though their quantitative recovery was not assured in these measurements.

LITERATURE CITED

Allison, L. E.; Bollen, W. B.; Moodie, C. D. In "Methods of Soil Analysis"; Black, C. A., Ed.; American Society of Agronomists, Inc.: Madison, WI, 1965; Chapter 89, p 1346.
Bartha, R. J. Agric. Food Chem. 1971, 19, 385.
Bartha, R. ASM News 1981, 46, 356.
Bartha, R.; Pramer, D. Adv. Appl. Microbiol. 1970, 13, 317.
Bleidner, W. E.; Baker, H. M.; Levitsky, M.; Lowen, W. K. J. Agric. Food Chem. 1954, 2, 476.
Bollag, J.-M.; Blattmann, P.; Laanio, T. J. Agric. Food Chem. 1978, 26, 1302.
Chisaka, H.; Kearney, P. C. J. Agric. Food Chem. 1970, 18, 854.
Geissbühler, H. In "Degradation of Herbicides"; Kearney, P. C.; Kaufman, D. D., Eds.; Marcel Dekker: New York, 1969; pp 79–111.
Geissbühler, H.; Kossmann, K.; Baunok, I.; Boyd, V. F. J. Agric. Food Chem. 1971, 19, 365.
Herrett, R. A. In "Degradation of Herbicides"; Kearney, P. C.; Kaufman, D. D., Eds.; Marcel Dekker, New York, 1969; pp 113–145.
Hill, G. D.; McGahen, J. W.; Baker, H. M.; Finnerty, D. W.; Bingeman, C. W. Agron. J. 1955, 47, 93.
Hsu, T.-S.; Bartha, R. Soil Sci. 1974, 116, 444.
Hsu, T.-S.; Bartha, R. J. Agric. Food Chem. 1976, 24, 121.
Hsu, T.-S.; Bartha, R. Appl. Environ. Microbiol. 1979, 37, 36.
Kirkland, J. J. Anal. Chem. 1962, 34, 428.
Marinucci, A. M.; Bartha, R. Appl. Environ. Microbiol. 1979, 38, 1020.
Parris, G. E. Environ. Sci. Technol. 1980, 14, 1105.
Still, C. C.; Hsu, T.-S.; Bartha, R. Bull. Environ. Contam. Toxicol. 1980, 24, 550.
Still, G. G.; Mansager, E. R. Weed Res. 1969, 9, 218.
Webley, D. J.; McKone, C. E. Misc. Rep. Trop. Pestic. Res. Inst. 1964, No. 441.
Worobey, B. L.; Webster, G. R. B. J. Agric. Food Chem. 1982a, 30, 161.
Worobey, B. L.; Webster, G. R. B. J. Agric. Food Chem. 1982b, 30, 164.

Formation of Two Thermal Degradation Products of β-Carotene

Philip N. Onyewu, Henryk Daun,* and Chi-Tang Ho

β-Carotene was heated at 210 °C for 4 h, and the thermal degradation products were separated by liquid column and thin-layer chromatography. Two compounds were tentatively identified as 3,7,10-trimethyl-1-1,12-bis(2,6,6-trimethylcyclohex-1-enyl)dodeca-1,3,5,7,9,11-hexaene and 3,6-dimethyl-1,8-bis(2,6,6-trimethylcyclohex-1-enyl)octa-1,3,5,7-tetraene. A mechanism for their formation is proposed.

Several studies have reported the formation of volatile compounds, mainly toluene, xylene, ionene, and 2,6-dimethylnaphthalene, as thermal degradation products of carotene. The summary of these studies is shown in Table I. The mechanism for the formation of toluene, xylene, and dimethylcyclodecapentaene from β-carotene has been proposed by Edmunds and Johnstone (1965) and advanced by Schwieter et al. (1969). It is said to involve the formation of a four-membered ring intermediate. This mechanism explains the formation or expulsion of toluene, xylene, and dimethylcyclodecapentaene from β-carotene but fails to show what is the remaining part of the carotene molecule after the expulsion.

