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Photoinduced Additions of Pesticides to Biomolecules. 2. Model Reactions of DDT and Methoxychlor with Methyl Oleate[1]

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Upon sunlight irradiation, photoinduced additions of pesticides to biomolecules of plant cuticles have been presumed. In order to check this hypothesis, model UV irradiations of DDT (2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane) and methoxychlor (2,2-bis(4-methoxyphenyl)-1,1,1-trichloroethane) in the presence of methyl oleate, as an example of octadecenoic acids often occurring in plant cuticles, were performed. DDT and methoxychlor were extensively added to the C–C double bond of methyl oleate via radical mechanisms. Besides chlorinated stearic acids, several addition products were formed, offering new possibilities to produce "bound residues" in plants.

After spraying, pesticides first contact plant cuticle by absorption with subsequent distribution therein (especially lipophilic compounds) and can be directly affected by sunlight. Components of plant cuticles include alkanes, alkanols, fatty acids, triterpenes, and sterols (epicuticular wax) and a biopolymer of hydroxy fatty acids (cutin).

In my research, photoinduced reactions of pesticides in the presence of biomolecules of plant cuticles are of main interest with special attention to photoaddition reactions producing "bound residues" (Schwack, 1986, 1987). In order to establish the photochemical reactivities of DDT (2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane) and methoxychlor (2,2-bis(4-methoxyphenyl)-1,1,1-trichloroethane) in the presence of plant cuticle constituents, model photoreactions with cyclohexene have been undertaken, which afford addition products in high yields (Schwack, 1984). This paper presents the results of photoinduced addition of DDT and methoxychlor to methyl oleate as an example of octadecenoic acids often occurring in plant cuticles.

EXPERIMENTAL SECTION

Gas Chromatography. A Hewlett-Packard GC 5830A gas chromatograph with FID equipped with a fused silica SE 54 capillary column (25 m × 0.3 mm (i.d.), carrier gas nitrogen) in combination with a 188500A terminal was used. The temperature program was 100 °C, 1 min isothermal, and then 100–300 °C at 5 °C/min.

Mass Spectrometry. Electron impact mass spectra (MS) were recorded on a LKB 2091 mass spectrometer at 15 eV to avoid strong fragmentations. Spectra are reported as mass (^{35}Cl), chlorine isotopes pattern (Cl_n), and relative intensity (percent).

^{1}H NMR Spectroscopy. A Bruker WM 400 spectrometer was used. Signals are reported (δ) downfield from tetramethylsilane as internal standard (δ 0.00).

IR Spectroscopy. IR spectroscopy was carried out with a Beckman IR 4240 spectrometer using capillary films on NaCl plates.

DDT (Zeidler, 1874) and methoxychlor (Zepp et al., 1976) were synthesized following the literature cited and recrystallized four times from *n*-hexane.

Degradation rate analyses were carried out by HPLC as previously described (Schwack, 1984).

Photolyses. DDT (250 mg, 0.71 mmol) and methoxychlor (250 mg, 0.72 mmol) were dissolved in 5 g of methyl oleate (99%, Sigma Chemical Co.), and the resultant mixture was irradiated in a quartz tube for 5 h using a 150-W high-pressure mercury lamp (TQ 150, Hanau Quarzlampen GmbH) equipped with a quartz glass water-cooling jacket. The UV light was filtered by a glass filter WG 295 ($\lambda > 280$ nm, Schott) before reaching the samples. Under these conditions 18% photodegradation of DDT and 24% photodegradation of methoxychlor occurred.

Product Isolation. The reaction mixtures (in 0.5-g aliquots were chromatographed on Bio Beads S-X8 (Bio Rad Lab) with methylene chloride as eluant (glass column, 2 cm (i.d.) × 85 cm, flow rate 1.5 mL/min) to separate the higher molecular weight addition products (first eluted) from the starting materials, using an UV (254-nm) detector (gel permeation chromatography). The combined addition products were separated by preparative TLC (silica gel 60 F_{254}, Merck) with the solvent systems 95:5 *n*-hexane–diisopropyl ether for the DDT products and 96:4 *n*-hexane–ethanol for the methoxychlor products. After rechromatography under the same conditions the addition products were all obtained as colorless oils. Purity control analyses were performed by HPLC using a 5-μm SiO_2 (LiChrosorb (Merck) column (4.6 mm (i.d.) × 25 cm)

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eluted with *n*-hexane (DDT products) or 0.025% ethanol in *n*-hexane (methoxychlor products).

