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The Complete Assignment of the ^{1}H and ^{13}C nmr Spectra of the Alkali Metal Salts of Salinomycin and Narasin

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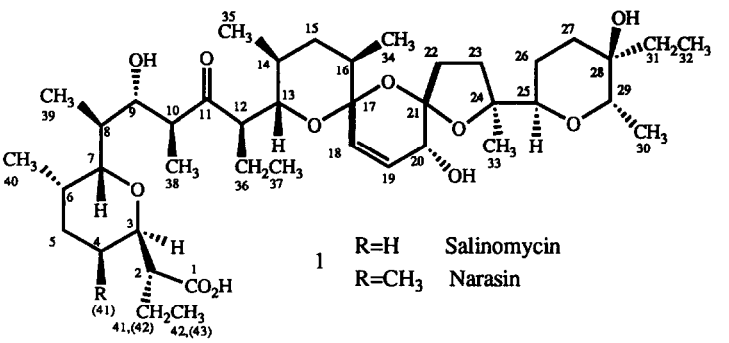
ABSTRACT *The preparation of the alkali metal salts of salinomycin and narasin, apart from lithium narasin, is described. The complete assignment of their ^{1}H spectra based on two dimensional COSY and NOESY spectra is reported. These asignments are then used in two dimensional $^{13}C/^{1}H$ correlations via direct and long range couplings to assign the ^{13}C spectra. Assignments in the ^{13}C spectrum of sodium salinomycin differ in important details from those in a previous report.*

INTRODUCTION

Salinomycin (1, R=H) and narasin (1, R=Me) are two of the most important commercially employed ionophoric antibiotics. Their efficacity arises from their ability to mediate the transport of metal ions, notably sodium and potassium, across biological membranes. They accomplish this by forming a complex with the alkali metal ion through the ether oxygen atoms thus shielding the charge on the metal ion from the hydrophobic interior of the membrane. We have previously reported a ^{23}Na and ^{39}K nmr study of the transport of sodium and potassium ions across model biological membranes by these ionophores.[1]



1 R=H Salinomycin
R=CH_3 Narasin

Crucial to their success in metal ion transport is their ability to accommodate alkali metal ions. Anteunis and Rodios studied the ^{1}H spectra of the parent acids, their sodium salts[2] and 17-epi-deoxy-(O-8) -

salinomycin.[3] They showed that these molecules have two hinge regions at either end of the central spirotricyclic system where they flex in order to accommodate the metal ion. These are at C(10)-C(11)-C(12) and at C(24)-C(25). Studies of the solvent dependence of the conformational equilibrium of narasin were reported by Caughey et al.[4] who showed that the conformations adopted by this molecule at the hinges were dependent on solvent polarity. More recently the same group reported the ^1H nmr spectra of narasin as the free acid, the free anion and the sodium and potassium salts in methanol solution.[5] The vicinal coupling constant about the C(12)-C(13) bond was shown to vary with the form in which the molecule was presented and with solvent, reflecting a substantial conformational change about the bond in that hinge region.

Carbon-13 nmr spectra should be good indicators of the conformations of these molecules since ^{13}C chemical shifts are very sensitive to the local stereochemical environment of a particular carbon atom. The problem in dealing with these compounds, however, is their complexity (42 or 43 carbons) and thus spectral assignment is difficult. Three reports exist in the early ^{13}C literature of such assignments which are for narasin, for salinomycin and for its sodium salt.[6-8] The assignments in these reports rely heavily on comparisons with chemical shifts in simpler molecules, on anticipated shift changes in derivatives and on the spectra of doubly labelled derivatives, all of which give sufficient scope for error.

The reports detailed above show the ability of nmr spectroscopy to give significant information on the conformations of salinomycin and narasin. Because these conformations are important in determining the kinetics and thermodynamics of the complexation reactions we decided to examine and assign the ^1H and ^{13}C spectra of the complete series of alkali metal salts of both ionophores. Nowadays the powerful range of two dimensional nmr techniques available makes assignments much more straightforward and reliable than when the earlier spectra were recorded. Our results confirm the assignments in the ^1H spectra reported above, show several serious errors in the early ^{13}C assignments, and extend the spectral assignments to almost the whole range of alkali metal salts of these ionophores.

