Ionene: A Thermal Degradation Product of β-Carotene

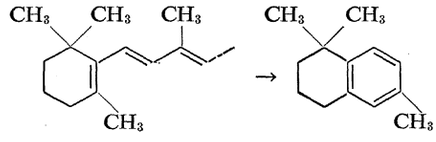
Abstract. *Ionene has been identified as a thermal degradation product of β-carotene. The 5-percent yield of ionene represents the largest amount of any compound thus far identified from the degradation. Experimental evidence has been obtained indicating the presence of ionene in a marine sediment subjected to the same temperature used to degrade β-carotene.*

In an earlier paper (*1*), the carotenoids occurring in recent aquatic sediments have been proposed as probable precursors of certain members of the aromatic series of hydrocarbons. These speculations were based on statements in the literature (*2*, *3*) that, on mild thermal treatment, certain pure carotenoids yielded small amounts of aromatic hydrocarbons of low molecular weight. Three hydrocarbons always obtained were toluene, *m*-xylene, and 2,6-dimethylnaphthalene. These three compounds most likely are formed by cyclization of the polyene chain. While toluic acid was obtained from bixin and azafrin (*2*), no compounds indicative of the end groups of such common carotenoids as β-carotene have been identified. Furthermore, no information was available concerning the fate of the 95 to 98 percent of the carotenoid not converted to the mentioned products. Accordingly, this investigation had as its objective the determination of optimum conditions for the degradation to products of low molecular weight and their characterization.

When a carotenoid is heated to its melting point, the compound is destroyed rapidly. It has been the experience in this laboratory that maintaining a solution of β-carotene at 119°C for a long period of time equally disrupts the molecule.

For the studies reported, the degradation was carried out by heating a 1-percent solution of β-carotene in benzene at 188°C for 72 hours. Dilute solutions were used to minimize intermolecular polymerization and to simulate the dispersed state of carotenoids in aquatic sediments. After removal of the solvent, the residue was a tacky, amber-colored substance. The molecular weight, as determined by osmometry with chloroform and benzene, was 460 and 425, respectively, compared to 537 for β-carotene.

A pale-yellow oil constituting 28 percent of the degraded β-carotene was collected by azeotropic distillation with ethylene glycol. The gas chromatographic analysis of this distillate showed that it was comprised of an array of compounds. The oil was separated into its components by fractional distillation, adsorption chromatography, and preparative gas-liquid chromatography. The four principal components were toluene, *m*-xylene, 2,6-dimethylnaphthalene, and a $C_{13}H_{18}$ compound. The latter, a colorless liquid, was shown by means of ultraviolet, infrared, and nuclear magnetic resonance to be ionene, that is, 1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene:



The identification was confirmed by comparison with an authentic sample of ionene synthesized from α-ionone by the method of Bogert and Fourman (*4*).

The weight-percent yields of these four degradation products of β-carotene are listed in Table 1. It will be seen that, of the compounds thus far identified, ionene is quantitatively the major constituent. The isolation and characterization of the remaining products are in progress.

In a related paper (*5*), it has been shown that heat treating organic-rich aquatic sediments under conditions comparable to those used for the degradation of β-carotene generated, among other hydrocarbons, toluene and probably *m*-xylene. These volatile hydrocarbons were isolated from the sublimate of a freeze-dry operation.

The residue from one of these experiments, namely, the Santa Barbara Basin sediment, was examined to ascertain whether the less volatile products of carotenoid degradation, particularly 2,6-dimethylnaphthalene and ionene, also had been generated.

The dry sediment was extracted with ether for 3 days in a Soxhlet extractor. A distillate fraction boiling at 135°C/0.5 mm was collected from the extracted material. The material was chromatographed on silica gel and about 1 mg of a colorless oil was washed through with pentane. In elution characteristics the material corresponded to 2,6-dimethylnaphthalene and ionene. Gas-liquid chromatographic analysis of the fraction showed a number of peaks, some of which corresponded in retention time to those observed for the equivalent distillate fraction of β-carotene. The presence of 2,6-dimethylnaphthalene and ionene also was tentatively confirmed. Since an equivalent unheated sediment sample has not been fully studied, as yet, it remains to be determined whether these compounds are, like toluene and the xylenes, generated by the heat treatment of the sediment, and whether they are, in fact, derived from the carotenoid pigments naturally present (*6*).

