Synthesis and Herbicidal Activity of Geometrical Isomers of Methyl [[[1-[5-[2-Chloro-4-(trifluoromethyl)phenoxy]-2-nitrophenyl]-2-methoxyethylidene]amino]oxy]acetate (AKH-7088)

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The *E* and *Z* geometrical isomers of methyl [[[1-[5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrophenyl]-2-methoxyethylidene]amino]oxy]acetate (Code No. AKH-7088) were separately synthesized, and their molecular conformations were determined by two-dimensional NMR spectroscopy. Greenhouse testing showed no significant difference between the herbicidal effects of the two isomers on broadleaf weeds such as velvetleaf, cocklebur, and morningglory in soybeans, in postemergent applications of 0.035–0.28 kg/ha. Mixtures of the two isomers in various proportions exhibited practically the same biological effects as those of each isomer alone. Soybeans exhibited excellent tolerance to the isomers and their mixtures.

Methyl(*E,Z*)-[[[1-[5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrophenyl]-2-methoxyethylidene]amino]oxy]-acetate (Code No. AKH-7088) is a novel compound with selective herbicidal effects discovered in 1984 and currently under development as a herbicide by Asahi Chemical Industry Co., Ltd. (Hayashi and Misumi, 1984, 1987). It is a postemergence herbicide for selective control of a wide spectrum of broadleaf weeds in soybeans. The preceding paper reported the syntheses and herbicidal activities of AKH-7088, a mixture of *E* and *Z* isomers (Hayashi, 1990).

In this paper, we report the separate syntheses of geometrical *E* and *Z* isomers of AKH-7088 (Figure 1), $Z \rightleftarrows E$ isomerization in oxime ether, and the structural identification of both isomers by two-dimensional NMR and compare their biological activities.

EXPERIMENTAL SECTION

Apparatus. Melting points (degrees Celsius) were determined with a Mettler FP61 melting point apparatus and are uncorrected. Infrared spectra were recorded on a Shimadzu IR-400 infrared spectrometer. Mass spectra were recorded on a JEOL DX-303. NMR spectra were recorded on a JEOL GX-270. Chemical purities were determined on a Jasco liquid chromatograph, Models 880-PU and 875-UV.

Syntheses of Compounds. *5′-[2-Chloro-4-(trifluoromethyl)phenoxy]-2′-nitro-2-methoxyacetophenone (1).* A solution of 34.5 g (0.10 mol) of 3′-[2-chloro-4-(trifluoromethyl)phenoxy]-2-methoxyacetophenone prepared from 3-chloro-4-fluorobenzotrifluoride and 3′-hydroxy-2-methoxyacetophenone (Hayashi, 1990) in 300 mL of dichloromethane was cooled to about −5 °C. To the resulting solution was added a mixed acid cooled to about 0 °C composed of 50.0 g (0.50 mol) of concentrated sulfuric acid (98%) and 6.75 g (0.105 mol) of concentrated nitric acid (98%) dropwise over a period of 30 min. The reaction mixture was then poured cautiously into 300 mL of ice–water, followed by extraction with dichloromethane. The separated dichloromethane layer containing the reaction product was washed with water until a neutral aqueous layer was obtained and dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure afforded 38.2 g of a reddish brown viscous oil. The obtained crude product was purified by chromatography on a silica gel column. The purified product was eluted from the column with a toluene–hexane gradient system. As a result, there was obtained 27.3 g (70% yield) of yellowish crystals, mp 95–96 °C. Anal. Calcd for $C_{16}H_{11}ClF_3NO_5$: C, 49.31; H, 2.84; N, 3.59. Found: C, 49.25; H, 3.00; N, 3.42.

