MAGNETIC RESONANCE IN CHEMISTRY, VOL. 31, 90–93 (1993)

Three-Bond ^{13}C–^{1}H Coupling Constants for Chrysanthemic Acid and Phenothrin Metabolites: Detection by Two-Dimensional Long-Range ^{13}C–^{1}H *J*-Resolution Spectroscopy

Tetsu Ando* and Nozomu Koseki
Department of Applied Biological Science, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183, Japan

Robert F. Toia and John E. Casida
Pesticide Chemistry and Toxicology Laboratory, Department of Entomological Sciences, University of California, Berkeley, California 94720, USA

Two-dimensional long-range ^{13}C–^{1}H *J*-resolution spectroscopy (LRCJR) was used to measure three-bond ^{13}C–^{1}H coupling constants $|^{3}J(C,H)|$ for *trans*- and *cis*-chrysanthemic acid, methyl *trans*-pyrethrate and some microsomal metabolites of the *trans*-chrysanthemate biophenothrin. The carbon of the methyl *cis*-disposed to an attached proton shows a larger $^{3}J(C,H)$ value than does the *trans*-carbon for the dimethyl-substituted cyclopropane and epoxide rings. The reverse situation applies for the analogous dimethyl vinyl grouping. The $^{3}J(C,H)$ values are not altered on conversion of one of the olefinic geminal methyl groups to a hydroxymethyl or methoxycarbonyl functionality, but increase on transformation to an aldehyde. These $^{3}J(C,H)$ values are in agreement previous results from long-range C–H COSY experiments, and provide a useful method for determining the stereochemistry of chrysanthemic acid derivatives.

KEY WORDS Long-range ^{13}C–^{1}H coupling constants Long-range ^{13}C–^{1}H *J*-resolution spectroscopy Chrysanthemic acid Pyrethroid 2D NMR

INTRODUCTION

(1*R*)-*trans*-Chrysanthemic acid is the acid moiety of pyrethrin I, one of the most useful natural insecticides, and of several synthetic pyrethroids including the 3-phenoxybenzyl ester, biophenothrin (Fig. 1). The acid moiety of these compounds is readily oxidized to an epoxy derivative on incubation with cytochrome P_{450}-dependent microsomal oxidases or on irradiation with light in air.[1,2] The geminal dimethyl groups of the cyclopropane ring and of the olefinic side-chain present in the starting materials are retained in most of the metabolites and photoproducts.

In a previous paper[3] we assigned the ^{13}C NMR resonances for these four methyl groups by two different experiments. One utilized ^{13}C–^{1}H correlation spectroscopy (C–H COSY), and the interpretation was based on the methyl ^{1}H signal assignments achieved by homonuclear 2D NMR techniques; the other utilized long-range C–H COSY measurements with some long delay times in the pulse sequence. The latter assignments were based on the assumption that the three-bond ^{13}C–^{1}H coupling constants $[^{3}J(C,H)]$ for nuclei in a *cis* relationship on a cyclopropane ring and on an epoxide ring are larger than when they are in a *trans* relationship following the Karplus relationship, but that the reverse situation applies to a C=C bond.[4] To test the validity of

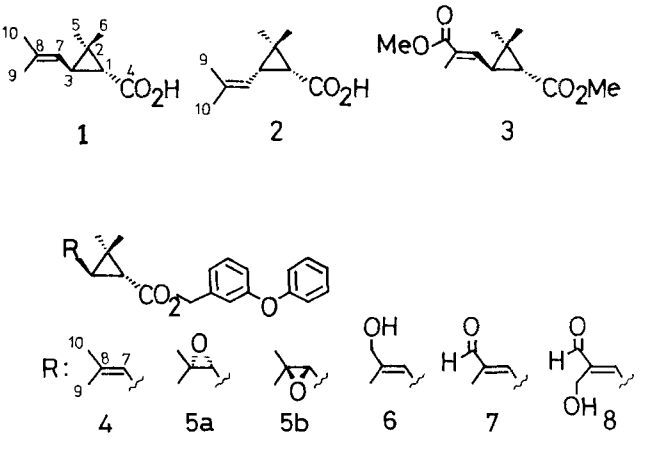

Figure 1. Structures of *trans*-chrysanthemic acid (**1**), *cis*-chrysanthemic acid (**2**), methyl *trans*-pyrethrate (**3**), biophenothrin (**4**) and its oxidized derivatives **5–8**, showing the numbering system used.

* Author to whom correspondence should be addressed.

