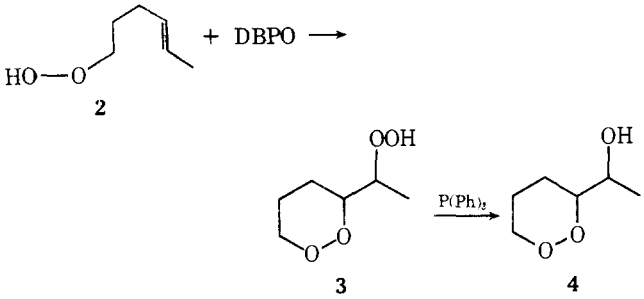
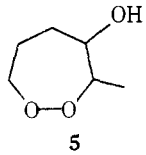
hydrogen attached to carbon. Thus the work of Howard and Ingold[5] and Walling[6] suggests that the relative rate of H atom abstraction by *tert*-butoxy radicals from ROO*H* compared to abstraction of an allyic H is approximately 50:1. The method reported here involves the formation of the requisite unsaturated peroxy radical by H atom transfer to *tert*-butoxy radicals from the corresponding hydroperoxide.

When the unsaturated hydroperoxide **2** (5.2 mmol)[7] is allowed to stand for 2 days with di-*tert*-butylperoxyoxalate (DBPO)[8] (2.0 mmol) in O_2 saturated benzene (500 ml) at 23° (four half-lives), a mixture of compounds is obtained which includes the two cyclic peroxides **3** and **4**. Although it



is possible to isolate these compounds directly by chromatography on silica gel it has been found more convenient to first reduce the hydroperoxide species in situ using triphenylphosphine (3.9 mmol). In this manner, the peroxy alcohol **4**, obtained analytically pure[9] as a mixture of threo and erythreo isomers, is isolated from the reduction reaction by silica gel chromatography in 30% overall yield.[10]

Although the NMR does not distinguish between the structure **4** and the corresponding seven-membered ring cyclization product **5**, double irradiation experiments show

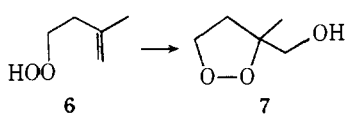


that the methyl group is clearly coupled to the proton α to the hydroxyl group suggesting **4** as the structure. Further confirmation of the structure assignment is provided by oxidation of the peroxy alcohol **4** with *N*-chlorosuccinimide, dimethyl sulfide[11] to a peroxy ketone which shows only a singlet in the α carbonyl region (2.28) of the NMR.

A mechanism consistent with our observations is presented in Scheme II.

According to this scheme, *tert*-butoxy radicals generated from DBPO homolysis abstract the hydroperoxy hydrogen yielding the peroxy radical. Cyclization and O_2 trapping leads ultimately to the peroxy hydroperoxide **3**.

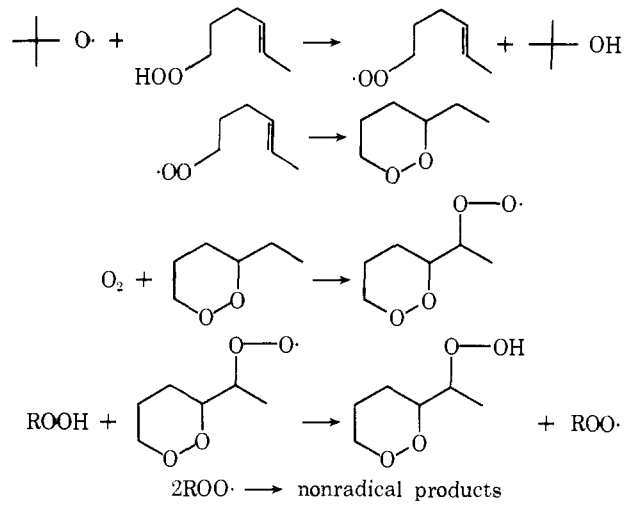
Treatment of hydroperoxide **6** with DBPO in benzene followed by triphenylphosphine reduction leads to the five-membered peroxy alcohol **7** in ≈25% overall yield. Peroxy



radicals thus appear to be subject to the same influences which cause carbon and alkoxy radicals to cyclize to five- rather than six-membered rings.[12]

The method reported here appears to be generally applicable to a systematic study of unsaturated peroxy radical cyclizations. In particular, model systems for radical cyclization leading to prostaglandin type products are currently under investigation in our laboratories.

