160. *Researches on Polyenes. Part VIII.*[1] *The Structures of Lagosin and Filipin.*

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The antibiotic lagosin, formerly known as "antibiotic A 246," is shown to be the 1→27-lactone of all-*trans*-3,5,7,9,11,13,14,15,26,27-decahydroxy-2-1′-hydroxyhexyl-16-methyloctacosa-16,18,20,22,24-pentaenoic acid. This conclusion follows the isolation of every carbon atom in the molecule as the degradation products formic acid, acetaldehyde, 11-methyldodecanoic acid, and 2-hexyltridecanoic acid, the latter being identified by mass-spectrometry and confirmed by rational synthesis. Filipin is probably 14-deoxylagosin.

The antibiotic lagosin, formerly given the code name A 246, was obtained [2] from an isolate of *Streptomyces* species present in a sample of soil collected in Lagos, Nigeria. Its relation to other antibiotics, especially filipin and fungichromin, is discussed below.

Molecular Formula and Functional Groups.—Lagosin formed fine hair-like needles from aqueous methanol, decomp. at 230—240°, α_D^{20} −146° ± 5° (*c* 0·369 in MeOH). It showed absorption maxima typical of a pentaene (Fig. 1). Over palladium, platinum, or rhodium it absorbed hydrogen, giving a perhydro-derivative which largely (50—65%) crystallised on treatment with dioxan. The melting point of the perhydro-compound (originally [3] given at 156—157°, later obtained as high as 160—162°) is less reliable than its rotation, α_D^{20} +3·5° ± 1° (*c* 1·98 in MeOH). Treatment with hydrazine in ethanol gave a product which under 1000-power magnification appeared to be crystalline. With these two exceptions, not one derivative of lagosin containing more than half of the molecule has yet been obtained crystalline. Thus, the prognosis for a structural determination by *X*-ray analysis was unfavourable, as incorporation of a heavy atom in a macrocrystalline derivative would probably have proved impossible. Even an *X*-ray determination of molecular weight was mechanically impossible for the hydrazide and difficult, and very difficult, respectively, for perhydrolagosin and lagosin. As lagosin, now known to have ten hydroxyl groups, is non-volatile, mass-spectrography could make no direct contribution, and classical chemical degradation was the most promising method of structural elucidation.

The analytical values obtained for lagosin and perhydrolagosin could be fitted to one or two formulæ in each of four sets, corresponding to 12—15 oxygen atoms per molecule, so that the establishment of this number, *i.e.*, of the approximate molecular weight, was the first problem. (These limits were set by the observed absorbance value and the molecular extinction coefficient of model compounds, *e.g.*, dodeca-2,4,6,8,10-pentaene.[4]) The methods first used involved perhydrolagosin, which is less sensitive and insoluble than the antibiotic itself. From *X*-ray data [5] a value of 810 ± 15 was obtained. This was partially confirmed by the results of equivalent-weight determinations, which fell in the

[1] Part VII, Malhotra and Whiting, *J.*, 1960, 3812.
[2] Ball, Bessel, and Mortimer, *J. Gen. Microbiol.*, 1957, **17**, 96.
[3] Dhar, Thaller, and Whiting, *Proc. Chem. Soc.*, 1958, 148.
[4] Nayler and Whiting, *J.*, 1955, 3037.
[5] White and Hodgkin, personal communications.

range 760—850. These involved the lactone or ester function, evident from the intense band at 1710 and 1725 cm.$^{-1}$, respectively, in both lagosin and perhydrolagosin (Nujol). Direct titration with alkali at 85° in aqueous ethylene glycol gave the best potentiometric titration curves, but even these were unsharp and hard to interpret accurately. Back-titration of solutions in an excess of alkali gave very obscure and variable results. The *X*-ray result thus seemed the more accurate value, and led to formulæ based on O_{14}, *i.e.*, $C_{41}H_{76-80}O_{14}$ and $C_{41}H_{66-70}O_{14}$ for perhydrolagosin and lagosin, respectively, which were assumed during most of the work described below. Unfortunately these methods were affected by the same error; perhydrolagosin was later found to separate from dioxan with solvent of crystallisation which is largely lost under the fairly mild conditions of drying for analysis (60°/0·01 mm.) but largely retained under the milder drying conditions used for preparing the samples used above (25°/10 mm.). When chemical results inconsistent with the C_{41} formula had accumulated (see below), re-examination of the original *X*-ray photograph showed [5] the diffraction pattern corresponding to a second, smaller lattice, weakly superimposed on the main pattern. Other crystals of the perhydro-compound showed this smaller lattice as predominant, although no specimen could be obtained free from the larger pattern. As the density could be determined only for mixed samples, the derived molecular weights, 670 and 770, were only approximate, but the difference corresponded roughly to one molecule of dioxan. This was then shown to be present in a substantial part of the recrystallised perhydro-derivative (*a*) by measurement of the loss in weight when a sample which had been dried at room temperature was heated to 100°, and (*b*) by gas–liquid chromatography of a methanolic solution, when a peak corresponding to 0·62 mol. of dioxan was observed. From the lower molecular weight, a $C_{35}O_{12}$ formula could be deduced; and this was also proved independently and almost simultaneously by the identification of known degradation products.

Molecular weights of 702 and 714, both ±35, were obtained [6] by measuring the depression in vapour pressure of a methanolic solution. These at least served to eliminate the early O_{14} formulæ, although the interpretation of results obtained in this way on a dioxan solvate is not obvious.

The hydrogen uptake corresponded to about 1 mole/140 g. It was first necessary to decide how many equivalents were involved. As lagosin was undoubtedly a pentaene and perhydrolagosin was saturated to bromine and permanganate and showed very little absorption even near 2000 Å (Fig. 2), the minimum number was five. The spectrum of lagosin itself near 2000 Å (Fig. 2) suggested that no second chromophore, not even an isolated ethylenic linkage was present, and this became certain when the model substance, 2-methyldodeca-2,4,6,8,10-pentaene-1,12-diol, became available. Any tri- or tetra-substituted ethylenic grouping would have sharply increased end-absorption, while any mono- or di-substituted ethylenic bond could hardly have resisted hydrogenation. No reasonably reactive carbonyl or masked carbonyl group could be present in lagosin, as no condensation occurred when lagosin was heated with 2,4-dinitrophenylhydrazine in pyridine or perhydrolagosin was treated with an acidic solution of the reagent. An epoxide group labile to hydrogenation would also probably have been labile to acetic acid–acetic anhydride–pyridine, and this was ruled out by the acetoxyl determinations described below. Accordingly, five mol. were indeed absorbed in the reaction leading to the isolable perhydro-derivative; this meant that the observed uptake and the larger (~800) molecular weight for lagosin (*ca.* 9% high for 5 mol.) could be rationalised only on the assumption of partial hydrogenolysis of the allylic hydroxyl groups. This was not inconsistent with *ca.* 60% yields of a perhydro-derivative with the original number of hydroxyl groups, but the discrepancy was one of the factors throwing doubt on the larger molecular weight. The uptake is on the contrary 3—4% low on the basis of the lower molecular weight now accepted, perhaps because the specimens hydrogenated were not completely pure.

[6] Clark, personal communication.

The hydrazide from perhydrolagosin was originally prepared in order that its nitrogen content, accurately determined on a large specimen, should indicate the molecular weight; and this result did indeed lead at once to a set of O_{12} formulæ. Unfortunately these were not accepted at the time because of the extraordinarily poor crystalline form of the hydrazide, the fact that the C, H, O, and N percentages added up to only about 99%, and our fear that the reaction might be more complex than simple hydrazinolysis of a lactone.

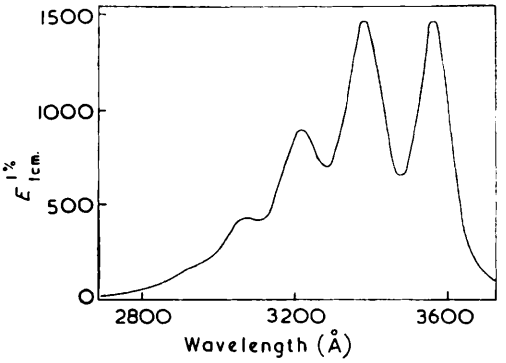


FIG. 1. Absorption spectrum of lagosin in alcohol.

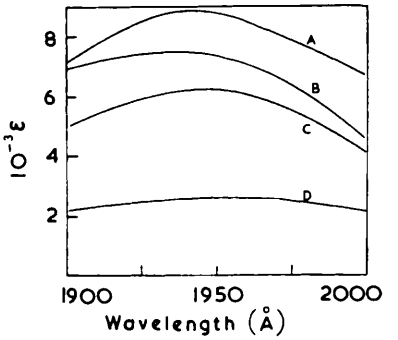


FIG. 2. Absorption spectra of lagosin derivatives in alcohol.

(A) Lagosin.
(B) Lagosin — perhydrolagosin.
(C) 2-Methyltrideca-2,4,6,8,10-pentaene-1,12-diol.
(D) Perhydrolagosin.

None of the other reactions of the functional groups present was suitable for equivalent-weight determination. Zeisel determinations were negative; Kuhn–Roth oxidation indicated about three *C*-methyl groups; Karl Fischer determinations indicated <0·5 mol. of water.

Once the molecular weight of 670 and the $C_{35}O_{12}$ formulation were established it became profitable to consider the analytical data in more detail. Only analyses in which all three elements were determined on the same sample at the same time (Table 1), with a

TABLE 1.

Combustion analyses (%) of lagosin derivatives.

	C	H	O	N	Total
Perhydrolagosin	61·68, 61·58, 61·50, 61·28	9·95, 9·95, 10·01, 10·00	28·08, 28·42, 28·06, 28·32	—	99·71, 99·95, 99·57, 99·60
Mean	61·51	9·98	28·22		99·71
$C_{35}H_{68}O_{12}$ requires	61·73	10·06	28·19		
$C_{35}H_{68}O_{12}.C_4H_8O_2$ requires	60·92	9·96	29·13		
$C_{35}H_{66}O_{12}$ requires	61·91	9·79	28·28		
$C_{35}H_{66}O_{12}.C_4H_8O_2$ requires	61·07	9·72	29·21		
Lagosin	62·20	8·79	28·88		99·87
$C_{35}H_{58}O_{12}$ requires	62·66	8·72	28·62		
$C_{35}H_{56}O_{12}$ requires	62·85	8·44	28·70		
Perhydrolagosinic hydrazide	58·49, 58·38	10·15, 10·17	26·47, 26·26	3·87, 3·95	99·00, 98·76
$C_{35}H_{72}O_{12}N_2$ requires	58·96	10·18	26·93	3·93	
$C_{35}H_{70}O_{12}N_2$ requires	59·13	9·93	27·01	3·94	

total of 99·5—100·0%, were considered, although the other analyses obtained were consistent with the deductions made. Most weight was given to the analyses of perhydrolagosin, although when solvation was known to interfere it was necessary to use dioxan content as a parameter.

