

A Study on the Evolution of 3,4-DCA and TCAB in Some Selected Soils [Part I]

A New Method of Synthesizing
¹⁴C-ring-labeled and Non labeled TCAB

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數種土壤中에서 3,4-DCA 및 TCAB의 變化에 관한 研究〔第一報〕

¹⁴C-環標識 및 非標識 TCAB의 新合成方法

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SUMMARY

Much attention has been paid to the fact that quite a few herbicides such as phenylcarbamates, phenylureas, and acylanilides form azo compounds known as carcinogens by virtue of the microorganisms in soil. In consequence, many investigators synthesized TCAB, an azo compound, starting from 3,4-dichloronitrobenzene for the related studies. However, the authors were under the necessity of synthesizing ¹⁴C-ring-labeled TCAB from ¹⁴C-ring-labeled 3,4-DCA available, in addition to making up for the disadvantage of dechlorination in the reduction of 3,4-dichloronitrobenzene. The new method is as follows: TCAB, ¹⁴C-ring-labeled and non-labeled, was produced by aerial oxidation of 3,4-DCA catalyzed by CuCl with pyridine as solvent at 60°C for 5-12 hrs, giving 80.2% yield. The procedure for purification was described in detail. The identities of TCAB isomers were confirmed by means of autoradiography, TLC, GLC, IR, and MS.

Introduction

The fate and persistence of pesticides released in the field bear great importance in relation to the environmental contamination and public health. The major pathways involved in the environmental chemistry of pesticides are biological meta-

bolism and photolysis. Above all, a number of herbicides composed of the substituted aniline moiety, such as phenylcarbamates, phenylureas, and acylanilides produce the aniline as one of their degradation products by micro-organisms in soil. ^(5,18) For one thing, the bulk of the 3,4-dichloroaniline (hereinafter 3,4-DCA) derived microbiologically from those herbicides is chemi-

cally bound to humic substances. (7,13) It also appears to undergo oxidation to CO₂ and chloride ion, condensation to form 3,3', 4,4'-tetrachloroazobenzene (hereinafter TCAB), (2,3,4,8,9,15,21,34) volatilization, (7,9) and mineralization. (7) The formation of azo compounds through the action of peroxidase, (2,15) especially, calls our attention in that certain azo compounds are known to be carcinogenic. (40) And hence, as part of the study on the evolution of 3,4-DCA and TCAB in various soils by means of radio-labeling in connection with public safety, the establishment of a new method synthesizing ¹⁴C-ring-labeled TCAB from ¹⁴C-ring-labeled 3,4-DCA was badly needed, since the method of Corbett et al. (14) and that of the oxidation of aniline with active manganese dioxide, (1,6,41) adopted by quite a few investigators, (3,4,8,11,12,13,20,22,28,29,31) turned out to be unsatisfactory. The purpose of this paper is to work out a new method of synthesis of non-labeled and ¹⁴C-ring-labeled TCAB as the first step toward the subsequent studies.

Materials and Methods

Purification of non-labeled 3,4-DCA

3,4-DCA purchased was recrystallized three times from chloroform. The persistent colored impurities were removed as follows; the sample was refluxed in 70% EtOH on a water-bath with charcoal treatment. The mixture was filtered by suction and the filtrate was poured into cold distilled water. The resulting white slurry was collected by suction filtration, washed with cold n-hexane, and dried. White crystals were obtained in pure form. The purity was established by GLC, TLC, and melting point determination (72~73°C).

Preparation of the sodium hypobromite solution: (38)

The solution was prepared by adding 10 gr (3.2ml) of bromine dropwise to a cold solution of 6.0 gr of NaOH in 75ml of distilled water immersed in an ice bath. Pyridine was refluxed over KOH pellets for being dry, and then distilled with careful exclusion of moisture. The

pyridine obtained has b. p. 115.5/760mm.

Mass spectra were obtained with a VARIAN MAT GmbH, CH 5 Massenspektrometer.

Infrared spectra were determined as KBr disks on a Perkin-Elmer 457 Grating Infrared Spectrophotometer.

