Table I. Products from the Elimination of Secondary Alkyl Tosylates at 55°

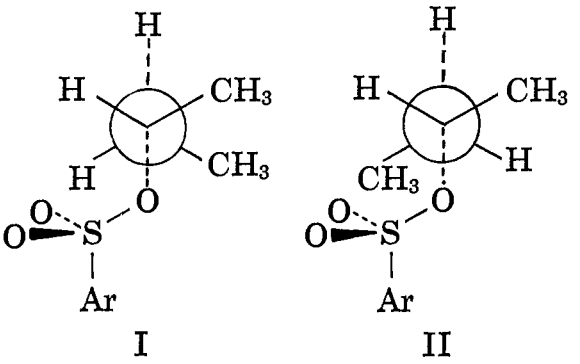
Expt	Alkyl group	Solvent	Base	% 1-ene	*trans*-2-ene/*cis*-2-ene
1	2-Butyl	EtOH	KOEt	35	1.95[a]
2	2-Butyl	*t*-BuOH	KOEt	54	0.80
3	2-Butyl	*t*-BuOH	KO-*t*-Bu	64	0.58
4	2-Pentyl	EtOH	KOEt	42	1.90[a]
5	2-Pentyl	*t*-BuOH	KOEt	62	0.57
6	2-Pentyl	*t*-BuOH	KO-*t*-Bu	73	0.38

[a] Data from ref 2.

state. The importance of this consideration is exemplified by the fact that in consideration of the steric requirements of halogen both the covalent bond radii and van der Waals radii must be considered.[4] The covalent bond radii appear to be the more important consideration since the steric requirement of chlorine is apparently greater than that of iodine.[5] Kinetic studies on tosylates[6] demonstrate that the E1cb character of the transition state is significantly greater in *t*-butyl alcohol than in ethanol. Product studies in DMSO,[2] when compared with the results in ethanol, reveal that with or without a factor for the effect of solvation[7] the steric requirement of the attacking base is relatively unimportant in related reactions.

That *t*-butyl alcohol inhibits C–X[8] stretching in the transition state in comparison with other solvents is demonstrated by the fact that the decrease in the *trans*-2-ene:*cis*-2-ene ratio reported here for tosylates parallels the effect observed in halide eliminations.[9,10]

Thus, we believe the evidence is strong that the transition state for tosylate eliminations in the *t*-butyl alcohol is basically one in which C–H stretching is of major importance with C–O stretching delegated to a minor role. Clearly this allows the elimination of the steric effect of base as a factor. However, this does allow the stereochemical requirements of the leaving group to assume a role in the transition state,[11] and the decision as to which transition state, I or II, is more energetically favorable then becomes much the same problem as determining the relative population in the ground state of the conformers from which structure I and II can be considered to be derived. Examination of Fisher–Hirschfelder–Taylor molecular models reveals that the C-1 as well as the C-4 methyl group interferes with free rotation of the tosylate group about the C–O bond. Thus, the preferred orientation of the tosylate would be to project to the side opposite C-1 as shown. From this, it is apparent that the C-4 methyl



group will interfere with free rotation about the S–O bond in structure II whereas it will not in structure I. Thus, structure I allows more rotational freedom to the tosylate group. This effect apparently must be more important than the methyl–methyl interactions developed in structure I. Extension of this phenomenon to the 2-pentyl system predicts that the *trans*-2-ene:*cis*-2-ene ratio would decrease as observed (expt 5 and 6) since tosylate–alkyl interaction is considered more important than alkyl–alkyl. However, as C–O bond stretching increases, the alkyl–alkyl interaction increases and at the same time the tosylate–alkyl interaction decreases; thus structure II becomes more and more energetically favorable and the *trans*-2-ene:*cis*-2-ene ratio increases (expt 3, 2, and 1, or 6, 5, and 4).

The above results suggest that the stereochemical requirement of leaving groups may be a factor when the degree of C–X stretching is very small; this condition is most likely to exist when the transition state has a great deal of E1cb character. By analogy, the size of attacking bases may possibly be a factor when the degree of C–H stretching is very small; this condition is most likely to exist when the transition state has a great deal of E1 character and/or a weak base is used.

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(4) (a) H. C. Brown and O. H. Wheeler, *J. Am. Chem. Soc.*, 78, 2199 (1956); (b) H. C. Brown and I. Moritani, *ibid.*, 78, 2203 (1956).
(5) A. J. Berlin and F. R. Jensen, *Chem. Ind.* (London), 998 (1960).
(6) C. H. DePuy and C. A. Bishop, *J. Am. Chem. Soc.*, 82, 2532 (1960).
(7) H. C. Brown and M. Nakagawa, *ibid.*, 78, 2197 (1956).
(8) Where X designates the leaving group.
(9) D. H. Froensdorf, M. E. McCain, and W. Dowd, unpublished results.
(10) D. H. Froemsdorf, M. E. McCain, and W. W. Wilkison, *J. Am. Chem. Soc.*, 87, 3984 (1965).
(11) A referee has suggested that the sulfone oxygens in the tosylate group are hydrogen bonded to alcohol, thus attributing the unique effect observed in *t*-butyl alcohol to the greater steric requirement of the solvated leaving group. Infrared studies of O–H and S–O stretching frequencies of carbon tetrachloride solutions containing both *t*-butyl alcohol and 2-butyl tosylate reveal that this is not an important consideration.