It was concluded that $C_{35}H_{58}O_{12}$ and $C_{35}H_{68}O_{12}$ were very probably correct for lagosin and perhydrolagosin, respectively; $C_{35}H_{56}O_{12}$ and $C_{35}H_{66}O_{12}$, respectively, constituted a barely acceptable alternative, and other formulæ could be ruled out. If we assume one ester (or lactone) group, the former pair would imply a monocyclic, and the latter a bicyclic, framework. No simple alkoxyl group could be detected. To determine the number of hydroxyl groups, obviously large, lagosin (two samples) and perhydrolagosin were treated with pyridine and acetic anhydride at 20° for 48 hr., polyacetates being obtained. That from lagosin (one sample) was purified by counter-current distribution, then chromatography on alumina, and the other two samples were subjected simply to chromatography. All showed infrared spectra without detectable absorption at 3500—3650 cm.$^{-1}$, so it seemed unlikely that any unreactive, presumably tertiary, hydroxyl group was present. The results of acetoxyl semimicro-determinations are given in Table 2.

TABLE 2.

Acetoxyl determinations (%) for lagosin derivatives.

	Found *	Found †
Lagosin peracetate	36·47, 36·16, 36·84	
Mean	36·49	36·92
$C_{35}H_{48}O_2(OAc)_{10}$ requires		39·44
$C_{35}H_{47}O_3(OAc)_9$ requires		36·99
C_{34} dioxo-ester peracetate	30·18, 29·68	
Mean	29·93	30·28
$C_{34}H_{47}O_4(OAc)_7$ requires		32·29
$C_{34}H_{46}O_5(OAc)_6$ requires		29·04
Perhydrolagosin peracetate	35·89, 35·69	
Mean	35·79	36·21
$C_{35}H_{58}O_2(OAc)_{10}$		39·03
$C_{35}H_{57}O_3(OAc)_9$ requires		36·64
$C_{35}H_{58}O_2(OAc)_{10}$ (70%) + $C_{35}H_{57}O_2(OAc)_9$ (30%)		38·49
$C_{35}H_{57}O_3(OAc)_9$ (70%) + $C_{35}H_{56}O_3(OAc)_8$ (30%)		36·01

* Based on potassium hydrogen phthalate. † Based on glucose penta-acetate (mean of three determinations).

There are formally four possibilities, based on $C_{35}H_{58}O_{12}$ and $C_{35}H_{56}O_{12}$ for lagosin and on nine or ten hydroxyl groups present. Of these, $C_{35}H_{58}O_{12}$ with nine hydroxyls implies a monocyclic structure with one ether (not an ethoxyl or methoxyl group) and one ester group, biogenetically implausible and eliminated by chemical evidence discussed later. Similarly, $C_{35}H_{56}O_{12}$ with ten hydroxyl groups would imply a bicyclic structure, necessarily monocarbocyclic. This also seemed unlikely biogenetically, and is eliminated by later evidence. The real alternatives are therefore $C_{35}H_{48}O_2(OH)_{10}$ (monocyclic) and $C_{35}H_{47}O_3(OH)_9$ (bicyclic) for lagosin, corresponding to straight carbon chains with a lactonic ring, respectively without and with a cyclic ether bridge, and only these will be considered in interpreting the acetoxyl analyses.

At first sight the $C_{35}H_{47}O_3(OH)_9$ formula is favoured, but consideration of systematic errors reverses this preference. First, it seems reasonable that with the technique and apparatus used the recovery of acetic acid may have been only about 99%, which would explain the slightly low values obtained for glucose penta-acetate. Recalculation of the results with this penta-acetate (mean of three concordant analyses) as standard was therefore necessary. There would remain three possible errors, all leading to low results, namely, partial dehydration under the acetylation conditions, incomplete acetylation, and retention of solvent by the glassy polyacetates. The first could be estimated in the favourable case of perhydrolagosin in two different ways; hydrogenation over platinum in ethanol resulted in an uptake of 0·29 mol., while the absorption spectrum in the region 2700—2000 Å indicated small quantities of unsaturated material. It was necessary to subtract from the observed spectrum that of perhydrolagosin itself and that of ten mol. of isopropyl acetate; the excess of absorption could then be plausibly assigned to four

chromophoric systems, reasonable on the basis of structure (I), and with probable absorption coefficients such that the total unsaturation would amount roughly to 0·4 mol., in agreement with the hydrogenation value. Calculated values for a 30 : 70 mixture of $C_{35}H_{56}O_2(OAc)_8$ and $C_{35}H_{57}O_2(OAc)_9$, corresponding to $C_{35}H_{57}O_3(OH)_9$ for perhydrolagosin (bicyclic), now proved to be slightly lower than the found values, although a similar mixture of $C_{35}H_{57}O_2(OAc)_9$ and $C_{35}H_{58}O_2(OAc)_{10}$ (monocyclic) still required values appreciably higher than those found. For lagosin and the C_{34}-dioxo-ester (II) the results, even without a correction for dehydration, were similar to, or slightly higher than, those required by the bicyclic structures. It therefore seemed probable, on the basis of these acetyl values alone, that the monocyclic formula for lagosin, $C_{35}H_{48}O_2(OH)_{10}$, was correct. If, however, the results were held to be consistent with a bicyclic structure based on $C_{35}H_{47}O_3(OH)_9$, it would be implied that the cyclic ether grouping resisted the action of cold acetic anhydride and pyridine. It is in any case clear that lagosin and its perhydro-derivative have the same number of reactive hydroxyl groups.

Lagosin and perhydrolagosin, examined as mulls, show a strong band at 852 cm.$^{-1}$, obviously analogous to that at 840 cm.$^{-1}$ in the spectrum of filipin. This latter had been interpreted [7] as due to " isoprenoid groupings," *i.e.*, presumably the R·CH=CR_2 out-of-plane deformation mode. As the band survives hydrogenation (in the case of lagosin at least), this is clearly not true. An alternative interpretation as due to a 1,2-epoxide grouping required consideration. Acetylation of either lagosin or perhydrolagosin completely removed the band, so that this hypothesis required the (reasonable) assumption of lability in the presence of acetic acid–acetic anhydride–pyridine, and could derive no support from the low acetyl values just mentioned. A reactive epoxide group, however, would also be expected to undergo reductive opening and lead to one more hydroxyl group in the perhydro-derivative, and it would be surprising if it resisted even the mild hydrogenation that gave perhydrolagosin. Nevertheless, further hydrogenation of perhydrolagosin was attempted in acetic acid at high concentration in the presence of much platinum. Absorption ceased after an uptake of 0·08 mol. at a rate less than that of benzoic acid under similar conditions. A reactive epoxide grouping is obviously absent; and this therefore also seems to be an incorrect assignment for the band, which is probably skeletal and connected with the $[CH(OH)\cdot CH_2]_5$ residue. The thiosulphate–phenolphthalein test [8] for reactive epoxide groups was also negative with both lagosin and perhydrolagosin; this is not, however, reliable in the presence of ester groups, which may be hydrolysed and absorb alkali.

Treatment of perhydrolagosin with triphenylmethyl chloride in pyridine failed to give a condensation product despite a search by counter-current distribution; some of the perhydrolagosin was recovered unchanged. As all the hydroxyl groups reacted with acetic anhydride and pyridine, it appeared probable that all were secondary.

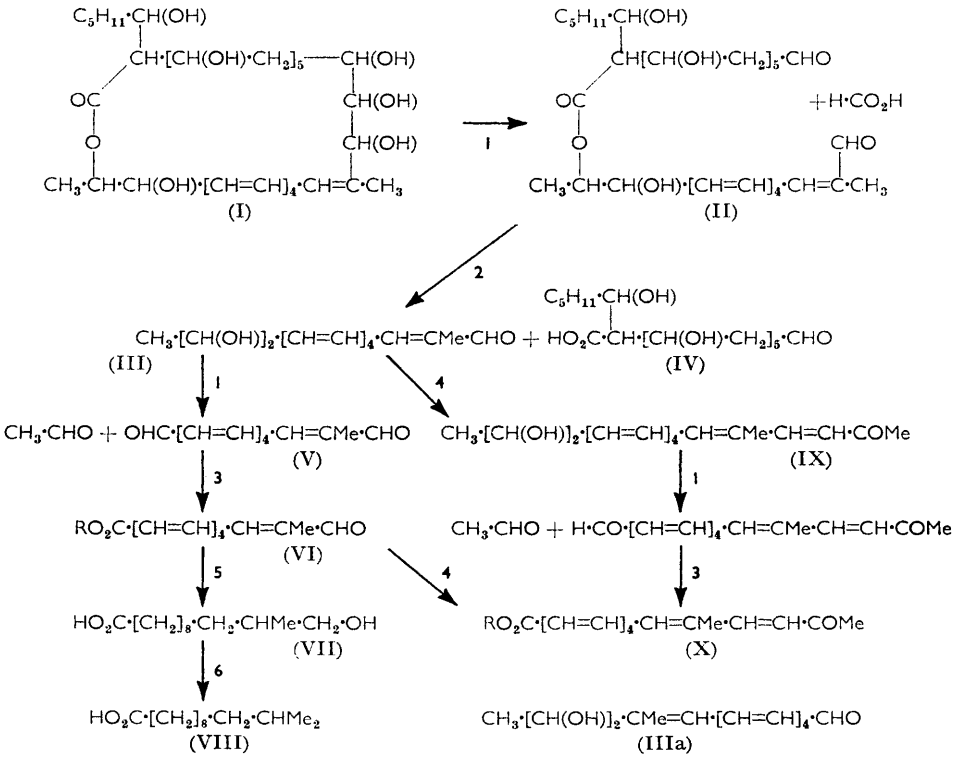
The Polyene Region.—Lagosin (and perhydrolagosin) reacted rapidly with periodate in acidic or in weakly alkaline solutions. One and two mol., respectively, were taken up within a few minutes and a further equivalent during several hours; side-reactions evident from the slow formation of iodine prevented the use of this reaction for equivalent-weight determination. The weakly alkaline solutions deposited a crystalline dicarbonyl compound, $C_{13}H_{14}O_2$, actually (V); the product(s) from the acidic fission, actually (II), were largely extractable by methylene dichloride, which on evaporation left a glassy residue. Mild alkaline hydrolysis converted this into a compound, $C_{15}H_{20}O_3$ (actually III) which underwent periodate oxidation to acetaldehyde, isolated as its 2,4-dinitrophenylhydrazone, and the C_{13} dicarbonyl compound (V) already obtained directly.

The dicarbonyl compound was oxidised by silver oxide and sodium hydroxide to an oxo-acid (actually VI), which resisted further, more vigorous, oxidation with the same

[7] Whitfield, Brock, Amann, Gottlieb, and Carter, *J. Amer. Chem. Soc.*, 1955, **77**, 4799.
[8] Ross, *J.*, 1950, 2257.

reagents. Hydrogenation of the dicarbonyl compound and the oxo-acid gave, respectively, a saturated glycol and a saturated hydroxy-acid. At first we were misled by the resistance of one carbonyl group to oxidation with silver oxide, which suggested a ketone, and by the bands at 1105 cm.$^{-1}$ in the two hydrogenation products, which are typical of secondary hydroxyl groups. Oxidation of the saturated glycol with chromic acid in acetone, however, gave a C_{13} crystalline dicarboxylic acid, while reduction of the hydroxy-acid with hydriodic acid and phosphorus gave 11-methyldodecanoic acid, identified by comparison with an authentic specimen. Clearly these products could only have been obtained from the pentaenedial (V). Condensation of this compound with acetone in the presence of ethoxide afforded confirmation, as the product obviously had a heptaenedione chromophore; we thank Professor B. C. L. Weedon for this helpful suggestion.

