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Metabolism of (*S*)-bioallethrin and related compounds in humans

Gabriele Leng *, Karl-Heinz Kühn, Brigitte Wieseler, Helga Idel

Institute of Hygiene, Heinrich–Heine University Düsseldorf, Moorenstraße 5, 40225 Düsseldorf, Germany

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Abstract

Chrysanthemate insecticides like (*S*)-bioallethrin, natural pyrethins, and related pyrethroids are subjected to extensive hydrolytic and oxidative degeneration by the mammalian metabolism, leading to a complex series of metabolites partially conjugated and finally eliminated in the urine. The major oxidation products of chrysanthemic acid, *cis*-(E)- and *trans*-(E)-chrysanthemumdicarboxcylic acid (*cis*-(E) and *trans*-(E)-CDCA), were synthesized and their structures were established by nuclear magnetic resonance spectrometry (H^1-NMR) and mass spectrometry (MS). Diastereoselective separation was by high performance liquid chromatography (HPLC) and capillary gas chromatography (GC). An analytical method for extraction and identification of CDCA from human urine was developed. Quantitation was by gas chromatography and electron-impact mass spectrometry (GC/MS). The limit of detection was 20 μg/l for *cis*-(E)-CDCA and 10 μg/l for *trans*-(E)-CDCA. To test the applicability of the presented method, urine samples of humans exposed to (*S*)-bioallethrin were investigated. Urinary peak excretion of *trans*-(E)-CDCA occurred within 24 h after exposure.

Keywords: (*S*)-Bioallethrin; Chrysanthemumdicarboxylic acid; Human metabolism; Synthesis; Biomonitoring method

1. Introduction

The synthetic pyrethroid insecticides have their origin in the important botanical insecticide pyrethrum, an extract obtained from the flowers of *Chrysanthemum cinerariaefolium*. The six natural esters of pyrethrum (pyrethrins, jasmolins and cinerins) are effective against a wide range of household and public health insects (Fig. 1). Nowadays, pyrethrum is formulated as aerosol or spray with synergists (e.g. piperonyl butoxide) for the use as a rapid knock-down or flushing agent in industry and in the home.

Purified pyrethrins are non-polar, viscous liquids susceptible to decomposition by ultraviolet light, oxygen, acid, and alkali. The replacement of specific structural elements found in pyrethrin I with moieties designed to increase the stability in the environment was an important strategy for the development of the first synthetic pyrethroids like allethrin. In such early analogs, the chrysanthemic acid moiety (CA; $X = CH_3$) was kept in-

* Corresponding author. Tel.: +49-211-8112607; fax: +49-211-8112919.

E-mail address: leng@uni-duesseldorf.de (G. Leng)

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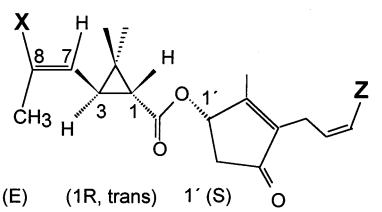
tact and only a minor change in the molecular structures of esterifying alcohols were made. Thus, in contrast to the natural pyrethrins, allethrin is less volatile and more stable to heat and light.

The presence of two chiral centers (C-1 and C-3) in the cyclopropane ring of CA produces two pairs of diastereomers, commonly designed *cis* or *trans* based on the orientation of the substituents in relation to the plane of the cyclopropane ring (Figs. 1 and 2). Whereas the acid moieties of the natural pyrethrins are exclusively in the [1R, trans] configuration, esters of synthetic CA were initially evaluated as racemic mixtures. Beside the stereochemistry of the acid moiety, the chiral center (C-1′) in the alcohol group plays also a significant role for a high insectical activity of pyrethroids. Among the possible eight stereoisomers in the racemic mixture of allethrin, (*S*)-bioallethrin ([1R, trans; 1′S]) is the most biologically active followed by the [1R, cis; 1′S]-isomer (WHO, 1989a). Consequently, (*S*)-bioallethrin is several times more effective against crawling and flying insects as allethrin or the natural pyrethrins.

This substantial increase in potency is responsible for the use of a single stereoisomer as active ingredient in many insecticide formulations. Furthermore, (*S*)-bioallethrin has served as an important model compound in numerous animal studies (Elliott et al., 1972; Class et al., 1990; Ando et al., 1991a).

Based on the binding assay on the animal gamma-aminobutyric acid (GABA) receptor-ionophore complex, ester pyrethroids are divided in two major classes: Type I pyrethroids without an α-cyano group and Type II pyrethroids bearing an α-cyano-3-phenoxybenzyl group in the alcohol moiety (Gammon et al., 1982; Lawrence and Casida, 1983). Like the parent compound allethrin, (*S*)-bioallethrin is classified as Type I pyrethroid. The Type I or 'T'-syndrome involves hyperactivity, tremor, convulsion and paralysis in mammals and insects. The primary mechanism of neurotoxic action is determined by an extended opening of the sodium channel in the nerve membranes, leading to prolonged depolarization and induction of repetitive nerve impulses (Aldrige, 1990; Vijverberg and van den Bercken, 1990; Naharashi, 1992). The overall results of such a transient increase in the sodium permeability is associated with pronounced repetitive activity in sense organs and in sensory nerve fibers (Van den Bercken, 1977). While all pyrethroids have essentially the same basic mechanism of action, however, the rate of relaxation differs substantially for



chrysanthemate: (CA)	cinerin I	X = CH_3, Z = CH_3
	jasmolin I	X = CH_3, Z = CH_2CH_3
	pyrethrin I	X = CH_3, Z = CH=CH_3
pyrethrate: (PA)	cinerin II	X = CO_2CH_3, Z = CH_3
	jasmolin II	X = CO_2CH_3, Z = CH_2CH_3
	pyrethrin II	X = CO_2CH_3, Z = CH=CH_3
(S)-bioallethrin		X = CH_3, Z = H, 1′ (S)
bioallethrin		X = CH_3, Z = H, 1′ (R/S)
cis/trans-allethrin		8 stereoisomers (racemic mixture)

Fig. 1. Structures of pyrethrin I, pyrethrin II (only trans configuration of CA and PA are found in nature) and allethrin (racemic mixture of *cis*-/*trans*-CA).