2-Decaprenyl-6-methoxyphenol, an Apparent Biosynthetic Precursor of Ubiquinone-10[1]

Sir:

A new phenolic substance, which is an apparent precursor of ubiquinone-10, has been isolated. Structural and synthetic data on this product are in agreement with structure V (n = 10), 2-decaprenyl-6-methoxyphenol[1] or 2-[3′-methyl-2-butenylenakis(3′-methyl-2′-butenylene)]-6-methoxyphenol. Four intermediates in the biosynthetic sequence from *p*-hydroxybenzoic acid (I) to ubiquinone-10 (VI, n = 10) are now evident.

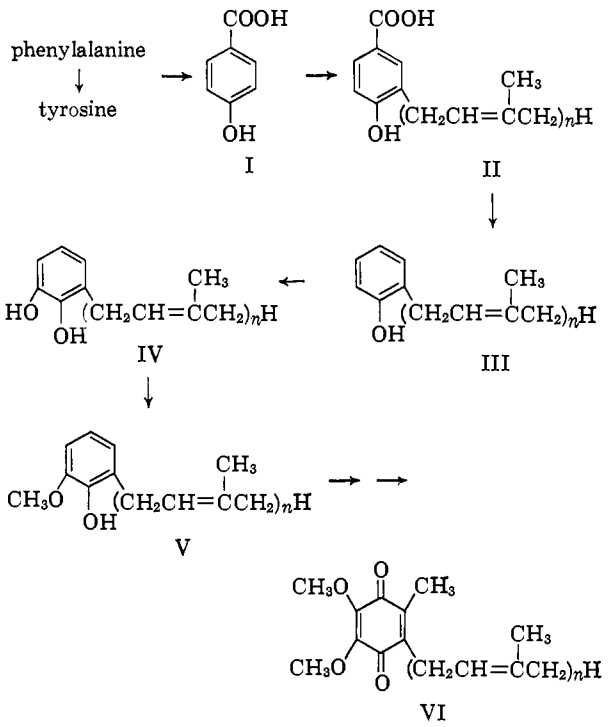
(1) (a) Coenzyme Q. LXXIII. (b) Nomenclature is based on a recommendation of an IUPAC–IUB Commission of Biochemical Nomenclature.

This newly recognized precursor, 2-decaprenyl-6-methoxyphenol (V, n = 10), and its 6-hydroxy analog (IV, n = 10) occupy positions in the biosynthetic pathway to ubiquinone-10 (VI, n = 10) which immediately follow 2-decaprenylphenol[2] (III, n = 10). It is apparent that 2-decaprenyl-6-methoxyphenol (V, n = 10) is formed from 2-decaprenylphenol (III, n = 10) by a two-stage process involving enzymatic introduction of a hydroxyl function *ortho* to the existing phenolic hydroxyl group followed by selective enzymatic methylation of the hydroxyl *meta* to the orienting alkyl side chain. This sequence of enzymatic transformations is known[3] to occur widely in numerous biosynthetic pathways in plants,[4] animals,[5] and bacterial systems.[6]

The isolation of the two precursors III and V allow the partial biosynthetic scheme I → V to be written. It is reasonable to assume the intermediacy of 3-decaprenyl-2-hydroxyphenol (IV) on the basis of the analogies cited; the evidence for the intermediacy of 3-decaprenyl-4-hydroxybenzoic acid (II) has been presented.[2] Obviously, there are several alternative pathways for the final two hydroxylations and two methylations to complete the sequence between 2-decaprenyl-6-methoxyphenol (V) and ubiquinone-10 (VI).

This sequence (Scheme I) as based on studies with

Scheme I



Rhodospirillum rubrum is very likely identical with, or nearly so, the sequence for mammalian tissue; this sequence is also presumably valid for ubiquinone-6, -7, -8, and -9 of microorganisms.

Fractionation of the lipid extract[7] obtained from *R. rubrum* was accomplished by column chromatography over silica gel. Elution with 2% ether in hexane yielded two fractions which contained phenolic substances as evidenced by a positive test with diazotized sulfanilic acid.[8]

From the second of these fractions was obtained 2-decaprenylphenol (III, n = 10, as previously reported.[2] Further separation of the first fraction containing diazotized sulfanilic acid sensitive material was effected by thin layer chromatography on silica gel G plates developed in benzene. A phenolic substance R_f 0.41 with mp 32–36° was isolated and shown to be homogeneous by chromatography in a variety of solvent systems.