EXPERIMENTAL

Salinomycin, supplied as the sodium salt, was a gift from Hoechst AG and narasin, as the acid, was a gift from Eli Lilly. We thank both companies for giving generous amounts of these materials which were used without further purification.

The alkali metal salts of the ionophores were prepared by the following general procedure. The ionophore as supplied (50-80mg) was dissolved in $CDCl_3$ (1ml) and stirred for 2 hours with a saturated aqueous solution of the alkali metal chloride containing excess solid chloride (ca 3ml). The chloroform layer was separated then dried and made neutral by filtration through a bed of the anhydrous carbonate of the alkali metal. The clear chloroform solutions were used without further purification.

Confirmation of the identity of the cesium salt of narasin was achieved as follows. The ^1H nmr spectrum of a known amount of narasin in chloroform was obtained. This solution was titrated with microlitre amounts of a known solution of cesium hydroxide in D_2O and the titration followed by ^1H nmr spectroscopy. As neutralisation proceeded the lines in the spectrum moved towards the positions previously observed for the cesium salt. At 50% neutralisation several of the lines (those that shifted the most) were considerably broadened. At 100% neutralisation the lines had sharpened and arrived at, or close to, the positions observed in the previously prepared cesium salt.

The lithium salt of narasin prepared under our conditions gave spectra that contained broadened lines possibly reflecting incomplete salt formation, or formation of a salt in which the coordinated oxygens were exchanging with non-coordinated oxygens.

Nmr spectra were obtained primarily on a Bruker MSL500 spectrometer operating in high resolution mode. Some spectra were obtained on a Bruker AM300 spectrometer. All spectra were referenced to internal TMS.

Two dimensional ^{1}H spectra were acquired typically by collecting 2048 FIDs of 4k data points, zero filling in the second dimension to 4k and Fourier transforming in magnitude mode with sinebell apodisation in both dimensions. Further noise reduction was achieved by symmetrisation about the diagonal. Some phase sensitive COSY spectra were obtained but offered little advantage in these systems. NOESY spectra were obtained using a typical 200msec mixing time. Digital resolution in both dimensions for the ^{1}H spectra was typically 1.7Hz per point. A typical 2D COSY spectrum is shown in Figure 1.

One dimensional ^{13}C spectra were acquired using WALTZ or DEPT sequences. Two dimensional $^{13}C/^{1}H$ correlated spectra with homonuclear ^{1}H decoupling were typically acquired by collecting 512 FIDs of 4k data points and zero filling to 1k or 2k in the second (^{1}H) dimension. Selection was made for either directly bound ^{1}J hydrogen-carbon correlations (J = 160Hz) or for ^{2}J hydrogen-carbon correlations (J = 12Hz).

RESULTS AND DISCUSSION

The mode of preparation of the alkali metal salts of the ionophores gave samples that had different ^{1}H and ^{13}C spectra. Whilst this suggests that different compounds have been prepared it does not prove it. Attempts to quantify the amount of alkali metal present in each salt by atomic absorption proved difficult because of the lack of a suitable solvent in which the salts would dissolve that was compatible with being put into a flame. Attempts to analyse the salts, after removal of chloroform under vacuum, by conventional C,H analysis gave results that were close to those expected but not within the normally accepted error limits for elemental analysis.

Eventually we turned to a titrimetric method to prove the identity of one of our samples. We selected what we believed to be the most difficult salt to form (i.e. that with the lowest formation constant) the cesium salt of narasin. If our preparative method gives the correct salt here, it should also do so for the cases where complex formation is more likely. Narasin, as the acid, was dissolved in chloroform and titrated with microlitre amounts of a D_2O solution of CsOH. The titration was followed by ^{1}H nmr. As the titration proceded the ^{1}H shifts altered, moving towards the positions observed for the previously prepared cesium salt. The resonances that moved the most also broadened showing maximum broadening at 50% neutralisation. The lines then sharpened and finished shifting at 100% neutralisation at virtually the identical chemical shifts to those found previously.

