

# Investigations by HPLC-electrospray mass spectrometry and NMR spectroscopy into the isomerisation of salinomycin

Adrienne L. Davis, James A. Harris,\* Charlotte A. L. Russell and John P. G. Wilkins

Unilever Research, Colworth House, Sharnbrook, Bedfordshire, UK MK44 1LQ

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HPLC-MS studies have indicated that certain polyether ionophore veterinary drugs are prone to degradation when stored as water-methanol solutions at ambient temperature. Salinomycin and narasin were particularly susceptible, disappearing completely within weeks to produce more polar species, which were identified as isomers of the original compounds. Lasalocid appeared to be stable under such conditions. Structural elucidation of the principal ultimate salinomycin isomerisation product was achieved by 2D NMR spectroscopy. This indicated that the isomerisation process consists of the opening of the spiro rings in the salinomycin structure with the concomitant formation of a furan moiety. The MS data indicated that the isomers retain the ability to complex alkali metal ions and may therefore retain their pharmacological activity. These discoveries may have implications both for the development of legislation covering acceptable levels of polyether ionophore residues in foodstuffs and also for analytical protocols designed to detect them.

## Introduction

The polyether ionophores are used intensively throughout the world in poultry production. They are coccidiostats, and may be incorporated in feedstuffs. They include lasalocid, monensin, narasin and salinomycin. Currently, there are no European Union (EU) maximum residue limits (MRLs) controlling their residues in food. However, their status is under review because of concerns over their toxicity and potential drug resistance problems.

We have been developing mass spectrometric methods for the determination of residues of these compounds in foodstuffs.<sup>1</sup> During the course of this work, we have observed that certain polyether ionophores isomerise in aqueous solution (such solutions are used, for example, during the preparation of spiked materials for recovery determination). The current work reports an investigation of this process.

The rates of isomerisation of lasalocid, monensin, salinomycin and narasin were studied by HPLC-MS, and the structure of the salinomycin isomer was determined by 2D NMR spectroscopy.

## Experimental aim

To investigate the isomerisation of polyether ionophores in aqueous solutions and to determine the structure of the principal ultimate isomer of salinomycin.

## Experimental

### Materials

Lasalocid (97% purity), monensin (90–95%), salinomycin (96%) and narasin (97%), all as sodium salts, were obtained from the Sigma Chemical Corporation (Poole, Dorset). Individual 1 mg ml<sup>-1</sup> stock solutions were prepared in methanol in glass volumetric flasks and stored at 4 °C for up to 3 months. Dilutions of these stock solutions were prepared (see below).

Solvents and reagents were of high purity. Deionised water (pH 6.3) was obtained from a Milli-Q Plus water purification system (Millipore, Watford, Hertfordshire).

### HPLC-electrospray MS

HPLC was performed using a Hewlett Packard (Bracknell, Berkshire) HP1050 system. The mobile phase was 0.02 M aqueous ammonium acetate (adjusted to pH 7.0 by the addition of 3–4 drops of 5% aqueous ammonia solution)-acetonitrile-methanol (20 + 60 + 20 v/v/v). Solvent delivery was at 1 ml min<sup>-1</sup>. Automated injections of 50 µl were made. The HPLC column was a Phenomenex (Macclesfield, Cheshire) Nucleosil 15 cm × 4.6 mm C<sub>18</sub> 5 µm column. Approximately 0.15 ml min<sup>-1</sup> of the eluent flow was delivered to the Micromass (Altrincham, Cheshire) Quattro I triple quadrupole MS (the rest being diverted to waste *via* a pneumatic splitter). The MS was equipped with a megaflo electro spray source operated in positive ion mode. The source temperature was 150 °C, and the nitrogen bath and sheath gas flows were 300 and 40 l h<sup>-1</sup> respectively. The cone voltage (CV) potential settings were 10 to 100 V. Various acquisition regimes were used; scanning (typically *m/z* 200–800) and/or selected ion recording (SIR) to monitor characteristic sodiated and ammoniated molecular ions plus diagnostic fragment ions<sup>1</sup> (mass window, 1.0 Da, dwell time, 0.1 s; interscan delay, 0.02 s). Data were acquired for 25 min.

