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OBSERVATIONS ON THE MASS SPECTROMETRY OF MONENSIN AND RELATED COMPOUNDS*

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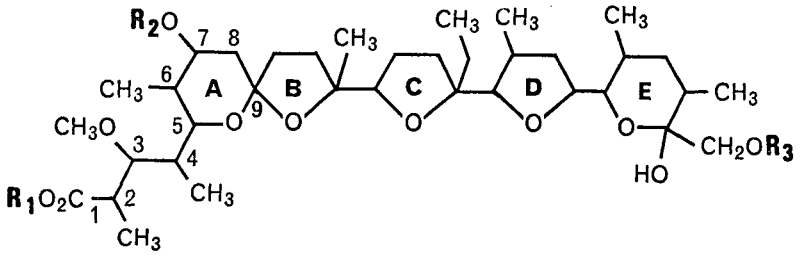
Abstract—Monensin and nigericin are structurally related compounds belonging to a family of biologically active, polycylic, monocarboxylic acids produced by *Streptomyces*. Partial interpretation of their mass spectra has been achieved with the aid of high resolution data and by correlation with spectra of derivatives. These results have been applied to structure elucidation of several minor factors produced along with monensin. An interesting aspect of the mass spectrometry of these compounds is that their alkali-metal salts are sufficiently volatile to yield mass spectra. Fragmentation of the salts is markedly different from that of the free acids and their derivatives. A scheme is proposed to account for the formation of a number of fragment ions which contain the metal atom.

THE POLYCYLIC carboxylic acids monensin (Ia)[1] and nigericin (IVa)[2] are members of a newly recognized family of biologically active compounds produced by *Streptomyces*.[3] Their structures have been determined recently by X-ray crystallography.[4,5] Independent investigation by chemical and spectroscopic methods provided corroborative results.[3,4,6] In this study the mass spectra of monensin, nigericin and several derivatives thereof were determined. This paper presents an interpretation of the mass spectral data and their application to the structure elucidation of two minor factors, IIa (factor B) and IIIa (factor C), formed along with monensin by the microorganism.[1,3]

Monensin and Related Factors

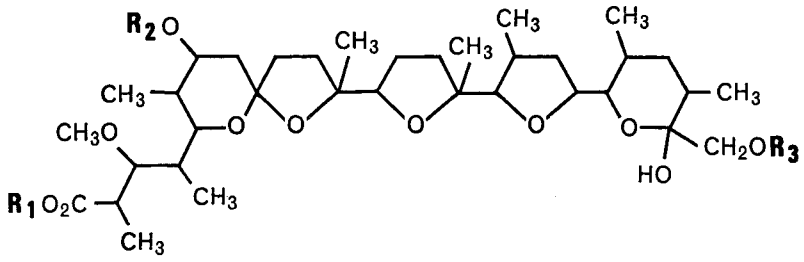
The mass spectrum (high-mass region) of monensin (Ia) is shown in Fig. 1. The molecular ion has not been observed for the free acid (Mol. wt. 670, $C_{36}H_{62}O_{11}$). The first peak occurs at *m/e* 634 ($C_{36}H_{58}O_9$)† and is due to the loss of two molecules of



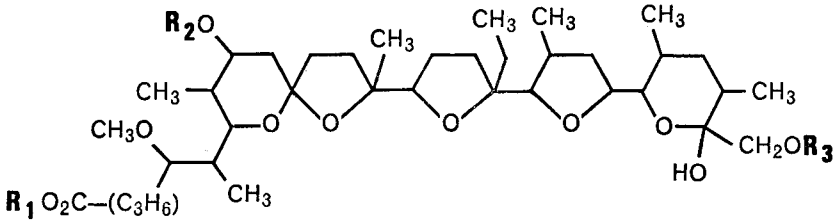
(Ia) $R_1 = R_2 = R_3 = H$
(Ib) $R_1 = CH_3$, $R_2 = R_3 = H$
(Ic) $R_1 = CH_3$, $R_2 = R_3 = CH_3CO$
(Id) $R_1 = Na$, $R_2 = R_3 = H$

* This paper was presented in part at the 155th National Meeting of the American Chemical Society, San Francisco, March 31–April 5, 1968, Abstract P-103.

† Elemental compositions of ions where given in parentheses were determined by accurate mass measurement. All experimentally determined masses were within ±5 m.M.u. of the calculated values.



(IIa) $R_1 = R_2 = R_3 = H$
(IIb) $R_1 = CH_3$, $R_2 = R_3 = CH_3CO$
(IIc) $R_1 = Na$, $R_2 = R_3 = H$



(IIIa) $R_1 = R_2 = R_3 = H$
(IIIb) $R_1 = CH_3$, $R_2 = R_3 = CH_3CO$
(IIIc) $R_1 = Na$, $R_2 = R_3 = H$

H_2O. Further loss of H_2O and OH gives rise to peaks at *m/e* 616 ($C_{36}H_{56}O_8$), *m/e* 599 ($C_{36}H_{55}O_7$) and *m/e* 598 ($C_{36}H_{54}O_7$). The methyl ester derivative Ib, however, does yield a detectable molecular ion at *m/e* 684 (Fig. 1).

The prominent peak at *m/e* 540 ($C_{34}H_{52}O_5$) represents further loss of oxygen substituents from the parent molecule along with two carbon atoms. Loss of another molecule of H_2O leads to the highly de-oxygenated ion at *m/e* 522 ($C_{34}H_{50}O_4$). Which carbon atoms are lost to form the C_{34} ion is uncertain, but the more likely sources would appear to be the carboxyl, methoxyl and hydroxymethyl functions. Neither these ions nor analogous ions displaced 14 mass units are found in the spectrum of Ib.

The ions which appear at *m/e* 329 ($C_{17}H_{29}O_6$) and *m/e* 427 ($C_{23}H_{39}O_7$) are formulated as B and C in Scheme 1.* Relevant to the formation of B and C is the observation that α-cleavage to yield cyclic oxonium ions is the most important fragmentation exhibited by α-alkylated derivatives of tetrahydrofuran.[7] Metastable peaks are present which correspond to the loss of H_2O from fragments B and C to give the abundant *m/e* 311 ($C_{17}H_{27}O_5$) and *m/e* 409 ($C_{23}H_{37}O_6$) ions, respectively. Each of the four peaks is shifted the requisite 14 mass units in the spectrum of Ib. Also shown in Scheme 1 is the cleavage of a bond adjacent to the methoxyl oxygen to give ion A. The latter is shifted to *m/e* 131 in the spectrum of Ib. In addition, the low-intensity peaks at *m/e* 511 and 483 may be due to the loss of ring E and an additional molecule of H_2O.