We believe that this experiment confirms the identity of the cesium salt of narasin and, therefore, the validity of our preparative method for the salts. The line broadening may well arise from a dynamic exchange process in which Cs^{+} ions are exchanging between molecules of the cesium salt and molecules of the free acid. This experiment has a potential use for following the kinetics of this process.

Because the lithium salt of narasin prepared under our conditions contained broadened lines consistent with some exchange process we do not report shifts for lithium narasin. The shifts for salinomycin acid that we quote are those obtained by Anteunis and Rodios.[2]

The ^{1}H spectra were assigned without reference to the shifts quoted in the previous papers by the following procedure using primarily the 2D COSY spectra. The three hydrogens at positions 2, 10 and 12 adjacent to carbonyl groups were identified from their chemical shifts. H(10) is distinguished from the other two because it

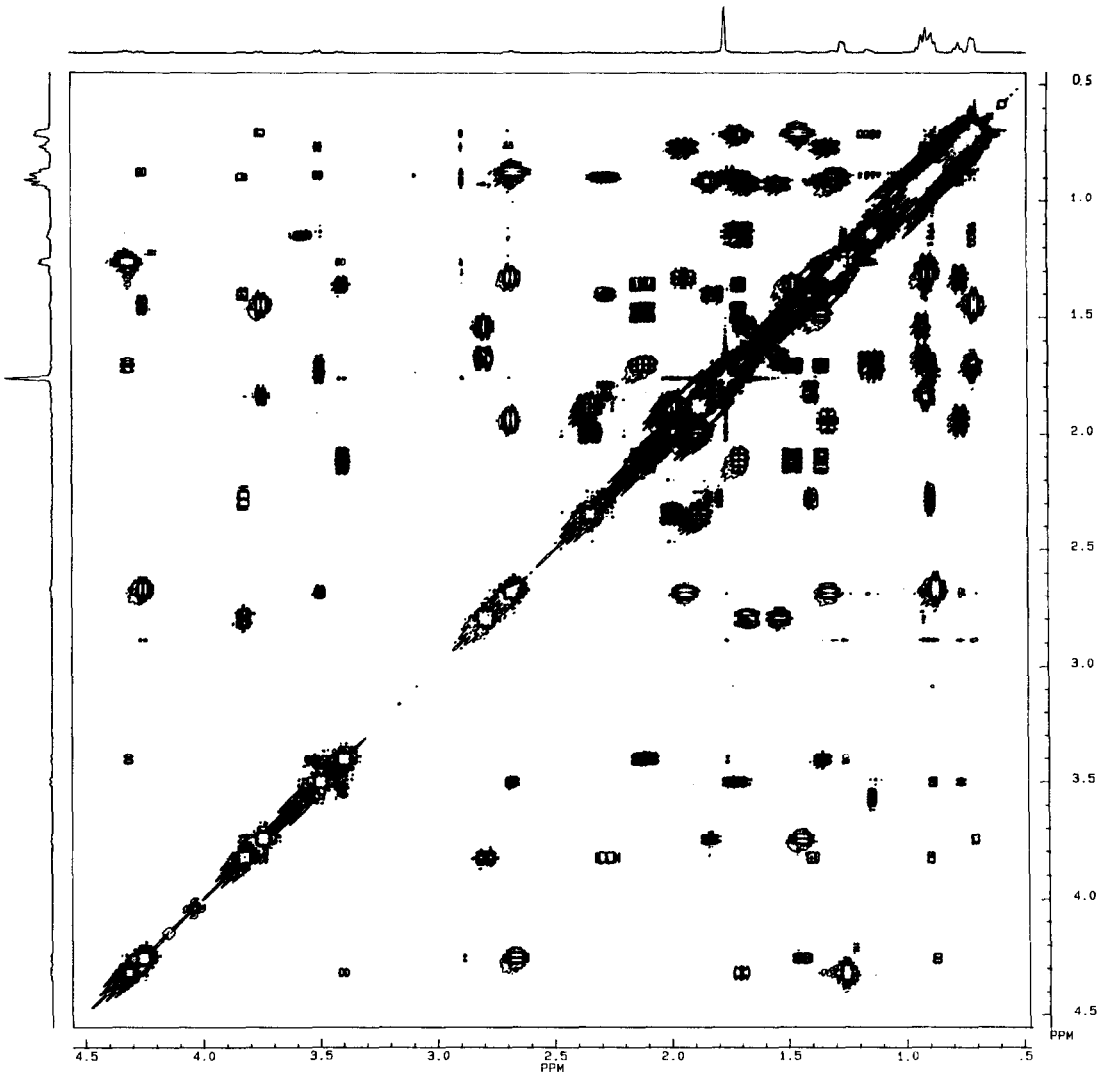


Figure 1
A typical section of a 2D COSY spectrum from the spectrum obtained for sodium narasin.