0749-1581/93/010090-04 $07.00

Received 22 October 1991
Accepted (revised) 10 September 1992

this assumption the ^3J(C,H) values have now been measured using a two-dimensional (2D) technique, specifically long-range ^{13}C–^1H J-resolution spectroscopy (LRCJR) as developed by Bax and Freeman[5] and improved by Seto *et al.*[6] In addition, the ^3J(C,H) values were measured for (1*R*)-*cis*-chrysanthemic acid, methyl (1*R*)-*trans*-pyrethrate and the 10-hydroxy and 10-oxo microsomal metabolites of biophenothrin.[1]

EXPERIMENTAL

Chemicals

The structures of the compounds studied and the numbering system used are shown in Fig. 1. (1*R*)-*trans*-Chrysanthemic acid (**1**), its *cis* isomer (**2**) and pyrethrum extract (20% pyrethrins) were supplied by Sumitomo Chemical (Osaka, Japan). Methyl pyrethrate (**3**) was obtained by methanolysis of a pyrethrin II mixture separated from the pyrethrum extract. ^1H and ^{13}C assignments for the acid moieties have been reported previously in detail[7,8] but H-5 and H-6 in **2** have been reassigned as reported in our previous 2D NMR analysis.[3] Biophenothrin (**4**), prepared by coupling the acid chloride of **1** and 3-phenoxybenzyl alcohol, was oxidized to obtain synthetic standards of the microsomal metabolites. A separable mixture of the diastereomeric epoxides **5a** and **b** was prepared, using *m*-chloroperoxybenzoic acid, and the 10-hydroxy (**6**) and 10-oxo (**7**) derivatives were formed using selenium dioxide. These oxidations, carried out as reported previously for the oxidation of (*S*)-bioallethrin,[1] and the NMR data for the acid moieties in **5**–**7** are almost the same as those for the corresponding (*S*)-bioallethrin derivatives.[1] The 9-hydroxy-10-oxo derivative **8** is a new compound obtained in the reaction of **3** with selenium dioxide; it is not observed as a microsomal oxidation product. The structure of **8** was confirmed by 2D NMR. In particular, the orientation of the functional groups on the double bond was determined by nuclear Overhauser effect spectroscopy (NOESY), and correlation peaks were observed between H-7–H-10 and H-3–H-9; ^1H NMR (ppm) of **8**: H-1 1.96 1H d (J = 5 Hz), H-3 2.51 1H dd (J = 10.5, 5 Hz), H-5 1.33 3H s, H-6 1.30 3H s, H-7 6.22 1H d (J = 10.5 Hz), H-9 4.46 2H s and H-10 9.38 1H s; ^{13}C NMR (ppm) of **8**: C-1 37.5, C-2 31.7, C-3 32.7, C-4 170.2, C-5 20.5, C-6 22.3, C-7 153.6, C-8 142.6, C-9 56.1 and C-10 194.5.

NMR spectroscopy

NMR spectra were recorded on a JEOL GX 270 Fourier transform NMR spectrometer for chloroform-*d* solutions, containing tetramethylsilane as internal standard, using a 5 mm dual-frequency ^1H–^{13}C probe (270 MHz for ^1H and 67.8 MHz for ^{13}C). Pulse widths were 6.1 μs (45°) for ^1H NMR and 4.2 μs (45°) for ^{13}C NMR. Two-dimensional LRCJR spectra were acquired with spectral widths of 12 700 (^{13}C) and 40.0 Hz (^1H) with the pulse sequence shown below,[6] 0.161 s acquisition time (Acq) and a 4096 × 128 data set;

^1H: sel —— sel BBD–BBD/sel

^{13}C: 90°_x–t_1–180°_y–t_1–Acq–PD

The optimum conditions for the measurements were determined by changing the sample concentration (20 and 200 mg ml^{-1}), probe temperature (25 and 40 °C), pulse delay (PD) (5, 10 and 12 s), and power level of the irradiation attenuator.

RESULTS AND DISCUSSION

The spectrum with the best signal-to-noise ratio was obtained with a highly concentrated sample (200 mg ml^{-1}) and a long PD (12 s) at elevated temperature (40 °C). Strong irradiation is preferred for the detection of long-range couplings, but it can also affect other protons resonating close to the irradiation centre. The power level that was used therefore depended on the location of the proton selected for irradiation.

The *trans*-acid **1** contains three methine protons (H-1, H-3 and H-7) resonating at 1.39, 2.10 and 4.90 ppm, respectively. Spectra suitable for ^3J(C,H) evaluation were obtained by irradiation of H-3 and H-7. Irradiation of the H-1 signal was precluded since it was too close to the H-5 (1.30 ppm) and H-6 (1.15 ppm) signals. The spectrum on the irradiation of H-3 revealed the ^3J(C,H) values between H-3 and the two geminal methyl carbons (C-5 and C-6) on the cyclopropane ring, and the spectra on irradiation of H-7 showed its couplings to C-9 and C-10 on the C=C bond (Fig. 2). The same relationships were obtained with the *cis*-acid **2**. In the case of **5a** and **b** the selective irradiation of H-1 and H-3 was difficult since they were also too close to the H-5 and H-6 signals.[3] Only H-7 irradiation gave reasonable spectra which showed the ^3J(C,H) values to C-9 and C-10 on the epoxide ring. Only H-7 was irradiated in measuring the ^3J(C,H) of values the two different types of geminal carbons (C-9 and C-10) on the C=C bond in **3** and **6**–**8**. Table 1 shows the results of the LRCJR experiments.

