tion with molecular oxygen makes inhibition data reported for whole cells seem more meaningful. Evidently Szczepkowski and Skarzynski (1952) were misled regarding cytochromes in this organism.

Furthermore, a close link exists between thiosulfate oxidation and the cytochromes in *Thiobacillus* X (Trudinger, 1961b). Extracts contained a thiosulfate oxidase which catalyzed the oxidation of thiosulfate to tetrathionate with one of the cytochromes (cytochrome 553.5) acting as the electron acceptor. When the extract containing soluble cytochromes and thiosulfate oxidase was combined with a particulate fraction (which also contained cytochrome), the oxidation of thiosulfate was accompanied by oxygen uptake. Oxygen uptake could be abolished by addition of cyanide. Thus the participation of a complete cytochrome chain in thiosulfate oxidation by *Thiobacillus* X is clearly established.

Since *T. thiooxidans* grows only on sulfur, the insolubility of which prevents it from acting in the cell-free systems so far studied, evidence for the participation of cytochromes and coenzyme Q in the electron transport system starting from sulfur is necessarily indirect. This evidence consists of inhibition of whole cell respiration on sulfur by cyanide, azide, and carbon monoxide, and the presence in cell-free extracts of a cyanide-inhibited cytochrome oxidase. Since, however, this is a unique organism growing on sulfur in an extremely acid environment and deriving all its carbon from CO_2, the presence of cytochromes and the high level of coenzyme Q found are themselves of interest. Whether the real oxidation substrate is sulfur or an adenosine phosphosulfate derivative (Peck, 1962) is a matter for further study.

The compounds of the coenzyme Q family have been found in many but not all bacteria. However, this is, to our knowledge, the first demonstration of coenzyme Q in an obligate chemosynthetic autotrophic bacterium. While the occurrence of coenzyme Q in the thiobacilli is perhaps not surprising in view of its widespread distribution among bacteria, its presence does strengthen the case for a cytochrome system in *T. thiooxidans*. Coenzyme Q has not been found in bacteria devoid of cytochromes, such as *Streptococcus* and *Clostridium*, but is universally present in cytochrome-containing organisms, including the strict anaerobe *Desulfovibrio desulfuricans* (Lester and Crane, 1959).

It is of some interest to compare the amount of coenzyme Q found in *T. thiooxidans* with that reported for other bacteria. The average value of 4.5 μmoles per gram for *T. thiooxidans* compares favorably with 4.3 μmoles per gram for *Rhodospirillum rubrum*, which is the highest value reported for bacteria (Lester and Crane, 1959). Such a high level of coenzyme Q would imply an important respiratory function.

References

Aubert, J. P., Milhaud, G., Moncel, C., and Millet, J. (1958), *Compt. rend. 246*, 1616.

Crane, F. L., Widmer, C., Lester, R. L., and Hatefi, Y. (1959), *Biochim. et Biophys. Acta 31*, 476.

Dixon, M., and Webb, E. C. (1958), Enzymes, New York, Academic Press, Inc., p. 166.

Klimek, R., Skarzynski, B., and Szczepkowski, T. W. (1956), *Acta Biochim. Polon. 3*, 261.

Lester, R. L., and Crane, F. L. (1959), *J. Biol. Chem. 234*, 2169.

Lester, R. L., Hatefi, Y., Widmer, C., and Crane, F. L. (1959), *Biochim. et Biophys. Acta 33*, 169.

Page, A. C., Gale, P., Wallick, H., Walton, R. B., McDaniel, L. E., Woodruff, H. B., and Folkers, K. (1960), *Arch. Biochem. Biophys. 89*, 318.

Peck, H. D. (1962), *Bacteriol. Rev. 26*, 67.

Postgate, J. R. (1956), *J. Gen. Microbiol. 14*, 545.

Starkey, R. L. (1935), *J. Gen. Physiol. 18*, 325.

Straus, W. (1954), *J. Biol. Chem. 207*, 733.