correlates to an adjacent methyl group. COSY continuities from H(10) then lead to the assignment of hydrogens at positions 7, 8, 9, 38 and 39. One of the remaining resonances next to a carbonyl group gave a COSY connection round the rear of a tetrahydropyran ring to H(7) thus enabling the identification of H(2) and H(12). This gave the assignments in the complete left hand side of the molecule as drawn. H(18), H(19) and H(20) fell out by inspection as did the remaining ethyl group. H(25) and H(29) were then distinguished by the presence of methyl(30). Four chemical shifts associated with the hydrogens at positions 22 and 23 remained. Distinct NOESY cross peaks associated with the proximity of methyl(34) and H(18), methyl(33) and *cis*-H(23) and *cis* hydrogens on carbons (22) and (23) allow distinction between H(18) and H(19) and between the four hydrogens on carbons (22) and (23). After assignment was complete it was satisfying that our assignments for narasin and the sodium salts of salinomycin and narasin agreed with previously published chemical shifts although our assignments are more complete.

All except six of the carbons in these compounds carry a directly bound hydrogen and the assignment of all of these carbons follows readily and unambiguously from the $^{13}C/^{1}H$ ^{1}J correlation. Of the six quaternary carbons the acid and ketone resonances are readily assigned on a chemical shift basis alone. The longer range (^{2}J) $^{13}C/^{1}H$ correlation for the potassium salt of salinomycin shows a correlation between methyl (33) and one of the non ketal quaternary carbons, which must be C(24). A similar experiment on lithium salinomycin showed a cross peak between C(19)-H and one of the ketal carbons. This did not assist in the assignment and so the chemical shifts given in the table are based on the assignments of *Seto et al.*[7]

It was found that the chemical shifts of some atoms were rather dependent on the cation present and others were virtually cation independent. These chemical shift changes show where the molecule undergoes the greatest conformational reorganisation to accommodate ions of different sizes. These changes should also reflect differences in the complexation behaviour of narasin and salinomycin, thereby providing information about the molecular recognition processes of these materials and the differences in selectivity between them.

The only structural difference between salinomycin and narasin is the methyl group on C(4) (Me(41)) in narasin, which must determine the overall selectivity and conformational differences between these two molecules and their complexes. This methyl group is equatorial and therefore is expected to hinder rotation about the C(2)-C(3) bond. This is borne out by the observation of +0.23ppm change in the chemical shift of C(2)-H in salinomycin going from the lithium salt to the cesium salt compared to a change of only +0.05ppm in narasin. A similar difference in the chemical shift in the ^{13}C spectra is also seen where there is a decrease of 3.2ppm in the salinomycin series compared to a maximum change of 1.6ppm in narasin. This points to rotation about the C(2)-C(3) bond being much less restricted in salinomycin than in narasin.

It has been suggested that Me(41) is a hindered rotor due to the presence of the adjacent side chain. Were this to be true we would expect to see appreciable NOESY cross peaks between Me(41) and C(42)-H. No such cross peaks were observed in our NOESY spectra.

The chemical shift of the acid carbon C(1) increases from 177.70ppm in narasin acid to 184.65ppm in sodium narasin. A similar change was reported earlier for the same carbon in salinomycin. We believe that this change is associated with loss of the acid hydrogen on salt formation. Similar changes are observed for salt formation in organic carboxylic acids and so there is no need to invoke a direct bond between the carboxyl group and the metal ion. The decrease in the chemical shift of this carbon along the series Na - Cs could be associated with the greater diffuseness of the charge on the metal ion.