### MS analysis of salinomycin and its ultimate isomerisation product

A freshly diluted aqueous 10 µg ml<sup>-1</sup> salinomycin solution, a similar solution that had been prepared 2 months earlier and stored at ambient temperature (20–25 °C) and a mixed solution made from equal volumes of these two solutions were analysed by HPLC electro spray MS (acquiring data from *m/z* 100–1000, with CV of 20, 30, 50 and 70 V).

## MS analysis of time course experiment

Duplicate dilutions of the stock solutions of the four polyether ionophores were prepared in water, to give 20  $\mu\text{g ml}^{-1}$  solutions (*i.e.*, 2% methanol). One of the duplicate solutions was stored at ambient temperature (20–25 °C) and the other at 4 °C. The solutions were analysed by HPLC-MS using SIR, within hours of preparation (day 0) and after 2, 4, 7 and 11 days. Quantification was performed *versus* standard solutions in 0.02 M aqueous ammonium acetate solution (pH 7.0)–acetonitrile (80 + 20 v/v), stored at 4 °C.

## NMR spectroscopy

To elucidate the structure of the isomer of salinomycin (compared to salinomycin), NMR spectra were measured on a Bruker AMX400 spectrometer (Rheinstetten/Karlsruhe, Germany) operating at a probe temperature of 294 K or 300 K using either a dual  $^1\text{H}/^{13}\text{C}$  5 mm probe or a multinuclear 5 mm inverse probe as appropriate. The solvent used was  $\text{D}_2\text{O}$ -exchanged acetonitrile- $d_3$  and spectra were referenced relative to internal tetramethylsilane (TMS).

Aqueous dilution of the stock solution was performed to give 50 ml of 40  $\mu\text{g ml}^{-1}$  salinomycin solution. This was stored at ambient temperature for 17 days. A similar aqueous dilution was then freshly prepared. The two solutions were analysed by HPLC-MS, then both solutions were evaporated to dryness using a freeze dryer. Residues were redissolved in 0.5 ml acetonitrile- $d_3$ . A 50  $\mu\text{l}$  aliquot was diluted to 5 ml with acetonitrile and analysed by HPLC-MS. The remaining extracts (approximate concentration, 4  $\text{mg ml}^{-1}$ ) were then analysed by NMR.

2D TOCSY experiments<sup>2</sup> were acquired using a multiple of the MLEV-17 sequence for spin locking flanked by two 2.5 ms trim pulses. The spin-lock field strength was 10 kHz, the mixing time was 10 ms and a relaxation delay of 1 s was employed. The HMQC experiment<sup>3</sup> was acquired with a 400 ms delay after the BIRD pulse, a dephasing/refocusing delay of 3.5 ms and a relaxation delay of 0.5 s between scans. GARP decoupling was applied during acquisition. The 2D HMBC experiment<sup>3</sup> was recorded with a 3.5 ms delay for the low-pass J filter, a 60 ms delay for evolution of long-range couplings and a relaxation delay of 0.5 s.

All 2D experiments were recorded phase sensitive in both dimensions (using TPPI for quadrature detection,<sup>4</sup> with the exception of the HMBC experiment which was recorded in mixed mode format.<sup>5</sup>

## Results and discussion

It should be noted that, when referring to pseudomolecular ions, *etc.*, M is based on the polyether ionophore free acid (*not* the sodium salt). Thus the sodiated pseudomolecular ion of salinomycin (free acid  $\text{C}_{42}\text{H}_{70}\text{O}_{11}$ ; nominal monoisotopic molecular weight, 750), described as  $[\text{M} + \text{Na}]^+$ , represents  $\text{C}_{42}\text{H}_{70}\text{O}_{11}\text{Na}^+$ , observed at  $m/z$  773.