Table 1. Thermal degradation products of β-carotene.

Compound	Yield (%)
Toluene	0.3
m-Xylene	0.4
2,6-Dimethylnaphthalene	1.3
Ionene	5.0

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Carotenoids in Sediments as a Function of Environment

Abstract. *The carotenoid content and xanthophyll/carotene ratio were determined for the surface horizons of each of nine recent aquatic environments. In general, the concentrations of carotenoids, expressed as a function of organic carbon, were higher in marine than in fresh-water sediments. Xanthophyll/carotene ratios were all above unity.*

Carotenoids are an ubiquitous class of red pigments present in almost all living plants and animals. Despite the fact that these highly unsaturated compounds are light- and oxygen-sensitive, they have been found to be present in recent sediments. The occurrence of carotenoids in lake sediments was first reported by Lyubimenko (*1*) in 1923 and in marine sediments by Trask and Wu (*2*) in 1930. Since that time, ca-

rotenoids have been shown to occur in the recent sediments of all environments studied: a cave, lacustrine deposits, sapropels, a river estuary, an intertidal sand, and marine sediments. The two oldest sediments found to contain carotenoids were a Searles Lake sediment (*3*) estimated to be 20,000 years old and an interglacial gyttja (*4*) estimated to be 100,000 years old. The general subject of carotenoids in sediments has been reviewed by Vallentyne (*5*).

Carotenoids are generally subdivided into two classes: carotenes, which are hydrocarbons, and xanthophylls, which are oxygen-containing compounds. The ratio, xanthophylls/carotenes (h/e ratio), varies between 3 and 10 in higher plants (*6*) and between 4 and 9 for algae (*5*). Carotenoids in recent sediments, however, are reported to have an h/e ratio of unity or below (*5*) with some exceptions.

One of us (*7*) has speculated on the role carotenoids might play as progenitors of petroleum. Knowledge of the distribution of carotenoids in sediments is an essential part of the experimental study of their role in petroleum genesis. Although many reports of the occurrence of carotenoids in sediments are to be found in the literature, no one has yet compared the carotenoid content of a series of recent sediments which were deposited under widely different environmental conditions. This preliminary report is of a systematic survey for a series of nine surface-horizon (0 to 15 cm) samples ranging in type from fresh-water swamp to deep marine.

Recent sediment samples were collected by this laboratory over a number of years. Tamarack Bog in northwestern Pennsylvania represents a northern fresh-water swamp; Okefenokee in Georgia, a subtropical fresh-water swamp with both cypress and savannah vegetation. The samples from Bellefontaine Marsh, the Mississippi Sound, and the Gulf of Mexico represent a traverse on the Gulf Coast from a brackish marsh through a salt-water lagoon to a shallow continental self. Santa Barbara Basin, San Nicolas Basin, and Outer Edge are from the Pacific continental borderland ranging from 8 to 225 km off the coast of California (*8*).

Samples were frozen in dry ice in the field immediately upon collection and kept at deep-freeze temperatures until used. Frozen cores were thawed, ground in a blender where necessary, and lyophilized. The dry material was passed through a No. 40 sieve in a glove box under nitrogen, and all subsequent operations were carried out under similar conditions.

Table 1. Carotenoid contents and h/e ratios of recent sediments.

