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Determination of Isomeric Olefinic Groups in Unsaturated Terpene Aminoethanols by Carbon-13 Nuclear Magnetic Resonance Spectrometry

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Natural abundance carbon-13 pulse Fourier transform NMR spectra were obtained for the pure and mixed isomers of 2-bis(3′,7′-dimethyl-2′,6′-octadienyl)aminoethanol, for *cis*- and *trans*-2-(3′,7′-dimethyl-2′,6′-octadienyl)aminoethanols and for the corresponding isomeric *N*-nitroso compounds. The successful application of the model spectroscopic technique to the quantitative analysis of the cis and trans octadienyl groups in the disubstituted aminoethanol has been extended to the analysis of mixtures of geometric isomers of the monosubstituted compounds of related structure. The method is based on the fact that the isomeric octadienyl groups of all the aminoethanols studied are easily distinguished by the difference in the ^{13}C NMR chemical shifts of the 3′-methyl carbons ($\Delta\delta$ in the range 6.4-7.2 ppm) and of the 4′-carbons ($\Delta\delta$ in the range 7.5-8.1 ppm). Advantages of the ^{13}C NMR method over the conventional gas chromatographic method are outlined and pertinent analytical data are presented for comparison.

During the course of a recent study (*1*) on the reaction of various fishery chemicals with nitrite, it was noted that mild nitrosation of an isomeric mixture of 2-bis(3′,7′-dimethyl-2′,6′-octadienyl)aminoethanol (BDOA) (Figure 1)—a fish toxicant—produced exclusively *cis*- and *trans*-2-nitroso-(3′,7′-dimethyl-2′,6′-octadienyl)aminoethanols (NDOA) (Figure 2) and that the composition of the cis–trans isomers of the nitrosamine product was solely controlled by the overall ratio of the cis/trans olefinic groups originally present in the BDOA mixture. This observation coupled with the existence of three geometric isomers (trans–trans, trans–cis, and cis–cis, Figure 1) of BDOA in the commercial product for use as a potential carpicide necessitated the development of an analytical method. This method would preferentially differentiate between the cis and trans octadienyl groups rather than the isomeric chemical entities of BDOA. Preliminary account of some proton NMR work in this regard has been presented in an earlier report (*2*). The latter technique showed little promise to be utilized as a method for the quantitative differentiation of the cis–trans geometry involving the aforementioned aminoethanols of fishery interest because of the appearance of systematic errors caused by peak overlaps in the ^{1}H NMR spectra. In this article ^{13}C NMR spectrometry of the three terpene-related aminoethanols is described. The distinct differentiability of carbon chemical shifts of 3′-methyl carbons and 4′-carbons for the cis–trans pairs of octadienyl groups should facilitate the quantitative assessment of the composition of cis and trans isomers of a mixture of the monosubstituted aminoethanol. In addition to NDOA, the monosubstituted aminoethanol chosen for study is its precursor secondary amine, 2-(3′,7′-dimethyl-2′,6′-octadienyl)aminoethanol (DOA) (Figure 3). This material has been found to coexist often with the carpicidal aminoethanol (BDOA) as major impurities in the commercial products apparently due to insufficient alkylation during its manufacture.

EXPERIMENTAL SECTION

By analogy to previously published procedures (*3*), the pure trans and the geometric mixtures of the investigated aminoethanols (DOA, BDOA, and NDOA) were synthesized at this laboratory from the corresponding octadienyl chlorides and ethanolamine hydrochloride (Aldrich Chemical Co., Milwaukee, WI). *cis*-DOA and *cis,cis*-BDOA were made available by repetitive fractional distillation of the relevant mixtures of isomers obtained above. *cis*-NDOA was separated from the trans compound by column adsorption chromatography on silica gel (J. T. Baker Chemical Co., Phillipsburg, NJ) followed by preparative reversed-phase high-performance liquid chromatography (acetonitrile–water, 60:40) using a Varian Model LC-5000 instrument. Pesticide grade BDOA was supplied by SCM Organic Chemicals, Jacksonville, FL. All other chemicals including deuterated chloroform were acquired as analytical reagent quality chemicals from commercial sources and used without further purification.

Natural-abundance ^{13}C NMR spectra were recorded at ambient temperature on a JEOL FX 90-Q Fourier transform nuclear magnetic resonance spectrometer operating at 22.5 MHz. The spectrometer was equipped with a Texas Instrument Model TI-980-B computer for pulse programming and data processing. Spectra were normally obtained with the proton noise decoupling technique. In some instances where the chemical shift assignments were desired, the off-resonance decoupling experiments were performed as needed. An internal deuterium solvent lock was used throughout this study to stabilize field/frequency. The typical operating parameters were as follows: 60° pulse width, 14 μs; repetition time, 15–20 s; acquisition time, 0.8 s; accumulations, 700 transients; data points, 8000.

Samples of the aminoethanols in 5-mm NMR tubes (Wilmad Glass Co., Buena, NJ) were prepared in deuterated chloroform (0.2–1 M solutions) containing 10% (v/v) tetramethylsilane (Me_4Si) as an internal standard. The chemical shifts were determined as δ values for spectral widths of 5000 Hz. They are expressed in parts per million (ppm) relative to the internal reference Me_4Si and are reported to the nearest 0.01 ppm with an accuracy of 0.03 ppm.