Confirmation that these molecules flex at the "hinge" regions to accommodate ions of different sizes is seen in the chemical shift changes observed for ^{1}H and ^{13}C resonances in these areas. This is seen most interestingly in the ^{1}H spectra of C(25)-H, C(26)-H and Me(33). For C(25)-H our data for narasin and that of Anteunis for salinomycin show a substantial decrease from ca 3.9 to ca 3.4ppm on going from the acid to the sodium salt. This change must be associated with a conformational change about the C(24-25) bond on complex formation. Analogously, large changes are seen in one of the C(26)-H and Me(33) chemical shifts on complex formation. Proceeding out along the metal series there is a decrease of 0.13ppm in salinomycin and 0.23 ppm in narasin from Na to Rb followed by a slight increase for the cesium salt.

The largest ^{13}C changes for the C(24-25) hinge region are observed for Me(33) where metal ion complexation increases the shift by 2.5ppm (narasin) and 1.8ppm (salinomycin) going from the acid to the sodium salt. There are respective decreases of 1.6 and 0.8ppm going from Na to Rb followed by a small increase on going from Rb to Cs. For C(26) the respective decreases on going from acid to Na salt are 2.1 and 1.0ppm with further small changes proceeding out along the alkali metal series. For C(23) the corresponding changes are +2.3ppm in both cases for acid to sodium salt followed by a small increase along the rest of the series.

These changes in the C(24-25) hinge region show the largest conformational changes to occur on going from acid to salt, but also confirm that further small changes in this region occur to help accommodate ions of different sizes.

The other hinge region identified by Anteunis and Rodios is the C(7-13) section of the molecule and this region also shows chemical shift changes associated with changing the cation. These changes are only seen for certain atoms and the pattern of changes varies from salinomycin to narasin. In the ^{1}H spectra the resonances of interest are from C(7)-H, C(9)-H and C(13)-H. In the carbon spectra the resonances of interest are from C(11) and C(13). C(13)-H is of particular interest as it shows large changes in both salinomycin and narasin. There is a large decrease in chemical shift on complexation with Na^{+} of ca 0.36 ppm for narasin and 0.33 ppm for salinomycin. The value for lithium salinomycin is a further 0.07 ppm lower. The chemical shift then returns to about its original position along the rest of the sequence. There is a gradual increase to the rubidium salt but then a large increase to the cesium salt. This implies that there is a large conformational change on complexation followed by a further large change as larger ions are incorporated and that this is an important site as the ligands adapt to ions of different sizes.

In the salinomycin series it is interesting to note changes in the intensity of the COSY cross peak between C(12)-H and C(13)-H. This is of low intensity for lithium salinomycin and not observed for the cesium salt, but much stronger for the Na, K and Rb salts. No similar trend is observed for narasin. This suggests a very small vicinal coupling between these hydrogens in the lithium and cesium salts of salinomycin with a dihedral angle of close to 90^{o} but away from 90^{o} for the other salts.

There is a small increase in chemical shift for C(9)-H with both ligands on going from H to Rb but there is a large shift change seen for C(9)-H and C(13)-H between the Rb and Cs salts of salinomycin. No similar abnormally large change is seen in narasin at either site. This change, together with the coupling constant change implied from the COSY result suggests a large change in the conformation at this centre on going to cesium salinomycin.

The ^{13}C spectra also show interesting changes in the chemical shifts for this region with differences between narasin and salinomycin. The sites where the largest effects are observed are C(11) and C(13). The

carbonyl group at C(11) is suggested by Anteunis and Rodios to be one of the metal ion complexation sites and as such is of considerable interest. This site shows an interesting trend in narasin with an initial increase of 1.5 ppm for H - Na and then a 2 ppm decrease to K with a further small decrease along the rest of the series. The pattern is similar but the changes are larger in salinomycin with an increase of 3.5 ppm from H to Na (Li is 1 ppm higher still) and a decrease of 3 ppm thereafter. These data are consistent with a substantial change in conformation on complexation and smaller but significant changes as the metal ion gets larger. The ^{13}C chemical shifts of C(13) for the narasin series show an increase of 2.3 ppm on complexation with sodium with a further 1 ppm increase across the series. This change could be due to rotation either about the the C(12-36) bond or the C(12-11) bond. However there are no changes seen at C(10) or at C(37) as might be expected were this the case. A more likely explanation is that the effects seen in the narasin series arise from changing γ–gauche interactions with the oxygens bonded to C(11), C(9) or C(13) which are all γ to the atoms where the greatest shift changes are observed. Interestingly there are no analogous shift changes at C(13) in the salinomycin series.