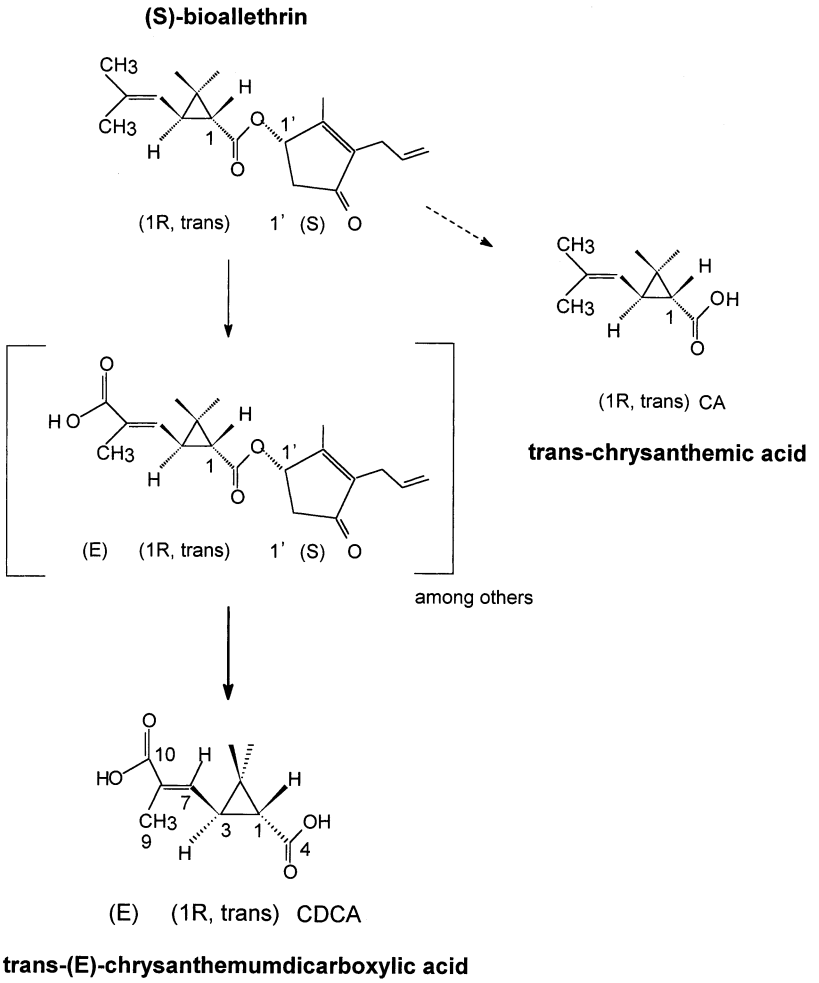


Fig. 2. Biooxidation of the isobutenyl moiety in the tentative metabolic pathway for (S)-bioallethrin in humans.

the various compounds. Allethrin and related pyrethroids are known to cause a transient itching or burning sensation in human skin (paresthesia).

However, due to the poor oral and dermal uptake and followed by a rapid metabolism, these insecticides are relatively safe for birds and mammals. The acute oral toxicity of allethrin and its isomers for rats is moderate to weak (LD_{50}: 378–2430 mg/kg body weight; WHO, 1989b).

Nevertheless, human exposure will be mainly through inhalation, often in combination with an oral uptake of residues of aerosol sprays and from other household applications (e.g. electro mate, mosquito coil). It is anticipated that their extensive use indoors, especially in narrow and poorly ventilated spaces has resulted in elevated insecticide concentrations, bearing a potential source of human exposure (Class and Kintrup, 1991).

Generally, when chrysanthemate insecticides are administered to mammals, detoxification takes place in part by hydrolysis, oxidation and finally conjugation, transforming the compounds into more water soluble urinary metabolites (Elliott et al., 1972; Class et al., 1990).

In previous animal studies, ester hydrolysis and oxidation of the (E)-methyl group of the isobutenyl side chain have been identified as major metabolic pathways of CA (Elliott et al., 1972;

Ueda et al., 1975; Brown et al., 1985) leading to the formation of the corresponding chrysanthemumdicarboxylic acid (CDCA) in high yields (Fig. 2). Furthermore, *trans*-(E)-CDCA is also a major product of the hydrolysis of pyrethric acid (PA; $X = CO_2CH_3$) and related natural esters of pyrethrum (pyrethrin II, jasmolin II and cinerin II) (Straudinger and Ruzicka, 1924; Crombie et al., 1957, 1970).

This is in contrast to the metabolism of sufficient photostable pyrethroids (e.g. permethrin, cyfluthrin, cypermethrin or deltamethrin) whereas the isobutenyl moiety is replaced by a halovinyl group. After a human exposure to these insecticides a significant amount of the unchanged halogenated acid moiety is present in the urine (Eadsforth et al., 1988; Woollen et al., 1992; Kühn et al., 1996; Leng et al., 1997a,b).

Moreover, oxidation and epoxidation followed by hydration of the alcohol moiety of (*S*)-bioallethrin leads to a series of more complex metabolites. Nevertheless, these epoxy and hydroxy derivatives are clearly unsuitable for a human biomonitoring without further study as they exhibited a remarkable species specificity in their metabolism (Class et al., 1990; Ando et al., 1991b).