Peak intensities were integrated in the absence of nuclear Overhauser effect (NOE) with gated decoupling, whereby the decoupler power was turned on during pulse and data acquisition

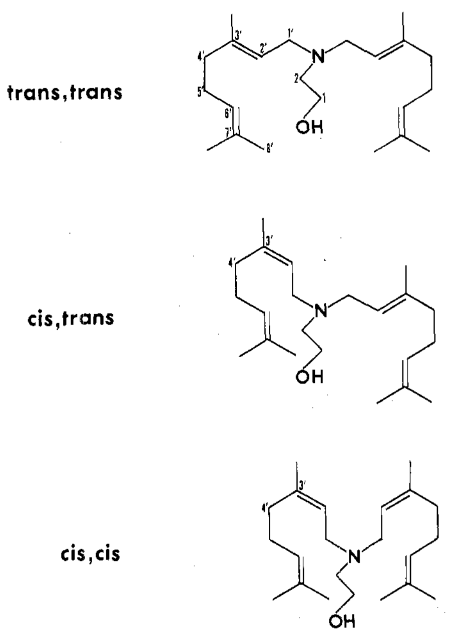

Figure 1. Structures of isomers of 2-bis(3',7'-dimethyl-2',6'octadienyl)aminoethanol (BDOA).

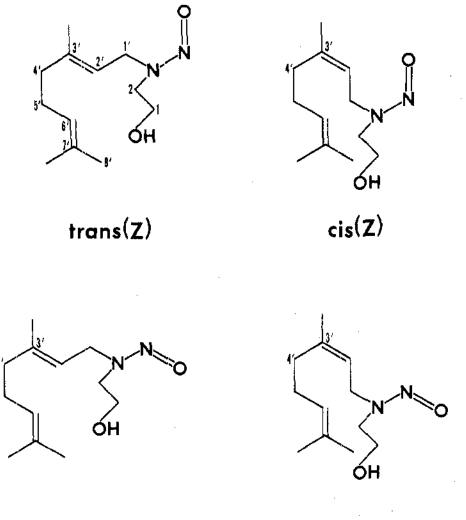

Figure 2. Structures of isomers of *N*-nitroso-2-(3',7'dimethyl-2',6'-octadienyl)aminoethanol (NDOA).

Figure 3. Structures of isomers of 2-(3',7'-dimethyl-2',6'-octadienyl)aminoethanol (DOA).

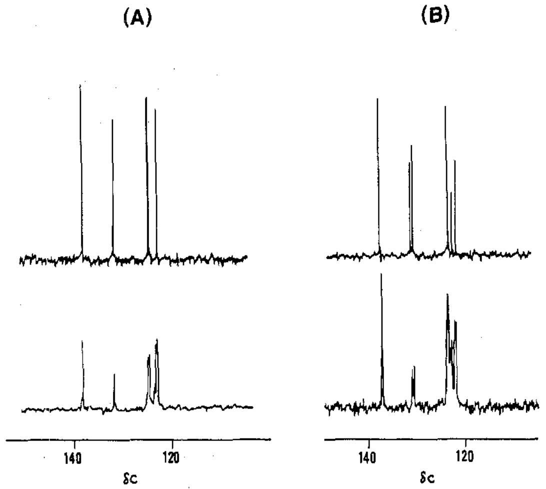

Figure 4. Olefinic carbon-13 resonances of BDOA: (A) (top) proton decoupled spectrum of a sample of pure *trans,trans*-BDOA, (bottom) off-resonance decoupled spectrum of a sample of pure *trans,trans*-BDOA; (B) (top) proton decoupled spectrum of a sample of pure *cis,trans*-BDOA, (bottom) off-resonance decoupled spectrum of a sample of pure *cis,trans*-BDOA.

for a time interval of 0.8 s and was turned off during a pulse delay (15–20 s interval). In a few experiments, peak areas were measured with the decoupler operating in the normal mode so that the NOE's for the carbons of interest were retained.

Spin–lattice relaxation times (T_1) for aminoethanol samples in deuterated chloroform at various concentrations (0.2–1 M) were determined by using the inversion recovery method (*4*). The coefficient of variation in T_1 measurements was within the range 3–5%.

For calibration, the spectra of samples of known composition and concentration were taken. Standard curves were constructed by plotting peak intensities against concentrations of the aminoethanol analytes.

RESULTS AND DISCUSSION

Since it is of importance to know the isomeric constituents of the highly mutagenic β,γ-unsaturated *N*-nitrosamine, NDOA (*5*), formed from the piscicidal aminoethanol (BDOA) mixture under conditions similar to the natural aquatic environment, we initiated a ^{13}C NMR spectroscopic study of the latter tertiary amine compound with an objective of delineating spectral characteristics of the cis and trans octadienyl groups in the BDOA molecule. Concurrently, the extent of magnetic nonequivalence experienced by the isomeric octadienyl groups in the different isomers of BDOA was evaluated in order to establish, if possible, some sorts of quantitative correlation between the ratios of isomers of both the reactant BDOA and the product NDOA. Before commencing spectral analyses of mixtures of isomeric structures, the spectra of isomerically homogeneous *cis,cis-* and *trans,trans*-BDOA were examined and the chemical shifts were assigned in each case. The assignments were made without ambiguity based on the off-resonance decoupled spectral information and on the reported chemical shift values of model compounds having analogous carbon structures. The hydroxyethyl and the octadienyl ^{13}C chemical shifts of BDOA can be assigned readily by comparison with those of respective 2-methylaminoethanol and 2-methyl-2-butene (*6*). Some representative partial spectra of *trans,trans*-BDOA are provided in Figure 4A to demonstrate the usefulness of the off-resonance method in the identification of the olefinic carbon resonances. The appearance of two doublets in the off-resonance spectrum is undoubtedly attributed to the 2'- and 6'-methine carbons, which are relatively more shielded than other olefinic carbons.