Gas-liquid chromatographic analyses were performed with an F & M Scientific 5750 Research chromatograph (Hewlett-Packard) equipped with a flame ionization detector. The column-packing was a 5% SE-30 (methyl) on 68-80 mesh Chromosorb W. Operating parameters were as follows: Stainless steel column of 9 ft × 1/8 in. outer diam (O. D.); injector temperature of 230°C; detector temperature of 230°C; nitrogen carrier flow of 23 ml/min. The analysis was made by temperature-programming from 80°C (post injection interval: 1 min) to 220°C (upper limit interval: 10min) at a rate of a linear temperature increase of 6°C per min.

For *column chromatography*, silicic acid (SiO₂ · xH₂O, 100 mesh, Analytic Reagent, Mallinckrodt Chemical Works, 2847) was activated by heating at 110°C for 16 hours.

TLC was performed with 5196494 X, Art. 5554, DC-Alufolien, Kieselgel 60 F₂₅₄ (25 Folien 20 × 20 cm), Schichtdicke 0.2mm, E. Merck, Darmstadt. A mixture of n-hexane and benzene (7 : 3 v/v) was used as the solvent system.

The *radioactivity* was measured with a Packard Model 3375 Tri-Carb Scintillation Spectrometer. ¹⁴C-ring-labeled 3,4-DCA (specific activity, 3.5 mCi/mmol; melting point, 72~73°C) was supplied by The Radiochemical Centre, Amersham in a sealed borosilicate glass ampoule. The ampoule was opened by lightly scratching the serration with the file provided and carefully snapping off the upper end. The specific activity was determined by placing 10μl of the sample in scintillation vials containing 10ml of toluene scintillation fluid being composed of 4 gr of PPO (2,5-diphenyl oxazole) and 0.5gr of POPOP {p-bis-[2-(5-phenyl oxazolyl)]-benzene} per liter. *The location of radioactive spots on chromatograms* was determined by autoradiography.

Results

Synthesis of non-labeled TCAB

A mixture of 1.62gr 3,4-DCA, 0.6gr CuCl, and 40ml pyridine was treated with air at 60°C for 5 hrs. After reacted, the mixture was evaporated to dryness on a rotary evaporator to remove pyridine. The residue was dissolved in benzene, charcoal and Celite 545 being added to it, and refluxed on water-bath. While hot, it was filtered by suction. The filtrate was passed through silicic acid column with benzene as eluant. The eluent was concentrated on a rotary evaporator, and orange-colored crystals were obtained.

Purification of non-labeled TCAB

The crude TCAB was suspended in 50% EtOH and refluxed on water-bath to dissolve the unreacted 3,4-DCA. The mixture was filtered by

suction while hot and the filtrate was discarded. This treatment was repeated three or four times. For the complete removal of the unreacted 3,4-DCA, the dried TCAB was redissolved in ethyl acetate, the solution transferred into a separatory funnel, and 2N-HCl was added to it. This mixture was vigorously shaken and allowed to stand. The lower aqueous layer was discarded. This procedure was repeated three or four times. The upper TCAB layer was washed with distilled water three or four times, dried over anhydrous MgSO₄, filtered through Whatman filter paper, and concentrated to dryness on a rotary evaporator. The resulting crystal has a melting point of 158–158.5°C. The purity was established by thin-layer and gas-liquid chromatography, melting point determination, and mass spectral analysis. The mass spectrum of TCAB is presented in Figure 1.

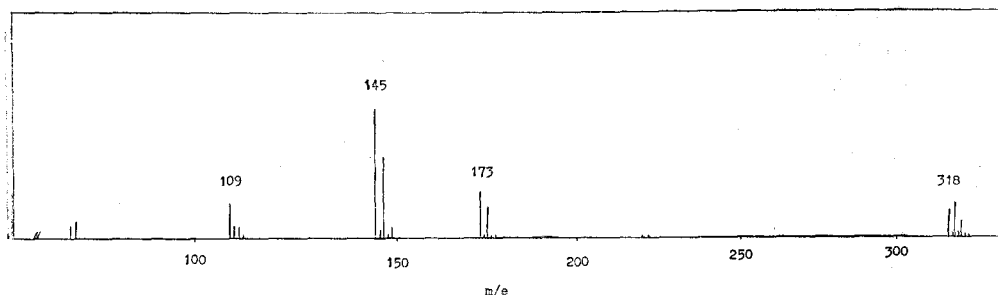


Figure 1. Mass spectrum of TCAB showing that cis- and trans-isomers are identical.

