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Phytadienes from the Pyrolysis of Pheophytin a

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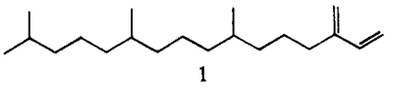
In the course of a study on organic compounds in polluted water,[1] a particularly facile pyrolytic reaction was observed when extracts containing pheophytin a were injected into a gas chromatograph which was operated with an injection port temperature of 250°. Since most thermal decompositions take place at much higher temperatures, a brief study of the pyrolytic behavior of this compound was undertaken.

Pheophytin a was pyrolyzed at 250, 350, and 400° directly onto a high-resolution gas chromatographic (gc) column the effluent of which was monitored with a fast-scanning computerized mass spectrometer. Four major fractions were observed and they were identified as various phytadiene isomers (**1**–**4**) from their mass spectra and gc retention indexes (see below for details). These data and the relative abundances of the various isomers are given in Table I. It can be seen that the relative yield of the pyrolysis products observed at the three temperatures is not significantly different and that at least 94% of these products are phytadienes. The remaining 5–6% were at least 15 different compounds and, judging from their gc retention times, all contained less than ten carbon atoms; they were not investigated further. In addition, any nonvolatile pyrolytic products which were not transmitted by the gas chromatograph were not studied.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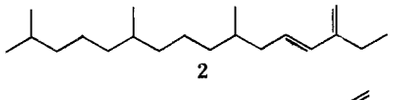
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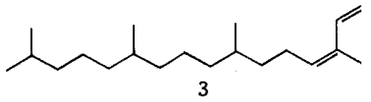
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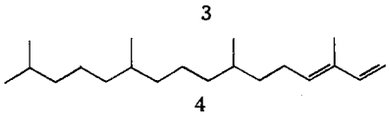
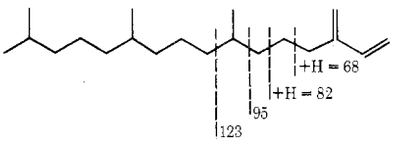

4

Table I
Compounds Identified in the Pyrolysate of Pheophytin a

Compd	Relative yield,[a] % 250°	350°	400°	Retention index (±1)	Mass spectrum, *m/e* (rel intensity)[b]
1[c]	61	63	64	1841	68 (100), 57 (86) 43 (80), 82 (73)
2[d]	2	2	1	1848	43 (100), 68 (90) 57 (84), 41 (79)
3	12	10	11	1863	43 (100), 82 (96) 68 (90), 57 (89)
4	20	19	18	1882	82 (100), 43 (78) 57 (77), 81 (71)
Others	5	6	6		

[a] Absolute total molar yield, relative to pheophytin a, is 40–60%. [b] See paragraph at end of paper regarding supplementary material. [c] Common name: neophytadiene. [d] Tentative structure.

The information which lead to these identifications is as follows. The mass spectra of the four major components were quite similar to each other. They all exhibited abundant ions at *m/e* 43, 57, 68, 82, 95, and 123 and a molecular ion at *m/e* 278. These ions are consistent with phytadienes and must originate by cleavage of the indicated bonds (using neophytadiene as an example). Ions at *m/e* 68 and



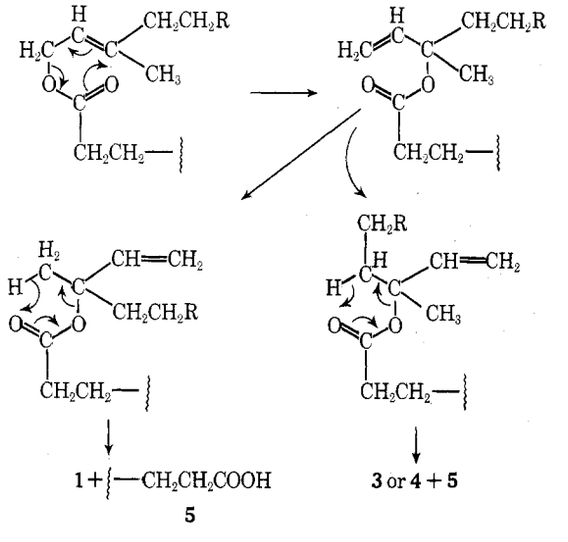
82 require the rearrangement of one hydrogen atom. The ions at *m/e* 95 and 123 indicate that the diene system is located at the formerly esterified terminus of the molecule. In addition, the mass spectrum of the most intense gc peak was identical with that of synthetic neophytadiene (**1**). In this way all four peaks were identified as phytadienes.

The exact positions of the double bonds could not, of course, be completely established by mass spectrometry. Fortunately, however, the gc retention indexes of several phytadienes isolated from zooplankton and identified by ozonolysis and infrared spectrometry have been reported.[2] The retention indexes of compounds **1**, **3** and **4** (see Table I) are identical with this reference data. The tentative structure **2** was assigned on the basis of gc retention characteristics (indicating a terminal methylene group) and a less abundant *m/e* 82 ion relative to the other isomers.

Although the above information is not sufficient to prove a reaction mechanism, the following suggestion (see Scheme I) nicely accounts for the identity and abundance of the products. This suggested mechanism involves two steps, the first step being a Cope-type rearrangement of the phytyl group and the second being the elimination of pheophorbide (**5**) by way of a six-membered cyclic transition state in which the carbonyl oxygen interacts with the various α-hydrogen atoms. The observed product distribution is close to that which would be expected based on the number of protons available. Since there are three primary protons and two secondary protons, the statistical product distribution should be 60% **1**, 20% **3**, and 20% **4**, and in fact these values are very close to those observed (see Table I).

