Diels–Alder Adducts of Fulvenes and Halogenated Dienes. Synthesis and Insecticidal Activity

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A series of eight adducts (1–8) of substituted fulvenes and polychlorinated cyclodienes was synthesized by Diels–Alder cyclization. The products isolated were the endo bicyclo adducts as determined by detailed ^1H and ^{13}C NMR spectral analysis. Steric hindrance of end-product bridge substituents coupled with bulky substituents at C_6 of the fulvenes led to one isomeric product in most cases. Compounds 1–8 demonstrated weak insecticidal action in *Musca domestica* as determined by topical LD_{50} and oral LC_{50} assays.

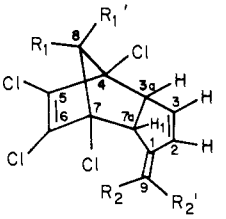
We had previously observed that pesticides of the halogenated diene class may induce genetic damage in mammalian cells.[1,2] These findings prompted an evaluation of the relative toxicities of various halogenated cyclodienes in insects as a function of mammalian toxicity, including genetic damage. A series of novel cyclodienes was synthesized in our laboratories utilizing various 6,6-disubstituted fulvenes as dienophiles reacting with polyhalogenated cyclopentadienes.[3] We report the synthesis and structural assignment of these compounds based on detailed (a) ^1H and ^{13}C NMR analysis, (b) elemental analysis, and (c) GLC evaluation and further describe the insecticidal action of our title compounds as determined by topical and oral toxicities in the house fly, *Musca domestica*.

Chemistry. The title compounds 1–8 (Chart I) were synthesized by Diels–Alder cycloaddition of the appropriately 6,6-disubstituted fulvene dienophiles with various polyhalogenated cyclopentadienes. Compounds 1–3 were prepared from hexachlorocyclopentadiene (**9**) with 6,6-dimethyl- (**10**), 6,6-diphenyl- (**11**), or 6-methyl-6-phenylfulvene (**12**), respectively, in yields of 61, 51, and 38%. Treatment of 5,5-dimethoxy-1,2,3,4-tetrachlorocyclopentadiene (**13**) with fulvenes **9**, **10**, or **11** gave adducts **4**, **5**, or **6** as the respective Diels–Alder addition products in yields of 55, 68, and 35%. Title compounds **7** and **8** were synthesized from 1,2,3,4-tetrachlorocyclopentadiene (**14**) prepared in 67% yield by reaction of **9** with zinc and acetic acid as described by Roedig and Hornig.[4] Reaction of **14** with dienophiles **10** or **11** gave **7** and **8** in respective yields of 43 and 51%.

The starting fulvenes were heated neat at 80 °C or were refluxed in benzene with the halogenated dienes. The products were isolated by separation of the crystalline precipitates or by fractional distillation of residual oils. Compounds 1–5 and 8 were determined to be discrete chemical compounds, while compounds 6 and 7 showed evidence of isomeric mixtures. Compound **6** was identified as a mixture, probably of R_2, R_2' syn and anti isomers, where the methyl or phenyl groups can exist syn or anti to the bridged bornyl ring system. In compound **7**, the combined absence of (a) bulky bridge substituents at C_8 and (b) bulky groups at R_2, R_2' lead to a mixture which combustion analyses suggested were molecular isomers (exo–endo).

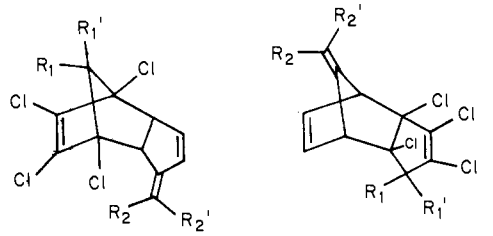
Diels–Alder cycloaddition reaction of fulvenes with dienes can occur by two modes of addition, the fulvene