Synthesis of ¹⁴C-ring-labeled TCAB

The ¹⁴C-ring-labeled 3,4-DCA purchased, 2.3 mg, was diluted with non-labeled 3,4-DCA, 17.7 mg, to give a total amount of 20mg. A mixture of 20mg the diluted 3,4-DCA, 25mg CuCl, and 1.5ml pyridine was treated with air at 60°C for 12 hrs. After reacted, the reaction mixture was evaporated to dryness by bubbling with a mild stream of air to remove pyridine. The residue dissolved in n-hexane was passed through the silicic acid column with n-hexane as eluant. The eluate was evaporated to dryness on a rotary evaporator and the remaining residue was redissolved in a small amount of benzene. The residue was developed on TLC plates of 0.25mm thickness with a mixture of n-hexane and benzene (7 : 3

v/v) as the solvent system. Bands corresponding to ¹⁴C-ring-labeled TCAB were scraped off, extracted with benzene and acetone successively, and evaporated to dryness on a rotary evaporator. CuCl and pyridine were added again to effect the reaction completely and the same procedure as above was followed.

Purification of ¹⁴C-ring-labeled TCAB

As the synthesized ¹⁴C-ring-labeled 3,3', 4,4'-tetrachlorohydrazobenzene), the following step was taken: to the 20mg of ¹⁴C-ring-labeled TCAB dissolved in 3 ml of ether, 2 ml of NaOBr solution were added and vigorously shaken for 10min on a Vortex-Genie,⁽⁸⁹⁾ The ether layer was taken with a spoid and evaporated to dryness with a mild stream of air. The residue was dissolved in

benzene, applied onto the TLC plates as a band and developed with a mixture of n-hexane-benzene (7:3 v/v) as the developing solvent. Bands corresponding to ^{14}C -ring-labeled TCAB were scraped off, extracted with acetone, and concentrated to a small volume with a stream of air. The homogeneity of the material was established by gas-liquid and thin-layer chromatography. The specific activity was $1.01\mu\text{Ci}/\mu\text{mole}$.

Separation of cis-TCAB from trans-TCAB

In spite of repeated and painstaking attempts to purify the ^{14}C -ring-labeled TCAB, the autoradiography showed that it was a mixture of two forms, that is, cis- and trans-TCAB (see Figure 2). For the continued research on the isomerization

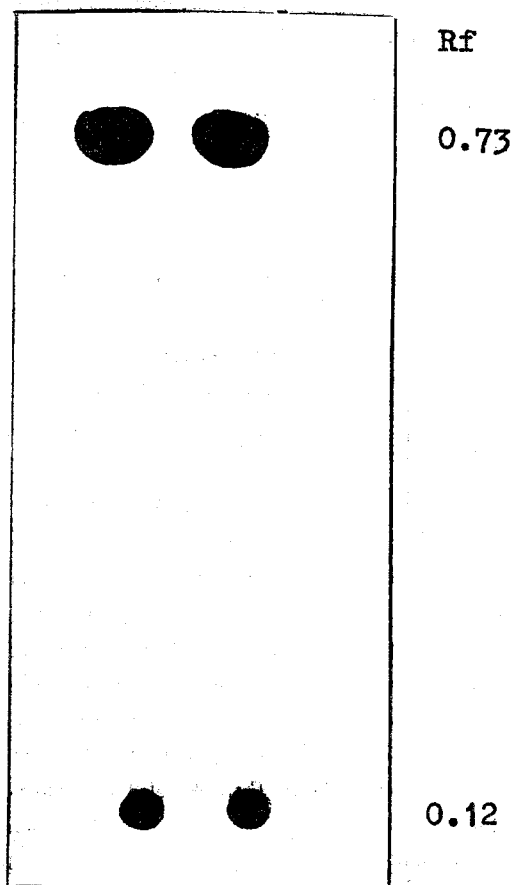


Figure 2. Autoradiogram of the purified ^{14}C -ring/labeled TCAB indicating the presence of cis-(lower) and trans-(upper) isomers.

of TCAB in soil, it was needed to separate the two forms in pure states. The following procedure turned out to be suitable for that purpose.⁽¹⁰⁾ In brief, non-labeled TCAB, 0.2 gr, was dissolved in 220 ml of glacial acetic acid and allowed to stand in the light for 69.5 hrs being stirred by a magnetic stirrer.