Such precedents suggests that the acid moiety in chrysanthemate insecticides is metabolized in humans and mammals by similar hydrolytic and oxidative routes, leading to the formation of CDCA as an important biomarker of internal burden.

2. Materials and methods

2.1. Designations

The structures of (*S*)-bioallethrin, *trans*-chrysanthemic acid (*trans*-CA) and its oxidation product *trans*-(E)-chrysanthemumdicarboxcylic acid (*trans*-(E)-CDCA) are given in Fig. 2. The stereoisomers about the cyclopropane ring are designated as cis or trans and of the side chain from oxidation of the chrysanthemate isobutenyl methyl groups as E. In the older literature the E stereochemistry of the olefinic linkage is often also indicated as $trans_o$ (Crombie et al., 1957).

2.2. Chemicals

The ethyl esters of *cis*/*trans*-CA, 2-phenoxybenzoic acid (2-PBA), selen dioxide, activated manganese dioxide, sodium cyanid, 3,3-dimethylacrolein (β-methyl-crotonaldehyde), ethyl α-bromopropionate, ethyl diazoacetate, tert. butyl methyl ether, ligronin, zinc, and copper powder were purchased from Sigma–Aldrich (Steinheim, Germany). (*S*)-bioallethrin and the natural pyrethrins were from Ehrensdorfer (Augsburg, Germany), potassium hydroxide, hexane, toluene, ethanol, methanol and propanol were from Merck (Darmstadt, Germany), conc. hydrochloric and conc. sulphuric acid were purchased from Baker (Deventer, The Netherlands), all other chemicals were from Promochem (Wesel, Germany).

2.3. Synthesis of cis-(E)- and trans-(E)-chrysanthemumdicarboxcylic acid

The required metabolite *trans*-(E)-CDCA and its *cis*-(E) isomer were prepared by two independent methods:

(A) A mixture of ethyl cis/trans-chrysanthemate (2.0 g of *cis*/*trans*-CA-Et) with selen dioxide (1.6 g of active SeO_2) in ethanol (20 ml) refluxed for 1 h lead to the formation of the corresponding aldehyde (1.0 g of *cis*/*trans*-[(E)-CHO]-CA-Et, Figs. 3 and 4) possessing only E configuration in the isobutenyl group (Crombie et al., 1970; Brown et al., 1985). The obtained aldehyde (0.1 g of *cis*/*trans*-[(E)-CHO]-CA-Et) was stirred with a mixture of sodium cyanid (0.08 g), acetic acid (0.03 g), and manganese dioxide (0.6 g) in 10 ml ethanol for 16 h at room temperature (Corey et al., 1968). After hydrolysis of the diethyl chrysanthemum-dicarboxylates (Crombie et al., 1957), the formatted diacids were subjected to purification and diastereoselective separation by preparative high performance liquid chromatography (HPLC).

The overall yield of (E)-*trans*-CDCA and (E)-*cis*-DCCA was in the order of 42%.

(B) An alternative route to avoid the toxic selen dioxide starts with a Reformatski reaction between β-methylcrotonaldehyde (11.0 g), ethyl α-

bromopropionate (25.0 g) and zinc (9.0 g) in toluene (25 ml) yielding the conjugated ethyl 2,5-dimethylhexa-2,5-dienoate (2,5-dimetylsorbate) (8.7 g) (Crombie et al., 1957). In a second step, 2,5-dimetylsorbate (8.5 g) and ethyl diazoacetate (10.7 g) in ligroin (20 ml) were used in an copper-catalyzed condensation (0.5 g of Cu) to build up the desired cyclopropane derivatives of CDCA (6.0 g of *cis*-(E)/*trans*-(E)-DCCA) (Harper and Reed, 1955; Crombie et al., 1957; Ettlinger et al., 1957). Again, after hydrolysis, diastereoselctive separation, and purification using preparative HPLC, the overall yields of the free diacids were in the same order of magnitude and have been proofed to be spectroscopically (gas chromatography and mass spectrometry (GC/MS)) identical with the separated diacids of the first preparation. The latter method has the additional benefit to prepare larger quantities of DCCA more easily.

The hydrolysis of natural pyrethrins with potassium hydroxide (5 g) in ethanol (50 ml) gave a mixture of *trans*-CA and *trans*-(E)-CDCA (Crombie et al., 1970). Subsequent esterification of the mixture with ethanol produced the corresponding esters having identical chromatographical proper-