Chart I



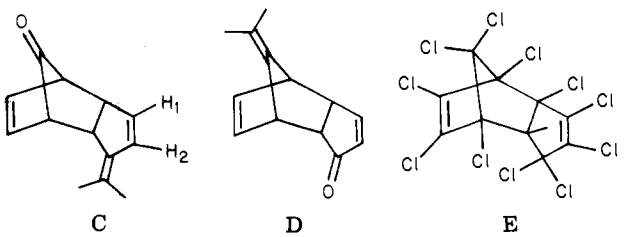
1, $R_1 = R_1' = Cl$; $R_2 = R_2' = CH_3$
2, $R_1 = R_1' = Cl$; $R_2 = R_2' = C_6H_5$
3, $R_1 = R_1' = Cl$; R_2 or $R_2' = CH_3$; R_2 or $R_2' = C_6H_5$
4, $R_1 = R_1' = OCH_3$; $R_2 = R_2' = CH_3$
5, $R_1 = R_1' = OCH_3$; $R_2 = R_2' = C_6H_5$
6, $R_1 = R_1' = OCH_3$; R_2 or $R_2' = CH_3$; R_2 or $R_2' = C_6H_5$
7, $R_1 = R_1' = H$; $R_2 = R_2' = CH_3$
8, $R_1 = R_1' = H$; $R_2 = R_2' = C_6H_5$

serving either as the diene or the dienophile. In the present cases, structures of type A or B are the possible cyclo-



A B

adducts. Houk and Luskus,[5] and Paddon-Row and Warrener[6] among others, have investigated reactions of this type. The ^1H and ^{13}C NMR spectra of the adducts prepared in this work are consistent in every case for structures of type A. Support for this conclusion comes from the report of Paddon-Row, Patney, and Warrener[7] in which they isolated and characterized adducts of dimethylfulvene and cyclopentadienone C and D. The



C D E

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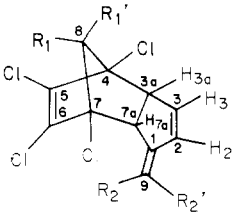
Table I. Proton Magnetic Resonance Chemical Shifts and Coupling Constants for Cyclodiene Adducts 1-8



	^{1}H NMR, δ (Me_4Si)							
no.	R_1 ($J_{R_1,R_1'}$, Hz)	R_1'	H_{7a} ($J_{3a,7a}$, Hz)	H_{3a} ($J_{3,3a}$, Hz)	H_2 ($J_{2,3}$, Hz)	H_3 ($J_{2,3a}$, Hz)	R_2	R_2'
1			3.93	3.93	6.46 (7.0)	5.71	1.96	1.78
2			4.46 (7.0)	3.94 (2.5)	6.41 (5.9)	5.88 (1.2)	7.21	7.21
3			4.06	4.06	6.20 (6.5)	5.71	2.3 or 7.21	2.3 or 7.21
4	3.61	3.53	3.73	3.73	6.43 (6.5)	5.65	1.91	1.76
5	3.50	3.45	4.30	3.85	6.41 (7.0)	5.90	7.23	7.23
6	3.66	3.57	3.83	3.83	6.11 (6.5)	5.83	2.28 or 7.21	2.28 or 7.21
7	2.58 (8.0)	2.33	3.60	3.60	6.36 (6.0)	5.72	1.93	1.75
8	2.51 (7.5)	2.30	4.33	3.85	6.40 (7.0)	5.93	7.26	7.26

proton NMR signals of H_1 and H_2 in the spectrum of C are very similar to the analogous vinyl proton signals in the compound prepared in this study.

The ^{1}H NMR chemical shifts and coupling constant assignments for the Diels–Alder adducts prepared in this study are listed in Table I.

^{1}H NMR (Table I). In each of the adducts **4–6** the methoxyl groups are magnetically nonequivalent. The more deshielded of each pair of methoxyl groups was assigned as the group syn to the norbornyl double bonds. In adducts 7 and 8 the hydrogens attached to the bridge methylene group C_8 are also nonequivalent and appear as an AB pattern. The more downfield of the protons at C_8 are assigned as being syn to the norbornenyl double bond. In the adducts containing one or two aryl groups, hydrogens H_{7a} and H_{3a} have differing chemical shifts. H_{7a}, being nearer a larger number of sp^2-hybridized carbon atoms than H_{3a}, is assigned as the more downfield of the two. In all of the adducts, vinyl protons H_2 and H_3 are magnetically nonequivalent. We assign H_2 as being downfield from H_3, consistent with the report of Paddon-Row et al.[7] In adducts **1**, **4**, and **7** the methyl groups R_2 and R_2' are nonequivalent.

