nance studies and Mr. D. P. Maier for mass spectral studies.

(14) IAESTE Student, Summer 1971. Department of Organic Chemistry, University of Geneva, 1211 Geneva 4, Switzerland.

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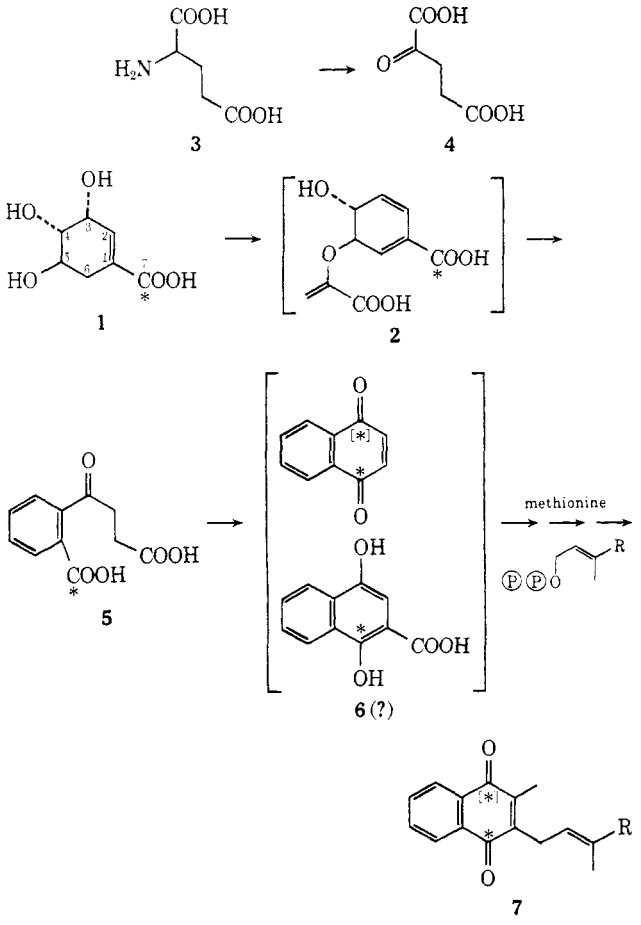
Biosynthesis of Menaquinones. Dissymmetry in the Naphthalenic Intermediate

Sir:

The biogenesis of bacterial menaquinones has been studied for many years and, in spite of experimental difficulties, intermediates and their sequence of involvement can now be identified with some degree of confidence (Scheme I). Experiments with numerous