CONCLUSIONS

Our results completely confirm the earlier, more limited assignments of ^{1}H chemical shifts by Anteunis and Rodios, [2,3] but call into question many of the ^{13}C assignments of Seto et al, [6,7] and Dorman et al.[8] The conformations of salinomycin and narasin free acids are clearly different from those of the alkali metal salts because of the substantial chemical shift changes found on complexation, particularly in the hinge regions. On proceeding down the series of alkali metal salts there are further chemical shift changes in the hinge regions that are larger than at the other almost unvarying sites in the molecules. The major conformational changes required to accommodate ions of different sizes take place in the hinge regions of the molecules.

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Table 1
The ^1H chemical shifts of the alkali metal complexes of salinomycin

Atom	H*	Li	Na	K	Rb	Cs
2	2.90	2.71	2.85	2.86	2.83	2.94
3	3.98	4.05	3.93	3.85	3.85	3.89
4d	1.93	1.85	1.89	1.89	1.88	1.92
4u		1.41	1.42	1.37	1.40	1.42
5d	1.60	1.84	1.88	1.95	1.93	1.89
5u		1.34	1.45	1.47	1.45	1.38
6	1.83	1.77	1.78	1.82	1.80	1.86
7	3.64	3.69	3.68	3.73	3.79	3.92
8	1.47	1.47	1.45	1.48	1.48	1.54
9	4.16	4.32	4.20	4.29	4.28	3.96
10	2.75	2.72	2.68	2.65	2.60	2.64
12	2.63	2.74	2.65	2.64	2.60	2.59
13	3.88	3.48	3.55	3.62	3.70	3.98
14	1.72	1.71	1.71	1.72	1.70	1.67
15d	1.61	1.70	1.68	1.65	1.62	1.62
15u	1.09	1.15	1.14	1.14	1.13	1.09
16	1.71	1.69	1.70	1.74	1.72	1.68
18	5.98	6.05	5.99	6.15	6.13	6.33
19	6.03	5.95	6.05	6.07	6.05	6.20
20	3.98	4.10	4.05	4.00	3.99	4.06
22d	2.40	2.27	2.31	2.41	2.36	2.28
22u	2.09	2.00	1.92	1.97	2.10	1.82
23d	2.23	2.00	1.95	1.89	1.88	1.97
23u	1.84	1.90	1.88	1.89	1.80	1.88
25	3.93	3.47	3.40	3.42	3.45	3.30
26d	1.54	2.25	2.25	2.09	2.05	2.10
26u	1.62	1.44	1.35	1.35	1.40	1.33
27d		1.77	1.70	1.67	1.68	1.70
27u		1.52	1.49	1.50	1.48	1.51
29	3.83	4.35	4.28	4.15	4.05	3.93
30	1.24	1.23	1.23	1.21	1.21	1.19
31d	1.39	1.31	1.28	1.33	1.39	1.40
31u	1.32	1.31	1.28	1.33	1.33	1.33
32	0.89	0.92	0.90	0.89	0.92	0.94
33	1.48	1.71	1.69	1.64	1.56	1.62
34	0.70	0.70	0.70	0.72	0.72	0.69
35	0.90	0.87	0.85	0.93	0.95	0.90
36d	1.93	1.98	1.90	1.94	1.94	1.93
36u	1.39	1.33	1.32	1.35	1.37	1.37
37	0.76	0.78	0.70	0.77	0.76	0.77
38	0.81	0.87	0.85	0.83	0.82	0.79
39	0.72	0.72	0.70	0.70	0.70	0.73
40	0.94	0.92	0.92	0.93	0.93	0.98
41d	1.54	1.48	1.49	1.48	1.46	1.49
41u	1.40	1.31	1.26	1.25	1.21	1.21
42	0.95	1.00	0.96	0.93	0.95	0.97

* These values are from the paper of Anteunis and Rodios. [2]

Table 2.
The ^{13}C chemical shifts of the alkali metal complexes of salinomycin.