(Z)-2-Methoxy-1-[2-nitro-5-[2-chloro-4-(trifluoromethyl)-phenoxy]phenyl]ethanone Oxime (2). A solution of 23.4 g (60 mmol) of compound **1**, 9.84 g (0.12 mol) of anhydrous sodium acetate, and 1.0 g (0.12 mol) hydroxylamine hydrochloride in 90 mL of ethanol was heated under reflux for 6 h. Sodium chloride was then removed by filtration, and the filtrate was subjected to evaporation–removal of most of the ethanol under reduced pressure. Subsequently, 100 mL of water and 150 mL of ether were added to effect extraction. The ethereal layer was separated, washed with water, and dried over anhydrous sodium sulfate, followed by evaporation–removal of the solvent under reduced pressure. There was consequently obtained 24.0 g of a reaction product composed of *E* isomer (36% by weight) and *Z* isomer (63% by weight). Separation and purification of each isomer by silica gel column chromatography with a toluene–acetone gradient system afforded 15.1 g (62% yield) of light brown crystals of the desired *Z* isomers **2**, mp 124–125 °C, and 8.5 g (35% yield) of a light yellow viscous oil of *E* isomer of the oxime, n^{30}_D 1.5278. Anal. Calcd for $C_{16}H_{12}ClF_3N_2O_5$: C, 47.48; H, 2.98; N, 6.92. Found (*Z* isomer): C, 47.55; H, 2.89; N, 6.63. Found (*E* isomer): C, 47.63; H, 3.07; N, 6.78. ^{1}H NMR ($CDCl_3$) (Z isomer): δ 3.12 (s, 3 H, CH_3), 4.40 (s, 2 H, CH_2), 6.98–8.08 (m, 6 H, aromatic), 11.39 (s, 1 H, OH). ^{1}H NMR ($CDCl_3$) (E isomer): δ 3.31 (s, 3 H, CH_3), 4.25 (s, 2 H, CH_2), 6.93–8.13 (m, 6 H, aromatic), 11.08 (s, 1 H, OH).

(E)-[[[1-[5-[2-Chloro-4-(trifluoromethyl)phenoxy]-2-nitrophenyl]-2-methoxyethylidene]amino]oxy]acetic Acid (3). A solution of 39 g (0.1 mol) of compound **2**, 5.0 g (61 mmol) of anhydrous sodium acetate, and 13.1 g (0.12 mol) of carboxymethoxylamine hemihydrochloride in 300 mL of ethanol was heated under reflux for 3 h. After completion of the reaction, sodium chloride was removed by filtration and the filtrate was subjected to evaporation–removal of most of ethanol under reduced pressure. Subsequently, 150 mL of water and 200 mL of dichloromethane were added to extract the reaction product. The dichloromethane layer was separated, washed with water, and dried over anhydrous sodium sulfate. HPLC analysis of the product showed that the *E/Z* ratio of isomers in the product was about 55/45 (by weight). Subsequently, to the dichloromethane solution containing the product was added 50.0 g

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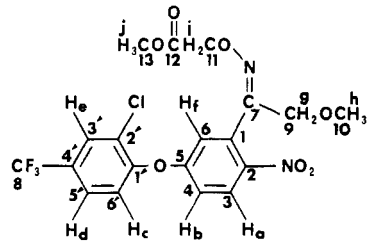
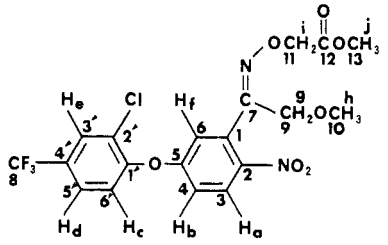


E isomer



Z isomer

Figure 1. Geometrical isomers of AKH-7088.

(0.1 mol) of concentrated sulfuric acid (98%) dropwise over a period of 10 min at about 0 °C. The reaction mixture was then warmed to 25–30 °C in 15 min and held at 25–30 °C for an additional 30 min. The *E*/*Z* ratio of the product was 73/27 (by weight). After completion of the isomerization reaction, the reaction mixture was poured cautiously into 300 mL of ice–water, followed by extraction with dichloromethane. The separated dichloromethane layer was washed with an aqueous sodium chloride saturated solution and with water and dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure afforded 44.4 g of reddish viscous residue and was followed by addition of 60 mL of methanol to precipitate the reaction product. The white precipitate was removed by filtration, washed with cold methanol, dried in vacuo, and purified by recrystallization from methanol and isopropyl ether, respectively, to give 26.8 g (58% yield, 99.3% purity) of white crystals of the desired *E* isomer, mp 119–120 °C. Anal. Calcd for $C_{18}H_{14}ClF_3N_2O_7$: C, 46.71; H, 3.04; N, 6.05. Found: C, 46.67; H, 3.23; N, 6.90. ^1H NMR ($CDCl_3$): δ 3.36 (s, 3 H, CH_3), 4.33 (s, 2 H, CCH_2O), 4.60 (s, 2 H, OCH_2CO), 6.96–8.28 (m, 6 H, aromatic), 10.16 (s, 1 H, OH).