Scheme I

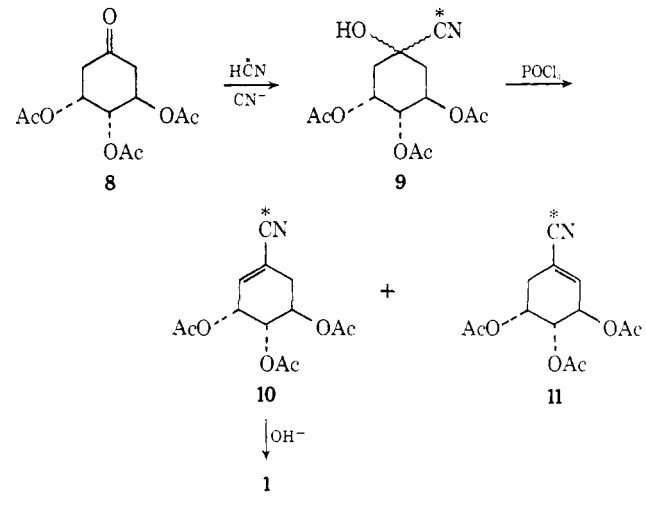


organisms allow definite assignment of the seven carbons of shikimic acid (**1**) to the benzenoid portion of the naphthoquinone nucleus,[1] with the carboxyl carbon forming one of the quinone carbonyls.[1b,c,f,h] The remaining three carbons of the naphthalene system are provided by C-2, -3, and -4 of glutamate (**3**)[1h,2] or its transamination product, 2-oxoglutarate (**4**),[3] C-2 becoming the other quinone carbonyl. Furthermore, the carbons (C-1 and -2) forming the ethylenic bridge of shikimic acid become the corresponding 9,10-bridge in the naphthoquinone.[1e,g] Efficient incorporation of *o*-succinylbenzoic acid (**5**)[1h,4] seems to provide strong evidence that the primary addition product of 2-oxoglutarate (or glutamate) and shikimate [or chorismate (**2**)[1a,g,4]] is immediately aromatized. The final sequence of cyclization and alkylation, however, remains in doubt. Several preformed naphthalenoid compounds, *i.e.*, 1,4-naphthoquinone,[1h] 2-methyl-1,4-naphthoquinone,[1h] α-naphthol,[1c-g,5] and 2-hydroxy-naphthoquinone,[1h] have been tested as precursors. In general, the results have been negative, although *S. aureus*,[1e] *A. aerogenes*,[1f] and *F. nigrescens*[6] will apparently incorporate 2-methyl-1,4-naphthoquinone and/or 1,4-naphthoquinone. A final restriction placed upon naphthoquinone biosynthesis is that the quinone oxygens must be derived from water.[7]

In view of the above state of knowledge we have directed our attention to the symmetry or dissymmetry of the as yet unidentified naphthalenic intermediate **6**. For example, if 1,4-naphthoquinone or 1,4-naphthohydroquinone were an intermediate, then a label introduced unsymmetrically would result in symmetrically labeled menaquinone. On the other hand, if no symmetrical intermediate were involved, the location of label in the menaquinone would allow one to define the orientation of the alkylations with respect to the carbonyl derived from shikimic acid. In order to ascertain the symmetry of the unknown intermediate, we have chosen the MK-9(II-H_2)-*Mycobacterium phlei* system, using [7-^{14}C]shikimic acid as the label source.

Specifically labeled shikimic acid was prepared (Scheme II) by addition of H^{14}CN to ketone **8**,[8] yielding

Scheme II



(1) (a) G. B. Cox and F. Gibson, *Biochem. J.*, **100**, 1 (1966); (b) I. M. Campbell, C. J. Coscia, M. Kelsey, and R. Bentley, *Biochem. Biophys. Res. Commun.*, **28**, 25 (1967); (c) E. Leistner, J. H. Schmitt, and M. H. Zenk, *ibid.*, **28**, 845 (1967); (d) J. R. S. Ellis and J. Glover, *Biochem. J.*, **110**, 22p (1968); (e) R. K. Hammond and D. C. White, *J. Bacteriol.*, **100**, 573 (1969); (f) M. Guerin, M. M. Leduc, and R. G. Azerad, *Eur. J. Biochem.*, **15**, 421 (1970); (g) M. M. Leduc, P. M. Dansette, and R. G. Azerad, *ibid.*, **15**, 428 (1970); (h) I. M. Campbell, D. J. Robins, M. Kelsey, and R. Bentley, *Biochemistry*, **10**, 3069 (1971); (i) K. H. Scharf and M. H. Zenk, *Chem. Commun.*, 576 (1971).

(2) D. J. Robins, I. M. Campbell, and R. Bentley, *Biochem. Biophys. Res. Commun.*, **39**, 1081 (1970).
(3) D. J. Robins and R. Bentley, *Chem. Commun.*, 232 (1972).
(4) P. Dansette and R. Azerad, *Biochem. Biophys. Res. Commun.*, **40**, 1090 (1970).
(5) B. S. Brown, G. R. Whistance, and D. R. Threlfall, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, **1**, 323 (1968).
(6) C. Martius and W. Leuzinger, *Biochem. Z.*, **340**, 304 (1964).
(7) C. D. Snyder and H. Rapoport, *Biochemistry*, **9**, 2033 (1970).
(8) R. Grewe and E. Vangermain, *Chem. Ber.*, **98**, 104 (1965).

an epimeric mixture of cyanohydrins **9**[9] which was directly dehydrated[8] to a 47:53 mixture of the two possible nitriles, **10** and **11**.[9] Preparative glpc[10a] separation furnished pure isomer **10** which was then hydrolyzed to [7-^{14}C]shikimic acid (**1**).