Table I. ^{13}C NMR Chemical Shifts of 2-Bis(3′,7′-dimethyl-2′,6′-octadienyl)aminoethanol (BDOA)[b]

carbon	carbon chemical shift δ, ppm		
	cis, cis	trans, trans	cis, trans[a]
1	58.79	58.79	58.79
2	55.10	55.10	55.10
1′	51.26	51.26	51.26
2′	122.7	121.6	121.6 (122.7)
3′	138.2	138.2	138.2
4′	32.29	39.93	39.93 (32.29)
5′	26.60	26.60	26.60
6′	124.2	124.2	124.2
7′	131.4	131.2	131.2 (131.4)
8′	25.68	25.68	25.68
3′-CH_3	23.51	16.31	16.31 (23.51)
7′-CH_3	17.66	17.66	17.66

[a] Values in parentheses correspond to the cis-carbon resonances. [b] Chemical shifts are relative to internal Me_4Si and recorded as δ values (ppm).

Table I lists the chemical shifts of all traceable peaks appearing on each of the spectra for the three isomers of BDOA. The data show the familiar features of common ^{13}C NMR spectra that the saturated carbon resonances lie in the 10–70 ppm region and the unsaturated olefinic carbons exhibit resonances in the 110–150 ppm region. A study of the chemical shift data for *cis,trans*-BDOA revealed each individual carbons to resonate indistinguishably from the corresponding carbons of a mixture of *cis,cis*-BDOA and *trans,trans*-BDOA as substantiated by their composite spectral data. With the exception of 2′-, 3′-, 4′-, and 3′-methyl-carbons, all other carbon chemical shifts are remarkably constant in the three BDOA isomers. Evidently the cis (or trans) octadienyl group in *cis,cis*(or *trans,trans*)-BDOA is magnetically equivalent to the very same group in *cis,trans*-BDOA. Experimentally the ^{13}C NMR spectrum of a sample of *cis,trans*-BDOA was noted to be invariably in superimposition with that of a sample prepared by mixing variable proportions of *cis,cis*-, *trans,trans*-, and *cis,trans*-BDOA.

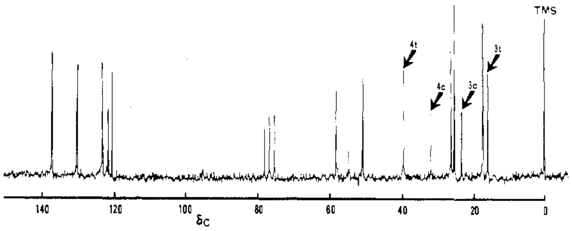

Figure 5. ^{13}C NMR proton decoupled spectrum of a mixture of *cis,cis*-, *trans,trans*-, and *cis,trans*-BDOA.

Table II. Quantitative Determination of Ratios of Cis/Trans Octadienyl Groups in Mixtures of Pure BDOA Samples

compn of BDOA mixture[a]	ratio of cis-octadienyl/trans-octadienyl		
	calcd[b]	obsd (^{13}C NMR integral)[c]	
		A ± std dev	B ± std dev
30:20:50	1.32	1.30 ± 0.05	1.35 ± 0.03
40:20:40	1.50	1.51 ± 0.06	1.48 ± 0.07
60:30:10	1.86	1.88 ± 0.10	1.85 ± 0.12
50:10:40	2.33	2.35 ± 0.08	2.33 ± 0.11
10:20:70	0.82	0.81 ± 0.03	0.81 ± 0.03
30:30:40	1.00	1.00 ± 0.06	1.02 ± 0.04

[a] Mixtures of pure BDOA samples were prepared by mixing pure isomers of BDOA in the ratio of cis,cis:trans, trans:cis,trans as shown. [b] Determined based on the known compositions of the BDOA mixtures. [c] 'A' represents the ratio measured from intensities of cis/trans 3′-methyl carbon resonances. 'B' represents the ratio measured from intensities of cis/trans 4′-carbon resonances. std dev = standard deviation.