The base peak in the spectrum of monensin occurs at *m/e* 323 ($C_{19}H_{31}O_4$). This ion is believed to comprise rings C, D and E, minus one molecule of H_2O. It is thus complementary to fragment B (Scheme 1). The analogous ion is found at *m/e* 309 in the spectrum of IIa. The *m/e* 323 ion is not a significant feature of the spectrum of the methyl ester Ib.

* The formulae are intended only to illustrate the basic fragmentations of the parent molecule and compositions of the resultant ions.

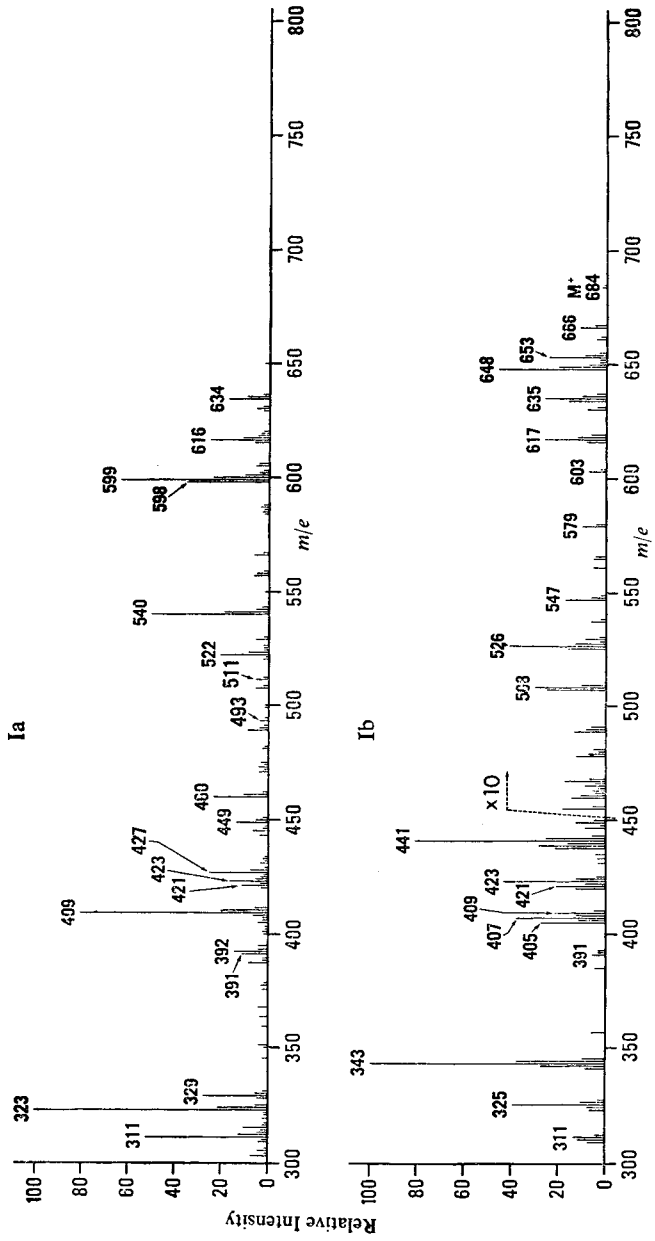
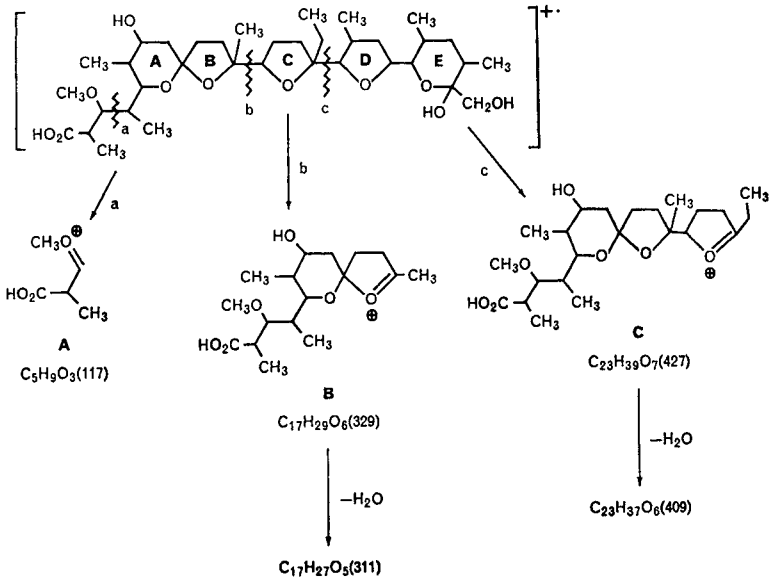


FIG. 1. Mass spectra of monensin free acid and monensin methyl ester.