Spectral data (ultraviolet, infrared, nmr, and mass spectra) allowed the assignment of the structure of this bacterial product as 2-decaprenyl-6-methoxyphenol (V, n = 10), a previously unknown substance. The ultraviolet absorption spectrum of the precursor, V (λ_{max}^{hexane} 273 and 279 mμ; $\lambda_{max}^{95\%EtOH}$ 274 and 279 mμ; $\lambda_{max}^{95\%EtOH+NaOH}$ 290 mμ), is very similar to that exhibited by 2-decaprenylphenol[2] (III, n = 10). The infrared spectrum shows hydroxyl absorption at 3500 cm^{-1}. The nmr spectrum[9] provided essential structural information: 3 H (aromatic), singlet at τ 3.35; 1 H (hydroxyl), singlet at τ 4.54; 10 H (vinyl), multiplet at τ 4.90; 3 H (methoxyl), singlet at τ 6.10; 2 H (benzylic), doublet at τ 6.68; alkyl multiplet at τ 8.0–8.4. The aromatic region of the nmr spectrum appeared unusual in that the three aromatic protons appeared as a singlet at τ 3.35. However, the nmr spectrum of 2-allyl-6-methoxyphenol[10] (*o*-eugenol) showed a corresponding three-proton singlet at τ 3.35, providing strong support for the structure assignment. A ten-unit isoprenoid side chain was indicated by integration of the nmr spectrum. This was confirmed by the mass spectrum[11] which showed the parent ion at *m/e* 805 corresponding to a monomethoxyphenol with a ten-unit isoprenoid side chain (mol wt calcd, 804.7).

The described spectra (ultraviolet, infrared, nmr) for the isolated product were indistinguishable from the spectra of newly synthesized 2-decaprenyl-6-methoxyphenol (V, n = 10). The isolated and synthetic products showed R_f 0.41 on silica gel G plates developed in benzene, and R_f 0.58 on alumina plates developed in chloroform.

Treatment of V with 3,5-dinitrobenzoyl chloride yielded a monobenzoate derivative: ν_{max}^{film} no OH absorption, 1755 cm^{-1} (C=O), 1545 and 1340 cm^{-1}

(2) R. K. Olsen, J. L. Smith, G. D. Daves, Jr., H. W. Moore, K. Folkers, W. W. Parson, and H. Rudney, *J. Am. Chem. Soc.*, 87, 2298 (1965).

(3) For general discussions, see, for example: "Biochemistry of Phenolic Compounds," J. B. Harborne, Ed., Academic Press Inc., New York, N. Y., 1964, pp 265, 321, 415–419; "Biogenesis of Natural Compounds," P. Bernfeld, Ed., The Macmillan Company, New York, N. Y., 1963, pp 455–461.

(4) See: S. Z. El-Basyouni, D. Chen, R. K. Ibrahim, A. C. Neish, and G. H. N. Towers, *Phytochemistry*, **3**, 485 (1964); B. J. Finkle and M. S. Masri, *Biochim. Biophys. Acta*, **85**, 167 (1964).

(5) A. B. Lerner, T. B. Fitzpatrick, E. Calkins, and W. H. Summerson, *J. Biol. Chem.*, **191**, 799 (1951); J. Axelrod and R. Tomchick, *ibid.*, **233**, 702 (1958).

(6) S. Gatenbeck and U. Brunsberg, *Acta Chem. Scand.*, **18**, 2061 (1964); G. Pettersson, *ibid.*, **19**, 1016 (1965).

(7) W. W. Parson and H. Rudney, *J. Biol. Chem.*, **240**, 1855 (1965).

(8) E. V. Truter, "Thin Film Chromatography," Interscience Publishers, Inc., New York, N. Y., 1963, p 160.

(9) Nmr spectra were taken with a Varian HR-60 spectrometer in CCl_4 solutions; chemical shifts are in τ units relative to tetramethylsilane as an internal standard.

(10) Available commercially from K & K Laboratories, Inc., Plainview, N. Y.

(11) The mass spectrum was determined using a CEC Model 103-C mass spectrometer with increased magnetic field, electron multiplier-Wien filter detection system, and 1-mm slits to permit unit resolution at *m/e* values as high as 1000. The sampling system is modified to permit direct sample introduction by means of a heated probe. We are indebted to Dr. Raffaele F. Muraca and Mrs. Julia A. Whittick for this determination.

($-NO_2$); nmr, 3 H (nitroaromatic), singlet at τ 0.78; 3 H (aromatic), multiplet at τ 3.40; 10 H (vinyl), multiplet at τ 4.98; 3 H (methoxyl), singlet at τ 6.23; 2 H (benzylic), doublet at τ 6.76; 69 H (alkyl), multiplet at τ 8.0–9.1.

