samples of the two isomeric acids XI and XII, paper chromatograms were run using 1-butanol saturated with 5 *N* ammonium hydroxide as the developing solvent. The oxidation product on the chromatograms showed a single, strongly absorbing spot when observed under ultraviolet illumination. It was indistinguishable by $R_f$ comparison from both of the two isomeric acids XI and XII.

| | Compound | $R_f$ | $R_f$[a] |
|---|---|---|---|
| 1 | Oxidation product | 0.68 | |
| 2 | 5-Ethoxy-2-methyl-3,4,6-trimethoxyphenylacetic acid | .67 | 0.67 |
| 3 | Mixture of 1 and 2 | .68 | |
| 4 | 4-Ethoxy-2-methyl-3,5,6-trimethoxyphenylacetic acid | | .64 |
| 5 | Mixture of 2 and 4 | | .65 |

[a] Separate experiment.

The infrared spectrum of the oxidation product was obtained directly from the oil, without solvent, using a Perkin–Elmer Infracord model 137.

**5-Ethoxy-6-methoxy-2-methylhydroquinone Dimethyl Ether (XIV).**—One gram of 5-ethoxy-6-methoxy-2-methylbenzoquinone (XIII) was dissolved in 5 ml. of ethanol containing a few drops of water. With stirring, 0.4 g. of sodium hydrosulfite was added in portions. The benzoquinone was reduced to the hydroquinone. An extra 0.1 g. of sodium hydrosulfite was added. Then, 6.3 g. of dimethyl sulfate was added. Finally, a solution of 2.0 g. of sodium hydroxide in 8 ml. of water was added over a 2-hour period at room temperature with stirring. After stirring at room temperature overnight, water was added to the reaction mixture, and it was extracted three times with ether. The combined ether extract was washed twice with 2 *N* sodium hydroxide solution and four times with water; it was dried over anhydrous magnesium sulfate, and concentrated under reduced pressure to yield 0.7 g. of crude 5-ethoxy-6-methoxy-2-methylhydroquinone dimethyl ether, as a pale yellow oil.

**5-Ethoxy-2-methyl-3,4,6-trimethoxybenzyl Chloride.**—The 0.7 g. (0.003 mole) of 5-ethoxy-6-methoxy-2-methylhydroquinone dimethyl ether (XIV) was suspended in a mixture of 35 ml. of concentrated hydrochloric acid and 1.0 ml. of formaldehyde. The mixture was stirred while gaseous hydrogen chloride was added. Gentle heating was maintained for 2.5 hours. The reaction mixture was diluted with water, and the mixture was extracted three times with ether. The combined ether extract was washed with water until the washings were neutral; it was dried over anhydrous magnesium sulfate, and the ether solution was concentrated under reduced pressure to yield 0.8 g. of crude 5-ethoxy-2-methyl-3,4,6-trimethoxybenzyl chloride as a nearly colorless oil. A small sample, when dissolved in ethanol–water solution, gave a precipitate of silver chloride when treated with silver nitrate.

**5-Ethoxy-2-methyl-3,4,6-trimethoxybenzyl Cyanide (XV).**—To 0.3 g. of potassium cyanide dissolved in 4 ml. of water was added 0.8 g. of 5-ethoxy-2-methyl-3,4,6-trimethoxybenzyl chloride in 30 ml. of ethanol. The mixture was refluxed with stirring for 4.25 hours. The ethanol was removed under reduced pressure. Water was added and the mixture was extracted three times with ether. The combined ether extract was washed well with water and dried over anhydrous magnesium sulfate. Removal of the ether by concentration under reduced pressure yielded 0.75 g. of 5-ethoxy-2-methyl-3,4,6-trimethoxybenzyl cyanide as a nearly colorless oil.

**5-Ethoxy-2-methyl-3,4,6-trimethoxyphenylacetic Acid (XI).**—A mixture of 0.75 g. of 5-ethoxy-2-methyl-3,4,6-trimethoxybenzyl cyanide (XV) and 17.9 g. of potassium hydroxide dissolved in 160 ml. of 50% methanol was refluxed with stirring under nitrogen for 10.5 hours. Ammonia was slowly evolved. The reaction mixture was concentrated under reduced pressure to remove methanol. More water was added to the concentrate, and the mixture was extracted four times with chloroform. The dried chloroform extract yielded 0.4 g. of pale yellow oil. The alkaline liquors were cooled and acidified to *p*H 2 with concentrated hydrochloric acid; no oil separated. The mixture was extracted with four portions of chloroform. After drying over anhydrous magnesium sulfate, the combined chloroform extract was concentrated under reduced pressure to yield 0.34 g. of 5-ethoxy-2-methyl-3,4,6-trimethoxyphenylacetic acid as a nearly colorless oil which crystallized. The product was distilled at 150–160° at 0.28 mm. pressure to give a colorless oil which crystallized when seeded; m.p. 60–62° (micro-block.).