The above assignments in the ^{1}H NMR spectra of the compounds prepared in this study are not consistent for structures of type B. In B, the vinyl (norbornenyl) protons would be expected as more nearly magnetically equivalent, as would be the methyl groups R_2 and R_2'. We would not expect the splitting pattern for the vinyl protons of B to appear as is observed in the present work.

^{13}C NMR (Table II). A detailed analysis of the ^{13}C NMR spectra of adducts **1–6** and **8** is presented in Table II to support our structural assignment of title compounds. Virtually all carbon atom signals have been assigned. Assignment of all proton-bound carbon absorbances was confirmed by off-resonance decoupling.

In the broad-band proton-decoupled spectrum of **4** there was absorption at δ 21.54 and 23.97, which we assigned to the methyl groups R_2 or R_2'. Peaks at δ 50.91 and 60.87 in the spectrum of **4** were assigned to C_{3a} or C_{7a} pending appropriate double-resonance studies. Peaks at δ 51.19 and 52.19 in **4** were assigned to the methoxyl groups R_1 or R_1'. In **4**, absorption occurs at δ 77.74 and 79.62, which is unchanged on removal of proton decoupling. We assign these peaks to the bridgehead carbons C_4 and C_7. Weak absorption in **4** at δ 112.63, which is also unchanged on removal of proton decoupling, we assign to C_8. In E, the doubly chlorinated bridging methylene group absorbs at δ 105.4. Relatively intense absorption in **4** at δ 126.23 and 136.85 we assign to C_2 and C_3. Since C_2 has one more β substituent than C_3, we expect C_2 to appear downfield from C_3.

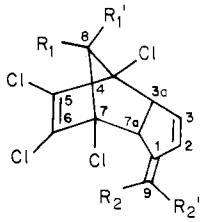
All carbons in **4** and **5** are assigned by aid of off-resonance decoupling, except the quarternary vinyl carbons C_1, C_5, C_6, and C_9. In the case of adducts **2–4**, **6**, and **8**, each of which contains one or two aryl groups, there is weak absorption at about δ 143. In adducts having no aryl substitution, no absorption as far downfield as δ 140 is observed. We therefore assign the peaks at δ 143 to the aryl carbon attached to C_9. Hawkes, Smith, and Roberts[8] report that the chemical shift of sp^2 carbon bound to hydrogen is similar to the chemical shift of sp^2 carbon bound to chlorine. Thus, we would expect to see both types of groupings absorb in the region from 125 to 140. With the data in hand, we do not make more refined assignments for these carbon atoms at the present time.

The assignment of the ^{13}C NMR spectra of adducts **1–3**, **6**, and **8** follow by close analogy with **4** and **5**. The reaction of dimethylfulvene with tetrachlorocyclopentadiene did not give a pure product **7**. Combustion analysis on **7** indicated a mixture of isomers. Due to the large number of peaks in the ^{13}C NMR spectrum of **7**, assignment was not possible.

Structure assignments were supported by selective heteronuclear decoupling experiments. Irradiation of the vinyl proton H_2 (δ 6.41) in compound **2** caused a sharp singlet absorption at δ 139.8 and a doublet absorption centered at δ 130.9. Irradiation of H_3 (δ 5.88) in **2** caused a singlet absorption at δ 130.9 and a doublet centered at δ 139.8. This allows assignment of the line at δ 139.8 in **2** to C_2, while absorption at δ 130.9 in **2** is assigned to C_3. Irradiation of H_{3a} (δ 3.94) in **2** caused a singlet absorption at δ 59.87 and a doublet at δ 51.56. Irradiation of H_{7a} (δ 4.46) in **2** reveals a singlet absorption at δ 51.56 and a doublet centered at δ 59.87. Thus, we are able to assign the chemical shift of C_{3a} at δ 59.87 and C_{7a} at δ 51.56. Based on this evidence, similar assignments are made for compounds **1** and **3–8**.