Atom	H*	Li	Na	K	Rb	Cs
1	177.2	185.20	184.85	184.06	183.21	182.36
2	48.9	52.81	51.10	51.76	51.20	49.56
3	74.9	76.79	75.98	75.72	76.00	76.56
4	20.1	20.38	19.86	20.02	19.97	20.42
5	26.4	27.71	26.89	26.53	26.36	26.27
6	28.0	28.48	28.04	27.98	28.03	28.24
7	75.2[a]	71.18	71.37	71.25	71.06	71.43
8	32.6[b]	34.98	35.86	36.28	36.30	36.36
9	68.7	67.10	68.00	67.92	67.78	68.21
10	49.2	51.53	49.71	48.82	48.88	49.78
11	214.5	218.96	218.06	215.21	215.28	215.14
12	56.5	55.73	55.89	55.26	55.48	56.24
13	71.7[a]	74.53	75.54	76.40	76.13	74.88
14	36.5[b]	32.37	32.43	32.79	33.02	33.17
15	38.6	38.92	38.67	38.35	38.16	38.75
16	40.7	40.22	40.55	41.40	41.54	41.56
17	106.4	98.56	98.98	99.96	99.97	99.86
18	121.6	122.10	122.11	121.92	122.04	123.42
19	132.4	130.94	133.61	133.06	133.35	132.64
20	67.2	66.79	66.62	67.46	67.78	66.96
21	99.2	107.31	106.98	107.82	107.68	107.49
22	36.2	37.86	36.85	34.70	35.36	35.14
23	30.2	32.11	32.52	32.67	32.77	33.46
24	88.5	88.54	88.47	88.57	88.29	87.63
25	73.7	75.47	74.71	74.01	73.84	74.96
26	21.9	20.38	19.86	19.25	19.31	19.95
27	29.3	30.44	29.36	28.04	27.90	28.88
28	70.9	70.13	70.25	71.25	71.20	71.43
29	77.2	77.39	76.56	76.50	76.81	76.56
30	14.5	14.61	14.63	14.68	14.67	14.90
31	30.6	32.06	32.21	32.06	31.74	31.96
32	6.3	6.74	6.50	6.19	6.16	6.77
33	25.8	27.69	27.59	27.71	26.84	27.90
34	17.9[c]	15.80	15.85	15.59	15.60	15.75
35	15.6[c]	17.09	17.57	18.11	18.31	18.28
36	22.7[d]	16.00	15.55	15.85	15.94	16.41
37	11.9[e]	12.93	13.15	13.22	13.22	13.61
38	12.8	11.92	12.05	12.43	12.60	12.75
39	7.0	7.12	6.75	6.64	6.71	7.14
40	11.2	12.10	10.74	10.98	11.24	12.21
41	16.6[d]	22.88	23.56	24.17	23.79	23.08
42	13.2[e]	12.77	12.44	12.26	12.30	12.94

* These values are from the paper of Seto et al [7] and are given here as published. [a-e] we believe these pairs of values should be exchanged.

Table 3
The ^{1}H chemical shifts of the alkali metal complexes of narasin.