Scheme II



Acknowledgment. This work was supported by a grant from the National Institutes of Health, Grant No. HL 17921-01.

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(10) Examination of the NMR of the crude cyclization product before triphenylphosphine reduction shows the absence of vinyl protons due to the starting hydroperoxide. The modest yields of analytically pure **4** obtained are most likely the result of side reactions during the reduction and/or decomposition of **4** during workup.
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Application of Carbon-13 Magnetic Resonance to Isoprenoid Biosynthesis. I. Ovalicin

Sir:

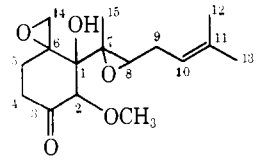
The sesquiterpene ovalicin[1] (**1**) isolated from culture filtrates of the fungus *Pseudorotium ovalis* STOLK, shows antibiotic as well as immunosuppressive and antitumor ac-

Table I. ^{13}C NMR Spectrum of Ovalicin[a]

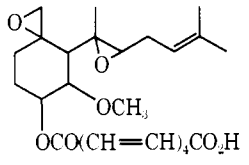
C-3	206.2 s[b,c]	C-8	56.5 d
C-11	135.0 s	C-14	51.0 t
C-10	117.8 d	C-4	36.4 t
C-2	85.9 d	C-5	30.0 t
C-1	78.2 s	C-9	26.8 t
C-6	60.2 s	C-13	25.4 q
C-7	60.0 s	C-12	17.7 q
C-16	58.9 q	C-15	14.1 q

[a] Recorded on Varian XL-100; spectral width 6000 Hz, acquisition time 0.6666 sec, pulse delay 6.00 sec, pulse width 35 μsec, 1392 transients, 0.85 *M* solution in $CDCl_3$, 12-mm sample tube. [b] TMS = 0.00 ppm. [c] Multiplicity in off-resonance decoupled spectrum: s = singlet, d = doublet, t = triplet, q = quartet.

tivity. The structure and stereochemistry of this substance was determined by a Sandoz group using a combination of chemical and X-ray crystallographic techniques. These workers also recognized the identity of ovalicin with the lettuce seed germination stimulant graphinone,[2] a metabolite of a *Graphium* sp. fungus. Ovalicin is closely related structurally to fumagillin (**2**)[3] an antibiotic metabolite of *Aspergillus fumigatus* with antiparasitic and carcinolytic properties. A preliminary study of fumagillin biosynthesis[4] showed that the terminal isopropylidene residue of fumagillin derived from labeling experiments with 2-^{14}C-mevalonate carried one-third of the incorporated radioactivity.



1



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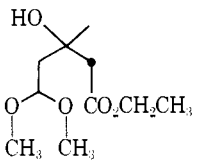


3

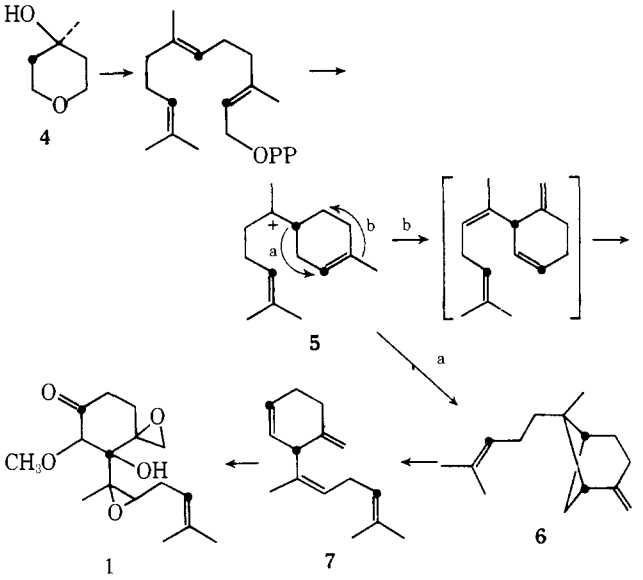
Ovalicin (**1**) and its relative fumagillin (**2**) possess a rare sesquiterpene carbon skeleton and may be formally considered as *o*-menthane derivatives. The biosynthetic problem presented by these substances involves the rigorous distinction among a number of plausible explanations which may be advanced a priori to account for their biological formation or biogenesis. Specifically one must account for the generation of the *o*-menthane skeleton of **1** from an intermediate such as cation **5**. We have applied the techniques of carbon-13 magnetic resonance to the solution of this problem and report our results below.

