1-Pentadecene Production in Tribolium confusum

Abstract. *Odoriferous glands of* Tribolium confusum *produce volatile compounds which are stored in special reservoirs. Besides the previously reported 2-methyl- and 2-ethyl-1,4-benzoquinones, a new compound, 1-pentadecene, was isolated. The acyclic $C_{14}H_{26}O$ ketone previously reported was not present. 1-Pentadecene is thought to facilitate absorption of the admixed quinones by* Tribolium *enemies.*

Tribolium confusum (the confused flour beetle) is one of several tenebrionid beetles which infest flour (*1*). They contain odoriferous glands which secrete a fluid that is stored in special reservoirs (*2*). Chemical analysis of this fluid established the presence of 1,4-benzoquinones, as well as other unknown compounds. Englehardt, Rapoport, and Sokoloff (*2*) reported these compounds as 2-methyl- and 2-ethyl-1,4-benzoquinone and an unknown oil of molecular weight 210. The latter was considered to be a terminally unsaturated acyclic ketone, $C_{14}H_{26}O$, on the basis of infrared, mass, and proton magnetic resonance (PMR) spectrometry. Although this colorless oil is produced in quantities larger than those of many other pheromones (1.08 mg per 1000 beetles) (*2*), the molecular weight of the ketone implied a volatility consistent with pheromonal activity. On this basis we investigated its structure and function.

Tribolium confusum was raised in plastic trays containing a mixture (4 : 4 : 1) of finely sifted whole wheat flour, white flour, and brewer's yeast (*3*). By sifting the flour every day or two through No. 20 and No. 35 screens, we could transfer eggs out of the culture and maintain a colony of adults. Immature forms of the beetle were raised in a moist atmosphere at 30°C, at which temperature the period of development from egg to adult is 1 month. Thus large numbers could be raised rapidly. All adults were maintained in the above food mixture at room temperature. Adults have a lifespan of about a year (*3*), and this colony of males and females was used several times for subsequent experiments.

Volatile compounds produced by these beetles were collected by drawing air through a cold trap connected to a manifold containing a series of 600-ml sintered glass funnels. Lids (with holes) were fitted to the funnels in order to prevent excessive air movement. The traps were maintained at −60°C with Dry Ice and isopropanol. Four to five thousand beetles were placed in each funnel, and the funnels were heated with a hair dryer for about 15 minutes. The stoppered funnels were then floated in ice water until the beetles stopped moving. This heating and cooling procedure was repeated three times.

Organic components were removed from the cold trap with three washings of specially purified methylene chloride (*4*). Vapor-phase chromatography of the crude mixture was accomplished on an Aerograph model 661 gas chromatograph equipped with a flame ionization detector and a stainless steel column (5 feet by ⅛ inch) packed with 5 percent SE 30 on Chromosorb W. This procedure revealed several fast-moving components established as quinones, and a slow-moving component assumed to be the compound with a molecular weight of 210 found by Englehardt (*2*).

Infrared spectroscopy of the crude mixture showed absorptions at 5.85 μm (carbonyl), 6.0 μm (benzoquinone carbonyl), 7.1 μm (benzoquinone), and 11.0 μm (terminal double bond). Proton magnetic resonance spectrums indicated a long-chain hydrocarbon (multiplet, $\delta = 0.9$ to 1.6 ppm), vinyl group ($\delta = 4.8$ and 5.0 ppm), methylbenzoquinone (singlet, $\delta = 1.5$ ppm; singlet, $\delta = 7.1$ ppm), and ethylbenzoquinone (triplet, $\delta = 1.1$ ppm, $J = 7$ hz; quartet, $\delta = 2.5$ ppm, $J = 7$ hz; multiplet, $\delta = 6.9$ ppm), and a methyl ketone (singlet, $\delta = 2.0$ ppm). The solution was rinsed from the PMR tube with CCl_4 and stripped of solvent; the infrared spectrum was taken again in CCl_4. This time no carbonyl absorption at 5.8 μm appeared in the infrared spectrum. The methyl ketone peak at 2.0 ppm was also missing from the second PMR analysis. All other peaks remained.

Further purification of the crude mixture from 10,000 *Tribolium* was carried out on a column (0.5 by 15 cm) packed with freshly prepared 80- to 200-mesh Alumina II with specially purified pentane (*4*) at 4°C. Separation was achieved by elution with 500 ml each of pentane, a mixture of anhydrous ether and pentane (1 : 9), and anhydrous ether. Removal of the solvent gave a colorless oil in the pentane fraction and yellow crystals in the two fractions containing ether. Vapor-phase chromatography of the purified oil with a mixture of known terminally unsaturated straight-chain olefins (C_9, C_{11}, C_{13}, C_{15}, C_{17}) (*5*) showed a single symmetrical peak at $C_{15}H_{30}$. Proton magnetic resonance data showed signals at chemical shifts of 0.9, 1.2 to 2.0, 4.8, and 5.0 ppm for both unknown and $C_{15}H_{30}$, and the infrared spectrums of the two were superimposable. Mass spectrums of the unknown and 1-pentadecene were identical (*6*). On this basis we conclude that the unknown oil of *T. confusum* is 1-pentadecene ($C_{15}H_{30}$), and not $C_{14}H_{26}O$ (*2*).

The presence and amounts of methyl- and ethylbenzoquinones were confirmed to be the same as those reported (*2*) by PMR and infrared spectroscopy and vapor-phase chromatography of collected material and freshly sublimed known quinones.

