($-NO_2$); 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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(12) W. W. Parson and H. Rudney, *Proc. Natl. Acad. Sci. U. S.*, **53**, 599 (1965).

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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.

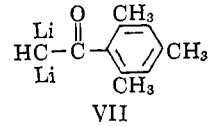
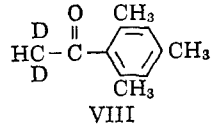
$C_6H_5CH(C_4H_9)CN$ (III) $\qquad$ $C_6H_5CD_2CN$ (IV)

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.

Studies are in progress not only on further reactions of dilithio salts II and VII but also on the preparation and reactions of 1,1-dilithio and 1,1,1-trilithio (or other trialkali) derivatives of other active hydrogen compounds having a functional group.

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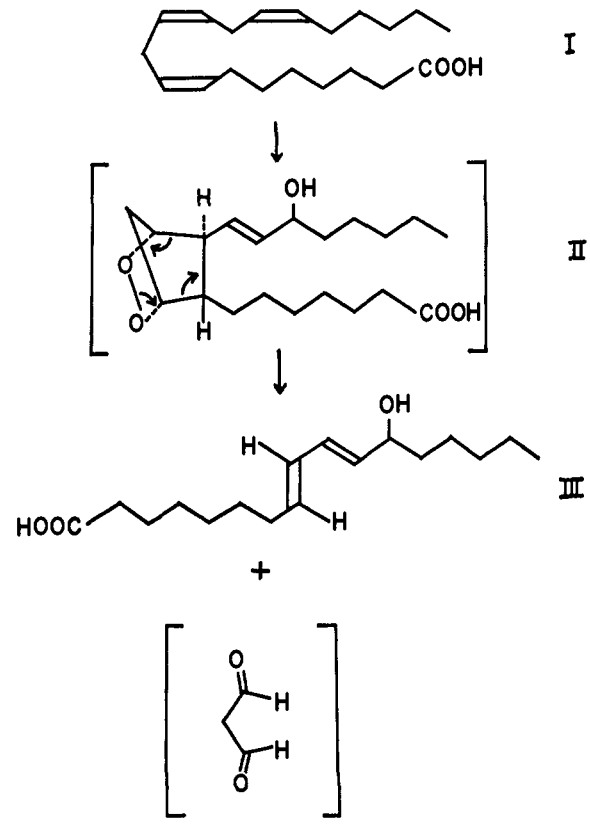
Novel Biological Transformations of 8,11,14-Eicosatrienoic Acid

Sir:

It was previously shown that 8,11,14-eicosatrienoic acid (I) is converted into prostaglandin E_1 by homogenates of the vesicular gland of sheep.[1,2] We now wish to report on the transformation of I into monohydroxy acids catalyzed by the same tissue. The hydroxy acids formed have been shown to consist of two C_{20} trienoic acids and one dienoic acid. The latter acid was identified as 12-hydroxy-*trans*,*trans*-8,10-heptadecadienoic acid (III), and it is suggested to be formed by a novel reaction involving elimination of three carbon atoms from the central part of the molecule.

Fifty micromoles of [2-^{14}C]8,11,14-eicosatrienoic acid[1] plus 75 μmoles of tetrahydrofolate were incubated for 60 min with the washed microsomal fraction and 30% of the high-speed supernatant obtained from a homogenate of 30 g of vesicular gland from sheep. Of the incubated radioactivity 10% was recovered as prostaglandin E_1, 11% as prostaglandin $F_{1\alpha}$, and 27% as monohydroxy acids by silicic acid chromatography. The monohydroxy acid fraction, which was eluted from the silicic acid column with ethyl acetate–benzene (10:90), was esterified by treatment with diazomethane. Further separation by preparative thin layer chromatography using silica gel G impregnated with silver nitrate and ethyl acetate–2,2,4-trimethylpentane (1:1) as solvent yielded two monohydroxytrienoate esters[3] and one monohydroxydienoate ester. The latter compound (isolated in 14% yield, based on incubated [2-^{14}C]8,11,14-eicosatrienoic acid) was acetylated and subjected to oxidative ozonolysis.[4] The product consisted of two main components which were identified using mass spectrometry in combination with gas–liquid partition chromatography[5] as 2-acetoxyheptanoic acid and monomethyl suberate. The methyl ester of the isolated compound was also hydrogenated and oxidized with chromic acid.[6] The product was identified as methyl 12-ketoheptadecanoate