Feeding experiments with *M. phlei* demonstrated maximum incorporation (0.25%) of [7-^{14}C]shikimic acid after 3 days of growth at 37°.[11] That the label remained intact after this time was shown by significant (55%) recovery of shikimate activity from the medium. Specifically, then, feeding of [7-^{14}C]shikimic acid (80 μCi/mmol, 80 mg) resulted in pure menaquinone (60 mg) of specific activity 1.4 μCi/mmol.

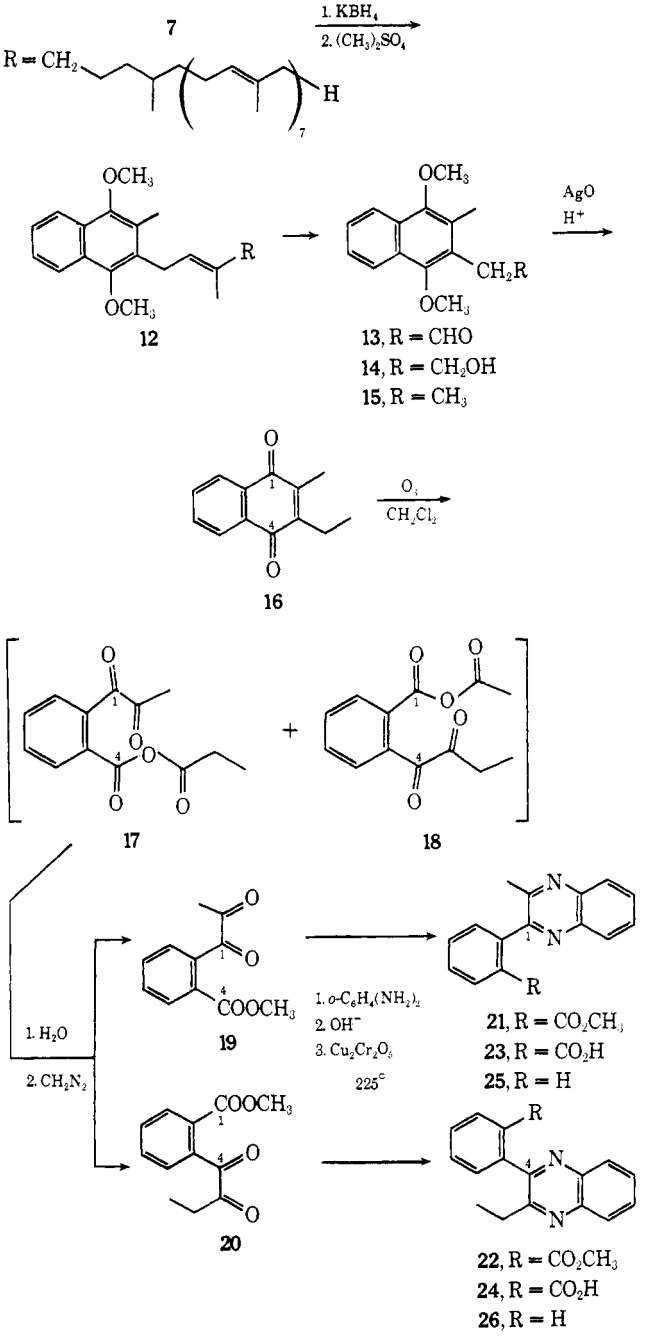
Independent determination of the label at C-1 and C-4 was accomplished by the degradation shown (Scheme III), the pivotal reaction being ozonolysis of quinone **16**. Difficulties encountered in ozonizing the menaquinone with the intact but fully saturated side chain required cleavage of the multiprenyl side chain at the $\Delta^{2'}$ position, most efficiently accomplished by OsO_4–H_5IO_6 oxidation[1h] of the dimethyl ether of MK-9(II-H_2)-hydroquinone **12**.[12] This was followed by borohydride reduction of the aldehyde **13** to alcohol **14** and thence to 2-methyl-3-ethyl-1,4-dimethoxynaphthalene (**15**) *via* the tosylate and treatment with lithium aluminum hydride. Oxidative demethylation[13] then generated quinone **16** in 37% overall yield from native quinone. Abnormal ozonolysis,[14] a known but neglected reaction of naphthoquinones, yielded a 3:2 mixture of anhydrides **17** and **18** which were directly hydrolyzed and esterified to the diketo esters **19** and **20**, separable by preparative glpc.[10b] After converting the α-diketone functions to quinoxaline derivatives, the esters **21** and **22** were hydrolyzed and the acids **23** and **24** were decarboxylated to yield 2-methyl- (**25**) and 2-ethyl-3-phenylquinoxaline (**26**), containing respectively C-1 and C-4 of MK-9(II-H_2).

