XLII, XL, and XLIII) coupling with an axial and an equatorial proton.[18]

Preparation of 6-Hydroxy-1-tetralone (XXXIV) from III.—A solution of 0.52 g. (0.0023 mole) of the crude dione III and 0.004 g. of *p*-toluenesulfonic acid monohydrate in 18 ml. of benzene was refluxed for 4.75 hr., cooled, and poured onto a column of 10 g. of acid-washed alumina. Elution with 1:4 acetone–hexane afforded 0.346 g. (91%) of crude, yellow crystalline phenol, m.p. 142–148.5°. Two recrystallizations from methanol–water raised the melting point to 150.5–152° (lit.[27a] m.p. 150°, lit.[27b] m.p. 149–152°, lit.[27c] m.p. 150–152°); $\lambda_{max}^{CHCl_3}$ 3.05, 5.99, 6.22, and 6.32 μ; λ_{max}^{EtOH} 229 (ϵ 12,000), and 279 mμ (ϵ 16,000) (lit.[27d] λ_{max}^{EtOH} 228 (ϵ 11,400) and 281 mμ (ϵ 17,600)).

The hydrazone derivative was obtained as pale yellow needles, m.p. 194.195.5° dec. (lit.[39] m.p. 195°); λ_{max}^{EtOH} 224 (ϵ 12,000) and 276 mμ (ϵ 17,600); λ_{max}^{Nujol} 3.00, 3.15, 6.19, 6.37, and 6.68 μ. The semicarbazone was prepared as usual and recrystallized from ethanol–water to m.p. 216–216.5° dec. (lit.[27c] m.p. 216.5–217.5°); λ_{max}^{EtOH} 227 (ϵ 12,400) and 288 mμ (ϵ 19,000); λ_{max}^{Nujol} 2.89, 3.15, 5.91, 6.15, 6.32, and 6.65 μ.

Preparation of 6-Hydroxy-1-tetralone from XXX.—A mixture of 0.603 g. (0.00251 mole) of ketol XXX, m.p. 190–192°, and 0.035 g. of *p*-toluenesulfonic acid monohydrate in 30 ml. of dry benzene was refluxed for 1.75 hr., cooled, and chromatographed to yield 0.333 g. (82%) of crystalline 6-hydroxy-1-tetralone (XXXIV), identified by m.p. and infrared spectrum.

Preparation of 6-Hydroxy-1-tetralone from XXXV.—A mixture of 0.061 g. (3.4×10^{-4} mole) of the hydroxydione XXXV, m.p. 114–115.5°, and 0.003 g. of *p*-toluenesulfonic acid monohydrate in 5 ml. of dry benzene was refluxed for 19 hr. After removal of the solvent, the crude product showed λ_{max}^{EtOH} 279 mμ (ϵ 5300). (An aliquot taken after 50 min. of reflux showed no strong absorption at 280 mμ.) Chromatography yielded a small amount (*ca.* 0.005 g.) of crystalline 6-hydroxy-1-tetralone (XXXIV), identified by m.p., m.m.p., and infrared spectrum. The acid-catalyzed aromatization of XXXV thus is much less facile than that of III or its progenitor XXX. It should also be noted that the sodium borohydride reduction product XL survives vigorous acid-catalyzed acetylation conditions without aromatizing, so it seems that both XL and the diacetate XLIII are more resistant to aromatization than III (although XL and XLIII have not been tested with *p*-toluenesulfonic acid in benzene).

Treatment of 2β-Acetoxy-3α-hydroxycholestane with *p*-Toluenesulfonic Acid in Benzene.—A 0.042-g. (9.8×10^{-5} mole) sample of 2β-acetoxy-3α-hydroxycholestane,[40] m.p. 112–114°, was dissolved in 5 ml. of dry benzene containing 6×10^{-4} g. of *p*-toluenesulfonic acid monohydrate and the mixture was refluxed for 25 min. When cool, the mixture was chromatographed on 1.0 g. of acid-washed alumina. There was eluted 0.037 g. of material which was identified as starting material by infrared spectrum and m.p. This substance, however, crystallizes very slowly and poorly, so that recovery of material of m.p. *ca.* 112–114° from the reaction (or upon recrystallization of known pure material) was not good. Accordingly, we cannot exclude the possibility of the presence of a small amount of material other than 2β-acetoxy-3α-hydroxycholestane, which escaped detection in the infrared spectrum. Further experiments were desirable, but were precluded by lack of additional model substance. In any case, in view of the conformational rigidity of hydroxyl and acetoxyl in XXIX as opposed to the more flexible relationship in 2β-acetoxy-3α-hydroxycholestane, the latter is not a completely adequate model.

