A NEW BIOLOGICALLY ACTIVE NAPHTHOQUINONE[1]

Sir:

Indications of apparent involvement of quinones as coenzymes in cellular metabolism[2,3] prompts us to report findings concerning a new naphthoquinone in *Mycobacterium phlei*. With extracts of *M. phlei* which have been irradiated to destroy naphthoquinones, restoration of oxidative phosphorylation is specifically dependent upon the addition of vitamin K_1 or a closely related 2,3-dialkyl-1,4-naphthoquinone.[4] The active substance[4] has now been identified as a naphthoquinone.

The naphthoquinone was extracted from washed cells (450 g., wet) by refluxing with 2,2,4-trimethylpentane–2-propanol (3:1). Extraction with acetone then gave a product which was chromatographed on Decalso. The naphthoquinone (10 mg.) was eluted as a yellow oil with petroleum ether–ether (49:1).

The absorption spectrum is identical in position and relative intensities ($\lambda_{max.}^{isooctane}$ 243, 249, 261, 270, 328 and a shoulder at 240 mμ) with those of vitamins K_1 and K_2 while the $E_{1\,cm.}^{1\%}$ indicated a maximum mol. wt. of 620. Comparison of the infrared spectrum with those of the homologs of vitamins K_1 and K_2 showed identity in the position of the peaks with the former but marked differences from the latter. The intensity of the C–H stretching and bending vibrations indicated more than 25 saturated carbon atoms in the molecule. The compound gives positive Dam–Karrer[5] and Almquist–Klose[6] tests, a negative Craven test[7] and is destroyed by light at 360 mμ.

Chromatography on vaseline-impregnated paper with methanol–2,2,4-trimethylpentane (3:1), solvent I, or methanol–2,2,4-trimethylpentane–2-propanol (3:1:1), solvent II, revealed a difference from all known K-homologs.[8]

Compound		R_f I	R_f II
Naphthoquinone ex *M. phlei*		0.06	0.10
K_1 series: side chain	C_5	.81	.88
	C_{10}	.53	.75
	C_{15}	.27	.56
	C_{20}	.17	.41
	C_{25}	.12	.25
	C_{30}	..	.14
Vitamin K_2		.15	.26

Comparison of the UV and IR spectra of the oily hydroquinonediacetate with those of the corresponding derivative of vitamin K_1 revealed differences similar to those between the parent compounds ($\lambda_{max.}^{isooctane}$ 233, 278 and 288 mμ).

(1) This investigation was supported in part by research grants E-827(C4) from the National Institute of Allergy and Infectious Diseases and CY-1696 (C5) from the National Cancer Institute, of the National Institutes of Health; and by the Milton Fund of Harvard University.

(2) A. F. Brodie, M. M. Weber and C. T. Gray, *Biochim. et Biophys. Acta*, **25**, 448 (1957).

(3) R. L. Lester, F. L. Crane and Y. Hatefi, THIS JOURNAL, **80**, 4751 (1958).

(4) A. F. Brodie, Abstracts of Papers, 132nd meeting, American Chemical Society, New York, New York, Sept. 1957, p. 52-C.

(5) H. Dam, P. Karrer, *et al.*, *Helv. Chim. Acta*, **22**, 310 (1939).

(6) H. J. Almquist and A. A. Klose, THIS JOURNAL, **61**, 1610 (1939).

(7) R. Craven, *J. Chem. Soc.*, 1605 (1931).

(8) We wish to express our appreciation to Dr. O. Isler, F. Hoffman LaRoche and Co., for a generous supply of these compounds.

The yellow oil has antihemorrhagic activity.[9] It restored both oxidation and phosphate esterification when added to light-treated extracts and was 3 times more active than vitamin K_1 in oxidation and 6 times in phosphorylation. The P/O ratio observed with the natural compound was 1.35, whereas it was only 0.68 with a concentration of vitamin K_1 which gave maximal restoration. Restoration also occurred with K_1-naphthoquinones, vitamin K_1 being the most active, while K_2-homologs containing 2 and 3 isoprene units showed only slight activity.[10]

Quinones isolated from beef heart mitochondria participate in electron transport[11] whereas a synthetic quinone was used to restore oxidation and phosphorylation[12] with mammalian mitochondria. The new naphthoquinone is unique in that it is found in mycobacterial extracts capable of phosphorylation and is more active than any other quinone tested in restoring oxidative phosphorylation. The monophosphate ester may exist as the active intermediate in oxidative phosphorylation.[2,4,13–16]

(9) We wish to express our appreciation to Dr. J. Vitale, Harvard School of Public Health, for assaying this compound.

(10) A. F. Brodie, *Federation Proc.*, **17**, 196 (1958).

(11) Y. Hatefi, R. L. Lester and T. Ramasarma, *ibid.*, **17**, 238 (1958).

(12) R. E. Beyer, *Biochim. et Biophys. Acta*, **28**, 663 (1958).

(13) J. S. C. Wessels, *Rec. trav. chim.*, **73**, 529 (1954).

(14) K. Harrison, *Nature*, **181**, 1131 (1958).

(15) V. M. Clark, G. W. Kirby and A. R. Todd, *Nature*, **181**, 1650 (1958).

(16) T. Wieland and F. Pattermann, *Angew. Chem.*, **70**, 313 (1958).

LEONARD WOOD MEMORIAL LABORATORY
DEPARTMENT OF BACTERIOLOGY AND IMMUNOLOGY
HARVARD MEDICAL SCHOOL — A. F. BRODIE
BOSTON, MASSACHUSETTS
DEPARTMENT OF CHEMISTRY — B. R. DAVIS
HARVARD UNIVERSITY — L. F. FIESER
CAMBRIDGE, MASSACHUSETTS

RECEIVED OCTOBER 24, 1958

THE PREPARATION OF PHOSPHORUS-CONTAINING HETEROCYCLES BY REACTION OF PHOSPHINE WITH ALDEHYDES

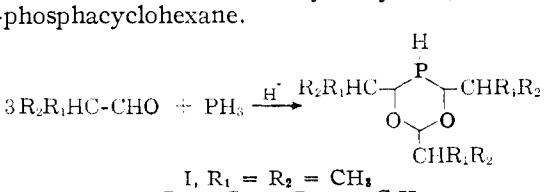
Sir:

The reaction of phosphine with aliphatic aldehydes has been investigated previously and the products usually obtained were tetrakis-(1-hydroxyalkyl)-phosphonium salts.[1–4]

$$4\ RCHO + PH_3 + HX \rightarrow (RCHOH)_4P^{(+)}X^{(-)}$$

R = H or *n*-alkyl group

We have found that this reaction takes a different course with alpha-branched aldehydes and leads to the formation of secondary phosphines which are derivatives of a novel heterocyclic system, 1,3-dioxa-5-phosphacyclohexane.



I, $R_1 = R_2 = CH_3$
II, $R_1 = C_2H_5$; $R_2 = n\text{-}C_4H_9$

(1) J. Messinger and C. Engels, *Ber.*, **21**, 326 (1888).

(2) A. Hoffman, THIS JOURNAL, **43**, 1684 (1921).

(3) A. Hoffman, *ibid.*, **52**, 2995 (1930).

(4) W. A. Reeves, F. F. Flynn and J. D. Guthrie, *ibid.*, **77**, 3923 (1955).