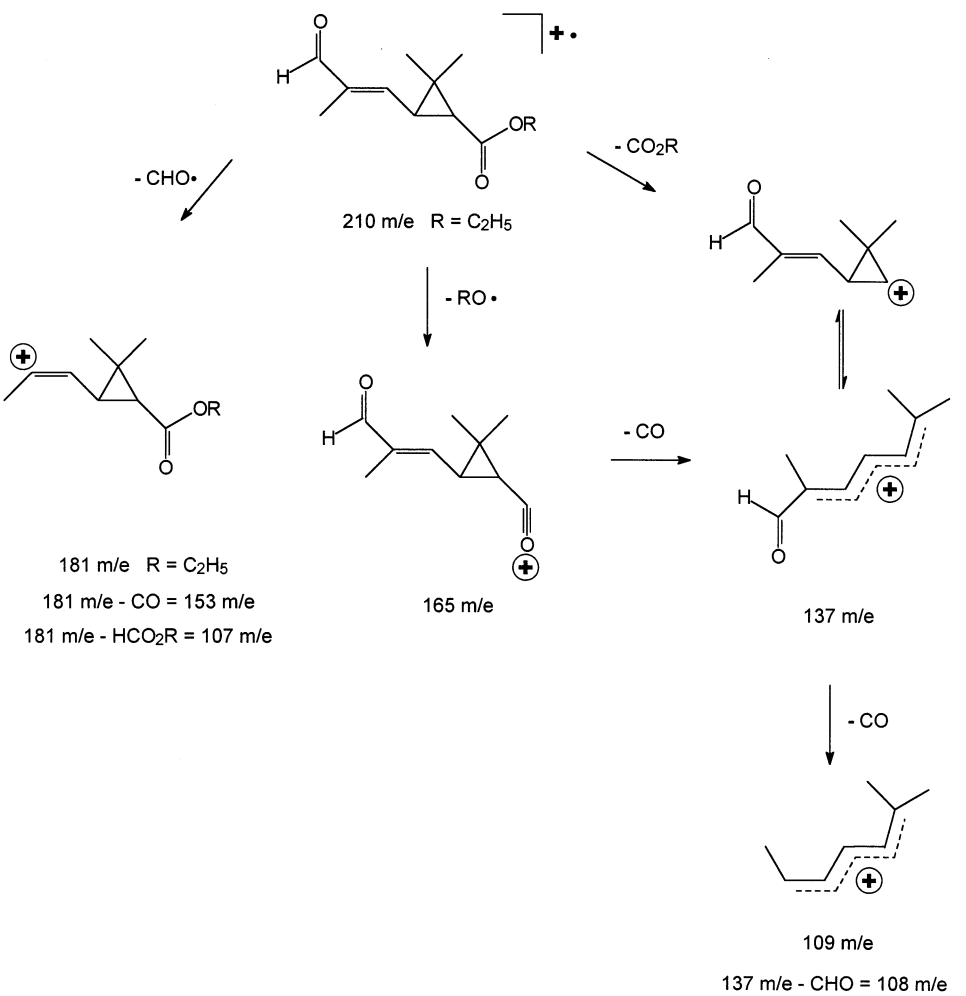


Fig. 3. Main fragmentations of the aldehyde (*cis*-/*trans*-[(E)-CHO]-CA-Et) formated as an intermediate from the SeO_2 oxidation of the isobutenyl methyl group of *cis*-/*trans*-CA-Et.

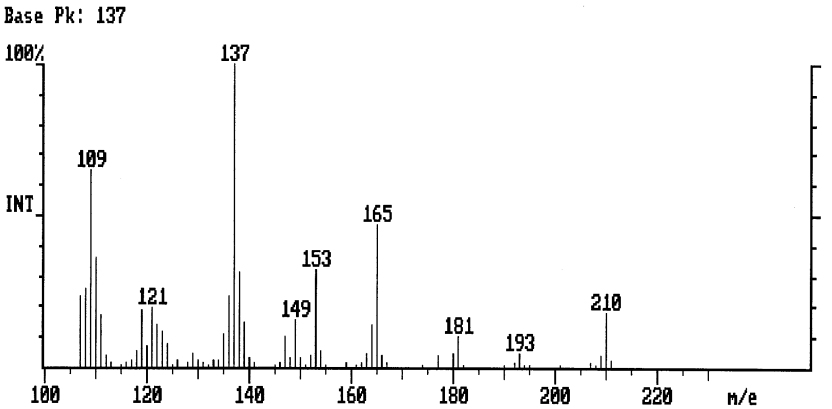


Fig. 4. ITD-mass spectra (full scan 107–220 m/e) showing the main fragmentations of the aldehyde (*cis*-/*trans*-[(E)-CHO]-CA-Et) indicated in Fig. 3.

ties and mass spectra (GC/MS) as the *trans*-(E) isomer of the first and second preparation.

For the development of an analytical method for the detection and quantitation of CDCA in biological matrices methyl, ethyl and n-propyl esters were prepared and subjected to GC/MS. Standards were solved in the corresponding alcohol and esterification was accomplished by adding concentrated sulphuric acid (Kühn et al., 1996).

Using electron impact (EI) mass spectroscopy, main fragmentations of the diesters of *trans*-(E)-CDCA were compared with those of pyrethric acid and related compounds showing similar pathways of decay (Pattenden et al., 1972).

2.4. Preparative HPLC

For diasteroselective separation, 100 mg of a mixture of the CDCA isomers was solved in 8 ml water/acetonitril (70/30%) and was injected onto a autoprep HPLC (Gilson–Abimed, USA) fitted with a 5-μm Kromasil 100-5, reverse phase C-18 steel column (Eka Nobel, Sweden; 250 mm × 20 mm) maintained at 25°C. The mobile phase was water/acetonitril (70/30%). The flow rate was 25 ml/min and the column eluent was monitored at 210 nm. The auto separation program was set from 5.0 to 5.4 min for the first and from 7.2 to 8.3 min for the second peak fraction. The fractions of separated isomers were concentrated under reduced pressure at 45°C.

2.5. Diastereoselective HPLC analysis

Solutions of the diacids were separately prepared in acetonitril and an aliquot of 2.5 μl were injected onto a HPLC (Merck–Hitachi, Germany) equipped with a 3.5-μm Zorbax SB, reverse phase C-18 steel column (Dupont De Nemours & Co., USA, 75 mm × 4.6 mm) maintained at 45°C. The mobile phase was 0.1% phosphoric acid (A)/acetonitril (B) with a gradient (% B): 10 (0.8 min), from 10 to 95 in 6.7 min, 95 (1.6 min). The flow rate was 3.0 ml/min and the column eluent was monitored at 210 nm with a DAD-UV detector (Gynkotek, Germany). Time of retention was 2.3 min for *trans*-(E)-CDCA and 2.7 min for *cis*-(E)-CDCA. The purity of the separated isomers was 91% for *cis*-(E)-CDCA and greater than 99% for *trans*-(E)-CDCA.

