tion of Pyr(1)Ala andPyr (3)Ala which are 2.2 and ~2.1, respectively.[27,28]

While causing a minimal alteration in the stereochemistry pyrazole–imidazole replacements appear to provide a means to assess the role of ionization in function of biologically active peptides.

We have evaluated in a systematic manner the relation between chain length and ability to regenerate active enzymes with S-protein of a series of S-peptide fragments.[6,21b] Removal of amino acid residues 15–20 had no effect on the protein-activating potential. Further shortening of the peptide chain from either the C- or N-terminus resulted in a decrease of activity. Only in one instance however was complete deactivation observed, namely when the histidine residue in position 12 was missing. On the basis of this and other evidence[8] it was concluded that histidine 12 is vital for enzymatic function. Two possible explanations come to mind for the role of this histidine. By virtue of its aromatic character it may make a profound contribution to the binding of the peptides to S-protein or because of its unique acid–base behavior it may be essential for function.

The dodecapeptide amides V and VI were synthesized in order to distinguish between these possibilities. We have added increasing amounts of peptide VI to S-protein up to peptide–protein ratios of 1500:1 without observing any activation. Similarly peptide V at ratios as high as 5500:1 was without effect. Thus, these two pyrazolylalanines cannot function in lieu of histidine 12 in the S-peptide–S-protein system. It remains to be determined whether this lack of activity is attributable to inability to function catalytically, inability to bind, or both.

(27) F. Schneider and W. Schaeg, *Z. Physiol. Chem.*, 327, 74 (1962).
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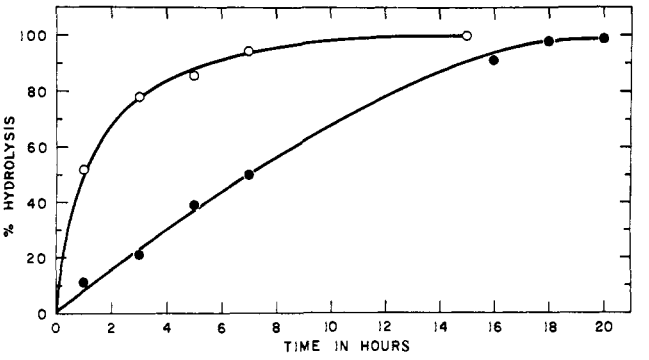

Figure 1. Rates of hydrolysis of β-(pyrazolyl-1)-L-alanine amide, ●, and β-(pyrazolyl-3)-L-alanine amide, ○, by aminopeptidase M. For experimental conditions see ref 21b.

Acknowledgment. The authors wish to express their appreciation to Mrs. Elaine Gleeson and Miss Judy Montibeller for technical assistance. They thank Dr. Frances M. Finn for the determinations of the ionization constants of the pyrazolylalanines, and Miss Marie Limetti for the results presented in Figure 1.

2-Multiprenylphenols and 2-Decaprenyl-6-methoxyphenol, Biosynthetic Precursors of Ubiquinones[1]

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Abstract: In the biosynthetic sequence of the conversion of *p*-hydroxybenzoic acid to ubiquinone, two intermediates have been isolated in pure form and structurally elucidated. These two compounds, 2-decaprenylphenol and 2-decaprenyl-6-methoxyphenol, are in the pathway to ubiquinone-10 of *Rhodospirillum rubrum*. Two other 2-multiprenylphenols, 2-tetraprenylphenol and 2-nonaprenylphenol, were also isolated and characterized. 2-Nonaprenylphenol is a precursor of ubiquinone-9 which is also known to be biosynthesized by *R. rubrum*. However, 2-tetraprenylphenol is somewhat surprising as a naturally occurring lower homolog; the corresponding ubiquinone-4 has not yet been reported from any source. Ubiquinone-4 may be a trace constituent of *R. rubrum*, but is as yet undetected. Radioactivity incorporation experiments have shown that all four of these 2-multiprenylphenols as isolated from *R. rubrum* are derived from *p*-hydroxybenzoic acid. Although none of the tocopherols or plastoquinones has been isolated from *R. rubrum*, the isolation of 2-tetraprenylphenol, showing its existence in nature, may forecast its precursor relationship to the tocopherols and plastoquinone-4 in other living systems.

