Tetrahedron Vol. 38, No. 5, pp. 639 to 644, 1982
Printed in Great Britain.

0040-4020/82/050639-06$03.00/0

INSECT PHEROMONE COMPONENTS

USE OF ^{13}C NMR SPECTROSCOPY FOR ASSIGNING THE CONFIGURATION OF C=C DOUBLE BONDS OF MONOENIC OR DIENIC PHEROMONE COMPONENTS AND FOR QUANTITATIVE DETERMINATION OF *Z/E* MIXTURES

RENZO ROSSI*
Dipartimento di Chimica, Università della Calabria, 87030—Arcavacata di Rende (CS), Italy

ADRIANO CARPITA and MARIA G. QUIRICI
Istituto di Chimica Organica, Facoltà di Scienze, M.F.N., Università di Pisa, Via Risorgimento, 35, 56100 Pisa, Italy

and

CARLO ALBERTO VERACINI
Istituto di Chimica-Fisica, Università di Pisa, Via Risorgimento, 35, 56100 Pisa, Italy

(*Received in the U.K.* 9 *July* 1981)

Abstract—Several insect sex pheromone components and structural analogues have been characterized by ^{13}C NMR spectroscopy. The evaluation of the difference between the chemical shift values of the allylic carbons present in the diunsaturated pheromone components and those of the carbons having the same position on the n-alkane chain of the corresponding saturated compounds was used to assign the configuration of the C=C double bonds present into such dienic compounds. The technique avoids the need to use the nuclear Overhauser effect; it allows quantitative evaluation, with good accuracy, of the stereoisomeric composition of mixtures of synthetic monoenic or dienic pheromone components such as (*Z*)- and (*E*)-9-tetradecen-1-yl acetate and (*Z*)- and (*E*)-9,11-dodecadien-1-yl acetate.

During our studies on the regio-, chemo- and stereoselective synthesis of monoenic and dienic sex pheromone components,[1-5] ^{13}C NMR was found very useful in elucidating the molecular structure of these compounds and their precursors, and in particular in assigning the configurations of their C=C double bonds.

In this note we wish to report a practically complete characterization of several sex pheromone components and their structural analogues by ^{13}C NMR spectroscopy, and to describe the use of this technique either to assign the configuration of disubstituted C=C double bonds present in dienic pheromone components, or to determine quantitatively the stereoisomeric composition of mixtures of synthetic monoenic or dienic pheromone components.

The compounds examined are shown in Table 1.

Compounds (I)–(IV) are potential insect attractants of Diptera Trypetidae such as *Anastrepha suspensa*.[2,6] Several other alcohols and acetates are pheromone components of Lepidoptera: (VI) for *Lobesia botrana*,[7] (VII) and (VIII) for *Diparopsis castanea*,[8] (IX) for *Laspeyresia pomonella*,[9] (X) for *Grapholita molesta*,[10] (XI) for *Archips argyrospilus*[11] and *A. mortuanus*,[11] (XIV) for *Spodoptera littoralis*,[8] (XV) for *Adoxophyes orana fasciata*,[12] *Pandemis pyrusana*,[13] *P. limitata*,[13] *A. mortuanus*,[11] *S. frugiperda*,[14] and *Agrotis ipsilon*,[15] (XVI) for *Ostrinia nubilalis*,[16] (XVIII) for *Lycorea ceres ceres*.[17] On the other hand, compound (XII) is a precursor of the sex pheromones of *Prionoxystus robiniae*[18] [Lepidoptera: Cossidae] or of *Attagenus elongatulus*[19] [Coleoptera: Dermestidae].

The assignment of the ^{13}C NMR chemical shift measured for all above mentioned compounds as well as those of other pheromone components previously reported in literature[20-22] are included in Table 1. Signals were assigned to individual carbons on the basis of the chemical shift values calculated using literature data[23-32] and by comparing logical series of related compounds. In particular, as regards the olefinic carbon atoms, the formula and the empirical parameter set reported by Dorman *et al.*,[25] as well as the correction terms reported by Batchelor *et al.*[26] were used. On the other hand, the calculation of the chemical shift values of the saturated carbon atoms was performed taking into account (i) the hydrocarbon data of Lindeman and Adams;[29] (ii) the empirical parameters associated to the presence of C=C double bonds into the molecule;[23,26,30] (iii) the parameters associated to the presence of functional groups.[22-24]

It must be noted that in the evaluation of the contributions due to the carbon atom chain, the parameters reported by Levy[24] are calculated taking into account that the functional groups substitute a terminal methyl group. On the other hand, the parameters reported by Wehrli[25] consider that the functional group substitutes an hydrogen atom. Thus, in the calculation of the chemical shift values it appeared necessary to use the Lindeman formula[23,29] considering separately the two different parameter sets. The results so obtained were sometimes rather different and caused contrasting assignments.

