tered by the American Chemical Society, and the National Institutes of Health (GM 24438) for financial support.

References and Notes

(1) Part 4 in the series "Cyclobutene Derivatives as Isoprene Equivalents in Terpene Synthesis". Contribution No. 3197 from the Department of Chemistry, Indiana University, Bloomington, Ind. 47401.

(2) G. A. Cordell, *Chem. Rev.*, **76,** 425 (1976).

(3) Review: E. E. Van Tamelen, *Bioorg. Chem.*, **5,** 51 (1976).

(4) (a) S. R. Wilson and L. R. Phillips, *Tetrahedron Lett.*, 3047 (1975); (b) S. R. Wilson and D. E. Schalk, *J. Org. Chem.*, **41,** 3928 (1976); (c) S. R. Wilson, L. R. Phillips, Y. Pelister, and J. C. Huffman, submitted.

(5) H. M. Frey, *Trans. Faraday Soc.*, **58,** 957 (1962).

(6) J. Shabtai and E. Gil-Av, *J. Org. Chem.*, **28,** 2893 (1963).

(7) (a) S. Akiyama and J. Hooz, *Tetrahedron Lett.*, 4115 (1973); (b) G. G. Eberhardt and W. A. Butte, *J. Org. Chem.*, **29,** 2928 (1964); (c) A. W. Langer, Jr., *Ann. N.Y. Acad. Sci., Ser. II*, **27,** 741 (1965); D. J. Peterson, *J. Org. Chem.*, **32,** 1717 (1967).

(8) It is interesting to note that the rate of proton abstraction from **6** is much faster than that from other methylenecycloalkanes (ring size 5, 6, 7, or 8) or 2-ethyl-1-butene: A. Schriesheim, R. J. Muller, and C. A. Rowe, Jr., *J. Am. Chem. Soc.*, **84,** 3164 (1962).

(9) Two allyllithiums related to **4** have been reported: E. Dumkelblum and S. Brenner, *Tetrahedron Lett.*, 669 (1973); D. Seebach, M. Kolb, and B. Grobel, *ibid.*, 3171 (1974).

(10) For a nice study of reactions of phenyllithium see V. Rautenstrauch, *Helv. Chim. Acta*, **57,** 2261 (1974).

(11) R. A. Benkeser, *Synthesis*, 347 (1971).

(12) K. J. Shea and S. Wise, *J. Org. Chem.*, **43,** 2710 (1978).

(13) (a) J. Hartmann, R. Muthukrishnan, and M. Schlosser, *Helv. Chim. Acta*, **57,** 2261 (1974). (b) The isomeric alcoholates are formed under kinetic control. Even the potassium salts[14] do not interconvert (KH, THF, 25 °C on the alcohol).

(14) S. R. Wilson, D. T. Mao, K. M. Jernberg, and S. T. Ezmirly, *Tetrahedron Lett.*, 2559 (1977).

(15) (a) C. Eaborn, "Organosilicon Compounds", Butterworths, London, 1960; (b) A. W. P. Jarvie, *Organomet. Chem. Rev., Sect. A*, **6,** 153 (1970).

(16) (a) A. Hosomi and H. Sakurai, *Tetrahedron Lett.*, 4041 (1977); (b) B. Au-Yeung and I. Fleming, *J. Chem. Soc. Chem. Commun.*, 79 (1977); (c) A. Hosomi and H. Sakurai, *J. Am. Chem. Soc.*, **99,** 1673 (1977); (d) B. Au-Yeung and I. Fleming, *J. Chem. Soc., Chem. Commun.*, 81 (1977); (e) D. Ayalon-Chass, E. Ehlinger, and P. Magnus, *ibid.*, 722 (1977); (f) M. J. Carter and I. Fleming, *ibid.*, 679 (1976); (g) G. Deleris, J. Dunogues, and R. Calas, *Tetrahedron Lett.*, 2449 (1976); (h) A. Hosomi and H. Sakurai, *Chem. Lett.*, 941 (1976); (i) A. Hosomi and H. Sakurai, *Tetrahedron Lett.*, 1295 (1976); (j) I. Fleming, A. Pearce, and R. L. Snowden, *J. Chem. Soc., Chem. Commun.*, 182 (1976); (k) R. Calas, J. Dunogues, J-P. Pillot, C. Biran, F. Piscotti, and B. Arreguy, *J. Organomet. Chem.*, **85,** 149 (1975); (l) G. Deleris, J. Dunogues, and R. Calas, *ibid.*, **93,** 43 (1975); (m) M. Laguerre, J. Dunogues, R. Calas, and N. Duffant, *ibid.*, **93,** C17 (1975); (n) I. Fleming, *Chem. Ind. (London)*, 449 (1975).

(17) P-J. Pillot, J. Dunogues, and R. Calas, *Tetrahedron Lett.*, 1871 (1976).

(18) A. Hosomi and H. Sakurai, *Tetrahedron Lett.*, 2589 (1978).

(19) The cyclobutyl cation shown as an intermediate in eq 3 could in principle isomerize to homoallyl or cyclopropylcarbinyl products, but none were observed: H. C. Brown and M. Borkowski, *J. Am. Chem. Soc.*, **74,** 1894 (1952).

(20) (a) L. H. Briggs and M. D. Sutherland, *J. Org. Chem.*, **7,** 397 (1942); (b) R. M. Silverstein, J. O. Rodin, and D. L. Wood, *Science*, **154,** 509 (1966); (c) R. M. Silverstein, J. O. Rodin, D. L. Wood, and L. E. Browne, *Tetrahedron*, 1929 (1966); (d) P. W. Atkinson and W. D. Crow, *Tetrahedron*, 1935 (1970); (e) Y-R. Naves, *Helv. Chim. Acta*, **31,** 29 (1948); (f) K. S. Baalsrud, D. Holme, M. Nestvold, J. Pliva, J. S. Sorensen, and N. A. Sorensen, *Acta Chem. Scand.*, **6,** 883 (1952); (g) K. L. Stevens, R. E. Lundin, and R. Teranishi, *J. Org. Chem.*, **30,** 1690 (1965); (h) A. Bhati, *Perfum. Essent. Oil. Rec.*, **53,** 685 (1962); *Chem. Abstr.*, **58,** 5988*b* (1963); (i) R. M. Carman and N. Dennis, *Aust. J. Chem.*, **20,** 157 (1967).

(21) Review: M. C. Birch, *Am. Sci.*, 409 (1978).

(22) (a) C. A. Reese, J. O. Robin, R. G. Brownlee, W. G. Duncan, and R. M. Silverstein, *Tetrahedron*, **24,** 4249 (1969); (b) S. Karlsen, P. Froyen, and L. Skattebol, *Acta Chem. Scand., Ser. B*, **30,** 664 (1976); (c) C. A. Reece, J. O. Rodin, R. G. Brownlee, W. G. Duncan, and R. M. Silverstein, *Tetrahedron*, **24,** 4249 (1968); (d) O. P. Vig, R. C. Anand, G. L. Kad, and J. M. Sehgal, *J. Indian Chem. Soc.*, **47,** 999 (1970); (e) J. A. Katzenellenbogen and R. S. Lenox, *J. Org. Chem.*, **38,** 326 (1973); (f) R. Riley, R. M. Silverstein, J. A. Katzenellenbogen, and R. S. Lenox, *ibid.*, **39,** 1957 (1974); (g) K. Mori, *Agric. Biol. Chem.*, **38,** 2045 (1974); (h) K. Mori, *Tetrahedron Lett.*, 2187 (1975); (i) C. Garvers and F. Scott, *ibid.*, 1625 (1976); (j) K. Mori, *Tetrahedron*, **32,** 1101 (1976).

(23) (a) J. Slutsky and H. Kwart, *J. Am. Chem. Soc.*, **95,** 8678 (1973); (b) for a review see C. W. Spangler, *Chem. Rev.*, **75,** 187 (1975).

(24) J. A. Verdone, J. A. Mangravite, N. M. Scarpa, and H. G. Kuivial, *J. Am. Chem. Soc.*, **97,** 843 (1975).

(25) W. von E. Doering and J. C. Gilbert, *Tetrahedron, Suppl.*, **7,** 397 (1966).

(26) The mixture **10a/11a** was reacted with methyl acrylate/$AlCl_3$ in $CHCl_3$ at room temperature. GC analysis showed that only Diels–Alder adduct **20**a was formed, although a number of more volatile byproducts prevented isolation of the material.

(27) Spectral data for **22b** matched those published for δ-terpinol: B. M. Mitzner and S. Lemberg, *J. Org. Chem.*, **31,** 2022 (1966).

(28) The tertiary carbonium ion derived by loss of water from the protonated alcohol **23** could cyclize to the bicyclic monoterpene β-pinene, but none could be detected.

(29) It should be noted that the relative thermodynamic stability [J. Shabtai and E. Gil-Av, *J. Org. Chem.*, **28,** 2893 (1963)] of **8, 9** is close to 85:15 and thus the observed product ratio from stannylation of 1 may be thermodynamic, not kinetic, due to facile [1,3] isomerization.[24]

(30) Cf. J. Iyoda and I. Shiihara, *J. Org. Chem.*, **35,** 4267 (1970).