After that time, the solution was agitated by a magnetic stirrer and 198 ml of distilled water were dropwise into it to isolate the trans-TCAB as slurry. The yellowish slurry of the trans-isomer was removed by suction filtration and 220 ml of distilled water were added to the filtrate (the cis-isomer portion). The diluted filtrate was extracted with 50 ml of chloroform on a separatory funnel. The chloroform solution was evaporated to dryness by bubbling with nitrogen gas and crystallized with petroleum ether. The crystal was collected and stored in a refrigerator. The same procedure was repeated for the remaining trans-TCAB.

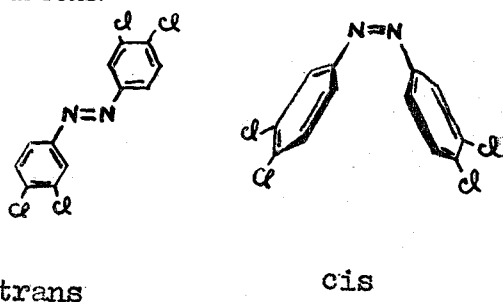


Figure 3. Spatial configurations of cis- and trans-TCAB.

Synthesis of TCAB by the method described by Corbett et al.⁽¹⁴⁾

3,4-Dichloro-nitrobenzene, 200 mg, was dissolved in ether and LiAlH_4 , 200 mg, was added with care in small amounts to it. The mixture was allowed to react for approximately 1 hr at room temperature. The reaction mixture then was heated on a water-bath for approximately 15 min; then, following removal of excess LiAlH_4 , the solution was filtered and dried on anhydrous MgSO_4 . TCAB was purified on a column of silica gel eluted with benzene. The material was recrystallized from n-hexane as bright-orange crystals.

Synthesis of TCAB by the method of the oxidation of aniline with active manganese dioxide^(1,6,41)

3,4-Dichloro-aniline, 1.62 gr, dissolved in 65 ml of benzene was refluxed for 20 hrs with 5.2 gr of

MnO₂ which had been previously activated at 120°C for 72 hrs. The reaction mixture was filtered by suction and the filtrate concentrated. The product was recrystallized from n-hexane.

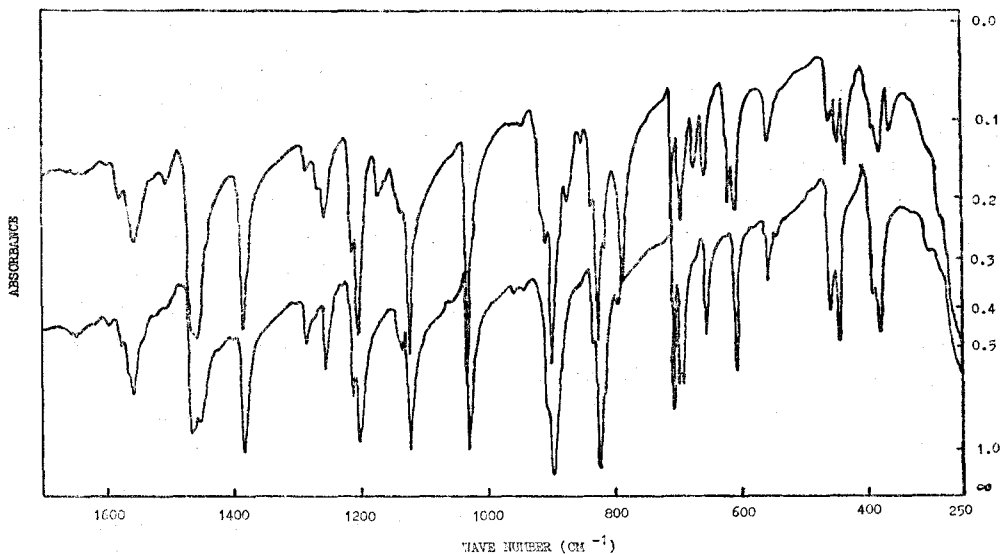


Figure 4. Infrared spectra showing a little difference between cis-(upper) and trans-(lower) TCAB.