Methyl (Z)-[[[1-[5-[2-Chloro-4-(trifluoromethyl)phenoxy]-2-nitrophenyl]-2-methoxyethylidene]amino]oxy]acetate (4, Z Isomer of AKH-7088). A solution of 10.1 g (25 mmol) of compound **2** and 1.6 g (30 mmol) of sodium methylate in 40 mL of dried *N,N*-dimethylacetamide was cooled to about 5 °C. To the resulting solution was added 3.8 g (25 mmol) of methyl bromoacetate dropwise over a period of 5 min. The reaction was allowed to proceed at room temperature for 3 h. Cold water (150 mL) and 150 mL of ether were then added to extract reaction product. The separated ethereal layer was washed with water and dried over anhydrous sodium acetate. The solvent was then removed under reduced pressure, to obtain crude product, which was purified by chromatography on a silica gel column. The purified product was eluted from the column with a toluene–acetone gradient system and then was recrystallized from ethyl acetate. As a result, there was obtained 6.9 g (58% yield, 99.4% purity) of colorless crystals, mp 99–100 °C. Anal. Calcd for $C_{19}H_{16}ClF_3N_2O_7$: C, 47.86; H, 3.38; N, 5.87; Cl, 7.43. Found: C, 47.72; H, 3.30; N, 5.69; Cl, 7.59.

Methyl (E)-[[[1-[5-[2-Chloro-4-(trifluoromethyl)phenoxy]-2-nitrophenyl]-2-methoxyethylidene]amino]oxy]acetate (5, E Isomer of AKH-7088). A solution of 23.1 g (50 mmol) of compound **3** and 0.76 g (4 mmol) of *p*-toluenesulfonic acid monohydrate in 200 mL of methanol was heated under reflux for 2 h. The catalyst was then titrated with 1 equiv of sodium hydroxide to prevent hydrolysis of the ester product during solvent removal. The methanol was distilled off under reduced pressure. The residue was dissolved in ethyl acetate, and the ethyl acetate layer was washed with water and dried over anhydrous sodium sulfate, followed by evaporation–removal of the solvent in vacuo. The product was purified by recrystallization from isopropyl ether and then ethyl acetate–hexane mixture, to afford 17.9 g (75% yield, 99.5% purity) of colorless crystals, mp 57.5–58.5 °C. Anal. Calcd for $C_{19}H_{16}ClF_3N_2O_7$: C, 47.86; H, 3.38; N, 5.87; Cl, 7.43. Found: C, 47.81; H, 3.29; N, 5.73; Cl, 7.35.

Structural Analysis by NMR. Each analytical-grade isomer, prepared as described above, was dissolved in deuterated chloroform ($CDCl_3$) containing tetramethylsilane (TMS) for proton and carbon-13 NMR measurements.

The one- and two-dimensional NMR spectra were obtained with a JEOL GX-270 (6.34 T) FT-NMR spectrometer operating at 270.05 MHz for proton and 67.8 MHz for carbon-13 measurements.

The normal one-dimensional proton NMR spectra were obtained with a 8.5-μs (90°) pulse and 16K data points to give 0.2 Hz/point digital resolution. Free induction decays were weighted to 0.3-Hz line broadening. Proton chemical shifts reported are referenced to TMS (0.00 ppm). The normal one-dimensional carbon-13 NMR spectra were obtained by using a 4.3-μs (90°) pulse and 32K data points to give 0.5 Hz/point digital resolution. Free induction decays were weighted to 1-Hz line broadening. Chemical shifts reported were referenced to TMS (0.00 ppm).

The proton homonuclear shift-correlated (*J*-correlated, COSY) spectra were obtained by using a sweep width of 1600 Hz, 1K data points, and 8.5-μs (90°) pulse. A total of 128 spectra were collected to provide the equivalent of a 1600-Hz sweep width in the second frequency dimension. The proton–carbon-13 shift-correlated (CHSHF) spectra were obtained by using a sweep width of 16 kHz, 2K data points, and 4.3-μs (90°) pulse. A total of 256 spectra were obtained to provide the equivalent of a 3.2-kHz sweep width in the proton frequency dimension. The δ values of chemical shift of carbon-13 NMR spectra shown in Table II as calculated data were obtained from the INKA data base provided by BASF.

Biological Testing. The separate *E* and *Z* isomers of AKH-7088 and their mixtures in various proportions were evaluated for postemergent herbicidal activity in a greenhouse. Each test formulation, as an emulsifiable concentrate, was prepared by the thoroughly mixing 10 parts by weight of the test compound, 10 parts by weight of Sorpol 3005X (surfactant), 40 parts by weight of xylene, and 40 parts by weight of cyclohexanone. All application composition, for the greenhouse test, were prepared by dilution of the concentrate in water at 200 L/ha.