(31) (a) For a review see J. A. Titov, *Russ. Chem. Rev. (Engl. Transl.)*, 267 (1962); (b) H. E. Hennis, *J. Org. Chem.*, **28,** 2570 (1963).

Structure of Ionomycin—a Novel Diacidic Polyether Antibiotic Having High Affinity for Calcium Ions

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Abstract: The structure and absolute configuration of ionomycin, $C_{41}H_{72}O_9$, a novel diacidic polyether antibiotic having high affinity for calcium ions, have been determined by X-ray and spectroscopic methods. X-ray analyses of orthorhombic forms ($P2_12_12$, $Z = 4$) of the isomorphous Cd and Ca salts and of a monoclinic form of the Cd salt containing two molecules per asymmetric unit ($P2_1$, $Z = 4$) indicate that the complexed ionophore adopts virtually identical conformations in the three independent solid-state environments. In both crystal forms, the ionophores are joined in pairs by two hydrogen bonds so as to form a "dimeric" globular structure having primarily lipophilic surfaces. Heptane and hexane molecules of crystallization are packed between the lipophilic "dimers" in the orthorhombic and monoclinic forms, respectively. The molecular structure of the salts of ionomycin contains a cisoid enolized β-diketone anion that, together with a carboxylate group and three other oxygen atoms, is octahedrally coordinated to the central divalent cation.

Introduction

The polyether antibiotics[2] display a range of interesting biological properties including antimicrobial activity, growth promotion in ruminants, and cardiovascular effects. These properties are related to the ability of the polyethers to form lipid-soluble complexes with inorganic cations and so transport them across hydrophobic barriers.

Ionomycin (**1**) is a new and especially interesting member of the polyether ionophores in that it chelates calcium (and

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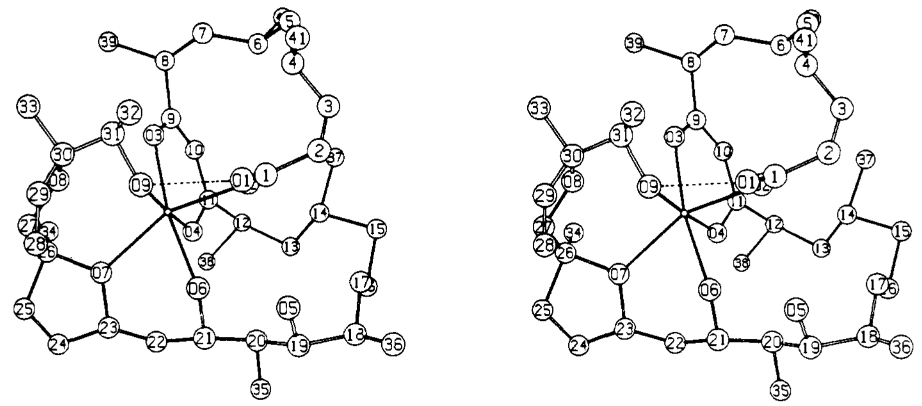


Figure 1. The structure and solid-state conformation in the orthorhombic crystal structure of the calcium and cadmium complexes of **1**.

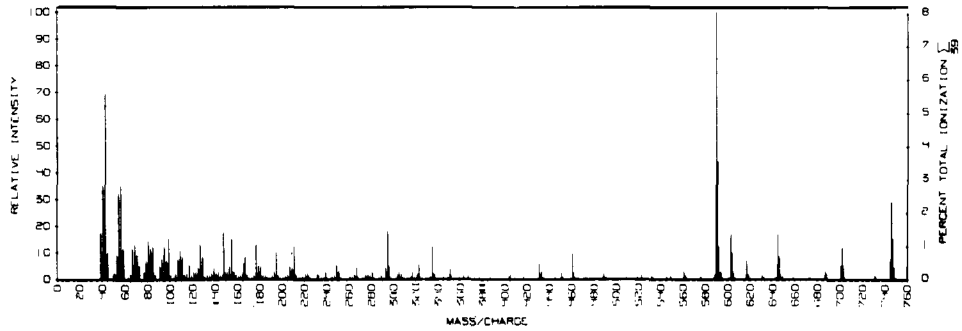


Figure 2. Low-resolution mass spectrum of Ca salt of **1**.

cadmium) as a *dibasic* acid, whereas the few other known ionophores which chelate dipositive ions do so as *monobasic* acids. The high affinity of **1** for calcium was early evident in the fact that the antibiotic was isolated as its calcium salt from hexane extracts of broth concentrates which had been adjusted to pH 12 with aqueous NaOH. A report on the ionophoric properties of **1** is in accord with this specificity for divalent cations.[3] The production of **1** by *Streptomyces conglobatus* sp. nov. Trejo and further details of isolation and initial characterization are being reported elsewhere.[4]

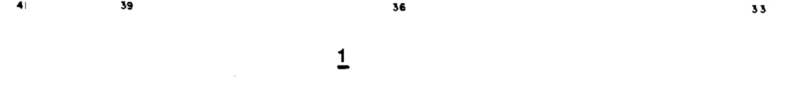
We report here the results of three X-ray analyses of the structure of **1** obtained for two crystalline modifications of the cadmium salt and one of the calcium salt. The X-ray data, together with ^1H and ^{13}C NMR data and high-resolution mass spectroscopic data, have led to the molecular structure assignment **1** for the parent acid, $C_{41}H_{72}O_9$.

The dianion of **1**, resulting from the ionization of the carboxyl and β-diketone groups, is octahedrally coordinated to the central divalent cation in the conformation observed in the solid state (Figure 1).

The existence of an orthorhombic crystal structure with one independent molecule per asymmetric unit ($P2_12_12$, $Z = 4$), as well as a monoclinic structure containing two crystallographically independent molecules per asymmetric unit ($P2_1$, $Z = 4$), afforded the opportunity to observe three independent solid-state conformations of the complexed ionophore. Both crystal forms of the cadmium salt have been analyzed, whereas only the orthorhombic form of the calcium salt (isomorphous with Cd) has been studied. The molecular conformations are very similar in all of these crystal structures. It is of interest that the two crystallographically independent molecules in the monoclinic structure are associated about a *noncrystallographic* twofold axis directed along $a = 12.132$ Å in virtually the same arrangement that obtains about the crystallographic twofold axis ($c = 12.079$ Å) of the orthorhombic crystal structure. The twofold related molecules in both crystal structures are joined by intermolecular hydrogen bonds between hydroxyl and carboxylate oxygen atoms, resulting in a "dimeric" structure in which only lipophilic surfaces are exposed. It is therefore not surprising that hexane and heptane are incorporated as solvents of crystallization in the crystal structures of the salts of **1**. The presence of the same "dimeric" lipophilic structure in the two independent crystal structures raises the possibility that it also exists in other dispersed lipophilic media.

Results

The structural assignment for **1** is based entirely on an interpretation of the X-ray and spectroscopic data; the chemical degradation of **1** has not been examined. Like other members of this class of antibiotics, **1** and its salts exhibit IR absorption due to carboxyl and hydroxyl groups.[4] However, **1** is distinguished from the others by virtue of its intense ultraviolet absorption[4] [300 nm (ϵ 21 600, CH_3OH) for the Ca salt; 280 nm



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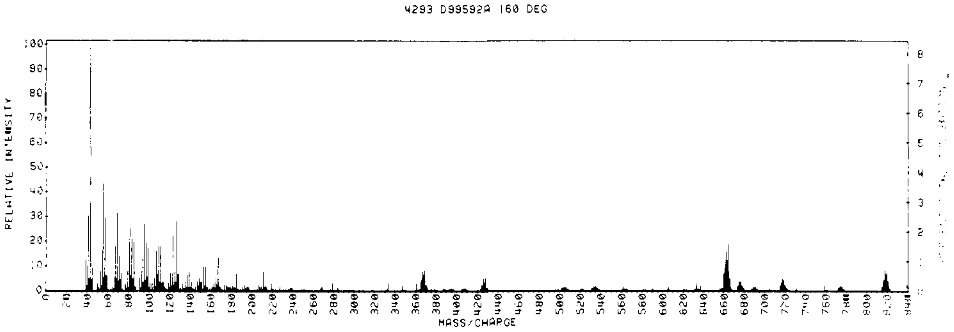

Figure 3. Low-resolution mass spectrum of Cd salt of **1**.