by gas chromatography and mass spectrometry.[6,7] The mass spectrum showed ions at *m/e* 298 (M), 267 (M − 31), 242 (β-cleavage with loss of $CH_2{=}CHCH_2CH_3$), 227 (α-cleavage with loss of $-(CH_2)_4CH_3$), and 185 (β-cleavage with loss of $-CH_2CO(CH_2)_4CH_3$). These experiments therefore show that the isolated compound was 12-hydroxy-8,10-heptadecadienoic acid. That both of the double bonds had the *trans* configuration was established by the infrared spectrum[8] exhibiting one absorption band at 10.13 μ but no absorption at 10.56 μ and was further supported by the ultraviolet absorption which showed λ_{max}^{EtOH} 231 mμ.

In another experiment, [3-^{14}C,15-^{3}H]8,11,14-eicosatrienoic acid[9] was incubated with the same system. The isolated 12-hydroxy-8,10-heptadecadienoic acid contained the tritium label at C-12 since oxidation of the hydrogenated derivative with chromium trioxide–pyridine complex[10] to 12-ketoheptadecanoic acid resulted in practically complete loss of the tritium label. It was also shown by degradation experiments[11] that the

(1) S. Bergström, H. Danielsson, D. Klenberg, and B. Samuelsson, *J. Biol. Chem.*, **239**, PC4006 (1964).
(2) D. A. van Dorp, R. K. Beerthuis, D. H. Nugteren, and H. Vonkeman, *Nature*, **203**, 839 (1964).
(3) This fraction was identified as a mixture of methyl 11-hydroxy-8-*cis*,12-*trans*,14-*cis*-eicosatrienoate (80–90%) and methyl 15-hydroxy-8-*cis*,11-*cis*,13-*trans*-eicosatrienoate (10–20%) by oxidative ozonolysis by mass spectrometric analysis of the derived saturated keto-esters, by their ultraviolet spectra (λ_{max}^{EtOH} 233 mμ), and by thin layer chromatographic comparison with methyl 15-hydroxy-8-*cis*,11-*cis*,13-*trans*-eicosatrienoate. It was also shown that incubation of linoleic acid with the same system gave 9-hydroxy-10-*trans*,12-*cis*-octadecadienoic acid (80–90%) and 13-hydroxy-9-*cis*,11-*trans*-octadecadienoic acid (10–20%). The structure of these acids was established by oxidative ozonolysis, mass spectrometry, and ultraviolet spectroscopy.
(4) M. Hamberg and B. Samuelsson, *J. Biol. Chem.*, **241**, 257 (1966).
(5) R. Ryhage, *Anal. Chem.*, **36**, 759 (1964).
(6) M. Hamberg and B. Samuelsson, *Biochem. Biophys. Res. Commun.* **21**, 531 (1965).
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(8) J. R. Chipault and J. M. Hawkins, *J. Am. Oil Chemists' Soc.*, **36**, 535 (1959).
(9) The labeled acid was prepared according to a previously reported procedure: D. Klenberg and B. Samuelsson, *Acta Chem. Scand.*, **19**, 534 (1965).
(10) G. E. Poos, G. E. Arth, R. E. Beyler, and L. H. Sarett, *J. Am. Chem. Soc.*, **75**, 422 (1953).
(11) The labeled 12-hydroxy-8,10-heptadecadienoic acid formed from [2-^{14}C]8,11,14-eicosatrienoic acid was hydrogenated, acetylated, and degraded by oxidation with permanganate in acetone followed by gas–liquid partition radiochromatography of the product as previously described; see E. Granström, U. Inger and B. Samuelsson, *J. Biol. Chem.*, **240**, 457 (1965). Only the C_{16} and C_{17} acids contained ^{14}C.