Four new biosynthetic precursors of ubiquinone have been isolated from the photosynthetic bacterium *Rhodospirillum rubrum* and structurally elucidated as 2-decaprenylphenol (III, n = 10),[5,6] 2-nonaprenylphenol (III, n = 9), 2-tetraprenylphenol (III,

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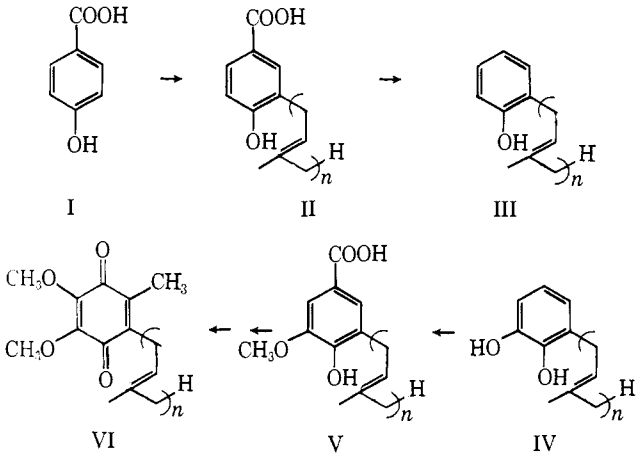
(2) Stanford Research Institute, Menlo Park, Calif.
(3) Western Reserve University, Cleveland, Ohio.
(4) Research Career Awardee, GM-K6-993, U. S. Public Health Service.

(5) The nomenclature in this paper is based on a recommendation of an IUPAC–IUB Commission of Biochemical Nomenclature, *Biochim. Biophys. Acta*, 107, 5 (1965). 2-Decaprenylphenol is also named 2-(3,7,11,15,19,23,27,31,35,39-decamethyl-2,6,10,14,18,22,26,30,34,38-tetracontadecaenyl)phenol (*Chemical Abstracts*) or 2-[3′-methyl-2-butenylenakis(3′-methyl-2′-butenylene)]phenol (IUPAC).
(6) 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).

n = 4), and 2-decaprenyl-6-methoxyphenol (V, n = 10).[5,7]

2-Decaprenylphenol (III, n = 10)[6] and 2-decaprenyl-6-methoxyphenol (V, n = 10)[7] are biosynthetic precursors of ubiquinone-10 (VI, n = 10).[5] 2-Nonaprenylphenol (III, n = 9) is an apparent precursor of ubiquinone-9 (VI, n = 9) which is also present in *R. rubrum*,[8] although in small concentration relative to Q_{10}. The isolation of 2-tetraprenylphenol (III, n = 4) may forecast the isolation of ubiquinone-4 (VI, n = 4), Q_4, which has not yet been reported as a natural product. It has been suggested[6,7] that appropriate 2-multiprenylphenols may also be biosynthetic precursors of the tocopherols and plastoquinones. Thus, 2-tetraprenylphenol may be a precursor not only of ubiquinone-4, but also plastoquinone-4[9] and some of the tocopherols. There is evidence[10] that some tocopherols are derived from precursors with unsaturated tetraprenyl side chains.



The biological significance[11] and the widespread occurrence in nature[12] of Q presumably enhanced the effort toward the elucidation of its biosynthesis. Mevalonic acid-2-^{14}C is incorporated into the isoprene side chain of Q in rat[13] and chick,[14] and in yeast[15] and various bacteria.[16] Acetate-^{14}C is incorporated into the Q side chain in rat liver,[13a,c,17] *Rhodospirillum rubrum*,[18] and other microorganisms.[16] In *R. rubrum*, 20% of the radioactivity arising from acetate-^{14}C and incorporated into Q was contained in the quinone ring.[18]