*Anal.* Calcd. for $C_{14}H_{20}O_6$: C, 59.14; H, 7.09. Found: C, 59.44; H, 7.24.

**3-Ethoxy-4-hydroxytoluene (XVII).**—A solution of 200 g. (1.2 moles) of 3-ethoxy-4-hydroxybenzaldehyde (XVI, commercially available) in 1800 ml. of glacial acetic acid and 4 g. of palladium oxide catalyst was hydrogenated until two equivalents of hydrogen was absorbed. The catalyst was removed by filtration and was washed with methanol. The solvents were removed under reduced pressure to leave a nearly colorless oil which was fractionated through a short Vigreux column at reduced pressure. The fraction boiling at 121–122° (24–25 mm.) weighed 164.13 g. and was essentially pure 3-ethoxy-4-hydroxytoluene.

*Anal.* Calcd. for $C_9H_{12}O_2$: C, 71.02; H, 7.95. Found: C, 71.42; H, 7.94.

**3-Ethoxy-4-methyl-6-nitrophenol.**—First, an ethereal nitric acid solution was prepared. To 250 ml. of anhydrous ether, was added dropwise 20 ml. of red fuming nitric acid (sp.gr. 1.59–1.6, Baker & Adamson) with stirring and cooling so that the temperature stayed at 3–5°. A solution of 55 g. (0.362 mole) of 3-ethoxy-4-hydroxytoluene (XVII) in 2.5 liters of anhydrous ether was cooled to 15°. To this solution, the ethereal nitric acid solution was added over a period of 2 hours with stirring and cooling so that the temperature remained at 5–10°. The reaction mixture was maintained at 10–15° for another hour and then was allowed to stand at room temperature overnight. The dark red colored reaction mixture was extracted with 1 liter of cold 2% sodium hydroxide solution and then with five 200-ml. portions of cold 2% sodium hydroxide solution to remove all red color. The alkaline extracts were combined and the ether removed *in vacuo* with heating. The alkaline solution was cooled and acidified to *p*H 2 with concentrated hydrochloric acid. The yellow-orange colored crystals which separated were collected and washed with cold water. A solution of the moist product in 300 ml. of warm ethanol was treated with charcoal and filtered; a rinse with 100 ml. of ethanol was used. To the filtrate, was added 600 ml. of water with stirring and cooling so that crystallization occurred. The yellow orange colored product was collected and washed with cold solvent mixture The 3-ethoxy-4-methyl-6-nitrophenol was dried *in vacuo* over potassium hydroxide and then *in vacuo* over phosphorus pentoxide; weight, 44.6 g.; m.p. 58–59° (micro-block). For analysis, a small sample was sublimed at 100° at 0.1 mm. pressure. The 3-ethoxy-4-methyl-6-nitrophenol melted at 59°.

*Anal.* Calcd. for $C_9H_{11}NO_4$: C, 54.82; H, 5.62; N, 7.10. Found: C, 54.83; H, 5.60; N, 6.84.

**3-Ethoxy-4-methoxy-5-nitrotoluene.**—To a filtered solution of 32.3 g. (0.164 mole) of 3-ethoxy-4-methyl-6-nitrophenol in 210 ml. of toluene, 58.7 g. (0.42 mole) of anhydrous potassium carbonate and 28.7 ml. (38.2 g., 0.31 mole) of dimethyl sulfate were added. The mixture was refluxed with stirring for 3 hours. An additional 28.7 ml. of dimethyl sulfate was added dropwise over a period of 1 hour. Refluxing was continued for an additional hour. The reaction mixture stood at room temperature overnight. The solution was decanted and the insoluble material was triturated with 200 ml. of toluene. The combined toluene solution was extracted with two 200-ml. portions of 2% sodium hydroxide solution and washed with two 200-ml. portions of saturated salt solution. After drying over anhydrous magnesium sulfate, the toluene solution was concentrated under reduced pressure to leave 62.3 g. of brown oil containing dimethyl sulfate. The oil was fractionated under reduced pressure; the fraction boiling at 175–178° at 17–18 mm. was then refractionated. The fraction boiling at 103–105° at 0.1–0.2 mm. pressure was essentially pure 3-ethoxy-4-methoxy-5-nitrotoluene; weight 27.7 g., m.p. 33° (micro-block).