To illustrate our general approach to the assignments of carbon chemical shifts, portions (olefinic regions) of the proton decoupled and off-resonance decoupled spectra of *cis,trans*-BDOA are shown in Figure 4B. A comparative analysis of these complementary spectra suggests that the extra two peaks (top spectrum) emerging 1.04 ppm and 0.2 ppm lower frequency than the respective signals at 121.63 ppm and 131.20 ppm must be due to the *cis*-2′-methine carbon (responsible for the signals as a doublet in the middle of the triple doublets shown in the bottom spectrum) and the nonprotonated cis-3′-carbon (no splitting occurred after off-resonance decoupling as indicated in the bottom spectrum). The above observation of certain degrees of separations of cis-carbon chemical shifts from the trans-carbon chemical shifts has rendered it possible to designate the most high frequency doublet around 121.63 ppm in the bottom spectrum of Figure 4A to the specific trans-2′-carbon instead of the trans-6′-carbon. It would be highly unlikely to envisage any differentiation between the cis- and trans-6′-carbon resonances on the grounds of their lack of involvement in geometrical isomerism. Upon reviewing the spectral data and the off-resonance splitting patterns (not shown here) for *cis,cis*-BDOA, we confirmed the shift assignments for the other two isomers and found the spectral parameters to be consistent within the same molecular system differing only by the cis–trans arrangement around the 2′,3′ double bond of the octadienyl group. It should be pointed out that, in contradiction to the observed trends of shifts to lower frequency for most cis carbons relative to the trans counterparts, the cis-4′-carbon resonates at 7.64 ppm higher frequency than the corresponding trans-carbon (Table I). Inspection of models discloses the former (cis-4′-carbon) to be situated in closer proximity with the two hydrogens attached directly to the 1′-carbon while the octadienyl group in a BDOA isomer adopts the cis geometry. The spectrum in Figure 5 displays the ^{13}C NMR signals of a typical mixture of BDOA comprising cis,cis, trans,trans, and cis,trans isomers sampled at random from the commercial batches of the fish toxicant.

Table II provides an example for the quantitative evaluation of the cis/trans ratios of the octadienyl groups in several samples of disubstituted (octadienyl) aminoethanol mixtures (BDOA). The peak integral values measured from the intensities of the two sets of cis/trans carbon chemical shifts at 23.51/16.31 ppm (3′-methyl-carbons) and 32.29/39.93 ppm (4′-carbons), respectively, allow direct dual estimation of the overall ratio of the cis–trans octadienyl groups present in the mixture of BDOA. While the ratios of three isomers of BDOA mixtures are understandably not correlative with those of the isomeric octadienyl groups, the observed cis/trans ratios of the same olefinic groups under analysis show relatively good agreement with the values calculated (Table II) indirectly from the known amounts of individual isomers in premixed samples and from the peak areas of gas chromatograms of unknown samples. Some considerations with respect to the definitive drawbacks inherent with the application of the latter methodology to the present case will be discussed later.

Following a similar approach taken in the chemical shift assignments for BDOA as described in the preceding paragraphs, ^{13}C NMR chemical shifts were determined for the cis- and trans-monosubstituted (octadienyl) nitrosoaminoethanols (NDOA), together with a mixture of these two isomers by analogy with those for diethylamine (*6*), diethylnitrosamine

Table III. ^{13}C NMR Chemical Shifts of *N*-Nitroso-2-(3′,7′-dimethyl-2′,6′-octadienyl)aminoethanol (NDOA)[c]

isomer	1	2	1′	2′	3′	4′	5′	6′	7′	8′	3′-CH_3	7′-CH_3
						cis						
(*E*)	58.88	46.25	51.67	118.1	142.3	31.58	26.51	123.7	131.8	25.81	22.87	17.84
(*Z*)	60.19	53.85	42.35	116.4	141.2	31.58	26.51	123.7	131.7	25.81	22.87	17.84
						trans						
(*E*)	58.88	46.25	51.67	117.5	143.1	39.69	26.51	123.7	131.8	25.81	16.47	17.84
(*Z*)	60.19	53.85	42.35	115.8	142.0	39.69	26.51	123.7	131.7	25.81	16.47	17.84
						cis–trans mixture[a]						
(*E*)	58.88	46.25	51.67	117.5	143.1	39.69	26.51	123.7	131.8	25.81	16.47	17.84
	b	*b*	*b*	(118.1)	(142.3)	(31.58)	*b*	*b*	*b*	*b*	(22.87)	*b*
(*Z*)	60.19	53.85	42.35	115.8	142.0	*b*	*b*	*b*	131.7	*b*	*b*	*b*
	b	*b*	*b*	(116.4)	(141.2)	*b*	*b*	*b*	*b*	*b*	*b*	*b*

[a] Values in parentheses correspond to the cis-carbon resonances. [b] Same values as those directly above. [c] Chemical shifts are relative to internal Me_4Si and recorded as δ values (ppm).

Table IV. Analysis of the Composition (as Cis/Trans Ratio) of the Isomeric Mixture of NDOA Formed in the Reaction of BDOA with Nitrite under Various Conditions