The counting results in Table I, typical of a number of experiments, clearly show that the carboxyl carbon of shikimic acid is incorporated into C-4 of MK-9-(II-H_2) in *M. phlei*. The specific activity retained in the 2-methyl-3-phenylquinoxaline (**25**), 3.6% of that

Table I. Radioactivity in Menaquinone MK-9(II-H_2) and Its Degradation Products after Feeding [7-^{14}C]Shikimic Acid to *M. phlei*

Compd	Specific activity, dpm/mmol	% of **7**
7	36,000	100
12	36,000	100
16	36,600	102
25 (C-1)	1,280	3.6
26 (C-4)	36,500	101

Scheme III



in the original menaquinone, MK-9(II-H_2) (**7**), remained unchanged after dilution with inactive material, column chromatography, recrystallization, and sublimation. The product was pure by glpc,[10c] and 2-ethyl-3-phenylquinoxaline (**26**) was absent to the limit of detection, <1%. Therefore, the small residual activity in **25** may result from (a) randomization of label from [7-^{14}C]shikimic acid into the aromatic ring, C-1, C-2, or 2-methyl of MK-9(II-H_2), or (b) minor participation of a symmetrical intermediate.

Returning to Scheme I, the label indicated by an asterisk, included to allow for possible symmetry, may now be removed since this position is essentially inactive. Symmetrical compounds such as 1,4-naphthoquinone are therefore excluded[15] as significant menaquinone precursors in *M. phlei*.

(9) Contrary to published reports [*cf.* ref 8 and H. J. Bestmann and H. A. Heid, *Angew. Chem., Int. Ed. Engl.*, **10**, 336 (1971)] these reactions were nonstereospecific. Confirmatory details are forthcoming: C. D. Snyder and H. Rapoport, in preparation.

(10) (a) 15 ft × 0.75 in., 5% on Chromosorb W, 60–80, column temperature 205°; (b) 10 ft × 0.25 in., 5% OV-17 on Chromosorb W, 80–100, column temperature 175°; (c) as in (b), column temperature 195°.

(11) A. F. Brodie and C. T. Gray, *J. Biol. Chem.*, **219**, 853 (1956).

(12) All new compounds were characterized as to purity by tlc or glpc, and their uv, ir, and nmr spectra support the assigned structures. Elemental compositions were established by mass spectra and combustion analyses.

(13) C. D. Snyder and H. Rapoport, *J. Amer. Chem. Soc.*, **94**, 227 (1972).

(14) E. Bernatek, "Ozonolyses in the Naphthoquinone and Benzofuran Series," Oslo University Press, 1960.

(15) 2-Hydroxy-1,4-naphthoquinone biosynthesis in plants also has been shown to proceed unsymmetrically: E. Grotzinger and I. M. Campbell, *Phytochemistry*, **11**, 675 (1972).