The natural abundance ^{13}C NMR spectrum of ovalicin was recorded, and peak assignments were made with the aid of off-resonance and single-frequency decoupling. The results are presented in Table I.

A sample of [4-^{13}C]mevalonolactone (**4**) was prepared by a modification of Cornforth's procedure.[5] [2-^{13}C]Acetyl chloride[6] was converted to ethyl acetate by reaction with absolute ethanol in the presence of triethylamine. This ethyl acetate was then metalated with lithium diisopropylamide[7] in tetrahydrofuran and the resulting lithium enolate was treated with acetoacetaldehyde dimethyl acetal to yield **3**. Conversion of **3** to [4-^{13}C]mevalonolactone (**4**) was accomplished by successive reduction with lithium aluminum hydride, acetylation, and performic acid oxidation[5] (48% yield based on acetyl chloride).

Four 500-ml DeLong flasks containing 100 ml of nutrient broth[1] were inoculated with *P. ovalis* and shaken at 30° for 7 days. Sodium [4-^{13}C]mevalonate (0.028 mol, 90% enriched) was added and incubation was continued for an additional 7 days. The culture filtrates were then extracted with methylene chloride and the extracts purified by PLC on silica gel to yield 9 mg of ovalicin. The proton decoupled ^{13}C NMR spectrum (12,041 transients) of ^{13}C enriched **1** showed an approximate three- to fourfold peak enhancement for the signals assigned to C-1, C-3, and C-10 of ovalicin. The magnitude of the peak enhancements, corresponding to ca. 3-4% ^{13}C enrichment at each of the respective carbons, allowed unambiguous assignment of the sites of labeling.[8]

Scheme I



The results of the ^{13}C NMR study demonstrate the intermediacy of mevalonate in the biosynthesis of ovalicin. The observed labeling pattern supports a biosynthetic scheme in which an initially generated bisabolene-like structure (**5**), formed by cyclization of farnesyl pyrophosphate, may undergo one of two possible rearrangements (Scheme I): pathway a in which there is a 1,3-migration of the eight-carbon side chain; or pathway b, a 1,3-migration of the ring methyl. Recently, Tanabe has also examined the biosynthesis of ovalicin by an alternative approach which utilized [1,2-^{13}C]acetate.[9] His results, first reported while this work was in progress, indicated, inter alia, that carbons 14 and 6 (carbons 13 and 1 using Tanabe's numbering[9b]) must originate from the same molecule of acetate. This is clearly inconsistent with pathway b. The combined results, therefore, strongly support pathway a and suggest that the cation **5** rearranges via intermediacy of β-bergamotene (**6**)[4,10,11] to the tetraene **7** which in turn undergoes appropriate oxidations to yield, ultimately, ovalicin.

Acknowledgments. We wish to thank Mr. William E. Hull for recording the ^{13}C NMR spectra. We are also indebted to Dr. Pietro Bollinger of Sandoz, A. G., Basel, Switzerland for a generous gift of ovalicin and strains of *P. ovalis*. This work was supported in part by a Cottrell Grant from the Research Corporation to D.E.C.