Biological Results and Discussion. The results of the biological evaluation of compounds **1–8** are presented in Table III. The cyclodiene adducts were tested for insecticidal activity in mixed sex adult house flies, *Musca domestica*. The multiple screening system included (a) determination of the topical toxicity of the compounds

Table II. ^{13}C NMR Chemical Shifts for Cyclodiene Adducts 1-8



^{13}C NMR, δ (Me_4Si)

no.	C_2	C_3	C_4	C_7	C_{7a}	C_{3a}	C_8	R_1 (b)	R_1 (b')	R_2 (b)	R_2 (b')
1	137.30	126.91	81.68	82.68	51.70	60.59	103.17			23.98 or 21.70	21.70 or 23.98
2	139.81	130.94	80.96	81.64	51.56	59.87	103.61			127-129	127-129
3	138.84	128.54		81.74	52.11	60.52	102.51			127-128 or 24.06	127-128 or 24.06
4	136.85	126.23	77.74	79.62	50.91	60.87	112.63	52.19 or 51.19	51.19 or 52.19	23.97 or 21.54	21.54 or 23.97
5	139.43	131.81	76.61	77.92	50.93	60.10	113.01	52.48 or 51.70	51.70 or 52.48	127-129	127-129
6[a]	138.46	129.24	77.78	79.62	51.41	60.83	112.88	52.68 or 51.85	51.85 or 52.68	24.13 or 128-129	24.13 or 128-129
8	138.17	132.44	71.76	72.68	54.62	63.36	64.33			127-129	127-129

[a] Mixture of isomers; other peaks present in spectrum. [b] Assignments supported by heteronuclear decoupling experiments (see ^{13}C NMR discussion in text).

Table III. Insecticidal Activity of Cyclodiene Adducts in Houseflies (*Musca domestica*)[a]

compd	topical application:[b] LD_{50}, μg/fly	oral:[c] LC_{50}, ppm
1	16	10 000
2	>20[d]	>12 000[e]
3	>20[d]	10 000
4	>20[d]	10 000
5	>20[d]	>12 000[e]
6	12	12 000
7	>20[d]	10 000
8	>20[d]	>12 000[e]
β-chlordane[f]	0.05	3.0
aldrin[f]	0.02	0.5
heptachlor[f]	0.04	

[a] Adult mixed-sex houseflies 3 ± 1 days old – 20 insects per test in duplicate runs assayed at 24 and 48 h relative to acetone controls. [b] 1.0-μL droplets applied to thoracic region in acetone. [c] Uniformly mixed with granulated sugar. [d] 0% mortality at 20 μg/fly. [e] 0% mortality at 12 000 ppm. [f] Source: USEPA standards.

expressed as the LD_{50} (μg/insect) and (b) the oral toxicity as determined by feeding experiments in granulated sugar and expressed as the LC_{50} (in ppm). Compounds 1–8 were compared to the pesticide standards of aldrin, β-chlordane, and heptachlor obtained from the U.S. Environmental Protection Agency.

These novel cyclodiene adducts demonstrated weak topical insecticidal action but were approximately three orders of magnitude weaker as topical pesticides than were heptachlor, chlordane, or aldrin. In the oral toxicity studies, a further reduced activity was observed as compared to various standard controls.

Compounds 1–8 represent systematic studies of substitutions at two sites on the molecule: (a) symmetrical attachment of chlorine (1–3), methoxy (4–6), or hydrogen (7 and 8) on the bridge carbon (C_8) of the norbornene ring and (b) dimethyl (1, 4, and 7), diphenyl (2, 5, and 8), or methyl and phenyl (3 and 6) additions to the exocyclic vinyl group.

Chlorination of the bridge carbon (C_8) is essential in the highly chlorinated insecticides aldrin and chlordane. The insect toxicity of both chemicals is usually lost if both bridge chlorines are replaced with alkoxyl groups or hydrogen.[9] LD_{50} values were unobtainable at the highest dose for most of our compounds, thereby precluding complete structure–activity analysis, but the dimethoxyl derivative 6 was more toxic than the dichloro analogue 3, in contrast with aldrin and chlordane.