HPLC–electrospray MS was used to characterise the freshly prepared, the 2 month old and the mixed fresh and aged 10  $\mu\text{g ml}^{-1}$  largely aqueous (water–methanol, 98 + 2 v/v) salinomycin solutions. (It was not convenient to prepare purely aqueous solutions because of solubility.) The main response from the fresh solution, observed at 15.3 min, was ascribed to unchanged salinomycin. (Some other minor responses were also observed. These have been identified as technical contaminants.<sup>1</sup> That from the aged solution, at *ca.* 2.8 min, exhibited a rather similar mass spectrum and was tentatively identified as an isomer of salinomycin (there was negligible response at the retention time of intact salinomycin). The

electrospray, positive ion mass spectra of the unchanged and the isomerised salinomycin, obtained with a cone voltage potential of 20 V, are both dominated by ammoniated  $[\text{M} + \text{NH}_4]^+$  and sodiated  $[\text{M} + \text{Na}]^+$  molecular ions at  $m/z$  768 and 773 (both with *ca.* 100% relative abundance). Additional responses in the spectrum of the isomer were observed at  $m/z$  733, with *ca.* 70% relative abundance, probably due to  $[\text{M} + \text{H} - \text{H}_2\text{O}]^+$ , and  $m/z$  809, with *ca.* 30% relative abundance, possibly due to the solvent adduct ion  $[\text{M} + \text{CH}_3\text{CN} + \text{NH}_4]^+$ .

The shorter retention time indicates that the apparent isomer is more polar than its parent. The overall MS response of the isomer peak was approximately 30% of that of the unchanged salinomycin peak.

HPLC–electrospray MS analysis of the 10  $\mu\text{g ml}^{-1}$  water–methanol (98 + 2 v/v) solutions of lasalocid, monensin, narasin and salinomycin, stored either at 20–25 °C or at 4 °C, over a period of 11 days, was undertaken to investigate the rates of conversion of the polyether ionophores (Fig. 1).

The responses for salinomycin and narasin in the water–methanol solutions stored at 20–25 °C had disappeared by day 11, and that for monensin had dropped by >30%. The reduction in the lasalocid response from the water–methanol solution was less (*ca.* 10%).

For the water–methanol solution of salinomycin stored at 20–25 °C for 2 days, three LC peaks in addition to that of salinomycin were observed. Their retention times (2.8, 4.3 and 5.3 min, with the 2.8 and 5.3 min peaks being most abundant) were shorter than that of salinomycin (15.3 min). The additional peaks all exhibited ions at  $m/z$  768 and 773, indicating that they were isomers of salinomycin. By day 11, the shortest retention time peak was the only significant peak observed.

For the water–methanol solution of narasin stored at 20–25 °C for 2 days, additional LC peaks were observed at 3.1, 4.7, 5.2, 6.5 and 7.4 min, with the 3.1 and the 6.5 min peaks being the most abundant (narasin eluted at 17.1 min). They all exhibited the  $m/z$  782 and 787 pseudomolecular ions of narasin. Again, by day 11, the shortest retention time peak was the only significant peak observed.

For the water–methanol solution of monensin stored at 20–25 °C, the rate of change was slower. By day 11, three minor additional LC peaks were observed at 2.5, 4.7 and 8.7 min, the major one being at 2.5 min (monensin eluted at 10.4 min).

As expected, the water–methanol solutions of polyether ionophores stored at 4 °C exhibited a similar behaviour, but a slower rate of change, with an approximately 60% drop in salinomycin and narasin levels and a 20% drop in monensin levels over the 11 days (lasalocid levels were again unchanged).

These experiments indicate that some polyether ionophores, particularly salinomycin and narasin, are unstable in water–methanol solution. No significant degradation was observed in the solvent containing acetonitrile and ammonium acetate at pH 7.0. The mechanism of degradation for salinomycin appears to