It was reported[17,19] that the quinone nucleus arises from phenylalanine in rat liver, though the incorporation was extremely low (0.001%). It was further shown[20,21] that the entire side chains of phenylalanine and tyrosine are lost in the biosynthesis of the ring portion of Q. Unlabeled *p*-hydroxybenzoic acid (HBA, I) caused a 99% decrease in the incorporation of tyrosine-U-^{14}C, indicating that *p*-hydroxybenzoate was a possible precursor.[20] *In vitro*, HBA-U-^{14}C is incorporated into Q by rat liver slices.[20,21] Formate-^{14}C was shown to be incorporated into the ring methyl and methoxyl groups of Q.[15,20,22] *p*-Hydroxybenzaldehyde-U-^{14}C and HBA-U-^{14}C are incorporated[22] into the quinone nucleus of Q in *R. rubrum*, *Azotobacter vinelandii*, Baker's yeast, and rat kidney, and the carbonyl carbon is lost since HBA-carbonyl-^{14}C led to no incorporation. The ring methyl and methoxyl groups were shown to come from methionine-methyl-^{14}C.[23]

An intermediate in the conversion of HBA to Q in *R. rubrum* was detected.[24] This intermediate, recognized chromatographically and of unknown structure, was termed "compound X." Evidence was presented that the carboxyl group is absent in compound X and that no methyl groups are present in the ring moiety. Compound X was also shown to have a multiprenyl side chain. The isolation and structural elucidation of compound X and related new products are detailed herein. Communications[6,7] reporting portions of this work have appeared.

The lipid fraction obtained from *R. rubrum*[23] was chromatographed on a silica gel column. Elution with 4% ether in hexane yielded a fraction which contained a phenolic substance as evidenced by a positive test with diazotized sulfanilic acid.[25] Preparative thin layer chromatography (silica gel G, benzene) allowed separation and purification of the phenolic substance which was indistinguishable in various chromatographic systems (Table I) from radioactive compound X isolated from *R. rubrum* incubated with HBA-U-^{14}C.[24]

Spectral data (ultraviolet, infrared, nmr) are consistent with the formulation of this substance as 2-decaprenylphenol (III, n = 10).[6] The infrared spectrum showed a phenolic hydroxyl absorption at 3400 cm^{-1}; the ultraviolet absorption (λ_{max}^{hexane} 272, 279 mμ; $\lambda_{max}^{95\%EtOH}$ 275 mμ; $\lambda_{max}^{95\%EtOH+NaOH}$ 291 mμ) is consistent with an *ortho*- or *meta*-substituted phenol structure. For example, 2-allylphenol,[26] *o*-cresol,[27] and *m*-cresol[27] show maxima at 272 and 279 mμ; *p*-cresol[27] has maxima at 279 and 286 mμ. The nmr spectrum corroborated

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Table I. Chromatography of 2-Multiprenylphenols

System	Compd X	X_{10}[a]	X_9[a]	X_4	X phytyl[a]	L_{10}	L_{10}[a]
Thin layer							
Silica gel G							
Benzene	0.32			0.22		0.41	0.41
10% ether–hexane	0.13			0.13		0.25	
1.5% methanol–benzene	0.47			0.38		0.61	
Chloroform	0.55			0.44		0.63	
Alumina							
Chloroform	0.52			0.40		0.58	0.58
1.5% methanol–benzene	0.46			0.39		0.65	
Silicone-impregnated paper							
1-Propanol–water (8:2)	0.76	0.75	0.79	0.92	0.91		
2-Propanol–water–ammonium hydroxide (8:1:1)	0.56, 0.63	0.55	0.63	0.92	0.88	0.18	

[a] Synthetic, see ref 29.

paper developed in 2-propanol–water–ammonium hydroxide (8:1:1). Components X_9 and X_{10} corresponded in R_f values to synthetic[29] 2-nonaprenylphenol, X_9 (III, n = 9),[28] and 2-decaprenylphenol, X_{10} (III, n = 10)[28] (Table I). Samples of the two components obtained by preparative paper chromatography were examined by mass spectrometry. The mass spectra established that component X_{10} was 2-decaprenylphenol (III, n = 10), and component X_9 was 2-nonaprenylphenol (III, n = 9). The mass spectrum of component X_{10} (R_f = 0.56), chromatographically indistinguishable from a synthetic specimen,[29] exhibited a molecular ion at m/e 774.5; the calculated molecular weight of X_{10} is 774.7. Component X_9 (R_f 0.63) showed a molecular ion at m/e 707; calcd for X_9, 706.6. Finally, chromatographic (Table I) and spectral (Table II) properties of components X_{10} and X_9 were indistinguishable from those of synthetic[29] 2-decaprenylphenol (III, n = 10) and 2-nonaprenylphenol (III, n = 9), respectively.