A similar mechanism has been suggested for the pyrolysis of certain allylic acetates.[3] For example, 2-acetoxy-*trans*-3-heptene pyrolyzes at 350° to give a mixture of 1,3- and 2,4-heptadiene. Isomerization of the ester was demon-

Scheme I



R = $(CH_3)_2CHCH_2CH_2CH_2CH(CH_3)CH_2CH_2CH_2CH(CH_3)CH_2$—

strated by isolation of 4-acetoxy-*trans*-2-heptene in the pyrolysate.[3]

It should be noted that tobacco smoke contains several phytadiene isomers, the most abundant of which is neophytadiene.[4] Since all of the phytadienes reported in Table I (except **2**) have been found in tobacco smoke, it seems likely that pyrolysis of the residual phytol esters originally present in tobacco as chlorophyll produces some of the phytadienes observed in smoke.

Besides tobacco, the only other reported occurrence of phytadienes is in zooplankton.[2] Since an injector port temperature of 250° is sufficient to produce phytadienes from pheophytin a, before reporting the presence of phytadienes in an extract it is important to demonstrate the absence of chlorophyll or its degradation products in that extract. In this respect it is possible that the phytadienes reported to be present in zooplankton[2] may have been an artifact.

Experimental Section

The samples were pyrolyzed directly into the gas chromatographic column using a CDS Pyroprobe 190 system. The sample (*ca.* 300 μg) was coated from solution (CH_2Cl_2) on a platinum ribbon (35 × 1.5 × 0.0127 mm); after solvent evaporation, the ribbon assembly was inserted into the injection port of the chromatograph (held at 240 ± 10°). After restabilization of the helium carrier gas flow (1–2 min), the ribbon was heated at 10°/msec to 250, 350, or 400° and held there for 2 sec. Pyrolysis products were immediately vaporized and swept onto the gc column. The estimated maximum residence time of the products at temperature was less than 100 msec; thus isomerization was avoided.

Samples were also pyrolyzed by injecting the solution (CH_2Cl_2) directly into the heated injection port. Injector temperatures of 250–320° were sufficient to pyrolyze the pheophytin a to give a low yield (5–10%) of phytadienes. Although the results of injector port pyrolyses were not as reproducible as those of the platinum ribbon system, the identities and distribution of products were approximately the same as shown in Table I.

Two gc columns were used: (a) 300 ft × 0.01 in. i.d. stainless steel, wall coated with SF-96 containing 5% Igepal 880 operated isothermally at 180° (110,000 theoretical plates), and (b) 150 ft × 0.02 in i.d. stainless steel, wall coated with OV-101; temperature programmed from 130 to 200° at 3°/min. Both columns gave identical retention indexes and the resolution was such as to verify that there were no more than four phytadienes present. The computerized combined gas chromatograph–mass spectrometer system has been described previously;[5] however, since capillary columns with carrier gas flow rates of 0.5–1.5 ml/min were used for this study and since the restrictors in the fritted glass interface between the gas chromatograph and mass spectrometer were adjusted for flow rates of 15–40 ml/min, it was necessary to add carrier gas after the column to bring the total gas flow into the interface up to these higher values. A flame ionization detector chromatogram was recorded in parallel to the gas chromatograph–mass spectrometer and was used for the quantitative values shown in Table I.

Pheophytin a was prepared by acid hydrolysis[6] of chlorophyll a which was, in turn, isolated from a mixed culture of green algae and blue-green algae (cultured in a modified Allen's medium[7] for 3 weeks). The cells were collected by centrifugation, and the chlorophyll a was isolated by chromatographic procedures.[6] The visible spectrum of the isolated pheophytin a (in ether) exhibited peaks at 410, 474, 506, 535, 562, 612, and 671 nm and was in agreement with published spectra.[8] Methyl pheophorbide a was prepared by Fisher's method,[6] and the mass spectrum was obtained by inserting the sample directly into the ion source at 380°; it showed characteristic ions at *m/e* 606, 576, 548, and 461 and agreed with the published mass spectrum of methyl pheophorbide a.[9]

Acknowledgments. Iwan Hirsan provided valuable assistance; the instrumentation was supported (in part) by National Institutes of Health Research Grant RR00317 from the Division of Research Facilities and Resources (K. Biemann, principal investigator).

Registry No.—1, 504-96-1; **2**, 51806-25-8; **3**, 21980-71-2; pheophytin a, 603-17-8.

Supplementary Material Available. Complete mass spectra of compounds **1–4** will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JOC-74-2634.

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A New, Practical Synthesis of L-2-Hydroxytryptophan and Its Derivatives

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DL-2-Hydroxytryptophan[2] (**3**) has been prepared by three- or four-step syntheses originating with the reaction products from ethyl 2-(*o*-nitrophenyl)acetate and diethyl methylenemalonate,[3] isatin and ethyl pyruvate,[4] or 3-chloromethyleneoxindole and diethyl formamidomalonate.[5] Yields, however, are modest (15–24% overall) and resolution, when the biologically important[3-6,8,9] L isomer is desired, poses difficulties.[4]

A one-step oxidation of L-tryptophan with peracetic acid in acetic anhydride[6,7] or aqueous hydrolysis (130°) of its symmetrical 2,2′-disulfide,[8] obtained by reaction with di-