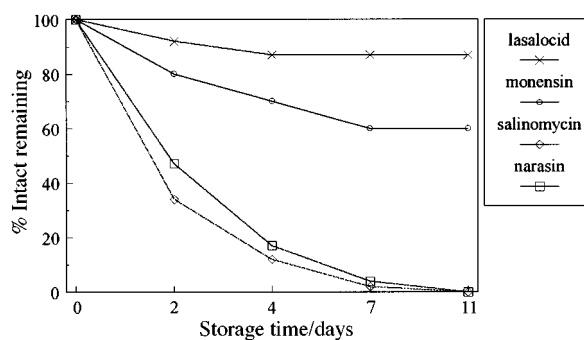


Fig. 1 Effect of storage at 20–25 °C on the concentration of various polyether ionophores in water–methanol (98 + 2 v/v), as determined by HPLC–electrospray MS (initial concentration *ca.* 10  $\mu\text{g ml}^{-1}$  = 100 %).

involve several relatively transient species, but results in one principal ultimate product (under the conditions and timescales described), which appears to be an isomer of salinomycin.

In order to investigate the mechanism of degradation, the ultimate isomerisation product of salinomycin was characterised by NMR. The structure and numbering scheme of salinomycin and its isomer are shown in Fig. 2.

Salinomycin itself has been previously studied by NMR spectroscopy<sup>6–8</sup> and <sup>13</sup>C NMR assignments have been published.<sup>6,7</sup> However, these assignments were made by comparison with shift data in smaller molecules,<sup>6</sup> or by comparison with data from the spectra of the alkali metal complexes,<sup>7</sup> not by 2D methods giving unambiguous results. Therefore, in order to aid interpretation of the NMR data for the isomer, the NMR spectra of salinomycin were acquired in the same solvent (acetonitrile-*d*<sub>3</sub>) and unequivocal assignments were obtained by 2D NMR spectroscopy, primarily the <sup>1</sup>H–<sup>13</sup>C correlation techniques HMQC and HMBC.

<sup>1</sup>H and <sup>13</sup>C NMR assignments for salinomycin are given in Tables 1 and 2 respectively. Long-range <sup>1</sup>H–<sup>13</sup>C correlations are listed in Table 3; all data given in Tables 1 and 2 are consistent with the correlations shown. In those cases where overlap in the <sup>13</sup>C dimension prevented identification of <sup>1</sup>H resonances in the HMQC spectrum [*e.g.*, C(8) and C(22)], uncertainties were resolved by observation of <sup>1</sup>H–<sup>1</sup>H correlations in a 2D TOCSY spectrum. The <sup>13</sup>C assignments given in

Table 2 are in agreement (allowing for solvent differences) with those given in the literature,<sup>7</sup> with the exception that C(17) and C(21) are interchanged.

NMR data for the isomer were obtained by the same methods and are also shown in Tables 1–3. Comparison of the spectra of salinomycin and the isomer immediately indicated that the isomerisation process had caused rearrangement of the spiro rings in the central part of the molecule. The carbon resonances C(17), C(21) and C(24) are all shifted very significantly, as are the two proton resonances at low field [H(18) and H(19)]. The coupling from H(18) to H(19) is reduced from 10.6 to 3.4 Hz and no coupling from H(18) and H(19) to H(20) is observed. In the HMBC spectrum of the isomer, H(18) and H(19) are observed to correlate to C(19) and C(18) respectively, and also to C(17) and C(20). The shifts of these carbons are 166.6, 108.8, 120.0 and 152.1 ppm [C(17)–C(20)]. These data, and the magnitude of the H(18)–H(19) coupling constant (3.4 Hz) strongly suggest the formation of a (substituted) furan moiety in the isomer.<sup>9,10</sup>

Further structural elucidation was achieved mainly by observing correlations from the Me protons in the HMBC spectrum as these were generally well resolved and had good S/N.