Most of the work done on thermal degradation of β-carotene (though not in food) has emphasized almost exclusively the volatile degradation products. Only very few works were done on the nonvolatiles. Rost (1976) reported the isolation of polycyclic aromatic hydrocarbons upon heat treatment of crude edible oils at both the neutralizing temperature of 260 °C and bleaching plus deodorizing temperature at 270 °C.

At very high temperatures (400 and 700 °C), small amounts of polycyclic aromatic hydrocarbons (PAH) are formed as pyrolysis products of β-carotene (Halaby and Fagerson, 1971). Ouyang et al. (1980) studied the nonvolatile compounds generated under conditions simulating palm oil deodorization at 210 °C. Conventional deodorization is carried out at 360–425 °F (182–218 °C) (Schwitzer, 1959; Swern, 1964). Apocarotene and apocarotenals were reported as being formed by Ouyang and co-workers (see Table I).

In light of the above-mentioned studies, it becomes important to investigate the nonpolar nonvolatile thermal degradation products (TDP) of β-carotene. The use of these deodorization conditions has both theoretical value and practical value. The fact is that in most developed countries (especially Europe and the United States) in order for edible oils to be acceptable to consumers, the oils must be refined and deodorized. Moreover, refined oils undergo further heat treatment during cooking and frying. In the course of thermal treatments, carotenoids are degraded and chemicals such as PAH could be formed. The nonpolar PAH, if formed, would be in the nonpolar nonvolatile fraction of the TDP of β-carotene. The previously developed model system (Onyewu et al., 1981) was used to study the formation of the nonvolatile thermal degradation products of β-carotene.

EXPERIMENTAL SECTION

Previously developed model system by Onyewu et al. (1981) was employed. Two grams of β-carotene (lot 181029,

Department of Food Science, Cook College, New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick, New Jersey 08903.

1148 *J. Agric. Food Chem.*, Vol. 30, No. 6, 1982

Onyewu, Daun, and Ho

Table I

thermal degradation conditions	compounds identified	reference
β-carotene and other carotenoids were heated	*m*-xylene, toluene, 2,6-dimethylnaphthalene	Kuhn and Winterstein (1932, 1933a,b)
a mixture of α- and β-carotene was heated at 260 °C for 24 h	2,6-dimethylnaphthalene	Jones and Sharpe (1948)
a 1% solution of β-carotene in benzene was heated at 188 °C for 72 h	toluene, *m*-xylene, 2,6-dimethylnaphthalene	Day and Erdman (1963)
a dispersed sample of β-carotene in water was heated in a sealed glass ampule at 188 °C for 72 h	toluene, *m*-xylene, dimethylnaphthalene	Mulik and Erdman (1963)
melted β-carotene was heated under vacuum at 240 °C for a prolonged period of time	toluene, *m*-xylene, *p*-xylene, 2,6-dimethylnaphthalene, ionene	Mader (1964)
β-carotene was heated under vacuum at 300 °C for 2 h	toluene, *m*-xylene, *p*-xylene, 2,6-dimethylnaphthalene, ionene	Edmunds and Johnstone (1965)
pyrolytic degradation of lycopene and β-carotene, heated to 250 °C for 10 min under CO₂	toluene, xylene, dimethylnaphthalene	Schwieter et al. (1969)
β-carotene in water was heated at 100 °C for 30 min	α-ionone, β-ionone	LaRoe and Shipley (1970)
β-carotene in benzene was heated at 188 °C for 72 h in the presence of air	α-ionone, β-ionone, toluene, *m*-xylene, dimethylnaphthalene	LaRoe and Shipley (1970)
β-carotene was pyrolyzed at 400 and 700 °C under nitrogen	polynuclear aromatic hydrocarbons	Halaby and Fagerson (1971)
β-carotene was heated at 190 and 220 °C for 10 min in the presence of nitrogen or air	toluene, *m*-xylene, ionene, 2,6-dimethylnaphthalene, β-cyclocitral, β-ionone, dihydroactinid iolide, 5,6-epoxy-β-ionone	Schreir et al. (1979)
thermal degradation of β-carotene during simulated palm oil deodorization	13-apo-β-carotenone, 15-apo-β-carotenal, 14′-apo-β-carotenal	Ouyang et al. (1980)
heated β-carotene under vacuum at temperatures from 200 to 350 °C for 24 h as a geothermal reaction model of carotenoids	toluene, xylene, ionene, 2,6-dimethylnaphthalene compounds with molecular weights of 138, 240, 346, and 444	Ishiwatari (1980)