Seeds of eight broadleaf species, one grassy weed species, and one crop were planted in pots each having a surface area 0.24 m^2 filled a sterilized upland soil. The test broadleaf species were smartweed (*Polygonum lapathifolium*; SW), sicklepod (*Cassia obtusifolia*; SP), velvetleaf (*Abutilon theophrasti*; VL), nightshade (*Solanum nigrum*; NS), morningglory (*Ipomoea spp.*; MG), pigweed (*Amaranthus retroflexus*; PW), prickly sida (*Sida spinosa*; PS), and cocklebur (*Xanthium strumarium*; CB); the grass was large crabgrass (*Digitaria ciliaris*; CG); the crop was soybean (*Glycine max* var. Williams; SOY).

Postemergence treatment was effected by applying the composition of a dosage of 0.035, 0.07, 0.14, and 0.28 kg/ha when the soybean and the weeds were, respectively, at the 1.5–2-leaf stage (trifoliates), 2–6.1-leaf stage. All treatments were replicated three times. On day 14, percent weed control was determined by visual estimation of percent plant growth reduction in treated as compared with nontreated plots. On day 21, percent crop injury was determined in the same manner.

RESULTS AND DISCUSSION

Synthesis. Etherification of oxime compound **2** with methyl bromoacetate by the above-described procedure resulted readily in *Z* isomer of AKH-7088 (compound **4**). By contrast, the *E* isomer obtained during compound **2** synthesis was converted to another as yet chemically unidentified compound by etherification under the same reaction conditions. NMR analysis of the unidentified compound resulted in assignment of methoxymethyl group