Methyl 9-[2,2-Bis(4-chlorophenyl)-1,1-dichloroethylidene]stearate (8) and Methyl 10-[2,2-Bis(4-chlorophenyl)-1,1-dichloroethylidene]stearate (7). MS (15 eV): *m/z* 614 (Cl_4, 1%, M^+), 578 (Cl_3, 14%, $M^+ - HCl$), 544 (Cl_2, 11%, $M^+ - Cl_2$), 542 (Cl_2, 6%, $M^+ - 2\,HCl$), 497 (Cl, 100%, $M^+ - Cl - CCl_2$), 419 (Cl_3, 3%), 373 (6%), 341 (4%), 295 (4%), 235 (Cl_2, 29%). IR (film): 2930, 2860, 1740 (C=O ester), 1590, 1490, 1470–1430, 1185, 1160, 1080, 1000, 750 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.07–7.58 (8 H, 2 AA'BB', aromatic protons, J = 8.5, 3, 2 Hz), 5.0 (1 H, s, $CCl_2CH(4\text{-ClPh})_2$), 3.65 (3 H, s, $COOCH_3$), 2.45 (1 H, m, $>CHCCl_2CH(4\text{-ClPh})_2$), 2.28 (2 H, t, CH_2COOR, J = 7.5 Hz), 1.15–1.35 (22H, m, $-(CH_2)-$), 0.88 (3 H, t, CH_2CH_3, J = 7 Hz).

Methyl 10-[2,2-Bis(4-chlorophenyl)-1,1-dichloroethylidene]-9-chlorostearate (9) and Methyl 9-[2,2-Bis(4-chlorophenyl)-1,1-dichloroethylidene]-10-chlorostearate (10). MS (15 eV): *m/z* 612 (Cl_4, 20% $M^+ - HCl$), 578 (Cl_3, 15%, $M^+ - Cl_2$), 576 (Cl_3, 5%, $M^+ - 2\,HCl$), 531 (Cl_2, 100%, $M^+ - Cl - CCl_2$), 496 (Cl, 18%), 495 (Cl, 36%), 487 (Cl_4, 2%), 471 (14%), 451 (Cl_3, 6%), 443 (Cl_4, 2%), 407 (Cl_3, 5%), 371 (15%), 241 (Cl_4, 14%), 235 (Cl_2, 19%). IR (film): 2930, 2860, 1740 (C=O ester), 1590, 1490, 1470–1430, 1185, 1160, 1080, 1000, 750 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.1–7.6 (8 H, 2 AA'BB', aromatic protons, J = 8.5, 3, 2 Hz), 5.01 (1 H, s, $CCl_2CH(4\text{-ClPh})_2$), 4.10 and 3.98 (0.5 H each, m, $>CHCl$), 3.65 (3 H, s, $COOCH_3$), 2.84 and 2.75 (0.5 H each, m, $>CHCCl_2CH(4\text{-ClPh})_2$), 2.28 (2 H, t, CH_2COOR, J = 7.5 Hz), 1.2–1.4 (20 H, m, $-(CH_2)-$), 0.86 (3 H, t, CH_2CH_3, J = 7 Hz).

Methyl 10-[2,2-Bis(4-methoxyphenyl)-1,1-dichloroethylidene]-9-chlorostearate (11) and Methyl 9-[2,2-Bis(4-methoxyphenyl)-1,1-dichloroethylidene]-10-chlorostearate (12). MS (15 eV): *m/z* 605 (Cl_2, 4%, $M^+ - Cl$), 604 (Cl_2, 1%, $M^+ - HCl$), 569 (Cl, 54%, $M^+ - Cl - HCl$), 535 (28%), 534 (30%), 533 (38%), 469 (Cl, 13%), 455 (Cl, 16%), 443 (Cl, 5%), 425 (Cl, 11%), 411 (Cl, 19%), 399 (Cl, 4%), 274 (Cl, 35%), 227 (100%). IR (film): 3000–3100, 2930, 2860, 1740 (C=O ester), 1605, 1585, 1505, 1470–1430, 1245, 1170, 1025 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.45 and 6.84 (4 H each, AA'BB', aromatic protons), 5.30 (1 H, s, $CCl_2CH(4\text{-MeOPh})_2$), 3.78 (6 H, s, $PhOCH_3$), 3.65 (3 H, s, $COOCH_3$), 3.7–3.9 (1 H, m, $>CHCl$, obscured by the methoxy signals), 2.58 and 2.44 (0.5 H each, m, $>CHCCl_2CH(4\text{-MeOPh})_2$), 2.30 (2 H, t, CH_2COOR, J = 7.5 Hz), 1.2–1.4 (20 H, m, $-(CH_2)-$), 0.87 (3 H, t, CH_2CH_3, J = 7 Hz).