2.6. Identification of the diacids by ^1H NMR

The configuration of the separated isomers were determined spectroscopically by ^{1}H NMR. Spectra were recorded on an AMX-400 (Bruker) instrument, using dilute solutions of CDCA in DMSO with tetramethylsilane (TMS) as internal standard. The cyclopropane ring protons (Fig. 2: H-1, H-3) and the olefinic proton (Fig. 2: H-7) in the isobutenyl moiety in both isomers constitute ABX-type spin systems. The appearance of the olefinic proton in the side-chain of the separated

diacids in the NMR spectra as a doublet of multiples (dm) has been used for the assignment of the overall configuration either as *trans*-(E)-CDCA (δ 6,44 ppm, dm) or *cis*-(E)-CDCA (δ 6,99 ppm, dm). This is in close agreement with the analysis of ^{1}H NMR spectra of *trans*-(E)-pyrethrate and related compounds derived from the natural pyrethrins (Bramwell et al., 1969; Elliott et al., 1972).

2.7. Diastereoselective GC/MS analysis

Generally, diastereoselective separation by capillary gas chromatography will be achieved for diethyl and dipropyl esters of *cis*-(E)- and *trans*-(E)-CDCA on a non-polar DB 5 ms (stationary phase: 5% diphenyl- 95% dimethyl polysilane) column (Table 1 and Fig. 7). After derivatization, an aliquot (2 μl) of the preparations was injected onto a PE Autosystem GC (Perkin–Elmer, USA) fitted with a 30-m DB 5 ms capillary column (J&W, USA; 0.25 mm i.d., 0.1 μm film) equipped with an ITD 800 ion trap detector (Finnigan MAT, USA). The carrier gas was helium (152 kPa). The temperature-programmable split/splittless injector Model 011 (QUMA, Germany) was set at 80°C (0.5 min) and programmed from 80°C at 15°C/s to 300°C, holding 300°C during GC run. The split flow was 15 ml/min. The split was open during injection, closed after 0.2 min for 2 min and remained open during GC run. The column was maintained at 70°C (1 min) and programmed from 70°C at 20°C/min to 140°C (1 min) and from 140°C at 10°C/min to 270°C (1 min). The transfer-line was set at 270°C and automatic gain control (AGC) was 29 000 μs. Electron impact (EI) ionization was at 70 eV and full scan mass chromatograms were recorded from 107 to 285 m/e. To enhance the sensitivity of the instrument multiple ion detection (MID) was from 123 to 197 m/e and from 242 to 282 m/e. Quantitation was achieved by internal calibration using 2-phenoxybenzoic acid (2-PBA) as internal standard. 2-PBA was monitored at 197 and 242 m/e for its ethyl and at 197 and 256 m/e for its propyl ester. Retention times and major mass ions of the derivatives of CDCA are indicated in Figs. 5 and 6 and Table 1.

Table 1
Key fragmentation patterns and retention times ($T_{(R)}$) of the formated derivatives of CDCA separated on a DB 5 ms column (MID-quantitation ions with sufficient high mass-to-charge ratios are indicated).

Derivatives	m/e	$T_{(R)}$ (min)
Dimethyl *cis*-(E)- and *trans*-(E) -chrysanthemumdicarboxylate	226, **183**, **167**, **135**, 107	6.3
Diethyl *cis*-(E)-chrysanthemumdicarboxylate	209, **181**, **153**, **135**, 139, 107	7.4
Diethyl *trans*-(E)-chrysanthemumdicarboxylate	254, **181**, **163**, **153**, 139, 107	7.6
Dipropyl *cis*-(E)-chrysanthemumdicarboxylate	223, **163**, **153**, **135**, 107	9.4
Dipropyl *trans*-(E)-chrysanthemumdicarboxylate	282, **195**, **163**, **153**, 135, 107	10.0

2.8. Sample collection

Urine samples (24 h or spot samples) of exposed subjects were collected in polyethylene bottles. Until preparation samples were stored at −21°C. Under these storage conditions, samples were stable for a minimum of 1 year.

2.9. Analysis of CDCA in human urine samples

A volume of 2 ml of an urine sample including the internal standard 2-PBO were placed in a glass centrifuge tube equipped with screw cap. After diluting with 2 ml 1 M KOH, the tightly sealed glass was placed in a water bath (50°C) for 1 h. The diacid metabolites and their conjugates were converted into free acids by adding 0.5 ml concentrated HCl to the urine sample (pH < 1). The acidified urine was treated with 2 ml tert. butyl methyl ether, vigorously shaken for 1 min and rolermixed for 5 min. Following centrifugation (7 × *g*, 10°C) for 10 min, the organic layer was transfered to a second centrifuge tube. This procedure was repeated once more.

In order to reduce the urinary matrix interference, the diacids were subjected to the following