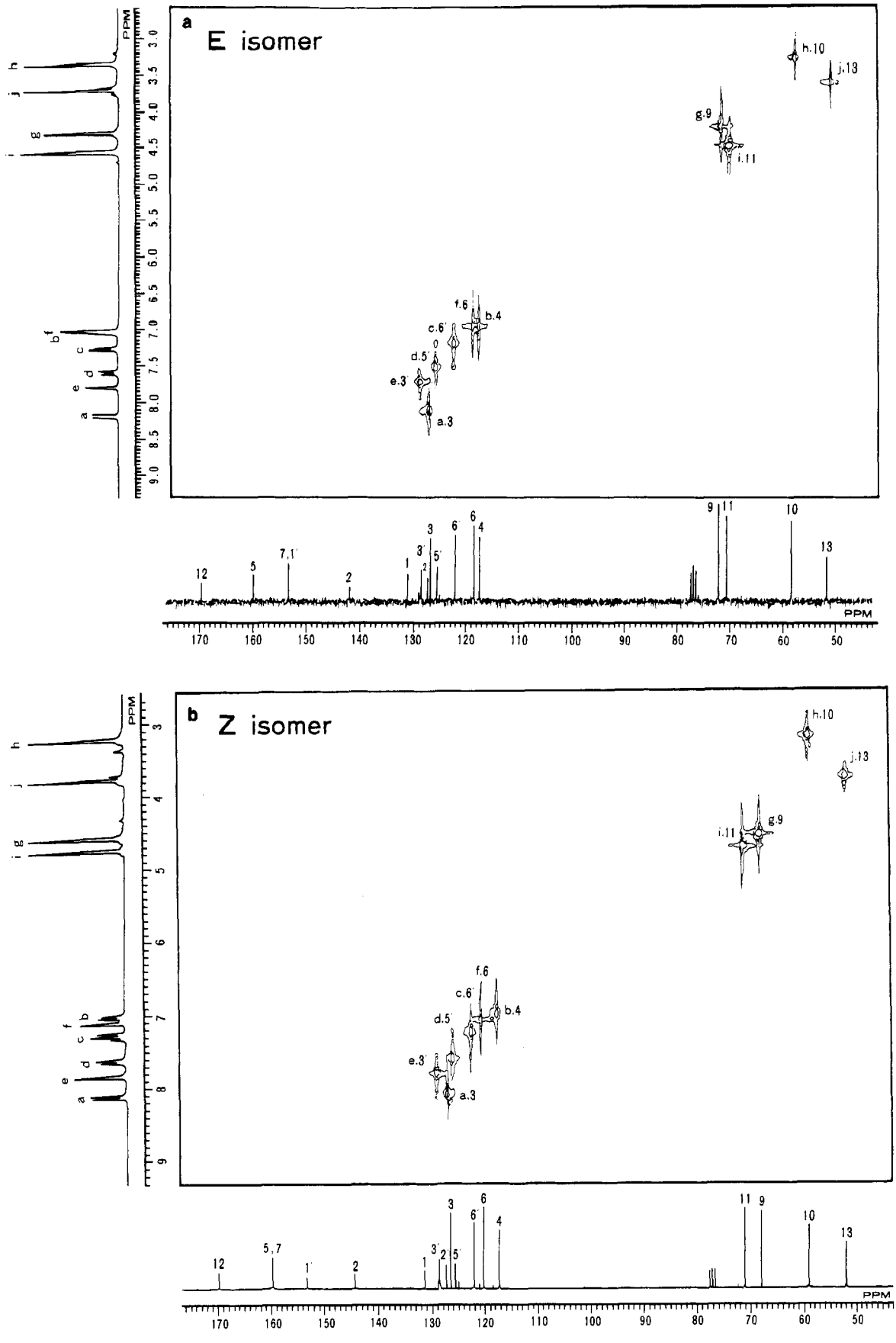


Figure 2. Contour plots of the chemical shift correlated (CHSHF) spectra of the *E* (a) and *Z* isomers (b) of AKH-7088 with projections along the carbon frequency dimension (abscissa) and the proton frequency dimension (ordinate).

in the molecule, but not the (methoxycarbonyl)methoxy group, which should be expected to form in oxime etherification. In its NMR spectrum, the peak assigned to the proton on the carbon adjacent to the carbon bearing the nitro group shifted toward high field and no oxime proton signal was observed. This apparently indicates the occurrence of a reaction between the nitro group and the hydrogen atom in the oxime moiety, resulting in the

Scheme I. Routes of Synthesis of the *E* and *Z* Isomers of AKH-7088

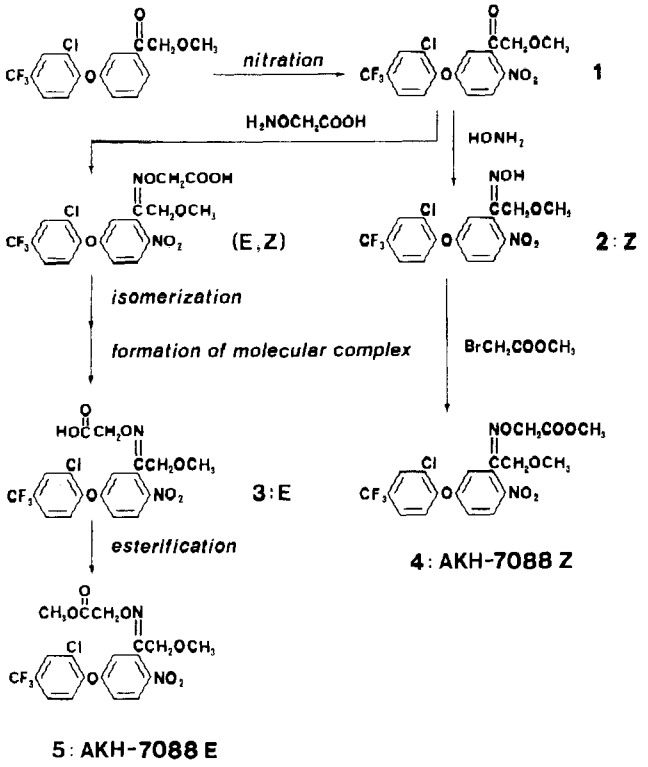


Table I. Equilibrium Constants of the Isomerization Reaction in Sulfuric Acid (25–30 °C, 30 min)

concn of sulfuric acid, %	equil const
90	1.4
95	2.2
98	2.7
104.5	3.5

formation of the unidentified compound. As the *E* isomer of AKH-7088 thus cannot be synthesized by etherification of this oxime compound, it must be synthesized through an other route such as that shown in Scheme I.

Isomerization in the Oxime Ether. $Z \rightleftharpoons E$ isomerization in oxime ethers induced by protonation (Johnson et al., 1981, 1982) or irradiation (Padwa and Albrecht, 1974) is a well-known phenomenon. In the present study, both were observed to result in this interconversion in compounds 3–5. In particular, isomerization in the mixture of compound 3 and its *Z* isomer by protonation in concentrated sulfuric acid was intensively investigated. In order to attain equilibrium of the isomerization reaction fairly rapidly, 5 mol equiv or more of acid was added to 1 mol equiv of oxime compound. The rate of the isomerization reaction was apparently temperature dependent, and the equilibrium constant of isomerization was acidity dependent; i.e., it varied in proportion to the concentration of sulfuric acid as shown in Table I.