*Anal.* Calcd. for $C_{10}H_{13}NO_4$: C, 56.86; H, 6.20; N, 6.63. Found: C, 57.23; H, 5.99; N, 6.97.

**5-Amino-3-ethoxy-4-methoxytoluene (XVIII).**—A solution of 54 g. (0.13 mole) of 3-ethoxy-4-methoxy-5-nitrotoluene in 250 ml. of methanol with 3 g. of palladium-on-charcoal (5%) catalyst was shaken under an initial pressure of 40 lb. of hydrogen until 3 equivalents of hydrogen had been absorbed. The catalyst was removed by filtration and was washed with methanol. The methanol was removed under reduced pressure to leave 23.3 g. of 5-amino-3-ethoxy-4-methoxytoluene as a reddish-tinted oil.

**6-Ethoxy-5-methoxy-2-methylbenzoquinone.**—A suspension of 23.3 g. of 5-amino-3-ethoxy-5-methoxytoluene (XVIII) in a mixture of 72 ml. of concentrated sulfuric acid and

222 ml. of water was cooled to −5°. A solution of 11.1 g. of sodium dichromate in 67.2 ml. of water was added dropwise over a 2-hour period with the temperature maintained at −10° to −5°. Stirring was continued for 3 more hours at −10° to −5°. After standing at room temperature overnight, the mixture was cooled to 0° and 22.2 g. of sodium dichromate in 134.4 ml. of water was added over a 2-hour period. Stirring was continued at 0 −2° for 1 hour and then for 2 hours at room temperature. The acidic reaction mixture was extracted with five 1-liter portions of ether. The combined ether extract was washed once with water saturated with sodium sulfate. The ether extract was dried over anhydrous magnesium sulfate and concentrated under reduced pressure to leave 7.1 g. of reddish colored oil. This oil was extracted with five 200-ml. portions of warm petroleum ether. Concentration of the extract under reduced pressure left 5.6 g. of reddish oil.

A small chromatographic column was prepared using 60 g. of alumina (Brockmann, Grade II) and petroleum ether. The reddish oil was placed on the column using petroleum ether. The column was developed using petroleum ether. A large orange-colored band moved quite rapidly down the column leaving purple, violet and yellow bands behind. The petroleum ether eluate was concentrated under reduced pressure to leave 5.17 g. of reddish oil. The oil was redissolved in petroleum ether; the solvent was slowly removed under reduced pressure to leave yellow-orange needles of 6-ethoxy - 5 - methoxy - 2 - methylbenzoquinone, m.p. 34–35° (micro-block). A small sample sublimed at 75–80° at 0.1 mm. pressure melted at 33–34° (micro-block).

*Anal.* Calcd. for $C_{10}H_{12}O_4$: C, 61.21; H, 6.17. Found: C, 61.11; H, 6.07.

**6-Ethoxy-5-methoxy-2-methylhydroquinone Dimethyl Ether (XIX).**—One gram of 6-ethoxy-5-methoxy-2-methylbenzoquinone was dissolved in a mixture of 5 ml. of ethanol and 1 ml. of water. With stirring, 0.4 g. of sodium hydrosulfite was added in portions. The benzoquinone was reduced to the hydroquinone. An additional 0.1 g. of sodium hydrosulfite was added. Then, 6.3 g. of dimethyl sulfate was added. Finally, a solution of 2 g. of sodium hydroxide in 8 ml. of water was added over a 2.5-hour period at room temperature with stirring. Some more sodium hydrosulfite was added during this time. Stirring was continued at room temperature overnight. Water was added to the reaction mixture; it was extracted three times with ether. The combined ether extract was washed three times with 2 *N* sodium hydroxide solution and four times with salt water. After drying over anhydrous magnesium sulfate, the ether extract was concentrated under reduced pressure to leave 0.7 g. of crude dimethyl ether of 6-ethoxy-5-methoxy-2-methylhydroquinone as a pale yellow oil.