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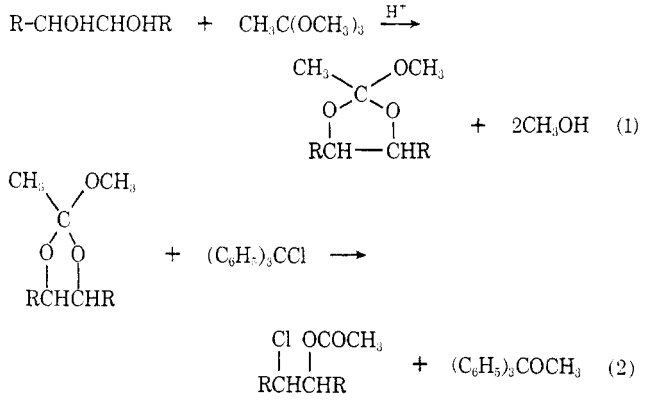
Conversion of Diols *via* Cyclic Orthoacetates to Acetates of Chlorohydrins by Treatment with Trityl Chloride

Sir:

In earlier papers, the conversion of 1,2-, 1,3-, and 1,4-diols to esters of the corresponding halohydrins was accomplished in two steps: (a) the acid-catalyzed reaction of diol with an α-keto acid to yield a ketal acid and (b) the reaction of the ketal acid (or the sodium salt thereof) with phosphorus pentachloride (or thionyl chloride) to yield the ester of the halohydrin.[1,2]

The overall yields from diol to halohydrin ester suffer because a high yielding method for step a was not developed. Furthermore, the use of phosphorus pentachloride (or thionyl chloride) places limitations on the other functionality that may be present. In this communication a new route for conversion of 1,2- and 1,3-glycols to esters of halohydrins is described which overcomes both of the limitations outlined above.

The new route is illustrated by eq 1 and 2.



As catalysts for ortho ester formation acids as mild as benzoic and chloroacetic acid are satisfactory.[3] Distillation of a mixture of the reactants affords about 2 equiv of methanol. The isolated yields of some typical cyclic ortho esters are listed in Table I.

On treatment of the cyclic ortho esters with trityl chloride in methylene chloride at reflux the acetates of the chlorohydrins are obtained in high yield (eq 2). The reactions are highly regiospecific and stereospecific. Essentially the same stereochemical results are obtained as in the ketal acid reactions described.[1,2] Our results are listed in Table II.

These results suggest a mechanism illustrated with 2-methoxy-2,4-dimethyl-1,3-dioxolane (**1**) which involves attack of the trityl cation on the methoxy group of the ortho ester,[4] followed by reaction of the ambident

(1) M. S. Newman and C. H. Chen, *J. Amer. Chem. Soc.*, **94**, 2149 (1972).
(2) M. S. Newman and C. H. Chen, paper submitted to *J. Org. Chem.*
(3) R. H. DeWolfe, "Carboxylic Ortho Acid Derivatives," Academic Press, New York, N. Y., 1970.
(4) Compare H. Meerwein, V. Hederich, H. Morschel, and K. Wunderlich, *Justus Liebigs Ann. Chem.*, **635**, 1 (1960).

Table I. Synthesis of Cyclic Ortho Esters from Diols

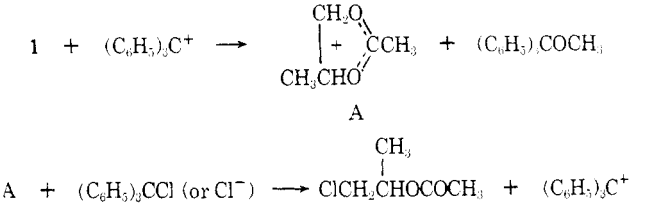
Diol $R_1CHOHCHOHR_2$	Compd, ortho ester[a] R_1CHO, OCH_3, R_2CHO, CH_3	Bp,[b] °C (P, mm)	Yield, %[c]
$R_1 = CH_3$; $R_2 = H$	**1**[d]	94–95 (14)	85
$R_1 = R_2 = CH_3$[e]	**2**[f]	37.0–37.5 (9.7)	88
$R_1 = C_6H_5$; $R_2 = H$[g]	**3**[d,h]	87.5–89.0 (1.3)	91
2,2-Dimethyl-1,3-propanediol	**4**	51 (10)	80
1,4-Butanediol	**5**	63–64 (20)	62