The most upfield Me proton resonance (at 0.759 ppm) was observed to have long-range correlations to three carbons, two of which were protonated carbons with shifts indicative of the

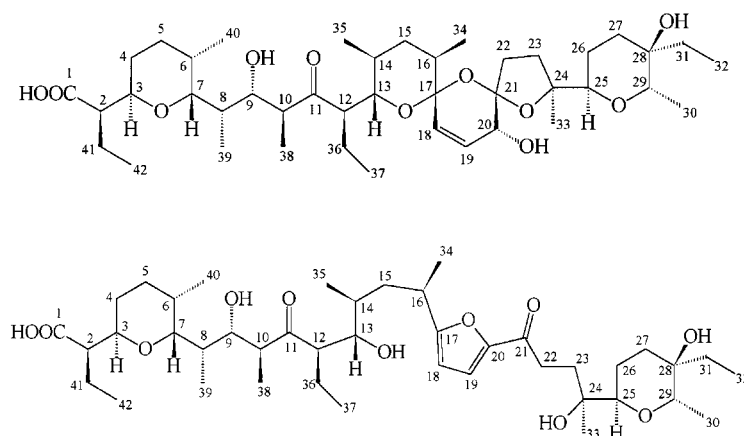


Fig. 2 The structure and numbering scheme of salinomycin (top) and its isomer (bottom).

Table 1 <sup>1</sup>H NMR data for salinomycin and its isomer<sup>a</sup>

Atom	Salinomycin	Isomer	Atom	Salinomycin	Isomer
1	—	—	22	2.280, 1.959	2.979, 2.789
2	— <sup>b</sup>	— <sup>b</sup>	23	2.113, 1.852	1.86, 1.83
3	3.885	3.87 <sup>c</sup>	24	—	—
4	1.921, 1.459	1.915, 1.467	25	3.618	3.459
5	1.919, 1.491	1.910, 1.475	26	1.45, 1.45	1.732, 1.545
6	1.842	1.832	27	1.63, 1.63	1.700, 1.613
7	3.667	3.671	28	—	—
8	1.490	1.510	29	4.002 <sup>c</sup>	3.778
9	4.119	4.11 <sup>c</sup>	30	1.252 d	1.238 d
10	2.911	3.051	31	1.326, 1.345	1.348, 1.348
11	—	—	32	0.902 t	0.901 t
12	2.804	2.715	33	1.579 s	1.146 s
13	3.689	3.821	34	0.735 d	1.315 d
14	1.742	1.412	35	0.931 d	0.906 d
15	1.684, 1.179	2.317, 1.304	36	1.923, 1.410	1.786, 1.161
16	1.677	3.142	37	0.817 t	0.774 t
17	—	—	38	0.847 d	0.838 d
18	6.115 dd <sup>d</sup>	6.364 d <sup>e</sup>	39	0.759 d	0.759 d
19	5.891 dd <sup>f</sup>	7.266 d <sup>e</sup>	40	0.962 d	0.958 d
20	4.041	—	41	1.35–1.44	1.35–1.45
21	—	—	42	0.929 t	0.923 t

<sup>a</sup> Chemical shifts are in units of ppm. Spectra were measured at 294 K and are referenced relative to internal TMS. <sup>b</sup> Not observed. <sup>c</sup> Broad resonance. <sup>d</sup> Coupling constants: 10.6, 2.4 Hz. <sup>e</sup> Coupling constant: 3.4 Hz. <sup>f</sup> Coupling constants: 10.6, 1.5 Hz.

attachment of oxygen, whilst the other had a shift very close to that observed for C(8) in salinomycin. The carbon directly attached to the upfield Me proton had a shift coincident with C(39) in salinomycin. Therefore, these isomer resonances could be identified as H(39), C(39), C(7), C(8) and C(9), with C(7) and C(9) distinguished by the correlation of H(7) to a further Me carbon, C(40). Correlations from C(40) enabled the methine carbon C(6) and the methylene carbon C(5) to be identified. C(7), C(6), C(5) and their attached protons have shifts nearly identical to the analogous resonances in salinomycin, strongly suggesting that this part of the molecule is unaltered in the isomer. This observation allowed C(2), C(3), C(4), C(41), C(42) and their attached protons [except H(2)] to be identified, all having shifts invariant from the analogous salinomycin resonances, as expected. The C(3) and C(41) resonances are both broad, as is C(2). H(2) and C(1) are not observable. Clearly, a dynamic process is broadening some of the signals relating to this part of the molecule.