obtained from Hoffmann-La Roche, Nutley, NJ) was placed in a pressure bottle (ACE 10-4722-01) containing 12 mL of glycerol. Then the bottle was flushed with nitrogen, closed with a glass stopper held tightly by a heavy spring wire clamp, and heated at 210 °C for 4 h. The control (glycerol only) was heated in the same manner.

After the sample was heated, the thermal degradation products (TDP) of β-carotene were isolated by extracting 3 times with a mixture of 20 mL of benzene and 20 mL of distilled water. The benzene solution of the TDP was dried with 2 g of anhydrous sodium sulfate (Na₂SO₄). The benzene that contained the TDP of β-carotene was completely evaporated with a rotary flash evaporator (Buchler Instruments, Fort Lee, NJ). Hexane (5 mL) was added to the residue, and the hexane-soluble products were extracted. TDP in hexane were separated and purified with column chromatography. The column bed was prepared by making a slurry of 30 g of partially deactivated alumina (neutral grade I, 80–200 mesh) and using hexane as the eluting solvent. The bed height was 17.5 cm in a glass column of 1.5-cm inside diameter. Partial deactivation was achieved by placing for 2 h a tray with a 1-cm layer of alumina close to a container with boiling water. The alumina was mixed at intervals of 15 min to assure uniform moisture absorption.

Further separation and purification were achieved by thin-layer chromatography (TLC). The developing solvent system for the TLC was hexane–benzene (99.5:0.5). Twelve unknows were obtained from the hexane-soluble portion of the TDP by preparative TLC on silica gel plates. Only two of these unknowns (occurring in the largest amount among the nonpolar fraction) were selected for characterization using ultraviolet, infrared, and mass spectrometry.

In the case of ultraviolet spectrophotometry, the samples were redissolved in petroleum ether and transferred to a

UV quartz cuvette in a Varian Techtron, Model 635 spectrophotometer. For the IR, the petroleum ether from the two selected samples was removed with nitrogen, and about 2 μL of carbon tetrachloride was added. Each sample in carbon tetrachloride was transferred with a 10-μL syringe to an ultramicrocavity cell with a 0.1-mm light path in a Beckman Acculab 4 IR spectrophotometer. A direct probe was employed for mass spectrometric analysis. A Hewlett-Packard 5985 mass spectrometer equipped with a 21 mx E series computer was used. The samples were redissolved in 5–8 μL of anhydrous diethyl ether. Each sample was transferred with a syringe to a capillary tube of about 0.5 in. in length. The solvent was evaporated at room temperature. The sample was placed in the outer chamber of the mass spectrometer of 0.05 torr and held for about 3 min to assure complete evaporation of the solvent. The sample was passed through a series of seals into the main chamber of 0.000001 torr. The chamber temperature was gradually increased to 175–200 °C. Samples were held for 10 min, to generate fragmentation ion spectra.