Acknowledgments.—The authors are grateful for the financial support provided by Public Health Service Research Grant A-5014. We wish to express our gratitude to Professor William S. Johnson of Stanford University for obtaining two of the n.m.r. spectra involved in this work and for his assistance, and that of Mr. Ronald J. McCaully of Harvard University, in the hydrogenation of pyrogallol.

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[CONTRIBUTION FROM THE MERCK SHARP & DOHME RESEARCH LABORATORIES DIVISION, MERCK & CO., INC., RAHWAY, N. J.]

Synthesis of the New 5-Phosphomethyl-6-chromanyl Acetate of Vitamin $K_{1(20)}$ by a Novel Cyclization Reaction[1]

BY ARTHUR F. WAGNER, AINO LUSI, ROBERT E. ERICKSON, BYRON ARISON, NELSON R. TRENNER, AND KARL FOLKERS

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A new type of phosphorylated chromanyl derivative, of current interest in studies of biological oxidative phosphorylation, has been synthesized by a novel cyclization reaction. Treatment of vitamin $K_{1(20)}$ with concentrated sulfuric acid, followed by reaction with water, yields the γ-hydroxy side-chain substituted derivative of vitamin $K_{1(20)}$. On reaction with acetyl chloride, the γ-hydroxy derivative is cyclized to the 5-chloromethyl-6-chromanyl acetate derivative of vitamin $K_{1(20)}$. This compound was converted, in several steps, to the 5-phosphomethyl-6-chromanyl acetate derivative of vitamin $K_{1(20)}$.

In a continuing study of phosphate derivatives of vitamin K in microbial oxidative phosphorylation, we have synthesized the 5-phosphomethyl-6-chromanyl acetate II of vitamin $K_{1(20)}$. This new structural type of phosphorylated chromanol (I) is likely to be more productive than previously reported hydroquinone monophosphates (III) and 6-chromanyl phosphates (IV) for studies of biological oxidative phosphorylation. Related data supporting a mechanism for one step in the synthesis of the 5-phosphomethyl derivative as well as its possible function in the formation of ATP from ADP by way of oxidative phosphorylation will be described in a subsequent publication.[2]

Anaerobic and acetylation techniques were recently introduced[3,4] to study a possible role for vitamin K in electron transport and coupled phosphorylation in light-inactivated, cell-free extracts of *Mycobacterium phlei*. Extension of these initial studies resulted in the isolation and identification of the new 6-chromanyl acetate V and later, the new and unexpected 5-chloromethyl-6-chromanyl acetate VI derivatives of vitamin $K_{1(20)}$ from the enzymic and acetylated reaction mixture.[5,6] Since it appeared likely that the chloro derivative VI was a nonenzymic product, the reaction of vitamin $K_{1(20)}$ with acetyl chloride was studied under a variety of conditions. This study revealed a new facet of vitamin K chemistry and provided the first experimental data to support a new concept[7] of "active phosphate" on the biosynthetic pathway from ADP to ATP.

Vitamin $K_{1(20)}$ and acetyl chloride do not react under strictly anhydrous conditions; in the presence of traces of moisture, however, the chloromethyl derivative VI was formed from these two reactants. Similarly, the

(1) (a) A preliminary report on this subject appeared as a Communication to the Editor in *J. Am. Chem. Soc.*, **85**, 1534 (1963). (b) This paper is Contribution L in the Coenzyme Q series.