The C_{15} dihydroxy-aldehyde could now have two possible structures, (III) and (IIIa). Condensation with acetone in the presence of ethoxide gave a hexaene-ketone (IX), which was successively oxidised with periodate and silver oxide to the hexaene-keto-acid (X; R = H). Its methyl ester proved to be identical with the compound prepared from the ester of the C_{13} aldehydo-acid (VI) by condensation with acetone. As the methyl group of the latter had been securely located next to the aldehyde, rather than the acid, group by hydrogenation and reduction to 11-methyldodecanoic acid, rather than 2-methyldodecanoic acid, the orientational problem was solved and the C_{15} dihydroxy-aldehyde must have structure (III).



Reagents: 1, KIO_4. 2, KOH. 3, Ag_2O–NaOH. 4, Me_2CO–NaOEt. 5, H_2–Pd. 6, HI–P; then Al–Ni + NaOH.

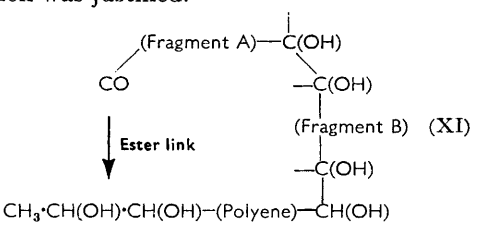
A number of other transformations of this part of the molecule were made, notably the preparation of 2-methyldodeca-2,4,6,8,10-pentaene-1,12-diol, which served as a useful model for the chromophore of lagosin itself. Unexpectedly, the α-glycol grouping in the

C_{15} dihydroxy-aldehyde was split by silver oxide in alkali, giving the aldehydo-acid (VI; R = H); we are not aware of a precedent for this reaction.

The C_{13} dialdehyde (V) has also been obtained from fungichromin by Cope and Johnson,[9] who proved its structure by oxidation and hydrogenation to 2-methyldodecanedioic acid. Silver nitrate and an excess of sodium hydroxide in their hands converted the dialdehyde into a diacid, whereas pre-formed silver oxide and sodium hydroxide in ours gave only the aldehydo-acid (VI; R = H), a surprising discrepancy.

The simplest interpretation of the degradation of lagosin by periodate and alkali successively to the dihydroxyaldehyde (III) was that the antibiotic was a macrocyclic lactone, with the whole polyene chain endocyclic. A partial formula (XI) indicates the position at this period.[2,3]

It was realised that a 1,2,3-triol grouping might have to be substituted for the two vicinal diol groups and fragment B, and that one of the postulated diol groups might be exocyclic rather than endocyclic if one hydroxyl group was in fact tertiary (see above). (At this time a $C_{41}O_{14}$ formula was still considered correct.) The macrocyclic structure was now confirmed directly. The product (II) obtained from lagosin and periodic acid showed both the ultraviolet spectrum of a pentaene-carbonyl compound, and (*inter alia*) the infrared band at 1725 cm.$^{-1}$ due to the ester linkage. It, and lagosin, were both treated with hydroxylamine hydrochloride and sodium ethoxide, and the alcoholic solutions were submitted to paper chromatography. That from the ester (II) gave a yellow spot, from the polyene fragment, and an invisible spot which became red on spraying with ferric chloride, due to the hydroxamic acid grouping. Despite the use of four different solvent systems, the yellow spot from lagosin and the red spot obtained on spraying proved inseparable. The attachment of the chromophore to the ester grouping *via* the main chain, and the rupture of that chain by periodate, were graphically revealed, and the simple interpretation was justified.



(XI)

The carboxyl group of lagosin evidently esterified one of the two hydroxyl groups present in the dihydroxy-aldehyde (III). First attempts to decide which was free involved treatment of lagosin with manganese dioxide at 20° and chloranil, *o*-chloranil, and tetrachlorodiphenoquinone under various conditions, it being expected that either one or two allylic secondary hydroxyl groups would be oxidised. The results were inconclusive. However, treatment of the ester (II) with manganese dioxide in chloroform at 20°, or with chloranil in chloroform under reflux, converted the spectrum from the type $[C{=}C]_5{\cdot}C{=}O$ into the type $O{=}C{\cdot}[C{=}C]_5{\cdot}C{=}O$, very like that of the dialdehyde (V). The new product was not that dialdehyde but an uncrystallisable substance eluted from alumina only by methanol. Unless acyl migration had occurred (and this seemed unlikely with such mild reagents) the allylic hydroxyl group in ester (II) must have been free, and that β to the polyene system must have been esterified in lagosin. Our conclusions about this part of the molecule were summarised in our first Note.[3]

The Side-chain.—At an early stage the presence of a *C*-pentyl group was established by nitric acid oxidation and paper chromatography of the steam-volatile products, as crystalline *p*-bromophenacyl hexanoate was isolated. A strong aldehydic smell after various alkaline treatments of lagosin and perhydrolagosin led to a suspicion that hexanal

[9] Cope and Johnson, *J. Amer. Chem. Soc.*, 1958, **80**, 1504.

was being formed, but this was confirmed, by the isolation of its 2,4-dinitrophenylhydrazone in 35% yield, only after we had heard [10] of the similar isolation of hexanal from fungichromin. A retroaldol fission was assumed and a 1-hydroxyhexyl side-chain could be located α to the carboxyl group.

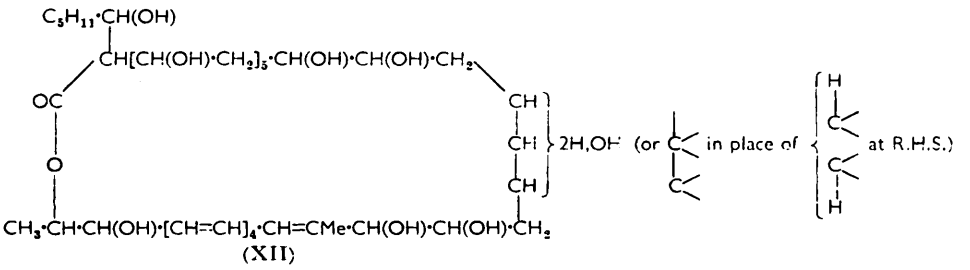
The Polyhydroxylic Region.—To learn about the rest of the molecule it seemed best first to establish the carbon skeleton; and this required removal of the large number of hydroxyl groups present. Some attempts were made to prepare a polytoluene-*p*-sulphonate from perhydrolagosin, but the amorphous product still contained hydroxyl groups (infrared spectrum) and gave no recognisable chromophore on treatment with sodium methoxide. Our main efforts involved reduction with hydriodic acid and phosphorus, a method little used (see Perlin and Purves [11]) with polyfunctional compounds since the classical work of Kiliani and Kleemann.[12] Like these authors, we obtained low yields of completely reduced materials. At first the total product from perhydrolagosin and periodate was reduced; even this gave a main fraction for which a structure that eventually proved correct was suggested by Professor Stenhagen (see below). More rationally, the dioxo-ester (II) was hydrolysed, the aldehyde (III) was removed by extraction, and the aqueous solution of a salt of acid (IV) was reduced successively with sodium borohydride, hydriodic acid–phosphorus, and Raney alloy. This gave a crude product which showed carbonyl bands attributable to a carboxylic acid and γ-lactone groups; the latter proved to be extremely stable towards further reduction. After separation of acidic and neutral fractions, the former still contained the lactone, while the latter consisted, from its infrared spectrum, largely of hydrocarbons. The mixture of acid and lactone was freed from polar contaminants by partition chromatography, esterified, and separated into ester and lactone fractions by chromatography on alumina. Finally it was hydrogenated, as the earlier sample had been found to contain unsaturated analogues, distilled, and sent to Professor E. E. Stenhagen. The elegant gas-chromatographic and mass-spectrographic work which led to the isolation of the main component (30% of the saturated ester fraction) and its tentative identification as methyl 2-hexyltridecanoate has been briefly described.[13] We prepared an authentic specimen of this compound by a conventional synthesis based on malonic ester from hexyl and undecyl alcohol. As commercial specimens of the hexyl alcohol needed were gas-chromatographically impure, the requisite hexyl bromide was prepared from crystalline sorbic acid. The final product proved to be identical in gas-chromatographic behaviour with that isolated from lagosin, and gave an almost identical (and exceedingly detailed) mass-spectrum. The very small differences were clearly due to residual impurities in the naturally derived sample.

Although the overall yield of pure methyl 2-hexyltridecanoate from the ester (II) was only 1·7%, there seems to be no reason to doubt the rationality of its formation. Much of the associated material was obviously chemically related to the saturated ester fraction, having its carboxyl group either lost by reduction to a methyl group or by decarboxylation, or present as a stable γ-lactone group. (In the product obtained directly from perhydrolagosin a much larger number of contaminants was present, and the ester finally isolated contained significant quantities of impurities which rendered the interpretation of its mass-spectrum somewhat tentative.) The hexyl group obviously came from the α-(1-hydroxyhexyl) group known to be present in lagosin (see above). The straight C_{11} chain is also independently proved by the evidence discussed below.

On the basis of partial formula (XI) and the earlier molecular formula for lagosin $C_{41}H_{66-70}O_{14}$, it was then possible to write a nearly complete structure (XII). (The lactone

[10] Cope, personal communication.
[11] Perlin and Purves, *Canad. J. Chem.*, 1953, **31**, 227.
[12] Kiliani and Kleemann, *Ber.*, 1884, **17**, 1296.
[13] Dhar, Thaller, Whiting, Ryhage, Ställberg-Stenhagen, and Stenhagen, *Proc. Chem. Soc.*, 1959, 154.

ring eliminates the acylic H_{70} formula.) This followed from the need to accommodate two, and not more than two, pairs of vicinal hydroxyl groups, no primary or tertiary hydroxyl group, and only three *C*-methyl residues; if the last restriction were relaxed some rearrangement of the C_5 residue between the vicinal glycol groupings would, of



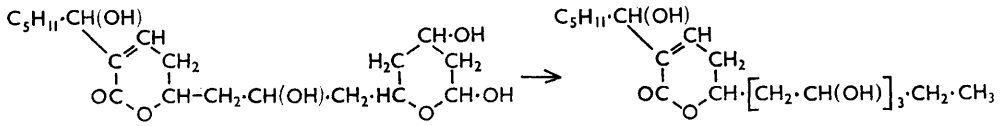
(XII)

course, be possible. Any such structure must, however, give, on periodic acid fission, a hydroxypimelic dialdehyde, a branched-chain isomer, or a cyclic analogue; and every attempt to isolate such a substance by, *e.g.*, counter-current distribution, or its transformation products, *e.g.*, pimelic acid after silver oxide oxidation and treatment with hydriodic acid and phosphorus, proved unsuccessful. These failures finally induced us to reopen the question of the molecular weight (see above). On the other hand, the formula $C_{35}H_{58}O_{12}$ equally led to a unique structure (I), which required the formation of formic acid in periodate fission; and this, as reported,[13] had not been found when fission was effected in aqueous dimethylformamide and the pH value of the solution was determined. When the fission of perhydrolagosin was effected in water, and the solution was extracted continuously with ether, formic acid ($\lessapprox$0·62 mol) was obtained, as compared with 0·85 mol. from glycerol under similar conditions. With this error rectified and the $C_{35}O_{12}$ formula acceptable crystallographically, structure (I) could be considered proved. This conclusion, however, involved acceptance of many negative arguments and drastic reductive degradation, and required confirmation.