Proceeding from a further Me resonance at 0.838 ppm, correlations to C(9), C(10) and C(11) are observed, identifying the Me group as H(38). C(8), C(9), C(10) and C(11) and their attached protons, where appropriate, have chemical shifts comparable with those in salinomycin, once again suggesting that this part of the molecule is unchanged. The ethyl sidechain consisting of C(36) and C(37) can be linked to this fragment by the correlation from one of the methylene protons on C(36) to the ketone carbonyl C(11). Correlations from H(37) to C(36) and C(12) allow C(37) and C(12) to be identified. No further linkages relating to this part of the molecule are observable in the HMBC spectrum. Correlations observed in the TOCSY spectrum were consistent with the above assignments, giving fragment 1 as shown in Fig. 3.

At the other end of the molecule [C(25) to C(29) and substituents], Me group resonances H(32) and C(32) can be identified by comparison of the shifts with those observed in salinomycin. Correlations from H(32) identify the methylene carbon C(31) and a quaternary carbon C(28). Correlations from the Me resonance H(30) to C(28) and C(29) allow C(30) and C(29) to be identified, whilst a methine carbon C(25) and a methylene carbon C(27) are identified by their correlations to H(29). Once again all of the  $^{13}\text{C}$  shifts are very similar to those observed in salinomycin, indicating that this part of the molecule is unchanged. The methylene carbon C(26) can

therefore be assigned by analogy to salinomycin. H(25) correlates to a quaternary carbon C(24) with a shift (73.8 ppm) indicative of an electronegative substituent, -OR. H(25) also correlates to an Me group C(33).

The Me proton H(33) correlates to C(25), C(24) and a methylene carbon C(23). Consequently, given these correlations and the structure of salinomycin, it can be deduced that C(25) is attached to a group  $-\text{C}(\text{OR})(\text{CH}_2\text{-R}')\text{Me}$ . The methylene protons H(23) and H(23') correlate to another

**Table 3** Long-range proton-carbon correlations for salinomycin and its isomer<sup>a</sup>

Salinomycin		Isomer	
Proton	Carbon	Proton	Carbon
3	2, 4, 5, 7		
7 (5,8)	5, 9, (8, 22), 40 7, 39	7	5, 8, 9, 40
9	7, 10, 11, (8, 22), 39		
10	9, 38	10	9, 38
12	11, 13, 36, 37		
13	11, 12, 15, 36	13	14, 15, (35, 36)
15	14, 16	15	13, 14, 16, 17, 35
		16	15, 17, 18, (26, 34)
18	17, 19, 20	18	17, 19, 20
19	17, 18, 21	19	17, 18, 20
20	18, 19, (8, 22)		
22	(23, 31)	22	21, 23, 24
(6, 23)	33	23	22, 24, 33
		25	24, 27, 33
		29	25, 27, 28, 30
30	28, 29	30	28, 29
		31	28, 29
32	28, 31	32	28, 31
33	(23, 31), 24, 25	33	23, 24, 25
34	15, 16, 17	34	15, 16, 17
(35, 42)	2, 13, 14, 15, 41	35	13, 14, 15
36	11	36	11
37	12, 36	37	12, 36
38	9, 10, 11	38	9, 10, 11
39	7, (8, 22), 9	39	7, 8, 9
40	5, 6, 7	40	5, 6, 7

<sup>a</sup> Measured in HMBC spectra at 400 MHz. Parentheses indicate ambiguous data.