Addition of methanol to the mixture of compound 3 and its *Z* isomer following this isomerization was found to result in the formation of a white precipitate containing compound 3 as its main component (Furuhashi, 1986). Recrystallization of the precipitate three times from methanol allowed the recovery of a molecular complex in pure form consisting of compound 3 and an equimolar amount of methanol, as shown by NMR analysis. This type of molecular complex did not form between the *Z* isomer of compound 3 and methanol. When the complex was dissolved in an organic solvent other than methanol, such as ethanol, acetone, ethyl acetate, ether, benzene, or chloroform, the methanol was readily dissociated from the complex. It thus seems apparent that the hydrogen bond between the oxygen of the carbonyl group and the hydrogen of the hydroxyl group in methanol contributes to the formation of the molecular complex. The preferential formation of the molecular complex facilitates effective recovery of compound 3, with only a small amount of its *Z* isomer and substantially no other impurities. In production of AKH-7088, therefore, nearly quantitative recovery of active ingredient can be accomplished by a combination of repeated isomerization and formation of the molecular complex.

Table II. Proton NMR Data for *E* and *Z* Isomers of AKH-7088

	E isomer		*Z* isomer	
proton	chem shift	coupling const, Hz	chem shift	coupling const, Hz
a	8.19 (d)	9.8	8.08 (d)	9.0
b	7.05 (dd)	9.8, 2.7	6.98 (dd)	9.0, 2.4
c	7.27 (d)	8.6	7.23 (d)	8.6
d	7.59 (dd)	8.6, 2.5	7.59 (dd)	8.6
e	7.79 (d)	2.5	7.80 (d)	
f	7.04 (d)	2.7	7.07 (d)	2.4
g	4.31 (s)		4.54 (s)	
h	3.35 (s)		3.19 (s)	
i	4.56 (s)		4.71 (s)	
j	3.70 (s)		3.75 (s)	

Table III. Carbon-13 NMR Data for the *E* and *Z* Isomers of AKH-7088

	E isomer		*Z* isomer		
carbon no.	chem shift (obsd)	coupling const, Hz	chem shift (obsd)	coupling const, Hz	chem shift (INKA)
1	131.39		131.47		121.3 ± 4.9
2	142.18		144.46		147.9 ± 0.6
3	127.10		126.74		128.5 ± 1.9
4	117.75		117.50		112.2 ● 0.4
5	160.15		159.81		150.6 ± 4.7
6	118.72		120.34		111.6 ± 3.0
7	153.66		159.77		153.3 ± 1.6
8	123.29 (q)	272.89	123.28 (q)	287.90	123.4 ± 0.2
9	72.38		68.08		65.5 ± 1.2
10	58.46		59.01		57.9 ± 0.0
11	70.82		71.16		73.0 ± 2.2
12	169.87		169.92		167.8 ± 2.3
13	51.76		51.89		52.0 ± 0.2
1′	153.43		153.47		152.4 ± 2.9
2′	127.38		127.40		125.3 ± 1.1
3′	128.70 (q)	3.92	128.69 (q)	3.92	128.3 ± 0.2
4′	128.77 (q)	34.24	128.75 (q)	33.26	129.6 ± 1.8
5′	125.84 (q)	3.92	125.86 (q)	3.92	125.4 ± 0.3
6′	122.40		122.36		120.2 ± 2.9

Assignment of Proton and Carbon-13 NMR Spectra. In the assignment of the normal one-dimensional 270-MHz proton NMR spectra, the absorptions due to the protons on the phenyl rings were first determined by homonuclear shift correlation (COSY). The NMR data are shown in Table II, and the proton assignment based on this data is shown in Figure 1. Absorptions a, b, and f were identified as representing resonances on the nitrobenzene ring, based on the observed chemical shift, which is empirically known to be characteristic of a proton adjacent to a nitro group, the observed cross peak between a and b, and the observed long-range coupling between b and f. Absorptions c–e were attributed to resonances on the other ring, since there were no cross peaks between them and the a, b, and f absorption group and were assigned on that ring in essentially the same man-

Table IV. Greenhouse Postemergent Herbicidal Activities and Selectivities of AKH-7088 *E* and *Z* Isomers Separately and in Mixtures of Various Proportions, as Percent Inhibition of Untreated Control Growth Determined for Weeds on Day 14 and for Soybean on Day 21 after Application