That 2-decaprenyl-6-methoxyphenol (V, n = 10) is a biosynthetic precursor to ubiquinone-10 (VI, n = 10) is implicit in the structural similarities of these compounds and in the close relationship of V to 2-decaprenylphenol (III, n = 10) which has been established[12] to be a precursor. Verification of this relationship was provided by radioactive incorporation experiments using [U-^{14}C]*p*-hydroxybenzoic acid (HBA)[12] as a marker. Collected cells of *R. rubrum* were suspended in buffer solution[12] and incubated with [U-^{14}C]HBA for 7.5 hr in light followed by 14 hr in darkness. The lipid material was chromatographed on silica gel and the 2-decaprenyl-6-methoxyphenol (V, n = 10) obtained was shown to be radioactive. A similar cell suspension was incubated with [U-^{14}C]HBA anaerobically in the dark and aliquots were analyzed at various time periods. Radioactivity associated with 2-decaprenyl-6-methoxyphenol (V) increased during the period 2–5.5 hr after addition of [U-^{14}C]HBA; during this period, radioactivity associated with 2-decaprenylphenol (III) decreased.

While these data do not directly establish the biosynthetic intermediacy of 2-decaprenyl-6-methoxyphenol (V, n = 10) in the conversion of 2-decaprenylphenol (III, n = 10) to Q, the data and the sequence (I → V) are clearly in accord with the radioactivity studies of Parson and Rudney,[12] and the interpretation is reasonable.

Studies are continuing on the biosynthetic transformations of V to ubiquinone-10 (VI), and on the synthesis of these precursors.

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Primary and Secondary Ionizations of α Hydrogens of Phenylacetonitrile by *n*-Butyllithium[1]

Sir:

We wish to report that phenylacetonitrile undergoes primary and secondary ionizations of its α hydrogens with *n*-butyllithium in tetrahydrofuran–hexane to form the mono- and dilithio salts, which may be represented as I and II, respectively.[2] These ionizations were accompanied by color changes of yellow and brown, respectively.

$C_6H_5CHLiCN$ (I) $\qquad$ $C_6H_5CLi_2CN$ (II)

That phenylacetonitrile undergoes primary ionization to form I to the extent of at least 91% was indicated by recovery of this percentage of the nitrile on treatment with slightly more than 1 equiv of *n*-butyllithium, followed by water; only a trace of material that might have arisen from addition of the reagent to the nitrile group was obtained. Moreover, the volume of *n*-butane evolved in a similar experiment was only slightly less than the calculated amount, and treatment of the resulting reaction mixture with *n*-butyl bromide afforded the monoalkyl derivative III[3] in 73% yield.

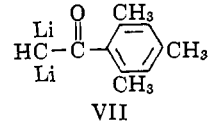
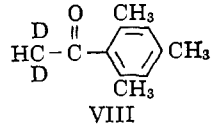
$C_6H_5CH(C_4H_9)CN$ (III) $\qquad$ $C_6H_5CD_2CN$ (IV)

Evidence for the twofold ionization of phenylacetonitrile to form dilithionitrile II was also obtained in three ways. Deuteration to give dideuterionitrile IV was effected by addition of phenylacetonitrile in THF to 2.25 equiv of *n*-butyllithium in THF–hexane followed, after 1 hr, by excess deuterium oxide. The product contained 89% of two deuterium atoms/molecule (determined by nmr). A blank experiment showed that no deuterium was acquired by phenylacetonitrile in the presence of lithium deuteroxide and deuterium oxide.

Treatment of phenylacetonitrile with 2.25 equiv of *n*-butyllithium in THF–hexane produced butane in only slightly less than the calculated amount, and alkylation of the resulting reaction mixture with *n*-butyl bromide and ethylene chloride afforded dibutyl derivative V[3] and cyclic product VI[4] in yields of 69 and 65%, respectively.

$C_6H_5C(C_4H_9)_2CN$ (V) $\qquad$  (VI)

Similarly, acetomesitylene underwent twofold ionization with excess *n*-butyllithium in THF–hexane to form dilithio salt VII;[2] subsequent deuteration with excess deuterium oxide afforded dideuterioacetomesitylene (VIII) in 60% yield. No deuterium was acquired by acetomesitylene in a blank experiment in the presence of lithium deuteroxide and deuterium oxide.

 (VII) $\qquad$  (VIII)

(1) Supported by the Army Research Office (Durham), and by the National Science Foundation.

(2) For the present purpose, only the carbanion resonance forms of these salts are represented, although other resonance forms may make a more important contribution to the structures.

(3) Identified by same boiling point and vpc retention times as an authentic sample.

(4) Identified by agreement of boiling point with reported value and by nmr and mass spectra.