Synthesis of *d-trans*-Chrysanthemumic Acid-1-C^{14} and Its Antipode on a Semimicro Scale

YOSHIHIKO NISHIZAWA[1] and JOHN E. CASIDA

Division of Entomology and Acarology, University of California, Berkeley, Calif.

dl-trans-Chrysanthemumic acid-1-C^{14} was prepared in 45% yield from 4 mmoles of ethyl glycinate-1-C^{14}. Yield of the *trans*-acid was increased by isomerization of methyl *cis*-chrysanthemumate-1-C^{14} with sodium *tert*-amylate. Geometrical isomers of the acid were separated by column chromatography, and optical isomers of the *trans*-acid were resolved as a quinine salt for the *l*-isomer and an *l*-α-methylbenzylamine salt for the *d*-isomer. Over-all yields of *trans*-chrysanthemumic acids-1-C^{14}, based on radioactivity from ethyl glycinate-1-C^{14} (2.7 mc. per mmole), were: 22.8% of impure *d*-isomer, 10.4% of pure *l*-isomer, and 7.0% of pure *d*-isomer with specific activities of 1.3 to 2.7 mc. per mmole.

Extracts of pyrethrum flowers contain, as their natural insecticidal constituents, esters of pyrethrolone and cinerolone with *d-trans*-chrysanthemumic acid (chrysanthemummonocarboxylic acid) and *d-trans*-pyrethric acid (chrysanthemumdicarboxylic acid monomethyl ester). Many of the favorable biological properties of the natural materials are also found for synthetic pyrethroids derived from synthetic chrysanthemumic acid (*dl-cis, trans*) esterified with *dl*-allethrolone, dimethylbenzyl alcohols, and other alcohols. The configuration of the chrysanthemumic acid isomer greatly affects the biological properties of its esters, so critical studies on their mode of action or inaction must utilize stereochemically pure compounds. In each case, the esters of these alcohols with natural chrysanthemumic acid (*d-trans*) were more insecticidal than esters with synthetic chrysanthemumic acid as a mixture of isomers (*dl-cis, trans*) or as the other individual resolved isomers (*5, 18, 19*). The toxicity of the eight isomers of allethrin to houseflies (*Musca domestica* L.) is synergized by piperonyl butoxide {α-[2-(2-butoxyethoxy)ethoxy]-4,5-methylenedioxy-2-propyltoluene} to different degrees for the different isomers (*13, 14*). Flies selected with pyrethrins developed highest resistance to the least toxic of the eight stereoisomers of allethrin (*4, 11*).

Certain types of toxicology and metabolism experiments on pyrethroids are greatly facilitated by the use of C^{14}-labeled compounds. Pyrethrum-C^{14} was prepared biosynthetically from carbon-14 dioxide (*20*) and randomly labeled pyrethrin-I and cinerin-I of about 0.05 mc. per mmole were chromatographically isolated (*8*). Pyrethrins I and II have also been prepared biosynthetically, in very low yields and specific activities, from mevalonic acid-2-C^{14}, acetic acid-2-C^{14}, and methionine-methyl-C^{14} (*9, 10, 15, 16*). Acree, Roan, and Babers (*2*) converted ethyl glycinate-2-C^{14} (25.5 mmoles, 0.074 mc. per mmole) to *dl-cis, trans*-chrysanthemumic acid-2-C^{14} in 52% yield, but the preparation contained impurities other than chrysanthemumic acid and the geometrical and optical isomers were not resolved (*1*). *dl-trans*-Chrysanthemumic acid-2-C^{14}, prepared according to the general procedure of Acree, Roan, and Babers (*2*) followed by fractional precipitation to concentrate the desired geometrical isomer, has been used to prepare radiolabeled pyrethrin-I combined with one of its diastereomers which was not resolved (*12*). Preparation of *d-trans*-chrysanthemumic acid-C^{14}, the common intermediate for preparation of radiolabeled pyrethrin-I, cinerin-I, and the most insecticidal isomer of the synthetic pyrethroids, is an essential step to future studies on radiolabeled pyrethroids.