The lipid extract of *R. rubrum* yielded two additional phenolic compounds. The infrared, ultraviolet, and

Table II. Spectral Data for 2-Multiprenylphenols

	Ultraviolet, λ_{max}, mμ					Nmr[a]					
	Hexane	95% EtOH	95% EtOH + NaOH	Infrared, cm^{-1}	Mass, m/e for M^+	Aromatic	OH	Vinyl	Methoxyl	Benzylic	Alkyl
Compound X	272 279	273	291	3400 (OH)		2.94–3.51 (m, 4)	5.30 (s, 1)	4.99 (m, 10)		6.65 (d, 2)	8.0–8.4
Component X_{10}	273 278				774.5						
Component X_9	273 278				707						
X_{10} (synthetic)[29]	273 278					2.86–3.42 (m, 4)	5.25 (s, 1)	4.91 (m, 10)		6.67 (d, 2)	7.75–8.5 (m, 69)
X_9 (synthetic)[29]	273 278					2.80–3.41 (m, 4)	5.26 (s, 1)	4.94 (m, 9)		6.68 (d, 2)	7.75–8.5 (m, 62)
Compound X_4	272 278				366	2.90–3.48 (m, 4)	5.21 (s, 1)	4.96 (m, 4)		6.70 (d, 2)	8.0–8.4 (m, 27)
X phytyl[28]	272 278					2.78–3.42 (m, 4)	5.15 (s, 1)	4.65 (t, 1)		6.64 (d, 2)	7.75–9.35 (m, 36)
Compound L_{10}	273 279	274 279	290	3500 (OH)	805	3.35 (s, 3)	4.54 (s, 1)	4.90 (m, 10)	6.10 (s, 3)	6.68 (d, 2)	8.0–8.4 (m, 69)
L_{10} (synthetic)[28]	273 279					3.37 (s, 3)	4.50 (s, 1)	4.88 (m, 10)	6.08 (s, 3)	6.67 (d, 2)	8.0–8.4 (m, 69)

[a] s = singlet; d = doublet; t = triplet; m = multiplet. The number in parentheses is the number of protons. Spectra were obtained using carbon tetrachloride solutions with a Varian Associates HR-60 spectrometer except for the spectrum of L_{10}, which was obtained with an HA-100 spectrometer.

the assignment of the structure as a 2-multiprenylphenol. The multiprenyl chain was evidenced by characteristic bands at τ 4.99 (vinyl) and 8.0–8.4 (alkyl); the ring attachment was revealed by the presence of a two-proton doublet at τ 6.65, and the hydroxyl proton appeared as a singlet at τ 5.30. The aromatic portion of the spectrum consisted of a complex multiplet at τ 2.94–3.51, which corresponded closely to the appropriate region of the spectrum of *o*-cresol; the spectra of *m*- and *p*-cresol differed significantly.

Compound X appeared homogeneous in several chromatographic systems (Table I), but was separated into two components (X_9 and X_{10})[28] on silicone-impregnated

(28) For convenience, the abbreviation X_n is used to designate the 2-multiprenylphenols (III) where "n" as in Q_n refers to the number of isoprene units contained in the side chain. Note that X_n designates

nmr spectra of one of these compounds (Table II) were essentially superimposable upon those of X_{10} except for differences in intensities as expected for a lower isoprenylog. The chromatographic behavior (Table I) of this compound is similar to that of 2-phytylphenol.[29] Therefore, this compound was assigned the structure 2-tetraprenylphenol, X_4 (III, n = 4). The mass spectrum (parent ion at m/e 366) confirmed this assignment.