Compounds 1–8 can be viewed as analogues of heptachlor, the most toxic insecticide of the heptachlor–chlordane group. The replacement of a substituted vinyl group for the chlorine of heptachlor caused drastic reductions in insect toxicity, possibly due to changes in molecular size and shape, electronegativity, penetration, or metabolism. The molecular profile of compounds 1–3 may be sufficiently different from heptachlor[10] to hinder interaction at the target site. The lack of the additional electronegative chlorine atom in compounds 1–3 explains some but not all of the reduced toxicity, since other analogues such as chlordene (topical LD_{50} = 1.0 μg/fly) exhibit marked insecticidal action. Oral toxicities were measured in the event that the topical toxicities were minimized by slow cuticle penetration. However, compared to chlordane, all experimental compounds were less toxic orally than dermally. This could be due in part to degradation in the gut. Finally, the title compounds may be metabolized rapidly by house flies. Cyclodiene analogues with reduced chlorine compositions are metabolized faster by house fly mixed-function oxidases than are heptachlor expoxide or dieldrin,[10,11] the metabolic products of heptachlor and aldrin oxidation. This metabolic transformation is a major factor in the eventual loss of toxicity.[10] The toxicity of nonchlorinated DDT analogues containing methyl and methoxyl substituents increased up to 250-fold in house flies when mixed-function oxidase was blocked with selective inhibitors. Thus, the alkyl and aryl adducts of compounds 1–8 may be attacked by house fly mixed function oxidase and detoxified.

Overall, the reduced toxicity of compounds 1–8, compared to heptachlor, is probably due to the combined effects of molecular size and metabolic instability. Although insect toxicities were reduced, these data augment structure–activity studies of other cyclodienes.[9,11] This may

facilitate research on nonhalogenated cyclodiene insecticides that possess a reduced stability and a reduced environmental hazard as compared to other compounds.[12] Compounds 1–8 are being further studied for genetic damage to mammalian cells.

Experimental Section

Biological Methods. All test compounds were within 0.3% of calculated values (C, H, and Cl; Galbraith Laboratories, Knoxville, Tenn.) and analyzed chromatographically by GLC for homogeneity on a Hewlett-Packard Model 402 gas chromatograph utilizing a 10% SE30 on Chromsorb W column with oven temperature 180 °C.

Contact toxicity was determined as LD_{50} by topical application of chemical dilutions to mixed-sex houseflies, *Musca domestica*, using reagent grade acetone as solvent. One-microliter droplets of each solution were applied with an ISCO microapplicator to the thoracic region of 20 adult flies, 3 ± 1 days of age. Dead and moribund flies were recorded at 24 and 45 h. Reconstituted powdered milk was offered as food during the observation period. Acetone only was administered to the control flies.

Oral toxicity was determined by feeding experiments on mixed-sex house flies. An appropriate volume of acetone dilutions of each chemical was uniformly mixed with granulated sugar, and the solvent was evaporated to provide a w/w% concentration in the food. The 48-h LC_{50} was determined for each exposure group containing 20 flies in a ventilated container. Dead and moribund flies were counted at 4 and 48 h. LD_{50} and LC_{50} values were interpolated from regression lines of probit mortality vs. $\log^{10}$ dose line fit.

Chemical Methods. All melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. ^1H and ^{13}C NMR were obtained with a Varian A-60 A spectrometer or Bruker Hx90-E NMR spectrometer and are reported with respect to Me_4Si. Detailed spectral analyses and coupling constants are reported in $CDCl_3$ in Tables I and II. IR data were obtained with a Beckman Model 4230 or Perkin-Elmer Models 457 and 337 infrared spectrophotometers. GLC analyses were performed on a Hewlett-Packard Model 402 flame-ionization gas chromatograph utilizing a 10% SE30 on Chromsorb W (6 ft × 1.0 mm i.d.) glass column with oven temperatures 180–200 °C. Analyses were supplied by Galbraith Laboratories, Knoxville, Tenn.