A semimicro scale (4 mmoles) procedure is reported for: preparation of *dl-cis,trans*-chrysanthemumic acid-1-C^{14} based on Acree, Roan, and Babers (*2*); chromatographic separation of the *cis* and *trans* isomers; isomerization of the *cis* isomer to the *trans* isomer based on Julia *et al.* (*17*); resolution of the *l-trans* isomer as a quinine salt and the *d-trans* isomer as a *l*-α-methylbenzylamine salt based on Campbell and Harper (*7*). Pure *d-trans*- and *l-trans*-chrysanthemumic acids-1-C^{14} (1.3 to 2.7 mc. per mmole) were obtained.

Experimental

Chemicals and Methods. Natural chrysanthemumic acid {*d-trans*, $[\alpha]_D$ + 24.1° (c, 6.600), b.p. 105° C./1.3 mm.} was obtained from a pyrethrum extract by the procedure of Staudinger and Ruzicka (*22*). Synthetic chrysanthemumic acid (*dl-cis,trans*, Sumitomo Chemical Co., Osaka, Japan) was recrystallized according to Campbell and Harper (*6*) to yield the *dl-cis*-acid (m.p. 113–115° C.) and the *dl-trans*-acid (m.p. 51–52° C.). *l-trans*-Chrysanthemumic acid {$[\alpha]_D$ − 24.9° (c, 4.460)} was obtained from the *dl-trans* acid by recrystallization of the quinine salt (*7*) to yield a melting point for the pure quinine-*l-trans* salt of 155–156° C. 2,5-Dimethyl-2,4-hexadiene (Sumitomo Chemical Co., Osaka, Japan) was purified according to Campbell and Harper (*6*) to yield a product with a b.p. of 134° C. Ethyl glycinate-1-C^{14} hydrochloride (2.7 mc. per mmole) was obtained from New England Nuclear Corp., Boston, Mass. Diazomethane was prepared according to Arndt (*3*). Quinine sulfate was converted to the free base and recrystallized from benzene (m.p. 176° C.). *l*-α-Methylbenzylamine (α_D − 37.5°, no solvent) was resolved from *dl*-α-methylbenzylamine (Aldrich Chemical Co., Milwaukee, Wis.) according to Theilacker and Winkler (*23*). This *l*-α-methylbenzylamine formed a salt with natural chrysanthemumic acid which on recrystallizing from 50% aqueous ethanol yielded a m.p. of 110–112° C.

All radioactive measurements were made with the Packard Tri-Carb Model 3003 liquid scintillation spectrometer. Optical rotations were measured at 25° C. with the ETL-NPL Automatic Polarimeter Type 143A (Bendix Ericsson, U. K. Ltd., Nottingham, England) using chloroform solutions for chrysanthemumic acid and no solvent for *l*-α-methylbenzylamine. Infrared spectra were determined as 10% chloroform solutions using the Beckman IR-4 infrared spectrometer. All melting points (uncorrected) were determined on microscope cover slips on a hot block by observing single crystals with a microscope.

Thin-layer chromatography (silica gel G, 0.25 mm. thickness) was used to ascertain the purity of *cis*- (R_f = 0.59) and *trans*-chrysanthemumic acids (R_f = 0.31) as resolved with a mixture of three parts of isopropyl acetate saturated with 10% ammonia aqueous solution and one part of methanol. Radioactive materials were detected by radioautography and known compounds by a spray of 20% phosphomolybdic acid in absolute ethanol (sensitivity limit: 0.1 μg.) (*21*).

[1] Present address, Pesticide Research Laboratory, Sumitomo Chemical Co., Osaka, Japan.