The other new compound appeared homogeneous in all chromatographic systems used (Table I). Spectral

pure compounds of definite structure and is not identical with the "compound X" of Parson and Rudney[24] and Olsen, *et al.*,[6] which has subsequently been shown to be a mixture of X_{10} and X_9. Similarly, the 2-multiprenyl-6-methoxyphenols (V) are designated L_n.

(29) G. D. Daves, Jr., H. W. Moore, D. E. Schwab, R. K. Olsen, J. J. Wilczynski, and K. Folkers, unpublished data.

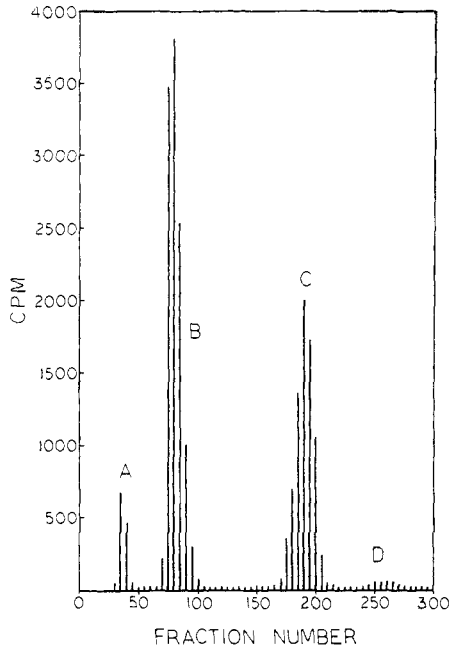

Figure 1. Distribution of radioactivity in the lipid extract obtained from *Rhodospirillum rubrum* incubated with *p*-hydroxybenzoic acid-U-^{14}C for 7.5 hr in the light, followed by 14 hr in darkness under anaerobic conditions.

data (ultraviolet, infrared, nmr, mass spectrum) and comparison with model compounds allowed the assignment of the structure as 2-decaprenyl-6-methoxyphenol,[7] L_{10} (V, n = 10).[28] The mass spectrum (*m/e* 805 for the parent ion, calcd, 804.7) established the assignment of a ten-unit polyisoprenoid side chain. The nmr spectrum was distinguished by the occurrence of a singlet at τ 3.35, corresponding in intensity to three protons. An equivalent three-proton singlet (τ 3.35) appears in the nmr spectrum of the closely related compound, 2-allyl-6-methoxyphenol (*o*-eugenol). Other examples of equivalent chemical shifts exhibited by vicinal protons on unsymmetrically substituted aromatic rings have been observed.[30]

Radioactive incorporation experiments have shown the existence in *R. rubrum* of radioactive compounds which corresponded chromatographically to each of the 2-multiprenylphenols (III, n = 4, 9, 10; V, n = 10). A cell suspension containing HBA (1.76×10^6 dpm) was incubated anaerobically for 7.5 hr in light followed by 14 hr in darkness. The lipid extract was chromatographed on a column of silica gel and every fifth fraction (15–20 ml) was investigated for radioactivity. Four distinct bands (Figure 1, A–D) containing radioactivity were detected. Careful comparison by paper chromatography of the material associated with these bands with authentic samples demonstrated significant radioactivity in fractions which corresponded to X, L, and Q. It was found that L_{10} was associated with band A, X_{10}, X_9, and X_4 with band B, and Q with band C. Thin layer chromatographic comparisons indicated that rhodoquinone[31] was associated with band D.

(30) See, for example: "NMR Spectra Catalog," N. S. Bhacca, L. F. Johnson, and J. N. Shoolery, Ed., Varian Associates, Inc., Palo Alto, Calif., 1962, Spectrum Numbers 257 and 333.

(31) H. W. Moore and K. Folkers, *J. Am. Chem. Soc.*, 87, 1409 (1965); 88, 567 (1966).

Experimental Section

General Comments. The ultraviolet absorption spectra were measured with a Cary Model 14M spectrophotometer. The infrared absorption spectra were determined with a Perkin-Elmer Model 221 spectrophotometer. The nuclear magnetic resonance spectra were determined on a Varian HR-60 high-resolution spectrometer with the chemical shifts expressed in τ units relative to tetramethylsilane as an internal standard.

