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.

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(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).

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

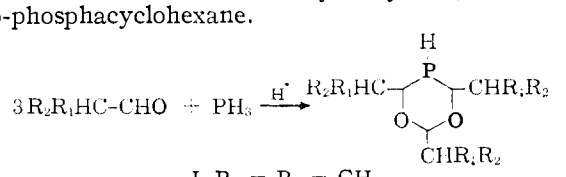
Sir:

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).

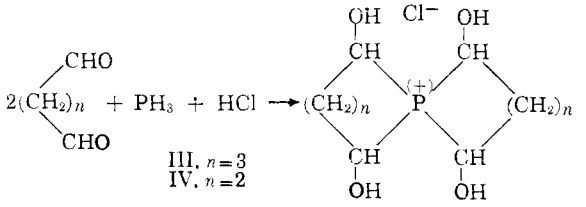
(2) A. Hoffman, THIS JOURNAL, **43**, 1684 (1921).

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

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

Presumably, these substances are formed rather than the phosphonium derivatives obtained from unbranched aldehydes because of steric hindrance toward further phosphine-aldehyde reaction in the disubstituted intermediates, $(R_1R_2CHOH)_2PH$.

We have also found that the phosphine-aldehyde reaction can be adapted to the preparation of spirophosphonium salts if suitable dialdehydes are employed. The application of this reaction to the preparation of simple heterocycles is obvious. A single spirocyclic phosphonium derivative has



been reported previously and required eleven steps in its preparation.[5] The stereochemical features of the spiranes reported here are interesting, and are being studied further.

The reaction of phosphine with isobutyraldehyde gave a 75% yield of 2,4,6-triisopropyl-1,3-dioxa-5-phosphacyclohexane (I), b.p. 110° (14 mm.), $n^{25}D$ 1.4602. Found: C, 61.82; H, 10.78; P, 13.26. Reaction with 2-ethylhexaldehyde gave a 90% yield of 2,4,6-(3-heptyl)-1,3-dioxa-5-phosphacyclohexane (II), b.p. 149–150° (0.02 mm.), $n^{24}D$ 1.4709. Found: C, 71.87; H, 12.35; P, 7.57. Glutaraldehyde gave a 65% yield of 1,5,7,11-tetrahydroxy-6-phosphazoniaspiro[5.5]undecane chloride (III), m.p. 167–168°. Found: C, 44.51; H, 7.55; Cl, 13.07; P, 11.58. Succinaldehyde[6] gave a 34% yield of 1,4,6,9-tetrahydroxy-5-phosphazoniaspiro[4.4]nonane chloride, m.p. 94–95°. Found: C, 39.49; H, 6.55; Cl, 14.65; P, 12.85; mol. wt., 92.0 (cryoscopic in water). These reactions were carried out by simultaneous addition of the aldehyde[6] and phosphine to a mixture of concentrated hydrochloric acid and tetrahydrofuran at room temperature. The derivatives I and II separated from the reaction mixture as upper phases.

Treatment of I with air in 2-propanol gave 2,4,6-triisopropyl - 1,3 - dioxa - 5 - phosphacyclohexane-5-inoic acid (59%), m.p. 159–160°. Found: C, 54.51; H, 9.44; P, 11.95; neut. equiv., 268.1. Hydrolysis of the acetal linkage in this phosphinic acid gave isobutyraldehyde (92%) and bis-(1-hydroxy-2-methylpropyl)-phosphinic acid (100%), m.p. 168–169°. Found: C, 45.78; H, 9.18; P, 14.73. Reaction of I with *p*-chlorophenylisocyanate in benzene solution catalyzed with triethylamine led to the formation of 5-(*p*-chlorophenylcarbamoyl) - 2,4,6 - triisopropyl - 1,3 - dioxa - 5 - phosphacyclohexane (42%), m.p. 162–163°. Found: C, 58.88; H, 7.89; P, 8.14.

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(5) F. A. Hart and F. G. Mann, *J. Chem. Soc.*, 4107 (1955).

(6) Succinaldehyde was added in the form of its acetal, 2,5-diethoxytetrahydrofuran.

MECHANISM OF ETHYLENE POLYMERIZATION WITH VANADIUM CATALYSTS

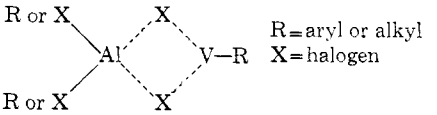
Sir:

In contrast to the usual heterogeneous, low-pressure catalysts, hydrocarbon soluble ethylene polymerization catalysts are formed by mixing aluminum bromide, an organoaluminum compound, and a hydrocarbon soluble vanadium halide. Thus, addition of 0.05 millimole of vanadium tetrachloride to a solution of 3 millimoles aluminum bromide, and 1 millimole of triphenylaluminum (or triisobutylaluminum) in 1 liter of cyclohexane formed a clear, pink solution. The subsequent addition of ethylene at 60° and atmospheric pressure gave 20–25 g. of polyethylene in 30 minutes. This polyethylene is linear (<1 CH_3—/1000 carbons), and highly saturated (<1 C=C/5000 carbons), with a melt index of 0.01.

The inorganic components of the pink solution were extracted into *N* sulfuric acid, and the vanadium was shown to be exclusively divalent by polarographic analysis.[1] In this catalyst the vanadium is reduced completely in less than one minute. By incremental addition of the vanadium halide to a cyclohexane solution, that is *M* in aluminum compounds ($3AlBr_3$ to $1AlR_3$), 0.05–0.1 *M* solutions of the soluble divalent vanadium species can be obtained. These solutions are very stable, retaining catalytic activity after months of storage in the absence of air and moisture.

Significantly, VCl_2 and VBr_2 are not cyclohexane soluble and are not catalysts for the low pressure polymerization of ethylene. Both compounds dissolve in a cyclohexane solution of aluminum bromide, but the resulting solutions are not catalytically active. Addition of an aluminum aryl or alkyl to these solutions provides the initiator and forms the active catalyst.

The solubility of the active catalyst, and of the divalent vanadium halides in an aluminum bromide solution in cyclohexane demonstrates complex formation between the aluminum and vanadium molecules. The active complex probably has the halogen bridged structure



found in the alkyl aluminum halide dimers,[2] and in the complex formed from triethylaluminum and bis-(cyclopentadienyl)-titanium dichloride.[3] Complexes of this type derive their stability from the Lewis acid character of the molecules and should be disrupted by Lewis bases. Addition of diethyl ether to the catalyst solution did, indeed, destroy it and the vanadium was precipitated as vanadium dibromide. Since the vanadium was added to the solution as the chloride (VCl_4) and later isolated as the bromide (VBr_2) the transfer of groups between aluminum and vanadium must take place very readily, and the species which precipitates is the *least soluble* of the possible divalent vanadium compounds.

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(2) L. O. Brockway and N. R. Davidson, *ibid.*, **63**, 3287 (1941).

(3) G. Natta, P. Corradini and I. W. Bassi, *ibid.*, **80**, 755 (1958).