Sample	Organic carbon (%)	Carotenoids (ppm, based on organic carbon)	Carotenoids (ppm of sediment, dry wt.)	Xanthophylls/carotenes (h/e)
Santa Barbara Basin: Pacific continental borderland	2.9	790	23	2.1
Gulf of Mexico: Continental shelf	0.6*	300*	1.8*	3.6*
Mississippi Sound	1.3†	220†	2.7†	3.0†
San Nicolas Basin: Pacific continental borderland	4.9	100	5.1	1.5
Okefenokee Swamp: savannah vegetation	51.3	65	33	2.4
Tamarack Bog	47.2	48	23	2.0
Okefenokee Swamp: cypress vegetation	48.2	36	18	1.3
Outer Edge: Pacific continental borderland	1.0	30	0.3	1.7
Bellefontaine Marsh, Gulf Coast	12.1‡	27‡	3.2‡	3.3‡

* Average of six determinations from two locations. † Average of five determinations from two locations. ‡ Average of four determinations from two locations.

The sediments were extracted five times with absolute methanol. For each extraction the mixture was allowed to stand for 24 hours with occasional shaking. After reduction of the volume by vacuum evaporation, an aliquot of the solution was saponified with 15-percent methanolic KOH overnight. The mixture was extracted with ether, the ether solution was evaporated, and the residue made up to volume in absolute methanol. Solutions were analyzed by integrating the areas under the spectral curves between 420 and 500 mμ. Results are expressed in β-carotene equivalents because this compound was used to prepare the standard curve. The h/e ratios were obtained by distributing the saponified material between hexane and 95-percent methanol. The organic carbon content of the sediments was determined by a modified Van Slyke-Folch wet combustion method.

Results are shown in Table 1 in order of decreasing carotenoid concentration expressed as parts per million of the organic carbon in the sediment. Excluding the sample from the Outer Edge of the Pacific continental borderland, the four highest carotenoid concentrations, 790 to 100 ppm, are found in marine environments, while the four lowest, 65 to 27 ppm, are from fresh-water or brackish-water environments. Since algae contain a larger percentage of carotenoid pigments per unit of total organic carbon than do the higher plants, the result is not unexpected. An explanation for the anomaly represented by the Outer Edge sample is not immediately evident, for little is known of this sediment in terms of specific environment (*8*).

In the traverse, Bellefontaine Marsh–Mississippi Sound–Gulf of Mexico, carotenoid concentration expressed as a function of organic carbon increased in the seaward direction, namely, 27 to 220 to 300 ppm. The three fresh-water swamp environments—Okefenokee Swamp under two types of vegetation and Tamarack Bog—were found to contain corresponding concentrations of carotenoids, namely, 65, 45, and 36 ppm.

As might be expected, no correlation was apparent between the type of environment and the carotenoid content expressed as parts per million of the total weight of sediment.

The h/e ratios of the sediments from all environments studied here were above one, namely, 1.3 to 3.6. Although this range generally differs from previous work to date (*5*), it is nearer the values exhibited by plants, namely, 3 to 10. Such h/e values imply that the xanthophylls are disappearing at a faster rate than the carotenes. From these data, no correlation can be made between h/e ratio and type of environment (*9*).

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Acquired Chromosomal Anomalies Induced in Mice by Injection of a Teratogen in Pregnancy

The experiments herein reported were undertaken to test whether a selected teratogen that can cause anomalies in 95 percent of fetuses can also cause chromosomal anomalies. 6-Amino nicotinamide was selected as the teratogenic agent, cleft palate as the deformity to be induced, and pregnant outbred females of the 15-year-old Phipps mouse colony as the biologic model to be used in the experiments. This agent, this defect, and this animal were chosen because much work had been done on chemical, morphologic, and embryological aspects of all three variables (*1*).

Pilot studies were made to establish the critical period for production of cleft palate by 6-amino nicotinamide in offspring of pregnant females of the Phipps mouse colony. Standard methods were modified and applied for the demonstration of chromosomal structure of cells of tissues adjacent to and remote from palatal defects induced by the administration of 6-amino nicotinamide to pregnant females around the 13th day of gestation. Chromosomal analyses were also carried out on maternal bone marrow, a tissue regularly in a high state of mitotic activity, to determine whether or not 6-amino nicotinamide injection had any pathogenic effect on maternal chromosomal patterns. For purposes of comparison similar studies were made of chromosomal patterns in cells of palates and other tissues from normal embryos of untreated mothers. Search was also made for presence or absence of chromosomal anomalies in cells from bone marrow of mothers of control embryos.