Table I. Mass Spectrum of **1**, Calcium Salt

found	C	H	O	Ca	calcd
746.7405	41	60	9	1	746.4646
731	40	67	9	1	
702	40	70	7	1	
701.4754	40	69	7	1	701.4669
701.4365	39	65	8	1	701.4305
687.4514	39	67	7	1	687.4513
687.4181	38	63	8	1	687.4181
645.4021	36	61	7	1	645.4043
631.3859	35	59	7	1	631.3859
617	34	57	7	1	
603.3543	33	55	7	1	603.3573
590.3512	32	54	7	1	590.3495
562.3520	31	54	6	1	562.3546
562.3142	30	50	7	1	562.3182

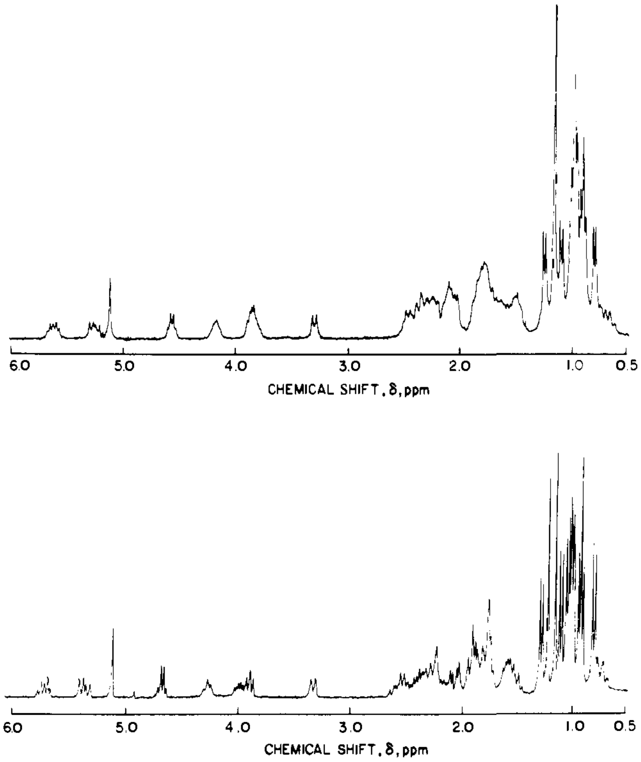

Figure 4. 270-MHz ^1H NMR spectra of the salts of **1** in deuteriochloroform (Me_4Si internal reference): calcium salt upper and cadmium salt lower.

(ε 18 600, CH_3OH) for the free acid] which can now be ascribed to the second acidic function—the cisoid enolized β-diketone group.

The electron-impact mass spectrum of the Ca salt (Figure 2) shows a M^+ at m/z 746 in agreement with the formula $C_{41}H_{70}O_9{}^{40}Ca$ derived from high-resolution mass spectral peak matching (calcd 746.4646, found 746.4705). Additional high-resolution measurements in the range m/z 562 to M^+ and metastable ion defocusing experiments further support this composition (Table I). The mass spectrum of the Cd salt (Figure 3) contains a cluster of M^+ at m/z 816–821 in the appropriate intensity ratio of the natural abundance of ^{110}Cd to ^{116}Cd isotopes. Crystal density and X-ray unit cell volume measurements on the salt, after due allowance for solvent of crystallization, suggested a molecular weight of 746 or 1492 for the calcium salt and 819 or 1638 for the cadmium salt. The Rast technique gave a value of 740 for the calcium salt.

Pertrimethylsilylation of the calcium ionophore results in a mixture of calcium-free tetra- and pentatrimethylsilyl derivatives with M^+ of m/e 996 and 1068, respectively. Given the presence of the carboxyl group and the assumption that the second acidic function is a β-diketone, four oxygens and three double bond equivalents are accounted for. Further, it seemed probable on the basis of the pentatrimethylsilylation that, of the remaining five oxygen atoms, three were hydroxylic. The formula of **1** requires an additional three double bond equivalents.

The lowest field regions of the ^1H and ^{13}C magnetic resonance spectra (Figures 4 and 5) of the calcium salt made possible a further understanding of the nature of the unsaturation of **1**. One of the remaining three double bond equivalents is accounted for by the presence of one trans disubstituted double bond with coupled ($J = 14$ Hz) proton signals centered at δ 5.66 (1 H) and 5.29 ppm (1 H). Also present is a sharp one-proton singlet (δ 5.14 ppm) attributable to a hydrogen of the enolized β-diketone anion ($OCCH{=}CO^-$). If the six

Table II. Crystal Data

cation	solvate	a, Å	b, Å	c, Å	β, deg	space group	Z	d_o, g cm^{-3}	mol wt per unit cell
Ca^{2+}	heptane	22.12 (2)	18.85 (1)	12.11 (1)		$P2_12_12$	4	1.047[a]	3187
Cd^{2+}	heptane	22.152 (5)	18.688 (3)	12.079 (1)		$P2_12_12$	4	1.154[b]	3477
Cd^{2+}	hexane	12.132 (2)	33.991 (14)	13.191 (3)	116.0 (0)	$P2_1$	4	1.183[b]	3485

[a] Measured by flotation in aqueous cadmium iodide. [b] Measured by flotation in aqueous potassium bromide.

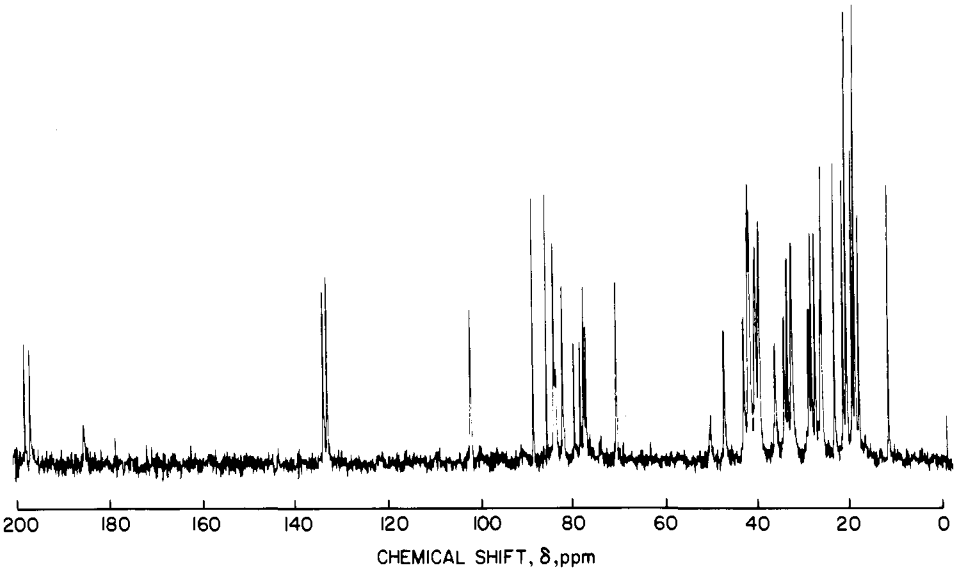

Figure 5. 25-MHz ^{13}C NMR spectrum of the Ca salt of **1** in deuteriochloroform.

Table III. Fractional Coordinates and Errors of Calcium Complex

atom no.	*x*	*y*	*z*	atom no.	*x*	*y*	*z*
C1	−0.080 (3)	−0.122 (3)	−0.327 (5)	C29	−0.124 (3)	−0.073 (4)	0.157 (6)
C2	−0.079 (2)	−0.152 (2)	−0.460 (4)	C30	−0.151 (3)	−0.142 (3)	0.092 (6)
C3	−0.108 (3)	−0.220 (3)	−0.481 (5)	C31	−0.162 (4)	−0.111 (4)	−0.021 (7)
C4	−0.165 (2)	−0.235 (3)	−0.439 (4)	C32	−0.205 (3)	−0.074 (4)	−0.068 (6)
C5	−0.193 (2)	−0.309 (3)	−0.448 (5)	C33	−0.196 (4)	−0.179 (4)	0.140 (7)
C6	−0.158 (3)	−0.366 (3)	−0.360 (6)	C34	−0.001 (3)	−0.270 (4)	0.162 (6)
C7	−0.192 (2)	−0.350 (3)	−0.243 (5)	C35	0.210 (3)	−0.155 (3)	−0.194 (5)
C8	−0.152 (3)	−0.394 (3)	−0.159 (5)	C36	0.157 (3)	−0.055 (3)	−0.513 (5)
C9	−0.101 (3)	−0.356 (4)	−0.135 (5)	C37	0.018 (3)	−0.404 (3)	−0.439 (5)
C10	−0.039 (3)	−0.385 (2)	−0.155 (4)	C38	0.123 (3)	−0.410 (3)	−0.104 (5)
C11	0.018 (3)	−0.349 (3)	−0.162 (5)	C39	−0.185 (3)	−0.392 (3)	−0.062 (5)
C12	0.078 (2)	−0.399 (3)	−0.197 (5)	C40	−0.154 (2)	−0.434 (3)	−0.409 (4)
C13	0.103 (3)	−0.366 (3)	−0.308 (6)	C41	−0.211 (3)	−0.185 (3)	−0.487 (4)
C14	0.050 (2)	−0.330 (3)	−0.400 (5)	O1	−0.099 (2)	−0.077 (2)	−0.292 (3)
C15	0.085 (2)	−0.294 (3)	−0.478 (4)	O2	−0.047 (1)	−0.178 (2)	−0.278 (2)
C16	0.123 (3)	−0.233 (3)	−0.473 (5)	O3	−0.097 (1)	−0.288 (2)	−0.094 (3)
C17	0.115 (2)	−0.174 (3)	−0.457 (4)	O4	0.031 (2)	−0.289 (2)	−0.115 (3)
C18	0.152 (3)	−0.117 (3)	−0.439 (6)	O5	0.081 (1)	−0.055 (1)	−0.333 (2)
C19	0.136 (2)	−0.085 (3)	−0.309 (5)	O6	0.047 (1)	−0.108 (2)	−0.135 (2)
C20	0.138 (3)	−0.138 (3)	−0.230 (5)	O7	0.021 (2)	−0.169 (2)	0.079 (3)
C21	0.112 (3)	−0.099 (3)	−0.125 (5)	O8	−0.099 (2)	−0.179 (2)	0.102 (3)
C22	0.122 (3)	−0.153 (3)	−0.014 (5)	O9	−0.106 (1)	−0.101 (2)	−0.076 (3)
C23	0.083 (3)	−0.141 (3)	0.073 (6)	Ca	−0.031 (1)	−0.195 (1)	−0.094 (1)
C24	0.106 (3)	−0.159 (3)	0.199 (5)	C1[a]	−0.500	0.000	−0.193
C25	0.050 (3)	−0.157 (4)	0.239 (6)	C2	−0.403	−0.011	−0.238
C26	−0.005 (4)	−0.186 (4)	0.181 (7)	C3	−0.461	−0.054	−0.262
C27	−0.060 (4)	−0.165 (4)	0.171 (7)	C4	−0.342	−0.036	−0.243
C28	−0.067 (3)	−0.079 (3)	0.182 (6)				