Discussion

In the investigations on TCAB formation from certain herbicides applied in soil, quite a few investigators synthesized the authentic compound, labeled or non-labeled, according to mostly the method described by Corbett et al.⁽¹⁴⁾ and to that of the oxidation of aniline with active manganese dioxide,^(1,41) respectively. Nevertheless, it was pointed out that synthesis of TCAB (the method of Corbett et al.) by LiAlH₄ reduction of the corresponding chlorinated nitrobenzenes was accompanied by reductive dechlorination. That is, the product contained traces of trichloroazobenzene and dichloroazobenzene on examination by GLC-mass spectrometry. Dechlorination of chlorinated nitroarenes by LiAlH₄ has been previously noted where chlorine is situated ortho to the nitro group and to a second chlorine

atom.⁽²⁸⁾ These by-products can give rise to confusion in the identification of degradation products in soil. The same phenomenon was also recognized by the authors in the course of synthesizing TCAB by the method of Corbett et al. Namely, on TLC, the product exhibited several impurities which were considered to be dechlorination products other than TCAB. The reason why the authors in this investigation were obliged to work out a new method was to synthesize TCAB free from dechlorination impurities, starting from the ring-labeled ¹⁴C-3,4-DCA available, not from ring-labeled ¹⁴C-3,4-dichloro-nitrobenzene.

In addition, LiAlH₄ in ether is very dangerous to handle. As an alternative, the authors tried to adopt another method. Previously, Barakat et al.⁽¹⁾ reported the oxidation of o- and p-chloro aniline and p-toluidine to corresponding azobenzenes by solid manganese dioxide in light petro-

leum solution in 87% yield.

Later, Wheeler et al.⁽⁴¹⁾ reported that they synthesized azobenzenes with active manganese dioxide in benzene solution in over 90% yield from p-fluoro-, p-chloro-, p-bromo- and p-iodo-aniline, p-anisidine, p-biphenylamine, m-chloro-, o-fluoro-, o-iodo- and o-ethyl-aniline and o-anisidine. In the case of 3,4-DCA, however, with all the strenuous and painstaking efforts, TCAB could not be obtained.

Finally, the authors found out the information that primary aromatic amines are readily oxidized to azobenzenes by cuprous chloride and oxygen when pyridine is used as the solvent.^(2,24,25,32,35,36,37) Up to the present, however, no one tried to employ this principle in the synthesis of TCAB for the research of herbicide degradations in soil. For the first time, Terentev et al.⁽³⁷⁾ obtained 4,4'-dichloroazobenzene from 4-chloroaniline in 73% yield by this aerial oxidation catalyzed by CuCl in pyridine. The authors attempted to employ the same principle in synthesizing TCAB from 3,4-DCA with some modifications. TCAB was obtained in 80.2% yield without any traces of dechlorination products which were seen in the method of Corbett et al.⁽¹⁴⁾ The only impurities present were the unreacted 3,4-DCA and the possible formation of 3,3',4,4'-tetrachloroazobenzene. These two compounds were readily eliminated with the 2 N-HCl⁽³⁸⁾ and sodium hypobromite solution, respectively, as shown. On the examination of the product on TLC, it proved that the non-labeled TCAB represented only one form which later turned out to be the trans isomer; whereas, the ring-labeled ¹⁴C-TCAB showed two forms, one of which constituted a small amount compared with the other as seen in the autoradiogram (Figure 2).

In the case of non-labeled TCAB synthesis, it was heated on a water-bath and obtained as crystalline forms. That was why the product was composed of only one form, trans isomer which is more stable, since the thermal conversion between cis- and trans-forms occurs only in the direction of cis-to trans-form.⁽¹⁹⁾ Meanwhile, be-

cause the ring-labeled ¹⁴C-TCAB was obtained in the form of acetone solution, the possibility can not be ruled out that the solution will contain cis- and trans-isomers at a certain ratio at the same time. The autoradiogram shows qualitatively the relative ratio. As a strong support to this observation, it was already found that solutions of azobenzene in MeOH on exposure to daylight or to a tungsten light in glass flasks give an equilibrium mixture of 27-28% cis- and 73-2% trans-azobenzene.⁽¹⁶⁾