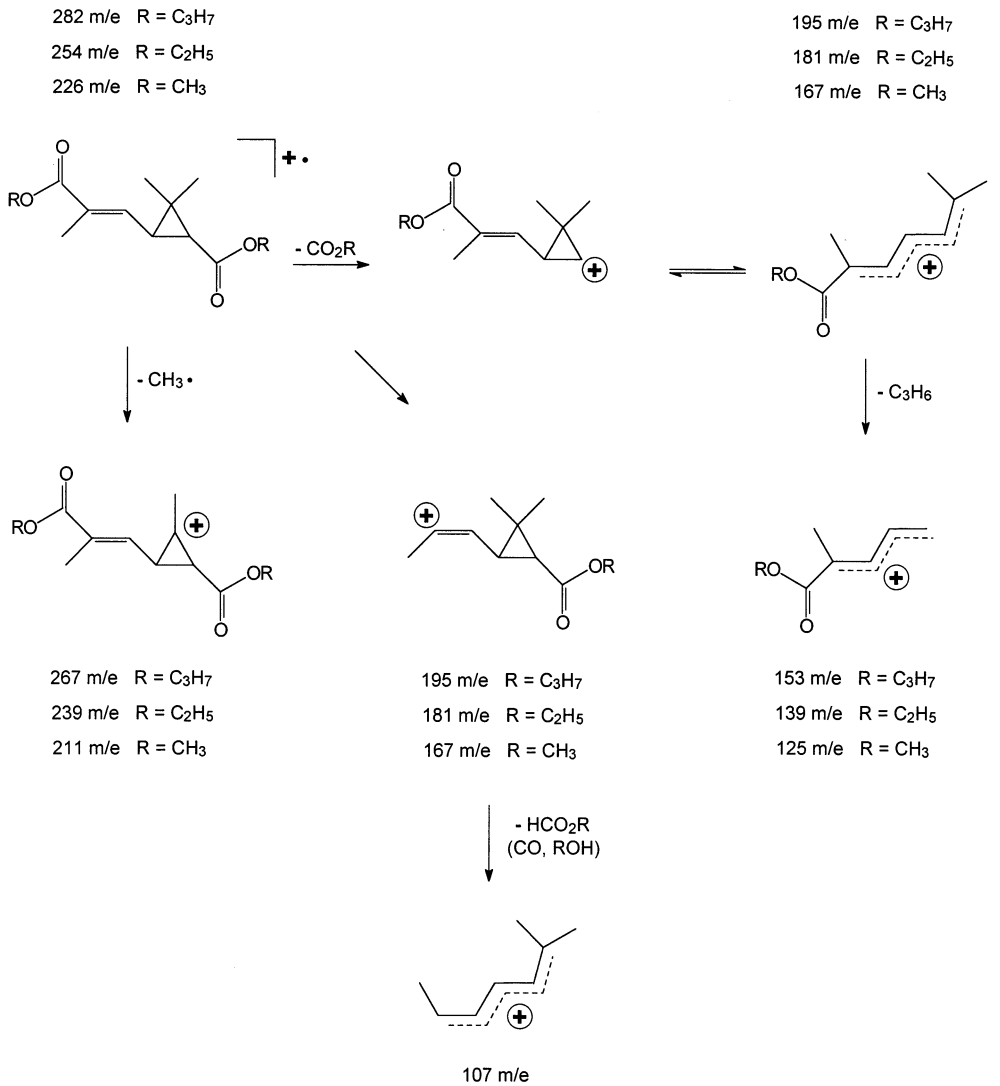


Fig. 5. Main fragmentations of dimethyl- (CH_3), diethyl- (C_2H_5) and dipropyl- (C_3H_7) *trans*-(E)-chrysanthemumdicarboxylates (*trans*-(E)-CDCA).

clean up steps. The combined organic layers were vigorously extracted by adding 2 ml 0.5 M NaOH and the organic layer were discharged after centrifugation. Again, after washing with 1.5 ml hexane, the aquateous phase was re-acidified by adding 0.2 ml concentrated HCl and extracted twice with 2 ml tert. butyl methyl ether. The collected organic layers were combined and reduced to dryness in a stream of nitrogen.

The residue was solved in 2 ml of the corresponding alcohol (methanol, ethanol or propanol). Esterification was accomplished by adding 0.4 ml concentrated H_2SO_4 and heating (60°C) the solution for 1 h in a water bath. After cooling, the mixture was diluted with 1 ml of

water and the derivatives were extracted twice with 1.5 ml hexane. By this procedure a lot of matrix interferences were kept back in the acidified alcoholic phase.

The collected organic layers were reduced in a gentle stream of nitrogen to a volume of 0.5 ml. An aliquot (2 μl) of the preparation was subjected to quantitative GC/MS.