Atom	H	Na	K	Rb	Cs
2	2.77	2.80	2.78	2.75	2.78
3	3.91	3.82	3.80	3.79	3.82
4	2.27	2.28	2.27	2.27	2.27
5d	1.72	1.79	1.82		1.84
5u	1.45	1.40	1.39	1.37	1.36
6	1.89	1.83	1.84	1.83	1.86
7	3.61	3.74	3.73	3.80	3.87
8	1.49	1.45	1.45	1.45	1.48
9	4.16	4.26	4.28	4.28	4.20
10	2.78	2.67	2.63	2.60	2.64
12	2.62	2.69	2.61	2.59	2.60
13	3.86	3.50	3.64	3.70	3.82
14	1.74	1.72	1.72	1.71	1.68
15d	1.64		1.64	1.63	1.63
15u	1.11	1.14	1.14	1.14	1.09
16	1.72	1.72	1.75	1.75	1.70
18	6.05	6.05	6.05	6.04	6.11
19	6.01	6.00	6.11	6.09	6.18
20	3.97	4.04	3.98	4.00	4.03
22d	2.39	2.34	2.41	2.36	2.31
22u	2.06	1.87	2.00	2.15	1.96
23d	2.24	1.98	1.87	1.89	1.88
23u	1.83	1.87	1.87	1.76	1.88
25	3.89	3.40	3.43	3.46	3.40
26d	1.65	2.11	2.06	2.01	2.03
26u	1.52	1.36	1.37	1.43	1.39
27d	1.63	1.70	1.68	1.70	1.69
27u	1.63	1.47	1.50	1.49	1.53
29	3.84	4.32	4.15	4.09	3.90
30	1.23	1.26	1.22	1.21	1.20
31	1.35	1.30	1.32	1.35	1.32
32	0.89	0.90	0.90	0.93	0.91
33	1.47	1.78	1.63	1.55	1.57
34	0.70	0.72	0.73	0.72	0.72
35	0.91	0.89	0.93	0.95	0.93
36d	1.95	1.94	1.95	1.95	1.92
36u	1.38	1.33	1.34	1.36	1.39
37	0.77	0.76	0.77	0.77	0.77
38	0.83	0.87	0.83	0.82	0.84
39	0.75	0.70	0.71	0.70	0.71
40	0.94	0.91	0.92	0.92	0.92
41	0.90	0.89	0.89	0.90	0.92
42d	1.63	1.67	1.62	1.60	1.54
42u	1.63	1.53	1.56	1.54	1.54
43	0.92	0.92	0.88	0.88	0.89

Table 4
The ^{13}C chemical shifts of the alkali metal complexes of narasin

Atom	H	Na	K	Rb	Cs
1	177.70	184.65	184.08	183.01	182.03
2	49.83	51.21	51.42	51.24	50.78
3	77.97	79.08	79.18	79.49	79.92
4	28.15	28.56	28.46	28.61	29.05
5	35.65	36.33	36.05	35.91	35.77
6	28.94	29.15	29.26	29.26	29.43
7	72.74	71.62	71.41	71.28	71.13
8	36.02	36.02	36.40	36.43	36.57
9	68.75	68.57	68.29	68.15	68.29
10	49.50	49.38	48.81	48.95	49.16
11	216.20	217.70	215.79	215.15	214.94
12	56.27	55.83	55.40	55.57	55.82
13	73.73	75.99	76.40	76.13	77.02
14	32.83	32.48	32.77	33.03	33.35
15	38.76	38.72	38.30	38.09	38.55
16	40.95	40.70	41.33	41.40	41.53
17	99.61	99.24	99.91	99.94	99.88
18	122.35	122.24	122.03	122.17	122.86
19	132.35	130.94	132.96	133.34	132.78
20	67.74	66.49	67.57	67.94	67.15
21	106.62	107.31	107.71	107.47	107.44
22	36.48	35.60	34.94	35.50	35.41
23	30.33	32.66	32.55	32.69	33.15
24	88.62	88.37	88.57	88.30	87.91
25	75.57	75.16	73.90	73.69	74.46
26	22.11	19.97	19.23	19.31	19.67
27	29.28	29.36	27.67	27.66	28.83
28	71.07	70.48	71.52	71.49	71.25
29	77.14	77.06	76.75	76.93	75.53
30	14.30	14.88	14.63	14.69	14.74
31	30.67	32.20	31.93	31.67	31.62
32	6.33	6.49	6.18	6.18	6.38
33	25.71	28.20	27.43	26.61	27.35
34	15.69	15.74	15.57	15.54	15.71
35	17.89	17.54	18.13	18.29	18.37
36	16.58	15.60	15.77	15.98	16.29
37	13.19	13.10	13.18	13.21	13.38
38	13.07	12.43	12.47	12.65	13.00
39	7.37	6.86	6.78	6.90	7.19
40	12.74	11.64	11.79	12.07	12.50
41	18.33	19.41	19.76	19.73	19.83
42	23.63	24.82	25.07	24.55	23.95
43	12.09	12.65	12.47	12.55	12.76