SCHEME 1

To determine the structural relationship of monensin and the minor factors IIa and IIIa, a comparison was made of the mass spectra of their diacetyl methyl ester derivatives, Ic, IIb and IIIb (Fig. 2). The molecular ion of Ic is found at *m/e* 768. Ions due to the loss of H_2O and CH_3CO_2H appear in the high-mass region. Fragment C of Scheme 1 is found at *m/e* 483 ($C_{26}H_{43}O_8$) due to the addition of CH_2CO and CH_2. Loss of CH_3CO_2H (m* 483 → 423, calcd. 370·8, obsd. 370·8) leads to the ion at *m/e* 423 ($C_{24}H_{39}O_6$). Fragment B of Scheme 1 is shifted to *m/e* 384 ($C_{20}H_{32}O_7$). By analogy with fragment C, it should be shifted to *m/e* 385, but apparently a hydrogen-transfer occurs, in addition to cleavage of the bond between rings B and C, which does not take place in the fragmentation of the free acid (Ia) or methyl ester (Ib). Further loss of 59 mass units gives rise to the base peak at *m/e* 325 ($C_{18}H_{29}O_5$). An intense peak at *m/e* 131 ($C_6H_{11}O_3$) corresponds to fragment A of Scheme 1 shifted by 14 mass units. The molecular ion of IIb appears at *m/e* 754. Fragment C appears at *m/e* 469 ($C_{25}H_{41}O_8$) in the spectrum of IIb. Fragment B is not shifted, however, and is found at *m/e* 384 ($C_{20}H_{32}O_7$). Therefore, IIb has one less CH_2 unit in the ring C portion of the molecule. This result, together with the n.m.r. data on the parent compound and a degradation product thereof,[3] demonstrates that a methyl group is attached to ring C as in structure II.* The molecular ion of IIIb is greater by 14 mass units. Here the difference is localized in fragment A which appears at *m/e* 145 ($C_7H_{13}O_3$). Thus the structure of this compound is best represented by structure III. Other data suggest that an ethyl group may be present on the carbon atom adjacent to the carboxyl group.[3]

* Physical, chemical and biological data indicate that monensin and the minor factors have the same gross structure.[3]

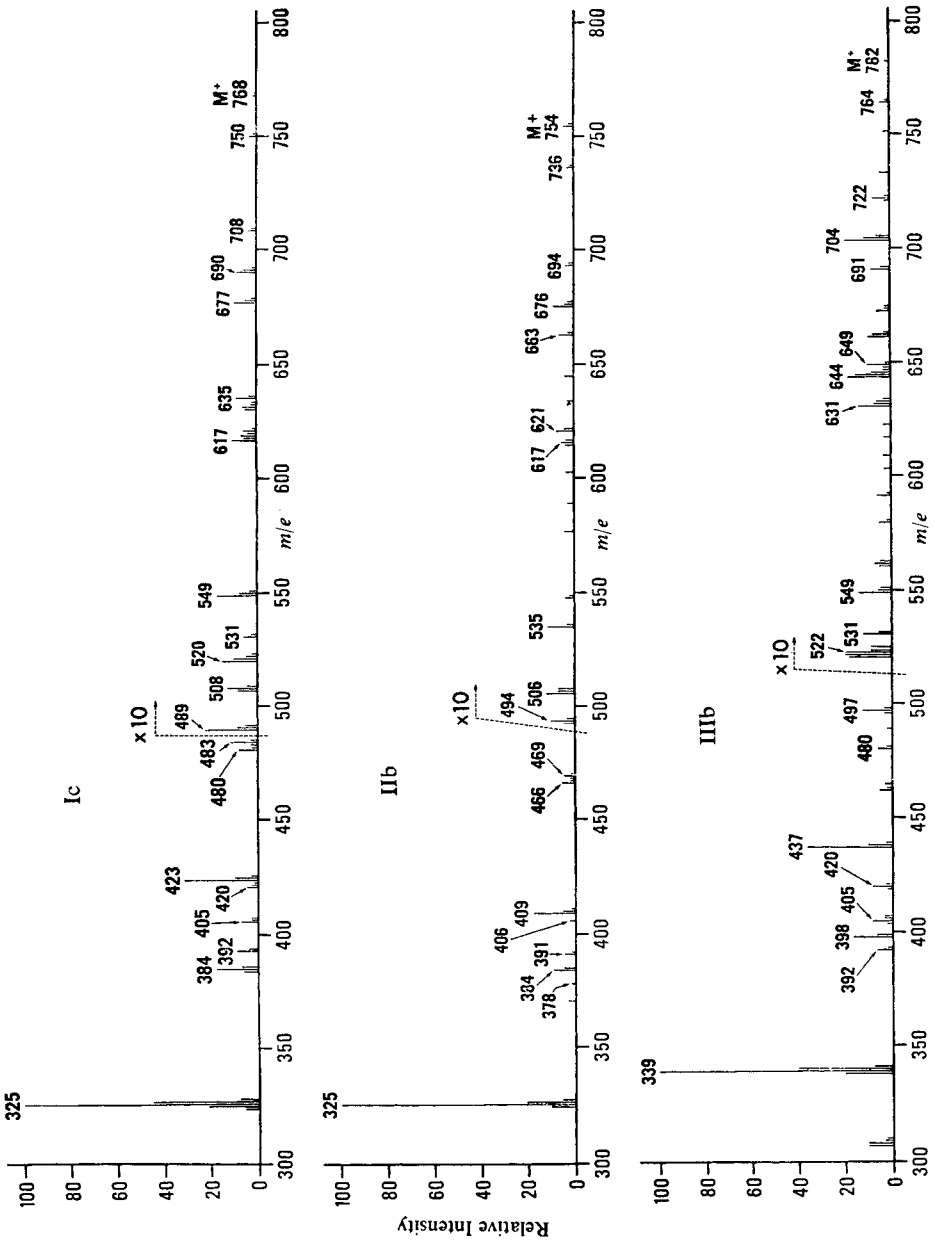


FIG. 2. Mass spectra of the diacetyl methyl ester derivatives of monensin, factor B and factor C.

Spectra of metal salts

Metal salts of monensin and related acids are characterized by low solubility in H_2O. They are soluble in most organic solvents, including hydrocarbons. The sodium salt of monensin (Id), for example, may be extracted from basic medium with $CHCl_3$.[1] This behavior may be rationalized by assuming that the salt molecule adopts a conformation in solution such as found in the crystalline state for the silver salt