E:*Z* ratio	kg/ha	CG	SW	SP	VL	NS	MG	PW	PS	CB	SOY
100:0	0.28	40	100	97	100	100	95	100	97	100	30
	0.14	30	98	60	100	80	80	97	90	99	25
	0.07	25	85	55	100	75	60	95	70	85	15
	0.035	20	80	30	100	70	60	90	60	65	10
75:25	0.28	35	100	98	100	100	85	100	90	95	30
	0.14	35	98	75	100	98	80	100	80	90	15
	0.07	35	85	55	100	95	70	100	75	80	15
	0.035	35	80	40	100	80	60	96	70	70	10
50:50	0.28	35	100	100	100	100	98	100	95	100	35
	0.14	30	98	85	100	100	75	100	90	95	20
	0.07	25	85	65	100	90	60	95	80	75	15
	0.035	25	70	55	100	75	50	85	65	60	9
25:75	0.28	30	100	90	100	100	90	99	92	100	30
	0.14	30	88	78	100	95	85	98	80	97	20
	0.07	30	80	60	100	90	60	90	80	75	15
	0.035	20	75	55	95	70	60	85	60	55	7
0:100	0.28	30	100	90	100	99	98	100	85	100	25
	0.14	25	95	85	100	95	90	100	78	90	20
	0.07	20	90	65	100	95	85	100	75	85	10
	0.035	5	80	60	98	85	60	90	65	80	10
leaf stage		4–5	3.9–5	1.1–2	3.3–4	2.9–4	5	2	2–3.1	4.1–6.1	1.5–2 trifoliates
height, cm		7–15	4.7–7	5–8	8–11	2–3	15–20	2–3	3–5	10–15	16–20

ner as described for a, b, and f. Similar results have been reported for other diphenyl ether herbicides (Lee, 1985).

Assignment of the protons g–j of the substituent group on the nitrobenzene ring was based on the presence of cross peaks between i and j and between g and h, and h and j were readily empirically assigned. Assignment of the carbon-13 NMR spectra was based on heteronuclear (^1H–^{13}C) two-dimensional shift correlation (CHSHF) as shown in Figure 2. The carbonyl carbon (12; *E*, 169.87 ppm; *Z*, 169.92 ppm), and trifluoromethyl carbon (8; *E*, 123.29 ppm, J_{CF} = 272.89 Hz; *Z*, 123.28 ppm, J_{CF} = 287.90 Hz), and the carbon bearing the trifluoromethyl group (4′; *E*, 128.77 ppm, J_{CCF} = 34.24 Hz; *Z*, 128.75 ppm, J_{CCF} = 33.26 Hz) were readily assigned due to their unique chemical environments. The assignments of the absorptions for the 10 hydrogen-bearing carbons (3, 3′, 4, 5′, 6, 6′, 9, 10, 11, 13) was readily achieved by two-dimensional analysis, since the proton NMR spectra of both isomers had been fully assigned. The results are summarized in Table III. The remaining five quaternary aromatic carbons (1, 1′, 2, 2′, 5) and one quaternary oxime carbon (7) were assigned by referring to data from the INKA data base of BASF and the report on other diphenyl ether herbicides (Lee, 1985).

Definitive identification of the two molecular conformations of the isomers, as shown in Figure 1, was achieved by detailed comparison of their carbon-13 NMR spectra, and in particular by the shift evident in Figure 2b toward a high field, of the peak assigned to the carbon fixed spatially on the sp^2 carbon (carbons 9 and 7, respectively), as compared with the corresponding peak in Figure 2a. In carbon-13 NMR spectroscopy, the shift of a peak toward high field is generally known to be attributable to the steric compression effect. Such an effect would be most likely between carbon 9 and proton i in the isomer of AKH-7088 having its oxime moiety extending geometrically in the same direction as its methoxymethyl group. The isomer which showed this peak shift was therefore identified as the zussammen, or cis isomer. That exhibiting no such shift may then be identified as the entgegen, or trans isomer. These two geometrical conformations were also confirmed by X-ray analysis (unpublished data). In the *Z* isomer, moreover, a chemical shift of the sp^2 carbon (7) toward low field, as compared with that in the *E* isomer, was also observed. A similar shift is found in (*Z*)-α-(1,2,4-triazol-1-yl)-2,4-dichloroacetophenone oxime (from the INKA data base of BASF).

The NMR spectra of AKH-7088 have thus been fully assigned with a combination of several two-dimensional NMR techniques, and the usefulness of these techniques in turn has been demonstrated by the precise determination of the geometrical conformations of the *E* and *Z* isomers of AKH-7088.

Biological Activity. The results of the pot test of the *E* and *Z* isomers separately and in mixtures of various proportions are shown in Table IV. The herbicidal activities against sicklepod and nightshade exhibited by the *E* isomer alone appeared to be slightly lower than those exhibited by the *Z* isomer, but the difference was not sufficient to be regarded as significant. The overall results indicate that, against these and all other weeds tested, the herbicidal activities of the *E* isomer alone, the *Z* isomer alone, and the mixtures of both are virtually the same.

With the isomers alone and with their mixtures, crop injury consisted of local and temporary crinkling and speckling, particularly on the youngest leaves, and disappeared rapidly. It was substantially the same for all of the compounds.

Thus no significant difference in biological activity was found between any of the tested compounds.