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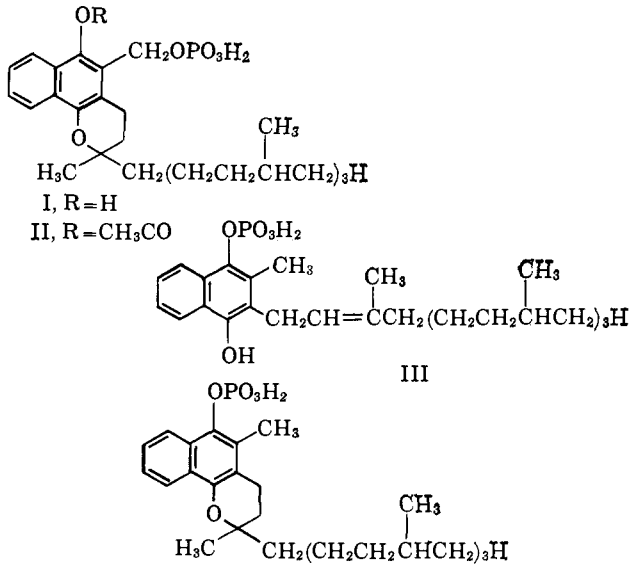
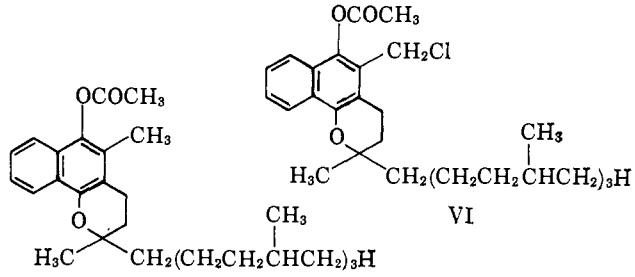


I, R=H
II, R=CH_3CO

III

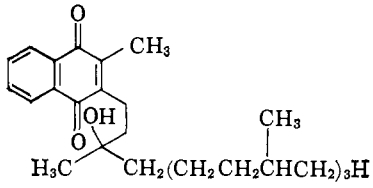
IV

reaction of vitamin $K_{1(20)}$ with acetyl chloride plus a trace of hydrochloric acid or sulfuric acid yielded the 5-chloromethyl-6-chromanyl acetate VI. Treatment of a mixture of vitamin $K_{1(20)}$ and dihydro vitamin $K_{1(20)}$ also gave the 5-chloromethyl-6-chromanyl acetate VI along with the expected diacetyldihydro vitamin $K_{1(20)}$. The results of all these experiments suggest that the reaction of vitamin $K_{1(20)}$ with acetyl chloride is catalyzed by strong acids.



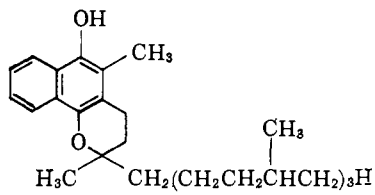
V VI

The reaction of vitamin $K_{1(20)}$ with concentrated sulfuric acid, followed by treatment with water, gave a number of products but only one in good yield. This major component, which was the only one to react with acetyl chloride to give the 5-chloromethyl-6-chromanyl acetate VI, was investigated further and assigned the structure of the γ-hydroxy side-chain substituted derivative VII of vitamin $K_{1(20)}$. The structural assignment was based on elemental analysis and ultraviolet and infrared spectra; the nuclear magnetic resonance spectrum of the γ-hydroxy derivative VII was also useful to the structural elucidation but its contribution to structural knowledge was not as critical as that to be acknowledged in the structural elucidation of the subsequent 5-substituted methyl derivatives. The ultraviolet absorption spectrum of the γ-hydroxy derivative VII was essentially identical with that of vitamin $K_{1(20)}$ except for a very slight shift to longer wave lengths; four consecutive absorption maxima at 244, 249, 264, and 273 mμ indicated the presence of a 1,4-naphthoquinone moiety, and the absence of weak maxima at 324 and 340 mμ suggested the absence of a 6-chromanol moiety. The infrared absorption spectrum of the γ-hydroxy derivative VII exhibited bands characteristic of the functional groups in vitamin $K_{1(20)}$ but, in addition, showed a band at 2.8 μ characteristic of a hydroxyl group. The nuclear magnetic resonance spectrum of the γ-hydroxy derivative VII established the presence of the 2-methyl substituent and an intact paraffinic isoprenoid side chain. Although the position of the hydroxyl group was not as rigorously established as those of the other functional groups, the well-documented acid-catalyzed hydrations of trisubstituted double bonds to yield tertiary alcohols and the facile dehydration of the hydroxy derivative to vitamin $K_{1(20)}$ constituted further supportive evidence for the γ-hydroxy formulation VII.