According to Beveridge et al.⁽¹⁰⁾, the geometry of cis-azobenzene giving the most consistent interpretation of the electronic spectrum is the propeller-shaped conformation wherein the phenyl rings are rotated approximately 30° out of the plane (see Figure 3). This is presumably due to the steric interaction of ortho hydrogens. Meanwhile, in the trans-isomer, the phenyl ring is coplanar with the azo group. This coplanarity is effectively inhibited in the cis-isomer owing to steric hindrance.⁽¹⁷⁾ The formation of azobenzenes from anilines depends on the position of chlorine substituents as well as on a number of other factors. Chlorination of the 2,5- or 2,6-positions stabilized the molecule and protected aniline from biochemical transformation.⁽⁸⁾

Whereas, pesticides partly composed of a chloroaniline unsubstituted in both ortho (2,6-) positions, or in adjacent ortho and meta (2,3- or 5,6) positions, are likely to give rise to azo compounds during decomposition in soil.⁽⁶⁾

Comparison of cis-and trans-TCAB

The geometrical structures of the two isomers are given in Figure 3.⁽³⁰⁾ The autoradiogram of the synthesized ¹⁴C-ring-labeled TCAB always exhibited two radioactive spots, one of which is much more active than the other (see Figure 2). In view of the fact that this material was well purified, these two spots will have to correspond to the geometrical isomers, cis- and trans-TCAB. The results of TLC and GLC of the products are shown in Table I and Table II, respectively.

In order to verify that these two materials

Table I. Rf values of products obtained from the TCAB synthesis

Compounds	Rf values
3,4-DCA	0.05
cis-TCAB	0.12
trans-TCAB	0.73

Table II. GLC results of TCAB isomers

TCAB isomers	Retention time(min.)
trans-TCAB	27
cis-TCAB	34.4

are geometrical isomers, their mass spectra were obtained separately. As the mass spectra of cis- and trans-TCAB were completely identical, only one is shown in Figure 1.

TCAB, cis and trans alike, exhibits a mass spectrum with a parent ion at m/e 318 with M, M+2, M+4, M+6, M+8 peaks indicating the presence of four chlorines. Other prominent peaks at m/e 173 (2Cl) ($M-C_6H_2Cl_2$), 145 (2Cl) ($M-C_6H_3Cl_2, N_2$), and 109 (1 Cl) ($M-C_6H_3Cl_2, N_2, HCl$) are in accord with the TCAB structure.

The ir spectra of these two isomers are given in Figure 4. In the spectra, in general, they are almost identical except that the intense absorption at $790cm^{-1}$ and the $-N=$ stretching vibration at $1508cm^{-1}$ were shown only by the cis TCAB^(26,27) Generally, the $-N=N-$ stretching vibration occurs between $1630cm^{-1}$ and $1575cm^{-1}$, but the observed absorption in these spectra at $1560cm^{-1}$ will be probably due to the reduction by chloro-substitution in the structure.⁽³⁰⁾ The C-Cl stretching vibrations are shown at $800-600cm^{-1}$.

要 約

Phenyl carbamates, phenyl ureas 및 acylanilides 등의除草劑는 土壤中에서 微生物의 作用에 依하여 發癌性 物質로 알려진 Azo 化合物을 形成하여 많은 注目を 끌고있다. 따라서 많은 研究者들은 이의 研究에 있어 azo 化合物의 一種인 TCAB

를 3,4-dichloro nitrobenzene으로부터 合成하였으나 脫鹽素化 作用 등의 缺點이 있고 또한 本 研究에서는 環標識 ^{14}C -3,4-DCA로부터 環標識 ^{14}C -TCAB를 合成할 必要가 있었으므로 本方法을 考案하였다. 即 3,4-DCA를 Pyridine에 녹인후 空氣存在下에서 $60^{\circ}C$ 로 5-12 時間 동안 $CuCl$ 과 反應시켜 80.2% 收率로 標識 및 非標識 TCAB를 合成하였으며 그의 精製過程을 詳細히 記述하였다.

또한 TCAB 異性體의 性質을 Autoradiography, TLC, GLC, IR 및 M.S로 檢討 確認하였다.

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