1,1,4,4-Tetrakis(4-methoxyphenyl)-2,2,3,3-tetrachlorobutane (13). MS (15 eV): *m/z* 618 (Cl_4, 0.5%, M^+), 548 (Cl_2, 2%, $M^+ - Cl_2$), 513 (Cl, 1%, $M^+ - Cl_3$), 477 (4%), 370 (3%), 274 (2%), 227 (100%), 212 (4%), 184 (5%), 169 (5%), 141 (3%), 121 (4%).

Methyl 9-Chlorostearate (14a) and Methyl 10-Chlorostearate (14b). Relative retention time (GC, methyl oleate = 1.0): 1.32. MS (GC/MS, 70 eV): *m/z* 332 (Cl, 1%, M^+), 300 (Cl, 4%, $M^+ - CH_3OH$), 296 (9%, $M^+ - HCl$), 264 (54%, $M^+ - HCl - CH_3OH$), 222 (16%), 220 (16%), 180 (14%), 153 (18%), 110 (28%), 97 (47%), 87 (50%), 74 (100%), 69 (53%), 55 (56%).

Methyl 9,10-Dichlorostearate (15, Two Diastereomers). Relative retention times (GC, methyl oleate = 1.0): 1.53, 1.54.

Authentic methyl 9,10-dichlorostearate was prepared by dropwise addition of 0.5 g of sulfuryl chloride (SO_2Cl_2; 3.7 mmol) to 1.0 g of pure methyl oleate (3.37 mmol) with stirring at room temperature. (*Precautions* are to be made using a well-ventilated fume cupboard.) This results in a violet reaction. After 10 min of additional stirring the mixture was diluted with ether, washed extensively with water, and dried with sodium sulfate. Upon evaporation of the solvent the product was isolated by column chromatography (SiO_2, 95:5 *n*-hexane–ether) and used for GC standard and comparison of spectra. Both diastereomers gave identical mass spectra. MS (GC/MS, 70 eV): *m/z* 366 (Cl_2, 3%, M^+), 335 (Cl_2, 1%, $M^+ - OCH_3$), 334 (Cl_2, 1% $M^+ - CH_3OH$), 331 (Cl, 1.4%, $M^+ - Cl$), 330 (Cl, 1.5%, $M^+ - HCl$), 294 (100%, $M^+ - 2\,HCl$), 263 (36%), 262 (40%), 245 (25%), 235 (5%), 220 (9%), 164 (9%), 130 (9%), 87 (18%), 74 (58%), 55 (6%). ^1H NMR (60 MHz, CCl_4): δ 3.8–4.1 (2 H, m, $CHClCHCl$), 3.6 (3 H, s, $COOCH_3$), 2.25 (2 H, t, CH_2COOR, J = 7 Hz), 1.2–2.0 (26 H, m, $-(CH_2)-$), 0.9 (3 H, t, CH_2CH_3).

RESULTS AND DISCUSSION

The photolyses of DDT (1) and methoxychlor (2) proceed via the homolytic cleavage of a trichloromethyl C–Cl bond, forming radicals **1a** and **2a**, respectively, as well as free chlorine atoms (Figure 1). For these radicals two reaction pathways are known from the literature: hydrogen transfer, affording DDD (3) or DMDD (4), and hydrogen elimination, giving DDE (5) and DMDE (6) (Mosier et al., 1969; MacNeil et al., 1972; Zepp et al., 1976, 1977). On plant surfaces a further possibility consists of radical additions to unsaturated biomolecules that are widely present in plant cuticle (Hull, 1974; Kollatukudy, 1976).

DDT Products. On irradiation of DDT in the presence of methyl oleate ($\lambda > 280$ nm), 80% of the photodegraded DDT was bound to the fatty acid and 20% was found as DDD (3).