Two types of column chromatography were utilized. A Florisil column was used to separate ethyl chrysanthemumate-1-C^{14} from radioactive impurities and from 2,5-dimethyl-2,4-hexadiene. This chromatography served to transfer the volatile ethyl chrysanthemumate from the hexadiene solvent to the petroleum ether–ether mixture (9 to 1), a solvent which was more readily removed with minimum loss of ethyl chrysanthemumate. Florisil (60- to 100-mesh, Floridin Co., Tallahassee, Fla.) was slurried in petroleum ether and added to a chromatographic tube which was packed to yield a 1.8- × 22-cm. column. 2,5-Dimethyl-2,4-hexadiene was eluted from this column with 100 ml. of petroleum ether, followed by ethyl chrysanthemumate with 100 ml. of petroleum ether–ether mixture (9 to 1), and impurities were eluted with 100 ml. of methanol.

A Celite column was used for separation of *cis*- and *trans*-chrysanthemumic acids, using a solvent system based on a paper chromatographic procedure for accomplishing this resolution (*1*). Thirty grams of Celite (Johns-Manville Celite analytical filter aid), heated at 150° C. for 24 hours and after cooling held in a desiccator over phosphorus pentoxide, was mixed with 15 ml. of 10% aqueous ammonia solution which had been previously saturated with isopropyl acetate. The resulting mixture was slurried in isopropyl acetate previously saturated with 10% aqueous ammonia solution and added to a chromatographic tube which was packed with air pressure to yield a column of 3 × 20 cm. Synthetic chrysanthemumic acid was dissolved in a small amount of isopropyl acetate saturated with 10% aqueous ammonia solution and introduced onto the column. Development and elution of the column were accomplished with 700 ml. of isopropyl acetate saturated with 10% aqueous ammonia solution, followed with 700 to 900 ml. of ethyl acetate and, finally, 300 to 400 ml. of methanol. Methyl and ethyl chrysanthemumate were eluted quickly before any other materials, and were followed by *cis*-chrysanthemumic acid which was mostly eluted with isopropyl acetate–ammonium hydroxide, and the remainder was found in the first few ethyl acetate fractions; *trans*-chrysanthemumic acid was eluted with ethyl acetate and polar impurities were eluted with methanol. Complete resolution of chrysanthemumate esters and the *cis*- and *trans*-chrysanthemumic acids was obtained with 100 to 300 mg. total weight of compounds (Figure 1), and only a slight overlap of the *cis* and *trans* acid peaks occurred when up to 500 mg. of the mixed acids were chromatographed, although 900 ml. of ethyl acetate were needed for complete elution of the *trans*-acid with the larger amounts. The separation efficiency of the column used with radioactive material was ascertained by cochromatography using the thin-layer procedure of aliquots of the C^{14}-fractions with unlabeled natural chrysanthemumic acid and *dl-cis*-chrysanthemumic acid. Infrared spectra and melting points were also used for differentiation of the *cis* and *trans* isomers as