BDOA sample[a]		reaction condition			cis-NDOA/trans-NDOA (*a*/*b*)			
						obsd		
comp, % (*x*:*y*:*z*)	cis/trans (octadienyl)	pH	temp, °C	time, h	expected *a*/*b*[b]	*a*/*b*[c] (CV)	*a*/*b*[d] (CV)	NDOA yield, mg/mol BDOA
				No. 1				
(23:34:43)	0.80 (0.79)	2	20	12	0.80	0.78 (3.3)	0.80 (2.9)	0.759
		3	20	12	0.80	0.79 (4.1)	0.77 (3.7)	14.02
		4	20	12	0.80	0.81 (4.2)	0.85 (3.1)	134.2
		5	20	12	0.80	0.76 (3.5)	0.79 (3.5)	8363
		6	20	12	0.80	0.83 (3.8)	0.80 (3.0)	4510
		7	20	12	0.80	0.80 (2.7)	0.78 (3.4)	2424
		5	50	6	0.80	0.61 (5.3)	0.59 (2.9)	12556
		5	90	3	0.80	0.49 (4.0)	0.49 (2.7)	17482
				No. 2				
(36:51:13)	0.74 (0.76)	5	20	12	0.74	0.73 (4.5)	0.75 (4.0)	7998
		5	50	6	0.74	0.52 (3.9)	0.50 (3.3)	13014
		5	90	3	0.74	0.40 (4.1)	0.43 (3.0)	17291
				No. 3				
(62:20:18)	2.45 (2.48)	5	20	12	2.45	2.51 (6.3)	2.47 (4.7)	81.08
		5	50	6	2.45	2.01 (4.1)	2.05 (3.9)	12673
		5	90	3	2.45	1.84 (5.4)	1.89 (2.3)	17596
				No. 4				
(19:47:34)	0.56 (0.55)	5	20	12	0.56	0.55 (3.5)	0.58 (2.0)	8507
		5	50	6	0.56	0.42 (2.8)	0.44 (3.5)	11985
		5	90	3	0.56	0.33 (3.6)	0.31 (2.6)	16890
				No. 5				
(40:22:38)	1.44 (1.41)	5	20	12	1.44	1.46 (4.5)	1.42 (5.3)	7984
		5	50	6	1.44	1.06 (3.2)	1.00 (3.0)	12383
		5	90	3	1.44	0.87 (2.9)	0.85 (3.2)	17649

[a] $(x:y:z)$ = percent composition of (cis,cis:trans,trans:cis,trans); cis/trans(octadienyl) = $(2x + z)/(2y + z)$, the cis/trans ratios in parentheses were obtained from NMR peak intensity measurements (Table II). [b] Expected *a*/*b* = cis/trans(octadienyl groups in a mixture of BDOA) in the absence of isomerization. [c] *a*/*b* = NMR integral ratio for the cis/trans 3′-methyl carbon resonances; CV = coefficient of variation (%). [d] *a*/*b* = NMR integral ratio for the cis/trans 4′-carbon resonances.

(*6*), and diethanolnitrosamine (*7*). Table III assembles the chemical shift data obtained from these assignments. A spectrum of a sample that contains the cis and trans nitroso compounds (NDOA) is shown in Figure 6. As anticipated, the presence of additional configurational isomers (*E* and *Z* rotamers, Figure 2) results in more spectral complexity in this system. Nevertheless, the reasonably well separated signals as seen in Figure 6 enabled us to assign unequivocally the chemical shifts to all carbons of NDOA. In consistence with the general NMR spectrometric properties of *N*-nitrosamines, the α- and β-carbons of an octadienyl group in the *E* rotamer (the nitroso oxygen is cis to the hydroxyethyl moiety) of NDOA are significantly less shielded than those in the *Z* isomer presumably owing to the shielding effect of the nitroso oxygen. Such an effect appears to diminish as the distance separating the octadienyl carbons from the *N*-nitroso group increases ($\Delta\delta(E\text{-}Z)$ for carbon-1′ 9.3 ppm, for carbon-2′ 1.7 ppm, and for carbon-3′ 1.1 ppm). The striking similarity between the spectra of BDOA and NDOA is reminiscent of closely related structures in which conceivably no considerable changes in the magnetic environment occur after displacing an octadienyl group by a nitroso group.

By use of the integrals computed by the instrumental data system for the two sets of cis/trans signals at 22.87/16.47 ppm (3′-methyl-carbons) and 31.58/39.69 ppm (4′-carbons) (examples are given in Table IV), the cis/trans ratios of NDOA in samples of varied composition can be quantitated with adequate reliability (Table IV). This technique is utilizable

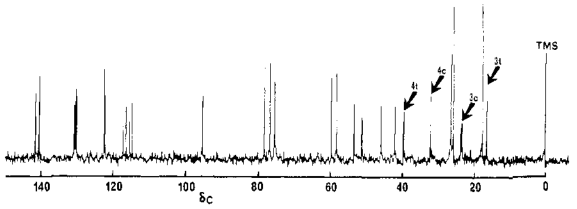

Figure 6. ^{13}C NMR proton decoupled spectrum of a mixture of *cis*- and *trans*-NDOA.

in the analysis of mixtures of reactants as well as products involved in the irreversible conversion of the piscicidal aminoethanol BDOA to the nitrosoaminoethanol NDOA. In this connection, we conducted an extensive study on the reaction of mixtures of BDOA having different compositions with nitrite in aqueous acetic acid solutions buffered at various pHs (*1, 8*). The nitrosamine products, after purification by column chromatography (*3*), were analyzed for contents of *cis*- and *trans*-NDOA by ^{13}C NMR. A summary of the results is given in Table IV. By comparison of the ^{13}C NMR data for the cis–trans octadienyl groups in BDOA with those for cis–trans NDOA, some degrees of correlation are obtained. In the light of previous experimental evidence (*8*) that selective α-cleavage of the $C_{1'}$–N bond of a mixture of the carpicide BDOA during nitrosation yields cis and trans *N*-nitrosamines (NDOA) as the only products containing the hydroxyethyl group, the ratio of the latter would be expected to be essentially equal to that of the total number of the individual cis vs. trans octadienyl groups contributed to the reactant (BDOA) mixture, which is measurable as demonstrated earlier from the ^{13}C NMR integrals of the 3′-methyl-carbons (or 4′-carbons) signals (Table II). In other words, the composition of a mixture of cis–trans nitrosoaminoethanols (NDOA) isolated from nitrosation of BDOA can be predicted on the basis of the known composition of the latter disubstituted (octadienyl) aminoethanols using the following relationship:

$$\frac{a}{b} = \frac{2x + z}{2y + z}$$

where a, b, x, y, and z represent, respectively, the percent composition of, *cis*-NDOA, *trans*-NDOA, *cis,cis*-BDOA, *trans,trans*-BDOA, and *cis,trans*-BDOA. It is noteworthy in this context that the observed and calculated a/b values for the nitrosamine analytes are in excellent agreement in those experiments where mixtures of BDOA were nitrosated at room temperature (Table IV). For studies carried out at higher temperature, the data (Table IV) show diverse discrepancies between the observed and calculated a/b values depending on the extent of concomitant cis–trans isomerization about the 2′,3′ double bond of the octadienyl group during nitrosation of the fish toxicant BDOA as supported by a separate study (*8*). Interestingly the a/b values obtained from the reactions employing the reactant BDOA in a fixed ratio (x:y:z = constant) were observed to remain practically unchanged over the entire pH range (2–7) studied so long as the nitrosation reactions were allowed to run at room temperature, although the variation of pH introduced substantial effects on yields of the isomeric nitrosamines, NDOA (Table IV).

Table V. ^{13}C NMR Chemical Shifts of 2-(3′,7′-Dimethyl-2′,6′-octadienyl)aminoethanol (DOA)[b]

	carbon chemical shift δ, ppm		
carbon	cis	trans	cis–trans mixture[a]
1	60.47	60.47	60.47
2	51.04	51.04	51.04
1′	46.87	46.87	46.87
2′	123.4	122.4	122.4 (123.4)
3′	138.1	138.1	138.1
4′	32.17	39.71	39.71 (32.17)
5′	26.60	26.60	26.60
6′	124.1	124.1	124.1
7′	131.6	131.3	131.3 (131.6)
8′	25.63	25.63	25.63
3′-CH_3	23.40	16.25	16.25 (23.40)
7′-CH_3	17.66	17.66	17.66

[a] Values in parentheses correspond to the cis-carbon resonances. [b] Chemical shifts are relative to internal Me_4Si and recorded as δ values (ppm).

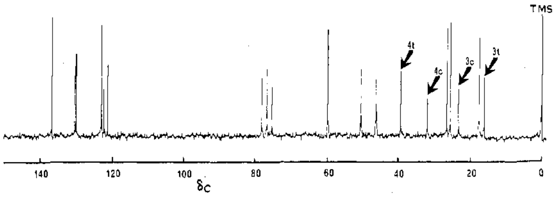

Figure 7. ^{13}C NMR proton decoupled spectrum of a mixture of *cis*- and *trans*-DOA.

The third type of compounds in this series under investigation consists of the cis- and trans-monosubstituted (octadienyl) aminoethanols (DOA) (Figure 3). They are related to BDOA and NDOA obviously because of their octadienylaminoethanol structure. Of particular importance is the fact that these secondary amines are viable precursors of NDOA on account of their potential reactivity toward nitrosation (*8*) and have been found as major contaminants in the commercial grade piscicide BDOA. Similar ^{13}C NMR spectral analyses were performed, as in the previous cases discussed so far, on *cis*- and *trans*-DOA along with a mixture of these isomers to give chemical shift data as compiled in Table V. As shown in Figure 7 the spectrum of a mixture of *cis*- and *trans*-DOA generally resembles that of a mixture of the disubstituted (octadienyl) aminoethanols (BDOA) (Figure 5) except for the α-carbon resonances, whose spectral signals appear at higher frequency (4.39 ppm for 1′-carbons and 4.06 ppm for 2′-carbons) than those of BDOA. This may be due to the adjacent N–H linkage shielding the α-carbons of DOA while the neighboring nitrogen–octadienyl moiety somewhat reduces the shielding effect on the corresponding α-carbons of BDOA.

Table VI compares the analytical results obtained by ^{13}C NMR spectrometric and gas chromatographic (GC) methods for the determination of compositions of geometrical mixtures of the three types of terpene aminoethanols involved in this study. Tabulated alongside are also some significant chemical shift difference data for the cis-trans pairs of monosubstituted (octadienyl) aminoethanols (NDOA and DOA) and the cis–trans octadienyl groups of the disubstituted (octadienyl) aminoethanol (BDOA). This is a manifestation of the magnitudes of differentiability of the isomeric carbon resonances that have been fully utilized in the development of the present method of analysis. Among the different aminoethanol types, the chemical shift differences fluctuate from 6.4 to 7.2 ppm for cis–trans 3′-methyl carbons and from 7.5 to 8.1 ppm for cis–trans 4′-carbons. Several BDOA samples of commercial piscicide quality known to contain DOA at low levels were selected for analytical evaluation (Table VI). Because of the low concentrations of DOA (less than 1%) that fall below the detection limit (0.05 M) of the instrumental system, it was necessary to effectively separate DOA from the bulk of BDOA by preparative pair ion high-performance liquid chromatography (*9*). The enriched DOA samples were then analyzed for the cis–trans composition as reported in Table VI. Ni-