The dioxo-ester (II) was hydrolysed to the dihydroxy-aldehyde (III) and the salt of a polyhydroxy-oxo-acid, which on the basis of these structures should be (IV). A compound obviously derived from this part of the molecule was obtained by concentration of the aqueous solutions and exhaustive extraction with butanol. After counter-current purification it was obtained as a glass, which showed no acidic properties and no infrared band suggesting a ketonic or aldehydic group. Its analysis and saponification value agreed with the formula $C_{19}H_{32}O_7$, which implies two double bonds or rings additional to the carbonyl (assumed lactone) grouping, and Kuhn–Roth oxidation gave a result (3·7% of *C*-methyl) implying that only one *C*-methyl group was present, presumably that of the 1-hydroxyhexyl side-chain.

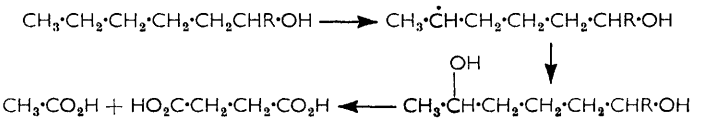
With the aim of further simplification, the removal of the (assumed) masked carbonyl group was examined. Huang-Minlon reduction gave no recognisable product, and sodium borohydride gave only an intractable boron complex. Condensation with ethanedithiol and treatment with Raney nickel, however, gave a total product (~5% of *C*-methyl) about half of which was obtained as one fraction after counter-current distribution. This compound, also neutral, gave two equivalents of volatile acid (6·7% as *C*-methyl) on Kuhn–Roth oxidation. Evidently the desired reaction, $-CO- \longrightarrow -CH_2-$, had taken place, and had generated a *C*-methyl group; it followed that the potential carbonyl group of the fission product had indeed been aldehydic, as required by structure (IV). The loss of two molecules of water from (IV) had not been expected, but lactonisation was obvious from the neutrality of the product isolated, and it is a reasonable *ad hoc* assumption that, under the fairly drastic conditions for solvent removal from the glassy product, one

of the two hydroxylic groups β to the lactone function might be dehydrated, giving, *e.g.*, structures (XIII) and (XIV) for the amorphous C_{19} lactones.



(XIII) (XIV)

It appeared that oxidation of these compounds might afford final confirmation of their structures, and hence that of acid (IV) and lagosin. These compounds, being stable to periodate and not polycyclic, must be without vicinal hydroxyl groups. When they, or indeed lagosin itself, were oxidised with dilute nitric acid, they should give no succinic (or higher dicarboxylic) acid. The oxidations were carried out under the usual vigorous conditions and the steam-volatile and the non-volatile fractions were separated; the acids from lagosin were esterified with and without the addition of a small quantity of glutaric acid to the acid fraction, and the esters were subjected to gas chromatography. It became clear that no glutaric acid (*i.e.*, $<0{\cdot}01$ mol.) was formed in the oxidation, and only a minute quantity (estimated at 0·015 mol.) of succinic acid. This could have been formed, inefficiently, from the 1-hydroxyhexyl side-chain, *e.g.*:



In similar conditions a substantial yield of succinic acid is to be expected from any molecule with two adjacent methylene groups.

The steam-volatile acids obtained provided further confirmation of structures (XIII) and (XIV). Each fraction was freed from nitric acid, etc., esterified, and subjected to gas–liquid chromatography. The mixture obtained from the lactone $C_{19}H_{32}O_7$ (XIII) contained the esters of the C_6, C_5, C_4, and C_3 acids in the ratio 1 : 0·55 : 0·38 : 0·33, while that from the lactone (XIV) contained the same esters in a ratio of 1 : 0·47 : 0·27 : 0·79. If the first mixture is derived from the 1-hydroxyhexyl side-chain, the second includes a much increased proportion of propionic acid, evidently derived from the ethyl group generated in converting (XIII) into (XIV). It follows that the main chain of lactone (XIII), and therefore of acid (IV), must terminate in the grouping $-CH(OH)\cdot CH_2\cdot CHO$. The postulated structures (IV) and (I) are again confirmed.[15]

The Relation between Lagosin and Other Antibiotics.—Lagosin is most closely related to fungichromin and filipin. For fungichromin Cope, Bly, Burrows, Ceder, Ciganek, Gillis, Porter, and Johnson [14] have recently proved a structure identical with that proposed by us [15] for lagosin in 1960. They used the same sequence of phosphorus–hydriodic acid reduction, gas-chromatographic purification, structure determination by mass-spectrography, and confirmation by total synthesis of the reduced product; but they applied it to a polyol containing the entire carbon skeleton of the antibiotic, instead of to the C_{19} polyhydroxy-acid we employed. At the cost of some practical difficulties in handling much larger molecules their method has two advantages over ours: it does not make use of, and so does not require, the vicinal glycol groups present in lagosin and fungichromin (but not in filipin); and it avoids the loss in yield observed when an acidic compound is used and due to partial reduction of the carboxyl residue. Obviously the completion of two independent proofs of the same gross structure adds to the confidence

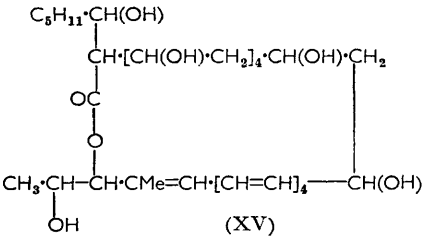
[14] Cope, Bly, Burrows, Ceder, Ciganek, Gillis, Porter, and Johnson, *J. Amer. Chem. Soc.*, 1962, **84**, 2170.

[15] Dhar, Thaller, and Whiting, *Proc. Chem. Soc.*, 1960, 310.

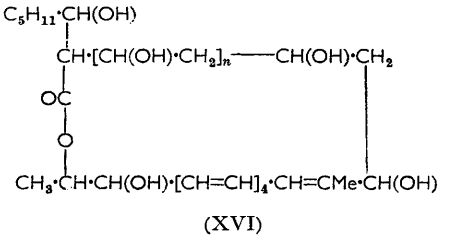
that can be felt in each, but there remains the question whether lagosin and fungichromin are identical. Published data suggest that fungichromin (originally formulated as $C_{35}H_{60}O_{13}$) forms a monohydrate more readily than lagosin does in our hands. Initial impressions, hard to state precisely but connected with rate of solution and crystallisation, were of some difference. Again, perhydrofungichromin melts a little higher than perhydrolagosin. Professor Cope and his collaborators were unable to distinguish between the two by *X*-ray methods or by paper chromatography, but they found fungichromin more lævorotatory in methanol (α_D $-176°$ against $-160°$, both $\pm 4°$, *i.e.*, $\Delta M_D = 54$—$162°$). Our rotation data in dimethylformamide obtained by photoelectric polarimetry showed more than the fluctuations expected instrumentally for each substance, and statistically the difference was hardly significant, but mean values were $-284{\cdot}5°$ and $-279{\cdot}5°$ for fungichromin and lagosin, giving ΔM_D *ca.* 34° in the same sense.

Unfortunately lagosin is undoubtedly polymorphic, and in addition it may well be readily convertible into other stereoisomers about the polyene chain; thus not all the specimens supplied to us have been paper-chromatographically homogeneous. These difficulties complicate the comparison, and we are compelled to agree with Professor Cope [14] that the question must be left open,* since the predicted changes in physical properties including optical rotation) consequent upon an inversion at one centre of asymmetry remote from the chromophore, especially in the interior of the $[CH(OH)\cdot CH_2]_5$ grouping, may be very slight. Should the two antibiotics ultimately be proved identical, the name " fungichromin " has priority.

For filipin we suggested in 1959 [13] that the C_{30} formula [7] was too small; and, after the C_{33} partial structure (XV) had been advanced by Berkoz and Djerassi,[16] we proposed [15] that filipin was a C_{35} molecule (XVI; $n = 5$), *i.e.*, monodeoxylagosin. Its infrared spectrum is in fact much more similar to (in fact, almost indistinguishable from) that of lagosin than would be likely if, *e.g.*, the lactone ring were closed on the allylic hydroxyl group. Djerassi, Ishikawa, Budzikiewicz, Shoolery, and Johnson,[17] while accepting most of the changes from (XV) to (XVI; $n = 5$), preferred the C_{37} variant (XVI, $n = 6$). They assumed that amorphous filipin polyacetate was a pure compound and compared the intensity of two regions in the nuclear magnetic resonance spectrum. The method was in effect standardised by using amorphous lagosin polyacetate, taken to be $C_{35}H_{48}O_2(OAc)_{10}$. When supplying the sample from which his polyacetate was made, we informed Professor Djerassi that our preparations of the latter did not give acetoxyl analyses in agreement with this formula; our experiments are now described in detail.



(XV)



(XVI)

Furthermore, Cope *et al.*[14] have since stated that " Acetyl analyses for an amorphous polyacetate of fungichromin indicated the presence of nine acetoxy-groups." Even Djerassi and Berkoz reported [16] low acetyl values for filipin polyacetate. That these antibiotics give, on acetylation, products containing less than the proportion of acetoxyl

* In our opinion two natural products of differing origin should be considered different unless, and until, they can be proved not to differ by criteria which would, beyond reasonable doubt, be sensitive to each of the smallest changes in structure or configuration that can be postulated.

[16] Berkoz and Djerassi, *Proc. Chem. Soc.*, 1959, 316.
[17] Djerassi, Ishikawa, Budzikiewicz, Shoolery, and Johnson, *Tetrahedron Letters*, 1961, 383.

naïvely expected is thus an experimental fact; as they are doubly β-hydroxy-esters it is hardly surprising. Djerassi *et al.* calculated [17] that for a deca-acetate of lagosin the ratio of high-field to low-field protons should be 58 : 20 = 2·90; corresponding values for a nona-acetate would be 57 : 19 = 3·00, while a dehydro-nona-acetate would give a ratio of 53 : 21 = 2·52. The experimental value was 2·92 ± 0·02, which was described as " demonstrating the reliability and accuracy of our procedure." All that can, in fact, be deduced from the ratio observed is that lagosin forms, along with an unknown, and possibly negligible, quantity of the deca-acetate, the other two products in a roughly calculable ratio (7 : 1 to 3 : 1; the range widens if some deca-acetate is present or if any systematic error at all is allowed for). These data can be reconciled with our analytical results without difficulty. Of course, if it could be assumed that the three products are formed to exactly the same extent on every acetylation, and to the same extent with filipin as with lagosin, the analogous experimental value for filipin (3·12 ± 0·02) would indeed constitute evidence in favour of formula (XVI; $n = 6$) (required, for complete acetylation, 62 : 20 = 3·10; for the other possibilities 61 : 19 = 3·21 and 57 : 21 = 2·71) as against (XVI; $n = 5$) (required, 57 : 19 = 3·00, 56 : 18 = 3·11 and 52 : 20 = 2·60). In practice this would be an unjustified assumption. However, the American workers did pronounce in favour of formula (XVI; $n = 6$) on the basis of these results. To economise hypotheses we favoured the alternative view that the two antibiotics had the same carbon skeleton, and we attempted to test this by comparing the absorbance ($E^{1\%}_{1\,cm.}$) value for filipin with those for lagosin and fungichromin, on the assumption that their extinction coefficients should be almost identical. Each of the three antibiotics was crystallised several times and the mean of the last three absorbance values at 3560 Å, carefully determined on the same instrument at the same time, was calculated. For lagosin and fungichromin, both available in quantity, these were 1454 and 1481, respectively, corresponding to extinction coefficients of 97,600 and 100,000 (mean 98,800). For filipin, available in much more limited amounts, the mean value was 1472, corresponding to $\varepsilon = 96{,}400$ if formula (XVI; $n = 5$) is correct, or 104,000 on the alternative hypothesis. These figures constitute in our view evidence in favour of our 1960 structure [15] (XVI; $n = 5$) which at any rate outweighs the nuclear magnetic resonance data on the amorphous polyacetates; a definitive conclusion requires work on filipin comparable with that on lagosin [3,13,15] and fungichromin [14] (the degradative methods of Cope *et al.*[14] would be appropriate).