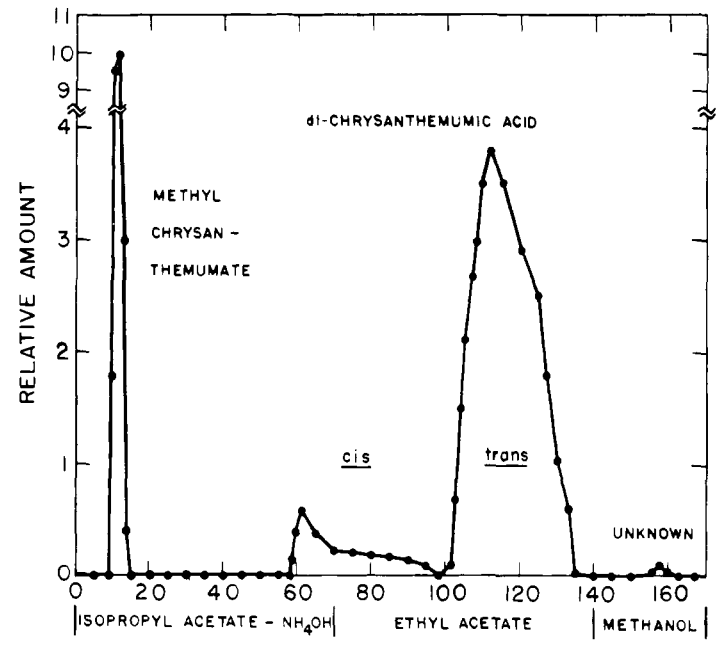

Figure 1. Separation on a Celite column of methyl chrysanthemumate-1-C^{14}, *dl-cis*-chrysanthemumic acid-1-C^{14}, *dl-trans*-chrysanthemumic acid-1-C^{14} and an unknown impurity

Relative amount based on c.p.m. × 10^4 per 5-μl. aliquot from each fraction. *dl-cis*-Chrysanthemumic acid-1-C^{14} (137 mg.) was converted to the methyl ester, isomerized as indicated in the text, and saponified. Total recoveries from the *cis*-acid were as follows: 25 mg. of methyl chrysanthemumate-1-C^{14} (expressed as chrysanthemumic acid-1-C^{14} equivalent); 15.0 mg. of *dl-cis*-chrysanthemumic acid-1-C^{14}; 68.5 mg. of *dl-trans*-chrysonthemumic acid-1-C^{14}; and 0.4 mg. (chrysanthemumic acid-1-C^{14} equivalent) of an unknown compound(s) eluting with methanol.

eluted. With both types of column used, 10-ml. fractions were collected with an automatic fraction collector. The position of eluting materials was ascertained both by radioactive measurements and by a spot test in which 2 μl. from each fraction were applied to filter paper and sprayed, after solvent evaporation, with 0.05% aqueous potassium permanganate to detect unsaturated compounds as brown spots on a pink background. (The sensitivity limit was 0.1 to 0.5 μg. for *cis*- and *trans*-chrysanthemumic acids and for 2,5-dimethyl-2,4-hexadiene, although the spot appeared more quickly with small amounts of the latter two materials.)

Preparation of *dl-cis-trans*-Chrysanthemumic Acid-1-C^{14}. Chrysanthemumic acid-1-C^{14} was prepared from 4 mmoles of ethyl glycinate-1-C^{14} according to Acree, Roan, and Babers (*2*).

Ethyl diazoacetate-1-C^{14} was prepared batchwise by using two 10-ml. separatory funnels. To ethyl glycinate-1-C^{14} hydrochloride (558 mg., 2.7 mc. per mmole) in one of the funnels were added, in order, sodium acetate (8.0 mg. in 2 ml. of water), sodium nitrite (420 mg.), and 1.5 ml. of 2,5-dimethyl-2,4-hexadiene. The temperature was controlled by swirling the reaction mixture in the separatory funnel in a water bath maintained at 15° C. Sulfuric acid (10%, 0.1 ml.) was then added dropwise to the swirling reaction mixture and, after thorough equilibration of the layers, the aqueous phase was drained into the second separatory funnel; the remaining hexadiene layer was passed by mild suction through a 1-gram column of anhydrous sodium carbonate. The first separatory funnel was immediately rinsed with an additional 1 ml. of hexadiene which was passed through the column. To the aqueous phase in the second funnel, 1.5 ml. of hexadiene were added, followed by sulfuric acid and equilibration as before. The hexadiene was passed through the column with a rinse and the procedure repeated a third time for the aqueous phase. The yellow color of the hexadiene phase (due to ethyl diazoacetate) was prominent during the first- and second-batch preparations, but the third hexadiene phase was almost colorless.

Ethyl chrysanthemumate-1-C^{14} was prepared by dropwise addition of the combined hexadiene extracts of ethyl diazoacetate-1-C^{14} to a refluxing mixture of 1 ml. of hexadiene and 0.5 mg. of copper powder. After the addition was completed, refluxing was continued for 30 minutes and the hexadiene volume was reduced to approximately 2 ml. under reduced pressure at 35° C. The resulting mixture was chromatographed on Florisil from which the hexadiene was eluted with petroleum ether (which also contained 0.9% of the radioactivity), the ethyl chrysanthemumate-1-C^{14} with petroleum ether–ether mixture (9 to 1) (which contained 66% of the radioactivity), and impurities with methanol (yielding 22% of the radioactivity).