VII

The reaction of the γ-hydroxy derivative VII with acetyl chloride yielded the 5-chloromethyl-6-chromanyl acetate VI. Elemental analysis of the product was consistent with the presence of one chlorine atom per molecule of vitamin $K_{1(20)}$ derivative. The reactivity of the chloro group was demonstrated by its reaction with alcoholic silver nitrate in ethanol and its replacement with a dibenzyl phosphate or acetate group after treating the derivative with the appropriate silver salt. Furthermore, reduction of the 5-chloromethyl-6-chromanyl acetate VI yielded the corresponding 6-chromanol VIII of vitamin $K_{1(20)}$. The ultraviolet absorption maxima of the 5-chloromethyl-6-chromanyl acetate VI were similar to those of the 6-chromanyl acetate V of vitamin $K_{1(20)}$,[8] but were slightly displaced to longer wave lengths due to the presence of the halogen atom on the 5-substituent. The infrared spectrum of the 5-chloromethyl-6-chromanyl acetate VI was similar to that of the 6-chromanyl acetate V. The nuclear magnetic resonance spectrum of the 5-chloromethyl-6-chromanyl acetate VI provided a key contribution to the structural argument by revealing the presence of a two-proton peak at 5.4 τ. This, coupled with the absence of the typical aromatic methyl signal, led directly to the Ar–CH_2–Cl formulation.

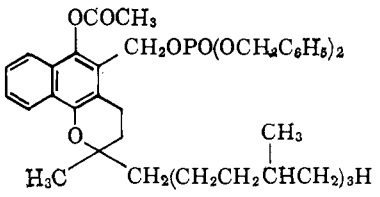


VIII

Treatment of the 5-chloromethyl-6-chromanyl acetate derivative VI with silver dibenzyl phosphate yielded the O,O-dibenzyl-O-{3,4-dihydro-2-*H*-naphtho[1,2-*b*]-pyran-5-ylmethyl} phosphate derivative IX of vitamin $K_{1(20)}$. The phosphoric acid triester IX was characterized by ultraviolet, infrared, and nuclear magnetic resonance spectroscopy. The 6-chromanyl functionality of the triester IX was evident from the ultraviolet and infrared absorption spectra, and the presence of the Ar–CH_2–O function was indicated by absorption at 5.05 and 5.20 τ in the nuclear magnetic resonance spectrum.

Selective cleavage of the benzyl groups in the triester IX to yield the 3,4-dihydro-2-*H*-naphtho[1,2-*b*]pyran-5-ylmethyl dihydrogen phosphate derivative II of vitamin $K_{1(20)}$ was accomplished by catalytic hydro-

(8) A. F. Wagner, P. E. Wittreich, B. Arison, N. R. Trenner, and K. Folkers, *J. Am. Chem. Soc.*, **85**, 1178 (1963).



IX

genolysis. The preferential cleavage of the benzyl groups was achieved, presumably because of steric effects which prevented attack by hydrogen at the highly substituted naphthopyranylmethyl substituent of the triester. The infrared and ultraviolet absorption spectra of the phosphoric acid derivative II were fully in accord with the structure. Interpretation of the nuclear magnetic resonance spectrum of II was equivocal because of certain bonding effects of the dihydrogen phosphate moiety. Reaction of the monoester II with diazomethane yielded the corresponding triester; the nuclear magnetic resonance spectrum of this product fully substantiated the structure II.

Experimental

3-(3-Hydroxy-3,7,11,15-tetramethylhexadecyl)-2-methyl-1,4-naphthoquinone.—An ice-cold mixture of 2.2 g. of vitamin $K_{1(20)}$ in 20 ml. of concentrated sulfuric acid was stirred until it became homogeneous. The solution was kept ice-cold for about an hour and then poured onto ice. The product was extracted with ether, and the extract was washed with water, dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residual oil (2.2 g.) was purified by chromatography on a column of 230 g. of silica gel packed in *n*-hexane. After preliminary elution was completed using *n*-hexane and *n*-hexane containing 1%, 3%, and 10% ether, the product was eluted with *n*-hexane containing 25% ether. The eluate was concentrated, and the residue (1.6 g.) was purified by chromatography on 200 g. of silica gel. About 0.9 g. of pure 3-(3-hydroxy-3,7,11,15-tetramethylhexadecyl)-2-methyl-1,4-naphthoquinone was obtained. The product was characterized by $\lambda_{max}^{isooctane}$ 244 ($E_{1\,cm}^{1\%}$ 380), 249 ($E_{1\,cm}^{1\%}$ 398), 264 ($E_{1\,cm}^{1\%}$ 366), 273 ($E_{1\,cm}^{1\%}$ 375), 325 mμ ($E_{1\,cm}^{1\%}$ 58); λ_{max}^{neat} 2.9, 6.0 μ.