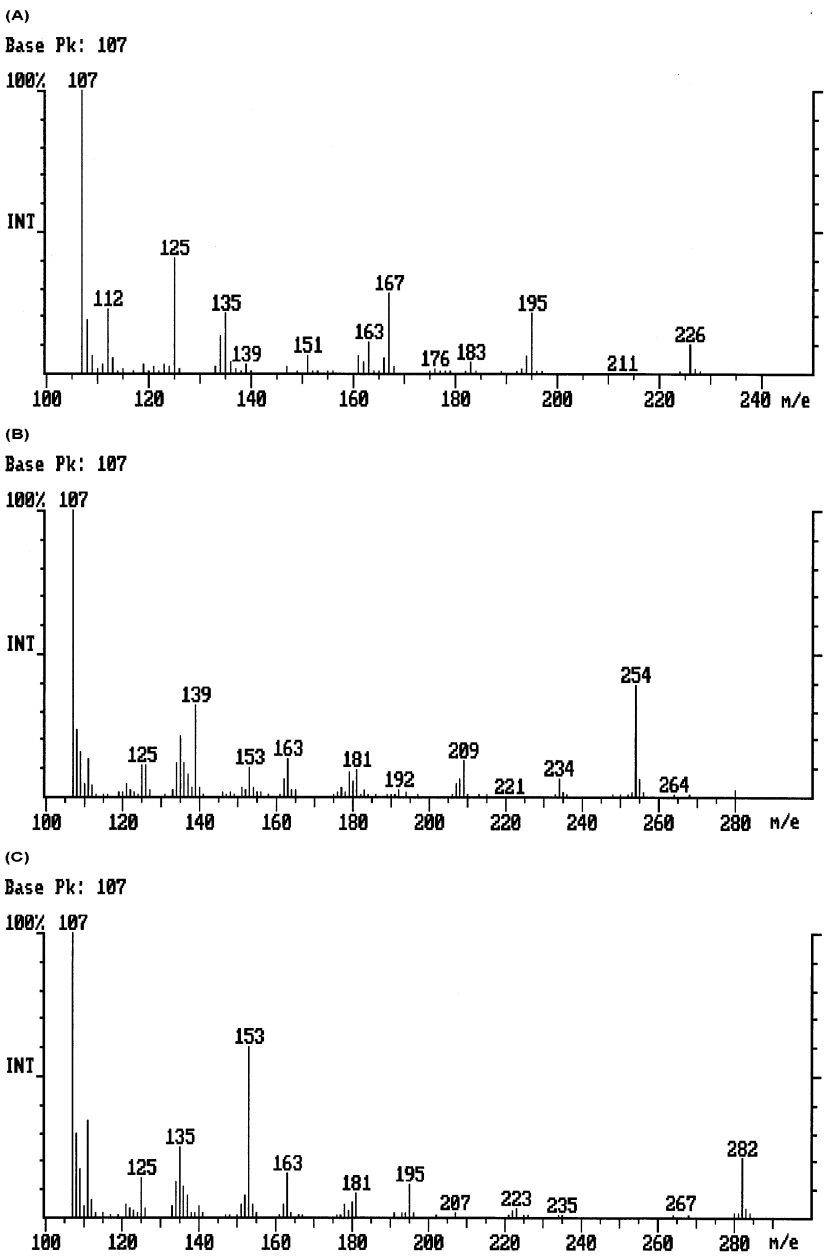


Fig. 6. ITD-mass spectra of (A) dimethyl-, (B) diethyl- and (C) dipropyl *trans*-(E)-chrysanthemumdicarboxylates (full scan 107–282 m/e) obtained by chemical synthesis showing main fragmentations indicated in Fig. 5.

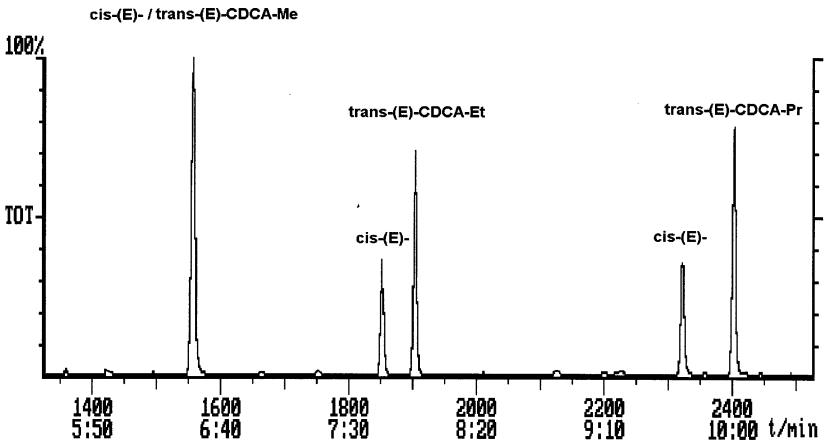


Fig. 7. Total ion chromatogram of the formated CDCA derivatives of standard solutions separated on a DB 5 ms column (standard solution containing 100 μg/ml of each diastereomere).

3. Results

After the preparation of the synthetical standards of *cis*-(E)- and *trans*-(E)-CDCA, the racemic mixture was subjected to diastereoselective preparative HPLC. Characterization of the separated and purified isomers was by nuclear magnetic resonance (^{1}H NMR) and derivatization followed by capillary GC/MS.

Diastereoselective gas chromatography was achieved for the diethyl and dipropyl esters of CDCA on a non-polar DB 5 ms capillary column (Fig. 7). The major ions and fragmentation pathways of the derivatives of CDCA produced by EI-mass spectrometry were discussed in Figs. 5 and 6. For quantitation, ion fragments showing the lowest matrix interferences were chosen (Table 1).

For a biological monitoring, urine samples of a person exposed to (*S*)-bioallethrin were subjected to hydrolytic cleavage. After extraction, CDCA, the major oxidation product of CA was transformed into appropriate ester derivatives. Separation and characterization was by diastereoselective gas chromatography with electron impact mass spectrometry (GC/MS). Quantitation was achieved by internal calibration using 2-PBA as internal standard. Full scan spectrum mode MS was used for the confirmation of the metabolites detected in the urine. For quantitation of CDCA, multiple ion detection (MID) using up to three characteristical fragment ions with sufficient high mass-to-charge ratios were employed.

Aliquotes of standard solutions of the free acid metabolites and the 2-PBA were added to 2 ml blank urine samples. For calibration purposes each of these samples were treated like urine samples of exposed people. Calibration curves of *cis*-(E)- and *trans*-(E)-CDCA were linear from 10 to 500 μg/l. The purity of the chemicals used was checked by analysis of blank water samples and possible background contaminations were recognized by subtracting from the calibration curves.

The limit of detection (LOD) was 20 μg/l for *cis*-(E)-CDCA and 10 μg/l for *trans*-(E)-CDCA with a signal-to-noise ratio (s/n) higher than 3:1. The average in-run coefficient of variation of spiked urine samples was 8% (50 μg/l, $n = 8$).

Internal calibration using 2-phenoxybenzoic acid (2-PBA, Aldrich) as internal standard was applied. In addition, no *cis*-to-*trans* conversion was observable and no CDCA was detectable in blank urine samples of non-exposed people.

In Fig. 8 mass chromatograms of two preparations of urine samples of a pest control operator are shown. For the sake of confirmation, two independent derivative reagents (ethanol and propanol) were applied. In both cases, only *trans*-(E)-CDCA was present in the urine in high yields

(204 ± 16 μg/l) whereas unmetabolized *trans*-CA was only detectable in trace amounts. Moreover, the concentration of the epimeric isomer *cis*-(E)-CDCA was below the limit of detection.