Photoinduced addition of DDT to methyl oleate is possible by radical attack of **1a** at the 9- or 10-position of oleic acid without any selectivity, affording the new radicals **1b** and **1c** (Figure 1). Following the rules of organic radical chemistry, hydrogen transfer, hydrogen abstraction, or radical chain reaction (chlorine transfer) may occur. By gel permeation chromatography and TLC, only two addition products could be isolated in the ratio of nearly 1:1. In accordance with previous results using cyclohexene (Schwack, 1984), the hydrogen transfer to the intermediate radicals **1b**/**1c** occurred in high percentages, yielding two positional isomers of "DDD-substituted" stearic acids **7** and **8** (Figure 1). Attempts to separate the isolated product into the two isomers by HPLC were unsuccessful with the columns and solvents used. From the point of view of the NMR and mass spectra it was also impossible to differentiate between **7** and **8**, both of which are undoubtedly expected.

The second, more polar, addition product of DDT and methyl oleate was formed by chlorine transfer to **1b**/**1c** (Figure 1), which may be interpreted as a radical chain reaction (with intact DDT molecules) or as a rate-determining step by free chlorine atoms. (Quantum yields have not been determined during these investigations.) Starting from the two radicals **1b** and **1c**, again, two positional isomers, **9** and **10**, are expected as diastereomers (threo, erythro). However, during HPLC (normal phase) the isolated product was eluted as a sharp and absolutely unresolved peak. The NMR spectrum showed doubled signals for the fatty acids methine protons 9-H and 10-H (intensity 1:1, slightly shielded), supporting the presence of diastereomers but not of positional isomers. Only mass spectrometry proved the presence of **9** *and* **10** addition products. Cleavage between C-9 and C-10 results in fragments at *m/z* 443 (Cl_4 pattern) for **9** and at *m/z* 487 (Cl_4 pattern) for **10** (Figure 2).