Anal. Calcd. for $C_{31}H_{48}O_3$ (468.69): C, 79.43; H, 10.32. Found: C, 79.14; H, 10.37.

5-Chloromethyl-3,4-dihydro-2-methyl-2-(4,8,12-trimethyltridecyl)-2-*H*-naphtho[1,2-*b*]pyran-6-yl Acetate.—Three grams of 3-(3-hydroxy-3,7,11,15-tetramethylhexadecyl)-2-methyl-1,4-naphthoquinone was treated with 15 ml. of acetyl chloride. The reaction mixture was protected from atmospheric moisture and was allowed to stand at room temperature overnight. The mixture was poured onto ice, and the product was extracted with ether. The extract was washed with water, dried over anhydrous magnesium sulfate and concentrated, and the product was purified by chromatography on 200 g. of silica gel packed in *n*-hexane. After impurities were eluted from the column with 1% ether in *n*-hexane, 1.85 g. of 5-chloromethyl-3,4-dihydro-2-methyl-2-(4,8,12-trimethyltridecyl)-2-*H*-naphtho[1,2-*b*]pyran-6-yl acetate was eluted with 3% ether in *n*-hexane. The product was characterized by $\lambda_{max}^{isooctane}$ 248 ($E_{1\,cm}^{1\%}$ 707), 278 ($E_{1\,cm}^{1\%}$ 61), 300 $E_{1\,cm}^{1\%}$ 96), 323 ($E_{1\,cm}^{1\%}$ 82), 338 mμ ($E_{1\,cm}^{1\%}$ 77); λ_{max}^{neat} 5.65 μ.

Anal. Calcd. for $C_{33}H_{49}O_3Cl$ (529.18): C, 74.89; H, 9.33; Cl, 6.70. Found: C, 74.82; H, 9.59; Cl, 6.26.

O,O-Dibenzyl-O-{6-acetoxy-3,4-dihydro-2-methyl-2-(4,8,12-trimethyltridecyl)-2-*H*-naphtho[1,2-*b*]pyran-5-ylmethyl} Phosphate.—A mixture of 780 mg. of 5-chloromethyl-3,4-dihydro-2-methyl-2(4,8,12-trimethyltridecyl)-2-*H*-naphtho[1,2-*b*]pyran-6-yl acetate, 780 mg. of silver dibenzyl phosphate, and 40 ml. of acetonitrile was refluxed for 2.5 hr. The mixture was cooled and filtered, and the filtrate was concentrated. The residue was dissolved in ether, and the solution was filtered and concentrated to yield 900 mg. of residual oil which was purified by chromatography on silica G (Research Specialties) packed in *n*-hexane. Impurities were eluted using *n*-hexane–ether mixtures, and the product was selectively eluted with 50% ether in *n*-hexane to yield 650 mg. of O,O-dibenzyl-O-{6-acetoxy-3,4-dihydro-2-methyl-2-(4,8,12-trimethyltridecyl)-2-*H*-naphtho[1,2-*b*]pyran-5-ylmethyl} phosphate, λ_{max}^{EtOH} 215 ($E_{1\,cm}^{1\%}$ 653), 247 ($E_{1\,cm}^{1\%}$ 497), 307 ($E_{1\,cm}^{1\%}$ 70), 322 ($E_{1\,cm}^{1\%}$ 61), 336 mμ ($E_{1\,cm}^{1\%}$ 61); λ_{max}^{neat} 5.65, 9.5–10.5, 14.4 μ.

Anal. Calcd. for $C_{47}H_{63}O_7P$ (770.95): C, 73.21; H, 8.24; P, 4.02. Found: C, 73.01; H, 8.09; P, 3.84.