Stereochemistry.—The ultraviolet spectrum of lagosin indicates that, as isolated, it is probably an all-*trans*-compound (high intensity and absence of a *cis*-peak). The infrared band at 1008 cm.$^{-1}$ (Nujol) is due to the *trans*-$[CH{=}CH]_4$ grouping. A 16-mono-*cis*-configuration cannot be excluded, and it is possible that *cis*-isomers may be formed, reversibly and to a small extent, on warming; this might account for the unusual behaviour of lagosin in requiring for dissolution much more solvent than is needed to maintain it in solution once dissolved.

The 26- and 27-hydroxyl groups were shown [18] to be in an *erythro*-relation by oxidation to *erythro*-αβ-dihydroxybutyric acid. Fungichromin has been shown [14] to have its corresponding hydroxyl groups in a similar relationship and their absolute configuration has been proved; as inversion so near the chromophore would produce a large shift in molecular rotation, the configurations in lagosin are undoubtedly the same.

The conformation of lagosin is of interest. If we assume an all-*trans*, all-*transoid* structure, which is necessitated at least as the dominant form by the electronic spectrum, the saturated region is, according to models, about the shortest chain which can strainlessly join the two ends of the polyene section. This gives a molecule in which a bow-and-bowstring effect is probable, so that the hydroxyl groups are rigidly oriented. If the polyvinyl alcohol region is syndiotactic, rather than isotactic, intramolecular hydrogen

[18] Berry and Whiting, following note.

bonding would be impossible. In contrast, rupture of the ring, as in the ester (II), would in any case allow such bonding. There is, in fact, a very considerable difference in polarity between lagosin, which is extremely hydrophilic, and ester (II) which can readily be extracted by methylene chloride. [Structure (XVI; $n = 5$) [15] would give filipin similarly a taut polyvinyl alcohol section, whereas (XVI; $n = 6$) [17] would allow more flexibility.]

Experimental

Lagosin was supplied as a pale yellow solid which after being washed with light petroleum and dried had $E^{1\%}_{1\,cm.}$ ~1200 at 3560 Å and was stable for many months, at least in sealed ampoules in nitrogen at −6° in the dark. It was crystallised by prolonged heating under reflux with methanol with exclusion of light and air until it dissolved, the solution being then evaporated to half its bulk and allowed to cool. The slowness of dissolution and crystallisation are characteristic; the crystals obtained in this way were thin needles, $E^{1\%}_{1\,cm.}$ rising on successive crystallisations to 1450—1480 at 3558 Å. Lagosin had m. p. 230—240° (decomp.), $[\alpha]_D^{20}$ −146° ± 5° (*c* 0·369 in MeOH), $[\alpha]_D^{24}$ −279·5° ± 3° * (*c* 0·479 in dimethylformamide) [Found: C, 62·2; H, 8·8; O, 28·95; N, 0·0; *O*-Me, 0·55; *C*-Me (micro), 7·15. $C_{35}H_{58}O_{12}$, as (I), requires C, 62·7; H, 8·7; O, 28·6; *C*-Me, 6·7%). A sample dried at 20°/10 mm. lost no weight when heated for 12 hr. at 60°/10^{-1} mm.; the latter conditions were used for the specimen analysed. The ultraviolet absorption is tabulated on p. 853; in the infrared region it had $\nu_{max.}$ (Nujol mull) 3270—3310, 1710, 1136, 1010, and 852 cm.$^{-1}$. On paper chromatograms (benzene–dimethylformamide–water; 100 : 100 : 75), lagosin is easily detected by its vivid green fluoroescence. Lagosin is insoluble in acetone, ethyl acetate, and chloroform, almost insoluble in water or propan-1-ol, slightly soluble in methanol and ethanol, and more soluble in aqueous alcohol than in either pure solvent. It dissolves freely in pyridine or dimethylformamide; distributed between ethyl acetate and ethylene glycol, lagosin was found entirely in the polar phase, whereas fairly equal distribution was observed between acetone and glycerol. Solutions in alcohol lost their absorption maxima rapidly on addition of dilute mineral acid, and a solution in glacial acetic acid lost 18% of its initial absorption intensity during 68 hr. at room temperature. The dark solutions obtained showed no selective absorption. Treatment with dilute alkali had no effect on the absorption spectrum of lagosin, but rapid loss of biological activity takes place.

Hydrogenation of lagosin.

Solvent	Catalyst	H_2 (mol.) absorbed (N.T.P.)	Yield (%)
Ethanol	Pd–$CaCO_3$ (2%)	4·4	54
,,	Lindlar catalyst [19]	4·75	57
,,	Adams catalyst [20]	4·75	60
Methanol	,, ,,	4·75	57
Acetic acid	,, ,,	5·3	73
Methanol	Rh–carbon (5%)	4·85	60

Perhydrolagosin.—Hydrogen uptake is recorded in the annexed Table; as a rule the lagosin was incompletely soluble in the solvent (*ca.* 600 c.c./g.) used, dissolving as hydrogenation proceeded. After filtration, evaporation of the solvent *in vacuo* left a colourless glass which was dissolved in hot dioxan and treated with charcoal. Perhydrolagosin crystallised slowly, on cooling, as acicular prisms, its m. p. rising slowly on repeated crystallisation from 148—153° to 160—162° when rhodium was used as catalyst; the rotation was almost constant at $[\alpha]_D^{20}$ +3·5—3·8° (in MeOH) [Found: OMe, 0·72; *C*-Me, 5·05; H_2O (Karl Fischer), 0·12%. C,H,O values tabulated in Table 1). Perhydrolagosin had $\lambda_{max.}$ 2160 Å (ε 100) (saturated ester grouping) and showed no rapidly rising absorption below 2000 Å, which indicates the absence of a C=C chromophore. It had $\nu_{max.}$ (in Nujol) 3400—3250, 1725, 1450, 1380, 1310, 1140, and 852 cm.$^{-1}$, $\nu_{infl.}$ 1685 cm.$^{-1}$.

Crystalline perhydrolagosin, dried over fresh P_2O_5 at 20° for 24 hr. (86·44 mg.), was heated

* A photoelectric polarimeter with tungsten light and a filter corresponding visually to the D lines was used for the dimethylformamide value.

[19] Lindlar, *Helv. Chim. Acta*, 1952, **35**, 446.
[20] Adams, Voorheer, and Shriner, *Org. Synth.*, Coll. Vol. I (1941), p. 463.

at 100° for 48 hr. at 0·05 mm. A loss of weight of 8·03 mg. was observed, consistent with the initial composition $C_{35}H_{68}O_{12},0{\cdot}79C_4H_8O_2$.

A solution of crystalline perhydrolagosin, dried at 20° for 1 month (50 mg.), was dissolved in methanol (0·3 c.c.) and chromatographed on Apiezon L at 100°. A peak with retention time (R.T.) 3·75 min. was observed; an authentic solution of dioxan in methanol showed, beside the solvent peak, one with R.T. = 3·8 min. After labelling each with *o*-xylene and comparison of peak areas, the composition $C_{35}H_{68}O_{12},0{\cdot}62C_4H_8O_2$ for this specimen could be deduced.

Perhydrolagosinic Hydrazide.—Perhydrolagosin (0·5 g.) in ethanol (8 c.c.) was treated with hydrazine hydrate (1 c.c.). After 4 days at room temperature a white gelatinous material separated (0·258 g.). "Recrystallised" from ethanol (15 c.c.) it melted at 141—144°. No crystals could be seen under the microscope except at 1000 magnification. For analytical results see Table 2.

Peracetyl-lagosin.—Lagosin (1·66 g.) was dissolved in dry pyridine (7 c.c.), and acetic anhydride (15 c.c.) was added. Nitrogen was passed through the mixture for 10 min. After 48 hr. in the dark at 20° the solution was evaporated *in vacuo* and the residue was dissolved in ether (75 ml.). After washing with water (4 × 25 c.c.) and drying, evaporation left a yellow glass (2·6 g.), $[\alpha]_D^{20}$ +40° ± 2° (*c* 1·014 in MeOH), $\lambda_{max.}$ 3590, 3400, and 3240 Å ($E_{1\,cm.}^{1\%}$ 654, 663, and 426). This was purified by counter-current distribution in water–methanol–benzene–hexane (1 : 9 : 5 : 5); after 53 transfers, the plot of optical density against tube number showed one symmetrical peak. Fractions 16—24, the central region, gave a yellow glass (1·42 g.), $[\alpha]_D^{20}$ +51·3° ± 4° (*c* 0·5852 in MeOH), $E_{1\,cm.}^{1\%}$ 870, 878, and 570 at the above wavelengths, $\nu_{max.}$ (in CS_2) 2915, 2860, 1739, 1331, 1222, and 1017 cm.$^{-1}$ (Found: C, 60·3; H, 7·5; acetyl, Table 2).

Peracetylperhydrolagosin.—(*a*) Perhydrolagosin (375 mg.), pyridine (3 c.c.), and acetic anhydride (5 c.c.) were set aside for 48 hr. at 20°. The product was isolated as above, and the crude product was chromatographed on alumina (grade H; deactivated with 5% of 10% acetic acid); benzene–ether (9 : 1) eluted the acetate as a colourless glass, $[\alpha]_D^{20}$ +18·7° ± 2° (*c* 1·1228 in MeOH) (Found: C, 60·05; H, 8·5; acetyl, Table 2. $C_{55}H_{88}O_{22}$ requires C, 59·9; H, 8·05%). It had a vague spectrum of appreciable intensity; when the very slight absorption contribution from perhydrolagosin and that of isopropyl acetate (10 mol.; appreciable only below 2200 Å) were subtracted, broad maxima were obtained at 2025, 2175, 2400, and 2585 Å (ε 1550, 830, 730, and 810, respectively, for *M ca.* 1100). If these are attributed to the chromophores AcO·C=C, C=C·CO_2R, C:C·C(CO_2R):C, and −C=C·C=C·CO_2R with ε 10,000, 10,000, 20,000, and 25,000, these would have to be present to the extent of, respectively, 15%, 8%, 3%, and 3%, amounting to 0·4 double bond per molecule. On microhydrogenation 199·1 mg. of the polyacetate in the presence of 33·2 mg. of pre-reduced platinic oxide in ethanol (2 c.c.) absorbed 1·156 c.c. (N.T.P.) of hydrogen, corresponding to 0·29 mol.

(*b*) Peracetyl-lagosin (182·5 mg.) was hydrogenated in methanol (35 c.c.) over platinic oxide (25·5 mg.). The uptake was equivalent to 19·6 c.c. (N.T.P.; after correction for the PtO_2 reduced), equivalent to 5·2 mol.; the amorphous product (182 mg.) had $[\alpha]_D^{20}$ +15° ± 1° (*c* 2·147 in MeOH). The two specimens had identical infrared spectra (CS_2), including $\nu_{max.}$ 2933, 2857, 1742, 1367, 1232, 1170, 1022, and 955 cm.$^{-1}$.