**Table 2**  $^{13}\text{C}$  NMR data for salinomycin and its isomer<sup>a</sup>

Atom	Salinomycin	Isomer	Atom	Salinomycin	Isomer
1	— <sup>b</sup>	— <sup>b</sup>	22	37.06 <sup>d</sup>	33.75
2	50.76 <sup>c</sup>	50.36 <sup>c</sup>	23	32.46 <sup>ef</sup>	33.41
3	76.42	76.66 <sup>c</sup>	24	89.15	73.77
4	20.67	20.62	25	75.15	74.70
5	27.04	27.00	26	21.26 <sup>cg</sup>	21.31
6	28.97	28.95	27	29.73 <sup>s</sup>	30.25
7	72.54	72.28	28	71.66	71.67
8	37.00 <sup>d</sup>	37.11	29	77.80	77.87
9	69.31	70.56	30	15.21	14.99
10	49.85	47.98	31	32.33 <sup>f</sup>	31.91
11	218.9 <sup>e</sup>	219.26	32	6.80	6.85
12	56.63	58.70	33	27.46 <sup>c</sup>	23.27
13	77.39	74.32	34	16.07	21.40
14	33.62	35.16	35	17.89	16.10
15	39.20	40.57	36	17.46 <sup>c</sup>	15.76
16	41.65	32.41	37	13.38	12.97
17	100.27	166.60	38	13.46	13.82
18	123.44	108.78	39	7.43	7.44
19	132.04 <sup>c</sup>	120.03	40	11.34	11.32
20	67.54 <sup>c</sup>	152.15	41	23.90	23.74 <sup>c</sup>
21	107.65	190.84	42	12.62	12.67

<sup>a</sup> Chemical shifts are in units of ppm. Spectra were measured at 294 K and are referenced relative to internal TMS. <sup>b</sup> Not observed. <sup>c</sup> Broad resonance. <sup>d</sup> Assignment Interchangeable. <sup>e</sup> Observable in 2D spectrum only. <sup>f</sup> Assignment Interchangeable. <sup>g</sup> Distinguished by comparison with data for the isomer.

methylene carbon, C(22), which in turn correlates to C(23) and C(24), as expected, and a carbonyl group C(21). This gives fragment 2 (see Fig. 3).

The final molecular fragment is constructed starting from H(13), which correlates to a methine carbon H(14) and a methylene carbon H(15). The chemical shifts of H(13) and C(13) are indicative that C(13) contains an electronegative substituent, -OR. Two Me signals, H(34) and H(35), correlate to C(15), one of which, H(34), also correlates to a further methine carbon, C(16). H(16) correlates to the previously identified furan carbon, C(17), giving fragment 3, as shown in Fig. 3 (other correlations listed in Table 3 are consistent with this structure).

In order to obtain the structure of the isomer, the three fragments must be linked together. The chemical shift of the carbonyl group C(21) indicates that it is probably either an  $\alpha\beta$ -unsaturated ketone or a ketone attached to an aromatic ring. Therefore, fragments 2 and 3 are linked by the C(20) to C(21) bond. In the TOCSY spectrum, a correlation is observed from H(12) to H(13), linking fragments 1 and 2. Two protons must be added to the structure in order to give the correct molecular mass. Consequently, C(13) and C(24) must each possess a hydroxy group, leading to the structure shown in Fig. 2. No attempts were made to establish the stereochemistry of the molecule; it is assumed that the stereochemistry of salinomycin has been maintained in the isomer. All  $^{13}\text{C}$  chemical shifts are fully consistent with the structure. In particular, C(18) to C(21) are in excellent agreement with previous data,<sup>9</sup> as indicated in Fig. 4 (shifts from the isomer outside parentheses).

A proposed mechanism for the formation of the isomer from salinomycin is shown in Fig. 5.

Having elucidated the structure of the ultimate isomerisation product of salinomycin, its electrospray mass spectra obtained at various CV potentials and those of unchanged salinomycin were re-evaluated. The greater abundance of the ion at  $m/z$  733 in the isomer spectrum indicates that the pseudomolecular ions of the salinomycin isomer are more prone to fragmentation/dehydration reactions than those of salinomycin (the stability of the sodiated pseudomolecular ion of salinomycin, which reflects the biological activity of salinomycin, has been reported.<sup>1</sup> The elevated abundance of the apparent solvent adduct ion at  $m/z$  809 may also indicate the more open structure of the ionised salinomycin isomer. It was not readily possible to rationalise any of the minor differences in the abundances of the lower mass ions (below *ca.*  $m/z$  650).