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Book Reviews

Progress in Polymer Science, Japan. Volumes 1 to 5. Wiley/Halsted, New York, N.Y. (also published in Japan by Kodansha Ltd., Tokyo). Volume 1: Edited by M. IMOTO and S. ONOGI. 1972. xii + 520 pp. $25.00. Volume 2: Edited by M. IMOTO and S. ONOGI. 1972. ix + 379 pp. $18.50. Volume 3: Edited by S. OKAMURA and M. TAKAYANAGI. 1972. xii + 388 pp. $18.50. Volume 4: Edited by K. IMAHORI and Y. IWAKURA. 1972. x + 278 pp. $14.00. Volume 5: Edited by K. IMAHORI and S. MURAHASHI. 1973. ix + 308 pp. $17.50.

Much of Japanese polymer research, especially that prior to 1970, has been available only in Japanese. These well-prepared volumes provide excellent English translations of review articles on selected and diverse topics emphasizing the outstanding research contributions of Japan's polymer scientists. Each volume consists of five to seven reviews about equally divided in subject matter between polymer synthesis and the structure and properties of polymers. Each review, written by the expert investigators, summarizes the work of both Japanese and other scientists. There are extensive figures, tables, and literature references. Each volume has a general index. To their credit, the editors and publishers have managed to minimize the me gap between submission of copy and publication. For example, Volume 5, published in 1973, consists of articles submitted in 1972 and containing references to the literature which appeared in that year.

The series is a valuable reference work not only as a convenient source of the Japanese contributions but also as a collection of up-to-date reviews in polymer science.

Volume 1: Structure and Reactivity of Vinyl Monomers in Radical Polymerization (T. Otsu); Effects of Metal Salts on Radical Polymerization (S. Tazuke); Cyclopolymerization of Divinyl and Dialdehyde Monomers (C. Aso, *et al.*); Conformational Statistics of Polymeric Chains (K. Nagai); Viscoelastic Properties of Concentrated Polymer Solutions (K. Osaki and Y. Einaga); Application of Polymer Rheology to Rubber Technology (K. Ninomiya, *et al.*); Secondary Structures of Synthetic Polypeptides and Proteins (K. Imahori).

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Volume 4: New Synthetic Routes to High Temperature Polymers by Cyclopolycondensation Reactions (N. Yoda, *et al.*); Reactions and Polymer Syntheses of Pseudoxazolones (Y. Iwakura, *et al.*); Syntheses and Reactions of Functional Polymers (M. Okawara, *et al.*); Domain Formation Mechanisms of Block and Graft Copolymers from Solutions (H. Kawai, *et al.*); Metalloenzyme Models (M. Hatano and T. Nozawa).

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An Introduction to Spectroscopic Methods for the Determination of Organic Compounds. Volume 2. Edited by F. SCHEINMANN (University of Salford, England). Pergamon Press, New York, N.Y. 1974. 354 pp. $8.50 (soft cover); $14.25 (hard cover).

This volume, which is the second of a two-part series on spectroscopic methods of structure determination (first volume not reviewed), is a generally well-written, practical discussion of spectroscopic techniques. Volume 2 covers mass spectrometry, uv, esr, and recent developments in nmr (lanthanides, nOe and a brief discussion of ^{13}C nmr). Volume 1 is concerned with general discussions of nmr and ir.

The book adopts rather a pragmatic approach to spectroscopic techniques, offering what the editor calls "adequate theory" which is indeed abbreviated. The examples and problems are interesting and informative and deal well with the actual mechanics of determining structures for spectroscopic data. There are a few annoying misprints and the American reader may find the use of joules instead of kcal somewhat troublesome. On the positive side, there are two full chapters near the end of the volume which deal specifically with integration of techniques in structure determination. A short final chapter, "Documentation of Molecular Spectra," adds interesting perspective on the collection, storage, retrieval, and availability of data.

In general, this volume offers a problem-solving approach to spectral methods; it covers major topics adequately, although the details of theory and instrumental operation are not all one might desire. The number and diversity of problems, examples, and references make this appear to be a useful text in a field where the variety of approaches precludes the selection of one "best" volume.

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