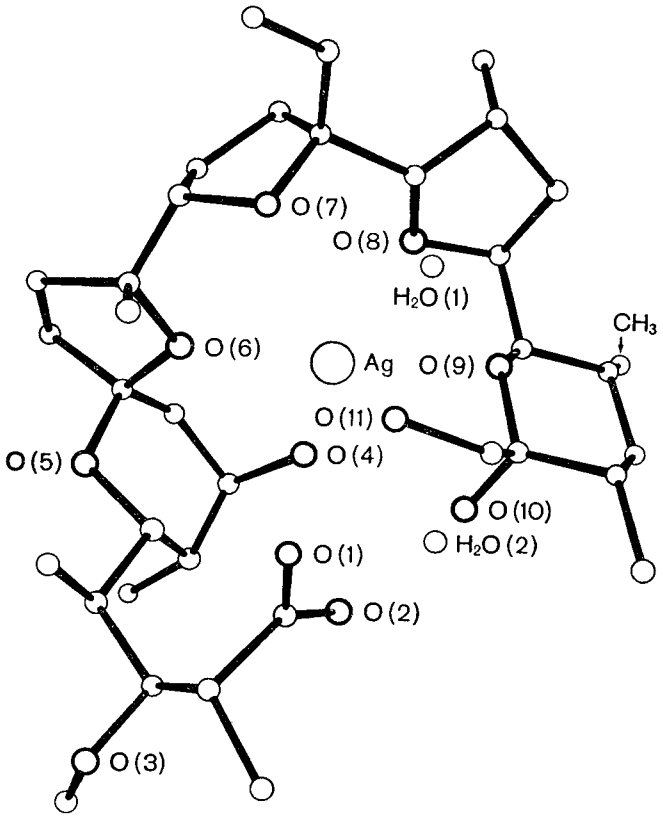


FIG. 3. Monensin silver salt.

(Fig. 3).[4] The metal ion is coordinated to oxygen, and the exterior of the molecule is hydrocarbon-like. The terminal hydroxyl groups hydrogen bond to the carboxyl group.

Alkali-metal salts of this group of compounds are volatilized in the mass spectrometer. Spectra of the sodium salts of monensin(Id) and the homologs, IIc and IIIc, are shown in Fig. 4.* In contrast to the free acids, the salts exhibit molecular ions, and these appear at *m/e* 692 ($C_{36}H_{61}O_{11}Na$), 678 and 706. They are accompanied by [M − 1] ions of equal or greater intensity. The base peak occurs at [M − 75]. In the spectrum of Id, ions appear at *m/e* 677 ($C_{35}H_{58}O_{11}Na$), 661 ($C_{35}H_{58}O_{10}Na$), 648 ($C_{35}H_{61}O_9Na$), 633 ($C_{34}H_{58}O_9Na$) and 617 ($C_{34}H_{58}O_8Na$). Their formation can be

* Mass spectra of divalent metal salts of several low molecular weight carboxylic acids have been described.[8]

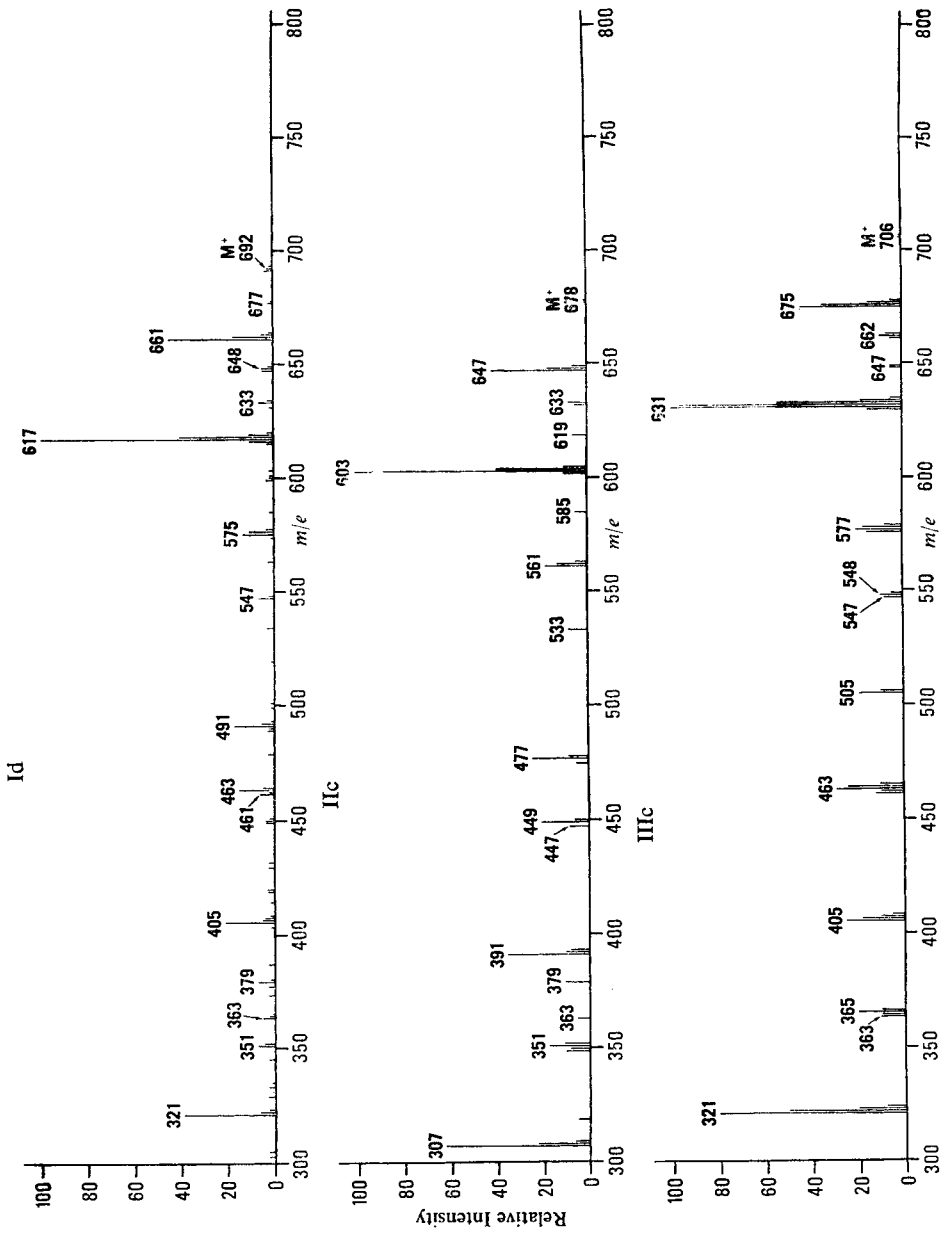


FIG. 4. Mass spectra of the sodium salts of monensin, factor B and factor C.

attributed to the loss of CH_3, CH_3O and CO_2 fragments. The homologs IIc and IIIc exhibit identical fragmentation patterns (with the appropriate 14-mass unit shifts) in the region above *m/e* 600.