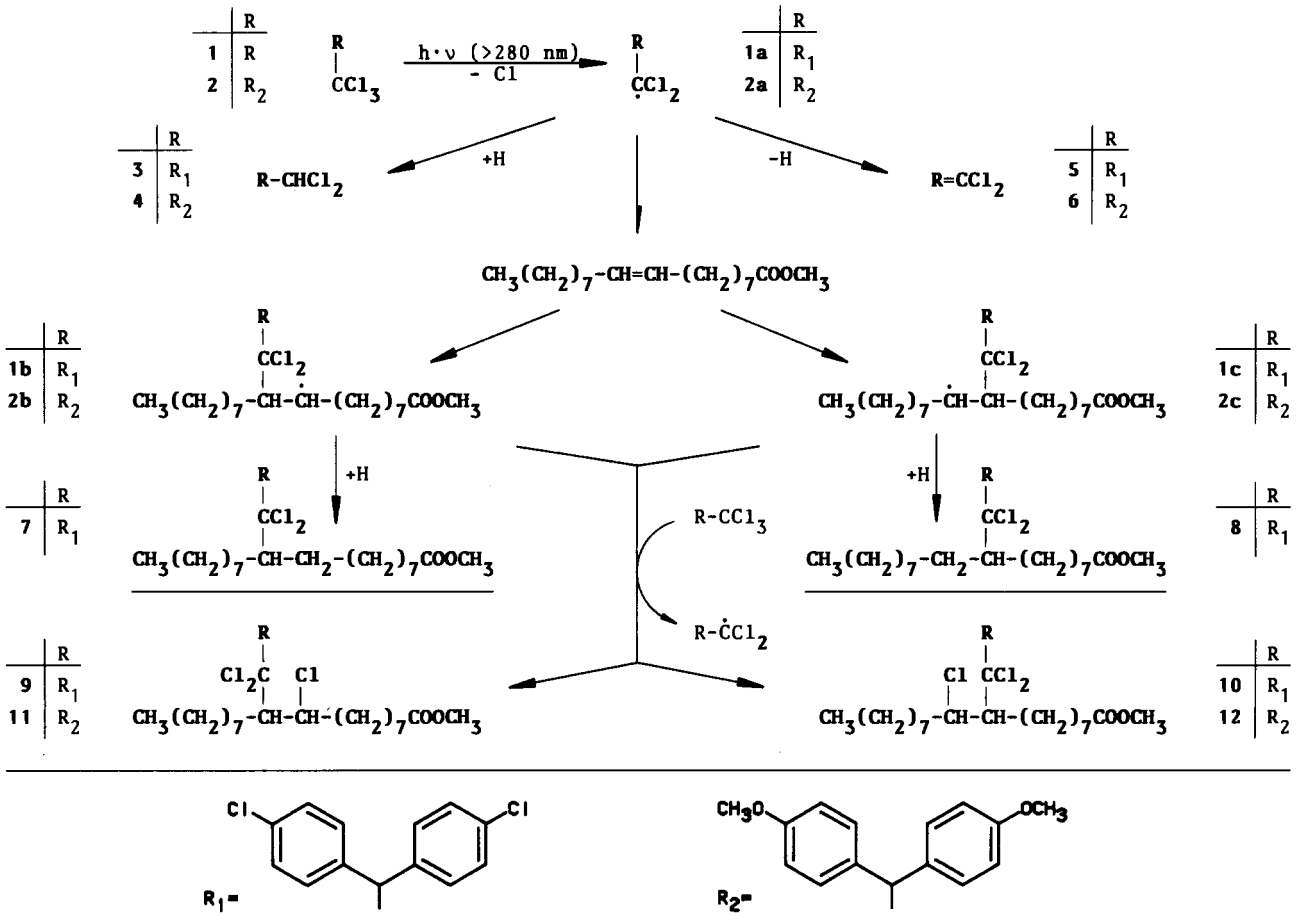

Figure 1. Photolysis of DDT and methoxychlor. Addition of the intermediate radicals to methyl oleate yielding different addition products.

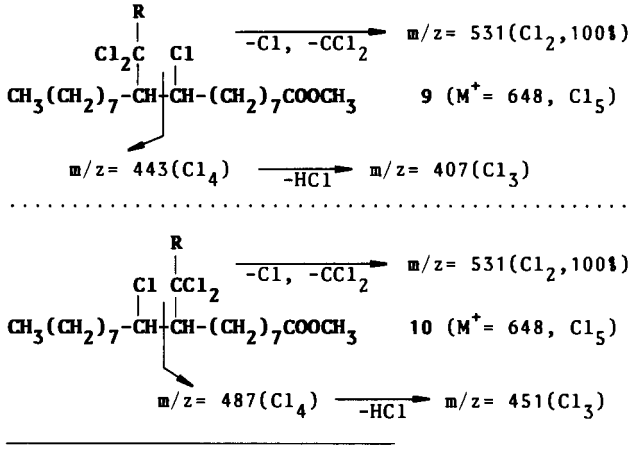

Figure 2. Proposed fragmentation of the positional isomeric addition products 9 and 10 of DDT and methyl oleate reported as mass (^{35}Cl) and chlorine isotope pattern (Cl_n).

In this context it may be of interest that *all* DDT olefin addition products isolated, so far, showed a very specific fragmentation during mass spectrometry, giving characteristic leading fragments as base peaks by loss of CCl_2 and Cl (Figure 2). This pattern may become important for the identification of "bound DDT residues" in plants. To emphasize this unique property of "bound" DDT derivatives it should be mentioned that the analogous methoxychlor addition products described below do not give this type of fragmentation. It is presumed that this type of fragmentation is dependent on the aromatic chlorine substituents of DDT.

Methoxychlor Products. After irradiation of methoxychlor (2) in the presence of methyl oleate, 47% of the photodegraded methoxychlor was bound to the fatty acid and 50% was recovered as DMDD (4). Two higher molecular weight reaction products have been isolated by gel permeation chromatography, but only the main one was proved to be an addition product of methoxychlor and methyl oleate. In agreement with the previous results (Schwack, 1984), methoxychlor only yielded the addition products 11 and 12 by chlorine transfer to the intermediate radicals **2b**/**2c** (Figure 1), but not products like 7 and 8.

As outlined above, 11 and 12 do not give a specific fragmentation during mass spectrometry, but only elimination of Cl and HCl. The base peak is the same as in the spectrum of methoxychlor (m/z 227). Cleavage between C-9 and C-10 followed by dehydrochlorination results in fragments as shown in Figure 3, proving the presence of both positional isomers 11 and 12.

The byproduct, isolated by TLC after gel permeation chromatography, could be identified by mass spectrometry as the dimer 13, a combination product of two DMDD radicals **2a** (Figure 4). Upon UV irradiation of methoxychlor-containing milk, similar methoxychlor dimerization products have been identified in the milk fat (Li and Bradley, 1969), but 13 has never been described before. The application of very short wave UV light by the authors cited could explain the different results.