RESULTS AND DISCUSSION

Characterization of Sample 1. The ultraviolet absorption range of this compound falls between 310 and 240 nm, and thus indicating the presence of a conjugated ene system (Silverstein et al., 1974; Lambert et al., 1976). Figure 1 shows the infrared spectrum of sample 1. The aromatic C–H stretching is shown by the band in the range 3100–2800 cm⁻¹. The absorption band in the 1650–1500-cm⁻¹ range is characteristic of conjugated olefinic double bond stretching. The mass spectrum of the compound is shown in Figure 2. The peak at *m/e* 378 is the molecular ion peak. The base peak is at *m/e* 69, and it is characteristic of methyl-substituted olefins according to McLafferty (1973). The *m/e* 69 peak corresponds to the
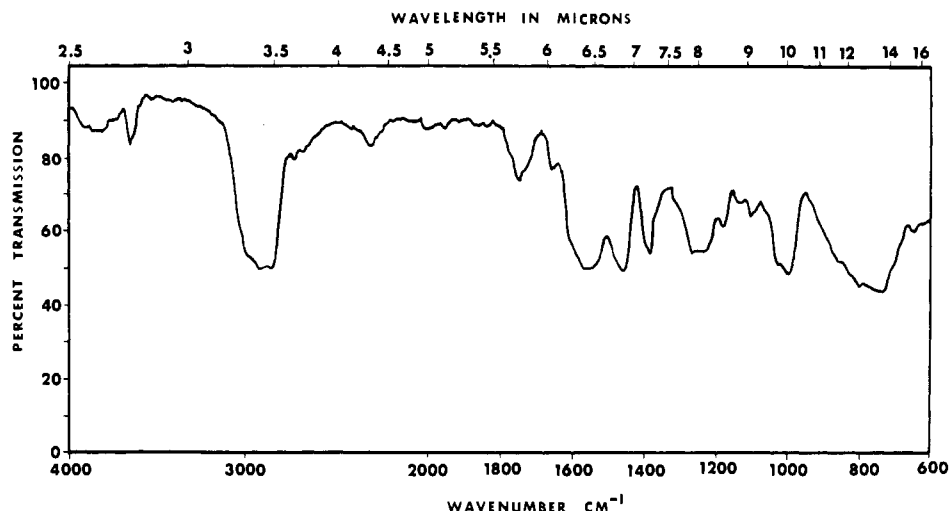
β-Carotene Degradation Product Formation

J. Agric. Food Chem., Vol. 30, No. 6, 1982   **1149**
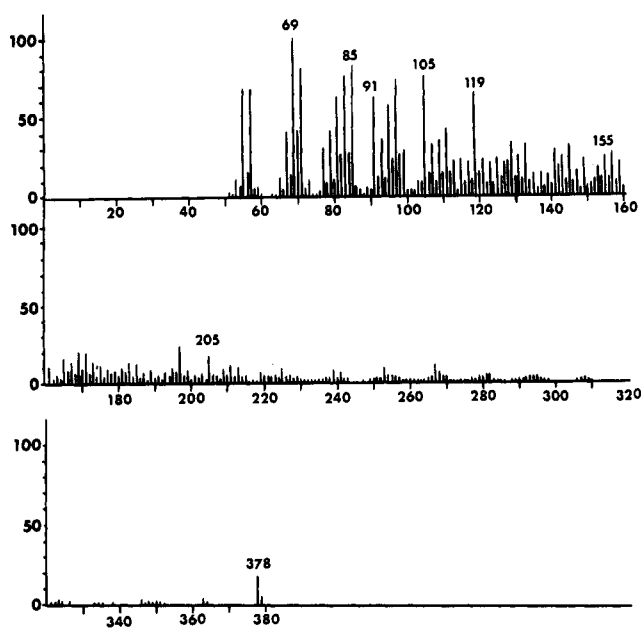

**Figure 1.** IR spectrum of sample 1.

Figure 2. Mass spectrum of sample 1.

loss of an isopentenyl radical from the molecule. The m/e 91 and 105 correspond to the expulsion of toluene and xylene fragment ions, respectively. The compound in question was tentatively identified as 3,6-dimethyl-1,8-bis(2,6,6-trimethylcyclohex-1-enyl)octa-1,3,5,7-tetraene. The structural formula of the compound is