[a] The following four atoms are due to heptane.

lowest field (δ >100 ppm) ^{13}C resonances of the calcium salt are attributed to the six sp^2 carbons of these groups [*OC*-*CH*=*CO*$^-$ (δ 198.7, 102.5, 197.5), -*COO*$^-$ (δ 186.1), -*CH*=*CH*- (δ 134.2, 133.4 ppm)], it follows that two rings are present in **1**.

A separate group of ^{13}C resonances in the range δ 89–71 ppm is attributable to seven sp^3 carbon atoms bonded to oxygen. Subtraction of the three carbons bearing hydroxyl groups leaves four oxygen-bonded carbons that are thus ethereal. This accounts for all of the oxygen in the structure. The proton spectrum from δ 3.3 to 4.6 ppm exhibits well-resolved signals at δ 4.6 (quartet, *J* = 6.4 Hz, 1 H), 4.18 (broad multiplet, 1 H), 3.87 (broad multiplet, 2 H), and 3.30 ppm (doublet, *J* = 9 Hz, 1 H) suggestive of five methine hydrogens bonded to these oxygen-bearing carbon atoms.

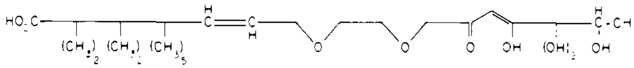

Figure 6. Schematic structure of **1** derived from spectroscopic data.

Well-resolved proton resonances from δ 0.77 to 1.22 ppm (270 MHz) were attributed to two tertiary methyl (δ 1.13 ppm, singlet, 6 H) and three secondary methyl groups [δ 0.77 (doublet, *J* = 7 Hz, 3 H), 1.07 (doublet, *J* = 6.9 Hz, 3 H), 1.22 ppm (doublet, *J* = 6.4 Hz, 3 H)]. Proton-proton spin de-

Table IV. Observed and Calculated Intensities of Selected Friedel Pairs of Reflections for the Orthorhombic Cd Salt of **1**

	$F^2(hkl)$		$F^2(\bar{h}\bar{k}\bar{l})$	
h k l	obsd[a]	calcd	obsd[b]	calcd
3 2 2	1255	1177	1619	1548
5 1 1	955	901	1149	1153
4 1 2	1574	1442	1264	1117
3 1 3	952	632	1312	933
3 2 4	1370	1732	1150	1426
4 4 3	1102	1143	905	901
3 4 4	1392	1703	1164	1459
6 6 1	1516	1304	1742	1558
2 8 1	1727	1600	1489	1309
1 3 5	1123	1109	1015	933
4 1 5	1323	1350	1050	992
3 3 5	1456	1631	1374	1428
8 6 1	1122	859	1301	1094
5 4 4	1826	1823	1588	1560
2 2 5	2071	1982	1751	1664
2 2 1	3590	4318	3205	3656
3 1 2	1988	2048	1644	1719
4 3 1	2946	2856	3269	3215

[a] The average of reflections hkl, $\bar{h}\bar{k}l$, $\bar{h}k\bar{l}$, and $h\bar{k}\bar{l}$. [b] The average of reflections $\bar{h}\bar{k}\bar{l}$, $hk\bar{l}$, $h\bar{k}l$, and $\bar{h}kl$.

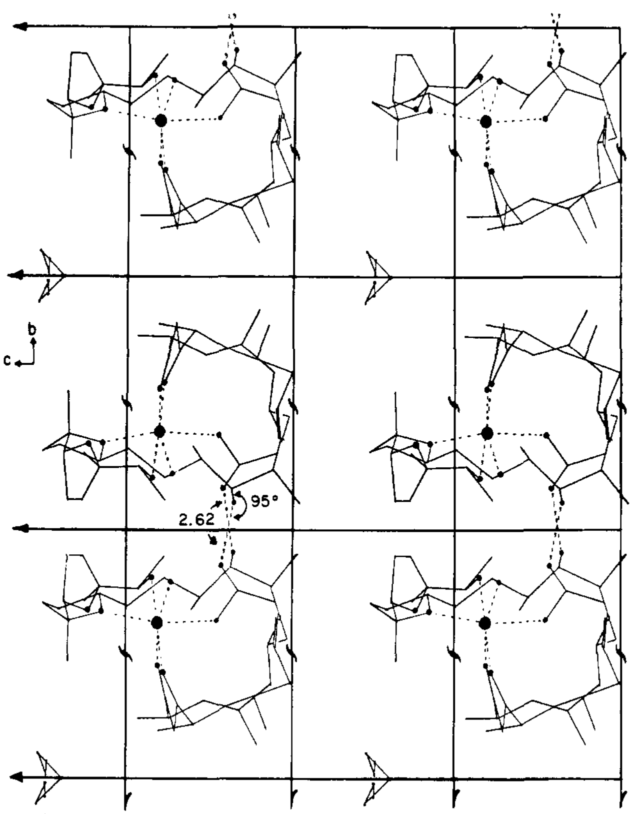

Figure 7. The molecular arrangement of the complexed ionophore in the orthorhombic crystal structure, viewed along *a*. Two hydrogen bonds across a twofold axis parallel to *c* join the molecules in pairs so as to conceal the hydrophilic molecular surface containing several of the oxygens (small black circles). The extended chain axis of the heptane solvent molecule, the central atom of which occupies a special position on the twofold axis, is approximately perpendicular to the drawing.

coupling measurements showed that the latter is coupled to the lowest field methine hydrogen (δ 4.6). There also appeared to be five other secondary methyl groups in the region δ 0.8-1.00 ppm (15 H), whereas the rest of the protons gave signals that primarily fell into two groups: δ 1.4-2.0 (ca. 14 H) and 2.0-2.7 ppm (ca. 11 H).

The structural features derived from the NMR and mass spectra are partially summarized in Figure 6.

Initial X-ray studies were focused on the monoclinic hexane-solvated cadmium salt containing two ionophores per asymmetric unit (Table II). The positions of the cadmium ions

Table V. Fractional Atomic Coordinates and Errors of the Orthorhombic Cadmium Complex of **1**