According to what has been previously observed for several monoenic sex attractants[21] it was noted that the olefinic carbons, which are easily recognized on the basis of their large chemical shift, afford signals of reduced interpretative value. Thus, their unequivocal assignment was not always possible. However, on the basis of the

Table 1. Assignment of the ^{13}C NMR chemical shifts of 1-alkanols, 1-alkyl acetates, alken-1-yl acetates, alkadien-1-ols and alkadien-1-yl acetates

Compound	$\delta^{13}C$ (ppm) of carbons in n position																		Ref.
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	$C_{1'}$	$C_{2'}$	
(4Z,6Z)-4,6-nonadien-1-ol (I)	62.3	32.4	23.8	130.7	124.1	122.6	134.0	20.8	14.1										a)
(4Z,7E)-4,7-nonadien-1-ol (II)	62.2	32.5	23.5	129.3	128.3	30.4	129.3	125.0	17.8										a)
(E)-4,8-nonadien-1-ol (III)	62.2	32.5	28.9	130.0	130.0	31.9	33.7	138.2	114.4										a)
(3Z,5Z)-3,5-nonadien-1-ol (IV)	62.0	30.9	126.8	123.0	126.1	132.8	29.5	22.7	13.7										a)
1-nonanol (V)	62.3	33.2	26.5	30.1	30.2	29.9	32.5	23.2	14.3										a),22
(ZE,9Z)-7,9-dodecadien-1--yl acetate (VI)	64.5	28.6	25.8	28.8	29.3	32.7	131.5	125.6	127.9	134.1	21.0	14.3							a)
(Z)-9,11-dodecadien-1-yl acetate (VII)	61.5	28.6	25.9	29.2- 29.3 - 29.6				27.7	132.1	129.1	132.6	116.5					170.6	20.9	a)
(E)-9,11-dodecadien-1-yl acetate (VIII)	64.5	28.6	25.9	29.1 - 29.3				32.5	130.8	135.2	137.1	114.1					170.6	20.9	a)

1-dodecyl acetate (XI)	68.4	29.0	26.3	29.7		30.1			29.7	32.3	22.9	14.2					172.2	21.1	21
(8E,10E)-8,10-dodecadien-1-ol (IX)	63.0	33.1	26.0	29.4	29.6		32.8	132.8	131.3	127.5	133.0	18.1							a)21
1-dodecanol (X)	63.2	33.2	26.2	29.9		30.1			29.8	32.4	23.0	14.2							a),21
(3Z,5Z)-3,5-tetradecadien-1-ol (XII)	62.0	30.9	126.6	123.1	126.2	133.0	27.5	29.2 - 29.4 -29.5				31.8	22.6	14.0					a)
1-tetradecanol (XIII)	62.0	32.9	25.9	29.6				29.8			29.5	32.0	22.8	14.1					a)
(9Z,11E)-9,11-tetradecadien-1-yl acetate (XIV)	64.5	28.6	25.9	29.4	29.7	29.2	30.1	27.6	129.8	128.5	124.6	135.9	25.9	13.7			170.8	20.9	a)
(Z)-9-tetradecen-1-yl acetate (XV)	64.6	28.7	26.0	29.8	29.3 - 29.4			27.2	129.8	129.7	27.0	32.0	22.4	14.0			170.9	21.0	a)
(E)-9-tetradecen-1-yl acetate (XVI)	64.5	28.6	25.9	29.2 - 29.4 -29.6			29.0	32.0	130.1	130.1	32.3	31.8	22.9	13.9			170.9	20.9	a)
1-tetradecyl-acetate(XVII)	64.5	28.8	26.1	29.4			29.8				29.5	32.0	22.8	14.1			170.9	20.9	a)
(7Z,11Z)-7,11-hexadecadien-1-yl acetate (XIX)	64.5	25.9	28.9	29.6	32.0	27.2	129.3-130.2		27.5	27.2	129.0-129.9		27.1	28.2	22.4	14.0	170.6	20.9	20
(7Z,11E)-7,11-hexadecadien-1-yl acetate (XX)	64.5	25.9	28.9	29.6	32.3	27.2	129.2-129.7		27.5	31.8	129.5-130.6		32.7	28.9	22.2	13.9	170.3	20.9	20
(7E,11Z)-7,11-hexadecadien-1-yl acetate (XXI)	64.5	25.8	28.7	29.5	32.4	32.7	129.8-130.3		31.9	27.4	129.0-130.0		27.0	28.8	22.4	13.9	170.7	20.9	20
(7E,11E)-7,11-hexadecadien-1-yl acetate (XXII)	64.5	25.9	28.7	29.5	31.8	32.3	129.8-130.5		32.8	32.8	129.6-130.2		32.3	28.7	22.2	13.9	170.7	20.9	20
1-hexadecyl acetate (XVIII)	64.5	26.6	28.7	29.4				29.8					29.4	32.1	22.7	14.1	170.6	20.9	a)