Szczepkowski, T. W., and Skarzynski, B. (1952), *Acta Microbiol. Polon. 1*, 93.

Trudinger, P. A. (1961a), *Biochem. J. (London) 78*, 673.

Trudinger, P. A. (1961b), *Biochem. J. (London) 78*, 680.

Vogler, K. G., LePage, G. A., and Umbreit, W. W. (1942), *J. Gen. Physiol. 26*, 89.

Vogler, K. G., and Umbreit, W. W. (1941), *Soil Sci. 51*, 331.

Wagner, H., Hörhammer, L., and Dingler, B. (1962), *J. Chromatog. 7*, 211.

Coenzyme Q. XXXVI. Isolation and Characterization of Coenzyme Q_{10} (H-10)

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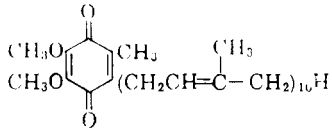
A new naturally occurring member of the coenzyme Q group has been isolated and crystallized from cells of *Gibberella fujckuroi*. Comparison of ultraviolet, infrared, and nuclear magnetic resonance spectra of this compound with spectra of coenzyme Q_{10} and related known compounds has revealed a structure differing from coenzyme Q_{10} in that the terminal unit in the ten-unit side-chain is isopentanyl, rather than isopentenyl. This new compound is designated coenzyme Q_{10}(H-10).

Coenzyme Q_{10} was described by Crane *et al.* (1957) after observations that a lipid in beef heart mitochondria was active in a succinoxidase system. This same quinone, which was designated ubiquinone (50), was described by Morton *et al.* (1957). It was shown to have structure I (Wolf *et al.*, 1958; Morton *et al.*, 1958).

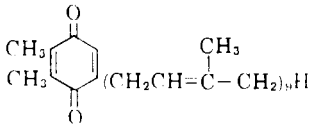
Interest in the biochemical role of this quinone has prompted investigation of the distribution of this quinone in a wide variety of potential source materials. Coenzyme Q_{10} has been found in all mammals that have been examined. There have also been reports of its presence in plants (Crane, 1959; Page *et al.*, 1959), microorganisms (Lester *et al.*, 1959; Page *et al.*, 1960; Erickson *et al.*, 1960), and an insect (Heller *et al.*, 1960).

In addition to coenzyme Q_{10}, there are four other naturally occurring coenzyme Q's (Q_6–Q_9) differing structurally from one another only in the number of isoprenoid units in the side-chain. Coenzymes Q_6–Q_9 occur most frequently in microbial species (Lester *et al.*, 1958), but Q_9 has been reported in some rodents (Linn

et al., 1959; Olsen and Dialameh, 1960; Green *et al.*, 1960).



Coenzyme Q_{10} I

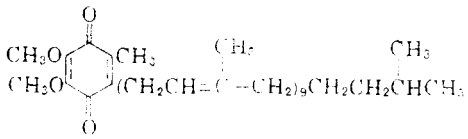


Plastoquinone II

Plastoquinone (II) (Crane, 1959; Trénner *et al.*, 1959), a structurally different quinone, is found in plants and appears to function in photosynthetic electron transport.

Diplock *et al.* (1960) have reported the presence in rat tissue of a quinone which was tentatively identified as a dihydroubiquinone-45 (side-chain reduced); detailed structural studies were not reported.

Cells of *Gibberella fujckuroi* have been used for the commercial production of gibberellic acid. We have obtained from these cells a quinone related to coenzyme Q_{10} in which the isoprenoid side-chain is partially reduced. This new quinone has been shown to have structure III. Since the terminal unit of the side-chain is reduced, the compound is designated coenzyme Q_{10}(H-10). Since there has been no nomenclature yet to designate such a compound, and a usable name is needed, the following rule has been used. As before,



Coenzyme Q_{10}*(H-10)* III

the subscript, 10, after the letter Q designates the number of isoprenoid units in the side-chain; now, the letter H in the parenthetical expression designates hydrogen or reduction (as frequently used in organic nomenclature) and the following number, 10, states that the tenth isoprenoid unit, counting from the nucleus, is reduced.