atom no.	*x*	*y*	*z*	atom no.	*x*	*y*	*z*
C1	−0.081 (2)	−0.128 (2)	−0.337 (4)	C29	−0.141 (2)	−0.056 (2)	0.139 (4)
C2	−0.089 (2)	−0.151 (2)	−0.457 (3)	C30	−0.161 (2)	−0.124 (2)	0.080 (4)
C3	−0.117 (2)	−0.223 (2)	−0.487 (3)	C31	−0.174 (2)	−0.123 (2)	−0.037 (4)
C4	−0.176 (2)	−0.234 (2)	−0.436 (3)	C32	−0.213 (2)	−0.070 (2)	−0.095 (4)
C5	−0.203 (2)	−0.310 (2)	−0.438 (3)	C33	−0.218 (2)	−0.158 (2)	0.132 (3)
C6	−0.167 (2)	−0.362 (2)	−0.348 (4)	C34	−0.020 (2)	−0.273 (2)	0.182 (4)
C7	−0.198 (2)	−0.351 (2)	−0.239 (3)	C35	0.195 (2)	−0.163 (2)	−0.196 (3)
C8	−0.163 (2)	−0.386 (2)	−0.138 (3)	C36	0.139 (2)	−0.053 (2)	−0.519 (3)
C9	−0.103 (2)	−0.347 (2)	−0.125 (3)	C37	0.008 (2)	−0.406 (2)	−0.437 (3)
C10	−0.048 (2)	−0.378 (2)	−0.151 (3)	C38	0.103 (2)	−0.406 (2)	−0.107 (4)
C11	0.009 (2)	−0.353 (2)	−0.157 (3)	C39	−0.202 (2)	−0.385 (2)	−0.030 (4)
C12	0.061 (2)	−0.396 (2)	−0.206 (3)	C40	−0.167 (2)	−0.440 (2)	−0.401 (4)
C13	0.091 (2)	−0.359 (2)	−0.303 (3)	C41	−0.226 (2)	−0.173 (2)	−0.472 (4)
C14	0.050 (2)	−0.338 (2)	−0.387 (3)	O1	−0.096 (1)	−0.081 (1)	−0.292 (2)
C15	0.076 (2)	−0.310 (2)	−0.500 (3)	O2	−0.056 (1)	−0.186 (1)	−0.282 (1)
C16	0.121 (2)	−0.238 (3)	−0.461 (4)	O3	−0.109 (1)	−0.281 (1)	−0.092 (2)
C17	0.095 (2)	−0.181 (2)	−0.462 (3)	O4	0.023 (1)	−0.288 (1)	−0.118 (2)
C18	0.137 (2)	−0.116 (2)	−0.434 (3)	O5	0.065 (1)	−0.052 (1)	−0.322 (2)
C19	0.124 (2)	−0.080 (2)	−0.318 (3)	O6	0.034 (1)	−0.104 (1)	−0.132 (1)
C20	0.125 (2)	−0.136 (2)	−0.221 (3)	O7	0.010 (1)	−0.169 (1)	0.075 (1)
C21	0.098 (2)	−0.104 (2)	−0.118 (3)	O8	−0.115 (1)	−0.172 (1)	0.113 (2)
C22	0.107 (2)	−0.158 (2)	−0.005 (3)	O9	−0.111 (1)	−0.100 (1)	−0.073 (2)
C23	0.067 (2)	−0.129 (2)	0.083 (4)	Cd	−0.0393 (1)	−0.1943 (1)	−0.0952 (2)
C24	0.087 (2)	−0.165 (2)	0.205 (4)	C1[a]	−0.500	0.000	−0.193
C25	0.030 (2)	−0.154 (2)	0.256 (3)	C2	−0.403	−0.011	−0.238
C26	−0.024 (2)	−0.187 (3)	0.181 (3)	C3	−0.461	−0.054	−0.262
C27	−0.074 (2)	−0.148 (2)	0.196 (3)	C4	−0.342	−0.036	−0.243
C28	−0.076 (2)	−0.068 (2)	0.177 (3)				

[a] The following four atoms are due to heptane.

Table VI. Geometries of Metal-Oxygen Coordination and Hydrogen Bonding in the Orthorhombic and Monoclinic Crystal Structures[a]

coordination bond	distance, Å				hydrogen bond	distance, Å			
	Ca_o	Cd_o	Cd_m	Cd_m		Ca_o	Cd_o	Cd_m	Cd_m
O2-M	2.28	2.30	2.4	2.3	O1—O9	2.66	2.69	2.5	2.3
O3-M	2.28	2.25	2.2	2.1	O6—O5	2.70	2.60	2.8	2.3
O4-M	2.26	2.25	2.4	2.3	O5—O1[b]	2.63	2.63	2.6	2.7
O6-M	2.44	2.38	2.1	2.5					
O7-M	2.45	2.38	2.4	2.7					
O8-M	2.82	3.06	3.2	3.1					
O9-M	2.44	2.40	2.5	2.3					
	angle, deg					angle, deg			
	Ca_o	Cd_o	Cd_m	Cd_m		Ca_o	Cd_o	Cd_m	Cd_m
O2-M-O3	91	87	87	76	O7-M-O6	73	73	71	82
O2-M-O7	152	156	162	162	O7-M-O4	88	89	87	90
O2-M-O6	80	83	91	80	O7-M-O9	96	93	96	100
O2-M-O4	95	92	93	86	O6-M-O4	94	97	97	86
O2-M-O9	83	87	85	81	O6-M-O9	91	87	88	83
O3-M-O7	117	117	112	121	O4-M-O9	174	176	175	163
O3-M-O6	166	170	177	153	C19-O5···O1[b]	105	96	103	90
O3-M-O4	77	81	84	80					
O3-M-O9	98	95	91	105					

[a] The subscript o refers to the orthorhombic crystal form. The subscript m refers to the two independent complexes in the asymmetric unit of the monoclinic form. [b] Intermolecular hydrogen bond.

were determined from a Patterson map and used to calculate a difference Fourier map ($R = 0.32$) that revealed the positions in both molecules of the nine oxygen atoms connected to a chain of atoms folded about the cation. The pseudo-twofold relationship of the two molecules about an axis parallel to a at $(y, z) = (0.4, 0.8)$, which became apparent at this stage, facilitated the interpretation of the subsequent cycles of structure factor-Fourier calculations that led to the molecular structure assignment, **1**. Although all of the 102 nonhydrogen atoms of the two independent ionophores were identified, no attempt was made to define the atomic positions of the hexane of crystallization which appeared as diffuse peaks between ionophores. This relatively large crystal structure was not refined completely; instead, all subsequent studies were focused on the orthorhombic structure of the isomorphous calcium and cadmium salts.

Patterson and difference Fourier analysis, simplified considerably through the assumption that the orthorhombic space group twofold axis corresponded to the pseudo-twofold axis of the monoclinic form, revealed all atoms of the ionophore, **1**, and a heptane molecule of crystallization. The central carbon atom of the latter lies on a twofold axis and accordingly there are only two heptane molecules with the four ionophores per unit cell. This hemiheptane solvate structure is consistent with the molecular weight of 796 $[C_{41}H_{70}O_9Ca + \frac{1}{2}(C_7H_{16})]$ calculated for the asymmetric unit from the measured crystal density and unit cell volume, as well as with the 6.1% weight loss observed at 100–130 °C during thermal gravimetric analysis (calcd, 5.8%).

The partially refined ($R = 0.18$) atomic coordinates from the orthorhombic structure of the calcium salt (Table III) were used as a basis for the more extensive refinements carried out with the larger intensity data set from the isomorphous cadmium salt. Several cycles of isotropic least-squares refinements (anisotropic Cd and O atoms) reduced the initial R factor of 0.22 to 0.11, at which point the anomalous components of the Cd scattering were introduced into the calculations. The absolute configuration shown in Figure 1 gave $R = 0.11$ directly and $R = 0.10$ after further least-squares refinements of coordinates and temperature factors; the same calculations for the enantiomorphic structure gave $R = 0.12$ directly and $R = 0.11$ after refinement. Measured differences in the intensities of several Friedel pairs of reflections that were expected to be sensitive to the choice of enantiomorph (Table IV) confirmed the absolute configuration shown for **1**. A difference map based on the final atomic coordinates for the Cd salt (Table V) showed no residuals greater than the $\sim$2.0 e/Å^3 peaks in the vicinity of the metal which probably are due to the neglect of absorption corrections. The only other appreciable residuals ($\sim$1.0 e/Å^3) occurred very near the positions of the heptane carbon atoms, none of which had been refined.

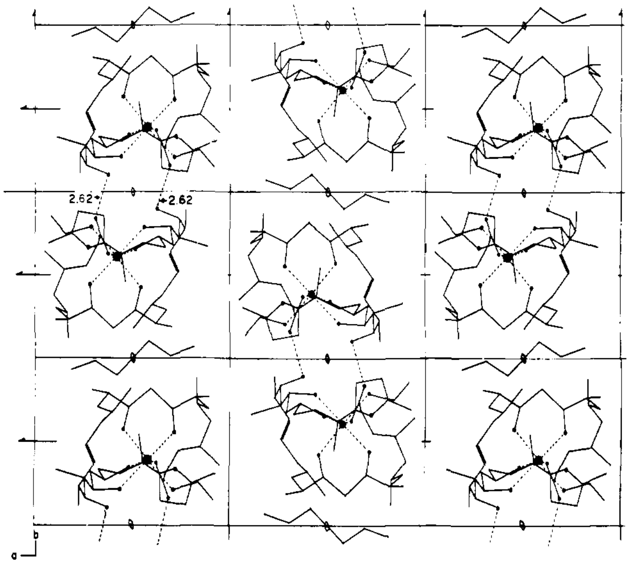

Figure 8. The molecular arrangement of the complexed ionophore in the orthorhombic crystal structure, viewed down the space group twofold axis which is parallel to c. The central atom of the heptane solvent of crystallization occupies a special position on the twofold axis (see Figure 9).