a) This work

literature data[26,30,33] the signal at higher field was assigned to the carbon nearer to the functional group. It was also observed that either for monoenic compounds or for conjugated and unconjugated dienic pheromone components the carbons in alpha position to disubstituted C=C double bonds show different chemical shift values in dependence on the (*E*)- or (*Z*)-configuration of the adjacent C=C double bond. In order to quantify for all considered diunsaturated compounds the effect of the (*Z*)- or (*E*)-configuration on the chemical shift values of the allylic carbons, the difference ($\Delta\delta$), between the observed chemical shift value of each allylic carbon of a diunsaturated compound and the chemical shift value of a carbon having the same position into the n-alkane chain of the corresponding saturated compound was evaluated. Table 2 summarizes all $\Delta\delta$ values found for the considered diunsaturated compounds. In this way it was possible to establish that carbons in allylic position to (*Z*)-double bonds are shifted 2.4 ppm upfield (standard deviation = 0.3 ppm) and that carbons in allylic position to (*E*)-double bonds are shifted 2.8 ppm downfield (standard deviation = 0.4 ppm) with respect to the analogous carbons of the corresponding saturated compounds. These $\Delta\delta$ values, 2.4 and 2.8 ppm were evaluated on the basis of fourteen and twelve data, respectively (Table 2). This type of dependence was very similar to that observed for several monounsaturated compounds.[21]

Thus, on the basis of these results it appeared possible to assign the configuration of C=C double bonds present into a dienic compound by analysis of its ^{13}C NMR spectrum or of that one of the corresponding saturated compound, and by evaluation of the above mentioned difference ($\Delta\delta$) of chemical shift values.

Taking into account these results it seemed also possible to evaluate quantitatively the stereoisomeric composition of mixtures of monoenic or dienic sex pheromone components. To this end, however, it appeared necessary to eliminate the effects perturbing the signal intensities in the ^{13}C spectra in order to establish that the peak areas were proportional to the number of nuclei and should reflect carbon concentration. The first technique used to obtain this result consisted of lowering the pulse flip angle to very small values and of gating the decoupler in such a way that it was on during data acquisition and off during a pulse delay. The typical experimental conditions used in this case to register the ^{13}C NMR spectra of pure (*Z*)- and (*E*)-9-tetradecen-1-yl acetate, (XV) and (XVI), or of mixtures of such stereoisomers were: acquisition time = 0.6 sec, pulse delay = 5 sec, offset = 45,207 Hz; spectral width = 5000 Hz; plot width = 500 Hz; pulse width = 18 μsec; no transients = 3000. The $CDCl_3$ solutions of the samples were deareated. For instance, by examination of the ^{13}C NMR spectrum of a 74:26 mixture of (XVI) and (XV), which had been previously quantitatively analyzed by glc it resulted that the concentration of (XVI) into the mixture was 76 ± 1%. Such evaluation was obtained by comparing the areas of the signals attributable to the C_8 and C_{11} allylic carbons of (XV) and (XVI). However, on the basis of the areas of the signals attributable only to the C_{11} allylic carbons (which were not enlarged and

Table 2. Effect of the configuration of 1,2-disubstituted C=C double bonds on the chemical shift of allylic carbons of dienic compounds