**4-Ethoxy-2-methyl-3,5,6-trimethoxybenzyl Chloride.**—The 0.7 g. of 6-ethoxy-5-methoxy-2-methylhydroquinone dimethyl ether (XIX) was added to a mixture of 35 ml. of concentrated hydrochloric acid and 1.0 ml. of formaldehyde. The suspension was stirred while gaseous hydrogen chloride was introduced. Gentle heating was maintained for 2.5 hours. The reaction mixture was diluted with water and the mixture was extracted three times with ether. The ether extract was washed four times with water. After drying over anhydrous magnesium sulfate, the ether extract was concentrated under reduced pressure to leave 0.75 g. of crude 4-ethoxy-2-methyl-3,5,6-trimethoxybenzyl chloride as a yellow colored oil. A small sample in ethanol–water solution gave a silver chloride precipitate when treated with silver nitrate.

**4-Ethoxy-2-methyl-3,5,6-trimethoxybenzyl Cyanide (XX).**—To 0.3 g. of potassium cyanide dissolved in 4 ml. of water was added 0.75 g. of 4-ethoxy-2-methyl-3,5,6-trimethoxybenzyl chloride in 30 ml. of ethanol. The mixture was refluxed with stirring for 4.5 hours. The ethanol was removed under reduced pressure. Water was added and the mixture was extracted three times with ether. The combined ether extract was washed three times with water. After drying over anhydrous magnesium sulfate, the ether extract was concentrated under reduced pressure to leave 0.75 g. of 4-ethoxy-2-methyl-3,5,6-trimethoxybenzyl cyanide as a nearly colorless oil.

**4-Ethoxy-2-methyl-3,5,6-trimethoxyphenylacetic Acid (XII).**—A solution of 0.75 g. of 4-ethoxy-2-methyl-3,5,6-trimethoxybenzyl cyanide (XX) and 17.9 g. of potassium hydroxide in 160 ml. of 50% methanol was refluxed with stirring under nitrogen for 11.5 hours. Ammonia was slowly evolved. The reaction mixture was concentrated under reduced pressure to remove methanol. More water was added to the concentrate which was then extracted with four portions of chloroform. The dried chloroform extract yielded 0.42 g. of pale yellow oil. The alkaline liquor was cooled and acidified to *p*H 2 with concentrated hydrochloric acid; an oil separated. The mixture was extracted with four portions of chloroform. After drying over anhydrous magnesium sulfate. the combined chloroform extract was concentrated under reduced pressure to leave 0.34 g. of nearly colorless oil which rapidly crystallized. The 4-ethoxy-2-methyl-3,5,6-trimethoxyphenylacetic acid was purified by sublimation. The product was distilled at 145–150° at 0.1 mm. pressure to yield a colorless oil which readily crystallized, m.p. 87–89° (micro-block).

*Anal.* Calcd. for $C_{14}H_{20}O_6$: C, 59.14; H, 7.09. Found: C, 58.89; H, 7.11.

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# Substitution, Oxidation and Group Participation in the Bromination of Indoles

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The structure of the dibromoskatole, resulting from the action of N-bromophthalimide on skatole in benzene, has been proved to be II by acid hydrolysis to 6-bromo-3-methyloxindole (III), an isomer of the bromination product IX of 3-methyloxindole, and by oxidative degradation to 2-acetamino-4-bromobenzoic acid (V). Electrophilic substitution of indoles in the 6-position has been shown, in the case of 2-phenylskatole (VI), to proceed *via* an unstable yellow perbromide intermediate (formulated as VII), rearranging rapidly to the 6-bromo compound VIII. In aqueous media, intramolecular participation of the carboxyl group of X, possibly by displacement on a bromonium intermediate XVI, has led to (5-bromo)-dioxindolespirolactones of type XI which have been hydrogenolyzed to oxindole-3-propionic acid (XII).

The action of brominating agents upon indoles has received comparatively little attention,[1,2] and has been studied mainly in non-aqueous media. The effect of water on the course of the reactions has not been generally recognized. In connection with the selective cleavage of tryptophyl peptide bonds by positive halogen[3-5] we had occasion to

(1) *Cf.* R. Brunck, *Ann.*, **272**, 206 (1893).

(2) W. C. Sumpter and F. M. Miller, "Heterocyclic Compounds with Indole and Carbazole Systems," **Interscience Publishers, Inc., New York, N. Y., 1954, pp. 29–31.**

(3) A. Patchornik, W. B. Lawson and B. Witkop, THIS JOURNAL, **80**, 4748 (1958).