1,2,3,4-Tetrachlorocyclopentadiene (14). Tetrachlorocyclopentadiene was prepared in 67% yield by reduction of hexachlorocyclopentadiene with zinc in acetic acid according to Roedig and Hornig. Crystals were obtained, mp 60–61 °C, lit.[4] 61 °C.

***endo*-4,5,6,7,8,8-Hexachloro-1,1-dimethylvinylidene-3a,4,7,7a-tetrahydro-4,7-methano-1*H*-indene (1).** 6,6-Dimethylfulvene (5.5 g, 0.052 mol; Pfaltz and Bauer, Stanford, Conn.) was mixed with 16.4 g (0.06 mol) of tetrachlorocyclopentadiene (14) with no added solvent, the mixture being held in an oil bath (110 °C). Within 2 min an exothermic reaction occurred and the reaction mixture turned to a dark brown-red color. Vacuum distillation gave 13.4 g (61%) of a light yellow viscous oil: bp 128–138 °C (0.05 mm); IR (neat) 1620 (C=C), 1450, 1262, 1260 cm^{-1}; GLC one component. Anal. ($C_{13}H_{10}Cl_6$) C, H, Cl.

***endo*-4,5,6,7,8,8-Hexachloro-1,1-diphenylvinylidene-3a,4,7,7a-tetrahydro-4,7-methano-1*H*-indene (2).** 6,6-Diphenylfulvene (18.5 g, 0.08 mol, Aldrich Chemical Co., Milwaukee, Wis.) and hexachlorocyclopentadiene (17.9 g, 0.08 mol) were dissolved in toluene (35 mL) and were held at reflux for 60 h. Removal of solvent at room temperature gave a pasty solid which was crystallized from hexane to give adduct 2 (17.9 g, 51%). Two recrystallizations from MeOH gave a white crystalline solid: mp 172.5–173.5 °C; IR (KBr) 1590 (C=C, Ar), 1430, 1245 cm^{-1}; GLC one product. Anal. ($C_{23}H_{14}Cl_6$) C, H, Cl.

***endo*-4,5,6,7,8,8-Hexachloro-1-methyl-1-phenylvinylidene-3a,4,7,7a-tetrahydro-4,7-methano-1*H*-indene (3).** 6-Phenyl-6-methylfulvene (8.0 g, 0.025 mol; Trans World Chemicals, Washington D.C.) was mixed with 4.2 g (0.03 mol) of hexachlorocyclopentadiene without added solvent. The mixture was held at 80 °C for 7 h, at which time the reaction mixture was dark brown. The crude mixture was triturated with 30 mL of hexane and, after 45 s, a white solid formed. The solid was collected, washed with 100 mL of hexane, and recrystallized from MeOH to give 4.6 g (38%) of white crystals, recrystallized from MeOH: mp 144–145 °C; IR (KBr) 1590 (C=C, Ar), 1420, 1240 cm^{-1}; GLC one product. Anal. ($C_{18}H_{17}Cl_6$) C, H, Cl.

***endo*-4,5,6,7-Tetrachloro-1-dimethylvinylidene-8,8-dimethoxy-3a,4,7,7a-tetrahydro-4,7-methano-1*H*-indene (4).** Dimethylfulvene (5.0 g, 0.047 mol) was added to 13.0 g (0.50 mol) of tetrachlorodimethoxycyclopentadiene (Aldrich Chemical Co., Milwaukee, Wis.) and held at 110 °C in an oil bath. In 2 min an exothermic reaction occurred with darkening of the product mixture. Heat was removed and the product was distilled under vacuum to give 4.8 g (55%) of 4, bp 135–141 °C (0.08 mm). On standing in the freezer, the distilled product solidified to a thick, tacky solid, mp 65–72 °C. Sublimation gave a waxy solid: mp 69–71 °C; IR (neat, oil) 1600 (C=C), 1605–1600 (br), 1445, 1270, 1180 cm^{-1}; GLC one product. Anal. ($C_{15}H_{16}Cl_4$) C, H, Cl.