There is a Karplus relationship for ^3J(C,H) as in the case of ^1H vicinal coupling, and the ^3J(C,H) value is estimated to be about 0.5–0.7 times the corresponding ^3J(H,H) value for a structure where a proton replaces carbon in the same configuration.[4] According to this rule, ^3J(C,H) values of 2–4 Hz (*trans*) and 4–7 Hz (*cis*) are predicted for cyclopropane rings with average ^3J(H,H) values of 4–6 Hz (*trans*) and 8–10 Hz (*cis*). Similarly, ^3J(C,H) values of 1–2.5 Hz (*trans*) and 1.5–3.5 Hz (*cis*) for epoxide rings correspond to ^3J(H,H) values of 2–3.5 Hz (*trans*) and 3–5 Hz (*cis*), and ^3J(C,H) values of 6–12.5 Hz (*trans*) and 3–8.5 Hz (*cis*) for C=C bonds correspond to ^3J(H,H) values of 12–18 Hz (*trans*) and 6–12 Hz (*cis*).[3] The ^3J(C,H) values for the analogous groupings in **1**, **2** and **5** (Table 1) coincide well with the predicted values, except for the *trans* ^3J(C,H) across the epoxide functionality of **5**. A value of *ca.* 0 Hz has also been observed with another epoxide.[6]

Carbons C-5 in **1** and C-6 in **2** on the cyclopropane ring, each *cis* to the irradiated proton, show larger ^3J(C,

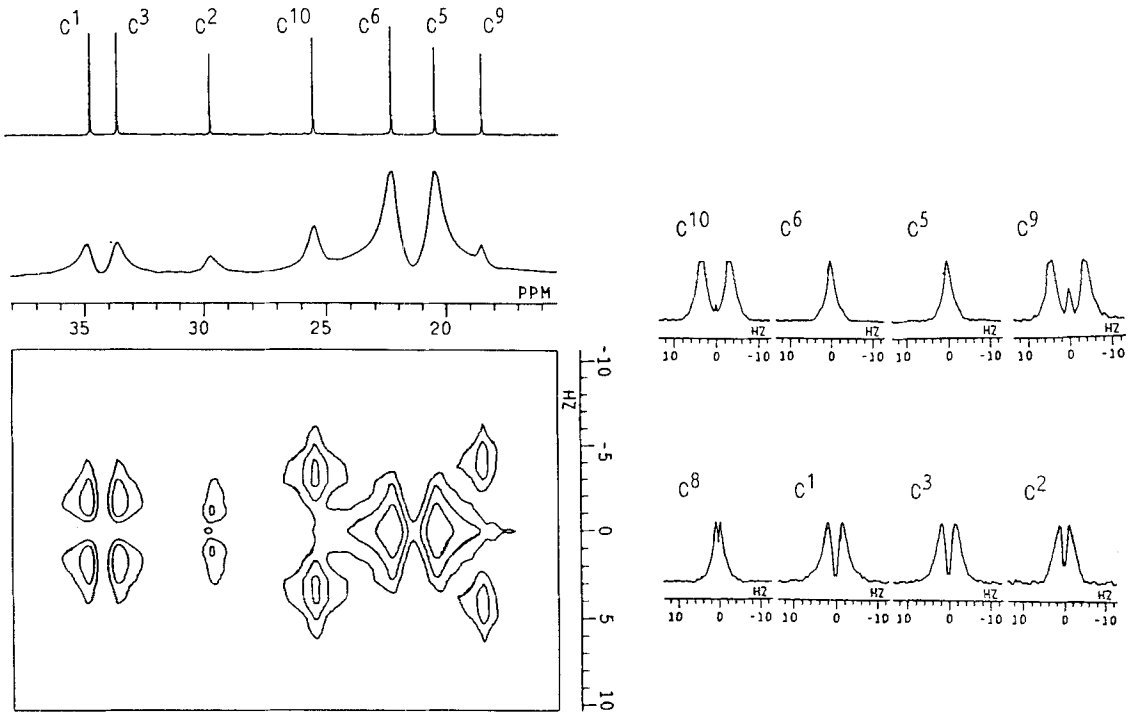

Figure 2. Partial LRCJR spectrum of *trans*-chrysanthemic acid (**1**) irradiating H-7, and its slice data.