Uptake of periodate.

	Acid solutions		Alkaline solutions			Acid solutions	
Time (min.)	Lagosin	Perhydro-lagosin	Lagosin	Perhydro-lagosin	Time (min.)	Lagosin	Perhydro-lagosin
0·25	0·79	0·71	2·04	1·35	15·0	1·27	1·28
0·33	0·85	—	—	—	60	1·76	—
0·5	0·90	0·87	—	1·57	75	—	1·60
1·0	1·01	—	2·60	1·94	300	—	1·45
5·0	—	1·13	—	2·72	900	2·03	—

Periodate Titration of Perhydrolagosin, etc.—Uptakes tabulated are the mean of two or three determinations, on a basis of M = 681 for lagosin; a correction of up to 12% may be needed for dioxan content of the perhydrolagosin used. Uptake values in the range 1·42—2·06 were obtained when after pre-treatment with sodium hydroxide the fission was effected in acidic solution for 0·25—5 min.

Equivalent-weight Determination of Lagosin.—This was attempted, after some failures, by

direct titration in 50% ethylene glycol at 80—85° in nitrogen. After each addition, a sharp rise in pH was observed, followed by a drift to a steady value; when these steady values were plotted against the volume added, inflexions were observed and equivalents could be calculated (Found: Equiv., 765, and in smaller-scale runs *ca.* 800—850. $C_{35}H_{68}O_{12}$ requires 680; $C_{35}H_{68}O_{12},C_4H_8O_2$ requires Equiv., 768).

2-*Methyldodecanedioic Acid*.—Perhydrolagosin (1·186 g.) in methanol (60 c.c.) was treated successively with aqueous 0·1N-sodium hydroxide (15 c.c.), water (60 c.c.) and aqueous 0·1M-sodium metaperiodate (60 c.c.). After 10 min. water was added and the mixture was extracted with methylene dichloride. Evaporation of the extract left a residue (308 mg.) which was shaken with silver oxide (from 2 g. of silver nitrate) in 1 : 9 aqueous methanol (25 c.c.) containing potassium hydroxide (150 mg.). Hydrochloric acid was added in excess and the precipitate filtered off and washed; isolation with ether gave an acid (162 mg.) which after several recrystallisations from hexane had m. p. 72—74° (Cope and Johnson [9] give m. p. 73—74·5° for the DL-acid).

12,13-*Dihydroxy*-2-*methyltetradeca*-2,4,6,8,10-*pentaenal* (III) (*Preparative Method*; with M. BERRY).—Lagosin (20 g.) was dissolved in boiling oxygen-free methanol (2·2 l.). The cooled solution was divided into two equal parts, and each was treated with periodic acid (27 g. of monohydrate in 400 c.c. of water) for 30 sec., then quenched by addition of ethylene glycol (100 c.c.). To each, water (2·5 l.) was added, and the mixtures were extracted with methylene chloride (500 c.c., then 6 × 250 c.c.). The combined extracts were washed with water, dried, and evaporated, leaving a glassy residue of the polyhydroxy-ester (II). This was dissolved in methanol (1 l.) and treated with potassium hydroxide (8 g.) in water (200 c.c.) (both solvents oxygen-free). After 5 min. water (2·5 l.) was added and the mixture was extracted with ethyl acetate (750 c.c., then 6 × 400 c.c.). After washing and drying, the solvent was evaporated at 20 mm. The residue crystallised from ethyl acetate as orange-yellow plates, m. p. 115—120° (4·17 g.; a further 0·6 g., m. p. 110—120°, was obtained from mother-liquors). An analytical specimen of the *aldehyde* had m. p. 117—121° (Found: C, 71·95; H, 7·95. $C_{15}H_{20}O_3$ requires C, 72·55; H, 8·05%), $\lambda_{max.}$ (in $CHCl_3$) 3740 and 3900 Å (ε 78,000 and 76,600, respectively), $\nu_{max.}$ (in $CHCl_3$) 3623, 2907, 1623, 1579, 1383, 1003, and 874 $cm.^{-1}$.

When the reaction times allowed for the oxidative cleavage and alkaline hydrolysis were increased, the yields fell; the above procedure resulted from small-scale investigations of both stages with spectroscopic assay.

The aldehyde gave an *oxime*, m. p. 185° (from ethyl acetate) (Found: C, 68·45; H, 7·85; N, 5·4. $C_{15}H_{21}NO_3$ requires C, 68·4; H, 8·05; N, 5·3%).

Oxidation. (*a*) The dihydroxy-aldehyde (200 mg.) in methanol (20 c.c.) was treated with aqueous 0·1425M-periodic acid (5 c.c.). After 15 min. the mixture was diluted with water and extracted with ether, the dried extract was evaporated, and the residue was chromatographed on deactivated alumina. The 2-methyldodeca-2,4,6,8,10-dodecapentaenedial (V) (130 mg.) formed needles, m. p. 145—147°, sublimes at $110/10^{-2}$ mm. (Found: C, 77·2; H, 7·05. Calc. for $C_{13}H_{14}O_2$: C, 77·2; H, 6·95%) (Cope and Johnson [9] give m. p. 140—144°).

(*b*) The dihydroxy-aldehyde (200 mg.) was dissolved in 1-methylpyrrolidin-2-one (10 c.c.) and treated with aqueous 0·1425N-periodic acid (5 c.c.). Nitrogen was passed through the solution and into aqueous 2,4-dinitrophenylhydrazine hydrochloride. The derivative which separated was collected, chromatographed, and crystallised from aqueous methanol; it had m. p. and mixed m. p. with acetaldehyde 2,4-dinitrophenylhydrazone, 164—167°.

Oxidation of Lagosin in Alkaline Solution.—Lagosin (310 mg.), methanol (20 c.c.), aqueous N-sodium hydroxide (2 c.c.), and water (10 c.c.) were warmed to 50° for 10 min., a clear dark solution being obtained. After cooling to 20°, water (30 c.c.) and aqueous 0·1M-sodium metaperiodate (20 c.c.) were added. After 10 min. the precipitate was collected, dried, and chromatographed on deactivated alumina, giving 2-methyldodeca-2,4,6,8,10-pentaenedial (V) (64 mg.) as needles, m. p. 145—147°, undepressed on admixture with a specimen obtained as above.

11-*Methyl*-12-*oxo*-2,4,6,8,10-*docosapentaenoic Acid* (VI; R = H).—The pentaenedial (150 mg.), freshly precipitated and washed silver oxide (from 1·5 g. of silver nitrate), and potassium hydroxide (50 mg.) were shaken for 24 hr. in 1 : 9 aqueous methanol (80 c.c.). The solution was filtered and the residue washed with aqueous methanol. The solution was made alkaline with aqueous sodium carbonate, and most of the methanol was evaporated under reduced

pressure in nitrogen. The neutral fraction was removed with methylene chloride, and the aqueous layer acidified and again extracted with methylene chloride. The last extract was shaken with 2N-sodium carbonate; acidification now gave the *oxo-acid* (114 mg.) which on crystallisation from chloroform–hexane formed needles, m. p. 195—198°, sublimes at 150°/10^{-2} mm. (Found: C, 71·35; H, 6·55. $C_{13}H_{14}O_3$ requires C, 71·55; H, 6·45%), $\lambda_{max.}$ (in $CHCl_3$) 2700 (infl.), 2790, 3590, 3780, and 3990 Å (ε 3400, 3500, 65,000, 111,000, and 105,000, respectively), $\nu_{max.}$ (in $CHCl_3$) 1674, 1615, 1104, 1042, 1004, and 872 $cm.^{-1}$. Its *methyl ester* (VI; R = Me) was prepared in 1% methanolic sulphuric acid at 20°; after chromatography on 1000 parts of alumina deactivated with 5% of 10% acetic acid and crystallisation from benzene–hexane it formed brownish needles, m. p. 161—163° (capilliary), sublimes at 110—120°/10^{-2} mm. (Found: C, 72·2; H, 6·5. $C_{14}H_{16}O_3$ requires C, 72·4; H, 6·95%), $\lambda_{max.}$ (in $CHCl_3$) 2700, 2785, 3215, 3590, 3780, and 3990 Å (ε 2900, 2800, 38,000, 78,000, 131,000, and 129,000, respectively), $\nu_{max.}$ 1701, 1663, 1610, 1342, 1296, 1124, and 1003 $cm.^{-1}$.

11-*Methyldodecanoic Acid*.—The above acid (218 mg.) was hydrogenated over 5% palladium–charcoal (200 mg.) in methanol (150 c.c.) (uptake 134 c.c. at N.T.P., 6·0 mol.). The oily residue (223 mg.) obtained on evaporation gave a single spot on paper chromatography in ethanol–ammonia–water (80 : 5 : 15) and had an infrared spectrum as expected for a saturated hydroxy-acid. This material (169 mg.) and red phosphorus (500 mg.) were heated under reflux for 7 hr. with hydriodic acid (5 c.c.; *d* 1·72) and propionic acid (5 c.c.). Most of the latter was removed by distillation and the residue was extracted with methylene chloride, which was washed with water and evaporated. The residue was dissolved in 2N-sodium hydroxide (20 c.c.), and nickel–aluminium alloy (1 g.) was added slowly; the mixture was finally heated on a water-bath for 1·5 hr. The acid fraction was isolated with ether and steam-distilled; the steam-volatile acid (65 mg.) was isolated with ether and distilled; the main fraction crystallised and, recrystallised from pentane, had m. p. 25—37° (17 mg.). Further crystallisation from aqueous methanol, then methanol, raised the m. p. to 37—40°, undepressed on admixture with an authentic specimen of m. p. 39—41°.

2-*Methyldodeca*-2,4,6,8,10-*pentaene*-1,12-*diol*.—The dialdehyde (95 mg.) was dissolved in methanol (24 c.c.) and treated with sodium borohydride (22 mg.) at 20° under nitrogen in the dark. After 10 min. more borohydride (19 mg.) was added and the precipitate was collected and crystallised from aqueous ethanol; the diol (44 mg.) formed yellow plates, m. p. 155—162° (Cope and Johnson [9] give m. p. 160—164°), $\lambda_{max.}$ (in EtOH) at 2420, 3040, 3180, 3335, and 3515 Å (ε 2860, 23,000, 51,000, 105,000, and 107,000, respectively). More vigorous reaction gave a product that showed bands at 2880 and 3025 Å not present for the diol itself.

5-*Methyloctadeca*-3,5,7,9,11,13,15-*heptaene*-2,17-*dione*.—The pentaenedial (55 mg.) in acetone (6 c.c.) was treated with ethanolic 5% potassium hydroxide (1·4 c.c.) and set aside for 45 min. at 20°. Benzene was added, and the solution was washed with dilute hydrochloric acid and water, dried (Na_2SO_4), and evaporated. When the residue was chromatographed on deactivated alumina the main red band yielded the *dione*, which formed orange-red needles, m. p. 152° (cap.), from benzene–hexane (Found: C, 80·5; H, 8·05. $C_{19}H_{22}O_2$ requires C, 80·8; H, 7·85%). In benzene it had $\lambda_{max.}$ 4085, 4305, and 4580 Å (ε 76,000, 123,000 and 121,000, respectively).