***endo*-4,5,6,7-Tetrachloro-1,1-diphenylvinylidene-8,8-dimethoxy-3a,4,7,7a-tetrahydro-4,7-methano-1*H*-indene (5).** 6,6-Diphenylfulvene (3.8 g, 0.035 mol) and tetrachlorodimethoxycyclopentadiene (10.0 g, 0.035 mol) were dissolved in xylene (30 mL) and held at reflux for 18 h. Sovent was removed at reduced pressure and room temperature to give a crude solid. The solid was recrystallized from CCl_4/MeOH (50:50) to give 9.3 g (68%) of white crystals and (2 × MeOH) a white solid: mp 161–162 °C; IR (KBr) 1590 (C=C, Ar), 1430, 1250, 1185 cm^{-1}; GLC one product. Anal. ($C_{25}H_{20}Cl_4$) C, H, Cl.

***endo*-4,5,6,7-Tetrachloro-1-methyl-1-phenylvinylidene-8,8-dimethoxy-3a,4,7,7a-tetrahydro-4,7-methano-1*H*-indene (6).** 6-Phenyl-6-methylfulvene (4.5 g, 0.028 mol) was mixed with 10 g (0.035 mol) of tetrachlorodimethoxycyclopentadiene without added solvent. The mixture was held at 75 °C for 6.5 h. The crude, viscous reaction mixture was distilled under vacuum to remove excess starting material. The viscous residue was taken up in hexane (10 mL) to give a yellow solution. White crystals formed from hexane. They were separated and crystallized from MeOH to give 4.6 g (35%) of product 6. Two more recrystallizations from MeOH gave mp 118–119.5 °C; IR (KBr) 1590 (C=C, Ar), 1420, 1280, 1250, 1190 cm^{-1}; GLC gave two methylphenylvinylidene isomers. Anal. ($C_{20}H_{18}Cl_4$) C, H, Cl.

***endo*-4,5,6,7-Tetrachloro-1,1-dimethylvinylidene-3a,4,7,7a-tetrahydro-4,7-methano-1*H*-indene (7).** Freshly distilled 6,6-dimethylfulvene (3.7 g, 0.025 mol) and compound 14 (7.8 g, 0.025 mol) were dissolved in benzene (5 mL) and warmed at 50 °C overnight. Solvent was removed and the resulting yellow-orange oil distilled to yield 43% of a viscous light yellow oil: bp 135–137 °C (0.12 mm); GLC gave a nonresolvable isomeric mixture; IR (neat oil) 1622 (C=C), 1450, 1275, 1255 cm^{-1}. Anal. ($C_{13}H_{12}Cl_4$) C, H, Cl.

***endo*-4,5,6,7-Tetrachloro-1,1-diphenylvinylidene-3a,4,7,7a-tetrahydro-4,7-methano-1*H*-indene (8).** 6,6-Diphenylfulvene (8.0 g, 0.035 mol) and freshly crystallized compound 14 (7.0 g, 0.034 mol) were dissolved in benzene (50 mL). The mixture was held at reflux for 2 days, concentrated to 15 mL, and 40 mL of CCl_4 added. A solid formed on standing at 4 °C, which on recrystallization gave 8.9 g (59%) of white crystals from MeOH: mp 201–202.9 °C; GLC one compound; IR (KBr) 1590 (C=C, Ar), 1430, 1262. Anal. ($C_{23}H_{16}Cl_4$) C, H, Cl.

Acknowledgment. The authors thank Dr. Edward Fairchild for his assistance in obtaining ^{13}C NMR spectra, Mr. Phillip Yip for aid in synthesis, and Dr. E. S. Mooberry and the North Carolina A & T State University for ^{13}C NMR spectra, which was supported by Public Health Service Grant 2-506-RR08019-07. This work was supported by Research Grant R805008010 from the U.S. Environmental Protection Agency.