Compound	Position of the allylic carbon into the carbon chain	Configuration of the C=C double bond	$\Delta\delta$ (a)
(3Z,5Z)-3,5-nonadien-1-ol (I)	2 7	Z Z	-2.2 -2.9
(4Z,6Z)-4,6-nonadien-1-ol (II)	3 8	Z Z	-2.7 -2.4
(4Z,7E)-4,7-nonadien-1-ol (III)	3 3	Z Z	-3.0 +3.0
(E)-4,8-nonadien-1-ol (IV)	3 6	E E	+2.4 +2.0
(7E,9Z)-7,9)-dodecadien-1-yl acetate (VI)	6 11	E Z	+2.7 -1.9
(9Z,11E)-9,11-tetradecadien-1-yl acetate (XIV)	8 13	Z E	-2.1 +3.1
(3Z,5Z)-3,5-tetradecadien-1-ol (XII)	2 7	Z Z	-1.9 -2.3
(E)-9,11-dodecadien-1-yl acetate (VIII)	8	E	+2.5
(Z)-9,11-dodecadien-1-yl acetate (VII)	8	Z	-2.3
(8E,10E)-8,10-dodecadien-1-ol (IX)	7 12	E E	+2.7 +3.9
(7Z,11Z)-7,11-hexadecadien-1-yl acetate (XIX)	6 13	Z Z	-2.6 -2.3
(7Z,11E)-7,11-hexadecadien-1-yl acetate (XX)	6 13	Z E	-2.6 +3.3
(7E,11Z)-7,11-hexadecadien-1-yl acetate (XXI)	6 13	E Z	+2.9 -2.4
(7E,11E)-7,11-hexadecadien-1-yl acetate (XXII)	6 13	E E	+2.5 +2.9

a) Each $\Delta\delta$ value correspondes to the difference between the experimental δ value of the allylic carbon present in the mentioned diunsaturated compound and the δ value of a carbon having the same position into the n -alkane chain of the corresponding saturated compound (See Table 1).

superimposed) the concentration of (XVI) into the mixture resulted in 75 ± 1%.

Best results as regards either precision or accuracy were however obtained by determination of ^{13}C NMR spectra of samples which contained a paramagnetic additive, $Cr(acac)_3$, dissolved in $CDCl_3$, which eliminates the nuclear Overhauser effect, introducing a powerful relaxation mechanism which shortens the T_1s of all carbons so that relaxation time differentials become insignificant.[34–37]

The analysis of the ^{13}C NMR spectra of deareated $CDCl_3$ solutions of (*Z*)- and (*E*)-9-tetradecen-1-yl acetate, (XV) and (XVI), containing Cr(III)acetylacetonate at a 0.05 M concentration showed that in the experimental conditions used [spectral width = 5000 Hz; offset = 45,207 Hz; plot width = 500 Hz; acquisition time = 0.6 sec, pulse width = 45 μsec, no transients = 3200] the correlation between peak areas and number of nuclei was good. In particular the peak areas of the two allylic carbons, C_8 and C_{11} at 27.2 and 27.0 ppm for (XV) and at 32.6 and 32.3 ppm for (XVI) was 0.99 : 1.

Good results were also obtained in the quantitative analysis of a synthetic mixture of (XV) and (XVI). On the basis of the signal areas corresponding to the C_8 and C_{11} allylic carbons present in the two stereoisomers it was possible to deduce that the percentage of (XVI) into the mixture was 74.0 ± 1.0%. Gas chromatographic analysis showed on the other hand that this mixture contained (XV) and (XVI) in 26.0 ± 0.2% to 74.0 ± 0.2% ratio.