Results and Discussion

Saponification of cells of *Gibberella fujckuroi* in the presence of pyrogallol, followed by extraction of the nonsaponifiable material with hexane, yielded an oily, orange-colored residue. Purification by chromatography, first on Florisil and then on a Decalso column, afforded several coenzyme Q-rich eluates from which an orange crystalline compound, m.p. 28–29°, was obtained.

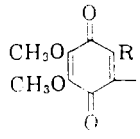
The close structural relationship between the new quinone (III) from *Gibberella fujckuroi* and coenzymes Q_9 and Q_{10} (I) was first noted as a result of the marked similarity between their ultraviolet spectra. The spectra of I (Linn *et al.*, 1959) and III, before and after treatment with sodium borohydride, were qualitatively and quantitatively identical. In addition, both compounds gave indistinguishable elemental analytical data and infrared spectra.

The difference in melting points (28–29° *vs.* 49.5–50.5°) of this quinone and coenzyme Q_{10} showed that the two compounds are not identical. Moreover, their papergram mobilities (Linn *et al.*, 1959) were quite different, the new quinone being less mobile than coenzyme Q_{10}.

Identity of the quinonoid nuclei of I and III was determined by comparison of their nuclear magnetic resonance (nmr) spectra. The absence of any aromatic protons established that the nucleus was totally substituted. Further interpretations of these data and nmr spectral data on solanesol (Erickson *et al.*, 1959), plastoquinone (Trenner *et al.*, 1959), and 2,3-dimethoxy-5-methyl-6-phytylbenzoquinone led to determination of the structure of the isoprenoid side-chain. The data are presented in Table I.

The area measurements of the resonance at 4.95 τ revealed the presence of only nine side-chain —CH= protons instead of ten as found in coenzyme Q_{10}. In addition to having all of the proton types of coenzyme Q_{10}, the new quinone showed, like 2,3-dimethoxy-5-methyl-6-phytylbenzoquinone, protons in the paraffinic region of 8.8–9.3 τ, indicating the presence of at least one reduced isoprenoid unit. The typical doublet at 9.10 and 9.20 τ of a six-proton area clearly points to the presence of two methyl functions of the type —CH(CH$_3$)—CH$_3$. This result, together with the presence of nine —CH= protons and no more than 5 protons in the 8.75 τ region, which is clearly a complex group of spin-spin coupled resonances, leads directly to the conclusion that a terminal —CH(CH$_3$)$_2$ function is present. The doublet separation of six cycles was similar to the known spin-spin coupling constant of an isopropyl group. In further support of this conclusion, it may be stated that if there were reduction of one carbon-carbon double bond located in mid-chain, there would be seven more paraffinic protons, which would fall in the 8.75 τ region; since an isopropyl function is certainly present, it follows that two reduced isoprenoid units would have fourteen paraffinic protons whose resonances would be found in the 8.75 τ region. This is far in excess of the number observed, and only one reduced unit is apparent. In the case of the 6-phytylbenzoquinone model, the 8.78 τ band is much larger in area than the paraffinic methyl doublet resonances at 9.08 and 9.19 τ; this result is in accord with the presence of a phytyl side-chain which has three reduced isoprenoid units.

Any consideration based on the absence of a five-methyl group and of the presence, in its place, of a branched group, as in IV, is excluded by the presence of the multiplet at 8.75 τ. Indeed, a slight but defi-



R = —CH(CH$_3$)$_2$
—CH$_2$CH=C(CH$_3$)$_2$
—CH$_2$CH$_2$CH(CH$_3$)$_2$

IV

nite shoulder is perceivable on the low field side of the band at 8.01 τ which is compatible with the presence of a five-methyl group. This effect is more clearly seen in the nmr spectrum of coenzyme Q_{10}. The presence of only two protons of the =C—CH$_2$—C= type at 6.80 and 6.94 τ clearly shows the presence of only one isoprenoid side-chain on the benzoquinone nucleus.

