TETRANACTIN, A NEW MITICIDAL ANTIBIOTIC. VI

DETERMINATION OF DINACTIN, TRINACTIN AND TETRANACTIN IN THEIR MIXTURES BY NMR SPECTROSCOPY

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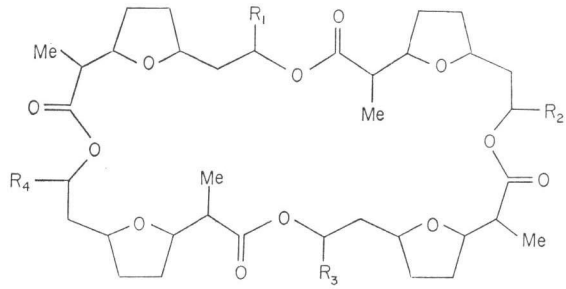
The macrotetrolide antibiotic tetranactin ($C_{44}H_{72}O_{12}$)[1] is produced by *Streptomyces aureus*, accompanied by its homologues dinactin ($C_{42}H_{68}O_{12}$) and trinactin ($C_{43}H_{70}O_{12}$) (Fig. 1). In the course of our investigation, an excellent method for quantitative determination of tetranactin and the homologues was established, based on complex formation of the antibiotics with sodium picrate[2]. However, for the estimation of the tetranactin homologues in a mixture, it is necessary to separate the components prior to analysis.

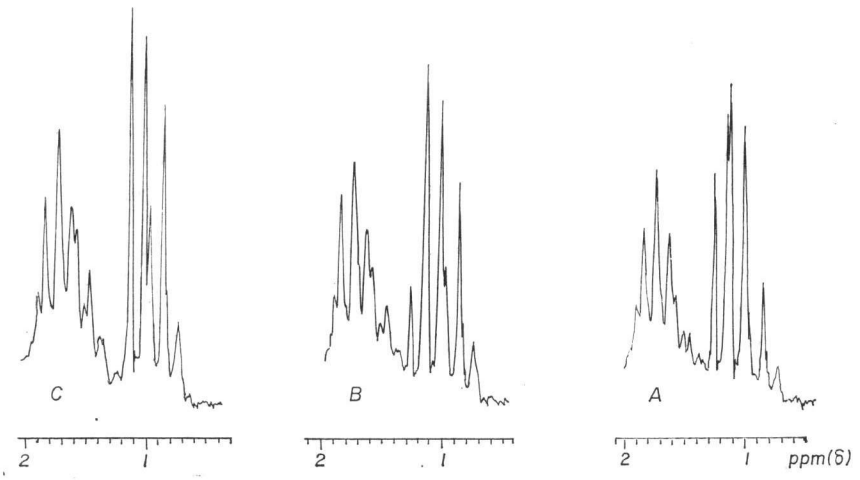
The homologues have characteristic difference in the NMR spectra[3,4]. As shown in Fig. 2, these differences are observed in the signals from the methyl protons in the residues R_1 (described in Fig. 1): methyl=δ 1.21 (doublet) and ethyl=δ 0.88 (triplet). The present note presents a simple method to determine the composition of mixtures of tetranactin homologues by NMR spectroscopy.

Fig. 1. Structures of tetranactin homologues.
Tetranactin: R_1, R_2, R_3, R_4=ethyl.
Trinactin: R_1, R_2, R_3=ethyl, R_4=methyl.
Dinactin: R_1, R_3=ethyl, R_2, R_4=methyl.



The standard samples used in this experiment were fractionated by column chromatography[5]. A Hitachi Perkin-Elmer model R-20A (60 MHz) NMR spectrometer was used with $CDCl_3$ solutions (*ca.* 0.4 ml) containing *ca.* 32 mg of the sample. Spectrometric conditions were as follows: sweep speed 250 sec/600 Hz, time constant 0.1 sec, H_1 level 5×10^8 μV, sensitivity 6.3×10, internal standard tetramethylsilane, at ambient temperature. Proton signals in the range of δ 0.5~2.0 were recorded ten times repeatedly on the same paper. For six signals in the spectra, the peak heights from base line were measured with slide calipers (vernier scale 0.05 mm). For convenience, observed spectra (averaged peak heights) were normalized to that of the doublet methyl proton signals (δ 1.08, peak Nos. 2 and 3, which are due to the four methyl groups, Me,

Fig. 2. NMR spectra of standard samples of tetranactin homologues (in $CDDl_3$; 60 MHz).
Spectra A, B and C are those of dinactin, trinactin and tetranactin, respectively.



in Fig. 1); consequently normalized spectra are independent of the concentration of the sample (Table 1).