It must be mentioned here that, in the present work, instrument resolution did not permit differentiation between ions which contain sodium and those which do not. For example, the formulae $C_{35}H_{58}O_{10}Na$ and $C_{37}H_{57}O_{10}$, which correspond to an ion of nominal mass 661, differ by only 2·4 millimass units. In this particular example, the latter formula is not reasonable and can be rejected. Similarly for the other ions mentioned above, the formulae given appear to be the more reasonable choices. The decision is not at all clear for the ions at lower masses, however.

Information bearing on this question was obtained by substituting other metals for sodium. Monensin free acid was treated with KOH and RbOH. Mass spectra of the products were obtained, the high-mass portions of which are shown in Fig. 5, along with that of the sodium salt for comparison. Both products appear to contain an appreciable amount of sodium salt.* The spectrum of the potassium preparation exhibits peaks due to sodium salt, but, in addition, there are corresponding peaks 16 mass units higher due to the presence of K^{39}.† Also, in the *m/e* 300 to 600 range of the spectrum, each significant peak due to sodium salt (Fig. 4) is accompanied by a [M + 16] peak of about the same intensity. A similar result was obtained with rubidium (Fig. 5).† Peaks are present 62 mass units higher relative to sodium salt due to Rb^{85} and 64 mass units higher due to Rb^{87} (for example, *m/e* 679, 681 and 723, 725).

TABLE 1. FRAGMENT IONS FROM THE SODIUM SALTS OF MONENSIN, FACTOR B AND FACTOR C

Compound	Ions (*m/e*)			
(Id)	575 ($C_{31}H_{52}O_8Na$)	547 ($C_{29}H_{48}O_8Na$)	491 ($C_{26}H_{44}O_7Na$)	405 ($C_{22}H_{38}O_5Na$)
(IIc)	561	533	477	391
(IIIc)	575	547	505	405
Compound	**Ions (*m/e*)**			
(Id)	379 ($C_{19}H_{32}O_6Na$)	351 ($C_{17}H_{28}O_6Na$)	321 ($C_{17}H_{30}O_4Na$)	
(IIc)	379	351	307	
(IIIc)	393	365	321	

* Analysis of the K and Rb salts for Na (emission spectrograph) showed the presence of about 10% and 3% Na salt, respectively. This is probably due to the presence in the starting free acid of Na salt from which the acid is prepared. The disproportionate intensity of peaks in the mass spectra due to Na salt may reflect a greater volatility of this salt, or it may be a consequence of metal exchange upon surfaces in the mass spectrometer. Such exchange in alkali-metal chelates of β-diketones has been reported recently.[9]

† Potassium consists mainly of the stable isotopes K^{39} (93·3%) and K^{41} (6·7%). The naturally occurring radioactive isotope K^{40} is present only to the extent of 0·011%. The naturally occurring isotopes of rubidium are Rb^{85} (stable, 72·8%) and Rb^{87} (radioactive, half-life $6·3 \times 10^{10}$ yr., 27·2%). Sodium exists naturally as a single stable isotope, Na^{23}. Peaks are found in spectra of the carboxylic acid salts at mass numbers corresponding to the metal ions. Sodium is particularly evident since its nominal mass does not coincide with carbon-containing fragments.

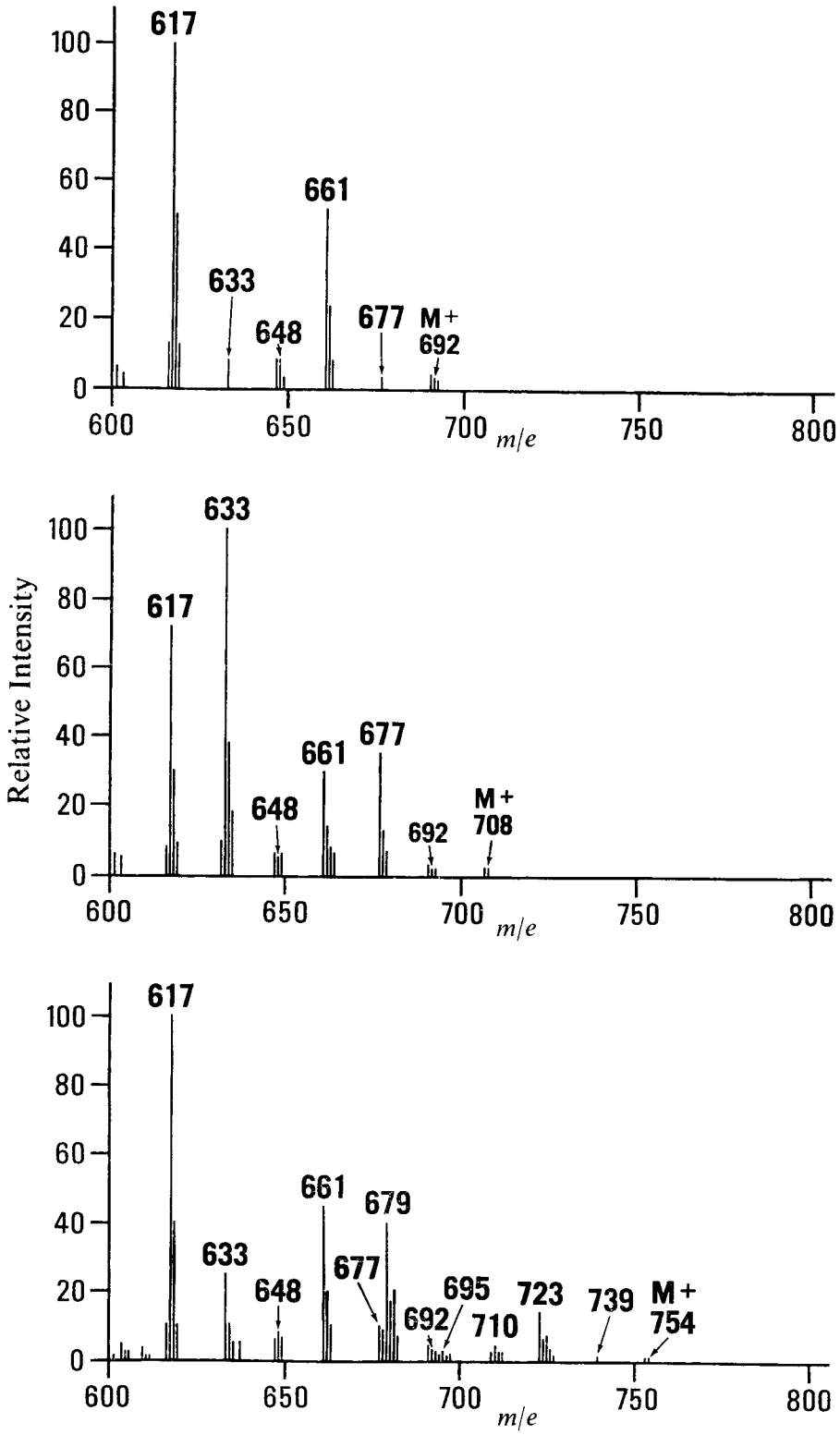
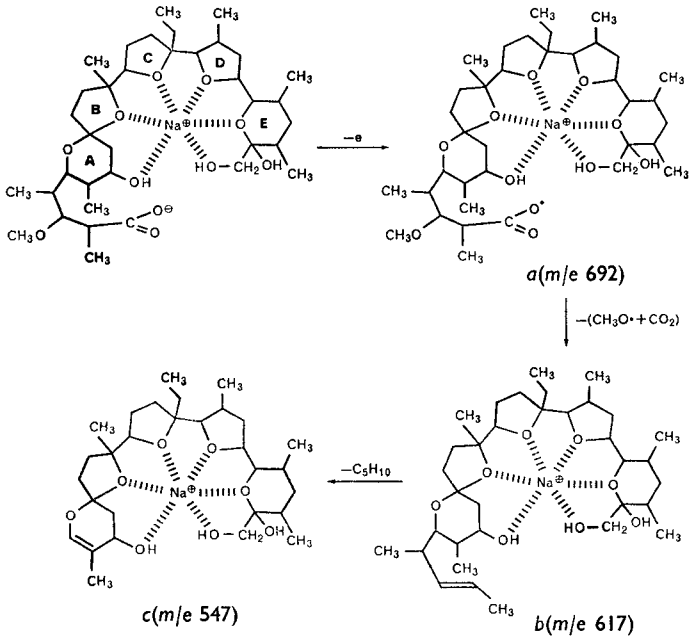