The structure of the isoprenoid side-chain, established by these data, is therefore the same as that for

TABLE I

COMPARISON OF THE NUCLEAR MAGNETIC RESONANCE SPECTRA OF COENZYME Q_{10} (H-10), COENZYME Q_{10}, AND 2,3-DIMETHOXY-5-METHYL-6-PHYTYLBENZOQUINONE

Substance	Shielding Number (t) and Relative No. of Protons	$-CH=$[a] Side-Chain	$=C-OCH_3$ Ring	$=C-CH_2-CH=$ Side-Chain	$=C-CH_3$ Ring	$=C-CH_2CH_2-C=$ Side-Chain	CH_3 1st $=C-CH_2$ Side-Chain	CH_3 2nd $=C-CH_2$ Side-Chain	CH_3 $=C-CH_2$ Side-Chain	All Others	Paraffinic CH_2 & CH Side-Chain	Paraffinic CH_3 Terminal
Co Q_{10} at 60 megacycles in CCl_4	t	4.97 (broad)	6.07 (singlet)	6.85 6.97 (doublet)	8.00 (shoulder)	8.03 (broad)	8.27 (broad)	8.33 (shoulder)		8.42 (broad)	—	—
	RNPO[b]	10	6[c]	2		37	3		33			
	RNPT	10	6	2		39	3		30			
Co Q_{10} (H-10) at 60 megacycles in CCl_4	t	4.95 (broad)	6.09 (singlet)	6.80 6.94 (doublet		8.01 (broad)	8.25 (broad)		8.39 (broad)		8.75 (multiplet)	9.10 9.20 (doublet)
	RNPO	8.6	6[c]	2		—	—		—		3.5 (*ca.*)[d]	6
	RNPT	9	6	2		37	3		24		5	6
2,3-Dimethoxy-5-methyl-6-phytylbenzoquinone at 40 megacycles in CCl_4	t	4.90 5.09 5.25 (triplet)	6.08 (singlet)	6.79 6.96 (doublet)	8.01 (singlet) (sharp)[e]	—	8.30 (broad)	—		—	8.78 (broad)	9.08 9.19 (doublet)
	RNPO	1	6[c]	2	5 (*ca.*)	—	5 (*ca.*)	—			17 (*ca.*)	12 (*ca.*)
	RNPT	1	6	2	3	—	5	—			19	12

[a] Italicized proton is the one giving the indicated resonance. [b] RNPO = Relative no. of protons observed. RNPT = relative no. of protons theory. [c] Arbitrarily assumed number of protons in the methoxyl resonance. [d] Much of this multiplet is spread over into the 8.39 broad resonance; therefore this value is a minimum. [e] Approximate because of overlap of broad adjacent resonances.

coenzyme Q_{10}, except that the terminal unit is isopentanyl instead of isopentenyl.

The length of the side-chain was confirmed by comparison of the papergram mobilities of catalytically reduced coenzyme Q_9, coenzyme Q_{10}, and this new quinone. The side-chain reduction products, as quinones, from the latter two compounds have the same mobility. The reduced product from coenzyme Q_9 moves at a faster rate.

Experimental

Saponification of Cells of Gibberella fujckuroi.—Six liters of fermentation broth was centrifuged, and the cellular paste was dried to a moist cake on a Buchner funnel. To the 800 g of moist cells was added 75 g of pyrogallol, 300 g of sodium hydroxide, 1.5 liters of methanol, and 1 liter of water. The mixture, contained in a 3-liter round-bottomed flask, was heated under reflux for 1 hour. After being cooled for one-half hour, the mixture was extracted successively with three 1.5-liter volumes of hexane. The pooled extracts were washed with three 500-ml portions of water and then dried over anhydrous sodium sulfate. Evaporation of the solvent from the dried hexane solution gave an oily, orange-colored residue which weighed 550 mg.