Ethyl chrysanthemumate-1-C^{14} was saponified by evaporation of all of the petroleum ether–ether mixture under reduced pressure, addition of 315 mg.

of sodium hydroxide dissolved in 2.9 ml. of 80% aqueous ethanol, and refluxing for 2 hours. After ethyl chrysanthemumate-1-C^{14} was saponified, the solvent was evaporated off under reduced pressure, and 3 ml. of cold 20% hydrochloric acid were added; the resulting mixture was extracted three times with equal volumes of ether. The combined ether layers were washed twice with 3 ml. of water and dried with sodium sulfate. Impure *dl-cis, trans*-chrysanthemumic acid-1-C^{14} was obtained in 73.5% yield based on weight (494 mg.) and 56% yield based on radioactivity from ethyl glycinate-1-C^{14}.

Chromatographic Separation of *dl-cis* and *dl-trans*-Chrysanthemumic Acid-1-C^{14} and Isomerization of the *cis*-Acid to the *trans*-Acid. The impure *dl-cis-trans*-chrysanthemumic acid-1-C^{14} (494 mg.) was separated on the Celite column to yield pure *dl-cis*-chrysanthemumic acid-1-C^{14} (137 mg., 2.7 mc. per mmole) and pure *dl-trans*-chrysanthemumic acid-1-C^{14} (222 mg., 2.7 mc. per mmole) for yields of 20.4 and 33.0%, respectively, based on the ethyl glycinate-1-C^{14} used.

Further *dl-trans*-chrysanthemumic acid-1-C^{14} was obtained by isomerization of the radiolabeled *cis* isomer according to Julia and coworkers (*17*). The methyl ester was prepared by addition of the *dl-cis*-chrysanthemumic acid-1-C^{14} (137 mg.) in 4 ml. of ether to a freshly prepared ether solution of diazomethane (4 ml., 2%, w./v.) at 5° C. After 30 minutes at 5° C., the excess diazomethane and ether were evaporated off under reduced pressure, and 4 ml. of sodium *tert*-amylate-benzene solution (prepared by refluxing 690 mg. of sodium and 4 ml. of *tert*-amyl alcohol in 10 ml. of benzene for 3 days until the sodium was completely dissolved) was added; the resulting mixture was refluxed for 4 hours and held for 18 hours at 25° C. The benzene and excess amyl alcohol were removed by evaporation under reduced pressure, 50 mg. of sodium hydroxide dissolved in 2.5 ml. of 80% aqueous ethanol were added, and the mixture was refluxed for 3 hours. The acids were recovered by evaporating the ethanol, acidifying, and extracting into ether. They were chromatographed (Figure 1) to yield *dl-trans*-chrysanthemumic acid-1-C^{14} (68.5 mg.), *dl-cis*-chrysanthemumic acid-1-C^{14} (15.0 mg.), and methyl chrysanthemumate-1-C^{14} (25-mg. chrysanthemumic acid equivalent based on radioactivity). Saponification of this ester and chromatography yielded an additional 3 mg. of *dl-cis-* and 12.5 mg. of *dl-trans*-chrysanthemumic acid-1-C^{14}.

dl-cis-Chrysanthemumic acid-1-C^{14} (18 mg.) and *dl-trans*-chrysanthemumic acid-1-C^{14} (303 mg.) of 2.7 mc. per mmole were therefore obtained in 2.7 and 45% yields, respectively, from ethyl glycinate-1-C^{14}.