The mass spectra were 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 express sincere appreciation to Dr. Raffaele F. Muraca and Mrs. Julia S. Whittick for obtaining the mass spectra and for assistance with their interpretation.

Thin layer chromatography was carried out on plates having a 0.3-mm layer of adsorbent. The plates were activated by heating at 130° in an oven for approximately 1.5 hr and were stored in a dry cabinet after cooling. Paper chromatography, unless specified otherwise, was carried out using silicone-impregnated Whatman No. 3MM paper.[32]

Radioactivity was measured with either a Nuclear Chicago gas-flow counter or a Nuclear Chicago liquid scintillation spectrophotometer. Samples for scintillation counting were prepared by adding an aliquot of the radioactive material, usually in 0.1–3 ml of a solvent such as hexane, methylene chloride, or ethanol, to a vial containing 10 ml of scintillation solution. The scintillation solution is prepared by dissolving 4.0 g of PPO (2,5-diphenyloxazole) and 0.2 g of POPOP [1,4-bis-2-(5-phenyloxazolyl)benzene] in 1000 ml of reagent grade toluene. When decompositions per minute (dpm) was required, two samples were prepared as described above. To one of the samples was added an aliquot of a ^{14}C standard, usually toluene-^{14}C.

Centrifugation of large-scale batches (60–90 l.) of *R. rubrum* was carried out with a Sharples centrifuge. In the radioactive experiments employing small batches of *R. rubrum*, centrifugation was performed with an International Centrifuge Model HR-1 employing the large head and at 8000 rpm and 0°.

Freshly distilled hexane was used throughout.

Purification of Radioactive Compound X.[24] Four grams of wet cells, obtained from a 4-day growth of *R. rubrum*, was suspended in phosphate buffer (pH 7.5), centrifuged, and resuspended in 100 ml of buffer. To this suspension was added HBA-U-^{14}C (8.8×10^5 dpm) in 0.1 ml of ethanol.[22] The suspension was divided into four portions contained in 50-ml erlenmeyer flasks and shaken for 2 hr in the dark at 30° in a Dubnoff shaker under an atmosphere of oxygen. The cells were collected by centrifugation, washed once with phosphate buffer, and extracted by shaking for 16 hr with 80 ml of ether–ethanol (3:1). The cells were filtered and washed with ether. The filtrate was evaporated to dryness *in vacuo*. The residue obtained was taken up in hexane and the resulting solution filtered to remove insoluble materials. The hexane solution was extracted with several portions of 95% methanol and dried over magnesium sulfate. The crude material was purified by thin layer chromatography on eight silica gel G plates developed in 1:1 chloroform–benzene, the region above Q being scraped to obtain radioactive compound X. Further purification was accomplished by thin layer chromatography (silica gel G, benzene; radioactivity was detected employing a Nuclear-Chicago counter).

The expression compound X is used to describe this purified material which was shown (see below) to be a mixture of X_{10} and X_9 with other impurities.

Purification of Unlabeled Compound X. *R. rubrum* was grown anaerobically in the light in 10-l. bottles as described previously.[23] Batches of 60–90 l. were grown during a single period. After a growth period of 3 days, the bottles were placed in the dark for 18 hr. The cells were collected by centrifugation in a Sharples centrifuge, extracted by shaking for 16 hr with 3 l. of ether–ethanol (*ca.* 4:1), and filtered. The extract was evaporated *in vacuo*, the residue obtained was taken up in hexane, and the resulting solution was filtered to remove insoluble material. The hexane solution was extracted several times with 95% methanol to remove chlorophyll and dried over magnesium sulfate. The brown oil accumuated from 400–500 l. of *R. rubrum* was chromatographed on a 4.5 × 90

(32) F. A. Crane and R. A. Dilley, *Methods Biochem. Analy.*, 11, 279 (1963).

cm column of silica gel (60–200 mesh). The column was developed by elution initially with hexane followed by successively increasing 1% increments of ether in hexane in 750- to 1000-ml portions. The materials eluted from the column were collected in 15- to 20-ml fractions. Crude compound X (17 mg) was eluted in fractions 295–365 with 4% ether in hexane. Compound X can be conveniently detected with either diazotized sulfanilic acid[25] or Gibbs[33] spray reagents. Further purification was effected on silica gel G thin layer plates developed in benzene; the upper band (R_f 0.32) yielded 4.5 mg of compound X. Spectral data are reported in Table II.