$$C_{28}H_{42}; M_r\ 378$$
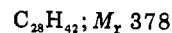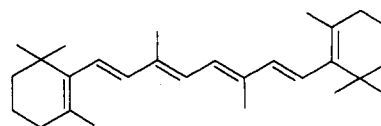
Characterization of Sample 2. The ultraviolet absorption of this sample is appreciable in the range 270–290 nm. This range is within those of the conjugated ene system as was indicated for sample 1. While the infrared spectrum of this compound (Figure 3) is very similar to that of sample 1, there is no indication of a functional group difference between the two samples other than chain length and the number of methyl group substituents. The mass spectrum is shown in Figure 4. The molecular ion peak is at m/e 444. The major fragment ion peaks show at m/e 91, 105, 155, 169, 205, 240, and 346. This sample's fragmentation pattern is very similar to the published spectrum of β-carotene (Schwieter et al., 1969). The m/e 205 peak, the base peak, and the m/e 240 peak result from cleavage of the double bonds in the polyene chain. The peaks at m/e 155, 158, and 169 indicate that fragments similar to 2,6-dimethylnaphthalene were generated. The m/e 158 peak corresponds to the loss of a dimethylcyclodecapentaene from the polyene chain of the molecule. The
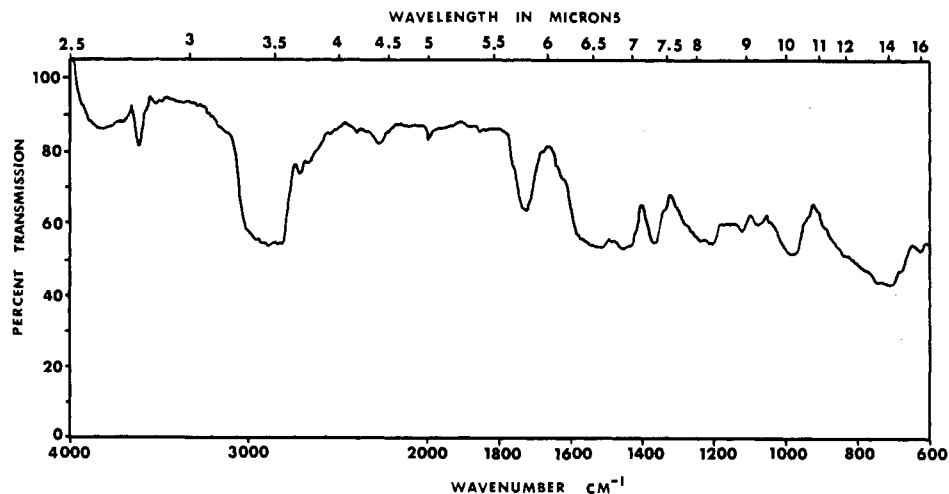

Figure 3. IR spectrum of sample 2.

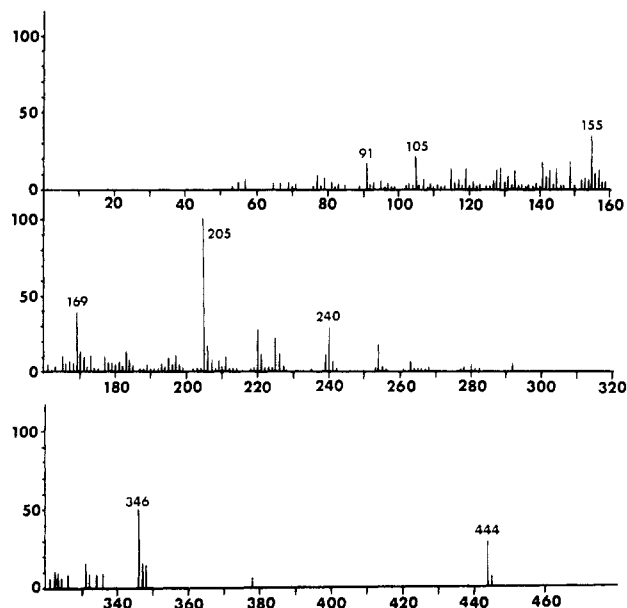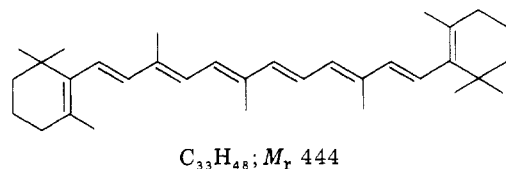1150   *J. Agric. Food Chem.*, Vol. 30, No. 6, 1982

Onyewu, Daun, and Ho

Figure 4. Mass spectrum of sample 2.