This type of ^{13}C NMR analysis was finally applied to determine the stereoisomeric composition of compounds which are not easily resolved by gas-chromatographic methods also employing high resolution glass-capillary columns. A typical example is the two sex pheromone components of the red bollworm moth, *D. castanea*, i.e. (*Z*)- and (*E*)-9,11-dodecadien-1-yl acetate, (VII) and (VIII) respectively.[8] The direct separation of such compounds may in fact be only accomplished either by gas chromatography on a smectic liquid crystal stationary phase,[38] or by reversed phase hplc on a column packed with octadecylsilane bonded silica particles.[3,4] A ^{13}C NMR spectrum of a $CDCl_3$ solution of a mixture of (VII) and (VIII) containing Cr(III) acetylacetonate at a 0.05 M concentration was registered in the following conditions: offset = 45,207 Hz; acquisition time = 0.8 sec; pulse width = 40 μsec; spectral width = 5000 Hz; no transients = 16,000; plot width: 500 Hz. The comparison between the peak areas attributable to the C_8 allylic carbons of (VII) and (VIII) (at 32.5 and 27.7 ppm respectively) showed that this mixture contained (VII) and (VIII) in ratio 25.5 : 74.5. A very similar ratio, 25.4 : 74.6, was calculated on the basis of a second ^{13}C NMR spectrum registered in different experimental conditions: offset = 45,207 Hz; acquisition time = 0.6 sec, pulse width = 45 μsec; plot width = 1000 Hz; no transients = 4000; spectral width: 5000 Hz. Hplc analysis[3,4] showed on the other hand that this stereoisomeric mixture contained (VII) and (VIII) in 25.3 ± 0.2 : 74.7 ± 0.2 ratio.

In conclusion, the above ^{13}C NMR method which appears general, rather precise, and accurate, represents an efficient tool to evaluate the stereoisomeric composition of compounds containing disubstituted C=C double bonds. Such method in some cases could constitute a valid alternative to other (quantitative) analytical techniques such as the gas–liquid chromatography or high-performance liquid chromatography.

EXPERIMENTAL

The ^{13}C NMR spectra were obtained by a proton noise decoupled ^{13}C Fourier transform operation using a computer-controlled system based on a Varian XL-100 spectrometer operating at 25.2 MHz. Deareated $CDCl_3$ solutions containing 15–20% of the sample were filled into standard 10 mm sample tubes. Chemical shifts were measured against internal TMS. The peak areas were measured by multiplying peak heights by half-band width or, alternatively, by cutting out the peaks and weighing the paper.

Compounds (I)–(IV), (VI)–(VIII), (XII)–(XVI) were synthesized in our laboratory by methods which have been recently reported.[1–5] Compounds (V), (IX), (X), (XVII) and (XVIII) were commercially available. Samples used for ^{13}C-measurements were all found over 99% pure by glc on high resolution glass capillary columns [25 m × 0.3 mm i.d. Carbowax 20 M or 25 m × 0.3 mm i.d. FFAP].

Glc analyses were performed on a DANI 3900 glass-capillary column dedicated gas-chromatograph using a FID detector (carrier gas N_2, 0.3 kg/cm^2; split 43 ml/min; temp. of detector, 260°).

Hplc analyses of (VII) and (VIII) and of their mixtures were carried out on a JASCO liquid chromatograph Mod. FAMILIC 100 N using a column packed with octadecylsilane bonded 5 micron (SC-01) silica particles; UV detector 232 nm; eluent: methanol/water (3 : 1), 6 μl/min substance load, 0.1 μl of a solution of such compounds (0.01 g) in methanol (1 ml). Calibration curves were obtained using samples of (VII) [UV (heptane): λ max (ϵ): 230 nm (29,941)] and (VIII) [UV (heptane): λ max (ϵ): 225 nm (32,792)] having 99.0 and 97.6% isomeric purity, respectively.

Acknowledgements—We thank the Italian National Research Council, Rome, for financial support. The "Centro C.N.R. per le Macromolecole Stereordinate ed Otticamente attive" (Pisa) is also gratefully acknowledged for use of its NMR facilities.

REFERENCES

[1] R. Rossi, A. Carpita, L. Gaudenzi and M. G. Quirici, *Gazz. Chim. Ital.* **110**, 237 (1980).

[2] R. Rossi, A. Carpita and M. G. Quirici, *Ibid.* **111**, 173 (1981).

[3] R. Rossi, A. Carpita and M. L. Gaudenzi, *Synthesis*, 359 (1981).

[4] R. Rossi, A. Carpita and M. G. Quirici, *Tetrahedron*, accepted for publication.

[5] R. Rossi, A. Carpita, M. G. Quirici and M. L. Gaudenzi, Communication presented at the 2nd European Symposium on Organic Chemistry, Stresa, 1–5 June, 1981.

[6] J. L. Nation, *Ann. Entomol. Soc. Am.* **65**, 1364 (1972); *Ibid.* **67**, 731 (1974).