6-Acetoxy-3,4-dihydro-2-methyl-2-(4,8,12-trimethyltridecyl)-2-*H*-naphtho[1,2-*b*]pyran-5-ylmethyl Dihydrogen Phosphate.—Hydrogen was bubbled through a solution of 450 mg. of O,O-dibenzyl-O-{6-acetoxy-3,4-dihydro-2-methyl-2-(4,8,12-trimethyltridecyl)-2-*H*-naphtho[1,2-*b*] pyran-5-ylmethyl} phosphate in 75 ml. of ethanol in the presence of 300 mg. of 5% Pd-on-Darco for about 3 hr. The reaction mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was dissolved in ether and the solution was extracted with aqueous 5% potassium bicarbonate. The product was isolated from the alkaline extract by acidification, followed by ether extraction. The ether solution was dried over anhydrous sodium sulfate, filtered, and concentrated; the residue (250 mg.) was dissolved in a small volume of petroleum ether. The product precipitated slowly and, after several days, 111 mg. of 6-acetoxy-3,4-dihydro-2-methyl-2-(4,8,12-trimethyltridecyl)-2-*H*-naphtho[1,2-*b*]-pyran-5-ylmethyl dihydrogen phosphate was collected by centrifugation. The amorphous product was characterized by $\lambda_{max}^{isooctane}$ 247 ($E_{1\,cm}^{1\%}$ 506), 336 ($E_{1\,cm}^{1\%}$ 65), 321 ($E_{1\,cm}^{1\%}$ 67), 308 mμ ($E_{1\,cm}^{1\%}$ 71); $\lambda_{max}^{CCl_4}$ 2.9–4.0, 5.65, 8.3–9.1, 9.5–10.1 μ.

Anal. Calcd. for $C_{33}H_{51}O_7P$ (590.71): C, 67.10; H, 8.70; P, 5.24. Found: C, 67.54; H, 8.49; P, 5.00.

5-Acetoxymethyl-3,4-dihydro-2-methyl-2-(4,8,12-trimethyltridecyl)-2-*H*-naphtho[1,2-*b*]pyran-6-yl Acetate.—A mixture of 100 mg. of 5-chloromethyl-3,4-dihydro-2-methyl-2-(4,8,12-trimethyltridecyl)-2-*H*-naphtho[1,2-*b*]pyran-6-yl acetate, 100 mg. of silver acetate, and 5 ml. of acetic acid was refluxed for 30 min. The reaction mixture was poured onto ice, and the product was isolated by extraction with ether. The ether extract was dried over anhydrous magnesium sulfate and concentrated. The residue was purified by preparative thin-layer chromatography on silica using isooctane–benzene–acetone (85:13:2) to yield 30 mg. of 5-acetoxymethyl-3,4-dihydro-2-methyl-2-(4,8,12-trimethyltridecyl)-2-*H*-naphtho[1,2-*b*]pyran-6-yl acetate, R_f 0.08–0.1; $\lambda_{max}^{isooctane}$ 247 ($E_{1\,cm}^{1\%}$ 556), 307 ($E_{1\,cm}^{1\%}$ 97), 320 ($E_{1\,cm}^{1\%}$ 87), 335 mμ ($E_{1\,cm}^{1\%}$ 82); λ_{max}^{neat} 5.6, 5.75 μ.

Anal. Calcd. for $C_{35}H_{52}O_5$ (552.77): C, 76.04; H, 9.48. Found: C, 75.91; H, 9.49.

Nuclear Magnetic Resonance Spectroscopy.—All the n.m.r. data reported herein were obtained through the use of a Varian Associates Model 4300B high resolution spectrometer equipped with superstabilizer and phase detector and operating at 60 Mc. All spectra were run using 5–10% solutions in carbon tetrachloride. The resonance positions were determined relative to an external benzene capillary and scaled by the use of side bands[9] generated by a frequency-countercalibrated Hewlett-Packard audiooscillator Model 200 CD. The shielding numbers were calculated using the equation $\tau = (\Delta\nu/\nu_0) + 3.50$ where $\Delta\nu$ is the observed resonance displacement from benzene in cycles per second and ν_0 is the spectrometer frequency in megacycles.[10]

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