## Conclusions

This work has demonstrated that certain polyether ionophore compounds are prone to isomerisation when stored in aqueous solutions at ambient temperature. Salinomycin and narasin isomerise more rapidly than monensin, whilst no significant isomerisation was observed for lasalocid. The effect of changing the pH of the aqueous solution was not studied, but it is very likely that this will accelerate the rate of isomerisation (and further hydrolytic degradation). NMR studies indicated

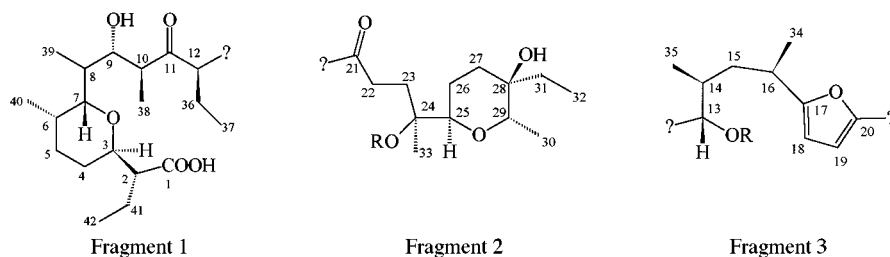


Fig. 3 Salinomycin fragments, as determined by 2D NMR spectroscopy.

that the principal ultimate isomerisation product of salinomycin differs from salinomycin itself by the opening up of the spiro rings and the formation of a substituted furan moiety. Narasin, which is a methylated homologue of salinomycin and which displayed a similar rate of degradation in aqueous solution, presumably undergoes a similar conversion.

It would be interesting to compare the observed degradation process with that pertaining in veterinary use. Given that the electrospray mass spectra of the isomers are dominated by sodiated molecular ions, it is likely that they retain at least some of their propensity to complex alkali metal ions, and thus preserve some of their biological activity. If they do retain

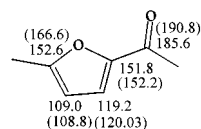


Fig. 4 Reported  $^{13}\text{C}$  chemical shift data<sup>9</sup> (shifts of analogous resonances from salinomycin isomer shown in parentheses).

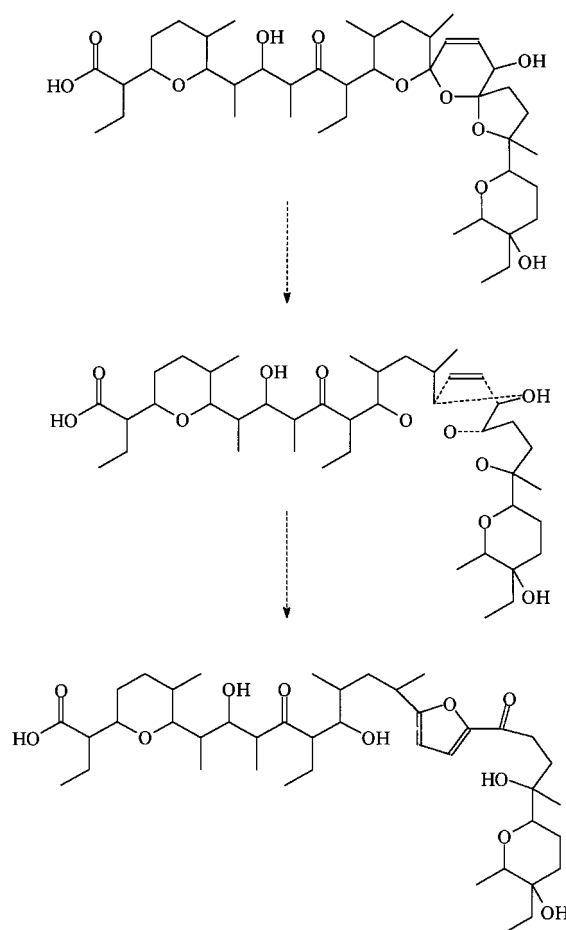


Fig. 5 Mechanism of formation of salinomycin isomer ( $\text{C}_{42}\text{H}_{70}\text{O}_{11}$ ; monoisotopic molecular weight, 750; average molecular weight, 750.01).

biological activity, it may be necessary to include them in residue monitoring studies.

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