[a] All cyclic ortho esters were new compounds which gave C and H analyses within ±0.3% of the theoretical. The nmr, ir, and mass spectral data were consistent with the assigned structures. [b] The boiling points listed are those of the cuts isolated by simple distillation. [c] The per cent yield (based on diol) of distilled material. [d] A mixture of diastereoisomeric forms not precisely analyzed. [e] D(−)-2,3-butanediol, $\alpha^{22}D$ −12.9° (neat, 1 dm). [f] $\alpha^{22}D$ −6.26° (neat, 1 dm). [g] $[\alpha]^{19.5}D$ −39.24° (*c* 0.0304, EtOH), 100% optical purity. [h] $\alpha^{19}D$ −51.8° (neat, 1 dm).

Table II. Reactions of Cyclic Ortho Esters with Trityl Chlorides

Ortho ester[a]	Products[b]	Yield, %[c]
1	**6**, $CH_3C(OCOCH_3)HCH_2Cl$[d]	89
2	**7**, $CH_3C(Cl)HC(OCOCH_3)HCH_3$[e]	90
3	**8**, $C_6H_5CHClCH_2OCOCH_3$[f–h]	93
4	**10**, $ClCH_2C(CH_3)_2CH_2OCOCH_3$	83
5	**11**, $Cl(CH_2)_4OCOCH_3$[i]	38

[a] Ortho esters were used as obtained, Table I. Reactions in CH_2Cl_2 unless otherwise noted. [b] These products had essentially the same properties as described in ref 2. [c] The yield of distilled material. [d] The product was shown by nmr analysis to consist of *ca.* 94% of **6** and 6% of 2-chloropropyl acetate. [e] L(+)-Erythro compound, $\alpha^{23}D$ +12.48° (neat, 1 dm), hence inversion has occurred. D(+)-2,3-Epoxybutane, $[\alpha]^{22}D$ +76.2° (*c* 0.0613, xylene), was obtained on treatment of **7** with KOH (see ref 2 for details). [f] $[\alpha]^{21}D$ +88.54° (*c* 0.0324, $CHCl_3$), $\alpha^{19}D$ −67.95° (neat, 1 dm). This compound ((*S*)-**8**) is mixed with about 5% of (*R*)-2-chloro-1-phenylethyl acetate (**9**) (see ref 2 for details of nmr analysis). [g] When run in CH_3CN the product (91% yield) consisted of about 88% of (*S*)-**8** and 12% of (*R*)-**9**. [h] (*R*)-(−)-styrene oxide, $[\alpha]^{22}D$ −21.29° (*c* 0.0324, $CHCl_3$), was obtained on treatment with KOH. [i] No attempts were made to optimize this yield or to identify the other products formed.



cation[5] A thus produced with trityl chloride (or chloride ion). The geometry of the latter reaction is that which would be expected from an SN2 type displacement at the carbon–oxygen bond being broken.

In a typical experiment which illustrates the mild conditions for reaction and the ease of isolation of product, a solution of 2.0 g of D(−)-2-methoxy-2,4,5-trimethyl-1,3-dioxolane (**2**) and 3.8 g (1 equiv) of trityl chloride in 6 ml of CH_2Cl_2 was refluxed for 1–2 hr.[6]

(5) S. Hünig, *Angew. Chem., Int. Ed. Engl.*, **3**, 548 (1964), C. V. Pittman, Jr., S. P. McManus, and J. W. Larsen, *Chem. Rev.*, **72**, 357 (1972).
(6) In the case of **3**, the reflux period was 10 hr.