Florisil Chromatography.—A column of activated magnesium silicate (Florisil) was prepared by pouring a mixture of 7.5 g of the adsorbent, 60–100 mesh, in hexane into a 9 mm i.d. glass chromatograph column. The excess solvent was drained from the column until only a thin layer remained above the Florisil. A solution containing 550 mg of the oily residue in 10 ml of hexane was added to the column and allowed to flow through it slowly. The solution was followed successively by 125 ml of hexane, 100 ml of 5% ethyl ether in hexane, and finally 100 ml of 50% ether in hexane. The third 100-ml eluate was concentrated *in vacuo* to a yellow oil. A solution of the oil in 10 ml of isooctane deposited white crystals at 5°. A second crop of crystals was obtained from the concentrated mother liquor. Evaporation of the final liquors gave an orange oil which was combined with similar material from another batch of cells. The pooled sample weighed 310 mg.

Chromatography on Decalso.—The 310 mg of material in 5 ml of hexane was added to a 9-mm diameter column of magnesium alumino silicate (Decalso), 50 mesh and finer, prepared in the same manner as the Florisil column. The column was washed with 125 ml of hexane and was then eluted with a total of 230 ml of 2% ether in hexane as follows: eluates 2 (100 ml), 3 (10 ml), 4 (11 ml), 5 (13 ml), 6 (12 ml), 7 (13 ml), 8 (12 ml), and 9 (60 ml).

Fractions 3–9 were evaporated to dryness. A solution of each residue in 1 ml of absolute ethanol was cooled to 5°. Solutions from fractions 5–8 deposited orange crystals which were combined and dried *in vacuo;* weight 32 mg, m.p. 28–29°.

Anal. Calcd. for $C_{59}H_{92}O_4$: C, 81.88; H, 10.72. Found: C, 81.46; H, 10.50.

An ethanol solution of the product gave an ultraviolet spectrum, λ_{max} 275 mμ, $E^{1\%}_{1cm}$ = 165. Treatment of the solution with sodium borohydride produced a shift in the ultraviolet maximum to 290 mμ, $E^{1\%}_{1cm}$ = 30. The infrared spectrum in chloroform was indistinguishable from that of coenzyme Q_{10}.

Coenzyme Q_{10} and the new quinone were paper chromatographed on circles of Whatman No. 1 paper impregnated with white petroleum jelly (Vaseline). Dimethylformamide (Merck, reagent)–water (97:3) solution saturated with Vaseline was the mobile phase. The chromatograms were developed radially. Zones on the air-dried papergrams were visible as dark areas when viewed in ultraviolet light. The two compounds gave zones with R_F values of 0.33 and 0.26, respectively. Comparisons of the mobilities of the monoethoxy and diethoxy analogs of Q_{10} and of this new quinone gave a value of 0.22 for the new quinone alone and slightly elongated zones at the same R_F for mixtures of the compound with the ethoxy analogs. Paper chromatography of the same compounds with 99:1 dimethylformamide–water as the developing solvent gave slightly elongated zones with R_F values of 0.43 and 0.45, respectively, for mixtures of the new compound and the mono and diethoxy coenzyme Q_{10} analogs.

Catalytic Hydrogenation of Coenzyme Q_{10}(H-10), Coenzymes Q_9 and Q_{10}.—Samples of approximately 20 mg of the compound from *Gibberella fujckuroi* and of coenzyme Q_9 and Q_{10} were each dissolved in 20 ml ethyl acetate and reduced catalytically with a platinum oxide catalyst. The products were isolated as oils and then chromatographed radially on Vaseline-treated Whatman No. 1 paper. Glacial acetic acid saturated with Vaseline was the mobile phase. Reduced coenzymes Q_9, Q_{10}, and the new compound gave ultraviolet-absorbing zones with R_F values of 0.07, 0.03, and 0.03, respectively. A mixture of the latter two compounds moved as a single substance. After chromatography (descending) for 68 hours in the same system, the quinones of perhydrogenated coenzyme Q_{10} and the new compound had moved 9.6 cm; the corresponding compound from coenzyme Q_9 had moved 22.8 cm.