5-*Methyl*-15-*oxopentadeca*-3,5,7,9,11,13-*hexaen*-2-*one*.—The C_{13} dihydroxy-aldehyde (100 mg.) was treated in acetone (5 c.c.) with ethanolic 5% potassium hydroxide (2·5 c.c.). After 45 min. at 20° the solution was poured into water and extracted with ethyl acetate. The extract was dried and evaporated, leaving the uncrystallisable C_{18} dihydroxy-ketone (110 mg.); this was treated in methanol (25 c.c.) with 0·1M-sodium metaperiodate solution (5 c.c.). After 15 min. water was added and the product was isolated with ethyl acetate. After chromatography on deactivated alumina the *keto-aldehyde* formed reddish needles (68 mg.), m. p. 142—143° (Found: C, 79·2; H, 7·7. $C_{16}H_{18}O_2$ requires C, 79·3; H, 7·5%), $\lambda_{max.}$ (in $CHCl_3$) 3100, 3915, 4145, and 4385 Å (ε 4940, 65,000, 107,000, and 105,000, respectively), $\nu_{max.}$ (in $CHCl_3$) 1669, 1582, 1361, 1140, 1109, 1008, 995, and 966 $cm.^{-1}$.)

Methyl 11-*Methyl*-14-*oxo*-2,4,6,8,10,12-*pentadecahexaenoate* (X; R = Me).—(*a*) The above keto-aldehyde (40 mg.) in 1 : 9 aqueous methanol (20 c.c.) containing potassium hydroxide (20 mg.) was shaken with silver oxide (from 0·5 g. of silver nitrate) for 24 hr. at 20°. The red acidic product (25 mg.) had m. p. 199—204° but failed to crystallise well; it showed $\nu_{max.}$ (in $CHCl_3$) 1678, 1622, 1582, 1359, 1104, and 873 $cm.^{-1}$ and the expected visible and ultraviolet absorption maxima. Its *methyl ester* (18 mg.) was prepared in methanolic sulphuric acid at

20°; after chromatography on alumina it formed yellow needles, m. p. 156—158° after a phase-change to plates at 137° (Found, after sublimation: C, 75·8; H, 7·45. $C_{17}H_{20}O_3$ requires C, 74·95; H, 7·4%).

(*b*) Methyl 11-methyl-12-oxo-2,4,6,8,10-docosapentaenoate (7 mg.) in acetone (1 c.c.) was treated with 3N-sodium methoxide solution (0·2 c.c.); after 45 min. at 20° the mixture was acidified and the neutral fraction was isolated with methylene dichloride. After chromatography the ester (X) (5 mg.) had m. p. 153—156°, undepressed on admixture with a specimen obtained by method (*a*) and having an identical ultraviolet absorption spectrum.

12,13-*Isopropylidenedioxy*-2-*methyl*-2,4,6,8,10-*tetradecapentaenal*.—The dihydroxy-aldehyde (300 mg.), dry acetone (10 c.c.), and anhydrous cupric sulphate (600 mg.) were shaken in the dark for 24 hr. The mixture was filtered and the acetone was evaporated; the residual *derivative* was chromatographed, and then separated from light petroleum as yellow needles (240 mg.), m. p. 78—79° (Found: C, 74·95; H, 8·15. $C_{18}H_{24}O_3$ requires C, 75·0; H, 8·35%), $\nu_{max.}$ (in $CHCl_3$) 1684, 1623, 1379, 1359, and 1171 cm.$^{-1}$. Attempted oxidation with silver oxide in aqueous-alcoholic potassium hydroxide led to no acidic material, the starting material being recovered in good yield.

Methyl 2-*Hexyltridecanoate*.—(*a*) The C_{34} dioxo-ester (II) (5 g.; see below) in methanol (45 c.c.) was treated with potassium hydroxide (600 mg.) in water (15 c.c.). After 4 hr. water was added and the C_{15} dihydroxy-aldehyde was removed by extraction with methylene dichloride; a small quantity of residual polyene-containing material was extracted with butanol. The aqueous layer was treated with sodium borohydride (1·2 g.) in water (12 c.c.). After 45 min. the solution was just acidified with acetic acid and evaporated to dryness *in vacuo*. Hydriodic acid (80 c.c.; *d* 1·7) and red phosphorus (1 g.) were added and the mixture was heated under reflux for 6 hr. Dilution, filtration, and isolation with chloroform gave an oil which showed strong absorption at 1778 cm.$^{-1}$ as well as at *ca.* 1720 cm.$^{-1}$ and was therefore heated for a further 12 hr. with hydriodic acid (40 c.c.) and phosphorus (0·5 g.), but with little improvement. The product (1·8 g.) was treated with Raney alloy (5 g.) and 2N-sodium hydroxide (25 c.c.), the alloy being added slowly at 20°, and the mixture was then heated 2 hr. at 100°. The resultant material was separated into acidic (800 mg.) and neutral fractions; the former was chromatographed on silica gel (100 g.) impregnated with aqueous 2-methoxyethanol (1 : 9), with light petroleum as eluant. The first 30 c.c. gave an oil (700 mg.) which was esterified with methanolic sulphuric acid. The crude ester still contained some lactone, having bands at 1739 and 1778 cm.$^{-1}$; it was freed completely from this impurity, though with considerable loss, by repeated chromatography on alumina. The ester (145 mg.) was shaken with hydrogen in the presence of platinic oxide (35 mg.) in methanol (uptake 20 c.c.), isolated, and distilled [b. p. 160° (bath temp.)/0·05 mm.].

This material was sent to R. Ryhage, S. Stälberg-Stenhagen, and E. Stenhagen, who reported that when submitted to gas–liquid chromatography on Reoplex 400 at 202·5° (efficiency ~5000 theoretical plates) it proved to be a complex mixture. The largest component (about 30% of the total) formed a symmetrical peak with a retention time of 9·2 min. This was isolated, rechromatographed on the same column, and examined in the mass spectrometer.

(*b*) Sorbic acid, m. p. 133·5—134° (35 g.), was hydrogenated and the resultant acid esterified with methanol and sulphuric acid. Working up and distillation gave methyl hexanoate (29·9 g.), b. p. 51—51·5°/14 mm., n_D^{20} 1·4045. Reduction with lithium aluminium hydride (6·54 g.) gave hexan-1-ol (17·7 g.), b. p. 69—70°/16 mm., n_D^{20} 1·4158. Treatment with hydrobromic acid (29 c.c.) and sulphuric acid (7 c.c.) gave n-hexyl bromide (13·9 g.) which was used to alkylate diethyl malonate; the product (10·4 g.) had b. p. 152—154°/19 mm., and (like the bromide) was gas-chromatographically pure. Undecan-1-ol (9 g.; Eastman, gas-chromatographically pure) was converted into the iodide (9·35 g.), b. p. 76—78°/0·01 mm., by treatment with iodine (6·75 g.) and red phosphorus (1·2 g.) at 180°.

A mixture of diethyl hexylmalonate (8 g.) and undecyl iodide (9·25 g.) was added to a solution of sodium (0·754 g.) in butanol (15 c.c.), and the mixture was heated under reflux for 12 hr. The neutral product was isolated and heated with an excess of ethanolic 20% potassium hydroxide at 100° for 5 hr. The resultant acid was isolated and heated, first at 10 mm., then at 0·01 mm., to 180—190° until decarboxylation was complete. After cooling aqueous sulphuric acid (20%) was added, and steam-volatile material was removed; the non-volatile acid was isolated with ether, dissolved in light petroleum, and extracted with a N-solution of potassium hydroxide in 80% methanol. Isolation of the acid from the aqueous-methanolic

solution and distillation gave the (±)-*acid*, b. p. 148—149°/0·01 mm., m. p. 29—30·5° (from acetone) (Found: C, 76·4; H, 13·0. $C_{19}H_{38}O_2$ requires C, 76·45; H, 12·8%). Its *amide*, prepared *via* the acid chloride, crystallised from methanol and had m. p. 110—111° (Found: C, 76·75; H, 12·85; N, 4·95. $C_{19}H_{39}NO$ requires C, 76·7; H, 13·2; N, 4·7%). Its *methyl ester*, prepared in 5% methanolic sulphuric acid at 65°, had b. p. 121/0·01 mm. (Found: C, 77·4; H, 12·95. $C_{20}H_{40}O_2$ requires C, 76·85; H, 12·9%).

The comparison between the two specimens of the ester was made at Uppsala by gas chromatography and by mass spectrometry. The two mass spectra were identical except for a very weak band in the naturally derived sample at $m/e = 326$, suggesting the presence of a trace of the next higher homologue (chemically an intelligible result on structure [I]). They were dominated by peaks at $m/e = 158$ and 228, interpreted as the ions $R{\cdot}CH{=}C(OMe)(OH)^+$ where $R = C_6H_{13}$ and $C_{11}H_{23}$, and at 87. Analogous bands were present in the spectrum of methyl 2-hexyldecanoate used as a control.

An essentially similar degradation was carried out earlier on perhydrolagosin, the periodate, borohydride, hydriodic acid–phosphorus, and Raney alloy treatments being effected without the isolation of intermediates. A hydrogenation step was not included. The methyl ester mixture was more complex and contained methyl decanoate and methyl stearate, as well as esters of C_{19} and C_{20} acids and components of larger molecular weight; however, the methyl 2-hexyltridecanoate fraction was the largest, and that structure was already proposed at this stage.

C_{34} *Polyhydroxy-ester* (II).—A solution of lagosin (5·5 g.) in methanol (600 c.c.) was treated with aqueous 0·1425M-periodic acid (220 c.c.) for 15 min. at 20°. Water (1·5 l.) was added, and the solution was extracted with methylene chloride until the extract was almost colourless. The combined extracts were washed well with water, dried, and evaporated, finally at 20°/0·1 mm. The yellow glass was distributed between ethylene glycol and ethyl acetate (50 transfers); fractions 18—35 showed similar absorption spectra, the plot of optical density against tube number showing a single smooth band. They were combined, water was added, and the ethyl acetate layers were repeatedly washed with water, dried, and evaporated. The residue, a yellow glass, had $\lambda_{max.}$ (in $CHCl_3$) 3720 and 3890 Å ($E^{1\%}_{1\,cm.}$ 914 and 887), and $\nu_{max.}$ (in $CHCl_3$) 3401, 2929, 1727, 1667, 1616, 1575, and 1002 $cm.^{-1}$ (Found: C, 61·5; H, 8·7. $C_{34}H_{54}O_{11}$ requires C, 63·9; H, 8·0%; thus water and/or ethylene glycol was probably still present). Attempted reduction with sodium borohydride gave an amorphous solid soluble only in 1-methylpyrrolidin-2-one, in which its absorption spectrum indicated a pentaene grouping. Acetylation as described for lagosin polyacetate gave a yellow glass.

Oxidation of this C_{34} ester (34 mg.) with chloranil (26 mg.) in boiling chloroform (6 c.c.) for 6 hr. gave a product which was chromatographed on deactivated alumina (5%) in chloroform. After removal of an unidentified band with maxima at 2930, 3745, 3935, and 4150 Å a fraction was eluted with chloroform–ether–ethanol (43 : 5 : 2) which had maxima at 3080, 3940, and 4145 Å and an inflexion 3765 Å.