H) values with H-3 than the *trans*-carbons (i.e. C-6 and C-5, respectively). Correlation peaks arising from long-range coupling were also easily observed for the *cis*-carbons in the previous long-range C–H COSY measurements.[3] Long-range coupling is not readily recognized with the *trans*-carbon of the epoxide, and a correlation peak with H-7 is observed only with the *cis*-carbon (C-10 in **5**). In contrast, the ^3J(C,H) values of the *trans*-carbons on the C=C bond in **1** and **2** are larger than those of the *cis*-carbons (i.e. C-9 and C-10 relative to H-7) and, accordingly, the long-range C–H COSY spectra[3] are also readily interpretable. The measurements with the delay adapted for the large (15 Hz) and small (6.3 Hz) ^3J(C,H) values showed strong correlation peaks with the *trans*- and *cis*-carbons, respectively.[3]

Although replacement of one of the olefinic geminal dimethyl groups with a hydroxymethyl or methoxycarbonyl functionality does not affect the ^3J(C,H)

Table 1. Three-bond ^{13}C–^1H coupling constants [^3J(C,H)] for *trans*- and *cis*-chrysanthemic acids (1 and 2), methyl pyrethrate (3) and biophenothrin derivatives 5–8

		trans Position			*cis* Position		
Compound	Irradiated proton	Carbon	Chemical shift (ppm)	^3J(C,H) (Hz)[a]	Carbon	Chemical shift (ppm)	^3J(C,H) (Hz)[a]
Cyclopropane							
1	H-3[b]	C-6	22.3	2.5	C-5	20.4	6.3
2	H-3	C-5	14.8	2.4	C-6	28.9	5.7
Epoxide							
5a	H-7	C-9	18.9	~0	C-10	24.5	2.6
5b	H-7	C-9	19.3	~0	C-10	24.4	2.6
Olefin							
1	H-7[c]	C-9	18.5	8.1	C-10	25.5	6.5
2	H-7	C-9	18.3	8.2	C-10	25.9	6.7
3	H-7	C-9	12.8	7.5	C-10	168.0	6.8
6	H-7	C-9	14.3	8.4	C-10	68.3	6.7
7	H-7	C-9	9.6	7.5	C-10	194.0	9.5
8	H-7	C-9	56.1	7.8	C-10	194.5	9.3

[a] Coupling constant measured by LRCJR when irradiating at the indicated proton.
[b] ^2J(C,H) and ^3J(C,H) values between H-3 and other carbons: C-1 3.7 Hz, C-2 1.7 Hz, C-4 4.9 Hz, C-7 1.7 Hz.
[c] ^2J(C,H) and ^3J(C,H) values between H-3 and other carbons: C-1 3.4 Hz, C-2 2.3 Hz, C-3 3.3 Hz, C-8 1.2 Hz (see Fig. 2).

values of C-9 and C-10, with an aldehyde group the ^3J(C,H) value of C-10 increases, as indicated by the data for **3** and **6–8**. An aldehyde carbon (C-10) located *cis* to H-7 on the C=C bond shows a larger ^3J(C,H) values than does the *trans*-carbon (C-9).

In summary, the ^3J(C,H) relationships noted in this study are in agreement with previous observations. They provide a basis for determining the stereochemistry of α,β-unsaturated carbonyl compounds and for the assignment of the NMR spectra of molecules containing substituted cyclopropyl or epoxy functionalities.

Acknowledgement

This study was supported, in part, by United States National Institutes of Health Grant P01 ES 00049.

REFERENCES

1. T. J. Class, T. Ando and J. E. Casida, *J. Agric. Food Chem.* **38**, 529 (1990).
2. L. O. Ruzo, I. H. Smith and J. E. Casida, *J. Agric. Food Chem.* **30**, 110 (1982).
3. T. Ando, N. E. Jacobsen, R. F. Toia and J. E. Casida, *J. Agric. Food Chem.* **39**, 600 (1991).
4. J. L. Marshall, *Carbon–Carbon and Carbon–Proton NMR Couplings* (*Methods in Stereochemical Analysis*, Vol. 2). Verlag Chemie International, Deerfield Beach, FL (1983).
5. A. Bax and R. Freeman, *J. Am. Chem. Soc.* **104**, 1099 (1982).
6. H. Seto, K. Furihata, N. Otake, Y. Itoh, S. Takahashi, T. Haneishi and M. Ohuchi, *Tetrahedron Lett.* **25** 337 (1984).
7. A. F. Bramwell, L. Crombie, P. Hemesley, G. Pattenden, M. Elliott and N. F. Janes, *Tetrahedron* **25**, 1727 (1969).
8. L. Crombie, G. Pattenden and D. J. Simmonds, *J. Chem. Soc., Perkin Trans. 1* 1500 (1975).