compound in question was hence tentatively identified as 3,6,10-trimethyl-1,12-bis(2,6,6-trimethylcyclohex-1-enyl)-dodeca-1,3,5,7,9,11-hexaene, a β-carotene-type compound with shortened polyene chain. The structural formula is

$$C_{33}H_{48}; M_r 444$$

The formation mechanism for these two compounds is proposed to follow the Edmunds and Johnstone (1965) scheme, modified by Schwieter et al. (1969) for the thermal loss of toluene from the carotene molecule. In this case toluene loss results in the formation of a $C_{33}H_{48}$ compound with a molecular weight of 444 as shown in Figure 5. Cyclization is started with an eight-electron system; rearrangement follows and then leads to the formation of a four-ring intermediate. The four-ring system then loses toluene to form a trimethylbis(2,6,6-trimethylcyclohex-1-enyl)dodecahexaene compound. Similarly, the $C_{28}H_{42}$ (M_r 378) compound is formed upon loss of a dimethylcyclodecapentaene. The cyclization here starts with twelve electrons instead of eight as in the formation of mass unit 444.

In this study, given the conditions of thermal treatment of β-carotene (210 °C, 4 h), it is evident that duo thermal competitive reactions were occurring during the heating. In one case a reaction involving the loss of toluene (mass unit 92) and the dimethylcyclodecapentaene (mass unit 158) in the polyene chain took place. In the other reaction, the parts of the degrading carotene isomerized and polymerized into more stable forms. This is confirmed in the resulting compounds with molecular weights 444 and 378 formed upon expulsion of mass units 92 and 158.

The formation of these two tentatively identified compounds is consistent with the Edmunds and Johnstone (1965) mechanism. Support for this hypothesis comes from the results of Schwieter et al. (1969) and Kjøsen et al. (1971), which show that in deuterated carotenes, toluene loss originated from the central part of the polyene chain. Only the 20- or 20'-methyl substituents are present in the expelled toluene, while the expelled dimethylcyclodecapentane contained not only the deuterium atoms (15,
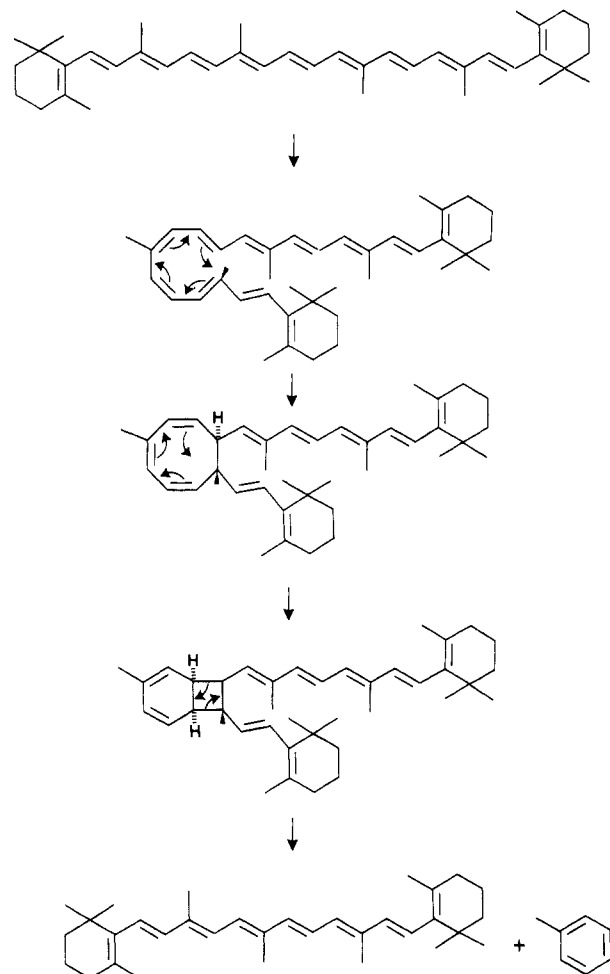

Figure 5. Proposed mechanism for formation of mass unit 444: 3,6,10-trimethyl-1,12-bis(2,6,6-trimethylcyclohex-1-enyl)dodeca-1,3,5,7,9,11-hexaene.