[7] H. Arn, E. Städler and S. Rauscher, *Z. Naturforsch.* **30**, 722 (1975).

[8] B. F. Nesbitt, P. S. Beevor, R. A. Cole, R. Lester and R. G. Poppi, *Nature New Biol.* **244**, 208 (1973).

[9] M. Beroza, B. A. Bierl and H. R. Moffit, *Science* **183**, 89 (1974).

[10] A. M. Carde, T. C. Baker and R. T. Carde, *J. Chem. Ecol.* **5**, 423 (1979).

[11] R. T. Carde, A. M. Carde, A. S. Hill and W. L. Roelofs, *Ibid.* **3**, 71 (1977).

[12] R. Sato and Y. Tamaki, *Appl. Ent. Zool.* **12**, 50 (1977).

[13] W. L. Roelofs, R. F. Lagier and S. C. Hoyt, *Environ. Entomol.* **6**, 353 (1977).

[14] R. L. Jones and A. N. Sparks, *J. Chem. Ecol.* **5**, 721 (1979).

[15] A. S. Hill, R. W. Rings, S. R. Swier and W. L. Roelofs, *Ibid.* **5**, 439 (1979).

[16] J. A. Klün and G. A. Junk, *Ibid.* **3**, 447 (1977).

[17] J. Meinwald and Y. C. Meinwald, *J. Am. Chem. Soc.* **88**, 1305 (1966).

[18] R. E. Doolittle, W. L. Roelofs, J. D. Solomon, R. T. Carde and M. Beroza, *J. Chem. Ecol.* **2**, 399 (1976).

[19] H. Fukui, F. Matsumura, A. V. Barak and W. E. Burkholder, *Ibid.* **3**, 539 (1977).

[20] H. Disselnkotter, K. Eiter, W. Karl and D. Wendisch, *Tetrahedron* **32**, 1591 (1976).

[21] A. Barabas, A. A. Botar, A. Gocan, N. Popovici and F. Hodosan, *Ibid.* **34**, 2191 (1978).

[22] J. D. Roberts, F. J. Weigert, J. I. Kroschwitz and H. J. Reich, *J. Am. Chem. Soc.* **92**, 1338 (1970).

[23] F. W. Wehrli and T. Wirtlin, *Interpretation of Carbon*-13 *NMR Spectra.* Heyden, London (1976).

[24] G. C. Levy and G. L. Nelson, *Carbon*-13 *Nuclear Magnetic Resonance for Organic Chemists.* Wiley-Interscience, New York (1972).

[25] D. E. Dorman, M. Joutelat and J. D. Roberts, *J. Org. Chem.* **36**, 2757 (1971).

[26] J. G. Batchelor, R. J. Cushley, J. H. Prestegard, *Ibid.* **39**, 1698 (1974).

[27] W. Höbold, R. Keck and R. Radeglia, *J. prakt. Chem.* **317**, 1054 (1975).

[28] O. Kajimoto and T. Fueno, *Tetrahedron Letters* 3329 (1972).

[29] L. P. Lindeman and J. Q. Adams, *Anal. Chem.* **43**, 1245 (1971).

[30] J. G. Batchelor, J. H. Prestegard, R. J. Cushley and S. R. Lipsky, *J. Am. Chem. Soc.* **95**, 6358 (1973).

[31] P. A. Couperas, A. D. H. Clague and J. P. C. M. Van Dongen, *Org. Magn. Resonance* **8**, 426 (1976).

[32] F. D. Gunstone, M. R. Pollard, C. M. Scrimglour, N. W. Gilman and B. C. Holland, *Chem. Phys. Lipids* **17**, 1 (1976).

[33] J. Bus and D. J. Frost, *Rec. Trav. Chim. Pays-Bas* **93**, 213 (1974).

[34] G. N. La Mar, *Chem. Phys. Lett.* **10**, 230 (1971).

[35] R. Freeman, K. G. R. Pachler and G. N. La Mar, *J. Chem. Phys.* **55**, 4586 (1971).

[36] D. F. S. Natusch, *J. Am. Chem. Soc.* **93**, 2567 (1971).

[37] J. N. Shoolery and W. C. Jan Kowsky, Application Note N.4 Varian Assoc., Palo Alto, Calif., 1975.

[38] R. Lester and D. R. Hall, *J. Chromatogr.* **190**, 35 (1980).