FIG. 5. High-mass portion of the spectra of sodium, potassium and rubidium salts of monensin.

The foregoing results provide direct evidence for the formation of fragment ions containing the metal and thus empirical formulae may be assigned to additional ions in the mass spectrum of monensin sodium salt (Fig. 4). These are listed in Table 1 along with the mass numbers of corresponding ions derived from IIc and IIIc. Comparison of values for Id, IIc and IIIc serves to indicate in a general way that portion of the molecule from which a given ion is derived. For example, the *m/e* 405 ion appears to include ring C but not the carboxyl–sidechain, whereas the opposite is true of the *m/e* 351 ion.



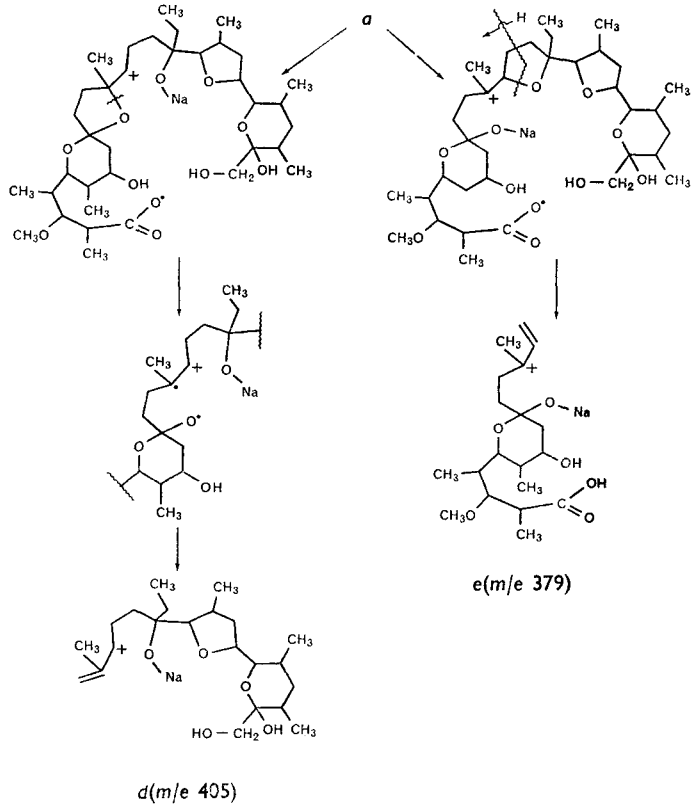
SCHEME 2.

Inspection of the values in Table 1 indicates that, with the possible exception of the *m/e* 351 ion, fragmentation of the salt does not yield ions analogous (that is, differing only by substitution of Na for H) to those formed from the free acid (Scheme 1). The empirical formula of the *m/e* 351 ion ($C_{17}H_{28}O_6Na$) and the *m/e* values for this fragment from IIc and IIIc suggest that it is related to ion B of Scheme 1. To rationalize formation of the other ions listed in Table 1 and the higher-mass ions mentioned earlier, the assumption is made that the sodium salt molecule in the gas phase may be represented as in Scheme 2 by analogy with the structure of the silver salt (Fig. 3).* Schemes 2 and 3 may not represent correctly in every detail the ion structures and their mode of formation. They are offered as at least a first approximation to account

* The sodium and silver salts of monensin have very similar, although not identical, crystal forms. The sodium, potassium and silver salts of nigericin are isomorphous (private communication from Prof L. K. Steinrauf). Thus it seems likely that the alkali-metal and silver salts of these compounds have similar structures in the crystalline state. Analogous complexation of sodium ion occurs with certain synthetic, cyclic polyethers.[10a] The latter exhibit specific influences on cation transport in rat liver mitochondria.[10b]

for the elemental compositions of the ions and the marked difference between fragmentation of the salt and the corresponding free acid.