The mode of metal-oxygen coordination bonding is identical in the four independently observed solid-state conformations of salts of **1**: one of the carboxylate oxygen atoms (O2), both oxygens (O3, O4) of the cisoid enolized β-diketone group, a cyclic oxygen (O7), and two hydroxyl oxygens (O6, O9) are octahedrally coordinated to the metal. The metal-oxygen distances (for calcium, average = 2.27 Å for the three anionic oxygens and 2.44 Å for the other three coordinated oxygens;

Table VII. Bond Distances and Angles in the Orthorhombic Calcium and Cadmium Complexes

	distance, Å			distance, Å			distance, Å			distance, Å	
bond	Ca	Cd	bond	Ca	Cd	bond	Ca	Cd	bond	Ca	Cd
C1-C2	1.70 (8)	1.53 (6)	C20-C35	1.67 (9)	1.66 (5)	C11-C12	1.68 (8)	1.53 (5)	C31-C32	1.32 (10)	1.51 (6)
C2-C3	1.46 (8)	1.52 (5)	C21-C22	1.70 (8)	1.72 (5)	C12-C13	1.59 (8)	1.51 (5)	O1-C1	1.04 (6)	1.08 (5)
C3-C4	1.40 (9)	1.47 (6)	C22-C23	1.38 (9)	1.50 (6)	C12-C38	1.51 (8)	1.52 (6)	O2-C1	1.41 (6)	1.40 (5)
C4-C5	1.53 (8)	1.54 (6)	C23-C24	1.65 (9)	1.69 (7)	C13-C14	1.75 (8)	1.41 (5)	O3-C9	1.38 (7)	1.32 (4)
C4-C41	1.51 (7)	1.66 (6)	C24-C25	1.33 (10)	1.43 (6)	C14-C15	1.39 (8)	1.56 (5)	O4-C11	1.29 (6)	1.36 (4)
C5-C6	1.70 (9)	1.67 (6)	C25-C26	1.52 (11)	1.62 (6)	C14-C37	1.62 (8)	1.71 (5)	O5-C19	1.37 (6)	1.38 (4)
C6-C7	1.63 (9)	1.50 (6)	C26-C27	1.28 (11)	1.35 (6)	C15-C16	1.44 (8)	1.75 (6)	O6-C21	1.46 (6)	1.44 (4)
C6-C40	1.42 (8)	1.59 (6)	C26-C34	1.59 (11)	1.61 (7)	C16-C17	1.14 (8)	1.21 (6)	O7-C23	1.47 (7)	1.46 (4)
C7-C8	1.59 (8)	1.59 (6)	C27-C28	1.63 (10)	1.53 (5)	C17-C18	1.38 (8)	1.58 (6)	O7-C26	1.40 (9)	1.54 (5)
C8-C9	1.36 (9)	1.51 (6)	C28-C29	1.30 (10)	1.53 (6)	C18-C19	1.73 (9)	1.59 (5)	O8-C27	1.23 (9)	1.43 (4)
C8-C39	1.38 (9)	1.56 (6)	C29-C30	1.64 (9)	1.54 (6)	C18-C36	1.48 (9)	1.57 (6)	O8-C30	1.35 (8)	1.42 (5)
C9-C10	1.49 (9)	1.39 (6)	C30-C31	1.51 (11)	1.44 (6)	C19-C20	1.38 (8)	1.58 (5)	O9-C31	1.41 (9)	1.52 (5)
C10-C11	1.45 (8)	1.34 (6)	C30-C33	1.34 (10)	1.55 (6)	C20-C21	1.57 (9)	1.50 (5)			

	angle, deg			angle, deg			angle, deg			angle, deg	
	Ca	Cd		Ca	Cd		Ca	Cd		Ca	Cd
O1-C1-O2	130 (5)	120 (3)	C17-C18-C36	125 (6)	117 (3)	C10-C11-C12	116 (4)	124 (4)	C25-C26-C34	113 (5)	110 (3)
O1-C1-C2	132 (6)	134 (4)	C19-C18-C36	107 (4)	105 (3)	C10-C11-O4	124 (4)	120 (3)	C27-C26-O7	103 (6)	113 (3)
O2-C1-C2	98 (3)	105 (2)	C18-C19-C20	112 (5)	112 (3)	C12-C11-O4	116 (4)	116 (2)	C27-C26-C34	111 (6)	126 (4)
C1-C2-C3	117 (5)	122 (4)	C18-C19-O5	98 (3)	108 (2)	C11-C12-C13	106 (4)	113 (3)	C34-C26-O7	94 (5)	102 (2)
C2-C3-C4	121 (4)	113 (3)	C20-C19-O5	119 (4)	107 (2)	C11-C12-C38	114 (4)	103 (3)	C26-C27-C28	114 (6)	123 (4)
C3-C4-C5	122 (6)	118 (3)	C19-C20-C21	103 (4)	110 (3)	C13-C12-C38	117 (5)	114 (3)	C26-C27-O8	131 (8)	105 (3)
C3-C4-C41	110 (5)	112 (3)	C19-C20-C35	111 (4)	111 (2)	C12-C13-C14	117 (4)	115 (3)	C28-C27-O8	101 (5)	101 (2)
C5-C4-C41	105 (4)	113 (3)	C21-C20-C35	103 (4)	109 (3)	C13-C14-C15	105 (4)	120 (3)	C27-C28-C29	100 (5)	103 (3)
C4-C5-C6	110 (4)	111 (3)	C20-C21-C22	108 (4)	112 (3)	C13-C14-C37	99 (3)	113 (3)	C27-O8-C30	124 (8)	117 (3)
C5-C6-C7	103 (4)	106 (3)	C20-C21-O6	104 (4)	107 (2)	C15-C14-C37	118 (5)	98 (3)	C28-C29-C30	113 (5)	106 (3)
C5-C6-C40	110 (4)	107 (3)	C22-C21 O6	97 (3)	101 (2)	C14-C15-C16	134 (7)	104 (3)	C29-C30-C31	100 (5)	120 (4)
C7-C6-C40	125 (5)	120 (3)	C21-C22-C23	115 (5)	107 (3)	C15-C16-C17	133 (7)	115 (4)	C29-C30-O8	93 (4)	101 (3)
C6-C7-C8	102 (4)	113 (3)	C22-C23-C24	118 (5)	109 (3)	C16-C17-C18	133 (8)	113 (4)	C29-C30-C33	118 (6)	113 (3)
C7-C8-C9	108 (5)	108 (3)	C22-C23-O7	124 (5)	105 (2)	C17-C18-C19	107 (4)	114 (3)	O8-C30-C31	114 (5)	115 (3)
C7-C8-C39	104 (4)	112 (3)	C24-C23-O7	99 (4)	95 (3)				O8-C30-C33	110 (5)	101 (3)
C9-C8-C39	104 (5)	113 (3)	C23-O7-C26	121 (7)	118 (4)				C31-C30-C33	118 (7)	105 (3)
C8-C9-C10	122 (6)	123 (4)	C23-C24-C25	92 (5)	95 (3)				C30-C31-C32	135 (9)	125 (4)
C8-C9-O3	129 (5)	114 (3)	C24-C25-C26	125 (7)	111 (3)				O9-C31-C30	111 (4)	96 (3)
C10-C9-O3	109 (4)	123 (3)	C25-C26-C27	134 (10)	109 (3)				C32-C31-O9	111 (5)	102 (3)
C9-C10-C11	131 (7)	134 (5)	C25-C26-O7	89 (5)	91 (3)						

for cadmium, 2.27 and 2.39 Å, respectively) and angles (Table VI) are very similar in all of these complexes.

The other cyclic oxygen, O8, is more than 2.8 Å from the metal, while the last two oxygens (O1 and O5), which are further than 3.75 Å from the metal, are joined by an *inter*molecular hydrogen bond (2.6 Å) between molecules related through a space group (or pseudo-) twofold axis. The two hydroxyls coordinated to the metal probably are also *intra*molecularly hydrogen bonded: O6- - -O5 and O9- - -O1. The folding of the long-chain structure **1** about the cation is strikingly evident in the fact that the last-mentioned hydrogen bond occurs between atoms at the extreme opposite ends of the molecule—a feature commonly found in the crystal structures of other members of this class of antibiotics.[2,5]

These and other comparisons indicate that there are no significant differences in the molecular conformations in all forms of **1** that have been examined. The calculated bond distances and angles for the orthorhombic structures are given in Table VII. Considerations of the dihedral angles about C-C bonds served uniquely to locate the trans disubstituted double bond between atoms C16 and C17 (dihedral angle C15-C16-C17-C18 = 171°). The seven atoms of the enolized β-diketone group, C8-C12, O3, and O4, are planar within experimental error (av dev = 0.05 Å; max dev = 0.13 Å).

Table VIII. ^{1}H NMR Spectra of Ionomycin^a

chemical shift, δ calcium salt^{b,c}	carbon number	proton type	chemical shift, δ cadmium salt^{b,d}
0.77 (d, 7) 0.86 (d, 6.1) 0.88 (d, 6.1) 0.94 (d, 6.1) 0.94 (d, 6.1) 0.97 (d, 6.1) 1.07 (d, 6.9)	35-41	$-CH_3$	0.76 (d, 6.7) 0.86 (d, ca. 6.3) 0.88 (d, ca. 6.3) 0.94 (d, 6.3) 0.95 (d, 6.3) 0.99 (d, 6.3) 1.05 (d, 6.3)
1.13 (s) 1.13 (s)	33, 34	-CH3	1.10 (s) 1.16 (s)
1.22 (d, 6.4)	32	-CH3	1.22 (d, 6.3)
3.3 (d, 9)	27	-CHO	3.28 (d, 10)
3.87 (J_T = 32)	19	-CHO	3.85 (t, 8)
3.87 (J_T = 32)	23	-CHO	3.94 (d, t, 5, 10)
4.18 (w_H = 23)	21	-CHO	4.34 (w_H = 27)
4.6 (q, 6.4)	31	-CHO	4.64 (q, 6.3)
5.14 (s)	10	-CH=	5.07 (s)
5.29 (d, d, 15, 9)	17	-CH=	5.33 (d, d, 15, 10)
5.66 (d, t, 15, ca. 7)	16	-CH=	5.68 (d, t, 15, 6.7)

[a] At 270 MHz in $CDCl_3$ containing Me_4Si. [b] Parts per million downfield from Me_4Si; apparent coupling constants in hertz. s = singlet, d = doublet, q = quartet, w_H = width of peak at half-height, and J_T = total width of coupled proton. [c] Approximately 67–70 protons were detected. Besides those listed, the following regions of the spectrum contained the additional number of protons specified; δ 0.6–1.4 (4 H), 1.4–2.0 (14 H), 2.0–2.7 (11 H). Three exchangeable protons were not detected. [d] Approximately 67–70 protons were detected. Besides those listed, the following regions of the spectrum contained the additional number of protons specified: δ 0.6–1.1 (5 H), 1.3–1.7 (4 H), 1.7–2.0 (9 H), 2.0–2.6 (10 H). One of the three exchangeable protons was found at δ 6.83.