The following steps, with modifications of standard techniques (*2*, *3*), formed the basis of procedure. Some pregnant animals were killed by ether inhalation 1 day after injection of 6-amino nicotinamide; most were killed 6 days after injection or as controls on the 19th day of gestation. Under sterile conditions, embryos were taken from the uterus and placed in a sterile petri dish with a few milliliters of nutrient (medium 199 containing 15-percent calf serum). The embryos were examined at this time to ascertain the presence or absence of cleft palate. The embryonic material was then processed for observation of cellular structure and chromosomal patterns of cells arrested in metaphase. Some pieces of palatal tissue, muscle, skin, and limb fragments were removed and processed separately as plasma clot preparations on cover slips in stationary roller tubes or Leighton tubes according to a method previously described by Ingenito and her colleagues (*2*). The remaining part of the embryo was then trypsinized, and the resulting cell suspension planted in similar culture tubes containing cover slips. In the early outgrowths—about 18 hours after explantation of trypsinized material, and about 36 hours after explantation for plasma clot preparations—the cells consist of two distinct types. One type is round and granular; the other is an elongated fibroblast-like cell with long processes. Within 1 to 2 days, the round cells show only a slight increase in number, whereas the fibroblast-like cells grow more rapidly out into a monolayer. At this time of most rapid proliferation, the cultures have reached their peak of mitotic activity, and the outgrowth on the cover slip is ready to be fixed and stained for chromosomal analysis.

The normal chromosomal pattern of cells cultured from the mouse consists of 40 similar-appearing chromosomes, with variations of 39 or 41 chromosomes occasionally encountered. One to 2 percent of these mitotic cells may exhibit polyploidy, and 3 to 4 percent may show fragmentation (Fig. 1 and Table 1).

When 6-amino nicotinamide is administered to pregnant females around the 13th day of pregnancy, about 95 percent of the embryos subsequently manifest cleft palate at section. The outgrowths from tissues of these embryos are composed of a loose meshwork of large vacuolated cells with coarse cytoplasmic granules and pyknotic nuclei (Fig. 2).

Microscopic study of chromosomal structure of these cells reveals that chromosomal anomalies have been produced by the teratogen used (Fig. 1), not only in cells grown from tissues adjacent to palatal defects, but also in cells grown from tissues in areas remote from the palate. Many more cells exhibiting polyploidy and fragmented chromosomes are observed in the offspring of test animals than in the offspring of normal controls. These findings are shown quantitatively in Table 1, the data of which also give clear indication that a time factor is involved. Polyploidy, for example, characterized almost half the mitotic cells examined within 24 hours after the injection of the teratogen; 9 percent of mitotic cells examined 6 days after injection; and in only about 2 percent of mitotic cells taken from control embryos. Not only were chromosomal anomalies found in cells cultured from embryonic tissues but the same kind of anomalies were demonstrated on examination of processed bone marrow of injected mothers 6 days after the administration of the teratogen (see Table 2).

Although the chemical explanation for the action of 6-amino nicotinamide in causing the chromosomal anomalies described is unknown, the epidemiologic (time-place-person) events in pregnancy are known. Had these events not been observed, the discovery of

Table 1. Normal and abnormal chromosomal patterns of mouse embryos 1 day and 6 days after injection of 6-amino nicotinamide.

Days after injection	Cells counted in metaphase	Normal pattern		Polyploidy		Fragmentation	
		No.	%	No.	%	No.	%
1	204	85	41.7	97	47.5	22	10.8
6	395	242	61.3	36	9.1	117	29.6
Control embryos	219	207	94.5	4	1.8	8	3.6