Referring to Scheme 2, removal of an electron from the salt molecule may occur to give the radical cation *a*. Fragmentation by loss of CO_2 and CH_3O from *a* leads to the abundant *m/e* 617 ion (*b*). Further loss of the 5-carbon chain and an additional hydrogen atom accounts for the composition of ion *c*. The *m/e* 575 ion is considered to arise also by fragmentation of the carboxyl side-chain with the loss of all but two carbon atoms of the chain and an additional hydrogen atom.



SCHEME 3.

A pathway for the formation of lower-mass fragments which contain Na is given in Scheme 3. The basic mechanism is illustrated by derivation of the *m/e* 405 ion (*d*, Scheme 3). It is proposed that C—O bond fission occurs in the molecular ion (represented by *a*) with retention of positive charge on carbon and formation of a sodium-oxygen bond. Homolytic fission of the adjacent C—O bond and cleavage of an 'allylic' C—C bond lead to the charge-stabilized ion *d*. The formation of *e* can be rationalized similarly except that a hydrogen transfer must be invoked to yield the observed empirical formula. Origin of the *m/e* 321 and 491 ions (corresponding to *d* and *e*, respectively) can be explained in like manner by cleavage of the appropriate bonds of rings C and D.

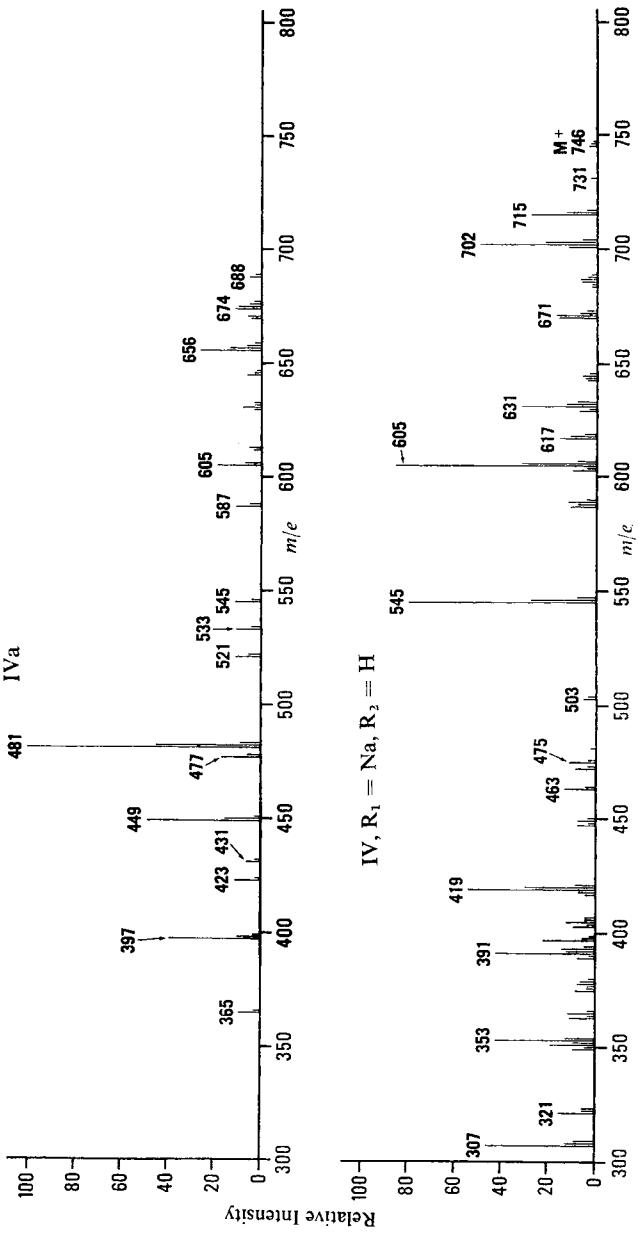
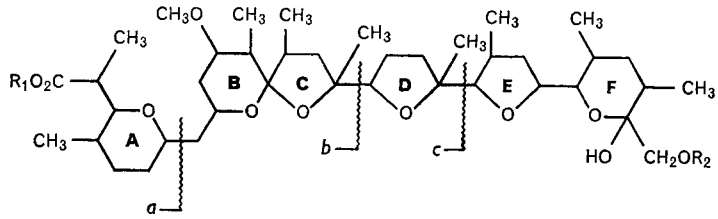


FIG. 6. Mass spectra of nigericin free acid and sodium salt.

Nigericin

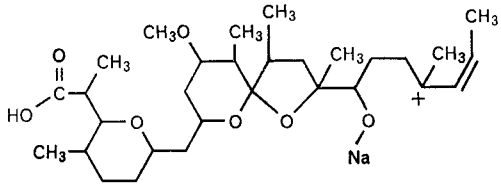
Nigericin (IV)[2] has been utilized for a number of years in the investigation of metabolic processes.[11] Recently the structure of its silver salt was determined by X-ray crystallography.[5] The conformation of the molecule in the crystal is similar to that of monensin (Fig. 3). Similarities are found also in the mass spectral behavior of these two substances.

Comparison of the spectra of nigericin free acid (IVa) (Fig. 6), the methyl ester (IVb) and the acetyl methyl ester derivative (IVc) shows that important fragmentations occur as shown in the structural formula.

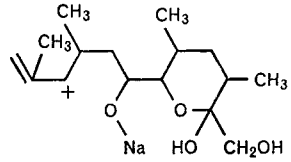


(IVa) $R_1 = R_2 = H$
(IVb) $R_1 = CH_3$, $R_2 = H$
(IVc) $R_1 = CH_3$, $R_2 = CH_3CO$

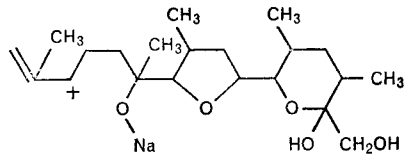
The molecular ion is not found in the spectrum of the free acid, IVa (Mol. wt. 724, $C_{40}H_{68}O_{11}$) (Fig. 6). The first peak occurs at *m/e* 688 due to the loss of two molecules of H_2O. The molecular ion is exhibited by the derivative IVc at *m/e* 780. The base peak in the spectrum of IVa occurs at *m/e* 481 and is due to ion *c*. Loss of 32 mass units (CH_3OH) (m* 481 → 449, calcd. 419·1, obsd. 419·1) gives rise to the *m/e* 449 peak. Cleavage of the bond between rings C and D yields ion *b* at *m/e* 397 which also undergoes loss of CH_3OH (m* 397 → 365, calcd. 335·6, obsd. 335·5) to give the *m/e* 365 ion. Appropriate shifts of these peaks are observed in the spectra of IVb and IVc.



m/e **545**



m/e **307**



m/e **391**

SCHEME 4. Fragment ions from nigericin sodium salt.