ACKNOWLEDGMENT

Thanks are expressed to H. Osada for his performance of the biological test.

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Received for review November 8, 1988. Accepted October 5, 1989.

Registry No. 1, 104460-23-3; 2, 104459-75-8; (*E*)-3, 104474-16-0; (*NS*)-3, 104459-85-0; 4, 124482-57-1; 5, 124482-58-2; 3′-(2-chloro-4-(trifluoromethyl)phenoxy]-2-methoxyacetophenone, 104460-45-9; 3-chloro-4-fluorobenzotrifluoride, 78068-85-6; 3′-hydroxy-2-methoxyacetophenone, 54794-31-9.

Metabolism of the Insecticidally Active $GABA_A$ Receptor Antagonist 4-*sec*-[3,4-$^3H_2$]Butyl-1-(4-cyanophenyl)-2,6,7-trioxabicyclo[2.2.2]octane

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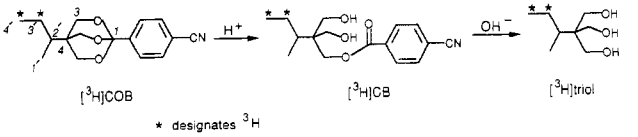
4-*sec*-[3,4-$^3H_2$]Butyl-1-(4-cyanophenyl)-2,6,7-trioxabicyclo[2.2.2]octane (referred to as [^{3}H]COB) was examined as an example of a new class of insecticidally active compounds that block the γ-aminobutyric acid gated chloride channel. Metabolites were identified by thin-layer cochromatography with standards from synthesis and by consideration of their hydrolytic and oxidative degradation products formed in situ on two-dimensional silica gel chromatoplates. Metabolism of [^{3}H]COB by mouse liver and housefly abdomen microsomes is dependent on fortification with NADPH. The *O*-methylene and *sec*-butyl sites are sensitive to oxidation. Each carbon of the *sec*-butyl group is individually functionalized with strong preference for the methylene site in the mouse but not the housefly microsomal system. *O*-Methylene hydroxylation initiates spontaneous cage opening to form an aldehyde that undergoes metabolic reduction, ultimately yielding the same cyanobenzoate ester of 2,2-bis(hydroxymethyl)-3-methylpentan-1-ol formed by direct hydrolysis. Houseflies injected with [^{3}H]COB form many if not all of the same metabolites, with major products being the aforementioned cyanobenzoate, the orthoester oxidized at the *sec*-butyl methylene site, and polar conjugates.

1,4-Disubstituted 2,6,7-trioxabicyclo[2.2.2]octanes are of interest as in vitro and in vivo probes for the γ-aminobutyric acid (GABA) gated chloride channel (Casida et al., 1985, 1988; Casida and Palmer, 1988) and as a new class of insecticidally active compounds (Palmer and Casida, 1985, 1987). 4-*tert*-Butyl-1-[^{3}H]phenyl-2,6,7-trioxabicyclo[2.2.2]octane (TBOB), used as a radioligand for the $GABA_A$ receptor–ionophore (Lawrence et al., 1985), is metabolized in houseflies, mice, and their microsomal oxidase systems to form cage-opened products and metabolites tentatively identified as involving modifications on both the 1- and 4-substituents (Scott et al., 1987). A newer radioligand probe is 4-*sec*-[3,4-$^3H_2$]-butyl-1-(4-cyanophenyl)-2,6,7-trioxabicyclo[2.2.2]octane (Nicholson et al., 1988) with enhanced biological activity relative to TBOB, i.e., housefly topical LD_{50} of 45 μg/g alone or 1.1 μg/g on pretreatment with the microsomal oxidase inhibitor piperonyl butoxide (PB), mouse intraperitoneal LD_{50} of 0.56 mg/kg, and 10-fold higher potency at the receptor target (Casida et al., 1988). This cyanoorthobenzoate (COB) undergoes cage opening in acid to form the cyanobenzoate (CB), which is hydrolyzed by base to the triol. This report considers the metabolic



fate of [^{3}H]COB in microsomal oxidase systems from mice and houseflies and in houseflies in vivo. Metabolites were identified by thin-layer chromatography (TLC) involving cochromatography with authentic standards or they were tentatively identified by comparisons of the hydrolytic and oxidative degradation products formed in situ on two-dimensional silica gel chromatoplates.

MATERIALS AND METHODS

Designations. Abbreviations are used as follows: 1′, 2′, 3′, and 4′ = sites undergoing metabolism in the *sec*-butyl group; AB = the aminobenzoate from hydrolysis of 1-(4-amidophenyl)-

0021-8561/90/1438-0850$02.50/0