4. Discussion

In the present study it was demonstrated that *trans*-(E)-CDCA is suitable as a biomarker for internal (*S*)-bioallethrin burden. This is in close agreement with the results of numerous animal studies in which similar urinary metabolites have been analyzed after oral administration of (*S*)-bioallethrin and related insecticides to rats (Elliott et al., 1972; Ueda et al., 1975; Brown et al., 1985; Class et al., 1990).

Maximum peak excretion of *trans*-(E)-CDCA was within the first 24 h after exposure. In addition, after 72 h the concentration of the metabolite fell below the limit of detection. The rapid renal elimination of (*S*)-bioallethrin is in close agreement with human exposure studies using the pyrethroid insecticides cypermethrin and

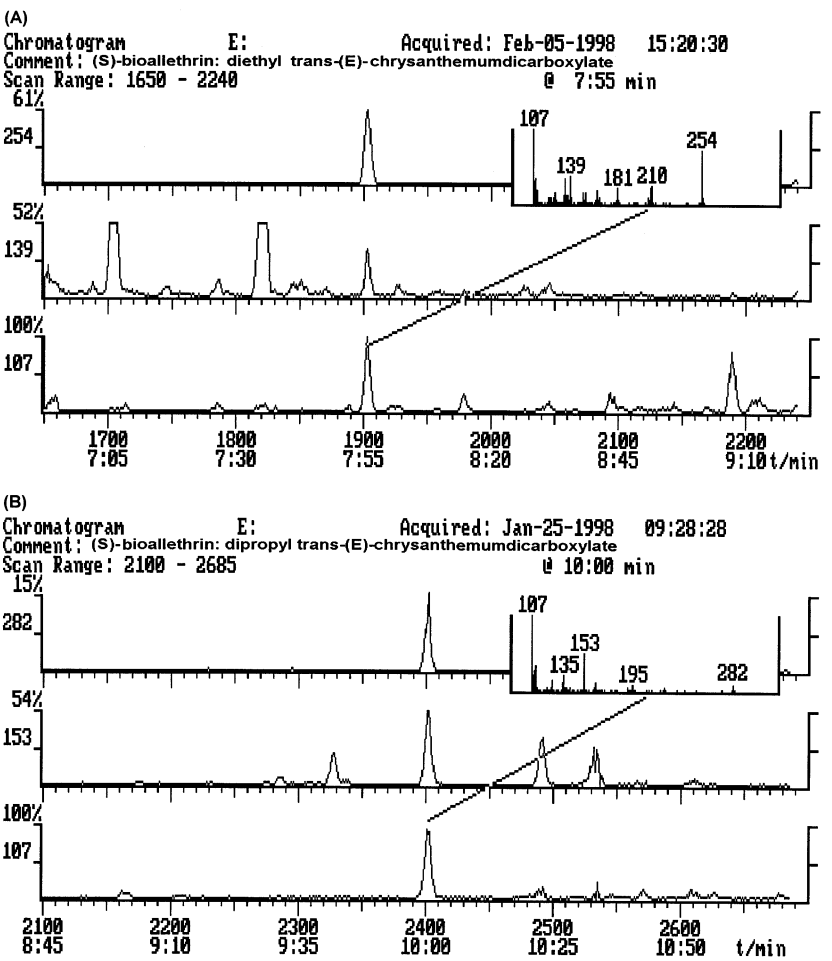


Fig. 8. Mass chromatograms (full scan) of a real 24-h urine sample from a pest control operator exposed to (*S*)-bioallethrin: trans-(E)-CDCA (204 μg/l) derivatized as (A) diethyl and (B) dipropyl ester. Major ion fragments and retention times are virtually identical to those of the corresponding *trans*-(E)-CDCA standard given in Figs. 6 and 7. Within the limit of detection, the epimeric *cis*-(E)-CDCA is not present in the urine.

cyfluthrin. There, peak excretion occurred within the first 24 h after oral, dermal (Eadsforth and Baldwin, 1983; Eadsforth et al., 1988; Woollen et al., 1992; Leng et al., 1997a) or inhalative administration (Leng et al., 1997b).

Furthermore, in urine samples of persons not exposed to the insecticides concerned, no CDCA could be found within the limit of detection.

In addition, the storage stability of the metabolites is essential for biological monitoring. Storage experiments demonstrated that *trans*-(E)-CDCA is stable for more than a year at −21°C. The same was shown with metabolites of the halovinyl type pyrethroid cyfluthrin and cypermethrin (Leng et al., 1997a).

Moreover, in the metabolism of the chrysanthemate pyrethroid resmethrin in rats some unanticipated isomerizations of CDCA has been detected (Ueda et al., 1975). Clearly, such an epimeric conversions is only expectable after an exposure to single diastereomeres (e.g. bioresmethrin, (*S*)-bioallethrin) not employing a racemic mixture. In the present study, however, an epimerization of (*S*)-bioallethrin by the human metabolism at C-3 of the cyclopropane ring leading to the excretion of significant amounts of *cis*-(E)-CDCA could not be observed.

Nevertheless, high resolution capillary gas chromatography employed for the diastereoselective separation and quantitation is necessary since chrysanthemate insecticides can show a very complex pattern of urinary metabolites (Class et al., 1990). A sufficient low matrix interference was established for fragment ions of CDCA with higher mass to charge ratios. Although of lower abundance, the best choices are the corresponding molecular ions of diethyl or dipropyl esters of *trans*-(E)-CDCA. Since a lot of recently synthesized pyrethroids (e.g. resmethrin, tetramethrin, cyphenothrin, empenethrin, phenothrin, prallethrin) are derived from *cis*- and/or *trans*-CA moiety, both diastereoisomers, *cis*-(E)- and *trans*-(E)-CDCA, seems to play a key role for the development of a human biomonitoring.

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