15') but also the 20- and 20'-methyl substituents. Our results are consistent with these findings. It is interesting to note that not only were these two compounds tentatively identified in this study formed in largest amounts (in the nonpolar nonvolatile fraction) but also there is no evidence for chemical involvement of glycerol in their formation.

CONCLUSION

(1) Two β-carotene-type compounds were tentatively identified as 3,7,10-trimethyl-1,12-bis(2,6,6-trimethyl-cyclohex-1-enyl)dodeca-1,3,5,7,9,11-hexaene and 3,6-dimethyl-1,8-bis(2,6,6-trimethylcyclohex-1-enyl)octa-1,3,5,7-tetraene, the thermal degradation products of β-carotene in a model system. These compounds were colorless. (2) The formation of these two compounds during processing of foods has not been previously reported in the literature. Ishiwatari (1980) reported a compound with mass unit 444 from vacuum pyrolysis of β-carotene. However, the compound with mass unit 378 has not been found in the available literature. (3) A hypothesis is proposed that these compounds are formed based on the scheme by Edmunds and Johnstone (1965) of the thermal loss of toluene and dimethycyclodecapentaene from the polyene chain of carotenes.

LITERATURE CITED

Day, W. C.; Erdman, J. G. *Science (Washington, D.C.)* 1963, *141*, 808.
Edmunds, F. S.; Johnstone, R. A. W. *J. Chem. Soc.* 1965, 2892.
Halaby, G. A.; Fagerson, I. S. *Proc., SOS/70, Int. Congr. Food Sci. Technol., 3rd, 1970* 1971, 820.
Ishiwatari, M. *J. Anal. Appl. Pyrolysis* 1980, *2*, 153.

Jones, R. N.; Sharpe, R. W. *Can. J. Res.* **1948**, *26*, 728.

Kjøsen, H.; Liaaen-Jensen, S.; Enzell, C. R. *Acta. Chem. Scand.* **1971**, *25*, 1, 85.

Kuhn, R.; Winterstein, A. *Ber. Dtsch. Chem. Ges.* **1932**, *65*, 1873.

Kuhn, R.; Winterstein, A. *Ber. Dtsch. Chem. Ges.* **1933a**, *66*, 429.

Kuhn, R.; Winterstein, A. *Ber. Dtsch. Chem. Ges.* **1933b**, *66*, 1733.

Lambert, J. B.; Shurvell, H. F.; Verbit, L.; Cooks, R. G.; Stout, G. H. "Structural Analysis"; Macmillan: New York, 1976; pp 151–468.

LaRoe, E. G.; Shipley, P. A. *J. Agric. Food Chem.* **1970**, *18*, 174.

Mader, I. *Science (Washington, D.C.)* **1964**, *144*, 533.

McLafferty, F. W. "Interpretation of Mass Spectra", 2nd ed.; W. A. Benjamin: New York, 1973.

Mulik, J. D.; Erdman, J. G. *Science (Washington, D.C.)* **1963**, *141*, 806.

Onyewu, P. N.; Daun, H.; Ho, C. T., presented at the 41st Annual IFT Meeting, Atlanta, GA, 1981.

Ouyang, J. M.; Daun, H.; Chang, S. S.; Ho, C. T. *J. Food Sci.* **1980**, *45*, 1214.

Rost, H. E. *Nippon Kagaku Kaishi* **1976**, 162.

Schreir, P.; Drawert, F.; Bhiwaparkar, S. *Chem. Mikrobiol., Technol. Lebensm.* **1979**, *6*, 90.

Schwieter, U.; Englert, G.; Rigassi, N.; Vetter, W. *Pure Appl. Chem.* **1969**, *20*, 365.

Schwitzer, M. K. "Continuous Processing of Fats"; Chemical Publishing Co., Inc.: New York, 1959; pp 186–262.

Silverstein, R. M.; Bassler, G. C.; Morril, T. C. "Spectrometric Identification of Organic Compounds", 3rd ed.; Wiley: New York, 1974; Chapters 1–5.