3,5-Dinitrobenzoate of Compound X. Five milligrams of compound X was dissolved in 0.1 ml of pyridine. To this solution was added approximately 20 mg of 3,5-dinitrobenzoyl chloride. The mixture was stirred briefly and then heated on the steam bath for 3 min. The reaction mixture was poured into water and extracted with ether. The ether extract was washed twice with water, twice with sodium bicarbonate solution, and several times with water, and dried over magnesium sulfate. The drying agent was removed and the solvent evaporated *in vacuo* to yield 5.3 mg of a yellow oil. This material was purified by preparative thin layer chromatography on three silica gel G plates developed in benzene. There was obtained 3.5 mg of a yellow oil which solidified upon standing several days in the refrigerator. Spectral data showed: infrared ν_{max}^{film} no OH absorption, 1750 cm^{-1} (carbonyl), 1540 and 1340 cm^{-1} (nitro); nmr, τ 0.79 (nitro aromatic, 3 H), 2.69–2.85 (phenol aromatic, 4 H), 5.0 (vinyl, 9–10 H), 6.80 (benzyl, 2 H), 7.95 and 8.36 (alkyl, *ca.* 65 H).

Comparison of Unlabeled Compound X with Radioactive Compound X. The unlabeled compound X was indistinguishable from radioactive compound X in eight chromatography systems (Table I). The comparisons were made in the following manner. To a major portion of the plate or paper radioactive compound X was applied while in the remaining portion unlabeled compound X was applied as a single spot. The chromatogram was developed in the appropriate solvent system and the radioactivity counted on a Nuclear-Chicago counter. The portion of the plate or paper containing the spot of unlabeled compound X was developed with diazotized sulfanilic acid as a spray reagent, and the corresponding region on the remainder of the plate or paper was removed. The plate or paper was reprocessed to determine the amount of radioactivity remaining. When the radioactivity was greatly diminished or absent, it was assumed that the two materials had the same R_f values.

Isolation of 2-Decaprenylphenol, X_{10} (III, n = 10), and 2-Nonaprenylphenol, X_9 (III, n = 9), from Compound X.[23] Both compound X and radioactive compound X, when applied to silicone-impregnated paper[32] and developed in 2-propanol–water–ammonium hydroxide (8:1:1), could be separated into two major components (R_f 0.55 and 0.65) in comparable amounts. Preparative paper chromatography of 10 mg of isolated compound X was carried out as follows. A solution of 10 mg of compound X in hexane was applied as a line on a 20-cm-wide strip of silicone-impregnated paper.[32] The paper was developed (descending) in the above described solvent system, the edge of the paper was sprayed with Gibbs reagent[33] to detect the bands, and the paper containing the two components was cut into small pieces and extracted several times with ether. The residues obtained after removal of the solvent contained large amounts (70–100 mg) of silicone oil. The silicone oil was removed by applying the materials to Whatman No. 4 paper (not silicone impregnated) and developing the paper in 2-propanol–water–ammonium hydroxide (8:1:1). In this system the 2-multiprenylphenols migrated at the solvent front, and elution as described above from the papers yielded, in each instance, approximately 10 mg of material. Thin layer chromatography (silica gel G, benzene) furnished samples (~1 mg) of each of the two compounds, judged to be essentially pure by paper chromatography. Each compound possessed identical ultraviolet maxima; λ_{max}^{hexane} 273, 278 mμ.

(33) (a) K. Randerath, "Thin Layer Chromatography," Academic Press Inc., New York, N. Y., 1963, pp 151, 176; (b) J. Gibbs, *J. Biol. Chem.*, 72, 649 (1927).