Table VI. Comparison of Methods for Determining the Composition of Samples Containing Geometric Isomers of Terpene Aminoethanols

		difference in chemical shift $\Delta\delta$ (t-c), ppm		method[b]			
				^{13}C NMR		GC	
no.	sample[a]	C-3'-CH_3	C-4'	comp, %	*a/b*	comp, %	*a/b*
			BDOA				
1	cis	−7.20	+7.64	42.6	0,74	44.1	0.79
	trans			57.4		55.9	
2	cis	−7.20	+7.64	35.5	0.55	36.3	0.57
	trans			64.5		63.7	
3	cis	−7.20	+7.64	47.8	0.92	48.0	0.92
				52.2		52.0	
4	cis	−7.20	+7.64	5.9	0.06	7.0	0.07
	trans			94.1		93.0	
			DOA				
5	cis	−7.15	+7.54	77.6	3.46	76.4	3.24
	trans			22.4		23.6	
6	cis	−7.15	+7.54	30.7	0.44	32.1	0.47
	trans			69.3		67.9	
7	cis	−7.15	+7.54	25.5	0.34	24.9	0.33
	trans			74.5		75.1	
8	cis	−7.15	+7.54	41.0	0.69	39.5	0.65
	trans			59.0		60.5	
			NDOA				
9	cis	−6.40	+8.11	78.0	3.55	78.3	3.62
				22.0		21.7	
10	cis	−6.40	+8.11	29.9	0.43	28.2	0.39
	trans			70.1		71.8	
11	cis	−6.40	+8.11	26.7	0.36	27.7	0.38
	trans			73.3		72.3	
12	cis	−6.40	+8.11	41.4	0.71	40.9	0.69
	trans			58.6		59.1	

[a] DOA samples 5, 6, 7, and 8 (isolated from respective BDOA samples 1, 2, 3, and 4) were nitrosated at room temperature to give NDOA samples 9, 10, 11, and 12, respectively. For BDOA, 'cis''trans' are referred to octadienyl groups. [b] Data are average values of dual determinations based on 3'-CH_3- and 4'-carbon resonance integrals. Coefficient of variation ranges from 2.6 to 4.1 for the ^{13}C NMR method and from 3.3 to 5.2 for the GC method.

Table VII. Spin-Lattice Relaxation Times (T_1) of Isomeric Carbons in Terpene Aminoethanols[a]

	3'-methyl-carbon		4'-carbon	
compd	δ, ppm	T_1, s	δ, ppm	T_1, s
		BDOA		
cis	23.51	3.03	32.29	1.27
trans	16.31	3.67	39.93	1.57
		NDOA		
cis	22.87	3.36	31.58	1.36
trans	16.47	4.04	39.69	1.79
		DOA		
cis	23.40	3.51	32.17	1.28
trans	16.25	3.96	39.71	1.81

[a] At 22.5 MHz, room temperature, 0.5 M in deuterated chloroform; chemical shifts are relative to internal Me_4Si and recorded as δ values (ppm) Coefficient of variation for T_1 measurements: 3–5%.

Table VIII. Peak Intensity Ratios (*a/b*) and Spin-Lattice Relaxation Time Ratios (T_{1a}/T_{1b}) for Cis-Trans Carbons of Terpene Aminoethanols at Various Sample Concentrations[a]

	BDOA		NDOA		DOA	
concn, M	*a/b*	T_{1a}/T_{1b}	*a/b*	T_{1a}/T_{1b}	*a/b*	T_{1a}/T_{1b}
0.2	0.38	0.81	0.32	0.73	0.34	0.70
0.4	0.30	0.79	0.37	0.77	0.31	0.68
0.6	0.34	0.80	0.34	0.75	0.35	0.71
0.8	0.33	0.79	0.38	0.75	0.36	0.73
1.0	0.34	0.82	0.31	0.76	0.35	0.70

[a] At 22.5 MHz, room temperature, in deuterated chloroform solutions; values are mean values of three determinations, coefficient of variation, 2–6% for *a/b* measurements and 3–5% for T_{1a}/T_{1b} measurements; all samples were prepared by mixing known amounts of cis and trans isomers in a ratio of 1:2; the *a/b* values are average values of dual determinations based on 3'-methyl- and 4'-carbon resonance integrals

trosation of the DOA samples obtained in the above manner yielded, upon purification, samples of corresponding isomeric *N*-nitrosamines (NDOA). For the latter isomers, the cis/trans intensity ratio, *a/b*, should agree with that of DOA especially in a situation where possibilities of cis–trans isomerization do not exist during nitrosation. This is actually observed in Table VI. Comparisons of the data from the ^{13}C NMR and GC methods show fairly good agreement between the two methods. The inability of the former technique to attain high sensitivity analysis appears to be compensated by its simple sample preparation procedure, reliable performance of the instrument, and, above all, the high degree of reproducibility. The average coefficient of variation (or relative standard deviation) for replicate analyses was 3.73% (Table II, Table IV, and Table VI).