We propose that the ketonic peak found by Englehardt was an artifact found in laboratory air. Acetone used in rinsing glassware was evaporating in our laboratory while the collection apparatus was operating. Blank samples exhibited the same PMR absorptions for methyl ketone as samples collected from the beetles.

Quinones are present in the defensive secretions of tenebrionid beetles and many other insect species (*7*). Relatively long-chain alkanes (C_9, C_{11}, C_{13}) (*8*) and terminally unsaturated alkenes (C_9, C_{11}, C_{13}) (*9*) have also been found, but this is the first report of 1-pentadecene. The function of the olefins is thought to be similar to that proposed for octanoic acid in the whipscorpion *Mastigoproctus giganteus* (*10*), that is, that of a spreading agent for the quinones, which aids their absorption by, and effect on, various enemies. Although *Tribolium* faces few competitors other than fungi in its present grain habitat, 1-pentadecene is proposed as having had this surfactant function in the past history of *Tribolium*. Quinones are considered to have a present-day function of inhibiting fungal growth (*2*).

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Tay-Sachs Disease: Prenatal Diagnosis

Abstract. *Fifteen pregnant women with a 25 percent risk of delivering a child with Tay-Sachs disease were monitored by amniocentesis and hexosaminidase A assays of amniotic fluid, uncultured amniotic cells, and cultured amniotic cells. Tay-Sachs disease was diagnosed prenatally in six fetuses; the diagnosis was confirmed in one child after birth and in five fetuses after therapeutic abortion. Prenatal diagnosis indicated the absence of Tay-Sachs disease in nine other fetuses; this diagnosis was confirmed postnatally in six, three are still in utero.*

Tay-Sachs disease is a fatal cerebral degenerative disorder transmitted in an autosomal recessive manner involving the accumulation of a specific lipid, ganglioside GM_2. The primary enzymic defect appears to be the absence of the enzyme β-D-*N*-acetylhexosaminidase A (*1*) which apparently participates in cleaving the terminal *N*-acetylgalactosamine residue from the stored ganglioside (*2*). Its absence leads to massive cerebral ganglioside storage, profound mental and motor deterioration, and death by age 2 to 4 years.

Hexosaminidase A is absent in all tissues studied from patients with Tay-Sachs disease (*1*). A partial reduction of the enzyme occurs in blood serum of heterozygous carriers (*3*). The enzymic defect persists in cultured skin fibroblasts after many cellular generations (*1*, *4*). The enzyme is present in normal amniotic cells (*1*), an indication that the prenatal diagnosis of Tay-Sachs disease is possible by hexosaminidase assay. Indeed, Schneck *et al.* (*5*) diagnosed Tay-Sachs disease prenatally in one fetus at 20 weeks of pregnancy, and found deficient hexosaminidase A in amniotic fluid and uncultured amniotic cells; they terminated the pregnancy, and confirmed the diagnosis by analysis of fetal tissues.

In order to be useful, prenatal diagnosis must be free of error. We now present data on 15 women who had previously delivered one or more af-

Table 1. Clinical data in high-risk pregnancies.

Case	Location	Amniocentesis* (weeks)	Prenatal fetal diagnosis	Outcome of pregnancy
1	California	18	Not Tay-Sachs	Clinically normal girl at 12 months of life; intermediate reduction of serum hexosaminidase A at 9 months.
2	New York	18	Probably heterozygous	Normal boy at 7 months; intermediate reduction of serum hexosaminidase A at 7 weeks.
3	Israel	25	Normal homozygote	Normal girl at 9 months; good activity for hexosaminidase A in serum at birth.
4	Israel	28	Normal homozygote	Normal boy at 11 months; good activity for hexosaminidase A in leukocytes at 3 months, normal activity of hexosaminidase A in cultured skin fibroblasts.
5	South Africa	28	Tay-Sachs disease	Affected girl with signs and symptoms of Tay-Sachs disease, bilateral cherry red spots at 9 months, and nearly absent serum hexosaminidase A.
6	California	18	Normal homozygote	Normal girl at 9 months; good activity for hexosaminidase A in serum at birth.
7	California	17	Normal homozygote	Good activity of hexosaminidase A in serum at birth.
8	California	16, 17	Tay-Sachs disease	Fetus aborted by saline infusion at 18 weeks; absent hexosaminidase A in organs, serum, urine, and cultured skin fibroblasts; 49-fold increase in cerebral ganglioside GM_2, neuronal lipidosis, and cytoplasmic membranous bodies in neurons.
9	New York	16	Normal homozygote	Due March 1971.
10	Colorado	17 19	Tay-Sachs disease	Fetus aborted by saline infusion at 20 weeks; neuronal lipidosis; cytoplasmic membranous bodies in neurons, 35-fold increase in cerebral ganglioside GM_2, and absent hexosaminidase A in skin.
11	New York	17	Normal homozygote	Due March 1971.
12	New York	18	Tay-Sachs disease	Fetus aborted by saline infusion at 20 weeks; neuronal lipidosis; 15-fold increase in cerebral ganglioside GM_2, absent hexosaminidase A in visceral organs.
13	New York	18	Normal homozygote	Due April 1971.
14	Australia	17	Tay-Sachs disease	Fetus aborted by saline infusion at 20 weeks; neuronal lipidosis, 16-fold increase in cerebral ganglioside GM_2, absent hexosaminidase A in visceral organs.
15	Baltimore	17	Tay-Sachs disease	Fetus aborted by saline infusion at 21 weeks; neuronal lipidosis, 22-fold increase in cerebral ganglioside GM_2, absent hexosaminidase A in visceral organs.

* Dates given are weeks after first day of last menstrual period.