Resolution of *d-* and *l-trans*-Chrysanthemumic Acid-1-C^{14} by Selective Crystallization. *dl-trans*-Chrysanthemumic acid-1-C^{14} (303 mg., 2.7 mc. per mmole) and quinine (584 mg.) were dissolved in 1.0 ml. of warm absolute ethanol. Crystals which formed within 18 hours at 25° C. were filtered off (306 mg., m.p. 145–158° C.) and recrystallized twice by dissolving in 2 ml. of warm 50% aqueous ethanol and holding at 25° C. for 18 hours (205 mg., m.p. 155–156° C.). This pure salt was decomposed by adding to it 2 ml. of 2*N* hydrochloric acid, and the liberated, labeled compound was recovered by extraction three times with 5-ml. portions of ether. After washing the ether twice with 5 ml. of water and drying the ether phase with sodium sulfate, pure *l-trans*-chrysanthemumic acid-1-C^{14} (70 mg., $[\alpha]_D$ − 25.1° (c, 3.500), 2.7 mc. per mmole) was obtained.

The absolute ethanol filtrate from the separation of the impure quinine salt of the *l*-acid was evaporated to dryness with an air stream. The residual quinine salt was decomposed as described above to yield impure *d-trans*-chrysanthemumic acid-1-C^{14} (178 mg., $[\alpha]_D$ + 11.8° (c, 3.560), 2.7 mc. per mmole). This optical rotation indicated that the impure *d-trans*-acid-1-C^{14} contained 129 mg. of *d-* and 49 mg. of *l*-acid. Addition of 139 mg. of natural chrysanthemumic acid to the impure *d-trans*-chrysanthemumic acid-1-C^{14} (2.7 mc. per mmole) gave 317 mg. of impure *d-trans*-acid-1-C^{14} {$[\alpha]_D$ + 17.4° (c, 6.340)} which, on the basis of optical rotation, contained 83% *d*-acid (1.3 mc. per mmole) and 17% *l*-acid (2.7 mc. per mmole). The impure *d-trans*-acid-C^{14} and *l*-α-methylbenzylamine (228 mg.) were dissolved in 1.6 ml. of warm 50% aqueous ethanol. Crystals which formed within 18 hours at 25° C. were recrystallized twice by dissolving in 1.6 ml. of 50% aqueous ethanol and holding at 25° C. for 18 hours to give the pure *l*-amine-*d*-acid salt (174 mg., m.p. 110–112° C.). Decomposition of this salt by the method indicated above gave pure *d-trans*-chrysanthemumic acid-1-C^{14} {98 mg., $[\alpha]_D$ + 24.7° (c, 1.962), 1.3 mc. per mmole}.

All filtrates from recrystallization were combined, the ethanol was evaporated, and the salts were decomposed as indicated above to recover impure *d-trans*-acid-1-C^{14} {250 mg., $[\alpha]_D$ + 11.2° (c, 2.495), 1.96 mc. per mmole based on direct radioactive measurement}. This impure *d-trans*-acid-1-C^{14} therefore contained 29% *l*-isomer (2.7 mc. per mmole) and 71% *d*-isomer (1.66 mc. per mmole), so that 60% of the total C^{14} was present as the *d-* and 40% of the total C^{14} as the *l*-isomer.

Discussion

Over-all yields based on radioactivity from ethyl glycinate-1-C^{14} (2.7 mc. per mmole) were as follows: 7.0% *d-trans*-chrysanthemumic acid-1-C^{14} (1.3 mc. per mmole); 10.4% *l-trans*-chrysanthemumic acid-1-C^{14} (2.7 mc. per mmole); 22.8% impure *d-trans*-chrysanthemumic acid-1-C^{14} (1.66 mc. per mmole); for a total recovery of 40.2% of the radioactivity as partially or completely resolved *trans*-chrysanthemumic acid antipodes. This method of resolution could be repeated by again fortifying the residual impure *d-trans*-acid-1-C^{14} with natural chrysanthemumic acid in the same manner and isolating the *l*-α-methylbenzylamine salt to recover further pure *d-trans*-chrysanthemumic acid-1-C^{14} of a lower specific activity.

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