Nuclear Magnetic Resonance Data.—All the nmr data were obtained through the use of a Varian Associates Model 4300B high-resolution spectrometer that was equipped with super-stabilizer and phase detector and operating at both 40 and 60 megacyles. All spectra were determined by placing 5–10% solutions in carbon tetrachloride in a spinning Wilmad precision bore tube. The resonance positions were determined relative to a benzene capillary as an external reference and scaled by the use of side-bands (Arnold and Packard, 1951) generated by a frequency-counter-calibrated Hewlett-Packard audio oscillator Model 200 CD. The shielding numbers τ were calculated from the equation $t = (\Delta N/N_0) + 3.50$ where ΔN is the observed resonance displacement from benzene in cycles per second and N_0 is the spectrometer frequency in megacycles. Resonance band areas were determined by averaging repetitive planimeter measurements.

References

Arnold, J. T., and Packard, M. E. (1951), *J. Chem. Phys. 19*, 1608.

Crane, F. L. (1959), *Plant Physiol. 34*, 128.

Crane, F. L., Hatefi, Y., Lester, R. L., and Widmer, C. (1957), *Biochim. et Biophys. Acta 25*, 220.

Diplock, A. T., Edwin, E. E., Green, J., Bunyan, J., and Marcinkiewicz, S. (1960), *Nature 186*, 554.

Erickson, R. E., Brown, K. S., Jr., Wolf, D. E., and Folkers, K. (1960), *Arch. Biochem. Biophys. 90*, 314.

Erickson, R. E., Shunk, C. H., Trenner, N. R., Arison, B. H., and Folkers, K. (1959), *J. Am. Chem. Soc. 81*, 4999.

Green, J., Edwin, E. E., Diplock, A. T., and Bunyan, J. (1960), *Biochem. Biophys. Res. Comm. 2*, 388.

Heller, J., Szarkowska, L., and Michalek, H. (1960), *Nature 188*, 491.

Lester, R. L., and Crane, F. L. (1959), *J. Biol. Chem. 234*, 2169.

Lester, R. L., Crane, F. L., and Hatefi, Y. (1958), *J. Am. Chem. Soc. 80*, 4751.

Linn, B. O., Page, A. C., Jr., Wong, E. L., Gale, P. H., Shunk, C. H., and Folkers, K. (1959), *J. Am. Chem. Soc. 81*, 4007.

Morton, R. A., Gloor, U., Schindler, O., Wilson, G. M., Chopard-dit-Jean, L. H., Hemming, F. W., Isler, O., Leat, W. M. F., Pennock, J. F., Rüegg, R., Schweiter, U., and Wiss, O. (1958), *Helv. Chim. Acta 41*, 2343.
Morton, R. A., Wilson, G. M., Lowe, J. S., and Leat, W. M. F. (1957), *Chem. and Ind.* 1649.
Olsen, R. E., and Dialameh, G. H. (1960), *Biochem. Biophys. Res. Comm. 2*, 198.
Page, A. C., Jr., Gale, P. H., Koniuszy, F., and Folkers, K. (1959), *Arch. Biochem. Biophys. 85*, 474.
Page, A. C., Jr., Gale, P. H., Wallick, H., Walton, R. B., McDaniel, L. E., Woodruff, H. B., and Folkers, K. (1960), *Arch. Biochem. Biophys. 89*, 318.
Trenner, N. R., Arison, B. H., Erickson, R. E., Shunk, C. H., Wolf, D. E., and Folkers, K. (1959), *J. Am. Chem. Soc. 81*, 2026.
Wolf, D. E., Hoffman, C. H., Trenner, N. R., Arison, B. H., Shunk, C. H., Linn, B. O., McPherson, J. F., and Folkers, K. (1958), *J. Am. Chem. Soc. 80*, 4752.