In the latter, the base peaks are due to ion *b*. In the spectrum of IVa, a peak which occurs at *m/e* 171 and which is shifted to *m/e* 185 in IVb and IVc is accounted for as ion *a*.

The mass spectrum of nigericin sodium salt (IV, R_1 = Na, R_2 = H) is shown in Fig. 6. The molecular ion is present at *m/e* 746, accompanied by an [M − 1] peak of greater intensity. The strong peak at *m/e* 702 is considered to arise by loss of CO_2. Further interpretation of the spectrum can be achieved by applying the same arguments that were used in rationalizing the spectrum of monensin sodium salt. Thus, for example, the abundant *m/e* 545 ($C_{30}H_{50}O_7Na$), *m/e* 391 ($C_{21}H_{36}O_5Na$) and *m/e* 307 ($C_{16}H_{28}O_4Na$) ions can be formulated as in Scheme 4. The *m/e* 419 ion ($C_{22}H_{37}O_6Na$) may arise by cleavage of the bond between rings C and D and would thus be analogous to the *m/e* 351 ion formed from monensin sodium salt (see discussion above). The elemental composition of the *m/e* 605 ion ($C_{32}H_{54}O_9Na$) suggests that it arises by fragmentation of ring A with the loss of the carboxyl side-chain and the carbon atoms of the ring.

EXPERIMENTAL

Mass spectra were recorded on a CEC Model 21-110A-1 spectrometer using a direct inlet system with ion-source temperatures in the range 200 to 230°. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The n.m.r. spectra were taken on a Varian Associates Model HR-60 spectrometer. Chemical shifts are reported as δ values in ppm relative to TMS = 0.

Monensin, factor B and factor C were obtained in pure form by column chromatography of the sodium salts over silica gel using EtOAc as the eluant.[3] The free acids were obtained by acidification of an aqueous MeOH solution of the sodium salts, followed by extraction with Et_2O and recrystallization from petroleum ether -Et_2O. Nigericin was obtained in these Laboratories as the sodium salt and similarly converted to the free acid.

Monensin methyl ester (Ib). A solution of monensin in Et_2O was treated with excess ethereal CH_2N_2. After several minutes the solution was evaporated to dryness. The product consisted of four components (t.l.c.). Column chromatography (silica gel, 1:3 EtOAc-Benzene) of the mixture led to the isolation of *monensin methyl ester* (Ib) as the major component (*ca.* 60%). The minor products were shown to be methyl ether derivatives of Ib by n.m.r. and mass spectrometry. The ester could not be induced to crystallize. i.r. spectrum ($CHCl_3$): γ_{max} 3425, 3279 (OH) and 1721 cm^{-1} (C=O). n.m.r. spectrum ($CDCl_3$): ester CH_3 at δ 3·71 ppm. Found: C, 65·13; H, 9·57. $C_{37}H_{64}O_{11}$ requires C, 64·88; H, 9·42%.

Diacetylmonensin methyl ester (Ic).[4] A solution of monensin sodium salt (1·00 g) in pyridine (10 ml) and acetic anhydride (10 ml) was allowed to stand overnight at room temperature. The solution was poured into water and extracted with Skellysolve F and then with ether. The combined extracts were washed three times with 10% hydrochloric acid, twice with water and once with saturated sodium chloride solution. Evaporation of the solvent gave a colorless gum residue. The infrared spectrum of the residue indicated the presence of anhydride and therefore the product was treated with a dilute solution of sodium bicarbonate in aqueous dioxane at room temperature for two hours. The solution was diluted with water and extracted three times with ether. The combined extracts were washed with water and dried with anhydrous sodium sulfate. The product obtained upon evaporation of the solvent was crystallized from ether-Skellysolve B to give *diacetylmonensin* (0·357 g), m.p. 80 to 100°. i.r. spectrum ($CHCl_3$): γ_{max} 3448 (OH) and 1727 cm^{-1} (C=O). Titration (66% DMF): pKa 7·6. n.m.r. spectrum ($CDCl_3$): acetyl CH_3 at δ 2·12 and 2·20 ppm. Found: C, 63·60; H, 9·05. $C_{40}H_{66}O_{13}$ requires C, 63·64; H, 8·81%.

The product from acetylation of 5·0 g of monensin sodium salt was dissolved in ether and treated with an ethereal solution of diazomethane. Evaporation of the solvent gave a dark orange-colored, gummy residue. The residue was dissolved in petroleum ether-ether (3:1) and the solution was filtered to remove undissolved material. The orange-colored filtrate was treated with charcoal. Removal of the charcoal by filtration through a Celite pad gave a colorless solution which was concentrated to one-fourth of the original volume and set aside at room temperature. There was obtained 1·1 g of crystalline diacetylmonensin methyl ester (Ib), m.p. 113 to 114°. i.r. spectrum

($CHCl_3$): 3546 and 1724 cm^{-1}. n.m.r. spectrum ($CDCl_3$): acetyl CH_3 at δ 2·07 and 2·13 ppm, ester CH_3 at δ 3·75 ppm. Found: C, 63·79; H, 8·96. Calc. for $C_{41}H_{68}O_{13}$: C, 64·04; H, 8·91%.

Diacetyl methyl ester derivatives of factor B and factor C (II*b*, III*b*). The derivatives were prepared in the same manner as given for monensin. The product from factor B was purified by column chromatography. The product from factor C was a single spot on t.l.c. The n.m.r. and mass spectra were consistent with the formation of diacetyl methyl ester derivatives.

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