The formation of 13 demonstrates the more rapid photolysis of methoxychlor than of DDT, which was found to be at least 300-fold in dilute water solutions compared to DDT (Zepp et al., 1977) and about 1.5-fold in this study. Yielding 13 appears to be the result of higher actual concentrations of free DMDD radicals **2a** than of **1a** derived

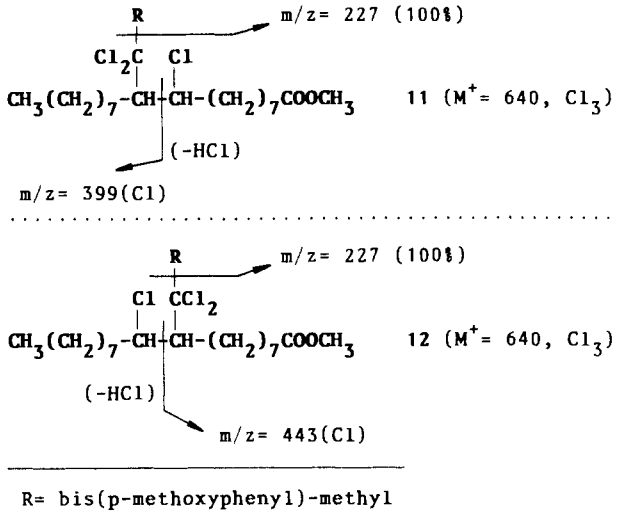

Figure 3. Proposed fragmentation of the positional isomeric addition products **11** and **12** of methoxychlor and methyl oleate reported as mass (^{35}Cl) and chlorine isotope pattern (Cl_n).

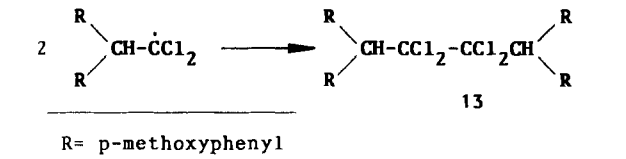

Figure 4. Methoxychlor dimer from the combination of DMDD radicals.

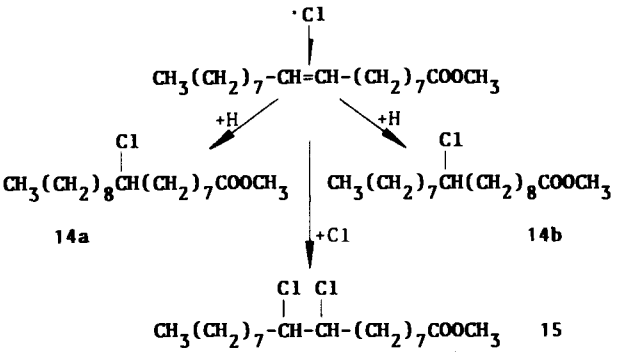

Figure 5. Methyl mono- and dichlorostearates formed by the addition of chlorine atoms derived from the photolysis of DDT and methoxychlor.

from the irradiation of DDT. Such dimerization competes with addition to olefins.

Chlorinated Fatty Acids. The free chlorine atoms formed by photolysis of DDT and methoxychlor (Figure 1) do not produce hydrogen chloride but add nearly quantitatively to the methyl oleate double bond, yielding different chlorinated fatty acids. In accordance with earlier studies (Schwack, 1984), the methyl monochlorostearates **14a** and **14b** (Figure 5) predominated, but separation of both isomers by GC was not possible. The same complications were found by Korhonen (1981) with several chlorinated fatty acids.

In contrast to the results with cyclohexene, the methyl dichlorostearate **15** was found in remarkably higher amounts (monochloro to dichloro ratio 10:1). Using GC, **15** is separated into two diastereomers with a peak ratio of 1:2.

CONCLUSIONS

The results presented in this study suggest that sunlight-induced additions of DDT and methoxychlor to unsaturated fatty acids of plant waxes and cutins may readily occur on a large scale. Addition products have to be termed as "bound residues", which should be extractable if the reactions proceed with components of plant waxes but will be nonextractable if DDT and methoxychlor are bound to the insoluble cutin polymer.

Chlorinated hydroxy fatty acids have been found in the cutin of grapes (Brieskorn, 1981/83), indicating that photoinduced radical chlorine transfer from chlorinated pesticides is generally possible, as well, under natural outdoor (sunlight) conditions, as was shown by model reactions with DDT and methoxychlor in this study.

Registry No. **7**, 113322-68-2; **8**, 113322-67-1; **9**, 113322-69-3; **10**, 113322-70-6; **11**, 113322-71-7; **12**, 113322-72-8; **13**, 76174-76-0; **14a**, 78898-45-0; **14b**, 78898-51-8; **15**, 33094-27-8; DDT, 50-29-3; methoxychlor, 72-43-5; methyl oleate, 112-62-9.

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