Table IX. ^{13}C NMR Spectrum of Ionomycin, Ca Salt^a

chemical shift, δ^b	carbon number	carbon type	calcd chemical shift, δ^c
12.5 (q)	35	$-CH_3$	11
18.8, 19.6, 19.6, 20.2 21.4, 21.4 22.2, 24.0, 26.7		$-CH_3$	19 (37), 19 (41), 20 (40), 18 (38) 18 (39), 17 (33), 17 (34), 19 (32), 16 (36)
27.0 (d), 28.2 (d), 29.0 (d)		-CH-	26 (6), 27 (4), 29 (14)
29.6, 33.1 (t), 33.2 34.1 36.8 (t), 34.8, 40.1, 40.3, 40.9, 42.2, 42.4, 42.6 43.6 (t), 47.8 (t)		-CH- and/or $-CH_2-$	32 (2), 33 (22), 36 (3), 37 (15), 38 (20), 40 (8), 40 (12), 40 (13), 42 (7), 43 (5), 43 (18)
71.0 (d)	21	-CHOH	68
78.1 (d), 82.6 (d) 84.1 (d), 84.5	19, 23, 27, 31	-CHO	75 (31), 76 (19)
86.1 (s), 89.0 (s)	26, 30	-CO-	
102.5 (s)	10	-CH=	
133.4 (d), 134.2 (d)	16, 17	-CH=	
186.1 (s)	1	$-CO_2^-$	
197.5 (s), 198.7 (s)	9, 11	-C=O and -OC=	

[a] At 25.15 MHz in $CDCl_3$ containing Me_4Si. [b] Parts per million downfield from Me_4Si (multiplicity in off-resonance proton decoupled spectrum). [c] For carbon indicated in parentheses; calculated chemical shifts for aliphatic carbons are based on additive influences of neighboring carbons (ref 9 and 10). Tentative assignments and groupings are based on proximity to these calculated values.

The packing arrangement of the approximately spherical complexes (spherical radius ≃ 7 Å) in the orthorhombic structure is shown in Figures 7 and 8. The complexes are joined in pairs through the *inter*molecular O5- - -O1 hydrogen bond so as to "cover" the only hydrophilic surface of the ionophores. The exposed surfaces of the resulting "dimeric" structure therefore are primarily aliphatic in nature. The heptane molecules of crystallization are packed on twofold axes in spaces between these lipophilic "dimers".

The packing of the same lipophilic "dimeric" structure

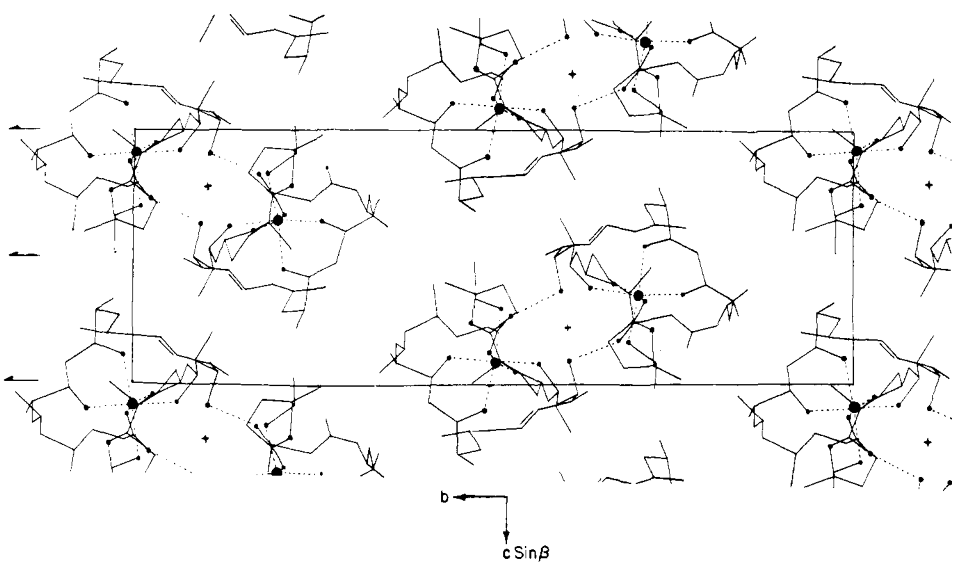

Figure 9. The molecular arrangement of the complexed ionophore as the Cd salt hexane solvate in the monoclinic crystal, viewed down the *a* axis. The two molecules of the asymmetric unit are joined through two hydrogen bonds about a pseudo-twofold axis normal to the drawing, and passing through the cross (+). .Virtually the same "dimeric" lipophilic structure is evident about the space group twofold axis in the orthorhombic structure (Figure 8).

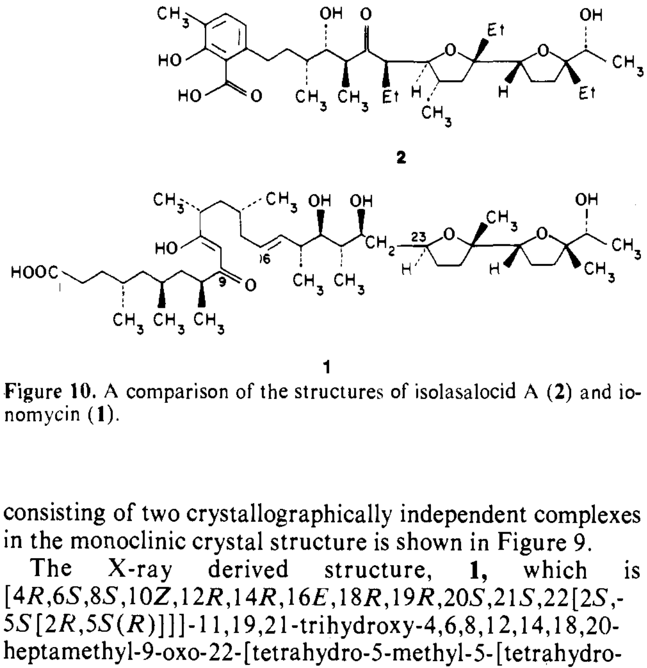

Figure 10. A comparison of the structures of isolasalocid A (**2**) and ionomycin (**1**).

consisting of two crystallographically independent complexes in the monoclinic crystal structure is shown in Figure 9.

The X-ray derived structure, **1**, which is [4*R*,6*S*,8*S*,10*Z*,12*R*,14*R*,16*E*,18*R*,19*R*,20*S*,21*S*,22[2*S*,5*S*[2*R*,5*S*(*R*)]]]-11,19,21-trihydroxy-4,6,8,12,14,18,20-heptamethyl-9-oxo-22-[tetrahydro-5-methyl-5-[tetrahydro-5-(1-hydroxyethyl)-5-methyl-2-furanyl]-2-furanyl]-10,16-docosadienoic acid, contains all of the structural features anticipated from the spectroscopic data (Figure 6). Tables VIII and IX present an interpretation of the ^{1}H and ^{13}C magnetic resonance spectra based on structure **1**.

The structure **1** determined for ionomycin is novel among the polyether antibiotics in that it contains two anionic functions as discussed previously. However, ionomycin bears several distinct similarities to the lasalocids. Ionomycin and the lasalocids have a higher ratio of carbon to oxygen than the other known polyethers, and neither contains ketal or hemiketal groups that are otherwise a general feature of this class of antibiotic. Starting with carbon 9 of ionomycin, the oxygenation pattern is identical with that in the lasalocids, and the alkylation pattern is very similar. The most striking similarity is with isolasalocid A,[6] **2**, in that the absolute configurations of the five asymmetric centers of **1** from C23 to the end are the same as those assigned for the corresponding asymmetric centers of **2** (Figure 10).

It is of interest that lasalocid also forms a "dimeric" structure in which the hydrophilic surfaces of two monomers face each other. These "dimers", which persist in nonpolar but not polar solvents, are unsymmetrical in all cases studied,[5] whereas in **1** the dimers in the two independent crystals have twofold symmetry. The subtle packing effect manifest in the change from an *orthorhombic*, crystallographically symmetric, *heptane*-solvated "dimeric" structure to a *monoclinic, hexane*-solvated "dimeric" structure for salts of **1** is under further investigation.