C_{19} *Polyhydroxy-oxo-lactone* (XIII).—The C_{34} ester (II) (5 g.) was hydrolysed as described under methyl 2-hexyltridecanoate (*a*). After removal of the C_{15} dihydroxy-aldehyde (III) the aqueous layer was acidified with acetic acid and reduced to 20 c.c. *in vacuo*. The concentrate was repeatedly extracted with butanol, and the extract was washed with a little water and evaporated at 0·1 mm., giving a light yellow gum (2 g.). Material so obtained (4·5 g.) was purified by distribution (101 transfers) between 1 : 1 methanol–water and ether; a plot of solid content against tube number indicated a number of minor constituents and one major component, isolated by evaporation of fractions 62—85 as a colourless glass (2·51 g.) insoluble in aqueous alkali [Found: C, 61·45; H, 8·55; *C*-Me, 3·64, 3·86. $C_{19}H_{32}O_7$ requires C, 61·3; H, 8·6%; this corresponds to (IV) $- 2H_2O$. Analogous formulæ from lagosin = $C_{35}H_{56}O_{12}$ would be $C_{19}H_{30}O_7$ which requires C, 61·55; H, 8·15, or $C_{19}H_{32}O_8$ which requires C, 58·75; H, 8·3%. Structure (XIII) requires *C*-Me, 4·04%].

C_{19} *Polyhydroxy-lactone* (XIV).—The foregoing lactone (902 mg.) in dry dioxan (15 c.c.) was treated with ethane-1,2-dithiol (3 c.c.; excess), and dry hydrogen chloride was passed through the solution for 1 hr. After 72 hr. at 20° the dioxan and ethanedithiol were removed *in vacuo* and the gummy residue was washed with water and dried in a desiccator. 3 : 2 v/v Ethanol–dioxan (60 c.c.) and Raney nickel (7 g.) were added and the mixture was heated under reflux for 10 hr. The solids were removed by filtration and washed with ethanol; the filtrate and washings were treated with charcoal, filtered, and evaporated. The residue (475 mg.) was dissolved in chloroform and chromatographed on silica gel (50 g.), giving as the main fraction

a glass (411 mg.) [Found: C, 61·2; H, 9·2; *C*-Me, 5·06. Calc. for $C_{19}H_{34}O_6$, as (XIV): C, 63·7; H, 9·8; *C*-Me, 8·4%]. Thus this product was impure, probably containing much unreduced oxo-lactone. A similar reduction of 2·026 g. of the latter, with 15 g. of Raney nickel, gave a crude product which, instead of being chromatographed, was subjected to counter-current distribution between 50% aqueous methanol and ether. After 100 transfers, fractions 59—78 gave the main fraction which was isolated as a gum (970 mg.) (Found: *C*-Me, 6·72%). This material was used in the oxidations described below.

Oxidation of Lagosin by Nitric Acid.—(*a*) The antibiotic (2 g.) was treated with concentrated nitric acid (40 c.c.) at 20° for 1 hr., then heated under reflux for 1 hr. Water (200 c.c.) was added, and the mixture was distilled until 100 c.c. of distillate had been collected; this was diluted to 200 c.c. and again distilled. After one repetition of this process the final distillate was extracted with ether and the extract was washed with water, dried, and evaporated. Diazomethane was added to the residue; chromatography on Apiezon L (2 m. at 120°) indicated the presence of hexanoic, valeric, and butyric acid.

The non-volatile fraction from the oxidation was evaporated *in vacuo*. Water was added and distilled off three times. The final residue (930 mg.) was dissolved in methanol (20 c.c.) and divided into two equal parts. To one, glutaric acid (111 mg., 0·3 mol.) was added; each was treated with an excess of ethereal diazomethane, and the solutions were used for gas–liquid chromatography with tritolyl phosphate (2 m.; 20%) at 140°. Both solutions showed a very small peak in the same position as authentic dimethyl succinate, but only one a (large) peak for dimethyl glutarate. No other peak was present, and the quantities of the succinate and glutarate originally present were estimated at 0·015 and $<0·01$ mol., respectively.

(*b*) In a similar experiment lagosin (3 g.) was oxidised with concentrated nitric acid (50 c.c.) at 20° for 1 hr., then under reflux for 3 hr. The solution was diluted to 200 c.c., and 100 c.c. was steam-distilled off. After two repetitions the acidic material present (2·65 millimoles) was identified by paper chromatography (Whatman No. 1) with butanol and aqueous ammonia (1 : 1); spots (Bromocresol Green) at R_F 0·57, 0·43, 0·29, and 0·19 (very weak) were identified, by comparison with standards, as hexanoic, valeric, butyric, and propionic acid. Similarly the derived hydroxamic acids were chromatographed with benzene–water–acetic acid (4 : 4 : 3) and with pentyl alcohol–acid–water (4 : 1 : 5); again the spots agreed with the C_6—C_3 normal acids. Finally the bulk of the acidic product (1·8 millimoles) was chromatographed in streak form on Whatman paper (No. 3 MM) with the butanol–ammonia mixture, and the main hexanoic zone was cut out and eluted with the same solvent. The eluate was made alkaline and evaporated to dryness, then treated with ethanol (0·5 c.c.) and 4-bromophenacyl bromide (19 mg.), made just acid, and heated to 70° for 2 hr. The solution was again evaporated, pyridine (0·1 c.c.) and ether (5 c.c.) were added, and the mixture was warmed for 5 min.; ether was added in excess and the whole was passed down a short column of active alumina. The eluate was evaporated and the residue was recrystallised twice from dilute ethanol, giving 4-bromophenacyl hexanoate, m. p. and mixed m. p. 70—71° (Judefind and Reid [21] report 71·6°).

Oxidation of the Two C_{19} *Lactones by Nitric Acid.*—The polyhydroxy-lactones before (XIII; 0·45 g.) and after (XIV; 0·46 g.) removal of the carbonyl group, each purified by counter-current distribution, were each treated with 10 c.c. of water and 15 c.c. of concentrated nitric acid. Each was heated to 100° for 45 min., diluted with water (200 c.c.), and distilled till 100 c.c. was collected. Each steam-distillate was treated with zinc dust (10 g.) and concentrated sulphuric acid with stirring. When the reactions were complete each mixture was continuously extracted with ether. Each extract was treated with diazomethane, and the methyl esters were chromatographed on Apiezon L (2 m.) at 100°. The peaks were cut out and weighed; after correction for the molar response factors they indicated C_6—C_3 acids in the ratio 100 : 55 : 35 : 33 from lactone (XIII), and 100 : 47 : 27 : 79 from lactone (XIV). In the conditions used, methyl acetate was not separable from ether.

The non-steam-volatile fractions again gave only a minute peak in the position of dimethyl succinate.

Identification of Formic Acid.—Perhydrolagosin (1·05 g.) was shaken for 24 hr. with a solution of sodium metaperiodate (0·8 g.) in water (30 c.c.), partly dissolving. The resultant suspension was extracted continuously with ether for 24 hr., and the extract was titrated, with shaking and in an atmosphere of nitrogen, with 0·104M-sodium hydroxide (9·5 c.c. required,

[21] Judefind and Reid, *J. Amer. Chem. Soc.*, 1920, **42**, 1048.

0·62—0·68 mol., according to the dioxan content assumed). In a similar experiment, glycerol (0·192 g.) and sodium metaperiodate gave an extract requiring 16·8 c.c. of alkali, equivalent to 0·83 mol. Both the neutralised solutions were evaporated to dryness *in vacuo*, and the residues, and sodium formate (122 mg.), were separately treated with methanolic 4% sulphuric acid (10 c.c.), then after 24 hr. with methanolic 1·15M-hydroxylamine (10 c.c.) (obtained from the hydrochloride and an excess of sodium methoxide. After 1 hr. the three reaction mixtures were subjected to chromatography on the same piece of Whatman No. 1 paper with pentan-1-ol–water–acetic acid (4 : 1 : 5) as solvent, the paper being sprayed with ferric chloride. A blank experiment, without glycerol or perhydrolagosin, was run simultaneously. The three formic acid derivative preparations each showed a spot at R_F 0·24—0·26 of comparable intensity; the mixture derived from perhydrolagosin showed an additional unidentified spot at R_F 0·59. The blank experiment led to no spot.

Attempted Hydrogenation of Perhydrolagosin.—Perhydrolagosin (100·4 mg.) in acetic acid (3 c.c.) was stirred in hydrogen with platinic oxide (30·1 mg.). The uptake was extremely slow and ceased after 7 hr., then amounting to 0·267 c.c. (0·08 mol.). In a comparative experiment benzoic acid (17·4 mg.) had absorbed 0·63 ml. (0·19 mol.) after 7 hr., and uptake continued.

Attempted Triphenylmethylation of Perhydrolagosin.—(*a*) A solution of triphenylmethyl chloride (100 mg.) and perhydrolagosin (200 mg.) in pyridine (5 c.c.) was heated for 3 hr. to 100°. Water (10 c.c.) was added and the resultant suspension was extracted with ether and filtered. The ethereal extract was washed with dilute hydrochloric acid, dried, and evaporated, leaving triphenylmethanol, m. p. and mixed m. p. 162°. The residue (170 mg.) was crystallised from dioxan, m. p. 153—155°, undepressed on admixture with perhydrolagosin.

(*b*) Triphenylmethyl chloride (2 g.), perhydrolagosin (0·9 g.), and pyridine (10 c.c.) were heated for 9 hr. to 100° and the mixture was cooled, diluted with water, and extracted with butanol. The butanol extract was washed with hydrochloric acid and water and evaporated *in vacuo*. The viscous residue was treated with ethylene glycol; a solid (450 mg.) remained insoluble; on crystallisation it gave triphenylmethanol (150 mg.). The glycol solution was distributed between ethylene glycol and ethyl acetate (50 transfers) and the fractions were assayed by ultraviolet absorption spectrometry. Only the least polar showed aromatic absorption, and they yielded more triphenylmethanol. From the most polar fractions perhydrolagosin (350 mg.) was recovered. It was concluded that perhydrolagosin was practically inert to triphenylmethyl chloride.

Hydroxamic Acid from Lagosin; Proof of Macrolide Structure.—Lagosin (46 mg.) was treated with a methanolic solution of hydroxylamine from 5N-sodium methoxide (2·25 c.c.) and 1·67N-hydroxylamine hydrochloride (5 c.c.). After 4 hr. at 20° the solution was evaporated under reduced pressure and the residue was dissolved in ethanol–water (3 : 2). The ethanolic solution was submitted to paper chromatography with (*a*) ethanol–aqueous ammonia (*d* 0·88)–water (16 : 1 : 3), (*b*) butanol–acetic acid–water (4 : 1 : 5), (*c*) ethyl acetate–acetic acid–water (3 : 1 : 1), and (*d*) benzene–acetic acid–water (9 : 7 : 9). Each chromatogram showed a yellow spot due to the polyene chromophore, R_F 0·70, 0·80, 0·73, and 0·0, respectively. When sprayed with alcoholic ferric chloride, each yellow spot became bright red. In a similar experiment with the C_{34} ester (II) the product was chromatographed with system (*b*) and with benzene–acetic acid–water (2 : 2 : 1), the yellow spots having R_F 0·86 and 0·65, respectively; when sprayed with ferric chloride solution, however, these yellow spots did not become red; instead red spots appeared at R_F 0·69 and 0·41, indicating that in ester (II) the hydroxamic acid residue and the polyene chromophore were in different molecules after treatment with hydroxylamine.

Analyses.—Acetyl and *C*-methyl determinations (except the *C*-methyl value for lagosin) were our own semimicro-estimations; hydrogenation, periodate titrations, and equivalent-weight determinations were also our own. Other analyses were done commercially.

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