Scale factors for the normalized spectra of dinactin and trinactin compared to tetranactin were estimated separately with binary mixtures of standard samples by the least-squares method. These factors may reflect the differences of intramolecular magnetic environments among the homologues (Table 2). In most cases, the estimated compositions were with an average error of about 3 mole % (Table 3). Unfractionated samples (Nos. 12 and 13), for which qualitative analyses were carried out by thin-layer chromatography, were treated as ternary and binary mixtures,

Table 1. Normalized NMR spectra of standard samples.

Peak No. (δ)	Sample		
	A	B	C
1 (0.88)	4.035	6.679	7.960
2 (1.02)	9.269	9.419	9.615
3 (1.13)	10.732	10.581	10.386
4 (1.28)	7.575	3.806	0.727
5 (1.59)	3.580	4.328	4.547
6 (1.74)	7.866	7.467	7.193

The averaged peak heights of six signals in each spectra were normalized to that of doublet methyl proton signals (δ 1.08, peak Nos. 2 and 3). The samples A, B and C are the standard samples of dinactin, trinactin and tetranactin, respectively.

Table 2. Estimation of scale factors (f_a and f_b) for the normalized NMR spectra of dinactin and trinactin, respectively, on the basis of tetranactin.

		Sample No.					
		1	2	3	4	5	6
Normalized NMR spectra	Sig. No. 1	4.952	6.222	7.358	7.715	7.153	7.287
	2	9.378	9.477	9.592	9.594	9.510	9.504
	3	10.622	10.523	10.408	10.406	10.490	10.496
	4	5.995	3.905	1.886	1.383	2.516	2.123
	5	3.733	4.075	4.365	4.512	4.347	4.423
	6	7.745	7.578	7.398	7.178	7.314	7.326
Added (Found) mole %	A	80.01 (80.6)	50.68 (51.6)	21.13 (20.2)	0.00 (—)	0.00 (—)	0.00 (—)
	B	0.00 (—)	0.00 (—)	0.00 (—)	21.34 (22.6)	61.30 (61.6)	50.31 (49.1)
	C	19.99 (19.4)	49.32 (48.4)	78.87 (79.8)	78.66 (77.4)	38.70 (38.4)	49.69 (50.9)
Scale factor		f_a=0.799			f_b=0.893		

respectively. Recovery test on an enriched sample (No. 14=dinactin 3.12 mg+sample No. 13, 29.85 mg) was satisfactory; namely the result indicated that the composition of sample No. 13 was 14.4 mole % (dinactin) and 85.6 mole % (tetranactin). Although standard deviations for the averaged peak heights are within 1 % in these measurements, accurate results were not obtained for mixtures which contained components in less than 10 mole %; for these mixtures standard samples should be added to obtain more accurate results.

In this experiment, a small desk top computer (Seiko S-301) was employed for data processing, and five signals (excluding the peak No. 3) were selected as the optimum for a simple simultaneous determination of tetranactin homologues in their mixtures. This method could be applied to NMR spectrometers capable of accumulation of spectra and calculation successively, in order to obtain more accurate results with less labor.

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Table 3. Results of determination.

		Sample No.							
		7	8	9	10	11	12	13	14
Normalized NMR spectra	Sig. No. 1	4.590	5.112	5.418	6.362	7.130	6.108	7.564	7.304
	2	9.247	9.340	9.333	9.440	9.482	9.444	9.596	9.575
	3	10.753	10.660	10.667	10.560	10.518	10.556	10.404	10.425
	4	6.887	6.070	5.673	3.865	2.457	4.177	1.445	2.081
	5	3.695	3.999	3.971	4.130	4.242	4.144	4.445	4.382
	6	7.793	7.735	7.700	7.532	7.268	7.507	7.321	7.403
Added (Found) mole %	A	80.22 (82.6)	60.61 (61.9)	50.33 (51.7)	33.53 (34.0)	10.48 (11.7)	— (42.1)	— (12.9)	+3.12 mg (23.0)
	B	19.78 (17.4)	39.39 (38.1)	49.67 (48.3)	32.80 (34.2)	37.94 (35.8)	— (27.4)	— (—)	— (—)
	C	0.00 (—)	0.00 (—)	0.00 (—)	33.67 (31.8)	51.58 (52.5)	— (30.5)	— (87.1)	— (77.0)

Unfractionated samples (Nos. 12 and 13) were treated as ternary and binary mixtures, respectively. Recovery test on an enriched sample (No. 14=dinactin 3.12 mg+sample No. 13, 29.85 mg) was satisfactory; the result indicates that the composition of sample No. 13 was 14.4 mole% (dinactin) and 85.6 mole% (tetranactin).

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