Swern, D., Ed. "Bailey's Industrial Oil and Fat Products", 3rd ed.; Interscience: New York, 1964; p 901.

Preparation and Biological Activity of Potential Inhibitors of Insect Juvenile Hormone Biosynthesis

Gary B. Quistad,* Luana E. Staiger, and David C. Cerf

Fluoromevalonolactone (tetrahydro-4-(fluoromethyl)-4-hydroxy-2*H*-pyran-2-one, FMev) is an inhibitor of juvenile hormone production in lepidopterous insect species. Numerous structural analogues of FMev were synthesized and bioassayed on the tobacco hornworm (*Manduca sexta*) in order to assess anti-juvenile hormone activity. Such activity was greatest with FMev while the difluoro analogue (tetrahydro-4-(difluoromethyl)-4-hydroxy-2*H*-pyran-2-one) was about 9-fold less active. Three other analogues show reduced anti-juvenile hormone activity, which may result from metabolic conversion to FMev. A number of structurally unrelated hypocholesterolemic agents were also assayed as potential inhibitors of juvenile hormone biosynthesis, but only negative results were obtained.

Although the theoretical basis for insect control by anti-juvenile hormones (AJH agents) has been widely discussed [e.g., Slama (1978)], relatively few compounds with AJH activity have been reported. Several derivatives of abietic acid and hydrofluorene are alleged by Murakoshi et al. (1975, 1977) to cause precocious metamorphosis (an AJH effect) in *Bombyx mori*, but this activity was not always definitive since treatment of some larval stages resulted in prolonged larval development (an agonistic or juvenile hormone effect). This property of antagonistic and agonistic effects from the same antihormone compound is also evidenced when larval *Manduca sexta* are treated with ETB [ethyl 4-[2-[(*tert*-butylcarbonyl)oxy]]-butoxybenzoate (Staal, 1977)] where the elicited morphological response is dose dependent, a fact that suggests severe limitations for usage of such compounds for practical insect control. The most unequivocal AJH effects have been described for the precocenes (Bowers et al., 1976; Slama, 1978), which are clearly AJH agents for certain Hemiptera and Orthoptera. The biological effects of prococenes are attributed to selective destruction of the source of biosynthesis of juvenile hormones, the corpora allata (Schooneveld, 1979), by formation of a highly reactive epoxide within the gland (Soderlund et al., 1980;

Brooks et al., 1979). Matolcsy et al. (1980) have also reported AJH activity against the cotton bug, *Dysdercus*, for a precocene analogue that lacks the chromene ring.

The similarities between the biosynthesis of juvenile hormones in insects (Schooley et al., 1973) and cholesterol in mammals suggest the possibility of finding mutual inhibitors of several enzymatic steps. Indeed, this hypothesis was tested previously by Matolcsy et al. (1974, 1975), who unsuccessfully assayed analogues of mevalonolactone and 3-hydroxy-3-methylglutaric acid (HMG) as insect anti-juvenile hormones. Our hope when we initiated this work was that prudent selection of known hypocholesterolemic agents for insect bioassay might reveal compounds with AJH activity. As we have reported previously (Quistad et al., 1981), tetrahydro-4-(fluoromethyl)-4-hydroxy-2*H*-pyran-2-one (fluoromevalonolactone, FMev) is an AJH in Lepidoptera, in addition to being a known hypocholesterolemic agent (Tschesche et al., 1963). FMev was synthesized first by Tschesche and Machleidt (1960), who shortly thereafter patented it (Tschesche et al., 1963) as useful for treating disorders caused by excessive cholesterol biosynthesis (e.g., atherosclerosis). FMev is known to inhibit the biosynthesis of cholesterol from both acetate and mevalonate in rat liver (Singer et al., 1959; Brauser and Hermann, 1965), and it is also alleged to block transformation of cystolic HMG–CoA to cholesterol in rat hepatocytes (Barth, 1978). In this paper we report the structure–activity relationships for a number of FMev analogues

---

Departments of Biochemistry and Insect Research, Zoecon Corporation, Palo Alto, California 94304.