Characterization of Vitamin $K_9(H)$ from *Mycobacterium phlei*

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A newly characterized naphthoquinone has been isolated from *Mycobacterium phlei*. Spectral data and analytical and papergram results revealed that it is closely related structurally to vitamin $K_{2(45)}$, but differs in that one of the side-chain isoprenoid units is reduced. Nuclear magnetic resonance data also show that the saturated isoprenoid unit is not located at either end of the side-chain. This compound is designated vitamin $K_9(H)$ by the same nomenclature used for the new coenzyme Q_{10}(H-10).

Brodie *et al.* reported in 1958 a new naphthoquinone isolated from extracts of *Mycobacterium phlei* which was active in restoring oxidative phosphorylation to light-inactivated preparations. Their comparisons by paper chromatograms led to the conclusion that the quinone from *M. phlei* is different from all known vitamin K homologs which were available to them and including six members of the vitamin K_1 series, that is, $K_{1(5-30)}$ and vitamin $K_{2(35)}$. Comparison of the infrared spectrum of this quinone from *M. phlei* with the homologs of vitamin K_1 and K_2 showed identity in the position of peaks with the former but marked differences from the latter.

A crystalline naphthoquinone derivative related to vitamin K_2 was isolated from the acetone-soluble fat fraction of *Mycobacterium tuberculosis* (Brevannes) by Noll in 1958. It was shown later by Noll *et al.* (1960) that this compound and vitamin $K_{2(45)}$, synthesized from menadione and solanesol (Shunk *et al.*, 1959), are identical.

On the basis of specific spectral characteristics and quantitative infrared spectroscopy as well as paper chromatography, Noll (1960) concluded that a sample of vitamin K from *M. phlei*, provided by Brodie, and vitamin $K_{2(45)}$ from *M. tuberculosis* are identical.

Our interest in enzyme preparations from *Mycobacterium phlei* for studies concerning the mechanism of oxidative phosphorylation and the apparent involvement of naphthoquinone derivatives in this biochemical process led us to isolate and newly characterize a biologically active[1] vitamin K from *Mycobacterium phlei*. In addition to this interest, we wished to clarify the nature of the vitamin K obtained from *M. phlei*.

Results and Discussion

The nonsaponifiable material obtained from *Mycobacterium phlei* cells was purified by chromatography on a Decalso column with *n*-hexane and ether as eluting solvents (Table I). Eluate fractions contained material having an ultraviolet absorption spectrum characteristic of vitamin K.

After further purification by solvent fractionation followed by chromatography on Decalso columns with isooctane as solvent (Tables II, III), the product was isolated as an orange-colored oil.

Comparison of the ultraviolet absorption spectrum with spectra of authentic vitamins $K_{1(20)}$ and $K_{2(45)}$ (Table IV) revealed that the isolated compound was a member of the vitamin K group. In addition, the strikingly similar quantitative absorptions of the product from *M. phlei* and vitamin $K_{2(45)}$ strongly suggested that the two compounds were of nearly equal molecular weight. The elemental analysis was consistent with the assignment of a vitamin K structure to this com-

TABLE I
CHROMATOGRAPHY ON DECALSO, COLUMN 1

Developing Solvent	Vol. of Fraction (ml)	Color	Weight of Residue (g)
400 ml *n*-hexane	(1) 200	Yellow	0.26
	(2) 200	Yellow	0.46
	(3) 150	Pale yellow	1.07

TABLE II
CHROMATOGRAPHY ON DECALSO, COLUMN 2

Developing Solvent	Volume of Fraction (ml)	Color	Weight of Residue (mg)
2800 ml isooctane	(1) 1000	Colorless	
	(2) 300	First yellow band	24.4
	(3) 800	Second yellow band	138.9
	(4) 700	Pale yellow	242.6
600 ml ether	(5) 600	Yellow	313.8

* Coenzyme Q. XXXVII.

† Oxidative Phosphorylation in Fractionated Bacterial Systems. IX.

[1] Unpublished data.