Experimental Section

Hemiheptane Solvate of Ionomycin Ca Salt. The calcium salt of ionomycin (20 mg) was dissolved in 0.4 mL of *n*-heptane and 2 drops of CH_2Cl_2. The solution was heated in order to drive off the CH_2Cl_2 and crystallization was allowed to proceed through slow evaporation of solvent at room temperature for 2 days.

Cd Salt of Ionomycin. The calcium salt of ionomycin (0.2 g) was dissolved in 8 mL of 10% $Cd(OAc)_2 \cdot 2H_2O$ in methanol. After 30 min, 20 mL of water was added and the mixture was extracted with CH_2Cl_2. The extract was concentrated in vacuo and the residue retreated twice with $Cd(OAc)_2$ solution as above. The resulting material was recrystallized from CH_2Cl_2-*n*-hexane, giving the monoclinic hemihexane solvate. The mass spectrum showed a molecular ion at *m/z* 820 ($C_{41}H_{70}O_9{}^{114}Cd$); no *m/z* 746 (Ca salt) was detected. Owing to instability of the solvate, satisfactory elemental analysis was not obtained. However, the presence of hexane was suggested by the excessive molecular weight of the asymmetric unit (1742) derived from unit cell volume and crystal density measurements (1637 calcd for $C_{82}H_{140}O_{18}Cd_2$). Crystals of the orthorhombic hemiheptane solvate were obtained by recrystallization from CH_2Cl_2-*n*-heptane.

X-ray Analysis. All unit cell parameters and intensities were measured diffractometrically using monochromatic Cu Kα radiation (λ = 1.542 Å). All intensities were measured using the θ-2θ variable scan rate technique. Background counts, B_1, B_2 were measured for $^1/_{20}$ the scan time at each end of the scan range. Intensities were converted to F^2 without correction for absorption. Standard deviations were assigned according to $\sigma(I) = r(C + 100(B_1 + B_2))^{1/2}$ where C is the total reflection count and r is the scan rate. Intensities, I, were considered as "observed" if $I \geq 3\sigma(I)$. For the monoclinic cadmium structure, 3106 independent reflections were measured out to $2\theta = 90°$. Of these, the 1998 "observed" reflections were used to solve the structure. Partial refinement by Fourier and isotropic least-squares methods (anisotropic Cd) based on the 1078 most intense reflections was terminated at $R = 0.12$.

The orthorhombic crystals are less stable and tend to desolvate and crack upon standing. Specimens used for data collection were coated with an epoxy cement. Even so, of the 2873 reflections measured out to $2\theta = 100°$ for the calcium salt, only 1226 were "observed." In view of the limited data, refinements were terminated ($R = 0.18$) after a few cycles of isotropic least-squares analysis (anisotropic Ca and O atoms). Somewhat larger crystals ($0.3 \times 0.34 \times 0.18$ mm) were obtained for the similarly unstable orthorhombic Cd salt. Of the 3481 reflections measured out to $2\theta = 115°$, 2554 were "observed." Only the 1729 most intense reflections were used for least-squares refinements. Program limitations also precluded simultaneous refinement of all atomic parameters in any least-squares cycle. At early stages of refinement only the coordinates were refined; thereafter the coordinates and temperatures factors of different halves of the molecule alternately were refined, then fixed during consecutive cycles.

The R factor mentioned above and throughout the text is the conventional index $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. The quantity minimized during least-squares analysis was $\Sigma w(|F_o|^2 - |F_c|^2)^2$ with $w = 1/\sigma^2$. Atomic scattering factors, including the anomalous components for cadmium, were taken from the International Tables for X-ray Crystallography.[7]

Spectroscopy. The 70-eV electron-impact low-resolution mass spectra were obtained on an AEI MS-902 double-focusing mass spectrometer using a direct insertion solid probe with a source temperature ~160 °C above ambient. Data were acquired via frequency-modulated analog tape, which was subsequently processed on a PDP-11 computer using published programs.[8] Manual peak matching of masses was done with the wide-range accessory of the MS-902.

^{1}H NMR spectra were measured on a Varian Associates XL-100-15 100-MHz spectrometer. Dr. G. Gray of Varian Associates obtained the 25.16-MHz proton-decoupled and off-resonance ^{13}C NMR spectra on a XL-100-12 Fourier transform spectrometer. We are grateful to Professor Joseph Fried of the University of Chicago for obtaining the 270-MHz ^{1}H NMR spectrum.

Supplementary Material Available: Tables of atomic coordinates for the Cd monoclinic crystal structure, temperature factors, and observed and calculated structure factors for each of the three crystal structures (49 pages). Ordering information is given on any current masthead page.

References and Notes

(1) (a) The Squibb Institute for Medical Research; (b) University of Minnesota.
(2) This group of antibiotics has recently been reviewed by Westley: J. W. Westley, *Adv. Appl. Microbiol.*, **22**, 177–223 (1977).
(3) C.-M. Liu and T. E. Hermann, *J. Biol. Chem.*, **253**, 5892 (1978).
(4) W.-C. Liu, D. S. Slusarchyk, G. Astle, W. H. Trejo, W. E. Brown, and E. Meyers, *J. Antibiot.*, **31**, 815 (1978).
(5) C. C. Chiang and I. C. Paul, *Science*, **196**, 1441 (1977).
(6) J. W. Westley, J. F. Blount, R. H. Evans, Jr., A. Stempel, and J. Berger, *J. Antibiot.*, **27**, 597 (1974).
(7) "International Tables for X-ray Crystallography", Vol. III, Kynoch Press, Birmingham, England, 1968.
(8) P. J. Black and A. I. Cohen, paper presented at the Twentieth Annual Conference on Mass Spectrometry and Allied Topics, Dallas, Texas, June 4, 1972.
(9) D. M. Grant and E. G. Paul, *J. Am. Chem. Soc.*, **86**, 2984 (1964).
(10) G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists", Wiley, New York, 1972, p 47.

A Novel Zwitterionic Structure and an Unusual Sugar Ring Conformation in 5-Iodo-5′-amino-2′,5′-dideoxyuridine, an Antiviral Nucleoside[1]

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Abstract: The three-dimensional structure of 5-iodo-5′-amino-2′,5′-dideoxyuridine, a potent inhibitor of herpes simplex virus, was determined by X-ray crystallography. The crystals belong to the orthorhombic space group $P2_12_12_1$ and the cell dimensions are $a = 7.892$ (1), $b = 9.332$ (1), and $c = 15.749$ (2) Å. Intensity data were measured with a diffractometer and the structure was solved by the heavy-atom method. Least-squares refinement, which included hydrogen atoms, converged at $R = 0.047$. The structure is zwitterionic, with a protonated 5′-NH_2 group and a negative charge on N(3) in the pyrimidine ring. The glycosyl bond is in the anti conformation ($\chi_{CN} = 53.6°$) and the exocyclic -$CH_2NH_3^+$ group is gauche-trans. The deoxyribose ring has the unusual O(1′) endo pucker. ^{1}H NMR spectroscopy was used to determine the conformation in solution. The spectra indicate an anti conformation about the glycosyl bond and equal contributions of the three staggered side-chain rotamers. The sugar ring may consist of a 36:64 equilibrium mixture of ^3E and ^2E conformers. It is pointed out, however, that the conventional interpretation may be inadequate, and that there may be a significant contribution of an O(1′) endo puckered ring.

Modification of the structure of nucleosides has produced anticancer, antiviral, and antibacterial agents.[3a–e] Examples of alterations of the base moiety include 5-fluoro-2′-deoxyuridine, 5-iodo-2′-deoxyuridine, 5-ethyl-2′-deoxyuridine, 5-(2-iodovinyl)-2′-deoxyuridine, 6-azauridine, and 5-azacytidine, whereas alteration of the pentose moiety has produced compounds such as 1-β-D-arabinofuranosylcytosine, 9-β-D-arabinofuranosyladenine, 1-β-arabinofuranosylthymine, (*S*)-9-(2,3-dihydroxypropyl)adenine ((*S*)-DHPA), and 9-(2-hydroxyethoxymethyl)guanine (acycloguanosine).

Nucleosides in which a sugar hydroxyl had been replaced by an amino group had been shown to possess biological activity.[4] 5′-Amino-5′-deoxythymidine and 3′-amino-3′-deoxythymidine were found to have antiviral and antineoplastic activity, respectively.[5] Since the thymidine analogue 5-iodo-2′-deoxyuridine is a potent antiviral agent, the corresponding 5′-amino analogue of 5-iodo-2′-deoxyuridine (5-iodo-5′-amino-2′,5′-dideoxyuridine; AIU, AIdUrd) as well as a number of other 5- and 5′-substituted analogues were synthesized by Lin et al.,[5,6] and AIU was found to have good antiviral activity with an unusual lack of toxicity to mammalian cells and experimental animals. Therefore, it is of considerable interest to obtain details of the structure and conformation of AIU, both in the crystal as well as in solution.

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