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Antiestrogens and Antiestrogen Metabolites: Preparation of Tritium-Labeled (±)-*cis*-3-[*p*-(1,2,3,4-Tetrahydro-6-methoxy-2-phenyl-1-naphthyl)phenoxy]-1,2-propanediol (U-23469) and Characterization and Synthesis of a Biologically Important Metabolite

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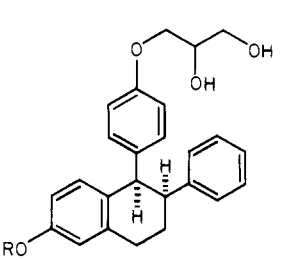
Received April 25, 1979

The Upjohn antiestrogen (±)-*cis*-3-[*p*-(1,2,3,4-tetrahydro-6-methoxy-2-phenyl-1-naphthyl)phenoxy]-1,2-propanediol (**2b**, U 23469) has been prepared in tritium-labeled form by reduction of an unsaturated dihydronaphthalene precursor with carrier-free tritium gas over a palladium catalyst followed by alkylation with 3-iodo-1,2-propanediol. After extensive chromatographic purification, the final material was obtained with a specific activity of 13 Ci/mmol and a radiochemical purity of 94%. In vivo studies with immature rats show that [^{3}H]**2b** is slowly converted to a more polar metabolite that is selectively accumulated in the nuclear fraction of the uterus where it is bound to the estrogen receptor. Chromatographic comparisons indicate that this metabolite is the demethylated analogue **2c**, a compound that has an affinity for estrogen receptor more than 300 times greater than that of **2b**. These studies suggest that the demethylated analogue **2c** may be a biologically important metabolite of **2b** that is involved in the action of this antiestrogen.

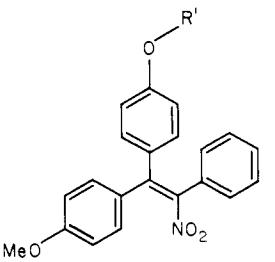
Antiestrogens are compounds that block, at least in part, the action of estrogens in target tissues.[1] While the pursuit of compounds with such activity was initially prompted by the search for effective contraceptive agents for the human female, interest has refocused on these compounds because of their potential for controlling the growth of estrogen-dependent neoplasms, particularly tumors of the breast.[2] In fact, recent clinical trials have shown antiestrogen treatment to be as effective as other forms of hormone additive or ablative therapy in human breast cancer.[2]

From recent studies on antiestrogens, it is clear that the molecular basis of their action is complex, involving interactions with both cytoplasmic and nuclear receptor sites.[1,3] Furthermore, the duration of action of the better-known antiestrogens is much longer than that of the estrogens with which they are normally compared, and it appears that the biological action of the antiestrogens may be mediated not only by the compounds administered but also by certain metabolites that may, in fact, be more potent than the parent compound.[3-5]

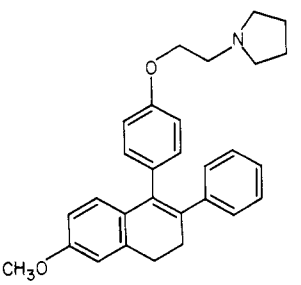
In order to facilitate studies on the molecular action of antiestrogens, we have endeavored to prepare several members of this class in high specific activity, tritium-labeled form to permit their interaction with target tissues and receptors to be followed directly. We have recently described the preparation of a Parke-Davis antiestrogen CI 628[6] in tritium-labeled form and investigated its interaction with receptor in the immature rat uterus and its in vivo metabolism.[5] Other studies with this radiolabeled antiestrogen[7] and radiosynthetic studies on a closely related antiestrogen CI 680[6,8] have appeared, as have studies utilizing [^{3}H]tamoxifen.[6,9]



U-23469 (**2b**), R = CH_3
metabolite (**2c**), R = H



CI 628, R' = CH_2CH_2-c-$N(CH_2CH_2)_2$
CI 680, R' = CH_2CH_2-CH_2NMe_2



nafoxidine; U 11,100A

In this report, we describe the preparation, in tritium-labeled form, of an Upjohn antiestrogen, (±)-*cis*-3-[*p*-1,2,3,4-tetrahydro-6-methoxy-2-phenyl-1-naphthyl)phen-

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