Unlike the ^{13}C NMR method, the GC method frequently (especially in dealing with aminoethanol compounds) requires tedious chemical derivatization and time-consuming optimization of chromatographic conditions in addition to the painstaking selection of proper GC columns for achieving efficient resolution of isomers. Furthermore, while the GC method provides measurements merely for the chemical entities *x*, *y*, and *z* (Table IV), the ^{13}C NMR method is unique in a sense that it is capable of permitting direct quantitation of the composition of cis/trans octadienyl groups (*a/b* in Table IV) in a mixture of BDOA.

Table VII contains the spin–lattice relaxation times (T_1) of the two sets of isomeric carbons in the three terpene aminoethanols studied. The data clearly demonstrate the similarity of T_1's between the cis–trans pairs of carbons. Under conditions used (Table VII), the respective cis/trans T_1 ratios in BDOA, NDOA, and DOA are 0.83, 0.83, and 0.88 for 3'-methyl carbons, and are 0.81, 0.76, and 0.71 for 4'-carbons. This would imply that preferential saturation of any of the isomeric carbons seems to be minimal particularly under the experimental conditions employed throughout this study. Since we dealt with samples of variable concentration (0.2–1 M) in all analyses, it was deemed appropriate to study the effect of aminoethanol concentrations on the observed values of peak intensity ratios (*a*/b) and spin–lattice relaxation time ratios (T_{1a}/T_{1b}) for cis–trans carbons of interest. Table VIII shows the results of such a study. The change in analyte concentration apparently has little influence on both the peak intensity and spin–lattice relaxation time ratios. To establish calibration curve data, standard solutions (0.2, 0.4, 0.6, 0.8, and 1.0 M) of the terpene aminoethanol in deuterated chloroform were prepared from either a pure isomer or mixed isomers. The peak intensities were found to vary linearly with concentrations over the entire range (0.2–1.0 M) (slope, 1.0, correlation coefficient, 0.996).

All analyses performed in this study are results of three replicate determinations. The precision of analytical data reflects the accuracy in the acquisition of peak integral values (see Tables II, IV, and VI for precision data). That the gated

Table IX. Compositions of Cis-Trans Mixtures Determined with and without Gated Decoupling

premixed sample	composition (% cis)		
	known	measd[a] gated decoupling	
		with	without
BDOA			
1	74.30	75.04	73.95
2	45.81	43.88	44.70
3	18.27	19.12	18.93
NDOA			
1	37.09	36.73	38.25
2	60.34	60.16	59.86
3	40.41	39.97	42.01
DOA			
1	29.48	30.31	30.40
2	53.52	54.09	55.19
3	25.63	25.05	24.80

[a] The measured values are average values of dual determinations based on 3′-methyl- and 4′-carbon resonance integrals. Coefficient of variation: with gated decoupling, 2-4%; without gated decoupling, 2-6%.

decoupling technique eliminates the residual NOE during data acquisition for integrals has been substantiated by the spectral analysis of a homogeneous sample containing a pure isomer. On the basis of the uniformity of NOE's received by the structurally similar cis–trans carbons, one would expect no appreciable effects of the different modes of decoupling (with vs. without gated) on the relative peak intensities of cis–trans carbon resonances. The data in Table IX illustrate that the two decoupling methods, with and without gated decoupling modes, can be used in the quantitative analysis of the composition of cis–trans mixtures of terpene aminoethanols. Both decoupling methods yielded comparable results (Table IX).

The generality of the ^{13}C NMR spectral behavior with the three types of unsaturated terpene aminoethanols indicates that the technique developed in the present study can be applied to the quantitative characterization of isomeric octadienyl groups in other closely similar compounds. This method provides a means for studying stereospecific interactions of the fish toxicant BDOA and the nitrosamine NDOA with biological systems.

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Computer Prediction of Substructures from Carbon-13 Nuclear Magnetic Resonance Spectra

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A program that searches a data base of assigned ^{13}C NMR spectra to find reference structures that model an unknown is described. The program exhaustively searches the data base for substructure–subspectrum pairs that are consistent with the spectrum of the unknown. A test to evaluate the interpretive character of library search programs is suggested. The test criterion is met by the interpretive procedure described. Retrieved reference compounds are organized on the basis of the substructure predictions. The spectroscopist–user interactively displays the structural diagrams of the retrieved reference structures.

New applications of computer technology are continuing to influence the practice of chemistry. The computer is increasingly applied as a tool to assist the chemist in elucidation and verification of chemical structures. ^{13}C NMR spectrometry is one area receiving increasing attention because of the well-defined nature of chemical shifts, multiplicities, and spectrum–structure correlations (assignments). In developing computer tools in ^{13}C NMR, three main avenues have been pursued—pattern recognition, artificial intelligence, and the search of spectroscopic libraries. The boundaries between these categories are sometimes not distinct. Pattern recognition has been used to interpret localized structural features such as functional groups (*1*) and to interpret global features such as chemical class and skeletal features (*2*).

Artificial intelligence applications may be considered to be those methods that use a "knowledge base" of spectra–structure correlations that is at least more abstract than a reference library. (Because the boundary between artificial intelligence and other techniques is often hazy, we have chosen to define it to reflect the various reported applications of the method to computer-assisted structure elucidation.) Carhart and Djerassi have developed a program to elucidate the structure of acyclic monofunctional amines (*3*). Mitchell and Schwenzer described spectrum–structure correlations derived from a data base using the Meta-DENDRAL approach (*4*).

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