Isolation of 2-Tetraprenylphenol, X_4 (III, n = 4). In the later fractions containing compound X and in the fractions immediately following a second diazotized sulfanilic acid sensitive material was detected.[25] Separation of this material from compound X was achieved by preparative thin layer chromatography on silica gel G plates developed in benzene. In this system, the newly detected substance had a slightly lower R_f value (Table I). Spectral data which establish this compound as 2-tetraprenylphenol are contained in Table II.

Isolation of 2-Decaprenyl-6-methoxyphenol, L_{10} (V, n = 10). A diazotized sulfanilic acid sensitive material[25] (18 mg) was isolated from fractions 160–190 (column chromatography) which were eluted with 2–3% ether in hexane. After further purification on silica gel G plates developed in benzene, a compound, mp 32–36°, was obtained and appeared homogeneous in a number of chromatographic systems (Table I). Spectral data which establish this compound as 2-decaprenyl-6-methoxyphenol, L_{10} (V, n = 10), are presented in Table II.

3,5-Dinitrobenzoate of 2-Decaprenyl-6-methoxyphenol, L_{10} (V, n = 10). To 6 mg of L_{10} dissolved in 0.1 ml of pyridine was added 15 mg of 3,5-dinitrobenzoyl chloride. The mixture was heated for 3 min on a steam bath, poured into water, and extracted once with ether. The ether extract was washed twice with water, twice with a solution of sodium bicarbonate, and several times with water. The ethereal solution was dried and the solvent removed *in vacuo* to yield 4.5 mg of a light yellow oil. Thin layer chromatography on silica gel G plates developed in benzene (R_f 0.35) and in 10% ether in hexane (R_f 0.15) showed one major spot with an R_f value in each case lower than that of L_{10}. Spectral data showed: infrared, ν_{max}^{film} no hydroxyl absorption, 1755 cm^{-1} (carbonyl), 1545 and 1340 cm^{-1} (nitro); nmr: τ 0.78 (singlet, nitro aromatic, *ca.* 3 H), 3.40 (multiplet, phenol aromatic, *ca.* 3 H), 4.98 (multiplet, vinyl, 10 H), 6.23 (singlet, methoxyl, 3 H), 6.76 (doublet, benzylic, 2 H), 8.04–9.10 (multiplet, alkyl, *ca.* 67 H).

Investigation of the Distribution of Radioactivity in the Lipid Extract of *R. rubrum* Incubated with *p*-Hydroxybenzoic Acid-U-^{14}C (HBA-U-^{14}C). To approximately 30 g of wet cells of *R. rubrum* suspended in phosphate buffer (pH 7.3) contained in a 500-ml ground-glass-stoppered erlenmeyer flask was added 1.7×10^6 dpm of *p*-hydroxybenzoic acid-U-^{14}C in 0.4 ml of ethanol. The flask was filled with buffer solution, flushed with nitrogen, and stoppered. The suspension was placed in light for 7.5 hr and then maintained in darkness for 14 hr. The cells were collected and worked up as described above. The crude lipid fraction was chromatographed on a 3 × 40 cm column of silica gel. Elution of the column was carried out initially with hexane, followed by gradually increasing the eluting power of the solvent by the addition of ether and, subsequently, methanol. The fractions were collected in tubes in volumes of 15–20 ml. Every fifth tube was removed; the solvent was evaporated under a stream of nitrogen, 2 ml of hexane was added, and a 1-ml aliquot was withdrawn and submitted for scintillation counting. The results are depicted graphically in Figure 1. That the radioactivity contained in radiochromatogram band A was associated with 2-decaprenyl-6-methoxyphenol, L_{10} (V, n = 10), was shown by paper chromatographic comparison with authentic material. Similarly, band B was shown to be resolvable into three bands containing significant radioactivity attributable to X_{10}, X_9, and X_4. Band C was shown to possess radioactivity associated with Q. For these determinations silicone-impregnated paper developed by descending chromatography in 2-propanol–water–ammonium hydroxide (8:1:1) was used.

Pooled fractions 240–270 (band D) had a leucomethylene blue sensitive material with an R_f value identical with that of rhodoquinone on silica gel G plates developed in 1:1 chloroform–benzene.

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